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Monographs on Medicinal Plants along Ganga River
Solanum nigrum L.



**GNAMAMI
GANGE**



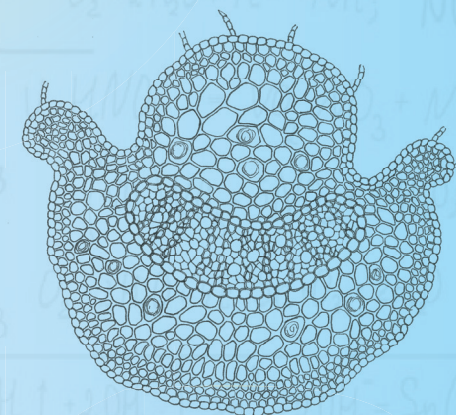
Monographs on Medicinal Plants
along Ganga River

Solanum nigrum L.

Focusing on

Botanical, Phytochemical, Scientific Validation and
Insilico Analysis Including Medicinal Importance
and Soil Properties

Volume 08



Monographs on Medicinal Plants along Ganga River

Solanum nigrum L.

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Botanical, Phytochemical, Scientific validation and Insilico
analysis including Medicinal importance and Soil properties

Volume 08

Sponsored by



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Department of Water Resources, River
Development & Ganga Rejuvenation,
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Implemented by Patanjali Organic Research Institute Pvt. Ltd. (PORI)
Food & Herbal Park, Village-Padartha, Laksar Road, Haridwar-249407, Uttarakhand (India)



ABOUT THE EDITOR

Dr. Acharya Balkrishna, Co-founder of Patanjali Organic Research Institute, is a highly ascetic entrepreneur with a diverse personality who is a specialist in Yoga, Ayurveda, Sanskrit language, Indian sacred books, and the Vedas. Dr. Balkrishna has become a significant source of inspiration for Traditional Medicinal Practitioners and a globally recognized celebrity after dedicating his life to the resurrection of ancient healing and living practices. His maverick leadership as Co-Founder & Managing Director of Patanjali Ayurved Limited, along with overseeing Patanjali Food and Herbal Park and Divya Pharmacy, has propelled Ayurveda into a global business phenomenon with a massive following. Additionally, he has been decorated with prestigious awards such as 'Ayurveda Expert', 'Manav Ratan', 'Bharat Gaurav', 'Indian of the Year', 'Ten Versatile and Dynamic Young Men of India', 'Bheeshma Pusaka', 'Lokmanya Tilak', and 'Transformational Business Leader' for his exceptional knowledge, passion, and service to mankind. According to a study published by Stanford University in the USA and Elsevier in Europe, Dr. Balkrishna has been recognized among the top 2% of scientists worldwide for his research on Ayurveda and Yoga.

With his early age passion for plants, he has become the most renowned & respected herbal specialist for health and nutrition. For the research of novel herbal medication formulations, he explored four rare plants: 'Sanjeevani', 'Somlata', 'Swarnakshiri', 'Swarnadraka', and 'Astavavarga plants'. He has taken many initiatives for Biodiversity conservation, presently working for the establishment of Patanjali Herbal Garden & herbarium, working on the compilation of a unique multivoluminous project i.e., 'World Herbal Encyclopaedia', containing the descriptions of ~50,000 medicinal plant species with the largest collection of plant paintings and drawings. This is being done to strengthen traditional medicine systems of the world. Additionally, Dr. Balkrishna has made significant contributions to the socio-economic development of tribal communities and the upliftment of rural communities with FPOs, CLFs, and SHGs. Furthermore, his endeavors have been directed towards exploring and conserving floral diversity, phytochemical and insilico analysis, investigating soil geochemistry, understanding complex plant-microbe relationships, and monitoring water quality from Gomukh to Gangasagar.

He has published more than 400 research articles in national and international journals received around 20 patents and authored more than 200 books on Yoga, Ayurveda, Agriculture, Herbal Medicine, and Information Technology and edited more than 40 unpublished ancient Ayurveda manuscripts. With

the vision of ensuring universal health for the last two decades, more than 1.5 million patients with several persistent, chronic, and non-communicable diseases have been effectively treated. He established Patanjali Ayurved College and the University of Patanjali with the humanitarian goal of assisting youngsters in achieving their goals and serving the country. Additionally, he has been involved in various government and non-government initiatives. He is also a key proponent of agricultural transformation through organic practices, working to boost agricultural productivity, increase farmer income, and ensure equitable access to a safe, affordable, and nutritious diet year-round. Apart from this, Dr. Balkrishna is actively participating in driving agricultural transformation through the 'Patanjali Farmer Samridhi Programme' by well-trained staff and around one lakh trainers. His generous personality is also reflected through his actions like helping the nation with emergency needs like post-disaster needs assessment, providing free shelter, food, and education to orphan children, and free OPD services at Patanjali Yogpeeth. With his humanitarian attitude, Dr. Balkrishna continues his phenomenal journey of making world records, uplifting mankind through medicine & lifestyle improvement, reviving sustainable agriculture, and preserving nature's gifts in the form of literature and a sustainable living approach.





Vision of Honourable Prime Minister

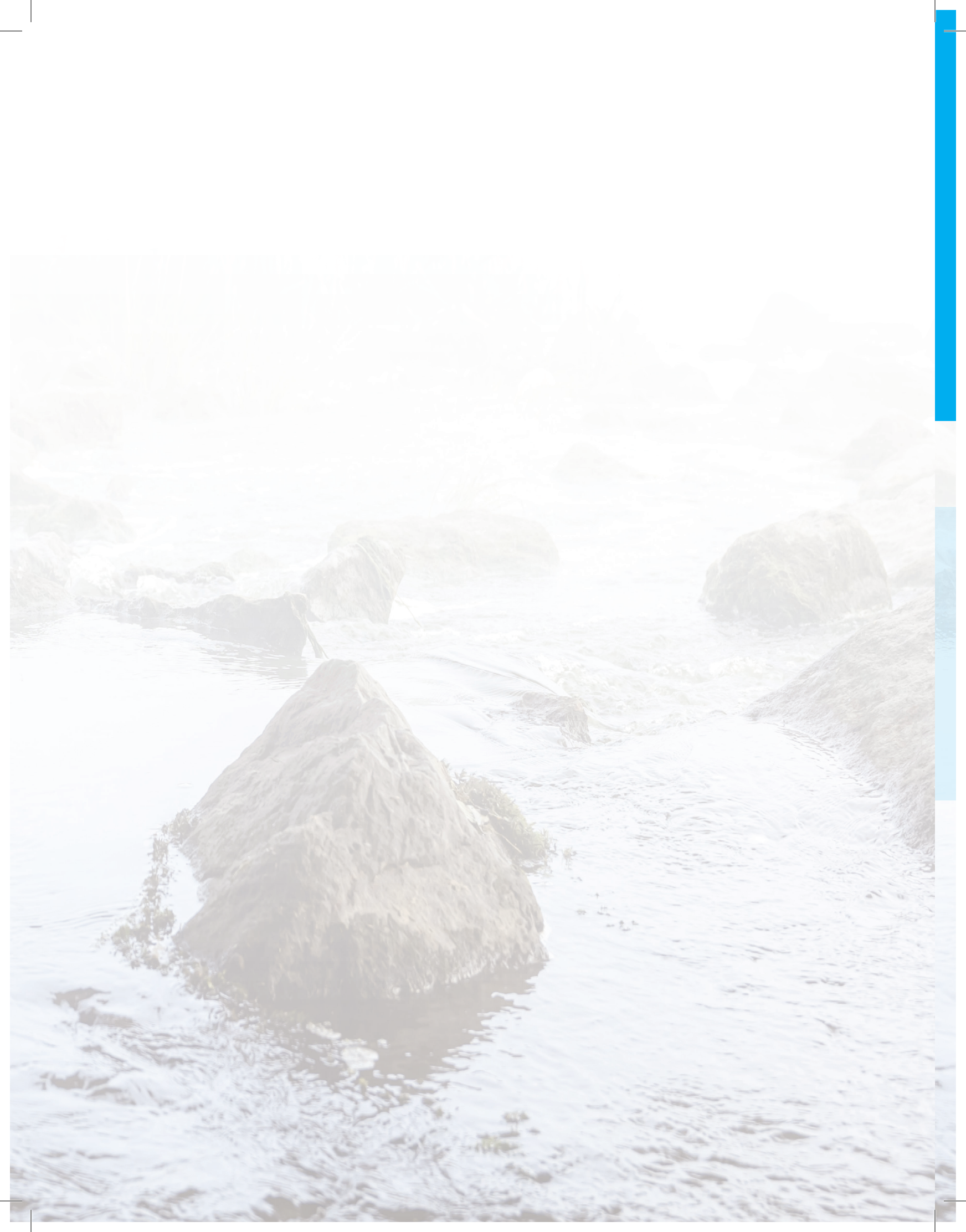
Shri Narendra Modi on Medicinal Plants



1. India's rich biodiversity of medicinal plants can provide affordable healthcare solutions.
2. Medicinal plants are nature's gift to humanity; we must protect and utilize them wisely.
3. Let us revive and promote the use of medicinal plants in everyday life.
4. India's biodiversity of medicinal plants is a treasure trove waiting to be explored.
5. Medicinal plants have been an integral part of our cultural heritage, offering natural remedies for various ailments.
6. The knowledge embedded in our traditional systems like Ayurveda and the use of medicinal plants can address global health challenges sustainably.
7. We must promote research and innovation in harnessing the potential of medicinal plants for healthcare and economic development.



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Vision of Union Minister for Jal Shakti

Shri Chandrakant Raghunath Patil

“

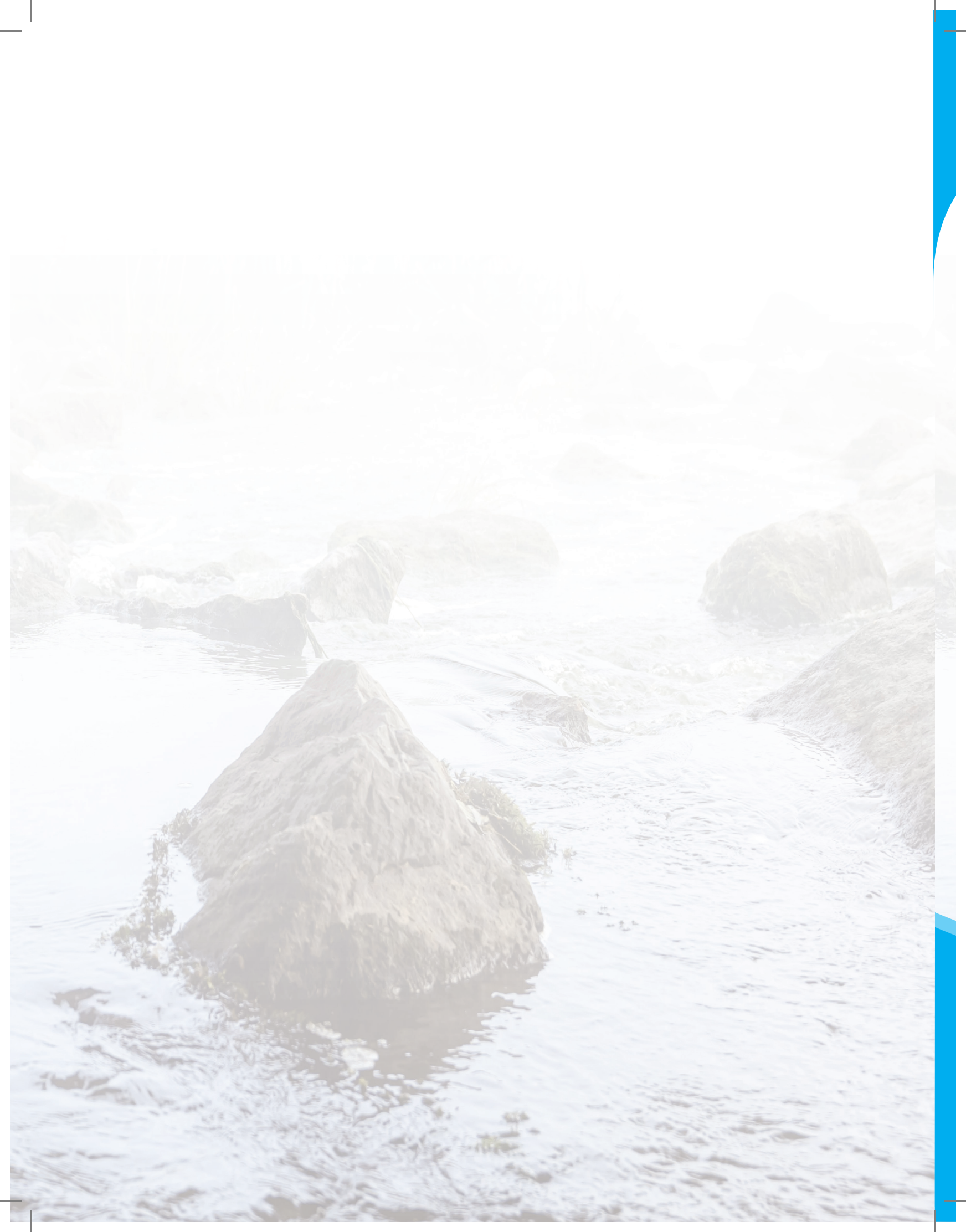
1. We will understand the importance of water, conserve it and ensure rich water resources for future generations.
2. Conservation and enhancement of water resources of our country is a sacred goal. I will work with my dedication and devotion to achieve this.
3. Proper access to water is a strong step towards women's empowerment.
4. Jal Shakti is Nari Shakti, as women are the most affected by water scarcity due to their household responsibilities in rural India.

”

<https://pib.gov.in/PressReleaseDetail.aspx?PRID=2024324>

<https://www.newsonair.gov.in/c-r-patil-emphasizes-water-access-as-essential-for-womens-empowerment-at-catch-the-rain-2024-workshop-in-delhi/>

<https://www.indiatvnews.com/gujarat/will-transform-jal-shakti-into-rashtra-shakti-cabinet-jal-shakti-minister-c-r-patil-after-portfolio-appointment-pm-modi-2024-06-10-936272>



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Uttarakhand Forest Department & Biodiversity Board

Uttar Pradesh Forest Department & Biodiversity Board

Bihar Forest Department & Biodiversity Board

Jharkhand Forest Department & Biodiversity Board

West Bengal Forest Department & Biodiversity Board

Ganga Task Force (GTF)

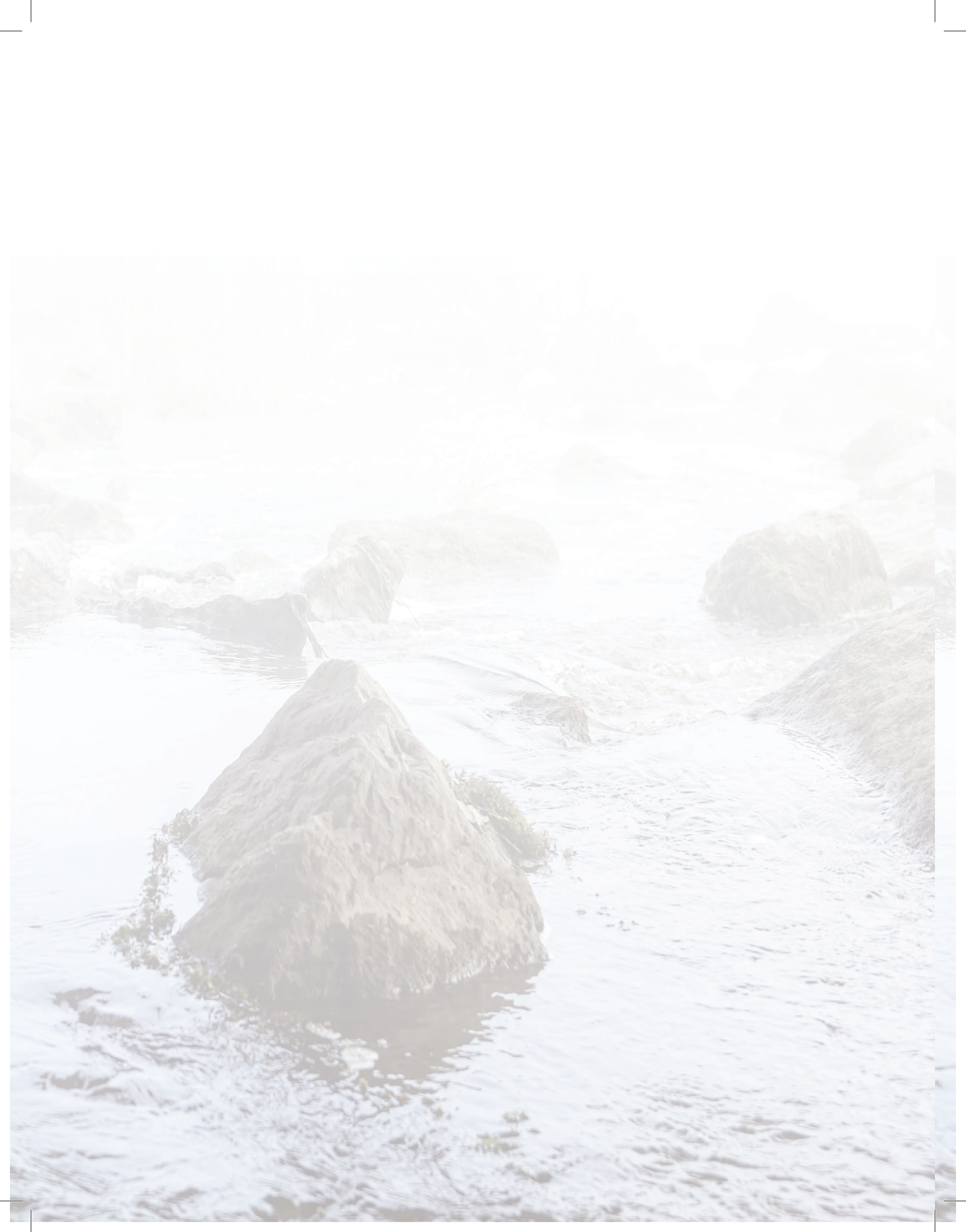


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NATIONAL MISSION FOR CLEAN GANGA



सत्यमेव जयते
75
आज़ादी का
अमृत महोत्सव

भारत सरकार
जल शक्ति मंत्रालय
जल संसाधन,
नदी विकास और गंगा संरक्षण विभाग
GOVERNMENT OF INDIA
MINISTRY OF JAL SHAKTI
DEPARTMENT OF WATER RESOURCES,
RIVER DEVELOPMENT & GANGA REJUVENATION



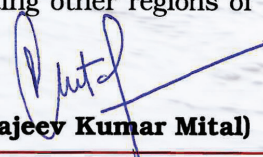
MESSAGE

The Ganga, a river deeply revered in our culture, is both a source of spiritual inspiration and home to diverse ecosystems, including riparian buffers along its banks that connect terrestrial and aquatic systems. These buffers play a crucial role in supporting amphibious life, stabilizing the riverbanks, and maintaining ecosystem services. Therefore, under the "Aviral Ganga" approach for biodiversity conservation and "Arth Ganga" for enhancing livelihoods, a project on floral diversity has been initiated through Namami Gange Mission – II, in collaboration with Patanjali Organic Research Institute (PORI), Haridwar, Uttarakhand.

The project "Scientific Exploration of Floral Diversity Near the Ganga Riverbanks for Ethnobotanical Purposes along with their Conservation and Economic Development of the Region via Skill Development Programs" aims to provide valuable scientific insights into the region's ethnobotanical wealth while promoting sustainable conservation practices. The 18-month study, conducted from Gaumukh to Gangasagar along the Ganga banks, reveals the floral profile, ethno medicinal aspects, applications, livelihood perspectives, and the role of biodiversity in ecosystem resilience, highlighting the symbiotic relationship between conservation and socio-economic development. Findings presented through publications, including project reports, monographs, exploration of ethno medicinal plants, and plant wealth along river Ganga India, will benefit stakeholders, including local communities' well-being.

It gives me immense pleasure to extend my appreciation to the entire team for successfully executing the project, which advances our understanding of the unique floral diversity along the Ganga and its role in ethnobotanical purposes, conservation, and integrating science with local knowledge. The project's focus on environmental stewardship and skill enhancement for sustainable livelihoods is commendable.

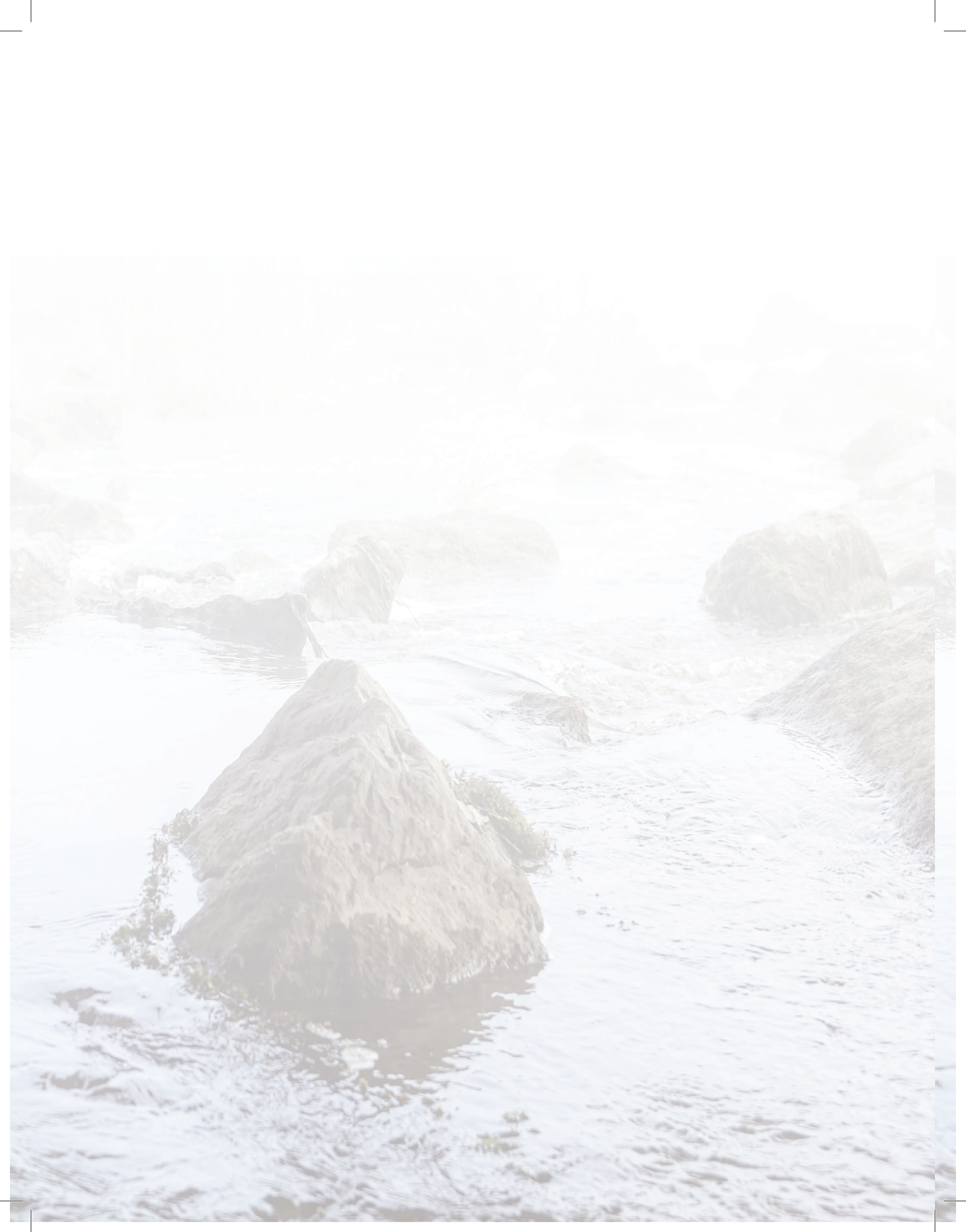
This excellent work documenting the floral diversity along the banks may pave the way for the rejuvenation of the Ganga and set a precedent for biodiversity conservation and socio-economic improvement, potentially benefiting other regions of India as well.


(Rajeev Kumar Mital)



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FOREWORD

*I*n the timeless flow of the sacred Ganga River, lies a profound treasure trove of nature's healing bounty. As we embark on this journey through the pages of this book on Medicinal Plants along the Ganga River, we are reminded of the ancient wisdom that has sustained our civilization for millennia. The Ganga, revered not only for its spiritual significance but also for its ecological richness, nurtures a diverse array of medicinal plants. The plant, meticulously documented in this comprehensive work, are not merely botanical specimen but living reservoir of phytochemicals that hold immense therapeutic potential.

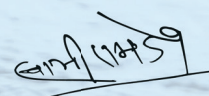
The authors have meticulously documented the plant exploration and botanical study, soil properties analysis, phytochemical analysis, and insilico analysis of the plants found in this region. Their dedication and deep respect for nature's wisdom are evident throughout the pages, making this monograph an invaluable resource for all who seek to deepen their understanding of the healing potential found in the lap of the Himalayas.

Moreover, the inclusion of phytochemical and bioinformatic aspects adds a modern scientific perspective to this ancient knowledge. By unraveling the chemical constituents and molecular mechanisms behind the therapeutic effects, this monograph bridges the gap between traditional wisdom and contemporary scientific advancements, paving the way for evidence-based herbal medicine.

I extend my heartfelt appreciation to Acharya Balkrishna Ji and his devoted team of expert scientists for their unwavering commitment for preserving and disseminating the wisdom of the Medicinal Plants along Ganga River. Their work not only contributes to the scientific community but also instills a deep reverence for nature and its intricate web of life.

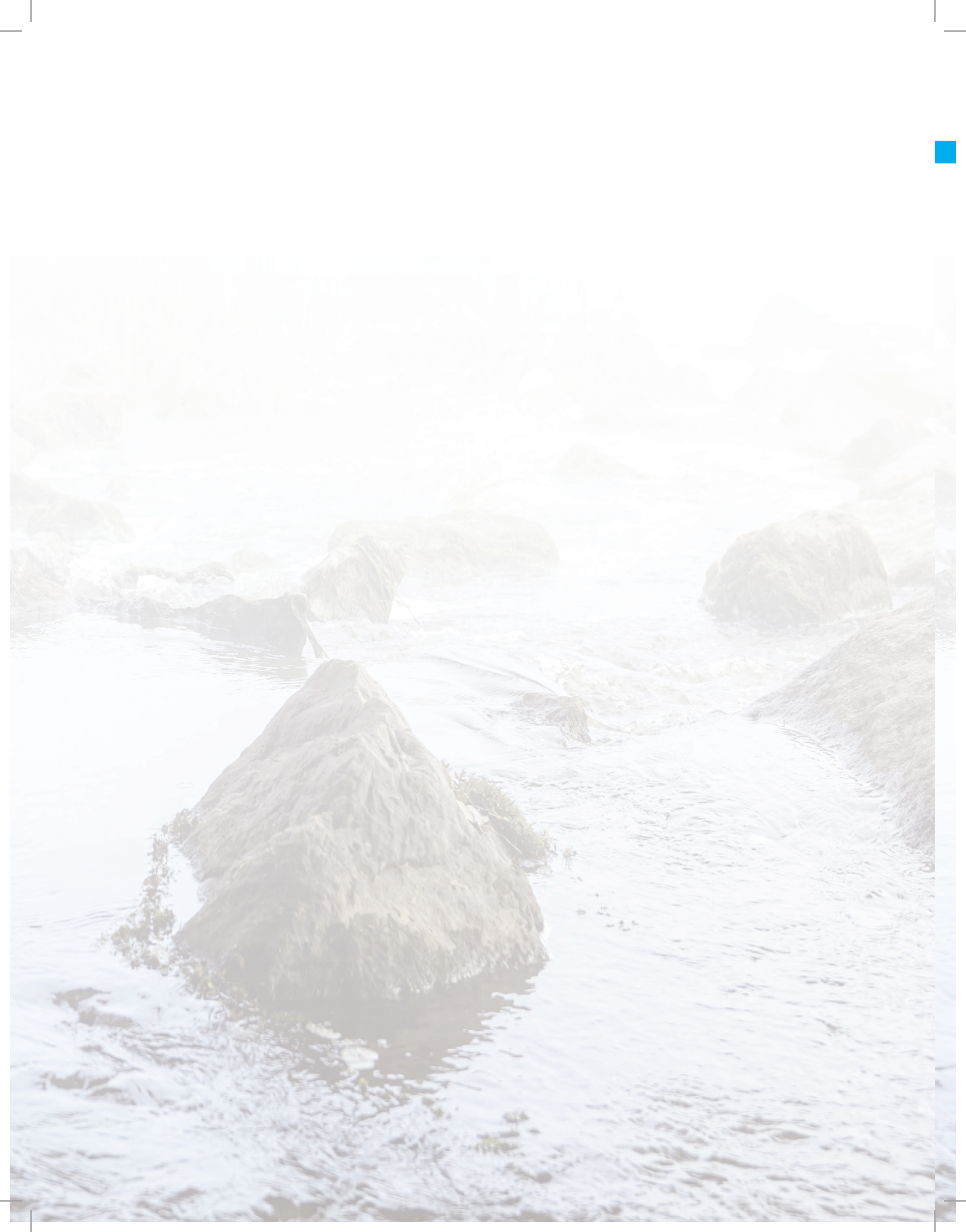
May this monograph serve as a guiding light for researchers, practitioners, and enthusiasts alike, illuminating the path towards a deeper appreciation of nature's pharmacy along the revered Ganga River.

With heartfelt gratitude and blessings,



- Swami Ramdev





PREFACE

The Ganga River, revered as the lifeblood of India, flows through the heart of the country, nourishing not only the land but also the spirit of the people. It is not just a geographical entity; it is a symbol of life, purity, and sustenance. From its origin in the pristine glaciers of Uttarakhand to its expansive delta in West Bengal, the Ganga traverses diverse landscapes, each rich in its own unique flora and fauna. For centuries, it has supported a wide variety of ecosystems, each harbouring plant species that are integral to the ecological balance, cultural heritage, and traditional practices of the region. This monograph series is dedicated to exploring and documenting the indigenous plants that thrive along this sacred river, from the high-altitude regions of Uttarakhand to the fertile plains of West Bengal. These plants are not merely biological entities; they are part of the cultural and spiritual fabric of the communities that have lived along the Ganga for generations. They are used in traditional medicine, rituals, and daily life, and they play a crucial role in the local economies.

The journey from Uttarakhand to West Bengal covers a vast array of ecosystems, from the alpine forests of the Himalayas to the mangroves of the Sundarbans. At each of the 26 sites we studied, the selected plant species reflect the unique environmental conditions and cultural practices of the region. This series of monographs is a culmination of an extensive research initiative aimed at understanding and preserving the botanical wealth of the Ganga basin. A total of 26 key sites along the river, spanning the length from its source in Uttarakhand to its mouth in the Bay of Bengal, were explored, analysed, and documented. At each of these sites, the indigenous plant species were studied which are adapted to the unique environmental conditions present. The exploration focuses on ten plant species that are not only emblematic of the region but also hold significant ecological, medicinal, and cultural value.

Each monograph in this series provides a comprehensive overview of the plant species, detailing its botanical characteristics, ecological role, traditional uses, and cultural significance. The data for distribution of these plants along the Ganga, highlighting the environmental factors that influence their growth and survival is primarily focussed. To achieve a comprehensive understanding, the selected sites were explored for their botanical diversity, phytochemical properties of the plants, and soil properties examination of each area. Additionally, *in silico* analysis was conducted to assess the plants' potential in combating several diseases. The study also investigated the ethnomedicinal and pharmacological uses and applications of these plants, complemented by a bibliometric analysis to evaluate existing research and knowledge. This exploration also presents as a fresh insight to classical literature of Ayurveda in the form of "Shlokas" and therefore extending beyond the identification of individual species; it adds a fresh perspective to the existing literature and delves into uncovering the intricate web of life that sustains these plants and the human communities that rely on them.

Patanjali Group recognized the critical need to explore the diverse botanical landscape of the Ganga River basin and to conduct scientific, evidence-based research to highlight its ecological and medicinal significance. With this vision, Patanjali Organic Research Institute embarked on an initiative to systematically document and analyse the indigenous plant species found across this region. The goal is to develop scientifically validated insights into the botanical diversity, phytochemical properties, and ethnomedicinal uses of these plants, thereby creating a comprehensive understanding of the current status and challenges associated with preserving this rich natural heritage.

We express our deepest gratitude to Param Pujya Swami Ramdev Ji for his unwavering leadership and support throughout this endeavour, as well as for his invaluable guidance in every aspect of our work. My best wishes to the dedicated and highly skilled experts, who have meticulously gathered data and conducted extensive research on the diverse botanical heritage of the Ganga River basin, utilizing cutting-edge scientific techniques and methodologies. Nature holds immense potential for sustainable practices and this project is a significant contribution to the preservation of these indigenous plant species, ultimately benefiting both ecological balance and the well-being of the communities that rely on them.

आचार्य बालकृष्ण

Dr. Acharya Balkrishna



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Classical Ayurvedic Insight

Solanum nigrum L.

Vedic name : (Yukpañcakam kṛṣṇaphalam) (युक्पञ्चकम् कृष्णफलम्)

Botanical name : *Solanum nigrum* L.

Utpatti sthānam

Uṣṇata kaṭibandhīye samaśītoṣṇake'pi ca.

Kākamācītarurbhāti śrīlāṅkāyāñca bhārate.

Trīṣatapurūṣocchrāyāt sahasrapurūṣocchrite.

Tṛṇaghāsādirūpeṇa vāṭikāsu tathaiva ca. (Saumitreya Mahodadhi: 1-2)

Distribution

It is widely distributed throughout the tropical and warm temperate regions of the world. It is found throughout India and Sri Lanka to an elevation of 750-2100 m as a weed in open disturbed habitats such as gardens, cultivated lands and on roadsides. ^[1-5]

Vānaspatika vivaraṇam

Kāyika lakṣaṇam-

Kedāreṣu tathā mārgaprāntabhāgeṣu labhyate.

Hastalambiśarīrācca trihastalambita kvacit.

Naikaprakārako'romā śākīyo vārṣikastathā.

Kāṇḍantu saralaprāyaṃ śūnyaromatayoditam.

Āho viralaromaivaṃ kaṇṭakai rahitaṃ sadā.

Dvivibhājitaśākhābhi sampṛktañcāpi dṛśyate.

Anekaṃ patrajātantu sapādāṅgulakocchritāt.

Caturaṅgulakocchrāyaṃ sārddhadvyaṅgulakāyatam.

Saralaṃ cā'ṇḍanīkāśamāho dīrghāyatatvata.

Aṇḍābhabhallanīkāśaṃ prāyeṇa niśitāgrakam.

Āho lambāgrataścaivamaroma tanurūpadhṛk.

Pūrṇaṃ vā'spaṣṭarūpeṇa danturaṃ parṇavṛntake.

Saṅkucitaṃ vibhāseta parṇavṛntantu tasya vai.

Aṅgulocchrāyirūpañca saṃlagnaromaśaṃ tathā.

Kvacicca granthilaṃ bhāti puṣpīyalakṣaṇe puna. (Saumitreya Mahodadhi:3-9)

Botanical Description

1. Vegetative Characters

A variable annual, glabrous, branched herb 0.30-1 m tall. Stem erect, glabrous or sparsely pubescent and much divaricately branched. Leaves simple, numerous, 2.5-9 cm long and 5 cm wide, ovate or oblong ovate-lanceolate, sub-acute or acuminate, glabrous, thin, entire or coarsely dentate, tapering in to the petiole. Petioles 2 cm long, appressed pubescent or glandular. ^[1-5]

Puṣpīya lakṣaṇam

Puṣpaṃ hr̥svaṃ tathā śvetaṃ bhāti klāntinataṃ sadā.
Tripuṣpitāt kvaciccā'ṣṭapuṣpīte chatrasannibhe.
Sasīmākṣe sthitaṃ cāpi tatprasūnālivṛntakam.
Triyavalambitānnūnamaṅgulalambitantathā.
Tanukañcāpi vidyeta puṣpavṛntaṃ tathaiva ca.
Triyavalambitārambhādardhayavavilambitam.
Bhūyīṣṭhatanu bhāseta bāhīkadalapuñjakam.
Sārdhayavasamucchrāyaṃ caṣakākārasambhṛtam.
Aromaṣaṃ tathā prāyo'romaṣaṃ pratibhāsate.
Bāhyadalāni pañcaiva dīrghāyatavapūṃṣyapi.
Kuñṭhitāgrāṇi tiṣṭhanti cā'rdhayavasamucchrītau.
Cīrasthāyīni tānyeva phale bhānti viśeṣata.
Dalapuñjaṃ yavocchrāyāccaturyavavilambi ca.
Śvetaṃ dīrghāyataṃ prāyonīśītāgraṃ tathā puna.
Vinamrapālijāte vai vibhājītavapurdharam.
Puṃskesarāḥ sadā pañca spaṣṭāśca pītavarṇata.
Puṃstantuścipītatvena cā'rdhayavavilambitāt.
Pādonayavakocchrāyo mūlabhāge ca romaśa.
Prasūnadhūlikośastu sapādayavalambita.
Dīrghāyataśca kuñṭhāgraṃ śīrṣaṃ danturameva ca.
Chidrābhyāṃ sphuṭitaṃ bhāti tathā tatrā'ṇḍakāśaya.
Golābho'romaśaścāpi vartikā vellanākṛti.
Ādhāre romaśā bhāti vartikāgraṃ samuṇḍitam.
Phaladavṛntamapyatra spaṣṭarūpeṇa sampratī.
Vikṣepitamivā''bhāti phalantu vartulantathā.
Prāyeṇa triyavavyāsāccaturyavasuvigraham.

Pāke śoṇitavarṇaṇca pītavarṇantathā'thavā.
 Vṛntākakṛṣṇavarṇābhamaṁ cirasthāyisvarūpiṇā.
 Bāhikadalapuñjēna samyutam sarasam bhavet.
 Anekānyatra bījāni hrasvāni pītavarṇata.
 Svalpakhātayutānyevam caṣakākāravanti ca.
 Pādonayavakavyāsasammitāni sum phalam.
 Varṣaparyantamābhāti phālgunāṣāḍhamadhyata. (Saumitreya Mahodadhi: 10-26)

2. Floral Characters

Flowers small, white, borne in drooping, umbellate 3-8 - flowered cymes. Peduncles 6-20 mm long, slender. Pedicels 6-10 mm long, very slender. Calyx 3 mm long, cup-shaped, glabrous or nearly so. Sepals 5, oblong, obtuse, 1.25 mm long, persistent on fruit. Corolla 2-8 mm long, white, divided into 5 oblong, subacute, reflexed lobes. Stamens 5, exposed, yellow. Filaments 1-1.5 mm long, flattened, hairy at the base. Anthers 2.5 mm long, oblong, obtuse, notched at the apex and dehiscing by 2 apical pores. Ovary globose, glabrous. Style cylindric, hairy, pubescent at the base. Stigma capitate. Fruiting pedicel strongly deflexed. Fruits berries, globose, 5-8 mm in diameter, red, yellow-or purplish-black when ripe with persistent calyx. Seeds small, numerous, disc-shaped, 1.5 mm in diameter, yellow, minutely pitted. Flowering / Fruiting throughout the year mostly February and July. ^[1-5]

Plant Anatomy

Prayojyāṅga

Yukpañcakābhidhaḥ kṛṣṇaphalo'tra pādapo'khilaḥ.
 Prayojyāṅgaṁ mataṁ vaidyairatnaḥsamracanocyate. (Saumitreya Mahodadhi:1)

Antaḥsamracaneḥṣaṇe

(a) Mūlam

Tvakprotake samālokyāḥ paṁkticatuṣṭayaśritāḥ.
 Sparśarekhīyakā lambāḥ kośikā, majjakośikā.
 Bṛhatī dṛśyate ceṣallambā ca tanubhittikā.
 Kośikāvaraṇaiḥprotā dṛḍhotakīyatantuṣu.
 Aṇḍākārata āgolāḥ sandṛśyā majjakośikāḥ.
 Ekalā dvividhā vātra ghaṭakatrayasamśritāḥ.
 Yadā kadā samālokyā santi maṇḍakaṇāstathā.
 Cūrṇātukṣāarakasyātra vṛtīyakaṇamiśritāḥ.
 Mṛdutaḥkośikā dṛśyāḥ kośikāḥ poṣavāhakāḥ.

Tanubhittīyakā saikabhujīyaiḥ pravinirmitāḥ.
 Kośikānicayaiḥ poṣavāharaśmivrajastathā.
 Paṃktibaddhaḥ samālokyo maṇḍakaṇasamanvitaḥ.
 Nyagvāhā nirmitā dṛśyā vāhikābhistathā punaḥ.
 Mṛdutakaiścatuḥkalpasamūhagā hi vāhikāḥ.
 Arīyakramasaṃbaddhā avāgvāhavrajastathā.
 Mṛdulaḥ sthūlabhittīyaścūrṇātukṣārakasya ca.
 Savṛtīyāḥ kaṇāḥ sūkṣmā dṛśyante sūkṣmikāyane.
 Yukpañcakasya saṃproktā mūlāntāracanā dhruvam. (Saumitreya Mahodadhi: 2-10)

Plant Parts

Whole plant

Microscopic Characters

Root

Cork consists of 2-4 rows of tangentially elongated cells. Cortex is large, slightly elongated, thin-walled cells having patches of lignified sclerenchyma fibres. Cortical cells are oval to round. Starch grains are single or with two or rarely 3 components. Some parenchymatous cells contain microspheroidal crystals of calcium oxalate. Phloem consists of thin-walled, polygonal cells. Phloem rays are uniseriate, filled with starch grains. Xylem is composed of vessels and parenchyma. Vessels are arranged in groups of 2-4 in radial rows. Xylem parenchyma is thick-walled containing microspheroidal crystals of calcium oxalate.

[1], [6-8]

(b) Kāṇḍabhāgaḥ

Adhicarmaprabhāgo'traikastarīyaśca kośikāḥ.
 Ghanākārata ālokyā vellanākṛtayastu tāḥ.
 Sustaritopacarmābhairvidyamānā samāvṛtāḥ.
 Dvītīyakākhyikā majjakośikā iha vīkṣitāḥ.
 Catuḥkalpastarīyā vai sthūlakoṇotakāḥ param.
 Daśakalpastarīyāste sparśarekhīyalambakaiḥ.
 Aṇḍākṛtyabhidṛśyābhiḥ kośikābhirvinirmitāḥ.
 Mṛdvībhiḥ kāsucinmajjakośikāsu kaṇāḥ khalu.
 Cūrṇātukṣāratattvasya kaṇā maṇḍakaṇā api.
 Aṇḍākārata ālokyā golākārāntaratvacā.
 Ekastarīyakā madhye parirambhavrajasya ca.
 Valayāśca pṛthagbhūtā uta vyūhacatuṣkagāḥ.

Saṃvahanakapūlāśca bahiḥ pravāhiṇastathā.
 Saṃyuktā muktarūpāśca dṛśyante te yathāyatham.
 Staracatuṣṭayaprotā tatraidhikā'valokyate.
 Nyagvāhavāhikā laghvyaḥ kendritārīyakāstathā.
 Majjabhāgo bṛhaddṛśyastanubhittīyakastathā.
 Mṛdulakośikāśṛṣṭo laghvantaḥ kośikīyakaḥ. (Saumitreya Mahodadhi: 11-19)

Stem

Epidermis is single layered, cubical to barrel-shaped cells, covered with thick slightly striated cuticle. Secondary cortex is composed of 2-4 layered collenchyma, but 4-10 layered in angular parts tangentially elongated and composed of oval parenchymatous cells. Some cortical cells contain crystals of calcium oxalate and simple, oval to round starch grains. Endodermis is single layered. Pericycle consists of ring of patches of fibres either isolated or in groups of 2-4. Vascular bundles are collateral, conjoint and open. Cambium is 2-4 layered. Xylem vessels are arranged radially smaller being towards centre. Pith is large and composed of thin-walled, parenchymatous cells with small intercellular spaces. ^{[1], [6-8]}

(c) Patram

Parṇavṛntaprabhāge'tra proccanimnādhicarmabhāk.
 Golākārata ālokyo hyaṇḍākāraḥsamīkṣaṇe.
 Asau samāvṛto dṛśyaḥ suvistr̥topacarmabhiḥ.
 Sthūlakoṇotakā atra dvitrastārīyakā matāḥ.
 Ubhayastaragā mṛdvyaḥ kośikāḥ ṣaṣṭstārīyakāḥ.
 Tanubhittīyakā antaḥkośikāspadasaṃśritā.
 Saṃvahanakapūlāśca cāpākārāḥ sukendragāḥ.
 Pravāhiṇa ime dṛśyā ubhayato yathāyatham. (Saumitreya Mahodadhi: 20-23)

Leaf

Petiole: Upper and lower epidermis is round to oval cells and covered with striated cuticle. Collenchyma is 2-3 layered on both surfaces. Parenchyma is 4-6 layered, thin-walled with small intercellular spaces. Vascular bundles are arc-shaped, bicollateral and placed centrally. ^{[1], [6-8]}

(d) Paṭalabhāgaḥ

Prṣṭhādhareṇa saṃyuktaḥ paṭalaḥ paridṛśyate.
 Proccanimnādhicarmātra caikastārīyakantathā.
 Aṇḍākārata ālokyāḥ sparśarekhīyalambikāḥ.
 Kośikā āvṛtāḥ sthūlopacarmanicayairiḥ.
 Asamakośikīyāni randhrāṇyatrobhayastare.
 Pravikīrṇāni nīcaistu stareṣu bahulānyapi.

Stambhotakaprabhāgo'tra caikastarīyako mataḥ.

Mṛdutakā ihocchūnāḥ ṣaṭkalpakastarīyakāḥ.

Antarākośikīyāni sthānāni haritānyapi.

Mṛdutakābhidhānāyā stambhotakīyasamḥateḥ.

Sārpilē sthūlane nīcairvāhikāḥ suvyavasthitāḥ.

Kāmācīpaṭalāntaḥsthā samracaneti coditā. (Saumitreya Mahodadhi: 23-29)

Lamina: Lamina is dorsiventral. Upper and lower epidermis is single layered, composed of oval to tangentially elongated cells covered with thick cuticle. Stomata are anisocytic and scattered on both surfaces but more abundant in lower surface. Palisade is single layered. Spongy parenchyma is 4-6 layered containing chloroplasts with intercellular spaces. Vessels are arranged below palisade parenchyma with spiral thickenings. ^{[1], [6-8]}

(e) Phalam

Bāhyabhittiḥphale dṛśyā tanvī kargalasannibhā.

Tanmadhyaphalabhittistu majjabhāgapariśritā.

Bījāṇḍanyāsa ālokya stambhīyo bījasamḥatiḥ.

Bījāṇḍāsanasaṃghena param kālāntare pṛthak.

Phalamajjaprabhāgo'tra pratiṣṭhitaḥ pravīkṣyate.

Kākamācīphalasyāntaḥ samracaneti dṛśyate. (Saumitreya Mahodadhi: 30-32)

Fruit

Fruit shows thin, papery epicarp, pulpy mesocarp and exile placentation. Seeds remain attached to the placenta but afterwards separate from it and lie free in pulp of fruit. ^{[1], [6-8]}

(f) Cūrṇam

Īśaddhārityasamkāśaṃ cūrṇakamavalokyate.

Vāhikānāṃ kaṇāścāpi sārpiḥsthūlanānyapi.

Kiñcittīkṣṇāgrakhaṇḍāni kṣatigrastāni santi ca.

Ekalakośikīyāni romāṇyaṇḍākṛtestathā.

Golākārāśca saṃyuktā dṛśyā maṇḍakaṇā api.

Ghaṭakatrayasaṃyuktāḥ prasūkṣmadarśikāyane. (Saumitreya Mahodadhi: 33-35)

Powder: Powder contains trichomes and epidermal peel.

Powder

Creamish-green in colour. It shows fragments of vessels with spiral thickening. It also contains a few broken pieces of pointed, unicellular hairs, single, oval to round and compound with three components of starch grains. ^{[1], [6-8]}

Medicinal Uses

Ophthalmic disorders

Kākamācīphalaikena ghṛtayuktena buddhimāna.

Dhūpayet pillarogārttaṃ patanti kṛmayo'cirāt. (Vṛ.Mā. 61: 291)

Ophthalmic diseases- Medicated fumigation of *Solanum nigrum* (Garden nightshade) fruits mixed with *Ghṛta* (Clarified butter) helps to alleviate the worms in patient suffering from *Pilla* (eye disorder) diseases.

[9] (Vṛ.Mā. 61:291)

Thoracic disorders

Vāyasīśākaṃ.....śasyate vātakāse.....(Ca.Ci. 18: 81-82)

Cough- The vegetable prepared from *Solanum nigrum* (Garden nightshade) leaves is wholesome in treating cough. [10] (Ca.Ci. 18:81-82)

Reproductive disorders

Kākamācīśiphāṃ puṣye kāmaṃ niṣpīḍya kanyayā.

Rasaṃ snātā pibennārī labhed garbhamanuttamam. (Vai.Ma. 13: 12)

For conception- *Solanum nigrum* (Garden nightshade) root juice obtained in *Puṣyanakṣatra* helps to attain pregnancy if taken by the female during the ovulatory period of cycle. [11] (Va. Ma. 13:12)

Śyāmāparūṣaphalinī kākamācīśiphāḥ pṛthak.

Piṣṭā nābheradho liptā garbhanikrāmaṇapradāḥ. (Vai.Ma. 13: 28)

External application of the paste prepared from *Solanum nigrum* (Garden nightshade) root below the navel region of the women in second stage of labour helps to ease delivery. [11] (Va. Ma. 13:28)

Dermatological disorders

Piṣṭvā ca kākamācī.....kuṣṭhanullepanaḥ. (Ca.Ci. 7:96)

Leprosy- External application of the paste prepared from *Solanum nigrum* (Garden nightshade) leaves are useful to alleviate leprosy. [10] (Ca.Ci. 7:96)

Indrāṇīśākaṃ kākāhvāṃ.....Pṛthagālepanaṃ kuryād dvadvaśaḥ sarvaśo'pi vā.

Pradehāḥ sarva evaite deyaḥ svalpaghṛtāplutāḥ. (Ca.Ci. 21: 90-92)

Erysipelas Topical application of the paste prepared from *Solanum nigrum* (Garden nightshade) leaves, *Albizia lebbek* (Indian siris) flower and *Vitex negundo* (Five leaved-chaste tree) leaf mixed with little *Ghṛta* (Clarified butter) is beneficial in treating erysipelas. [10] (Ca.Ci. 21:90-92)

Svarasena kākamācyāḥ piṣṭā śuṇṭhī jayet koṭhān. (Vai.Ma. 16: 140)

Urticaria- Application of the paste prepared after rubbing *Zingiber officinale* (Dry ginger root) with *Solanum nigrum* (Garden nightshade) juice is useful in treating erysipelas, urticaria and other diseases.

[11] (Va. Ma. 16:140)

Svarasena kākamācyāḥ piṣṭā śuṇṭhī jayet koṭhān.

Munitarudalarasapiṣṭā sā ca karīṣasya vā svarase. (Vai. Ma. 16:140)

Application the paste prepared from *Zingiber officinale* (Dry ginger root) pounded with *Solanum nigrum* (Garden nightshade) juice or *Zingiber officinale* (Dry ginger root) pounded with *Rakta Vaka Vṛkṣa* is helpful to alleviate urticaria. ^[11] (Va. Ma. 16:140)

Generalised body disorders

.....savāyasīmūlakavetranimbam.

Śākārthinām śākamiti praśastam.

Bhojye purāṇaśca yavaḥ saśāliḥ. (Ca.Ci. 12: 63)

Inflammation- Intake of vegetable prepared from *Solanum nigrum* (Garden nightshade) is wholesome diet. ^[10] (Ca.Ci. 12:63)

Rejuvenation and virilification

Guḍena pippalībhīrvā maricenānvitā śṛtā.

Kākamācī bhaved balyā tadrāsena haviḥ śṛtam. (Vai.Ma. 4: 3)

Rejuvenation- Intake of decoction prepared from whole plant of *Solanum nigrum* (Garden nightshade) along with jaggery, *Piper longum* (Long pepper) and *Piper nigrum* (Black pepper) or regular intake of *Ghṛta* (Clarified butter) processed from *Solanum nigrum* (Garden nightshade) juice helps to attain the rejuvenating properties. ^[11] (Va. Ma. 4:3)

Alexipharmic Treatment

Kākādanīkākamācyo svaraseṣvathavā kṛtam. (Su.Ka. 7: 31)

Rat poisoning- The *Ghṛta* (Clarified butter) processed with the juice of *Kākādanī* and *Solanum nigrum* (Garden nightshade) is useful to alleviate the toxic effects caused due to rat poisoning. ^[12] (Su.Ka. 7:31)

Part Used

Whole plant.

Dose

Juice 5-10ml, fruit powder 1-3gm or as directed by the physician.

Toxicity

There is no any complication if this plant is consumed in an appropriate dose. It may cause abdominal, intestinal diseases, cephalgia, irritation and vomiting if taken in excessive dose. ^[13] (HMH: 97)

Note

It is very useful in treating inflammation and arthritic diseases. It should be used under the supervision of physician due to its toxic properties. ^[14] (HA-4: 1042)

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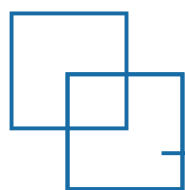
Abbreviation

A.Hṛ.Ci.	:	AṣṭāṅgaHṛdayaCikitsā
A.Hṛ.U.	:	AṣṭāṅgaHṛdaya Uttara Tantra
As.Hr.Ut.	:	AṣṭāṅgaHṛdaya Uttara Tantra
Bhā.Pra.Ci.	:	BhāvaprakśaCikitsā
Śā.Sa.Ut.Kha.	:	ŚāraṅgadharaSamhitā Uttara Khaṇḍa
Ca.Ci.	:	Caraka SamhitāCikitsāSthāna
Ca.Da.Ci.	:	CakradattaCikitsā
Ca.Si.	:	Caraka Samhitā Siddhi Sthāna
Ca.Sū.	:	Caraka SamhitāSūtraSthāna
Ck.Dt.Ci.	:	CakradattaCikitsā
Ga.Ni.Kā.Ci.	:	GadanigrahaKāyacikitsā
Ga.Ni.Kau.Taṃ.	:	GadanigrahaKaumaryatantra
Ga.Ni.Śā.Ta.	:	GadanigrahaŚālākya Tantra
Ga.Ni.Śa.Taṃ.	:	GadanigrahaŚalyatantra
Hā.Sa.	:	HārāitaSamhitā
Hā.Saṃ.	:	HārāitaSamhitā
Kā.Saṃ.Khilasthāna	:	KāśayapaSamhitāKhilasthāna
Rā.Mā.	:	RājaMārtaṇḍa
Ra.Ra.Sa.	:	Rasa Ratna Samuccaya
Śā.Ni.Guḍūcyādivarga	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Śā.Sa.U.Kha.	:	ŚāraṅgadharaSamhitā Uttara Khaṇḍa
Śā.Saṃ.Ma.Kha.	:	ŚāraṅgadharaSamhitā Madhyam Khaṇḍa
Sd.Bh.Mm. Ch. Gr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaGrahaṇāiCikitsā
Sd.Bh.Mm. Ch. Nd.Vr.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Nāḍāivraṇa Cikitsā
Sd.Bh.Mm. Ch.Nt.Rg.	:	Siddha BheṣajaMaṇimālāCaturthaGuccha Netra RogaCikitsā
Sd.Bh.Mm.Ch. Ars.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaArśaCikitsā
Sd.Bh.Mm.Ch. Vrn.Ci.	:	Siddha BheṣajaMaṇimālāCaturthaGucchaVraṇaCikitsā
Sh.Gr.Ng. Gd.Vg.	:	ŚāligrāmaNighaṇṭuGuḍūcyādivarga
Su.Ci.	:	SuśrutaSamhitāCikitsāSthāna

Su.Ka.	:	SuśrutaSamhitā Kalpa Sthāna
Su.Śā.	:	SuśrutaSamhitāSārīraSthāna
Su.Ut.	:	SuśrutaSamhitā Uttara Tantra
Vai.Jī. Caturthavilāsa	:	Vaidya JāivanamCaturthavilāsa
Vai.Jī. Dvitiyavilāsaḥ	:	Vaidya JāivanamDvitiyavilāsaḥ
Vai.Ma.	:	Vaidya Manoramā
Vaṃ. Se. Āmavātarogādhikāraḥ	:	VaṃgasenaSamhitāĀmavātarogādhikāraḥ
Vaṃ.Se. Vātavyādhidhikāraḥ	:	VaṃgasenaSamhitāVātavyādhidhikāraḥ
Vaṃ.Se.Karṇarogaḥ	:	VaṃgasenaSamhitāKarṇarogādhikāraḥ
Vaṃ.Se.Medarogaḥ	:	VaṃgasenaSamhitāMedorogādhikāraḥ
Vaṃ.Se. Netrarog	:	VaṃgasenaSamhitāNetrarogādhikāra
Vaṃ.Se. Śothādhikāraḥ	:	VaṃgasenaSamhitāŚothādhikāraḥ
Vaṃ.Se. Strīrogaḥ	:	VaṃgasenaSamhitāStrīrogaḥ
Vaṃ.Se.Vātavyādhīḥ	:	VaṃgasenaSamhitāVātavyādhidhikāraḥ
Vṛ.Mā.	:	Vṛndamādhava

CHAPTER
01

Plant Exploration and Botanical Study



INTRODUCTION

Water is the key resource required to sustain life on this planet. It is found in most of the earth as in the ocean, rivers, ponds, streams, ice, etc. The river Ganga is an important source of water in India that makes an important river system from the Himalaya to the Bay of Bengal. The Indian civilization and economy depended on this river system due to its water availability throughout the year (Paul and Sinha, 2013). The river Ganga alone accounts for 25% of India's total water resources (Paul, 2017). Globally more than 300 million people from India, Nepal, and Bangladesh depend on the river Ganga (Gopal, 2000). This is the thirtieth longest river in the world and covers a basin area of 861,404 km² (Rahaman, 2009a). This basin is the most populated area in the world with an average density of 520 persons/km² (Das and Tamminga, 2012). The basin is very rich in heritage, cultural, and religious values. India's about one-fourth area drains by the river Ganga.

The river originates from the Gangotri glacier at Gomukh (30° 36' N; 79° 40' E; 3800 m) in the Uttar Kashi district of Uttarakhand under the name of Bhagirathi; and another tributary *i.e.*, Alaknanda which is originated from the Bhagirath-Kharak (30° 49' N; 79° 17' E) and Satopanth (30° 45' N; 79° 21' E) glaciers. Both the tributaries join at Devprayag from where the name Ganga started. The river has a total length of 2600 km from its main source Gomukh.

Up to Haridwar it flows in the hills after here it enters the plain area. From here it flows southwards, passing through the plains of Uttar Pradesh starting from Bijnor district. Up to Ballia it continuously flows in Uttar Pradesh through different districts after here it enters Bihar near Chausa of Buxar district. It flows up to Katihar district in Bihar and from here it enters the Sahebganj district of Jharkhand. Then it turns southwards and enters West Bengal from the Murshidabad district. About 40 km from the Farakka, it divides into two streams; the left stream flows eastwards toward Bangladesh while the right stream, known as Bhagirathi, continues to flow south through West Bengal. Its name changed to Hooghly when it flows in the west and south-west of Kolkata. The



Hoogly at Diamond Harbor flows southward and is split into two streams before reaching the Bay of Bengal (Rahaman, 2009b).

The annual water discharge from the river Ganga is about 18700 m³/s which is the fifth highest in the globe (Paul, 2017). The maximum flow of the Ganga exists within the catchment area with a mean maximum flow is 468.7×10^9 m³ which is a total of 25.2 % of the total water resources of India (Sarkar et al., 2012). The main source of the river Ganga is the melting of snow in the Himalayas and monsoon rains. The water of the Ganga is regularly used by the living being for drinking. Outdoor bathing has a spiritual significance for Hindus in which millions of people take a holy dip at least once a year throughout the course of the river, from Gangotri to Ganga Sagar.

The Ganga basin supports biodiversity, species richness, and uniqueness, attracting people from different parts of the earth. Plant communities have been a major magnetism for investigation since time immemorial. Vegetation composition and assemblages of an area form a significant habitat that contributes to the structure and function of such ecosystems. The vegetation pattern along the Ganga varies according to the seasonal changes, flood level, and species composition differs by the function of water supply and different soil types, which has a sharp influence on plant species distribution. Macrophytes such as submerged, emergent, and free-floating aquatic plants are known to accumulate and bioconcentrate heavy metals producing an internal concentration several folds greater than their surroundings (Chen et al., 2008; Allen-Diaz et al., 2008). The river basin has large numbers of medicinal plant species. The natives depend on the river for water and medicinal importance to fulfill their requirements from the beginning.

