Indian Journal of Natural Products and Resources
Vol. 16(4), December 2025, pp 644-655
DOI: 10.56042/ijnpr.v16i4.19300

Morpho-anatomical, physicochemical and antibacterial evaluation of the underexplored aerial part of the plant *Gonostegia hirta* (Hassk.) Miq. of different ecotones

Sreya Dutta, Susmita Roy*, Ayan Samadder, Deepak Kumar, Kalyan Hazra, Rajosik Bose, Simmi Mall, Amit Kumar Dixit and Gajji Babu

Central Ayurveda Research Institute, 4 CN Block, Sector 5, Saltlake, Calcutta 700091, West Bengal, India

Received 30 April 2025; revised received 14 October 2025; accepted 30 October 2025

Edible plants from natural sources are receiving wide attention as a rich source of bioactive chemicals and an easily accessible resource that may contribute to the treatment of various diseases. *Gonostegia hirta* is an edible plant with folk claims used for wound healing, digestive aids and anti-inflammatory effects. In the present study, aerial parts of *G. hirta*, i.e. leaves and stem, were collected from three different geographical regions of India- Nagaland, Arunachal Pradesh and Uttarakhand. The morphological and anatomical characterisation of the plant was carried out by macroscopy, microscopy and powder drug analysis. The aerial parts were washed, pressed, shade-dried and pulverised, followed by preparation of the methanolic extract. The antibacterial activity of the methanolic extract was evaluated on five bacterial strains- *Enterocococcus faecalis, Staphylococcus aureus* (Gram-positive cocci), *Salmonella sp*., *Pseudomonas aeruginosa*, and *Escherichia coli* (Gram-negative bacilli) by micro-broth dilution assay. The size and shape, colour, surface, odour and taste, along with plant anatomy, including powder characters of plants collected from three different regions of India, were comparable with slight variations. Methanolic extracts of all regions showed antibacterial activity on a broad antibacterial spectrum, with a better effect on Gram-positive strains. Comparatively, this study validates the local practices of *G. hirta* preparation, suggesting that methanolic extracts could be effective for treating certain bacterial strains of clinical importance, in addition to being the first ever report of comparative geographical variations with respect to antibacterial activity.

Keywords: Antibacterial, *G. hirta*, Pharmacognosy, Physicochemical

IPC code; Int. cl. (2021.01)– A61K 36/00, A61K 127/00, A61K 135/00, A61P 31/00

Introduction

Natural diversification of plant phytocompounds encompasses the wide range of chemical compounds synthesised by plants for defence against predators, attraction of pollinators, and environmental interactions. These secondary metabolites, including alkaloids, flavonoids, glycosides, phenolics, phyto-sterols, and terpenoids, are crucial for plant interactions but are not directly involved in growth or reproduction. The diversity of these compounds arises from evolutionary processes like natural selection and genetic drift, as well as environmental factors such as climate and soil composition. Seasonal variations in phytocompounds also occur, enhancing plants adaptability and resilience in various ecosystems, which have important implications for human health and agriculture. *Gonostegia hirta* (Hassk.) Miq. of the Urticaceae family is a perennial plant that grows up to 1 meter tall, blooms during June to July, with seeds maturing from August to September, in sandy loam and clay soils with mild alkalinity and in both partial shade and full sunlight. Commonly found in northern India's high altitudes, including Assam and Sikkim, this plant is consumed as a leafy vegetable by the '*Galo'* tribe of Arunachal Pradesh and has been studied for its nutritional value[1]. This plant is also used as traditional medicine in the treatment of asthma. The raw paste of the whole plant is used to relieve inflammation of the skin due to fire burns among the local women. This plant is also used as traditional medicine in the treatment of asthma[2].

A study reported on the characterisation of phenolic compounds, carotenoids and vitamins present in the leaves. The phytochemical investigations and identifications of secondary metabolites of *G. hirta* were demonstrated by a group of researchers. Phytochemical analysis revealed the presence of different classes of secondary metabolites like flavonoids, terpenoids, and saponins[3, 4].

*Correspondent author
Email: roy740@gmail.com

Several studies have explored *G. hirta* as an edible plant, whereas the biological activities are less explored. Very few biological activities were studied earlier on the leaves of this plant. According to some researchers, the plant was found to have good antioxidant capacities, as presented by DPPH, FRAP, ABTS, and hydroxyl radical scavenging capacity. Paste and decoction of the plant leaves have shown good response against the gastrointestinal system[5]. A study with methanolic extract exhibits significant antipyretic activity with the reduction of yeast-induced pyrexia temperature[6]. Paste of the fresh leaves is sometimes used by tribal people of the northeast region for restoration of bone health[7].

Due to rising antibiotic resistance, newer and safer treatment alternatives for bacterial infections are in high demand. The interest in herbal medicines is increasing as their effectiveness has been proven in treating parasitic infections, their easy and cheap availability, and their lesser toxic effects. Other species of the *Euphorbia* genus have been extensively studied for their efficacy against bacterial infections. The leaves of *G. hirta* are rich in alkaloids and flavonoids, the compounds known for their potential antibacterial activities[4]. To date, limited studies have been conducted to explore the antibacterial properties of plant extracts of *G. hirta*. Understanding the antibacterial characteristics of such extracts is crucial in addressing global health challenges, promoting sustainable practices, and meeting consumer demands for natural solutions, especially to extrapolate their potency to combat antibacterial resistance. By focusing on research and development, we can unlock the full potential of the plant kingdom. Having said that, the authors of the present study have seized the opportunity to investigate the antibacterial properties of the leaf extracts of *G. hirta*. Hence, the present study exclusively highlighted the antibacterial assay of the extract of the aerial part and the pharmacognostic characterisation, which may be a guideline for authentication of the plant in future studies.

Materials and Methods

Chemicals, reagents, bacteriological media, bacterial strains

Chemicals and reagents were purchased from E Merck Pvt. Ltd., Mumbai, and microbiological culture media were procured from Himedia Laboratories Pvt. Ltd. For antibacterial study, following bacterial strains of *Enterococcus faecalis*, *Staphylococcus aureus* (Gram-positive cocci) and *E. coli, Salmonella sp., Pseudomonas aeruginosa* (Gram-negative bacilli) were selected. These bacterial cultures were procured from MTCC [*S. aureus*- MTCC 96; *E. coli*- MTCC 1563; *Salmonella* sp.- MTCC 1162; *P. aeruginosa*- MTCC 2474], Chandigarh, India, except *Enterococcoccus faecalis* [ATCC 29212], which was collected from the Microbiology laboratory of JM Biotech (Pvt.) Ltd., Kolkata, India.

Plant material collection, authentication

The collection of plant samples for biodiversity studies is a vital part of experimental work. Key protocols included selecting suitable locations, recording adjacent vegetation and soil textures, and ensuring proper storage, transportation, and authentication of samples. To find sites with abundant plants of interest, we reviewed literature and herbarium specimens, followed by field surveys at three locations. Field notebooks and habitat images were documented for reference. We used the quadrat sampling method to study the environmental gradient due to the widespread vegetation. A 3 x 3 m² quadrat was defined, and we recorded the species within it using the nested quadrat approach[8,9]. Ten healthy specimens of *G. hirta* were collected in mid-September 2021 from three locations, authenticated, and assigned reference numbers.

