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Monographs on Medicinal Plants along Ganga River
Achyranthes aspera L.



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along Ganga River

Achyranthes aspera L.

Focusing on

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

Volume 5



Monographs on Medicinal Plants along Ganga River

Achyranthes aspera L.

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

Volume 05

Sponsored by



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Department of Water Resources, River
Development & Ganga Rejuvenation,
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"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" of The
Ministry of Jal Shakti, Government of India
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

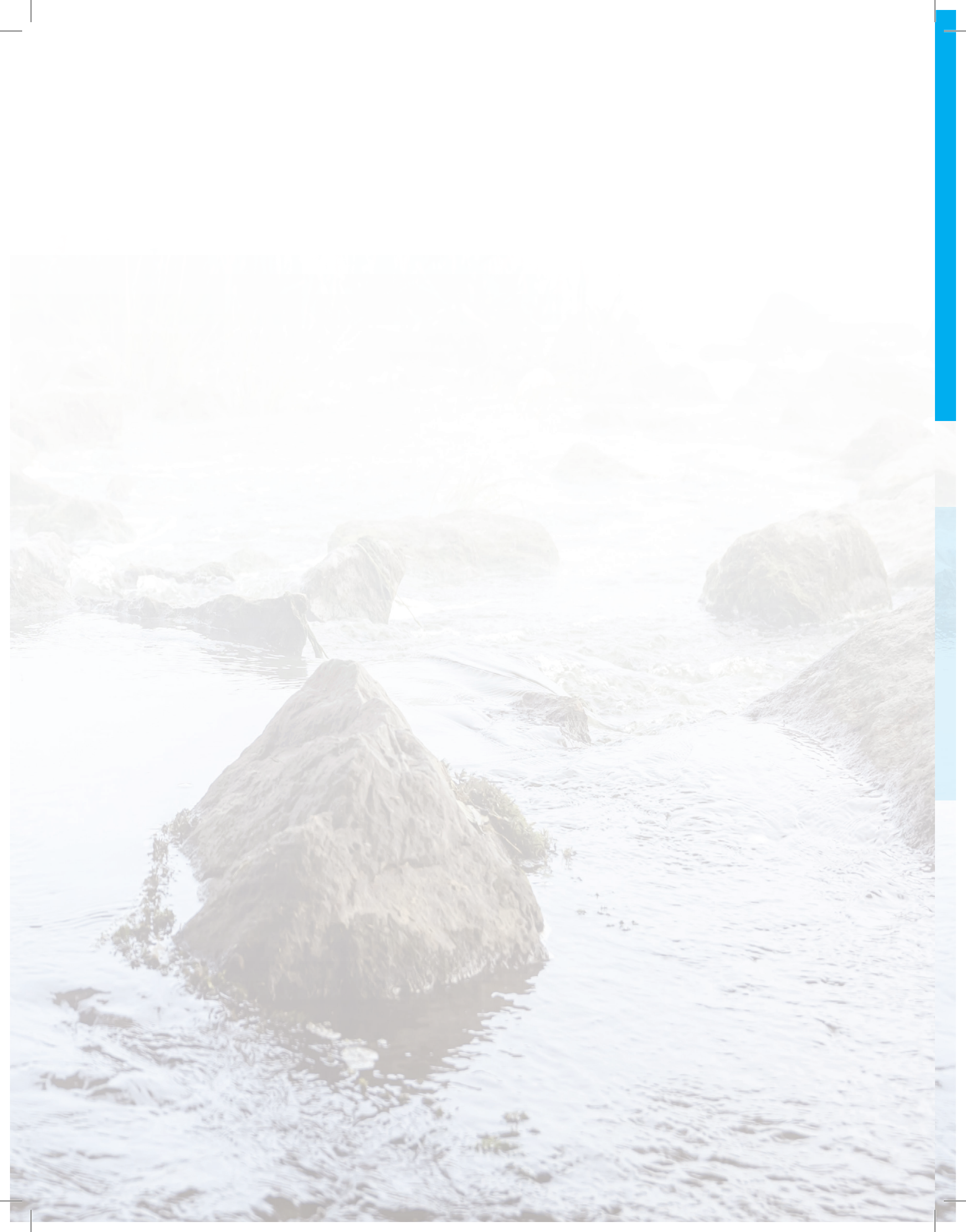
Shri Narendra Modi on Medicinal Plants



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

“

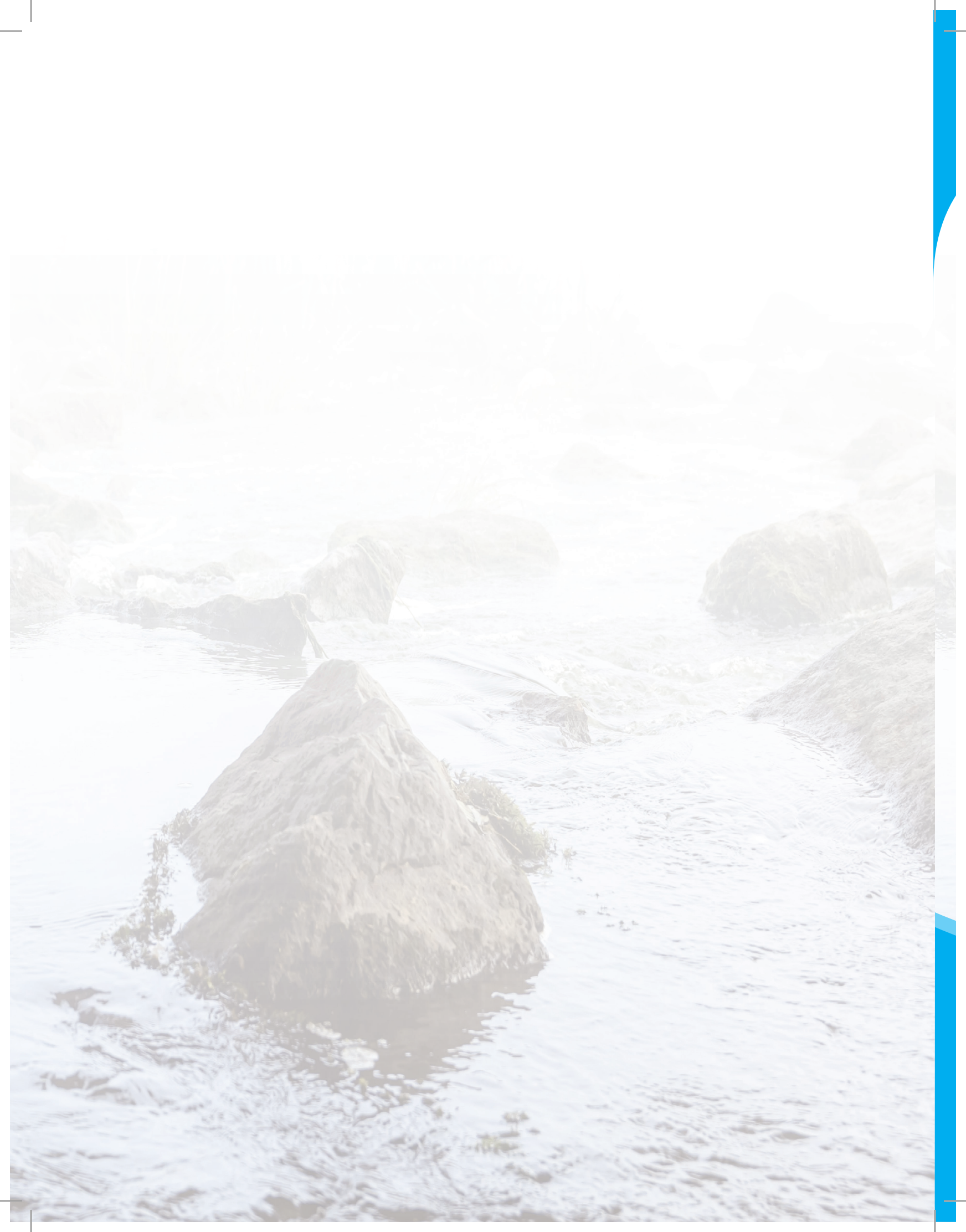
1. We will understand the importance of water, conserve it and ensure rich water resources for future generations.
2. Conservation and enhancement of water resources of our country is a sacred goal. I will work with my dedication and devotion to achieve this.
3. Proper access to water is a strong step 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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- ▶ Sunil Kumar, Environmental Engineer
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Ministry of Environment, Forest and Climate Change (MoEFCC)

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)

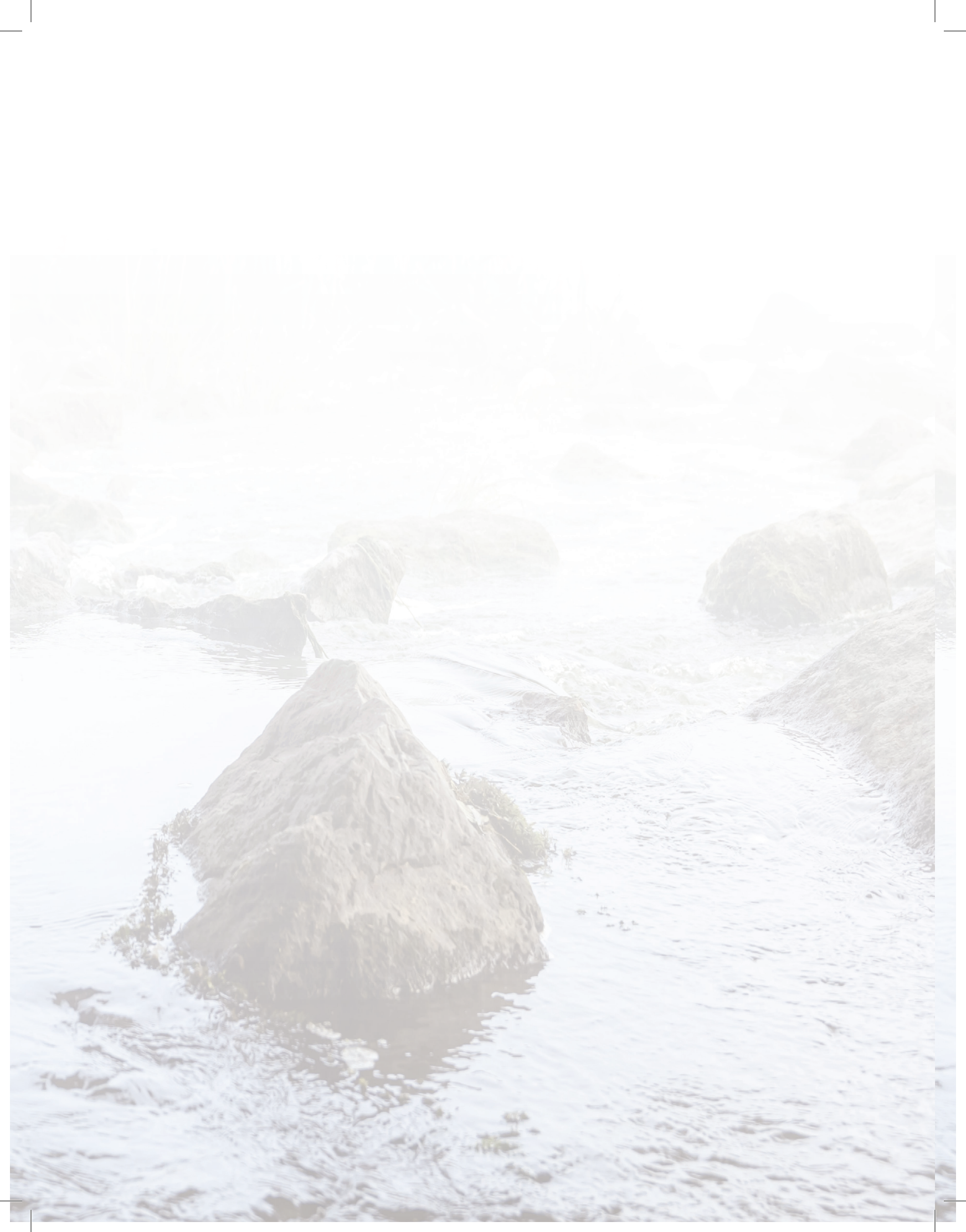


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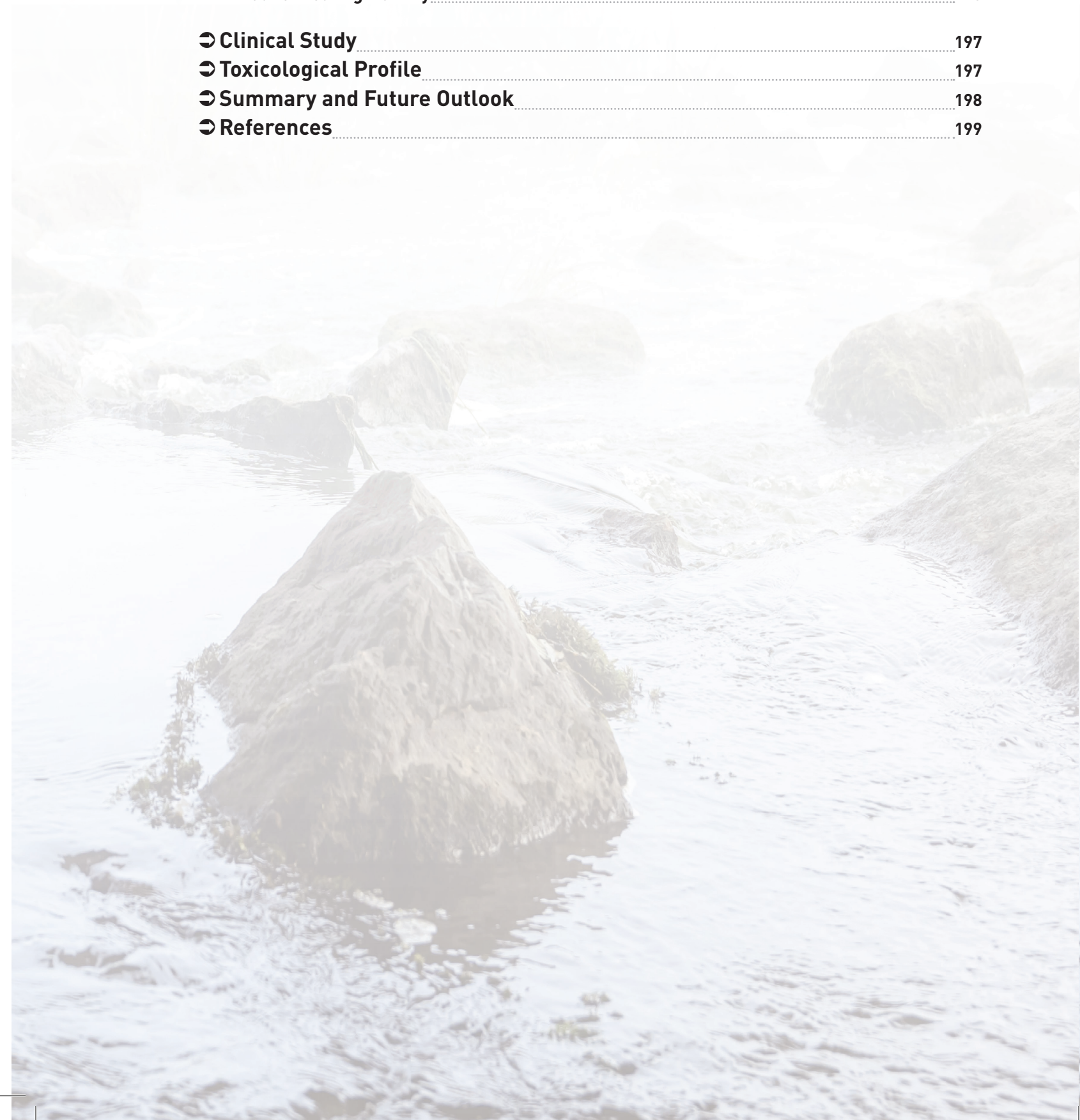
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DIRECTOR GENERAL
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सत्यमेव जयते
75
आज़ादी का
अमृत महोत्सव

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



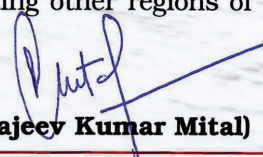
MESSAGE

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.

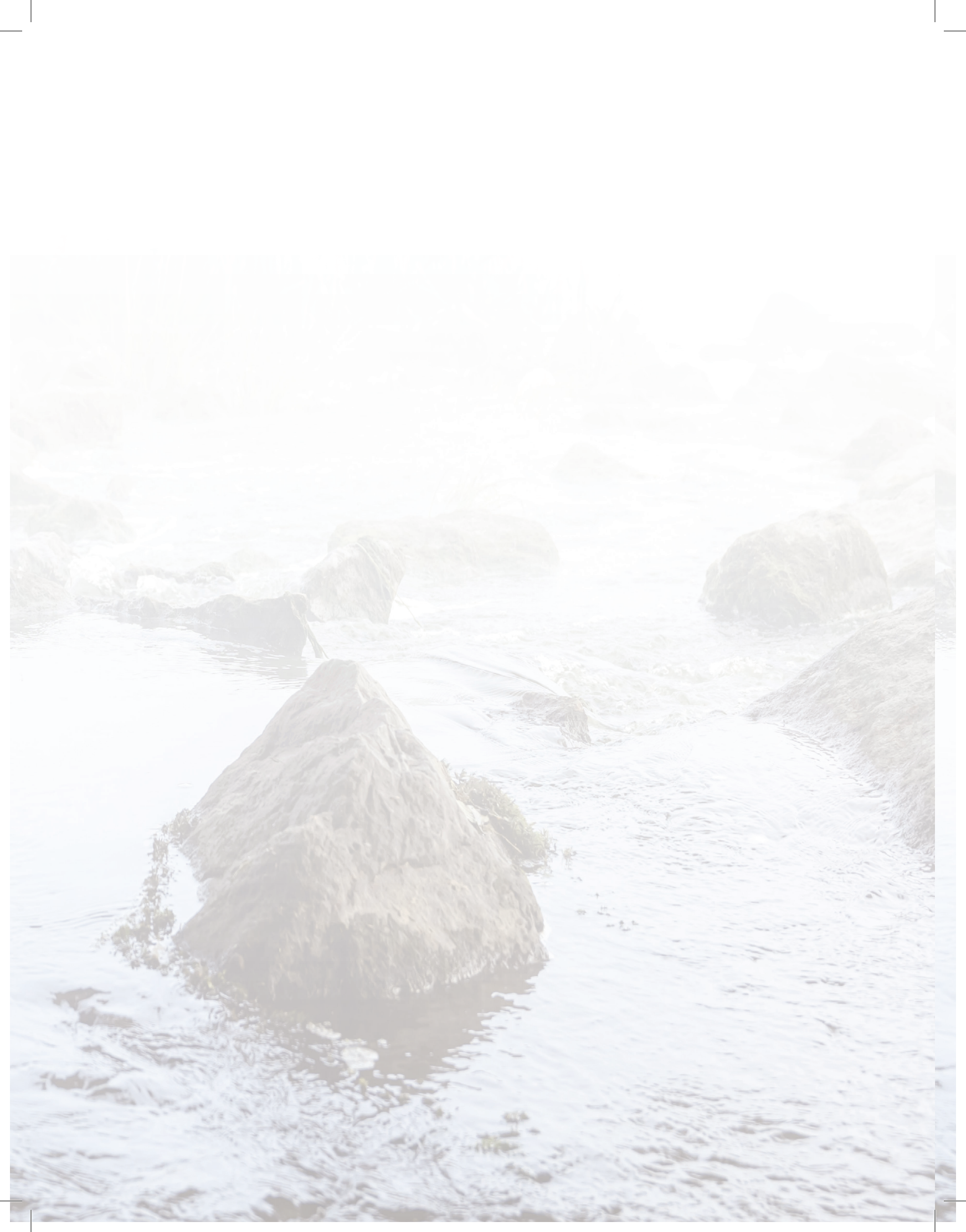
This excellent work documenting the floral diversity along the banks may pave the way for the rejuvenation of the Ganga and set a precedent for biodiversity conservation and socio-economic improvement, potentially benefiting other regions of India as well.


(Rajeev Kumar Mital)



राष्ट्रीय स्वच्छ गंगा मिशन
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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.

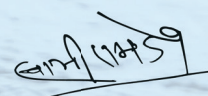
The authors have meticulously documented the plant exploration and botanical study, soil properties analysis, phytochemical analysis, and insilico analysis of the plants found in this region. Their dedication and deep respect for nature's wisdom are evident throughout the pages, making this monograph 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.

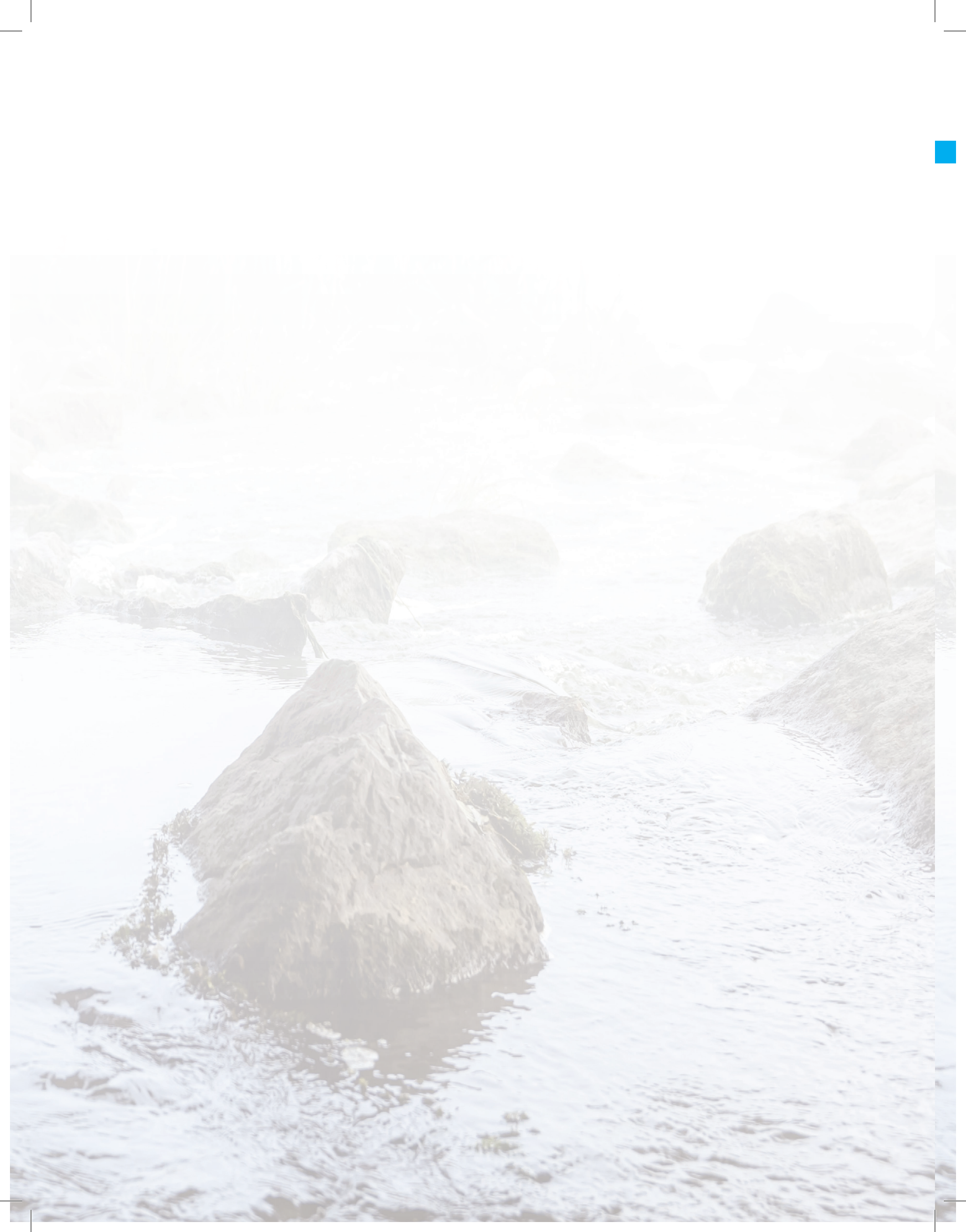
May this monograph serve as a guiding light for researchers, practitioners, and enthusiasts alike, illuminating the path towards a deeper appreciation of nature's pharmacy along the revered Ganga River.

With heartfelt gratitude and blessings,



- Swami Ramdev





PREFACE

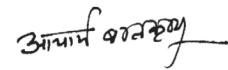
The Ganga River, revered as the lifeblood of India, flows through the heart of the country, nourishing not only the land but also the spirit of the people. It is not just a geographical entity; it is a symbol of life, purity, and sustenance. From its origin in the pristine glaciers of Uttarakhand to 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, insilico 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.



Dr. Acharya Balkrishna



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Classical Ayurvedic Insight

Achyranthes aspera L.

Vedic name : (Apāmārgakaḥ kharakaṇiśaḥ) (अपामार्गकः खरकणिशः)

Botanical name : *Achyranthes aspera* L. Syn. *Achyranthes australis* R.Br.

Paricayaḥ

Utpatti Sthānam

Apatṛṇatvarupeṇa varṣartau bhāratāvanau.

Maleśiyā pharīkādaḥ laṃkāṣṭreliyaḥbhūmiṣu.

Philipīne ca cīnādaḥ pādapaḥ proccakāspade.

Amerikāvanau tāivāne'yaṃ kila jāyate. (Saumitreyā Mahodadhi: 1-2)

Distribution

It is distributed throughout the tropical world. It can be found in many places growing as an introduced species and a common weed. It is an invasive species in some areas, including many Pacific Islands environments. ^[1-4]

Vānaspatika Vivaraṇam

1. Kāyika lakṣaṇam-

Kharamaijikanāmāyaṃ rūḍhaḥ kāyikavīkṣaṇe.

Apāmārgataruḥ śveto vārṣiko vyāmalambitaḥ.

Kāṣṭhīyaḥ kāṇḍa ādhāre saraktanīlavarṇabhāk.

Pāṭalābhaśca koṇīyaḥ śākhīyo romaśastathā.

Rjuḥ parvasu cocchūnaḥ sthūlādatyantadurbalaḥ.

Rjuḥ pañcāṅgulocchrāyaṃ vṛttaṃ dīrghāyatantathā.

Patramasya vibhinnañca parṇavṛntayutaṃ kila.

Taraṅgāyitakorañca niśitāgrañca romaśam. (Saumitreyā Mahodadhi: 3-6)

Botanical Description

1. Vegetative Characters

It is an annual or perennial, erect, herbaceous, suffrutescent or diffuse shrub, upto 1 m height. Stem woody at base, often tinged purple reddish, pinkish, angular, ribbed, erect, herbaceous, pubescent, swollen at the nodes, stout to very weak, sub-glabrous to densely tomentose, the nodes shrunken when dry. Leaves simple, 2.5-12.5 cm long and 1.5-7 cm wide, exstipulate, opposite, base cuneate, leaf blade ovate-lanceolate or ovate-oblong with rounded apex, very variable in shape, shortly petioled, margins undulate, acute or acuminate to almost round and very obtuse, gradually or abruptly narrowed below, both surfaces hairy. ^[1-4]

2. Puṣpīya Lakṣaṇam

Hastalambaśca kakṣīyapuṣpakrama udāhṛtaḥ.
Atrāpāmārgakasyāddhā tatpauṣparupamucyate.
Sahapatrāṇi pītāni kuntākārāṇi santi ca.
Dviyavākārayuktāni romaśūnyāni tāni vai.
Ādhāreṣu dvipakṣā tu triyavā sahapatrikā.
Kaṇṭakitā kaṭhorā ca yavamātraṃ sakorakam.
Ācchāditam bhavet pakṣam parito dalapuñjakam.
Śvetamāho bhavet pītam raktaṃ vā nīlavarṇakam.
Vibhaktaṃ pañcakhaṇḍyāñca dvivṛtilambitam tathā.
Bāhyaparidalānyasya kuntākārāṇi madhyataḥ.
Ekaspaṣṭaśīrāyuñji pītāgrāṇi ca pārśvataḥ.
Śīrādvayena yuktāni pumaṅgāni tathā'sya tu.
Puñkesaraparītāni bandhyapuñkesarasya ca.
Ekāntare tu vidyante dviyavāni viśeṣataḥ.
Ādhāre saha-jātā ca puntantuvitatistathā.
Kośāścātra parāgāṇām dvikośīya udāhṛtaḥ.
Aṇḍāśayastathā dīrghaḥ susampīditavigrahaḥ.
Vartikā tantulā namraṃ tadagraṃ tanu vidyate.
Bījāṇḍamekamevā'sya tathaikena ca dīrghataḥ.
Ādhāriṇaiva bījāṇḍavṛntena saha vartate.
Phalaṃ vṛhisamucchrāyaṃ hrasvaṃ dīrghāyataṃ punaḥ.
Aṇḍākārādṛtistatra cirasthāyitayoditaiḥ.
Tat paridalakhaṇḍaistu sahapatrīsamāvṛtam.
Dīrghāyataṃ tathā bījamekalaṃ viparītakam.
Raktābhababhruvarṇaṇca śīrṣe śuṇḍitakāyakam.
Ādhāreṣu savṛttaṃ tat varṣāsu sumasaṃbhavaḥ. (Saumitreya Mahodadhi: 7-19)

2. Floral Characters

Inflorescence is axillary or slender spikes, at the base it is dense and finally elongating to 8-34 cm. Bracts lanceolate or narrowly deltoid-lanceolate, pale or brownish membranous, 1.75-5 mm, glabrous. Bracteole 1.5-4.5 mm, spiny, shiny, rigid, base 2-winged. Wings 1.5-2 mm, membranous, margin entire. Perianth whitish or pale green to red or purple, 5 segments, 3-7 mm in length. The outer perianth longest, lanceolate, very acute, with a distinct midrib and 2 obscure to distinct lateral nerves, narrowly or moderately pale-margined. Androecium consists of 5 stamens, alternating with sub-quadrate pseudo-staminodes, 2.5-3.5

mm in length. Filaments connate at the base. Anthers 2 celled. Ovary oblong, sub-compressed. Style filiform. Stigma capitulate, slender, 1-4 mm. Ovule single, pendulous from a long basal funicle. Fruits easily disarticulating oblong, ovoid utricle, small, 2.5 to 3 mm long, detach from plant with perianth and bracteoles. Seeds single, inverse, oblong-ovoid, sub-cylindric, truncate at apex, rounded at the base, reddish brown. Fruiting and flowering takes place in August to November. ^[1-4]

Plant Anatomy

Prayojyāṅgam

Kharamañjikanāmāpāmāgeko'khilapādapaḥ.

Prayojyāṅgam mataṁ vaidyairantaḥsaṁracanocyate. [1] (Saumitreyā Mahodadhi)

Antaḥsaṁracaneḥṣaṇe

(a) Mūlam

Aṣṭakalpata ālokyāstvakprotakīyakośikāḥ.

Starīyā āyatākārāḥ sparśarekhīyalambakāḥ.

Tanubhittimayā majjakośikānicayāstathā.

Navakalpastarā dṛśyā aṇḍākārata āyatāḥ.

Tanubhittīyakā mṛdvyaḥ kośikāstatra santi ca.

Ekalāstā dvitīyākhyā aśmarīkośikāstataḥ.

Ṣaṭkalpato dvitīyākhyasthūlanasya tathotakāḥ.

Valayairnirmītā dṛśyāḥ saṁvahanakasaṁjjñakāḥ.

Cālanīnālikāgucchā mṛdule poṣavāhake.

Samālokyā avāgvāhāḥ sāmānyatattvanirmītāḥ.

Vāhikāḥ saralā gartayuktā atra bhavanti ca.

Madhyāṁśaraśmayaścātra trikalpakośikāstrtāḥ.

Tāsu bhavanti vai majjakośikāparidhau tathā.

Cūrṇātukṣāarakasyātra kaṇā naike saṁīkṣaṇe. (Saumitreyā Mahodadhi: 2-8)

Plant Parts

Whole plant

Microscopic Characters

Root

Cork cells are 3-8 layered, rectangular, tangentially elongated and thin-walled. Secondary cortex consists 6-9 layers, oval to rectangular, thin walled and parenchymatous cells. Secondary cortex contains a single or groups of stone cells and followed by 4-6 discontinuous rings of anomalous secondary thickening composed of vascular tissues. Small patches of sieve tubes are found in phloem parenchyma. Xylem is composed of usual elements. Vessels are simple pitted. Medullary rays are 1-3 cells wide. Crystals of calcium oxalate are present in cortical region and numerous in medullary rays. ^[1, 5-8]

(b) Kāṇḍabhāgaḥ

Daśakalpata ālokyāḥ kāṇḍabhāgāḥ samucchritāḥ.
Nīcairāpatārāste ca vellanākṛtayastathā.
Adhicarmaprabhāgo'traikastarapaṃktisaṃyutaḥ.
Kośikāḥ pañcakalpā vai tvagromagranthisaṃkulāḥ.
Catuḥkalpata ālokyāḥ pragolākāraśīrṣakāḥ.
Vṛntakaiḥ kośikīyaiste sthūlopacarmaṇāvṛtāḥ.
Daśakalpaṃ tathā majjakośikāstarasaṃcayaḥ.
Sṛṣṭo mṛdutaḥkīyābhistābhīrasau vilokyate.
Sthūlakoṇotakā majjakośikāsu samīkṣitāḥ.
Saṃvahanotake vṛddhā nyagvāhāḥ ṣaṭ prakalpataḥ.
Apūrṇāśca tathā poṣavāhā valayitā iha.
Dvītīyakeṣu teṣvaddhā'pūrṇā valayakasthitiḥ.
Avāgvāho dvītīyākhyāḥ sāmānyatattvasaṃyutaḥ.
Tatrānupasthitāḥ santi tantavo hyatha vāhikāḥ.
Valayākṛtayo gartayuktāḥ sarpilā matāḥ.
Pralambāstantavo gartakośikāvaraṇairyaṇyutāḥ.
Mṛdulakośikābhistā vinīmitā bhavanti ca.
Prasūkṣmavālukāleśā adhicarmavraje punaḥ.
Majjamadhye tathā majjakośikāsu virājitāḥ. (Saumitreyā Mahodadhi: 9-18)

Stem

Stem shows 6-10 prominent ridges, which diminish downwards upto the base where it becomes almost cylindrical. Epidermis is single layered, covered by thick cuticle having uniseriate, 2-5 celled, covering trichomes and glandular with globular head, 3-4 celled stalk. Cortex is 6-10 layered and composed of parenchymatous cells. Cortex is collenchymatous. Vascular tissues show anomalous secondary growth having 4-6 incomplete rings of xylem and phloem. Secondary phloem consists of usual elements form

incomplete rings. Secondary xylem consisting of usual elements, fibres being absent. Vessels are annular, spiral, scalariform and pitted. Fibres are pitted, elongated and lignified. Pith is wide consisting of oval to polygonal, parenchymatous cells. Micro-sphenoidal silica crystals are present in some epidermal, cortical and pith cells. ^[1, 5-8]

(c) Patrāṇi (Parṇavṛntam)

Arddhacandrākṛtiprekṣye paridhau parṇavṛntakam.
 Saṃlagnamadhicarmātraikastarīyakamīkṣyate.
 Sthūlabhittīyakantacca bharaṇotakasamḥatiḥ.
 Srṣṭāmṛdutaḥkīyākyatanubhittīyakośakaiḥ.
 Tanmadhyavartini kṣetre cūrṇātukṣāarakasya ca.
 Guccakaṇāsthā pañca saṃvahanakapūlakāḥ. (Saumitreyā Mahodadhi: 19-21)

Leaf

Petiole: Petiole shows crescent-shaped outline, having single-layered epidermis with thick cuticle and ground tissues consisting of thin-walled, parenchymatous cells. It contains rosette crystals of calcium oxalate and 4-5 vascular bundle are situated in mid region. ^[1, 5-8]

(d) Madhyaśirā

Adhimadhyāśirāñcādhicarma naikastarīyakam.
 Talakadvayake dṛśyaṃ sthūlakoṇotakāsthā.
 Pañcakalpastarīyāḥ syurūrdhvaṃ dvitrāśca kalpataḥ.
 Nīcaiḥ starīyakā bhānti, bharaṇotakasamḥatiḥ.
 Tanubhittīyakā mṛdvīkośikāsu bhavantiyapi.
 Saṃvahanakapūlāśca nyagvāhavāhikāsthā.
 Adhyedhikaṃ tathā paścādalpastarīyakasya vai.
 Poṣavāhavajrasyekṣyā parirambhavrajasthā.
 Dvitrastarābhisamṣuktaḥ sthūlabhittīyako mataḥ.
 Kośikāvaraṇavṛtāvihīnāḥ kośikāḥ punaḥ.
 Cūrṇātukṣāarakasyāpi pragucchā bharaṇotake.
 Vitatā abhidṛśyante prasūkṣmadarśikāyane. (Saumitreyā Mahodadhi: 22-27)

Midrib: Epidermis is single layered, on both surfaces. Collenchyma is 4-5 layered on upper side and 2-3 layered on lower side. Ground tissue consists thin-walled, parenchymatous cells having a number of vascular bundles. Each vascular bundle shows below xylem vessels, thin layers of cambium and followed by phloem. Pericycle is 2-3 layered of thick-walled, non-lignified cells and rosette crystals of calcium oxalate found scattered in ground tissues. ^[1, 5-8]

(e) Paṭalabhāgaḥ

Ekastarīyako dṛśyaḥ paṭalaḥ sparśarekhikaḥ.
Kośikā adhicarmākhyā lambā sthūlopacarmabhiḥ.
Tvagromabhistathā dṛśyā samāvṛtāḥ samānataḥ.
Kāṇḍamubhayataḥ parṇamadhyotakavrajastathā.
Spaṇjīmṛdutake samyag vibhājito'bhidṛśyate.
Catuḥkalpastaraprotāḥ stambhotakavrajastviha
Bṛhanmṛdutakavrāta ūrdhvago lambakaḥ param.
Āyato'sau tathā nimnastaravrāte vilokyate.
Spaṇjīmṛdutakavrātaḥ pañcakalpastarānvitaḥ.
Prasthūlakasamavyāsimṛdulakośikāvrajaiḥ.
Vinirmīto, vicitrā vai bṛhatyaḥ kośikā iha.
Cūrṇātukṣāarakasyātra kaṇāḥ stambhotakā api.
Praspañjīmṛdulākhyāsu kośikāsu bhavantyapi.
Asamakośikiye cāniyatakośikīyake.
Randhrāṇi vidyamānāni staravrāte bhavanti vai.
Ityantāracanā tasya sūkṣmataḥ samudīritā. (Saumitreya Mahodadhi: 28-35)

Lamina: Lamina is single layered, tangentially elongated epidermis cells covered with thick cuticle having covering trichomes. Which are similar to those of stem found on both surfaces. Mesophyll is differentiated in to palisade and spongy parenchyma. Palisade is 2-4 layered, thick parenchyma larger, slightly elongated in upper, while smaller and rectangular in lower surface. Spongy parenchyma is 3-5 layered, thick, more or less isodiametric parenchymatous cells. Idioblast contains large rosette crystals of calcium oxalate distributed in palisade and spongy parenchyma cells. Stomata are anisocytic and anomocytic in both surfaces. [1, 5-8]

(f) Cūrṇam

Atreṣatpītavarṇābhaṃ cūrṇam tatrādhicarmagāḥ.
Kośikā lambikā dṛśyāstanubhittīyakāyatāḥ.
Aparāstantavastatra vāhikā valayānvitāḥ.
Sarpākārāśca tadrūpaiḥ sthūlanairgartaniṣṭhitāḥ.
Pāṃktiko romasandohaḥ samucchrito'bhidṛśyate.
Cūrṇātugucchakāstrijmakaṇā lakṣyāḥ samīkṣaṇe. (Saumitreya Mahodadhi: 36-38)

Powder

Light yellow in colour. It contains fragments of elongated, rectangular, thin-walled epidermal cells, aseptate fibres, vessels with annular, spiral, scalariform and pitted thickening, uniseriate hair with bulbous base, rosette and prismatic crystals of calcium. [1, 5-8]

Medicinal Uses

Cephalic disorders

Pratyakpuṣpā śirovirecanānām. (Ca.Sū. 25:40)

According to Caraka Saṁhitā it is used as Śirovirecaka dravya for the treatment of cephalic disorders. ^[9]
(Ca. Su. 25:40)

Nirguṇḍīlāṅgalikārkasādhitaṁ tailamabhyāṅgāt.

Śirasorujāḥ samagrā yadi vā'pāmārgaphalasiddham. (Ga.Ni.Śā.Ta. 1:125)

According to Śoḍhala Nighaṇṭu massage of oil processed with *Achyranthes aspera* (Prickly chaff-flower) fruit is useful in cephalgia. ^[10] (Gd.Ni.Sh.T. 1:125)

Śirasorujāḥ samagrā yadi vā'pāmārgabījasaṁsiddham. (Rā.Mā. 1:24)

According to Rāja Martaṇḍa massage or arrhine of oil processed with *Achyranthes aspera* (Prickly chaff-flower) seed is useful in headache and other cephalic disorders. ^[11] (Ra.Ma. 1:24)

Ophthalmic disorders

Apāmārgasya śiphā ghrṣṭā madhunā saindhavena ca.

Tāmrapātre bhṛtā netre hanti pīḍāṁ tadudbhavām. (Ga.Ni.Śā.Taṁ. 3:460)

According to Śoḍhala Nighaṇṭu application of collyrium prepared from *Achyranthes aspera* (Prickly chaff-flower) root triturated with rock salt and honey is useful in ophthalmalgia. ^[10] (Gd.Ni.Sh.T. 3:460)

Ear disorders

Mārgakṣārajaleṇa tat kṛtakalkenasādhitaṁ tilajam.

Apaharati karṇanādaṁ bādhiryaṁ cāpi pūraṇataḥ. (Vṛ.Mā. 59:30)

Apāmārgakṣārajale tat kṣāraṁ kalkitaṁ kṣipet.

Tena pakvaṁ jayettelaṁ bādhiryaṁ karṇanādakam. (Śā.Saṁ.U.Kha. 11:143)

According to Śāraṅgadhara and Vṛndamādhavainstillation of ear drops prepared from *Achyranthes aspera* (Prickly chaff-flower) alkaline, leaf paste and oil is useful in deafness, tympanitis and tinnitus. ^[13]
(Vṛ.Md. 59:30), ^[14] (Sh.Sm.Ut.Kh. 11:143)

Tilatailaṁ tvapāmārgatailaṁ karṇeṣu bindutaḥ. Prakṣipet tasya gaccheyuḥ karṇagā vyādhayo'khilāḥ. (Saumitreyā Mahodadhīḥ)

Instillation of 2-3 ear drops prepared from *Sesamum indicum* (Sesame) oil and *Achyranthes aspera* (Prickly chaff-flower) oil is useful in ear disorders. ^[1] (Saumitreyā Mahodadhīḥ)

Thoracic disorders

Apāmārgashtitaḥ kṣāraḥ śvāsakaṣṭāñca dāruṇam. Vināśayet tathā kāsaprabhāvaṁ śvāsarogīṇām. (Saumitreyā Mahodadhīḥ)

Intake of 65-125 mg *Achyranthes aspera* (Prickly chaff-flower) alkaline is useful in dyspnea and cough. ^[1]
(Saumitreya Mahodadhi)

Yadi kāsanimittena vakṣaḥśūlaṃ na jāyate. Mūlaphāṇṭen kāryaṃ ca vamaṇaṃ
hitakārakam. (Saumitreya Mahodadhiḥ)

Intake of *Achyranthes aspera* (Prickly chaff-flower) root infusion expels out *Doṣa* through vomiting and
gives relief in chest pain. ^[1] (Saumitreya Mahodadhi)

Apāmārgasya cūrṇantu maricacūrṇa saṃyutam. Māṣamātraṃniṣeveta kāsastasya
praśāmyati. (Saumitreya Mahodadhiḥ)

Intake of powder prepared from an equal quantity of *Achyranthes aspera* (Prickly chaff-flower) root and
Piper nigrum (Black pepper) alleviates cough. ^[1] (Saumitreya Mahodadhi)

Abdominal disorders

Jalapītamapāmārgamūlaṃ hanyādvisūcikām. (Bhā.Pra.Ci. 6:110)

According to Bhāvaprakāśa intake of *Achyranthes aspera* (Prickly chaff-flower) root paste is useful in
cholera. ^[15] (Bh.Pr.Ci. 6:110)

Parṇasvarasasevā tu śastā prāvāhike gade. (Saumitreya Mahodadhiḥ)

Intake of 1-2 ml leaf juice is useful in dysentery. ^[1] (Saumitreya Mahodadhi)

Ano-rectal disorders

Apāmārgamūlaṃ ca taṇḍulodakena sakṣaudramaharaḥ. (Su.Ci. 6:13)

According to Ācārya Suśrutaintake of *Achyranthes aspera* (Prickly chaff-flower) root paste mixed with
honey and rice water is useful in hemorrhoids. ^[16] (Su.Ci. 6:13)

Apāmārgasyabījānāṃ kalkastaṇḍulavāriṇā.

Pīto raktārśasāṃ nāśaṃ kurute nātra saṃśayaḥ. (Śā.Saṃ.Ma.Kha. 5:19)

According to Śāraṅgadharma Saṃhitāintake of 10-12 gm *Achyranthes aspera* (Prickly chaff-flower) seed
paste with rice water is useful in hemorrhoids. ^[14] (Sh.Sm.Md.Kh. 5:19)

Kalkamapāmārgamayam vimiśraya sujātatakreṇa.

Suvasanavilulanagalitam piba rudhirārśaḥsu bheṣajam lalitam. (Si.Bhe.Ma.Caturtha
guccha, Arśa ci.: 4:33)

According to Siddhabheṣaja-Maṇi-Mālāintake of *Achyranthes aspera* (Prickly chaff-flower) whole plant
paste alongwith buttermilk is useful in hemorrhoids. ^[17] (Sd.Bh.Mm.Ch. Ars.Ci. 4:33)

Mayūravalkakiṇḍjalkavarīvāsāśṛtaṃ jalam.

Raktārśasāṃ praśamanaṃ syāt palāṇḍurivāsitaḥ. (Vṛ.Mā. 5:8)

According to Vaidya ManoramāIntake of decoction prepared from *Achyranthes aspera* (Prickly chaff-
flower), *Mesua ferrea* (Cobras saffron), *Asparagus racemosus* (asparugus) and *Adhatoda vasica* (Malabar
nut) is useful in hemorrhoids. ^[13] (Vr.Ma. 5:8)

Hepatosplenic disorders

Apāmārga śamīmūlaṃ piṣṭvā takreṇa pāyayet.

Kāmalāṃ śvayathuṃ pāṇḍuṃ karṣamātraṃ niyacchati. (Ra.Ra.Sa. 19:109)

According to Rasaratna Samuccayaintake of 10-12 gm paste prepared from an equal quantity of *Achyranthes aspera* (Prickly chaff-flower) and *Prosopis cineraria* root alleviates jaundice, inflammation and anaemia. ^[18] (R.R.S. 19:109)

Renal and Urinary bladder disorders

Tilāpāmārgakadalīpalāśayavasambhavaḥ kṣāraḥ peyo'vimūtreṇ śarkarāsvaśmarīṣu ca.
(A.Hr.Ci. 11:31-32)

According to Aṣṭāṅga Hṛdayaintake of *Sesamum indicum* (Sesame) , *Achyranthes aspera* (Prickly chaff-flower), *Musa sapientum* (Banana), *Butea monosperm,a* (Forest flame) and *Hordeum vulgare* (Barley) alkaline alongwith sheep urine is useful in calculus. ^[19] (As.Hr.Ci. 11:31-32)

Mūtrakṛcchraṃ jayet pītā kṣīreṇa śikharīśiphā.

Ajākṣīreṇ saṃpeṣya pītā nīlījaṭā tathā. (Vṛ.Mā. 7:4)

According to Vaidya Manoramā intake of *Achyranthes aspera* (Prickly chaff-flower) root rubbed in goat urine is useful in dysuria. ^[13] (Vd.Mn. 7:4)

Reproductive system disorders

Anyairanākhyāya kadācidāśu mūrdhanyapāmārgaraso'bhiṣiktaḥ. (Ga.Ni.Kau.Taṃ. 1:54)

According to Śoḍhala head massage of *Achyranthes aspera* (Prickly chaff-flower) juice is useful in leucorrhea. ^[12] (Gd.Ni.Kau.T. 1:54)

Pāṭhālāṅgalisīmḥasyamayūrakajaṭāḥ pṛthak.

Nābhibastibhagālepāt sukhaṃ nārī prasūyate. (Ga.Ni.Kau.Taṃ. 4:23)

Apāmārgaśikhāṃ yonimadhye niḥkṣīpya dhāyryate.

Sukhaṃ prasūyate nārī bheṣajasyāśya yogataḥ. (Vaṃ.Se.Strīrogaḥ233)

Mūlaṃ pratyakpuṣpyāḥ pāṭhāyāśca viniveśitaṃ guhye.

Strīṇāṃ duṣprasavānāṃ prasavaṃ kurute sukkenaiva. (Ga.Ni.Kau.Taṃ. 4:21)

According to Śoḍhalatopical application of paste prepared from *Achyranthes aspera* (Prickly chaff-flower) root, leaf and twig promotes normal delivery. ^[12] (Gd.Ni.Kau.T. 4:23), ^[20] (Vg.Sn.Str.Rg. :233), ^[12] (Gd.Ni.Kau.T. 4:21)

Apāmārgapatradvayaṃ yonimadhye niviṣṭaṃ kṣaṇād yoniśūlaṃ nihanti.

Sudurvāramapyevameva prayukto..(Ga.Ni.Kau.Ta. 6:15)(Rā.Mā.31:35)

According to Śoḍhala topical application of *Achyranthes aspera* (Prickly chaff-flower) leaf paste is useful in vaginal pain. ^[12] (Gd.Ni.Kau.T. 6:15), ^[11] (Ra.Ma. 31:35)

Piṣṭvā lipte yonau mūlaṃ kharamañjarī punarnavayoḥ.

