Indian Journal of Natural Products and Resources
Vol. 16(1), March 2025, pp. 160-176
DOI: 10.56042/ijnpr.v16i1.13623

Macro-microscopic identity, phytochemical screening and pesticidal efficacy of *Blumea mollis* (D.Don) Merr. aerial parts

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Received 09 September 2024; revised received 30 January 2025; accepted 25 February 2025

Blumea mollis (D.Don) Merr. is an indigenous herb used for diarrhoea and skin diseases and having antiseptic and insect repellent properties. The current study is carried out on the pharmacognostical and phytochemical evaluation and pesticidal activity of B. mollis stem and leaves. Micro-morphological profiling assists the authentication of the plant in its crude drug as well as an ingredient in formulations. As the plants possess natural insecticidal properties it can be used to control insect pests and it offers a more environmental friendly pest management rather than depending on synthetic chemicals. Macroscopic, microscopic, quantitative analysis of leaf, powder microscopy, histochemical tests and qualitative screening of phytochemicals and fingerprinting by TLC and HPTLC were done. For pesticidal efficacy of B. mollis, a preliminary field trial was conducted using pot culture experiment in a Completely Randomised Design against sucking pest complexes using chilli as a test crop. The organoleptic characters, presence of multicellular trichomes, glandular trichomes, anisocytic and anomocytic stomatal types, lignified stone cells, bordered pitted vessels, rosette crystals of calcium oxalates, etc. were noted as the diagnostic features of the plant. The spots obtained and R_f values of HPTLC can be used to differentiate *B. mollis* from other species and from adulterants or substitutes even in powdered form. *B. mollis* extract 5% proved effective in reducing the leaf curl symptom in chilli and was proved better than the commonly used botanicals such as neem oil 2%, pongamia oil 2%, *Andrographis paniculata* 5% and chemical pesticides like Spiromesifen 22.9% SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha and wettable Sulfur $3g^{-1}$. The outcomes of this research work will be supportive in the standardisation of AYUSH formulations containing *B. mollis* as an ingredient.

Keywords: HPTLC, Natural pesticides, Pharmacognosy, Plant microscopy, Standardization

IPC code; Int. cl. (2021.01)- A61K 36/00, A01N, A01P

Introduction

Blumea mollis (D.Don) Merr. (Syn.: *Blumea axillaris* DC.)[1] is a valuable traditional plant belonging to the family Asteracea. It is widely found throughout the paleotropics[2] and is diversified mostly in Africa, Australia and Asia. Within the genus *Blumea*, nearly 36 species are reported to occur in Peninsular India, of which 19 are indigenous to South India[3]. *Blumea* species are reported to have medicinal, insecticidal and anti-bacterial properties[4,5]. Among the species, *B. mollis* is a common herb widely scattered in the south-west plains of India and in the Himalayas at an altitude of 2000 ft.

B. mollis leaf is used in traditional medicine for the cure of diarrhoea and skin diseases[6]. The boiled plant is used by Mundas for treatment of diarrhoea[7]. The plant has antiseptic properties and the leaves are used in ophthalmic diseases, ear troubles, headache, as haemostatic, blood coagulation, and insect repellents[8]. The phytochemical screening of plant by GC-MS showed more than 30 compounds like linalool, copaene, γelemene, allo-ocimene, γ-terpinene estragole and alloaromadendrene[4]. Isolation of structures of 5-hydroxy-7-ethoxy-carvaotanacetone and 5-hydroxy-7-tigloyloxy-carvaotanacetone, *n*-triacontane, *n*-hentriacontane, chrysanthenone, caryophyllene oxide, 2,3-dimethoxy-p-cymene, etc., reported in oil and chrysanthenone isolated from essential oil[9]. The essential oil of the plant also yielded α-pinene, camphene, -pinene, α-terpinene, humulene, eugenol, isoborneol, β-bisabolene, etc[8].