Aerial parts of *G. hirta,* i.e. leaves and stem, were collected from Itanagar, Nagaland and Mandi in the month of September. The plant materials (Leaves, Stem) were washed with aqueous 70% (v/v) ethanol, and dried at an ambient temperature (24-27°C), then supplied to Kolkata and authenticated by Dr Manishi Nath Roy, Ex Scientist from Pharmacognosy Department, CARI, Kolkata[10]. Authenticated samples have been kept at the Department for future reference with a designated number.

Plant material processing

The samples were washed, pressed, and shade-dried until the moisture content fell below 2.40%, measured with an Aczet MB 50 IR moisture balance. The dried material was then pulverised through a 20 mesh sieve to obtain coarse powder. Live records and field data were preserved for future reference. A small portion of the air-dried plant sample, i.e. leaves and stem, was used for macroscopic, organoleptic and anatomical (transverse section) studies, while the rest of the plant materials were shade dried and pulverised

with a grinder (Bajaj Twister 410526). The whole and powdered plant samples were stored at room temperature in airtight and light-resistant containers. The fine (sieved in 85) and coarse powder samples were used for powder analysis, physicochemical, phytochemical and chromatographical examinations according to the standard method as per the guidelines of Ayurvedic Pharmacopoeia of India[11]. Details of sample collections and sample designations are mentioned in Table 1.

Optimisation of extraction solvents

The extraction of medicinal herbs is a crucial step in accurately determining the quality and quantity of phytocompounds present in the plant[12]. Selecting an incorrect solvent for extraction can lead to an inaccurate phytocompound profile of the plant, resulting in either the absence of certain phytochemicals or only partial presence of others[13]. To optimise the extractability, solvents with varying polarities, ranging from low polar to high polar through medium polar, were used. Hexane was used as a low-polar solvent, chloroform and ethyl acetate were used as medium-polar solvents and ethanol, methanol and water were used as high-polar solvents, keeping the extraction methods identical for each case[14].

Table 1 — Details of collection and designation of the plant samples under investigation

S No	Location of the plant leaves collection	Reference Number	Plant sample designation
1	Galuki, Nagaland (25º67′64″ N, 93º72′39″E)	CALP/NIF09G	G
2	Itanagar, Arunachal Pradesh (27º08′44″ N, 93º60′53″E)	CALP/NIF09I	I
3	Mhamizer, Uttarakhand (29º52′06″N, 79º44′16″E)	CALP/NIF09M	M

G= Galuki, Nagaland, I= Itanagar, Arunachal Pradesh, M= Mhamizer, Uttarakhand

Preparation of extracts for antibacterial study

Given that methanol was found to be the best extraction media (shown in Table 2), the same has been used for extraction for antibacterial activity. An amount of 1 g of pulverised plant sample was subjected to Soxhlet extraction using 50 mL of methanol for 3 hours[15]. Finally, the extract was filtered off using Whatman filter paper (No. 41). The filtrate was transferred to a tarred flat-bottom evaporating dish and evaporated under vacuum to get a solidified mass. The solid extract was preserved under refrigeration at 4℃ in an airtight amber colored vial for further use in an antibacterial study.

Preparation of plant extract for TLC fingerprinting

As the methanolic extract of the plant materials gave the maximum extractive value, the same was used for the fingerprinting analysis by HPTLC. For this, coarsely powdered plant material (1 g) was extracted with 50 mL of methanol using a Soxhlet apparatus. The extract was filtered and labelled as G, I & M and kept for further investigations.

Macroscopy, microscopy and powder drug analysis

The morphological characteristics of the aerial plant parts (leaves and stem) were studied, and the photographs were taken with the help of Radical, RSMR 3 stereozoom microscope[16]. The dried samples (aerial part) were treated with warm water for 2 to 4

Table 2 — Physico-chemical eevaluation of *G. hirta* aerial part[a]

Physicochemical parameters	Results in Weight percentage		
	G	I	M
Loss on drying at 105°C	11.35±0.02	12.65±0.04	12.77±0.03
Total ash	13.20±0.11	13.10±0.05	13.10±0.04
Acid-insoluble ash	7.15±0.08	6.95±0.07	6.70±0.06
Water-soluble ash	5.32±0.01	6.12±0.03	5.65±0.03
Sulphated ash	3.32±0.02	3.35±0.08	3.30±0.01
pH value (10% aq. Suspension)	6.42±0.03	6.36±0.01	6.37±0.03
Hexane extracts	1.72±0.11	1.32±0.02	1.01±0.0.01
Chloroform extracts	1.23±0.11	1.34±0.03	1.21±0.03
Ethyl acetate extracts	11.96±0.12	11.12±0.13	11.98±0.07
Methanol extracts	20.01±0.07	21.56±0.07	18.12±0.12
Ethanol extracts	8.45±0.05	11.95±0.15	12.56±0.08
Water extracts	11.54±0.13	12.65±0.12	11.48±0.13

[a]values are expressed as Mean ± SD

hours, and free-hand transverse sections (T.S.) were made using a sharp razor blade. The sections were treated with phloroglucinol in concentrated HCl and 0.02N iodine reagent. Photomicrographs of all the sections in different magnifications were taken with an Olympus CX 21i microscope, attached to Magcam DC 14 (14 MP1/2.3" CMOS sensor)[17]. Dried powdered samples (aerial part, approximately ~2 g each) were separately treated with different solutions, i.e., aqueous saturated chloral hydrate (for maceration), 50% glycerin, phloroglucinol in conc. HCl (for staining lignified tissues) and 0.02 N iodine reagent (for staining starch grains), mounted on slides with 50% glycerin and observed under a digital microscope (Olympus CX21i). The photomicrographs of different cellular structures and inclusions were taken using a Magcam DC14 digital camera attached to the microscope[18].

Physicochemical evaluation

The physico-chemical parameters like loss on drying, ash values and extractive values of the plant parts were determined in accordance with standard protocols[14].

TLC fingerprinting

The methanolic extract of plants (G, I and M), each of 2 µL, were applied in the form of an 8 mm band, at 15 mm from the bottom edge of a 10 x 10 cm reactivated glass-supported precoated silica gel $60F_{254}$ TLC plate, with the help of ATS-4 applicator attached to a CAMAG HPTLC system. The mobile phase plays a crucial role during the TLC analysis for the exact measurement of analytes. A solvent system that would give a well-resolved peak with appropriate and significantly separated R_f values was highly desired. During optimisation of the mobile phase, we also tried gradient elution, which was sometimes proven to be a better option[19]. In view of this, a number of mobile phases were tried, and it was found that the solvent composition of Hexane: Chloroform: Acetone: Ethyl acetate: Methanol: Acetic acid (1:2:1:8:2:0.5, v/v) was found to be the best for development up to 90 mm. The plate was developed in a pre-saturated twin trough chamber. The developed plate was dried for 10 min at an ambient temperature. Images of the developed plate were captured under 254 nm and 366 nm. Densitometric scanning of the developed plate at 254 nm was performed for obtaining chromatograph[20].