Among all the medicinal plants *Solanum nigrum* was considered in this study. The *Solanum nigrum* is one of the largest and most variable species and worldwide weeds of arable land, gardens, rubbish tips, and soils rich in nitrogen, in moderately light and warm situations which occur from sea to montane levels. It is commonly known as blackberry, blackberry nightshade. In Sanskrit “kakamachi”, “kakahva”, in Hindi “makoi”. Its antiproliferative, antiseizure, antioxidant, antiviral, anti-inflammatory, and hepatoprotective effects are used for diverse activities using different parts of this plant, including the seeds, roots, leaves, and stem. It has diverse morphology and anatomical variations. On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *S. nigrum* around the Ganga River.

Genus *Solanum* L.

The genus *Solanum* belongs to the family Solanaceae. This genus was first described by Linnaeus and published in Sp. Pl.: 184.1753. This genus was the largest genus with a total of 23 species first described by the Linnaeus in 1753; all the species were described from European or African material. Later, French botanist Michel-Félix Dunal included 235 species in his thesis (Dunal, 1813). Dunal (1816) revised his previous work and included a total of 321 species under this genus. Many of these additional taxa were based on specimens collected by Alexander von Humboldt and Aimé Bonpland in tropical South America (Knapp, 2007). The combination of large numbers of species with relatively poorly circumscribed groups within the genus has meant that *Solanum* taxonomy has proceeded in a piecemeal fashion until relatively recently (Knapp et al., 2013).

This genus is cosmopolitan. Currently, it has approximately 1,400 species which makes it one of the largest genera of flowering plants (Frodin, 2004). The genus is mainly tropical and there are comparatively few species of *Solanum* in cool temperate regions. South and Central America, as well as Australia, have the highest species diversity; Africa has less species richness but a significant number of endemic species (Sarkinen et al., 2018). This taxon has a major social and economic importance; it includes several plants cultivated in many countries. Among the various plants that are being used as raw

materials to produce steroidal drugs, the *Solanum* holds an important place due to its quick growth and low initial investment in its commercial cultivation (Singh et al., 2019).

Every species in the genus *Solanum* is primarily utilized for medical purposes. Most of the species are used for the treatment of various diseases like toothache, removal of tooth worms, cough, asthma, sore throat, worm infestation, arthritis and other joint pain, ear infections, wound healing, and as contraceptives. The leaves are used to cure rheumatic, joint swelling and pain, skin disease, and tuberculosis and they are also used for dropsy, nausea, and nervous disorders. The flower and fruit decoction are useful in cough, and erysipelas. A few species such as *S. lycopersicum*, *S. melongena*, *S. nigrum*, *S. torvum*, *S. tuberosum* and *S. violaceum* are utilized as vegetables in different countries (Singh et al., 2019; Balkrishna et al., 2022).

Etymology

The genus belongs to the family “Solanaceae” which is based on the genus *Solanum*. The name *Solanum* is derived from the Latin word ‘sol’, which means ‘sun, the plant of the sun’. It was first applied by Plinius to a plant also called nightshade (Quattrocchi, 2000).

Habitat and Distribution

The genus *Solanum* is cosmopolitan. According to POWO (2024) it is native to Afghanistan,

Alabama, Alaska, Albania, Alberta, Aldabra, Algeria, Altay, Amur, Andaman Is., Angola, Argentina Northeast, Argentina Northwest, Argentina South, Arizona, Arkansas, Aruba, Assam, Austria, Azores, Bahamas, Baleares, Baltic States, Bangladesh, Belarus, Belgium, Belize, Benin, Bismarck Archipelago, Bolivia, Borneo, Botswana, Brazil North, Brazil Northeast, Brazil South, Brazil Southeast, Brazil West-Central, British Columbia, Bulgaria, Burkina, Burundi, Buryatiya, California, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Cayman Is., Central African Repu, Central American Pac, Central European Rus, Chad, Chatham Is., Chile Central, Chile North, Chile South, China North-Central, China South-Central, China Southeast, Chita, Christmas Is., Colombia, Colorado, Comoros, Congo, Connecticut, Cook Is., Corse, Costa Rica, Cuba, Cyprus, Czechoslovakia, Delaware, Denmark, Desventurados Is., District of Columbia, Djibouti, Dominican Republic, East Aegean Is., East European Russia, East Himalaya, Easter Is., Ecuador, Egypt, El Salvador, Equatorial Guinea, Eritrea, Ethiopia, Fiji, Finland, Florida, France, Free State, French Guiana, Gabon, Galápagos, Gambia, Georgia, Germany, Ghana, Great Britain, Greece, Guatemala, Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Guyana, Hainan, Haiti, Hawaii, Honduras, Hungary, Idaho, Illinois, India, Indiana, Inner Mongolia, Iowa, Iran, Iraq, Ireland, Irkutsk, Italy, Ivory Coast, Jamaica, Japan, Jawa, Juan Fernández Is., Kansas, Kazakhstan, Kentucky, Kenya, Kermadec Is., Khabarovsk, Kirgizstan, Korea, Krasnoyarsk, Kriti, Krym, Kuril Is.,

Kuwait, KwaZulu-Natal, Laos, Lebanon-Syria, Leeward Is., Lesotho, Lesser Sunda Is., Liberia, Libya, Louisiana, Madagascar, Madeira, Maine, Malawi, Malaya, Mali, Maluku, Manchuria, Manitoba, Marianas, Marquesas, Marshall Is., Maryland, Massachusetts, Mauritania, Mauritius, Mexican Pacific Is., Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Michigan, Minnesota, Mississippi, Missouri, Mongolia, Montana, Morocco, Mozambique, Myanmar, Namibia, Nansei-shoto, Nebraska, Nepal, Netherlands, Netherlands Antilles, Nevada, New Brunswick, New Caledonia, New Guinea, New Hampshire, New Jersey, New Mexico, New South Wales, New York, New Zealand North, New Zealand South, Newfoundland, Nicaragua, Nicobar Is., Niger, Nigeria, Niue, Norfolk Is., North Carolina, North Caucasus, North Dakota, North European Russi, Northern Provinces, Northern Territory, Northwest European R, Norway, Nova Scotia, Ohio, Oklahoma, Oman, Ontario, Oregon, Pakistan, Palestine, Panamá, Paraguay, Pennsylvania, Peru, Philippines, Pitcairn Is., Poland, Portugal, Primorye, Prince Edward Is., Puerto Rico, Qinghai, Queensland, Québec, Rhode Is., Romania, Rwanda, Réunion, Sakhalin, Samoa, Sardegna, Saskatchewan, Saudi Arabia, Selvagens, Senegal, Seychelles, Sicilia, Sierra Leone, Sinai, Society Is., Socotra, Solomon Is., Somalia, South Australia, South Carolina, South China Sea, South Dakota, South European Russia, Spain, Sri Lanka, Sudan, Sulawesi, Sumatera, Suriname, Swaziland, Sweden, Switzerland, Tadzhikistan, Taiwan,



Fig. 1 Global distribution of genus *Solanum*

Tanzania, Tasmania, Tennessee, Texas, Thailand, Tibet, Togo, Tokelau-Manihiki, Tonga, Transcaucasus, Trinidad-Tobago, Tuamotu, Tubuai Is., Tunisia, Turkey, Turkey-in-Europe, Turkmenistan, Turks-Caicos Is., Tuva, Uganda, Ukraine, Uruguay, Utah, Uzbekistan, Vanuatu, Venezuela, Venezuelan Antilles, Vermont, Victoria, Vietnam, Virginia, Washington, West Himalaya, West Siberia, West Virginia, Western Australia, Western Sahara, Windward Is., Wisconsin, Wyoming, Xinjiang, Yakutskiya, Yemen, Yugoslavia, Zambia, Zaïre, Zimbabwe; and introduced into Antipodean Is., Ascension, Bermuda, Caroline Is., Chagos Archipelago, Cocos (Keeling) Is., Gilbert Is., Kerguelen, Laccadive Is., Line Is., Nauru, Ogasawara-shoto, Rodrigues, St. Helena, Tristan da Cunha, Tuvalu, Wake Is. (Fig. 1)

Botanical Characteristics

Herbs, shrubs, trees, or vines, sometimes trailing or climbing. Stem terete or angled, branched or unbranched, sparsely to densely pubescent with simple, uniseriate, multicellular. Leaves simple or pinnate, alternate or in pairs; petiole usually canaliculate and alate; lamina ovate to lanceolate, occasionally rhomboidal, base cuneate or cordate, margin entire to sinuate dentate, apex acuminate, acute or obtuse, sparsely to densely pubescent. Inflorescence cymose, cymes umbellate to helicoid, compressed to lax, 2 to 36-flowered, pedunculate rarely solitary; peduncle simple or forked, erect or reflexed at maturity, glabrescent to pubescent; pedicel erect or reflexed at maturity, glabrescent

to pubescent. Flowers are usually bisexual. Calyx campanulate or cupuliform, stellate, with broadly triangular to ovate-lanceolate sepal lobes. Corolla white, purple, stellate to rotate, recurved, pubescent, hairy; lobes 5, spreading or recurved. Stamen 5 or more, equal or unequal; filaments fused, uniseriate, multicellular hairy; anther bilocular, basifixed, oblong or attenuated, yellow to brown. Ovary 2 (occasionally 3-4)-locular; style straight or geniculate, hairy; stigma globular, terminal, capitate or bifid. Fruits berry, 2-locular, many-seeded, globose to ovoid, black, brown, red, orange. Seed small, obovate to obliquely ovate, white, yellow, or brown.

Ethnomedicinal Importance

Several species of the *Solanum* genus, which are utilized in dietary supplements and traditional medicine, can be found in tropical and subtropical regions. *S. lycopersicum* fruits are used to treat skin and cardiovascular diseases, cancer, burns, scalds and sunburn, rheumatism and severe headaches, filarial worm swellings, incipient leprosy spots, and toothache. *S. nigrum* has been valued for its ethnobotanical significance because of its application in traditional medicine to treat a range of illnesses. The leaves and bitter berries with pungent have been traditionally used against severe ulcers, heart diseases, piles, dysentery, gastritis, and stomachache (Chidambaram et al., 2022). In Italy, the aerial part of *S. nigrum* is used as an antispasmodic, sedative, and analgesic drug (Chen et al., 2022).

S. tuberosum is used to treat burns, chilblains, skin cracks, sores, and swelling of eyelid, and the leaves are used to cure cough and headaches [Umadevi et al., 2013]. Various parts of *S. sisymbriifolium* (wild tomato), are used to cure numerous diseases, such as hypertension, diarrhea, and respiratory and urinary tract infections. In Central and South America people used to treat veterinary and human diseases [More, 2019]. The whole plant of *S. villosum* is used to treat nappy rash, wounds, leukorrhea, and cold sores. The leaves are used to cure stomachache, tonsillitis, and cardiac diseases, and unripe fruit soothes the toothache and squeezes on babies' gums to ease pain during teething [Zahara et al., 2019]. The whole-dried plant powder of *S. trilobatum* and *S. xanthocarpum* are used to treat respiratory diseases [Govindam et al., 1999].

The roots of *S. surattense*, are a key component of the Ayurvedic preparation 'dashmularishta', which is given to nursing moms as a tonic. The entire plant is used for treating leprosy, dropsy, and cough by the folklore [Tekuri et al., 2019]. Different parts of the *S. incanum* are also widely used in the treatment of skin problems, including skin infections, ringworm, burns, sores, rashes, wounds, warts, ulcers, and benign tumors. In addition, the plant is used to treat ailments like stomach problems, snake bites, chest pain, skin wounds of cattle, etc. Leaves, fruits, roots, and seeds boiled in butter taken orally are also used for stomach disorders [Dakone et al., 2016].

Chemical Constituents

Solanum genus has been documented for its phytochemicals. In the features, 66 medicinal species of *Solanum* in their geographical distribution, traditional uses, and 670 isolated chemical constituents, including 134 steroidal saponins, 63 steroidal alkaloids, 13 pregnane glycosides, 128 terpenes, 75 flavonoids, 31 lignans, 31 other types of alkaloids, 66 sterols, 52 phenolic compounds, 20 coumarins and coumestans, 4 coumarinolignoids, 23 fatty acids and esters and 30 other compounds have been investigated by Kaunda et al. [2019]. Some of the major phytoconstituents have been mentioned here:

Steroidal Saponins- Chlorogenone, solakhasoside, foliumin A, torvoside N, degalactotigonin, diosgenin gentiobioside, (22R, 23S, 25R)-3 β ,6 α , 23-trihydroxy5 α -spirostane 6-O- β -d-xylosyl-(1'''-3'')-O-[β -d-quinovosyl (1''-2')]-O-[α -l-rhamnosyl (1'-3')] -O- β -d-quinovoside, nuatigenosido, (3 β ,5 α ,14 β ,25R)-3-hydroxyspirost-8-en-11-one, torvoside Q, dioscin, methylprotodioscin, indioside D, 26-O- β -d-glucosyl-22-methoxyfurost-5-ene-3 β ,26-diol 3-O- α -l-rhamnosyl-(1-2)- β -d-glucoside, isonuatigenin-3-O- β -solatriose, chlorogenin, chrysogenin, laxumin A, lyconoside Ia, lyconoside IV, aculeatiside A, (22R,23S,25S)-3 β ,6 α ,23-trihydroxy-5 α -spirostane 6-O- β -d-xylosyl-(1-3)-O- β -d-quinovoside, solagenin 6-O- β -d-quinovoside, neochlorogenin, yuccagenin, indioside L, borassoside D, indioside I, zingiberoside A1, solanolactoside A, solanolide, torvoside J, gekogenin, sisalagenin,

5 soladulcosides B, abutiloside L, anguivioside A, anguivioside I, 6-O- α -l-rhamnosyl-(1'-3')- β -d-quinovosyl-(22S,23R,25S)-3 β ,6 α ,23-trihydroxy-5 α -spirostane, 6-O- β -d-Xylosyl-(1'-3')- β -d-quinovosyl-(22S,25S)-3 β ,4 β ,6 α -trihydroxy-5 α -spirostane.

Steroidal alkaloids- Demissine, solasodiene, solanoside A, solamargine, γ -solamargine, khasianine, solasonine, 12-hydroxysolasonine, solasodine, N-hydroxysolasodine, putuline, anguivine, isoanguivine, robeneoside A, lobofrutoside, [22R, 25R]-16 β -H-22 α -N-spirosol-3 β -ol-5-ene 3-O- α -l-rhamnosyl-(1-2)-[α -l-rhamnosyl-(1-4)]- β -d-glucoside, solaculine A, β -solamarine, tomatidenol, dehydrotomatine, tomatidine 3-O- β -d-glucoside, solaverol A, tomatidine, tomatine, 22-imido-3-[4'-(6'-deoxy- α -l-mannoside)- β -d-glucoside]-5-dehydro spirostane, leptinidine, solanine, α -chaconine, jurubine, deacetylveralosine, soladunalinidine, caavuranamide, [22R,25R]3 β -amino-5 α -spirosolane, isojuripidine, spiraloside C, esculeoside A.

Pregnane glycosides- Solanigroside A, solanigroside B, 5 α -pregn-16-en-3 β -ol-20-one lycotetraoside, [5 α]-3-hydroxypregn-16-en-20-one, hypoglaucin H, 16-dehydropregnenolone, 16-dehydropregnenolone 3-O- α -l-rhamnosyl-1-2) β -d-glucosiduronic acid, torvpregnanoside A, 5 α -pregn-16-en-3,20-dione-6 α -ol-6-O-[α -l-rhamnosyl-(1-3)- β -d-quinovoside], torvpregnanoside B, ganaxolone, allopregnanolone, pregnanolone.

Triterpenes- Betulinic acid, lupeol, cycloeucalenone, 24-oxo-31-norcycloartanone, friedelin, ursolic acid, 2 α ,3 β -dihydroxyursolic acid, daturaolone, carbenoxolone, β -amyrin, oleanolic acid, 2 α -hydroxyoleanolic acid, 3 β -acetoxy-11 α ,12 α -epoxyoleanan-13 β ,28-olide, solanoglycosydane I.

Diterpenes- Phytol, kaur-16-ene, solaneroside A, tricalysioside U.

Sesquiterpenes- Roseoside, (6E,10E)-5,12-dihydroxy- β -nerolidol 5-O- β -d-glucoside, amarantholidoside IV, solavetivone, 13-hydroxysolavetivone, lubiminoic acid, α -farnesene, nerolidol, anhydro- β -rotunol, caryophyllene, α -gurjunene, α -guaiene, α -calacorene, solafuranone, solajiangxin B, septemlobin D, blumenol A, dehydrovomifoliol, boscialin, 1 β -hydroxy-1,2-dihydro- α -santonin, lyratol A, solajiangxin A, -hydroxysolajiangxin E, nardoeudesmol A, solanerianone A, selina-3 β ,4 α ,11-triol 10 β ,12,14-trihydroxy-allo-aromadendrane, teferidin, ferutinin, septemlobin A, thujopsene, α -cedrene, α -hexylcinnamaldehyde, β -cadinene.

Monoterpenes- Decanal, 2,4-decadienal, terpinen-4-ol, linalool, limonene, β -cyclocitral, β -ionone, 1, 2-dihydro-1,1,6-trimethylnaphthalene, loliolide, hotrienol, neroloxide, nerol, 8-hydroxylinalool, (R)-linalyl β -d-glucoside, p-cymenene, apiole, α -terpinen-7-al, 1,3,8-p-menthatriene.

Flavonoids- Vitecetin, quercetin, kaempferol 7-O-rhamnoside, rutin, kaempferol 3-rutinoside-7-rhamnoside, afzelin, astragalin, kaempferol 3-O-[α -

apiofuranosyl-(1-2)]- α -rhamnoside, tiliroside, kaempferol, camelliaside C, narcissin, isorhamnetin 3-glucoside, populnin, quercetin 3-O-robinoside, kaempferol 3-O-(6'-O-cis-p-coumaroyl)-O- β -galactoside, myricetin-3-galactoside, pelanin, peonanin, pelargonidin 3-rutinoside, cyanidin 3-O-(2'-O-xylosyl) rutinoside, biochanin A-7-O- β -d-apiofuranosyl-1-5) β -d-apiofuranosyl-1-6) β -d-glucoside, vitexin, isoquercitrin, 5-hydroxy,7,2',3',5'-tetramethoxyfavone, baicalin, manghaslin, rhamnocitrin, 3-O-methylquercetin, tamarixin.

Lignans- Isolariciresinol, 5-methoxyisolariciresinol, (+)-lyoniresinol 3-O-d-glucoside, alangilignoside C, liriodendrin, melongenamide A, cannabisin D, grossamide, cannabisin F, grossamide K, pinioresinol-4-O- β -d-glucoside, syringaresinol-4'-O- β -d-glucoside, simulanol, balanophonin, tribulusamide A, clemastanin B.

Other alkaloids- Xylogranatinine, cernumidine, cernidine, 3-indolecarboxylic acid, L-valyl-L-isooleucyl-L-leucine, 2-methyltetrahydro- β -carboline, acetamide, stearamide 4-coumaroyltyramine, N - t r a n s - f e r u l o y l t y r a m i n e , cafeoylputrescine, aurantiamide acetate, N - (3 - a m i n o p r o p y l) - N - [4 - [[3 - (3 , 4 - d i h y d r o x y p h e n y l) - 1 - o x o p r o p y l] a m i n o] b u t y l] - 3 , 4 - d i h y d r o x y b e n z e n e p r o p a n a m i d e , soya-cerebroside I.

Sterols- Cilistol G, capsisteroid A, β -sitosterol, campesterol, cholesterol, γ -sitosterol, 7-oxositosterol, (3 β)-7-hydroxystigmast-5-en-3-yl β -dglucoside,

stigmasterol, brassicasterol, poriferasterol monoglucoside, 7-oxostigmasterol, clerosterol, 7-sitoster-3 β -ol, stigmasta-5,24(28)-dien-3-ol, avenasterol, γ -tocopherol, lanosterol, 9,11-dehydroergosterol peroxide, tumacone A, SN-1, 9 α ,11 α -epidioxyergosta-6,22-dien-3 β -ol, carpesterol, carpesterol methyl ether, toptriol, cholecalciferol, tumaquenone, abutiloside A.

Phenolic compounds- 4-cafeoylquinic acid, (1R,3R,4S,5R)-3-(acetyloxy)-5-[[[(2E)-3-(3,4-dihydroxyphenyl)-1-oxo-2-propen-1-yl]oxy]-1,4-dihydroxycyclohexanecarboxylic acid, chlorogenic acid, neochlorogenic acid, rosmarinic acid, 3,5-dicafeoylquinic acid, (Z)-neochlorogenic acid, gallic acid, 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid, cafeic acid, p-coumaric acid, 2,4,6-trimethoxyphenol, propionylsyringol, cis-p-coumaric acid ethyl ester, erythro-1,2-bis-(4-hydroxy-3-methoxyphenyl)-1,3-propanediol, ethyl cafeate, methyl salicylate, p-hydroxybenzoic acid, quinic acid, salicylic acid, α -carotene, foliaxanthin, physoxanthin, (E)-coniferaldehyde, (E)-2,3-dihydroxycyclopentyl-3-(3',4'-dihydroxyphenyl)acrylate, eugenol.

Coumarins and coumestans- Scopolin, scopoletin, coumarin, fraxetin, umbelliferone, 7-hydroxy-6,8-dimethoxy-3-(4'-hydroxy-3'-methoxyphenyl)-coumarin, cleosandrin, arteminorin A, indicumin E, aesculetin, 6,7-dimethoxycoumarin, 1'-O-7-esculetin-4'-O-1'-ethylenglycol- β -d-glucose, 9-hydroxy-2',2'-dimethyl [5',6':2,3]- coumestan, solalyratin A.

Coumarinolignoids- Indicumine A, indicumine B, indicumine C, indicumine D.

Fatty acids and esters- Hexadecanoic acid, octadecanoic acid, linoleic acid, lignoceric acid, corchorifatty acid B, linolenic acid, 9(Z),11(E)-octadecadienoic acid, decosahexaenoic acid, oleic acid, eicosapentaenoic acid, palmitoleic acid, arachidonic acid, myristic acid, gamma-linolenic acid, 9-oxo-(10E,12Z)-octadecadienoic acid, eicosanoic acid, octacosanoic acid,

Others- Puerariafuran, 1,2-benzenedicarboxylic acid, 1, 4-dimethylbenzene, n-nonane, n-octanol, methyl hexadecanoate, dodecane, undecanal, nonanal, eicosane, methyl-9,12-octadecadienoate, hexadecane, 9,17-octadecadienal, 4-hydroxy-4-methyl-2-pentanone, ethyl- α -D-arabinofuranoside, solalyratin B, 5-hydroxymethyl furfural, solanesol, 3-hydroxymethyl-7-methoxywutaifuranol, phenylmethyl 2-O- β -D-xylosyl- β -D-glucoside, zizybeoside I.

Species

Genus *Solanum* is known to contain a total of 1239 taxonomically accepted species (POWO, 2024) that are as-

1. *S. abitaguense* S.Knapp in Brittonia 38: 290 (1986)
2. *S. abortivum* Symon in J. Adelaide Bot. Gard. 8: 93 (1985)
3. *S. absconditum* Agra in Syst. Bot. 33: 556 (2008)
4. *S. abutilifolium* Rusby in Mem. Torrey Bot. Club 4: 229 (1895)
5. *S. abutiloides* Bitter & Lillo in Repert. Spec. Nov. Regni Veg. 12: 136 (1913)
6. *S. acanthodapis* A.R.Bean in Austrobaileya 6: 250 (2002)
7. *S. acaule* Bitter in Repert. Spec. Nov. Regni Veg. 11: 391 (1912)
8. *S. accrescens* Standl. & C.V.Morton in Publ. Field Mus. Nat. Hist., Bot. Ser. 18: 1075 (1938)
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Solanum nigrum L.

Solanum nigrum is the one of the biggest and most diverse species groups of the genus *Solanum*. It is more usually referred to as the section Morella (Maurella (Dun.) Dumort.; Morella (Dun.) Bitt.), but since the generic type is *S. nigrum*, the correct name for this section is *Solanum* (Seithe, 1962). *Solanum nigrum* and its allied species, commonly known as black nightshades, are weeds that grow everywhere from the sea to the montane levels of arable land, gardens, trash piles, and soils rich in nitrogen (Edmonds et al., 1997). The plant *S. nigrum* is also referred to as ‘ganake soppu’ in Kannada, ‘makoi’ in Hindi, ‘kachchipandu’ in Telugu, ‘munatakali’ in Tamil, ‘piludi’ in Gujarati, and ‘black nightshade’ in English (Mandal et al., 2023).

Although *Solanum nigrum* has been used for a long time in Indian Ayurvedic medicine, it is evident that the term does not differentiate between *S. nigrum* and *S. villosum* in terms of therapeutic usage. The plants are recognized for their antiseptic and antidiarrhetic qualities.

They are also thought to be febrifuges and anti-inflammatory, just like other members of the family. They have been used in the treatment of many different diseases and complaints in many areas of the Old World (Särkinen et al., 2018).

General overviews of black nightshade taxonomy and systematics have been published (Edmonds, 1977, 1978, 1979; Chakrabarti and Sharma, 1994), including geographically focused taxonomic treatments for South America (Edmonds, 1972), North America (Schilling, 1981), Australia (Henderson, 1974), Africa (Edmonds and Chweya, 1997; Olet, 2004; Manoko, 2007; Edmonds, 2012), Europe (Wessely, 1960) and the Indian subcontinent (Schilling and Andersen, 1990) and detailed cytological, molecular and morphological studies (Saarisalo-Taubert, 1967; Venkateswarlu and Rao, 1972; Edmonds, 1982, 1983, 1984; Nandhini and Paramaguru, 2014; Särkinen et al., 2018).

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobinota
Super Division	-	Spermatophyta
Division	-	Mangoliophyta
Class	-	Magnoliopsida
Subclass	-	Asteridae
Order	-	Solanales
Family	-	Solanaceae
Genus	-	<i>Solanum</i>
Species	-	<i>nigrum</i>

Common Names

Makoya, Kakamachi, Kali makoy (Hindi); Garden nightshade, Black nightshade (English); Dhvansamaci (Sanskrit); Pitkachi (Assamese); Gudakamai (Bengali); Mako, Peelak, Mamoli (Punjabi); Kamoni (Marathi).

Global Distribution

The species *S. nigrum* is found Temperate Eurasia, Macaronesia, North and Northeast Tropical Africa. According to POWO (2024) it is native to Afghanistan, Albania, Algeria, Altay, Amur, Andaman Is., Assam, Austria, Azores, Baleares, Baltic States, Bangladesh, Belarus, Belgium, Bulgaria, Canary Is., Cape Verde, Central European Rus, Chad, China North-Central, China South-Central, China Southeast, Chita, Corse, Cyprus, Czechoslovakia, Denmark, Djibouti, East Aegean Is., East European Russia, East

Himalaya, Egypt, Eritrea, Ethiopia, Finland, France, Germany, Great Britain, Greece, Gulf States, Hungary, India, Inner Mongolia, Iran, Iraq, Ireland, Irkutsk, Italy, Japan, Kazakhstan, Khabarovsk, Kirgizstan, Korea, Krasnoyarsk, Kriti, Krym, Kuril Is., Kuwait, Laos, Lebanon-Syria, Libya, Madeira, Manchuria, Morocco, Myanmar, Nepal, Netherlands, Nicobar Is., North Caucasus, North European Russi, Northwest European R, Norway, Oman, Pakistan, Palestine, Poland, Portugal, Primorye, Romania, Sakhalin, Sardegna, Saudi Arabia, Selvagens, Sicilia, Sinai, South China Sea, South European Russi, Spain, Sudan, Sweden, Switzerland, Tadzhikistan, Taiwan, Thailand, Tibet, Transcaucasus, Tunisia, Turkey, Turkey-in-Europe, Turkmenistan, Ukraine, Uzbekistan, Vietnam, West Himalaya, West Siberia, Western Sahara, Yemen, Yugoslavia; and introduced into Alabama, Alaska, Alberta, Antipodean Is., Ascension, Borneo, British Columbia, California, Cape Provinces, Chatham Is., Colorado, Connecticut, District of Columbia, Florida, Georgia, Iowa, Jawa, Kerguelen, Maine, Malaya, Manitoba, Maryland, Massachusetts, Mexico Northwest, Missouri, Montana, Nevada, New Guinea, New Hampshire, New Jersey, New Mexico, New South Wales, New York, New Zealand North, New Zealand South, Newfoundland, Norfolk Is., North Carolina, Northern Territory, Nova Scotia, Oklahoma, Ontario, Oregon, Pennsylvania, Philippines, Queensland, Québec, South Australia, South Carolina, St. Helena, Sumatra, Tasmania, Texas, Tristan da Cunha, Utah, Victoria, Virginia, Washington, Western Australia, Wisconsin (Fig. 2).

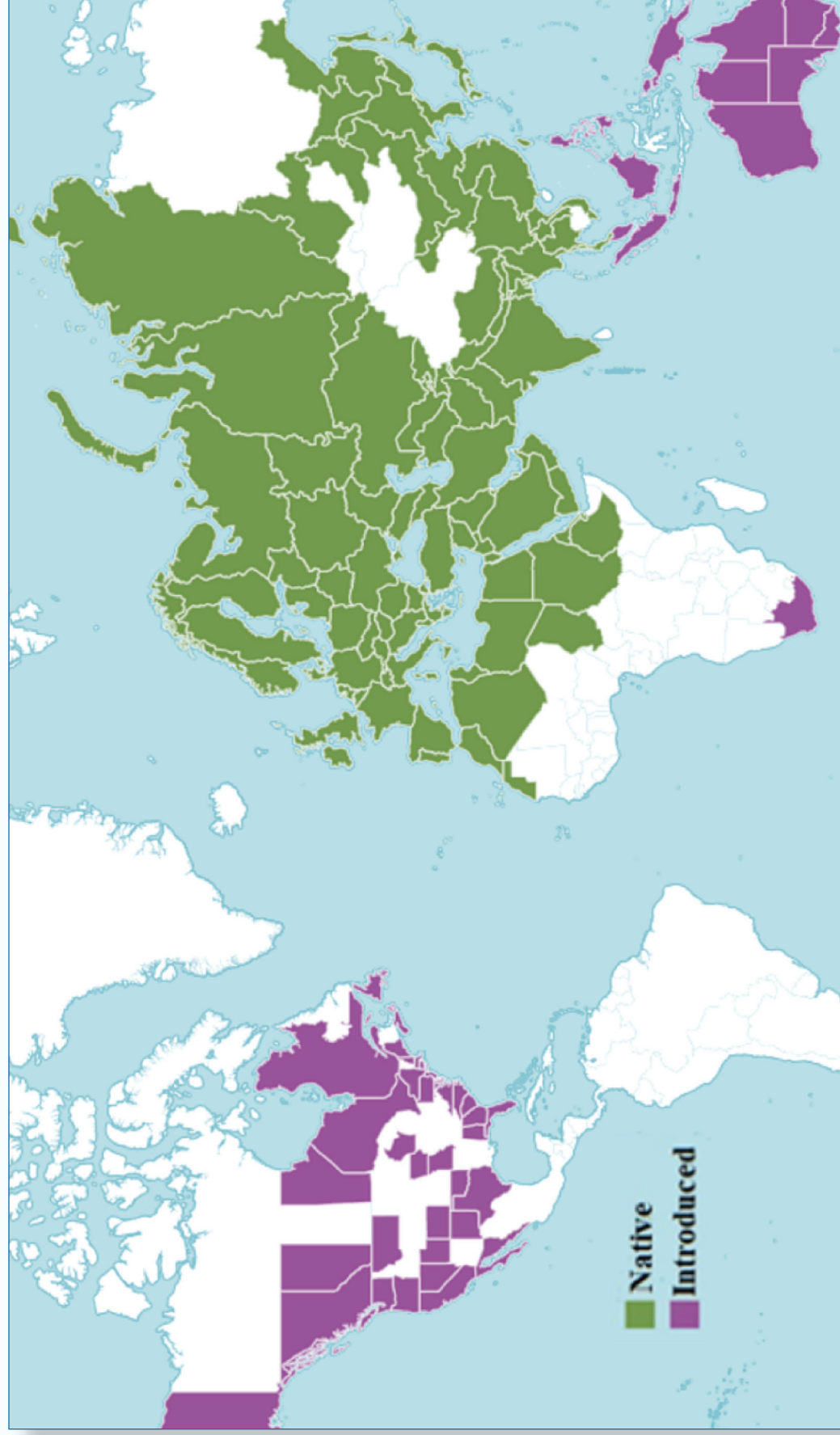


Fig. 2 Global distribution of *S. nigrum*

Distribution in India

S. nigrum grows in wooded areas. They are distributed from temperate to tropical regions and from sea level to high altitudes. They are widely distributed in throughout the India including Annamalai Nagar (Tamil Nadu), Gorakhpur, Jaunpur (Uttar Pradesh), Hassan (Karnataka), Jabalpur (Madhya Pradesh), Mannanore forest (Andhra Pradesh), Pune (Maharashtra) and West Bengal, etc. (Bekele, 2019; Singh, et al., 2022).

Ethnopharmacology and Traditional Uses

S. nigrum is commonly edible medicinal plant. The whole plant is used as an alternative, antiperiodic, antiphlogistic, aphrodisiac, diaphoretic, diuretic, emollient, febrifuge, laxative, narcotic, purgative, sedative, stimulant and tonic and different parts like stem, leaves, fruits used to cure many diseases such as cancerous sores, boils, leukoderma, and wounds. The leaves are used as poultice to cure rheumatic and gouty joints, skin diseases, in the treatment of tuberculosis and promotes excessive sweating (diaphoresis), treat dropsy, nausea and nervous disorders (Bekele, 2019). In Uttarakhand, the local and tribal people use it as pain relieving agent, leaves are used to treat heart problem, blood coagulation, indigestion, skin disease, jaundice, and diabetes (Padalia, 2015).

Flowers and fruits decoction are useful in cough and erysipelas. Fruits juice treat diarrhea, ophthalmopathy and hydrophobia.

The seeds are used to treat and dipsia and the part of roots useful in otopathy, rhinopathy and hepatitis (Saleem et al., 2009). In Africa and Algeria, whole plant decoction is used to cure burns and dermal affections. In India the whole plant and leaf are useful in treatment of stomachache, stomach ulcer, rabies, wound cough while root juice is used to treat asthma and whooping cough (Jain et al., 2021).

The infusion of *S. nigrum* is used in dysentery, stomach complaints and fever. Plants juice is used on ulcers and other skin diseases. Seed is used to cure asthma, tonic, laxative, appetite stimulant. The herb was traditionally used to treat tuberculosis. It is used as antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic. This plant has a high nutritional value and can provide minerals, vitamins, proteins, hormones, and other nutrients (Kumar et al., 2012)

The usage of herbal treatment, particularly Ayurvedic plants, is not only widespread but also expanding globally. The Ayurvedic herbal pharmaceutical industry has experienced tremendous development due to its efficacy, safety, and inexpensiveness. In Ayurveda *Solanum nigrum* is indicated for various disorders like *Shotha* (inflammation), *Vrana* (wound healer), *Visarpa* (herpes), *Vrishanashotha* (antitoxic), *Yakritshotha* (inflammation of liver), *Udararoga* (ascitis), *Mukharoga* (diseases of mouth), *Kanharoga* (throat disorders), *Nasaroga* (nasal disorder), *Karnashoola* (ear ache), *Netraroga* (eye disorders), *Shwitra* (leukoderma / vitiligo), *Agnimandya* (appetizer), *Chhardi* (antiemetic), *Arsha* (anti-hemorrhoid), *Jeerna pravahita*

(chronic amoebiasis), *Atisara* (anti-diarrhea), *Pleehavridhi* (spleenomegali), *Hridroga* (heart diseases), *Raktabharadhikya* (antihypertensive), *Kushtha* (skin diseases), *Vatarakta* (gout), *Amavata* (anti-rheumatoid), *Vrikkaroga* (kidney disorders), *Prameha* (antidiabetic), *Jeerna jwara* (chronic fever), *Samanya daurbalya* (general debility), and *Kshaya* (antitubercular) [Jani et al., 2010; Kumar et al., 2012].

Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *S. nigrum* were investigated

across the Ganga River. The Ganga began from the Gomukh in Gangotri glacier and ended at Gangasagar in the Bay of Bengal. A total of 26 sites were selected with 2 sites ranging around 100 km in distance while the whole distance is about 2600 km (Table 1). The plant diversity was assessed up to 10 km on both sides of each site. The plants were identified and deposited in the Patanjali Research Foundation Herbarium with an acronym of PRFH for future records. A bulk sample of the plant was taken from each site in a large polybag which was deposited in the analytical laboratory for phytochemical profiling.

Table 1 Different studied sites and their GPS coordinates along with *S. nigrum* status

Site	Locality	GPS Coordinates			Status
		Altitude (m)	Latitude (N)	Longitude (E)	
S1	Gomukh	4023	30.80	79.15	Absent
S2	Gangotri	3415	30.98	78.93	Absent
S3	Uttarkashi	1158	30.73	78.44	Absent
S4	Devprayag	830	30.15	78.60	Present
S5	Haridwar	314	29.97	78.17	Present
S6	Bijnor	225	29.37	78.13	Present
S7	Narora	174	28.20	78.38	Absent
S8	Budaun	164	28.05	79.12	Present
S9	Farrukhabad	151	27.37	79.63	Present
S10	Bithoor	126	26.61	80.27	Present
S11	Dalmau	115	26.07	81.03	Present
S12	Prayagraj	98	25.45	81.85	Present
S13	Mirzapur	80	25.15	82.58	Present

Site	Locality	GPS Coordinates			Status
S14	Varanasi	81	25.32	83.01	Present
S15	Ballia	67	25.76	84.15	Present
S16	Revelganj	52	25.78	84.67	Present
S17	Patna	53	25.61	85.14	Present
S18	Barh	47	25.48	85.72	Present
S19	Bahachouki	55	25.30	86.36	Absent
S20	Farka	42	25.23	87.09	Present
S21	Sahebganj	16	25.25	87.65	Absent
S22	Farakka Bar- rage	30	24.82	87.90	Absent
S23	Murshidabad	18	24.18	88.27	Absent
S24	Mayapur	11	23.43	88.39	Absent
S25	Hoogli	9	22.91	88.40	Present
S26	Gangasagar	4	22.19	88.19	Absent

Local Occurrence

The plant was recorded in all the sites, but it was collected for the analytical studies only from the sites 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 25, and 26 due to these sites being the source of its bulk collection. While in the remaining sites as 1, 2, 3, 7, 19, 21, 22, 23, 24, and 26 it was only representative. Hence, it may be concluded that these sites require more conservation of this species.

Taxonomic Treatment

Solanum nigrum L., Sp. Pl.: 186. 1753.; C.B.Clarke in Hook.f., FL Brit. India 4: 229. 1883; Duthie, Fl. Gangetic Plain 2: 124. 1911; Haines, Bot. Bihar Orissa 2: 640.1922; Deb, J.

Econ. Taxon. Bot. 1: 49. 1980; Uniyal & al., Fl. Pl. Uttarakhand, Checkl.: 203. 2007; Sinha & Sukla, Fl. Uttar Pradesh 2: 213.2020.

Synonyms

The Plants of the World Online database mentioned it with its eight homotypic and 83 heterotypic synonyms (POWO, 2024) as-

Homotypic Synonym

1. *S. humile* Salisb. in Prodr. Stirp. Chap. Allerton: 134 (1796), nom. superfl.
2. *S. morella* Desf. in Observ. Pl. Angers: 113 (1818), nom. superfl.
3. *S. morella* subsp. *nigrum* (L.) Rouy in G.Rouy & J.Foucaud, Fl. France 10: 364 (1908), nom. superfl.

4. *S. nigrum* var. *genuinum* Hassl. in Trab. Mus. Farmacol. 21: 104 (1909), not validly publ.
5. *S. nigrum* var. *humile* Macloskie in Rep. Princeton Univ. Exped. Patagonia, Botany 8: 707 (1905), not validly publ.
6. *S. nigrum* var. *legitimum* Neill. in Fl. Nied.-Oesterr.: 535 (1858), not validly publ.
7. *S. vulgatum* Baumg. in Fl. Lips.: 120 (1790), nom. superfl.
8. *S. vulgatum* var. *nigrum* (L.) Spenn. in Fl. Friburg. 2: 427 (1826), nom. superfl.
11. *S. chenopodium* Raf. in Autik. Bot.: 107 (1840)
12. *S. chlorocarpum* Schur in Enum. Pl. Transsilv.: 478 (1866), not validly publ.
13. *S. cuneifolium* Dunal in A.P.de Candolle, Prodr. 13(1): 53 (1852), not validly publ.
14. *S. decipiens* Opiz in B.V.von Berchtold & P.M.Opiz, Oekon.-Techn. Fl. Böhm. 3(2): XXIV (1843)
15. *S. decurrens* Wall. ex Dunal in A.P.de Candolle, Prodr. 13(1): 50 (1852), not validly publ.
16. *S. diffusum* Vell. in Fl. Flumin.: 83 (1829)
17. *S. exaratum* Raf. in Autik. Bot.: 107 (1840)
18. *S. extremiorientale* Ignatov in Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol. 91(3): 113 (1986)
19. *S. foetidum* Rottb. in Acta Lit. Univ. Hafn. 1: 287 (1778), nom. illeg.
20. *S. fontanesianum* Schrank in Pl. Rar. Hort. Monac.: t. 18 (1817)
21. *S. ganchouenense* H.Lév. in Repert. Spec. Nov. Regni Veg. 11: 295 (1912)
22. *S. guineense* var. *nepalense* Dunal in A.P.de Candolle, Prodr. 13(1): 49 (1852)
23. *S. hirsutum* Kit. ex Kanitz in Linnaea 32: 440 (1863), nom. illeg.
24. *S. judaicum* (L.) Besser in Prim. Fl. Galiciae Austriac. 1: 183 (1809)
25. *S. memphiticum* var. *repandum* Dunal in A.P.de Candolle, Prodr. 13(1): 47 (1852)
26. *S. morella* var. *schultesii* (Opiz) Rouy & Foucaud in Fl. France 10: 365 (1908)

Heterotypic Synonyms

1. *S. acutifolium* Kit. ex Kanitz in Linnaea 32: 440 (1864), nom. illeg.
2. *S. astroites* G.Forst. in Fl. Ins. Austr.: 89 (1786)
3. *S. atriplicifolium* Desp. ex Dunal in Solan. Syn.: 12 (1816), not validly publ.
4. *S. besserianum* Weinm. in Elench. Pl. Hort. Pawlowsk.: 100 (1824), not validly publ.
5. *S. bidentatum* Raf. in Autik. Bot.: 108 (1840)
6. *S. cechicum* Opiz in Lotos 4: 94 (1854)
7. *S. cestrifolium* Jacq. ex Weinw. in Elench. Pl. Hort. Pawlowsk.: 100 (1824)
8. *S. cestrifolium* Jacq. ex Spreng. in Syst. Veg., ed. 16. 1: 680 (1824)
9. *S. cestrophyllum* Dunal in A.P.de Candolle, Prodr. 13(1): 186 (1852)
10. *S. chenopodiifolium* H.Lév. in Repert. Spec. Nov. Regni Veg. 12: 531 (1913)

27. *S. moschatum* C.Presl in J.S.Presl & C.B.Presl, Delic. Prag.: 77 (1822)
28. *S. nigrum* var. *atriplicifolium* G.Mey. in Chloris Han.: 265 (1836)
29. *S. nigrum* var. *atriplicifolium* N.H.F.Desp. in Fl. Sarthe: 189 (1838), nom. illeg.
30. *S. nigrum* subvar. *atriplicifolium* (G.Mey.) Schinz & Thell. in H.Schinz & R.Keller, Fl. Schweiz, ed. 3, 2: 295 (1914)
31. *S. nigrum* var. *chlorocarpum* (Spenn.) Schur in Enum. Pl. Transsilv.: 478 (1866)
32. *S. nigrum* f. *chlorocarpum* (Spenn.) A.Braun ex Döll in Rhein. Fl.: 413 (1843)
33. *S. nigrum* subsp. *chlorocarpum* (Spenn.) Arcang. in Comp. Fl. Ital.: 497 (1882)
34. *S. nigrum* var. *elbaensis* Täckh. & Boulos in Publ. Cairo Univ. Herb. 5: 102 (1974)
35. *S. nigrum* subsp. *europaeum* Fil. in Kul't. Fl. SSSR 10: 370-386 (1958)
36. *S. nigrum* var. *flavidum* S.Z.Liou & W.Q.Wang in Fl. Liaoningica 2: 1158 (1992)
37. *S. nigrum* var. *flavum* Rouy in G.Rouy & J.Foucaud, Fl. France 10: 366 (1908)
38. *S. nigrum* var. *glabrum* Lowe in Man. Fl. Madeira 2: 73 (1872)
39. *S. nigrum* var. *hebecaulon* Lowe in Man. Fl. Madeira 2: 73 (1872)
40. *S. nigrum* var. *hirsutum* G.Mey. in Prim. Fl. Esseq.: 109 (1818), nom. illeg.
41. *S. nigrum* var. *incisum* Täckh. & Boulos in Publ. Cairo Univ. Herb. 5: 101 (1974)
42. *S. nigrum* *inerme* K.Koch in Linnaea 17: 283 (1843)
43. *S. nigrum* f. *judaicum* (L.) Miq. in Fl. Ned. Ind. 2: 637 (1857)
44. *S. nigrum* var. *judaicum* L. in Sp. Pl.: 186 (1753)
45. *S. nigrum* f. *longepedunculatum* Hassl. in Trab. Mus. Farmacol. 21: 105 (1909)
46. *S. nigrum* f. *luridum* Wessely in Feddes Repert. Spec. Nov. Regni Veg. 63: 311 (1960)
47. *S. nigrum* var. *luteovirescens* N.H.F.Desp. in Fl. Sarthe: 189 (1838)
48. *S. nigrum* var. *macrocarpum* Schur in Enum. Pl. Transsilv.: 478 (1866)
49. *S. nigrum* subsp. *moschatum* (C.Presl) Arcang. in Comp. Fl. Ital.: 497 (1882)
50. *S. nigrum* var. *ochrocarpum* Lej. in A.L.S.Lejeune & R.J.Courtois, Comp. Fl. Belg. 1: 194 (1828)
51. *S. nigrum* f. *pallidum* Wessely in Feddes Repert. Spec. Nov. Regni Veg. 63: 311 (1960)
52. *S. nigrum* f. *paludosum* Miq. in Fl. Ned. Ind. 2: 637 (1857)
53. *S. nigrum* var. *perennans* Bertol. in Fl. Ital. 2: 634 (1836)
54. *S. nigrum* var. *schultesii* (Opiz) Rouy in Acta Horti Gothob. 10: 201 (1935)
55. *S. nigrum* subsp. *schultesii* (Opiz) Wessely in Feddes Repert. Spec. Nov. Regni Veg. 63: 311 (1960)
56. *S. nigrum* subf. *sinuatodentatum* Hassl. in Trab. Mus. Farmacol. 21: 105 (1909)
57. *S. nigrum* var. *stenopetalum* Döll in Rhein. Fl.: 412 (1845)
58. *S. nigrum* f. *stenopetalum* A.Braun ex

- Döll in Rhein. FL.: 412 (1843)
59. *S. nigrum* var. *stylosum* Witasek in Anales Univ. Chile 124: 456 (1909)
 60. *S. nigrum* var. *subhastatum* Hassl. in Trab. Mus. Farmacol. 21: 104 (1909)
 61. *S. nigrum* var. *tauschii* [Opiz] Rouy in G.Rouy & J.Foucaud, Fl. France 10: 365 (1908)
 62. *S. nigrum* var. *vulgare* L. in Sp. Pl.: 186 (1753)
 63. *S. nigrum* var. *vulgatum* Dunal in A.P.de Candolle, Prodr. 13(1): 50 (1852), nom. illeg.
 64. *S. nigrum* var. *vulgatum* L. in Sp. Pl., ed. 2.: 266 (1762)
 65. *S. paludosum* Dunal in A.P.de Candolle, Prodr. 13(1): 57 (1852), nom. illeg.
 66. *S. parviflorum* Moris ex Badarò in Giorn. Fis. Chim. Storia Nat. Med. Arti, Dec. 2, 7: 364 (1824), nom. illeg.
 67. *S. peregrinum* E.P.Bicknell in Bull. Torrey Bot. Club 42: 332 (1915)
 68. *S. probstianum* Polgar in Mitt. Naturf. Ges. Solothurn 12: 30 (1938), nom. provis.
 69. *S. pseudoflavum* Pojark. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 17: 338 (1955)
 70. *S. pterocaulum* var. *deppei* Dunal in A.P.de Candolle, Prodr. 13(1): 52 (1852)
 71. *S. reineggeri* Opiz in B.V.von Berchtold & P.M.Opiz, Oekon.-Techn. Fl. Böhm. 3(2): XIX (1843)
 72. *S. repens* Noronha in Verh. Batav. Genootsch. Kunsten 5(4): 26 (1790), not validly publ.
 73. *S. rhinozerothis* Blume in Bijdr. Fl. Ned. Ind.: 695 (1826)
 74. *S. rottboellianum* Roem. & Schult. in Syst. Veg., ed. 15[bis]. 4: 668 (1819)
 75. *S. roxburghii* Dunal in A.P.de Candolle, Prodr. 13(1): 57 (1852), not validly publ.
 76. *S. schultesii* Opiz in B.V.von Berchtold & P.M.Opiz, Oekon.-Techn. Fl. Böhm. 3(2): XXIV (1843)
 77. *S. speciosum* Dunal in A.P.de Candolle, Prodr. 13(1): 52 (1852), not validly publ.
 78. *S. stenopetalum* A.Braun in Index Seminum [B, Berolinensis] 1853(App.): 8 (1853)
 79. *S. tauschii* Opiz in B.V.von Berchtold & P.M.Opiz, Oekon.-Techn. Fl. Böhm. 3(2): 20 (1842)
 80. *S. villosum* var. *glabrescens* Opiz ex Roem. & Schult. in Syst. Veg., ed. 15[bis]. 4: 592 (1819)
 81. *S. viride* Schur in Enum. Pl. Transsilv.: 478 (1866), nom. illeg.
 82. *S. vulgare* (L.) Hegetschw. in Fl. Schweiz: 219 (1839)
 83. *S. vulgatum* var. *chlorocarpum* Spenn. in Fl. Friburg. 3: 1074 (1829)

While another database i.e., World Flora Online (WFO, 2024) mentioned it with a total of following ninety-six synonyms.

1. *S. acutifolium* Kit. ex Kanitz in Linnaea 32: 440. 1863. nom. Illeg
2. *S. atriplicifolium* Desp. ex Dunal in Solan. Syn. : 12 (1816)

3. *S. atriplicifolium* var. *atriplicifolium*
4. *S. bidentatum* Raf in Autik. Bot. 108. 1840
5. *S. cechicum* Opiz in Lotos 4: 94. 1854
6. *S. cestrifolium* Jacq. ex Spreng in Syst. Veg. ed. 16, 1: 680 (1825)
7. *S. chenopodiifolium* H.Lév in Repert. Spec. Nov. Regni Veg. 12: 531. 1913. nom. Illeg
8. *S. chenopodium* Raf in Autik. Bot. 107. 1840
9. *S. chlorocarpum* Schur in Enum. Pl. Transsilv. 478. 1866
10. *S. cuneifolium* Dunal in Prodr. [A. P. de Candolle] 13(1): 53. 1852
11. *S. decipiens* Opiz in Oekon.-techn. Fl. Böhm. (Bercht. & Opiz) 3(2): XXIV. 1843
12. *S. deppei* Dunal in Prodr. [A. P. de Candolle] 13(1): 52. 1852
13. *S. exaratum* Raf in Autik. Bot. 107. 1840
14. *S. ganchouenense* H.Lév in Repert. Spec. Nov. Regni Veg. 11: 295. 1912
15. *S. guineense* var. *nepalense* Dunal in Prodr. [A. P. de Candolle] 13(1): 49. 1852
16. *S. hirsutum* Kit. ex Kanitz in Linnaea 32: 440. 1863 nom. Illeg
17. *S. humile* Nocca
18. *S. humile* Salisb in Prodr. Stirp. Chap. Allerton 134. 1796. nom. Illeg
19. *S. judaicum* (L.) Miq in (L.) Miq., (W.) Miq., (J.) Miq., (D.) Miq., and (B.) Miq., Fl. Ned. Ind. 2: 637. 1857., 1857
20. *S. judaicum* Besser in Prim. Fl. Galiciae Austriac. 1: 183. 1809
21. *S. judaicum* Hack. ex Dunal in Prodr. [A. P. de Candolle] 13(1): 53. 1852
22. *S. luridum* Wessely in Wessely and (O.) Wessely, Repert. Spec. Nov. Regni Veg. 63: 311. 1960., 1960
23. *S. memphiticum* var. *repandum* Dunal in Prodr. [A. P. de Candolle] 13(1): 47. 1852
24. *S. morella* Desv in Observ. Pl. Angers 113. 1818
25. *S. morella* subsp. *Morella*
26. *S. morella* subsp. *nigrum* (L.) Rouy in Fl. France [Rouy & Foucaud] 10: 364. 1908
27. *S. moschatum* C.Presl in Delic. Prag. 77. 1832
28. *S. nigrum* Tausch ex Dunal in Prodr. [A. P. de Candolle] 13(1): 53. 1852
29. *S. nigrum* f. *chlorocarpum* A.Braun ex Döll in Rhein. Fl.: 413 (1843)
30. *S. nigrum* f. *genuinum* Döll
31. *S. nigrum* f. *judaicum* Miq in Fl. Ned. Ind. 2: 637. 1857
32. *S. nigrum* f. *luridum* Wessely in Repert. Spec. Nov. Regni Veg. 63: 311. 1960
33. *S. nigrum* f. *pallidum* Wessely in Feddes Repert. Spec. Nov. Regni Veg. 63: 311 (1960)
34. *S. nigrum* f. *paludosum* (Dunal) Miq in Fl. Ned. Ind. 2: 637. 1857
35. *S. nigrum* f. *stenopetalum* A.Braun ex Döll in Rhein. Fl.: 412 (1843)
36. *S. nigrum* subsp. *australiense* Filov
37. *S. nigrum* subsp. *europaeum* Filov in Kul't. Fl. SSSR 10: 370-386. 1958 nom. Illeg

38. *S. nigrum* subsp. *schultesii* (Opiz) Wessely in Feddes Repert. Spec. Nov. Regni Veg. 63: 311. 1960
39. *S. nigrum* subvar. *atriplicifolium* (Desp. ex Dunal) Schinz & (Thell.) in Fl. Schweiz [Schinz], ed. 3, 2: 295. 1914. nom. Illeg
40. *S. nigrum* var. *aegyptiacum* Lam. ex C.C.Gmel L. ex C.C.Gmel in Hort. Carlsruh. 253 1811., 1811
41. *S. nigrum* var. *atriplicifolium* Desp. ex Filov in Kult. Fl. SSSR (Zhukovskii) 10: 370-386. 1958. nom. Illeg
42. *S. nigrum* var. *atriplicifolium* Desp. ex G.Don in Gen. Hist. 4: 412. 1837. nom. Illeg
43. *S. nigrum* var. *atriplicifolium* Dunal in Prodr. [A. P. de Candolle] 13(1): 50. 1852. nom. Illeg
44. *S. nigrum* var. *atriplicifolium* G.Mey in Chloris Han. 265. 1836
45. *S. nigrum* var. *chlorocarpum* F.Muell
46. *S. nigrum* var. *chlorocarpum* Koch in Syn. Fl. Germ. Helv., ed. 2: 600. 1846
47. *S. nigrum* var. *chlorocarpum* Spenn in Fl. Friburg. 3: 1074. 1829
48. *S. nigrum* var. *flavidum* S.Z.Liou & W.Q.Wang in Fl. Liaoningica 2: 1158. 1992
49. *S. nigrum* var. *frutescens* A.B.
50. *S. nigrum* var. *genuinum* (Döll) Döll
51. *S. nigrum* var. *genuinum* Godr in Fl. Lorraine 2: 133. 1843
52. *S. nigrum* var. *genuinum* Hassl in Trab. Mus. Farm. Fac. Cienc. Med. Buenos Aires 21: 104. 1909
53. *S. nigrum* var. *glabrum* Lowe in Man. FL. Madeira 2: 73 [1872]
54. *S. nigrum* var. *griseocarpum* Filov
55. *S. nigrum* var. *hebecaulon* Lowe in Man. FL. Maderia 2: 73. 1872
56. *S. nigrum* var. *hirsutum* G.Mey in Prim. Fl. Esseq. 109. 1818
57. *S. nigrum* var. *humile* F.M.Bailey in F.M.Bailey, Queensland Philos. Soc. 3: 1-4. 1881., 1881
58. *S. nigrum* var. *incisum* Täckh. & Boulos in Publ. Cairo Univ. Cairo Herb. 5: 101. 1974 ['1972']
59. *S. nigrum* var. *inermis* K.Koch in Linnaea 17: 283. 1843
60. *S. nigrum* var. *judaicum* L in Sp. Pl. 186. 1753
61. *S. nigrum* var. *legitimum* Neill in Fl. Nied.-Oesterr 535. 1858 nom. Illeg
62. *S. nigrum* var. *macrocarpum* Schur in Enum. Pl. Transsilv. 477-478. 1866
63. *S. nigrum* var. *paludosum* (Dunal) Miq in (J.) Miq., (D.) Miq., (D.) Miq., and (B.) Miq., Fl. Ned. Ind. 2: 637. 1861., 1861
64. *S. nigrum* var. *perennans* Bertol in Fl. Ital. 2: 634 [1836]
65. *S. nigrum* var. *schultesii* (Opiz) Rouy in Acta Horti Gothob. 10: 201. 1935
66. *S. nigrum* var. *stenopetalum* (A.Braun ex Döll) Döll in Fl. Baden [Döll] 768. 1859.
67. *S. nigrum* var. *stenopetalum* (A.Braun ex Döll) Döll
68. *S. nigrum* var. *uliginosum* (Blume) Miq (J.) Miq., (D.) Miq., (D.) Miq., and (B.) Miq. In Fl. Ned. Ind. 2: 637. 1861., 1861

69. *S. nigrum* var. *uniflorum* Miq Miq in FL. Ned. Ind. 2: 638. 1861., 1861
70. *S. nigrum* var. *vulgare* L in Sp. Pl. 186. 1753
71. *S. nigrum* var. *vulgatum* Dunal in Prodr. [A. P. de Candolle] 13(1): 50. 1852
72. *S. nigrum* var. *vulgatum* L in Sp. Pl., ed. 2. 1: 266. 1762
73. *S. paludosum* (Dunal) Miq (D.) Miq in FL. Ned. Ind. 2: 637. 1857., 1857
74. *S. paludosum* Dunal in Prodr. [A. P. de Candolle] 13(1): 57. 1852
75. *S. parviflorum* Moretti ex Badarò in Giorn. Fis. Chim. Storia Nat. Med. Arti Dec. 2, 7: 364. 1824. nom. Illeg
76. *S. peregrinum* E.P.Bicknell in Bull. Torrey Bot. Club 42: 332. 1915
77. *S. probstianum* Polg in Mitteil. Naturfor. Gesellsch. Solothurn 12: 30. 1938
78. *S. pseudoflavum* Pojark in Bot. Mater. Gerb. Inst. Komorova Akad. Nauk S.S.S.R. 17: 338. 1955
79. *S. reineggeri* Opiz in Oekon.-techn. FL. Böhm. (Bercht. & Opiz) 3(2): XIX. 1843
80. *S. repens* Noronha in Verh. Batav. Genootsch. Kunst. 5(Art. 4): 84. 1791
81. *S. roxburghii* Dunal in Prodr. [A. P. de Candolle] 13(1): 57. 1852
82. *S. schultesii* Opiz in Oekon.-techn. FL. Böhm. (Bercht. & Opiz) 3(2): XXIV. 1843
83. *S. stenopetalum* (A.Braun ex Döll) A.Braun in Ind. Sem. Hort. Berol.: 18. 1853
84. *S. suffruticosum* Schousb. ex Willd in Enum. Pl. (Willdenow) 1: 236. 1809
85. *S. tauschii* Opiz in Oekon.-techn. FL. Böhm. (Bercht. & Opiz) 3(2): XX. 1843
86. *S. uliginosum* (Blume) Miq (L.) Miq., (W.) Miq., (J.) Miq., (D.) Miq., and (B.) Miq in FL. Ned. Ind. 2: 637. 1857., 1857
87. *S. uniflorum* Miq. Miq in FL. Ned. Ind. 2: 638. 1857., 1857
88. *S. villosum* Opiz ex Dunal in Prodr. [A. P. de Candolle] 13(1): 51. 1852
89. *S. viride* Schur in Enum. Pl. Transsilv. 478. 1866. nom. Illeg
90. *S. vulgare* Hegetschw in FL. Schweiz 219. Dec 1838-Jan 1839. nom. Illeg
91. *S. vulgare* Kittell in Taschenb. FL. Deutschl. 285. 1837
92. *S. vulgatum* (L.) Spenn in FL. Friburg. 2: 427. 1826
93. *S. vulgatum* Baumg in FL. Lips. 120. 1790. nom. Illeg
94. *S. vulgatum* var. *chlorocarpum* Spenn in FL. Friburg. 3: 1074. 1829
95. *S. vulgatum* var. *nigrum* (L.) Spenn in FL. Friburg. 2: 427. 1829
96. *S. vulgatum* var. *vulgatum*

Botanical Description

Herb, 0.25-1 m tall. Stem decumbent to erect, branched, sparsely hairy; branches and shoots subglabrous or pubescent to glandular-villous; hairs appressed or patent. Leaves opposite or alternate; petiole 1-2 cm long, appressed pubescent or glandular inuate to irregularly dentate; lamina 2.5-10 x 1.5-5.5 cm, oval, elongated or egg-shaped in outline, base sub-truncate or rounded to

cuneate, oblique, $\pm$ decurrent into the petiole, margin entire, bluntly toothed or slightly lobed, apex acute or obtuse, acuminate, sparsely hairy. Inflorescence simple, umbellate, lax, and often extended cymes, 3-10 flowered; peduncle slender, erect, or ascending, ascending to deflexed; pedicel up to 5 mm long, slender, decurved to ascending, much shorter, recurved in fruit. Calyx campanulate, ovate; lobes obovate, semi-elliptic or ovate to $\pm$ triangular, rounded to acute, glabrous to

pubescent. Corolla stellate, much exceeding the calyx in length; lobes oblong or ovate to lanceolate, rarely linear, $\pm$ acute, triangular-acute. Anther yellow; filaments as long or shorter than the anthers, pilose. Ovary glabrous; style $\pm$ included pubescent at the base; stigma capitate. Fruits berry, up to 8 mm in diameter, globose to subovoid, black or orange red. Seeds 1.5-2 mm in diameter, discoid, minutely reticulate-foveolate (Fig. 3A, B).