Various pharmacological actions like anti-bacterial, antioxidant, anticancer, anti-microbial, anti-

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inflammation, hepatoprotective, wound healing, platelet aggregation, anti-obesity etc. have been recorded from the plant[5,10]. The essential oil from leaves showed potent antibacterial activity against *Aeromonas* sp., *Escherichia coli* and *Collectotrichum capsica*[11]. Antifungal activity of essential oil from leaves was also found active against the fungus *Sclerotium rolfsii* and showed antiviral effects against tobacco mosaic virus[11]. Anti-inflammatory and anti-pyretic activities of *B. mollis* was reported by Devi *et al.*,[12] on carrageenan-induced rat paw oedema.

As the traditional promises on various herbal drugs have been validated scientifically, modern medicine is also looking for plant-derived medicines which highlights the importance of natural herbs in healthcare systems. The most used natural source for herbal drug preparation is the medicinal plants. Therefore, quality assessment of herbal plants is necessary to validate their authenticity and efficacy in the treatment of respective traditional descriptions. For the standardisation of drugs, widely employed primary steps are pharmacognostic studies and phytochemical analysis which help to explore the bioactive compounds in the plant and its pharmacological activity and the preparation of new herbal drugs. Hence the current study was concentrated on the pharmacognostical, physico-chemical characterization and pesticidal activity of *B. mollis*.

Materials and Methods

Collection and authentication

The plant sample of *Blumea mollis* aerial part was collected in December 2023 from Kollam district, Kerala. The collected plants were identified and authenticated from the Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Chennai, Tamil Nadu and stored in raw drug museum of the Department (PCOG-BWP88).

Macro-morphology

Morphologically observed and documented with the naked eye and magnifying lens. Size was measured using ruler graduated in millimetres for the measurement of length, width and thickness of crude materials; the colours were observed under daylight; using a small portion of sample and a pinch of powder identified odour and taste[13].

Microscopy

The dried plant material was submerged in water for 2 hours and free hand sections were taken by standard procedures. The section were cut into thin transverse section using a sharp blade and the sections were stained with 0.4% Safranin and 0.4% Astra blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 Colour digital camera under bright field light. Magnifications were indicated by scale bar.

Histochemical studies

Plant sections were treated following the standard procedures[14]:

Crystals

The section was mounted in water and one end of the cover slip was irrigated with acetic acid. While looking through the microscope, the water within the cover slip was replaced using a piece of filter paper at the opposite end of the cover slip. Formation of air bubbles indicated Calcium carbonate crystals. If no air bubbles were formed, the experiment was repeated with conc. HCl, where in dissolution of crystal and formation of needles of Calcium sulphate indicated the presence of Calcium oxalate crystals.

Fats, Fatty oils volatile oils and resins

About 1 to 2 drops of Sudan-IV was added to the section and allowed to stand for a few minutes. Presence of fatty oil substances were indicated by orange-red/pink/red coloured globules; while red coloured irregular contents indicated resin.

Starch

A drop of iodine solution (2%) was added to the sections. Formation of blue coloured granules in the cells indicated presence of starch grains.

Phenolic compounds

A drop of ferric chloride solution was added to the section. Formation of bluish black coloured contents in the cells indicated presence of phenolic compounds like flavonoids/tannins etc.

Mucilage

A drop of reagent ruthenium red was added to the section. Formation of of pink to red coloured contents indicated presence of mucilage.

Lignified cell walls

A drop of phloroglucinol was added to the section and allowed to stand for about 2 min or until almost dry. A drop of 50% HCl was added to the section and observed the colour of cell walls after few minutes. Cell walls stained with pink to cherry red indicated presence of lignin.

Suberized or cuticular cell walls

A drop of reagent Sudan red III was added to the section and allowed to stand for a few minutes. Cell walls stained with orange-red or red indicated presence of suberin or cutin deposition over cell wall.

Alkaloids

A drop of Wagner's reagent was added - the presence of yellow to reddish brown coloured contents confirmed alkaloids.

Quantitative Microscopy

Leaf fragments of about 5 x 5 mm in size were placed in a test-tube containing about 5 mL of saturated aqueous solution of chloral hydrate and heated in water bath for 10-15 minutes. Then it was kept on slide and mount it in glycerin. Examine under a microscope with a 4x objective and a 10x eyepiece, equipped with a camera lucida. The quantitative parameters obtained during microscopic observation of epidermal peelings of leaves were recorded. The number of epidermal cells per mm^2, number of stomata per mm^2, stomatal index, leaf vein-islet and vein-termination number were determined based on standard pharmacopoeia procedures[15].