Antibacterial activity of drug extracts

The antibacterial activities of extracts of aerial parts of *G. hirta* from three regions, namely Galuki (G), Itanagar (I) and Mhamizer (M), were studied by micro-broth dilution assays.

For the determination of antibacterial activity of drug extracts, a micro-broth dilution test was performed as established in CLSI standard methods, with the following modifications. The stock concentration of all extracts was prepared as 500 mg/mL in a non-toxic concentration of DMSO (not exceeding 25% aqueous concentration; data not shown). The serial dilutions of the test extracts in Mueller-Hinton Broth (MHB) were prepared in a concentration range from 0.49 mg/mL to 250 mg/mL (w/v). Then, 100 µL of each dilution was transferred into the wells of a 96-well microplate. Bacterial suspension (100 µL) was inoculated in each well to obtain final concentrations of 5×10^4 CFU/mL of test bacteria and a final volume of 200 µL per well. Finally, the microplate was incubated with a sterile film cover for 24 h at 37°C, and then 50 µL of inoculum from each well of the 96-well plate was spread over Mueller-Hinton agar (MHA) plates by the spread plate technique. MH agar plates were incubated at 37°C overnight for cross verification of the result. The lowest dilution of test drugs, at which no growth was observed in the MHA plate, was considered the Minimum Bactericidal Concentration (MBC). Standard antibiotics were used as the positive control.

Results and Discussions

Macroscopic features

The stem is dried, fragmented, of different sizes, brown, with ridges and grooves, mildly pubescent, stout, and without pith. Cylindrical, slightly curved with nodes and internodes (1.5-4 cm), varying in size, 0.2-0.3 cm in thickness, with a distinct hollow centre. The outer surface is rough, ridged and grooved, dark brown, and the inner surface appears creamish-yellow. Odour is not distinct, taste mucilaginous, slightly bitter. The stem samples from G (Nagaland) and I (Arunachal Pradesh) are more or less the same in morphology, but the stem samples from M (Uttarakhand) slightly differ in stem size, i.e., they are compressed and very thin (0.1 - 0.2 cm in diameter) with a dull white inner surface. (Fig. 1a-c).

The dried leaves are medium-sized and sessile, lanceolate to ovate-lanceolate, entire, acuminate,

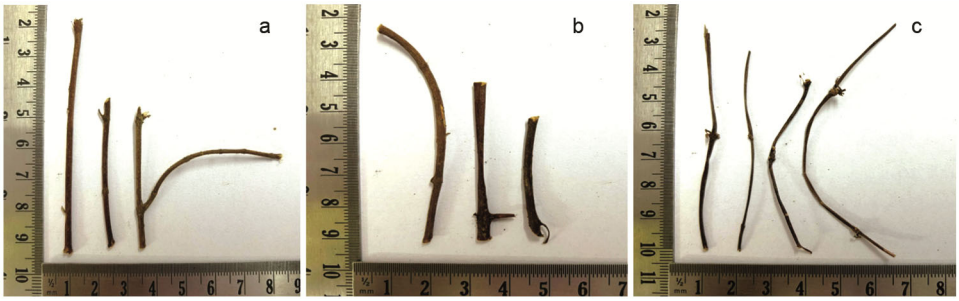

Fig. 1 — Fragmented Dried Stem of *G. hirta* from three locations, a) G (Nagaland), b) I (Arunachal Pradesh) and c) M (Uttarakhand).

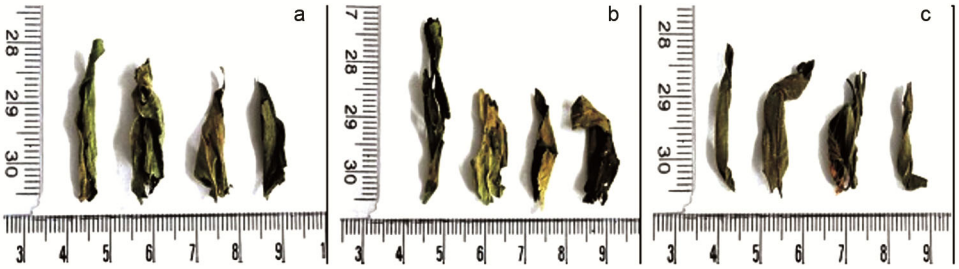

Fig. 2 — Dried Leaves of *G. hirta* from three locations, a) G (Nagaland), b) I (Arunachal Pradesh), and c) M (Uttarakhand).

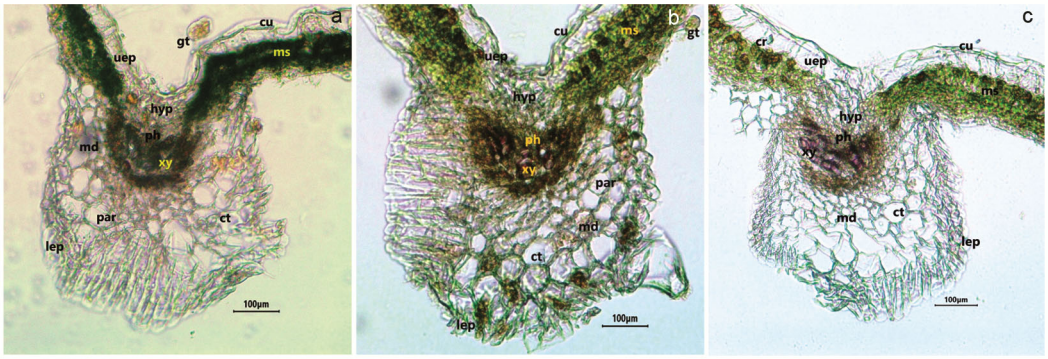

Fig. 3 — Transverse section of dried leaves of *G. hirta* through the midrib of three locations, a) G (Nagaland), b) I (Arunachal Pradesh), and c) M (Uttarakhand) [cu: cuticle, uep: upper epidermis, lep: lower epidermis, hyp: hypodermis, ph: phloem, xy: xylem, md: mucilage duct: ct: cortex, par: parenchyma, ms: mesophyll]

2- 3 cm long, 1-1.5 cm in diameter, brittle and easily detached from the rachis in dry conditions. The upper surface is smooth and dark green, while the lower surface is light green. Odour and taste are not distinct. The leaf samples from G (Nagaland), I (Arunachal Pradesh), and M (Uttarakhand) are largely similar in morphology (Fig. 2a-c).

Microscopic features in the transverse section of leaves

A transverse section (T.S.) of the midrib is concave on adaxial surface and broadly convex on abaxial surface showing rectangular upper epidermal cells (uep) covered with cuticle (cu) with few covering and glandular trichomes (gtr) followed by a few cell layered collenchymatous hypodermis (hyp); the outer cortex is made up of thin walled parenchymatous cells (par) filled with starch grains (st) and prismatic crystal of Ca-oxalate (pc); crescent patch of secondary vascular bundles are present at centre; pericycle (per) of rectangular elongated cells; vascular bundle is exarch; the phloem tissues are present on the upper outer periphery, some pericyclic sclerenchymatous fibres present in vascular region; interfascicular region is present in between vascular bundles; the lower cortex is wide, parenchymatous with mucilage deposits (md); the lower epidermis (lep) covered with cuticle (Fig. 3).