Navasūtāyāḥ śūlaṃ yonigataṃ sakalamapanayataḥ. (Vṛ.Mā. 13:40)

According to Vaidya Manoramā topical application of paste prepared from *Achyranthes aspera* (Prickly chaff-flower) and *Boerhavia diffusa* (Hog weed) root is useful in postpartum pain. ^[13] (Vr.Ma. 13:40)

Apāmārgadalālepaḥ sadyo badhnāti śoṇitam.

Atipravṛttamapyāśu setubandha ivodakam. (Vr.Mā. 16:120)

According to Vaidya Manoramā topical application of *Achyranthes aspera* (Prickly chaff-flower) leaf paste is useful in hemorrhage. ^[13] (Vr.Ma. 16:120)

Mūlaṃ pratyakpuṣpyāḥ pāṭhāyā vā niveśitam guhye.

Strīṇāṃ duṣprasavānāṃ prasavaṃ kurute sukhena khalu. (Rā.Mā. 31:27)

According to Rāja Mārtaṇḍatopical application of *Achyranthes aspera* (Prickly chaff-flower) root paste promotes normal delivery. ^[11] (Ra.Ma. 31:27)

Mūlakalkapradehena garbhasrāve'srarodhanam. (Saumitreya Mahodadhiḥ)

Topical application of *Achyranthes aspera* (Prickly chaff-flower) root paste is useful in hemorrhage caused due to abortion. ^[1] (Saumitreya Mahodadhi)

Dermatological disorders

Pralepāt sidhmayātyastamapāmārgasya bhasmanā.

Kevalena yathāpāpaṃ candraśekhara bhasmanā. (Vr.Mā. 11:60)

According to Vaidya Manoramā topical application of *Achyranthes aspera* (Prickly chaff-flower) ash along with *Brassica campestris* (Field mustard) oil is useful in *Sidhma*. ^[13] (Vr.Ma. 11:60)

Śikharirasena supiṣṭaṃ mūlakabījaṃ pralepataḥ sidhmam.

Kṣāreṇa kadalyā vā rajanīmīśreṇa nāśayati. (Vr.Mā. 51:21)

According to Vṛnda Mādhavatopical application of raddish seed pounded with *Achyranthes aspera* (Prickly chaff-flower) juice is useful in *Sidhma*. ^[13] (Vr.Md. 51:21)

Niṣpīḍitaiḥ karapuṭena mayūrakasya patrairviniḥsarati yaḥ svaraso'tisāndraḥ.

Tatpūrite satatanaryidapi kṣaṇena raktaṃ niruddhamiva tiṣṭhati śastraghāte.
(Rā.Mā. 26:10)

Apāmārgasya saṃsiktaṃ patrotthana rasena tu.

Sadyovraṇeṣu raktaṃ hi pravṛttaṃ paritiṣṭhati. (Ga.Ni.Śa.Taṃ. 4:29), (Ca.Da.Ci. 44:52)

According to Śoḍhalatopical application of *Achyranthes aspera* (Prickly chaff-flower) leaf juice is useful in hemorrhage and wound caused due to an injury. ^[11] (Ra.Ma. 26:10), ^[12] (Gd.Ni.Sh.T. 4:29), ^[21] (Ca.Da.Ci. 44:52)

Apāmārgo'śvagandhā ca tālapatrī suvarcalā.

Utsādane praśasyante kākolyādiśca yo gaṇaḥ. (Su.Sū. 37:30)

According to Ācārya Suśrutatopical application of paste prepared from *Achyranthes aspera* (Prickly chaff-flower), *Withania somnifera* (Winter cherry), *Curculigo orchioides* (Black musli), black salt and medicinal herbs of *Kakolyadi Gaṇa* (group of medicinal herbs) is useful in wound. ^[16] (Su.Su. 37:30)

Mayūraḥ rājavṛkṣo nimbaḥ kośātakī tilāḥ.

Bṛhatī kaṇṭakārī ca haritālāṃ manaḥśilā.

Śodhanāni ca yojyāni taile dravyāṇi śodhane. (Su.Sū. 37:17-18)

According to Ācārya Suśrutatopical application of oil processed with *Achyranthes aspera* (Prickly chaff-flower), *Cassia fistula* (Pudding pipe tree), *Azadirachta indica* (Margosa tree) bark is useful in wound. ^[16]
(Su.Su. 37:17-18)

Pratyakpuṣpīphalayutaistilaiḥ piṣṭaiḥ pralepayet. (Vr.Mā. 47:2)

According to Vṛnda Mādhavatopical application of *Achyranthes aspera* (Prickly chaff-flower) fruit powder alongwith *Sesamum indicum* (Sesame) paste is useful in sinus caused due to *Vāta*. ^[13] (Vr.Md. 47:2)

Suvarcikāśaimdhavacitrakeṣunikumbhatālītala rūpikāsu.

Phaleṣvapāmārgabhavēṣu caiva kuryāt samūtreṣu hitāya tailam. (Su.Ci. 17:25)

According to Ācārya Suśrutatopical application of oil processed from *Sajji Kshar*, rock salt, *Plumbago indica* (White leadwort), *Baliospermum montanum* (Wild castor), *Emblica officinalis* (Indian goose berry) root, *Calotropis gigantea* (Madar), *Achyranthes aspera* (Prickly chaff-flower) seed paste and cow urine promotes healing. ^[16](Su.Ci. 17:25)

Tilatailamapāmārgamūlenāmbhonvitena yat.

Siddham tatsveditaḥ śastraghāto na kurute vyathām.(Rā.Mā. 26:7)

According to Rāja Mārtaṇḍatopical application of oil processed from *Achyranthes aspera* (Prickly chaff-flower) root and water is useful in pain caused due to an injury. ^[11] (Ra.Ma. 26:7)

Palaṃ śikharibījānāṃ madhunaḥ kavalagrahaḥ.

Sarpiḥ palaṃ vipaktavyaṃ vraṇānmalaharo haret.

(Si.Bhe.Ma. Caturthaguccha, Vraṇa ciḥ 9)

According to Siddha Bheṣaja Maṇimālātopical application of *Ghrita* (Clarified butter) processed from *Achyranthes aspera* (Prickly chaff-flower) seed and honey is useful in wound. ^[17] (Sd.Bh.Mm.Ch. Vrn.Ci. :9)

Pralepātsidhma yātyastamapāmārgasya bhasmanā.

Kevalena yathā pāpaṃ candraśekharabhasmanā. (Vai. Ma. 11:60)

According to Vaidya Manoramātopical application of *Achyranthes aspera* (Prickly chaff-flower) ash is useful in *Sidhma*. ^[22] (Vd.Mn. 11:60)

Apāmārgadalālepaḥ sadyo baghnāti śoṇitam.

Atipravṛttamapyāśu setubandha ivodakam. (Vai. Ma. 16:120)

According to Vaidya Manoramātopical application of *Achyranthes aspera* (Prickly chaff-flower) paste is useful in hemorrhage caused due to wound. ^[22] (Vd.Mn. 16:120)

Bhasmapradehato lābho vraṇe śīśnasya kīlake. (Saumitreya Mahodadhiḥ)

Topical application of *Achyranthes aspera* (Prickly chaff-flower) ash with Arsenic di-sulphide (Realgar) is useful in wound and skin disorders. ^[1] (Saumitreya Mahodadhi)

Tatkalkasya pralepastu kaṇṭajanyavraṇaṃ haret. (Saumitreyā Mahodadhīḥ)

Topical application of *Achyranthes aspera* (Prickly chaff-flower) paste is useful in wound caused due to thorn. ^[1] (Saumitreyā Mahodadhī)

Psychological disorders

Kākajamghā cāpāmārgaḥ. kokilākṣaḥ suparṇikā.

Kvātheā nīdrākaraḥ śīghraṃmūlaṃ vāṃ bandhayecchikham. (Hā.Sa.3:15:6)

According to Hārītatntake of decoction prepared from *Achyranthes aspera* (Prickly chaff-flower) and *Peristrophe bicalyculata* (Panicked peristrophe) whole part is useful in insomnia. ^[23] (Hā. Sa 3:15:6)

Apāmārgasya pañcāṅgakvātho vai kākajamghayā.

Naikakāraṇasaṃjātāmanīdrāṃ nāśayed drutam. (Saumitreyā Mahodadhī)

Intake of decoction prepared from *Achyranthes aspera* (Prickly chaff-flower), *Peristrophe bicalyculata* (Panicked peristrophe) and *Hygrophila spinosa* (Marsh barbel) is useful in insomnia. ^[1] (Saumitreyā Mahodadhī)

Alexipharmic treatment

Atyantaśaityena janasya jātaṃ kampaṃ mahāntaṃ śīthilīkaroti.

Jalīyasāntrāsasamāgate punarvījaṃ tathā parṇamīhaiva yujyate.

(Saumitreyā Mahodadhīḥ)

Intake of decoction prepared from seed and leaf is useful in hydrophobia. ^[1] (Saumitreyā Mahodadhī)

Puṣpīyaśūken saśarkareṇa vā vaṭī kṛtā'larkaviśapraśāntaye. (Saumitreyā Mahodadhīḥ)

Intake of tablet prepared from *Achyranthes aspera* (Prickly chaff-flower) flower is useful in rabies. ^[1] (Saumitreyā Mahodadhī)

Sarpavṛścikadaṃśīyaviśapṛkṭaprabhāvataḥ.

Apāmārgasya pañcāṅgaprayoga īṣyate punaḥ. (Saumitreyā Mahodadhīḥ)

Intake of decoction prepared from *Achyranthes aspera* (Prickly chaff-flower) whole part is useful in snake poisoning. ^[1] (Saumitreyā Mahodadhī)

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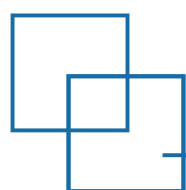
Abbreviation

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. Dvitīyavilāsaḥ	:	Vaidya JāivanamDvitīyavilāsaḥ
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 depends 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 $18700 \text{ m}^3/\text{s}$ 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 of $468.7 \times 10^9 \text{ m}^3$ 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, *Achyranthes aspera* was considered in this study. It is an annual herb distributed throughout the tropical and subtropical world. It is commonly known as “chaff flower”, “prickly chaff flower” and “devil’s horsewhip”. In Sanskrit, “Adhoghanta”, “Adhvashalya”, “Aghamargava”, “Aghata”, “Apamarga”, “Apangaka”, “Chamatkara”, “Dhamargava”. In Hindi “Apang”, “Chirchra”, “Chirehitta”, “Latjira” and “Onga”. Antimicrobial, anti-tumor, and anti-cancerous properties are used for diverse ailments using different sections of this plant, including the seeds, roots, leaves, and stems. It has diverse morphology and anatomical variations. On this basis, the current chapter is focused on the morphology, taxonomy, anatomy, and distribution assessment of *A. aspera* around the Ganga River.

Genus *Achyranthes* L.

Achyranthes L. commonly known as chaff flower belongs to the family Amaranthaceae. This genus includes a group of perennial or annual herbs, mainly growing on the edges of forests, hillsides, or riverbanks. Firstly, this genus was described by Carolus Linnaeus in 'Species Plantarum' in 1753 with *A. aspera* as the type species. Botanically, it is recognized by its elliptical or ovate leaves with acuminate apex, and undivided inflorescence with reflexed flowers. The generic name, *Achyranthes* comes from two ancient Greek words "Achyro" meaning "chaff or husk" and "anthas" meaning "flower". It contains approximately 14 species, which are widely distributed in tropical and subtropical regions. Among them, *A. bidentata* and *A. aspera* are the two most commonly used medicinally species and have been used for thousands of years and even mentioned in Vedic kinds of literature. Various parts of these plants, including the leaves, juice, and seeds, can be used as medicines, and the dried root of *A. bidentata* has been well-mentioned in Chinese, Japanese, and Korean Pharmacopoeias.

Many ancient medical books, along with a series of modern toxicity tests, have shown that *A. bidentata* and *A. aspera* are non-toxic for oral and external uses, while the usually emphasized contraindication in pregnancy is due to their ability to induce abortions. In Shennong's 'Classic of Materia Medica', the *A. bidentata*, is an upper herb that is bitter and sour and acts on the liver and kidney meridians (China Pharmacopoeia

Commission, 2015). Interestingly, *A. bidentata* is generally prepared with yellow rice wine or salt water to enhance the actions of enriching the kidney and nourishing the liver, strengthening the sinews and bones (Chinese Herbalism Editorial Board, 1999; He et al., 2017). *A. aspera* has occupied a pivotal position in Indian culture and folk medicine, and it has been used in all the Indian traditional systems of medicine, including Ayurveda, Unani, Siddha, and Homoeopathy (Perumalsamy et al., 1998). According to Ayurveda, it is bitter, pungent, laxative, stomachic, and carminative (Pandey et al., 2013). Besides its application in human health, *A. bidentata* has also been used to extend silkworm age in the southeast coastal area of China (Li and Liu, 1990), and *A. aspera* has been reported to be capable of removing urolith in goats and expelling the placenta after parturition and can be applied as an anthelmintic in cattle (Khan, 2009). In addition, *A. bidentata* and *A. aspera* have also been incorporated as active ingredients into various products, including health care products, health wine, sex pills, creams, toothpaste, and sanitary napkins worldwide (He et al., 2017).

Pharmacologically, the genus *Achyranthes* shows biological effects on the immune system, nervous system, bone metabolism, and reproduction, along with various biological activities such as blood-activating, antitumor, anti-oxidation, anti-inflammation, anti-arthritis, and so on. Up to the study, a total of 133 compounds from the genus

have been reported (He et al., 2017). Among these reported compounds, the four classes such as polysaccharides, polypeptides, triterpenoid saponins, and ketosteroids are the main bioactive ingredients. In Chinese Pharmacopoeia, the β -ecdysterone is recorded as the index component for the quality control of *Achyranthis bidentatae* Radix (China Pharmacopoeia Commission, 2015). In addition, it is worth mentioning that the epithet "*bidentata*" is still being misspelled as "bidentate".

Etymology

The genus belongs to the family "Amaranthaceae" which is based on the genus "*Amaranthus*" derived from the Latin word '*amaranthus*' and the Greek word '*amarantos*' which means 'unfading'; referring to the lasting quality of the flowers, persistent bracts, and sepals (Patil, 2007). The genus name "*Achyranthes*" is derived from the Greek '*achyron*' which means 'chaff, husk, straw' and '*anthos*' which means 'a flower'; due to the chaffy or scarious nature of the perianth of the flower.

Habitat and Distribution

The genus *Achyranthes* is a small herb or shrub distributed throughout the tropical and subtropical regions of the world; abundantly found in Africa and Asia. Currently, according to the Plants of the World Online (POWO, 2023) Database, it has 14 accepted species including both medicinal and ornamental that are native

of Afghanistan, Aldabra, Algeria, Andaman Is., Angola, Assam, Bangladesh, Benin, Bismarck Archipelago, Borneo, Botswana, Burkina, Burundi, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caroline Is., Central African Repu, Chad, Chagos Archipelago, China North-Central, China South-Central, China Southeast, Christmas I., Cocos (Keeling) Is., Comoros, Congo, Djibouti, East Himalaya, Egypt, Eritrea, Ethiopia, Free State, Gabon, Gambia, Ghana, Gilbert Is., Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Hainan, Hawaii, India, Iran, Italy, Ivory Coast, Japan, Jawa, Kazan-retto, Kenya, Korea, KwaZulu-Natal, Laccadive Is., Laos, Lebanon-Syria, Lesotho, Lesser Sunda Is., Liberia, Line Is., Madagascar, Madeira, Malawi, Malaya, Maldives, Mali, Maluku, Marianas, Marquesas, Marshall Is., Mauritania, Mauritius, Morocco, Mozambique, Mozambique Channel I, Myanmar, Namibia, Nansei-shoto, Nauru, Nepal, New Guinea, Nicobar Is., Niger, Nigeria, Norfolk Is., Northern Provinces, Northern Territory, Ogasawara-shoto, Oman, Pakistan, Panamá, Philippines, Pitcairn Is., Queensland, Rodrigues, Rwanda, Réunion, Samoa, Sardegna, Saudi Arabia, Selvagens, Senegal, Sicilia, Sierra Leone, Society Is., Socotra, Solomon Is., Somalia, South China Sea, Spain, Sri Lanka, Sudan, Sulawesi, Sumatera, Swaziland, Taiwan, Tanzania, Thailand, Tibet, Tokelau-Manihiki, Tuamotu, Tubuai Is., Tunisia, Tuvalu, Uganda, Vietnam, Wallis-Futuna Is., West Himalaya, Western Australia, Western Sahara, Yemen, Zambia, Zaire, Zimbabwe; and introduced in Alabama, Aruba, Austria, Azores, Bahamas, Belize,

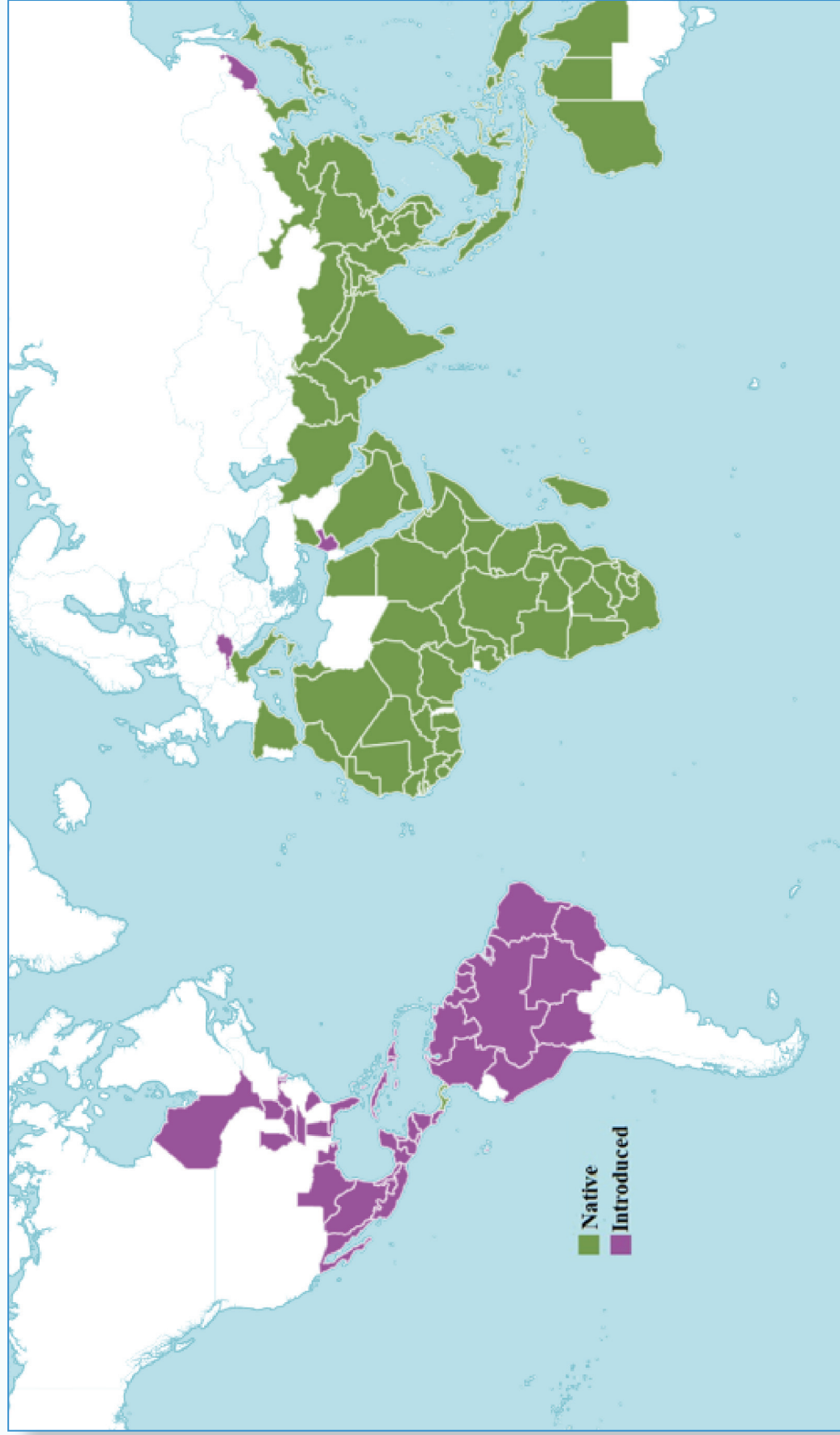


Fig. 1 Global distribution of genus *Achyranthes*

Bolivia, Brazil North, Brazil Northeast, Brazil Southeast, Brazil West-Central, Colombia, Cook Is., Costa Rica, Cuba, Dominican Republic, El Salvador, Fiji, Florida, French Guiana, Galápagos, Guatemala, Guyana, Haiti, Honduras, Illinois, Jamaica, Kentucky, Kermadec Is., Leeward Is., Louisiana, Maryland, Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Netherlands Antilles, New Caledonia, New Zealand North, Nicaragua, Niue, Ohio, Ontario, Palestine, Peru, Primorye, Puerto Rico, Seychelles, South Carolina, Southwest Caribbean, St. Helena, Suriname, Tennessee, Texas, Tonga, Trinidad-Tobago, Turks-Caicos Is., Vanuatu, Venezuela, Venezuelan Antilles, West Virginia, Windward Is. (Fig. 1). In India, 5 species viz. *A. aspera* L., *A. bidentata* Blume, *A. coynei* Santapau, *A. porphyristachya*, Wall. ex Moq., *A. shahii* M.R. & S.M.Almeida are recorded that are mentioned in Flowering Plants of India, Dicotyledons, vol-I (Acanthaceae- Avicenniaceae) (Karthikeyan et al. 2009). All the reported species are found in wastelands and roadsides throughout the country mainly in the southern, northern, and western parts.

Historical Background

Firstly, this genus was described by Carolus Linnaeus in '*Species Plantarum*' in 1753 with *A. aspera* as the type species. Later on Standley in 1915, described several species under this genus. Linnaeus's genus included several species which were then referred to as three genera. In earlier studies, this genus included several species of genus *Alternanthera*. So,

both genera were subject to nomenclatural confusion. Standley (1915) designated *Achyranthes repens* L. as the lectotype species of *Achyranthes*, while the later this species was transferred to *Centrostachys* Wall. and the species that were under *Alternanthera* Forssk. transferred in *Achyranthes* Bullock (1957) confirmed that Standley's lectotypification was incorrect and that the type species of *Achyranthes* is *A. aspera* L. The generic concepts of *Achyranthes* and *Alternanthera* then returned to those prior to 1915. The plant published by Linnaeus in 1753 was similarly typified in *Genera Plantarum* in 1754 as *Achyranthes repens*. Later on, the generic description given by Linnaeus better applied for the typified species. In 1896 a genus *Stachyarpagophora* Vaill. ex M.Gómez was separated, which was mentioned by Linnaeus under *Celosia*; now synonymous taxa of the *Achyranthes*. Linnaeus himself interpreted and transferred *A. repens* in *Illecebrum* Ruppius ex L. in 1762 and renamed it *Illecebrum achyrantha* L. now the synonym of *Alternanthera pungens* Kunth. Due to this transference, the nomenclature type was missed hence, he designated another type as *Achyranthes aspera* L.

Achyranthes has several heterotypic synonyms as *Achiranthos* P.Browne in Civ. Nat. Hist. Jamaica: 180 (1756), *Cadelari* Adans. in Fam. Pl. 2: 268 (1763), *Cyathula* Lour. in Fl. Cochinch.: 101 (1790), *Cadelaria* Raf. in Fl. Tellur. 3: 39 (1837), *Amorgine* Raf. in New Fl. 4: 44 (1838), *Stachyarpagophora* Vaill. ex M.Gómez in Anales Inst. Segunda Enseñ. 2: 312 (1896). In many studies, this genus is

separated based on stamen tube length which is not an authentic character to separate plants among closest relationships. Wallich in 1824 established a new genus *Centrostachys* Wall. with *Centrostachys aquatica* (R.Br.) Moq. as a type which was based on *Achyranthes aquatica* R.Br. *Centrostachys aquatic* considered the genus distinct from his *Achyranthes*, while some authors again merged them in *Achyranthes*.

Botanical Characteristics

Herbs, rarely shrubs or trees, with opposite, entire leaves. Flowers in elongating bracteate spikes, solitary in the axils of the bracts, usually sharply deflexed along the inflorescence axis in fruit, bracteoles spinous-aristate with the excurrent midrib. Tepals 4-5, 1-3 nerved, narrowly lanceolate, the outer 2 slightly longer. Stamens 2-5, the filaments monadelphous below, alternating with quadrate to quadrate-spathulate staminodes which are commonly furnished with a variably developed dorsal scale. Ovary 1-ovulate. Style slender, stigma capitate. Fruit capsule, irregularly ruptured by the thin walls, apex firm, falling with the perianth and bracteoles.

Ethnomedicinal Importance

Achyranthes plants have been widely used for thousands of years for the treatment of various diseases due to the presence of a large number of different classes of bioactive ingredients. *A. aspera* is the most widely used species among *Achyranthes*

plants in their traditional systems of medicine that have been utilized all over the world mainly in India, South Korea, Bangladesh, Pakistan, Sri Lanka, Philippines, and Kenya. All the plant parts are mostly used in the treatment of various ailments including gynaecological disorders, asthma, ophthalmia, odontalgia, haemorrhoids, abdominal tumour, difficult labour, wound healing, and insect and snake bites.

A. bidentata is the most abundant plant used in China; the reports of Chinese Pharmacopoeia and “Zhonghua Bencao” say that it activates the blood and regulates menstruation enriching the kidney and nourishing the liver, strengthening the sinews and bones, increasing urine excretion and relieving strangury, and conducting fire back to its origin, thus can be used for the treatment of various diseases, including amenorrhea, dysmenorrhea, lumbago, gonalgia, paraplegia, edema, stranguria, headache, dizziness, odontalgia, oral ulcer, hematemesis, and epistaxis [Chinese Herbalism Editorial Board, 1999; China Pharmacopoeia Commission, 2015].

Commonly, the *A. bidentata* is used in combination with the dried floret of *Carthamus tinctorius* L. and *Angelica sinensis* (Oliv.) (diels) to treat gynaecological disorders. The liver and kidney deficiency are controlled by the combination of *A. bidentata* with *Eucommiae Cortex* and *Rehmanniae Radix* *Praepapata* (Lei, 2012). *A. rubrofusca* is the most powerful to treat diabetes (Geetha et

al., 2011). *A. japonica* is widely used to treat osteoarthritis, arthritis, edema, delayed menses, contraception, and induction of abortion in Korea (Bang et al., 2012; Lee et al., 2012).

Chemical Constituents

Achyranthes plants have been proven to be a rich source of various natural compounds having different chemical compositions. He et al. (2017) updated more than 133 identified chemical compounds that have been arranged into the groups of triterpenoid saponins, ketosteroids, sterols, alkaloids, flavonoids, anthraquinones, and organic acids along with these it is also a rich source of polysaccharides and polypeptides. In Chinese Pharmacopoeia, a chemical i.e., β -ecdysterone recorded as the index component for the quality control of *Achyranthis Bidentatae Radix* (China Pharmacopoeia Commission, 2015).

Polysaccharides and polypeptides - fructose, glucose, galactose, arabinose, rhamnose, glucuronic acid, and galacturonic acid, β -D-fructofuranosyl, (1 $\rightarrow$ 4)-D-glucuronic acid, (1 $\rightarrow$ 4)-D-galacturonic acid residues, L-arabinose and L-rhamnose.

Triterpenoid saponins - Oleanolic acid; betulinic acid; momordin Ib; momordin Ib; Oleanolic acid 3-O- β -D-glucuronopyranoside-6-O-methyl ester; oleanolic acid 3-O- β -D-glucuronopyranoside-6-O-butyl ester; zingibroside R1; oleanolic acid 3-O-[3'-O-(2'-hydroxy-1'-carboxyethoxycarboxypropyl)]- β -D-glucopyranoside; achyranthoside G; 28-deglucosyl achyranthoside D methyl

ester; niuxi saponin A; oleanolic acid 3-O- β -D-glucopyranoside; achyranthoside IV; bidentatoside II; chikusetsusaponin-1; chikusetsusaponin IVa; chikusetsusaponin IVa methyl ester; chikusetsusaponin IVa ethyl ester; chikusetsusaponin IVa butyl ester; achyranthoside C; achyranthoside E; achyranthoside F; achyranthoside C dimethyl ester; achyranthoside E dimethyl ester; β -D-glucopyranosyl 3 β -[O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-O- β -D-glucopyranuronosyloxy] oleanolate; chikusetsusaponin V; pseudoginsenoside RT1; achyranthoside E trimethyl ester; achyranthoside H; achyranthoside C butyl dimethyl ester; achyranthoside E butyl dimethyl ester; 18-(β -D-glucopyranosyloxy)-28-oxoolean-12-en-3 β -yl 3-O-(β -D-glucopyranosyl)- β -D-glucopyranosiduronic acid methyl ester; momordin IIa; chikusetsusaponin V methyl ester; chikusetsusaponin V butyl ester; achyranthoside D, 3-O-[2'-O- β -D-glucopyranosyl-3'-O-(2'-hydroxy-1'-carboxyethoxycarboxypropyl)]- β -D-glucopyranosyl oleanolic acid 28-O- β -D-glucopyranoside; achyranthoside D trimethyl ester; sulfachyranthoside B; sulfachyranthoside D; bidentatoside I; achyranthoside B; achyranthoside A; achyranthoside A trimethyl ester; niuxi saponin B; achyranthoside I; α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-(β -D-glucopyranosyluronic acid)-(1 $\rightarrow$ 3)- β -D-oleanolic acid-28-O- β -D-glucopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside; hederagenin-28-O- β -D-glucopyranosyl ester; β -D-glucopyranosyl 3 β -[O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-

O - β -D-glucopyranuronosyloxy] machaerinate; β -D-glucopyranosyl 3 β -[O- α -L-galactopyranosyl-(1 $\rightarrow$ 2)-O- α -D-glucopyranuronosyloxy]machaerinate.

Ketosteroids - Ecdysterone; inokosterone; 25S-inokosterone; 25R-inokosterone; polypodine B; stachysterone C; podecdysone C; achyranthesterone A; stachysterone A; rhapontisterone B; stachysterone D; shidasterone; 25, 26-dehydroponasterone A; rubrosterone; (20R, 22R)-2 β , 3 β , 20, 22, 26-pentahydroxy-cholestan-7, 12-dien-6-one; (20R,22R)-20-O-22-O-(5'-hydroxymethyl)-furfurylidene-2 β , 3 β , 25-trihydroxy-14 β -methyl-18-nor-5 β -cholesta-7, 12-dien-6-one; (20R,22R,24S)-20-O,22-O-(5'-hydroxymethyl)-furfurylidene-2 β , 3 β , 14 α , 25-tetrahydroxy-5 β -ergost-7-en-6-one; (20R, 22R, 25R)-20-O,22-O-(5'-hydroxymethyl)-furfurylidene-2 β , 3 β , 5 β , 14 α , 26-pentahydroxycholest-7-en-6-one; (25S)-20, 22-O-(R-ethylidene)inokosterone; 20,22-O-(R-3-methoxycarbonyl)propylidene-20-hydroxy ecdysone.

Sterols - β -sitosterol; stigmasterol, α -spinasterol, β -sitosteryl glucoside; stigmasteryl glucoside; α -spinasterol-3-O- β -D-glucoside; Ergosta-7, 22-diene-3 β , 5 α , 6 β -triol.

Alkaloids - Achyranthine; betaine; betaine hydrochloride; betaine hydrate; coptisine.

Flavonoids - Isoquercetin; astragalin; baicalin; wogonin; hyperoside; rutin.

Anthraquinones - Emodin; physcion; chrysophanol.

Organic acids - Palmitic acid; succinic acid; oxalic acid; γ -aminobutyric acid; caffeic acid; nonanedioic acid; tianshic acid; linoleic acid; trans-13-docasenoic acid; N-hexacos-17-enoic acid; N-hexacos-11-enoic acid; N-hexacos-14-enoic acid.

Other chemicals - Eugenol; hydroquinone; para-benzoquinone; asarone; α -ionone; spathulenol; allantoin; geniposide; 5-hydroxymethyl furaldehyde; N-butyl- β -D-fructopyranoside; (S)-N-feruloyl normetanephine; N-trans-feruloyltyramine; N-cis-feruloyltyramine; N-cis-feruloyl-3-methoxytyramine; nerol; tetracontanol-2; pentatriacontanone; 10-tricosanone; 10-octacosanone; 4-tritriacontanone; pentatriacontane; hexatriacontane; hentriacontane; tritriacontane; N-hexacosanyl N-decaniate; (9E)-8, 11, 12-trihydroxyoctadecenoic acid methyl ester; tritriacontanol; 27-cyclohexylheptacosan-7-ol; 4-methylheptatriacont-1-en-10-ol; 16-hydroxy-26-methylheptacosan-2-one; 36, 47-dihydroxyhenpentacontan-4-one.

Species

Genus *Achyranthes* is known to contain a total of 14 taxonomically accepted species (POWO, 2023) that are as-

1. *A. ancistrophora* C.C.Towns.
2. *A. arborescens* R.Br.
3. *A. aspera* L.
4. *A. bidentata* Blume
5. *A. coynei* Santapau
6. *A. diandra* Roxb.

7. *A. fasciculata* (Suess.) C.C.Towns.
8. *A. mangarevica* Suess.
9. *A. marchionica* F.Br.
10. *A. margaretarum* de Lange
11. *A. mutica* A.Gray ex H.Mann
12. *A. shahii* M.R.Almeida & S.M.Almeida
13. *A. splendens* Mart. ex Moq.
14. *A. talbotii* Hutch. & Dalziel

The recent database of World Flora Online (WFO, 2023) mentioned it with a total of 12 accepted species as-

1. *A. arborescens* R.Br.
2. *A. aspera* L.

3. *A. bidentata* Blume
4. *A. diandra* Roxb.
5. *A. fasciculata* (Suess.) C.C.Towns.
6. *A. mangarevica* Suess.
7. *A. marchionica* F.Br.
8. *A. margaretarum* de Lange
9. *A. mutica* A.Gray ex H.Mann
10. *A. shahii* M.R.Almeida & S.M.Almeida
11. *A. splendens* Mart. ex Moq.
12. *A. talbotii* Hutch. & Dalziel

Among them, four species namely *A. aspera* L., *A. bidentata* Blume, *A. coynei* Santapau and *A. shahii* M.R.Almeida & S.M.Almeida are native to India.

Achyranthes aspera L.

A. aspera is an annual herb distributed throughout the tropical and subtropical world. It can be found in many places as a common weed and grows as an introduced species while sometime as invasive species including many Pacific Islands environments. It has been used for thousands of years in medicinal practices and to some extent as food constituent also. The name of this plant is different in various regions of the world. In English, it is known as “chaff flower”, “prickly chaff flower” and “devil’s horsewhip”. In Kenya, it is called “snake’s tail”. In Sanskrit “Adhoghanta”, “Adhvashalya”, “Aghamargava”, “Aghata”, “Apamarga”, “Apangaka”, “Chamatkara”, “Dhamargava”. In Hindi “Apang”, “Chirchra”, “Chirehitta”, “Latjira” and “Onga”. In Persian “Kharevazhun”. In Punjabi “Kutri”. In Indonesia “Jarong”. In African Countries

“Grootklits”. It has a wide range of varieties and cultivars which vary in flavour and uses. It has nine accepted infraspecific taxa as *A. aspera* var. *aspera*, *A. aspera* var. *borbonica* (Willd. ex Schult.) C.C.Towns., *A. aspera* var. *indica* L., *A. aspera* var. *late-ovata* Boerl., *A. aspera* var. *porphyristachya* (Wall. ex Moq.) Hook.f., *A. aspera* var. *pubescens* (Moq.) M.Gómez, *A. aspera* var. *rubrofusca* (Wight) Hook.f., *A. aspera* var. *sicula* L., and *A. aspera* var. *velutina* (Hook. & Arn.) C.C.Towns. It is usually herbaceous in nature but sometime it shows shrubby habit and height ranges from 1-2 m. The leaves might be thick, ovate, elliptic, or obovate depending upon the variety to variety and habitat. Flower colour ranges from greenish to white. Most of the varieties of this shows perennial habits while some are annual (Srivastav et al., 2011; Rehman et

al., 2018). All the varieties have essential oil which are variable among varieties depending on growing conditions, geographic origins, harvesting time, etc.

It is frequently found in waste areas along roadsides, foot paths, railroads and sand dunes. It often infests fence rows, open woodland, and the borders of forests and plantations. Its introduction risk is very high because it can easily transport to new habitats due to its spiny fruits that can easily detach and stick to clothes, fur, and feathers. So that its seeds easily transported to new habitats by birds, mammals, and humans. It has very wide range to adaptability (Holm et al., 1979). It was introduced in Commonwealth of the Bahamas, is an island country within the Lucayan Archipelago of the West Indies in the North Atlantic from where it was collected by N.L. Britton and C. T. Millspaugh in 1920 (Britton and Millspaugh, 1920). In 1924 this was reported from the Puerto Rico (including Mona, Vieques and Culebra), the US Virgin Islands (St. Croix and St. Thomas) and the British Virgin Islands (Tortola) by N.L. Britton (Britton and Wilson, 1924). In Kenya, Tanzania, and Uganda it is reported as an invasive species (CABI Invasive Species Compendium, 2011). It was introduced on Pacific Islands (including Hawaii, Samoa, Mariana Islands, Cook Islands, Fiji, and French Polynesia) where it was mentioned as an invasive species (PIER, 2012). It was also reported from Florida where it is mentioned as a “potential problematic species” (USDA-NRCS, 2012).

History and Vedic Literatures

A. aspera is native to South-east Asia. It has grown here from ancient times. Etymologically the species epithet “*aspera*” comes from Greek language which means “rough”, as the stem and leaves of this herb are very rough, so it is called “*aspera*”. This suggestion is uncertain while some authors stated that the name is due to its symmetry above the plan. The history of this plant is steeped in legend. It is assumed that the Alexander the Great (356-323 B.C.E) brought it to Greece. During 1700s it brought from South Asia to France and USA (Rehman et al., 2018). It takes a gentle position in Indian medicinal systems as ‘Miracle Herb in Indian Traditional Healing’. Ethnobotanically, the detailed study on this plant focussed and progressed since 1990. It is known as ‘Apamarga’ which is mentioned in ‘Sadbakalpadruma’ and composed by AP + Ā + Mrj + Karane Ghaj that means wipes; that means it cleans the body by removing disease from throughout the body (Prasad and Subhaktha, 2001). Historically, it was mentioned in Yajurveda, Atharvaveda, and Ayurveda, along with its medicinal uses. In Yajurveda (35.11), for this plant the statement has been mentioned as “Apamarga, please protect us from harmful deeds and bad nightmares”. In contemporary scenarios, this plant operates in line with the assertion by boosting willpower and mind control (Chand, 1995; Sharma, 1968; Prasad and Subhaktha, 2001).

In Atharvaveda the name ‘Apamarga’ is mentioned eleven times. It is considered that this plant is the Lord of all plants on earth because it has the capability to increase life span

as it combats diseases. In Atharvaveda Kanda IV. 17.6-8, it has been mentioned as very useful to combat the disease caused by thirst and hunger, defects of the organs, speech, sterility, physical debility, and all other ailments. Along with these in Kanda IV. 18.7-8 and 19.4-5, it is described that it can sweep away chronic diseases, the evil habit of using foul language and the ailments which cause severe pain as well as decrease in vitality. It is described in Kanda VII 65.1 for eliminating all “dosas” and purging sins from the body. According to Kanda VII. 65-3, if people who are crippled and whose teeth are black and nails are malformed, consuming this plant can get rid of all the “dosas” that are left over (Sharma, 1968; Chand, 1995; Prasad and Subhaktha, 2001).

Ayurveda is the contribution of Maharishi Caraka, Susruta and Vagbhata who wrote the big compendium individually as ‘Caraka Samhita’, ‘Susruta Samhita’ and ‘Astanga Hrdaya Samhita’ respectively which are collectively known as ‘Brhathrayi’. In ‘Caraka Samhita’ the Apamarga placed under Krimighna, Vamanopaga and Sirovirecanopaga groups which is mentioned a total of 27 times with four different names as Apamarga, Pratyakpuspi, Mayuraka and Sikhari (C.Su.4/11 and 13) (Singh and Chuneekar, 1972; Sharma, 1981; Prasad and Subhaktha, 2001). According to this literature, it is best for Siroviracana (C.Su. 25/40). In ‘Susruta Samhita’ it is mentioned under the group of Varunadigana, Viratarvadigana, and Arkadigana where it is mentioned about twenty-nine times with four different names as Kharamanjari, Pratyakpuspi, Mayuraka and Vasir (Su. Su. 38/8,10 and 16) (Singh and

Chuneekar, 1972; Ambikadutta, 1995; Prasad and Subhaktha, 2001). ‘Astanga Hrdaya Samhita’ followed both earlier mentioned ‘Samhitas’ in which the Apamarga placed under Tiktavarga (bitter group) and use similar synonyms which are placed twenty-six times (A.Hr. Su. 10/29) (Gupta, 1951; Singh and Chuneekar, 1972; Prasad and Subhaktha, 2001). Along with these literatures it was well mentioned in other literatures.

In ‘Astanga Samgraha’, the Apamarga mentioned thirty-six times with seven names as Kinihi, Kharamanjari, Nandi, Mayuraka, Sikhari, Vasir and Pratyakpuspi which are placed under the Sodhanadigana as Sirovirecana dravya (A.S.Su.14/5), the Tikta Skanda drugs (bitter group of drugs) (A.S.Su.18/23) (Murthy, 1996; Uniyal, 1981; Prasad and Subhaktha, 2001). In ‘Bhavaprakasa’ it is mentioned for its Tiktsna gunas and Katu rasa properties with seven synonyms as Sikhari, Adhahsalya, Mayuraka, Markati, Durghraha, Kinihi and Kharamanjari. In this manuscript, it is mentioned for improving digestion, useful in ‘Cardi’ (vomiting), disease of ‘Kapha’, ‘Medas’ (fat) and ‘Vata’. Along with these it is very useful in ‘Hrdroga’, ‘Adhmana’ (flatulence), ‘Arsa’ (piles), ‘Kandu’ (itching), ‘Udarasula’ (pain in abdomen), ‘Udara’ (enlargement of abdomen) and ‘Apaci’ (scrofula) (B.P.Guducyadivarga, 219 & 220) (Murthy, 1998). Fruits of Apamarga are said to be sweet both in ‘Rasa’ (taste) and ‘Vipaka’ (taste after digestion) which are not easily digestible and cause constipation, increases ‘Vata’; dryness, mitigate ‘Rakta’ and ‘Pitta Dosa’ (B.P Guducyadivarga/223) (Murthy, 1998; Prasad and Subhaktha, 2001).

In Ayurvedic literatures, the Nighantus (lexicon) play important role that are helpful to identify the uses of medicinal plants and plant medicinal properties. Many numbers of Nighantus are available today, in which the Apamarga described with various names. As in 'Raja Nighantu' which is written by 'Narahari', mentioned it under the 'Guducyadi varga' (group) with twenty-one names as Adhahsalya, Durabhigraha, Durgraha, Kandakanta, Kanti, Katurmajjarika, Kharamanjari, Kinihi, Ksavak, Kubja Malakanta, Markati, Markatippali, Mayuraka, Nandi, Panktikantakah, Parakpuspi, Pratyakpuspi, Saikharika, Sikhari, and Vasirah (Guducyadivarga: 386-388) (Ganesh, 1925; Prasad and Subhaktha, 2001).

Classification

Kingdom	-	Plantae
Subkingdom	-	Tracheobinota
Super Division	-	Spermatophyta
Division	-	Mangoliophyta
Class	-	Mangoliophsida
Subclass	-	Caryophyllidae
Order	-	Caryophyllales
Family	-	Amaranthaceae
Genus	-	<i>Achyranthes</i>
Species	-	<i>aspera</i>

Common Names

Bonsoth, Obhat, Prickly Chaff Flower, Agharo, Aghara, Chirchira, Durabhigraha, Dhanushka, Pratyakpushpi, Mayur, Latjira,

Aghado, Katalaati, Khujumpere, Apamarga, Kharamanjari, Chaff-flower, Prickly Chaff Flower, Kharamanjari, Apamarkkam, Uttarenu, Churchura.

Global Distribution

The species *A. aspera* abundantly found throughout the world mainly in tropical and subtropical regions. In Africa it is distributed in Cameroon, Ethiopia, Ghana, Kenya, Mali, Nigeria, Tanzania and Zaire. In Asia it is reported from Arabia, Afghanistan, China, India, Japan, Java, Malaysia, Nepal, Northern Myanmar, Pakistan, the Philippines, Sri Lanka and Taiwan. Currently, according to the Plants of the World Online (POWO, 2023) Database it is native of Afghanistan, Aldabra, Algeria, Andaman Is., Angola, Assam, Azores, Bangladesh, Benin, Bismarck Archipelago, Borneo, Botswana, Burkina, Burundi, Cambodia, Cameroon, Canary Is., Cape Provinces, Cape Verde, Caroline Is., Central African Repu, Chad, Chagos Archipelago, China South-Central, China Southeast, Christmas I., Cocos (Keeling) Is., Comoros, Congo, Djibouti, East Himalaya, Egypt, Eritrea, Ethiopia, Free State, Gabon, Gambia, Ghana, Gilbert Is., Guinea, Guinea-Bissau, Gulf of Guinea Is., Gulf States, Hainan, India, Iran, Italy, Ivory Coast, Jawa, Kazan-retto, Kenya, KwaZulu-Natal, Laccadive Is., Laos, Lebanon-Syria, Lesotho, Lesser Sunda Is., Liberia, Line Is., Madagascar, Madeira, Malawi, Malaya, Maldives, Mali, Maluku, Marianas, Marshall Is., Mauritania, Mauritius, Morocco, Mozambique, Mozambique Channel I, Myanmar, Namibia, Nansei-shoto,

Nauru, Nepal, New Guinea, Nicobar Is., Niger, Nigeria, Norfolk Is., Northern Provinces, Northern Territory, Ogasawara-shoto, Oman, Pakistan, Panamá, Philippines, Pitcairn Is., Queensland, Rodrigues, Rwanda, Réunion, Samoa, Sardegna, Saudi Arabia, Selvagens, Senegal, Sicilia, Sierra Leone, Society Is., Socotra, Solomon Is., Somalia, South China Sea, Spain, Sri Lanka, Sudan, Sulawesi, Sumatera, Swaziland, Taiwan, Tanzania, Thailand, Tokelau-Manihiki, Tuamotu, Tubuai Is., Tunisia, Tuvalu, Uganda, Vietnam, Wallis-Futuna Is., West Himalaya, Western Australia, Western Sahara, Yemen, Zambia, Zaire, Zimbabwe; and introduced in Alabama, Aruba, Austria, Bahamas, Belize, Bolivia, Brazil North,

Brazil Northeast, Brazil Southeast, Brazil West-Central, Colombia, Cook Is., Costa Rica, Cuba, Dominican Republic, El Salvador, Fiji, Florida, French Guiana, Galápagos, Guatemala, Guyana, Haiti, Hawaii, Honduras, Jamaica, Japan, Kermadec Is., Leeward Is., Louisiana, Marquesas, Maryland, Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Netherlands Antilles, New Caledonia, New Zealand North, Nicaragua, Niue, Palestine, Peru, Puerto Rico, Seychelles, South Carolina, Southwest Caribbean, St. Helena, Suriname, Texas, Tonga, Trinidad-Tobago, Turks-Caicos Is., Vanuatu, Venezuela, Venezuelan Antilles, Windward Is. (Fig. 2).

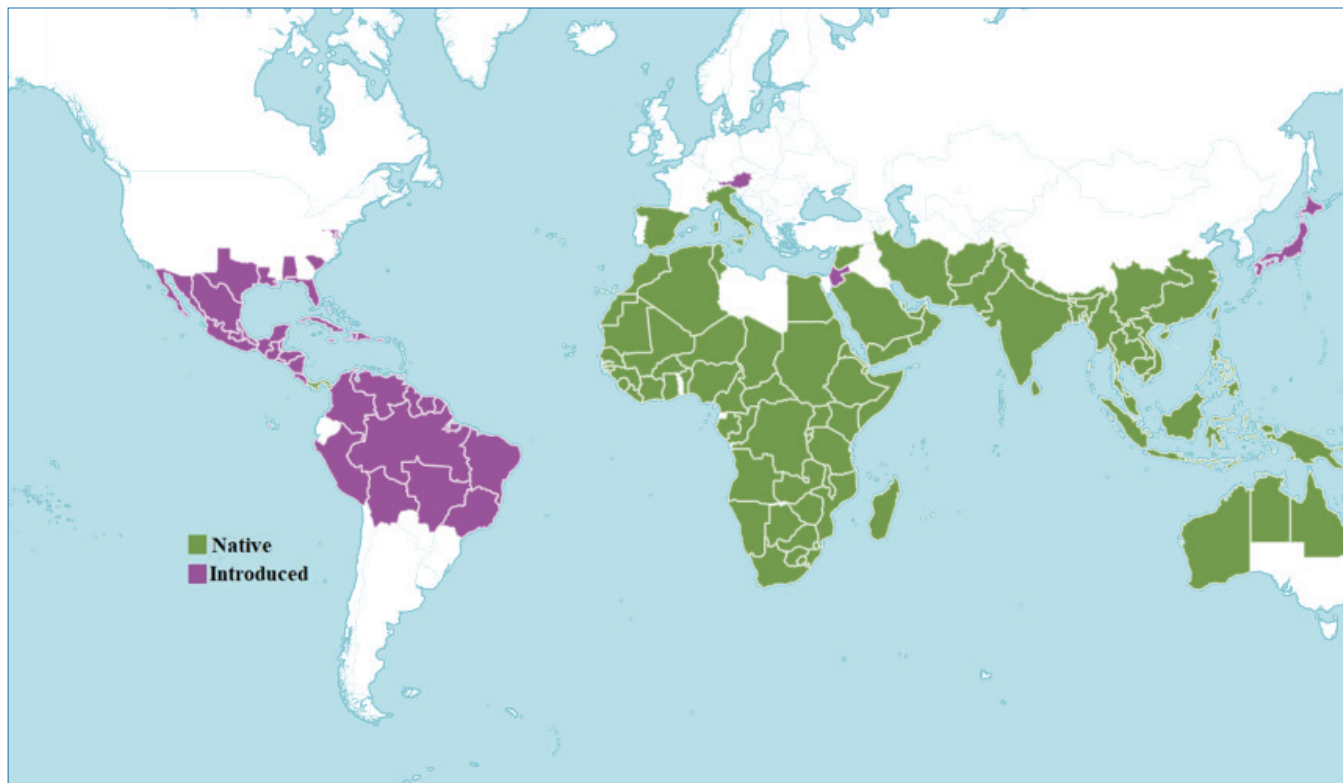


Fig. 2 Global distribution of *A. aspera*

Distribution in India

It is distributed throughout India, up to 1219 m along roadsides and waste places as well as on hills up to 900 m. India biodiversity organization mentioned it in Assam, Bihar, Gujarat, Karnataka, Madhya Pradesh, Odisha, Rajasthan, South India, and Uttar Pradesh. In eFlora of India discussion it is mentioned in Flora of Maharashtra, Peninsular India, Flora of Panipat, Hooghly, Govindghat Ghangriya Trek, Gujrat, Bengal.