Powder microscopy

A pinch of the powdered sample was mounted on a microscopic slide with a drop of 75% glycerol after clearing with saturated solution of chloral hydrate[16]. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

Phytochemical extraction

About 30 g of coarse powder (Sieve 40) of dried *B. mollis* samples kept inside a thimble and loaded to the Soxhlet extractor. Methanol was used as the extraction solvent.

Phytochemical analysis

Qualitative phytochemical analysis of alkaloids, phenols, flavonoids, tannins, terpenoids, glycosides, saponins, steroids, quinones, and fatty acids was carried out as per standard pharmacopeial procedures[17].

Estimation of phenol

Total phenol was estimated using Folin-Ciocalteau reagent and 20% Na_2CO_3 as per the record of Meda *et al.*[18].

Estimation of flavonoids

Total flavonoids were estimated by colourimetric assay (aluminum chloride). The reaction mixture was prepared as per the standard procedure[19]. The absorbance was noted at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as µg of QE/mg of extract[19].

Estimation of alkaloid

Estimation of alkaloid were carried out as per the protocol given by Fazel *et al.*[20]. The absorbance were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer[20].

Estimation of terpenoids

Estimation of terpenoids were done using vanillin-glacial acetic acid (5%) and perchloric acid solution and absorbance was measured at 548 nm using a UV-visible spectrophotometer[21].

Estimation of tannins

The sample was subjected to colorimetric assay using Folin-Ciocalteu method and the absorbance was read at 700 nm[22].

Estimation of glycosides

Glycoside content was estimated using Baljet's reagent. The absorbance was measured at 495 nm in spectrophotometer[23].

TLC/HPTLC

All the chemicals and solvents used were AR grade (Merck). Plant material extracted with methanol visualizing the developed spots in TLC reagent containing 1 g of vanillin, 5 mL sulphuric acid and 95 mL ethanol (VSA) was used. The mobile phases used were *n*-Hexane and Ethyl acetate (8:2 *v/v*); aluminium plate (Merck) pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness was used. 15 µL of each sample were applied on track as 6 mm band and at 8 mm from the base of a 10 x 10 cm of pre-coated silica gel 60 F_{254} TLC plate (E. Merck) of 0.2 mm thickness by using automatic TLC applicator (ATS-4). The plate was developed in a suitable solvent system in a twin trough chamber till the solvent rises to a distance of 8 cm from the application position. The plate was observed through TLC Visualizer under UV at 254 and 366 nm and photos were documented. Finally, the plate was dipped in vanillin-sulphuric acid reagent and heated in a hot air oven at 105°C until the colour of the spots appeared and photo was documented under white light and the Rf values were recorded by the help of Vision CATS software.

Before derivatization the plate was scanned under UV at 254 and 366 nm using deuterium and mercury lamps respectively. After derivatization the plate was scanned at 540 nm using a tungsten lamp. The fingerprint data were recorded by Vision CATS software[13].

Pesticidal efficacy evaluation

A preliminary field trial was conducted at Krishi Vigyan Kendra Kollam, Kerala, India to evaluate the pesticidal efficacy of *B. mollis* during the year 2023. A pot culture experiment was laid out in a Completely Randomised Design (CRD) to test the pesticidal efficacy of *B. mollis* against sucking pest complexes using chilli as a test crop. Seven treatments were implemented, consisting of four botanical treatments (T1: 2% Neem oil emulsion, T2: 2% Pongamia oil, T3: 2% *Andrographis paniculata* leaf extract, and T4: 2% *B. mollis* leaf extract), two recommended doses of chemicals (T5: 3% Wettable sulfur and T6: Spiromesifen 22.9% SC 96 g ai/ha (active ingredient per hectare) + Thiamethoxam 25 WG 50 g ai/ha), and one control. Each treatment was replicated thrice. The botanicals and chemicals were prepared according to the following specified methods.