The T.S. of the lamina shows upper epidermis (uep) with cuticle; a few glandular trichome (gtr) present, followed by small palisade (ps) parenchyma cells and round spongy parenchyma (sp) cells; some cells with brown content and few vascular bundles are

present in between these palisade and spongy cells; followed by the lower epidermis. The microscopic features are more or less the same in the leaf samples of three geographical regions, but trichomes are very few in samples from M (Uttarakhand) (Fig. 4).

Microscopic features in the transverse section of the stem

T.S. of stem shows few layers of cork cells (ck) followed by rectangular epidermal cells, followed by thin walled parenchymatous cells filled with profuse starch grains forming the outer cortical (ct) region; some mucilage ducts (md) are present in this region; followed by the 2-3 cell layered thick walled stone cells (st) in patches; inner cortex parenchymatous; the vascular bundle is endarch; xylem vessels are separated by uniseriate to bi-seriate medullary rays (mr); the phloem (ph) is present on the outer side of the metaxylem (mx); Pith mostly absent, as represented in following diagrammatic sketch of whole stem section. Only traces are present in samples of Arunachal Pradesh.

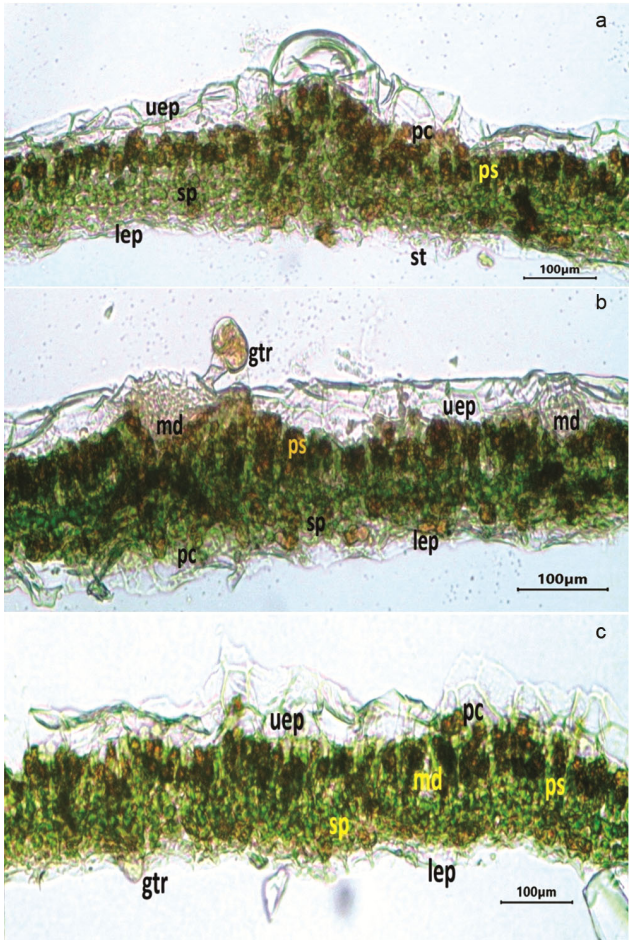

Fig. 4 — Transverse section of dried leaves of *G. hirta* through the lamina of three locations, a) G (Nagaland), b) I (Arunachal Pradesh), and c) M (Uttarakhand) [gtr: glandular trichome, uep: upper epidermis, lep: lower epidermis, pc: prismatic crystals, ps: palisade parenchyma, sp: spongy parenchyma: md: mucilage duct]

The microscopic features are more or less same in the stem samples of three geographical regions, but upper cortex is wider (7-8 cell layered), lower cortex is smaller (2-3 cell layered), stone cell layers are wider (3 - 4 layers); metaxylem elements are fewer than protoxylem elements, and more medullary ducts are present in samples from Uttarakhand (M) (Fig. 5).

Powder characters

Presence of cork-cell fragments, groups of stone cells, fragments of spiral, reticulate and pitted vessels; tangentially elongated parenchyma cells; fibres with xylem parenchyma; fragments of lamina with oval brown content; spongy parenchyma; anomocytic stomata; fibres; covering and few glandular trichome and prismatic crystals of ca-oxalate.

The microscopic features are largely similar in the leaf samples of three geographical regions, except for a few features, i.e., the presence of very few glandular trichomes and fibres with xylem parenchyma, found in the samples of Arunachal Pradesh (I) (Fig. 6a-c).

As a whole, the aerial parts, i.e. stem and leaves of *G. hirta* are more or less morphologically the same among the three different locations except for the length of internodes and diameter of stem. Microscopically, three samples are more or less the same except for the thickness of different cell layers, presence and amount of glandular trichomes and medullary ducts.

Physico-chemical evaluation

Evaluation of the physico-chemical parameters of the plant samples represented in Table 2. The total ash value is in the range of 13%, water soluble and acid insoluble ash contents are in the range of 5-7% which indicates that the physiological ashes present in the plant tissues are quite high. While looking at the loss on drying, it is observed that the trapped moisture in the plant sample is a little bit on the higher side, which suggests that it should be stored carefully in an airtight container to avoid any air contact; otherwise, the materials get spoiled easily. The extractive values of different solvents for the plant samples revealed maximum and minimum extraction by methanol and hexane, respectively. These observations of extractive values are in close agreement with previously reported data[2]. Based on the best phytoconstituents