Ethnopharmacology and Traditional Uses

Tribal and rural people have been using the *A. aspera* plant for a wide range of medical conditions since ancient times. It has long been connected to conventional Indian medicine and culture. Nearly all conventional medical systems, including Ayurveda, Unani, and Siddha, employ it. This plant was found across the old world's tropical and subtropical regions, according to Maiden's 1889 book "The Useful Native Plants of Australia." Ayurveda claims that it works well for curing many different illnesses, such as *Sirovirecana*, *Pandu*, *Krimi*, *Karnaroga*, and *Pandu* (Prasad and Subhaktha, 2001). Acharya Charaka classified Apamarga into three categories: *Krimighna*, *Vamanopaga*, and *Sirovirecanopaga*. In the Charaka Samhita, he used the four synonyms *Pratyakpuspi*, *Mayuraka*, *Sikhari*, and *Apamarga* to allude to Apamarga in about twenty-seven different places (Sharma, 2000). Susruta also depicts Apamarga in *Varunadigana*, *Viratarvadigana*,

and *Arkadigana*. Using four synonyms, such as *Kharamanjar*, *Pratyakpuspi*, *Mayuraka*, and *Vasir*, he referenced this drug around 29 times (Shastri, 1995).

As one of the most adaptable medicinal herbs with a broad range of biological activity, *A. aspera* is an herbaceous plant that is widely recognised in India and other countries. Traditional healers hold this plant in great esteem and utilise it to treat a wide range of illnesses, including beriberi, colic, bronchial infections, toothaches, convulsions, blood abnormalities, diabetes mellitus, menstruation issues, and reproductive issues. People with dropsy are given this herb in India. The seeds are administered to patients with hydrophobia, snakebites, ophthalmia, and skin conditions (Dhale and Bhoi, 2013). Medication created from blooming spikes prepared by lightly sugaring them is administered to those attacked by crazy dogs (Iwu, 2014). An important component of the plant's ash is potash, which is used in washing.

Pneumonia is treated with boiling crushed plant in water. The root is infused to treat digestive issues. A decoction made from the whole plant is used to treat inflammatory diseases of the body. A decoction of the roots might help to treat digestive issues. For diarrhea, consume 2–5 gm of dried leaf powder with honey. Leaf juice is a helpful treatment for scabies and pruritus. Toxic bites are treated externally with leaf paste. Abdominal issues and bleeding piles respond well to whole plant ash. The root of *A. aspera* is used as a mouthwash and to treat halitosis.

Another application for twig infusion is as a toothache wash. For night blindness, root extract is used as an eye drop before bed (Raji, 2013). When combined with water to form a paste, the blooming spikes or seeds can be used externally to treat snake and reptile bites. Additionally, skin conditions and night blindness are treated with it (Nadkarni, 2009).

Ground root is administered with water to patients who have been bitten by snakes until they become aware again and vomit. It is recommended to inhale this plant's vapors in combination with *Smilax ovalifera* roots to increase appetite and treat a variety of stomach ailments (Bhattaraj, 1992). Its leaves and seeds have emetic, hydrophobic, carminative, reversing edema, digestive, and pharyngitis, making it beneficial for hemorrhoids. The plant's ash is applied topically to treat warts and ulcers. To relieve strained back pain, crushed leaves are applied to the affected area (Singh et al., 1986). The roots pasted in water is used for corneal opacities and ophthalmia. Wasp bite pain can be relieved with a paste made of fresh leaves (Gupta, 2010). The herb helps with rheumatism, scabies, liver problems, and other skin conditions. Additionally, it has calming effects (Anonymous, 2007).

Crushed leaves are administered topically to wounds. To treat diarrhea in infants, one teaspoon of crushed root is given with water sugar three times a day. For a few days, seeds cooked in milk are consumed to lower blood pressure. When treating asthma, one teaspoon of roasted seed powder is taken orally till the

condition improves. The treatment for urinary tract infections involves giving one table spoon of root powder twice a day. To treat cholera, a decoction of 15 gm of root powder is administered twice a day for three days. When treating asthma, one teaspoon of ground-up roasted seeds is taken orally till the condition improves. After sprinkling chilli powder on the bite site, a paste made from leaves and roots is used to cure animal and dog bites. This process takes thirty minutes. For bug bites and scorpion stings, an external paste made of roots and leaves is administered (Singh et al., 2018).

Selection of the Sites and Characteristics Studied

The distribution, morphological variations, and association of *A. aspera* 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 twenty-six sites were selected with two sites ranging between around 100 km in distance while the whole distance is about 2600 km (Table 1). 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 *A. aspera* 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	Present
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	Absent
S12	Prayagraj	98	25.45	81.85	Absent
S13	Mirzapur	80	25.15	82.58	Absent
S14	Varanasi	81	25.32	83.01	Absent
S15	Ballia	67	25.76	84.15	Absent
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	Present
S20	Farka	42	25.23	87.09	Absent
S21	Sahebganj	16	25.25	87.65	Present
S22	Farakka Barrage	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	Absent
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, 7, 8, 9, 10, 16, 17, 18, 19, and 21, due to these sites being the source of its bulk collection. For the remaining sites as 1, 2, 3, 11, 12, 13, 14, 15, 20, 22, 23, 24, 25, and 26 it was only a representative. Hence, it may be concluded that these sites require more conservation of this species.

Taxonomic Treatment

A. aspera L., Sp. PL: 204. l 753; Hook.f., Fl. Brit. India 4:730. 1885; Duthie, Fl Gangetic Plain (repr. ed.) 2: 140. 1960. Fl. Uttar Pradesh 2: 367.2020.

Synonyms

The Plants of the World Online database mentioned it with its two homotypic Synonyms (Powo, 2023) as-

1. *Centrostachys aspera* (L.) Standl. in J. Washington Acad. Sci. 5: 75 (1915)
2. *Stachyarpagophora aspera* (L.) M.Gómez in Anales Inst. Segunda Enseñ. 2: 312 (1896)

While another database i.e., World Flora Online (WFO, 2023) mentioned it with a total of following twenty-seven synonyms-

1. *A. acuminata* hort. ex Schinz
2. *A. aspera* f. *subgrandifolia* Suess.
3. *A. aspera* var. *aspera*
4. *A. aspera* var. *australis* (R.Br.) Domin

5. *A. aspera* var. *canescens* F.M.Bailey
6. *A. aspera* var. *obtusifolia* (Lam.) Suess.
7. *A. aspera* var. *simplex* Millsp.
8. *A. asperoides* P.Lima
9. *A. australis* R.Br.
10. *A. canescens* R.Br.
11. *A. daito-insularis* Tawada
12. *A. ellipticifolia* Stokes
13. *A. fruticosa* Desf.
14. *A. grandifolia* Moq.
15. *A. obovata* Peter
16. *A. obovatifolia* Stokes
17. *A. okinawensis* Tawada
18. *A. robusta* C.H.Wright
19. *A. sicala* Roth
20. *A. tenuifolia* Steud.
21. *Cadelaria punctata* Raf.
22. *Centrostachys aspera* (L.) Standl.
23. *C. australis* Standl.
24. *C. canescens* Standl.
25. *C. grandifolia* Standl.
26. *C. indica* (L.) Standl.
27. *Stachyarpagophora aspera* (L.) M.Gómez

Varieties of *Achyranthes aspera* L.

It has a total of nine varieties, are accepted in Plants of the World Online database (POWO, 2023) as-

1. *A. aspera* var. *aspera*
2. *A. aspera* var. *borbonica* (Willd. ex Schult.) C.C.Towns.

3. *A. aspera* var. *indica* L.
4. *A. aspera* var. *late-ovata* Boerl.
5. *A. aspera* var. *porphyristachya* (Wall. ex Moq.) Hook.f.
6. *A. aspera* var. *pubescens* (Moq.) M.Gómez
7. *A. aspera* var. *rubrofusca* (Wight) Hook.f.
8. *A. aspera* var. *sicula* L.
9. *A. aspera* var. *velutina* (Hook. & Arn.) C.C.Towns.

While the World Flora Online (WFO, 2023) database mentioned only seven varieties as-

1. *A. aspera* var. *borbonica* (Willd. ex Schult.) C.C.Towns.
2. *A. aspera* var. *indica* L.
3. *A. aspera* var. *porphyristachya* (Wall. ex Moq.) Hook.f.
4. *A. aspera* var. *pubescens* M.Gómez
5. *A. aspera* var. *rubrofusca* (Wight) Hook.f.
6. *A. aspera* var. *sicula* L.
7. *A. aspera* var. *velutina* (Hook. & Arn.) C.C.Towns.

Botanical Description

Herb, perennial, sometimes woody and suffrutescent, occasionally flowering in the first year, up to 2 m tall, stiffly erect to subscandent or straggling and prostrate, simple to much branched. Stem stout to very weak, distinctly to obscurely 4-angled, striate or sulcate, subglabrous to densely tomentose. Leaves 5-22 × 1.3-8 cm, elliptic, oblong or ovate, acute or acuminate to almost round and

very obtuse, gradually or abruptly narrowed below, indumentum varying from subglabrous through subglabrous above and densely appressed-canescens below to densely tomentose on both surfaces; petioles of main stem leave 3-25 mm long, shortening above and below. Inflorescence at first dense, finally elongating to 8-34 cm; peduncles 1-6 cm long; bracts lanceolate or narrowly deltoid-lanceolate, pale or brownish-membranous, 1.75-5 mm long, glabrous; bracteoles 1.5-4.5 mm long, basal wings 1/3-1/4 the length of the spine and adnate to it, typically tapering off above but not rarely rounded or truncate. Perianth whitish or pale green to red or purple; segments 5, 3-7 mm long, outer pair longest, narrowly lanceolate to lanceolate, very acute, with a distinct midrib and 2 obscure to distinct lateral nerves, narrowly or moderately pale-margined. Stamens 5, filaments 1.5-4.5 mm long, alternating with subquadrate pseudostaminodes; typically the apex of the latter curves slightly inwards as a narrow, crenate or entire, often very delicate flap, while from the dorsal surface arises afimbriate-ciliate scale extending across the width of the pseudostaminode; not rarely, however, this is reduced to a “stag’s-horn” process at the centre of the dorsal surface or a shallow, dentate rim, or even becomes small and filiform – or else subapical or apical so that the pseudostaminode appears simple. Style slender, 1-4 mm long. Fruit capsule, oblong-ovoid, 1-3 mm long. Seed filling the capsule, oblong-ovoid, smooth (Fig. 3A, B).



Fig. 3A Plant habit



Fig. 3B Plant illustration

Chromosome number

The basic chromosome numbers are 7 ($x=7$). Polyploidy is common in naturally occurring plant (Tandon and Rao, 1966; Kumar, 1982). The common ploidy level is seven ($2n=42$), so the gametophytic chromosome counts are 21 ($n=21$) (Pal, 1964; Sharma, 1970; Bir and Sidhu, 1979; Morton, 1993, de Lange, et al. 2004). But in some cytotypes they have been counted 24 ($n=24$) and 48 ($n=48$) (Kumar, 1982; Bir and Sidhu, 1979). Bhat et al. (2019) first time reported 28 ($2n=28$) diploid chromosomes from the plant. The cytotypes of this plant varies as it was reported for its intraspecific octaploid with $2n=56$ (Kumar, 1982), decaploid with $2n=70$ (Romano et al., 1986) and dodecaploid with $2n=84$ (Van Loon, 1974). Thus, this plant shows a lot of chromosomal diversity.

Anatomical Features

Structure of the Leaf

The leaves are arranged in decussate opposite pattern on the stem that bear secondary

branches in the axils which ultimately give rise the branches and leaves of third order by following the same pattern as on the primary stem. The leaves surface is covered by long filamentous multicellular hair on both the surfaces. The transverse section of the leaf shows the anatomical characteristics as position of cells, stomata, and vascular bundles. The epidermal cell layer is simple not wavy. The stomata are usually present on the lower surface of the leaves, but rarely few stomata reported on upper surface. In detailed study of petiole, the stomata are totally absent. A few layers of collenchyma are present below the epidermis. Below collenchyma the mesophylls tissues including both spongy and palisade tissue are present. A clear ring of four collateral vascular bundles presents in the midrib. The vascular bundle splits in half in the petiole region, and the two halves separate to form an arc of bundles. The arc having normally a total of five bundles but it may vary from three and seven due to union and division of the lateral ones on each side (Fig. 4).

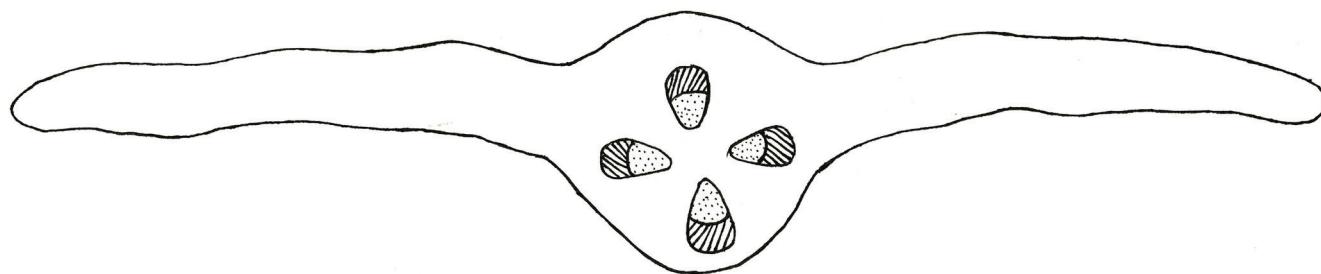


Fig. 4 Transverse section of the leaf, showing the arrangement of bundles in the midrib

Structure of the stem

Transverse section of the stem shows alternate groove and ridges due to the presence of ribs of collenchyma tissue below epidermis. The epidermal cells are very elongated opposite to the ridges while opposite to grooves they are small. The stem is totally covered with the hairs. The hairs vary accordingly the plant habit and climatic conditions. Below the epidermis, the cortex layer exist that is chlorenchymatous in nature with intercellular

spaces. The stomata also present in the stem part in grooves. The endodermis is unclear. Inside the endodermis two to three layers of pericycle is present, the outer layer is made up of a chain of sclerenchymatous fibres. The T.S. of stem just below node shows similar arrangement of vascular tissue in a ring as a typical dicotyledons' but the two collateral bundles are present inside the peripheral ring (Fig 5). These two collateral bundles are lie opposite to each other.

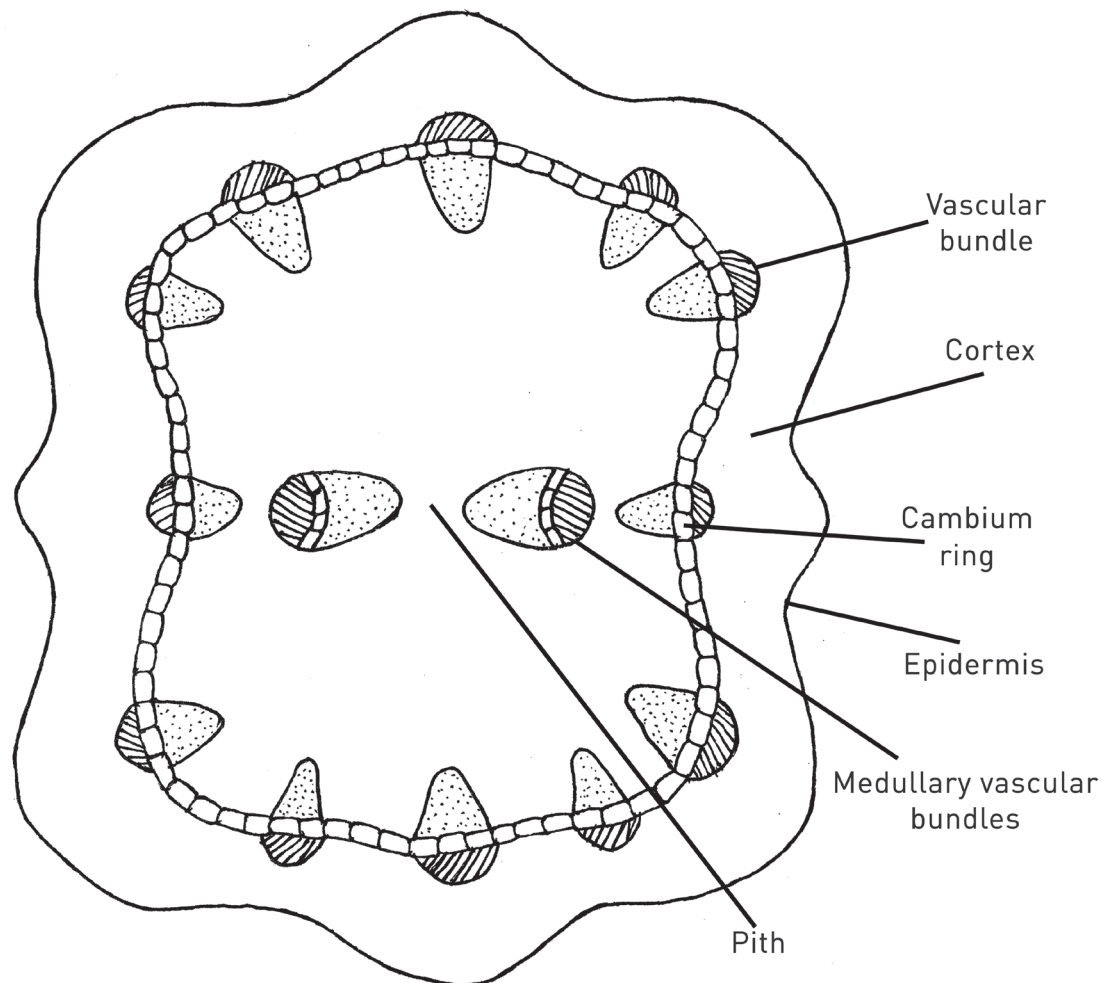


Fig. 5 T.S. of stem just below the node that showing the arrangement of vascular bundles

The internode, which is present below the inflorescence the two medullary bundles remain separate throughout the length while in other internodes they are just separated above or below a node. The vascular bundles are in outer ring are collateral, open and endarch. However, in the maximum portion of the internode the two bundles fuse together

to form an amphixylic type of single bundle. The phloem in the middle of a mature bundle consists of a big band of parenchymatous tissues, which are elongated transversely between the two xylem patches. The xylem divided into outer protoxylem and metaxylem or secondary xylem. The later develop centripetally (Fig. 6).

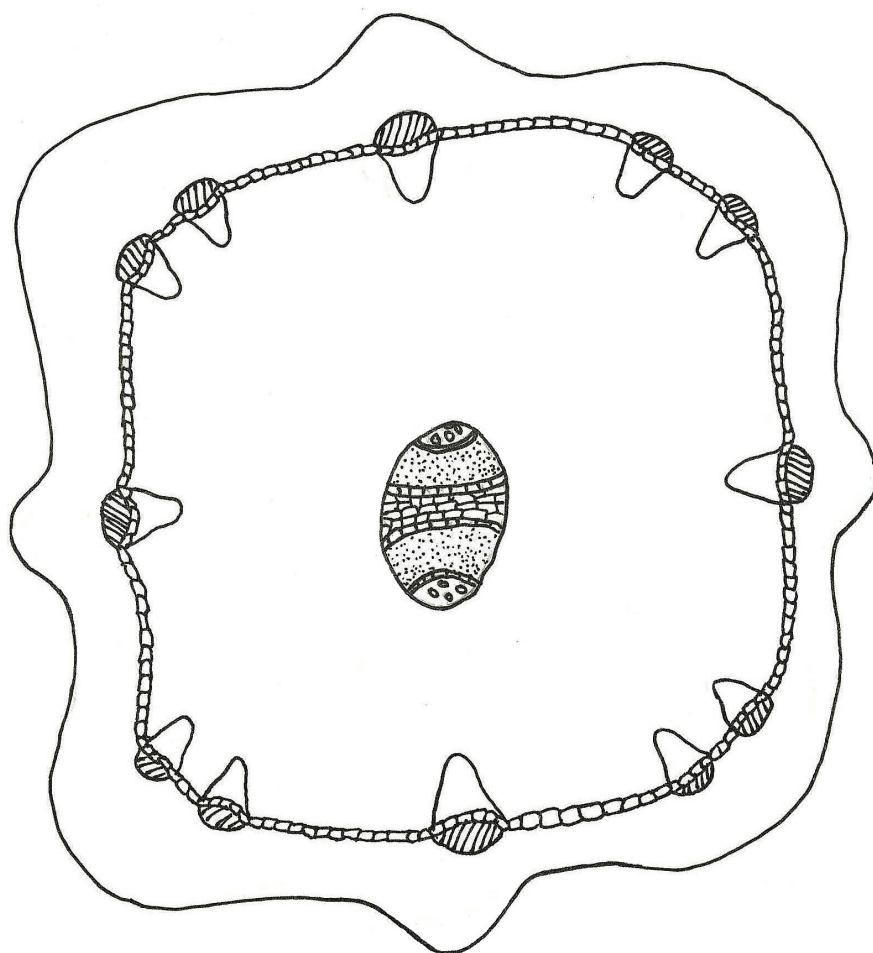


Fig. 6 T.S. of stem through the middle of an internode, showing the union of the two collateral medullary bundles to form a single bundle of amphixylic type

The presence of cambium in between xylem and phloem indicate that this plant undergoes secondary growth. The cambium along with the vascular bundles also present in the

outside of the phloem where it joined the two ends of the phloem.

The T.S. of young leaf just behind the growing apex, where primary vascular

bundles still differentiates and sieve tubes are freely scattered shows the medullary bundles without parenchyma (Fig. 7). At maturity the distinct central parenchymatous patch is formed by division of few parenchymatous cells present in the original phloem, it is responsible for the pushing of sieve tubes outwards. The bundles change into amphixylic bundle in the internode from two quite free normally orientated collateral bundles near

the node. The two free medullary bundles approach each other and meet after that the xylem of both are joined and become continuous. The phloem begins to cut into the xylem carrying the cambium before it. The parenchymatous appearance appear in the middle of the phloem. After complete division at maturity the medullary bundles are formed from this single amphixylic bundle (Fig. 8).

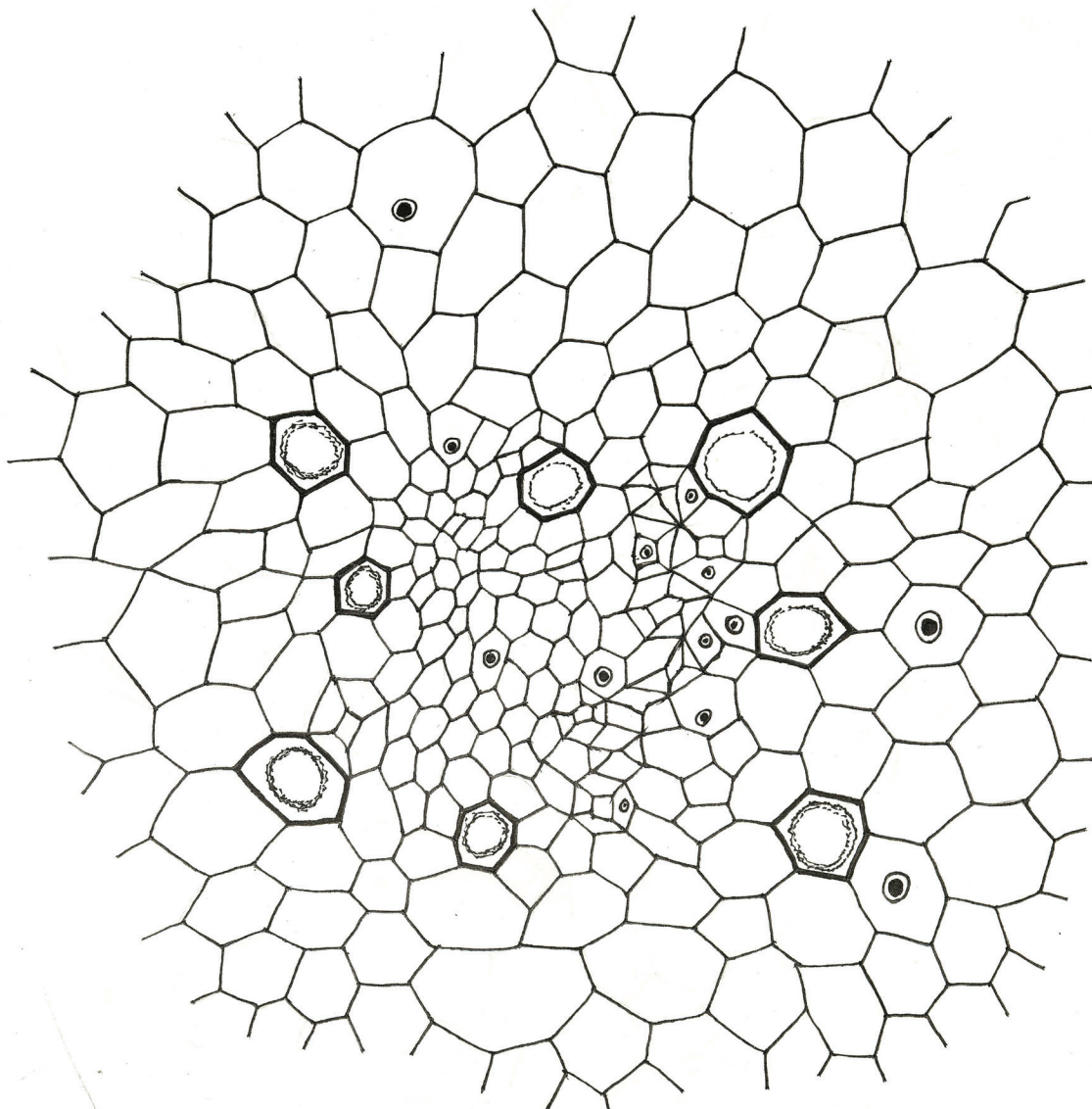


Fig. 7 T.S. of young leaf just behind the growing apex (young stage)

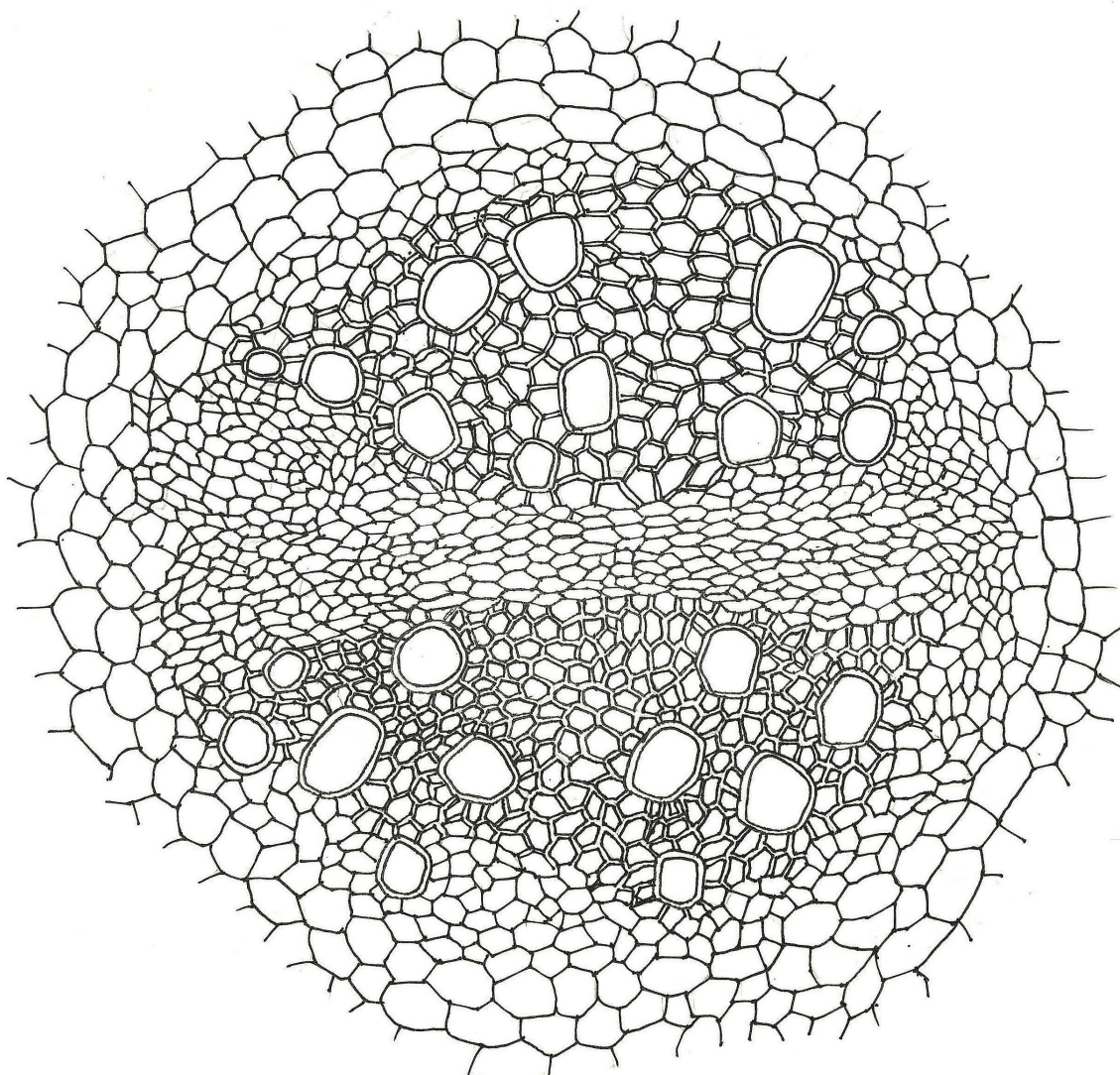


Fig. 8 T.S. of young leaf just behind the growing apex (mature stage)

The medullary bundles vary in various locality. In the genus *Achyranthes*, the collateral medullary bundles commonly present inside the primary vascular bundle. These bundles are opposite to each other in internodes.

Secondary Growth

The cambium in *Achyranthes aspera* is composed of multi-storeyed short fusiform cells which are 215–287 μm in length (Rajput and Rao, 2000). In transverse views, the

cambium initially appears in 2-3 layered but at maturity when the xylem and phloem start to develop it appear 3-6 layered. Each cambial ring developed into two unique alternative segments, some of which produced exclusively xylem fibres centripetally and others which produced phloem parenchyma centrifugally. The cambium firstly formed a complete cylinder and then after bidirectionally differentiated into inside xylem and outside phloem. After this, it cease to divide and a new

ring develops at a distance about two to three cell layers external to the phloem and having the activity of cambium (Rajput and Rao, 2000).

The phloem made up of sieve tube members, companion cells and axial parenchyma whereas rays completely absent. The phloem parenchyma having largest cells including a companion cell which are present at the margin of each sieve tube. In sieve tubes, the callose deposition is in functional elements while in non-functional areas it heavily accumulates. The xylem rings are not complete but they appeared as overlapping segments. These are composed of vessels, tracheid, nucleated xylem fibres and axial parenchyma while rays were absent as the phloem (Rajput and Rao, 2000). The vessels might be solitary or multiple of 2-3 radial cells. They have alternate bordered pits with a simple perforation plate in transverse to slightly oblique end walls. The length and width of vessel varies between 147–163 μm and 39–58 μm respectively.

Embryology

First time the embryological or cytological features of *A. aspera* were defined by Kajale (1937) and he studied from the initiation of the flower bud to the formation of the seed. The flowers developed in acropetal succession. Firstly, the bracts appear on the floral axis, followed by the bracteoles, and after that the perianth appear. The stamens arise firstly as an annular outgrowth after some times the primordia of the anthers separated. The nucellus formed at the base of the floral axis

from which the ovary wall arises as a cup like structure. The wall narrows at the top and form a hollow style and stigma. After some times, the glandular hairs developed. After that a long basal funnellis developed at the base of nucellus which is differentiated in to two integuments in a basipetal order. The ovule begins to curve just as the primary archesporium differentiates.

Male Gametophyte

In a transverse section, the principal archesporium in each of the four lobes of an anther consists of 2-4, usually 3 cells. The anther longitudinally forms a plate which is made up of 8-10 cells long and 2-4 cells broad. These cells periclinally divided into two layers; the outer layer is just below the epidermis forms the fibrous endothecium of the mature anther while the inner again divided periclinally resulting in the inner layer and functions as the tapetum and the outer layer degenerate very early. Initially the tapetal cells are uninucleate but they become binucleate as the pollen-mother cells enter the first reduction division. As the pollens going to tetrad formation and pollens mature, the tapetum is degenerated. Simultaneously the anther walls differentiates and the primary sporogenous cells divide mitotically twice and form microspores mother cells. These cells followed the heterotypic and homotypic division and form four nuclei which are connected by secondary spindle fibres that disappear as the cytokinesis started. These four nuclei separated as the cytokinesis goes to furrowing. This furrow

started from the periphery and extend to the centre, resulting in four pollen grains separated or developed. These pollen grains vary in their shape, size that ranges between 14-20 μ . The tube nucleus has an amorphous form and is already deteriorating. The male gametes seem to be little more than naked nuclei because they lack a clear cytoplasmic coating. The gametes are quite lengthy and curved in different shapes.

| Female Gametophyte

The fully differentiated ovule is of campylotropous type but it shows the characters of anatropous ovule; hence, it may be defined as ana-campylotropous. It has a long basal funicle ovule that hangs its top with the chalazal face placed against one side of the top of the ovary. Due to the division of epidermal cells, this most distinctive characteristic of this ovule is that it bears a cap. The thickness of cap varies from 2-3 layers of cells. The ovule is bi-integumented, the inner is two cells thick throughout except at the micropylar end where it is thick and takes part in micropyle formation, while the outer integument is three cells thick. The archesporium in the ovule differentiated into a group of 2-5 cells, these all cells are not always of hypodermal origin, sometimes sub-hypodermal archesporial cells have been developed. Normally the megaspore mother cell give rise to the dyad. The dyad cells which are in the chalazal side divided homotypically resulting in 3 megaspores. Among them the

chalazal megaspore develops into the embryo sac while the other two are degenerated. The egg is a flask shaped structure having much vacuolated cytoplasm, the synergids are hooked in shape. The polar nuclei fused before fertilization. The secondary nucleus always lies in the chalazal half of the embryo-sac. The antipodal cells are three.

| Fertilization

Four pollen tubes form from the germinating pollen grains that are captured by the stigma's glandular hairs, move along the hollow style, and enter the egg apparatus through the nucleus and micropylar apex of the embryo sac. The second male gamete fuse with the secondary nucleus thus the primary endosperm nucleus is a definite product of triple fusion. Both gametes undergo a form shift and become more or less spherical during fertilisation.

| Endosperm and Perisperm

After fertilization the nucleus of primary endosperm divided to 16 nuclei before the first transverse division of the egg. At the micropylar end the wall formation start which is extended right up to the chalazal end. It becomes cellular and developed into the growing embryo. At maturity inside the seed, it has 1-2 layers of cells that surrounds the radicle in a cap-like fashion. The mature seed's centre contains perisperm, which is a significant portion of the ovule's nucellus. In the perisperm cells the starch is abundantly deposited.

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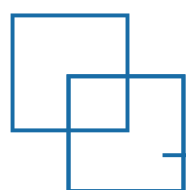
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CHAPTER 02

Bibliometric Analysis



INTRODUCTION

Achyranthes aspera L. is a medicinal plant of the family Amaranthaceae with various traditional therapeutic applications, found in Asia, South America, and Africa. *Achyranthes aspera* plant is a well-known medicinal plant with roots, root bark, latex, leaves, and flower parts which are used for various medicinal purposes to fight several human and animal diseases. In Indian traditions, *A. aspera* holds cultural and medicinal significance, finding use in Ayurveda, Unani, Siddha, and Homeopathy (Lin et al., 2024). The decoction prepared from the roots of this plant is used to treat cough, fever, stomach pain, pneumonia, and diabetes. The plant is also used to cure tonsillitis, head wounds, ringworm, diarrhea, and asthma. This plant possesses antibacterial, antifungal, thyroid-stimulating, antiperoxidative, anti-inflammatory, antiarthritic, immunomodulatory, wound healing, anti-obesity, anticonvulsant, treat post-partum complications, hemorrhoids, swelling, strained back, anticancer and hepatoprotective properties. This plant is rich in phytochemicals such as saponins, phenolic compounds, flavonoids, alkaloids, steroids, and terpenoids have been reported to occur in this plant (Sinan et al., 2020; Lin et al., 2024). Moreover, pharmacological studies have demonstrated that whole plants possess anti-asthmatic, anticancer, anti-diabetic, antioxidant, nephroprotective, and wound healing properties (Singh et al., 2021). These attributes render *A. aspera* an interesting subject for researchers examining novel therapeutic agents and exploring various approaches to modern medicine.

Bibliometric analysis is a fundamental technique in modern scientific analysis, providing a comprehensive evaluation of the scholarly area encompassing a particular subject or topic (Verano-Tacoronte et al., 2024). The present study examines the research landscape of *A. aspera* through bibliometric analysis, exploring the extensive literature, research trends, and knowledge dissemination associated with this versatile and widely studied plant species. The bibliometric analysis enables the investigation of both quantitative and qualitative extents within scholarly publications, providing researchers with the means to scrutinize patterns, trends, and the evolutionary trajectory of scientific literature related to *A. aspera* (Jing et al., 2023). This analytical approach involves



examining publication metrics, citation patterns, author collaborations, geographical distributions, and thematic emphases within the body of scientific literature (Vieira, 2022). Scientifically analyzing bibliographic data of *A. aspera* provides researchers with a comprehensive understanding of the extent and scope of research conducted on this plant species.

The bibliometric analysis is considered a valuable tool to navigate and comprehend the dynamic research environment associated with this multifaceted plant species, providing researchers with meaningful insights to track knowledge progression and stimulate innovation in various related fields. The objective of this study was to conduct a comprehensive bibliometric analysis of scholarly literature dedicated to *A. aspera* research, to gain insights into past research trends, identify significant contributors, understand citation patterns, and delineate collaborative networks in the field. The primary aim was to offer a thorough perspective on the existing knowledge landscape surrounding *A. aspera*, facilitating the identification of gaps and guiding future research efforts. Additionally, the study focused on strategic *decision*-making in diverse areas such as agricultural practices, environmental conservation, and pharmaceutical applications.

Insights with Data Source and Tools

The study examines the research landscape of *A. aspera* L. through a bibliometric analysis, aiming to provide a thorough understanding of the scope, impact, and evolving trends within academic publications focused on this botanical species. The scholarly articles used for this comprehensive analysis were sourced from Dimensions.ai, with specific parameters including the exact phrase "*Achyranthes aspera*" and the publications covered from 2002 to 2023. Various analytical tools were employed, including Microsoft

Excel for data organization and initial assessments. Additionally, tools such as lens.org and VosViewer (Version 1.6.19) were utilized for a more in-depth bibliometric analysis, examining citation networks, co-authorship patterns, and generating visual representations of bibliographic data (Cavalcante et al. 2021; Sarjidan et al., 2023). The focused areas of the study were determined by utilizing the integrated tools within Lens.org, enabling a thorough exploration of key research domains.

Comprehensive Data-driven Insights

A comprehensive analysis of the scholarly publications related to *A. aspera* between 2002 and 2023 offers a thorough insight into their worldwide reach, collaborative networks, and impact. A total of 528 scholarly articles have been published on this subject by 547 entities across 55 nations, involving the contributions of 1961 authors. Particularly, 317 authors have exhibited specialized expertise through independent publications. These scholarly works found their place in 372 esteemed journals, indicating a diverse dissemination platform. The research on *A. aspera*, with an average of 10.90 citations per document, has demonstrated a substantial impact within the scholarly community. Over time, the trajectory of research appears to have shifted, potentially indicating a progression from foundational taxonomy and ecological studies to applied research encompassing agronomy, pharmacology, ecology, and molecular biology. The widespread global contributions underscore broad interest and potential for collaboration across diverse regions. Collaborative networks among authors and organizations from different countries suggest a transdisciplinary approach in examining various facets of this species. Bibliometric analysis has elucidated the multifaceted understanding and significance of *A. aspera*, pointing towards opportunities for innovative applications in agriculture, medicine, pharmacology, and environmental science.

Temporal Evolution and Growth Analysis

A total of 528 documents were discovered after extensive searches across bibliographic databases via Dimensions.ai. These demonstrated a variety of study forms, including 505 articles, 12 chapters, 8 proceedings, and 3 preprints. Spanning from 2002 to 2023, the dataset revealed fluctuations and trends in scholarly output over two decades. The research landscape began modestly with five publications in 2002, gradually increasing to 11 by 2009, indicating a growing interest in exploring the properties and applications of *A. aspera* L. A significant surge in research output was observed from 2010 to 2013, with the number of publications more than double from 16 in 2010 to 38 in 2012. This period signified the emergence of the plant as a prominent subject of study within the scientific community. Following this rapid growth phase, the publication count stabilized around the late 2010s, with fluctuations observed in subsequent years. Despite variations, the overall trend remained steady, indicating sustained interest and continued research activity. The growth analysis revealed patterns of exponential growth ($R^2=0.7842$), particularly during the early and mid-2010s, followed by a plateauing in the latter years of the dataset. In the year 2021, the highest volume of scholarly articles was published compared to other years (Fig. 1). While growth may have stabilized, the sustained level of research output indicated a mature

and established research field with continued relevance and interest among researchers. Throughout the timeline, periodic fluctuations in research output suggested cyclical patterns influenced by factors such as funding, scientific

trends, and research priorities. Understanding these trends is crucial for contextualizing research efforts, identifying emerging areas of inquiry, and informing future directions in the study of *A. aspera* L.

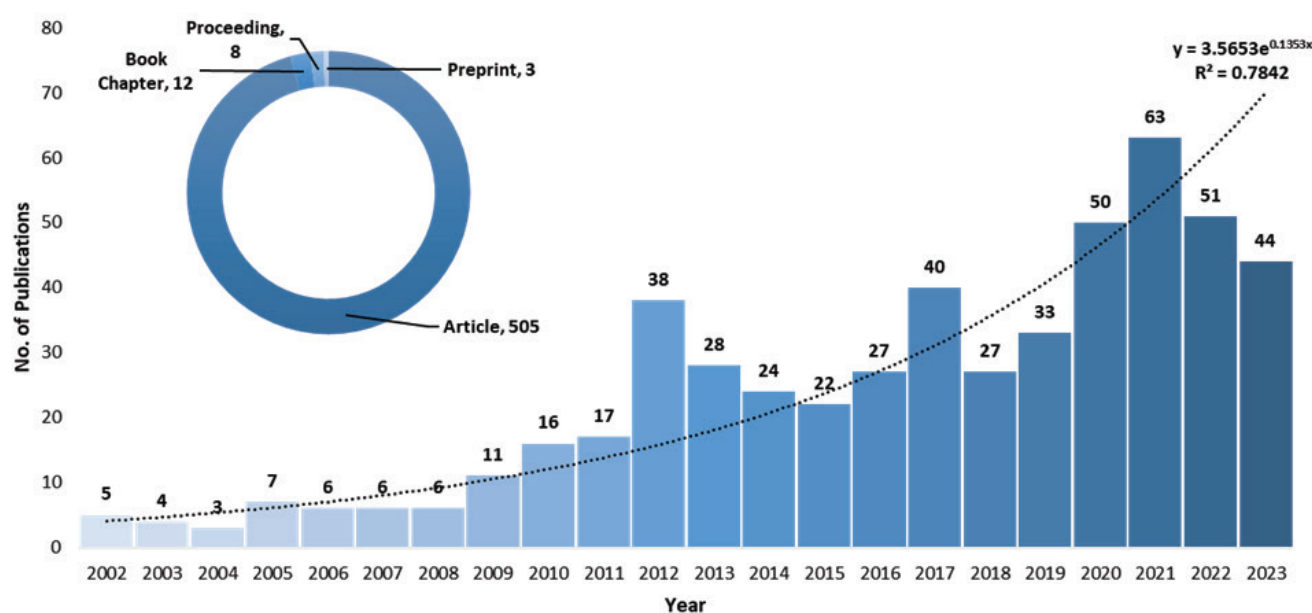


Fig. 1 Publication trends and distribution types in *A. aspera* research

Citation Analysis

Citation analysis offered valuable insights into the impact and distribution of research within the domain of *A. aspera* L. Spanning from 2002 to 2023, the dataset illustrated the number of citations received by publications related to *A. aspera* L. Initially, from 2002 to 2009, there were fluctuations in the number of citations, while the peak in citations was observed in 2012 (763 citations) and 2005 (549 citations), reflecting heightened interest and widespread recognition of research contributions (Fig. 2). However, citation numbers fluctuated in subsequent years, with a gradual decline observed towards the

latter half of the decade. Despite fluctuations, the overall trend suggested enduring impact and influence, serving as a measure of knowledge distribution and utilization within the scientific community. The stabilization and decline in citations towards the latter years of the dataset might have indicated shifting research focuses or reduced interest in the topic. Citation analysis remained crucial for assessing visibility, identifying seminal works, and evaluating the trajectory of research within the scholarly landscape. As the field evolves, ongoing citation analysis will continue to inform future research directions and assess the impact of scholarly endeavors in the domain of *A. aspera* L.