Fig. 3A Plant habit



Fig. 3B (a) Plant, (b & c) Flower, (d) Open corolla, (e) Stamen, (f) Anther, (g) Stigma, (h) T.S of ovary, (i) L.S of ovary

Chromosome number

In *S. nigrum*, there are 12 basic chromosomes ($x=12$). This species exhibits diploid ($2n=24$), tetraploid ($2n=48$), and hexaploid ($2n=72$) polyploidy with haploid numbers of 12, 24, and 36 respectively (Tandon and Rao, 1964). *S. nigrum* is currently recognised as being hexaploid, with $2n=6x=72$ (Symon, 1981; Bukenya-Ziraba, 1996; Kumar and Pushpangadan, 2005; Melo et al., 2011). The naturally occurring tetraploid is crossed with the diploid to create an artificial

hexaploid species. The triploid is then raised to the hexaploidy level by treating it with colchicine.

Anatomical Features

Structure of the Petiole

The cross sections of the petiole showed an oval form with two ears on the lateral side. The epidermis was coated in a cuticle and was uniseriate. Numerous uniseriate, unicellular, or multicellular trichomes are present in

the epidermis. Furthermore, the cortex is situated under the epidermis. The crescent-shaped vascular bundles, including xylem and

phloem, are situated at the core of the pith (Fig. 4).

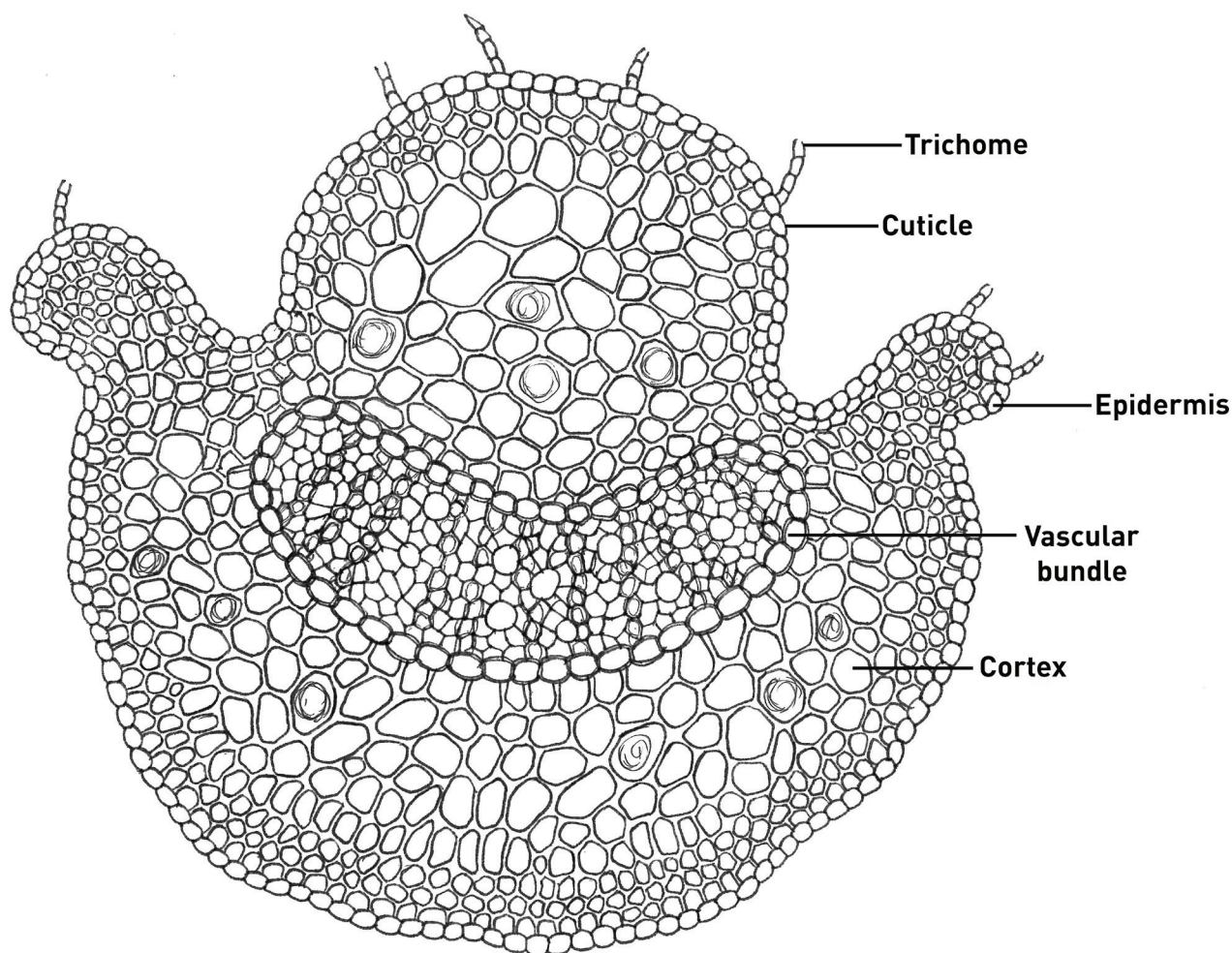


Fig. 4 T.S. of the petiole

| Structure of the Leaf

The stomata complex is visible in the leaf's epidermis; these stomata are anomocytic, meaning they lack subsidiary cells surrounding the guard cells, which have a kidney-shaped form, and the anticlinal walls of the epidermal cells are wavy (Fig. 5). The upper and lower uniseriate epidermis make up the transfer portion of the leaf. The upper epidermis has

ovule-shaped cells and is coated with cuticle. There are spongy layers and palisade layers in the leaf known as the bifacial mesophyll. The leaf's midrib area resembles a pear, the vascular bundle in the centre is crescent-shaped, and the epidermis is coated in multicellular trichomes and eglandular uniseriate structures (Fig. 6).

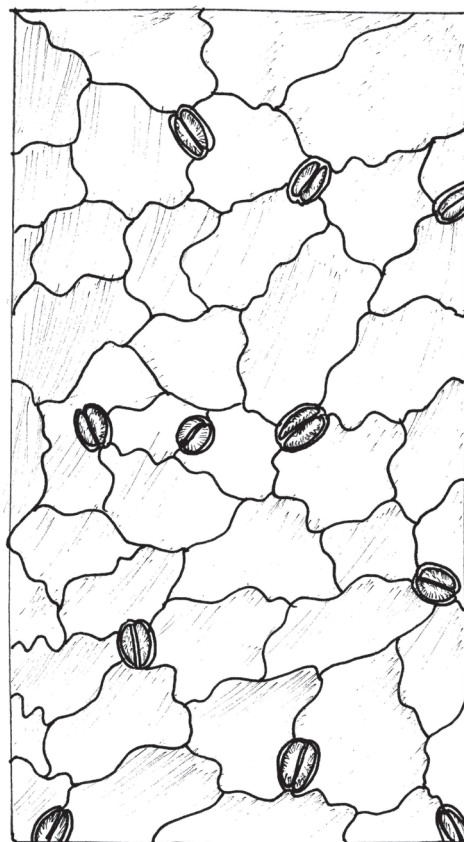


Fig. 5 Surface view of epidermis showing anomocytic types of stomata

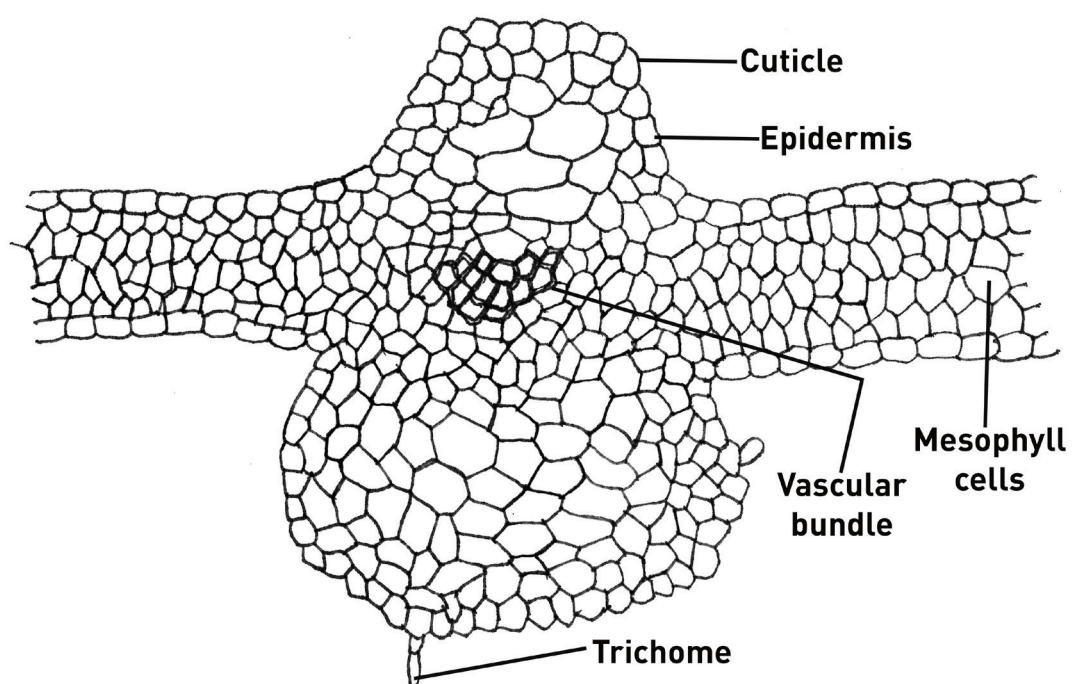


Fig. 6 T.S. of leaf midrib

Structure of the Stem

The outline stem has a tetragonal form, with a uniseriate epidermis coated in a cuticle that has uniseriate trichomes in its cross section. There are two layers of cortex located under the epidermis: parenchymatous tissues make up the inner layer, while collenchymatous

tissue makes up the outer layer. The vascular bundles on the inner side of the cortical layer are open, collateral, and provide shape to the stems. The pith, which is packed with circular parenchyma cells, is situated in the centre of the stem (Fig. 7).

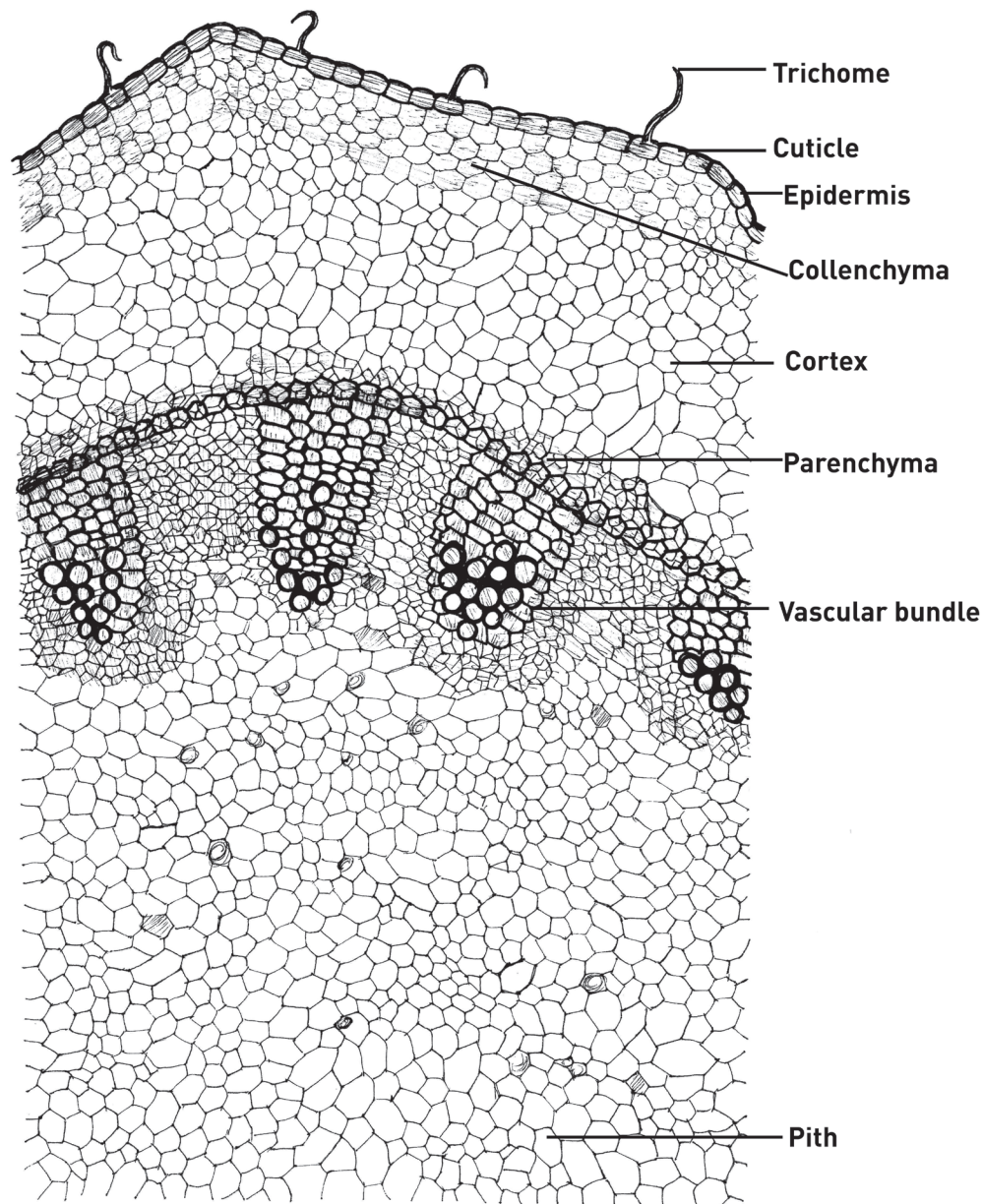


Fig. 7 T.S. of stem

Structure of the Root

The outline root has a round shape. The periderm covers the root, which is then followed by many rows of longitudinal and ovate parenchymatous tissues called the cortex. The cells of the cortex are scattered

across the usual schizogenous intercellular spaces. There are two types of vascular tissue: phloem and xylem. The pith included parenchyma cells and was situated in the centre of the root (Fig. 8).

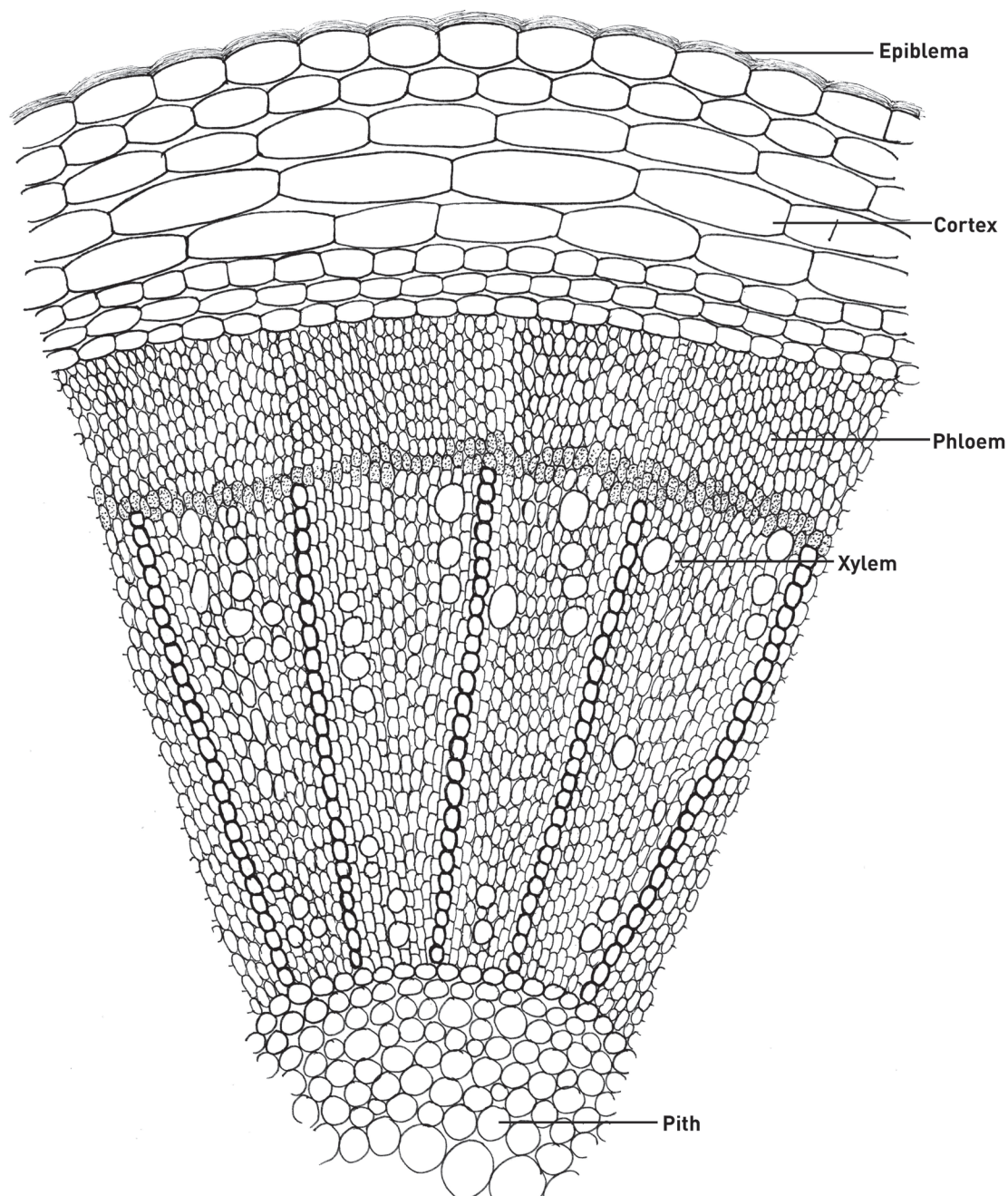


Fig. 8 T.S. of root

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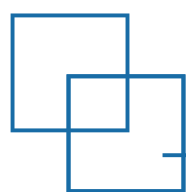
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CHAPTER
02

Bibliometric Analysis



INTRODUCTION

Solanum nigrum L., commonly referred to as 'Maku' or black nightshade, is a herbal plant native to Southeast Asia and a member of the Solanaceae family. It flourishes as a weed in moist habitats and can grow in various soil types. Traditionally, *S. nigrum* has been exclusively used to treat a range of disorders, including fever, pain, hepatitis, and inflammation (Yang et al., 2021). Although many traditional medicine systems around the world depend on *S. nigrum* for treating numerous diseases, modern therapeutic applications have yet to acknowledge its significance (Nawaz et al., 2021). Within herbal medicine, this plant is utilized for its numerous properties such as antioxidant, anti-inflammatory, anti-ulcerogenic, diuretic, hepatoprotective, antitumor, hypotensive, neuroprotective, immunomodulatory, antibacterial, liver-protective, antitumorigenic, and antipyretic (Mani et al., 2022). These diverse activities result from a variety of biological compounds that have been extensively identified. This plant contains bioactive compounds such as solasonine, other alkaloids, carbohydrate, glycones, solasodine, and solamargine. It contains steroidal saponins, steroidal alkaloids, flavonoids, coumarin, lignin, organic acids, volatile oils, polysaccharides, and other ingredients (Chen et al., 2022). It is in great demand in the pharmaceutical industry, since it has tolerance to an adverse environment (Muthuvel et al., 2020). It is of cold property having good effects of dispersing blood stasis and detumescence, clearing away heat, as well as detoxification and has been commonly used for the treatment of canker sores, skin eczema, urinary tract infections, bacterial dysentery, prostate, and chronic bronchitis, etc. for thousands of years (Chen et al., 2022). In addition, in modern clinical practice, *S. nigrum* is commonly combined with other drugs for the treatment of cancers, such as lung cancer, cervical cancer, breast cancer, esophageal cancer, stomach cancer, liver cancer, and bladder cancer. In India, the leaves and berries of this plant are commonly consumed as food or vegetable after cooking (Wang et al., 2017).

Bibliometric analysis serves as a basis in contemporary scientific inquiry, offering a holistic assessment of the scholarly domain surrounding a specific subject (Ellegaard and Wallin, 2015). This study investigated the research landscape of *S. nigrum* through



bibliometric examination, through a vast array of literature, research trajectories, and knowledge dissemination pertaining to this highly versatile and extensively studied plant. Through bibliometric analysis, researchers can investigate both the quantitative and qualitative dimensions within academic publications, empowering them to discern patterns, trajectories, and the evolutionary path of scientific discourse regarding *S. nigrum* (Karakose et al., 2022). This methodological approach involves a thorough investigation of publication metrics, citation dynamics, collaborative networks among authors, geographical distribution of research activities, and thematic focal points across the collection of scientific literature (Mejia et al., 2021). Through systematic analysis of bibliographic data related to *S. nigrum*, scholars gain an encompassing understanding of the breadth and depth of research activities of this botanical species.

The objective of this bibliometric analysis was to systematically map and evaluate the research landscape of *S. nigrum*, as a plant of significant medicinal and agricultural importance. This study aimed to identify key trends, prolific authors, important publications, and major research themes. By analyzing publication outputs, citation patterns, and collaboration networks, the analysis sought to determine the evolution of research focus, highlight gaps in current knowledge, and provide insights into emerging areas of interest. This comprehensive overview served as a valuable resource for researchers, funding agencies, and policymakers involved in the study and application of *S. nigrum*. Furthermore, strategic decision-making in various domains, including agricultural practices, environmental conservation, and pharmaceutical applications, was emphasized.

Insights with Data Source and Tools

The study examined the research landscape of *S. nigrum* through an extensive bibliometric analysis to provide a comprehensive understanding of the scope, impact, and evolving trends in academic publications related to this plant species. To achieve this, scholarly articles were sourced from Dimensions.ai, using the specific search phrase "*Solanum nigrum*" to cover publications

from 2000 to 2023. Microsoft Excel was used for organizing and conducting initial assessments of the data, offering a robust platform for managing and pre-processing large datasets. For more advanced bibliometric analysis, the study employed tools such as Lens.org and VosViewer (Version 1.6.19). Lens.org enabled an in-depth exploration of top fields of study, while VosViewer was essential for creating

visual representations of bibliographic data. These tools were selected for their proven efficiency in similar studies (Yu et al., 2020; Hajkowicz et al., 2023). The integrated features of Lens.org were particularly valuable in identifying thematic areas within the research on *S. nigrum*. This enabled a thorough exploration of key research domains related to

this species, revealing significant trends and patterns in academic literature. Through this comprehensive approach, the study provided valuable insights into the development and impact of research on *S. nigrum*, highlighting its scientific significance and the evolution of scholarly interest over the years.

Comprehensive Data-driven Insights

A bibliometric analysis of scholarly publications on *S. nigrum* from 2000 to 2023 offers detailed insights into the global reach, collaborative networks, and impact of research on this species. During this period, 1732 scholarly articles were published, authored by 6389 researchers from 1609 organizations spread across 86 countries. This wide geographical distribution highlights the extensive international interest in *S. nigrum*. Among the contributors, 117 authors distinguished themselves by publishing independently on *S. nigrum*, emphasizing their specialized expertise and significant contributions to the field. These articles are published in 933 esteemed journals, representing the diversity and wide dissemination of research on this plant. The average citation rate of 19.24 citations per document reflects the considerable impact of these publications within the academic community, indicating the relevance and influence of the research conducted on *S. nigrum*. The research scope has broadened significantly, encompassing applied research in horticulture, pharmacology, chemistry, botany, traditional

medicine, ecology, and molecular biology. Global contributions and the extensive collaborative networks among authors and organizations highlight the widespread interest and potential for international collaboration in *S. nigrum* research. These collaborations are crucial for advancing knowledge and encouraging innovation in agriculture, medicine, pharmacology, and environmental science.

Temporal Evolution and Growth Analysis

A total of 1732 documents were discovered after extensive searches across bibliographic databases via Dimensions.ai. These demonstrated a variety of study forms, including 1648 articles, 54 chapters, 17 preprints, 13 proceedings. Spanning from 2000 to 2023, the dataset showed increasing trends in scholarly output over two decades. The research landscape began with 18 publications in 2000, increasing gradually, indicating a growing interest in exploring the properties and applications of *S. nigrum*.

L. The growth analysis revealed patterns of exponential growth ($R^2=0.9566$). In the early 2000s, the number of publications remained relatively modest, with an average of around 21 publications per year from 2000 to 2005. However, a noticeable increase began in 2006, reaching 41 publications, and this upward trajectory continued over the subsequent years. By 2010, the number of publications had risen to 66, marking a significant growth in research activity. This trend sustained

momentum, with peaks in 2013 and 2014, recording 67 and 78 publications respectively. The year 2022 marked the highest number of publications at 177, reflecting a substantial surge in research interest, which slightly dipped to 164 in 2023 (Fig. 1). Overall, the data indicates a robust and accelerating growth in the scholarly examination of *S. nigrum*, highlighting its increasing prominence in scientific research.

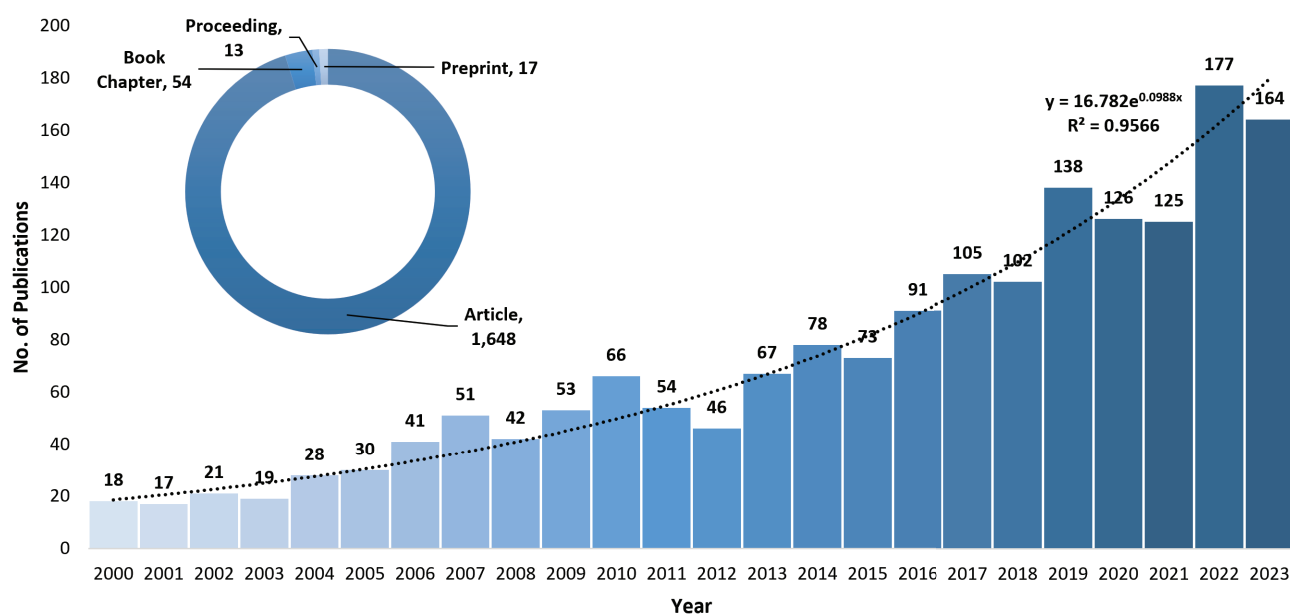


Fig. 1 Publication trends and distribution types in *S. nigrum* research

Citation Analysis

Citation analysis is a fundamental tool in bibliometric research, offering insights into the impact and dissemination of scholarly work within a specific field or topic. Applying this method to the research landscape of *S. nigrum*, the data revealed exciting trends over the past two decades. The citation analysis of the research landscape on *S. nigrum* from 2000 to 2023 reveals significant fluctuations

in scholarly attention. The early 2000s saw modest citation counts, with a gradual increase from 473 in 2000 to 923 in 2004. A surge occurred in 2007 with 2,492 citations, indicating a period of heightened research interest. This trend continued with peaks in 2010 and 2019, reaching 2,901 and 2,880 citations respectively. Such peaks suggest pivotal publications or advancements in research during these years. The data also highlights periods of relative decline, such as

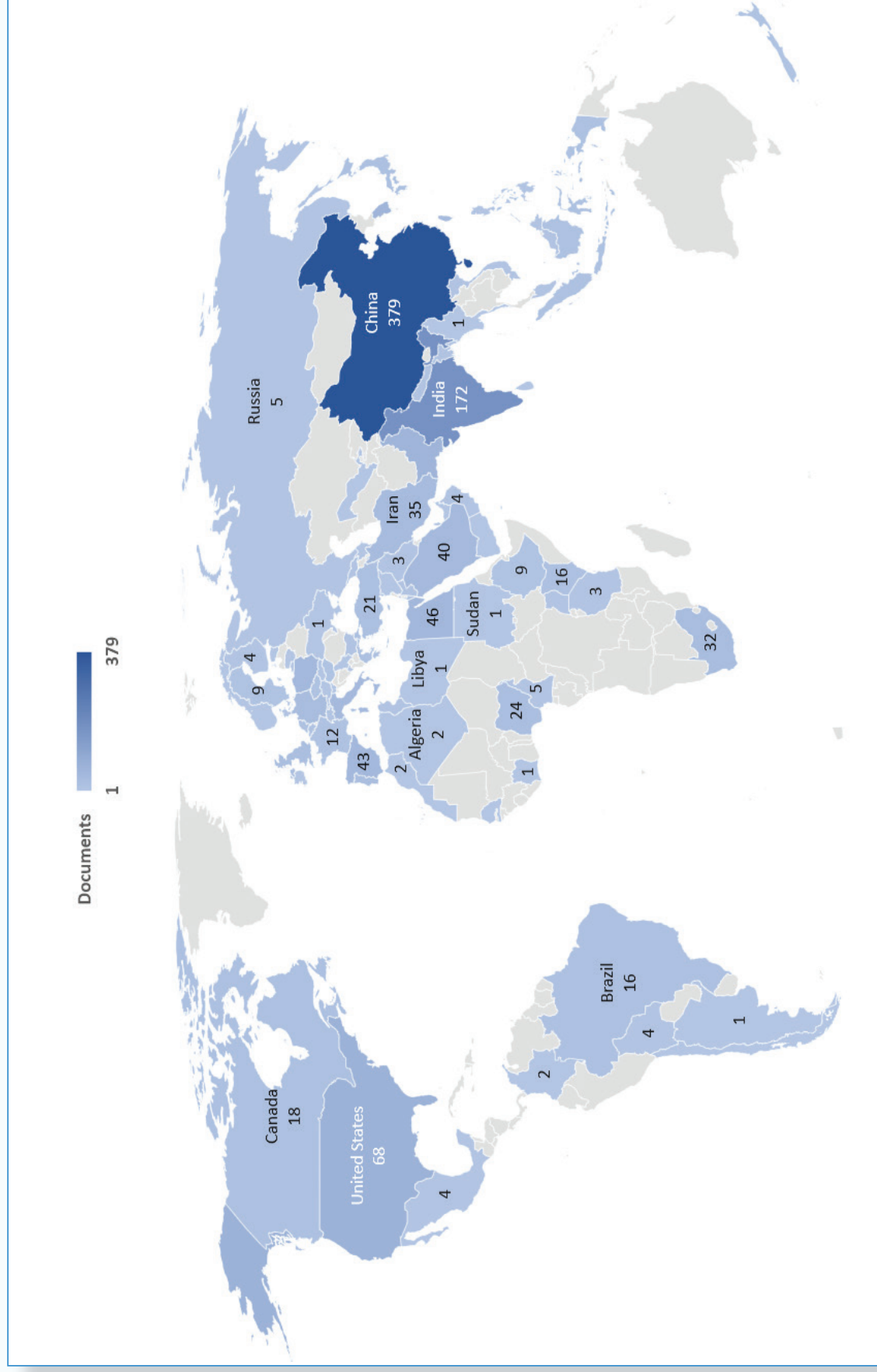


Fig. 3 Global distribution of research on *S. nigrum*

in 2022 with only 678 citations and a sharp drop to 173 in 2023 (Fig. 2). These variations could reflect changes in research focus, publication rates, or the influence of key studies in the

field. Overall, the citation trend showed the dynamic nature of research interest and impact in the study of *S. nigrum* over the past two decades.

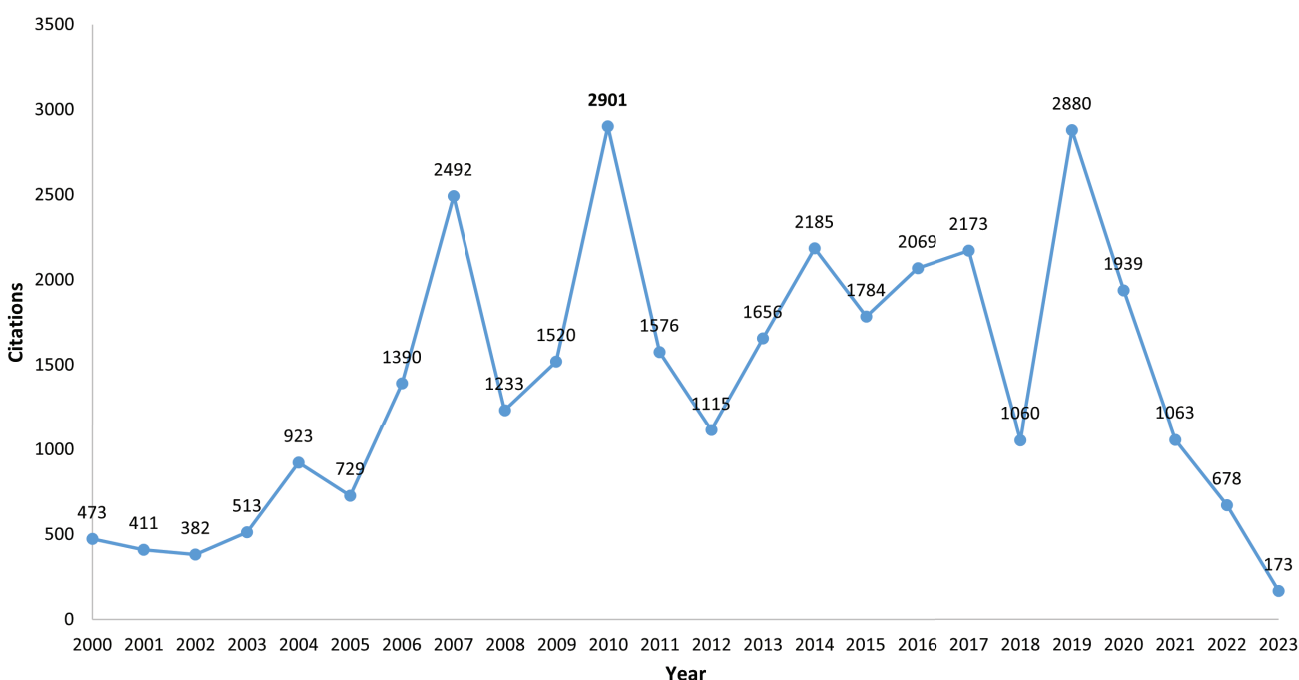


Fig. 2 Citation trends for *S. nigrum* research over time

Country-wise Publication Analysis

The bibliometric analysis of the research landscape on *S. nigrum* revealed a significant difference in the number of publications and citations across various countries. China leads the research efforts with 379 documents and an impressive 10,720 citations, indicating a strong research output and influence in this field. India followed with 172 documents and 4,765 citations, showcasing its substantial contribution to the literature on *S. nigrum* (Fig. 3). The United States, with 68 publications and 1,794 citations, and Pakistan, with

58 documents and 1,992 citations, also demonstrate remarkable research activities. South Korea has produced 56 documents, receiving 1,532 citations, while Egypt (46 documents, 683 citations), Poland (45 documents, 684 citations), and Spain (43 documents, 1,357 citations) show moderate research involvement. Italy and Saudi Arabia each have 40 publications, with 883 and 802 citations respectively, reflecting their efforts in *S. nigrum* research. This analysis highlights China and India as the dominant contributors, with other countries also playing significant roles in advancing the knowledge on *S. nigrum*.

Most Prominent Authors

The bibliometric analysis of the research landscape on *S. nigrum* reveals several prominent authors. Among all authors, Shuhe Wei stands out with the highest number of documents (36) and citations (770), indicating significant contributions and impact. Lijin Lin and Huiping Dai also show substantial involvement with 26 and 23 documents, respectively, but Huiping Dai has a higher citation count (297 vs. 209). Jorge Teixeira and Fernanda Fidalgo, despite having fewer

documents (15 and 14), have remarkably high citations (589 and 582), reflecting influential work in fewer publications. Pei Zhou, with 13 documents and 528 citations, also demonstrates significant impact (Fig. 4). Other contributors include Ming'An Liao, Lidia Skuza, and Kye-Taek Lim, each with substantial publications and citations, highlighting their roles in advancing the understanding of *S. nigrum*. These authors collectively shape the research landscape through their prolific output and impactful work, paving the way for further advancements in the field.

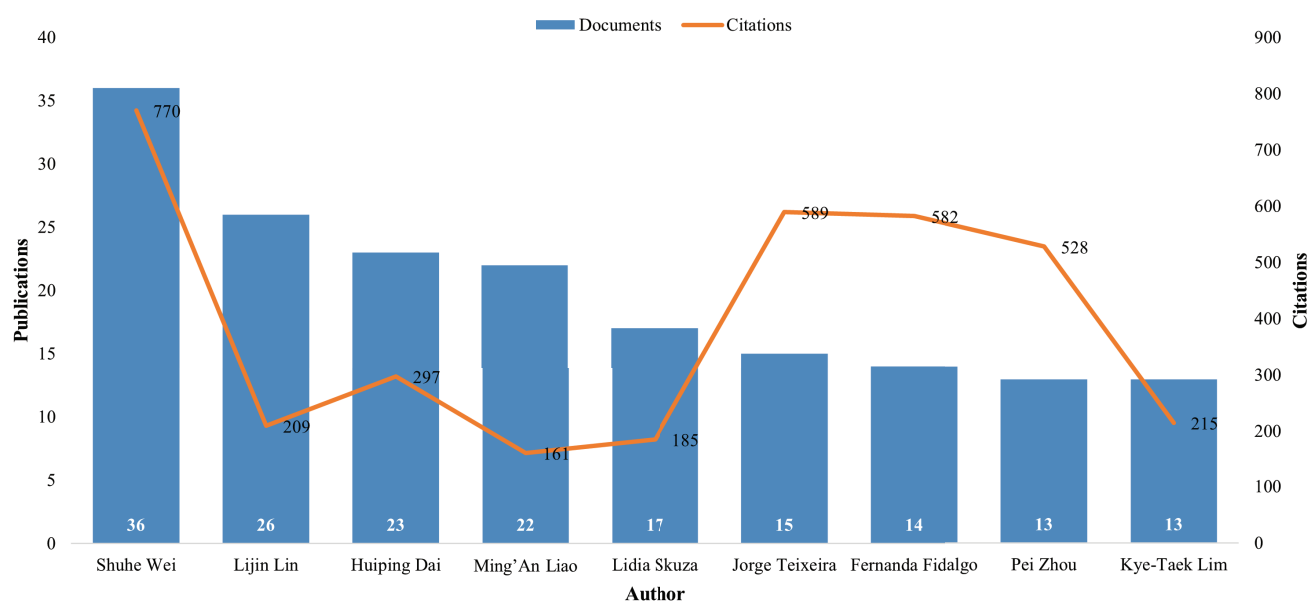


Fig. 4 Most prominent authors based on research contributions to *S. nigrum*

Highly Cited Articles

The top 10 highly cited research articles on *S. nigrum* provide a comprehensive overview of its diverse applications and properties (Table 1). The most cited work published in Plants (2019) is “Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal”, collected 628 citations

[Aryal et al., 2019]. Another prominent study from 2015 in Spectrochimica Acta Part A “Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their antibacterial activity”, garnering 470 citations (Ramesh et al., 2015). Other influential articles cover topics such as bioremediation of heavy metals by hyperaccumulator endophytic bacteria in Bioresource Technology, 2010 and the health

risks of soil heavy metal contamination associated with artisanal gold mining in Ecotoxicology and Environmental Safety, 2017 (Guo et al., 2010; Xiao et al., 2017). These articles collectively represented the diverse applications and ecological significance of *S. nigrum* in phytoremediation, pharmacology, and environmental safety.

Table 1 Top 10 highly cited research articles published on *S. nigrum*

Rank	Title	Source	Year	Citations	Reference
1	Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal	Plants	2019	628	Aryal et al., 2019
2	Green synthesis of ZnO nanoparticles using <i>Solanum nigrum</i> leaf extract and their antibacterial activity	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy	2015	470	Ramesh et al., 2015
3	Bioremediation of heavy metals by growing hyperaccumulator endophytic bacterium <i>Bacillus</i> sp. L14	Bioresource Technology	2010	325	Guo et al., 2010
4	Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China	Ecotoxicology and Environmental Safety	2017	320	Xiao et al., 2017
5	Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator <i>Solanum nigrum</i> L.	Bioresource Technology	2008	263	Sun et al., 2008
6	Interaction of Cd-hyperaccumulator <i>Solanum nigrum</i> L. and functional endophyte <i>Pseudomonas</i> sp. Lk9 on soil heavy metals uptake	Soil Biology and Biochemistry	2014	258	Chen et al., 2014
7	Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence	British Journal of Pharmacology	2020	232	Lin et al., 2020
8	Native Bacterial Endophytes Promote Host Growth in a Species-Specific Manner; Phytohormone Manipulations Do Not Result in Common Growth Responses	PLOS ONE	2008	230	Long et al., 2008

Rank	Title	Source	Year	Citations	Reference
9	Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen	Journal of Ethnopharmacology	2007	224	Al-Fatimi et al., 2007
10	Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant <i>Salmonella typhi</i>	Phytotherapy Research	2004	213	Rani and Khullar, 2004

Most Active Journals

The present study showed the top journals of *S. nigrum* research based on their impact factor, publications, and citations. The "Plant Disease," by American Phytopathological Society with impact factor 4.21 lead the list with 24 publications and 509 citations, indicating a significant focus on plant pathology related to *S. nigrum*. The journal "Chemosphere" by Elsevier with impact factor 8.94 with 22 documents and 1033 citations, highlighted its relevance in environmental and toxicological studies. The "Journal of Hazardous Materials", "Ecotoxicology and Environmental Safety" and "Journal of Ethnopharmacology" also showed

substantial contributions, with 21 and 20 publications, with impact factor 14.22, 6.29 and 4.36 respectively, and exceeding 900 citations, reflecting the plant's role in environmental safety and toxicology (Table 2). Other journals include the "International Journal of Phytoremediation" with 19 documents and 467 citations, emphasizing remediation studies, and "Environmental Science and Pollution Research" with 17 publications and 497 citations. Journals "Weed Research," "Plos One," and the "Journal of Agricultural and Food Chemistry" also contributed significantly, indicating diverse research interests ranging from agronomy to general scientific studies related to *S. nigrum*.

Table 2 Top journals in *S. nigrum* research with highest published document and citations

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
1	Plant Disease	American Phytopathological Society (APS)	4.21	24	509	21.21
2	Chemosphere	Elsevier	8.94	22	1033	46.95
3	Journal of Hazardous Materials	Elsevier	14.22	21	975	46.43

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
4	Ecotoxicology and Environmental Safety	Elsevier	6.29	20	1271	63.55
5	Journal of Ethnopharmacology	Elsevier	4.36	20	1269	63.45
6	International Journal of Phytoremediation	Taylor & Francis	3.91	19	467	24.58
7	Environmental Science and Pollution Research	Springer	5.19	17	497	29.24
8	Weed Research	Wiley	3.16	15	399	26.60
9	Plos One	Public Library of Science (PLOS)	3.75	12	644	53.67
10	Journal of Agricultural and Food Chemistry	American Chemical Society (ACS)	5.89	12	495	41.25

Top Productive Organizations

The examination of scientific contributions made to the *S. nigrum* ecology demonstrated diverse trends in recognition and effect among various successful groups. The Institute of Applied Ecology leads the research landscape on *S. nigrum* with a substantial contribution of 47 documents, garnering 1829 citations, indicating a high impact and significant influence in this field. Sichuan Agricultural University follows with 35 publications, though with a markedly lower citation count

of 281 (Table 3). The University of Chinese Academy of Sciences with 31 documents receiving 1171 citations. Similarly, Shanghai Jiao Tong University has 23 documents with 1082 citations, demonstrating strong research output and influence. Other contributors included Shaanxi University of Technology, University of Porto, and King Saud University, each producing between 15 and 21 documents and achieving varying citation counts, reflected their respective impacts on the research landscape of *S. nigrum*.

Table 3 Top organizations' contributions to *S. nigrum* research

Rank	Organization	Country	Documents	Citations	Citations per Document
1.	Institute of Applied Ecology	China	47	1829	38.91
2.	Sichuan Agricultural University	China	35	281	8.03
3.	University of Chinese Academy of Sciences	China	31	1171	37.77
4.	Shanghai Jiao Tong University	China	23	1082	47.04
5.	Shaanxi University of Technology	China	21	321	15.29
6.	University of Porto	Portugal	18	731	40.61
7.	King Saud University	Saudi Arabia	17	288	16.94
8.	Chonnam National University	South Korea	16	310	19.38
9.	University of Szczecin	Poland	16	185	11.56
10.	Shandong University	China	15	623	41.53

Top Fields of Study

The bibliometric analysis of *S. nigrum* research highlighted several primary fields of study based on document counts, revealing a multifaceted interest in this plant. The study examined the prevalence of terms within prominent academic fields by analyzing a dataset comprising 2405 scholarly documents sourced from Lens.org. The most represented field was Biology, with 1557 documents, indicating a comprehensive exploration of the plant's genetics, physiology, ecology, and overall biological characteristics. Subsequently, research specifically tagged

under *S. nigrum* comprised 1545 documents, reflecting a focused interest in the species itself, including its classification, species-specific traits, and various applied research topics. Botany accounted for 922 documents, addressing the plant's structural, genetic, and ecological characteristics. The Chemistry field, with 718 documents, mainly focused on the chemical composition of *S. nigrum*, including secondary metabolites and alkaloids, emphasizing the importance of chemical analysis in understanding, and utilizing the plant's properties. Research in Horticulture (538 documents) covered the cultivation and breeding of *S. nigrum*, focusing

on improving yield, pest resistance, and optimal growing conditions, underscoring the plant's agricultural importance and potential for crop improvement. A substantial number of documents in Traditional Medicine (408) suggested the significant role of *S. nigrum* in traditional medicinal practices, investigating its ethnobotanical uses, efficacy, and safety. Agronomy (320 documents) examined the practical aspects of growing *S. nigrum*, including soil management, crop rotation, and sustainable farming practices, highlighting ongoing efforts to optimize agricultural practices. The field of *Solanum* (309 documents) included broader studies within the genus, exploring comparative analyses, evolutionary studies, and phylogenetics,

underscoring the importance of understanding *Solanum nigrum* within its genus' context. Biochemistry (308 documents) research likely examined the biochemical pathways and molecular mechanisms within *S. nigrum*, including enzyme functions and metabolic pathways. Medicinal research involving *S. nigrum*, documented in 288 papers, focused on its potential health benefits, therapeutic applications, and clinical studies, highlighting the translational potential of the plant from traditional to modern medicine (Fig. 5). The research landscape of *S. nigrum* spanned a wide array of disciplines, reflecting the plant's biological importance, medicinal potential, agricultural value, and environmental applications.

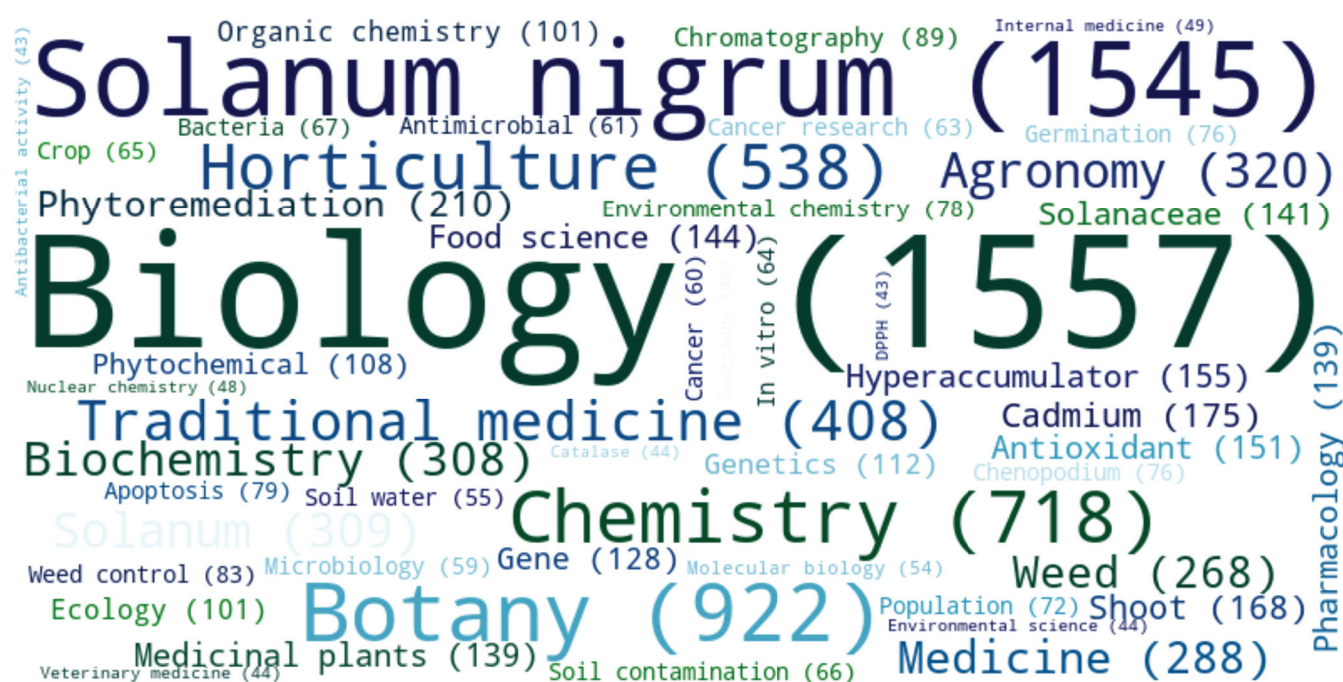


Fig. 5 Terms co-occurrence analysis within prominent terms

Research Collaboration Networks

Authors' Collaboration Network

The study involved an exhaustive examination of 6389 authors, examining a considerable number of researchers within the specified subject domain. Author similarities were evaluated through co-authorship, with particular emphasis on their publications. The network encompassed authors who had authored a minimum of 7 documents on *S. nigrum*, each with a minimum citation count of 7 citations per document. The analysis identified 4 clusters within the network, indicating interconnected and collaborative author groups. Cluster 1 comprised 5 authors, while Clusters 2, 3, and 4 each consisted of 2

members (Fig. 6). Especially, Lijin Lin emerged as the most influential author, boasting the highest total link strength of 66, emphasizing his influential role and extensive connections within the research community. Furthermore, Ming'an Liao and Shuhe Wei also stood out as prominent authors, with total link strengths of 52 and 43, respectively. These findings exhibited a diverse and dynamic research community marked by varying degrees of specialization and collaboration. The differing link strengths highlighted the breadth of expertise and interconnectedness among researchers, representing a robust network where individual contributions significantly contribute to the collective advancement of knowledge.

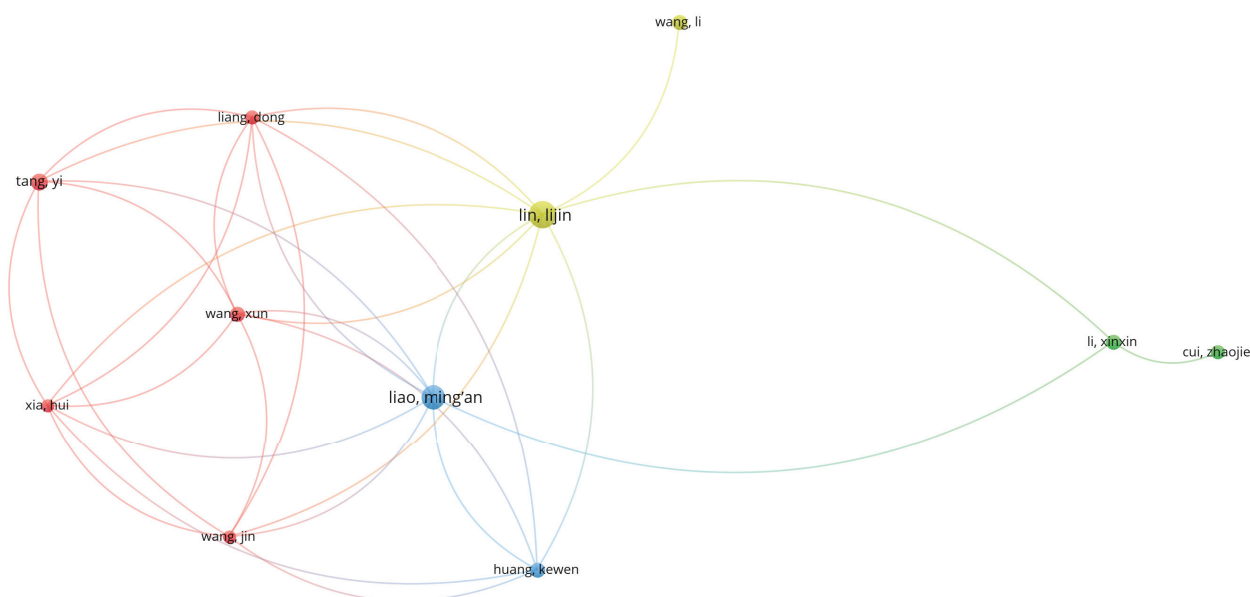


Fig. 6 Collaborative network among the authors on *S. nigrum* research

Journal-wise Collaboration Network

The assessment of collaboration among journals dedicated to *S. nigrum* research involved scrutinizing a dataset containing 933 journals. This examination revealed 248 interconnections among 56 journals through citation analysis. To qualify for inclusion in the network, journals had to meet specific criteria; publishing a minimum of 5 documents on *S. nigrum*, each receiving at least 5 citations per document. This rigorous necessity ensured the inclusion of influential and relevant journals, resulting in a network that identified 6 distinct clusters.

Cluster 1 emerged as the largest, comprising 12 journals, while Clusters 2 and 3 each

encompassed 11 journals. Clusters 4, 5, and 6 consisted of 10, 7, and 5 journals, respectively (Fig. 7). Among these clusters, "Ecotoxicology and Environmental Safety" showed the highest connection strength at 151, highlighting its pivotal role in promoting collaborative efforts within the research community. Furthermore, the journals "Journal of Hazardous Materials" and "Chemosphere" demonstrated significant influence, with total link strengths of 141 and 134, respectively. This network analysis offers valuable insights into the thematic strengths and collaborative potential among journals engaged in *S. nigrum* research. By delineating these interconnections, the study enriches our understanding of the scholarly landscape, uncovering the intricate web of collaboration and specialization that characterizes this field.

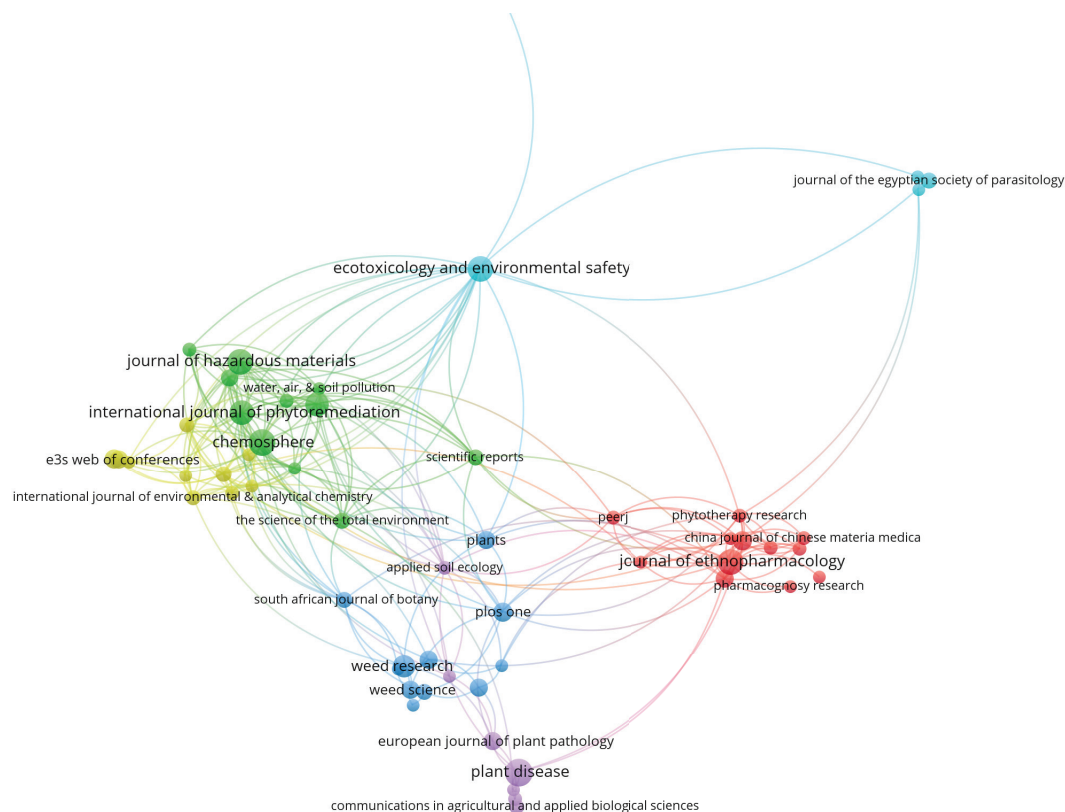


Fig. 7 Journal-wise collaboration network analysis on *S. nigrum* research

Organizational Collaborative Network

A study explored the collaborative network among organizations engaged in *S. nigrum* research using citation analysis. The investigation involved 1609 organizations, revealing relationships among 18 entities. To qualify for inclusion, each organization had to publish a minimum of 10 documents and receive at least 10 citations per document related to *S. nigrum* research. The resulting network showed 3 distinct clusters, comprising a total of 87 connections (Fig. 8). Cluster 1 emerged as the largest with 9 organizations, showcasing significant inter-organizational cooperation. Clusters 2 and 3, with 5 and 4 organizations respectively,

exhibited closely-knit connections within them. The Institute of Applied Ecology stood out with the highest overall connection strength, boasting 724 links, emphasizing its pivotal role in the network. Subsequently, the University of Chinese Academy of Sciences and the Shaanxi University of Technology, showed link strength of 524 and 356 connections respectively. Understanding this collaborative network is essential for encouraging successful partnerships and advancing research on *S. nigrum*. Organizations can gain valuable insights from these networks to strategically bolster collaborative efforts and propel research advancement, providing a roadmap for enhancing cooperation and maximizing the impact of research initiatives.