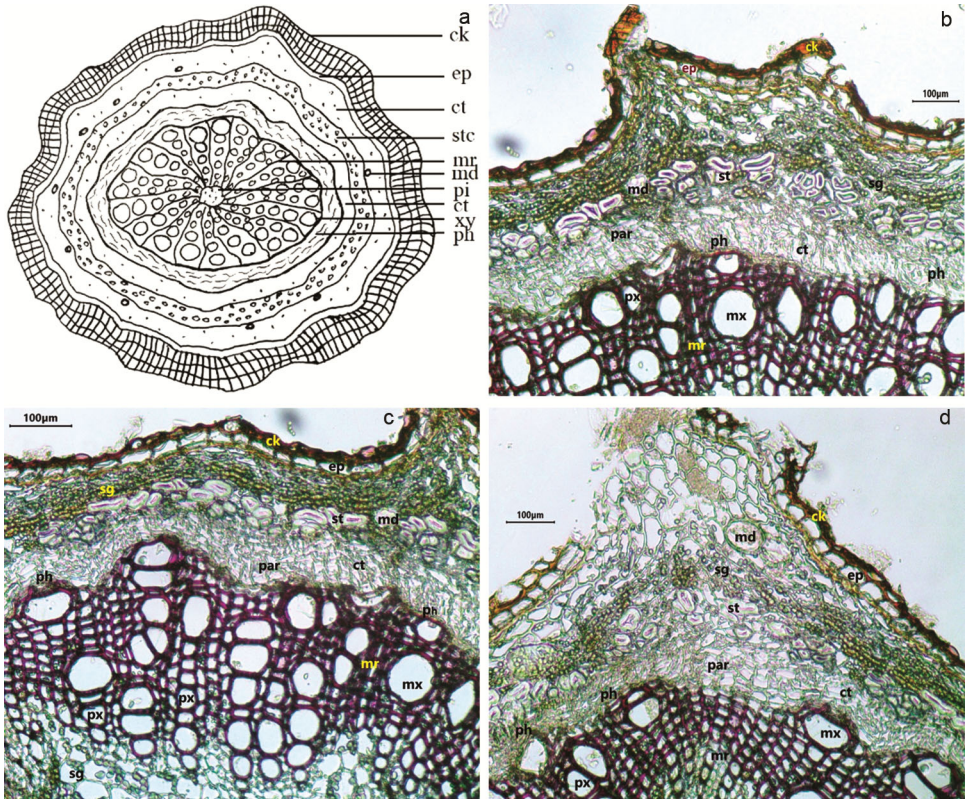

Fig. 5 — Transverse section of dried stem of *G. hirta* of three locations, a) Diagrammatic sketch of whole stem section, b) G (Nagaland), c) I (Arunachal Pradesh), and d) M (Uttarakhand) [ck: cork, ep: epidermis, st: stone cells, sg: starch grains, ph: phloem, xy: xylem, md: mucilage duct, ct: cortex, par: parenchyma, per: pericycle, mx: mesophyll, px: protoxylem, mr: medullary rays]

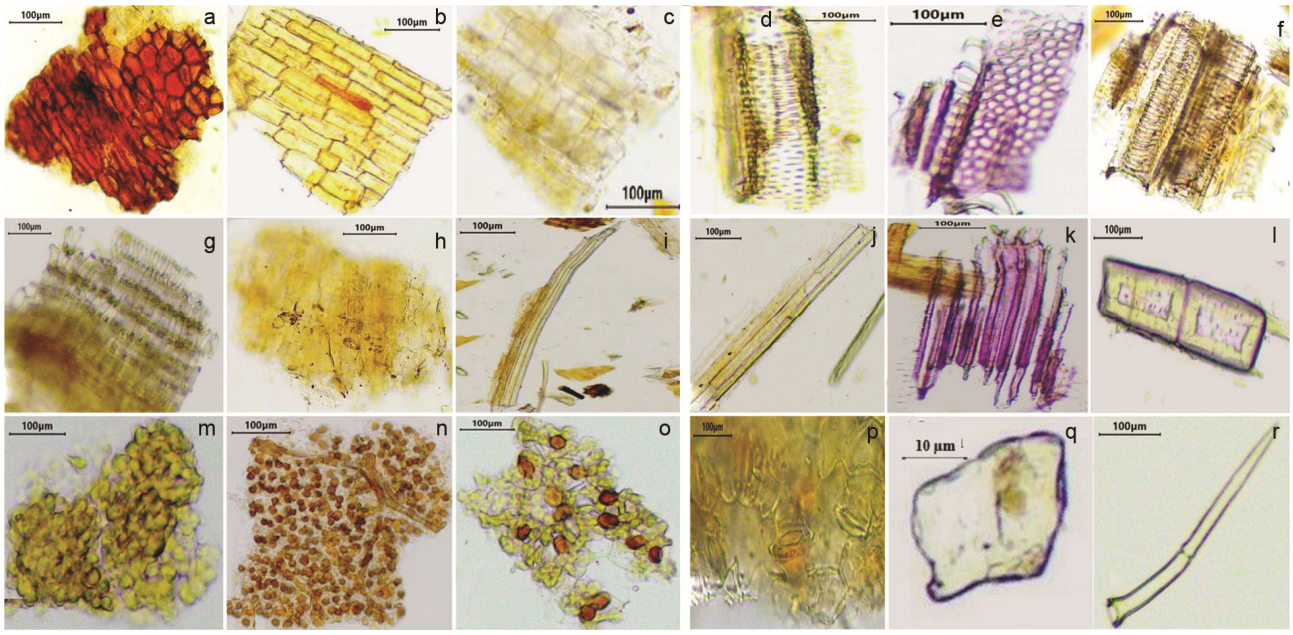

Fig. 6a — Photomicrographs of powder microscopy of aerial parts of *G. hirta* (X 100) of G (Nagaland) a) Groups of cork cells, b) Groups of tangentially elongated parenchyma cells, c) Ground parenchyma cells with cell content, d,e) Fragmented pitted vessels, f, g) Fragmented spiral vessels, h) Vessels with annular thickening, i, j, k) Fibers, l) Stone cells, m) Spongy mesophyll cells, n,o) Fragments of lamina with oval brown content, p) Anomocytic stomata, q) Prismatic crystals of Ca-oxalate, r) Covering trichome.

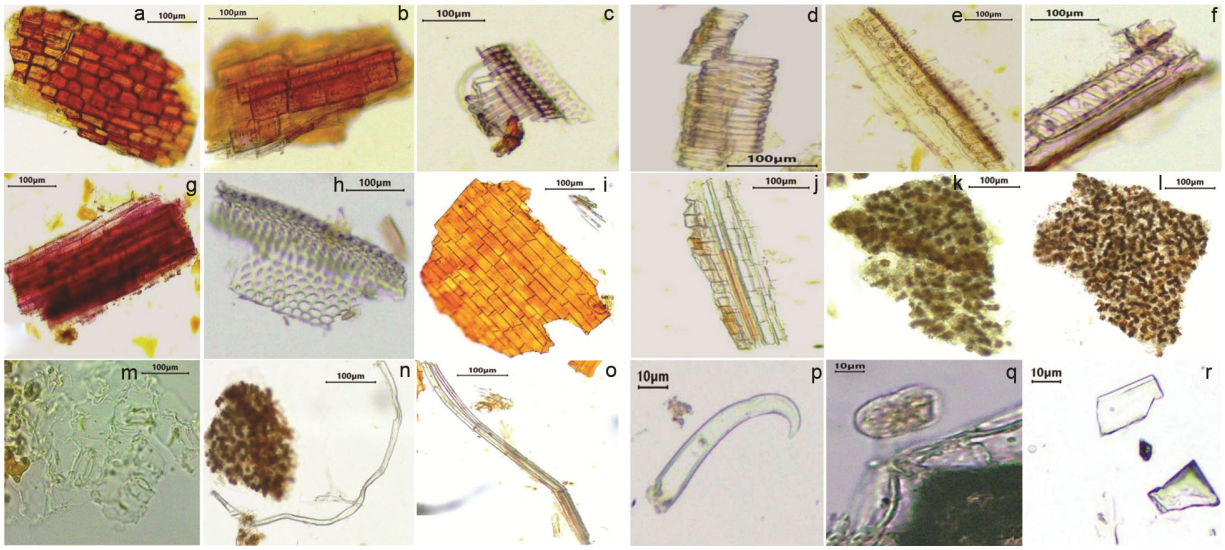

Fig. 6b — Photomicrographs of powder microscopy of aerial parts of *G. hirta* (X 100) of I (Arunachal Pradesh). a) Fragments of cork cells, b) Groups of stone cells, c, d, e) Fragmented spiral vessels, f) Fragmented reticulate vessels, g-h) Fragmented pitted vessels, i) Tangentially elongated parenchyma cells, j) Fiber with xylem parenchyma, k-l) Fragments of lamina with oval brown content, m) Anomocytic stomata, n-o) Fibers, p) Covering trichome, q) Glandular trichome, r) Prismatic crystals of Ca-oxalate.