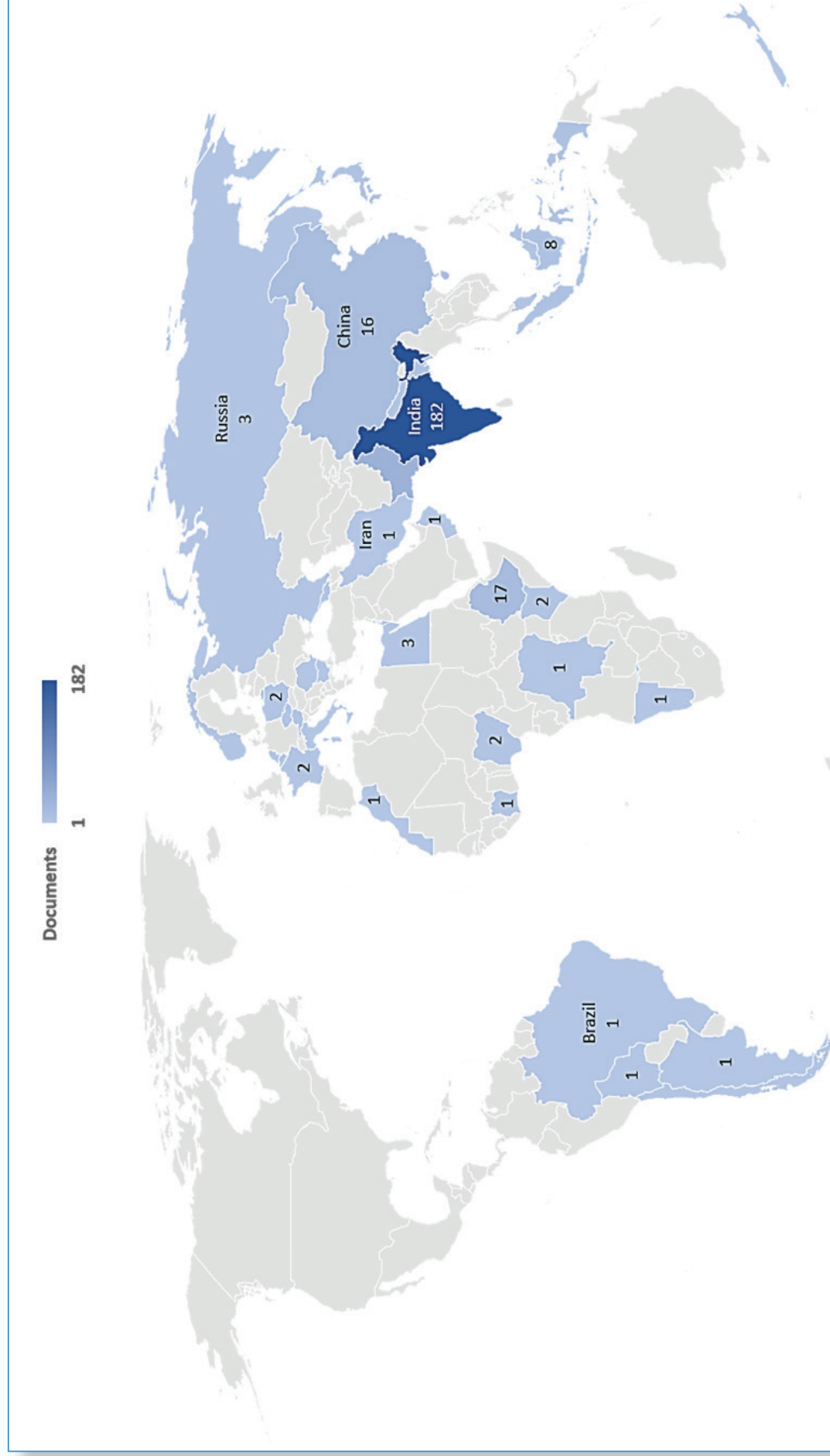


Fig. 3 Global distribution of research on *A. aspera*

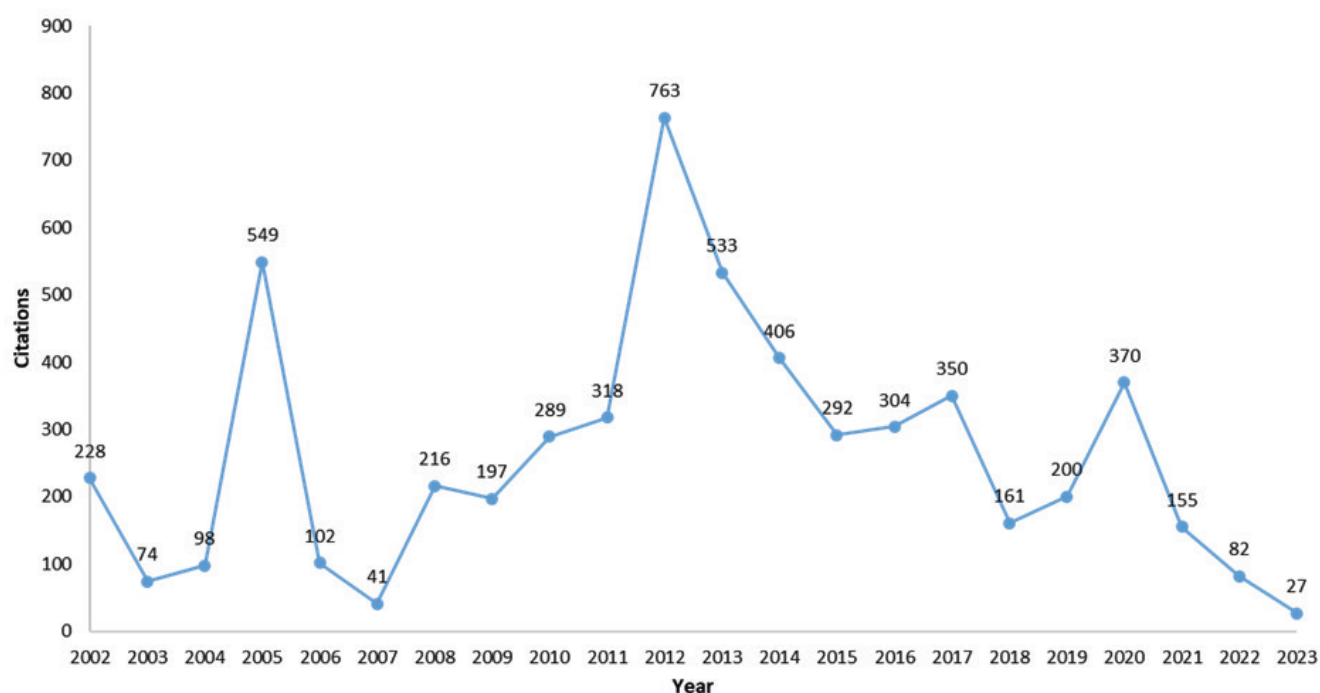


Fig. 2 Citation trends for *A. aspera* research over time

Country-wise Publication Analysis

In the examination of publication output based on countries, insights were gleaned into the geographical distribution of research activity concerning *A. aspera* (Fig 3). India emerged as the leading contributor to research on *A. aspera* L., with 182 publications, reflecting its significance in traditional medicine and Indian pharmacology. Following India, Pakistan demonstrated remarkable research interest with 34 publications, indicating a growing recognition of the plant's medicinal properties within Pakistani pharmacological contexts. Ethiopia contributed 17 publications, highlighting its engagement in exploring the pharmacological properties and traditional uses of the plant within African contexts.

China also showed research interest with 16 publications, reflecting the global relevance of *A. aspera* L. within traditional Chinese medicine and modern pharmacology. Other countries also contributed well including Bangladesh, Japan, South Africa, and Austria (Fig. 3). Despite having lesser outputs, they could have burgeoning research communities or specialized study areas. This country-wise publication analysis underscored the global scope of research on *A. aspera* L. and the collaborative efforts among researchers worldwide to explore its therapeutic potential and pharmacological properties. It emphasized the plant's importance in traditional medicine systems across different regions and informed collaborative opportunities for future interdisciplinary studies and knowledge exchange initiatives.

Most Prominent Authors

Among all authors, Rina Chakrabarti, whose 21 documents and 666 citations established her as a leading figure in the field, demonstrated substantial scholarly output and impact. Following closely was Jaigopal Sharma, with 13 documents and 172 citations, indicating significant engagement with research on *A. aspera* L. Sarita Kumar's work, comprising 8 documents and 95 citations, also highlighted a commendable level of scholarly activity and impact within the research landscape. Additionally, Aarti Sharma's 7 documents and 77 citations contributed meaningfully

to the understanding and discourse surrounding. Among the prominent authors in the field, Pushplata Tripathi and Neelesh Kumar each contributed significantly with 6 scholarly documents with 76 and 44 citations, respectively (Fig. 4). Moreover, R. Chakrabarti, Zafar Iqbal, Ayub Khan, and Fazli Khuda each had 5 documents with 72, others with 61 citations each respectively, demonstrating significant engagement and impact within the research community focused on *A. aspera* L. Collectively, these authors represented the forefront of research events pertaining to *A. aspera* L., shaping the research landscape and driving innovation and discovery in the field through their scholarly output and citation impact.

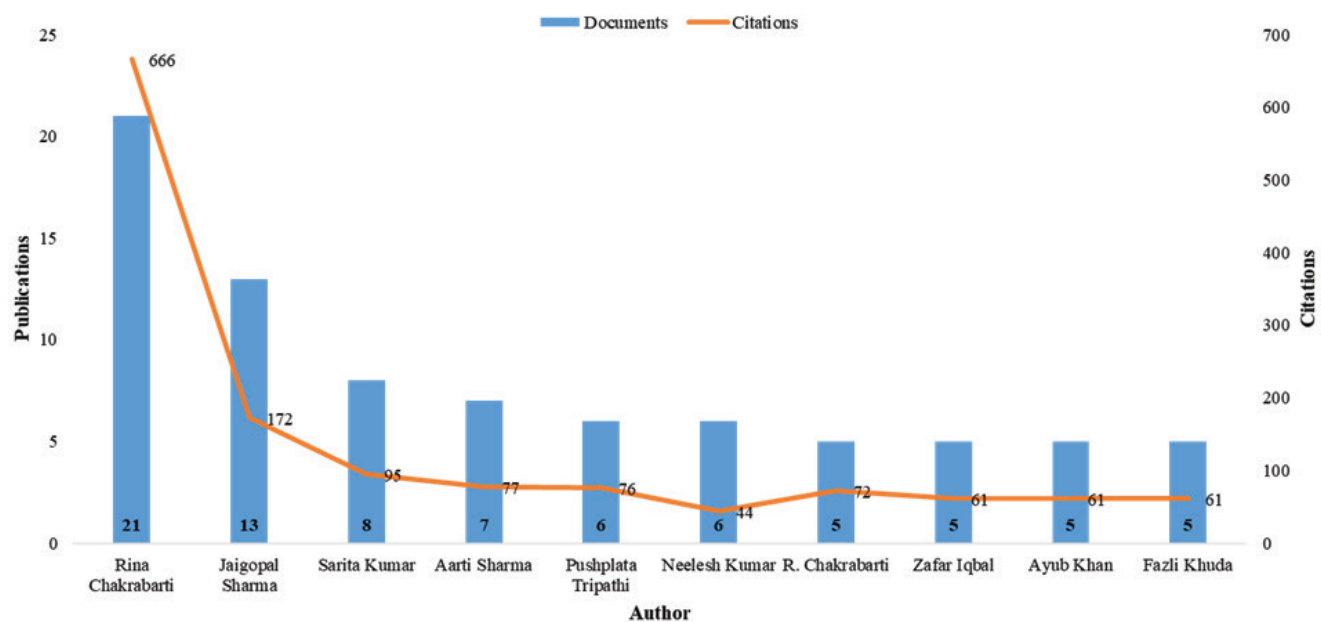


Fig. 4 Most prominent authors based on research contributions to *A. aspera*

Highly Cited Articles

The top 10 highly cited research articles on *A. aspera* provide a comprehensive overview of its diverse applications and properties.

The most highly cited article published in 2005 was "Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*" with

289 citations (Rao et al., 2005). Additionally, "Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India" (2013) gained 130 citations, indicating its substantial contribution to understanding traditional medicinal practices (Bhatia et al., 2013). "Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide" (2010) received 113 citations, demonstrating its relevance in the context of ecological management (Kaur et al., 2010). "Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa*, *A. speciosa* and *A. aspera*" (2002) is prominent with 111 citations, reflecting its impact on pharmacological research (Gokhale et al., 2002). Moreover, "Larvicidal activity

of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)" (2008) has 106 citations, underscoring its significance in pest control strategies (Bagavan et al., 2008). "Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia" (2011) received 105 citations (Taye et al., 2011). Other articles have citations less than 100 indicating their importance in ethnopharmacological research (Table 1). These highly cited articles collectively represent significant contributions to the literature on *A. aspera* L., guiding further research and informing practice within the field.

Table 1 Top 10 highly cited research articles published on *A. aspera*

Rank	Title	Source	Year	Citations	Reference
1	Effect of <i>Achyranthes aspera</i> on the immunity and survival of <i>Labeo rohita</i> infected with <i>Aeromonas hydrophila</i>	Fish & Shell-fish Immunology	2005	289	Rao et al., 2005
2	Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India	Journal of Ethnopharmacology	2013	130	Bhatia et al., 2013
3	Phytotoxic effects of volatile oil from <i>Artemisia scoparia</i> against weeds and its possible use as a bioherbicide	Industrial Crops and Products	2010	113	Kaur et al., 2010
4	Preliminary evaluation of anti-inflammatory and anti-arthritic activity of <i>S. lappa</i> , <i>A. speciosa</i> and <i>A. aspera</i>	Phytomedicine	2002	111	Gokhale et al., 2002
5	Larvicidal activity of saponin from <i>Achyranthes aspera</i> against <i>Aedes aegypti</i> and <i>Culex quinquefasciatus</i> (Diptera: Culicidae)	Parasitology Research	2008	106	Bagavan et al., 2008

Rank	Title	Source	Year	Citations	Reference
6	Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia	Asian Pacific Journal of Tropical Bio-medicine	2011	105	Taye et al., 2011
7	Medicinal plants for women's health-care in southeast Asia: A meta-analysis of their traditional use, chemical constituents, and pharmacology	Journal of Ethnopharmacology	2013	87	de Boer et al., 2013
8	Ethnomedicinal plants used for treating epilepsy by indigenous communities of sub-Himalayan region of Uttarakhand, India	Journal of Ethnopharmacology	2013	86	Sharma et al., 2013
9	Stimulation of immunity in Indian major carp <i>Catla catla</i> with herbal feed ingredients	Fish & Shellfish Immunology	2005	83	Rao et al., 2005
10	Evaluation of in vivo wound healing activity of methanol extract of <i>Achyranthes aspera</i> L.	Journal of Ethnopharmacology	2012	81	Fikru et al., 2012

Most Active Journals

The present study showed the top journals of *A. aspera* research based on their impact factor, publications, and citations. Journal of Ethnopharmacology by Elsevier had emerged as the topmost journal, boasting an impressive impact factor of 5.90 and publishing 16 documents, with 699 citations on this topic. Subsequently, the Ancient Science of Life from Phcognet (Phalcon Publishing), maintained a significant presence, contributing 8 documents and garnering 76 citations, marking its considerable influence in *A. aspera* research. The journal Fish & Shellfish Immunology by Elsevier had an impact of 5.53 with 7 documents and 471 citations. The

Pakistan Journal of Pharmaceutical Sciences by PCSIR (Pakistan Council of Scientific and Industrial Research) and Asian Journal of Pharmaceutical and Clinical Research herbs had an impact factor of 1.30 and 1.53, both with 7 published documents and 74 and 11 citations respectively, underscoring its considerable contribution to this area of research. Moreover, the journals Asian Pacific Journal of Tropical Biomedicine, Journal of Pharmaceutical Research International, Research Journal of Pharmacy and Technology, and Journal of Drug Delivery and Therapeutics contributing 6 documents (Table 2). Overall, the study's results assist readers and researchers in selecting relevant and valuable materials for their *A. aspera* research.

Table 2 Top journals in *A. aspera* research having the highest published document and citations

Rank	Source	Publisher	Impact factor	Documents	Citations	Citations per Document
1	Journal of Ethnopharmacology	Elsevier	5.90	16	699	43.69
2	Ancient Science of Life	Phcognet (Phalcon Publishing)	-	8	76	9.50
3	Fish & Shellfish Immunology	Elsevier	5.53	7	471	67.29
4	Pakistan Journal of Pharmaceutical Sciences	PCSIR (Pakistan Council of Scientific and Industrial Research)	1.30	7	74	10.57
5	Asian Journal of Pharmaceutical and Clinical Research	Medknow Publications	1.53	7	11	1.57
6	Asian Pacific Journal of Tropical Biomedicine	Wolters Kluwer - Med-pharm	2.40	6	254	42.33
7	Journal of Pharmaceutical Research International	JBPT Publisher (Jagdish Bhagwan Publishers and Traders)	-	6	13	2.17
8	Research Journal of Pharmacy and Technology	Y and Y Publishers	-	6	13	2.17
9	Journal of Drug Delivery and Therapeutics	The Pharma Research Society	-	6	7	1.17
10	Pharmaceutical Biology	Taylor & Francis Online	3.069	5	156	31.20

Top Productive Organizations

The examination of scientific contributions made to the *A. aspera* ecology demonstrates diverse trends in recognition and effect among various successful groups. The study's findings list the top organizations according to how much research they have done on *A. aspera*. With 28 publications, 751 citations, and an average of 27.86 citations per article, the University of Delhi dominated the field of study. Following this, Delhi Technological University ranked second and contained 14

documents with 142 citations. Each of the two institutions had a considerable influence, with 8 documents, 139 and 136 citations, and average citation counts of 17.78 and 18.88 for King Saud University and Banaras Hindu University, respectively. Another organization with 6 documents each including University of Madras (197 citations), Periyar University (109 citations), Bharathiar University (88 citations), Anna University, (78 citations), and Punjabi University (72) have treasured contributions (Table 3).

Table 3 Top organizations' contributions to *A. aspera* research

Rank	Organization	Country	Documents	Citations	Citations per Document
1.	University of Delhi	India	28	751	27.86
2.	Delhi Technological University	India	14	142	10.57
3.	Addis Ababa University	South Africa	9	379	40.50
4.	King Saud University	Saudi Arabia	8	139	17.78
5.	Banaras Hindu University	India	8	136	18.88
6.	University of Madras	India	6	197	35.33
7.	Periyar University	India	6	109	19.83
8.	Anna University, Chennai	India	6	78	15.33
9.	Bharathiar University	India	6	88	15.33
10.	Punjabi University	India	6	72	12.00

Top Fields of Study

The present study analyzed the occurrence of terms in significant academic domains using a dataset consisting of 819 scholarly documents sourced from lens.org. The research results were based on document frequency, brought

attention to a wide range of subjects. *Achyranthes aspera* had the highest document count at 646, indicating significant scholarly attention to this area of study. Traditional medicine followed closely with 450 documents, showing a substantial focus on the exploration of traditional healing practices. Biology emerged

as a key field, with 418 documents, reflecting a broad spectrum of research within the biological sciences. Medicine was well-represented with 272 documents, underscoring the depth of inquiry into medical practices and treatments. Chemistry, with 244 documents, indicated a substantial focus on the chemical composition of substances and their applications. Botany, with 168 documents, emphasized the study of plants, while the 141 documents on medicinal plants highlighted dedicated research on their therapeutic properties. The presence of 132 documents on pathology indicated a specific focus on the study of diseases. Alternative

medicine was another noteworthy field, with 126 documents highlighting a keen interest in non-conventional healthcare approaches. Fields such as phytochemical (106 documents), pharmacology (96 documents), and biochemistry (82 documents) demonstrated the diversity of research within the chemical and medical sciences (Fig. 5). These findings emphasized the multidimensional nature of scholarly research, with a particular emphasis on traditional medicine, biology, and chemistry. The dataset reflected a rich tapestry of exploration across diverse fields, contributing to the collective understanding of these subjects.

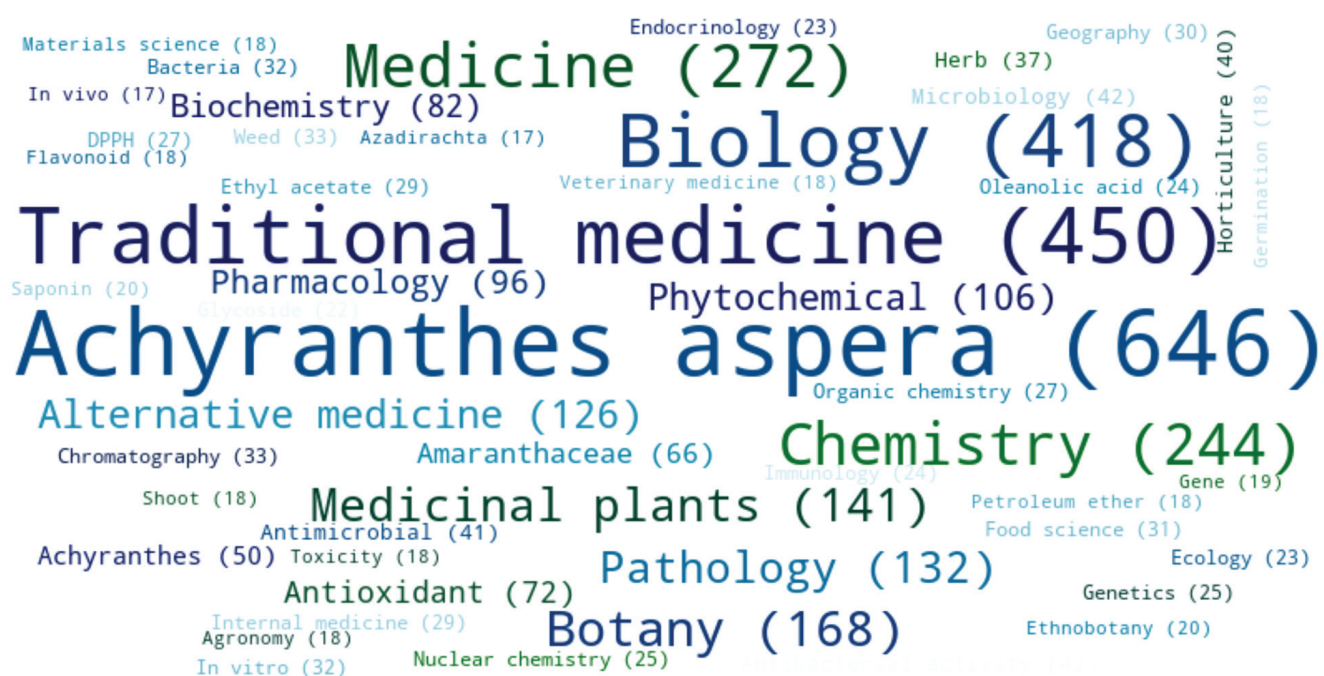


Fig. 5 Terms co-occurrence analysis within prominent terms

Research Collaboration Networks

Authors' Collaboration Network

The investigation included a thorough examination of 1961 authors, revealing a significant presence of researchers in the specified subject domain. Similarity among authors was measured through co-authorship, primarily focusing on their publications. The network encompassed authors who had published a minimum of two documents on *A. aspera* and met a citation threshold of at least two citations

per document. The analysis identified five clusters within the network, illustrating interconnected and collaborative author groups. Clusters 1-3 each comprised three members, while Clusters 4 and 5 consisted of two members each (Fig. 6). Especially, Rina Chakrabarti emerged as the most prominent author, boasting the highest total link strength of 44. Jaigopal Sharma closely follows, holding a corresponding total link strength of 38. These findings reveal a diverse research community characterized by varying degrees of specialization and collaboration.

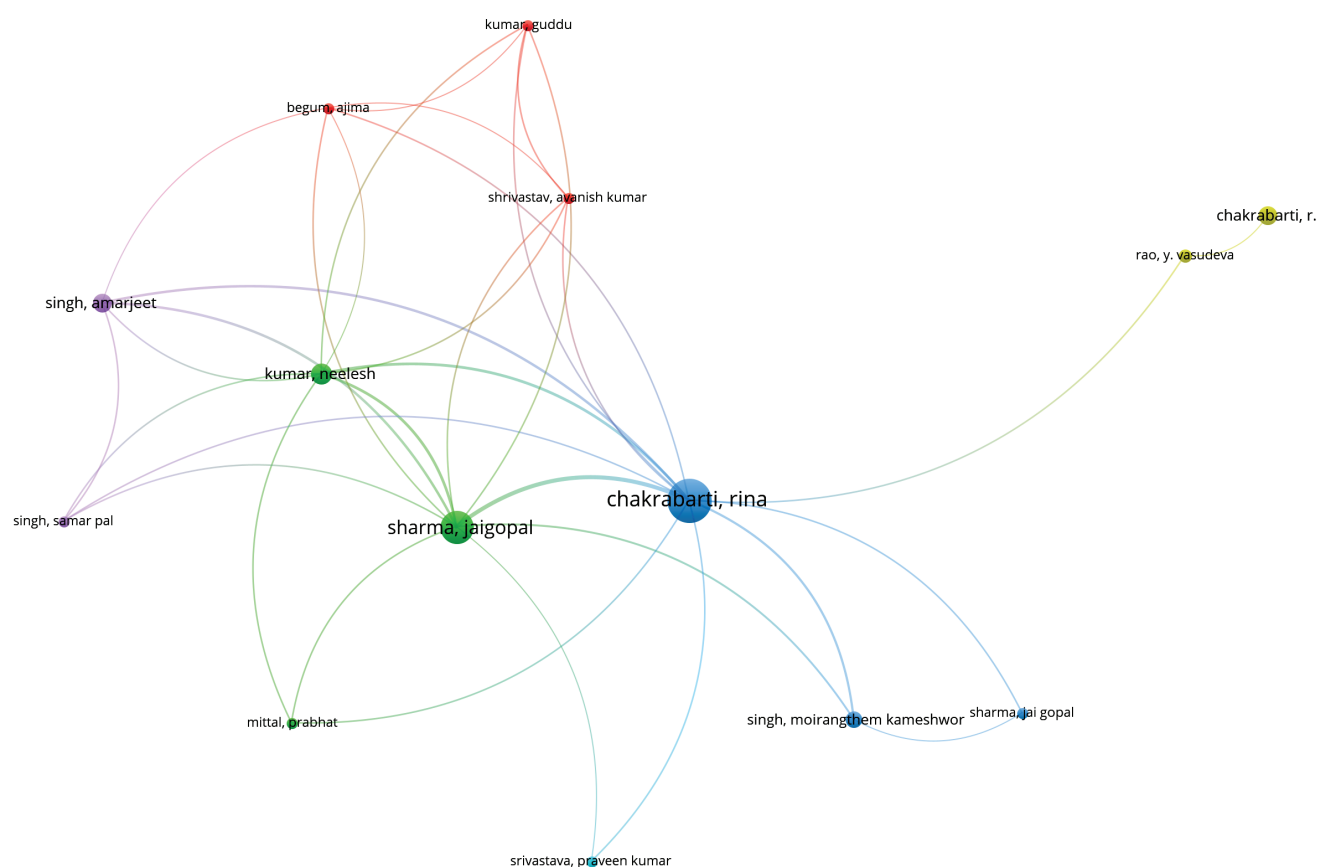


Fig. 6 Collaborative network among the authors on *A. aspera*

Journal-wise Collaboration Network

In the examination of collaboration among journals dedicated to *A. aspera* research, a dataset of 372 such journals were analyzed, revealing 45 interconnections among 21 journals through citation analysis. Journals included in the network had to meet the criteria of having published a minimum of three documents on *A. aspera*, each with a citation threshold of at least three citations per document. The resulting network identified six distinct clusters, with cluster 1 being the largest, consisting of six journals, followed by cluster 2 and cluster 3, each containing four journals. Clusters 4 and 5 comprised

three journals each, while Cluster 6 had one journal. These clusters highlighted thematic concentrations within *A. aspera* research, highlighting specific areas of scholarly focus. Primarily, the Journal of Ethnopharmacology demonstrated the highest connection strength at 22, indicating its significant role in facilitating collaborative efforts. Moreover, the journals “Journal of Drug Delivery and Therapeutics”, and “Pharmaceutical Biology” exhibited prominent influence, with total link strengths of 29 and 25, respectively (Fig. 7). This network analysis provides insights into thematic strengths and collaborative potential among journals engaged in *A. aspera* research, contributing to a deeper understanding of the scholarly landscape within this field.

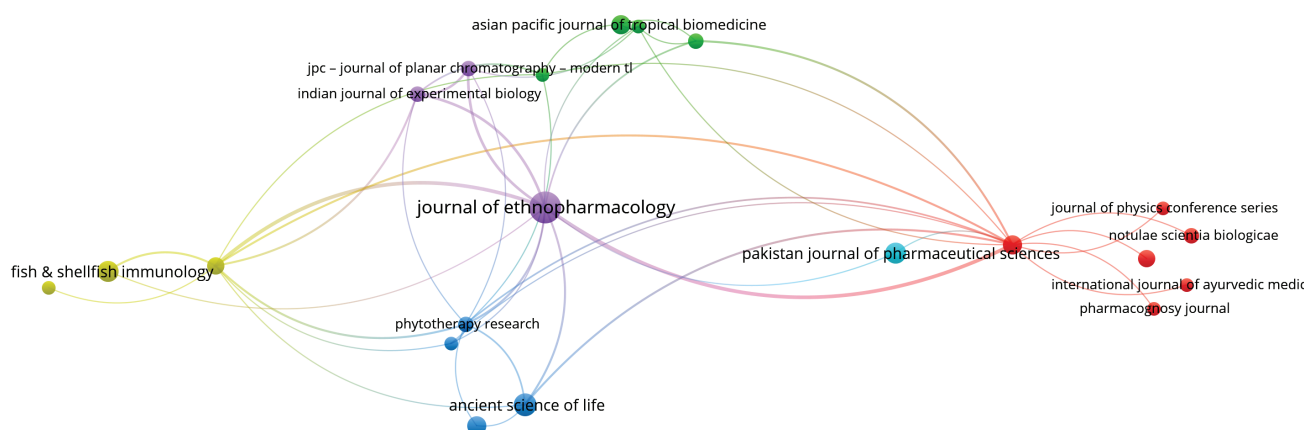


Fig. 7 Journal-wise collaboration network analysis on *A. aspera* research

Organizational Collaborative Network

Utilizing citation analysis, an investigation into the organizational collaboration network within the domain of *A. aspera* research was

undertaken. This comprehensive examination encompassed 547 organizations, elucidating relationships among 19 entities. The criteria set required a minimum of four documents and four citations pertaining to *A. aspera* research. The resulting organizational collaboration

network revealed four distinct clusters (Fig. 8). Cluster 1, consisting of eight organizations, emerged as the largest and showcased a remarkable degree of inter-organizational cooperation. Clusters 2 and 3 comprised five and four organizations respectively, highlighting closely-knit connections among them. Cluster 4 comprised two organizations, demonstrating close connections between them. The University of Delhi exhibited the highest overall connection strength, with 124,

followed by Delhi Technological University and Banaras Hindu University with 111 and 26 links respectively. Understanding this collaborative network is essential for fostering successful partnerships and advancing research on *A. aspera*. Stakeholders such as researchers, institutions, and policymakers stand to gain valuable insights from these networks to strategically bolster collaborative efforts and propel research advancement.

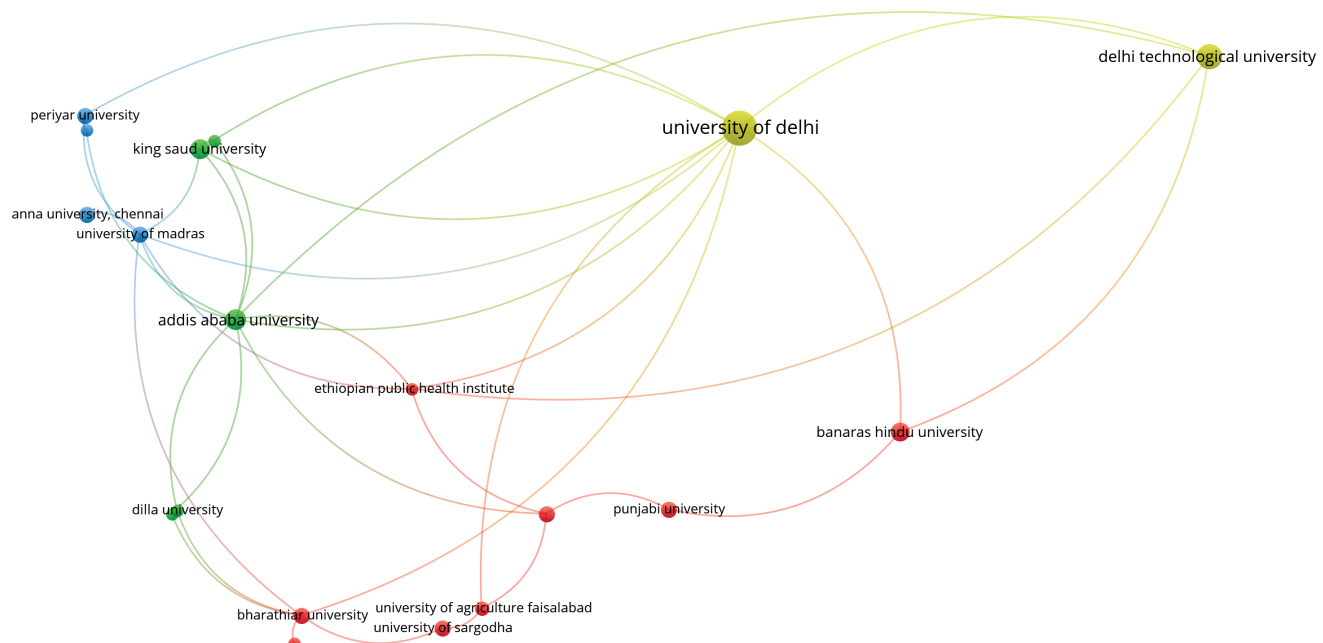


Fig. 8 Clusters of collaborative institutions for *A. aspera* research

Country-wise Collaboration Network

In the assessment of country-wise collaboration networks within the domain of *A. aspera* research, a comprehensive analysis was conducted involving 55 countries, resulting in the identification of 12 linkages.

The inclusion criteria mandated a minimum of four documents on *A. aspera* with at least four citations. The study revealed four distinct clusters through citation analysis, each characterized by varying levels of collaboration and research focus. Clusters 1 and 2 comprised five and three countries respectively,

indicating significant collaboration between them. Clusters 3 and 4 each consisted of two countries, indicating more concentrated collaboration networks (Fig. 9). Moreover, India and Ethiopia demonstrated the strongest connection strength, with 219 and 61 links

respectively, underscoring their central roles in this analysis. This analysis highlights the importance of collaborative research in enhancing our understanding of *A. aspera* by elucidating the global landscape and interconnectedness of scientific endeavors.

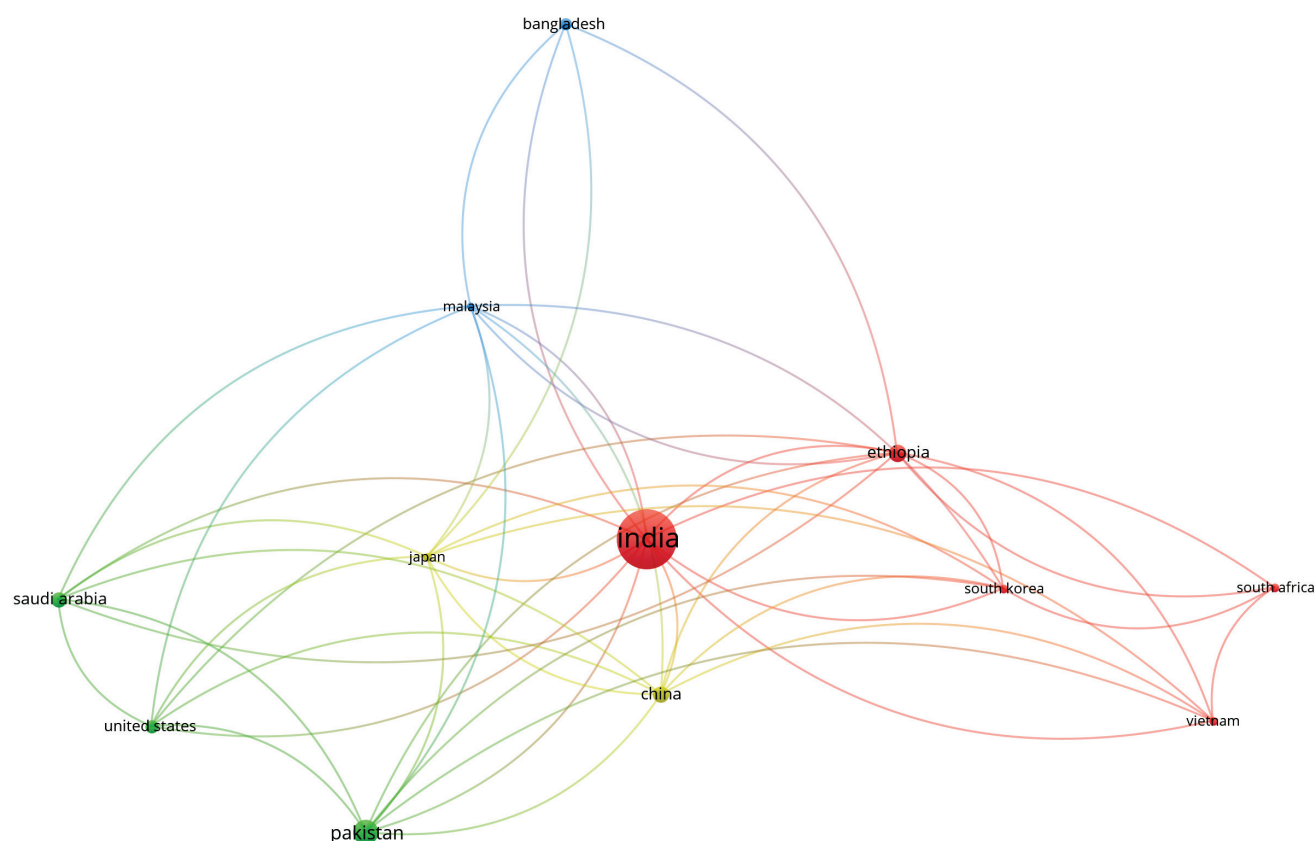


Fig. 9 Country-wise collaboration network in *A. aspera* research

Conclusion

In the present study, the bibliometric analysis conducted into the research landscape of *Achyranthes aspera* L. between 2002 and 2023 yielded comprehensive insights into the global reach, collaborative dynamics, and impact of scholarly publications on this subject. With 528 articles authored by 547 entities

across 55 nations and involving 1961 authors, the breadth and depth of research efforts were evident. India emerged as a significant contributor, reflecting its rich heritage in traditional medicine and pharmacology. Temporal evolution revealed a remarkable surge in scholarly output in 2021, while

citation analysis underscored key periods of heightened interest, particularly in 2012 and 2005. The prominence of authors like Rina Chakrabarti and influential works such as those by Rao et al. (2005) underscored the impact of individual contributions and landmark publications. Journal of Ethnopharmacology emerged as a pivotal platform for disseminating research findings, highlighting its role in shaping the discourse around *A. aspera*. The University of Delhi stood out as a powerhouse in research productivity, reflecting institutional excellence in this domain. Moreover, the collaborative networks

analysis elucidated the interdisciplinary nature of *A. aspera* research, suggesting fertile ground for future collaborations and knowledge exchange. The identification of highly cited articles, active journals, and productive organizations served as a roadmap for researchers, policymakers, and practitioners, facilitating informed decision-making and fostering continued advancement in the field. Overall, this bibliometric analysis offered valuable insights into the scholarly landscape of *Achyranthes aspera*, paving the way for further exploration, collaboration, and innovation in this vital area of study.

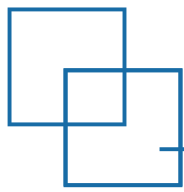
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CHAPTER 03

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 rhizospheric soil) were collected from a total of 26 sites. The numbering of the sites, name of sites along with the 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 site 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

Site	Temperature (°C)	Altitude (m)
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 temperature 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 ml = 1 cm³).
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/cm³) = 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.
3. Initially the instrument was calibrated with buffer solution of known pH 4, 7.0, and 9.2.
4. The electrodes were washed with distilled water and wiped dry with a tissue paper.
5. Then, the electrode was dipped in the sample and the readings were taken.
6. 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 reclamation.
6.5-8.7	Normal	Optimum for most crops
8.8-9.3	Alkaline	Requires application of organic manures
>9.3	Alkali (Sodic)	Requires gypsum for amelioration

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 (Chopra and Kanwar, 1976; Richards, 1954).

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

Procedure

1. 25 g of the soil samples was taken in a 100 ml beaker.
2. Added 50 ml distilled water.
3. Intermittent stirring was done with a glass rod for 30 minutes.
4. The samples were left overnight to obtain a clear supernatant.
5. 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. (Jackson, 1973; Walkley and Black, 1934).

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 litre with distilled water.
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.

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 gm 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.

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 $\text{K}_2\text{Cr}_2\text{O}_7$ used for oxidation of organic carbon in soil = $(X-Y)/2$ ml
5. 1 ml of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ = 0.003 g C
6. % Organic Carbon in Soil = $\frac{X-Y}{2} \times 0.003 \times 100/W$

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 litre.
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 litre.
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 one litre of distilled water and to it 40 ml mixed indicator was added. Then, the pH was adjusted to 4.5-5.0 by adding 2.5 % 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 litre.
 - 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 one litre 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 litre.
4. Reagent A: Dissolve 12 g of ammonium paramolybdate in 250 ml distilled water. Separately, 0.2908g of potassium antimony tartrate ($\text{KSbO}_4 \cdot \text{C}_4\text{H}_4\text{O}_6$) was dissolved in 100 ml of distilled water. Both these dissolved reagents were added to 1 litre of 5 N sulphuric acid. Both were mixed thoroughly and diluted with distilled water to 2 litres.
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 produced 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. 10 ml 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) = $X \text{ (AU)} \times 100$
10. Available P (Kg/ ha) = $\text{ppm} \times 2.24$

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 and 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 (CH_3COONH_4): 77.09 g/l of ammonium acetate was dissolved in distilled water and the volume was made up to 1 litre. The pH of the solution was adjusted to 7 with ammonium solution or acetic acid.
2. 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 litre.
3. Working standard solution of K: The stock solution was diluted 100 times to get 10 ppm K solution.

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

1. 5 g of soil was weighed in a 150 ml conical flask.
2. To this, 25 ml of neutral normal ammonium acetate solution was added.
3. The mixture was shaken for 5 minutes on an electric shaker and then filtered through Whatman No. 1 filter paper.
4. 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.

Calculations

1. Weight of soil sample taken = 5 g
2. Volume of the neutral normal CH_3COONH_4 solution added = 25 ml
3. Dilution = 5 times
4. Reading of K (ppm) in flame photometer = Y
5. In ppm K = $Y \times \text{total dilution} - A$
6. In kg/ha = $A \times 2.24 - C$

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

1. 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 litre.
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. 10g 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.
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 $\times 2.24$

Calculations

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 HNO₃ (Suprapure grade) add 1 ml of H₂O₂ (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 50ml 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 CaCl₂.2H₂O 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 Ca²⁺ through CaCl₂.2H₂O helps avoiding dissolution of CaCO₃ 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 litre. This solution was stable for several months.

Apparatus and equipment required: 1 litre 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 .
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 = $A \times 2$ mg/kg

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).

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

1. Colony forming Units (CFU/ml) = Number of colonies × Dilution factor
2. 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

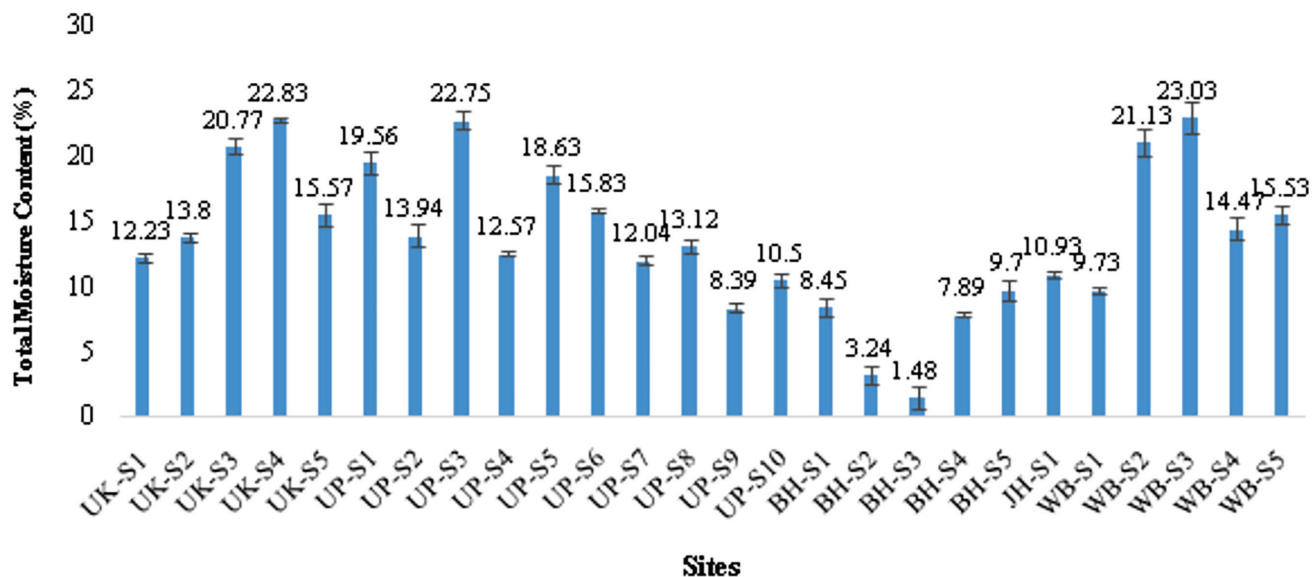


Fig. 1 Total moisture content of different sampling sites

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

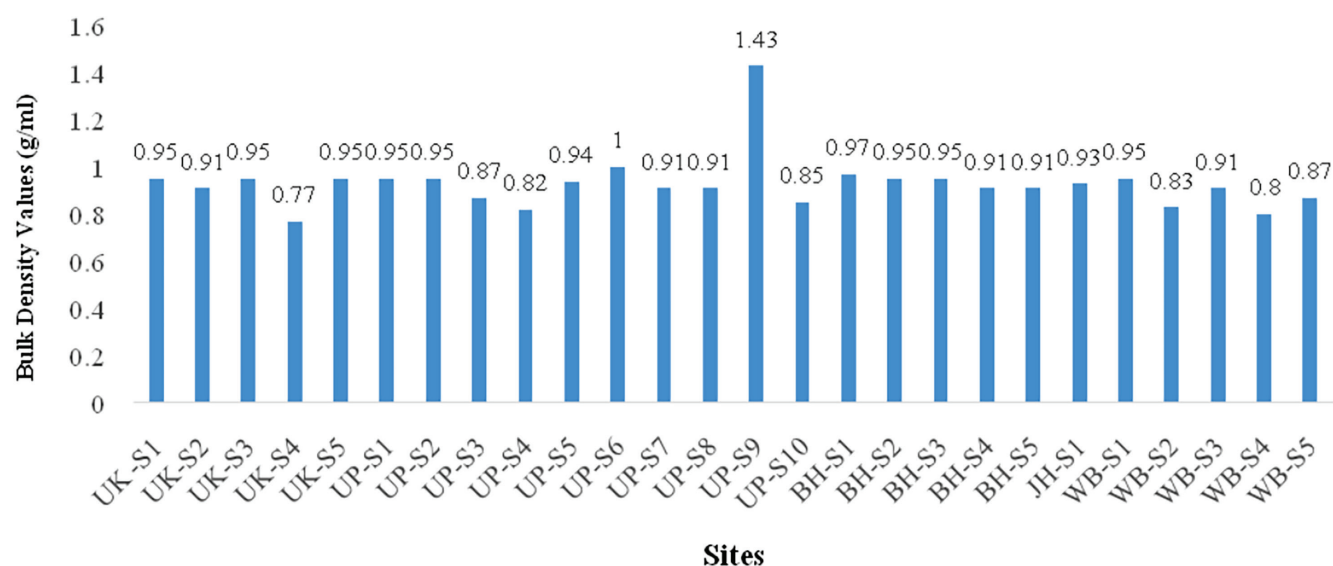


Fig. 2 Soil bulk density of different sampling sites

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

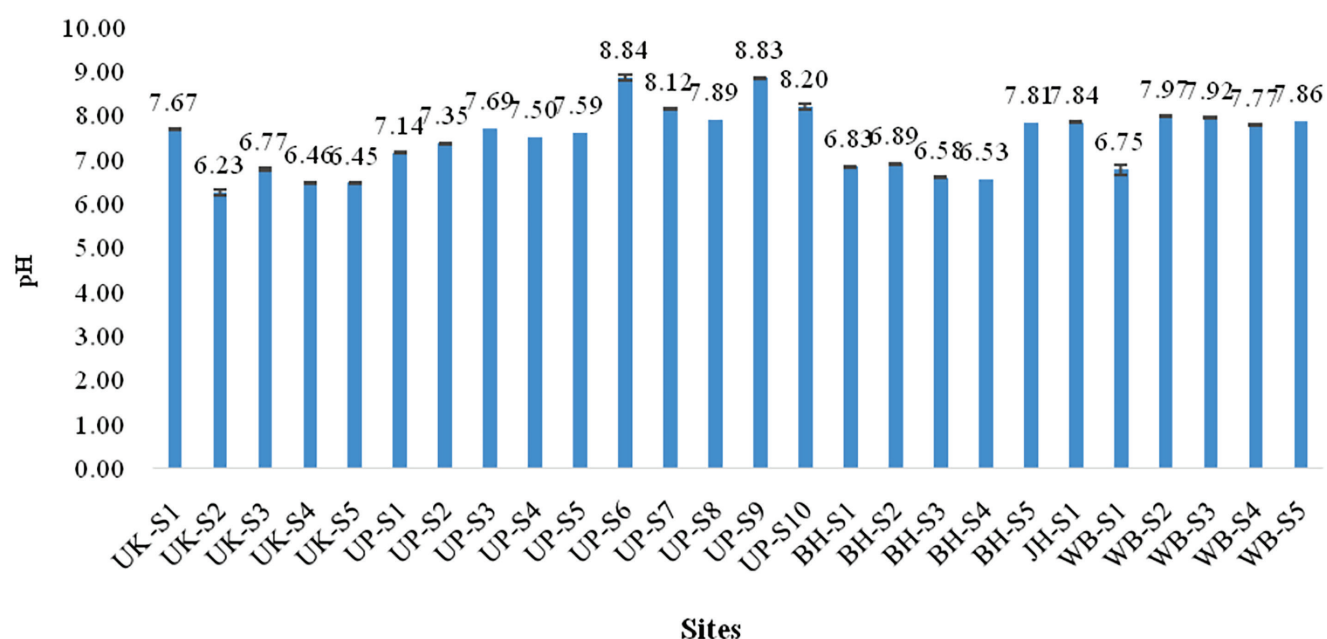


Fig. 3 Soil pH of different sampling sites

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

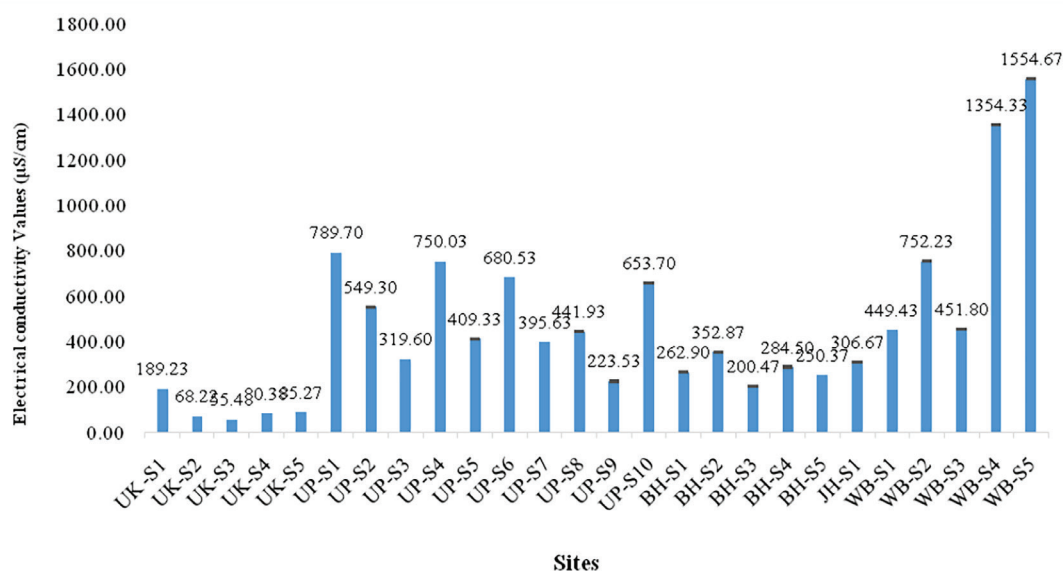


Fig. 4 Soil electrical conductivity at different sampling sites

The electrical conductivity of almost all the soil samples were within the prescribed limits of having EC below 800 μ S/cm. Soil samples from WB-S4 and WB-S5 showed the highest electrical conductivity value of 1354.33 $\pm$ 2.52 μ S/cm and 1554.67 $\pm$ 2.52 μ S/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 (Rawls et al., 2004; Allison, 1965). The organic carbon content as measured for the 26 soil samples is shown in Fig. 5