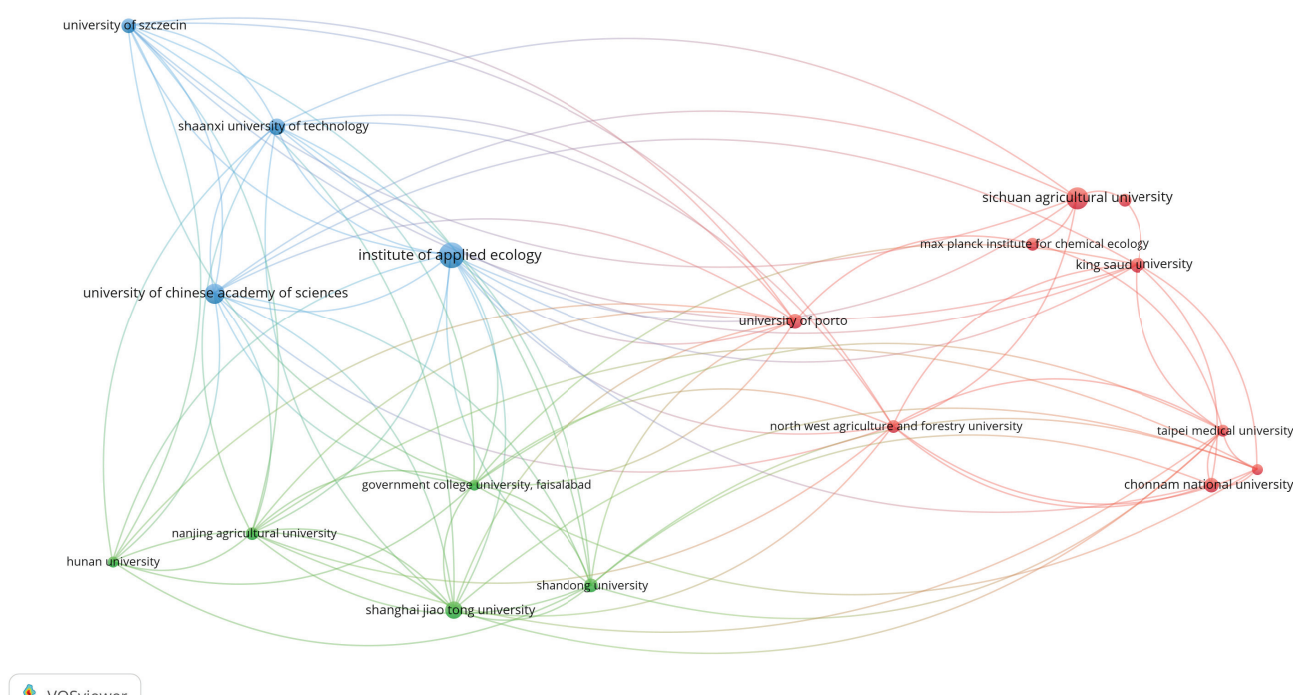


Fig. 8 Clusters of collaborative institutions for *S. nigrum* research

Country-wise Collaboration Network

A detailed assessment of collaboration networks among countries in the domain of *S. nigrum* research was undertaken, involving 47 nations, and revealing 493 connections. To meet the criteria for inclusion, each country had to have published at least 5 documents on *S. nigrum*, each with a minimum of 5 citations per document. Through meticulous citation analysis, 6 distinct clusters were identified, each showing varying degrees of collaboration and research emphasis. Clusters 1, 2, and 3 comprised 17, 9, and 8 countries respectively, indicating significant collaborative efforts within these groups. Cluster 4, consisting of 7 countries, revealed more concentrated

collaboration networks. Meanwhile, Clusters 5 and 6 comprised 3 countries each (Fig. 9). The analysis highlighted robust connection strengths of China and India, boasting 1,663 and 694 links respectively, thus highlighting their pivotal roles within the collaborative network. This analysis accentuates the significance of collaborative research in enriching our comprehension of *S. nigrum*, illuminating the global landscape and interconnectedness of scientific pursuits. It emphasizes how international partnerships can propel research in this domain, offering valuable insights for researchers, institutions, and policymakers seeking to nurture and leverage global collaborations to advance knowledge and innovation in *S. nigrum* research.

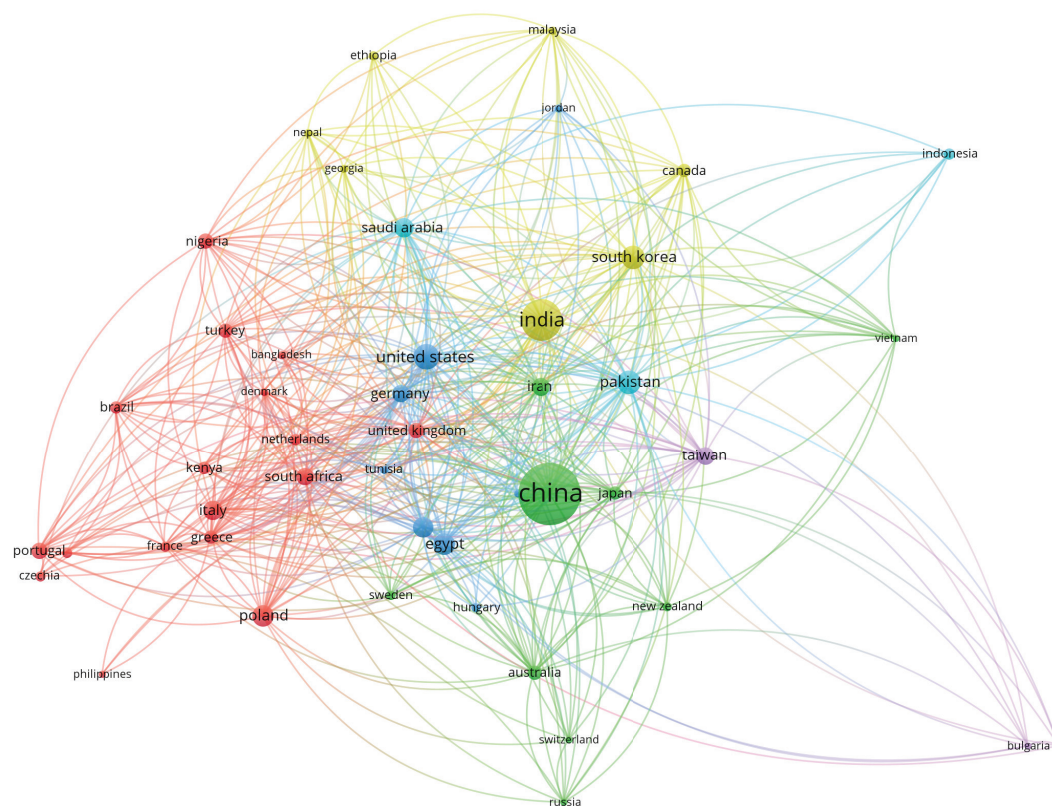


Fig. 9 Country-wise collaboration network in *S. nigrum* research

Conclusion

The present bibliometric analysis on *S. nigrum* from 2000 to 2023 offers a detailed overview of the global research landscape, collaboration dynamics, and scholarly impact in this area. With 1732 articles authored by 6389 researchers from 1609 organizations across 86 countries, the study revealed extensive international involvements. The China emerged as the leading contributor with 379 publications and 10,720 citations, reflecting significant research output and influence. A surge in publications occurred in 2022, and citation analysis highlighted periods of intense research activity, especially in 2010. Shuhe Wei is identified as a prominent author with 36 papers and 770 citations. The most cited article, published in *Plants* in 2019, examined the antioxidant potential of wild vegetables from Nepal, amassing 628 citations. "Plant Disease," with an impact factor of 4.21, is the leading journal, featuring 24 articles and 509 citations, highlighting the importance of plant pathology in *S. nigrum* research. The Institute of "Applied Ecology" is highlighted for its significant contributions, with 47

documents and 1829 citations. "Biology" is the most represented field of study, encompassing 1557 documents, indicative of extensive research into the plant's genetics, physiology, and ecology. Moreover, Influential authors like Lijin Lin and their collaborative networks represented the interconnected nature of research efforts in this domain. The analysis also identifies key journals, organizations, and countries development research dissemination and collaboration. Furthermore, collaborative networks among authors, journals, organizations, and countries explored research quality, increase impact, facilitate resource sharing, and promote innovation by combining diverse expertise and perspectives. Overall, the present study represented the pivotal role of collaborative efforts in advancing knowledge and promoting interdisciplinary perspectives on *S. nigrum*. As interest and research in *S. nigrum* continue to grow, this bibliometric analysis serves as a valuable guide for future research directions and collaborations in this field.

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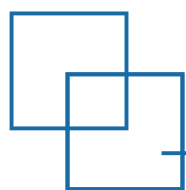
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CHAPTER 03

Soil Properties



INTRODUCTION

Soil is a complex matrix generated by the weathering of rocks that compose the Earth's crust's outer layer. It is generated through the dynamic interplay of minerals, organic matter, air, water, and other living species. It promotes plant growth due to its nature and the availability of nutrients. Plant development and yield are enhanced when the soil is nutrient rich (The State of Queensland, 2013). Soils, in addition to serving as a medium for plant growth, play an important role in gaseous exchange by absorbing and releasing gases such as carbon dioxide, oxygen, and methane. They can also hold water, which is an important resource for plant growth. Soils are host to various living organisms such as animals, fungi, bacteria, and other organisms because of their qualities. As a result of the presence of numerous living creatures, organic matter is constantly recycled through breakdown and accumulation. Soil is also a valuable basis for construction material from a commercial perspective (Soil Science Society of America, 2023).

Soil is made up of biotic and abiotic constituents, which comprise live creatures as biotic factors and minerals, organic matter, gas, and water as abiotic ones. All the components are responsible for plant growth and serve a significant role in preserving soil quality (Needelman, 2013). The percentages of each component vary amongst soil types, such as sandy, clayey, loamy, silty, and so on. The quantities of distinct components in soil are determined by the sites where the soil is available and the parent material from which it forms. The area where the soil is available and the parent material from which it forms determines the respective proportions of individual components in the soil. For instance, there are many different types of soils available in India. The first type is the alluvial soil which covers 46% of the Indian soil area. Rocks of the Himalayas are the parent material, and this kind of soil formation occurs by silt deposition by Indo-Gangetic-Brahmaputra Rivers. The next kind is the black soil which is formed from volcanic rocks in the Deccan Plateau. These soils are rich in clay and cover 16.6% of the total soil cover in India. The other types of soil include red and yellow soil, desert soil, laterite soil, peaty and marshy soil, alkaline soil, and mountain soil (Bhattacharyya et al., 2013).



The percentage composition, quantity, and quality of the components or nutrients in the soil all affect how fertile it is. The fertility of the soil in turn determines the type of crop that can grow in it and its yield and nutritional parameters. Ogundola et al., (2021) found different concentrations of essential oil from the shoots of *Solanum nigrum* L. that were grown in different types of soil. Due to the change in the oil content of the shoot, the free radical scavenging activity varied. It was reported in the study that plants grown of clay loam soil showed the highest radical scavenging activity. Similar results were obtained from extracts of plant shoots grown on clay loam soil for ABTS radical scavenging. Andrade et al., (2011) also reported a similar phenomenon in the case of *Piper dilatatum* Rich. They showed that the essential oil composition of plants collected from different regions of Amazon, Brazil during rainy seasons varied due to environmental variations including variations in soil type. Jimoh et al., (2019) reported that *Amaranthus caudatus* L. exhibited the highest phytochemical yield when grown in clayey loam soil. A similar thing was replicated for the antioxidant activity of the plant extract. Zargoosh et al., (2019) showed that the interaction of site and elevation played a significant effect on the antioxidant potential and total phenol content in *Scrophularia striata* Boiss. The antioxidative capacity was indirectly correlated to elevation, phosphorous, potassium, organic carbon, organic matter, and nitrogen levels for the first site. Similarly, the acidity content had the highest negative correlation with antioxidant capacity. The lime and sand percentage of soil was also negatively related to the phytochemical content.

It has previously been demonstrated that a plant's environment has an impact on the number of secondary metabolites it produces and, ultimately, on the phytochemical composition of that plant. (Walker et al., 2001; Srivastava and Shym, 2002; Dorri et al., 2009). The temperature and the humidity of the area in which a plant grows affect the phytochemical profile of the plant (Fox et al., 1999). How the environment regulates phytochemicals composition and quantity of plants is yet to be completely understood. Yet theories state that environmental factors may influence metabolic processes in plants which thereby affects the phytochemical content. At different altitudes, even the same plant shows different growth characteristics due to differences in light intensity, exposure to radiation, wind speed, humidity, water content in soil, and nutrient content in soils. The microbial composition of the soil may also affect the plant phytochemicals. Makgato et al., (2020) showed that inoculation of beneficial soil microbes like *Rhizobium* into soil can also stimulate plant phytochemical content. They showed that total phenolic

and flavonoid content increased because of *Rhizobium* inoculation in soil as compared to control. This occurred even though plant biomass and nitrogen fixation did not show any increase. Similarly, Egamberdieva et al., (2015) have reviewed in their book that plant growth promoting rhizobacteria helps plants by improving their salt and heavy metal tolerance, preventing plant diseases, and boosting soil fertility. In the case of medicinal plants specifically, they can enhance the phytochemical levels by inducing secondary metabolite production. It is thus beneficial to enrich soils with beneficial microbes which can further be utilized for commercial purposes. Thus, it is evident that environmental variations including humidity, temperature, radiations as well and soil microbiomes influence the secondary metabolite production of medicinal plants and hence their phytochemical production. These attributes are beneficial to understanding plant physiology and the best possible conditions for the cultivation of medicinal plants. In the current study, emphasize on studying the physicochemical variations in soil quality from different altitudes of sampling sites is given in Table 2. Soil physicochemical parameters like pH, electrical conductivity, organic carbon content, available nitrogen, phosphorous, potassium, sulphur and micronutrients, total moisture content, heavy metal, and bulk density have been studied to understand the quality at different altitudes and how they may influence vegetation in the different sites.

Sampling Sites

The bulk soil and plants (along with the of 26 sites. The numbering and name of the rhizospheric soil) were collected from a total sites along coordinates are given in Table 1.

Table 1 Study sites and coordinates

S.No.	States	Site	Site Code	Latitude	Longitude
1.	Uttarakhand	Gomukh	UK -S1	30.56790	79.03081
2.		Gangotri	UK-S2	31.03920	78.74655
3.		Uttarkashi	UK-S3	30.74619	78.48377
4.		Devprayag	UK-S4	30.1358	81.1218
5.		Haridwar	UK-S5	29.9169	81.5315

S.No.	States	Site	Site Code	Latitude	Longitude
6.	Uttar Pradesh	Bijnor	UP-S1	29.2857	82.3243
7.		Narora	UP-S2	28.1465	83.1315
8.		Badaun	UP-S3	27.9394	83.5612
9.		Farrukhabad	UP-S4	27.4104	84.496
10.		Bithoor	UP-S5	26.6159	85.1242
11.		Dalmau	UP-S6	26.3485	85.5979
12.		Prayagraj	UP-S7	25.2537	86.2076
13.		Mirzapur	UP-S8	25.8588	87.0908
14.		Varanasi	UP-S9	25.1518	87.396
15.		Ballia	UP-S10	25.3546	87.9107
16.	Bihar	Revelganj	BH-S1	25.4388	88.2213
17.		Patna	BH-S2	25.3808	88.3809
18.		Barh	BH-S3	25.2311	88.3895
19.		Bahachoki	BH-S4	25.1782	88.1959
20.		Farka	BH-S5	25.2323	78.8535
21.	Jharkhand	Sahibganj	JH-S1	25.1461	79.6282
22.	West Bengal	Farraka	WB-S1	24.8227	80.277
23.		Hazarduari	WB-S2	23.9969	81.1218
24.		Mayapur	WB-S3	23.4129	81.5315
25.		Hoogly	WB-S4	22.8491	82.3243
26.		Gangasagar	WB-S5	22.1774	83.1315

The temperature of a particular sites is dependent on its altitude as well as climate conditions. At the time of sample collection, the temperature of the sites as well as altitude

of the sites as shown in live GPS map via GPS map camera app was noted down. The altitude and temperature of different sites for sample collection are shown in the Table 2.

Table 2 Temperature and altitude of different sampling sites

Site	Temperature (°C)	Altitude (m)
UK -S1	09	3794
UK -S2	17	2506
UK -S3	18	1232
UK -S4	23	1014
UK -S5	34	265
UP-S1	29	217
UP-S2	31	179
UP-S3	25	162
UP-S4	27	136
UP-S5	28	113
UP-S6	31	91
UP-S7	33	91
UP-S8	32	91
UP-S9	38	65
UP-S10	34	67
BH-S1	25	69
BH-S2	25	36
BH-S3	28	30
BH-S4	30	18
BH-S5	25	23
JH-S1	25	17
WB-S1	28	32
WB-S2	30	14
WB-S3	27	8
WB-S4	30	9
WB-S5	31	3

Soil Analysis

Physicochemical Analysis of Soil

Total Moisture Content (%)

Soil moisture content, often known as water content, is an indication of the quantity of water in the soil. Moisture content is stated as a proportion of the mass of water contained in the pore spaces of soil to the solid mass of particles in that substance. The mass of the sample is determined using a reference temperature of $110 \pm 5^\circ\text{C}$. Almost, all soil tests detect the natural moisture content of the soil, which is critical knowledge for all soil mechanics. The natural moisture content indicates the condition of the soil in the field (Hossain et al., 2022).

Apparatus and equipment required: non-corrodible vented container, thermostatically controlled drying oven that maintains temperatures between 105°C to 115°C , Balance of sufficient sensitivity (sensitive to 0.01 g) and container handling apparatus.

Procedure

1. Clean, dry and weigh W_1 the container. The balance needs to be tared before it is used to measure the weight.
2. Weigh W_2 a sample of the specimen in the container.
3. Keep the container in the oven for 24 hours. Dry the specimen to a constant weight, maintaining the temperature between 105°C to 115°C . (The time will

vary with the type of soil, but 16 to 24 hours is usually sufficient.)

4. Record the final constant weight W_3 of the container with the dried soil sample. Peat and other organic soils should be dried at a lower temperature (approximately 60°C) for a longer period.

Calculations

1. Weight of the container = W_1 g
2. Weight of the container + Weight of the wet sample = W_2 g
3. Weight of the container + Weight of the dried sample = W_3 g
4. Weight of water in the soil sample = $W_2 - W_3 = M_w$ g
5. Weight of the dry soil = $W_3 - W_1 = M_s$ g
6. Moisture content in the given soil sample = $(M_w \text{ g} / M_s \text{ g}) \times 100\%$

Bulk Density

Bulk density is a commonly measured soil property by agriculturalists and engineers. High bulk density soils are soils with little pore space, so water infiltration is reduced, root penetration is inhibited, and aeration is restricted – reducing agricultural productivity. Low bulk density soils are easily compacted and may settle considerably to the detriment of roads, sidewalks, and building foundations (Bowen, 2016).

Apparatus and equipment required: Top load balance, soil spatula, 100ml graduated measuring cylinder, 2×50 ml beaker, paper towels and mud bucket.

Procedure

1. Add slightly more than 50 ml of the soil sample to 50 ml beaker.
2. Clean and thoroughly dry a 100 ml graduated cylinder. Weigh and record weight (A).
3. Slowly add soil sample to pre-weighed graduated cylinder to the 10 ml line. Compact the soil by dropping onto a padded surface like a book, notebook, etc. at least ten times from a height of about 2-3 inches.
4. Repeat this process in 10 ml intervals until you reach the 50 ml mark.
5. Use a soil spatula to level the top of the sample in the graduated cylinder and add soil with the spatula until the top of the soil sample is exactly even with the 50 ml line – this is the bulk volume of compacted soil (B) ($1 \text{ ml} = 1 \text{ cm}^3$).
6. Weigh and record graduated cylinder plus compact soil weight (C).
7. After drying the beaker, place any soil sample that is still in it back into the sample storage container.
8. Return 50 ml sample in graduated cylinder to 50 ml beaker. Remove all of sample within graduated cylinder.
9. Slowly pour approximately 25 ml of soil sample from beaker into water in the graduated cylinder. Gently stir soil/water mixture to remove any air bubbles. Add the second 25 ml of soil sample and stir again to remove air bubbles.

Calculations

1. Weight of 100 ml graduated cylinder = A
2. Bulk volume of the compacted soil = B
3. Weight of cylinder + compacted soil = C
4. Weight of soil sample = $C - A = D$
5. Bulk density (g/cm^3) = D/B

pH

Principle: The pH of sample is measured with a pH meter, in which the potential of a hydrogen ion indicating electrode (glass electrode) is potentiometrically measured against a calomel saturated reference electrode, which also functions as a salt bridge. Most pH meters now contain a single integrated electrode. The equipment must be calibrated with a standard buffer solution of known pH before measuring the pH of the soil. As temperature affects pH, the pH meter is set to according to the temperature of the solution (Varley, 1972; Jackson, 1973).

Reagents: Buffer solutions (pH 4.0, 7.0, and 9.2)

Equipment required: A balance, 100 ml beaker, measuring cylinder, glass rod, pH meter and ordinary tissue paper.

Procedure

1. 25 g of the soil was weighed in a 100 ml beaker and make it to 50 ml final volume by adding of distilled water. The mixture was stirred well for at least four times within a 30-minute period to allow the soil and water to reach equilibrium.
2. In the meantime, the pH meter was switched on.

- Initially the instrument was calibrated with buffer solution of known pH 4, 7.0, and 9.2.
- The electrodes were washed with distilled water and wiped dry with a tissue paper.
- Then, the electrode was dipped in the sample and the readings were taken.
- Finally, the electrodes were washed with distilled water and placed back into a beaker containing 4.00-7.00 pH buffer or 3-4M KCl.

Interpretation

pH	Category	Soil Rating/ Recommendation
<6.5	Acidic	Requires liming for reclama- tion
6.5-8.7	Normal	Optimum for most crops
8.8-9.3	Alkaline	Requires application of or- ganic manures
>9.3	Alkali (Sodic)	Requires gypsum for ame- lioration

Electrical Conductivity

Principle: A conductivity meter known as “Solu Bridge” is used to measure the electrical conductivity of a soil solution. It is based on the Wheat Stone Bridge principle, in which alternating current is utilized instead of direct current to prevent electrode polarization and electrolysis of the solution. In a branched circuit with the conductance cell having resistance R_x , two fixed resistances R_1 and R_2 and a variable resistance (R_v) are linked. The variable resistance (R_v) is adjusted until no current flows through it and the reading is taken. The resistance or conductance (R_v) is measured (Richards, 1954; Chopra and Kanwar, 1976).

Apparatus and equipment required: Weighing balance, 100 ml beaker, measuring cylinder, glass rod and conductivity meter.

Procedure

- 25 g of the soil samples was taken in a 100 ml beaker.
- Added 50 ml distilled water.
- Intermittent stirring was done with a glass rod for 30 minutes.
- The samples were left overnight to obtain a clear supernatant.
- The conductivity of the supernatant liquid was determined with the help of a conductivity meter.

Interpretation

EC (1:2 soil water; $\mu\text{S/m}$)	Soil Rating
Below 800	Normal.
800 - 1600	Critical for salt sensitive crops.
1600 - 2500	Critical for salt tolerant crops.
Above 2500	Injurious to all crops.

Organic Carbon

Principle: In the presence of concentrated sulphuric acid, a known weight of soil was treated with an excess of standard potassium dichromate solution. The heat of the sulphuric acid gently digests the soil at a low temperature, oxidizing the organic carbon in the soil to CO_2 . The excess potassium dichromate was titrated against a standard solution of ferrous ammonium sulphate in the presence of a diphenylamine indicator and sodium fluoride or phosphoric acid, which distinguishes the colour due to their flocculating effect. The hue of the suspension varies from violet to blue to vivid green at the terminal point (Walkley and Black, 1934; Jackson, 1973).

Chemicals and reagents used

1. Potassium dichromate solution (1 N $\text{K}_2\text{Cr}_2\text{O}_7$): 49.04 g of analytical grade $\text{K}_2\text{Cr}_2\text{O}_7$ was dissolved in distilled water and the volume was made up to 1 litre.
2. Concentrated sulphuric acid (H_2SO_4).
3. Orthophosphoric acid (85%) or sodium fluoride (NaF).
4. Diphenylamine indicator: 0.5 g diphenylamine indicator was dissolved in a mixture of 100 ml concentrated sulphuric

acid and 20 ml distilled water. This was stored in an amber colour bottle.

5. Ferrous ammonium sulphate solution [0.5 N, $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$]: 392 g analytical grade ferrous ammonium sulphate was dissolved in distilled water, to which 15 ml concentrated sulphuric acid was added and volume to made up to 2 liters with distilled water.

Apparatus required: 250 ml Erlenmeyer (Conical) flask, pipette, burette and measuring cylinder.

Procedure

This process is also known as Walkley and Black's rapid titration method (1934).

1. 2 g of dried, ground, and sieved soil was taken in a 250ml conical flask.
2. To it, 10 ml 1 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution was added and shaken gently to mix the contents.
3. Next, 20 ml of concentrated sulphuric acid was added while swirling the flask slowly as the reaction is exothermic and a lot of heat is produced.
4. The flask was kept on a dry tile or asbestos sheet for 30 minutes and left to attain room temperature.
5. After cooling, about 0.5 g of NaF or 5 ml

orthophosphoric acid, 100 ml of distilled water and 10 drops of diphenylamine indicator solution were added. These were shaken vigorously for complete mixing.

6. This was titrated against N/2 ferrous ammonium sulphate solution till the colour changes from violet to bright green through blue.
7. The volume of ferrous ammonium sulphate solution used for titration was noted down every time.
8. A blank titration was carried out without any soil.

Calculations

1. Weight of soil taken (W) = 2 g
2. Vol. of N/2 ferrous ammonium sulphate used for blank titration = X ml
3. Vol. of N/2 ferrous ammonium sulphate used to titrate excess nascent oxygen = Y ml
4. Vol. of 1N $K_2Cr_2O_7$ used for oxidation of organic carbon in soil = $(X-Y)/2$ ml
5. 1 ml of 1N $K_2Cr_2O_7$ = 0.003 g C
6. % Organic Carbon in Soil = $(X-Y)/2 \times 0.003 \times 100/W$

Interpretation

Organic Carbon (%)	Soil Rating
<0.4	Low
0.4-0.75	Medium
>0.75	High

Available Nitrogen

Principle: An excess of alkaline potassium permanganate is applied to a known weight of soil, extracting a relatively easily oxidizable fraction of organic nitrogen. Ammonia is absorbed in excess of boric acid, forming ammonium borate, which is titrated against standard acid to determine the amount of ammonium absorbed (Subbaiah, 1956).

Chemicals and reagents required

1. Potassium permanganate solution ($KMnO_4$): 3.2 g/l of potassium permanganate was dissolved in distilled water and the volume was made up to 1 liter.
2. Sodium hydroxide solution (NaOH): 25 g/l of sodium hydroxide pellets were dissolved in distilled water and the volume was made up to 1 liter.
3. 0.02 N sulphuric acid (H_2SO_4).
4. Mixed indicator: 0.066 g of methyl red and 0.099 g of bromocresol green were mixed in 100 ml of ethanol.
5. Boric acid: 25 g/l of boric acid was dissolved in 1 liter of distilled water and to it 40 ml mixed indicator was added. Then, the pH was adjusted to 4.5-5.0 by adding 25 % NaOH.

Apparatus and equipment required: Kjeldahl distillation assembly, measuring cylinder,

burette, balance, pipettes, and 100 ml conical flask.

Procedure

1. The Kjeldahl assembly was prepared by dipping the respective inlet tubes, one in 0.32% KMnO_4 and second in 2.5% NaOH reagent tanks.
2. 5 g soil sample was weighed and put into distillation tube carefully so that the soil sample does not stick to the sides of the tubes. Soil particles attached to the sides of the tube were washed down with distilled water.
3. The distillation tube was fixed in distillation unit and the sample was moistened with distilled water by pressing the dilution key.
4. 25 ml of 2.5% boric acid was taken in a conical flask and the receiving end of the distillation tube was dipped in it.
5. Next, 25 ml of KMnO_4 and 25 ml NaOH were added in the sample by pressing the respective keys.
6. The heating unit was switched on, and the process ran for 6 minutes.
7. The ammonia gas released from the sample was distilled and collected into the receiver containing acid. After complete digestion, the receiver flasks were removed.
8. The ammonium borate formed in the receiver flask was titrated against 0.02 N H_2SO_4 acid and note the volume of 0.02 N H_2SO_4 utilized. The colour changes from bluish green to wine red.

Calculations

1. Weight of soil taken = 5 g
2. Vol. of 2.5% boric acid taken = 25 ml
3. Vol. of 0.02 N H_2SO_4 used to titrate ammonium borate = X ml
4. 1 ml of 0.02 N H_2SO_4 = 0.00028 g of N
5. Available N (%) = 0.00028 g of N
6. Available N (ppm) = percentage N $\times$ 10,000
7. Available N (kg/ha) = ppm $\times$ 2.24

Interpretation

Available Nitrogen (kg/ha)	Soil Rating
<272	Low
272-544	Medium
>544	High

Available Phosphorous

Principle: The activity of Ca^{2+} in the soil solution and the pH of the soil regulates the solubility of calcium phosphate in it. The bicarbonate (HCO_3^-) activity in the soil is increased by the 0.5 M NaHCO_3 solution buffered to pH 8.5,

which reduces calcium activity. As a result, some phosphate from the surface of calcium phosphate gets dissolved in the soil. Similarly, due to the inactivation of Al and Fe, NaHCO_3 solution removes some phosphorous from Al and Fe phosphates. The precipitation of phosphate released from calcium phosphate

is prevented by low Ca^{2+} activity. The soluble phosphate forms heteropoly complexes with molybdate ion freed from ammonium molybdate solution when added to the soil extract. (Bray and Kurtz, 1945; Black, 1965).

Chemicals and reagents required

1. Standard phosphorous solution
 - i. Standard solution of P (100 ppm): 0.4387 g of KH_2PO_4 was dissolved in distilled water and the volume was made up to 1 liter.
 - ii. Standard solution of P (5 ppm): 5 ml of 100 ppm P solution was diluted to 100 ml with distilled water.
2. 0.5 M NaHCO_3 : 42 g of NaHCO_3 was dissolved in distilled water and the volume was made up to 1 liter after adjusting the pH to 8.5 with sodium hydroxide using a pH meter.
3. Sulphuric acid (5N): 139 ml of concentrated H_2SO_4 was diluted to 1 liter.
4. Reagent A: Dissolve 12 g of ammonium paramolybdate in 250 ml distilled water. Separately, 0.2908g of potassium antimony tartrate ($\text{KSbO}_3\cdot\text{C}_4\text{H}_4\text{O}_6$) was dissolved in 100 ml of distilled water. Both these dissolved reagents were added to 1 liter of 5 N sulphuric acid. Both were mixed thoroughly and diluted with distilled water to 2 liter.
5. Reagent B: 1.056 g of L-ascorbic acid was dissolved in 200 ml of reagent A and mixed. It was prepared freshly before use.

Apparatus and equipment required: 100 ml conical flasks, funnels, pipette, (1 and 5 ml),

25 ml volumetric flasks, 100 ml measuring cylinder, electric shaker, Whatman No. 1 filter paper and spectrophotometer.

Procedure

a) Preparation of a standard curve

The relationship between the intensity of the coloured solution of a substance and the percent transmittance or absorbance of the light rays flowing through the solution was depicted by a standard curve. It was used to figure out how much of a certain element is present in an unknown sample. 0, 0.5, 1, 2, 3, 4, 5 ml of P solution was taken in seven different 25 ml volumetric flasks to make the standard curve. It will produce a solution with a final concentration of 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 0.1 ppm, respectively. To it, 5 ml of extracting solution and 0.5 ml 5N H_2SO_4 were mixed and shaken well until the evolution of CO_2 stopped. Ten milliliters of distilled water was added while washing the neck of the flask and 4 ml of reagent B and the volume was made up to the mark with distilled water and mixed. All flasks developed a blue colour of variable intensity. The intensity of blue colour was read on spectrophotometer at 880 nm. A standard curve was plotted using P concentration against absorbance value to establish their relationship.

b) Analysis of the test sample

- 1) 1 g of soil sample was taken in a 100 ml flask.
- 2) To it, a pinch of Darco-G 60 and 20 ml of 0.5 N NaHCO_3 solution were added.

- 3) The flask was placed on an electric shaker and mixed at a constant speed for half an hour. Then the contents were filtered through Whatman No. 1.
- 4) A blank was prepared by following all the steps without addition of soil.
- 5) In case, the filtrate was not clear, a pinch of Darco-G was added.
- 6) 5 ml of the filtrate was taken in a 25 ml volumetric flask and proceed further for colour development as described under preparation of standard curve.

Calculations

1. Weight of soil sample taken = 1 g

2. Vol. of 0.5N NaHCO_3 solution added = 20 ml
3. First dilution = 20 times
4. Vol. of the filter taken for colour development = 5 ml
5. First volume made = 25 ml
6. Second volume = 5 times
7. Total dilution = $20 \times 5 = 100$ times
8. Concentration of P from spectrophotometer = X AU (absorbance units)
9. Available P in soil (ppm) = X (AU) $\times 100$
10. Available P (kg/ ha) = ppm $\times 2.24$

Interpretation

Available Phosphorus (kg/ha)	Soil Rating
>12.4	Low
12.4-22.4	Medium
22.4-50	High
>50	Very High

Available Potassium

Principle: In a neutral normal ammonium acetate solution, a known weight of soil was shaken. Potassium (K) ions absorbed on soil colloids exchange ammonium ions. The amount of exchangeable and water-soluble potassium in the extract was measured using a flame photometer. The transfer of non-exchangeable K to exchangeable form was hampered during ammonium acetate extraction because ammonium ions, like

K^+ , retain strongly charged layers together (Merwin and Peech, 1951; Black, 1965; Jackson, 1973).

Chemicals and reagents required

1. Neutral normal ammonium acetate solution ($\text{CH}_3\text{COONH}_4$): 77.09 g/l of ammonium acetate was dissolved in distilled water and the volume was made up to 1 liter. The pH of the solution was adjusted to 7 with ammonium solution or acetic acid.

- Standard solution of K (1000 ppm K): 1.91 g of potassium chloride (KCl) was dissolved in distilled water and the volume was made to 1 liter.
- Working standard solution of K: The stock solution was diluted 100 times to get 10 ppm K solution.
- 5 ml of the filtered extract was taken in a 25 ml volumetric flask and the volume was made up with distilled water. This solution was fed into the atomizer of the flame photometer and readings were noted down.

The amount of K in the test sample was calculated by using the dilution factor.

Apparatus and equipment required: A weighing balance, 150 ml conical flasks, a shaker, funnels, beaker, Whatman filter paper No 1, pipettes and a flame photometer.

Procedure

- 5 g of soil was weighed in a 150 ml conical flask.
- To this, 25 ml of neutral normal ammonium acetate solution was added.
- The mixture was shaken for 5 minutes on an electric shaker and then filtered through Whatman No. 1 filter paper.
- Dilution = 5 times
- Reading of K (ppm) in flame photometer = Y
- In ppm K = $Y \times \text{total dilution} - A$
- In kg/ha = $A \times 2.24 - C$

Calculations

Interpretation

Available Potassium (kg/ha)	Soil Rating
<137	Low
137– 337	Medium
>337	High

Available Sulphur

Principle: Soil was shaken with 0.15% CaCl_2 solution. During extraction, chloride ions displace adsorbed sulphate while calcium ions decrease soil organic matter extraction and hence eliminate contamination caused by extractable organic sulphur. The turbidity produced by the precipitation of sulphate as barium sulphate is measured on a

spectrophotometer at a wavelength of 420 nm. The turbidity is stabilized using gum acacia solution, which prevents the barium sulphate formed from settling (Lisle et al., 1994).

Chemicals and reagents required

- Extracting solution (0.15% CaCl_2): 1.986 g of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was dissolved in distilled water and volume made up to 1 liter.

2. Barium chloride (BaCl_2)
3. Gum acacia solution (0.25%): 0.25 g of gum acacia was dissolved in distilled water and diluted to 100 ml.
4. Standard sulphur solution (100 ppm): 0.5434 g of the reagent grade potassium sulphate (K_2SO_4) was dissolved in distilled water and diluted to 1 litre.

Apparatus and equipment required: 150 ml conical flask, funnel, pipettes, 25 ml volumetric flasks, electric shaker, and spectrophotometer and Whatman No. 42 filter paper.

Procedure

a) Preparation of standard curve--:

1. 0.25, 0.5, 1.0, 2.5 and 5.0 ml of 100 ppm S solution were taken in different 25 ml volumetric flasks. It was theoretically calculated to give 0, 1, 2, 4, 7, 10, 20 ppm concentration of sulphur in the final volume.
2. To every flask 10 ml 0.15% CaCl_2 solution and 1 g BaCl_2 were added.
3. Mixed for 1 minute to dissolve all the crystals.
4. Next, 1ml 0.25% solution of gum acacia was added.
5. The volume was made up to the mark for all the flasks and shaken properly for thorough mixing. Within 5-30 minutes after the development of turbidity, the standards were read on a spectrophotometer at 420 nm.
6. 0 absorbance at 0 ppm solution (blank) was adjusted.

7. A standard curve was plotted showing relationship between concentration of S (turbidity) and transmittance/absorbance readings.

b) Analysis of test samples--:

1. 10 g air dried soil was weighed and transferred to a 150 ml conical flask.
2. 50 ml of 0.15% CaCl_2 solution was added, and the mixture was shaken for 30 minutes on an electric shaker.
3. Filter the suspension through Whatman No. 42 filter paper.
4. 20 ml of the filtrate was taken in a 25 ml volumetric flask and the same steps as in case of standard curve were followed.
5. A blank was run with all the chemicals except the soil.
6. The sulphate concentration of unknown samples was determined from the standard curve.

Calculations

1. Weight of soil taken= 10 g
2. Volume of extractant added= 50 ml
3. First dilution= 5 times
4. Volume of aliquot taken= 20 ml
5. Final volume= 25 ml
6. Second dilution= 1.25 times
7. Total dilution= $5 \times 1.25 = 6.25$ times
8. ppm of S from standard curve= Y
9. ppm of S in soil= $Y \times 6.25$
10. S in kg/ha= ppm of S $\times 2.24$

Interpretation

Available Sulphur (ppm)	Soil Rating
<10 ppm	Deficient
>10 ppm	Sufficient

Heavy Metal

All the heavy metal i.e. Cr, Ni, As, Sr, Cd, Hg and Pb, are extracted with the help of ICP-MS (Make-Thermo Scientific instrument) (Retka et al., 2010).

Procedure

1. Take the sample and homogenize properly.
2. Take approximately 0.1 to 0.2 g sample in microwave vessels and add 4 ml of HNO_3 (Suprapure grade), add 1 ml of H_2O_2 (Suprapure grade).
3. Kept at room temperature 20-30 minutes for open digestion.
4. Afterwards samples were put in microwave for close digestion.
5. After digestion, samples were transferred into the 50 ml volumetric flasks and volume make up to the mark.
6. Samples were vortexed properly and run of ICP-MS against the Linearity.

Calculations

Calculate the concentration of the elements as follows.

Sample Conc.= (Sample reading-reagent blank reading $\times$ dilution factor)/ Sample Wt.

Micronutrient

Principle: All the four micronutrient cations i.e. Zn, Mn, Fe and Cu, are extracted by

shaking the soil with DTPA extracting solution containing 0.005M DTPA, 0.1 M TEA (Triethanol amine) and 0.01 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ buffered at pH 7.3. During this extraction, TEA gets protonated as HTEA^+ because of which micronutrient cations from the solid phase comes into solution and are chelated by the DTPA. Buffering of the extractant in the slightly alkaline pH range and inclusion of soluble Ca^{2+} through $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ helps avoiding dissolution of CaCO_3 and thus excludes from the estimation of the occluded micronutrients, which do not form a part of the pool that is available for absorption by plant roots. After that the contents of the micronutrients cations in the soil extract are estimated on atomic absorption spectrophotometer (Katyal and Sharma, 1991).

Chemicals and reagents required

1. DTPA extraction solution: This solution was prepared to contain 0.005M DTPA, 0.01M CaCl_2 and 0.1M TEA (Triethanolamine) and its pH was adjusted to 7.3. For preparing one litre of this solution, 13.3 ml $(\text{HOCH}_2\text{CH}_2)_3\text{N}$ i.e. TEA, 1.967 g DTPA and 1.47 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were dissolved in about 500 ml of deionized water. Sufficient time was given for DTPA to dissolve, and the contents are diluted to approximately 900 ml. The pH of the solution was then adjusted to 7.30 ± 0.05 by adding 6N HCl

while stirring and finally the contents were diluted to 1 liter. This solution was stable for several months.

Apparatus and equipment required: 1 liter volumetric flask, 100ml narrow mouth polyethylene bottles, pipettes, electric shaker, Whatman No. 1 filter paper and atomic absorption spectrophotometer.

Procedure

1. 12.5 g of air-dried soil sample was taken and transferred to a 100 ml narrow mouth polyethylene bottle.
2. 50 ml DTPA solution was added, and the bottle was closed with the stopper.
3. The bottle was shaken on an electric shaker for 2 hours at 25°C.

Interpretation

Content below which the soil is deficient	
Metal	mg/kg soil
Zn	0.6
Cu	0.2
Fe	4.5
Mn	3.5

Rhizosphere Soil Microbiology

Principle: The principle for the analysis of microbe's different media is used such as nutrient agar, Eosin Methylene blue, MacConkey Agar, Azotobacter, Azospirillum, Rhizobium, zinc solubilising, phosphate solubilising and potash mobilizer. The microbial count is usually expressed in CFU (colony-forming units) per gram or millilitre. The direct count method for enumerating bacteria in natural environments is widely used (Kirchman, 1982).

4. The contents were then filtered through Whatman No. 42 filter paper to obtain a clear solution.
5. Also, a blank was run with only DTPA solution without the soil.

Calculations

1. Weight of soil used for extraction= 12.5 g
2. Volume of the extractant used= 50 ml
3. Dilution= 2 times
4. Concentration of the given micronutrient in extract= A mg/kg
5. Available micronutrient in given soil sample= A × 2 mg/kg

Procedure

- Different growth medium is used to grow different types of microorganisms is given in Table 3.
- The medium is sterilized in an autoclave.
- Petri dishes are used to hold the growth media.
- A small number of bacteria is needed to inoculate the growth media.
- A 100 µl culture of sample is picked up with the help of pipette and transferred

- to the growth media and spread with the help of L-shaped spreaders.
- The inoculated growth media is incubated at the optimal temperature and conditions for the bacteria to grow.
- After incubation, the bacterial growth can be observed by looking closely at the colonial growth on the surface of a solid medium.

Table 3 Lists the various growth media that are used to cultivate the various kinds of microorganisms

Serial No.	Target Organism	Synthetic Media Used	Make
1.	Total Bacterial Count	Nutrient Broth	HiMedia (M002-500G)
2.	Total Fecal Count	MacConkey Agar	HiMedia (M008S-500G)
3.	Total Coliform Count	Eosin methylene Agar	HiMedia (M317-500G)
4.	Potash Mobilizer	Aleksandrow	HiMedia (M1997-500G)
5.	Zinc Solubilizer	Zinc sulphate	HiMedia (M2023-500G)
6.	<i>Rhizobium</i>	Rhizobium	HiMedia (M408-500G)
7.	<i>Azotobacter</i>	Azotobacter	HiMedia (M1944-500G)
8.	<i>Azospirillum</i>	Azospirillum + KOH	HiMedia (M1720-500G + M1720-500G)
9.	Phosphate Solubilizes	Pikovskaya	HiMedia (GM1719-500G)

Apparatus and equipment required: Weighing balance, spatula, distilled water, measuring cylinder, and a 1000 ml conical flask.

Calculation

Colony forming Units (CFU/ml) = Number of colonies × Dilution factor/ Volume of culture

Correlation Studies and Statistical Analysis

The tests have been performed in triplicates and the mean of values along with the standard deviation has been represented graphically. A total of 8 parameters have been studied for 26 sites and each parameter has shown considerable variation. The effect of one parameter on the other may be studied through correlation. A correlation coefficient

is an indicator of the relationship between two variables. The correlation coefficient is a statistical measure that indicates the strength and direction of the relationship between two variables. Its value ranges from -1 to +1, representing different degrees and types of correlation. When the correlation coefficient is closer to +1, it signifies a strong positive relationship between the variables. This means that as one variable increases, the other tends to increase as well. A correlation coefficient around 0 implies no linear relationship between the variables. Changes in one variable do not predict or affect changes in the other. As the correlation coefficient approaches -1, it denotes a strong negative relationship. This suggests that as one variable increases, the other tends to decrease.

Results and Discussion

Physicochemical Characterization of Soil

Moisture content is one of the most essential index qualities for determining the relationship between soil behaviour and index values. Soil moisture content expresses the phase relationships of water,

air, and solids in each volume or weight of material. The consistency of a specific soil, combined with its liquid and plastic limitations, is used to represent its relative consistency in cohesive soil (Hossain et al., 2022). The moisture content of different sampling sites is shown in Fig.1.

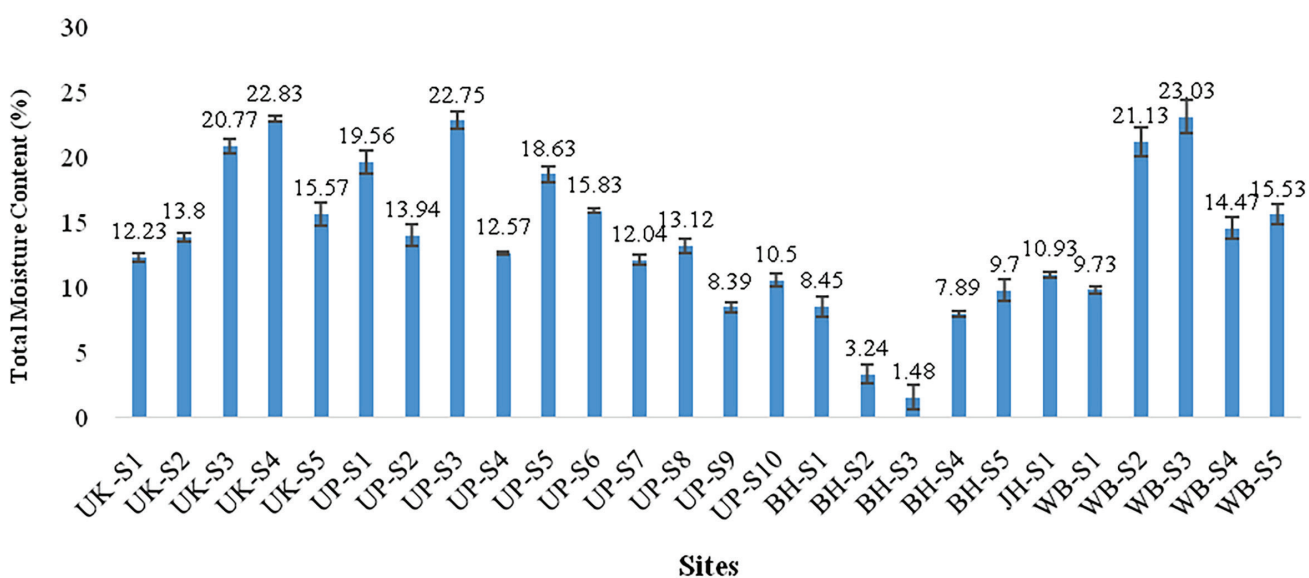


Fig. 1 Total moisture content of different sampling sites

The TMC% was found to be in the range of $1.48 \pm 0.94\%$ (lowest) to $23.03 \pm 1.25\%$ (highest). Soil sample from BH-S3 had very little moisture content i.e., $1.48\% \pm 0.94$. Seven of the soil samples had moisture content below 10%, while in most samples, moisture content was found to be within 20%. Sample from five sites had their TMC% over 20%.

Soil bulk density was another characteristic investigated. It is a measure of how thick or

firmly packed the soil is. The composition of the soil, the structure of the soil peds, the distribution of sand, silt, and clay particles, the volume of pore space, and how densely the particles are packed all influence soil bulk density. Bulk density indicates how easily roots can develop and water can filter through a profile's multiple soil strata (The Globe Program). The soil bulk density of different sampling sites is illustrated in Fig.2

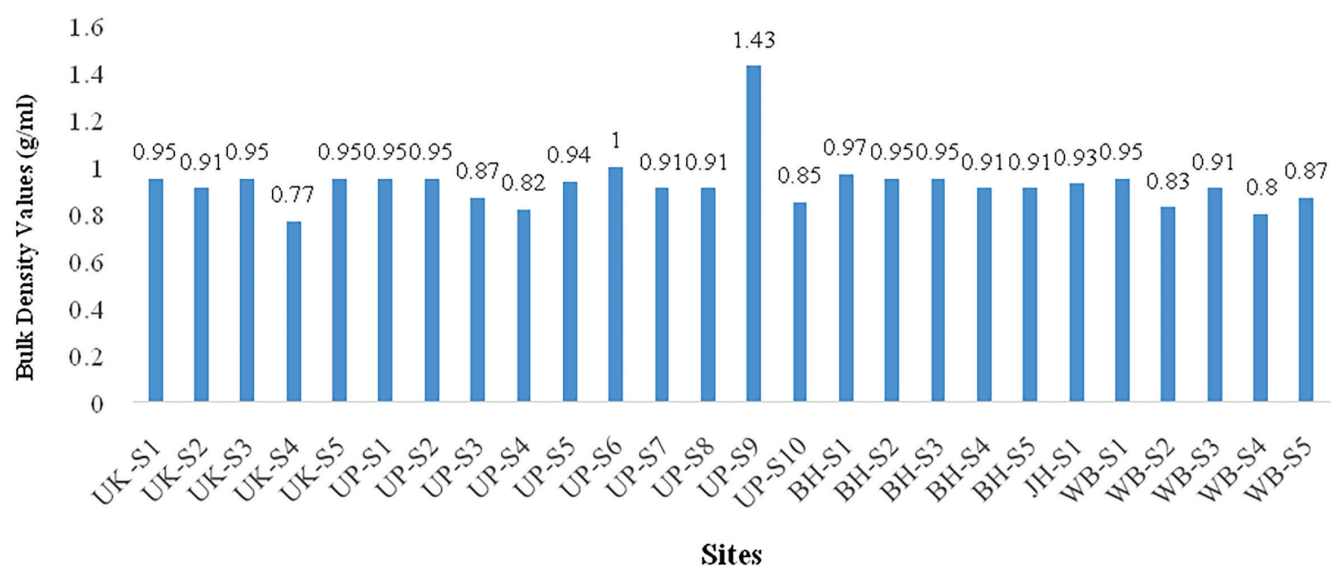


Fig. 2 Soil bulk density of different sampling sites

From the above data it was deduced that soil bulk density ranged from 0.77 g/ml to 1.43 g/ml. Soil bulk density of UP-S9 was highest i.e. 1.43 g/ml but that of UK-S4 was lowest i.e. 0.77 g/ml. Six of the sites had their bulk density below 0.90 g/ml while the remaining sites had their bulk density values above 0.90 g/ml.

The pH of soil is an important physical attribute that determines species richness

and density (Gough et al., 2000). This happens as soil pH determines the amount of nutrients that are soluble in soil water and their availability to plants. Some nutrients are more available under acidic conditions while some are available under alkaline condition. However extreme condition of acidity or alkalinity are harmful for the soil as well as plant growth. The pH of the different sampling sites is shown in Fig.3.

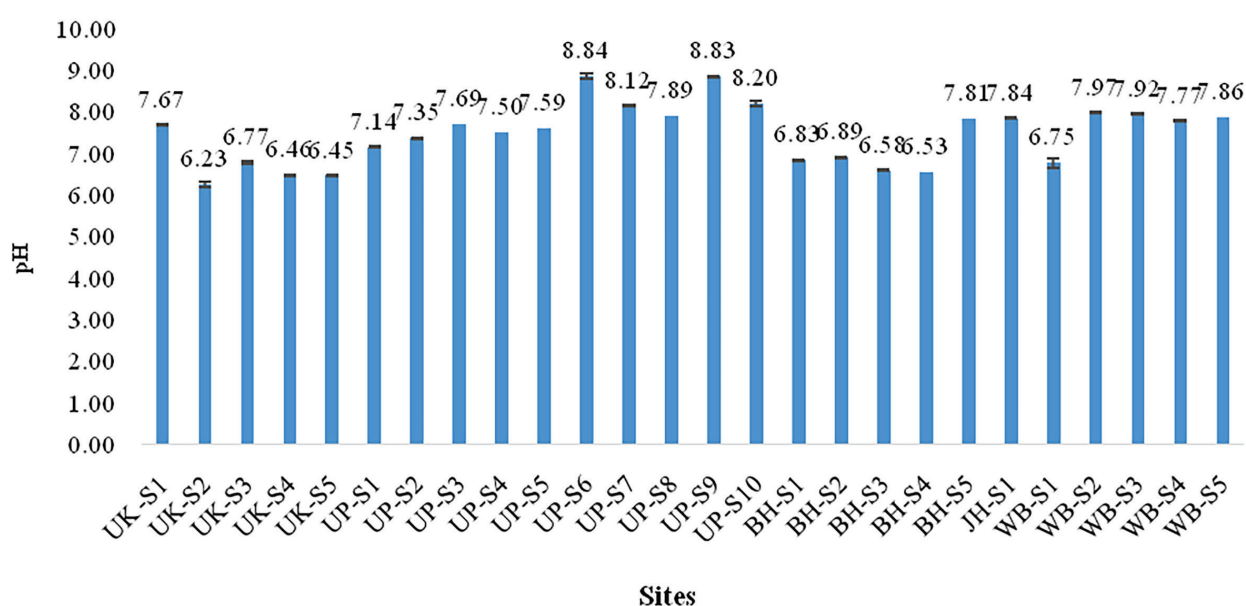


Fig. 3 Soil pH of different sampling sites

The pH of the sample sites ranged from as low as 6.23 ± 0.06 to as high as 8.84 ± 0.01 . Soil samples from UK-S2, UK-S4 and UK-S5 were found to be acidic, with pH levels lower than 6.5. The pH of 9 sites was neutral, whereas the pH of the remaining sites was alkaline.

The next parameter studied, was electrical conductivity of the soil. Soil electrical conductivity is an indicator of soil salinity, clay

content and the nutrient concentration in the field (Heiniger et al., 2003). As shown above, electrical conductivity may be categorized in different divisions and that may be used to determine the salinity of the soil so that which crop could be sown may be decided. The electrical conductivity of soils from different sampling sites was determined and is represented in Fig. 4

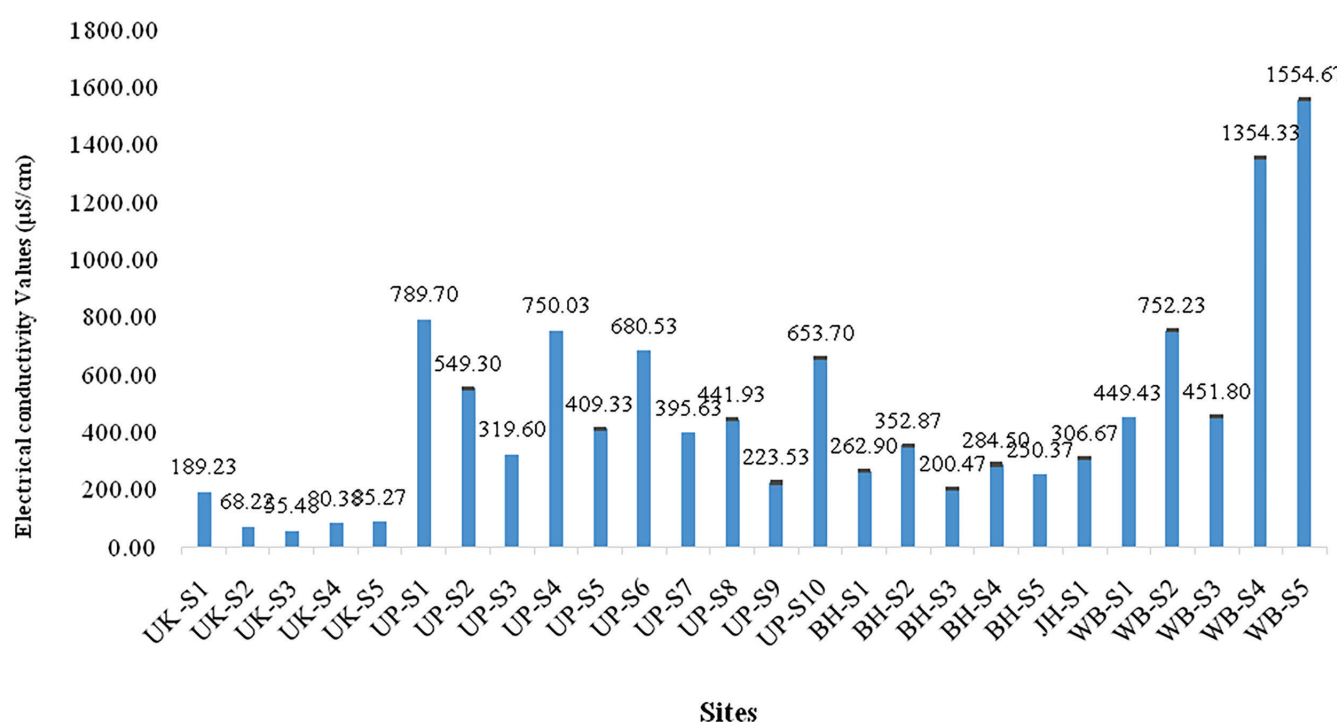


Fig. 4 Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below 800 µS/cm. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of 1354.33 ± 2.52 µS/cm and 1554.67 ± 2.52 µS/cm respectively. Thus, it may be said that the soil electrical conductivity was within the range at remaining all sampling sites and hence favourable for plant growth.