Soap solution

Six grams of ordinary bar soap was sliced into small pieces and dissolved in 100 mL of lukewarm water and was used for the preparation of one litre of various emulsions.

Neem oil emulsion (2%)

For the preparation of 1 L neem oil emulsion, 20 mL of neem oil was added to 100 mL soap solution as mentioned and made upto 1000 mL under constant agitation.

Pongamia oil emulsion (2%)

For the preparation of 1 L neem oil emulsion, 20 mL of neem oil was added to 100 mL soap solution as mentioned and made upto 1000 mL under constant agitation.

***Andrographis paniculata* extract (5%)**

For preparing 5% *A. paniculata* extract, 50 g of *A. paniculata* leaves were crushed in pestle and mortar and strained through muslin cloth. The extract was then mixed with 100 mL soap solution under constant agitation and final volume was made upto 1000 mL by adding water.

***B. mollis* extract (5%)**

For preparing 5% *B. mollis* extract, 50 g of *B. mollis* leaves were crushed in pestle and mortar and strained through muslin cloth. The extract was then mixed with 100 mL soap solution under constant agitation and final volume was made upto 1000 mL by adding water.

Wettable sulfur (3%)

For preparing the 3% solution, 3g of the dust formulation of wettable sulfur was weighed and dissolved in one litre of water by continuous agitation.

***Spiromesifen* 22.9% SC 96g *ai*/ha + *Thiamethoxam* 25 WG 50g *ai*/ha**

For preparing the combination pesticide spray solution, one mL of Spiromesifen 22.9% SC and 0.2 g of thiamethoxam 25 WG were mixed with 1000 mL of water under constant agitation.

Experiment layout

The experiment were conducted using Chilli seedlings of Ujwala variety, raised in pro trays upto 25 days old and transplanted into grow bags (35 x 20 x 20 cm) filled with a potting mixture prepared from sand, soil, and farmyard manure in a 1:2:1 ratio. The crop was cultivated following the package of practices recommended by Kerala Agricultural University (13 A). A consistent population of sucking pests, including aphids (*Aphis gossypii*) and mites (*Polyphagotarsonemus latus*), were maintained on these plants without any plant protection interventions. Three rounds of treatments were applied at 30, 60 and 90 days after planting coinciding with the vegetative and reproductive stage of the crop. Pretreatment observations on biometric, leaf curl symptoms and population of sucking pests were recorded.

Imposing treatments

Respective treatments were applied to the plant using a hand sprayer ensuring uniform coverage of both abaxial and adaxial surfaces of leaves. An untreated set of plants were maintained as check.

Assessment of growth parameters of chilli

The following biometric observations of the plants were recorded to assess the impact of different botanical treatments on plant growth, pest damage, and any other potential treatment effects.

Plant height/shoot length and internodal length

The growth parameters such as the shoot length and internodal length were measured using a measuring scale, and the mean length was calculated and expressed in centimetres. Observations were recorded at 40, 70, and 90 DAP (days after planting).

Yield

Per plant yield of the treatments were taken and expressed in kg/ha.

Observations on the incidence of pests

Pre and post treatment populations of sucking pests (mites and aphids) were observed on the day before the treatment application and on the 7th day after spraying. From each replication, one leaf each was selected from top, middle and bottom of the stem at random to assess the pest population. The count of aphids and mites were taken from both surfaces of the leaves using a hand lens and expressed as numbers per leaf. Populations of mites and aphids were subjected to statistical analysis. Three plants were selected randomly in each replication and scored for leaf curl symptoms of sucking pest complexes visually by following the standard scoring procedure as described by Niles (1980)[24] and is depicted below (Table 1).

The leaf curl index was calculated using the equation below[25].

$$\text{Leaf curl index} = \sum \frac{\text{Score} \times \text{Number of plant in that score}}{\text{Total number of plants}}$$

Further the data on biometric observations; plant height, internodal length and yield and observations on the incidence of pests in the field were statistically analysed.