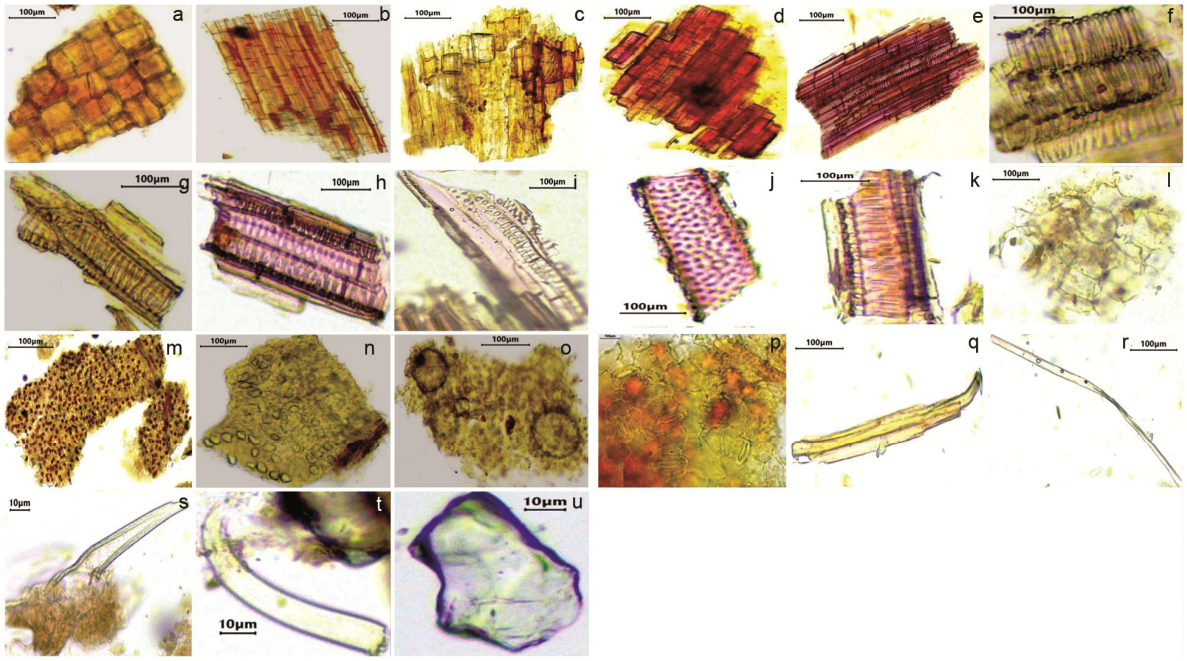

Fig. 6c — Photomicrographs of powder microscopy of aerial parts of *G. hirta* (X 100) of M (Uttarakhand). a) Cork cells, b) Tangentially elongated parenchyma cells, c-d) Groups of stone cells, e-f) Spiral vessels, g-h) Groups of reticulate vessels, i, j, k) Fragments of Pitted vessels, l) Groups of oval parenchymatous cells with content, m) Fragments of lamina with oval brown content, n) Prismatic crystals in lamina, o) Spongy mesophyll cells with medullary duct, p) Anomocytic stomata, q-r) Fibre, s-t) Covering trichomes, u) Prismatic crystal of Ca-oxalate.

yield in the methanol extract, the same was used for the subsequent fingerprinting analyses.

Qualitative TLC fingerprinting

The TLC conditions for the best separation of the phytoconstituents were optimised to gradient development, which gave well-separated peaks with the best resolution for the phytocompounds under investigation. Methanolic extract showed bands/spots at R_f 0.07, 0.16, 0.23, 0.38, 0.47, 0.65, 0.73 and 0.79 when visualised at 254 nm, 0.10, 0.17, 0.22, 0.28,

Table 3 — MIC values (mg/mL) of plant extracts of *G. hirta* from three different regions

Plant extracts	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*Salmonella sp.*	*E. coli*
G- Nagaland	125	125	Bacteriostatic effect	125	No effect
I- Arunachal Pradesh	62.5	125	Bacteriostatic effect	125	No effect
M- Uttarakhand	125	125	125	125	No effect

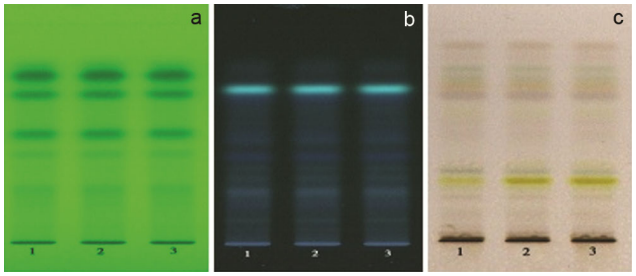

Fig. 7 — TLC profiles of methanolic extracts visualised at a) 254 nm, b) 366 nm, and c) visualised under white light after derivatisation (Tracks 1, 2, and 3 are for G, I and M, respectively).

0.31, 0.37, 0.44, 0.65, and 0.75 when visualised at 366 nm and 0.09, 0.20, 0.26, 0.30, 0.50, 0.70, 0.82 and 0.88 when visualised at white light after derivatisation (Fig. 7).

Antibacterial screening of extracts

The MIC values of plant extracts isolated from three regions of Nagaland, Arunachal Pradesh, Uttarakhand have been depicted in Table 3. The stock concentration of all test plant extracts was 500 mg/mL. The test extracts from three different regions exhibited significant growth inhibition in the concentration range of 62.5 to 250 mg/mL for *S. aureus*, 125 mg/mL and 250 mg/mL for *Enterococcus faecalis* and *Salmonella* sp. The effect of the plant extracts on *P. aeruginosa* was lower; the extract only from Uttarakhand showed bactericidal effect in the concentration of 125 mg/mL and 250 mg/mL. There was no effect of any extracts on *E. coli*.

A noteworthy observation is that the current study demonstrated that the morphological and internal microscopic characteristics derived from the analysis of transverse sections and powder microscopies were consistent throughout the three regions, regardless of the environmental condition, soil type and altitude. The plants of different locations did not show morphological variations, but there were notable variations in physicochemical characters. The observation of phytochemical differences was noted previously by a group of researchers[3].

When a plant species shows no visible differences in structure yet varies in phytochemical composition, it often indicates differences in secondary metabolites – compounds that are essential for medicinal properties, defence and adaptation. These compounds are essential for medicinal properties, defence, and adaptation. Genetic variations within the same species can lead to different phytochemical profiles without affecting morphology. Environmental factors like soil type, light, temperature, altitude, and humidity also influence these compounds. For example, plants in nutrient-poor soils may produce more alkaloids or phenolics as a stress response. Ecotypes are populations adapted to different environments with unchanged morpho-anatomy but varying chemical profiles. Chemotypes refer to distinct chemical variants within a species. Additionally, environmental triggers can alter gene expression affecting phytochemical pathways, and levels may fluctuate during different growth stages. Microorganisms associated with the plant can also impact phytochemical production without changing morphology. Understanding these aspects enhances our insight into the relationship between plant structure and chemical composition.