Soil organic carbon is a measure of the organic content present in soils. It is an important determinant of the hydraulic properties of soil like porosity, water retention and hydraulic conductivity. Thus overall, it is an important parameter determining food content in soil for uptake by plants (Allison, 1965; Rawls et al., 2004). The organic carbon content as measured for the 26 soil samples is shown in Fig. 5

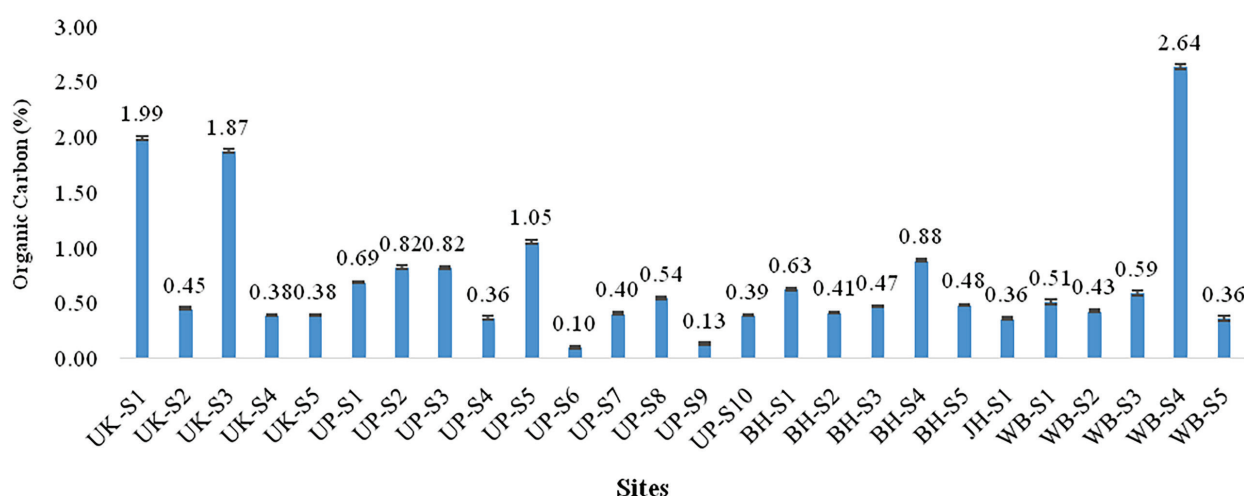


Fig. 5 Soil organic carbon content at different sites

According to the data, the organic carbon content of soil ranged from $0.10 \pm 0.01\%$ to $2.64 \pm 0.03\%$. UP-S6, which had the highest pH value of 8.84 ± 0.01 had the lowest organic carbon content of $0.10 \pm 0.01\%$. The highest quantity of organic carbon content was discovered in WB-S4 ($2.64 \pm 0.03\%$), which had an alkaline pH (7.77 ± 0.02). Soil samples from 8 different sites showed low organic carbon concentration, whereas soil samples from 11 different sites had medium organic carbon content. A total of 7 sites had high levels of organic carbon.

The next soil parameter studied was available nitrogen content. Nitrogen is the most important plant macronutrient, and its proper availability is a crucial determinant for plant growth and its optimal health. Researchers (Baričević and Zupančič, 2002) showed that increasing concentration of N added to soil resulted in increased concentration of alkaloids which is a critical component in extracts of medicinal plants. The available nitrogen content in soils from the different sites is shown in Fig. 6

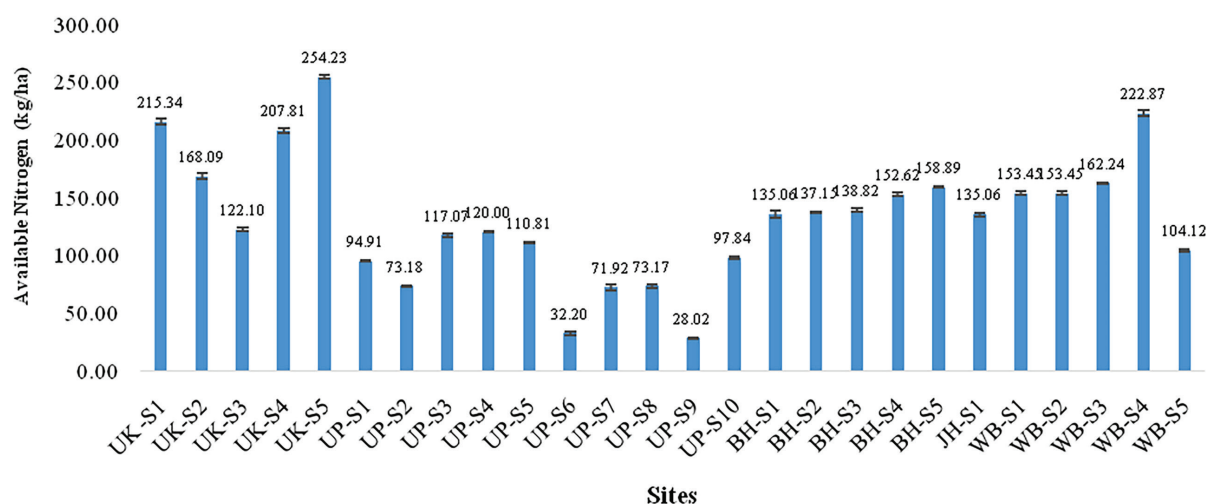


Fig. 6 Soil available nitrogen content at different sites

From the available data it was observed that site UK-S5 had the maximum available nitrogen content (254.23 ± 1.45 kg/ha) and as per soil categories the content was low. For all the other types of soil, the available nitrogen content was low. This could be because all these samples were randomly picked from different sites, which necessarily did not receive any fertilization.

The next parameter tested was that of phosphorous which is another important

plant macronutrient. Phosphorous is a macronutrient that controls the water use efficiency, winter hardiness and promotes root formation (Chakraborty and Prasad, 2021). In case of medicinal plants enough phosphorous in soils promotes development of leaf biomass which is the main part of the medicinal plant in most cases (Stewart, 2003). The concentration of available phosphorous in soils from the different sites is shown in Fig. 7

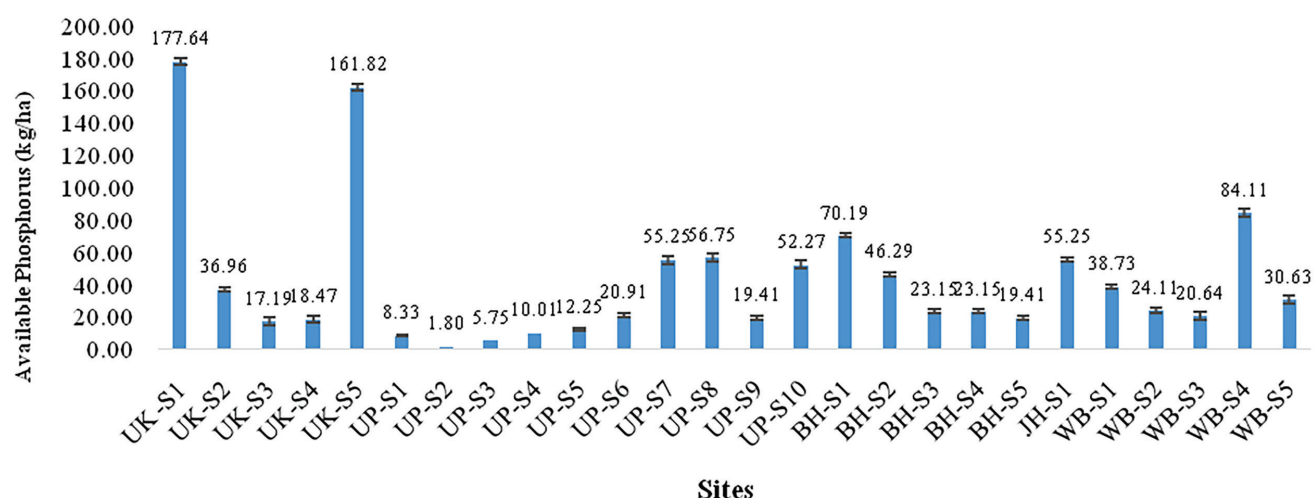


Fig. 7 Soil available phosphorus content at different sites

As per the data obtained and divisions demarcating phosphorous content in soil, the maximum P was recorded in site UK-S1 (177.64 ± 1.83 kg/ha) while a very less amount was recorded in site UP-S2 (1.80 ± 0.06 kg/ha). The remaining sites have enough amount of the P in soil. Four of the sites had available phosphorus content in low range, while 6 sites were having available phosphorus in medium range. Similarly, 4 sites had their range in high category and the remaining sites had available phosphorus in very high category.

The next parameter is the third major macronutrient for plants, potassium. In

general, potassium plays a critical role in the closing and opening of stomata and thus regulates the uptake of water by plants. It also regulates plant growth and yield (Perrenoud, 1977). Literature also suggests that proper potassium content in soil results in higher concentration of essential oils in medicinal plants. Thus, such plants when grown in properly fertilized soil will result in good quality of medicinal plants for maximum benefit of consumers. The potassium content of the different soil samples is shown in Fig. 8

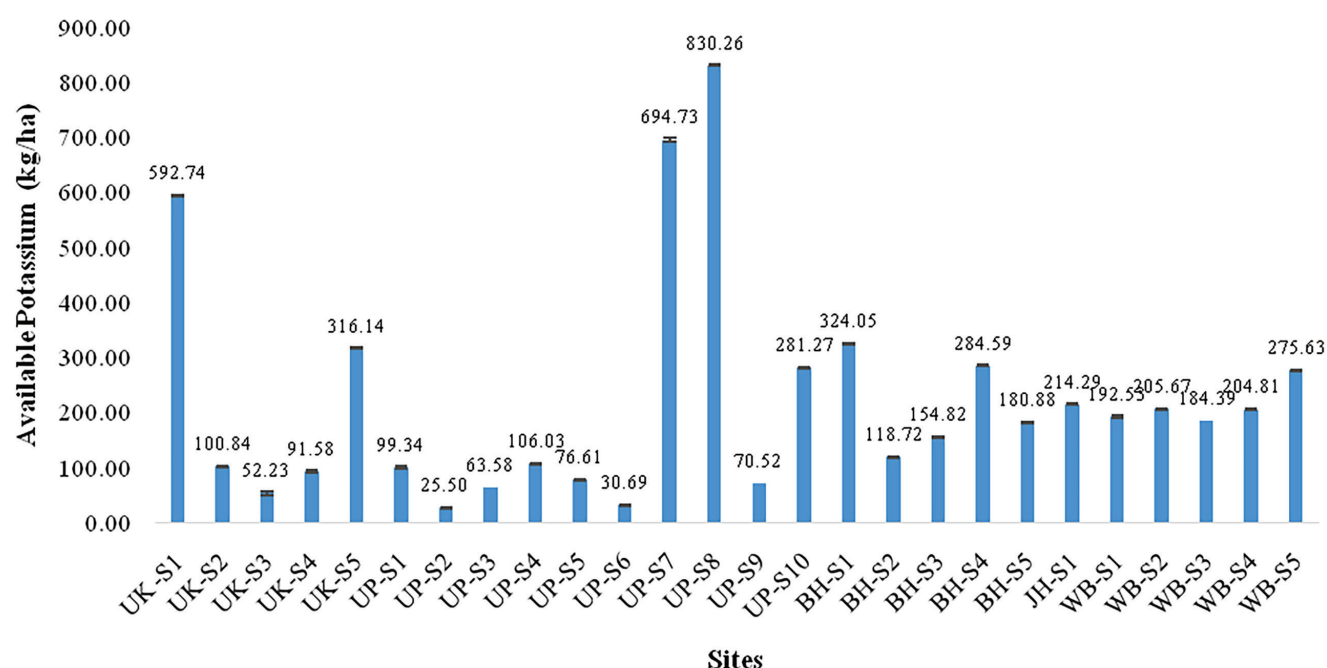


Fig. 8 Soil available potassium content at different sites

From the obtained results, it was seen that site UK-S1, UP-S7 and UP-S8 had high potassium content. As per the divisions of potassium content in soil, the potassium content was medium at twelve sites. The remaining sites had low potassium content.

Sulphur content in soil plays a role as it is up taken by plants for the formation of sulphur containing amino acids and hence the buildup of proteins, chlorophyll, and oils (Tabatabai, 1984). The sulphur content in the soil is shown in Fig. 9.

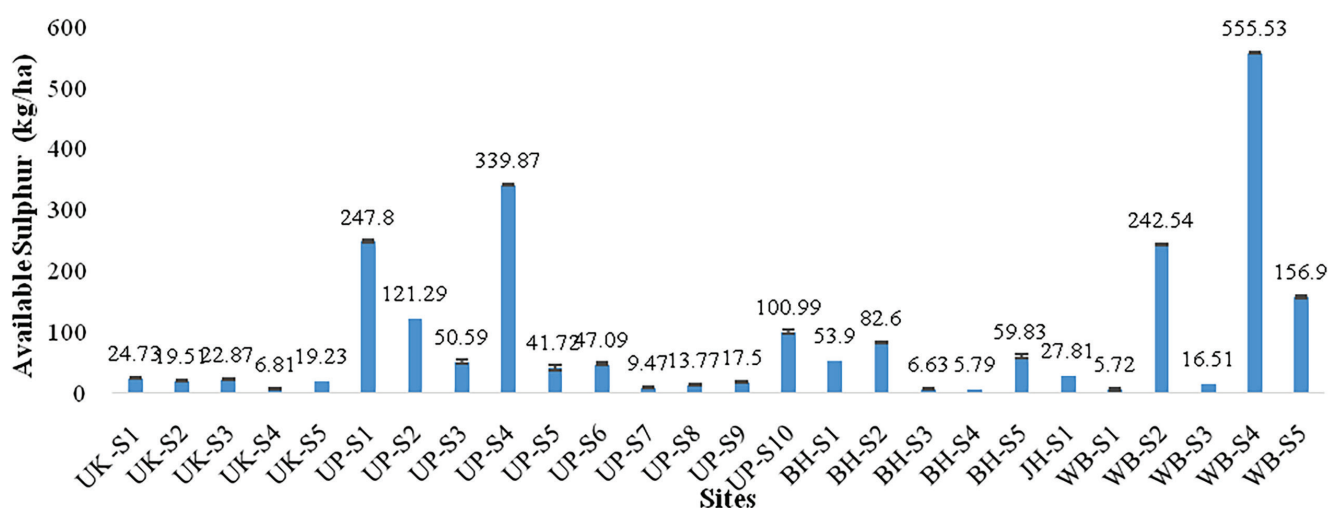


Fig. 9 Soil available sulphur content at different sites

For the results obtained for the sulphur content, it was found that 10 sites were sulphur deficient. The remaining sites had enough sulphur in their soil. Further, correlation studies will help us to understand the effect of pH, EC, or even microbial parameters on sulphur content and vice versa. Additionally, the impact of both high and low sulphur on the composition of phytochemicals can be examined.

Fig. 10a-g shows the different heavy metals content in all the sampling sites. The concentration of 7 heavy metals Cr, Ni, As, Sr, Cd, Hg and Pb were determined using ICP-MS. Their concentration further talks about the level of pollution and contamination in the soil sampling sites.

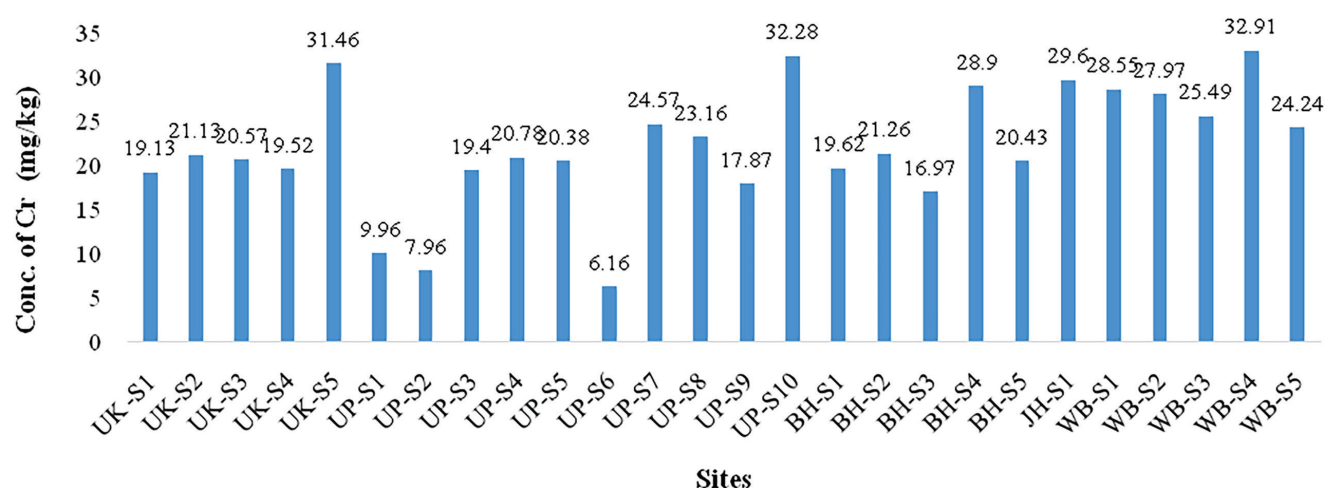


Fig. 10a Concentration of chromium at different sites



Fig. 10b Concentration of nickel at different sites

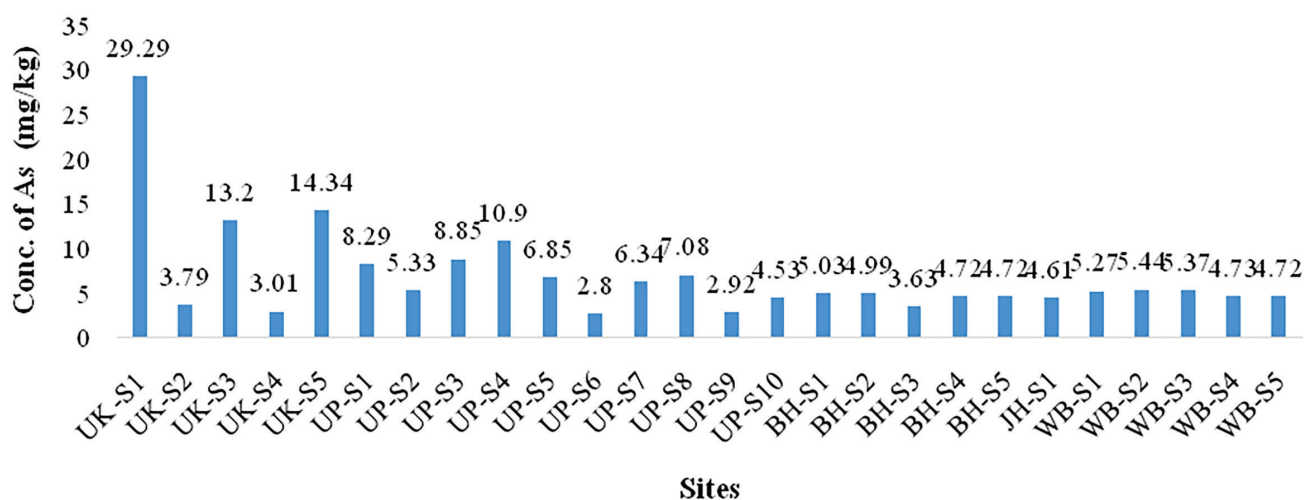


Fig. 10c Concentration of arsenic at different sites

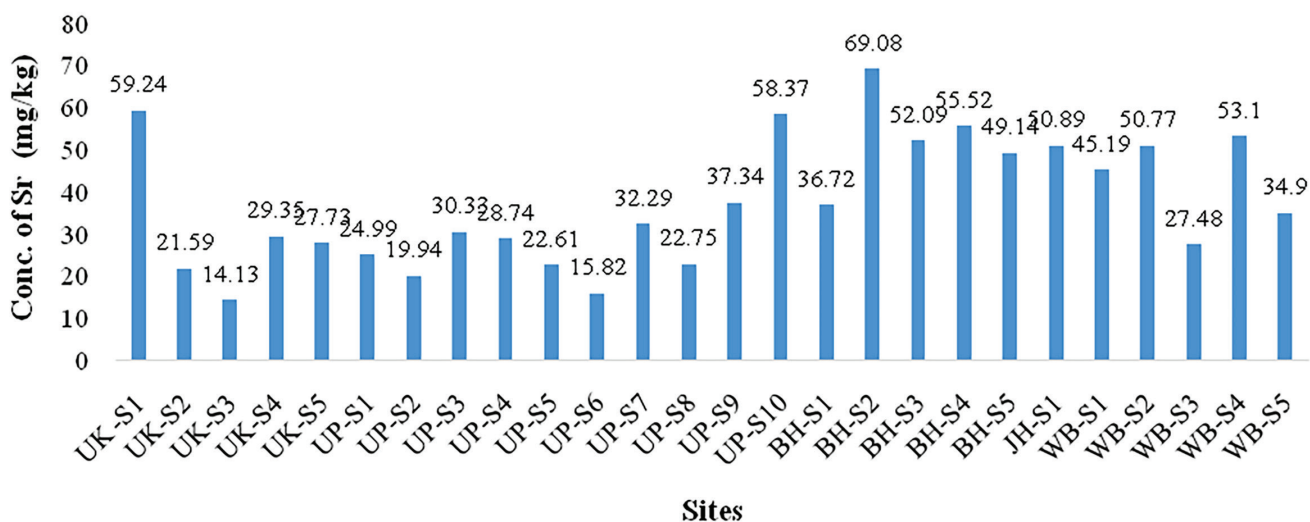


Fig. 10d Concentration of strontium at different sites

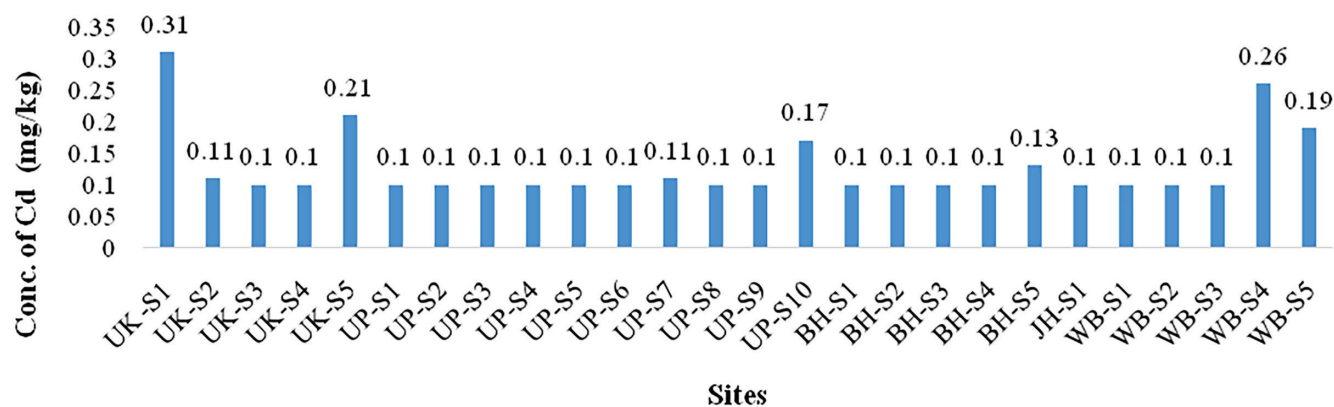


Fig. 10e Concentration of cadmium at different sites

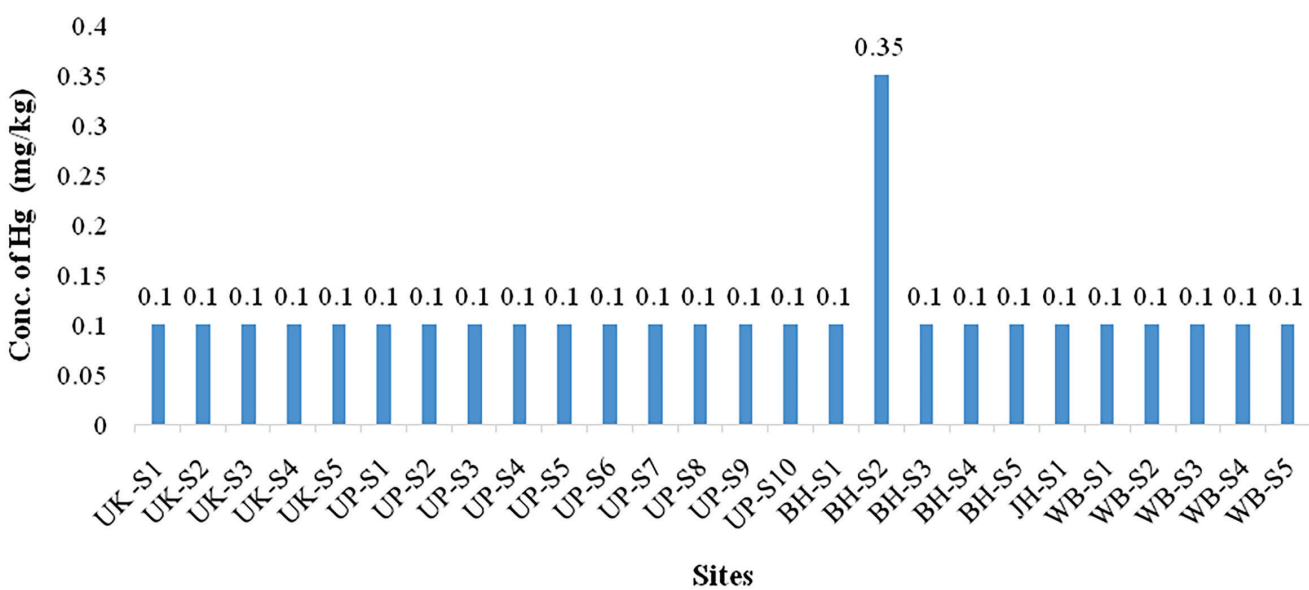


Fig. 10f Concentration of mercury at different sites

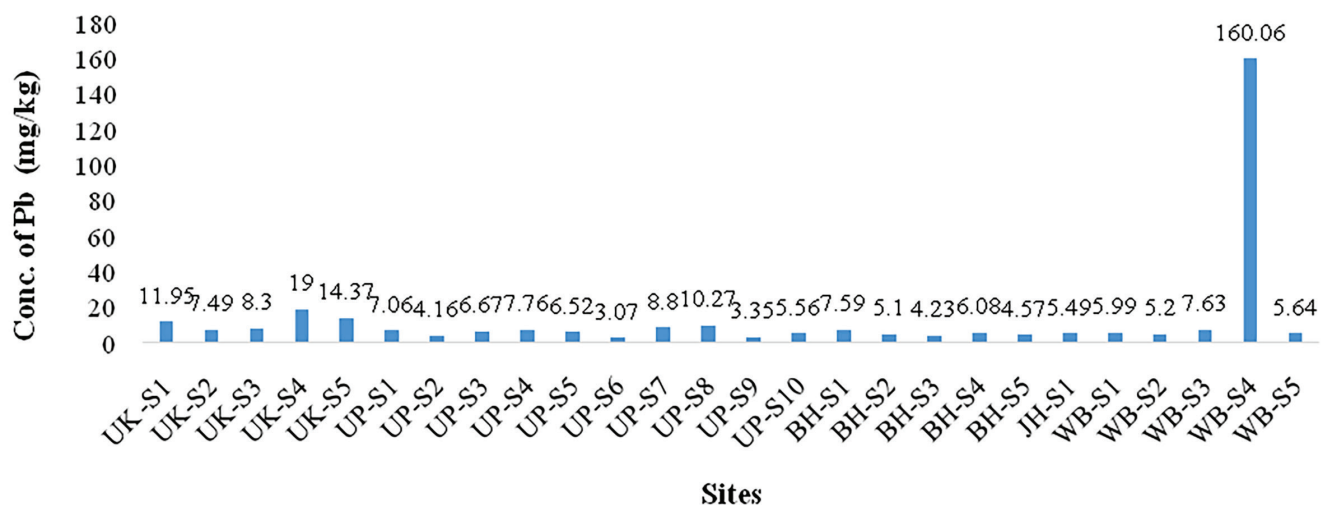


Fig. 10g Concentration of lead at different sites

Micronutrients are elements which are required by plants in very small quantities. They mainly act as co-factor of enzymes. When available to plants in the right quantity, they help in proper metabolism and bring about optimal plant growth and yield. The

concentration of the 4 micronutrients Zn, Fe, Mn and Cu were determined using atomic absorption spectrophotometer. The values obtained for all the sites are represented in Fig. 11 a, b, c and d.

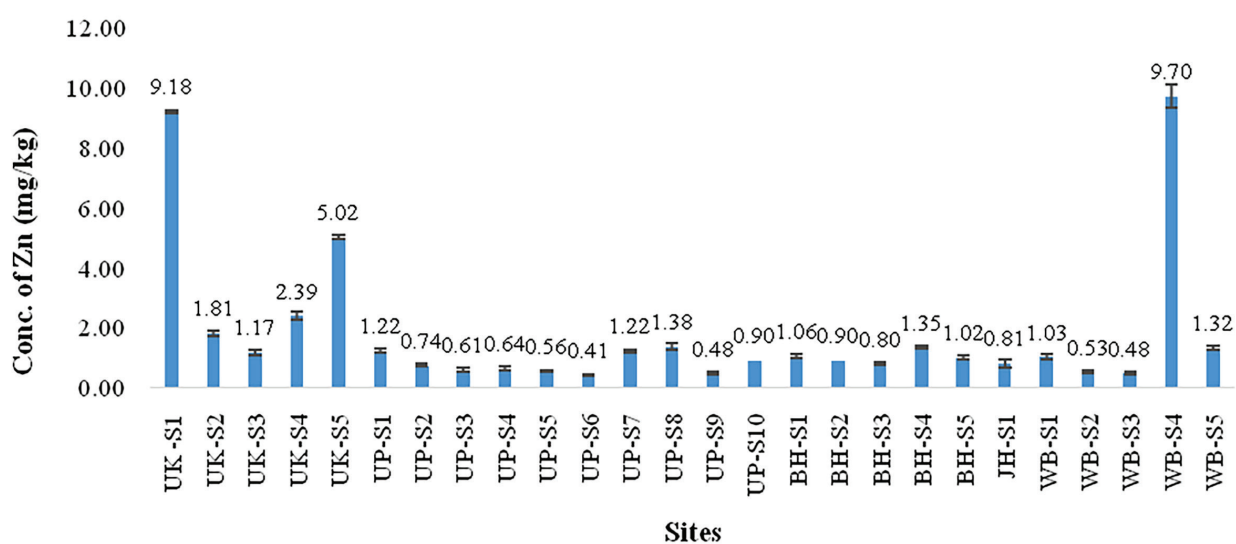


Fig. 11a Concentration of zinc at different sites

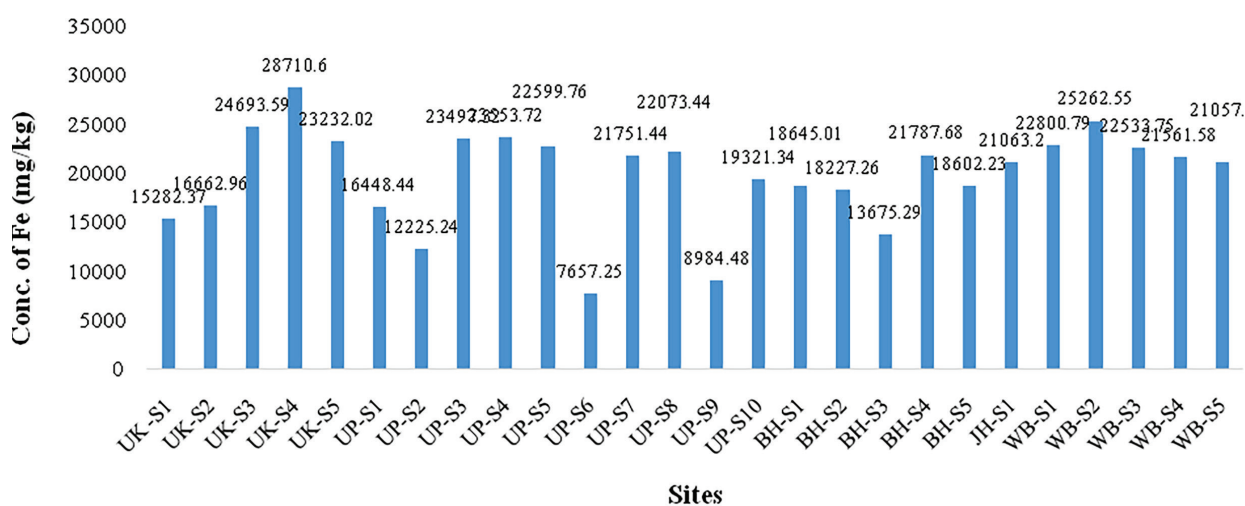


Fig. 11b Concentration of iron at different sites

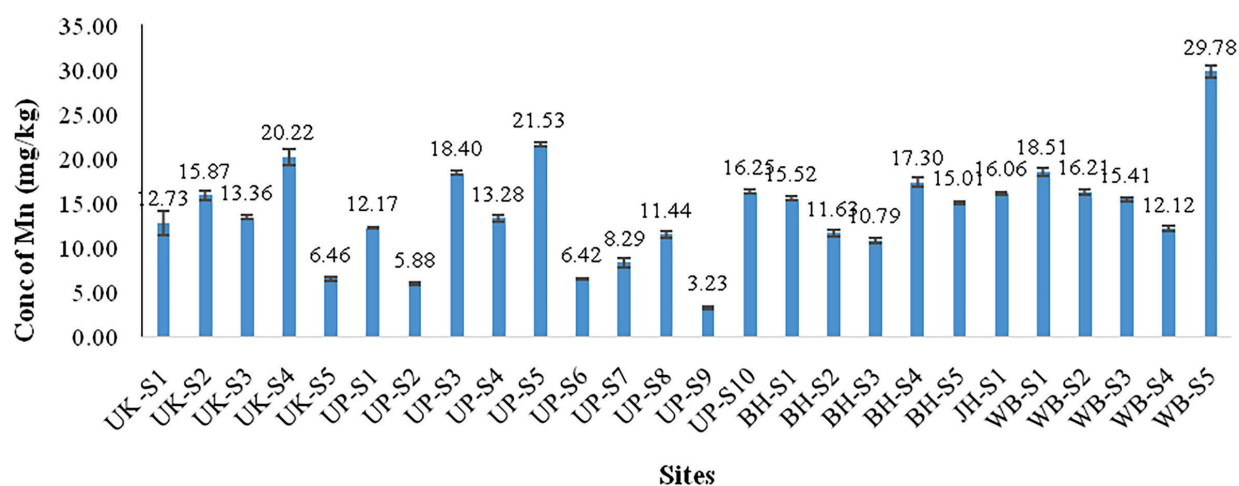


Fig. 11c Concentration of manganese at different sites

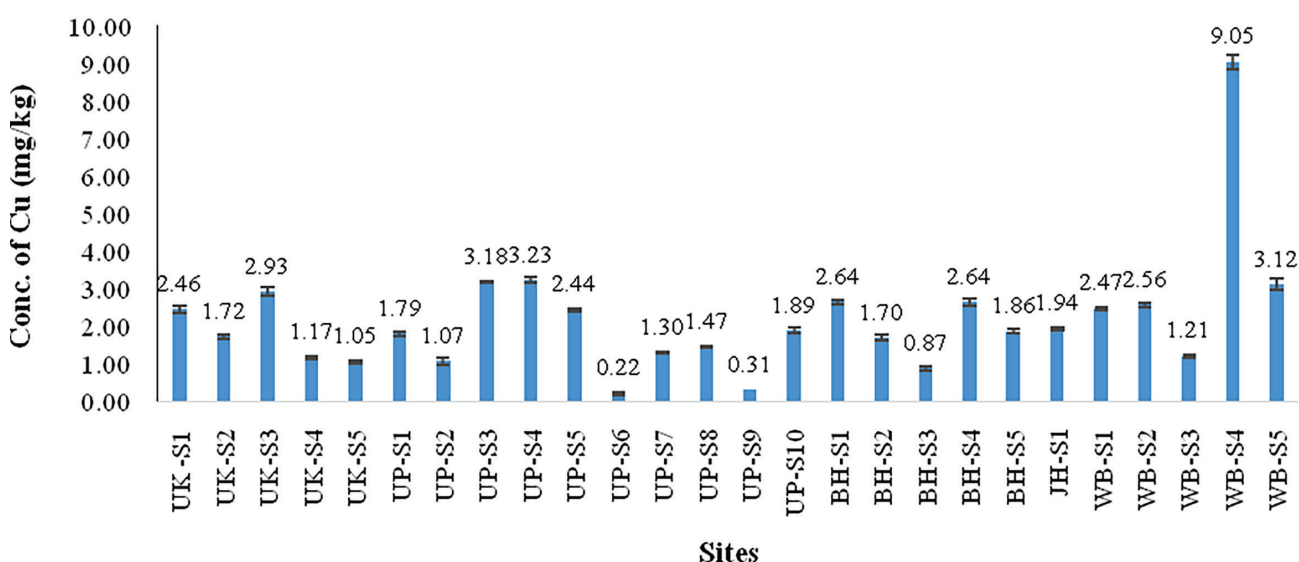


Fig. 11d Concentration of copper at different sites

As per the requirement of micronutrients by plants, there are different prescribed limits. The minimum amount of a particular micronutrient to be present in the soil is considered as the threshold. Deficient soils are those with concentrations below them, whereas sufficient soils have concentrations above them.

Though micronutrients are required by plants in very small quantities, they are important for proper metabolic functioning. Soils where micronutrients are present in insufficient quantities will result in plant having inadequate metabolism and hence,

less of phytochemical content too. Thus, cultivation of medicinal plants must be done in soils with proper nutritional content (Katyal et al., 1991).

Total and Differential Bacterial Count from Rhizosphere

Rhizosphere microbial testing involves analysing the microbial communities in the soil, rhizosphere, and roots of plants. The rhizosphere microbial test is performed in different media to calculate the bacterial count for the *Solanum nigrum* L. in different locations shown in Fig.12

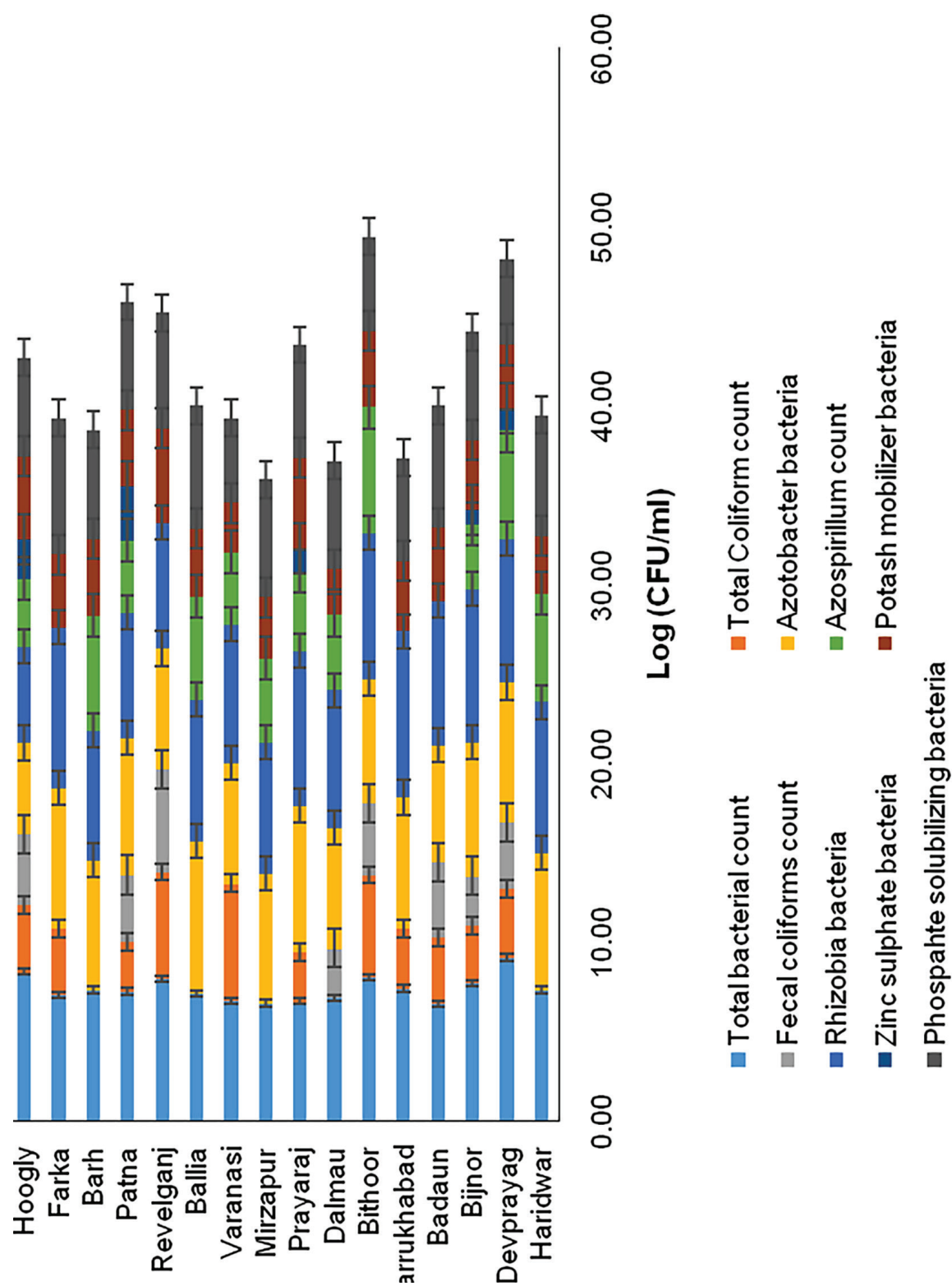


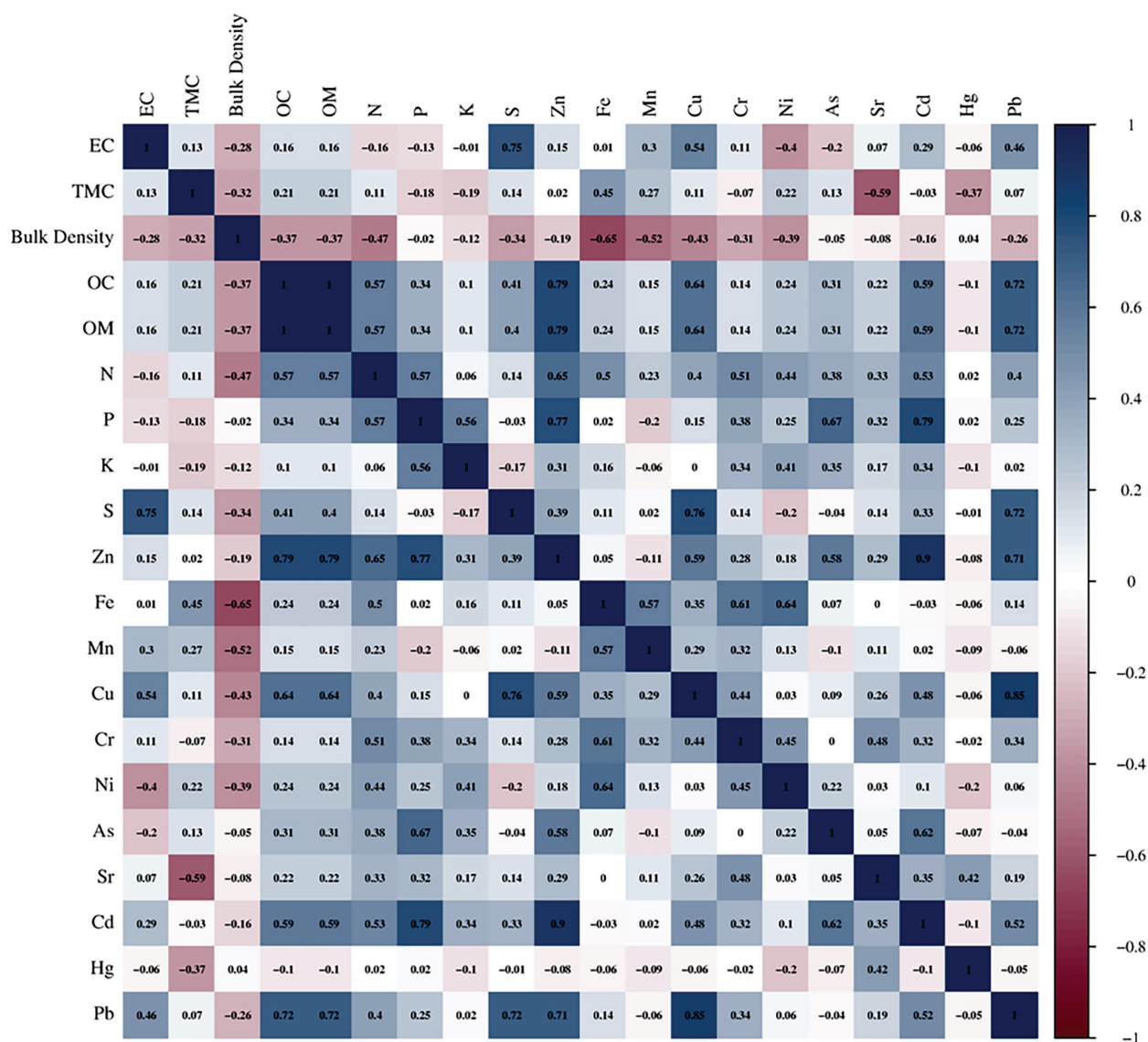
Fig. 12 Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Devprayag is 9.15753 cfu/ml and lowest in Mirzapur is 6.5971 cfu/ml. Whereas the total faecal and coliform count is highest in

Revelganj and Varanasi is 5.75927 and 6.5395 cfu/ml. The results of correlation studies have been represented as a matrix in Table 4.

Correlation Coefficient Matrix

Table 4 Correlation coefficient matrix



The correlation matrix in Table 6 clearly shows the micronutrient status at different sites and according to the correlation coefficient values of one parameter against the other, the values

have been colour coded. The colour coding key also shown above shows the colour codes as per the range of the correlation coefficient value. According to correlation coefficient

values of each parameter against the other, few observations can be made. These are relevant for every parameter from every site because the values were determined using information gathered for every site for every parameter.

- pH showed weak negative correlation with total moisture content, potassium, sulphur, and lead while it showed moderate positive correlation with electrical conductivity.
- A high degree of strong positive correlation between electrical conductivity and sulphur was observed, while moderate positive correlation was seen between the former and copper and lead.
- Total moisture content showed moderate positive correlation with iron; furthermore, bulk density showed weak negative correlation with mercury.
- Organic carbon showed negative correlation with mercury, moderate positive correlation with nitrogen, phosphorus, sulphur and cadmium and strong positive correlation with zinc, copper, and lead.
- Nitrogen showed a very strong positive correlation with zinc. Phosphorus showed the same with zinc, arsenic, and cadmium. Potassium had moderate positive correlation with zinc, chromium, nickel, arsenic, and cadmium.
- A strong positive correlation was seen between sulphur, copper, and cadmium. The same was also observed between zinc, cadmium, and lead. Iron, chromium, and nickel. Manganese and chromium shared moderate positive correlation between each other.
- Strong positive correlation was seen between copper and lead. Chromium, arsenic, and mercury had negative correlation amongst themselves. The same was also observed between nickel and mercury. A strong positive correlation was seen between arsenic and cadmium.
- Strontium showed moderate positive correlation between cadmium and mercury. The same was also seen between cadmium and lead. Mercury on the other hand showed negative correlation with lead.

Conclusion

From the physicochemical and microbial analysis of soil it was found that the parameters were different at each site. If the soil of each site is characterized as per the availability of nutrients and bacteria according

to the recommendations, then the best soil as per the area can be determined. The Table 5 shows the conclusive results of all the results obtained for different parameters from all the sites.

Table 5 Conclusive results for different parameters from all sites

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
UK -S1	AB	WR	AR	BR	Very AR	AR	WR	WR	WR	WR	WR	WR	WR	AB	WR	WR	WR	WR
UK-S2	BR	WR	WR	BR	AR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S3	WR	WR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S4	BR	WR	AR	BR	WR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UK-S5	BR	WR	BR	BR	Very AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S1	WR	WR	WR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S2	WR	WR	AR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S3	AB	WR	AR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S4	WR	WR	BR	BR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S5	AB	WR	AR	BR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S6	AB	WR	BR	BR	WR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S7	AB	WR	WR	BR	Very AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S8	AB	WR	WR	BR	Very AR	AR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S9	AB	WR	BR	BR	WR	BR	BR	BR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR
UP-S10	AB	WR	BR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
BH-S1	WR	WR	WR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S2	WR	WR	WR	BR	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	AB	WR
BH-S3	WR	WR	WR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S4	WR	WR	AR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
BH-S5	AB	WR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
JH-S1	AB	WR	BR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S1	WR	WR	WR	BR	AR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S2	AB	WR	WR	BR	AR	WR	WR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S3	AB	WR	WR	BR	WR	WR	BR	BR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S4	AB	CSSC	AR	BR	Very AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR
WB-S5	AB	CSSC	BR	BR	AR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR	WR

The given parameter range is expressed in CSSC [Critical for Salt-Sensitive Crops]: This range is vital for salt-sensitive crops. WR (Within Range): The parameter falls within the acceptable range. AR (Above Range): The parameter exceeds the recommended range. BR (Below Range): The parameter is below the recommended range. These parameter ranges are associated with the studies conducted by the Katyal et al., 1991, Hossain et al., 2022, Bowen, 2016, Varley, 1972; Jackson, 1973, Chopra and Kanwar, 1976; Richards, 1954, Jackson, 1973; Walkley and Black, 1934, Subbaiah, 1956, Bray and Kurtz, 1945; Black, 1965, Black, 1965; Merwin and Peech, 1951; Jackson, 1973, Lisle et al., 1994 and Retka et al., 2010.



The comprehensive investigation can also provide a better understanding of how soil quality factors, environmental fluctuations, and

altitudinal variations affect the phytochemical composition of medicinal plants.

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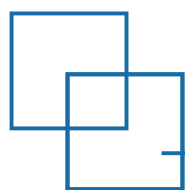
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CHAPTER
04

Traditional and Ethnomedicinal Applications



INTRODUCTION

Plants have been utilized for therapeutic purposes from ancient times, predating the notion of “medicine” and “medical science” (Mandal et al., 2023). Approximately 80% of people worldwide utilize herbal medicine, and the World Health Organization (WHO) supports the integration of herbal medications into national healthcare initiatives. These medications are thought to be safer than contemporary synthetic drugs since they are readily accessible, reasonably priced, and have withstood the test of time (WHO, 2013). However, a barrier to the broader acceptance of medicinal plants is the paucity or insufficiency of available scientific data regarding the ethno-medicinal aspects of these substances (Kamboj, 2000). Due to its distinctive principles and practices, the traditional system of Indian Medicine is becoming more and more popular globally (Bodeker, 2002). It is being widely used, researched, and investigated due to its potential to treat various ailments (Jagtap et al., 2013). The genus *Solanum* L. is found throughout the world’s temperate and tropical zones and has over 1500 species, making it the largest and most complex genus within this family (Edmonds and Chweya, 1997; Mandal et al., 2023). This genus is valuable economically since it has a large number of cultivated species that produce edible fruits and vegetables (Muthoni et al., 2012), in addition to several medicinal ones (Pereira et al., 2014; Modilal et al., 2015). In addition to being native to Europe, Asia, and North America, *S. nigrum* is also brought to South America, Australia, and Africa as a member of the Solanaceae family. It stands for one of the genus’s biggest and most diverse species groups. It sometimes referred to by its English name, “black nightshade,” is an herbaceous plant that grows widely around the globe, from tropical to temperate climates (Särkinen et al., 2018). It is a minor food crop in many underdeveloped nations; the shoots and berries are utilized for a variety of local and therapeutic purposes in addition to being fruits and vegetables (Jagatheeswari et al., 2013). It is sometimes referred to as “makoi” locally, utilized in Indian medicine as a sedative, analgesic, antiperiodic, antiphlogistic, diuretic, and purgative (Nyeem et al., 2017). While the plant extracts are said to have anti-inflammatory, antispasmodic,



and vasodilator properties, as well as the leaves, stem, and roots are used as a poultice or wash to treat leukoderma, wounds, and malignant sores. According to scientific research, many pharmacological studies on *S. nigrum* indicated that it was employed for centrally mediated depressive activities, hepatoprotective, cytoprotective, antioxidative, antibacterial, molluscicidal, larvicidal, and ulcer healing activities in addition to its anticancer potential (Singh, 2017). It is mostly used as a vegetable in soup preparation around the globe. Numerous scholarly investigations have examined the nutritional composition of the “vegetable black nightshade,” presenting proof that this species is a nutrient-dense vegetable (Akubugwo et al., 2007). Further, the different chemical compounds found in *S. nigrum* are the source of its pharmacological actions. Glycoalkaloids, glycoproteins, polysaccharides, and polyphenolic substances such gallic acid, catechin, PCA (protocatechuic acid), caffeic acid, epicatechin, rutin, and naringenin are its active ingredients (Ravi et al., 2009). It is extensively utilized in many traditional medical systems across the world for a variety of diseases, but it has received little attention for its modern therapeutic applications (Jain et al., 2011).

Traditional and Ayurvedic Benefits

Since ancient times, medicinal systems, more or less, have all used plant-based medicine in some capacity and thus medicinal plants are important in both conventional and contemporary systems. Because of the numerous negative consequences of using synthetic medications and antibiotics, as well as their exorbitant cost, their use has increased as a result of numerous studies and applications. Eighty percent of people still utilize traditional folk remedies made from natural materials in poor nations (Farnsworth et al., 1985). Native Americans have long had rich customs of using therapeutic plants near natural resources

(Gogoi and Islam, 2012). Traditional medicine frequently uses *S. nigrum* as a treatment to cure a variety of illnesses. The berries are excellent in treating liver, heart, and eye diseases as well as piles, fever, and dysentery. They also have sedative, diaphoretic, diuretic, hydragogue, and expectorant qualities (Rastogi and Mehrotra, 1991). The leaves are hypotensive and are used to treat open wounds (Ye, 1984). In folk medicine, berries have been used to cure stomach ulcers in South Africa, Europe, China, and India (Ikram and Hussain, 1918). There have been reports that the hepatoprotective effect of *S. nigrum* is enhanced by the

fruits. One potential mode of action has been proposed to be the inhibition of lipid peroxidation and free radical scavenging (Sultana et al., 1985). In Ayurveda, *S. nigrum* is indicated for various disorders like 'Shotha' (Inflammation), 'Vrana' (Wound healer), 'Visarpa' (Herpes), 'Vrishanashotha' (Antitoxic), 'Yakritshotha' (Inflammation of Liver), 'Udararoga' (Ascitis), 'Mukharoga' (Diseases of mouth), 'Kantharoga' (Throat Disorders), 'Nasaroga' (Nasal Disorder), 'Karnashoola' (Ear ache), 'Netraroga' (Eye disorders), 'Shwitra' (Leukoderma/vitiligo), 'Agnimandya' (Appetizer), 'Chhardi' (Anti-emetic), 'Arsha' (Anti-hemorrhoid), 'Jeerna Pravahita' (Chronic amoebiasis), 'Atisara' (Anti-diarrheal), 'Pleehavridhi' (Splenomegaly), 'Hridroga' (Heart diseases), 'Raktabharadhikya' (Anti-hypertensive), 'Kushtha' (Skin diseases), 'Vatarakta' (Gout), 'Amavata' (Anti-rheumatoid), 'Vrikkaroga'

(Kidney disorders), 'Prameha' (Anti-diabetic), 'Jeerna Jwara' (Chronic fever), 'Samanya Daurbalya' (General debility) and 'Kshaya' (Anti-tubercular) (Jani, 2010). Additionally, it has been used traditionally for a long time to treat a variety of illnesses, including pain, fever, and inflammation (Acharya and Pokhrel, 2006; Zakaria et al., 2006) and enteric diseases (Rani and Khullar, 2004). It is multifunctional, having anti-tumorigenic, antioxidant properties (Lee and Lim 2003), anti-inflammatory (Zakaria et al., 2006), hepatoprotective (Raju et al., 2003) diuretic, antipyretic agent (Zakaria et al., 2006; Li et al., 2007), anti-bacterial (Rani and Khullar, 2007) mycotic infection (Kambizi and Afolayan, 2001), cytotoxicity, anti-convulsant and anti-ulcerogenic (Sikdar and Dutta, 2008; Jani and Ahir, 2010). It is also used against sexually transmitted diseases (Atanu et al., 2011), as depicted in Table 1.

Table 1 Some important preparations used against various diseases in ancient Ayurveda

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
1.	Ophthalmic disorders	Kākamācīphalaikena ghṛtayuktena bud-dhimāna.Dhūpayet pillarogārttaḥ patanti kṛmayo'cirāt- medicated fumigation of <i>S. nigrum</i> (Garden nightshade) fruits, mixed with <i>Ghṛta</i> (Clarified butter), used to remove worms in patients suffering from <i>Pilla</i> (eye disorder) diseases.	Tiwari, 2007
2.	Thoracic disorders	Vāyasīśākaḥ.....śasyate vātakāse.....- vegetable prepared from <i>S. nigrum</i> (Garden nightshade), used against cough.	Śāstrī and Chaturvedi, 2011

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
3.	Reproductive disorders	Kākamācīśiphāḥ puṛye kāmaḥ niṛpīrya kanyayā. Rasaḥ snātā pibennārī labhed garbhamanuttamam- <i>S. nigrum</i> root juice, obtained in <i>Puṣyanak'ātra</i> , helps to attain pregnancy if taken by the female during the ovulatory period of the cycle; Śyāmāparūṛaphalinī kākamācīśiphāḥ pṛthak. Piṛṛā nābheradho liptā garbhan-ikrāmaḥapradāḥ- external application of a paste prepared from <i>S. nigrum</i> root, below the navel region of the women in the second stage of labor, helps to ease delivery.	Sharma, 2012a; Sharma, 2012b
4.	Dermatological disorders	Piṛṛvā ca kākamācī.....kuṛṛhanul-lepanaḥ- external application of a paste prepared from <i>S. nigrum</i> leaves, useful in leprosy; Indrāṛīśākaḥ kākāhvāḥ.....Pṛthagālepanaḥ kuryād dvadvaśaḥ sarvaśo'pi vā.Pradehāḥ sarva evaite deyāḥ svalpaghṛtāplutāḥ- topical application of a paste prepared from <i>S. nigrum</i> leaves, <i>Albizia lebbek</i> (Indian siris) flower and <i>Vitex negundo</i> (Five leaved-chaste tree) leaf, mixed with little <i>Gh'ita</i> (Clarified butter), beneficial in erysipelas; Svarasena kākamācyāḥ piṛṛā śuṛṛhī jayet koṛhān- application of a paste prepared after rubbing <i>Zingiber officinale</i> (Dry ginger root) with <i>S. nigrum</i> juice, useful in treating erysipelas, urticaria, and other diseases; Svarasena kākamācyāḥ piṛṛā śuṛṛhī jayet koṛhān.Munitarudalarasapiṛṛā sā ca karīṛasya vā svarase- application of a paste prepared from <i>Z. officinale</i> pounded with <i>S. nigrum</i> juice or <i>Z. officinale</i> pounded with <i>Rakta Vaka V'ik'a</i> , used against urticaria.	Śāstrī and Chaturvedi, 2011b; Śāstrī and Chaturvedi, 2011c; Sharma, 2012c; Sharma, 2012d
5.	Generalized body disorders	savāyasīmūlakavetranimbam. Śākārthināḥ śākamiti praśastam.Bhojye purāṛaśca yavaḥ saśāliḥ- vegetable prepared from <i>S. nigrum</i> , useful for inflammation.	Śāstrī and Chaturvedi, 2011d

S. No.	Diseases/ Conditions	Mode of Administration/Uses	References
6.	Rejuvenation and Virilification	Guṛena pippalībhīrvā maricenānvitā śṛtā. Kākamācī bhaved balyā tadrāsena haviṛ śṛtam - a decoction prepared from <i>S. nigrum</i> whole plant along with jaggery, <i>Piper longum</i> (Long pepper) and <i>P. nigrum</i> (Black pepper) or regular intake of <i>Ghṛta</i> (Clarified butter) processed from <i>S. nigrum</i> juice used to attain the rejuvenating properties.	Sharma, 2012e
7.	Alexipharmic treatment	Kākādanīkākamācyo svaraseṛvathavā kṛtam - <i>Ghṛta</i> (Clarified butter) processed with the juice of <i>Kākādanī</i> and <i>S. nigrum</i> , used to alleviate the toxic effects caused due to rat poisoning.	Chunekar and Yadava, 2005

Therapeutic Potential

In traditional Indian medicine, *S. nigrum* plays a significant role. Infusions are used to treat fever, diarrhea, and stomach issues. The plant's juice is applied topically to treat ulcers and other skin conditions. In addition to being used to cure asthma and "excessive thirst," the fruits are used as tonic, laxative, and appetite stimulants. The plant has historically been used to cure tuberculosis. In Telangana, it is called Peddakashapandlakoora. The leaves of this plant are used to treat mouth ulcers that happen during winter periods. It is known as 'manathakkalikeerai' in Tamil Nadu and 'kagesoppu' in Karnataka, and apart from its use as a home remedy for mouth ulcers, it is used in cooking like spinach. The boiled extracts of leaves and berries are also used to alleviate liver-related ailments, including jaundice. The juice from its roots is used to

treat whooping cough and asthma in Assam (Baghbahadorani, 2016; Miraj, 2016a). *S. nigrum* is a commonly used plant in Oriental medicine, where its benefits include hepatoprotection, antipyretic, antioxidant, anti-tumorigenic, and anti-inflammatory properties (Miraj, 2016b). In addition to the other parts of the entire plant, the berries and leaves are the principal portions used for medicinal purposes. The leaves are said to cause diaphoresis and are used as a poultice for rheumatoid and gouty joints, skin conditions, and anti-tubercular medication. Additionally, leaves are utilized for neurological problems, nausea, and dropsy. For cough, the berry and flower infusion are beneficial. These are diuretics and a treatment for pulmonary tuberculosis and bronchitis. The berry juice is used to treat hydrophobia and ophthalmopathy. The

berries are utilized for their diuretic, cathartic, and tonic effects. Additionally, they help with skin disorders and inflammations. The roots are beneficial for hepatitis, osteopathy, ophthalmopathy, and rhinopathy. The entire plant is used for swelling, coughing, asthma, diuretic, expectorant, cardiogenic, digestive,

laxative, diaphoretic, sedative, and antiseptic purposes. Furthermore, the herb is useful in treating nephropathy, ophthalmopathy, hemorrhoids, cardiopathy, leprosy, dropsy, and general debility as mentioned in Table 2 (Nadkarni and Nadkarni 1976; Akhtar, 1992).