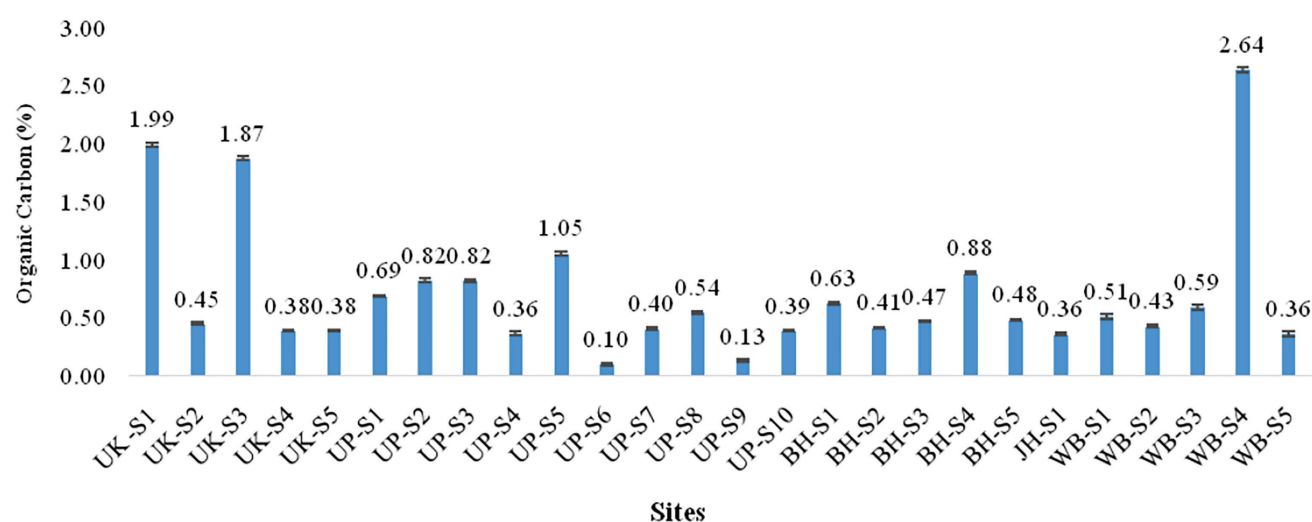


Fig. 5 Soil organic carbon content at different sites

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 $\pm$ 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 $\pm$ 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

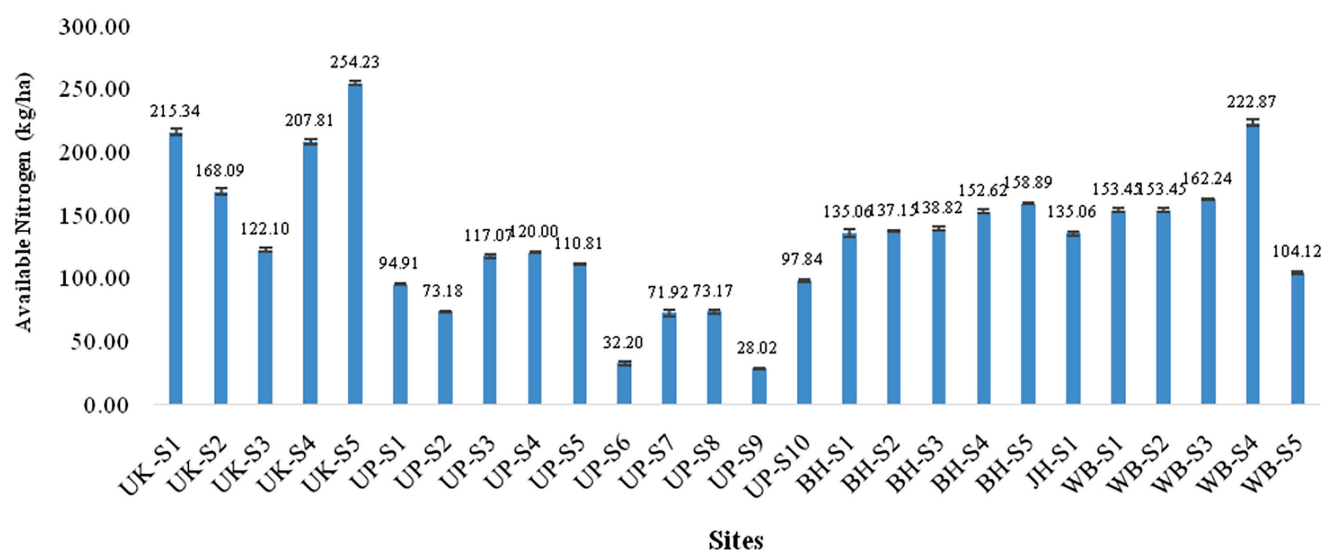


Fig. 6 Soil available nitrogen content at different sites

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

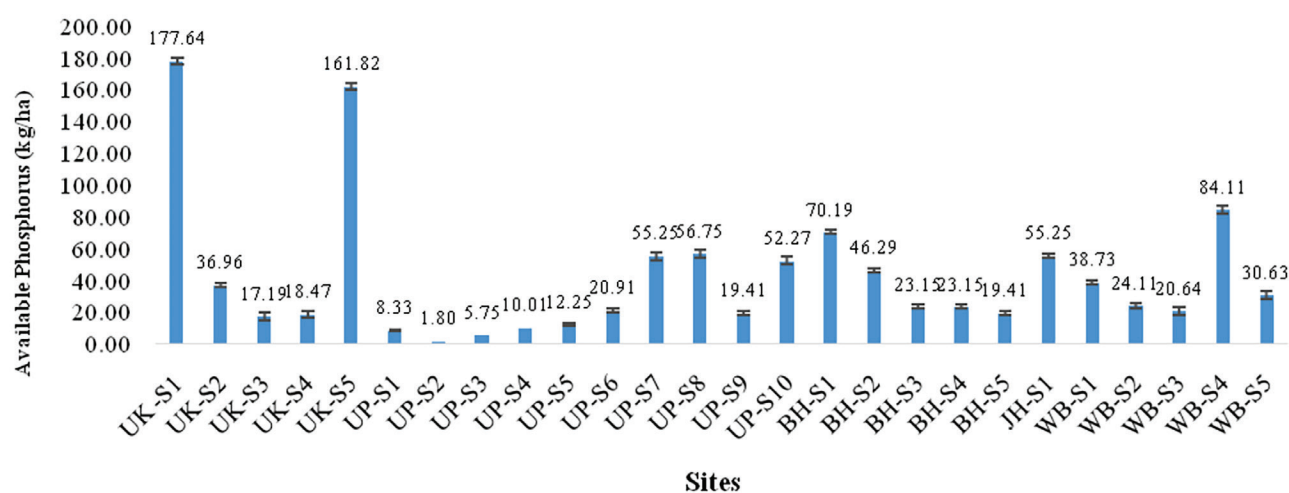


Fig. 7 Soil available phosphorus content at different sites

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

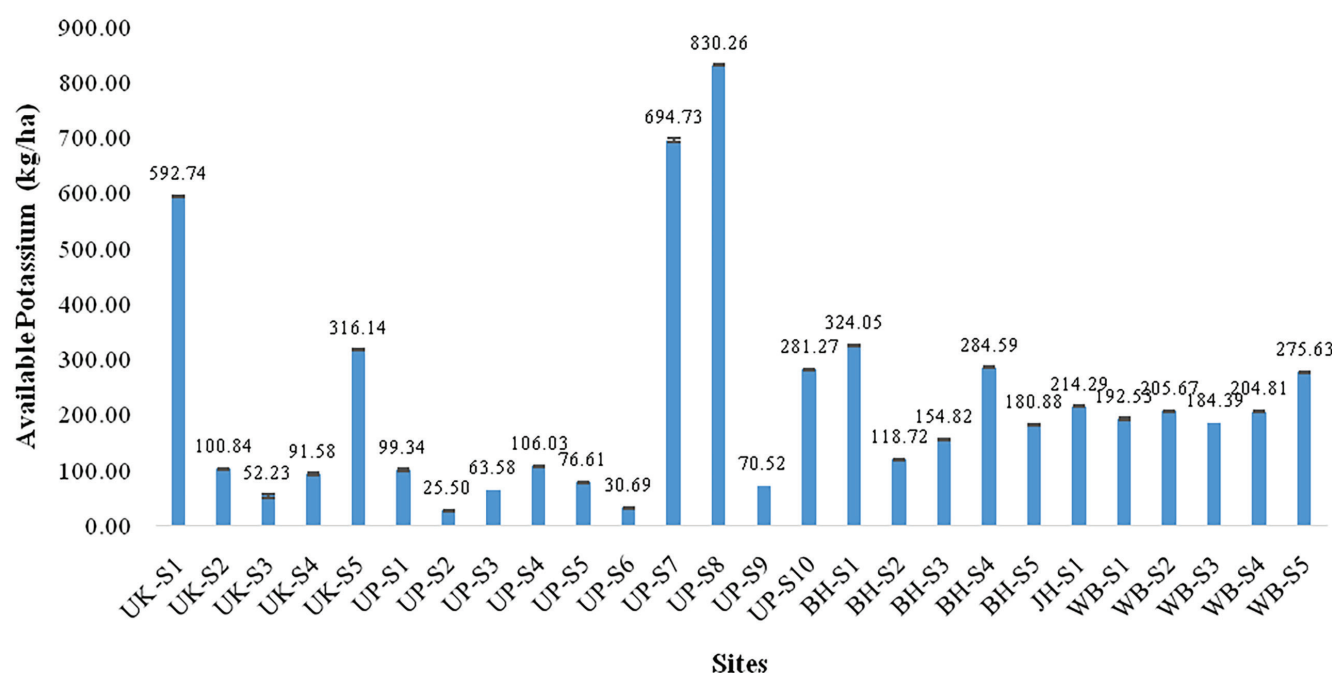


Fig. 8 Soil available potassium content at different sites

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

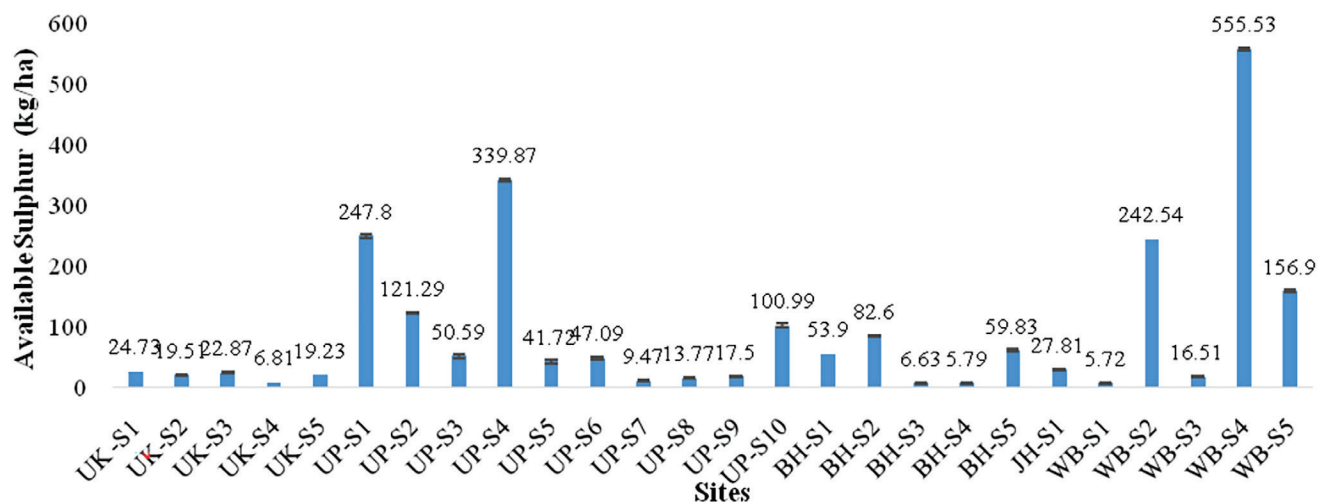


Fig. 9 Soil available sulphur content at different sites

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. 10 a-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.

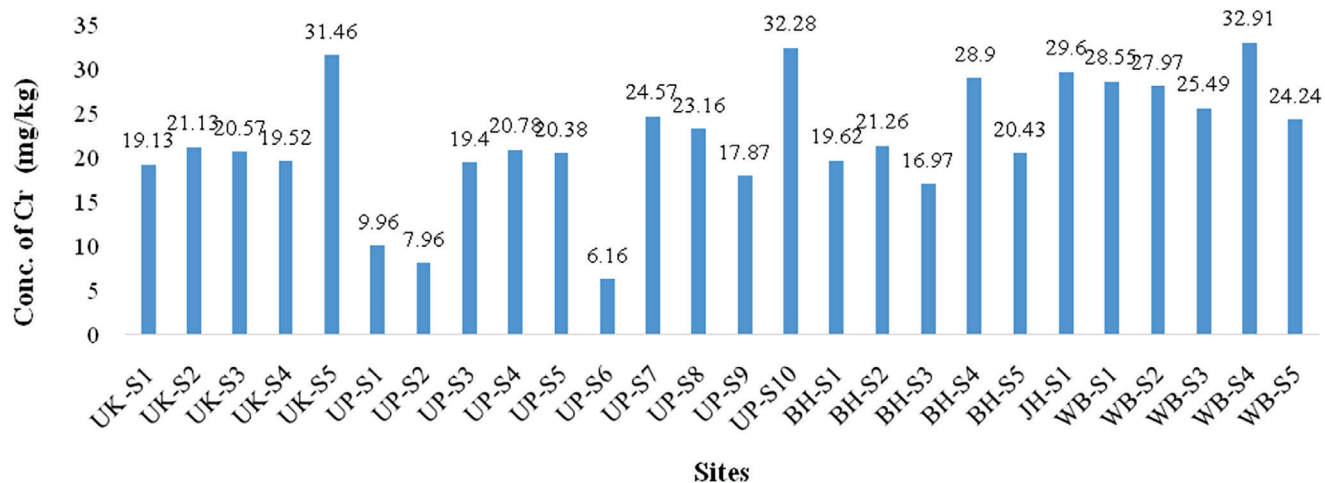


Fig. 10a Concentration of chromium at different sites

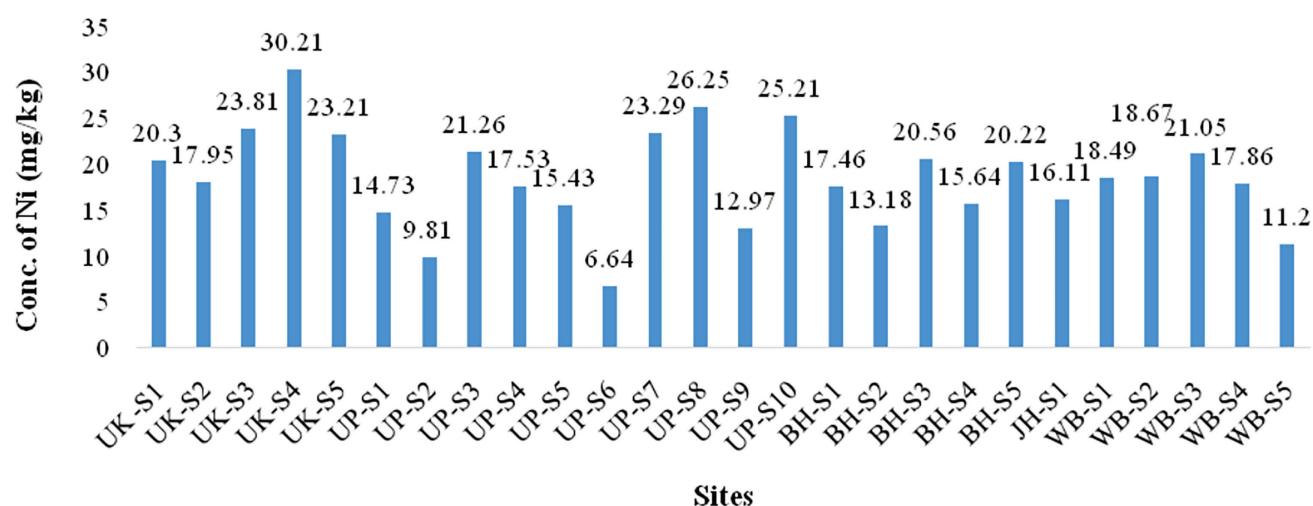


Fig. 10b Concentration of nickel at different sites

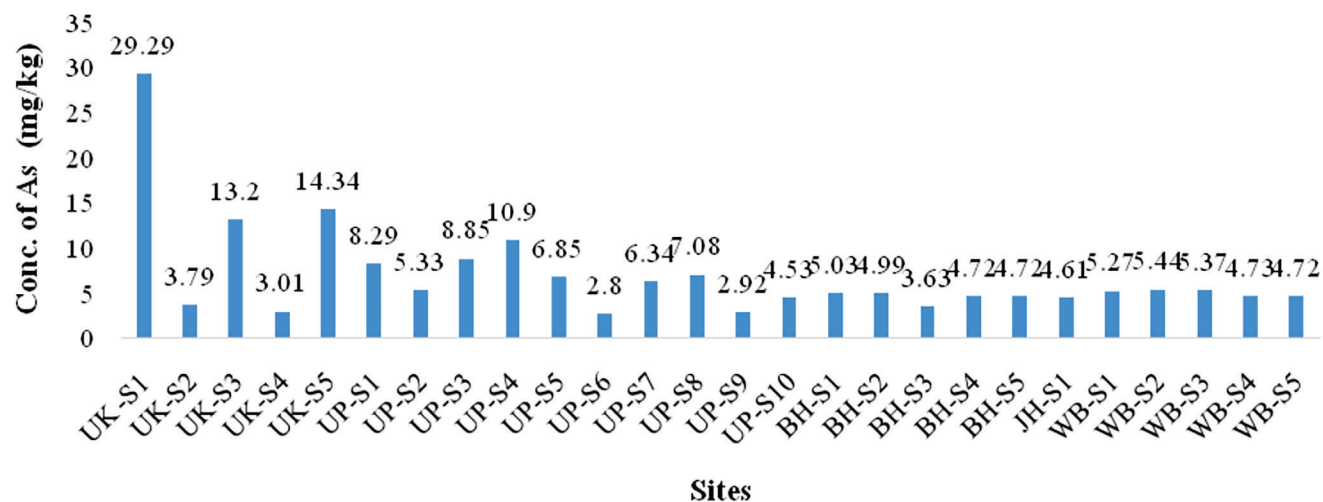


Fig. 10c Concentration of arsenic at different sites

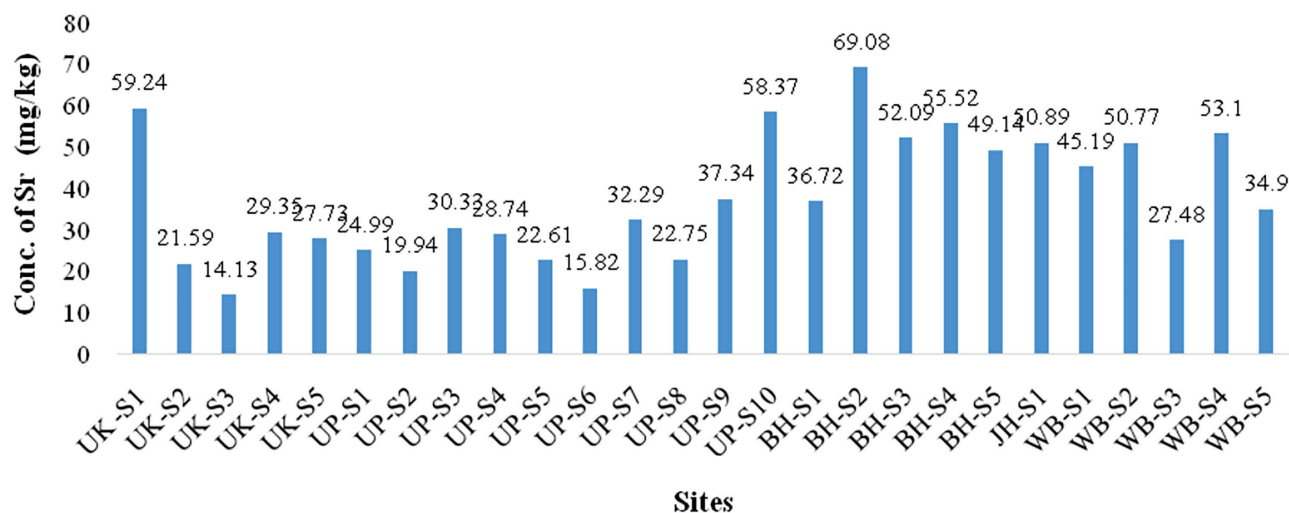


Fig. 10d Concentration of strontium at different sites

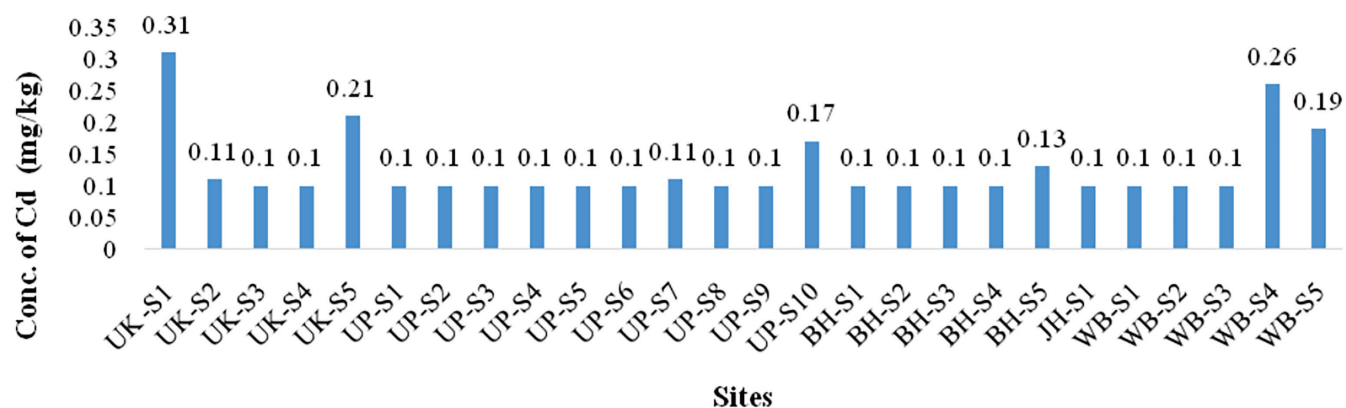


Fig. 10e Concentration of cadmium at different sites

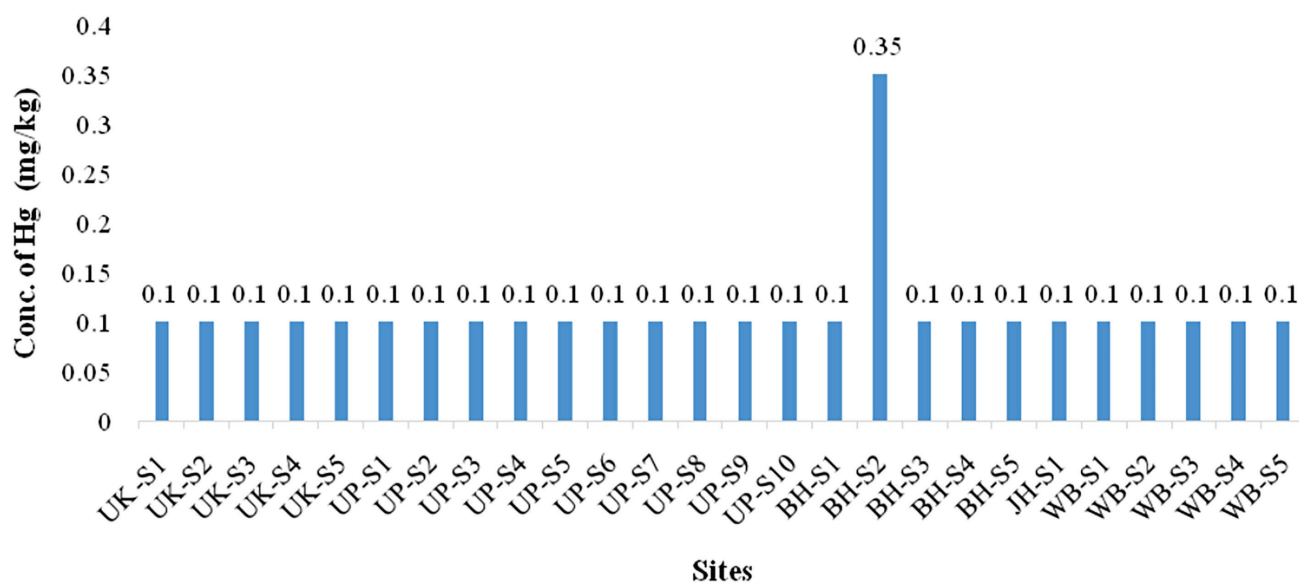


Fig. 10f Concentration of mercury at different sites

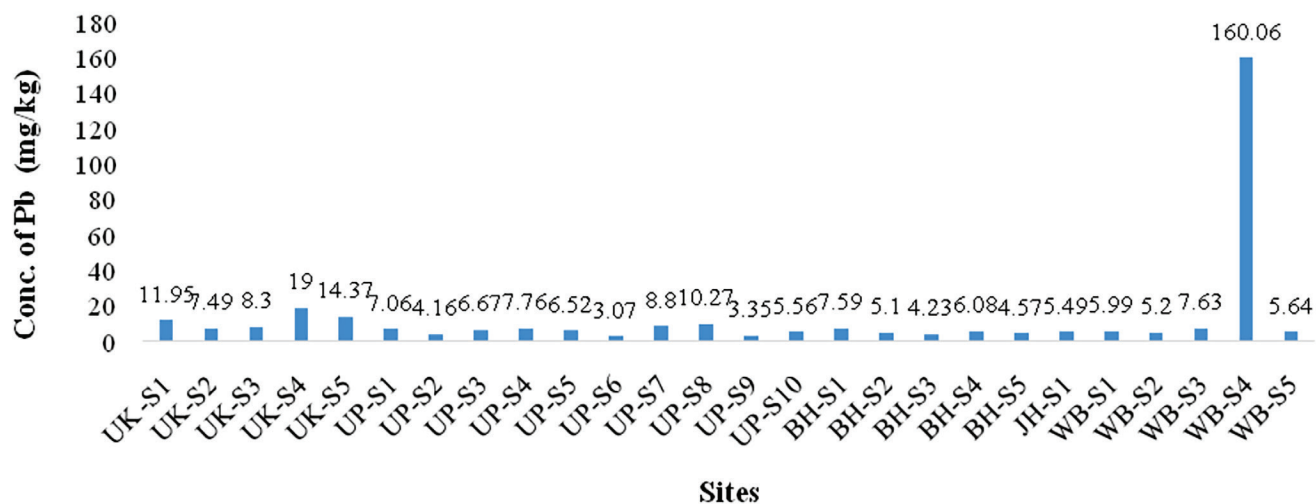


Fig. 10g Concentration of lead at different sites

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 four 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.

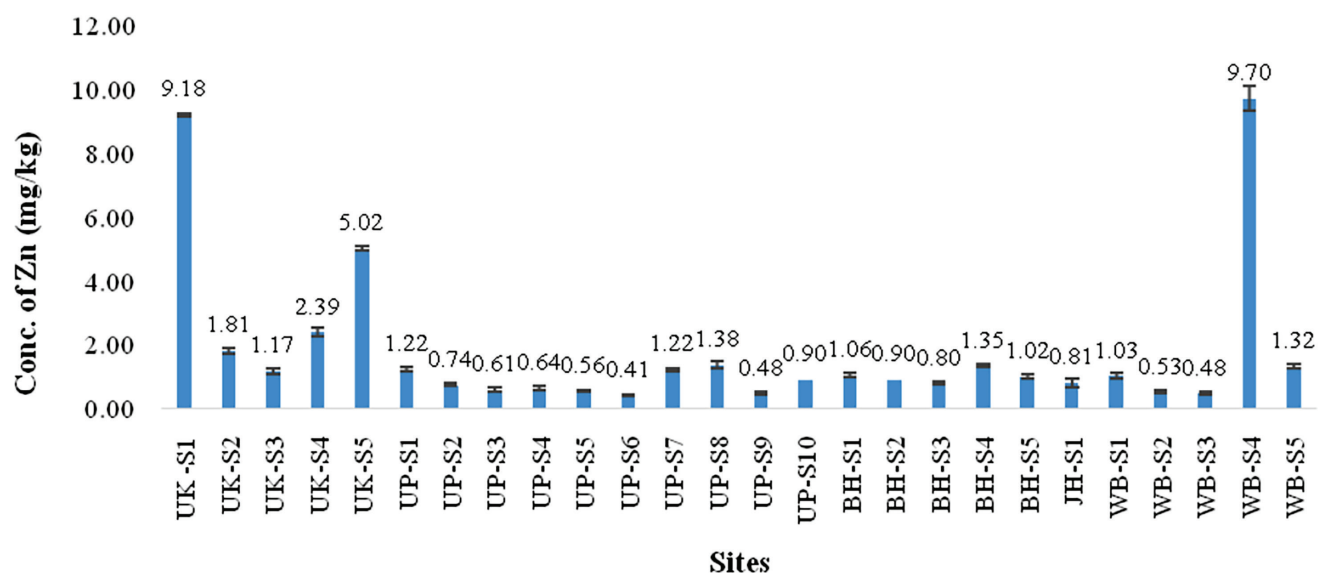


Fig. 11a Concentration of zinc at different sites

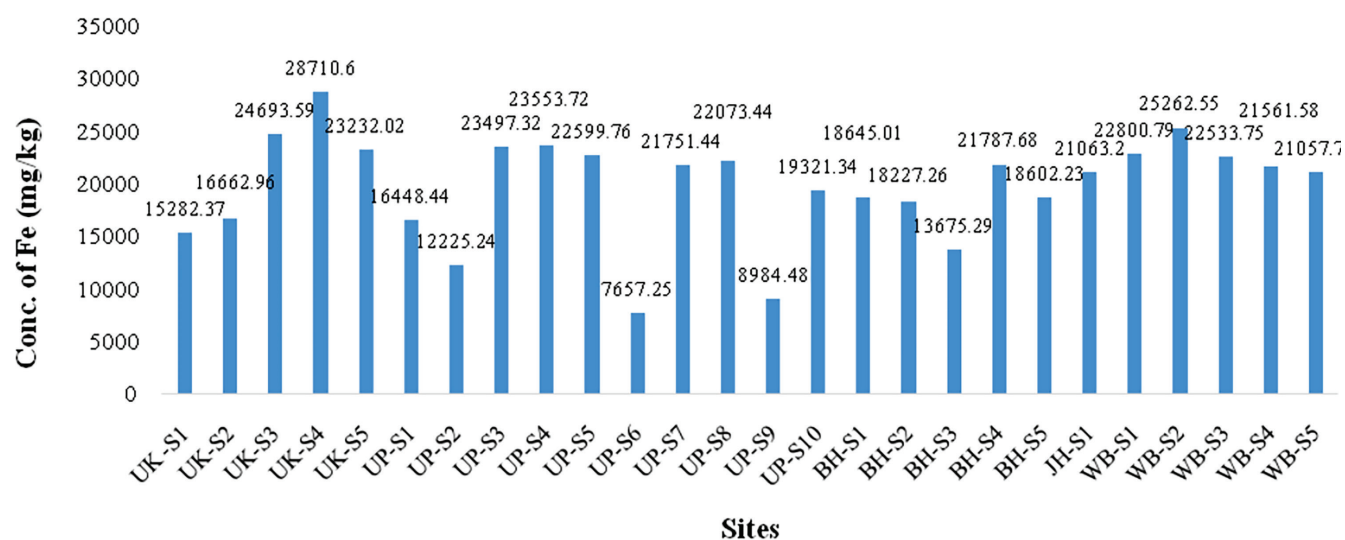


Fig. 11b Concentration of iron at different sites

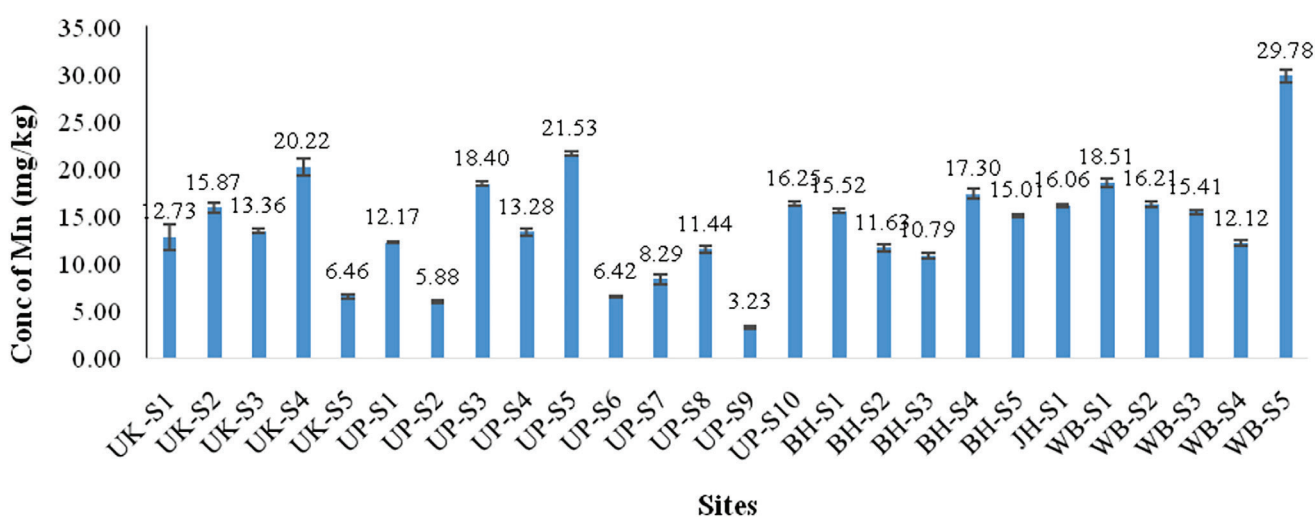


Fig. 11c Concentration of manganese at different sites

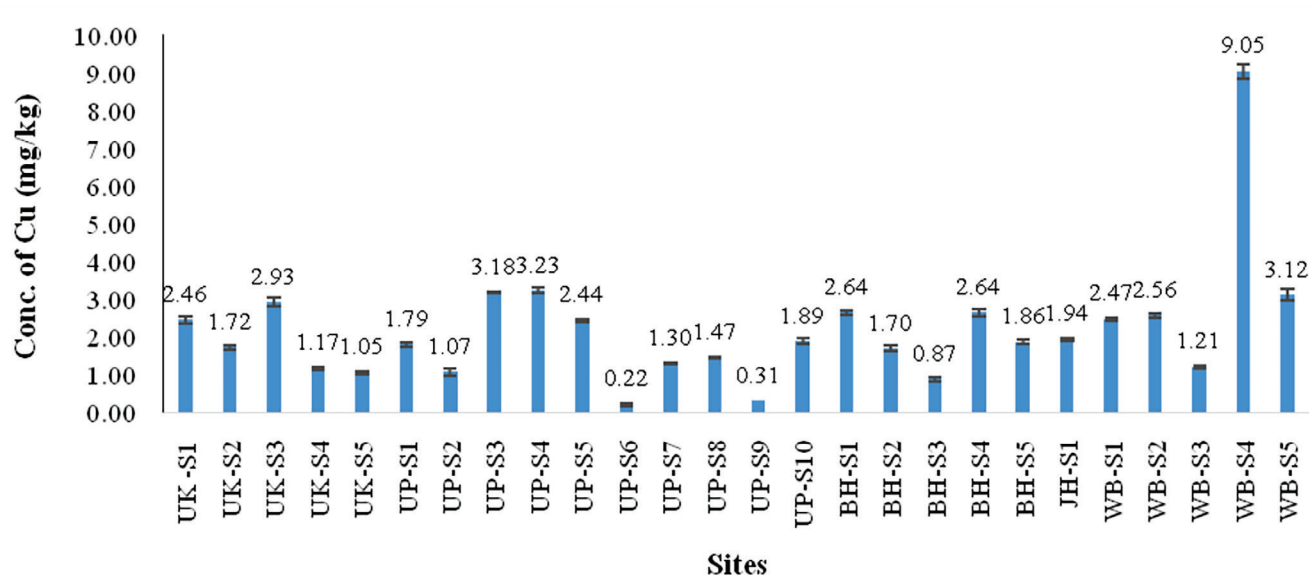


Fig. 11d Concentration of copper at different sites

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 *Achyranthes aspera* L. in different locations shown in Fig. 12

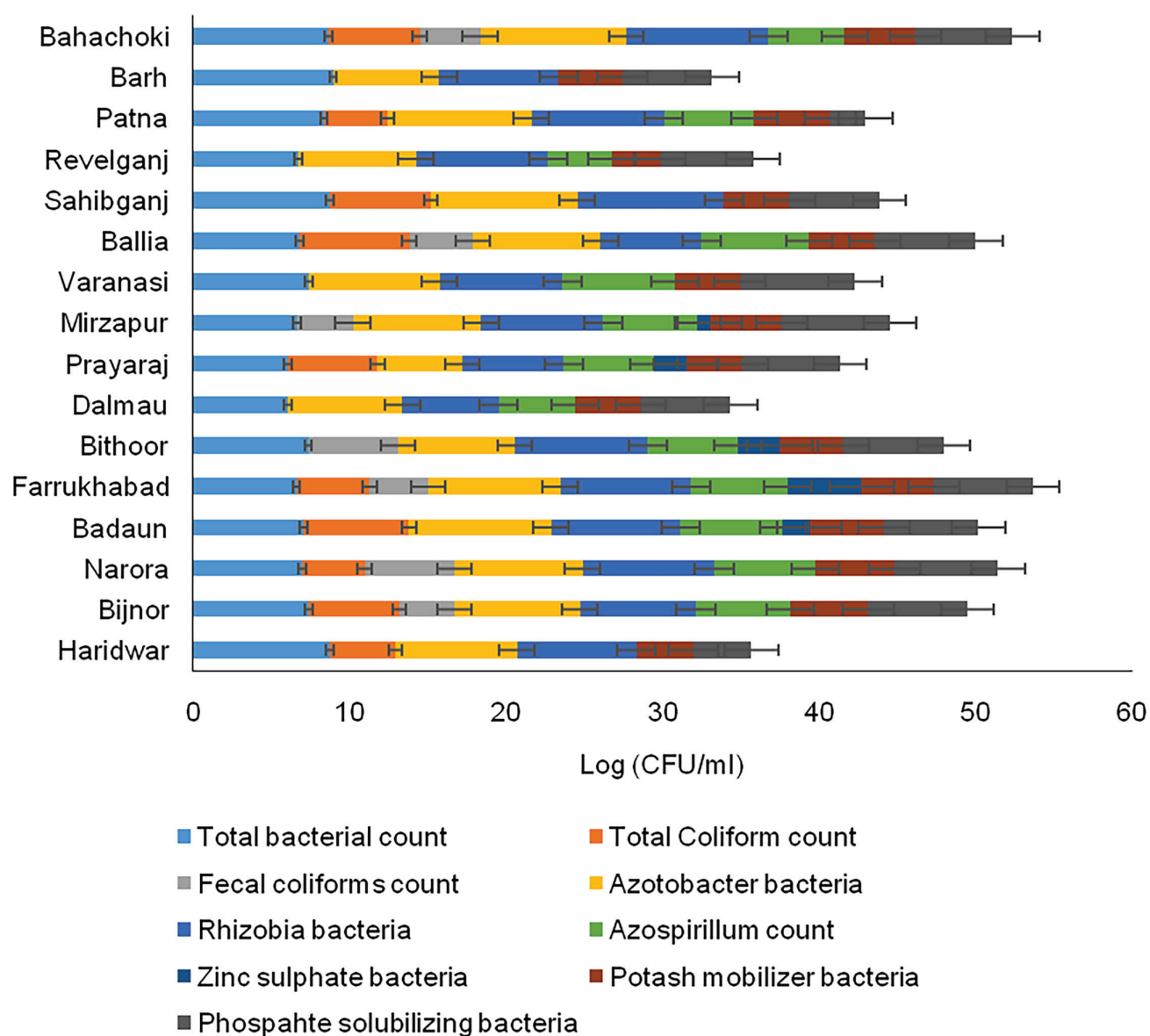


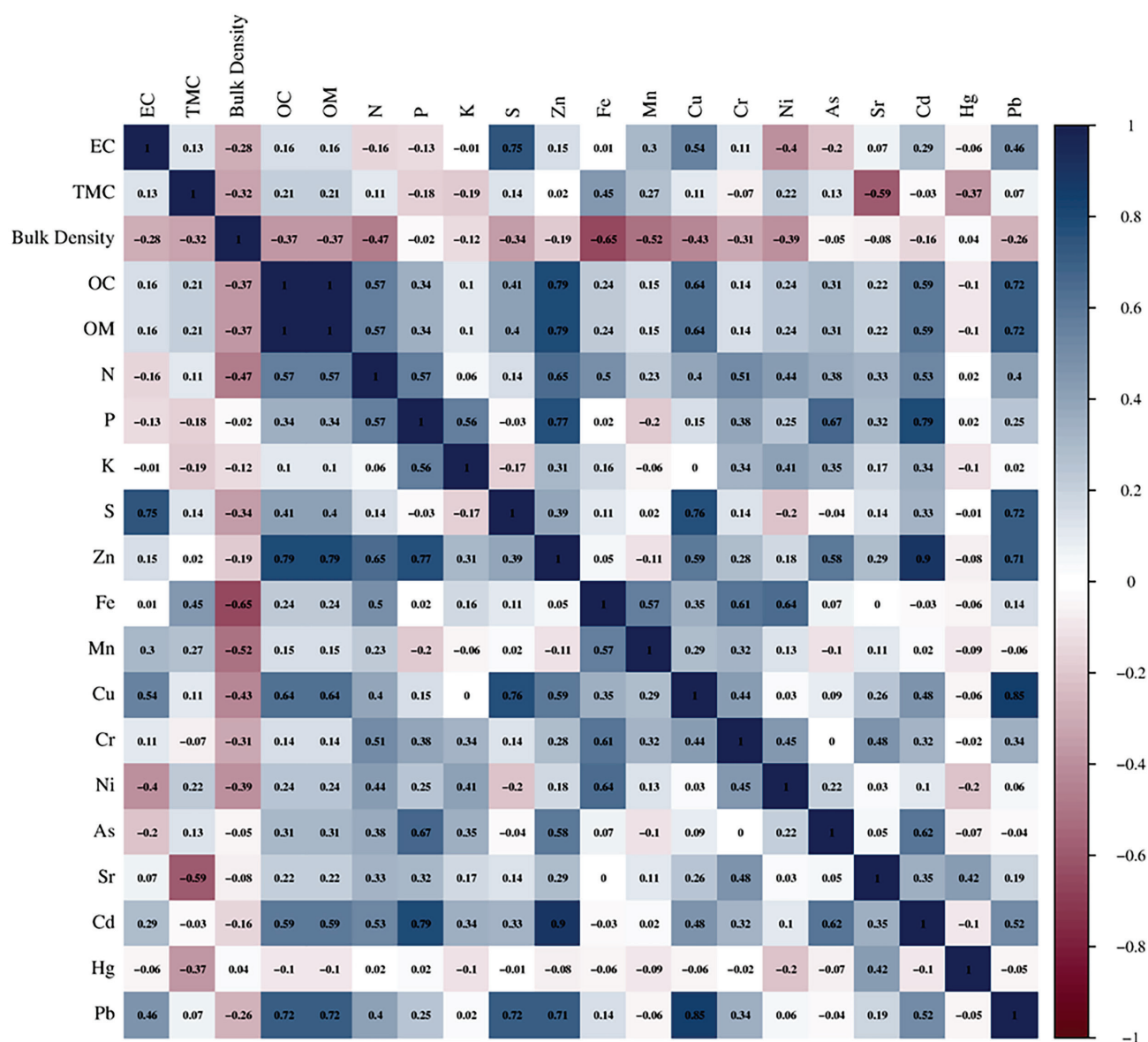
Fig. 12 Total and differential bacterial count

The total rhizosphere microbial test is performed for the identification of total bacterial count which is found highest in Barh 8.9488 cfu/ml and lowest in Prayagraj is 6.0199 cfu/ml. Whereas the total faecal and

coliform count is highest in Bithoor and Ballia i.e., 5.73399 and 6.9918 cfu/ml. respectively. 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
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

Sites	pH	EC	OC	N	P	K	S	Zn	Fe	Mn	Cu	Cr	Ni	As	Sr	Cd	Hg	Pb
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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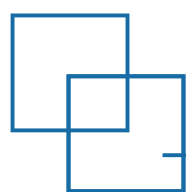
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CHAPTER
04

Traditional and Ethnomedicinal Applications



INTRODUCTION

People have handed down their knowledge of plants from generation to generation since prehistoric times. Herbal treatments play an important part in therapy in all traditional medical systems. Herbal medicine exemplifies the efficacy of broad therapeutic variation. Plants have been used for medicine more than any other material from the beginning of time because they are widely available, inexpensive, and tailored to an individual's urgent requirements (Srivastav et al., 2011). Plants have an almost endless ability to create secondary metabolites with antimicrobial properties, such as tannins, terpenoids, alkaloids, glycosides, and phenols. According to estimations, 14-28% of higher plant species are utilized medicinally, and 74% of pharmacologically active plant-derived compounds were discovered through ethnobotanical uses of the plants (Singh and Singh, 2017).

Achyranthes aspera L. is a member of the Amaranthaceae family and is commonly referred to as "chirchira or chirchita." Since ancient times, tribal and rural people have utilized this plant to treat a variety of ailments. Chirchira has long been associated with Indian culture and traditional medicine. It is used in almost every traditional medicinal system, such as Ayurveda, Unani, and Siddha (Singh et al., 2018). This plant was discovered in all the tropical and subtropical parts of the ancient globe, according to the book published in 1889, "The Useful Native Plants of Australia" (Maiden, 1889). In India, the plant is given to treat dropsy. Patients suffering from hydrophobia, snakebites, ophthalmia, and skin disorders are fed the seeds (Dhale and Bhoi, 2013). Those bitten by crazy dogs are given medications made from flowering spikes that have been prepared by putting a little sugar on them (Iwu, 2014). The plant's ash contains a substantial amount of potash, which is used in laundry. This plant has also been collected and studied for its physicochemical properties and secondary metabolites at various elevations in Uttarakhand's Garhwal Himalayas. With the help of techniques like HPTLC, HPLC, quantification of the marker component were also performed on plants collected at various heights (Dubey, 2007; Balkrishna et al., 2023).