The studies on the biological activities of *G. hirta* are limited. In a study[21], leaf extracts of *G. hirta* showed antipyretic activity and analgesic activity, effects comparable to those of conventional alternatives[22]. They explored the phytochemical composition of *G. hirta* and proved their antioxidant activity. This is also in line with our recent study, where the presence of alkaloids, flavonoids, terpenoids, and phytosterols has been established in the methanolic extract of *G. hirta* leaves[3]. However, there is a very limited study to date depicting any effect of this plant on bacterial strains. India, being a rich source of biodiversity, offers numerous herbal formulations for therapeutic purposes, including infections and inflammations. Due to rising concerns of antibacterial resistance and increasing demand for newer, safer alternatives, unexplored and easily available plants can be established as potent substitutes as antibacterial agents. A survey evaluated 78 plant species of ethno-medicinal importance from the Darjeeling hills for antibacterial activities and

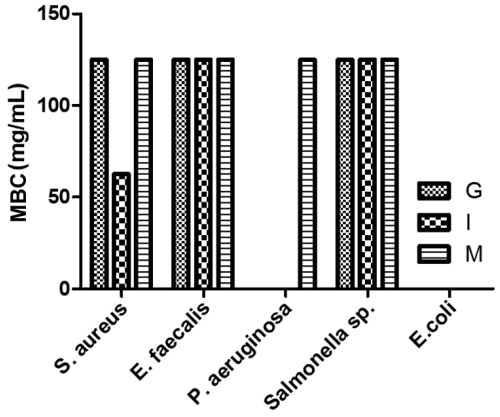

Fig. 8 — MBC of methanolic extract of *G. hirta* against different bacterial strains.

showed diverse effects; most importantly, *Pouzolzia hirta* (synonym of *G. hirta*) showed antibacterial effect on Gram-positive bacteria[23]. Many studies have worked on plant extracts isolated from North-east India, a biodiversity hotspot and showed that many of the plant extracts had potential to work against bacteria[23-25]. According to previous investigation, the exploration of phytochemical components exposed the presence of alkaloids like europine, allosamidine, flavonoids like epigenin, quercetin in the *G. hirta* leaves, among which allosamidine and europine have reported antibacterial properties. The botanical characterisation showed that plant samples collected from all three different geographical regions do not differ much; the trichomes were very few in the stem sample from Uttarakhand. The thicknesses of the upper and lower cortical layers, stone cells, and the numbers of metaxylem elements and medullary ducts differ in leaf samples from Uttarakhand. Conversely, the sample from Arunachal Pradesh showed very few glandular trichomes and fibres with xylem parenchyma as powder characters.

However, these features did not reflect any effect on its biological effect in the form of antibacterial activity, whereas the variations in phytochemicals may be attributed to bacterial strains. For instance, plants collected from Uttarakhand contain a greater amount of flavonoids than those of the other two places. Probably, this may be the plausible reason for showing the greater antibacterial activities, because flavonoids show good antibacterial activity[26]. Flavonoids such as morusin and auriculoside, whose presence in the plant of Uttarkhand is the probable chemical moieties, exhibit antibacterial activities.

The plant sample from Arunachal Pradesh showed a slightly more effect on bacterial strains, especially *S. aureus*, emphasising the scope of the alkaloids like Haplophytine, Heliotrine, and Retronecine as antibacterial agents, along with other alkaloids predominantly present in this sample. On the other hand, among Gram-positive and Gram-negative strains, the effect of all samples was more on Gram-positive ones in comparison to Gram-negative; the effect on *P. aeruginosa* was mostly bacteriostatic and no effect on *E. coli* (data not shown). The antibacterial effect of plant extracts on Gram-positive and Gram-negative bacteria varies depending on the bacterial cell wall structure and phytochemical composition of plant extracts. Gram-positive bacteria are generally more susceptible due to their simpler cell wall structure, which allows phytochemicals to more easily penetrate and disrupt their cellular processes, while Gram-negative bacteria, with their additional outer membrane, often show greater resistance to the same phytochemicals[27,28]. Alkaloids and terpenoids often exhibit antibacterial activity, with many studies showing a stronger effect against Gram-positive bacteria compared to Gram-negative bacteria[29,30]. It is due to their thicker peptidoglycan layer, which is more easily disrupted by the alkaloid molecule compared to the thinner peptidoglycan layer in Gram-negative bacteria with their additional outer membrane. On the other hand, the lipophilicity (fat-solubility) of a flavonoid can influence its activity against Gram-positive bacteria, with more lipophilic flavonoids showing greater effectiveness[31,32]. Here, in this study, the greater effect of the extract on Gram-positive strains supports this concept, where interaction between the cell wall and phyto-constituents plays a crucial role in exerting antibacterial activity. However, there is no significant difference in the MIC of *G. hirta* from different geographical regions. A graphical representation of quantitative MIC values across the different geographical regions has been depicted in Fig. 8.

Conclusion

In conclusion, the study confirms that in a previously conducted study, the predominant phytochemicals present in the methanolic extracts of *G. hirta* plants are mostly alkaloids, flavonoids and terpenoids, which may have a greater effect on Gram-positive bacteria. The alkaloid content is higher in the sample from Arunachal Pradesh, and the antibacterial effect of this sample is slightly more pronounced. This present study emphasises that by correlating the previous study of alkaloidal contents and the

antibacterial efficacy, we can streamline the phytochemicals responsible for exhibiting the biological efficacies. The study explicitly showed that the pharmacognostic study and its evaluation may serve as a guideline to authenticate the plant aerial parts. The present study indicates that the edible or non-edible, whatever the herb is, needs to be explored for its medicinal values for maximum utilisation of natural resources. In the era when scientists are looking for an alternative to the synthetic antibacterial, the present study may open up a path for further detailed investigations on natural sources.

Conflict of interest

The authors of the present work declare that there is no conflict of interest associated with this manuscript.

References

1 Brahma J and Narzary D, Bioactive and nutraceutical compound manipulation from the leaves of some wild edible medicinal plants in Chirang District of Assam, India, *Am J Ethnomed*, 2015, **2**(6), 98-183.

2 Acharya B R S, Joshi R A, Srivastava A, Prajapati U B, Srivastava J, *et al.*, The nutraceutical studies of *Gonostegia hirta* (Blume) Miq. a traditional vegetable from Northeast India, *J Pharmacogn Phytochem*, 2024, **13**(3), 265-269, doi: 10.22271/phyto.2024.v13.i3d.14965.

3 Hazra K, Kumar D, Mondal S, Arya D, Bharadwaj Y, *et al.*, The diversity of the phytochemical array observed in the medicinal plants *Gonostegia hirta* (Blume. ex Hassk.) Miq., *Ind J Nat Prod Resour*, 2025, **16**(1), 113-122, doi: 10.56042/ijnpr.v16i1.4187.

4 Phurailatpam A, Senjam R and Mayanglambam C, Morpho chemical characterisation of some underutilised vegetables under different shade conditions, *J Pharmacogn Phytochem*, 2021, **10**(3), 260-266.