Table 2 Medicinal importance along with their preparation and mode of administration *Solanum nigrum* L.

S. No.	Parts Used	Preparations	Disease treated and Therapeutic Use	References
1.	Whole plant	-	Ringworm, inflammation, joint pain, constipation, earache, bleeding piles, paralysis, insanity, diarrhea, urinary problems, liver disorders, dysentery, fever, pyorrhea, diuretic expectorant, analgesic, sedative, diaphoretic, skin diseases, burns, itching, pain, cough	Boulos, 1983; Muthu et al., 2006; Khan et al., 2009; Leporatti and Ghedira 2009; Saleem et al., 2009; Gaur and Sharma, 2011; Bargali et al., 2013; Singh and Attri, 2014; Padhan and Panda et al., 2015; Kumari et al., 2016; Patra et al., 2017; Sharma et al., 2017; Ningombam and Setty, 2023
		Decoction	Anti-spasmodic, cough, dysentery, jaundice, rheumatism, goiter, piles, urine complications, skin diseases, burns	
		Juice	Jaundice	
		Sap	Erysipelas	
		Extract	Liver cirrhosis	
2.	Shoots	Paste	Skin diseases	Samanta, 2019
3.	Leaves, Berries	-	Stomachache, fever	Ningombam and Setty, 2023
4.	Berries	-	Pneumonia, toothache, tumour	Ningombam and Setty, 2023
5.	Whole plant, Fruits	Decoction and extract	Diabetes	Begum et al., 2018

S. No.	Parts Used	Preparations	Disease treated and Therapeutic Use	References
6.	Roots and Leaves	Decoction	Foot infection	Puri et al., 2019
7.	Fruits	Mixed with water	Fever, indigestion	Boulos, 1983; Parinitha et al., 2004; Moshi, 2009; Phondani et al., 2009; Gaur and Sharma, 2011; Mehra et al., 2014; Rawat, 2016; Singh et al., 2017; Alamgeer et al., 2018; Das, 2018; Rahman et al., 2018; Wagh, 2020
		-	Diarrhea, leprosy, piles, eye diseases, dysentery, fever, nocturnal enuresis	
		Poultice	Blisters	
		Extract	Liver problems, piles, dysentery, eye ailments	
		Juice	Pimples, corrosive ulcers, suppurating syphilitic ulcers, pustules, ringworm, wound, eczema, leukoderma, gastritis, acidity	
		Diluted infusion of berries	Blindness, conjunctivitis, glaucoma, trachoma, cataract	
		Paste	Headache	
		Paste made with mango seeds	Ulcers	

S. No.	Parts Used	Preparations	Disease treated and Therapeutic Use	References
8.	Leaves	Juice	Cuts and boils, ringworm, cuts, wound, mouth ulcers, ear pain, fever, skin disease	Ignaci-muthu, 2006; Moshi, 2009; Ramya and Jayakumararaj, 2009; Hills, 2010; Bartwal et al., 2011; Singh et al., 2013; Bhatia et al., 2014; Chauhan et al., 2014; Pande and Joshi, 2015; Panda et al., 2016; Nyeem et al., 2017; Singh et al., 2017; Singh et al., 2017; Agarwal, 2019; Chauhan et al., 2021; Ru-pam and Singh, 2022; Kumar and Singh, 2023
		-	Fever, eye troubles, rabies	
		Pounded	Ringworm, warts	
		Cooked with onion bulbs and cumin seeds	Stomachache, gastric ulcer	
		Paste	Pimples, psoriasis and other skin diseases, rabies, wounds	
		Extract	Whooping cough, ear pain	
9	Roots	-	Intermittent fever, for easy delivery	Acharya et al., 2006; Pratap et al., 2015
		Decoction	Fever	
10.	Leaves and Branches	Paste	Jaundice, fever	Bisht, 2013
11.	Ripe fruits	-	Diuresis	Sahoo, 2020
12.	Roots, Leaves, and Fruits	-	Leukoderma	Uprety et al., 2016
13.	Leaves and Fruits		Skin diseases	Singh et al., 2014
14.	Stem	Extract	Diarrhea, fever	Chauhan et al., 2021
15.	Roots and Seeds	-	Liver-related problems	Mehra et al., 2014
16.	Fruits, Leaves, Whole plant	-	Boil, fever, goiter, piles, sores, sprain, stomachache, swelling, aphthous ulcer, urine disorders	Singh and Dubey, 2012

Ethnomedicinal and Folk Treatments along the Ganga Basin of India

India is one of the world's 12 mega biodiversity centres and is also home to many medicinal plants. About 65% of Indians get their medical care via the conventional system in their nation (Arya, 2014). Plant-based traditional medicinal systems serve almost 80% of people globally for their basic medical needs, and they are quite successful in healing diseases (Owolabi et al., 2007). The hilly state of Uttarakhand is adorned with verdant woods, evergreen rivers, and a varied terrain that spans from low-lying plains to high-altitude glaciers. It is one of the nation's biggest reservoirs of biodiversity due to the wide variances in the flora and fauna (Bartwal et al., 2011). The Himalayas stretch for over 2500 kilometres in a huge arc from west to east (Mehra et al., 2014). Because of its difficult topography, unpredictable climate, and delicious fruits that draw people in as a rich source of nutrition, Uttarakhand is one of the places where wild edible fruiting plants grow well (Meyers et al., 2003). The people of Uttarakhand employed a variety of edible and medicinal plants for a variety of purposes. According to information on ethnomedicinal plants in Uttarakhand, India, the indigenous population, particularly women and members of tribal communities, regularly uses the historically used medicinal herbs for health purposes. This is because, in comparison to contemporary medicine, these are more widely available, less expensive, and have no negative effects (Samy, 2000). *S. nigrum* is used by the

inhabitants of Nainital to cure skin conditions, chronic illnesses, diarrhea, eye conditions, and liver disorders (Saleem et al., 2009). Moreover, the Jaunsari tribes utilized the leaves of this plant, known as 'khalarkoi' or 'bhomolan', to treat fever, skin conditions, and acne (Bartwal et al., 2011). Additionally, the leaves and branches of this plant were employed as a paste to cure fever and jaundice in the Chamoli district (Bisht et al., 2013). Further, the people of the district Tehri Garhwal have used the edible fruits of *S. nigrum* as well as its stem extract to treat fever and diarrhea and leaves extract helps to relieve earache (Chauhan et al., 2021).

Likewise, the state of Uttar Pradesh is home to many tribes, making it somewhat unique. The traditional knowledge of plant species utilized for food, drink, colouring, gums, resins, and medicines is held by the tribal people. In addition to being passed down from generation to generation, this knowledge was crucial to the preservation and sustainable use of biodiversity. Additionally, they are aware of the *in-situ* conservation of various plant resources, such as sacred groves. Traditional medicinal techniques based on plants Around 25% of people worldwide still use traditional plant-based medical systems. According to estimates from the World Health Organization, more than 80% of people on the planet mostly use traditional medicine (Ali and Shankar, 2014). Historically, numerous societies have utilized ethnomedicinal plants

as a source of medicine. The existence of plants and plant-derived products having therapeutic qualities has been linked to the widespread usage of herbal remedies (Verma and Yadav, 2021). Numerous ethnobotanical and tribal communities employed a wide range of medicinal plants to treat a wide range of illnesses. They are used as tinctures, decoctions, concoctions, teas, and other medicinal formulations for treating a variety of ailments. *S. nigrum* leaves are utilized by the inhabitants and tribes of Uttar Pradesh to treat fever and eye problems. A leaf infusion is used as an anti-spasmodic. In Saharanpur, those who have whooping cough take leaf extract orally (Kumar and Singh, 2023). In a different study, people of Sonbhadra utilized a 50 ml decoction of the plant's leaves, known as 'makoi', to treat liver and skin conditions (Singh et al., 2012). Its root preparation is used to treat fever in Gorakhpur (Pratap et al., 2012). In the Bundelkhand region, patients with dropsy are given boiled leaves, tender shoots, and fresh plant extract, helps in curing liver cirrhosis (Unial et al., 2011). *S. nigrum* is also used to treat dropsy, gonorrhoea, piles, enlargement of liver and spleen, dysentery, stomach complaints, and fever in other parts of Uttar Pradesh, including Pilibhit, Purnapur Tehsil, Bisalpur Tehsil, Daulapur village, Kuanrpur village, Kajarbojhi village, Shahjahanpur, Mala, and Deoria forest (Pilibhit), Budaun, Bareilly, Etah, Aligarh, Hanthras, Firozabad, Mainpuri, Mathura Moradabad, Meerut, Bijnor, Rampur, Jyotiba Phule Nagar, Bagpat, Muzaffernagar, and Saharanpur. Further, its berries are beneficial for heart conditions and

vision issues. The leaves stimulate hunger and can be used to treat ringworm infections, urinary disorders, asthma, and several skin conditions (Prakash et al., 2017).

Approximately 70% of rural Bihar residents also rely on the traditional medicinal plants. Many healers and practitioners of traditional medicine make their formulations and administer them to patients (Ranjan et al., 2018). Situated in the subtropical portion of the temperate zone, the state is completely landlocked. Bihar has a transitional climate, economy, and culture because it is situated between the humid West Bengal in the east and the subhumid Uttar Pradesh in the west. These are similarly made up of reeds, grass, and shrubs (Singh and Prakash, 1996; Paranjape, 2001; Singh et al., 2013). The people and tribes of Bihar used a wide variety of medicinal plants to treat a wide range of illnesses. Numerous plants also use it as a vegetable. The locals utilized fruits to treat diarrhea and leaf paste to treat psoriasis and other skin conditions (Singh et al., 2013). To relieve headaches, they apply a paste of the whole plant to the forehead (Singh and Singh, 2015). It is also used to treat gout, rheumatism, and skin conditions. The herb's juice is administered for persistent liver enlargement. It can alleviate joint discomfort, age-related symptoms, as well as eye and ear ailments (Ranjan et al., 2018). They are also utilized against gonorrhoea, as sedatives, diuretics, and to treat skin conditions (Tiwari and Sourabh, 2012).

Furthermore, Jharkhand has a high biodiversity of therapeutic plants. It is a well-

known fact that Jharkhand has a richer and more varied flora than some other states. Out of Jharkhand's entire land area, around 40% is covered by forests. The local and tribal people are familiar with a variety of plants that have medicinal values. There are over thirty-two tribal communities in Jharkhand. These people, according to their traditional knowledge, frequently use therapeutic plants. In rural Jharkhand, traditional healers serve as the main sources of healthcare. An effort was undertaken to learn as much as possible about the several medicinal plants that the people of Jharkhand mostly use. The preservation of these natural resources is equally crucial. In addition to being used to treat a variety of illnesses, these medicinal plants are also planted or farmed as attractive plants, shade-giving plants, timber-yielding plants, construction plants, medicinal plants, vegetables, fruits, etc. in fields and residences. A variety of plant elements, such as entire plants, leaves, stems, roots, tubers, bark, flowers, fruits, and seeds are used by the locals (Anand, 2019). Jharkhand is home to a vast floral diversity as well as several tribal groups. In Jharkhand, scheduled tribes make up 26.30% of the state's total population. The Munda, Oraon, Kharia, Ho, and Santhal are the major tribes of Jharkhand. The Chhotanagpur Plateau and Santhal Parganas, which include the districts of Ranchi, Gumla, Lohardaga, Palamau, Hazaribagh, Singhbhum, Giridih, Dhanbad, Sahibganj, Godda, Pakud, Dumka, and Deoghar, are the primary locations for the tribes (Singh, 2008). Jharkhandis, sometimes refer *S. nigrum* as "makoi", and use this herb

to stop nose bleeding (Anand et al., 2014). Additionally, the people of Hazaribag use its berries as a home remedy for fever, diarrhea, and issues of eye pupils (Lal, 2012). Its leaves and fruit decoction are used by the rural residents in district Chatra as a veterinary medicine. It is used to treat eczema, skin conditions, and itching. It lessens the production of gas in the stomach. Despite complaints about its toxicity, it is grown as a food crop in America, Africa, and other parts of the world (Kumari et al., 2019). Another area of Jharkhand uses *S. nigrum* against nasal haemorrhage. 100 g of mustard oil is cooked with 25 g of dry fruit and after being filtered, the oil is applied to the forehead (Ghosh, 1971). Since the beginning of civilization, humans have relied mostly on plants for their food, shelter, and medical needs (Dubey et al., 2004). Herbal formulations constitute the foundation of the age-old traditional medical systems, such as Ayurveda, Unani, Siddha, and homeopathy, among others (Das and Bhakat, 2018). According to the most recent Indian census, which was conducted in 2011, West Bengal's population has increased from an estimated 87.8 million in 2009 to 91.3 million (Vineeta et al., 2018). A sizeable section of the state's population, particularly the indigenous people who live in surrounding forests, such as the Gonds, Kol, Santal, Oraon, Munda, Lodha, Mech, Bedia, Bhumij, and Mahali, are heavily dependent on plant resources for their primary healthcare (Vineeta et al., 2018). Yje natives of West Bengal use one significant medicinal herb *S. nigrum*, sometimes referred to as 'kakmachi', and used to treat a variety of

diseases. Skin diseases are treated using its young shoot paste (Samanta, 2019). Segments of the *S. nigrum* aerial portions are utilized as sedatives in Alipurduar, West Bengal (Das and Sen, 2022). This herb was utilized for wounds, jaundice, abdominal swellings, stomach pain, headaches, gonorrhoea, piles, dysentery,

boils, and eye infections among communities in Northern Bengal (Dar et al., 2011; Amjad et al., 2015; Singh and Dubey, 2015). Its roots are used as an antiseptic, anti-inflammatory, and for wound healing. It is also referred to as 'parbotatai penek' (Bose, 2011).

Conclusion and Future Perspectives

S. nigrum, a popular plant in Eastern medicine, has been proven to have a variety of properties, including antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic. This plant has several medicinal applications, including antiseptic, anti-inflammatory, expectorant, cardiotonic, digestive, diuretic, laxative, diaphoretic, sedative, swelling, cough, asthma, and the treatment of cardiopathy, leprosy, haemorrhoids, nephropathy, ophthalmopathy, dropsy, and general debility. It is also utilized

in ancient Ayurveda to cure a variety of diseases, as well as by various tribes and locals in different places. Considering the plant's helpful properties, it can be supported as a safe, very significant medicinal herb for the general public. It is a fascinating plant with the potential to produce a variety of treatments. Therefore, more research is needed to separate the active components from the *S. nigrum* extract to properly create drugs and carry out more clinical trials to address the health issues.

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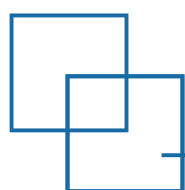
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CHAPTER
05

Phytochemical Analysis



INTRODUCTION

Solanum nigrum, also known as 'black nightshade', is a plant species of the Solanaceae family, native to Eurasia and introduced in the America, Australasia, and South Africa. It is a highly variable species with many varieties and forms described. It thrives in diverse climates and can be found in various habitats such as forests, fields, gardens, and even wastelands. Due to its adaptability, black nightshade has acclimatized in many regions while often considered a weed in agricultural settings (Edmonds and Chweya, 1997; Chen et al., 2022).

Traditionally, black nightshade has been used for various purposes, including stomach irritation, cramps, spasms, pain, and nervousness (Chen et al., 2022). In Oriental medicine, it is antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic. Some studies have shown that the plant inhibits the growth of cervical carcinoma in mice, and its active ingredient, solanine, inhibits the proliferation of different cancer cells *in-vitro*, such as breast cancer and pancreatic cancer (Kong et al., 2020). The plant contains a chemical called solanine, which is poisonous to humans, with the green fruits containing the highest amounts of solanine and being the most toxic parts. However, the fully ripened fruit and foliage of black nightshade are cooked and eaten in some areas. The steroidal alkaloids contained in *S. nigrum* are also the basis of its antitumor activity (Chen et al., 2022).

Sampling Sites

Table 1 Sampling sites for the *Solanum nigrum*, collected from various state-specific locations

S. No.	Sample registration code
1.	Bijnor, Uttar Pradesh
2.	Narora, Uttar Pradesh
3.	Badaun, Uttar Pradesh
4.	Farrukhabad, Uttar Pradesh



S. No.	Sample registration code
5.	Bithoor, Uttar Pradesh
6.	Dalmau, Uttar Pradesh
7.	Prayagraj, Uttar Pradesh
8.	Mirzapur, Uttar Pradesh
9.	Varanasi, Uttar Pradesh
10.	Ballia, Uttar Pradesh
11.	Revelganj, Bihar
12.	Patna, Bihar
13.	Barh, Bihar
14.	Farka, Bihar
15.	Farraka, West Bengal
16.	Gaumukh, Uttarakhand
17.	Gangotri, Uttarakhand

Phytochemical Analysis

Phytochemical components like tannin (by titration), total saponins (by gravimetry), total polyphenols, and total flavonoids (by UV-visible spectrophotometer), were determined for their respective contents via in-house protocols using API standards and literature, developed in Chemical Science Division, Drug Discovery and Development Department, Patanjali Research Foundation, Haridwar. Advanced methods and techniques were employed for the identification and quantification of secondary metabolites and active components within the collected samples. High-performance thin layer chromatography (HPTLC) served to detect marker compounds and provide a chromatographic fingerprint of the plant

sample. The hyphenated techniques have been proven as a powerful tool for the identification and structural characterization of known compounds. Hence, for further identification and quantification of compounds, high-performance liquid chromatography (HPLC) and ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC/MS-QToF) were employed.

Determination of Tannin Content

To begin the experiment, samples weighing 1-10 g were taken and mixed with 50 ml of milli-Q water. The mixture was shaken and

sonicated for 30 minutes. Then, the volume was adjusted to 100 ml and filtered. From this, 10 ml was taken and mixed with 750 ml of milli-Q water. Next, 25 ml of indigo sulphonic acid was added and shaken. The mixture was titrated with a 0.1N potassium permanganate solution until a golden yellow color appeared, indicating the end point. Finally, a blank test was conducted without any sample.

Determination of Saponin Content

A 5 g sample was mixed with 50 ml of a solvent consisting of a 1:1 ratio of methanol to water. The mixture was heated under reflux for 1 hour, then cooled and filtered. This process was repeated 3 times. The filtrate from each repetition was combined, concentrated, and evaporated until dry. Next, 25 ml of petroleum ether was added, and the mixture was refluxed for 10 minutes. After cooling, the ether layer was separated. Then, 10 ml of methanol and 100 ml of acetone were added to the remaining residue and the mixture was filtered. The filter paper with the residue was dried at 80°C for 1 hour and weighed.

Determination of Total Polyphenol Content

A 1 ml sample was taken in a test tube and 1 ml of Folin–Ciocâlteu reagent was added. The mixture was incubated for 5 minutes. Then, 1 ml of a 10% sodium carbonate solution was added. The test tube was kept in the dark for an hour. After that, the absorbance was measured at 760 nm using a UV-Visible spectrophotometer. The same steps were repeated for gallic acid to create a linear plot.

Determination of Total Flavonoid Content

A 1 ml sample was placed in a test tube. Then, 0.4 ml of 10% aluminum chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol were added to the sample. The mixture was left at room temperature for 30 minutes. After that, the absorbance was measured at 450 nm using a UV-Visible spectrophotometer. These steps were repeated for the quercetin dihydrate sample to create a linear plot.

HPTLC Fingerprinting

This technique has been recognized for its effectiveness in exploring the qualitative aspects of secondary metabolites in various plant species. As per WHO Technical Report Series, No. 1010, [2018], HPTLC chromatographic pattern, generally refer as “fingerprints”, are used for identification of phytochemicals. The band or spots obtained during the test are characteristics of particular

herb. A color image of typical TLC fingerprint provides a clearer guide to the users.

Sample Preparation

To analyze each batch, approximately 1 g of sample was dissolved in 10 ml of methanol. The samples were then shaken and sonicated for 20 minutes. Afterward, the solution was

centrifuged at 5000 rpm for 5 minutes. The resulting clear solution was used for analysis.

Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttenez, Switzerland), equipped with

Automatic TLC Sampler (ATS 4), TLC scanner 4 and TLC visualizer. Data processing acquisition and visualization were achieved using win-CATS software (version 1.4.10). The chromatographic conditions for the HPTLC analysis were as follows:

Stationary phase	TLC Silica gel 60 F ₂₅₄ aluminum sheet (1.0554.0007)
Mobile phase	Toluene: ethyl acetate: formic acid (5:5:1 v/v/v)
Saturation time	15 minutes
Migration distance	80 mm
Band length	8 mm
Injection volume	10 µL
Visualization	254 nm and 366 nm and under white without derivatization

High-Performance Liquid Chromatography (HPLC)

Sample and Standard preparation

Sample: About 500 mg of sample from each batch individually was dissolved in 10 ml of hydro methanol (20:80) which was further sonicated for 30 minutes, centrifuged for 5 min at 9000 rpm and filtered using 0.45 µm nylon filter. The filtered solution was used for analysis.

Standard: Pure scopoletin was dissolved in methanol to prepare a solution having a concentration of 50 ppm.

Analytical and Instrumentation Conditions

Separation was achieved using a Shimadzu C8 (3.0×100 mm, 3 µm,) column, subjected to binary gradient elution. The two solvents used for the analysis consisted of water containing 0.1% orthophosphoric acid in water with adjusted pH value (2.5) by diethyl amine (solvent A) and acetonitrile (solvent B). The flow rate was set 0.5 ml/min during the analysis. Five microliters of standard and test solution were injected. Wavelength was set at 325 nm.

Gradient program used

Time (Min)	A%	B%
0	98	02
4	98	02
8	90	10
15	85	15
20	85	15
25	80	10
35	65	35
45	05	95
46	98	02
50	98	02

Identification of Compounds by Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (UPLC/MS-QToF)

The objective of this analysis was to identify the major compounds in *Solanum nigrum* test sample [PRF/CHI/1123/1202].

Sample Preparation

A 500 mg of the powdered sample was dissolved in 5 ml methanol and sonicated for 30 min. The solution was centrifuged at 10000 rpm for 5 minutes and filtered through 0.22 µm nylon filter.

Analytical and Instrumentation Conditions

Analysis was performed on a Xevo G2-XS QToF (Waters Corporation, USA) with

Acquity UPLC- I Class and Unifi software. Separation was carried out using Acquity UPLC HSS-T3 (100 × 2.1 mm, 1.8 µm) column (China) with the flow rate of 0.3 ml/min using gradient elution of 0.1% v/v formic acid in water (mobile phase A) and 0.1% v/v formic acid in acetonitrile (mobile phase B). The column was maintained at 35 °C throughout the analysis and sample temperature was kept at 20°C. Detection was carried out by Xevo G2-XS QToF. One microliter of test solution was injected in UPLC/MS-QToF and chromatograms were recorded in positive and negative ionization mode.

Gradient program

Time (min)	Flow (ml/min)	Mobile phase A%	Mobile phase B%
0	0.3	95	5
2	0.3	95	5
10	0.3	85	15
40	0.3	65	35
50	0.3	40	60
55	0.3	20	80
60	0.3	20	80
61	0.3	95	5
65	0.3	95	5

Xevo G2-XS QToF Parameters

Parameter	Polarity (+ve)	Polarity (-ve)
Ionisation type	ESI	ESI
Mode	MS ^E	MS ^E
Mass range (m/z)	50-1200 m/z	50-1200 m/z
Scan time	0.5 s	0.5 s
Cone Voltage	40 V	40 V
Capillary	1.0 kV	2.0 kV
Low CE	6.0 eV	6.0 eV
High CE	15-60 eV(ramp)	15-60 eV(ramp)
Source temperature	120 °C	120 °C
Desolvation Temperature	500 °C	500 °C
Cone gas flow	50 L/h	50 L/h
Desolvation gas flow	900 L/h	900 L/h
Lock Spray (Leucine Enkaphalin)	556.2766 m/z	554.2620 m/z
Lock mass scan time	0.5 s	0.5 s
Lock mass interval	30 s	30 s

Results and Discussion

Phytochemical Analysis

The quantitative phytochemical analysis of samples collected from different locations revealed the presence of tannins, saponins, total phenolics, and total flavonoids in varying concentrations (Table 2). The tannin content ranged from 0.019 to 0.061% w/w, saponin

content varied from 2.725 to 15.020% w/w, total polyphenol content was between 0.002 to 0.016% w/w, and total flavonoid content was consistently less than 0.001% w/w across all samples. The variations in phytochemical content among the samples indicate potential environmental and genetic influences on the plant's phytochemical profile.

Table 2 Phytochemical analysis of *Solanum nigrum* collected from different locations

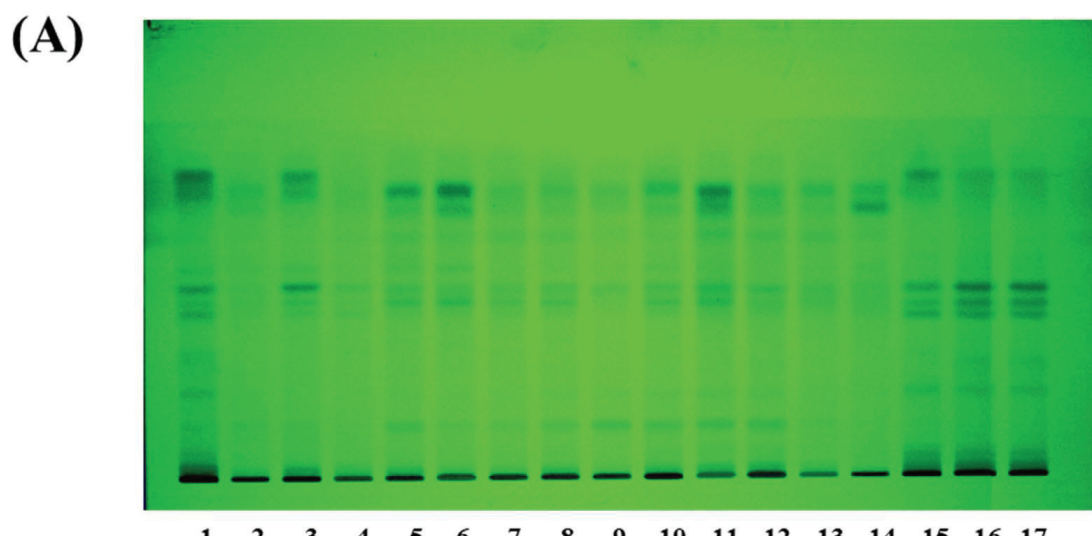
S. No.	Samples	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Bijnor, Uttar Pradesh	0.019	15.020	0.007	<0.001
2.	Narora, Uttar Pradesh	0.020	5.870	0.003	<0.001
3.	Badaun, Uttar Pradesh	0.020	12.580	0.002	<0.001
4.	Farrukhabad, Uttar Pradesh	0.038	8.445	0.003	<0.001
5.	Bithoor, Uttar Pradesh	0.020	5.080	0.006	<0.001
6.	Dalmau, Uttar Pradesh	0.039	8.259	0.003	<0.001
7.	Prayagraj, Uttar Pradesh	0.039	4.237	0.002	<0.001
8.	Mirzapur, Uttar Pradesh	0.040	2.725	0.003	<0.001
9.	Varanasi, Uttar Pradesh	0.059	9.120	0.004	<0.001
10.	Ballia, Uttar Pradesh	0.059	8.377	0.002	<0.001
11.	Revelganj, Bihar	0.060	9.550	0.004	<0.001

S. No.	Samples	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
12.	Patna, Bihar	0.058	6.528	0.016	<0.001
13.	Barh, Bihar	0.061	9.066	0.003	<0.001
14.	Farka, Bihar	0.057	5.784	0.004	<0.001
15.	Farraka, West Bengal	0.040	3.527	0.005	<0.001
16.	Gaumukh, Uttarakhand	0.040	11.893	0.007	<0.001
17.	Gangotri, Uttarakhand	0.056	13.340	0.005	<0.001

HPTLC Fingerprint Analysis

The HPTLC analysis of *S. nigrum* methanolic extracts showed distinct chromatographic patterns under multiwavelength detection (254 nm and 366 nm UV light). The presence of high-intensity bands across all samples suggests a

rich diversity of chemical compounds. These results provide a preliminary indication of the complex phytochemical composition of the plant and underline the importance of further detailed analyses to identify the specific compounds responsible for the observed bands (Fig. 1).



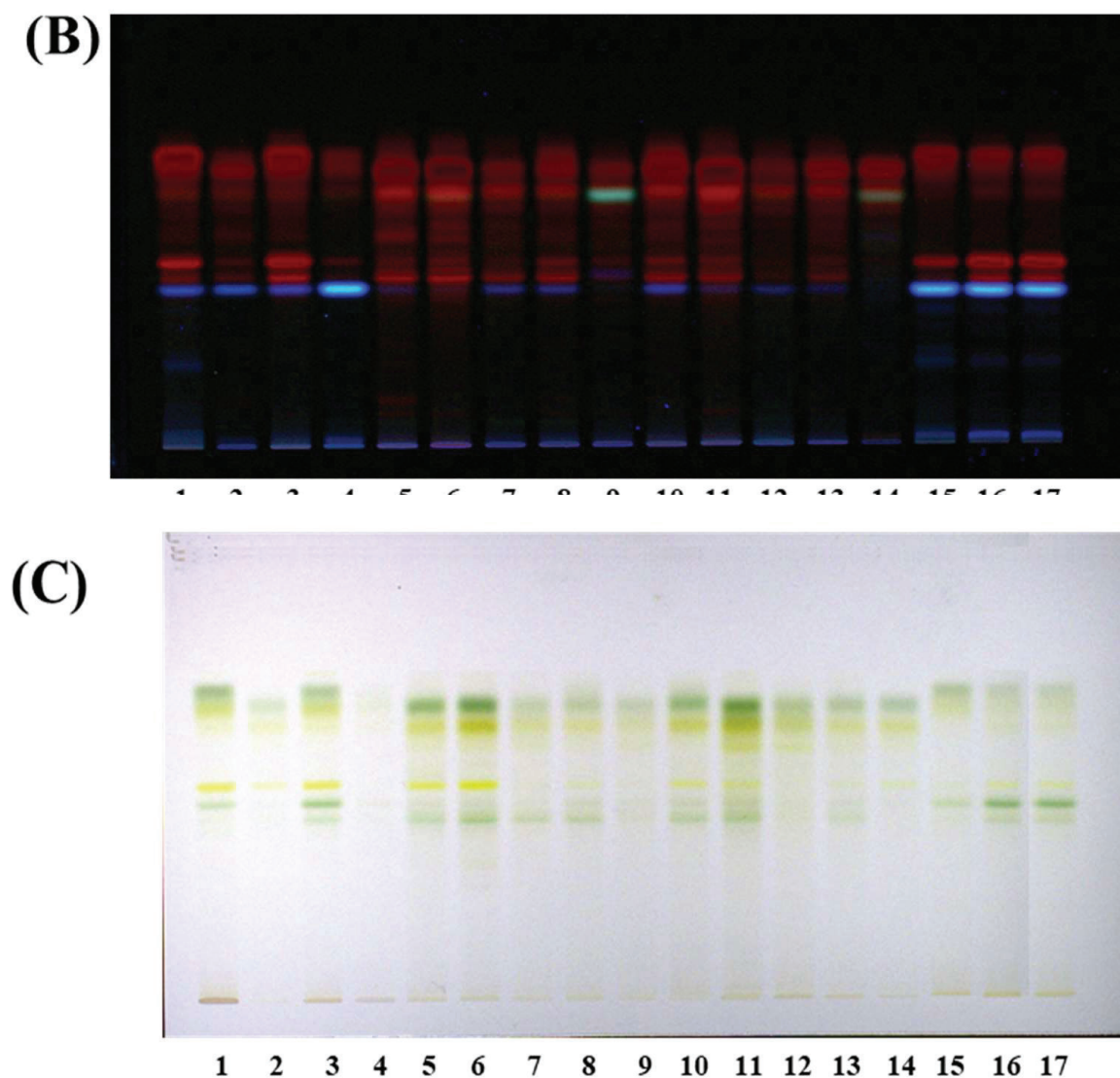


Fig. 1 HPTLC fingerprinting of methanolic extract of *Solanum nigrum*.
A. 254 nm, **B.** 366 nm and **C.** Under white light without derivatization

The HPTLC fingerprint patterns observed suggest a high degree of chemical diversity, which is consistent with the plant's reported pharmacological activities. The presence of multiple high-intensity bands indicates the presence of bioactive compounds that may contribute to the therapeutic effects of the plant. The qualitative data from HPTLC, combined with the quantitative insights from HPLC and UPLC/MS-QToF, provide a

robust framework for understanding the phytochemical basis of *Solanum nigrum*'s bioactivity.

HPLC Analysis

HPLC analysis using scopoletin as the reference compound provided quantitative data on the presence of this specific compound across samples (Table 3). The chromatograms

displayed characteristic peaks corresponding to scopoletin, indicating its presence in varying amounts. This analysis not only confirms the presence of scopoletin but also highlights the differences in its concentration

across different geographical locations, which could be attributed to environmental factors affecting the biosynthesis of secondary metabolites in the plant (Fig 2, 3).

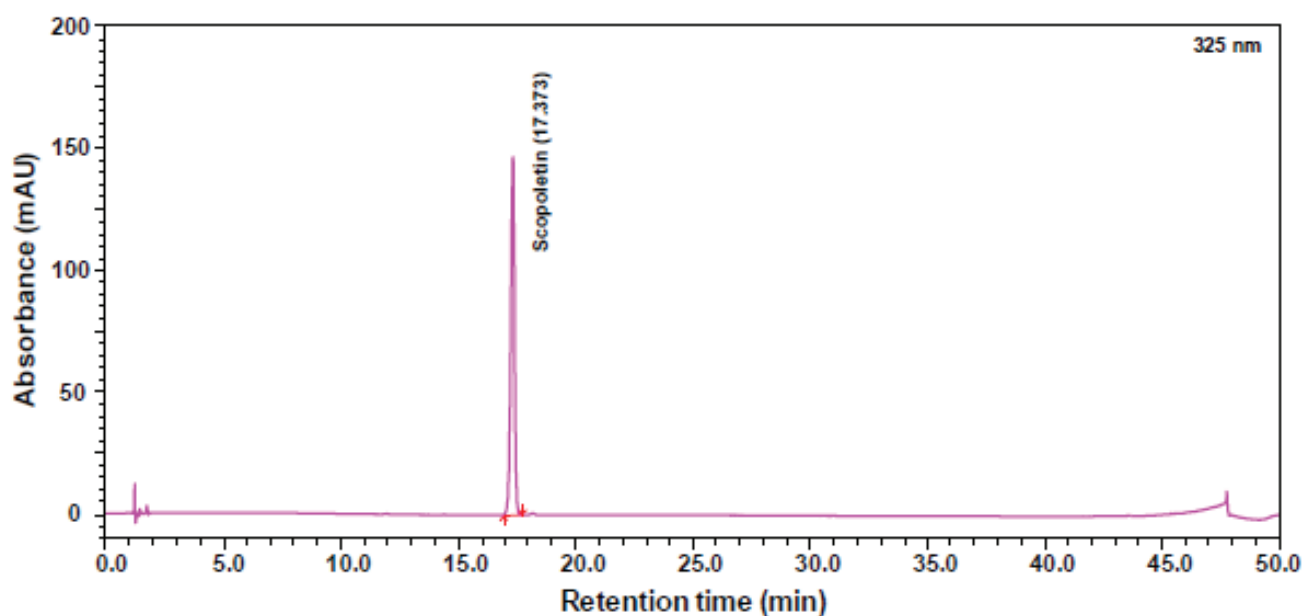


Fig. 2 HPLC chromatogram of reference standard Scopoletin at 325 nm

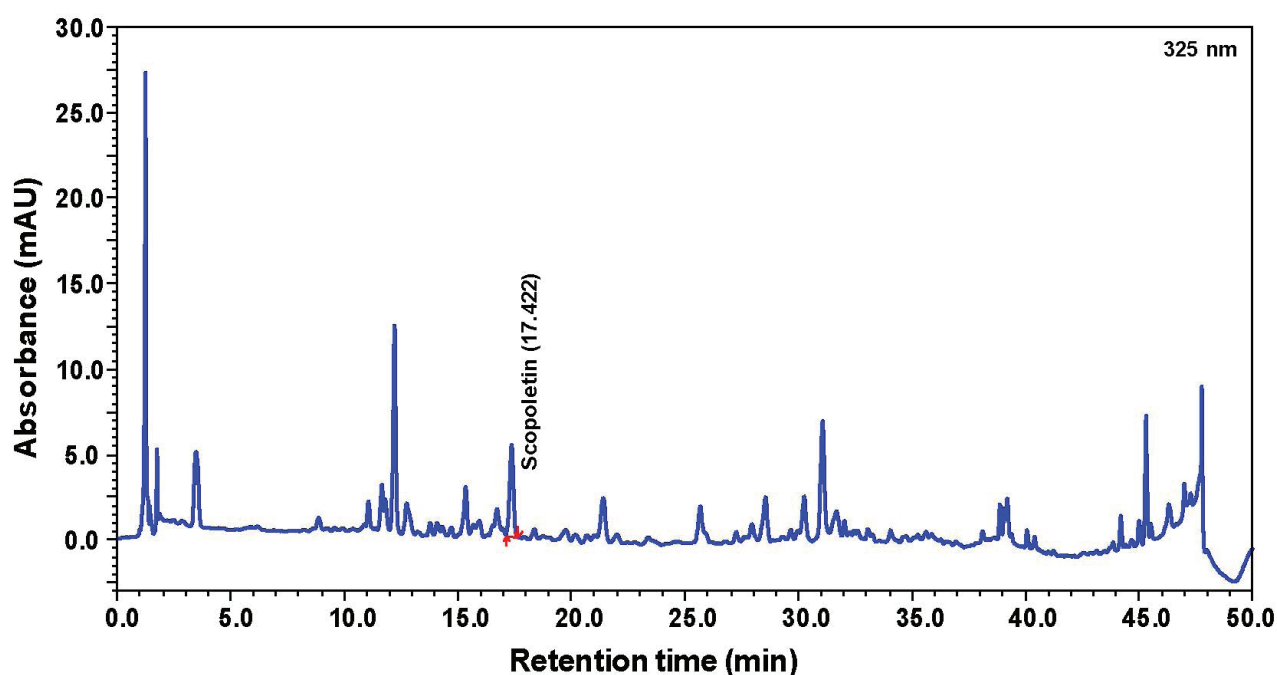


Fig. 3 HPLC chromatogram of *Solanum nigrum* (PRF/CHI/1123/1203) at 325 nm

Table 3 Phytochemical content in *Solanum nigrum* collected from different locations

S. No	Internal code	Samples	Scopoletin (µg/mg)
1	PRF/CHI/1123/1202	Bijnor, Uttar Pradesh	0.034
2	PRF/CHI/1223/1242	Narora, Uttar Pradesh	0.013
3	PRF/CHI/1123/1203	Badaun, Uttar Pradesh	0.039
4	PRF/CHI/1123/1204	Farrukhabad, Uttar Pradesh	0.011
5	PRF/CHI/1123/1205	Bithoor, Uttar Pradesh	0.019
6	PRF/CHI/1123/1206	Dalmau, Uttar Pradesh	ND
7	PRF/CHI/1123/1207	Prayagraj, Uttar Pradesh	ND
8	PRF/CHI/1123/1208	Mirzapur, Uttar Pradesh	ND
9	PRF/CHI/1123/1209	Varanasi, Uttar Pradesh	0.011
10	PRF/CHI/1123/1210	Ballia, Uttar Pradesh	ND
11	PRF/CHI/1123/1211	Revelganj, Bihar	0.010
12	PRF/CHI/1123/1212	Patna, Bihar	ND
13	PRF/CHI/1123/1213	Barh, Bihar	0.010
14	PRF/CHI/1123/1214	Farka, Bihar	ND
15	PRF/CHI/1123/1215	Farraka, West Bengal	ND
16	PRF/CHI/1123/1216	Gaumukh, Uttarakhand	ND
17	PRF/CHI/1123/1217	Gangotri, Uttarakhand	0.071

Scopoletin is a well-known coumarin frequently identified in various traditional medicinal plants for its anti-inflammatory, antimicrobial, and antioxidant properties. The varying concentrations of scopoletin across different samples of *S. nigrum* highlight the influence of geographical and environmental factors on phytochemical synthesis. This variation could have implications for the standardization and quality control of *S. nigrum*-based herbal products.

UPLC/MS-QToF Analysis

UPLC/MS-QToF analysis was employed to identify the major compounds present

in *Solanum nigrum* samples. The high-resolution separation achieved in this method allowed for the identification of 27 compounds, including phenylalanine, tryptophan, chlorogenic acid, quercetin derivatives, and several steroidal glycosides like dihydroxysola sodinesolatrioside and solaradixine. The accurate mass measurements and retention times provided a comprehensive profile of the phytochemicals present, demonstrating the utility of this technique in unravelling the complex chemical composition of plant extracts.

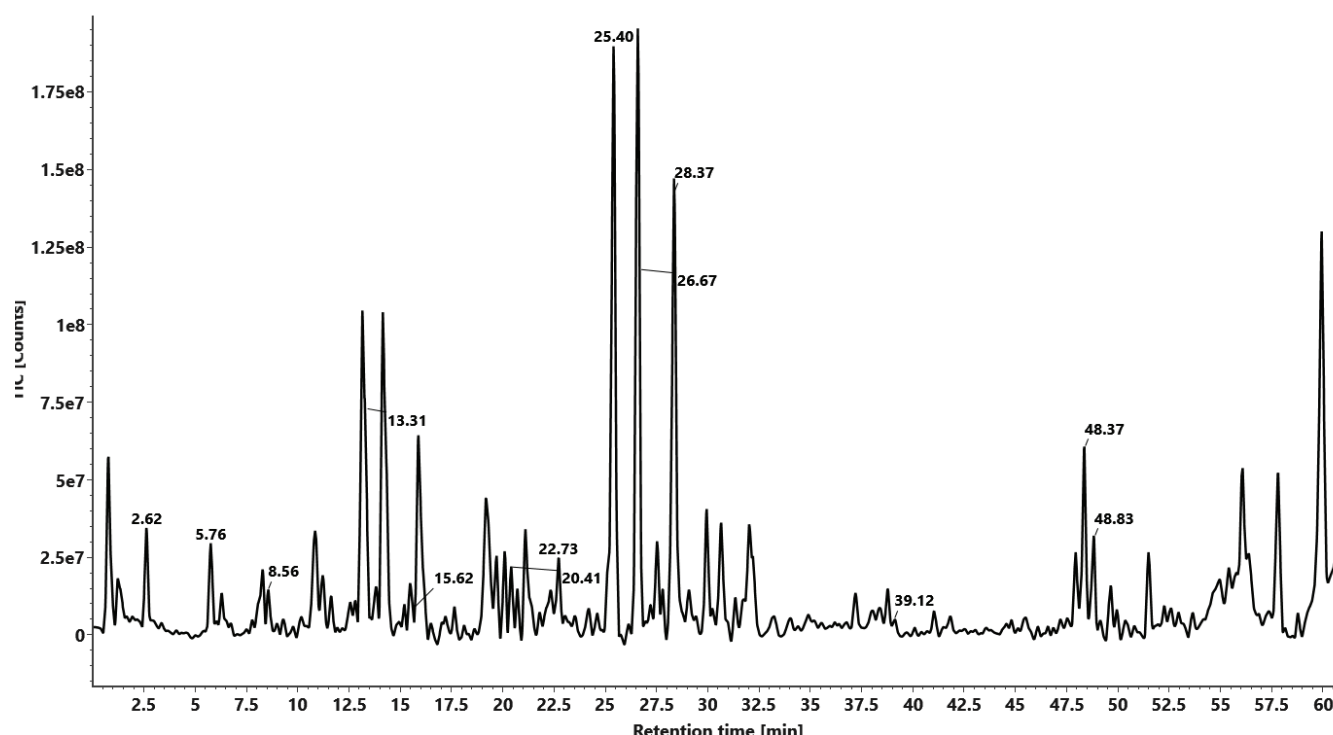


Fig. 4 TIC chromatogram of *Solanum nigrum* (PRF/CHI/1123/1202) in positive ionization mode

Table 4 Compound-dependent parameters of analytes/identified compounds in *Solanum nigrum* (PRF/CHI/1123/1202) in positive ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observe m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Phenylalanine	$C_9H_{11}NO_2$	165.0790	166.0847	-1.5	2.62	514018	+H
2	Tryptophan	$C_{11}H_{12}N_2O_2$	204.0899	205.0957	-1.4	5.76	377691	+H
3	Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	355.1007	-1.7	8.56	369506	+H
4	Quercetin 3-O-rutinoside-7-O-glucoside	$C_{33}H_{40}O_{21}$	772.2062	773.2175	4.0	11.13	238406	+H
5	Quercetin 3-glucosyl-(1-6)-galactoside	$C_{27}H_{30}O_{17}$	626.1483	627.1560	0.4	12.83	800877	+H
6	Dihydroxysolasodine solatrioside	$C_{45}H_{73}NO_{18}$	915.4828	916.5004	10.4	13.31	9976017	+H
7	N-cis-Feruloyloctopamine	$C_{18}H_{19}NO_5$	329.1263	352.1146	-0.9	15.62	45678	+Na
8	Solaradixine	$C_{51}H_{83}NO_{21}$	1045.5458	1046.5653	12.2	18.60	337035	+H

S. N.	Component name	Formula	Neutral mass (Da)	Observe m/z	Mass error (mDa)	RT (min)	Response	Adducts
9	α -Solamarine	$C_{45}H_{73}NO_{16}$	883.4929	884.5087	8.5	20.41	3216927	+H
10	Solaverine I	$C_{45}H_{73}NO_{16}$	883.4929	884.5082	8.0	20.74	2187857	+H
11	Isorhamnetin-3-O- β -rutinoside	$C_{28}H_{32}O_{16}$	624.1690	647.1607	2.5	21.31	702380	+Na
12	N-trans-Coumaroyltaramine	$C_{17}H_{17}NO_3$	283.1208	284.1267	-1.4	21.45	772748	+H
13	N-trans-Ferulytyramine	$C_{18}H_{19}NO_4$	313.1314	314.1372	-1.5	22.73	1337030	+H
14	Solasonin	$C_{45}H_{73}NO_{16}$	883.4929	884.5074	7.2	25.40	21165092	+H
15	Solamargine	$C_{45}H_{73}NO_{15}$	867.4980	868.5165	11.2	26.67	24371302	+H
16	Tigonin	$C_{56}H_{92}O_{27}$	1196.5826	1197.6114	21.5	28.37	16090373	+H
17	Solasodine	$C_{27}H_{43}NO_2$	413.3294	414.3356	-1.1	39.12	914993	+H
18	Degalactotigonin	$C_{50}H_{82}O_{22}$	1034.5298	1057.5338	14.8	48.37	4510519	+Na
19	Nigrumnin I	$C_{55}H_{90}O_{25}$	1150.5771	1173.5866	20.3	48.83	1407105	+Na

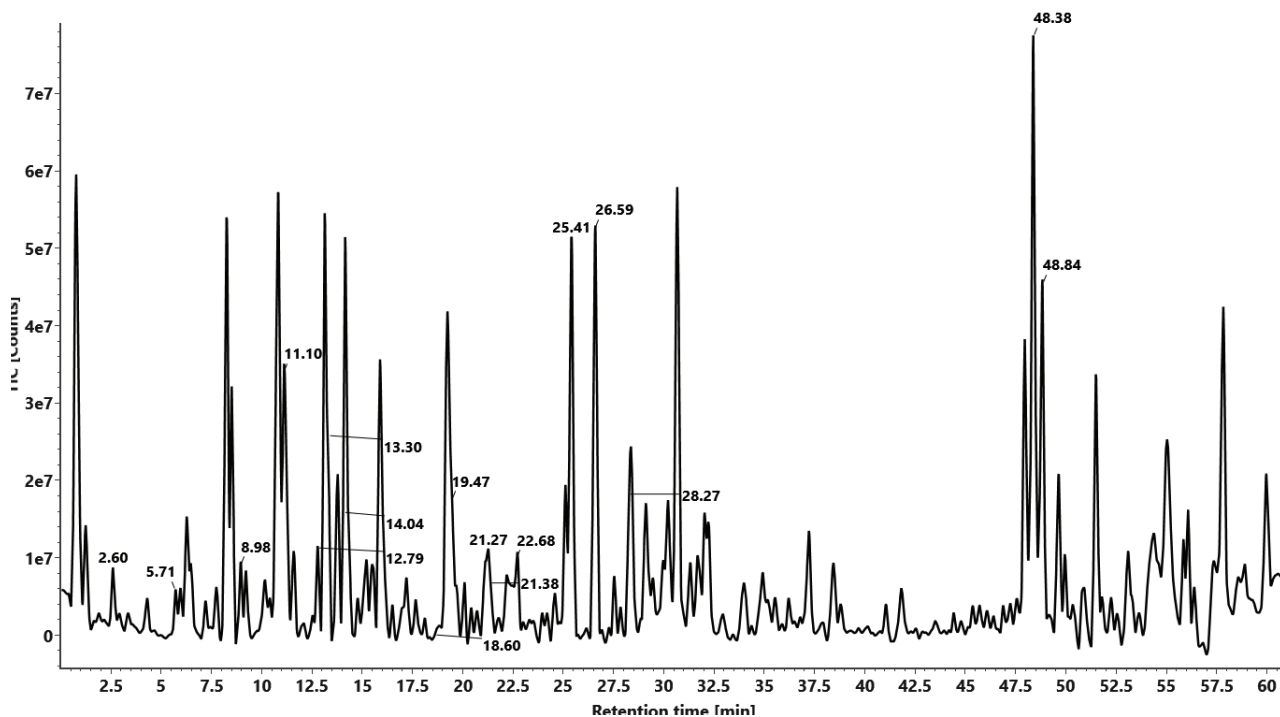


Fig. 5 TIC chromatogram of *Solanum nigrum* (PRF/CHI/1123/1202) in negative ionization mode

Table 5 Compound-dependent parameters of analytes/identified compounds in *Solanum nigrum* (PRF/CHI/1123/1202) in negative ionization mode

S.N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Phenylalanine	C ₉ H ₁₁ NO ₂	165.0790	164.0716	-0.1	2.60	1194404	-H
2	Tryptophan	C ₁₁ H ₁₂ N ₂ O ₂	204.0899	203.0827	0.1	5.71	994213	-H
3	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.0951	353.0878	0.0	8.98	1804340	-H
4	Caffeic acid	C ₉ H ₈ O ₄	180.0423	179.0351	0.1	9.21	2023205	-H
5	Quercetin 3-O-rutinoside-7-O-glucoside	C ₃₃ H ₄₀ O ₂₁	772.2062	771.1989	0.0	11.10	946754	-H
6	Quercetin 3-glucosyl-(1-6)-galactoside	C ₂₇ H ₃₀ O ₁₇	626.1483	625.1411	0.1	12.79	5265555	-H
7	Dihydroxysolasodinesolatrioside	C ₄₅ H ₇₃ NO ₁₈	915.4828	960.4825	1.5	13.30	7663111	+HCOO
8	Scopoletin	C ₁₀ H ₈ O ₄	192.0423	237.0403	-0.2	13.42	3777	+HCOO
9	Quercetin 3-rhamnosyl-(1-2)-galactoside	C ₂₇ H ₃₀ O ₁₆	610.1534	609.1472	1.1	14.04	1256267	-H
10	Sinapic acid	C ₁₁ H ₁₂ O ₅	224.0685	223.0613	0.1	15.11	1471106	-H
11	N-cis-Feruloyltopamine	C ₁₈ H ₁₉ NO ₅	329.1263	328.1192	0.1	15.56	947275	-H
12	Isochlorogenic acid B	C ₂₅ H ₂₄ O ₁₂	516.1268	515.1191	-0.4	16.97	483928	-H
13	Isochlorogenic acid A	C ₂₅ H ₂₄ O ₁₂	516.1268	515.1198	0.3	17.66	725041	-H
14	Solaradixine	C ₅₁ H ₈₃ NO ₂₁	1045.5458	1090.5448	0.8	18.60	163556	+HCOO

S.N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
15	Isochlorogenic acid C	C ₂₅ H ₂₄ O ₁₂	516.1268	515.1195	0.0	19.47	4280012	-H
16	α-Solamarine	C ₄₅ H ₇₃ NO ₁₆	883.4929	928.4917	0.6	20.42	1442240	+HCOO
17	Solaverine I	C ₄₅ H ₇₃ NO ₁₆	883.4929	928.4919	0.7	20.74	888500	+HCOO
18	Isorhamnetin-3-O-β-rutinoside	C ₂₈ H ₃₂ O ₁₆	624.1690	623.1620	0.2	21.27	3736160	-H
19	N-trans-Coumaroyltaramine	C ₁₇ H ₁₇ NO ₃	283.1208	282.1135	-0.1	21.38	797193	-H
20	N-trans-Ferulytyramine	C ₁₈ H ₁₉ NO ₄	313.1314	312.1239	-0.2	22.68	1262408	-H
21	Solasonin	C ₄₅ H ₇₃ NO ₁₆	883.4929	928.4921	1.0	25.41	18503424	+HCOO
22	Solamargine	C ₄₅ H ₇₃ NO ₁₅	867.4980	912.4976	1.4	26.59	18375278	+HCOO
23	Solanigroside C	C ₅₁ H ₈₂ O ₂₆	1110.5094	1155.5120	4.3	28.27	1515607	+HCOO
24	Degalactotigonin	C ₅₀ H ₈₂ O ₂₂	1034.5298	1079.5303	2.3	48.38	22160444	+HCOO
25	Nigrumnin I	C ₅₅ H ₉₀ O ₂₅	1150.5771	1195.5793	3.9	48.84	13642534	+HCOO

The identification of a broad range of phytochemicals through UPLC/MS-QToF analysis underscores the multifaceted therapeutic potential of *S. nigrum*. Phenylalanine and tryptophan, identified in the analysis, are essential amino acids involved in various metabolic processes. Chlorogenic acid, known for its antioxidant properties, and quercetin derivatives, recognized for their

anti-inflammatory and anticancer activities, further substantiate the medicinal value of the species. The steroidal glycosides identified, including dihydroxysolasodinesolatrioside and solaradixine, are noteworthy for their potential therapeutic applications, particularly in the context of anti-inflammatory and anticancer effects [Chester et al., 2017].

Conclusion

The aim of our study was to investigate the presence and composition of secondary metabolites in *Solanum nigrum* plant samples collected from various locations along the Gangetic course. In line with our objectives, we found that the samples contained several phytochemicals including tannins, saponins, polyphenols, and flavonoids. The analysis revealed interesting patterns in the distribution of these compounds among the samples.

Firstly, the content of tannins and total polyphenols was consistently observed across all the samples, indicating a stable presence of these compounds throughout the study area. This suggests that tannins and polyphenols may play a significant role in the physiological and ecological functions of the plants growing along the Gangetic course. On the other hand, the content of saponin exhibited high variability among the samples, with the highest levels (>12% w/w) found in the samples collected from Bijnor and Badaun in Uttar Pradesh. This discrepancy in saponin content could be attributed to local environmental factors, genetic variations, or other factors influencing

the biosynthesis of saponins in these specific locations. Interestingly, the samples showed the lowest content of flavonoids (<0.001% w/w) compared to the other phytochemicals analysed. This finding suggests that the presence of flavonoids might be relatively scarce or less pronounced in the plants growing along the Gangetic course.

To gain a more comprehensive understanding of the phytochemical profile, we employed high-performance thin layer chromatography (HPTLC), which revealed a diverse range of phytochemicals in the samples. Furthermore, we utilized high-performance liquid chromatography (HPLC) to determine the exact content of the scopoletin compound in the samples. Consistent with our previous findings, the Badaun sample from Uttar Pradesh exhibited the highest concentration of scopoletin. This compound, known for its various biological activities, may contribute to the medicinal properties associated with the plants growing in this region. Lastly, the study also employed advanced hyphenated techniques, UPLC/MS-

QToF, for the identification and quantification of a total of 27 compounds. This advanced methodology allowed for a highly detailed and accurate characterization of the chemical constituents present in the species.

Overall, the comprehensive phytochemical profiling of *Solanum nigrum* using HPTLC, HPLC, and UPLC/MS-QToF analyses provides valuable insights into the plant's chemical composition and

potential therapeutic applications. The correlation of these findings with existing literature highlights the significance of these phytochemicals in contributing to the medicinal properties of *Solanum nigrum*. Further studies focusing on the bioactivity of these identified compounds could pave the way for the development of novel therapeutic agents derived from *S. nigrum*.

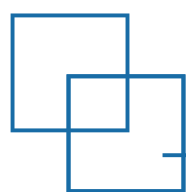
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CHAPTER

06

Insilico Analysis Against Nipah Virus Infection



INTRODUCTION

Nipah virus infection is a highly infectious and zoonotic disease, with fruit bats (Genus *Pteropus*) as its natural reservoir (Thomas et al., 2019). The causative agent behind the disease is Nipah virus (NiV) of family Paramyxoviridae and genus Henipavirus which was first discovered in a massive encephalitis outbreak in Malaysia in 1998 (Hassan et al., 2018). The virus most commonly manifests in the form of respiratory distress, encephalitis and occasionally, myocarditis. In some instances, patients suffered with drowsiness, lethargy, mental confusion and in the extreme episode, coma. In addition, some patients were confronted with late-onset encephalitis, almost after a year of initial infection (Liew et al., 2022). The virus has a high death rate in humans. Apart from that, the probability of transmission among humans and absence of a standard vaccine has prompted the World Health Organization (WHO) to recognize it as a global health problem and has placed it as priority in research and development activities (Skowron et al., 2022). Recent incidences of NiV encephalitis have been witnessed in Bangladesh, Malaysia, Singapore, Philippines, and India (Alam et al., 2022). At molecular level, the viral genome is an 18.2 kb negative sense single-stranded RNA which translates into six structural proteins nucleocapsid, phosphoprotein, matrix protein, fusion protein, glycoprotein and RNA polymerase. These structural proteins have a crucial role synthesis of viral RNA, adherence, membrane fusion and host cell invasion (Pillai et al., 2020).

The NiV glycoprotein, a type II membrane protein, mediates cellular/host attachments by interacting with host cell surface receptors, such as ephrin B2 or B3 (Kalbhor et al., 2021; Loomis et al., 2021). The complexes of NiV glycoprotein and human receptor cell surface proteins Ephrin-B2 (EFNB2) and Ephrin-B3 (EFNB3) have been extensively explored to develop antivirals and vaccines to hinder the process of fusion (Priyadarsinee et al., 2022). For the above-mentioned role of NiV glycoprotein in mediating the NiV infection, the present study aims to target the same for the development of natural, risk-free therapeutic options.

Solanum nigrum L., commonly known as 'Black Nightshade', is a member of the Solanaceae family distributed in Europe, Asia, North America, South America, Australia,



and Africa [Campisi et al., 2019]. The plant is utilised as a traditional medicine for pain, inflammation, fever, malaria, and enteric diseases and is known to possess antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic, antibacterial, antifungal, cytotoxic, anti-convulsant, and antiulcerogenic properties [Birru et al., 2022]. Additionally, *Solanum* species, including *S. nigrum*, are generally used against herpes virus (human herpes virus type 1: HHV-1) [Ngbolua et al., 2020], have shown in silico efficacy against hepatitis C virus genotype 1 RNA-directed RNA polymerase [Mboto et al., 2020], and have demonstrated efficacy against anti-hepatitis B virus [Parvez et al., 2022]. In Asian countries, its role has been documented for the treatment of tumors [Chen et al., 2022], and Ayurveda has mentioned the role of its leaves and berries for therapeutic purposes [Goel et al., 2022]. One study highlights the role of *S. nigrum* as a siddha medicine against urinary tract infections [Kumarasamy and Radhakrishnan, 2022]. Accumulating evidence suggests the potent anti-cancer potential of the herb against a plethora of cancers, including cervical cancer [Patel et al., 2009], breast cancer [Khan et al., 2016], endometrial cancer [Tai et al., 2012], and ovarian cancer [Wang et al., 2015]. The medicinal properties of the plant are attributed to the various key secondary metabolites, such as alkaloids, terpenoids, steroids, flavonoids, tannins, glycosides, and saponins, harbored by it [Sivaraj et al., 2020].