Traditional and Ayurvedic Benefits

Traditional natural medicine having roots in India's Vedic civilization is called Ayurveda. The prevention and treatment of several human illnesses is the most widely used application of Ayurveda in modern society. It could provide the safest and healthiest way of life. Nature provides many healing herbs that may be used on a wider scale and are most effective against all diseases [Pandey et al., 2013]. An essential and commonly available Ayurvedic herb called 'Apamarga' (*Achyranthes aspera* L.) is referenced in early Vedic texts. The Lord of all currently existing plant life, according to the *Atharvaveda*, is Apamarga. It revives, cures sterility, and strengthens the body. According to Ayurveda, it is effective for treating a variety of diseases, including Karnaroga, Krimi, Pandu, and

Sirovirecana [Prasad and Subhaktha, 2001]. The categories of Krimighna, Vamanopaga, and Sirovirecanopaga have been used by Acharya Charaka to classify Apamarga. He referred to Apamarga in around twenty-seven distinct places in Charaka Samhita using the four synonyms Pratyakpuspi, Mayuraka, Sikhari, and Apamarga [Sharma, 2000]. Varunadigana, Viratarvadigana, and Arkadigana are more works by Susruta that depict Apamarga. He mentioned this medication about 29 times, using four synonyms 'Kharamanjaro', 'Pratyakpuspi', 'Mayuraka', and 'Vasir', for instance [Shastri, 1995]. In ancient Ayurvedic literature, there are various therapeutic aspects, some of which are described here 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.	Cephalic disorders	Pratyakpuṣpāśirovirecanānām- Śirovirecaka Dravya, used in cephalic disorders, according to Charaka Saḍhitāit; Śirasorujaṣsamagrāyadivā'pāmārgaphalasiddhm- Oil processed with the fruits, useful in cephalgia, as per Śoḍhala Nighaḍḍu; Śirasorujaṣsamagrāyadivā'pāmārgabījaṣsiddham- Rāja Martaḍḍa massage or arrhine of oil processed with seeds, for headache and other cephalic disorders.	Mishra, 2009a; Śāstrī, et al., 2011; Tripathi, 2012a
2.	Ophthalmic disorders	Tāmrāpātrebṣṭānetrehantipīṣāṣṭadudbhavām- Śoḍhala Nighaḍḍu, application of collyrium prepared from the roots triturated with rock salt and honey, used in ophthalmalgia.	Tripathi, 2012b

S. No.	Diseases/ Conditions	Mode of Administration/ Uses	References
3.	Ear disorders	Mārgakṣārajaleṇa tat kṣṭakalke-nasādhitaṣṭilajam. Apaharati-karṣanādaṣbādhiryaṣcāpipūraṣataṣ- Śāraṅgad-hara and Vṇdamādhava instillation of ear drops prepared from <i>A. aspera</i> (Prickly chaff-flower) alkaline, leaf paste, and oil, useful in deafness, tympanitis, and tinnitus.	Tiwari, 2007a
4.	Abdominal disorders	Jalapītamapāmārgamūlaṣhanyādvīsūcikām- Described in Bhāvaprakāśa, root paste, useful for cholera.	Tripathi, 2013a
5.	Anorectal diseases	Apāmārgamūlaṣ ca taṣṣulodakenasakṣaudra-maharaṣ- root paste mixed with honey and rice water, prescribed by Āchārya Suśruta, against hemorrhoids; Apāmārgasyabījānāṣkalkastaṣṣu-lavāriṣā.Pītoraktārśasāṣnāṣaṣku-rutenātrasaṣśayaṣ- 10-12 g seed paste with rice water, against hemorrhoids, mentioned in Śāraṅgad-hara Saḍhitā; Kalkamapāmārga-mayaṣvimiśrayasujātatakreṣ. Suvasanavilulanagalitaṣpibarudhirārśaṣsub-heṣajaṣlalitam- According to Siddhabheḍa-ja-Maḍi-Mālā, whole plant paste along with buttermilk, useful in hemorrhoids.	Chunekar, et al., 2005a; Misra, et al., 2012; Tripathi, 2013b
6.	Hepato-splenic disorders	Apāmārgaṣamīmūlaṣpiṣṣvātakreṣapāyayet. Kāmālāṣśvayathuṣpāṣṣuṣkarṣamātraṣniyaccha-ti- 10-12 g equal quantity of <i>A. aspera</i> and <i>Prosopis cineraria</i> roots, for jaundice, inflammation, and anemia.	Bhatta, 2008a
7.	Renal and Urinary bladder disorders	Tilāpāmārgakadalīpalāṣayavasambha-vaṣkṣāraṣpeyo'vimūtreṣśarkarāṣvaśmarīṣu-ca- <i>Sesamum indicum</i> (Sesame), <i>A. aspera</i> , <i>Musa sapientum</i> (Banana), <i>Butea monosperma</i> , (Forest flame), and <i>Hordeum vulgare</i> (Barley) alkaline along with sheep urine, used in calculus, described in AḍḍāṅgaHḍḍaya; Mūtrakṣcchraṣjayetpītākṣīreṣaśikharīṣiph. Ajākṣīreṣsaṣpeṣyapītānīlījaṣātathā- Vaidya Manoramā suggested roots with goat urine, against dysuria.	Tripathi, 2009; Sharma, 2012a

S. No.	Diseases/ Conditions	Mode of Administration/ Uses	References
8.	Reproductive system disorders	Anyairanākhyāyakadāśumūrdhanyapāmārgaraso'bhiṣiktaṣ- described in Sodhala, head massage with juice in leucorrhea; Pāṣhālāṣgalisiṣhāsyamayūra-kajaṣāṣpṣthak.Nābhibastibhagālepāt-sukhaṣnārīprasūyate;Apāmārgaśikhāṣyonimadhyeniṣkṣipyadhāyryate. Sukhaṣprasūyatenārībheṣajasyāsyayogataṣ;Mūlaṣpratyakpuṣpyāṣpāṣhāyāścaviniveśitaṣguhye. Strīṣāṣduṣprasavānāṣprasavaṣkurutesukhenaiva- topical application of roots paste, twigs and leaves, to promote delivery, described in Sodhala; Apāmārgapatradvayaṣyonimadhy-eniviṣṣaṣkṣāṣādyoniśūlaṣnihanti.Sudur-vāramapyevamevaprakukto- leaves paste, applied topically for reducing vaginal pain; Piṣṣvālipteyonaumūlaṣkharamaṇjarīpunarnavayoṣ.Navasūtāyāśśūlaṣyonigataṣsakalamapan-ayataṣ- prescribed by Vaidya Manorama, topical application of <i>A. aspera</i> and <i>Boerhavia diffusa</i> roots paste, against postpartum pain; Apāmārgadalālepaṣsadyobadhnātiśoṣitam.Atipravṣṭtama	Tripathi, 2009; Mishra, 2009b; Sharma, 2012b; Sharma, 2012c; Tripathi, 2012c; Tripathi, 2012d; Tripathi, 2012e; Tripathi, 2012f
		pyāśusetubandhaivodakam- application of leaves paste, against hemorrhages; Mūlaṣpratyakpuṣpyāṣpāṣhāyāścaviniveśitaṣguhye. Strīṣāṣduṣprasavānāṣprasavaṣkurutesukhenakhalu- prescribed by RājaMārtaḍḍ, topical application of roots paste, to promote normal delivery.	

S. No.	Diseases/ Conditions	Mode of Administration/ Uses	References
9.	Derma- tological disorders	Pralepātsidhmayātyastamapāmārgasyabhasmanā.Kevalenayathāpāpaścandraśekhar-abhasmanā- in <i>Sidhma</i>, topical application of plant's ash along with mustard oil; Śikharirase-nasupiṣṣaṣmūlakabījaṣpralepataṣsidhmam. Kṣāreṣakadalyāvārajanīmiśreṣanāśayati- according to VṛndaMādhav, topical application of radish seed pounded with the juice of <i>A.aspera</i>; Apāmārgasyasaṣsiktaṣpatrotthenarasenatu. Sadyovraṣeṣuraktaṣ hi pravṣttaṣparitiṣṣhati- topical application of leaves juice, used in hemorrhages and wounds caused by injury; Apāmārgo'śvagandhā ca tālapatrīsuvarcalā. Utsādanepraśasyantekākolyādīścayogaṣaṣ- prescribed by Acharya Sushruta, topical application of the paste of <i>A. aspera</i>, <i>Withania somnifera</i> (Winter cherry), <i>Curculigo orchioides</i> (Black musli), black salt, and medicinal herbs of <i>Kakolyadi Gaṇa</i>, for wounds; Mayūrakorā-javṣkṣonimbaṣkoṣātakītilāṣ.Bṣhatīkaṣṣakārī ca haritālaṣmanaṣśilā.Śodhanāni ca yojyāni-tailedravyāṣīśodhane- topical application of oil processed with <i>A. aspera</i>, <i>Cassia fistula</i> (Pudding pipe tree), <i>Azadirachta indica</i> (Margosa tree) bark, in wounds, described by Acharya Sushruta; Pratyakpuṣpīphalayutaistilaiṣpiṣṣaiṣpralepayet- according to VṛndaMadhava, topical application of <i>A. aspera</i> fruits powder, with <i>Sesamum indicum</i> paste, useful in sinus caused due to <i>Vata</i>; Suvarcikāsaṣdhavacitrakeṣunikumbhatālītalarūpikāsu.Phaleṣvapāmārgabhaveṣucaivakuryātsamūtreṣuhitāyatailam- according to Acharya	Chunekar, 2005b; Chunekar, 2005c; Chunekar, 2005d; Bhatta, 2008b; Tiwari, 2007b; Tripathi, 2012g; Sharma, 2012d; Sharma, 2012e

S. No.	Diseases/ Conditions	Mode of Administration/ Uses	References
		Sushruta, application of oil, processed from <i>Sajji Kshar</i> , rock salt, <i>Plumbago indica</i> (White leadwort), <i>Baliospermum montanum</i> (Wild castor), <i>Embllica officinalis</i> (Indian gooseberry) root, <i>Calotropis gigantea</i> (Madar), <i>A. aspera</i> (Prickly chaff-flower) seed paste and cow urine, used to promote healing; Palaśśikharibī-jānāśmadhunaśkavalagrahaś.Sarpiśpalaśvipa-ktavyaśvraśānmalaharoharet - mentioned in Siddha BheḍajaMaḍimālā, topical application of <i>Ghrita</i> (Clarified butter), processed from <i>A. aspera</i> (Prickly chaff-flower) seed and honey, useful in wounds.	
10.	Psychological disorders	Kākajaśghācāpāmārgaś.kokilākśaśsuperśikā. Kvātheānidrākaraśśīghraśmūlaśvāśbandhayechikham - According to Harita, decoction of <i>A. aspera</i> and <i>Peristrophe bicalyculata</i> , used against insomnia.	Pandey, 2010

Therapeutic Potential

People have used plants for their healing properties since the beginning of time. Many powerful and efficient drugs are now made from a variety of plants (Hossain et al., 2013). The usage, maintenance, and appreciation of wild plants are important aspects of traditional knowledge in many human cultures. Thus, traditional practices like gathering plants for food, sharing knowledge, and keeping it within the community all contribute to the survival of several civilizations (Toledo et al., 2009). Because of the tremendous increase in the use of plant-based health products in both developed and developing countries, the market for herbal products has increased dramatically globally in recent years (Pandey

et al., 2013). There has been a discernible shift towards herbal medicines in recent years due to the significant cumulative and irreversible adverse effects of modern drugs. Natural resources and the related traditional knowledge, however, are becoming more and more rare because of urbanization, population growth, and continuing exploitation of these herbal assets (Pande, 2007).

The herbaceous plant *Achyranthes aspera* L. is well-known in India and other nations as one of the most versatile medicinal herbs with a wide spectrum of biological activities. Since ancient times, tribal and rural people have utilized this herb to treat a variety of diseases. It has been used virtually in all traditional

medicinal practices, including Ayurveda, Unani, and Siddha (Agharkar, 1991; Dwivedi, 2004). This herb is highly regarded by traditional healers and used for the management of various diseases such as beriberi, colic, bronchial infections, toothache, convulsions, blood disorders, diabetes mellitus, menstrual

problems, fertility disorders, etc., as summarized in Table 2. Different plant parts are used in combination with other plants for treating different ailments. Also, it has several therapeutic properties such as diuretic, antifertility, astringent, antiperiodic, etc.

Table 2 Medicinal importance along with their preparation and mode of administration of *Achyranthes aspera* L.

S. No.	Parts used	Preparations	Diseases Treated and Therapeutic Uses	References
1.	Whole plant	Boiled for 20-30 min. and recommended at night	As a diuretic in renal dropsy and general anasarca	Nadkarni, 2005
		Decoction, 10-20 g	Colic	Bhutya, 2011
		Decoction, 2 tablespoons, 3 times a day	Beriberi	Dwivedi, 2004
		Infusion, thrice a day	Bronchial infection	Dwivedi et al., 2008
		Juice, thrice a day	Toothache	Rangari, 2006
		Decoction with roots, leaves, and fruits	Stomach pain	Asolkar et al., 2002
		Ash alone or mixed with water and jaggery	Ascites	Balkrishna, 2022
		Juice	Blood disorders	Shukal, 2014
		Decoction with leaves juice	Skin eruption	Bhutya, 2011
		Smoke as well as powder with honey	Bronchial asthma	Chauhan, 1999
		Decoction	Chills	Lassak and McCarthy, 2011

S. No.	Parts used	Preparations	Diseases Treated and Therapeutic Uses	References
		Ash with honey, twice a day	Cough	Chopra, 1933
		Fumes with roots of <i>Smilax ovalifolia</i>	Anorexia	Pandey, 2012
		Paste mixed with the bark of <i>Alyxiastellate</i> and fruits of <i>Foeniculum vulgare</i> , applied over the body	Convulsions	Wart, 2006
2.	Roots	Decoction, twice a day	Pneumonia, stomach disorders, anemia	Dwivedi et al., 2008; Ahmed, 2010; Haq, 2012
		Extract, at night	Menstrual disorders, constipation	Singh and Singh, 1981; Haq, 2012; Verma, 2014
		Powder, 2 teaspoonfuls, once at night	Bowel complaints, stomachic, digestive	Quisumbing, 1951; Chopra, 1933
		Paste with water	Antifertility	Dwivedi et al., 2008; Nehra, 2014
		Powder with water	Leprosy, consciousness in epileptic conditions, scorpion sting	Dwivedi et al., 2008; Verma, 2014; Balkrishna, 2022
		Infusion, thrice a day	Mild astringent	Nadkarni, 2005
		Powder, twice a day or with black pepper, thrice a day for 15 days	Bleeding in delivery, rheumatism, gout	Dwivedi, 2003; Verma, 2014

S. No.	Parts used	Preparations	Diseases Treated and Therapeutic Uses	References
		Paste with honey, make pills, once daily with water	Diabetes mellitus	Verma, 2014
		A piece tied to the right forearm, twice a week	Displacement of umbilicus	Verma, 2014
		Powder 3 g with 1 g powder of <i>Picrorhiza kurroa</i> , 1 h before meal	Abdominal pain	Balkrishna, 2022
3.	Stem	As toothbrush	Pyorrhea	Verma, 2014
4.	Roots and Leaves	Paste	Antidote	Jain and Singh, 2010
5.	Leaves	Juice with opium, twice a day	Syphilitic sores, gonorrhea	Nadkarni, 2005; Rangari, 2006
		Poultice	Carbuncles, abscesses	Harrington, 2001
		Crushed	Pimples, swelling	Singh, 2003
		Juice with water at bedtime	Piles, boils, skin eruption, bowel complaint, stomachache	Nadkarni, 2005
		Mixed with jaggery or black pepper and garlic, make pills, take twice a day	Antiperiodic	Dwivedi et al., 2008
		Decoction of powder, twice a day	Diarrhea and dysentery	Nadkarni, 2005
		Juice	Bronchial asthma, cold, and headache	Bradacs, 2008; Verma, 2014
		Powder, half teaspoon, 3 days	Stomach pain	Jain and Singh, 2010
		Paste	Boils and blisters	Verma, 2014
		Decoction	Cuts and wounds	Verma, 2014
6.	Seeds	Raw with water	Expectorant	Dwivedi et al., 2008

S. No.	Parts used	Preparations	Diseases Treated and Therapeutic Uses	References
		Raw, twice, or thrice a day	Bleeding piles, brain tonic	Dwivedi et al., 2008
		Powder, with black pepper and honey, twice a day for 1 month	Asthma	Verma, 2014
		Powder, 5 g or 1 teaspoon 2 times daily	Dysentery, snake bite	Verma, 2014
7.	Flowers	Paste	Menorrhagia	Rangari, 2006
8.	Whole plant, and roots	-	Dilation of blood vessels, depression of the heart, the amplitude of respiration	Srivastav et al., 2011
9.	Roots and seeds	Smoke	Cough	Haq, 2012

*[-] Not mentioned

Ethnomedicinal Uses and Folk Treatments along the Ganga Basin of India

The Ganga basin is the most populated river basin in the world, supporting half of India's population, including two-thirds of the destitute (Kumar, 2017). The Ganga basin, which drains an area of 8,61,452 square kilometers, or slightly more than one-fourth (26.3%) of the nation's total geographical area, is primarily located in India. It is the largest river basin in the nation. Uttar Pradesh, Madhya Pradesh, Rajasthan, Bihar, West Bengal, Uttarakhand, Jharkhand, Haryana, Chhattisgarh, Himachal Pradesh, and Delhi are among the states included in it (India Water Resources

Information System, 2023). In India, along the Ganga basin, has a large tribal population that has existed for centuries, and they have a traditional knowledge system that addresses many important topics as well as the health problems that affect their communities. The indigenous people obtain their healing through their own herbal remedies, local practitioners, and their traditional knowledge and comprehension of nature (Prakash, 2015).

The Uttarakhand Himalayas are a rich reservoir of biodiversity, with numerous

plant and wildlife species. It is home to many indigenous communities, including Bhotias, Boxas, Gujjars, Tharus, Rajis, and Jaunsaries, who have extensive traditional knowledge of medicinal plants used to treat a variety of human health issues. In addition to these, many other forest-dwelling communities and local people have unique knowledge of the medicinal plants found in the region. Several researchers have documented ethnomedicinal plants used by various indigenous communities in Uttarakhand's sub-Himalayan region to treat various human health-related ailments (Sharma et al., 2014). In the district of Udham Singh Nagar, the crushed leaves of the plant are applied topically on boils by the Tharu community (Bhandary and Chandrashekar, 2002). Similarly, in the Garhwal region, infusion of the roots is used for the treatment of malarial fever, as well as extract of the leaves is used to facilitate delivery. Also, a decoction of the plant is used against dropsy and bronchitis (Kumar et al., 2011).

The Terai region of Uttar Pradesh is known for its high levels of humidity and rich phytodiversity, which can lead to the development of numerous dermatological infections. The Tharu tribes use the ash of the plant mixed with clarified butter for the management of boils and blisters, spermatorrhea, and eye diseases, as an

antidote for snake bites, insect and scorpion bites, dysentery, fever, to increase sexual power, for easy delivery, stomachache, cough, bronchitis, asthma, headache, tooth problems, thorn injury, piles, diabetes, jaundice, fever, hemorrhoids, constipation, eye inflammation, as an anti-fertility and against spleen enlargement (Kumar et al., 2013). Further, in Western Uttar Pradesh, this herb is used by traditional healers for the above-mentioned ailments (Khan and Khan, 2005).

Additionally, the biodiversity of medicinal plants is abundant in Jharkhand. Approximately 40 percent of Jharkhand's total area is covered by forests. There are 32 tribal communities in Jharkhand. Traditional people use these plants as medicinal ones. The primary healthcare provider in rural Jharkhand refers to traditional healers as 'vaidyas' or 'kavirajas'. The stems and leaves are used by the traditional healers of Jharkhand for the management of otorrhea, wounds, and injuries (Lal and Singh, 2012). In the region of Santhal Pargana, the tribal people used *A. aspera* as a remedy for diabetes and fever (Choudhary, 2020). Further, in the Ranchi district, the local people use this plant for piles, boils, skin eruption, colic, snake bites, dysentery, and leprosy (Guptaa et al., 2014).

Conclusion and Future Perspectives

The use of plants or plant products as medicine held a special position in life from the prehistoric era. Due to the phytochemical components of medicinal plants, their use in treating various disorders stretches back to antiquity. The research clearly shows that the locals are quite familiar with the plants in their surroundings, which is crucial for developing contemporary sustainable technologies. According to the literature, *A. aspera* is a very significant plant because of both its wide range of therapeutic benefits and its significant chemical constituents, including ecdysterone, achyranthine, betaine, pentatriacontane, 6-pentatriacontanone, hexatriacontane, and tritriacontane. Numerous pharmacological properties of the plant include spermicidal, anti-allergic, cardiovascular, nephroprotective,

antiparasitic, hypoglycemic, analgesic, and antipyretic effects. Several traditional applications, such as antiperiodic, purgative, and laxative, in several forms of gastrointestinal problems and bodily discomfort that are currently being researched, are also recorded. However, much more study is required to understand their therapeutic potential and stop the development of crippling illnesses. A system based on conventional and natural medicines is what modern medicine recognizes as the best way to cope with the knowledge contained in plants. It is possible to improve the current healthcare system in a much-needed way by fusing recent developments in contemporary medical sciences with natural human pharmacopeia.

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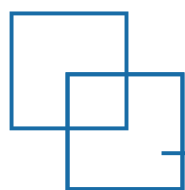


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CHAPTER
05

Phytochemical Analysis



INTRODUCTION

Achyranthes aspera, commonly known as “prickly chaff flower”, is a medicinal plant with a long history of traditional use in various regions. Geographically, it thrives in tropical and warmer areas, found in countries across tropical Asia, Africa, Australia, and America. In India, it grows abundantly as a weed along roadsides, vacant agricultural lands, and uncultivated areas. This plant is recognized for its acrid and bitter taste, making it a popular choice in indigenous medicine and traditional healing practices (Ndhlala et al., 2015).

Phytochemical analysis of *A. aspera* has identified a range of compounds in various parts of the plant, contributing to its medicinal properties. These compounds include flavonoids, phenolic compounds, alkaloids, saponins, and more. The plant is valued for its diverse pharmacological activities, such as anti-inflammatory, antimicrobial, antifertility, antilithiatic, and wound-healing properties. Traditional medicine extensively uses various parts of this species, including the roots, seeds, leaves, flowers, and fruits, for treating conditions like wounds, skin infections, diarrhea, snake bites, ulcers, and more (He et al., 2017).

The classification places it in the Amaranthaceae family, characterized by its stiff, erect annual herb with angular, ribbed stems and thick leaves. The plant's traditional uses encompass a wide array of therapeutic applications, from treating asthma, cough, and edema to addressing piles, skin eruptions, and digestive issues. Its roots, seeds, and shoots are particularly valued for their medicinal properties, with research highlighting its antiperiodic, diuretic, purgative, hepatoprotective, and anti-inflammatory activities (Ndhlala et al., 2015). This plant's rich phytochemical profile and extensive traditional usage underscore its importance in herbal medicine and the need for further exploration of its therapeutic potential.



Sampling Sites

Table 1 Samples of the *Achyranthus aspera* were collected from various state-specific locations

S. No.	Samling sites
1.	Prayagraj, Uttar Pradesh
2.	Mirzapur, Uttar Pradesh
3.	Varanasi, Uttar Pradesh
4.	Ballia, Uttar Pradesh
5.	Patna, Bihar
6.	Barh, Bihar
7.	Sahibganj, Jharkhand
8.	Gaumukh, Uttarakhand

Phytochemical Analysis

The quantification of phytochemical constituents, including tannin (via titration), total saponins (via gravimetry), total polyphenols, and total flavonoids (via UV-visible spectrophotometer), was conducted following established protocols and standard methodologies. 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

A 1–10 g sample was taken and mixed with 50 ml of Milli-Q water. The mixture was then shaken and sonicated for 30 minutes before being brought up to a volume of 100 ml and filtered. Subsequently, 10 ml of the filtered solution was combined with 750 ml of Milli-Q water. Following this, 25 ml of indigo sulphonic acid was added, and the solution was shaken. The mixture was then titrated with 0.1N potassium permanganate solution until a golden yellow colour endpoint was reached. A blank run was conducted without the sample.

Determination of Saponin Content

A 5 g sample was taken and combined with 50 ml of a 1:1 (methanol: water) solvent. The mixture was then refluxed for 1 hour, cooled, and filtered. This process was repeated three times. The filtrates were combined, concentrated, and evaporated to dryness. Subsequently, 25 ml of petroleum ether was added, and the solution was refluxed for 10 minutes, then cooled and the ether layer was decanted. Following this, 10 ml of methanol and 100 ml of acetone were added, and the solution was filtered. The filter paper with residue was dried at 80°C for 1 hour and then weighed.

Determination of Total Polyphenol Content

A 1 ml sample was placed in a test tube and mixed with 1 ml of Folin–Ciocâlteu reagent,

then incubated for 5 minutes. Following this, 1 ml of a 10% sodium carbonate solution was added, and the mixture was kept in the dark for 1 hour. The absorbance was measured at 760 nm using a UV-visible spectrophotometer. These steps were repeated for gallic acid to establish linearity.

Determination of total flavonoid content

A 1 ml sample was placed in a test tube, followed by the addition of 0.4 ml of 10% aluminium chloride, 0.4 ml of sodium acetate, and 3 ml of ethanol. The mixture was then left at room temperature for 30 minutes. Absorbance was measured at 450 nm using a UV-visible spectrophotometer. These steps were repeated for quercetin dihydrate to establish linearity.

HPTLC Fingerprinting

The application of HPTLC in the study reflects a prevalent methodology documented in the literature for profiling secondary metabolites. The use of HPTLC not only enables the rapid separation of compounds but also aligns with the commonly reported diverse chemical fingerprints found in *Ricinus* species. 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 patterns, generally refer as “fingerprints”, are

used for the identification of phytochemicals. The bands or spots obtained during the test are characteristic of a particular herb. A colour image of a typical TLC fingerprint provides a clearer guide to the users.

Sample Preparation

About 1 g of sample from each batch was individually dissolved in 10 ml of methanol. The samples were shaken and sonicated for 20 min. The solution was centrifuged for 5 min at 5000 rpm. The clear solution thus obtained was used for analysis.

Methodology and Analytical Conditions

Analysis was performed on CAMAG HPTLC (Muttenez, Switzerland), equipped with an 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 F254 aluminium sheet (1.0554.0007)
Mobile phase	Chloroform: methanol (9: 1)
Saturation time	15 minutes
Migration distance	70 mm
Band length	8 mm
Injection volume	15 μ l
Visualization	254 nm and 366 nm and under white light after derivatization (Iodine vapor)

High-Performance Liquid Chromatography (HPLC)

Sample and Standard Preparation

Sample preparation: Each batch was individually sampled with approximately 1.0 g of material, which was then diluted with 25 ml of hydro-methanol (20:80) solution. The samples were sonicated for 4 hours, followed by centrifugation at 9000 rpm for 5 minutes and filtration using a 0.45 μ m nylon filter. The resulting filtered solution was utilized for analysis purposes.

Standard preparation: The standard betadysone was dissolved in methanol to create a 1000 ppm standard stock solution. A volume of 0.025 ml was taken from the standard stock

solution (1000 ppm) to prepare a 25-ppm standard working solution.

Analytical and Instrumentation Condition

Separation was achieved using a Shodex C18-4E (5 μ m, 4.6 $\times$ 250 mm) column, subjected to binary gradient elution. The mobile phase consisted of a mixture of 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile (80:20). The flow was set at 1.0 ml/min during the analysis. Ten microliters of standard and test solution were injected. The wavelength was set at 247 nm. The run time was 30 minutes.

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

This analysis aimed to identify the major compounds in the *Achyranthus aspera* test sample (PRF/CHI/1223/1288).

Sample Preparation

500 mg powdered sample was dissolved in 10 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 x 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 40°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)	Mobilephase A %	Mobilephase B%
0	0.3	95	5
10	0.3	95	5
20	0.3	80	20
35	0.3	60	40
40	0.3	40	60
55	0.3	20	80
56	0.3	95	5
60	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 the samples exhibited the presence of tannins,

saponins, total phenolics, and total flavonoids in diverse percentages as reflected accurately in the table given below (Table 2).

Table 2 Phytochemical analysis of *Achyranthus aspera* collected from different locations

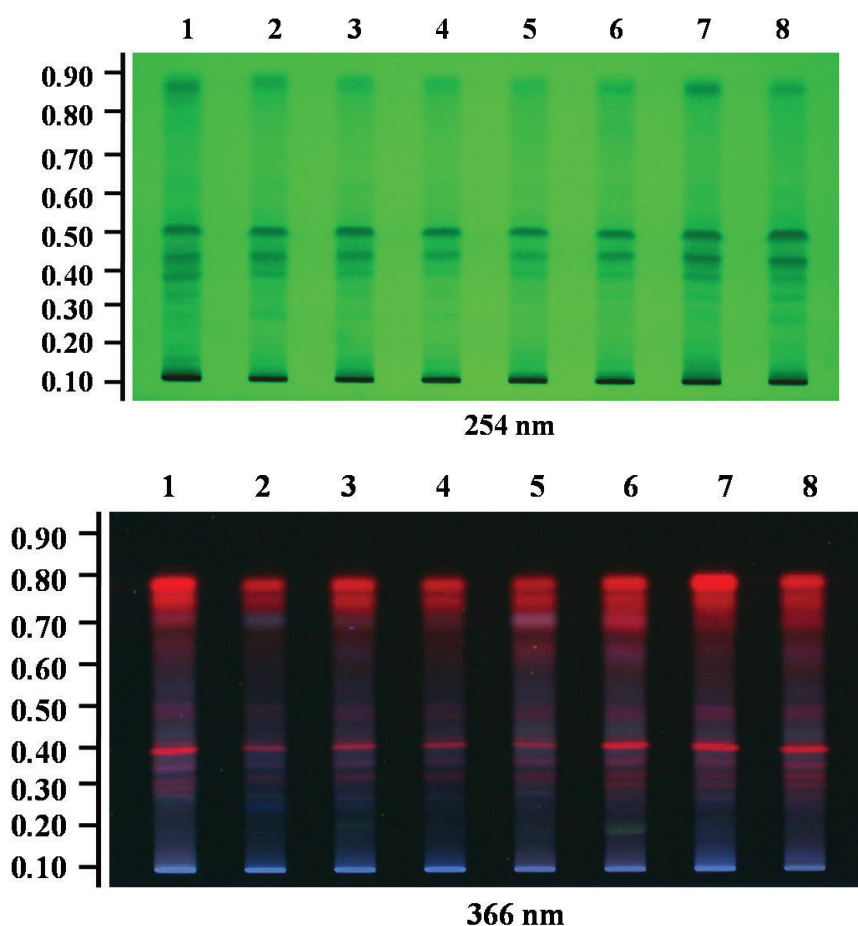
S. No.	Samples	Tannin content (%w/w)	Saponin content (%w/w)	Total polyphenol content (%w/w)	Total flavonoid content (%w/w)
1.	Prayagraj, Uttar Pradesh	0.040	6.267	0.121	0.069
2.	Mirzapur, Uttar Pradesh	0.040	3.547	0.176	0.066
3.	Varanasi, Uttar Pradesh	0.060	5.047	0.212	0.080
4.	Ballia, Uttar Pradesh	0.060	9.664	0.143	0.090
5.	Patna, Bihar	0.060	6.043	0.108	0.043
6.	Barh, Bihar	0.059	4.283	0.151	0.050
7.	Sahibganj, Jharkhand	0.040	6.707	0.116	0.053
8.	Gaumukh, Uttarakhand	0.060	9.778	0.170	0.101

The phytochemical analysis of *A. aspera* revealed the presence of several bioactive compounds, including tannins, saponins, total polyphenols, and total flavonoids. The tannin content ranged from 0.040% to 0.060%, with the highest content observed in samples from Varanasi, Ballia, and Patna. Saponin content varied significantly across the samples, with the highest concentration (9.778%) found in the Gaumukh sample, while the Mirzapur sample exhibited the lowest concentration (3.547%). The total polyphenol content was highest in the Varanasi sample (0.212%) and lowest in the Patna sample (0.108%). Total flavonoid content ranged from 0.043% to 0.101%, with the highest concentration in the Gaumukh sample.

These variations in phytochemical content can be attributed to the geographical differences and environmental conditions at the sampling sites. Similar studies have reported that environmental factors, such as soil type, climate, and altitude, significantly influence the phytochemical composition of medicinal plants (Raghavendra et al., 2016).

HPTLC Fingerprint Analysis

The HPTLC fingerprint reveals distinct bands of phytochemicals observed to be consistent at 254 nm, 366 nm, and under white light after derivatization with iodine vapor, although there were variations in the intensity of the bands (Fig. 1).



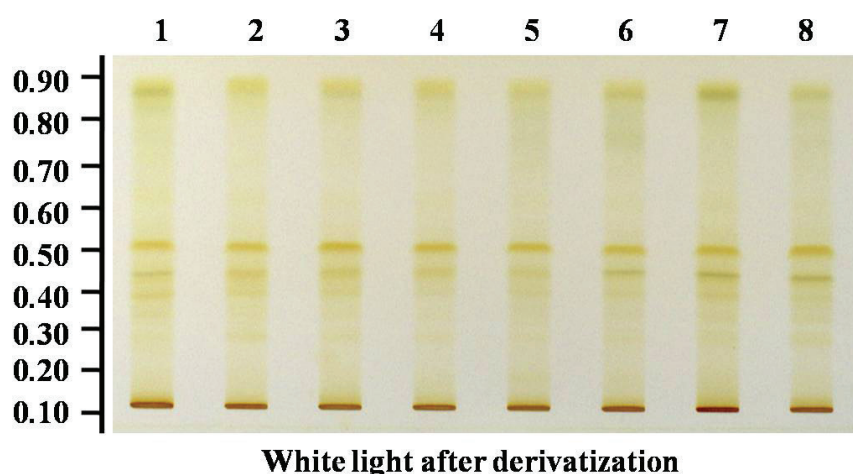


Fig 1. HPTLC fingerprinting of *Achyranthes aspera* L. at (a) 254 nm (b) 366 nm, and (c) under white light after derivatization (Iodine vapor)

- **UV Detection at 254 nm:** Several distinct bands were observed, indicating the presence of diverse phytochemicals, including flavonoids and phenolic acids. The intensity of these bands varied, with some samples showing higher concentrations of specific compounds.
- **UV Detection at 366 nm:** Under UV light at 366 nm, the chromatograms revealed additional bands corresponding to compounds with fluorescent properties, such as flavonoids and alkaloids. The fluorescence intensity was particularly strong for compounds like quercetin and kaempferol derivatives.
- **After Derivatization:** Post-derivatization with Iodine vapor enhanced the visibility of various compounds. Bands corresponding to flavonoids, tannins, and saponins became more pronounced, facilitating their identification.

These findings align with the HPTLC profiles of other medicinal plants, where similar

bands have been associated with therapeutic activities. The presence of specific bands corresponding to flavonoids and phenolic acids supports the antioxidant and anti-inflammatory properties of *A. aspera*. This method is widely recognized for its ability to provide a comprehensive profile of phytochemicals, which can be used for quality control and authentication of herbal medicines. The consistency of the bands at different wavelengths confirms the presence of characteristic phytochemicals, supporting the plant's traditional medicinal uses.

HPLC Analysis

The HPLC study utilized reference standard beta-ecdysone to analyse various plant samples. The highest concentration of beta-ecdysone (0.375 µg/mg) was detected in *A. aspera* L. with sample code Varanasi, while the lowest concentration (0.097 µg/mg) was found in a sample from Mirzapur, Uttar Pradesh. Table 3 below presents the data illustrating

the different percentages of the compound in the analysed samples. Additionally, Fig. 2 and 3 display the corresponding chromatograms

for both the reference standard and the plant samples collected from eight distinct sites.

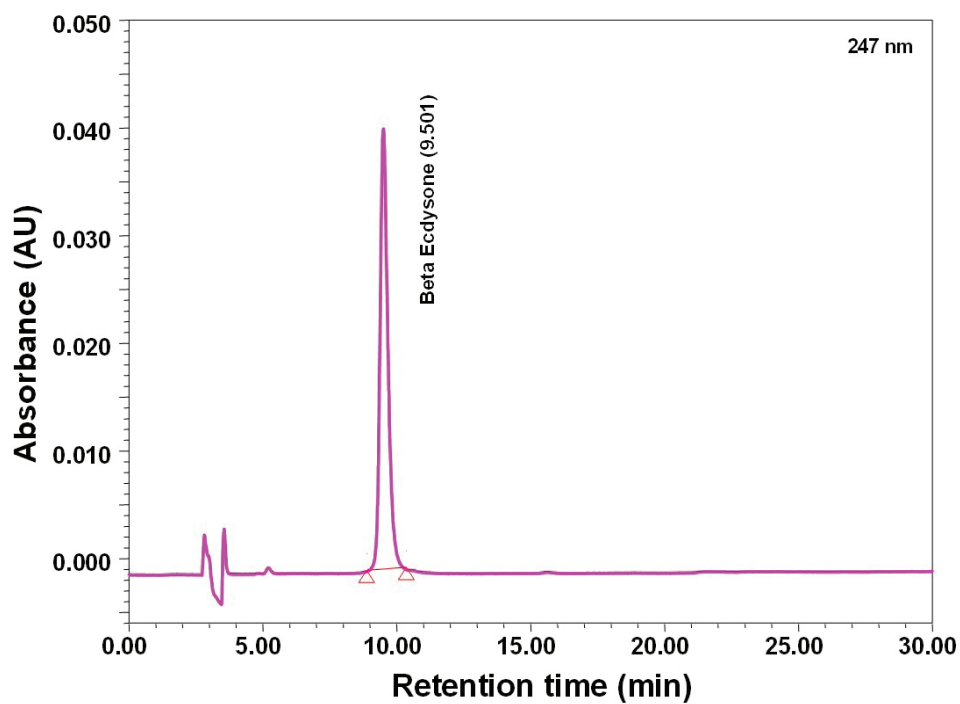


Fig. 2 HPLC chromatogram of standard Beta-Ecdysone at 247 nm

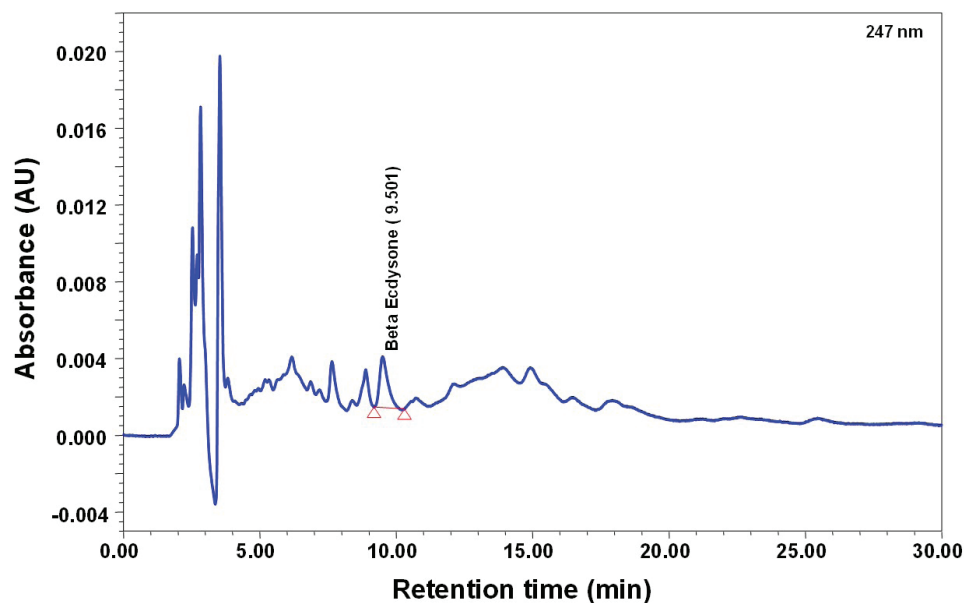


Fig. 3 HPLC chromatogram of *Achyranthes aspera* L. [PRF/CHI/1223/1288] at 247 nm

Table 3 Content of phytochemicals (in $\mu\text{g}/\text{mg}$) present on *Achyranthus aspera* collected from the different locations

S. No.	Internal code	Samples	Beta-Ecdysone ($\mu\text{g}/\text{mg}$)
1	PRF/CHI/1223/1285	Prayagraj, Uttar Pradesh	0.149
2	PRF/CHI/1223/1286	Mirzapur, Uttar Pradesh	0.097
3	PRF/CHI/1223/1287	Varanasi, Uttar Pradesh	0.375
4	PRF/CHI/1223/1288	Ballia, Uttar Pradesh	0.232
5	PRF/CHI/1223/1289	Patna, Bihar	0.242
6	PRF/CHI/1223/1290	Barh, Bihar	0.354
7	PRF/CHI/1223/1291	Sahibganj, Jharkhand	0.215
8	PRF/CHI/1223/1292	Gaumukh, Uttarakhand	0.192

Beta-ecdysone is a phytoecdysteroid known for its potential health benefits, including anti-inflammatory and anabolic effects (Lafont and Dinan, 2003). The variation in beta-ecdysone content among the samples highlights the influence of geographical and environmental factors on its biosynthesis.

UPLC/MS-QToF Analysis

The UPLC-QToF analysis resulted in a high-resolution separation with high sensitivity, leading to the identification of 34 compounds. The TIC chromatogram and the list of identified compounds, along with their parameters in both positive and negative modes, are presented below.

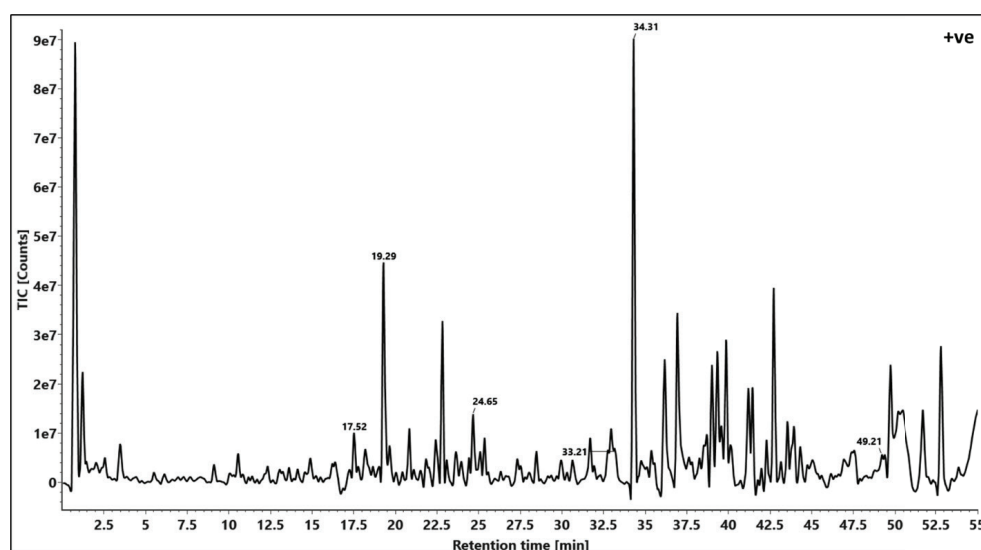
**Fig. 4** TIC chromatogram of *Achyranthus aspera* (PRF/CHI/1223/1288) in positive ionization mode

Table 4 Compound-dependent parameters of analytes/identified compounds in *Achyranthes aspera* (PRF/CHI/1223/1288) in positive ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass error (mDa)	RT (min)	Response	Adducts
1	Pariphyllin B	C ₅₀ H ₈₀ O ₂₁	1016.5192	1017.5261	-0.3	17.52	36187	+H
2	Rutin	C ₂₇ H ₃₀ O ₁₆	610.1534	611.1591	-1.6	18.43	93739	+H
3	Viscumneoside II	C ₂₅ H ₂₆ O ₁₃	534.1373	535.1431	-1.5	18.70	150602	+H
4	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	464.0955	465.1006	-2.1	18.95	47509	+H
5	Beta-Ecdysone	C ₂₇ H ₄₄ O ₇	480.3087	481.3154	-0.6	19.29	908205	+H
6	N-trans-Feruloytyramine	C ₁₈ H ₁₉ NO ₄	313.1314	314.1387	0.0	24.65	1318752	+H
7	Chikusetsusaponin V	C ₄₈ H ₇₆ O ₁₉	956.4981	979.4851	-2.2	33.21	10827	+Na
8	Momordin IIa	C ₄₈ H ₇₆ O ₁₈	940.5032	963.4934	1.0	34.31	375367	+Na
9	[20R,22R]-28,38,20,22,26-Pentahydroxy-cholestan-7,12-dien-6-one	C ₂₇ H ₄₂ O ₆	462.2981	485.2885	1.1	49.21	389370	+Na

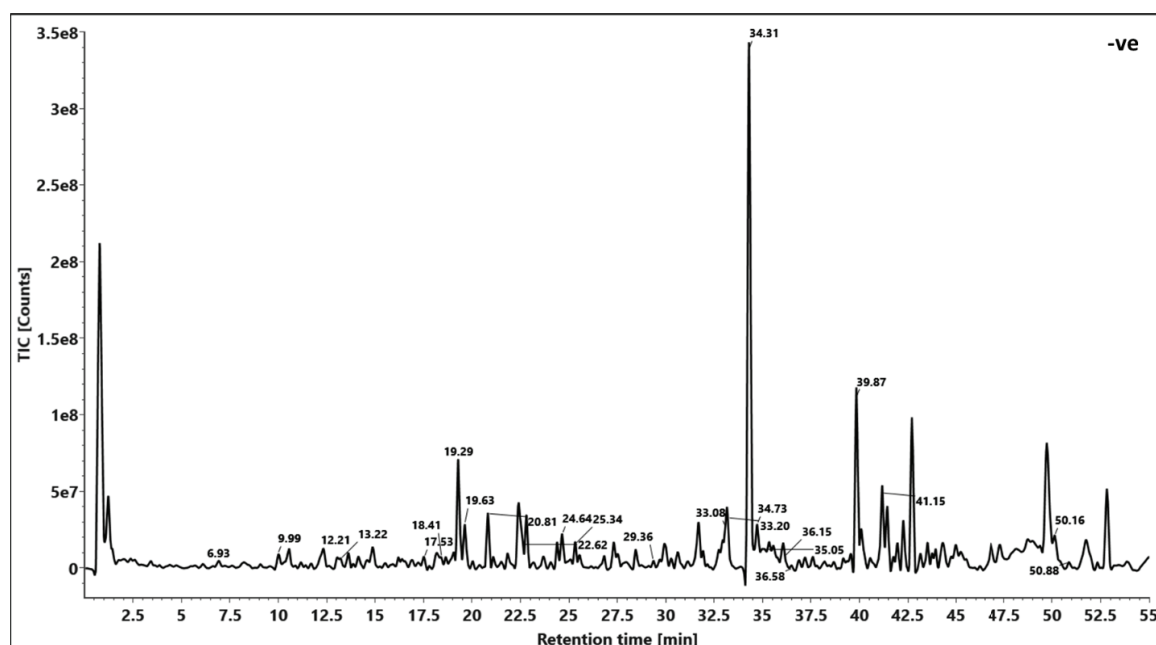


Fig. 5 TIC chromatogram of *Achyranthus aspera* (PRF/CHI/1223/1288) in negative ionization mode

Table 5 Identified compounds in *Achyranthus aspera* (PRF/CHI/1223/1288) in negative ionization mode

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass Error (mDa)	RT (min)	Response	Adducts
1	Uralenneoside	C ₁₂ H ₁₄ O ₈	286.0689	285.0620	0.4	6.93	693382	-H
2	2-Hydroxy-5-[[2-O-D-Apio-Beta-D-Furanosyl-Beta-D-Xylopyranosyl] Oxy] Benzoic Acid	C ₁₇ H ₂₂ O ₁₂	418.1111	417.1038	0.0	9.99	1551767	-H
3	Orcinol gentiobioside	C ₁₉ H ₂₈ O ₁₂	448.1581	447.1515	0.7	12.21	502990	-H
4	Tuberonic acid glucoside	C ₁₈ H ₂₈ O ₉	388.1733	387.1662	0.2	13.22	768455	-H
5	Pariphyllin B	C ₅₀ H ₈₀ O ₂₁	1016.5192	1061.5175	0.0	17.53	1179745	+HCOO
6	Rutin	C ₂₇ H ₃₀ O ₁₆	610.1534	609.1456	-0.5	18.41	742019	-H
7	Viscumneoside II	C ₂₅ H ₂₆ O ₁₃	534.1373	533.1305	0.4	18.69	504256	-H
8	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	464.0955	463.0886	0.4	18.93	539099	-H
9	Beta-Ecdysone	C ₂₇ H ₄₄ O ₇	480.3087	525.3069	0.0	19.29	7374035	+HCOO
10	Polypodine B	C ₂₇ H ₄₄ O ₈	496.3036	541.3017	-0.2	19.41	814089	+HCOO
11	Acteoside	C ₂₉ H ₃₆ O ₁₅	624.2054	623.1983	0.2	19.63	4953047	-H
12	Isoacteoside	C ₂₉ H ₃₆ O ₁₅	624.2054	623.1973	-0.8	20.81	2680803	-H

S. N.	Component name	Formula	Neutral mass (Da)	Observed m/z	Mass Error (mDa)	RT (min)	Response	Adducts
13	Kaempferol-3-rutinoside	C ₂₇ H ₃₀ O ₁₅	594.1585	593.1517	0.5	22.62	916955	-H
14	N-trans-Feruloytyramine	C ₁₈ H ₁₉ NO ₄	313.1314	312.1249	0.7	24.64	2013532	-H
15	Hmp-hmpep	C ₁₉ H ₂₁ NO ₅	343.1420	342.1352	0.5	25.34	910289	-H
16	Achybidensaponin I	C ₄₈ H ₇₆ O ₁₉	956.4981	955.4907	-0.1	26.83	605070	-H
17	Ginsenoside Ro	C ₄₈ H ₇₆ O ₁₉	956.4981	955.4908	0.0	29.36	575093	-H
18	Achyranthoside E dimethyl ester	C ₄₈ H ₇₄ O ₁₉	954.4824	999.4803	-0.3	30.32	296790	+HCOO
19	28-Deglucosyl chikusetsusaponin V	C ₄₂ H ₆₆ O ₁₄	794.4453	793.4372	-0.8	30.55	303627	-H
20	Tribulusamide A	C ₃₆ H ₃₆ N ₂ O ₈	624.2472	623.2400	0.1	33.08	916096	-H
21	Chikusetsusaponin V	C ₄₈ H ₇₆ O ₁₉	956.4981	955.4906	-0.2	33.20	1970327	-H
22	Momordin IIa	C ₄₈ H ₇₆ O ₁₈	940.5032	985.5020	0.6	34.31	17206762	+HCOO
23	Momordin II	C ₄₇ H ₇₄ O ₁₈	926.4875	925.4806	0.3	34.52	426829	-H
24	Chikusetsusaponin IVa	C ₄₂ H ₆₆ O ₁₄	794.4453	793.4376	-0.3	34.73	3562271	-H
25	Achyranthoside B	C ₄₇ H ₇₀ O ₂₀	954.4460	953.4388	0.0	35.05	11574527	-H
26	Achyranthoside E	C ₄₆ H ₇₀ O ₁₉	926.4511	925.4440	0.1	36.15	1819460	-H
27	Achyranthoside III	C ₄₇ H ₇₀ O ₂₀	954.4460	953.4391	0.3	36.58	743859	-H
28	Momordin Ia	C ₄₂ H ₆₆ O ₁₃	778.4503	777.4433	0.2	39.87	10374389	-H
29	Oleanolic acid 3-O-β-D-glucuronopyranoside	C ₃₆ H ₅₆ O ₉	632.3924	631.3847	-0.5	40.54	452606	-H
30	Achyranthoside IV	C ₄₁ H ₆₀ O ₁₅	792.3932	791.3864	0.5	41.15	1378941	-H
31	Oleanolic acid	C ₃₀ H ₄₈ O ₃	456.3604	455.3533	0.2	50.16	1408896	-H
32	Hederagenin-28-O-β-D-glucopyranosyl ester	C ₃₆ H ₅₈ O ₉	634.4081	633.4015	0.7	50.88	290059	-H
33	Oleanolic acid 28-O-β-D-glucopyranoside	C ₃₆ H ₅₈ O ₈	618.4132	617.4061	0.2	55.55	605531	-H

The UPLC-QToF analysis of *A. aspera* highlighted the presence of various bioactive compounds known for their pharmacological properties. The identified compounds, such as pariphyllin B, rutin, isoquercitrin, and beta-ecdysone, contribute to the plant's therapeutic potential.