5 Chetry L B, Basar K, Taye K, Taka T, Tsering J, *et al.*, Medicinal plants used against gastrointestinal disorders among the Adi Tribe of Eastern Himalaya, *NeBIO-Int J Environ Biodiver*, 2018, **9** (1), 93-101.

6 Jamoh L, Sharma A, Kalita P and Tag H, Pharmacological activity of leaves extract of *Gonostegia hirta* Blume Ex Hasskarl Miquel, a wild food used by the local people of Arunachal Pradesh, *Ind J Ravishankar Univ*, 2016, **29**(1), 176-177.

7 Chettri B, Khar T U and Bhutia S, Ethnobotanical survey of medicinal plants for bone fracture treatment in Lingmoo, Sikkim, *J Appl Pharm Res*, 2025, **13**(1), 162-169, doi: 10.69857/joapr.v13i1.909.

8 Certner M, Lucanova M, Sliwinska E, Kolar F and Loureiro J, Plant material selection, collection, preservation, and storage for nuclear DNA content estimation, *Cytometry Part A*, 2022, **101**(9), 737-748, doi: 10.1002/cyto.a.24482.

9 Vogl C R, Vogl-Lukasser B and Puri R K, Tools and methods for data collection in ethnobotanical studies of homegardens, *Field Methods*, 2004, **16**(3), 285-306.

10 Rocha R P, Melo E C and Radünz L L, Influence of drying process on the quality of medicinal plants: A review, *J Med Plants Res*, 2011, **5** (33), 7076-7084, doi: 10.5897/JMPRx11.001.

11 Anonymous, Ayurvedic Pharmacopoeia of India. Min. of Health & Family Welfare, Govt. of India, New Delhi, 2006, 12-14.

12 Hazra K, Mitra A, Singh R, Singh A and Hazra J, Rationalisation of extractive protocol by high-performance thin-layer chromatographic–densitometric quantification of berberine in multiple hydroalcoholic extract of *Tinospora cordifolia* stem, *J Planar Chromat*, 2021, **34**(2), 157-163, doi: 10.1007/s00764-021-00098-5.

13 Hazra K, Kumar D, Debnath S, Mondal S, Batule M, *et al.*, Dynamicity and extractability of hydro-alcoholic solvents for *Tinospora cordifolia* stem: an investigation for target-oriented traditional drug discovery based on biologically active phytocompounds, *Vegetos*, 2025, **38**, 931-941, doi: 10.1007/s42535-024-00835-1.

14 Anonymous, Ayurvedic Pharmacopoeia of India. Min. of Health & Family Welfare, Govt. of India New Delhi, 2008, 242-244.

15 Anonymous, Quality control methods for herbal materials, World Health Organization Geneva, 2011, 141-142.

16 Hazra K, Dutta S, Mandal A K, Ravte R K, Mitra A, *et al.*, Pharmacognostical and phytochemical blueprint of *Abroma augusta* L. stem bark, *Ind J Nat Prod Resour*, 2021, **12**(2), 271-28, doi: 10.56042/ijnpr.v12i2.24981.

17 Hazra K, Kumar D, Mitra A, Dutta S, Sarkar S, *et al.*, Phytopharmacognostic profiling of *Prunus cerasoides* Buch. -Ham. ex D. Don, heartwood, *Ind J Nat Prod Resour*, 2024, **15**(1), 146-155, doi: 10.56042/ijnpr.v15i1.4232.

18 Kokate C K, Purohit A P and Gokhale S B, Pharmacognosy, (Nirali prakashan Pune), 2006, 122-241.

19 Hazra K, Kumar D, Dutta S, Subhose V, Meena A K, *et al.*, Improvised TLC and conceptualisation of coordinate position: A notion for qualitative profiling of complex herbal formulations, *Acta Chromat*, 2025, **37**(2), 1-11, doi: 10.1556/1326.2025.01304.

20 Reich E and Schibili A, *High Performance Thin Layer Chromatography*, (Thieme Publication New York), 2006, 247-249.

21 Jamoh L, Hui P K, Debmalya D and Tag H, Ethnobotany and nutritional potential of *Gonostegia hirta* (Blume ex Hassk.) Miq. (Urticaceae) from Arunachal Pradesh, India, *Pleione*, 2017, **11**, 329–335.

22 Li Y, Hu Z, Chen X, Zhu B, Liu T, *et al.*, Nutritional composition and antioxidant activity of *Gonostegia hirta*: An underexploited, potentially edible, wild plant, *Plants*, 2023, **12**(4), 875-881.

23 Saha J, Sarkar P K and Chattopadhyay S, A survey of ethnomedicinal plants of Darjeeling hills for their antimicrobial and antioxidant activities, *Ind J Nat Prod Resour*, 2011, **2**, 479-482.

24 Parekh J and Chanda S, *In-vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants, *Turk J Biol*, 2007, **31**(1), 53-58.

25 Naik S S, Thilagaraj W R, Gangadharan P and Leela K V, Comparative study of antibacterial activity between selected international and indian essential oils against selected pathogenic bacteria, *J Pure Appl Microbiol*, 2024, **18**(1), doi: 10.22207/JPAM.18.1.23.

26 Cushnie T T and Lamb A J, Antimicrobial activity of flavonoids, *Int J Antimicrob Agents*, 2005, **26**(5), 343-356, doi: 10.1016/j.ijantimicag.2005.09.002.

27 Sharifa A A, Neoh Y L, Iswadi M I, Khairul O, Abdul Halim M, *et al.*, Effects of methanol, ethanol and aqueous extract of *Plantago major* on Gram positive bacteria, Gram negative bacteria and yeast, *Ann Microsc*, 2008, **8**, 42-44.

28 Koohsari H, Ghaemi E A, Sheshpoli M S, Jahedi M and Zahiri M, The investigation of antibacterial activity of selected native plants from North of Iran, *J Med Llife*, 2015, **8**(2), 38-43.

29 Sulaiman M, Jannat K, Nissapatorn V, Rahmatullah M, Paul A K, *et al.*, Antibacterial and antifungal alkaloids from Asian angiosperms: Distribution, mechanisms of action, structure-activity, and clinical potentials, *Antibiotics*, 2022, **11**(9), 1146-1154, doi: 10.3390 /antibiotics11091146.

30 Dias K J, Miranda G M, Bessa J R, Araújo A C, Freitas P R, *et al.*, Terpenes as bacterial efflux pump inhibitors: A systematic review, *Front Pharmacol*, 2022, **13**, 103-110, doi: 10.3389/fphar.2022.953982.

31 Yuan G, Guan Y, Yi H, Lai S, Sun Y, *et al.*, Antibacterial activity and mechanism of plant flavonoids to gram positive bacteria predicted from their lipophilicities, *Sci Rep*, 2021, **11**(1), doi: 10.1038/s41598-021-90035-7.

32 Shamsudin N F, Ahmed Q U, Mahmood S, Ali Shah S A, Khatib A, *et al.*, Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation, *Molecules*, 2022, **27**(4), 93-99, doi: 10.3390/molecules27041149.