As a result of the previously mentioned problem, a comprehensive in silico study was conducted with targeted protein NiV glycoprotein associated with NiV infection. Furthermore, results have established a strong candidature named daidzin to be used as a drug-like molecule for preventing NiV infection. To elucidate their dynamic and thermodynamic properties and to support the molecular docking results, molecular dynamics simulation was performed on daidzin. Furthermore, to ensure daidzin's safety and efficacy in the treatment, its drug-likeness, physicochemical properties, and ADMET were investigated.

Etiology of Nipah Virus Infection

NiV has a tendency to spread in the geographical area of carrier bats, i.e., Bangladesh, Malaysia, India and Singapore and its transmission to animals has been attributed to loss in the habitat of bats (Bruno et al., 2022). The virus can also transmit to the human body via urine and saliva of carrier bats (Talukdar et al., 2023). In one Malaysian instance, pigs were found to be the most frequent source of human infections, while in Bangladesh ingestion of bat saliva contaminated date palm sap was the most responsible route. In addition, Bangladesh and India witnessed person-to-person transmission among healthcare workers (Kumar et al., 2019). Generally, elevation has a negative influence on NiV outbreak, whereas, it is positively influenced

by human-induced land changes and rainfall (Jagadesh et al., 2022).

Reported Therapeutic Uses

The herb has been reportedly employed for thousands of years to relieve blood stasis and detumescence, canker sores, skin eczema, urinary tract infections, bacterial dysentery, prostate, and chronic bronchitis, etc. (Chen et al., 2022). Further, the plant is a traditional remedy for joint and muscle pains, ulcers, fevers, eye infections, and is reported to possess laxative, diuretic, sedative, emollient, and tonic effects (Mohyuddin et al., 2022). In India, it is used to treat cardalgia and gripe, owing to its antibacterial and anti-dysenteric characteristics (Mandal et al., 2023). The broad-spectrum therapeutic uses of *S. nigrum* are detailed in Table 1.

Table 1 List of therapeutic uses of *S. nigrum*

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Aerial part, Root, Whole plant	Liver diseases	MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z; MESH:D008107, UMLS:C0023895, DOID:409, ICD-11:SA0Z	ISBN:9788172362089; ISBN:9770972795006, ISBN:9788172362089; ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172360481

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Flow- er, Root, Seeds	Contraceptive agents	MESH:D003270, UM- LS:C0009871, MESH:D003270, UMLS:C0009871, MESH:D003270, UMLS:C0009871	ISBN:9788172360481, ISBN:9770972795006, ISBN:9788172360481
Fruit, Leaves, Root, Whole plant	Diuretics	MESH:D004232, UMLS:C0012798, ICD-11:XM4D06; MESH:D004232, UMLS:C0012798, ICD-11:X- M4D06; MESH:D004232, UMLS:C0012798, ICD-11:X- M4D06; MESH:D004232, UM- LS:C0012798, ICD-11:XM4D06; MESH:D004232, UMLS:C0012798, ICD-11:XM4D06	ISBN:9770972795006; IS- BN:9770972795006, ISB- N:9788172360481; A Re- port On Medicinal Plants Of Kachchh (Gujarat), ISBN:9770972795006, ISBN:9788172361266, ISBN:9788172363130, Medicinal Plants Of Nag- pur And Wardha Forest Divisions (Maharash- tra); ISBN:9770972795006, ISBN:9788172360481; ISBN:9770972795006, ISBN:9788173717024
Leaves, Root, Seed	Abdominal pain	MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4; MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4; MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4; MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4	ISBN:9770972795006, ISBN:9789327275590; Contribution To The Medi- co-Botany Of East Godavari And West Godavari Dis- tricts Of Andhra Pradesh, ISBN:9789327275590; ISBN:9789327275590; Contribution To The Medi- co-Botany Of East Godavari And West Godavari Dis- tricts Of Andhra Pradesh, ISBN:9770972795006, ISBN:9788172360481

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Analgesics	MESH:D000700, UMLS:C0002771, ICD-11:XM49F7	ISBN:9770972795006
Leaves, Root, Seed	Anemia	MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z; MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z; MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z; MESH:D000740, UMLS:C0002871, DOID:2355, ICD-11:3A9Z	ISBN:9789327275590; ISBN:9770972795006, ISBN:9788172361266, ISBN:9789327275590, Me- dicinal Plants Of Nagpur And Wardha Forest Divi- sions (Maharashtra), The Ayurvedic Pharmacopoeia of India Part-1 Volume-9; ISBN:9789327275590; ISBN:9770972795006, ISBN:9788171360536, ISBN:9788173717024
Leaves	Antifungal agents	MESH:D000935, UMLS:C0003308, ICD-11:XM83G4	ISBN:9770972795006, ISBN:9788172362089
Leaves	Antihyperten- sive agents	MESH:D000959, UMLS:C0003364, ICD-11:XM2PT6	ISBN:9770972795006
Leaf, Root, Whole plant	Anti-in- flammatory agents	MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1; MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1; MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1; MESH:D000893, UMLS:C0003209, ICD-11:XM7XD1	ISBN:9788172360481; ISBN:9770972795006; ISBN:9788172360481; ISBN:9770972795006, ISBN:9788172360481, ISBN:9788173717024
Leaves	Antineoplas- tic agents	MESH:D000970, UMLS:C0003392; MESH:D000970, UMLS:C0003392	ISBN:9770972795006; ISBN:9788172360481

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaf, Root	Antirheumatic agents	MESH:D018501, UMLS:C0003191, ICD-11:XM95N2; MESH:D018501, UMLS:C0003191, ICD-11:XM95N2	ISBN:9770972795006, ISBN:9788172360481; ISBN:9770972795006
Leaf, Root	Appetite stimulants	MESH:D019167, UMLS:C0376447; MESH:D019167, UMLS:C0376447; MESH:D019167, UMLS:C0376447	ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172361266; ISBN:9770972795006
Leaf, Root, Seed	Arthralgia	MESH:D018771, UMLS:C0003862, ICD-11:ME82; MESH:D018771, UMLS:C0003862, ICD-11:ME82; MESH:D018771, UMLS:C0003862, ICD-11:ME82	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590
Leaf, Root	Arthritis	MESH:D001168, UMLS:C0003864, DOID:848, ICD-11:FA2Z; MESH:D001168, UMLS:C0003864, DOID:848, ICD-11:FA2Z	ISBN:9788172362089; ISBN:9770972795006
Leaf, Root, Seed	Asthma	MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23; MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23; MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23; MESH:D001249, UMLS:C0004096, DOID:2841, ICD-11:CA23	ISBN:9770972795006, ISBN:9789327275590; ISBN:9770972795006, ISBN:9788172361266, ISBN:9789327275590, Medicinal Plants Of Nagpur And Wardha Forest Divisions (Maharashtra); ISBN:9789327275590; ISBN:9770972795006

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root, Seed	Astringents	MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6; MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6; MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6; MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006
Leaves	Common cold	MESH:D003139, UMLS:C0009443, DOID:10459, ICD-11:CA00	ISBN:9770972795006
Root, Seed	Cystitis	MESH:D003556, UMLS:C0010692, DOID:1679, ICD-11:GC00; MESH:D003556, UMLS:C0010692, DOID:1679, ICD-11:GC00	ISBN:9789327275590; ISBN:9789327275590
Leaves	Digestive system diseases	MESH:D004066, UMLS:C0012242, ICD-11:DE2Z	ISBN:9770972795006
Leaves, Root, Seed	Dyspepsia	MESH:D004415, UMLS:C0013395, ICD-11:DD90.3; MESH:D004415, UMLS:C0013395, ICD-11:DD90.3; MESH:D004415, UMLS:C0013395, ICD-11:DD90.3; MESH:D004415, UMLS:C0013395, ICD-11:DD90.3	ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9788171360536, ISBN:9788173717024
Leaves	Eczema	MESH:D004485, UMLS:C0013595	ISBN:9770972795006, ISBN:9788172360481

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root	Edema	MESH:D004487, UMLS:C0013604, ICD-11:MG29.1; MESH:D004487, UMLS:C0013604, ICD-11:MG29.1; MESH:D004487, UMLS:C0013604, ICD-11:MG29.1	ISBN:9770972795006, ISBN:9788172360481; A Report On Medicinal Plants Of Kachchh (Gujarat), ISBN:9770972795006, ISBN:9788172361266, Medicinal Plants Of Nagpur And Wardha Forest Divisions (Maharashtra); ISBN:9770972795006, ISBN:9788172360481, ISBN:9788172363130
Leaves, Root, Seed	Endophthalmitis	MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21; MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21; MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21; MESH:D009877, UMLS:C0014236, DOID:4692, ICD-11:9C21	ISBN:9770972795006, ISBN:9788172360481, ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9788173717024
Leaves, Root, Seed	Expectorants	MESH:D005100, UMLS:C0015314; MESH:D005100, UMLS:C0015314; MESH:D005100, UMLS:C0015314; MESH:D005100, UMLS:C0015314	ISBN:9789327275590; ISBN:9770972795006, ISBN:9788172361266, ISBN:9788172363130, ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9770972795006, ISBN:9788173717024

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root, Seed	Flatulence	MESH:D005414, UMLS:C0016204, ICD-11:ME08; MESH:D005414, UMLS:C0016204, ICD-11:ME08; MESH:D005414, UMLS:C0016204, ICD-11:ME08	ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590
Leaves, Root, Seed	Gallbladder emptying	MESH:D016473, UMLS:C0085085; MESH:D016473, UMLS:C0085085; MESH:D016473, UMLS:C0085085	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590
Leaves, Root, Seed	Gonorrhea	MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z; MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z; MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z; MESH:D006069, UMLS:C0018081, DOID:7551, ICD-11:1A7Z	ISBN:9770972795006, ISBN:9788172360481; ISBN:9770972795006; ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172363130
Leaves, Root, Seed	Heart diseases	MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z; MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z; MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z; MESH:D006331, UMLS:C0018799, DOID:114, ICD-11:BC4Z	ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006
Leaves, Root, Seed	Hematologic diseases	MESH:D006402, UMLS:C0018939, DOID:74, ICD-11:3C0Z; MESH:D006402, UMLS:C0018939, DOID:74, ICD-11:3C0Z; MESH:D006402, UMLS:C0018939, DOID:74, ICD-11:3C0Z; MESH:D006402, UMLS:C0018939, DOID:74, ICD-11:3C0Z	ISBN:9770972795006, ISBN:9788172360481, ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Whole plant	Hypotension	MESH:D007022, UMLS:C0020649, ICD-11:BA2Z; MESH:D007022, UMLS:C0020649, ICD-11:BA2Z	ISBN:9770972795006; ISBN:9770972795006
Leaves	Infection	MESH:D007239, UMLS:C3714514, ICD-11:1H0Z	ISBN:9770972795006
Leaves, Root, Seed	Jaundice	MESH:D007565, UMLS:C0022346, ICD-11:ME10.1; MESH:D007565, UMLS:C0022346, ICD-11:ME10.1; MESH:D007565, UMLS:C0022346, ICD-11:ME10.1; MESH:D007565, UMLS:C0022346, ICD-11:ME10.1	ISBN:9770972795006; A Report On Medicinal Plants Of Kachchh (Gujarat), ISBN:9770972795006, ISBN:9788172361266, Medicinal Plants Of Nagpur And Wardha Forest Divisions (Maharashtra); ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172363130, ISBN:9788173717024
Leaves, Root, Seed	Kidney calculi	MESH:D007669, UMLS:C0022650, ICD-11:GB70.0; MESH:D007669, UMLS:C0022650, ICD-11:GB70.0; MESH:D007669, UMLS:C0022650, ICD-11:GB70.0	ISBN:9789327275590; A Report On Medicinal Plants Of Kachchh (Gujarat), ISBN:9789327275590; ISBN:9789327275590
Leaves, Root, Seed	Kidney diseases	MESH:D007674, UMLS:C0022658, DOID:557, ICD-11:GC2Z; MESH:D007674, UMLS:C0022658, DOID:557, ICD-11:GC2Z; MESH:D007674, UMLS:C0022658, DOID:557, ICD-11:GC2Z; MESH:D007674, UMLS:C0022658, DOID:557, ICD-11:GC2Z	ISBN:9789327275590; ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9788172360481

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root	Laxatives	MESH:D054368, UMLS:C0282090, ICD-11:XM62T3; MESH:D054368, UMLS:C0282090, ICD-11:XM62T3; MESH:D054368, UMLS:C0282090, ICD-11:XM62T3	ISBN:9770972795006; A Report On Medicinal Plants Of Kachchh (Gujarat), ISBN:9770972795006, ISBN:9788172361266, ISBN:9788172363130, Medicinal Plants Of Nagpur And Wardha Forest Divisions (Maharashtra); ISBN:9770972795006, ISBN:9788173717024
Leaves, Root, Seed	Leukorrhea	MESH:D007973, UMLS:C0023533, DOID:3766, ICD-11:MF3A; MESH:D007973, UMLS:C0023533, DOID:3766, ICD-11:MF3A; MESH:D007973, UMLS:C0023533, DOID:3766, ICD-11:MF3A; MESH:D007973, UMLS:C0023533, DOID:3766, ICD-11:MF3A	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006, ISBN:9780387706375, ISBN:9788173717024
Leaves, Root, Seed	Neoplasms	MESH:D009369, UMLS:C0027651, DOID:162, ICD-11:2E2Z; MESH:D009369, UMLS:C0027651, DOID:162, ICD-11:2E2Z; MESH:D009369, UMLS:C0027651, DOID:162, ICD-11:2E2Z	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590
Na	Abdominal neoplasms	MESH:D000008, UMLS:C0031149	ISBN:9788172360481
Leaves, Root, Seed	Nephritis	MESH:D009393, UMLS:C0027697, DOID:10952, ICD-11:GB40; MESH:D009393, UMLS:C0027697, DOID:10952, ICD-11:GB40; MESH:D009393, UMLS:C0027697, DOID:10952, ICD-11:GB40	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves, Root	Oxytocics	MESH:D010120, UMLS:C0030094; MESH:D010120, UMLS:C0030094	ISBN:9770972795006; ISBN:9770972795006
Leaves	Postpartum hemorrhage	MESH:D006473, UMLS:C0032797, ICD-11:JA43	ISBN:9770972795006
Leaves	Pruritus	MESH:D011537, UMLS:C0033774, ICD-11:EC90	ISBN:9770972795006, ISBN:9788172360481
Leaves, Root, Seed	Scabies	MESH:D012532, UMLS:C0036262, DOID:8295, ICD-11:1G04; MESH:D012532, UMLS:C0036262, DOID:8295, ICD-11:1G04; MESH:D012532, UMLS:C0036262, DOID:8295, ICD-11:1G04; MESH:D012532, UMLS:C0036262, DOID:8295, ICD-11:1G04	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9788171360536, ISBN:9788173717024
Leaves, Root	Snake bites	MESH:D012909, UMLS:C0037379; MESH:D012909, UMLS:C0037379; MESH:D012909, UMLS:C0037379	ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172361266; ISBN:9770972795006
Leaves, Root, Seed	Splenomegaly	MESH:D013163, UMLS:C0038002, ICD-11:ME10.01; MESH:D013163, UMLS:C0038002, ICD-11:ME10.01; MESH:D013163, UMLS:C0038002, ICD-11:ME10.01; MESH:D013163, UMLS:C0038002, ICD-11:ME10.01	ISBN:9770972795006, ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006
Leaves	Sprains and strains	MESH:D013180, UMLS:C0038048	ISBN:9788172360481
Leaves, Root	Stomach diseases	MESH:D013272, UMLS:C0038354, DOID:76, ICD-11:DA4Y; MESH:D013272, UMLS:C0038354, DOID:76, ICD-11:DA4Y	ISBN:9770972795006; ISBN:9770972795006, Me- dicinal Plants Of Nagpur And Wardha Forest Divisions (Maharashtra)

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Tocolytic agents	MESH:D015149, UMLS:C0040349	ISBN:9770972795006
Leaves, Root	Urination disorders	MESH:D014555, UMLS:C0042035, ICD-11:GB0Y; MESH:D014555, UMLS:C0042035, ICD-11:GB0Y; MESH:D014555, UMLS:C0042035, ICD-11:GB0Y	ISBN:9770972795006; ISBN:9770972795006; ISBN:9770972795006, ISBN:9788172363130, ISBN:9788173717024
Leaves, Root, Seed	Urologic diseases	MESH:D014570, UMLS:C0042075; MESH:D014570, UMLS:C0042075; MESH:D014570, UMLS:C0042075	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590
Leaves, Root, Seed	Vomiting	MESH:D014839, UMLS:C0042963, ICD-11:MD90.1; MESH:D014839, UMLS:C0042963, ICD-11:MD90.1; MESH:D014839, UMLS:C0042963, ICD-11:MD90.1; MESH:D014839, UMLS:C0042963, ICD-11:MD90.1	ISBN:9789327275590; ISBN:9789327275590; ISBN:9789327275590; ISBN:9770972795006
Leaves, Root	Wound healing	MESH:D014945, UMLS:C0043240; MESH:D014945, UMLS:C0043240	ISBN:9770972795006; ISBN:9770972795006
Plant Exudate, Root	Exanthema	MESH:D005076, UMLS:C0015230; MESH:D005076, UMLS:C0015230	ISBN:9770972795006; ISBN:9770972795006
Root, Seed	Anthelmintics	MESH:D000871, UMLS:C0003158, ICD-11:XM4EC0; MESH:D000871, UMLS:C0003158, ICD-11:XM4EC0; MESH:D000871, UMLS:C0003158, ICD-11:XM4EC0	ISBN:9770972795006; ISBN:9770972795006; ISBN:9788173717024
Root	Stress, physiological	MESH:D013312, UMLS:C0449430, ICD-11:6E40.4	ISBN:9770972795006

Plant Part	Therapeutic use	Therapeutic use identifiers	References
Root	Urinary tract infections	MESH:D014552, UMLS:C0042029, DOI:0080784, ICD-11:GC08	ISBN:9770972795006, ISBN:9788172361266, Medicinal Plants Of Nagpur And Wardha Forest Divisions (Maharashtra)
Root	Night blindness	MESH:D009755, UMLS:C0028077, DOI:8499, ICD-11:MC1Y	ISBN:9788172360481
Root	Leprosy	MESH:D007918, UMLS:C0023343, DOI:1024, ICD-11:1B20	A Report On Medicinal Plants Of Kachchh (Gujarat)
Root	Eye diseases	MESH:D005128, UMLS:C0015397, DOI:5614, ICD-11:9E1Z	ISBN:9770972795006

After conducting an extensive study of the reported traditional medicinal uses of *S. nigrum*, it was found that this plant exhibits efficacy against a variety of ailments and holds potential for future exploration. Consequently, we have opted to utilize *S. nigrum* in addressing NiV infection, aiming to contribute to the alleviation of the impact of viral diseases.

Materials and Methods

Protein Structure Retrieval and Preparation

The crystal structure of NiV glycoprotein in complex with human cell surface receptor, ephrin B2 (PDB ID: 2VSM), obtained via X-RAY diffraction method at a resolution of 1.80 Å was retrieved from RCSB Protein Data Bank (www.rcsb.org). In order to generate accurate predictions, any of the associated water molecules or heteroatoms

were removed from the structures with the assistance of PyMol (www.pymol.org). NiV attachment glycoprotein chain A (Fig. 1) was selected as target protein for this study by using UCSF Chimera software (www.cgl.ucsf.edu). Further, the protein structures were repaired using protein repair and analysis server (www.protein-science.com) by adding missing hydrogens, side chains and secondary structure elements. The final structure was saved in PDB format for further studies.

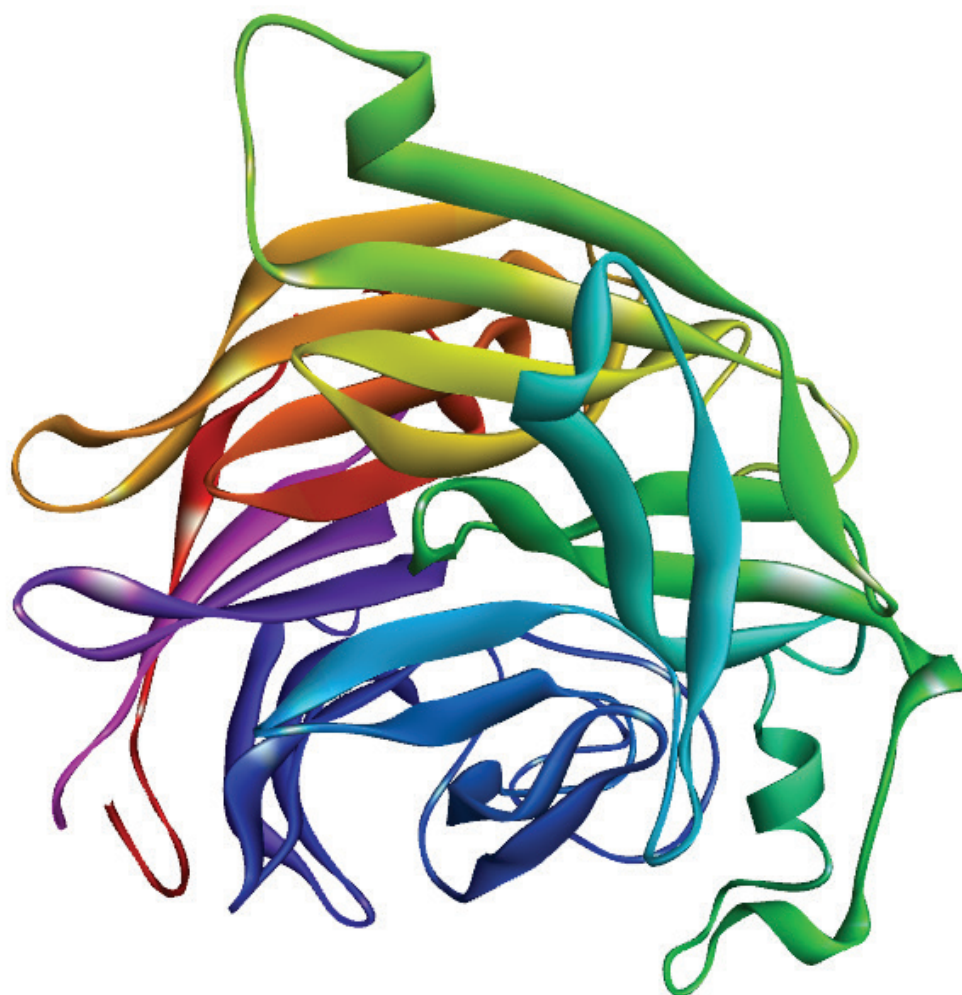


Fig. 1 Nipah virus GP chain A

Phytochemical Library Preparation

Extensive literature survey was performed to obtain a list of 135 phytoconstituents of *S. nigrum*. The three dimensional structures of the compounds in SDF format were retrieved from PubChem database (www.pubchem.ncbi.nlm.nih.gov). In addition, the SDF files were converted into PDB format by utilizing Open Babel GUI (www.openbabel.org), in order to facilitate the process of molecular docking.

Rule of Five (R05)

For initial prescreening of phytoconstituents, an online web-based platform, ADMET lab 2.0 (<https://admetmesh.scbdd.com/>) was utilised to predict the drug-likeness of the compounds. For small molecules, it is an empirical rule of thumb to assess their physicochemical aspects, in order to refine their drug ability. In the present study, we analyzed several properties including molecular weight (MW), partition coefficient (LogP), number of hydrogen bond acceptors (nHA) and number

of hydrogen bond donors (nHD). As these attributes form the foundation for the Lipinski rule of five, any molecule deviating from the threshold values was eliminated from further analysis (Lipinski, 2004).

Molecular Docking

In the current study, PyRx software (<https://pyrx.sourceforge.io/>) was utilised for the molecular docking study. The prepared PDB structures of target proteins and plant phytoconstituents were imported into the software and were processed to generate the respective PDBQT files. Blind molecular docking was performed with grid box sizes $x = 61.0423797226$; $y = 61.8005298615$; $z = 53.2407892609$. Moreover, the protein-ligand complexes were prepared using PyMol. These complexes were imported to Discovery Studio Visualizer v21.1.0.20298 (<https://discover.3ds.com/discovery-studio-visualizer-download>) for visualization of various interactions formed between the target and phytoconstituents.

Bioavailability Radar and Bioactivity Score

Drug-likeness of screened candidate with best binding energy was analyzed in a comprehensive way using SwissADME web-based tool (www.swissadme.ch). In order to do so, physiochemical properties such as size (MW), lipophilicity (XLOGP3), polarity (TPSA), solubility (LogS), flexibility (fraction Csp3), and saturation (rotatable bonds) were investigated and a bioavailability radar diagram representing these parameters was

obtained (Lipinski et al., 2012). The region highlighted in pink delineates the optimal parameter values, and substantial deviations from these values on a significant scale imply that the ligand may not possess favorable oral bioavailability (Daina et al., 2017). Additionally, the bioactivity scores were determined by using Molinspiration software (www.molinspiration.com). Parameters analyzed include Ion channel modulator (ICM), nuclear receptor ligand (NRL), protease inhibitor (PI), enzyme inhibitor (EI), G protein-coupled receptor ligand (GPCR ligand).

In silico ADMET analysis

After molecular docking studies of all the compounds with targeted protein of NiV, pharmacokinetics parameters such as ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) of the best candidate were investigated using pkCSM webserver (<https://biosig.lab.uq.edu.au/pkcsml/prediction>). ADMET constitutes a crucial aspect of drug design and development, encompassing the assessment of significant pharmacokinetic parameters (Olasupo et al., 2020). ADMET properties include absorption (water solubility, Caco2 permeability, human intestinal absorption, skin permeability, P-glycoprotein substrate and P-glycoprotein I inhibitor); distribution: (VDss, fraction unbound, BBB permeability and CNS permeability); metabolism (CYP2D6 and CYP3A4 substrate; CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 inhibitors); excretion (Total Clearance and Renal OCT2 substrate); Toxicity (AMES toxicity, Max. tolerated dose,

Oral Rat Acute Toxicity, Oral Rat Chronic Toxicity, Hepatotoxicity and Skin Sensitisation] (Xiong et al., 2021).

Molecular Dynamics Simulation

On the basis of molecular docking analysis, top phytochemical from *S. nigrum* was further selected for molecular dynamics (MD) simulation to examine its stability and intermolecular interactions using Desmond molecular dynamics study software (Bowers et al., 2006). Using the protein preparation wizard of the Desmond-maestro interface, all protein-ligand contacts were preprocessed and improved. The system was configured

for each complex using the SPC model of solvent, an orthorhombic box simulation box was calculated using the buffer method with a distance of $10 \times 10 \times 10$ Å. Later, counter ions (Na^+ or Cl^-) were added for neutralization, system's concentration was set to 0.15 M and OPLS3e force field was applied to the system. This generated system was simulated for 100 ns in a minimized system in the NPT ensemble. After simulation, the trajectory was analyzed using parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), protein-ligand interactions, radius of gyration of the lead compound, and ligand torsion analysis using the simulation interaction diagram utility of the Desmond.

Results and Discussion

Despite of cutting edge research and development taking place to produce innovative solutions to combat viral infections, past four decades has witnessed many new viral outbreaks across the different parts of the globe, resulting in a surge in mortality, morbidity, as well as economic loss (Sharma et al., 2019). The NiV is a highly pathogenic zoonotic virus causing severe respiratory illness or fatal encephalitis. In this infection, the glycoprotein plays a critical role in the initial stages of infection, acting as the key for the virus to enter host cells and initiate the disease process. The G protein on the surface of the nipah virus particle serves as the attachment protein (Hauser et al., 2021). It binds specifically to cellular receptors, primarily ephrin-B2 and ephrin-B3. These

receptors are present on a wide range of cell types, including epithelial and endothelial cells, as well as neurons (Yang and Kar, 2023). Following attachment, the G protein undergoes a conformational change triggered by receptor binding. This change activates the fusion protein on the viral envelope, promoting fusion between the viral membrane and the host cell membrane. Through this fusion process, the viral contents are delivered into the cytoplasm of the host cell, initiating viral replication and ultimately cell death (Cheliout Da Silva et al., 2021). The sole objective of this study is to identify potential compounds from *S. nigrum* with good binding affinity against NiV glycoprotein protein, using diverse computational tools.

Preliminary Screening of Drug-likeness

In the pursuit of creating a highly effective drug with minimal adverse effects, preliminary screening on the basis of drug-likeness is a crucial step. To conduct this preliminary screening, Lipinski's rule of five was utilised for evaluating all the phytochemicals derived from *S. nigrum*. Lipinski's rule of five outlines five key parameters that influence a drug's oral bioavailability. A drug candidate is considered more likely to have good oral absorption if it adheres to most, ideally all of the criteria. According to the rule, A compound demonstrates favorable oral bioavailability when its molecular weight (MW) ≤ 500 Da, hydrogen bond donors (HbD) ≤ 5 , hydrogen bond acceptors (HbA) ≤ 10 , and an octanol-water partition coefficient log P ≤ 5 (Lipinski, 2004). According to various

studies, molecules with higher MW generally have difficulty penetrating the intestinal wall, limiting their absorption into the bloodstream. Similarly, increased number of hydrogen bond donors and acceptors can form increased interactions between the drug and water molecules, hindering intestinal absorption. Finally, a high logP value suggests the drug might favor partitioning into fat tissues over dissolving in water for absorption (Roskoski, 2023; Truong et al., 2021). Compounds that violated more than one parameter were excluded, while the remaining compounds were identified as ligands for the subsequent docking study. As illustrated in Table 2, out of the total 135 phytoconstituents, 127 candidates successfully passed Lipinski's rule and were carried forward for further analysis. 99 compounds depicted 0 Lipinski's violations whereas 28 compounds satisfied the criterion with 1 violation.

Table 2 Screening of phytoconstituents based on Lipinski's rule

Phytoconstituents	LogP	MW	nHA	nHD	Lipinski	Violations
alpha-hydroxyisobutyric acid	-0.361	104.05	3	2	Accepted	0
D-lactic acid	-1.047	90.03	3	2	Accepted	0
Ethanedioic acid, bis(trimethylsilyl) ester	3.075	234.07	4	0	Accepted	0
2-Deoxy ribose 0,0',0''-tris(trimethylsilyl)-	3.847	350.18	4	0	Accepted	0
1-O-pentadecylglycerol, bis(trimethylsilyl) ether	8.73	446.36	3	0	Accepted	1
DL-Malic acid, O-trimethylsilyl-,bis(trimethylsilyl) ester	4.292	350.14	5	0	Accepted	0
Pyroglutamic acid, bis(trimethylsilyl)	3.335	273.12	4	2	Accepted	0
Isovanillic acid	1.37	168.04	4	2	Accepted	0

Phytoconstituents	LogP	MW	nHA	nHD	Lipinski	Violations
Azelaic acid, bis(trimethylsilyl) ester	4.931	332.18	4	0	Accepted	0
L-(+)-tartaric acid, bis(trimethylsilyl) ether, bis(trimethylsilyl) ester	5.129	438.17	6	0	Accepted	0
1H-indole-3-carboxylic acid,2-ethoxy-1-(trimethylsilyl)-5-[(trimethylsilyl)-oxy]-,	5.252	393.18	5	0	Accepted	0
Cholestrol trimethylsilyl ether	8.68	458.39	1	0	Accepted	1
Stigmasterol trimethylsilyl ether	8.731	484.41	1	0	Accepted	1
Sarsasapogenin	5.444	416.33	3	1	Accepted	1
Solanidine	5.524	397.33	2	1	Accepted	1
(+)-pinoselinol	2.192	358.14	6	2	Accepted	0
(+)-syringaresinol	1.993	418.16	8	2	Accepted	0
(+)-medioresinol	2.072	388.15	7	2	Accepted	0
beta-sitosterol	7.663	414.39	1	1	Accepted	1
Solasodine	5.075	413.33	3	2	Accepted	1
Diisooctyl phthalate	7.494	390.28	4	0	Accepted	1
4-nitroguaiacol	1.58	169.04	5	1	Accepted	0
Flavone	3.844	222.07	2	0	Accepted	0
5-Decenedioic acid,5,6-dimethyl,dimethyl ester	3.045	256.17	4	0	Accepted	0
Z,E-2methyl-3,13-octadecadien-1-ol	7.243	280.28	1	1	Accepted	1
2,4-di-tert-butylphenol	4.832	206.17	1	1	Accepted	0
2,6,6,10-tetramethyl-undeca-8,10diene-3,7-dione	3.1	236.18	2	0	Accepted	0
N-trans-feruloyltyramine	2.538	313.13	5	3	Accepted	0
(R)-3-[4-hydroxy-3-methoxyphenyl]-N-[2-[4-hydroxyphenyl]-2-methoxyethyl]acrylamide	2.137	343.14	6	3	Accepted	0
(E)-ethyl caffeate	2.383	208.07	4	2	Accepted	0
ethyl 4-hydroxy-3-methoxycinnamate	2.633	222.09	4	1	Accepted	0
3-O-acetylbetulinic acid	6.342	498.37	4	1	Accepted	1
chlorogenic acid	-0.162	354.1	9	6	Accepted	1
caffeic acid	1.43	180.04	4	3	Accepted	0

Phytoconstituents	LogP	MW	nHA	nHD	Lipinski	Violations
methylsinapate	2.117	238.08	5	1	Accepted	0
drummondol	0.386	240.14	4	2	Accepted	0
2 α ,9-dihydroxy-1,8-cineole	0.602	186.13	3	2	Accepted	0
tryptophol acetate	0.035	278.09	7	3	Accepted	0
4-amino3-methoxyphenol	0.455	139.06	3	3	Accepted	0
BENZOFURAN, 2,3-DIHYDRO	2.318	120.06	1	0	Accepted	0
1,6-ANHYDRO-BETA-D-GLUCOPYRAN- NO	-1.943	162.05	5	3	Accepted	0
2-PYRAZINOL	-1.013	96.03	3	1	Accepted	0
1,2-BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER	4.337	278.15	4	0	Accepted	0
cyclopentasiloxane, decamethyl-	5.23	370.09	5	0	Accepted	0
L-proline, ethyl ester	0.523	143.09	3	1	Accepted	0
2-ethyl-1-butanol, methyl ether	2.609	116.12	1	0	Accepted	0
octadecane, 3-ethyl-5-(ethyl butyl)	11.071	366.42	0	0	Accepted	1
5-keto-2,2-dimethylheptanoic acid, ethyl ester	2.118	200.14	3	0	Accepted	0
Z-8-methyl-9-tetradecenoic acid	5.527	240.21	2	1	Accepted	0
9,12,15-octadecatrienoic acid	6.156	278.22	2	1	Accepted	1
valeric acid, 4-pentadecyl ester	7.655	312.3	2	0	Accepted	1
2-methyl-4,6-dinitro phenol	2.133	198.03	7	1	Accepted	0
retrorsine	1.18	351.17	7	2	Accepted	0
O-(3, 5-dichloro-2,4- dihydroxy benzo- yl) benzoic acid	3.964	325.97	5	3	Accepted	0
linoleic acid	6.652	280.24	2	1	Accepted	1
tetracosanoic acid	9.98	368.37	2	1	Accepted	1
1-acetoxy pinosresinol	2.095	416.15	8	2	Accepted	0
Triethyl phosphate	0.93	182.07	4	0	Accepted	0
4-Trifluoroacetyltetradecane	6.402	310.21	2	0	Accepted	1
3,7,11,15-Tetramethyl2-hexadecane	8.708	280.31	0	0	Accepted	1
daidzin	0.109	416.11	9	5	Accepted	0
genistin	0.064	432.11	10	6	Accepted	1

Phytoconstituents	LogP	MW	nHA	nHD	Lipinski	Violations
daidzein	2.795	254.06	4	2	Accepted	0
genistein	2.506	270.05	5	3	Accepted	0
apigenin	3.307	270.05	5	3	Accepted	0
(+)-isolariciresinol	1.507	360.16	6	4	Accepted	0
transcinnamic acid	2.363	148.05	2	1	Accepted	0
ferulic acid	1.803	194.06	4	2	Accepted	0
Solanocapsine	4.086	430.36	4	4	Accepted	1
Diosgenin	5.556	414.31	3	1	Accepted	1
2-Pyrrolidinecarboxylic acid-5-oxo-, ethyl ester	0.045	157.07	4	3	Accepted	0
3, 4-dihydroxhbenzoic acid	1.041	154.03	4	3	Accepted	0
p-hydroxybenzoic acid	1.51	138.03	3	2	Accepted	0
6,7-dimethoxycoumarin	1.38	206.06	4	0	Accepted	0
6-methoxy-7-hydroxycoumarin	1.191	192.04	4	1	Accepted	0
6,8- dimethoxycoumarin	1.683	206.06	4	0	Accepted	0
umbelliferone	1.311	162.03	3	1	Accepted	0
vanillin	1.096	152.05	3	1	Accepted	0
vanillic acid	1.396	168.04	4	2	Accepted	0
syringic aldehyde	0.864	182.06	4	1	Accepted	0
ethyl 3,4-dihydroxybenzoate	1.972	182.06	4	2	Accepted	0
ethyl 3,4- dihydroxyphenylacetate	1.534	196.07	4	2	Accepted	0
α -Pinene	4.125	136.13	0	0	Accepted	1
Dehydro-1,8-cineole	2.677	152.12	1	0	Accepted	0
1,8-Cineole	2.582	154.14	1	0	Accepted	0
Linalool	2.978	154.14	1	1	Accepted	0
6-Camphenone	2.172	150.1	1	0	Accepted	0
Tetrahydrolinalool	3.489	158.17	1	1	Accepted	0
trans-p-Mentha-2,8-dien-1-ol	2.365	152.12	1	1	Accepted	0
trans-Verbenol	2.505	152.12	1	1	Accepted	0
Pinocarvone	2.239	150.1	1	0	Accepted	0
Borneol	2.719	154.14	1	1	Accepted	0

Phytoconstituents	LogP	MW	nHA	nHD	Lipinski	Violations
Umbellulone	2.284	150.1	1	0	Accepted	0
Terpinen-4-ol	3.06	154.14	1	1	Accepted	0
Methyl salicylate	2.338	152.05	3	1	Accepted	0
Myrtenol	2.554	152.12	1	1	Accepted	0
Myrtenal	2.544	150.1	1	0	Accepted	0
Safranal	3.059	150.1	1	0	Accepted	0
trans-Carveol	2.437	152.12	1	1	Accepted	0
β -Cyclocitral	3.239	152.12	1	0	Accepted	0
Carvotanacetone	2.742	152.12	1	0	Accepted	0
2-Phenyl ethyl acetate	2.208	164.08	2	0	Accepted	0
Ethyl salicylate	2.821	166.06	3	1	Accepted	0
Thymol	3.153	150.1	1	1	Accepted	0
Carvacrol	3.259	150.1	1	1	Accepted	0
p-Vinyl-guaiacol	2.251	150.07	2	1	Accepted	0
2-Phenyl ethyl propanoate	2.734	178.1	2	0	Accepted	0
(E)- β -Damascenone	4.05	192.15	1	0	Accepted	0
Geranyl acetone	4.447	194.17	1	0	Accepted	0
(E)-Ethyl cinnamate	3.051	176.08	2	0	Accepted	0
(E)- β -Ionone, 5,6-epoxide	2.591	208.15	2	0	Accepted	0
(E)-Nerolidol	5.179	222.2	1	1	Accepted	0
(2Z,6E)-Farnesol	5.979	222.2	1	1	Accepted	0
Methyl eudesmate	1.958	226.08	5	0	Accepted	0
iso-Longifolol	4.155	220.18	1	0	Accepted	0
(5Z,9E)-Farnesyl acetone	6.697	262.23	1	0	Accepted	1
Lupeol	7.291	426.39	1	1	Accepted	1
p-hydroxycinnamic acid	1.923	164.05	3	2	Accepted	0
Oxetane, 3-(1-Methylethyl)-	1.407	100.09	1	0	Accepted	0
1-[2-(1,3-Cyclopentadien-1-Yl)Ethyl]	1.435	163.14	1	0	Accepted	0
1-Methyl-1-(2-Tridecyl)Oxy-1-Silacyclopentane	7.24	298.27	1	0	Accepted	1
1-Methyl-1-(3-Tetradecyl)Oxy-1-Silacyclopentane	7.479	312.28	1	0	Accepted	1

Phytoconstituents	LogP	MW	nHA	nHD	Lipinski	Violations
Cis,Cis,Cis-7,10,13-Hexadecatrienal	5.051	234.2	1	0	Accepted	0
Octanoic Acid, 2-Dimethylaminoethyl Ester	3.22	215.19	3	0	Accepted	0
4,8,12,16-Tetramethylheptadecan-4-olide	7.327	324.3	2	0	Accepted	1
Methyl [Z]-5,11,14,17-Eicosatetraenoate	6.722	318.26	2	0	Accepted	1
lunasin	4.138	288.16	4	0	Accepted	0

Molecular Docking Analysis

Molecular docking plays a vital role as a computational tool in the field of drug discovery. It is a computational technique used in drug discovery to simulate the interaction between a small molecule (ligand) and a larger biomolecule (receptor) like a protein. It essentially predicts how well a ligand fits into the binding site of the receptor, providing valuable insights for drug design and development (Chen et al., 2020). It was performed on the ligands that had undergone the prescreening. In the current study, the selected targeted protein is docked against screened phytoconstituents to examine their binding affinity. The docking results of top 10 lead compounds against each studied protein are detailed in Table 3. On the basis of Lipinski violation and docking results evaluation, it was found that daidzin was able to bind the

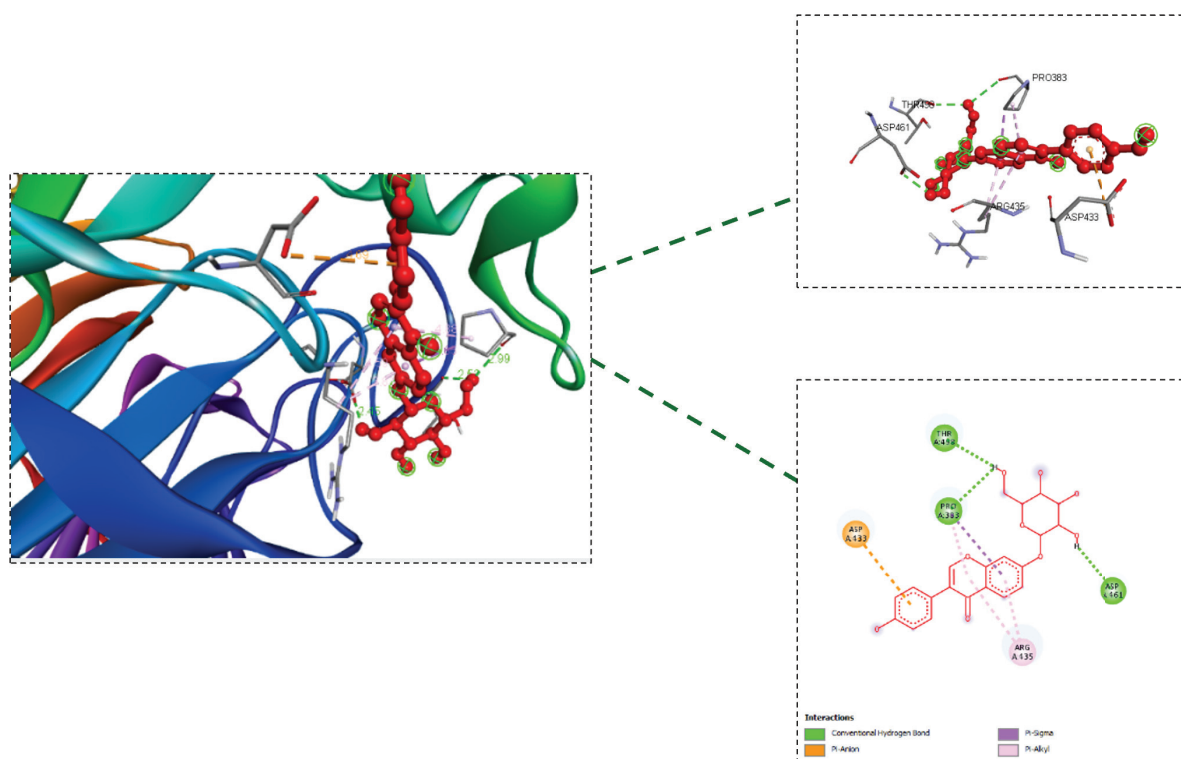
target protein with highest binding affinity of -8.8 kcal/mol and 3 hydrogen bonds with residue THR A:498, PRO A: 383 and ASP A: 461 at distances of 2.45, 2.52 and 2.99 Å (Table 4; Fig. 2). In addition, daidzin showed 0 Lipinski violations, making it a suitable candidate for further analysis. Daidzin is a major isoflavone, with several pharmacological activities such as anti-inflammatory, anti-cancer, anti-urolithiatic and anti-epileptic (Yao et al., 2021; Tan et al., 2022; Yuan et al., 2023). In addition, studies have discussed the anti-viral efficacy of daidzin against nipah virus-related Japanese encephalitis virus and Dengue virus (Zhang et al., 2012 and Asman et al., 2020). In a similar study, targeting SARS-CoV-2, Daidzin depicted a bind affinity of -8.3 kcal/mol towards its methyltransferase (Chandra et al., 2022). This evidence supports the molecular docking results obtained in the present study.

Table 3 Molecular docking results of top 10 phytoconstituents

Phytoconstituents	Binding Affinity (kcal/mol)
Daidzin	-8.8
(-)-Epicatechin-3-gallate	-8.6
Silymarin	-8.6
Solanocapsine	-8.6
Solasodine	-8.6
Genistin	-8.6
Diosgenin	-8.5
Solanidine	-8.5
1-acetoxy pinoresinol	-8.3
Lupeol	-8.3

Table 4 Hydrogen bond interactions formed by top compound

Phytoconstituent	Hydrogen bonds	Residue	Distance (Å)
Daidzin	3	THR A: 498, PRO A: 383, ASP A: 461	2.45, 2.52, 2.99

**Fig. 2** Illustration of Niv GP-Daidzin complex

Bioavailability Radar and Bioactivity Score

Bioavailability is an important parameter of the drug development process (Wei et al., 2022). In this study, bioavailability was evaluated for candidate with best binding affinity i.e

daidzin. As shown in Fig. 3, daidzin showed size, lipophilicity, flexibility, instauration, and insolubility within the permissible range, however, the polarity of the computed deviated minutely from the set boundary. Over all, the compound depicted acceptable bioavailability parameters with a score of 0.55.

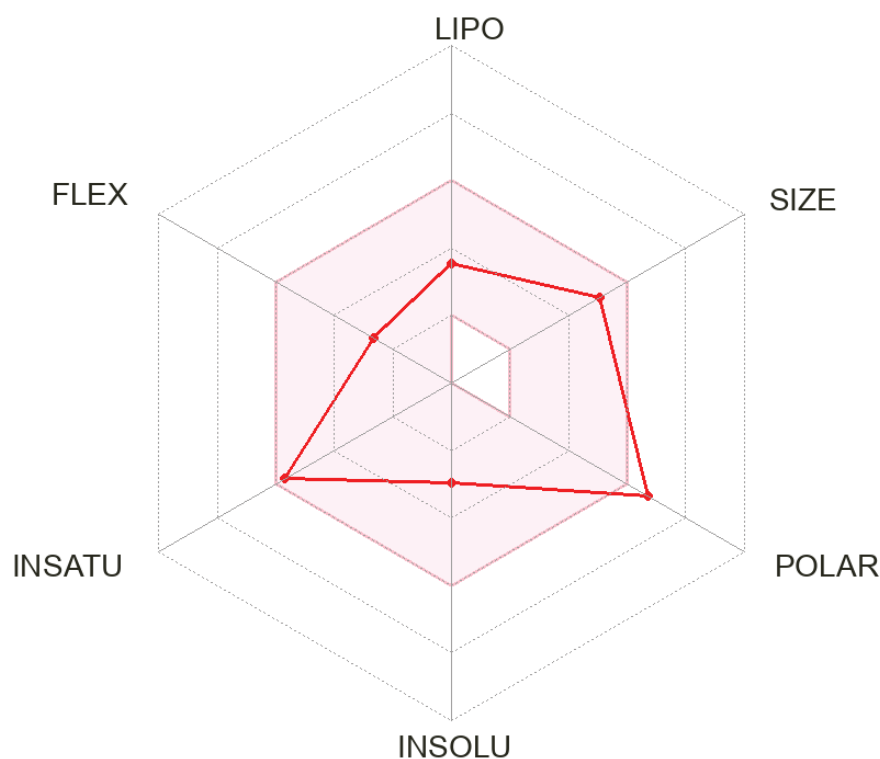


Fig. 3 Bioavailability radar diagram of Daidzin

In addition, the bioactivity scores for Daidzin such as G protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand and protease inhibitor were assessed using Molinspiration online tool. In general, organic molecules are considered active when bioactivity score is

>0, moderately active if score is between -5.0 to 0.0 and inactive when the score is <-5.0 (Kulkarni et al., 2021). As illustrated in Fig. 4 It was found that Daidzin depicted potent nuclear receptor ligand activity and moderate rest of the activities.

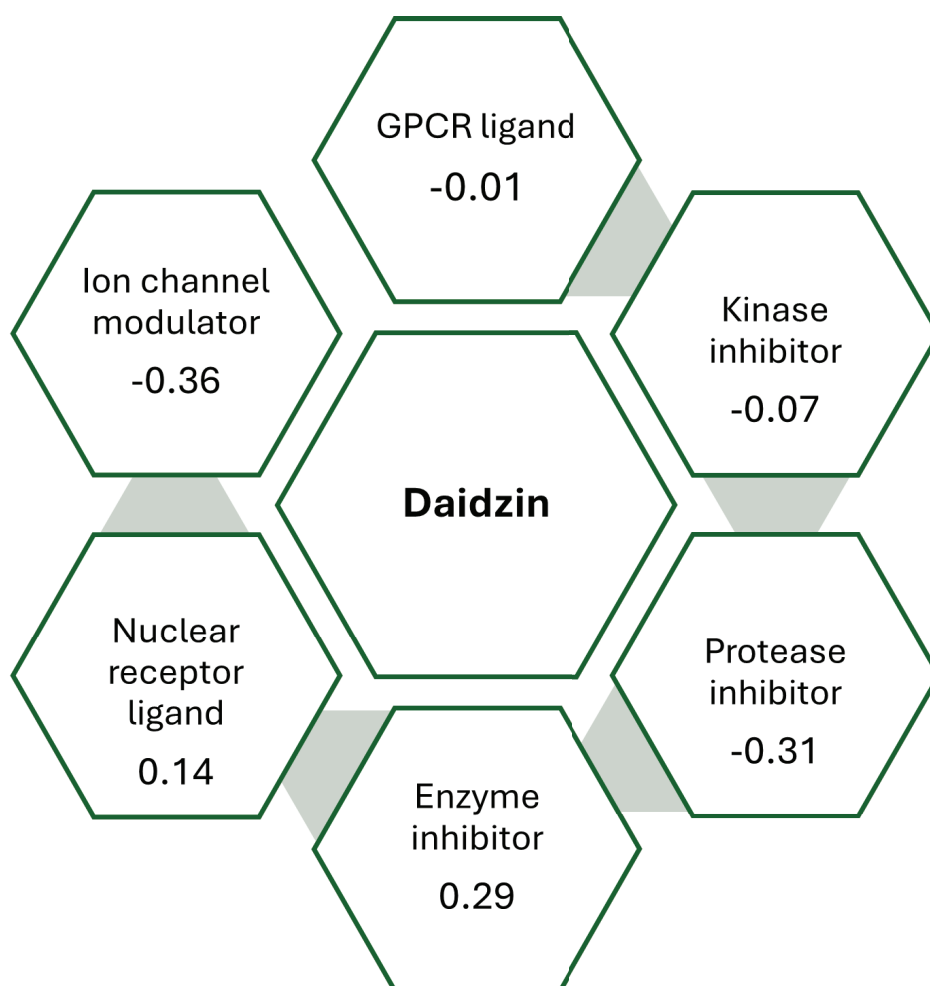


Fig. 4 Bioactivities of Daidzin

***In silico* ADMET Analysis**

Administering medication orally is the most convenient method for patients. When a drug is taken orally, it must be absorbed through the epithelial lining of the small intestine (Azman et al., 2022). In the drug discovery pipeline, a critical bottleneck lies in identifying promising lead compounds with favorable pharmacokinetic (ADME) and toxicological (T) properties. Traditionally, these properties are assessed through extensive *in vitro* and *in vivo* experiments, which are time-consuming,

resource-intensive, and often require large quantities of the candidate drug. *In silico* ADMET analysis offers a powerful alternative approach to evaluate these properties computationally, playing a crucial role in modern drug discovery (Pradeepkiran et al., 2021). The ADMET results presented in Table 5 revealed that the compound Daidzin demonstrated significant parameters that make it suitable for use as a drug. It can easily dissolve in the body and meets the ADMET criteria and drug-likeness standards.

Table 5 *In Silico* ADMET analysis of daidzin

Property	Model Name	Predicted Value
Absorption	Caco2 permeability (log Papp in 10 ⁻⁶ cm/s)	0.24
	human intestinal absorption (% Absorbed)	59.319
	Skin Permeability (log Kp)	-2.736
	P-glycoprotein substrate	Yes
	P-glycoprotein I inhibitor	No
	P-glycoprotein II inhibitor	No
Distribution	VDss (log L/kg)	-0.166
	Fraction unbound	0.199
	BBB permeability (log BB)	-1.232
	CNS permeability (log PS)	-3.584
Metabolism	CYP2D6 substrate	No
	CYP3A4 substrate	No
	CYP1A2 inhibitor	No
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	No
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	No
Excretion	Total Clearance (log ml/min/kg)	0.104
	Renal OCT2 substrate	No
Toxicity	AMES toxicity	No
	Max. tolerated dose (log mg/kg/day)	0.488
	hERG I inhibitor	No
	hERG II inhibitor	Yes
	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.738
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	4.717
	Hepatotoxicity	No
	Skin Sensitisation	No
	<i>T. Pyriformis</i> toxicity (log ug/L)	0.285
	Minnow toxicity (log mM)	3.902

Molecular dynamics simulations

On the basis of molecular docking, bioavailability, bioactivity, and ADMET analysis, daidzin fulfilled all the parameters and was therefore, subjected to molecular dynamics simulations for 100 ns. The dynamic behaviors or molecular stability of daidzin-NiV glycoprotein complex was observed through root-mean square deviation (RMSD), root mean square fluctuation (RMSF), protein-ligand interaction analysis, and ligand torsion analysis.

RMSD of Daidzin-NiV Glycoprotein Complex

RMSD analysis gives idea whether the simulation has equilibrated or not, with acceptable fluctuations ranging between 1 to 3 Å. Fig. 5 depicts RMSD plots for simulated protein-daidzin complex. As presented in the figure, the RMSD trajectory of c-alpha and Daidzin fit protein complex depicted perfectly overlapped trajectory from 2.60 ns to 66.70 ns, indicating towards stable complex formation. Post that, the trajectories were stable with minor fluctuations, until the end of the 100 ns simulation period.

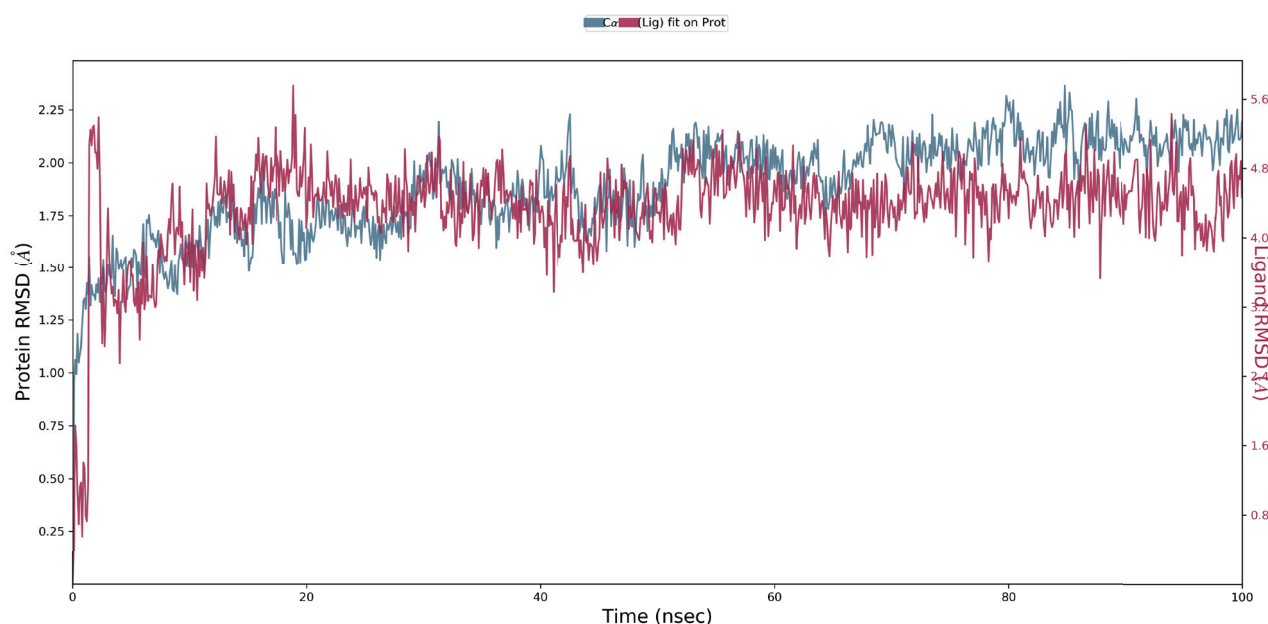


Fig. 5 RMSD Plot of Daidzin-NiV glycoprotein complex

RMSF of NiV Glycoprotein

RMSF is a measure of the displacement of the reference structure over the number of atoms. In Fig. 6, peaks indicate areas of the protein that fluctuate the most during the simulation. Typically, the tails (N- and C-terminal) fluctuate more than any other

part of the protein. Secondary structure elements like alpha helices and beta strands are usually more rigid than the unstructured part of the protein, and thus fluctuate less than the loop regions. The protein residues that interact with the ligand are marked with green-colored vertical bars. Generally, RMSF

value >2.5 Å points towards less stable bonds (Fatriansyah et al., 2022). The RMSF values for most of the protein residues, including those responsible for making protein-ligand

contacts were less than 2.5 Å. The peaks, indicating RMSF surpassing this threshold were occupied by SER 213, SER 325, GLY 328, THR 583 and HIS 603.