Results

Morphology

Leaves

Fresh leaf arranged in alternate, up to 15 cm long and up to 4 cm width, petiolate up to 4 cm long and up to 2.5 mm width, oblanceolate with serrate margin, lateral vein alternate, venation reticulate, adaxial side having more numbers of long white trichomes in younger leaf and less in abaxial side, tender leaves pubescent; dried leaf upper surface is dark greyish brown and lower surface velvet brown in colour; surface rough, fracture crumble; odour aromatic, taste slightly salt with pungent.

Table 1 — Score for the calculation of leaf curl index

Score	Category	Symptom
0	No damage	No symptom
1	Less damage	1 to 25% leaves/plant show curling
2	Moderate damage	26 to 50% leaves/plant show curling
3	Heavy damage	51 to 75% leaves/plant show curling, heavily damaged, malformation of growing points and reduction in plant height
4	Complete damage	> 75% leaves/plant show curling, severe and complete destruction of growing points, drastic reduction in plant height, defoliation and severe malformation

Stem

Young fresh stem long and cylinder, 2 cm in width; dried stem 2 to 4 mm in width and longitudinal wrinkles, fracture short, greyish brown in colour. odour aromatic, taste slightly mucilaginous (Fig. 1).

Anatomy

Stem

Diagrammatic TS of stem shows circular in outline; epidermis bears numerous covering and glandular trichomes; beneath the epidermis is narrow collenchymatous hypodermis and parenchymatous cortex, centrally located wide zone of parenchymatous pith encircled by a band of phloem and discontinuous group of xylem elements caped by group of sclerenchymatous pericyclic fibre and thin walled xylem fibre on lower side (Fig. 2a).

Detailed TS shows, squire to rectangular thin-walled, single layered epidermis covered by thin cuticle with numerous uniseriate, multi-cellular, covering and multicellular head glandular trichomes; followed by a narrow band of 2 or 3 layer of lamellar collenchymatous hypodermis broken at place of air cavities, and 4 to 8 cells layer of thin walled parenchymatous cortex consists oval to circular cells with intercellular space underneath this lie collateral open vascular bundle consists a ring of phloem tissue with usual elements capped with discontinues group of lignified, thick-wall with narrow lumen pericyclic fibres; cambium distinct, discontinues group or together of xylem consists, endarch with usual elements, xylem fibre and tracheids are thin-wall, lignified with wide lumen; central wide zone of thin-walled parenchymatous pith embedded with rosette crystals of calcium oxalate, oil globule and a few simple starch grains (Fig. 2b).

Petiole

Diagrammatic TS shows slightly convex on upper side and broadly convex on lower side with wing projection on each margin of upper side; epidermal cells with numerous trichomes followed by a band of thick-walled hypodermis, thin walled cortex and an arc of discontinuous group of bicollateral vascular bundles arranged bigger in centre and smaller on the edges of the wing embedded in ground tissue.

Detailed TS shows single layer of epidermis embedded with brownish content and oil globules, epidermis bearing uniseriate, multi-cellular stalk with covering, uni and multicellular head glandular trichomes with oil like content followed by two layers of

Fig. 1 — *Blumea mollis* Merr. Dried aerial part.

collenchymatous hypodermis on both the epidermis. Cortex having thin-walled parenchymatous tissue with brownish content and oil globules, wide zone of parenchymatous ground tissue embedded with pitted parenchyma, bicollateral vascular bundle, rosette crystals, brownish content and oil globules; vascular bundle consists group of radically arranged xylem vessels with uniseriate medullary rays caped on both side thin walled phloem tissue with usual elements and thick walled lignified pericyclic fibres (Fig. 3a).

Leaf

Detailed TS of the leaf passing through midrib shows bi-convex, slightly on upper and broadly on lower; both the epidermii are single layered and bears uniseriate, multi-cellular stalk with covering, uni and multicellular head glandular trichomes covered by thin cuticle; in the midrib region both the epidermis is followed by two layer of lamellar collenchymatous hypodermis continued on each side thin walled parenchymatous tissue embedded with brownish content and oil globule; ground tissue consists of thin walled parenchyma cells with intercellular spaces embedded with bicollateral vascular bundle formed of group of radically arranged xylem vessels with uniseriate medullary rays, caped on both side thin walled phloem tissue with usual elements and thick walled lignified pericyclic fibres enclosed with a layer of bundle sheath tissue (Fig. 3b).