- Pariphyllin B has been documented for its antitumor and anti-inflammatory activities.
- Rutin is well-known for its antioxidant, anti-inflammatory, and vascular protective effects.
- Isoquercitrin and viscumneose II are flavonoids that exhibit significant antioxidant and anti-inflammatory properties.
- Beta-ecdysone is a phytoecdysteroid with various pharmacological effects,

including anabolic, adaptogenic, and hepatoprotective activities.

The identification of these compounds aligns with the findings of previous studies, corroborating the medicinal value of *A. aspera*. For instance, Ndhlala et al. (2015) and He et al. (2017) have reported similar phytochemical profiles in their investigations, underscoring the plant's potential in traditional and modern medicine.

Furthermore, the diversity of identified compounds indicates a complex phytochemical composition that may contribute to the plant's broad spectrum of therapeutic effects. This comprehensive profiling enhances our understanding of *A. aspera*'s medicinal properties and supports its use in herbal formulations and potential drug development.

Conclusion

The phytochemical analysis of *Achyranthus aspera* L. leaf samples collected from 8 diverse locations across the Ganga River basin provides valuable insights into the chemical diversity of this medicinal plant species. Through comprehensive testing for tannin, saponin, polyphenol, and flavonoids, alongside HPTLC fingerprinting at short and long wavelengths, and HPLC quantification of beta-ecdysone, a detailed characterization of the leaf samples was achieved.

The consistent binding pattern of phytochemicals observed at 254 nm, 366 nm, and under white light following derivatization with iodine vapor highlights the robustness

of the analytical techniques employed in this study. Despite this consistency, variations in the intensity of bands suggest inherent differences in phytochemical composition among the sampled locations, potentially influenced by environmental and geographical factors.

Of particular significance is the detection of beta-ecdysone, a bioactive compound known for its pharmacological properties. The highest concentration of beta-ecdysone was recorded in *Achyranthes aspera* L. with sample code Varanasi, while the lowest concentration was found in a sample from Mirzapur, Uttar Pradesh. These findings

underscore the importance of geographical location in influencing the production of secondary metabolites in the plant species, which could have implications for its medicinal efficacy and ecological adaptation. The UPLC-QToF analysis provided valuable insights into the phytochemical richness of the plant species, highlighting its potential as a source of bioactive compounds for therapeutic applications. Future studies should focus on isolating these compounds and exploring their specific pharmacological activities to further validate the plant's traditional uses and uncover new medicinal applications.

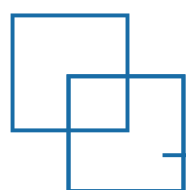
In conclusion, this study contributes to our understanding of the chemical diversity of *Achyranthes aspera* within the Ganga River basin, highlighting the need for further investigation into the factors shaping phytochemical variability across different geographical regions. Such knowledge is essential for optimizing the therapeutic potential of medicinal plants and for conservation efforts aimed at preserving biodiversity and traditional medicinal knowledge in the region.

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CHAPTER
06

Insilico Analysis Against Cervical Cancer



INTRODUCTION

Cervical cancer (CC) ranks as the fourth most form of malignancy and contributor to female mortality worldwide (Xia et al., 2023). According to the GLOBOCAN 2020, around 604,000 women cases were reported globally, resulting in approximately 341,000 deaths. The low-income countries are the most affected ones due to prevalent limitations in healthcare. The general symptoms depicted by the affected patients includes, foul smell discharge, bleeding in between periods, post-menopausal bleeding, urinary urgency, backache, and lower abdominal pain (Reichheld et al., 2020). The most encountered subtypes of CC are squamous cell carcinoma and adenocarcinoma with a proportion of 70 and 25% out of all cases, respectively (Lohmussaar et al., 2021). Among the existing treatments, surgery is the most utilized option. Chemo-radiotherapy is considered for treatment based on stage of the disease, which again compromises with the living quality of the patient and is also a financial burden (Du et al., 2021). Additionally, investigating anticancer drugs with minimal side-effects has become essential due to adverse effects induced by chemotherapeutic agents (Kumar et al., 2022).

As suggested by the existing literature, human papilloma virus (HPV) is the causative agent behind almost all cases of CC. The most common HPV strains linked to this progression are HPV16 and HPV18 (Chatterjee et al., 2019). HPV is a non-enveloped and double-stranded DNA virus, hosted by the Papovaviridae family. This 55 nm virus is comprised of an icosahedral capsid of 72 capsomeres (Salaria et al., 2022). E6 and E7 proteins are characterized as viral oncoproteins, since these are expressed all types of HPV (Juneja et al., 2021). Among these, E6 protein inactivates a range of host cellular proteins, including E6AP, p53, retinoblastoma family proteins, MAML1, and proteins containing PDZ domains that affects the cell proliferation and apoptosis processes. (Kolluru et al., 2019).

Plant-based medicines have built an important niche for themselves in the present century owing to their financial affordability, availability, and minimal side effects (Nguyen et al., 2021). *Achyranthes aspera*, commonly known as 'prickly chaff flower', is a member of family Amarathaceae. This erect or procumbent, annual, or perennial shrub plant



grows as a weed along roadside, prominently in the tropical and subtropical regions of the world, such as India, Baluchistan, Sri Lanka, tropical Asia, Africa, Australia, and America (Emon et al., 2020; Mengie et al 2021; Singh et al., 2021). The plant is extensively employed by the traditional healer for the treatment of asthma (Chinnasamy et al., 2019). In addition, various parts of the plant display positive effects on hydrophobia, snakebite, urinary calculi, influenza, rabies, piles, diarrhea, renal dropsy, gonorrhea, bronchitis, and abdominal pain (Emon et al., 2020). Apart from this, the plant is a storehouse to a plethora of phytochemicals, including saponin, flavonoid, terpenoid, steroid, phenolic compound, and tannin (Lanjwani et al., 2021; Christi et al., 2022). Based on the strong literature support regarding its therapeutic potential, *A. aspera* and its potential active compounds can provide valuable insights into the development of new drug for the treatment of CC.

As the consequence of the above-mentioned problem of CC, we have conducted a comprehensive *in silico* study, in which we used molecular docking to target the human papillomavirus oncoprotein E6 with phytoconstituents of *A. aspera*. Furthermore, to ensure ethyl iso-allocholate's safety and efficacy in the treatment, its drug-likeness, physicochemical and ADMET properties were investigated. In addition, to elucidate their dynamic and thermodynamic properties and to support the molecular docking results, dynamics simulation was performed on ethyl iso-allocholate.

Etiology of Disease

HPV is the major etiologic agent behind CC, which is usually transmitted by sexual contact, causing squamous intraepithelial lesions. These lesions, if not ameliorated within 6 to 12 months through immunological intervention can turn cancerous. Most lesions disappear after 6-12 months due to immunological intervention (Zhang et al., 2020). The HPV E6 and E7 proteins are considered major oncoprotein responsible for cell cycle regulation. E6 protein is a ligand for p53 and triggers telomeric activity in cancer cells. In addition, E7 oncoprotein binds to members of retinoblastoma family of tumor suppressor genes, which induce gene transcription and cell proliferation (Allouch et al., 2020). Some studies have highlighted role of aberrant methylation of integrated viral DNA in the development of CC (Yang et al., 2020). Additionally, factors such as illiteracy, low socioeconomic status, early marriage, oral

contraceptive pills, multiple sexual partners, and smoking are intimately linked with the progression of CC (Baskaran et al., 2019).

Reported Therapeutic Uses

The efficacy of *A. aspera* has been verified against many diseases. For instance, a study revealed the positive effect of leaf chloroform fraction on wound healing process and possess potent anti-inflammatory activity (Mengie et al., 2021). Moreover, the plant is a rich source of antioxidants which are beneficial for patients with diabetes and other oxidative stress related conditions (Mishra et al., 2021). An *in vitro* study described the potential of *A. aspera* in inhibiting pancreatic lipase, suggesting its anti-obesity action (Athesh et al., 2020). In addition, the extract from this plant ameliorated the symptoms of depression in mice (Gawande et al., 2022). Table 1 mentions various therapeutic roles of the plant.

Table 1 List of therapeutic uses of *A. aspera*

Plant part	Therapeutic use	Therapeutic use identifiers	References
Aerial part	Parasympatholytics	MESH:D010276, UMLS:C0030511, ICD-11:XM3A77	ISBN:9770972795006
Bark	Hypolipidemic agents	MESH:D000960, UMLS:C0086440	ISBN:9770972795006
Flower	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	ISBN:9770972795006
Flower	Menorrhagia	MESH:D008595, UMLS:C0025323, ICD-11:GA20.50	ISBN:9770972795006, ISBN:9780387706375
Flower	Rabies	MESH:D011818, UMLS:C0034494, DOID:11260, ICD-11:1C82	ISBN:9770972795006
Fruit	Anti-infective agents	MESH:D000890, UMLS:C0003204, ICD-11:XM4TV0	ISBN:9788172360481

Plant part	Therapeutic use	Therapeutic use identifiers	References
Fruit	Bites and stings	MESH:D001733, UMLS:C0005659	ISBN:9788172360481
Fruit	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	A Report on Medicinal Plants of Kachchh (Gujarat)
Fruit	Rabies	MESH:D011818, UMLS:C0034494, DOID:11260, ICD-11:1C82	ISBN:9770972795006
Fruit	Urinary tract infections	MESH:D014552, UMLS:C0042029, DOID:0080784, ICD-11:GC08	ISBN:9788172360481
Leaves	Cough	MESH:D003371, UMLS:C0010200, ICD-11:MD12	Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Diarrhea	MESH:D003967, UMLS:C0011991, DOID:13250, ICD-11:ME05.1	ISBN:9770972795006
Leaves	Diuretics	MESH:D004232, UMLS:C0012798, ICD-11:XM4D06	ISBN:9788172360481, Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Dysentery	MESH:D004403, UMLS:C0277526, DOID:12384, ICD-11:1A40.Z	ISBN:9770972795006
Leaves	Dysmenorrhea	MESH:D004412, UMLS:C0013390, ICD-11:GA34.3	Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Ear diseases	MESH:D004427, UMLS:C0013447, ICD-11:AC0Z	ISBN:9770972795006, ISBN:9788172360481
Leaves	Eczema	MESH:D004485, UMLS:C0013595	ISBN:9788172360481, Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Eye diseases	MESH:D005128, UMLS:C0015397, DOID:5614, ICD-11:9E1Z	ISBN:9788172360481
Leaves	Eye drops	MESH:D009883, UMLS:C0015399	ISBN:9770972795006
Leaves	Fever	MESH:D005334, UMLS:C0015967, ICD-11:MG26	ISBN:9770972795006
Leaves	Furunculosis	MESH:D005667, UMLS:C0016867, ICD-11:1B75.0	ISBN:9770972795006

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Gonorrhea	MESH:D006069, UMLS:C0018081, DOI:7551, ICD-11:1A7Z	ISBN:9770972795006
Leaves	Hemorrhage	MESH:D006470, UMLS:C0019080, ICD-11:MG27	ISBN:9788172363130
Leaves	Hemorrhoids	MESH:D006484, UMLS:C0019112, DOI:9746, ICD-11:DB60.Z	Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Hepatomegaly	MESH:D006529, UMLS:C0019209, ICD-11:ME10.00	ISBN:9770972795006
Leaves	Hyperhidrosis	MESH:D006945, UMLS:C0020458, ICD-11:EE00.Z	ISBN:9770972795006
Leaves	Insect bites and stings	MESH:D007299, UMLS:C0021568, ICD-11:EK50.0	ISBN:9788172363130
Leaves	Leprosy	MESH:D007918, UMLS:C0023343, DOI:1024, ICD-11:1B20	ISBN:9770972795006, ISBN:9788172360481
Leaves	Low back pain	MESH:D017116, UMLS:C0024031, ICD-11:ME84.2	ISBN:9770972795006
Leaves	Lung diseases	MESH:D008171, UMLS:C0024115, DOI:850, ICD-11:CB40.Y	Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Malaria	MESH:D008288, UMLS:C0024530, DOI:12365, ICD-11:1F4Z	ISBN:9770972795006, Standardization of Single Drugs of Unani Medicine Part - IV
Leaves	Menstruation disturbances	MESH:D008599, UMLS:C0025345, ICD-11:GA20.30	ISBN:9770972795006
Leaves	Rickettsia	MESH:D012281, UMLS:C0035583, ICD-11:XN4YH	ISBN:9770972795006
Leaves	Scorpion stings	MESH:D065008, UMLS:C0238417, ICD-11:NE61	ISBN:9770972795006
Leaves	Snake bites	MESH:D012909, UMLS:C0037379	ISBN:9770972795006
Leaves	Toothache	MESH:D014098, UMLS:C0040460, ICD-11:DA0A.Y	ISBN:9770972795006
Leaves	Typhoid fever	MESH:D014435, UMLS:C0041466, DOI:13258, ICD-11:1A07	ISBN:9770972795006
Leaves	Urination disorders	MESH:D014555, UMLS:C0042035, ICD-11:GB0Y	ISBN:9770972795006

Plant part	Therapeutic use	Therapeutic use identifiers	References
Leaves	Vitiligo	MESH:D014820, UMLS:C0042900, DOI:12306, ICD-11:ED63.0	ISBN:9770972795006
Leaves	Wasp sting	UMLS:C0413119, ICD-11:NE61	ISBN:9788172363130
Leaves	Wounds and injuries	MESH:D014947, UMLS:C0043251	ISBN:9770972795006, ISBN:9788172360481, Standardization of Single Drugs of Unani Medicine Part - IV
Plant exudate	Tetanus	MESH:D013742, UMLS:C0039614, DOI:11338, ICD-11:1C13	ISBN:9770972795006
Root	Abdominal neoplasms	MESH:D000008, UMLS:C0031149	ISBN:9788172360481
Root	Abdominal pain	MESH:D015746, UMLS:C0000737, SYMP:0000457, ICD-11:MD81.4	ISBN:9770972795006, ISBN:9788172360481
Root	Acne vulgaris	MESH:D000152, UMLS:C0702166, DOI:6543, ICD-11:ED80.Z	ISBN:9770972795006
Root	Anti-infective agents, local	MESH:D000891, UMLS:C0003205, ICD-11:XM4VG4	ISBN:9770972795006
Root	Antipyretics	MESH:D058633, UMLS:C0003419, ICD-11:XM1RS7	ISBN:9770972795006
Root	Asthma	MESH:D001249, UMLS:C0004096, DOI:2841, ICD-11:CA23	ISBN:9770972795006
Root	Astringents	MESH:D001252, UMLS:C0004110, ICD-11:XM0VK6	ISBN:9770972795006, ISBN:9788172361266, Medicinal Plants of Nagpur and Wardha Forest Divisions (Maharashtra)
Root	Burns	MESH:D002056, UMLS:C0006434, ICD-11:NE2Z	ISBN:9770972795006

After an extensive study of the reported traditional medicinal uses of *A. aspera*, 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 *A. aspera* in addressing CC, aiming to contribute to the alleviation of the impact of malignant diseases.

Materials and Methods

Protein Structure Retrieval and Preparation

The crystal structure of human papillomavirus oncoprotein E6 in complex with LXXLL peptide of ubiquitin ligase E6AP (PDB ID: 4GIZ; 2.55 Å resolution) was retrieved from RCSB protein data bank (<https://www.rcsb.org/>) (Fig. 1). To generate accurate predictions, any of the associated water molecules or heteroatoms were removed from the structures with the

assistance of PyMol (<https://pymol.org/2/>). Further, the protein structures were repaired using protein repair and analysis server (<https://www.protein-science.com/>) by adding missing hydrogens, side chains and secondary structure elements. In addition, chain A of the protein was selected for docking by utilizing Chimera (<https://www.cgl.ucsf.edu/chimera/>). The final structure was saved in PDB format for further studies.



Fig. 1 Protein structure of HPV oncoprotein E6; chain A

Phytochemical Library Preparation

Extensive literature survey was performed to obtain a list of 71 phytoconstituents present in *A. aspera*. The three-dimensional structures of the compounds in SDF format were retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). In addition, the SDF files were converted into PDB format by utilizing Open Babel GUI 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 utilized to predict the drug-likeness of the compounds. For small molecules, it is an empirical rule of thumb to assess their physicochemical aspects, 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 utilized for the high-throughput 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. 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 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 (<http://www.swissadme.ch/index.php>). To do so, physicochemical 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 bioactivity scores were determined by using Molinspiration software (<http://www.molinspiration.com/>). Parameters analyzed include ion channel modulator (ICM), nuclear receptor ligand (NRL), protease inhibitor (PI), enzyme inhibitor (EI), and G protein-coupled receptor ligand (GPCR ligand).

In silico ADMET Analysis

After molecular docking studies of all the compounds with targeted protein of HPV, pharmacokinetics parameters such as ADMET (absorption, distribution, metabolism, excretion, and toxicity) of the best candidate were investigated using ADMETlab 2.0 (<https://admetmesh.scbdd.com/>). 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 (P-glycoprotein substrate, P-glycoprotein inhibitor, human intestinal absorption, Caco-2 permeability, MDCK permeability, human oral bioavailability 20%, and human oral bioavailability 30%); distribution: (plasma protein binding, blood–brain barrier penetration, volume distribution, and fraction unbound in plasma); metabolism (CYP 1A2/2C19/2C9/2D6/3A4 inhibitor and CYP 1A2/2C19/2C9/2D6/3A4 substrate); excretion (clearance of a drug and half-life of a drug); toxicity (skin sensitization, carcinogenicity, respiratory toxicity, rat oral acute toxicity, eye irritation/corrosion, Ames test for mutagenicity and maximum recommended daily dose) (Xiong et al., 2021).

Molecular Dynamics Simulation

Based on molecular docking analysis, top phytochemical of *A. aspera* 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

Science and technology have paced forward immensely in the field of drug discovery and development to produce innovative solutions to combat cancer. Despite that, CC remains a

significant threat to a substantial proportion of women worldwide. This malignancy is the fourth most frequent cause of mortality among women globally (Xia et al., 2023).

Human papillomavirus (HPV), particularly high-risk strains like HPV16 and HPV18, is the leading cause of CC worldwide. While HPV infection is prevalent, not all infections progress to cancer, directing towards the role of viral oncoproteins like E6 in cervical carcinogenesis (Bhattacharjee et al., 2022). E6, a multifunctional protein encoded by HPV, primarily contributes to cancer development by inactivating the tumor suppressor protein p53 (Pal and Kundu, 2020). E6 achieves this by forming a complex with E6-associated protein (E6AP) to degrade p53 via the ubiquitin-proteasome system, preventing p53 from halting cell division or inducing apoptosis in response to DNA damage. Additionally, E6 promotes cell cycle dysregulation by targeting the retinoblastoma protein (pRb), further facilitating uncontrolled proliferation. Beyond p53 inactivation, E6 enhances oncogenesis by promoting DNA replication, evading apoptosis, and altering the immune response, allowing HPV-infected cells to survive and proliferate despite genetic abnormalities and immune surveillance (Peng et al., 2024). The chief objective of this study is to identify natural inhibitors from *A. aspera* using diverse computational tools. Thus, an attempt was undertaken to explore diverse phytoconstituents from *A. aspera*, targeting HPV E6 oncoprotein that plays a significant role in progression of CC.

Preliminary Screening of Drug-likeness

In the pursuit of creating a highly effective drug with minimal adverse effects, preliminary screening based on drug-likeness is a crucial step. Drug-likeness refers to the physicochemical properties that influence a molecule's ability to become a successful drug (Kar and Leszczynski, 2020). Molecules with poor drug-likeness characteristics are more likely to exhibit unfavorable pharmacokinetic properties, such as poor absorption, distribution, metabolism, and excretion, leading to failure in later development stages (Agamah et al., 2020). To conduct this preliminary screening, Lipinski's rule of five was utilized for evaluating all the phytoconstituents derived from *A. aspera*. A compound demonstrates favourable 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 $\log P \leq 5$ (Lipinski, 2004). 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 71 phytoconstituents, 59 candidates successfully passed Lipinski's rule and were carried forward for further analysis. Thirty-five compounds depicted 0 violation, whereas 23 compounds satisfied the criterion with 1 violation.

Table 2 Screening of phytoconstituents based on Lipinski's rule

Phytocompound	LogP	MW	Vol	nHA	nHD	LV
(+)-Lavandulol acetate	3.85	196.15	225.779	2	0	0
1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene	1.903	208.11	223.377	3	0	0
1,3- Dicyclohexylurea	3.158	224.19	244.439	3	2	0
13-Docosenamide, [z]-	8.263	337.33	403.582	2	2	1
13-Hydroxydocosanoic acid	3.922	273.27	313.869	3	4	0
1-Hexadecanol	6.773	242.26	294.082	1	1	1
1-Hexadecyl-2-Omethyl-glycerol	6.388	330.31	380.847	3	1	0
2,4,4-Trimethoxy-2,5-cyclohexadien-1-one	0.145	184.07	182.915	4	0	0
2-Acetyl-1-oleoylsn-glycerol	6.59	398.3	442.406	5	1	0
2E,4Z,6Z,8ZDecatetraenedioic acid	1.856	194.06	200.858	4	2	0
2-Hydroxymyristic acid	4.031	244.2	274.434	3	2	0
3-Carene	4.36	136.13	161.767	0	0	1
3-Eicosyne	8.754	278.3	349.203	0	0	1
4-Hydroxy lauric acid	3.085	216.17	239.843	3	2	0
4-Hydroxy palmitic acid	4.845	272.24	309.026	3	2	0
4-Methoxycinnamic acid	2.406	178.06	186.148	3	1	0
6-Methylfuro[2,3-C]pyrid-5-one	0.589	149.05	147.843	3	0	0
6-Octen-1-ol, 3,7-dimethyl-, propanoate	4.472	212.18	245.712	2	0	0
8-Methyl-alpha-ionone	3.844	206.17	243.025	1	0	0
9,12,15-Octadecatrienoic acid	6.156	278.22	326.919	2	1	1
9,12,15-Octadecatrienoic acid, 2,3-dihydroxy-propyl ester, [Z,Z,Z]	5.38	352.26	396.387	4	2	0
9-Hexacosene	11.891	364.41	455.616	0	0	1
9-Octadecenamide,[Z]	6.443	281.27	334.398	2	2	C
alpha-Amyrin	7.352	426.39	490.807	1	1	1
Ambelline	0.993	331.14	321.504	6	1	0
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy- methyl ester	4.616	292.2	327.153	3	1	0
beta-Methoxycinnamic acid	2.207	178.06	186.148	3	1	0
C16 Sphinganine	3.922	273.27	313.869	3	4	0
C8- Dihydroceramide	7.684	427.4	492.983	4	3	1
Calcifediol	5.126	400.33	459.55	2	2	1

Phytocompound	LogP	MW	Vol	nHA	nHD	LV
Caryophyllene	5.906	204.19	245.61	0	0	1
Cholest-5-ene, 3-bromo-(3beta)	8.476	448.27	457.969	0	0	1
Cis-2-methoxycinnamic acid	2.36	178.06	186.148	3	1	0
Cyclopropane, nonyl-	6.229	168.19	207.552	0	0	1
Dibutyl phthalate	4.337	278.15	298.714	4	0	0
Diisooctyl phthalate	7.494	390.28	437.082	4	0	1
DL-alpha tocopherol	9.624	430.38	502.698	2	1	1
Eicosane, 7-hexyl	11.971	366.42	458.252	0	0	1
Ethyl iso-allocholate	2.981	436.32	465.341	5	3	0
Heneicosanoic acid, 18-propyl, methyl ester	10.08	382.38	455.9	2	0	1
Heptacosane, 1-chloro	12.439	414.4	490.759	0	0	1
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	5.275	330.28	369.705	4	2	0
Hexadecanoic acid, ethyl ester	7.448	284.27	334.828	2	0	1
Methyl p-methoxycinnamate	2.688	192.08	203.444	3	0	0
N-(3-Hydroxy-7Ztetradecenoyl)- homoserine lactone	3.551	325.23	349.576	5	2	0
N-Acetylsphingosine	4.461	341.29	386.571	4	3	0
Patchouli alcohol	3.878	222.2	251.117	1	1	0
Phenol, p-tert-butyl	2.851	193.11	208.287	3	2	0
Phenylacetaldehyde	1.155	120.06	136.612	1	0	0
Phthalic acid, butyl isohexyl ester	5.075	306.18	333.306	4	0	0
Phytol	7.764	296.31	360.63	1	1	1
Phytol, acetate	8.311	338.32	401.376	2	0	1
Precocene I	3.718	190.1	206.03	2	0	0
Squalene	12.248	410.39	511.617	0	0	1
Stigmasterol	7.436	412.37	479.432	1	1	1
Terephthalic acid	1.89	166.03	160.346	4	2	0
Trans-2-hydroxycinnamic acid, methyl ether, methyl ester	2.627	192.08	203.444	3	0	0
Trans-cinnamic acid	2.363	148.05	160.062	2	1	0
Trichloroacetic acid, tetradecyl ester	7.742	358.12	345.869	2	0	1

MW: Molecular weight; nH-A: H-bone acceptor; nH-D: H-bone donor; LV: Lipinski violations

Molecular Docking Analysis

Molecular docking plays a vital role as a computational tool in the field of drug discovery. It was performed on ligands that had undergone the pre-screening. In the current study, the 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 benzenepropanoic acid 35-bis(11-dimethylethyl)-4-hydroxy- methyl ester and cholest-5-ene 3-bromo-(3beta) depicted same highest binding affinity of -10.2 Kcal/mol for the targeted protein, however both failed at forming any hydrogen bonds.

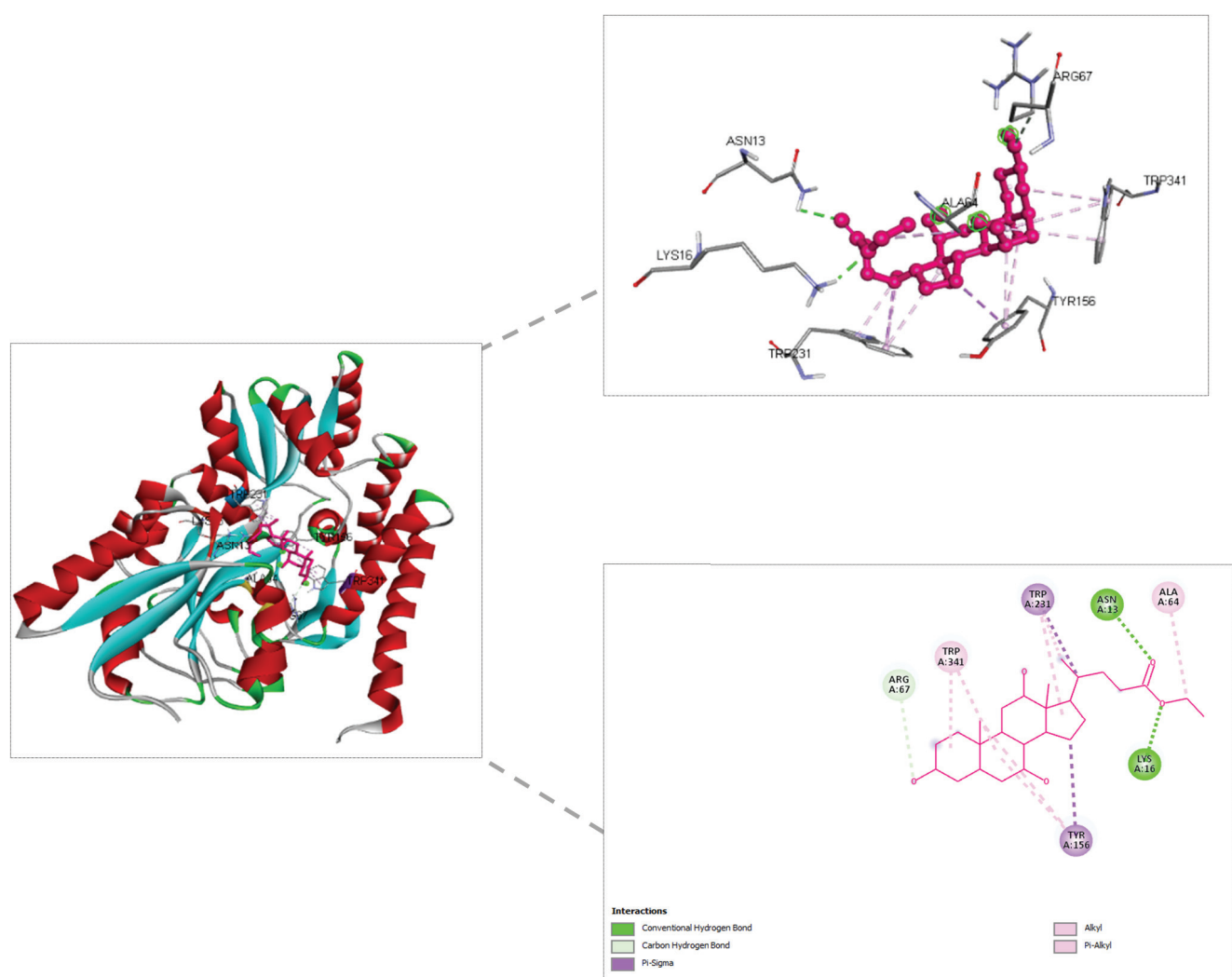
Furthermore, stigmasterol and calcifediol managed to show binding energies of -9.5 and -9.3 Kcal/mol, respectively but both also showed one Lipinski's violation. In addition, ethyl iso-allocholate displayed binding affinity of -9 Kcal/mol by forming hydrogen bond interactions with residues ASN A: 13 and LYS A:16 (Fig. 2). Also, ethyl iso-allocholate did not show any violation for Lipinski's rule. Moreover, ethyl iso-allocholate is a steroidal compound possessing numerous bioactivities such as, anti-inflammatory, antimicrobial, antiasthma, anti-arthritic, anticancer and is a diuretic (Javaid et al., 2020; Tyagi and Agarwal, 2017). Based on these findings, ethyl iso-allocholate was selected for further evaluation of additional drug-likeness parameters.

Table 3 Molecular docking results of top 10 phytoconstituents

Phytoconstituents	Binding Affinity (kcal/mol)
Benzenepropanoic acid 35-bis(11-dimethylethyl)-4-hydroxy- methyl ester	-10.2
Cholest-5-ene 3-bromo-(3beta)	-10.2
Stigmasterol	-9.5
Calcifediol	-9.3
Ethyl iso-allocholate	-9
Ambelline	-7.9
DL-alpha tocopherol	-7.4
Squalene	-7.3
Caryophyllene	-6.9
Dibutyl phthalate	-6.9

Table 4 Hydrogen bond interactions formed by top 5 compounds

Phytoconstituent	H-Bonds
Benzenepropanoic acid 35-bis(11-dimethylethyl)-4-hydroxy- methyl ester	-
Cholest-5-ene 3-bromo-(3beta)	-
Stigmasterol	TYR A:211
Calcifediol	ARG A: 67; GLU A: 112
Ethyl iso-allocholate	ASN A: 13; LYS A:16

**Fig. 2** Illustration of HPV E6 oncoprotein with ethyl iso-allocholate

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 the candidate with the best binding affinity *i.e.* ethyl iso-allocholate. 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 favourable oral bioavailability (Daina et al., 2017). As shown in Fig. 3, ethyl iso-allocholate showed all the parameters within the prescribed range. Overall, the compound depicted acceptable bioavailability parameters with a score of 0.55.

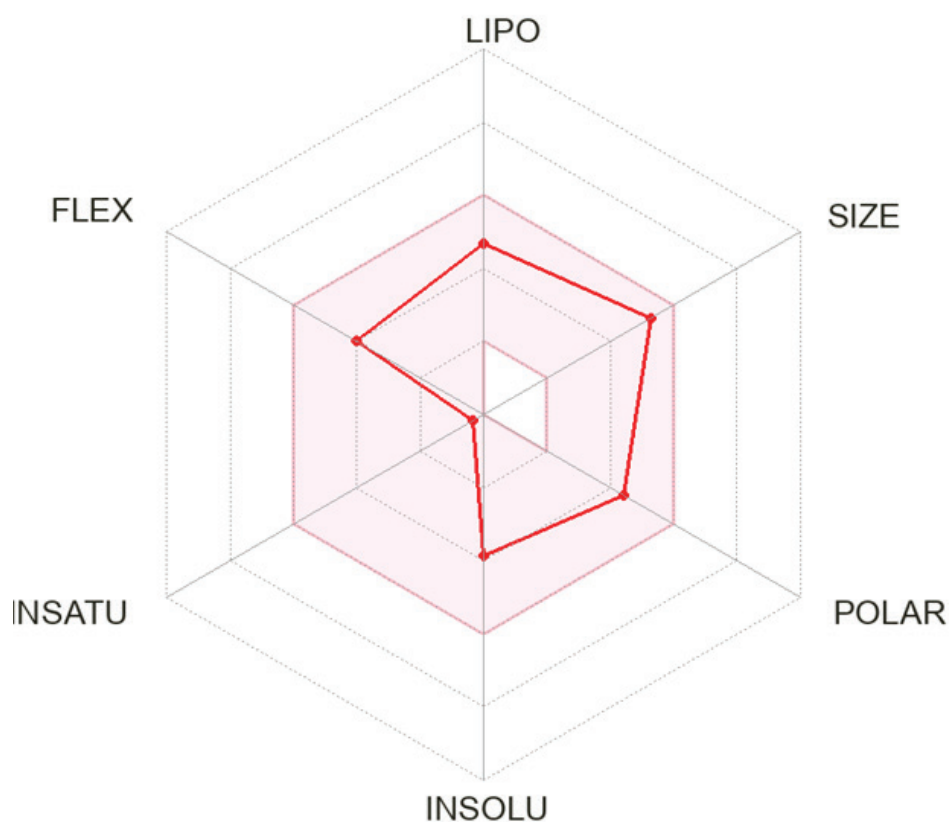


Fig. 3 Bioavailability radar diagram for ethyl iso-allocholate

In addition, the bioactivity scores for ethyl iso-allocholate 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). It was found that ethyl iso-allocholate possessed good nuclear receptor ligand, enzyme inhibition properties, ion channel modulation, protease inhibition and GPCR ligand activity (Fig. 4).

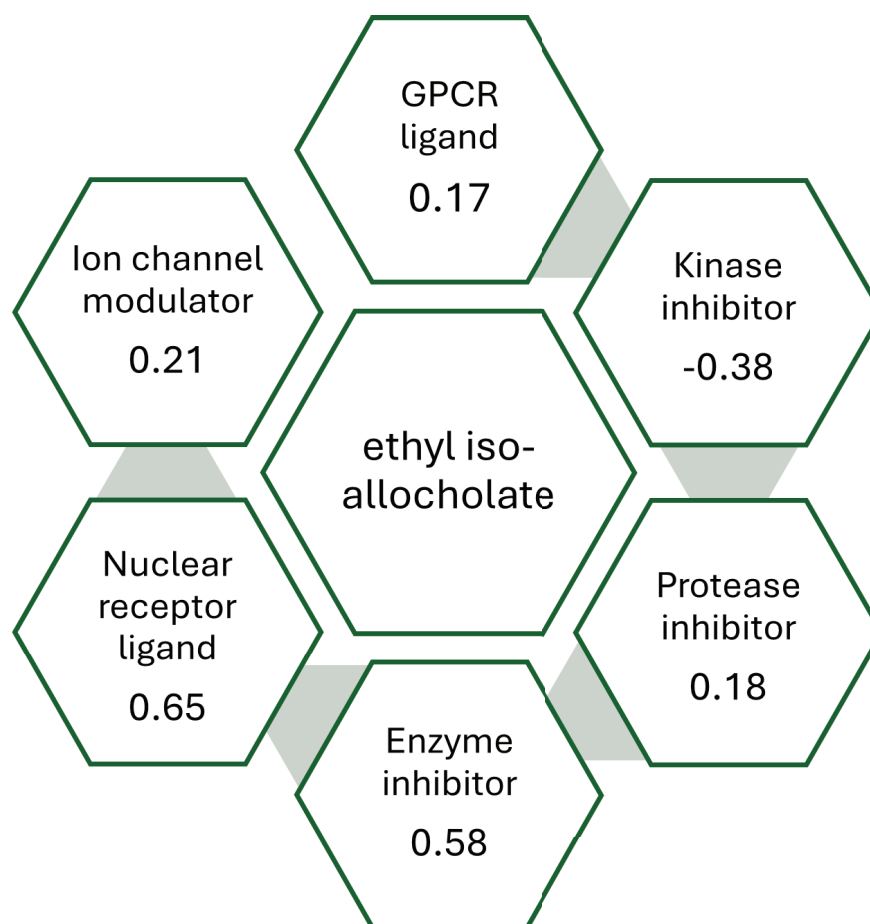


Fig. 4: Bioactivity scores of ethyl iso-allocholate

***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 favourable 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 ethyl iso-allocholate 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. All the parameters under absorption, including Caco-2 permeability (Caco-2), MDCK permeability (MDCK), probability of being Pgp-substrate, human intestinal absorption (HIA), human oral bioavailability 20% ($F_{20\%}$) and human oral bioavailability 30% ($F_{30\%}$)

except for P-glycoprotein inhibition were found to be excellent. P-glycoprotein (P-gp) is a transmembrane efflux pump expressed in various tissues throughout the body, including the intestinal epithelium and the blood-brain barrier. It plays a critical role in limiting drug absorption and distribution by actively transporting xenobiotics, including many therapeutic drugs, out of cells. Whereas a P-gp substrate is a molecule recognized and actively transported by P-gp. This leads to reduced intracellular drug accumulation at its site of action, potentially compromising its therapeutic efficacy (Seelig, 2020). Similarly, the compound depicted acceptable distribution and metabolism characteristics. VD_{ss} represents the theoretical volume in which a drug would need to be uniformly distributed to achieve its observed plasma concentration. A low VD_{ss} indicates high concentration in blood plasma, likely due to high plasma protein binding or water solubility. A high VD_{ss} suggests extensive tissue distribution, possibly due to high lipid solubility or tissue binding (Smith et al., 2015).

In silico models can predict the likelihood of a drug crossing the BBB based on its properties. A drug that readily crosses the BBB is more likely to reach its target in the central nervous system but may also lead to unwanted central nervous system side effects (Pandit et al., 2020). PPB refers to the fraction of a drug bound to plasma proteins like albumin, with only the unbound drug being pharmacologically active, meaning a high PPB results in less free drug available to exert its effect and may lead to a longer duration of action. In addition, FU is the unbound fraction of the drug in plasma, representing the pharmacologically active portion (Di, 2021). Furthermore, the compound exhibited a high clearance, but the half-life was found to be short. Lastly, the compound did not express AMES toxicity, rat oral acute toxicity (ROA), skin sensitivity (SkinSen), carcinogenicity, eye irritation (EC) and eye corrosion (EI). However, the compound showed respiratory toxicity and maximum recommended daily dose value (FDAMDD) was found to be poor.

Table 5 *In silico* ADMET analysis of ethyl iso-allocholate

	Property	Ethyl iso-allocholate
Absorption	Pgp-inh	0.862
	Pgp-sub	0.145
	HIA	0.014
	F(20%)	0.005
	F(30%)	0.109
	Caco-2	-4.751
	MDCK	0.000106477

		Property	Ethyl iso-allocholate
	Distribu- tion	BBB	0.742
		PPB	82.44%
		VDss	0.974
		Fu	7.58%
	Metabo- lism	CYP1A2-inh	0.025
		CYP1A2-sub	0.36
		CYP2C19-inh	0.017
		CYP2C19-sub	0.761
		CYP2C9-inh	0.065
		CYP2C9-sub	0.42
		CYP2D6-inh	0
		CYP2D6-sub	0.28
		CYP3A4-inh	0.131
		CYP3A4-sub	0.362
	Excretion	CL	17.353
		T12	0.2
	Toxicity	Ames	0.011
		ROA	0.105
		FDAMDD	0.869
		SkinSen	0.177
		Carcinogenicity	0.014
		EC	0.003
		EI	0.008
		Respiratory	0.835

Molecular Dynamics Simulations

On the basis of molecular docking, bioavailability, bioactivity, and ADMET analysis, ethyl iso-allocholate fulfilled all the parameters and was therefore, subjected to

molecular dynamics simulations for 100 ns. The dynamic behaviours or molecular stability of ethyl iso-allocholate-HPV E6 oncoprotein complex was observed through root-mean square deviation (RMSD), root mean square fluctuation (RMSF), protein-ligand interaction analysis, and Ligand torsion analysis.

RMSD of Ethyl Iso-allocholate-HPV E6 Oncoprotein Complex

RMSD analysis gives an 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-ethyl iso-allocholate complex. As clear from the figure, after fluctuating during an initial 18.30 ns, the c-alpha RMSD attained equilibrium from 18.70 ns until the end of the 100 ns simulation. In addition, the

ethyl iso-allocholate fit protein depicted an initial stabilization up to 17.40 ns, post which it fluctuated up to 17.20 ns. Further, the trajectory of ethyl iso-allocholate fit protein gained stability up to 38.60 ns, followed by minor fluctuations up to 45.40 ns. Finally, the trajectory attained equilibrium from 45.70 ns until the end of the simulation period. Also, the perfectly overlapped trajectories from 45.70 to 100 ns points towards formation of a stable complex.

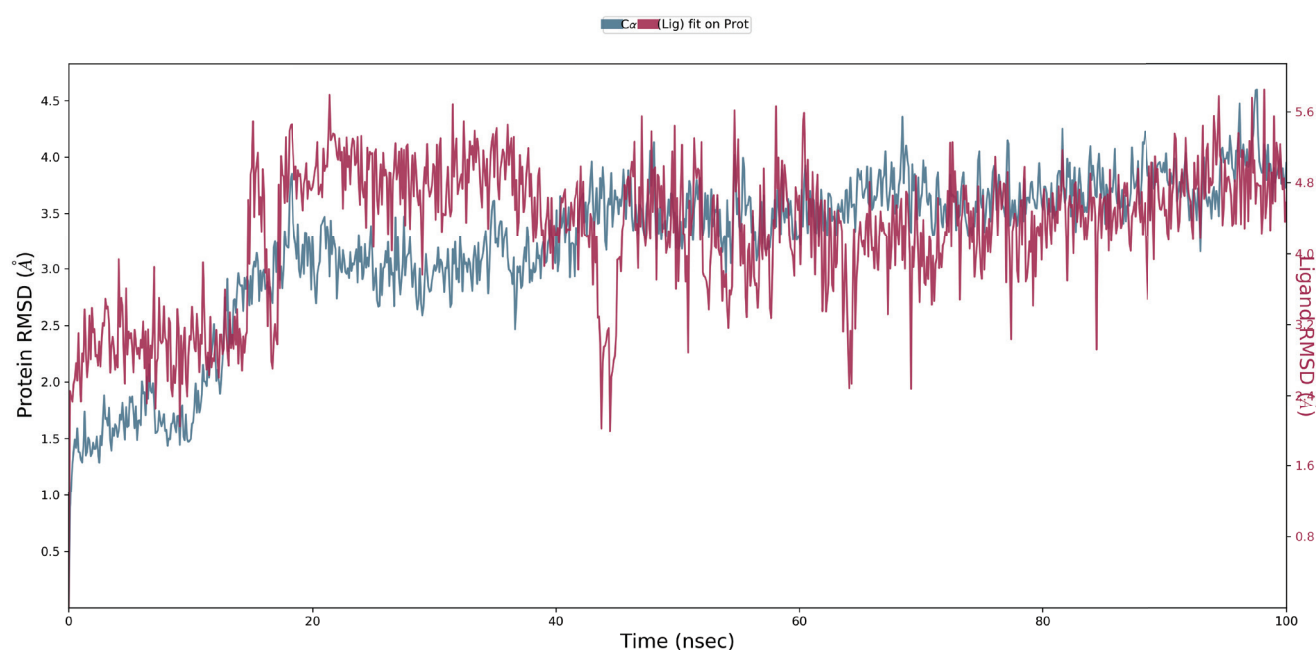


Fig. 5 RMSD Plot of ethyl iso-allocholate-HPV E6 oncoprotein complex

RMSF of HPV E6 Oncoprotein

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-coloured vertical bars. Generally, RMSF value >2.5 Å points towards less stable bonds (Fatriansyah et al., 2022). The protein RMSF

graph depicts six peaks, occupied by GLY 175, ASP 208, TYR 211, GLU 215, GLN 376 and GLY 380. In addition, the residues responsible for

making protein-ligand contacts showed RMSF values within the permissible range, depicting stable bonds.

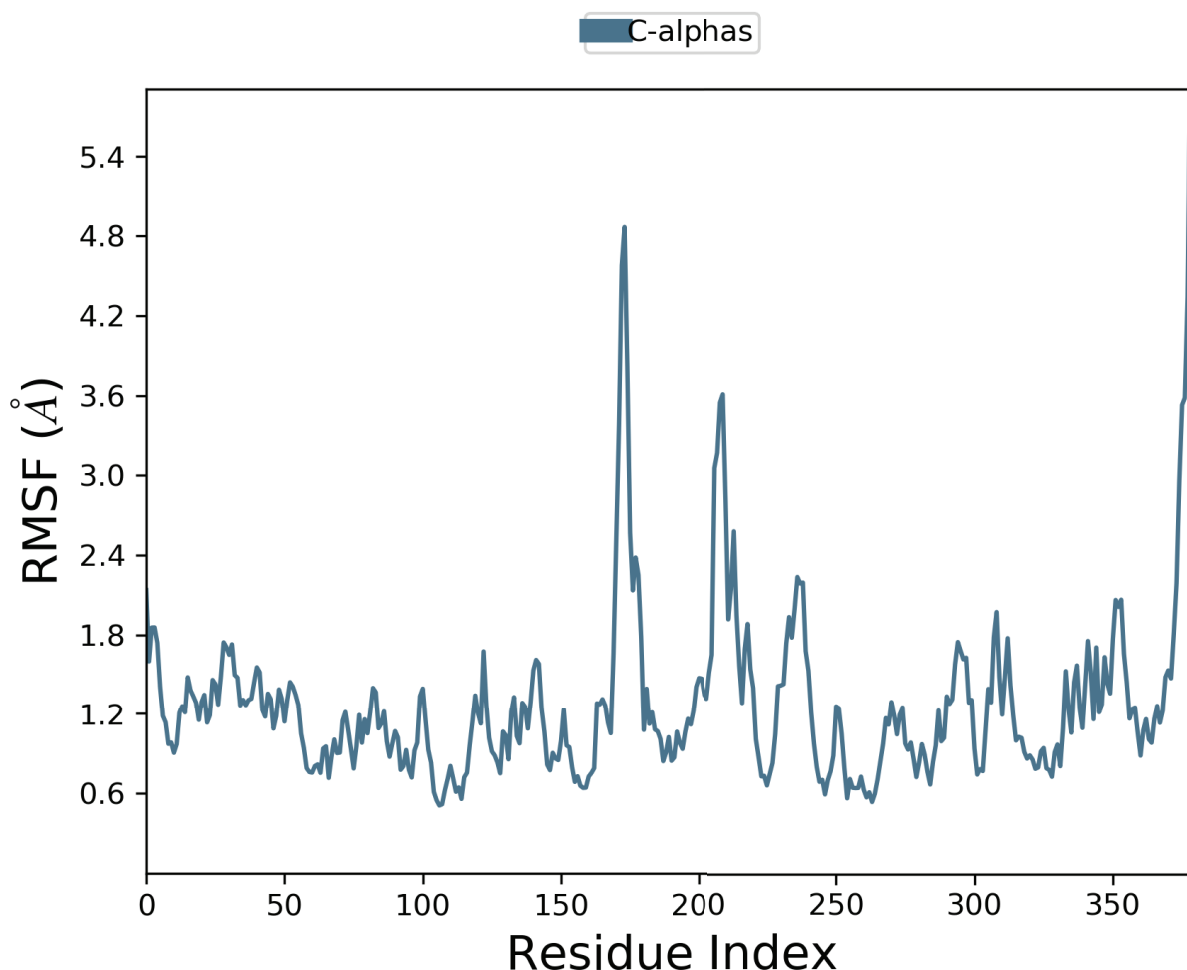


Fig. 6 RMSF Plot of HPV E6 oncoprotein

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 important due to their strong influence on drug specificity. As shown in the figure, the majority of the significant ligand-protein interactions determined with MD are hydrogen bonds

and hydrophobic interactions. The majority of the interactions formed in the complex were water bridges and hydrophobic bonds. In terms of hydrogen bonds interaction, GLU 154 formed the strongest hydrogen bond with interaction fraction of 0.316. Apart from that ASP 66, ARG 345, TRP 341, ARG 67 and ALA 64 also managed to formed hydrogen bonds to some extent.