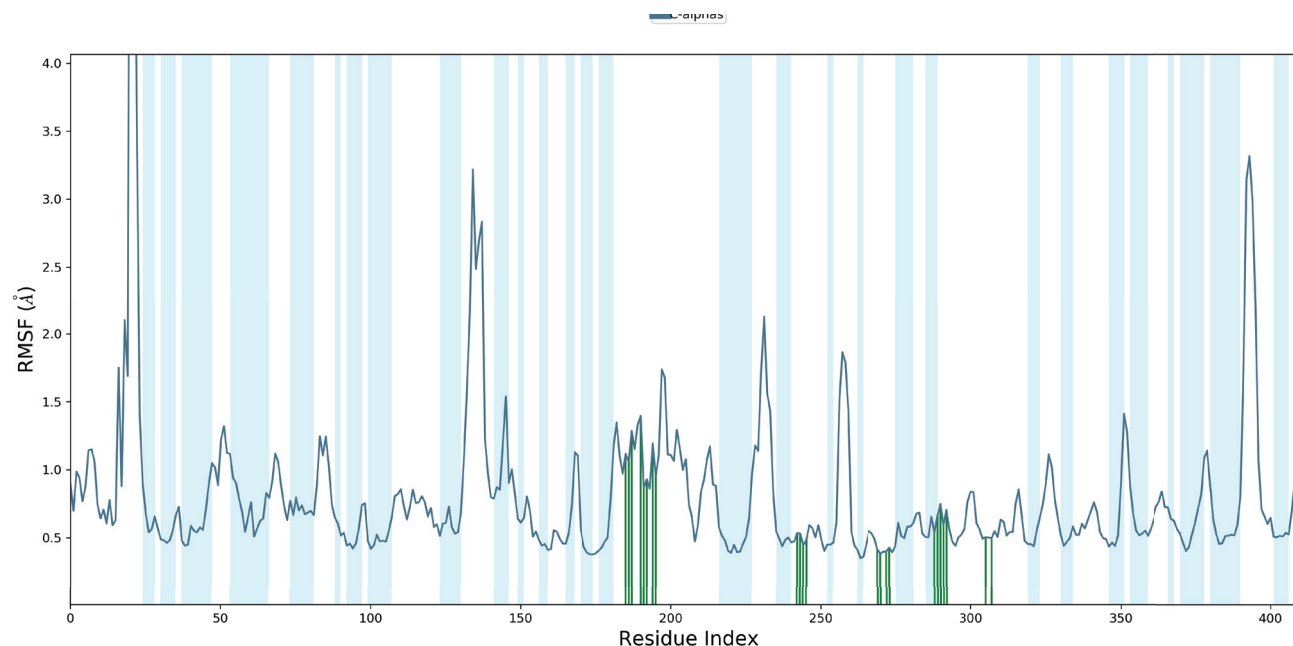


Fig. 6 RMSF Plot of NiV glycoprotein

Protein-Ligand Contacts Analysis

Protein interactions with the ligand can be monitored throughout the simulation. These interactions can be categorized into four types: hydrogen bonds, hydrophobic, ionic and water bridges. Consideration of hydrogen bonds is importance due to their strong influence on drug specificity. As shown in Fig. 7, the majority of the significant ligand-protein interactions determined with MD are hydrogen bonds and hydrophobic interactions. As shown in figure, the daidzin-

NiV glycoprotein complex predominantly depicted the formation of hydrogen bonds, water bridges and hydrophobic bonds. Among hydrogen bonds, the strongest one was depicted by ASN 378 with interaction fraction (IF) of 0.845, followed by LYS 386, ASN 481, ASN 482 and THR 498. Apart from this, ASN 381, ASP 433, ASP461 and TYR 377 also manages to form hydrogen bonds. The protein-ligand interactions maintained for more than 10% of the simulation time are detailed in Fig. 7.

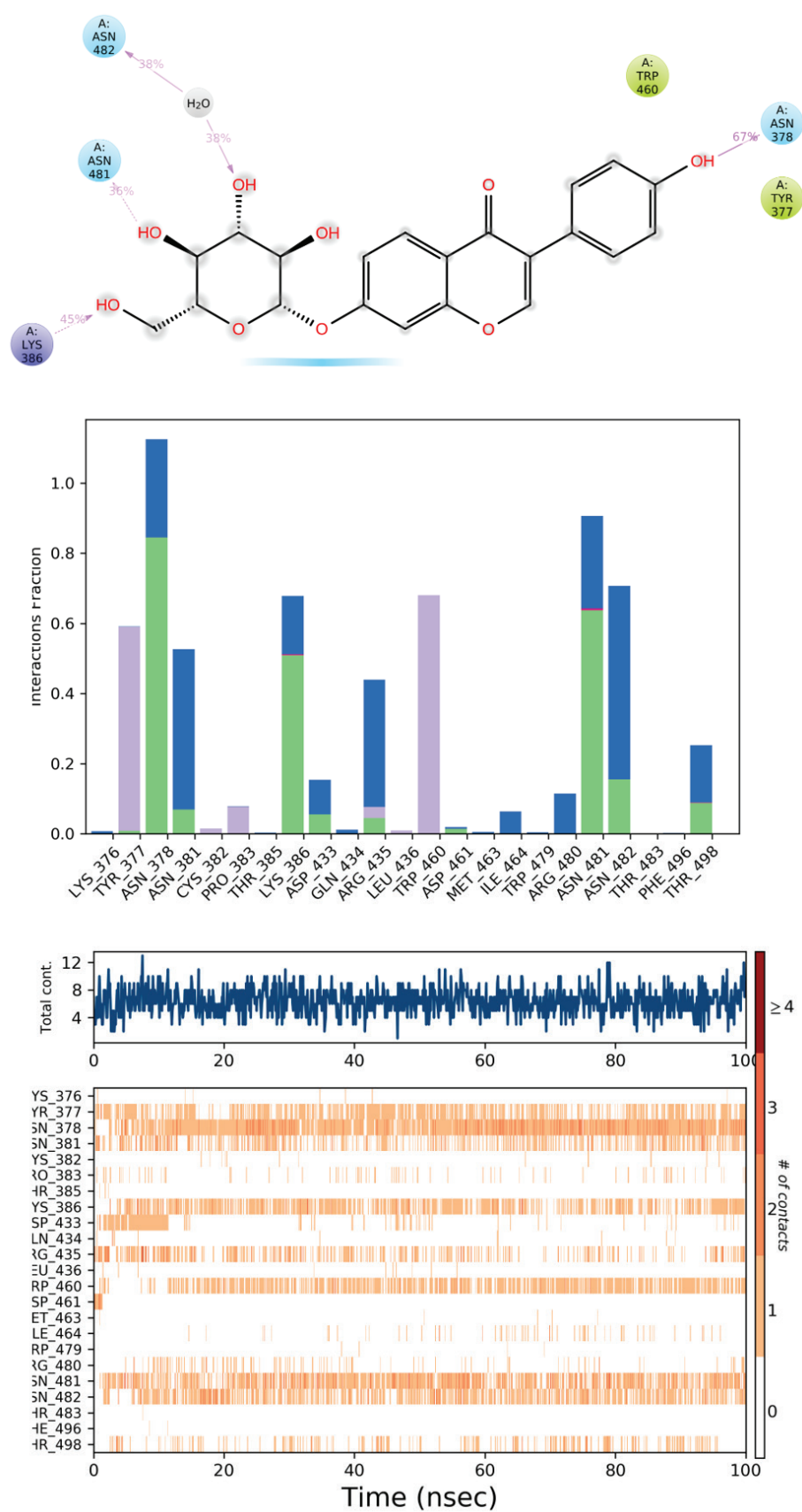


Fig. 7 Protein-Ligand interactions of Daidzin-NiV glycoprotein complex

Ligand Torsion Analysis

The torsional degree of freedom for the rotatable bonds of daidzin were analyzed. The compound exhibited nine rotatable bonds as shown in a color-coded manner in the ligand torsion analysis figure. Fig. 8 represents dial and bar plots of same color, where the dial plot exhibits the conformation of torsion throughout the simulation period, starting at

the center and evolving radially outwards. In addition, the bar plot briefs the results of dial plots and indicates the probability density of the torsion. In case the torsional potential information is available, it is shown at the y-axis of the bar plot, which may give information about the conformational strain the ligand undergoes in order to maintain a protein-bound conformation.

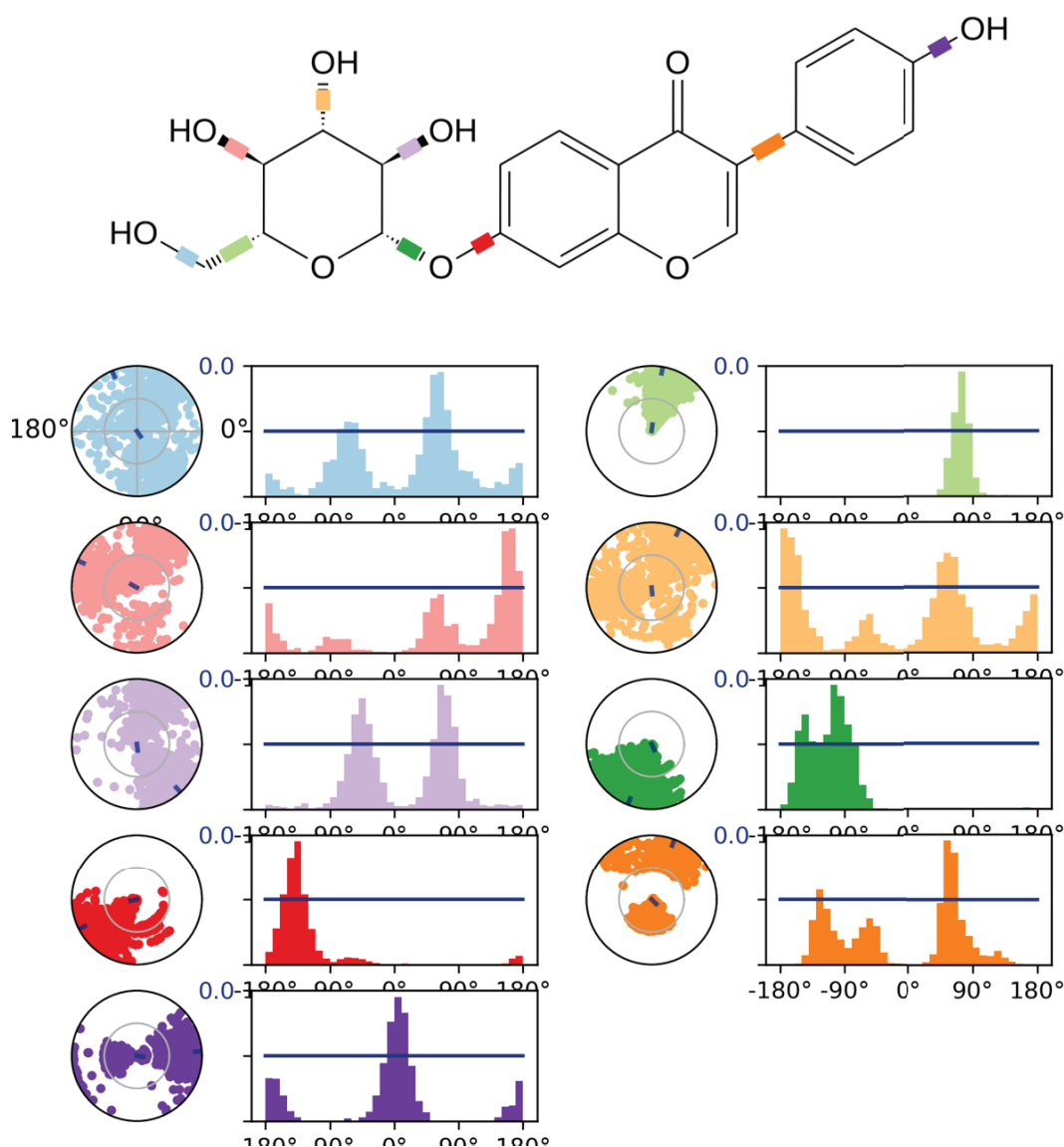


Fig. 8 Ligand torsion analysis of Daidzin

Conclusion

This comprehensive computational study has unveiled the substantial potential of bioactive phytoconstituents derived from the medicinal plant *S. nigrum* in targeting the NiV GP. Through the adept utilization of virtual screening, encompassing molecular docking analysis and molecular dynamics simulations, we have provided evidence of the therapeutic promise of daidzin in combating NiV infection. The molecular docking analysis has underscored the robust binding affinity of daidzin with the target protein, indicating its potential as an inhibitor for NiV GP-mediated infection. Furthermore, daidzin exhibited favorable pharmacokinetic properties, bioavailability, and bioactivities, affirming

its safety and suitability as a prospective drug candidate. The insights gained from molecular dynamics simulations have further substantiated the stability of the daidzin-NiV GP complex, shedding light on their potential as an effective therapeutic strategy against NiV infection. In essence, this research has shed light on the promising potential of daidzin as a natural therapeutic agent targeting NiV GP, a key protein in the pathogenesis of this viral infection. These findings provide a solid foundation for future investigations and the development of targeted therapies for NiV infection, fostering optimism for improved treatment options and enhanced outcomes for patients afflicted with this disease of concern.

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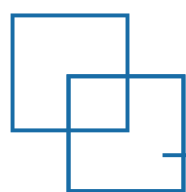
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CHAPTER 07

Reported Pharmacological Profile



INTRODUCTION

Plant-derived natural compounds are valuable sources for treating a variety of ailments. In the present period, knowledge and experience with herb use are being combined with cutting-edge technology to create a safe and attractive herbal product. As a result, herbal medications are widely used in many underdeveloped nations. Ethnomedicines are concerned with the use of various health-promoting cultural practices and natural products for illness prevention and treatment, as well as the maintenance of optimal physical and mental health (Nasim et al., 2022). *Solanum nigrum* L. ("black nightshade") is a widespread edible medicinal herb from the Solanaceae family that originated in Southeast Asia and is now extensively dispersed throughout temperate to tropical parts of Europe, Asia, and America. Historically, it has been used to treat cancer, acute nephritis, urethritis, leukorrhea, sore throat, toothache, dermatitis, eczema, carbuncles, and furuncles. *S. nigrum* has 188 chemical components that have been identified thus far. The principal bioactive ingredients are steroidal saponins, alkaloids, phenols, and polysaccharides. Investigations into the pharmacological activities of *S. nigrum* indicated that this edible medicinal plant has a wide variety of therapeutic potential, including anti-cancer, anti-inflammatory, antioxidant, anti-bacterial, and neuroprotective properties both *in vivo* and *in vitro* (Chen et al., 2022). A brief review on the biological activity and therapeutic potential of *S. nigrum* is presented in this chapter.

Pre-clinical Study

S. nigrum is a widely distributed plant with a long history of traditional medicinal use. From soothing inflamed joints to calming digestive woes, this unassuming nightshade has found its way into traditional remedies across cultures. The pre-clinical study embarks on a journey to unlock the secrets of *S. nigrum*. Through rigorous scientific investigation, we aim to shed light on its pharmacological effects and

potential toxicity, paving the way for a deeper understanding of its medicinal potential. By employing cutting-edge techniques and adhering to strict scientific protocols, we hope to provide a solid foundation for future clinical research and potentially reveal the hidden therapeutic treasures within this common yet enigmatic plant.



Anti-angiogenic Activity

Yang et al. (2016) reported the anti-angiogenic activity of water and polyphenol extracts of *S. nigrum* (0.1 to 2%) against human umbilical vascular endothelial cells (HUVEC) and HepG2 (human hepatoma) cells-induced angiogenesis-mediated tumor growth in nude mice through inhibition of AKT/mTOR pathway. Both extracts significantly ($p < 0.01$) reduced the tumor volume and tumor weight when compared with untreated cells. The large number of CD31-positive cells significantly ($p < 0.01$) decreased and, also inhibited tumor angiogenesis and vascular endothelial growth factor (VEGF)-induced phosphorylation of AKT and mTOR when compared with untreated group. Moreover, the extracts regulated VEGF-induced capillary tubule formation of HUVECs when compared with VEGF alone. The hemoglobin level was significantly lower and, also VEGF-induced phosphorylation of VEGFR2 suppressed in both extracts-treated matrigel when compared to HepG2 cell alone. Further, the water (2 mg/ml) and polyphenol (1 mg/ml) extracts significantly ($p < 0.01$) decreased the HepG2 cell viability and inhibited the activation AKT and mTOR in normal cultured HepG2 cells.

Anti-asthmatic Activity

Nirmal et al. (2012) investigated the anti-asthmatic effect of petroleum ether, ethanol and aqueous extracts (50, 100 and 200 mg/kg, b.w.) of *S. nigrum* berries against clonidine (1 mg/kg) and haloperidol (1 mg/kg)-induced catalepsy, milk (4 ml/kg)-induced leucocytosis

and eosinophilia as well as, mast cell stabilizing activity in mice and histamine (0.1 to 0.4 mg/ml)-induced contraction in guinea pig ileum (*in vitro*). The petroleum ether extract was found more significant ($p < 0.05$) in reduction of clonidine-induced catalepsy than other extracts but, none of tested extracts inhibited haloperidol-induced catalepsy when compared with untreated groups. Similarly, the petroleum ether extract significantly ($p < 0.05$) inhibited an increase in leukocyte and eosinophil counts due to milk allergen than other extracts. Moreover, the petroleum ether extract (200 mg/kg) highly protected the mast cell stabilizing activity with 57.7% protection than both extracts (34.2 and 29.7%) which was like disodium chromoglycate (59.3% at 200 µg/kg) used as standard. The petroleum ether extract (100 µg/ml) showed better relaxation of guinea pig ileum in histamine-induced contraction than other extracts.

Anti-biofilm Activity

Khaled et al. (2021) demonstrated the anti-biofilm activity of essential oils (25 to 200 µg/ml) from *S. nigrum* on biofilm forming *Proteus mirabilis* using agar well diffusion method. The oils at 100 mg/ml dose showed 32 mm zone inhibition for biofilm forming *P. mirabilis*. The oils at 200 µg/ml deactivated survival rate with 92%, while the bacteria with rough surface, condensed structure, and irregular shape of *P. mirabilis* were observed at 200 µg/ml of concentration for treated cells image. The morphology and cell wall damage in oils treated *P. mirabilis* was evidently proved by scanning electron microscope (SEM).

Anti-cancer Activity

Churiyah et al. (2020) evaluated the anti-cancer activity of ethanolic extract (12.5 to 400 µg/ml) of *S. nigrum* ripe fruits against human cancer cells: breast (MCF-7) and Vero by MTT assay. The extract exhibited an anti-cancer effect with IC_{50} of 40.7 µg/ml when compared with doxorubicin (0.14 µg/ml) used as standard towards MCF-7 cells as well as, cytotoxic effect with 298.96 µg/ml on Vero cells. Moreover, the extract induced MCF-7 cell apoptotic was insignificantly different ($p > 0.05$). The extract arrested cell cycle in S phase and continued to G2/M phase *i.e.*, extract and doxorubicin-treated MCF-7 demonstrated the number of cells expressed by area for both S and G2/M phase was not different significantly (20 µg/ml) with similar strength as doxorubicin (0.07 µg/ml) in cell cycle study.

Nawaz et al. (2021) assessed the anti-cancer effect of ethyl acetate, petroleum ether, chloroform, *n*-butanol, ethanol, and aqueous fractions (2.5 to 200 µg/ml) of *S. nigrum* against human cancer cells: liver (HepG2), cervical (HeLa) and baby hamster kidney fibroblasts (BHK) using MTT assay. Results displayed the potent cytotoxic activity of ethyl acetate fraction of *S. nigrum* against HepG2 cells with IC_{50} value of 7.89 µg/ml. Other fractions exhibited more potent anticancer activity against HepG2 cells followed by HeLa cells. In a nutshell, all fractions exhibited inhibitory effect with IC_{50} values ranging from 7.89 to 92.3 µg/ml towards tested cells, whereas highest effect observed for ethyl acetate (7.89 µg/ml) against HepG2 cells.

Gu et al. (2018) examined the anti-cancer potential of solanine A, 7 α -OH khasianine, 7 α -OH solamargine, 7 α -OH solasonine, solasodine, khasianine, β 2-solasonine, solasonine, solamargine and 12 β , 27-dihydroxy solasodine isolated from *S. nigrum* fruits were against human cancer cell lines: gastric (MGC803), liver (HepG2) and colon (SW480) using sulforhodamine B (SRB) assay. Only solanine A, solasodine, solasonine and solamargine exhibited anti-cancer effect with IC_{50} ranging from 5.02 to 23.79 µM towards tested cell lines, whereas highest effect found for solasodine (5.02 µM) against MGC803 cells as compared with KPT330 (0.14 to 0.38 µM) used as standard for MGC803, HepG2 and SW480 cells, respectively. However, other compounds were found to be inactive (> 50 µM) for tested cell lines.

Xiang et al. (2018) investigated the anti-proliferative activity of steroidal glycosides isolated from *S. nigrum* berries against human cancer cell lines: leukemia (HL-60), histiocytic lymphoma (U-937), T lymphocyte (Jurkat), immortalized myelogenous leukemia (K562) and liver (HepG2) using MTT assay. Among these glycosides, only [25S]-26-O- α -D-glucopyranosyl-cholest-5(6)-en-3 β , 26-diol-16, 22-dione-3-O- α -L-rhamnopyranosyl-(1/2)-[α -L-rhamnopyranosyl-(1/4)]- α -D-glucopyranoside showed weak anti-proliferative effect with IC_{50} value of 43.74 µM when compared to cisplatin (6.50 µM) used as positive control towards HepG2 cell line. However, other compounds did not exhibit effectiveness (> 50 µM) towards tested cell lines.

Gabrani et al. (2012) investigated the anti-proliferative activity of aqueous, methanol and ethanol extracts (0.3125 to 10 mg/ml) of *S. nigrum* against human cancer cell lines: Jurkat (leukemic T) and HL 60 (promyelocytic leukemia) using MTT assay. When compared with other extracts, only methanol extract was found more effective with IC_{50} of 3 and 4.8 mg/ml towards Jurkat T and HL 60 cell lines whereas, its effectiveness was found more pronounced on Jurkat cells.

Yuan et al. (2015) screened the anti-tumor potential of crude polysaccharides (31.25, 62.5 and 125 mg/kg, b.w.) from *S. nigrum* against erythrocyte membranes of tumor-bearing S180 and H22 in mice. Astragalus polysaccharide (100 mg/kg) was used as a standard drug. Polysaccharides (125 mg/kg, b.w.) improved S180 and H22 tumor-bearing mice erythrocyte membrane fluidity. Moreover, the extract significantly ($p < 0.05$, $p < 0.01$) improved the red blood cell membrane and S180 tumor-bearing mice sealing ability when compared with negative control group. H22 tumor-bearing mice increased the red cell membrane and sealing ability, where difference was found significant ($p < 0.05$).

Huang et al. (2018) reported the anti-tumor effect of polysaccharide (30, 60 and 120 mg/kg, b.w.) from *S. nigrum* against tumor growth in H22-bearing ICR mice through regulation of caspase-3 and Bcl-2 *in vivo*. Polysaccharide (120 mg/kg, b.w.) inhibited the tumor weight with percentage inhibition 42.60% when compared with cyclophosphamide (30 mg/kg) used as positive control (68.25%). Moreover,

polysaccharide significantly ($p < 0.05$) increased the index of thymus while, did not affect the spleen index when compared with model group. Further, infiltration into fat, extensive necrosis and apoptosis of tumor cells were found in lower dose of extract while, shrinking necrosis of tumor cells and thymic cell proliferation were found in medium dose of polysaccharide treated group. In addition, polysaccharide increased the protein expression of caspase-3 while, decreased the expression of Bcl-2 in tumor tissue when compared with model group.

Shi et al. (2019) evaluated the anti-tumor activity of aqueous extract along with solaoiacid and solasonine isolated from fresh ripe berries (SNFA), dried ripe berries (SNDA) and whole plant (SNLH) of *S. nigrum* against lung cancer (A549) cells using MTT assay and Lewis tumor bearing-male C57BL/6 mice *in vivo*. All extracts at 40 μ g/ml exhibited anti-tumor effect with percentage inhibitions as 50, 15 and 18%, whereas solaoiacid and solasonine showed effectiveness with IC_{50} of 2.36 and 8.7 μ mol/l towards lung cancer cells. Moreover, SNFA decreased the expressions of both mRNA levels of fatty acid synthase (FASN) and, estimated glomerular filtration rate (EGFR) by 30.5 and 38.7%. In *in vivo* study, SNFA (5, 10 and 20 mg/kg) inhibited tumor growth with 50.8% where average tumor weight was 3.85 after extract treatment while, average tumor weight in control group was 7.83. NSCLC deregulated 1469 proteins and 49 signaling pathways in response to SNFA treatment. SNFA upregulated the LDHC, HSD17B4, CAD and ACSS with absolute values

of logarithm of fold changes of 2.59, 0.81, 0.63 and 0.37, respectively.

Anti-colitis Activity

Joo et al. (2009) evaluated the anti-colitis activity of glycoprotein (10 and 20 mg/kg) isolated from *S. nigrum* fruits, on dextran sulfate sodium (DSS; 3%)-induced colitis in male A/J mice. Glycoprotein (20 mg/kg) significantly reduced ($p < 0.05$) the disease activity index, production of nitric oxide (NO), TBARS and LDH whereas, increased the colonic length ($p < 0.01$) when compared with DSS untreated group. Moreover, glycoprotein also decreased the activities of NF- κ B, AP-1, COX-2 and iNOS when compared with DSS treated group.

Anti-dermatitis Activity

Hong et al. (2020) evaluated the anti-dermatitis activity of ethanolic extract (1 and 10 mg/ml) of *S. nigrum* against 1-chloro-2,4-dinitrobenzene (DNCB)-induced atopic dermatitis in BALB/c mice (*in vivo*) and in TNF- α /IFN- γ -stimulated HaCaT cells (*in vitro*). The extract did not show any difference in body weight, liver weight, AST, and ALT levels while, significantly ($p < 0.05$) decreased the IgE concentration at 1 mg/ml when compared with control. Moreover, the extract significantly ($p < 0.05$, $p < 0.01$) reduced the epidermis thickness (10 mg/ml), CD8 and, mast cell number (1 mg/ml) when compared with control. However, the extract (1 to 100 μ g/ml) was found to be cytotoxic at 100 μ g/ml of concentration. In mRNA expression of TNF- α /IFN- γ -stimulated HaCaT cells, the extract

(50 μ g/ml) significantly ($p < 0.01$) decreased the levels of TARC and GM-CSF as well as, inhibited the CXCL-8 level in tested cells when compared with untreated group. In addition, the expressions of p-p38 (30 min) and p-NF- κ B (15 min) inhibited TNF- α /IFN- γ stimulation while, increased the p-p38 expression after 5 min by extract treatment.

Anti-diabetic Activity

Tai et al. (2016) demonstrated the anti-diabetic activity of aqueous extract (100 mg/kg; b.w.) from *S. nigrum* against advanced glycation end products (AGEs; 50 μ g/ml)-induced RAGE signaling and activation of hepatic stellate cells (HSCs) as well as, high-fat diet (HFD; 30%)/ethanol (10%)-induced hyperglycemia in male Wistar rats. Pioglitazone (10 mg/kg, b.w.) was used as positive control. The extract (10 μ M) inhibited enhancement of MMP-2 and α -SMA expressions caused by AGEs. Further, the extract significantly ($p < 0.05$) reduced insulin, blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, production of α -SMA when compared with HFD/ethanol untreated animal group. However, the extract effectively improved hepatic fibrosis induced by HFD/ethanol. Further, the extract significantly ($p < 0.05$) reduced total cholesterol, triglycerides levels but, HDL-C and FFA decreased by extract and increased by positive control. Similarly, the extract significantly ($p < 0.05$) increased the PPAR α , PGC-1 α , Nrf2 levels whereas, decreased the ACC, ChREBP, FAS mRNA levels, AGEs in liver when compared with untreated group.

Sohrabipour et al. (2013) investigated the anti-diabetic potential of aqueous extract (1 g/l) of *S. nigrum* fruits against streptozotocin-induced chronic diabetic (CD) and non-diabetic (ND) male Wistar rats. The extract decreased plasma glucose concentrations in CD rats but, there were no changes in ND rats. The extract caused plasma magnesium concentration increased in CD rats while, ND rats caused decrease in plasma magnesium level. Further, the extract caused plasma very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), total cholesterol and triglyceride concentrations return to normal levels whereas, plasma high-density lipoprotein level was significantly ($p < 0.001$) increased in CD and ND animals than other groups. In addition, the extract significantly ($p < 0.0001$) increased contractile responses to phenylephrine in the mesenteric beds of CD rats.

Umamageswari et al. (2017) evaluated the anti-diabetic effect of aqueous extract (200 and 400 mg/kg/day) of *S. nigrum* berries against alloxan-induced diabetes in albino Wistar rats *in vivo*. The extract (400 mg/kg) significantly ($p < 0.001$) reduced fasting blood glucose level at day 21 when compared with glimepride (0.1 mg/kg) used as positive control. Moreover, the extract (200 mg/kg) regenerate islets of Langerhans with infiltration of neutrophils whereas, positive control fully regenerated without inflammation.

Anti-hyperlipidemic Activity

Arulmozhi et al. (2010) conducted the anti-hyperlipidemic activity of aqueous fruit extract

(250 mg/kg; b.w.) of *S. nigrum* against ethanol (20%)-induced toxicity in male albino Wistar rats. Silymarin (25 mg/kg, b.w.) was used as standard drug. The extract significantly ($p < 0.05$) increased the final body weight while, decreased the liver weight when compared with ethanol-treated group. Moreover, the extract significantly ($p < 0.05$) reduced TBARS (plasma, liver, kidney), total cholesterol, LDL-C, VLDL-C, triglycerides, free fatty acids, phospholipids of plasma, total cholesterol, triglycerides and free fatty acids in liver and kidney whereas, enhance the reduced glutathione, vitamin C, E (plasma, liver, kidney), HDL-C of plasma and phospholipids of liver and kidney when compared with ethanol-treated group. In histopathological examination, the extract attained normal histology with mild congestion of central vein.

Anti-hyperglycemic Activity

Villaseñor and Lamadrid (2006) evaluated the anti-hyperglycemic effect of methanol extract of *S. nigrum* leaves against blood glucose levels using oral glucose tolerance test (OGTT) in male Swiss Webster mice. The extract insignificantly increased fasting blood glucose level when compared with control.

Anti-inflammatory and Anti-pyretic Activities

Xiang et al. (2018) reported the anti-inflammatory activity of steroidal glycosides (2.5 to 50 μ M) from *S. nigrum* berries against nitric oxide (NO) production-induced by lipopolysaccharide (LPS) in RAW 264.7 cell

line *in vitro*. The steroidal glycosides exhibited anti-inflammatory effect with IC_{50} ranging from 11.33 to 72.39 μ M, whereas (25R)-26-O- β -dglucopyranosyl-cholest-5(6)-en-3 β ,26-diol-16,22-dione-3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- β -dglucopyranoside showed highest effect (11.33 μ M) better than indomethacin (47.4 μ M) used as positive control towards LPS-induced NO production.

Gu et al. (2018) evaluated the anti-inflammatory effect of solanine A, 7 α -OH khasianine, 7 α -OH solamargine, 7 α -OH solasonine, solasodine, khasianine, β 2-solasonine, solamargine and 12 β , 27-dihydroxy solasodine (20 μ M) from *S. nigrum* fruits against lipopolysaccharide (LPS)-induced NO production in murine RAW264.7 macrophages. Among tested compounds, only solanine A exhibited anti-inflammatory effect with IC_{50} of 3.85 μ M towards NO production, where cytotoxic effect with 87.92% observed for murine RAW264.7 macrophages. However, other compounds were found to be inactive (>20 μ M).

Xiang et al. (2019) investigated the anti-inflammatory potential of (25R)-22 α N-4-nor-spirosol-5(6)-en-3 β -ol-6- α -l-3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- α -D-glucopyranoside, (25R)-22 α N-spirosol-5(6)-en-3 α -ol-7-oxo-3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- α -D-glucopyranoside, (25R)-22 α N-spirosol-4(5)-en-3 β -ol-6-

oxo-3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- α -D-glucopyranoside, solamargine and solasonine isolated from *S. nigrum* immature fruits against NO production-induced by lipopolysaccharide (LPS) in RAW 264.7 macrophages. Among these compounds, only (25R)-22 α N-4-nor-spirosol-5(6)-en-3 α -ol-6- α -l-3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)]- α -Dglucopyranoside exhibited anti-inflammatory effect with IC_{50} value of 23.42 μ M as compared with indomethacin (47.40 μ M) used as standard for NO production. However, other compounds were found to be inactive (>50 μ M).

Zakaria et al. (2009) demonstrated the anti-pyretic effect of aqueous extract (20, 100 and 200 mg/kg) of *S. nigrum* leaves against Brewer's yeast (BY)-induced pyrexia in Sprague-Dawley rats. The extract exhibited a significant ($p < 0.05$) reduction in body temperature of treated rats when compared to acetylsalicylic acid used as positive control.

Anti-microbial and Anti-viral Activities

Sunitha et al. (2017) reported the anti-microbial activity of alcoholic extract of *S. nigrum* leaves against caries causing bacterial strains: *Lactobacillus*, *Streptococcus mutans*, *S. oralis*, *S. salivarius* and *S. sanguis* using broth dilution method. The extract exhibited anti-microbial effect with MIC ranging from 25 to 100 mg/ml towards all tested strains whereas potent effect observed for *Lactobacillus* (25 mg/ml).

Javed et al. (2011) evaluated the anti-microbial potential of methanol, chloroform extracts and its n-hexane fraction (3.12 to 200 µg) of *S. nigrum* seeds against hepatitis C virus (HCV 3a)-infected liver (Huh-7) cells using MTT assay. The methanol extract showed effectiveness with 37% inhibition of HCV RNA at non-toxic concentration (100 µM). Similarly, a significant inhibition against HCV was shown by chloroform extract with more than 50% reduction (concentration of viral titer) and, n-hexane fraction minorly decreased the viral survival (95%) when compared with control (100%). In addition, chloroform extract dose-dependently inhibited HCV RNA expression and interferon, while GAPDH mRNA expression remains unaffected by extract when compared with control.

Anti-malarial Activity

Haddad et al. (2017) studied the anti-malarial activity of methanolic extract of *S. nigrum* fruits against *Plasmodium falciparum* (3D7 and K1 strains)-infected Raji cells *in vitro* and *P. berghei*-infected female albino Swiss mice *in vivo*. The extract exhibited anti-plasmodial effect with IC₅₀ value of 10.29 and 18.67 µg/ml towards 3D7 and K1 strains, where CC₅₀ value of 66.3 µg/ml for tested cells and indicated non-toxic effect. Moreover, the extract (100 mg/kg) suppressed the parasitemia of *P. berghei* with 60.68%.

Anti-nociceptive Activity

Zakaria et al. (2009) investigated the anti-nociceptive activity of aqueous extract (1

to 100%) of *S. nigrum* leaves against acetic acid-induced abdominal constriction and hot plate tests in male BALB/c mice as well as, formalin (5%)-induced pain in Sprague-Dawley rats. Acetylsalicylic acid (ASA; 100 mg/kg) and morphine sulfate (5 mg/kg) were used as standard drugs. The extract at 10 and 100% exhibited a significant ($p < 0.001$) anti-nociceptive effect (>80%), while 50% concentration of extract did not show any significant effect against acetic acid-induced abdominal constriction in mice. The extract at all concentrations attained a significant ($p < 0.05$) effect until the end of experiment but was less effective than morphine for hot plate test in mice. Also, the extract at all concentrations was found to produce a significant ($p < 0.05$) effect in the early and late phases of formalin-induced nociception in rats.

Anti-obesity Activity

Peng et al. (2020) investigated the anti-obesity activity of aqueous extract (0.5, 1 and 2%) and derived polyphenols (0.1 to 0.5 mg/ml) from *S. nigrum* against adipocytes 3T3L1 cells *in vitro* and high fat diet (HFD)-induced obesity in male C57BL/6 mice. The extract at 2% concentration significantly ($p < 0.05$) reduced the body weight, perirenal fat, epididymis fat, body fat, triglyceride, TC, HDL-C, LDL-C, aspartate aminotransferase and alanine aminotransferase levels. Moreover, the extract significantly ($p < 0.01$) decreased the hepatic expressions of GPAT, FaS, SREBP-1, HMG-CoR and SREBP-2 while, increased the levels

of AMPK-P, CPT-1 and PPAR α when compared with HFD treated group. The polyphenols (0.5 mg/ml) reduced the number of 3T3 cells to approximately 55% after treatment for 24 h. Polyphenols ameliorated all lipid contents to the level of pre-3T3-L1. In addition, polyphenols also significantly ($p < 0.05$) decreased the FaS, SREBP-1, HMG-CoR and SREBP-2 levels, while increased the levels of AMPK-P, CPT-1 and PPAR α in adipocytes when compared with untreated cells.

Chang et al. (2017) evaluated the anti-obesity activity of polyphenolic extract (0.5, 1 and 2%) of *S. nigrum* on oleic acid (OA) (0.5 mM)-induced hepatic steatosis of human hepatoma (HepG2) cells using MTT assay as well as, high fat diet (HFD)-induced obesity in male C57BL/6 mice. The extract at 4 mg/ml concentration showed ~38 to 42% reduction of HepG2 cell numbers. The extract at 2% significantly ($p < 0.05$) decreased the AST, HDL-C, LDL-C, total cholesterol, AST/ALT ratio, liver weight, TG, cholesterol, MD A, and superoxide dismutase levels whereas, increased the catalase level when compared with HFD untreated group. Moreover, the extract at 0.5 mg/ml concentration significantly ($p < 0.05$) attenuated the lipid accumulation when compared with untreated OA group. Further, the extract significantly ($p < 0.05$) increased AMPK-P whereas, decreased the relative expressions of FAS, SREBP-1, HMG-CoA, SREBP-2, CPT-1 and PPAR- α levels when compared with OA treated group. In addition, the extract (2%) decreased IL-6 and TNF- α expression levels when compared with those in the HFD treated group.

Antioxidant Activity

Campisi et al. (2019) conducted the antioxidant activity of methanol/water (80:20) and water extracts (0.025 to 0.4 mg/ml) of *S. nigrum* leaves using DPPH assay as well as, GSH and ROS levels in primary rat astroglial cells. Both extracts at higher dose showed a significant ($p < 0.05$ and $p < 0.001$) decrease with >80% which was like trolox (>80% at 30 mM) used as positive control for DPPH assay. The extracts at 1 mg/ml significantly ($p < 0.001$) restored the GSH and ROS levels in primary rat astroglial cells.

Meng et al. (2020) evaluated the antioxidant activity of crude powder along with petunidin-3-(cis-p-coumaroyl)-rutinoside-5-glucoside (PCRG) and petunidin-3-(p-coumaroyl)-rutinoside-5-glucoside (PTCRG) from *S. nigrum* fruits using DPPH, ABTS and FRAP assays. The crude powder and tested compounds exhibited inhibitory effect with IC_{50} values of 1.25, 0.52 and 0.52 μ M (DPPH), 1.49, 0.76 and 0.75 μ M (ABTS) and, 0.63, 0.29 and 0.29 μ M (FRAP), respectively.

Zaghlool et al. (2019) reported the antioxidant activity of ethanolic extract of *S. nigrum* using DPPH assay. The extract exhibited DPPH scavenging effect with IC_{50} value of 295.84 μ g/ml when compared with ascorbic acid (10 μ g/ml) used as standard.

Omojokun et al. (2019) investigated the antioxidant activity of alkaloidal extract of *S. nigrum* leaves using DPPH, OH, NO assays and Fe $^{2+}$ -induced lipid peroxidation in rat's penile tissues. The extract exhibited antioxidant

effect with EC_{50} of 55.77, 242.72 and 735.29 $\mu\text{g/ml}$ for DPPH, OH and NO assays, respectively, and 165.01 $\mu\text{g/ml}$ towards lipid peroxidation in rat's penile tissues.

Anti-seizure Activity

Wannang et al. (2008) investigated the anti-seizure activity of aqueous extract (5 to 60 mg/kg; b.w.) of *S. nigrum* leaves against electrically induced seizure in chicks and albino rats as well as, pentylenetetrazole (90 mg/kg) and picrotoxin (90 mg/kg)-induced seizure in albino mice and rats. The extract (60 and 40 mg/kg) showed anti-seizure effect with 75 and 100% protection when compared with control (0%) towards electrically induced seizure in chicks and rats. Moreover, the extract exhibited 100% protection for pentylenetetrazole-induced seizure in rats at 60 mg/kg and mice at 30 mg/kg. Also, the extract (60 mg/kg) attained effectiveness on picrotoxin-induced seizure with 75 and 100% in rats and mice, respectively.

Anti-ulcer Activity

Jainu and Devi (2006) investigated the anti-ulcer activity of methanol extract (200 and 400 mg/kg; b.w.) of *S. nigrum* against cold restraint stress (CRU), indomethacin (IND), pyloric ligation (PL), ethanol (EtOH) and acetic acid-induced gastric ulcer in male albino Wistar rats. The extract (400 mg/kg) exhibited anti-ulcer effect with 76.6, 73.8, 80.1 and 70.6% protection towards CRU, IND, PL and EtOH models, respectively, when compared with

omeprazole (78.8, 71.2 and 83% at 10 mg/kg) used as standard. In pylorus-ligated rats, the extract significantly ($p < 0.001$) decreased gastric juice volume and reverse the increased output of acid and pepsin secretion. Moreover, the extract also significantly ($p < 0.001$) inhibited the H(+)K(+)ATPase activity of gastric mucosa but, reduced the plasma gastrin hormone concentration when compared with EtOH untreated group.

Akhtar and Munir (1989) evaluated the anti-ulcer potential of powder (1, 2 and 4 g/kg) along with methanol and aqueous extracts (~4 g/kg) of *S. nigrum* aerial parts against aspirin-induced gastric ulcers in albino male Sprague-Dawley rats. The powder at 4 g/kg and methanol extract exhibited higher inhibition with 28.1 and 23.3% in aspirin-induced gastric ulcer followed by aqueous extract (3.2%) when compared with gefarnate (19% at 15 mg/kg) used as standard. Moreover, the extract significantly ($p < 0.01$) decreased pepsin levels but, powdered forms did not affect the pepsin concentration. Both extracts did not show any significant effect on hexosamine levels and gastric acid output in aspirin-ulcerated rats. Similarly, both extracts for acid output and methanol extract for pepsin concentration attained a significant suppression in normal rats when compared with control.

Cardioprotective Activity

Lin et al. (2022) demonstrated the cardioprotective effect of aqueous extract (100 and 300 mg/kg; b.w.) of *S. nigrum* against angiotensin-II (AngII; 100 nM)-induced cardiac hypertrophy and cardiac

function in cardiomyoblast cells of male Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) *in vivo*. The extract attenuated the AngII-induced cardiac hypertrophy as well as, suppressed the hypertrophic signaling mediators like Protein Kinase C- α , Mel-18, and IGF1R in dose-dependent manner while, HSF2 activation (restore deSUMOylation) leads to down-regulation of IGF-1R expression. In addition, the extract reduced heart size in treated SHRs rats when compared to control WKY rats.

Cercaricidal Activity

Ahmed and Ramzy (1997) evaluated the cercaricidal activity of water extract of *S. nigrum* leaves collected in Fayium (FLWE) against *Schistosoma haematobium*, *S. mansoni* and *Fasciola gigantica*. The extract exhibited cercaricidal effect with LC₁₀₀ of 10, 10 and 15 mg/l, respectively towards tested strains after 90 min exposure.

Cytoprotective Activity

Kumar et al. (2001) studied the cytoprotective activity of ethanol extract (10, 100 and 1000 μ g/ml) of *S. nigrum* against gentamicin (1 mM)-induced toxicity on Vero cells using Trypan blue exclusion and MTT assays. In trypan blue assay, the extract significantly ($p < 0.01$) reverted gentamicin toxicity where, cell viability was found to be 84.23% at 1000 μ g/ml dose. Moreover, tested cells exposed to extract exhibited a dose-dependent reduction of MTT revealing preservation of viability in presence of extract.

Effect on Bones

Kim et al. (2021) reported the effectiveness of ethanolic extract (50 to 100 mg/kg; *in vivo* and 5 to 40 μ g/ml; *in vitro*) of *S. nigrum* against ovariectomy (OVX)-induced osteoporosis in Sprague Dawley rats *in vivo* and RANKL-stimulated RAW 264.7 cells *in vitro*. 17 β -estradiol (E2; 100 μ g/kg; i.v.) was used as positive control. The extract (100 mg/kg) significantly ($p < 0.05$ and $p < 0.01$) increased bone mineral density, bone volume/total volume, trabecular number, trabecular area whereas, decreased the trabecular separation, tartrate-resistant acid phosphatase (TRAP), nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1) and CTsK positive cells. Moreover, the extract at 40 μ g/ml significantly ($p < 0.05$ and $p < 0.01$) reduced RANKL stimulated osteoclasts number, TRAP, pit area, formation of actin rings, protein, and mRNA expressions of NFATc1 and c-Fos as well as, decreased the expression of osteoclast-associated genes; *Acp5*, *Ctsfk*, cathepsin K, *Mmp9*, *Oscar*, *Atp6v0d2* and *Ca2*, without affecting the cell viability when compared with RANKL group. However, lactate dehydrogenase (LDH) was slightly increased after extract treatment.

Youn et al. (2008) evaluated the effectiveness of methanol extract (0.1 and 0.2 mg/ml) from *S. nigrum* on osteoclastogenesis regulated by receptor activator of nuclear factor NF- κ B ligand (RANKL)-induced osteoclast differentiation in bone marrow cells of C57BL/6 male mice *in vivo*. The extract at 0.1 and 0.2 mg/ml showed suppression in osteoclastogenesis of mice.

Enzyme Inhibitory Activity

Omojokun et al. (2019) evaluated the enzyme inhibitory activity of alkaloidal extract (0 to 30.51/133.69 µg/ml) of *S. nigrum* leaves against phosphodiesterase~5 (PDE~5) and arginase enzymes. The extract exhibited inhibitory effect with EC₅₀ of 133.69 and 26.92 µg/ml towards PDE~5 and arginase enzymes, respectively.

Hameed and Akhtar (2018) conducted the enzyme inhibitory potential of methanol extract, its n-hexane, ethyl acetate and water fractions (1 mg/ml) of *S. nigrum* against tyrosinase enzyme. The extract and its fractions exhibited inhibitory effect with percentage inhibitions ranging from >25 to ~60%, whereas highest effect found for ethyl acetate fraction (~60%) for tested enzyme.

Ogunsuyi et al. (2020) reported the enzyme inhibitory activity of aqueous and alkaloid extracts of *S. nigrum* leaves against monoamine oxidase (MAO) and acetylcholinesterase (AChE) enzymes. The aqueous extract exhibited potent inhibitory effect with IC₅₀ of 1.17 and 1.02 mg/ml where alkaloid extract attained effectiveness of 0.56 and 1.52 mg/ml towards AChE and MAO enzymes, respectively.

Estrogenic Activity

Jisha et al. (2011) assessed the estrogenic activity of defatted methanol extract (20 to 320 µg/ml and 100 to 500 mg/kg; b.w.) of *S. nigrum* dried fruits against estrogenicity in MCF-7 cell lines using hydroxy apatite (HAP) binding assay and, also on ovariectomized

mice by uterotrophic assay. The extract induced dose-dependent proliferation for MCF-7 breast cancer cells. However, higher concentrations (80 to 320 µg/ml) exhibited progressive cell growth inhibition. Moreover, extract significantly inhibited the binding of [3H]-E2 to cytosolic estrogen receptor with IC₅₀ of 40 µg/ml. In histological observation, the extract induced maximum height of luminal epithelial cells which indicated an increase (30.8%) over control (p<0.01). Also, the extract significantly increased the wet weight of uterus relative to control group (p<0.001) which was increased by 150% in ovariectomized mice. However, the extract did not induce any uterotrophic effect.

Gastroprotective Activity

Zaghlool et al. (2019) investigated the gastroprotective activity of ethanolic extract (200 mg/kg/day) of *S. nigrum* against pyloric ligation/indomethacin-induced ulceration in albino male rats. Omeprazole (20 mg/kg/day) was used as positive control. The pre-treatment of extract attained reduction in ulcer number, ulcer index, histamine, and gastrin contents about 37.89, 44.71, 44.27 and 41.17%, respectively, as well as elevation in GSH, NO contents and SOD activity to 617.70, 589.58 and 579.37%, respectively, when compared to gastric ulcer control group. *Solanum nigrum* pretreatment significantly increased mucin content to 245.52%, in comparison to the gastric ulcer control group. Also, it significantly decreased mucin content to 56.96%, in comparison to the omeprazole (1st standard

pretreatment) group. Further, the extract caused changes in gastric volume, titratable acidity, or acid output when compared to gastric ulcer group. Likewise, the extract exhibit elevation in PG-E2 content to 573.42% while, increased PG-I2 content insignificantly in comparison to gastric ulcer control. In addition, the extract significantly decreased the TNF- α and IL-1 β contents to 37.76 and 42.53% while, increased CBS and HO-1 activities to 271.3 and 230.65% in comparison to gastric ulcer group.

Hepatoprotective Activity

Liu et al. (2016) reported the hepatoprotective activity of aqueous extract (50, 75 and 100 μ g/ml) of *S. nigrum* against ethanol-induced acute hepatic injury in primary hepatocytes and adult male Kunming mice. Silymarin (200 mg/kg) was used as positive control. The extract at 100 μ g/ml significantly ($p < 0.05$) decreased the ALT, AST, and MDA level whereas, increased the SOD and GSH levels in hepatocytes and mice when compared with untreated group. Moreover, the extract significantly ($p < 0.01$) reduced the GSTA1 content in hepatocyte when compared with untreated group. Further, extract significantly ($p < 0.01$) reduced the GSTA1 contents in serum while, enhanced GSH-Px and GSTA1 levels in liver when compared with animal untreated group.

Akshatha et al. (2018) evaluated the hepatoprotective activity of aqueous and alcoholic extracts (200 and 400 mg/kg; b.w.) of *S. nigrum* leaves against N-nitrosodiethylamine (NDEA; 200 mg/kg)-induced hepatocellular carcinoma (HCC) in Wistar rats. Sorafenib

(11.4 mg/kg) was used as positive control. The alcoholic extract reduced the severity of lesions in the liver in treated rats. Moreover, both extracts also increased the activity of cleaved caspase 3, a marker of apoptosis (programmed cell death), in liver cells. Both the extracts at 400 mg/kg exerted reduced degeneration and distortion of hepatocytes and marked rejuvenation of hepatic architecture when compared with HCC group.

Hsu et al. (2009) evaluated the hepatoprotective activity of aqueous extract (1 and 2%) of *S. nigrum* against 2-acetylaminofluorene (AAF) and AAF/NaNO₂-induced hepatic injury and hepatocarcinogenesis in male Wistar rats. The extract at 2% significantly ($p < 0.05$) suppressed the increase of body weight and liver weight. Moreover, extract at 2% significantly ($p < 0.05$) reduced the serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, (C) γ -GT level and alpha fetoprotein (AFP) levels in plasma whereas, increased the GSTs and Nrf2 expressions in liver when compared with AAF-treated group. Further, the extract also increased the levels of GPx, SOD-1 and catalase to 2, 1.6 and 2.3-folds as compared to AAF treatment. The extract effectively alleviated the hepatic fibrosis and hepatoma induced by AAF/NaNO₂ treatment in rats resulted in a great increase of the survival rate.

Abdel-Rahim et al. (2014) studied the hepatoprotective activity semi-modified dried fruits and ethanolic extract (300 mg/kg) of *S. nigrum* against cadmium chloride (CdCl₂; 88

g/kg)-induced hepatic damage in albino male rats. Treatment with both fruits and extract ameliorate and improved the harmful effects in all above parameters either for blood (Hb, Hct, RBC, WBC count, MCHC, MCV and MCH values) and liver (ALT, AST, and total bilirubin) in CdCl₂-intoxicated rats.

Immunomodulatory Activity

Pu et al. (2020) investigated the immunomodulatory activity of homogeneous polysaccharide (SNLP-1) from *S. nigrum* stem against innate immune system in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages as well as, Lewis lung carcinoma (LLC) cell lines-induced tumor growth in female C57BL/6 mice. Treatment with SNLP-1 significantly ($p < 0.05$) reduced the production of nitric oxide, tumor necrosis factor- α (TNF- α) and interleukin (IL-6) and, induced mRNA and protein levels of critical nodes (except TRAM) in TLR4 signaling pathways. Moreover, SNLP-1 inhibited the tumor with 20.62 and 45.68% whereas, SNLP-1 significantly ($p < 0.05$ and $p < 0.01$) increased the spleen and thymus index as well as, CD4⁺/CD8⁺ ratio in peripheral blood higher. Further, SNLP-1 significantly ($p < 0.05$) decreased the production of IL-2, IFN- γ and TNF whereas, elevated the mRNA and protein expressions of TLR4, MyD88, TRAF6, p-NF- κ B and p-c-Jun when compared with LPS group.

Larvicidal Activity

Rawani et al. (2013) reported the larvicidal activity of crude (1-3%) and chloroform:

methanol extracts (1:1, v/v) of *S. nigrum* fresh, mature, green berries against instar larval forms (1st to 4th) of *Culex quinquefasciatus*. The chloroform: methanol extract exhibited potent larvicidal effect with values of 48.41, 54.20, 23.54 and 61.15 μ g/ml towards 1st, 2nd, 3rd, and 4th instar of *C. quinquefasciatus*, respectively, after 72 h. Moreover, the crude extract at 3% exert significantly ($p < 0.05$) higher mortality rate for instar larval forms.

Rawani et al. (2014) evaluated the larvicidal potential of glucosinolate from *S. nigrum* leaves against 1st to 4th larval forms of *Culex quinquefasciatus*. The compound exhibited larvicidal effect with LC₅₀ of 10.08, 12.22, 13.93 and 27.77 ppm after 72 h towards 1st to 4th instars larval forms of *C. quinquefasciatus*.

Rawani et al. (2010) investigated the larvicidal effect of crude extract of *S. nigrum* (0.1 to 0.5%) fresh, mature, green leaves against instar larval forms of *Culex quinquefasciatus*. The extract exhibited larvicidal effect with LC₅₀ values of 0.06, 0.02, 0.05 and 0.08% after 72 h treatment towards 1st, 2nd, 3rd, and 4th instar of *C. quinquefasciatus*.

Rawani et al. (2017) studied the larvicidal activity of phytosteroid isolated from chloroform: methanol extract (1:1; v/v) of *S. nigrum* mature leaves (25, 45 and 60 mg/l) against early 3rd instar larvae of *Culex vishnui* and *Anopheles subpictus*. The compound showed lethal effect with LC₅₀ values of 5.64 and 3.68 mg/l towards *Cx. vishnui* and *An. subpictus* after 72 h.

Molluscicidal Activity

Rawani et al. (2014) demonstrated the molluscicidal activity of petroleum ether, benzene, ethyl acetate, chloroform: methanol (1:1), acetone and absolute alcohol extracts (75, 100 and 150 ppm) of *S. nigrum* leaves against adult *Lymnaea acuminata* (an intermediate host of parasites causing fasciolopsiasis). All extracts exhibited molluscicidal effect with LC_{50} values ranging from 46.37 to 172.12 ppm whereas, chloroform: methanol extract exert highest effectiveness (46.37 ppm) towards tested snail after 72 h.

Ahmed and Ramzy (1997) evaluated the molluscicidal activity of leaves powder and aqueous extract of *S. nigrum* (prepared from Fayium and Giza origin) leaves and fruit against snail species (*Biomphalaria alexandrina*, *Bulinus truncatus* and *Lymnaea natalensis*). Both powder and extract exhibited molluscicidal effect with LC_{50} ranging from 10.5 to 63.1 mg/l towards tested strains after 24 and 48 h exposure, whereas highest effect observed for Fayium leaves aqueous extract (10.5 mg/l) against *B. truncatus* at 48 h.

El-Sherbini et al. (2009) tested the molluscicidal activity of petroleum ether, chloroform, acetone, methanol, and ethanol extracts from *S. nigrum* leaves against adult *Biomphalaria alexandrina*. All tested extracts exhibited molluscicidal effect with LC_{50} ranging from 4.2 to 70.75 ppm after 24 h where petroleum ether showed highest effect (4.2 ppm) towards *B. alexandrina*.

Ojewole (2004) studied the molluscicidal

activity of aqueous extract of *S. nigrum* aerial parts and leaves against *Schistosoma haematobium* and *S. mansoni* infections. Treatment with extract showed mild to moderate molluscicidal effect with 100-200 ppm when compared with niclosamide (0.20-0.8 ppm) used as standard against tested strains.

Neuroprotective Activity

Li et al. (2019) evaluated the neuroprotective potential of cannabisin F (12.5, 25 and 50 μ M) from *S. nigrum* against 1-methyl-4-phenylpyridinium (MPP⁺, 1 mM)-induced injury in SH-SY5Y cells. Cannabisin F (50 μ M) showed most significant ($p < 0.05$) neuroprotective effect with cell viability was improved by 12%. Moreover, cannabisin F suppressed the morphological characteristics of apoptotic cells. Further, it also reduced apoptotic ratio whereas, enhanced monodansylcadaverine (MDC) positive ratio (30.2%). Also, it induced protective autophagy to protect SHSY5Y cells from MPP⁺-induced apoptosis.

Ogunsuyi et al. (2018) reported the neuroprotective activity of aqueous extract (5 and 10%) of *S. nigrum* leaves towards scopolamine (SCOP, 2 mg/kg)-induced cognitive and neurochemical impairments in albino rats. The extract (10%) ameliorates the impaired memory function and also reversed the increasing activities of AChE, BChE and brain nitrite content ($p < 0.05$) as well as, elevation in MAO and MDA contents in SCOP-induced rats. Moreover, the extract significantly ($p < 0.05$) reversed the depletion

in brain GSH content which was induced by SCOP when compared with untreated group. Pre-treatment with extract ameliorated the reduction in activities of brain GST and SOD induced by SCOP.

Perez et al. (1998) evaluated the neuroprotective effect of ethanol extract (51, 127.5 and 255 mg/kg, b.w.) of *S. nigrum* fruits against pentobarbital (50 mg/kg)-induced sleeping time, Y-maze, traction and pentylenetetrazole (PTZ; 50 mg/kg) seizure tests in Wistar rats as well as, amphetamine (5 mg/kg)-induced toxicity, head-dip, evassive, rotarod, chimney and inclined tests in CD1 mice. The extract (255 mg/kg, b.w.) significantly ($p < 0.01$) increased

the duration of sleep and inhibited the spontaneous motility with 48.7% inhibition in locomotor test. Moreover, the extract significantly ($p < 0.01$) reduced the mean number of head dips and mean entry (Y-maze test) and, also caused a significant inhibition of residual curiosity in mice (Evassive test) when compared with control. In rotarod, traction, chimney and inclined screen tests, no motor incoordination was found at 255 mg/kg. A similar dose of 255 mg/kg of extract did not alter the flexor, extensor, and clonic phases of maximal electroshock (MES) seizures as well as did not exert any protection towards pentylenetetrazole-induced convulsion in rats.

Toxicological Study

Akshatha et al. (2018) tested the acute toxicity of aqueous and alcoholic extracts of *S. nigrum* leaves in Wistar rats. Both extracts did not show any toxic effect even at 2000 mg/kg doses in rats.

Umamageswari et al. (2017) reported the acute toxicity of aqueous extract from *S. nigrum* berries in albino Wister rats. The extract at 2000 mg/kg, b.w. did not produce any mortality,

while all animals were found safe, active, and healthy during the observation period.

Jainu and Devi (2006) evaluated the toxicity of methanol extract from *S. nigrum* in male albino rats of Wistar strain for 14 days. The extract at 4 g/kg, b.w. showed non-significant effect on RBC, WBC, Hb, HCT, MCV, blood sugar and urea when compared to control group indicating no adverse effect in treated rats.

Case Study

Oh et al. (2016) reported a case report of 72-year-old Korean man having seven-year history of diabetes suffering from acute interstitial nephritis (AIN), after ingestion of *S. nigrum* (orally at least two times/day

for 2 months) raw extract. The raw extract did not improve renal function while urine volume decreased from 2000 ml/day at 200 ml/day admission on day 8. In addition, patient complained of dyspnea. Therefore,

hemodialysis was initiated and, then planned a renal biopsy which revealed features of AIN, with diffuse interstitial infiltrate of

lymphocytes and eosinophils, some of which invaded the tubular cells.

Summary and Future Outlook

S. nigrum is a plant that grows in temperate and sub-tropical climates worldwide. This species has been used in traditional medicine for centuries in Ayurveda, Traditional Chinese Medicine, and other systems to treat various ailments. This plant has a high concentration of bioactive components, including phenolic compounds, flavonoids, alkaloids, and sterols, contributing to its pharmacological properties. Pre-clinical investigations of this species seem encouraging; nevertheless, more rigorous clinical trials are required to prove effectiveness and safety in humans. This species possesses antioxidant and anti-inflammatory properties, which reduce inflammation and discomfort caused by a variety of illnesses. Also, protects the liver from damage caused by poisons and illnesses as well as, limiting the development and multiplication of cancer cells. Furthermore,

this plant combats infectious diseases caused by bacteria and fungi. Likewise, this plant helps to reduce blood sugar and protect brain cells from damage and neurodegenerative illnesses. *S. nigrum* includes solanine, which is a poisonous glycoalkaloid. Consumption in excessive quantities can be poisonous. Proper preparation and dosing are critical. Furthermore, there is a need to investigate the mechanisms of action behind *S. nigrum*'s pharmacological effects, as well as perform well-designed clinical trials to assess its effectiveness and safety in certain circumstances. To assure the supply and quality of *S. nigrum*, sustainable production and harvesting procedures must be established. While further study is needed, *S. nigrum* may offer promise for managing a variety of health issues, particularly in areas with limited access to conventional medications.

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