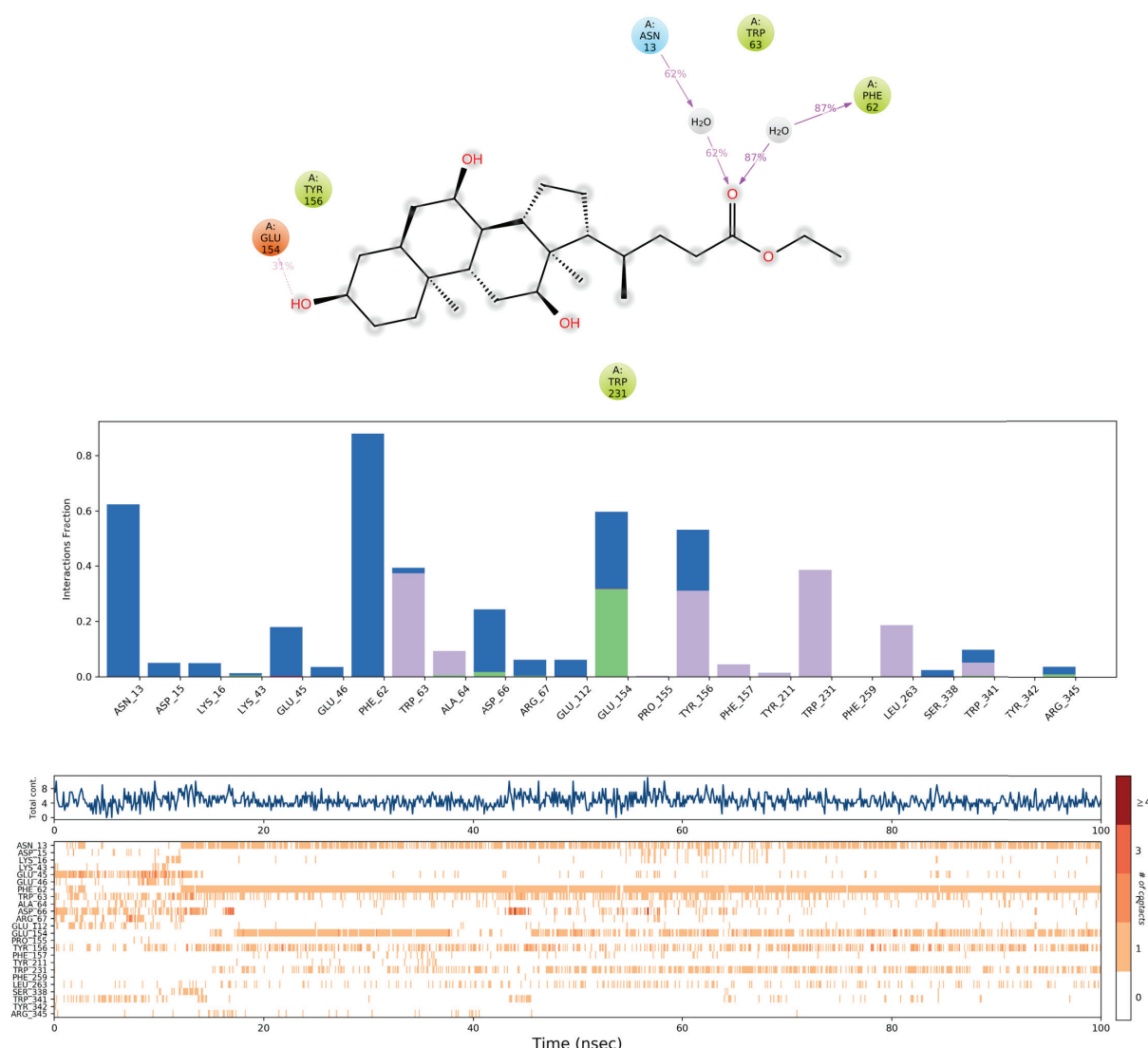


Fig. 7 Protein-ligand interactions of ethyl iso-allocholate-HPV E6 oncoprotein complex

Ligand Torsion Analysis

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

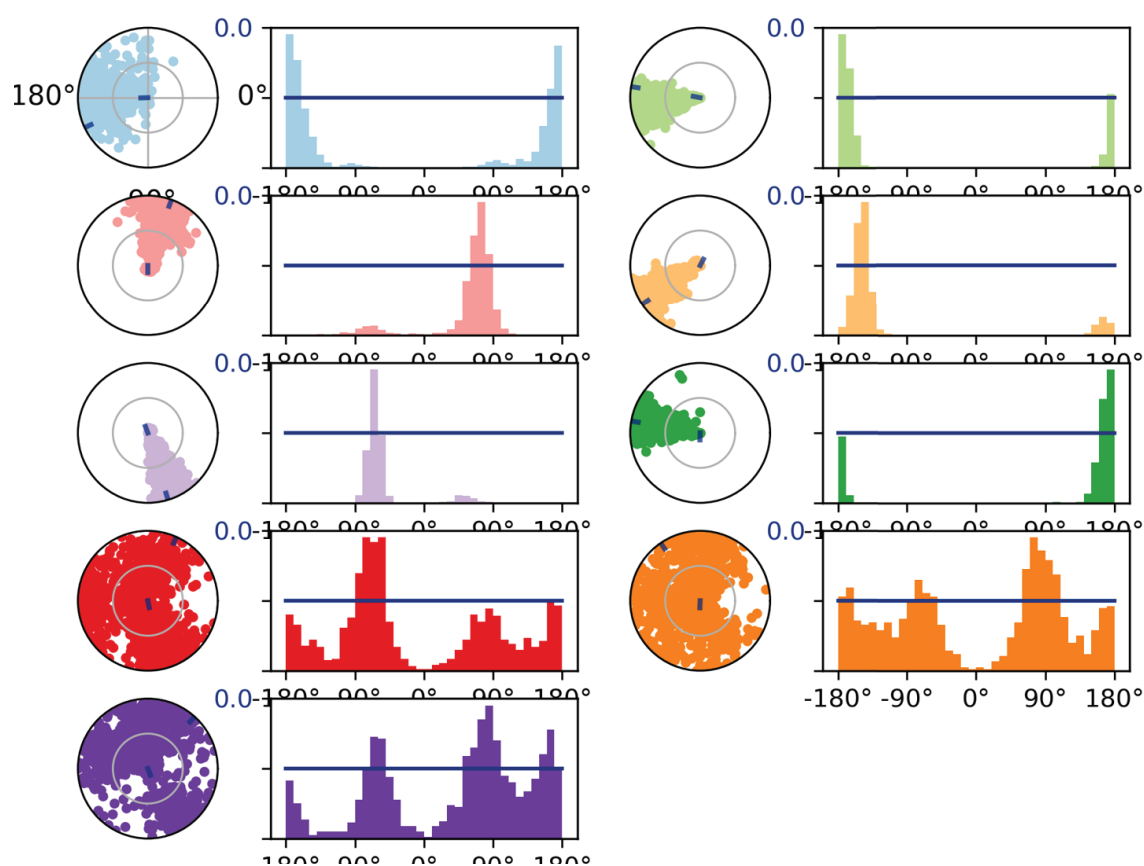
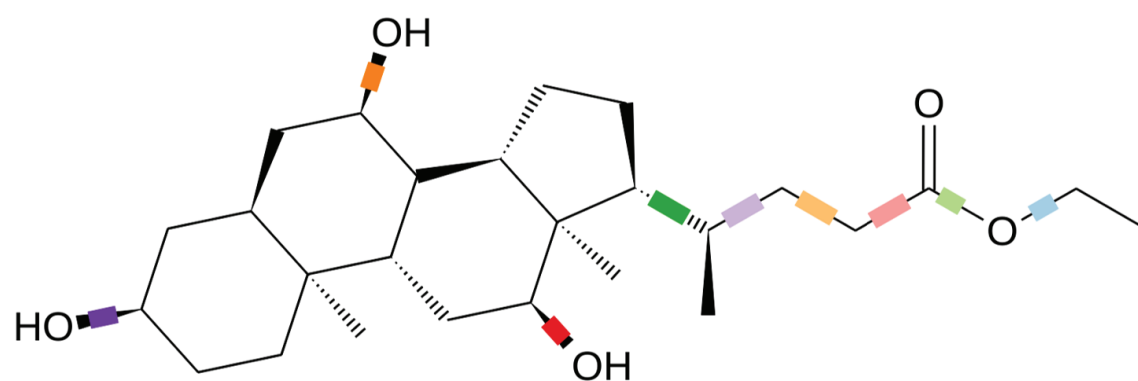


Fig. 8 Ligand torsion analysis of ethyl iso-allocholate

Conclusion

In conclusion, this comprehensive computational study has unveiled the substantial potential of bioactive phytoconstituents derived from the medicinal plant *A. aspera* in targeting the HPV E6 oncoprotein. Through the adept utilization of virtual screening, encompassing molecular docking analysis and molecular dynamics simulations, we have provided evidence of the therapeutic promise of ethyl iso-allocholate in combating CC. Our molecular docking analysis has underscored the robust binding affinity of ethyl iso-allocholate with the target protein, indicating its potential as an inhibitor for E6 oncoprotein-mediated CC progression. Furthermore, ethyl iso-allocholate exhibited favourable 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 ethyl iso-allocholate-E6 oncoprotein complex, shedding light on their potential as an effective therapeutic strategy against CC. In essence, this research has shed light on the promising potential of ethyl iso-allocholate as a natural therapeutic agent targeting E6 oncoprotein, a pivotal player in the pathogenesis of CC. These findings provide a solid foundation for future investigations and the development of targeted therapies for CC, fostering optimism for improved treatment options and enhanced outcomes for patients afflicted with this disease of concern.

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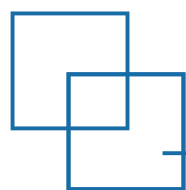
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CHAPTER 07

Reported Pharmacological Profile



INTRODUCTION

Achyranthes aspera L. and other members of the genus *Achyranthes* are credited with a range of biological activities. Pharmacological investigations carried out on *A. aspera*, and related species confirmed the therapeutic potential of these plants and their importance in traditional, classical, and modern medicine. *A. aspera* and other members of the genus possess anthelmintic, anti-arthritic, anti-biofilm, anti-nociceptive, anti-cancer, anti-diabetic, anti-fertility, anti-inflammatory, anti-microbial, anti-obesity, anti-oxidant, anti-pyretic, anti-ulcer, anti-urolithiatic, anti-viral, anxiolytic, bronchoprotective, cardiovascular system (CVS) protective, central nervous system (CNS) depressant, diuretic, effect on eyes, effect on reproductive system, enzymatic, gastroprotective, hepatoprotective, larvicidal, nephroprotective, and wound healing activities (Saini, 2016). They also act against lead acetate-induced nephrotoxicity. A brief review on the biological activity and therapeutic potential of *A. aspera* and other important members of the genus *Achyranthes* is presented in this chapter.

Pre-clinical Study

A. aspera is a medicinal plant that has been traditionally used in various systems of medicine, including Ayurveda and traditional Chinese medicine. It has a wide range of potential therapeutic properties and has been the subject of several preclinical studies. Pre-clinical studies provide valuable insights into the potential therapeutic properties of a substance. However, further research, including clinical trials in humans, is necessary to determine its safety and efficacy in medical applications.

Anthelmintic Activity

Ndhala et al. (2015) examined the anthelmintic activity of water and acetone extracts of *A. aspera* leaves collected from two different geographical locations (Ukulinga, South Africa and Ciaat, Eritria) against free-living transparent nematode *Caenorhabditis elegans*. Both extracts exhibited anthelmintic effect with MIC values of 1.17, 0.59 and 0.59, 0.29 mg/ml for *C. elegans*, as comparable to levamisole used as positive control (0.042 mg/ml).



Anti-arthritic Activity

Chinnasamy et al. (2019) reported the anti-arthritic activity of aqueous extract of *A. aspera* leaves against formaldehyde-induced arthritis in Wistar rats. The oral administration of extract exhibited significant ($p < 0.01$) and dose-dependent protection for formaldehyde-induced arthritis. At 21st day, the extract at 250 and 500 mg/kg doses showed inhibition of paw volume by 30 and 38.33%, respectively, while joint swelling by 27.2 and 36.36% on 14th day when compared to diclofenac sodium (10 mg/kg), which showed 36.61% inhibition for arthritis and joint swelling at 21st and 14th day.

Kothavade et al. (2015) investigated the anti-arthritic activity of saponin rich (SR; 50 and 100 mg/kg) fraction of *A. aspera* against adjuvant-induced arthritic rats. Treatment with fraction suppressed paw swelling, arthritic score and improved the pain threshold in motility and stair climbing tests. The fraction exerts modulation of SOD, CAT, GSH, MDA, and NO levels, whereas the fraction also decreased plasma levels of tumor necrosis factor- α and interleukin-6. There was a significant reduction in synovial hyperplasia, inflammatory cell infiltration, and bone destruction in the joints confirmed in histopathological study. Thus, therapeutic benefit of saponin rich fraction of plant could be beneficial in suppressing the progression of adjuvant-induced arthritis.

Anti-biofilm Activity

Murugan et al. (2012) reported the petroleum ether, benzene, methanol, aqueous extracts

of *A. aspera* for anti-biofilm activity against cariogenic isolate *Streptococcus mutans* by agar well diffusion assay. All extracts showed anti-biofilm effect with MIC values ranging from 62.5 to 500 mg/ml; whereas methanol extract exhibit minimum value of 125 mg/ml when compared with chlorhexidine and triclosan as 2 and 6.25 mg/ml, respectively for *S. mutans*.

Anti-cancer Activity

Singh et al. (2021) evaluated the anti-cancer activity of methanolic extract from *A. aspera* leaves on Dalton's Lymphoma (DL) in BALB/c mice (*in vitro*, *in vivo*). The extract suppressed cell proliferation, decreased mitochondrial membrane potential, changed morphological structure, and induced apoptosis. The extract promotes the release of cytochrome c by regulating Bcl-2 family proteins and then activated caspase-9/-3 to trigger cell apoptosis as well as inhibited protein kinase Ca (PKCa) pathway. Remarkably, for *in vivo*, the extract mediated suppression of DL growth in BALB/c mice was accompanied by attenuation of the PKCa pathway and induction of apoptosis. Thus, extract promotes mitochondrial apoptotic cascade in DL cells by suppressing the PKCa signaling pathway.

Subbarayan et al. (2012) reported the anti-cancer activity of methanol extract (50, 100 and 200 mg/kg) from the leaves of *A. aspera* against human pancreatic tumor growth in athymic mice by apoptosis. The extract showed no adverse reactions in treated mice. The extract reduced both

tumor weight and volume in tumor bearing mice and, significantly induced ($p < 0.001$) caspase-3 mRNA and, suppressed ($p < 0.05$) expression of the pro survival kinase Akt-1. TUNEL assay and immunohistochemistry study confirmed apoptosis induction by activation of caspase-3 and inhibiting Akt phosphorylation in treated mice.

Arora and Tandon (2014) examined the anti-cancer effect of ethanol and aqueous extracts of *A. aspera* on the growth of colon (COLO-205) cancer cell using MTT assay. Treatment with ethanol extract at 200 $\mu\text{g/ml}$ significantly decreased ($p < 0.01$ and $p < 0.001$) cell viability at 48 and 72 hrs, respectively. The aqueous extract showed more cytotoxic effect with IC_{50} of 165.7 $\mu\text{g/ml}$ towards tested cells, as compared to ethanol extract that was 184.1 $\mu\text{g/ml}$. Further, the aqueous extract markedly increased mRNA expression levels of caspase-9, caspase-3, Bax, p16, p21, along with decreased Bcl-2 expression. The cell cycle arrest at S phase was detected by flow cytometric analysis after treatment with aqueous extract.

Subbarayan et al. (2010) performed the anti-cancer activity of methanol extract (28, 33 and 42 g/ml) of *A. aspera* leaves against human pancreatic Panc (10.05), cancer cell line by clonogenic cell survival assay. The extract at 28, 33 or 42 $\mu\text{g/ml}$ showed the survival rate of 38, 11 and 2.5%, respectively, at 2 hrs., and IC_{50} value of 22 $\mu\text{g/ml}$ for Panc (10.05). Further, the extract suppressed the transcription of metalloproteases (MMP-1 and -2), inhibitors of MMPs (TIMP-2) and angiogenic factors (VEGF-A and VEGF-B).

Anti-diabetic Activity

Kumaran (2018) examined the anti-diabetic activity of aqueous-ethanolic extract (300 and 600 mg/kg, b.w.) of *A. aspera* seeds against streptozotocin (STZ)-induced diabetes in albino Wistar rats. Treatment with extract showed an increase in blood glucose, hemoglobin, and insulin levels. The extract significantly reduced total cholesterol and serum triglycerides levels, low-density lipoprotein (LDL), and very LDL, and the high-density lipoprotein level was significantly increased. There was a significant decrease in levels of superoxide dismutase and glutathione (GSH) peroxidase and, an increase in the levels of vitamin E, catalase, and reduced GSH in treated rats.

Talukder et al. (2012) reported the anti-diabetic activity of ethanolic extract (200 and 400 mg/kg, b.w.) of *A. aspera* against alloxan-induced diabetic albino Swiss mice. In oral tolerance test, extract exhibit dose-dependent decrease in plasma glucose level of normal mice. The extract at 400 mg/kg reduced elevated glucose level in the second week. Treatment with extract significantly decreased the level of lipid peroxidation products (TBARS and hydroperoxide), NO levels and enzyme catalase. Thus, plant showed significant anti-hyperglycemic effect in alloxan-treated mice that may be mediated by diminished oxidative stress.

Kumar A et al. (2011) evaluated the anti-diabetic activity of ethanolic extract of *A. aspera* leaves against streptozotocin (STZ)-induced diabetic albino rats. The ethanol extract attained significant ($p < 0.01$)

reduction in blood glucose level in STZ-induced diabetic rats.

Anti-inflammatory, Anti-nociceptive and Anti-pyretic Activities

Viswanatha et al. (2019) reported the anti-inflammatory effect of methanol extract of *A. aspera* aerial parts on lipopolysaccharide-induced generation of reactive oxygen species (ROS), nitrite and tumor necrosis factor- α in RAW 264.7 cell lines. The cytotoxicity was found very low with IC_{50} of 1914.2 μ g/ml by extract using MTT assay. The extract exhibited moderate inhibition with values of 201.58, 201.33 and 375.21 μ g/ml for generation of ROS, nitrite and TNF- α in LPS-activated RAW 264.7 cell lines, respectively, when compared with DPI and L-NAME used as reference drugs and attained good inhibition (4.62 and 44.59 μ g/ml, respectively) for ROS and nitrite generation.

Mengie et al. (2021) examined the *in vivo* anti-inflammatory study of aqueous, n-butanol and chloroform fractions of 80% methanol leaves extract (100, 200 and 400 mg/kg) of *A. aspera* on carrageenan-induced paw edema in albino Wistar rats. Among the tested fractions, the chloroform fraction at dose of 400 mg/kg showed highest percentage edema inhibition with 52.50% at 4 hrs., which was comparable to indomethacin (53.33%) used as standard drug for tested model. Further, the chloroform and n-butanol fractions at all tested doses also significantly ($p < 0.01$) inhibited the formation of inflammatory exudate and granuloma mass.

Vetrichelvan and Jegadeesan (2003) evaluated the anti-inflammatory activity of an alcohol extract (125, 250, 375 and 500 mg/kg) of *A. aspera* on carrageenan-induced hind paw edema and cotton pellet granuloma models in male albino rats. Treatment with extract at 375 and 500 mg/kg doses showed the maximum edema inhibition with values of 65.38 and 72.37% at 3 hr. for carrageenan-induced rat paw edema when compared with diclofenac sodium (60.87%) used as standard drug. In chronic test, the extract exhibited 40.03 and 45.32% reduction in granuloma weight of rats as compared to diclofenac sodium (53.21%).

Barua et al. (2010) investigated the anti-nociceptive activity of methanolic extract (300, 600 and 900 mg/kg) from leaves of *A. aspera* against acetic acid-induced writhing syndrome test, central/narcotic models like hot plate and tail flick tests in albino Swiss mice. The extract showed anti-nociceptive effect with dose-dependent reduction in writhing number ranging from 9.49 to 17.37% for acetic acid-induced writhing syndrome test. In addition, the extract exerts significant ($p < 0.01$) increase in reaction time for hot plate test as well as, 30 to 60 min. in tail flick tests.

Uma et al. (2010) reported the anti-nociceptive activity of ethanol extract at dose of 400 mg/kg from leaves of *A. aspera* using tail flick, hot plate, and formalin-induced pain tests in albino Wistar mice. Treatment with extract at tested dose reduced the formalin-induced pain in both phases (*i.e.* neurogenic and inflammatory) by 58.8 and 92.7%, respectively. In tail flick and hot

plate tests, the extract exerts significant analgesic effect at 30, 60, 90 and 120 min. when compared to control.

Goli et al. (2011) performed the anti-pyretic activity of methanol extract at doses of 100 and 200 mg/kg of *A. aspera* leaves against Brewer's yeast-induced pyrexia in albino Wistar rats. Treatment with extract at tested doses exert highly significant ($p < 0.01$) effect in maintaining normal body temperature and reducing yeast-induced elevated body temperature in rats.

Anti-microbial and Anti-viral Activities

Yadav et al. (2016) screened the anti-bacterial activity of aqueous extract of the *A. aspera* roots and stems (2.5, 5, 7.5 and 10%) towards *Streptococcus mutans* using cup plate assay. Both extracts exhibited maximum inhibition zone values of 16 and 21 mm at 10% and 250 μ l volumes of the extract against *S. mutans*.

Kaur et al. (2005) studied the anti-microbial activity of chloroform, methanol, aqueous and petroleum ether extracts of root and shoot of *A. aspera* against *Bacillus subtilis*, *Klebsiella* sp., *Pencillium*, *Phytophthora*, *Sclerouum* sp., and *Fusarium* sp. The chloroform and methanol extracts showed good antibacterial effect with zone inhibitions ranging from 9 to 16 mm against *Klebsiella* sp. while, petroleum ether root extract exert inhibitory effect against *B. subtilis*. Different concentrations (50-200 μ g)/100 μ l of all extracts attained antifungal effect ranging in between 9 to 13.33 mm for *Fusarium* sp. Further, the methanol and aqueous shoot extracts were found weakly

active against *Pencillium*, *Phytophthora* and *Sclerouum* sp.

Owk et al. (2014) reported the anti-microbial activity of chloroform, methanol, ethanol, and aqueous extracts from *A. aspera* roots against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptomyces pneumoniae*, *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* using agar dilution assay. All extracts exhibited moderate to good inhibitory effect with MIC values ranged from 62.5 to 1000 μ g for all tested microorganisms.

Ndhala et al. (2015) examined the anti-microbial potential of water and acetone extracts of *A. aspera* leaves collected from two different geographical locations (Ukulinga, South Africa and Ciaat, Eretria) against *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* by micro-dilution assay. Both extracts from Ciaat, Eretria exhibited good anti-microbial effect with MIC values ranging from 0.09 to 0.78 mg/ml towards all tested microorganisms except *E. coli*. Both extracts from Ukulinga, South Africa showed effectiveness with value of 0.78 mg/ml for *E. coli*, *K. pneumonia* and *C. albicans* and, other strains were found in between 1.17 to 6.15 mg/ml.

Mukherjee et al. (2013) evaluated the anti-viral activity of methanolic extract along with oleanolic acid of *A. aspera* roots against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) by MTT and plaque reduction assays. Both

extract and compound inhibited HSV and their plaque formation with EC_{50} values of 64.4 and 6.8 $\mu\text{g/ml}$ (HSV-1), and 72.8 and 7.8 $\mu\text{g/ml}$ (HSV-2), as compared to acyclovir (5 $\mu\text{g/ml}$) used as positive control (2.1 and 2.9 $\mu\text{g/ml}$). The extract (112.5 $\mu\text{g/ml}$) and compound (13.8 $\mu\text{g/ml}$) exhibited nearly 100% inhibition for HSV-1 virus. On the other hand, similar effect was observed with extract (120 $\mu\text{g/ml}$) and compound (14.5 $\mu\text{g/ml}$) towards HSV-2 virus. In plaque assay, compound was unable to inactivate the virus and there was no reduction of plaque count in cell when compared with control. Both extract and compound significantly induced level of pro-inflammatory cytokines IL-6 and IL-12 at 24 hrs. However, the extract and compound probably inhibited the early stage of multiplication (post infection of 2-6 h).

Anti-obesity Activity

Khan et al. (2015) investigated the anti-obesity activity of seed saponins (50, 75, 100, 125 and 150 mg/kg) from *A. aspera* on high cholesterol diet (HCD) fed albino rats. Treatment with saponins exert significant ($p < 0.05$) decrease of total cholesterol, total triglycerides, and LDL-C whilst, an increase in HDL-C level of treated rats was observed. The saponins also exert significant ($p < 0.01$) improvement of serum antioxidant status in treated rats. However, saponins showed mild hepatoprotective potential on hepatic functions of treated rats.

Rani et al. (2012) evaluated the anti-obesity effect of ethanol extract of *A. aspera* seeds (900 mg/kg) on pancreatic amylase, lipase (*in vitro*) and high-fat diet-induced obesity in

albino Swiss mice. Treatment with extract showed lipase inhibition with IC_{50} value of 2.34 mg/ml which was stronger than α -amylase (3.83 mg/ml) and, plasma triacylglycerol concentrations were significantly ($p < 0.01$) lower in mice. The extract also suppressed an increase in body, retroperitoneal adipose tissue, liver weights, and serum parameters, namely, total cholesterol, total triglyceride, and LDL-cholesterol level in mice.

Antioxidant Activity

Viswanatha et al. (2019) examined the antioxidant activity of methanol extract of *A. aspera* (aerial parts) through *in vitro* models such as DPPH, FRAP and ORAC methods. The extract exhibited good scavenging effect with IC_{50} value of 126.50 $\mu\text{g/ml}$ (DPPH); 759.65 mg of FeSO_4 equivalents/g (FRAP) and 979.4 Trolax equivalents/g (ORAC) assays, as compared to vitamin C (26.80 $\mu\text{g/ml}$ for DPPH, 1424.32 mg of FeSO_4 equivalents/g for FRAP and 3286.5 Trolax equivalents/g for ORAC).

Arora and Tandon (2014) screened the antioxidant activity of aqueous and ethanolic extracts of roots of *A. aspera* by DPPH and FRAP assays. In comparison to aqueous extract, the ethanol extract exhibited good antioxidant effect with FRAP value of 195 $\mu\text{M Fe (II)}$ /gram of dry extract and, DPPH scavenging effect with IC_{50} of 372.6 $\mu\text{g/ml}$, respectively.

Talukder et al. (2012) investigated the antioxidant activity of ethanol extract of *A. aspera* stem and leaves using DPPH, nitric oxide (NO) and hydrogen peroxide (H_2O_2)

radical scavenging assays. The extract exhibited radical scavenging effect with IC_{50} values of 243.7, 39 and 92 $\mu\text{g/ml}$ which was comparable to ascorbic acid used as positive control (55.9, 69.9 and 158.9 $\mu\text{g/ml}$) for DPPH, NO and H_2O_2 assays, respectively.

Anti-urolithiatic Activity

Sundararajan et al. (2022) evaluated the anti-urolithiatic activity of ethanolic extract of *A. aspera* seeds by inhibition of calcium oxalate crystallization. Treatment with extract showed 62% dissolution of calcium oxalate by titrimetric method. The extract exhibit calcium palat crystallization inhibition with 83% using turbidity method.

Aryal et al. (2019) investigated the anti-urolithiatic activity of methanol extract (0.5 and 1.0 mg/ml) of *A. aspera* against calcium oxalate (CaOX) crystals. The growth of CaOX was highly inhibited by 42.17% as compared to cystone (43.35%) used as standard. In addition, the extract at 1.0 mg/ml showed ~53 and ~58% reductions in nucleation and aggregation of CaOx crystals when compared to cystone (63.28%).

Diuretic Activity

Asif et al. (2014) evaluated the diuretic activity of crude aqueous extract (10, 30 and 50 mg/kg) of *A. aspera* (dried seeds) on excretion of urinary electrolytes in normal albino rats. The extract showed dose-dependent increase in urinary flow. The extract at tested doses exhibited diuretic

effect with 34, 40 and 46%, respectively in treated rats. There was a significant ($p < 0.001$) natriuretic effect observed. In addition, the extract increased excretion of urinary K^+ and saliuretic index values as well as, dose-dependent increase in the excretion of Na^+ and K^+ in the urine samples and, insignificant pH of fresh urine samples.

Effect on Eyes

Deshmukh et al. (2012) investigated the eye irritation potential of aqueous extract of *A. aspera* leaves, using Hen's Egg Chorioallantoic Membrane Test (HET-CAM) (*in vitro*) and acute eye irritation test (*in vivo*) in rabbits. Treatment with aqueous extract at 0.3 ml showed absolutely no effect of hemorrhage, lysis, or coagulation or not even the death of subject after 2 hrs in HET-CAM assay. The extract attained irritation score of ≤ 0.55 outcome and as a result did not qualify for category 1 and 2 of the classification criteria. In addition, the aqueous extract of plant was found to be non-irritant and did not exert any eye irritation potential.

Effect on Reproductive System

Sandhyakumary et al. (2002) screened the impact of feeding ethanolic extract of *A. aspera* roots on reproductive functions of male albino rats. The extract significantly reduced ($p < 0.05$) sperm counts, weight of epididymis, serum level of testosterone and testicular activity of 3β -hydroxysteriod dehydrogenase, whereas extract did not affect the motility of sperm and activity of HMG CoA reductase. Cholesterol

level in the testis, incorporation of labeled acetate into cholesterol, 17-ketosteroids in urine and hepatic and fecal bile acids were increased. The results suggest that ethanolic extract of *A. aspera* caused reproductive toxicity in male rats and the action may be by suppressing the synthesis of androgen.

Shibeshi et al. (2006) reported the anti-fertility effect of methanolic extract (1, 3, and 5.5 g/kg) of *A. aspera* leaves on fetal abortion, uterine and pituitary weights, serum lipids and hormones in female Wistar rats. The extract at dose of 5.5 g/kg significantly ($p < 0.05$) reduced survival of fetuses. Further, the extract at 3 g/kg did not show significant abortifacient effect. The extract significantly ($p < 0.05$) increased pituitary and uterine wet weight compared to control. The extract did not significantly influence serum concentration of the ovarian hormones and various lipids except significant hypolipidemic effects on high density lipoproteins (HDL).

Vasudeva and Sharma (2006) conducted the anti-fertility activity of ethanol extract of *A. aspera* root in proven fertile female albino Wistar rats. Treatment with extract at 200 mg/kg dose exhibited anti-implantation activity with 83.3% in treated rats. In addition, the extract attained an increase in uterine weight, diameter of uterus, thickness of endometrium and vaginal epithelial of treated rats.

Anuja et al. (2011) determined the spermicidal activity of 58 kDa *Achyranthes* protein (Ap) isolated from *A. aspera*, on human and rat sperm (*in vitro*). Protein (Ap) exerts significant reduction in sperm viability

and hypo-osmotic swelling. The number of acrosomes reacted cells were found to be high and, caused agglutination of the sperms. Also, a significant reduction in the activity of membrane bound 5' nucleotidase and acrosin enzyme observed. Thus, protein isolated from the roots of plants possess spermicidal property.

Teshome et al. (2021) evaluated the anti-fertility activity of ethanolic extract (250, 500 and 1000 mg/kg) of leaves of *A. aspera* on developmental toxicity of female albino Wistar rat embryos and fetuses. The extract at 1000 mg/kg significantly reduced the number of implantation sites, morphological score, and somites in day 12 embryos. Also, the number of branchial bars was significantly reduced at 1000 mg/kg while, retarded development of hindlimb, forelimb, optic and olfactory systems was detected at a high dose. In near-term fetuses, a significant reduction of litter weight and crown-rump length at 1000 mg/kg dose was observed. However, external anomalies were not detected for all offspring.

Enzyme-inhibitory Activity

Prakash and Sagar (2021) investigated the enzyme inhibitory effect of methanol, acetone, and aqueous extracts from leaves of *A. aspera* on α -amylase and urease enzymes inhibition. All extracts exhibited good inhibitory effect with IC_{50} of 0.58, 0.82 and 1.06 mg/ml (α -amylase) and, 0.56, 0.79 and 1.01 mg/ml (urease), respectively, as compared to acarbose (0.53 mg/ml) and thiourea (0.51 mg/ml) used as positive controls for α -amylase and urease, respectively.

Gastroprotective Activity

Das et al. (2011) examined the gastroprotective potential of ethanolic leaves extract (200, 400 and 600 mg/kg) of *A. aspera* on pylorus ligation and chronic ethanol-induced ulcer in albino Wistar rats. Treatment of extract significantly ($p < 0.01$) reduced the gastric volume, pH, total acidity ($p < 0.05-0.01$) and free acidity ($p < 0.01-0.001$) in pylorus ligation induced ulcer in rats when compared to vehicle control group. In chronic ethanol-induced ulcer, the extract at 400 and 600 mg/kg significantly ($p < 0.01-0.001$) decreased volume of gastric content and pH whereas, extract exhibit moderate protection ($p < 0.05-0.01$) by decreasing the stomach acidity in treated rats as compared to vehicle control.

Rahman et al. (2017) evaluated the gastroprotective activity of crude extract (Aa. Cr) of *A. aspera* against castor oil-induced diarrhea in BALB/c mice, K^+ -induced contraction in isolated rabbit jejunum and guinea pig ileum. The extract at 3 and 10 mg/kg doses increased fecal output, like castor oil, whereas extract at 30, 100, 300, and 700 mg/kg exhibit protection towards castor oil-induced diarrhea in mice. The extract caused spasmogenic effect on rabbit jejunum and guinea pig ileum preparations, which was partially inhibited by atropine while, completely blocked by cyproheptadine preincubation. Also, the extract relaxed high K^+ (80 mM)-induced contraction in rabbit jejunum. Treatment with extract inhibited CCh (100 μ g/kg)-induced bronchospasm in rats,

like aminophylline. The extract relaxed high K^+ and CCh (1 μ M)-induced contractions in guinea pig trachea and caused rightwards parallel shift of CCh concentration-response curves at the lower concentrations, followed by non-parallel shift at the higher concentrations.

Ahmed et al. (2021) investigated the petroleum ether, dichloromethane, ethanol extracts (200 and 400 mg/kg) of *A. aspera* against castor oil-induced diarrhea, prostaglandin-E2 induced enteropooling and gastrointestinal motility tests in albino rats. All extracts exhibited significant inhibition of diarrheal episodes ranging in between 60.20 to 93.97% for castor oil-induced diarrheal rats. The extract diminished movement of charcoal in the gastrointestinal tract by decreasing the distance travelled by charcoal meal. The volume of intestinal fluid was reduced in prostaglandin-E2-induced enteropooling in rats. In prostaglandin-E2-induced enteropooling model, the volume of intestinal fluid was reduced by all extracts as compared to atropine sulphate used as standard drug.

Hepatoprotective Activity

Tahiliani and Kar (2000) demonstrated that hepatoprotective activity of aqueous extract (200 mg/kg, b.w.) of *A. aspera* leaves on serum thyroid hormone concentrations and glucose levels in male Wistar rats. The extract exerts a significant increase in serum T3 and T4 concentrations and T3/T4 ratio along with an increase in body weight and hepatic protein content. Although, the hepatic superoxide dismutase (SOD) and catalase (CAT) activities

showed a non-significant increase, while hepatic lipid peroxidation (LPO) significantly decreased in treated rats. Thus, the plant could be both prothyroidic and anti-peroxidative in nature and might be used for the treatment of hypothyroidic subjects.

Fahim and Sathi (2018) examined the hepatoprotective activity of methanol extract (10, 20, and 30 mg/kg) of *A. aspera* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino Wistar rats. Treatment with extract increased the levels of SGPT and decreased bilirubin level in CCl_4 -induced rat as compared to dexamethasone used as standard drug.

Sharma and Chaudhary (2016) investigated the hepatoprotective activity of aqueous roots extract (200 and 400 mg/kg) of *A. aspera* against sodium arsenate induced hepatotoxicity in albino Swiss mice. Treatment with extract significantly improved ($p < 0.001$) the LPO level while CAT, SOD, GSH, GST and GPx levels in treated mice. Also, the extract produced effective ($p < 0.001$) recovery in the hepatic enzyme levels including AST, ALT, ACP, and ALP. In histological study, the extract at 400 mg/kg dose maintained normal liver architecture and was able to reduce the fibrosis, congestion, incidence of inflammatory cells infiltration.

Larvicidal Activity

Sharma et al. (2022) reported the larvicidal activity of silver nanocomposites prepared from *A. aspera* (AA-AgNC) leaves and stem extracts on morphology, behavior, and development of early fourth instar of *Aedes aegypti*. The AA-

AgNC-prolonged development of larvae and decreased emergence of adults, on the larval behavior of *A. aegypti* which did not show any noticeable behavior change except the induced mortality after 24 hr. Intense damage to the cuticle membrane and slight contraction in the internal membrane of anal papillae were found. Morphologically, the midgut appeared disorganized, darkly pigmented, and shrunk. Histological investigation of the mid-gut revealed significantly disordered internal architecture with lysed cells, damaged peritrophic membrane and microvilli, disintegrated epithelial layer, and a ruptured and displaced basement membrane. Thus, AA-AgNCs could be recommended for *A. aegypti* management as a safe and effective intervention.

Sharma et al. (2017) evaluated the larvicidal potential of n-hexane extracts from leaves and stem of *A. aspera* against early fourth instars of *Aedes aegypti*. The n-hexane extract exhibited larvicidal effect with LC_{50} values of 0.068 and 0.082 mg/ml towards tested strain. Thus, plant leaves and stem extracts could be suggested as efficient and eco-friendly control agent for *A. aegypti*.

Bagavan et al. (2008) investigated the larvicidal effect of saponin (1000 ppm) from *A. aspera* against *Aedes aegypti* and *Culex quinquefasciatus*. The saponin exhibited significant effect with LC_{50} values of 18.20 and 27.24 ppm, respectively for tested strains.

Nephroprotective Activity

Jayakumar et al. (2009) studied the nephroprotective activity of methanolic

extract of *A. aspera* (200 mg/kg, b.w.) against lead acetate-induced nephrotoxicity in albino Wistar rats. Treatment with extract significantly ($p < 0.05$) decreased the kidney weight and BUN (blood urea nitrogen), uric acid and serum creatinine levels as well as, increased the protein level in serum as compared to control (isotonic saline; 0.5ml/day i.p). The extract exerts insignificant changes in marker enzyme activities. In addition, normal kidney architecture was found in histopathological findings.

Gehani et al. (2019) examined the nephroprotective activity of aqueous and alcoholic extracts (300 mg/kg b.w.) of *A. aspera* on adenine-induced chronic kidney disease (CKD) in male Wistar rats. Treatment with extracts significantly ($p < 0.05$) restored changes in serum total protein, SGPT, SUN, uric acid, creatinine, calcium, phosphorus, and albumin by 70th day of experiment. Thus, plant extracts administration for 42 days orally was much better in restoring the changes in biochemical parameters in adenine induced CKD Wistar rats.

Neuroprotective Activity

Gawande et al. (2017) studied the anti-convulsant activity of methanolic extract of *A. aspera* roots (2.5, 5 and 10 mg/kg) against maximal electroshock (MES), pentylenetetrazol (PTZ), picrotoxin and bicuculline-induced seizure in albino Swiss mice. The extract at doses of 5 and 10 mg/kg exhibit significant enhancement in the seizure threshold treated mice towards PTZ-

, picrotoxin- and bicuculline-induced seizure models, whereas the extract failed to show protection in MES-induced seizure in mice. Moreover, the extract significantly enhanced GABA level in hippocampus and cortex as compared to saline treated group. However, the extract was devoid of any sign of acute toxicity as well as neurotoxicity.

Viswanatha et al. (2017) screened the effect of petroleum ether, methanol, aqueous extracts (200, 400 and 800 mg/kg) of *A. aspera* (aerial parts) using three different experimental models in albino Swiss mice such as actophotometer, maximal electroshock (MES) and pentylenetetrazole (PTZ), GABA levels in brain; actophotometer NMDA-induced lethality and six-hertz-seizure test. In both MES and PTZ-induced models, only methanol extract (200 and 400 mg/kg) showed significant and dose-dependent protection in mice. Further, the methanol extract was found to be mild and dose-dependent and attained saturation effect after 400 mg/kg dose extract administration. Alongside, the methanol extract at same dose showed a significant increase in GABA levels of brain compared to control flumazenil (5 mg/kg, i.p.). However, the methanol extract shown insignificant protection towards NMDA-induced mortality and did not show significant influence on locomotor effect.

Gawande et al. (2022) investigated the anti-depressant-like effect of methanolic extract of the roots of *A. aspera* (2.5, 5 and 10 mg/kg) against chronic unpredictable mild stress (CUMS)-induced depression in male albino

Swiss mice. Treatment with extract and betaine (standard) improved sucrose preference, increased grooming frequency and latency in splash test and, ameliorated depression-like condition in CUMS mice for Porsolt test as well as, also decreased the elevated plasma corticosterone and reversed the effect of CUMS on TNF- α , IL-6 and BDNF immunocontent in mice. The extract diminished self-care and motivational behavior in splash test and increased immobility time in FST.

Goel et al, (2017) demonstrated the anti-depressant effect of methanol root extract (5, 10 and 20 mg/kg) of *A. aspera* using forced swimming (FST) and tail suspension (TST) tests in albino Swiss mice as well as, serotonin, dopamine 5-HIAA and DOPAC levels in the hippocampus. Treatment with extract at 20 mg/kg dose showed a reduction in the duration of immobility for both tests, compared to imipramine (15 mg/kg) used as standard drug. The extract significantly ($p < 0.05$) increased hippocampal serotonin levels, DOPAC and dopamine levels in the hippocampus as well as, significantly decreased the serotonin metabolite levels (5-HIAA) in treated mice.

Barua et al. (2012) evaluated the anti-depressant activity of methanol extract (100, 300 and 600 mg/kg) of *A. aspera* leaves using experimental models of anxiety such as hole board (HB), open field (OF), elevated plus maze (EPM) and light/dark exploration (LDE) tests in albino Swiss mice. The extract significantly increased the number and duration of head poking in HB test. Also, the extract increased the time spent and number of entries in

open arm for EPM test. The extract produced an increase in time spent and number of crossings but decreased the duration of immobility in light box for LDE test. In OF test, the extract also exhibited an increase in number of rearing, assisted rearing and squares crossed.

Gawande and Goel (2015) examined the nootropic potential of methanolic extract of *A. aspera* (25, 50 and 100 mg/kg/day) using radial arm maze, passive shock avoidance and novel object recognition tests in female albino Swiss mice. Treatment with extract improved the learning and memory as evident by decreased working memory errors, reference memory errors and latency time in RAM test, step through latency in PSA test and increased recognition index in novel object recognition were found when compared to betadine and piracetam (standard drug) treatment. Further, the extract also enhanced glutamate level and reduced acetylcholinesterase activity in hippocampus and cortex as compared to saline treated group.

Viswanatha et al. (2019) examined the neuroprotective potential of methanol extract from aerial parts of *A. aspera* (400 and 800 mg/kg) on ischemia-reperfusion (I/R)-induced brain injury in Wistar rats. Treatment with extract showed significant decrease in the fall off latencies for both PFT and HWT tests. The pre-treatment of extract exhibit protection towards I/R-induced altered antioxidant enzyme (SOD and catalase) activities and maintained MDA and GSH levels closer to sham control. There was a significant reduction in glutathione

level with increased lipid peroxidation. The pro-inflammatory cytokines (TNF- α , IL6, and ICAM-I) levels were significantly increased and decreased to the IL-10 level. I/R insult increased the brain volume and aggravated cerebral infarct formation. Histopathological examination of the brain revealed vascular congestion, cerebral edema, leukocyte infiltration, and brain tissue necrosis. The seven days' pretreatment with 800 mg/kg of extract attained significant protection towards I/R-induced functional, morphological, biochemical, and histopathological alterations in Wistar rats.

Chitra et al. (2017) investigated the neuroprotective role of *A. aspera* hydroalcoholic extract (200 and 400 mg/kg, p.o.) on haloperidol-induced Parkinson's symptoms in Wistar rats. There was a significant reduction in the degree of catalepsy after 60 min. with extract at 200 and 400 mg/kg doses. In the rotarod test, the extract improved the retention time and fall-off time was improved in hang test. Further, the extract significantly ($p < 0.05$, and 0.01) increased the dopamine, DOPAC, and homovanillic acid levels. Treatment with extract reduced the TBARS level and, SOD, CAT, GPx, and GSH activities were also significantly decreased in haloperidol-treated rats. Thus, the plant possesses a protective role for haloperidol catalepsy and exerting antioxidant property.

Wound-healing Activity

Mengie et al. (2021) examined the wound healing potential of aqueous, n-butanol and

chloroform fractions (5 and 10% ointments) obtained from 80% methanol extract of *A. aspera* leaves on excision and incision wounds in albino Wistar rats. In the excision wound model, 10% w/w ointment of chloroform fraction exhibit significant ($p < 0.01$) effect of wound contraction in most post-wounding days and, reduced period of epithelialization in treated rats when compared to nitrofurazone used as positive control. On the other hand, 5% and 10% w/w ointments of aqueous and n-butanol fractions attained insignificant wound healing effect. However, ointment's all fraction at tested doses exerted significant ($p < 0.01$) increase in tensile strength as compared to nitrofurazone (0.2% ointment) (80.89%) for incision wound model.

Fikru et al. (2012) performed wound healing potential of topical application from methanol extract (2.5, 5 and 10% (w/w)) of *A. aspera* leaves on incision and excision wound models of albino rats. The extract at 2.5-10% w/w significantly ($p < 0.05$) increased the percentage wound contraction starting from day 9 and, required significantly short epithelization time. Treatment with extract exhibit significantly ($p < 0.05$) higher total DNA content in wound of rats. In incision wound model, extract at 5 and 10% (w/w) exhibit higher breaking strength which was comparable to silver sulphadiazine (1%) used as standard drug.

Barua et al. (2012) investigated the healing efficacy and antioxidant potential of methanol extract (2.5, 5.0 and 7.5% (w/w)) from *A. aspera* leaves in full thickness burn-wound model in albino rats. Treatment with

extract showed the rate of reduction of the wound area to be 89.93% as compared to himax (76.99%), used as positive control. The extract attenuates significantly ($p < 0.05$) higher protein, hydroxyproline and vitamin C content of the granulation tissues. Also, the extract also increased antioxidant parameters like CAT, SOD and GSH concentration in

granulation tissue. Gelatin zymography analysis of the granulation tissue attained an increase in expression of both MMP-2 and 9 when compared to the control wounds after 7 days of treatment. Histopathological analysis attenuated collagen deposition, fibroblast proliferation and formation of epidermis.

Clinical Study

A. aspera is a medicinal plant traditionally used in various medicine systems for its potential therapeutic properties. While there is a lack of extensive clinical research on *A. aspera*, some preliminary studies have explored its pharmacological properties and potential health benefits. Additionally, clinical evidence on the effectiveness of *A. aspera* (10% mouthwash), aqueous chlorhexidine gluconate (0.2%) and *Punica granatum* oral

rinse towards salivary *Streptococcus mutans* count was observed in 60 children aged between 8 to 12 years. The mouthwash showed significant ($p < 0.01$, $p < 0.05$) reduction in *S. mutans* count and plaque score, reported by Goel et al. (2016). However, more rigorous clinical trials are necessary to establish its safety, effectiveness, optimal dosage, and potential side effects in humans.

Toxicological Profile

A. aspera, is a medicinal plant that has been used in traditional systems of medicine, such as Ayurveda and traditional Chinese medicine, for various purposes. While it possesses several potential therapeutic benefits, it is important to consider the toxicological profile of *A. aspera* as well. Anuja et al. (2010) reported the spermatotoxicity of a 58-kDa protein (Ap; 25 mg/kg) isolated from *A. aspera* in male albino Swiss mice. The Ap exerts significant decrease in the sperm motility and sperm count and increased sperm abnormality. The testicular HMG CoA reductase activity and cholesterol content was

significantly increased as well as insignificant reduction in 3 β -HSD, 17 β -HSD, glucose-6-phosphate dehydrogenase, and serum testosterone levels. However, insignificant alterations in the activities of AST and ALT was observed. Alelign et al. (2020) investigated the acute and/or sub-acute toxicity profiles of *A. aspera* leaves in albino Wistar non-pregnant female rats. In acute study, extract did not showed mortality at 2000 mg/kg body weight and no visible signs of acute toxicity for 14 days were observed. Similarly, the extract did not show any mortality and weight changes at 2000

mg/kg b.w. in sub-acute study. The extract exhibited mild acute injury on liver tissues and some minor tubular inflammation confirmed by liver histopathology study. Further, the extract also decreased levels of ALP and did not show effect on AST levels. Gopal et al. (2022) assessed the sub-acute toxicity effect of ethanolic extract (AAE; 1000 mg/kg, p.o.) of *A. aspera* in female young virgin Wistar rats. Repeated dose for 28-days at 1000 mg/kg/oral, found to be safe and, did not alter the normal behavior of the treated rats. The extract did not alter hematological parameters and serum biochemical parameters. The level of ALP, glucose was less, and cholesterol level was very higher and, found statistically significant when compared to control group animals. Hormonal levels of estrogen, progesterone was altered while, T3, T4 and TSH levels not altered. Histopathology of hepato, renal and

cardiac were devoid of toxicity in nature. There was no adverse effect level of plant extract at 1000 mg/kg per day. Reddy and Kamble (2014) examined the acute and sub-acute toxicity of methanol extract of *A. aspera* on various organs system in albino Swiss mice. In acute study, the extract at 250 and 500 mg/kg did not show any toxic symptoms or mortality in mice. The extract at 25 and 50 mg/kg did not show any external manifestation of toxic symptoms, other behavioral patterns, and mortality even after 30 days in sub-acute study. Also, the extract did not produce any statistically significant changes in the body weights, food, and water intake, hematological, bone marrow studies of differential leucocytes count and chromosomal morphology but, significant changes were found in biochemical, histopathological and body weights. The whole plant powder extract of plant found, to be non-toxic.

Summary and Future Outlook

The history of the use of *A. aspera*, commonly known as prickly chaff flower goes back several millennia to ancient written Ayurvedic documents such as *Charaka Samhita* and *Sushruta Samhita*. The prophylactic and curative properties of *A. aspera* and related species from the same genus were known to ancient physicians. Over the years, scientific research has focused on understanding its phytochemical composition and potential therapeutic properties. This group of plants became an important component in the curative arsenal of several ancient cultures. Since *A. aspera* and allied species are used

in several ethnomedicinal preparations, they have been the subject of intensive studies across the globe. *A. aspera* exhibits diverse pharmacological properties and has the potential to be developed as a source of novel therapeutic agents. Some of the extracts exhibited anti-cancer, hepatoprotective and CNS related properties. Besides, several parts of *A. aspera*, particularly the leaves and roots, are shown to possess various pharmacological properties. Despite the promising findings, there are still many gaps in our understanding of *A. aspera*. Future research should focus on isolating and characterizing

bioactive compounds responsible for their therapeutic effects. Moreover, clinical trials are necessary to evaluate its safety and efficacy in humans. Additionally, investigating the molecular mechanisms underlying its various pharmacological activities would

provide valuable insights and pave the way for targeted drug development. However, further research is required to fully understand its mechanism of action, optimize its dosage forms, and evaluate its efficacy and safety in clinical settings.

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