Lamina - shows single layer of upper and lower epidermal cells of them bigger in size an upper, epidermis bearing few covering and glandular trichomes covered by externally with thin cuticle and embedded with stomata they being few in the upper and more on the lower side; underneath the upper epidermis lies a layer of palisade cells embedded with brownish content; the remaining cells of the mesophyll composed 3 to 6 rows of spongy parenchyma tissue embedded with rosette crystals of calcium oxalate, oil globule and centrally located obliquely cut vascular bundles; a row of lower mesophyll tissue embedded with brownish content air cavities and mucilage contents (Fig. 3c).

Quantitative microscopy

The quantitative analysis of mature leaves shows upper and lower epidermis having wavy epidermal walls within anisocytic and few anomocytic stomata;

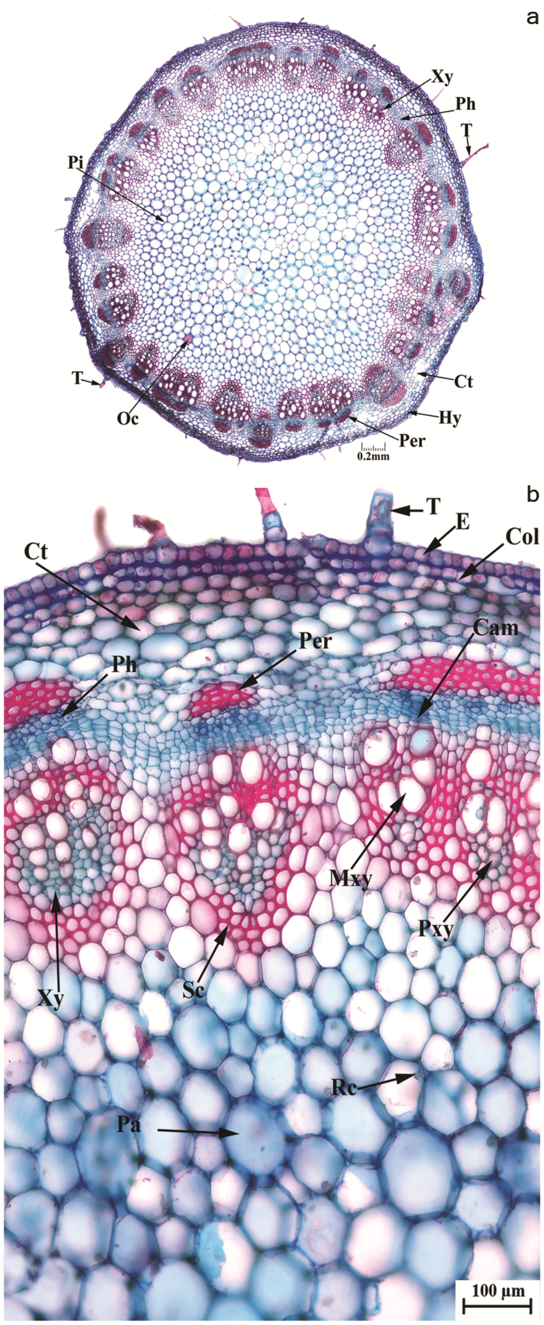


Fig. 2 — TS of *B. mollis* stem. a) Diagrammatic view, and b) Detailed TS of stem. Cam - cambium; Col - collenchyma; Ct - cortex; E - epidermis; Hy - hypodermis; Mxy - meta xylem; Oc - oil cell; Pa - parenchyma; Per - pericycle; Ph - phloem; Pi - pith; Pxy - protoxylem; Rc - rosette crystals; Sc - sclerenchyma cells; T - trichome; Xy - xylem.

comparatively very less number of stomata distributions was observed in upper epidermis. The stomatal number, stomatal index, epidermal numbers, palisade ratio, vein isolate and vein termination numbers indicated in Table 2 (Fig. 4).

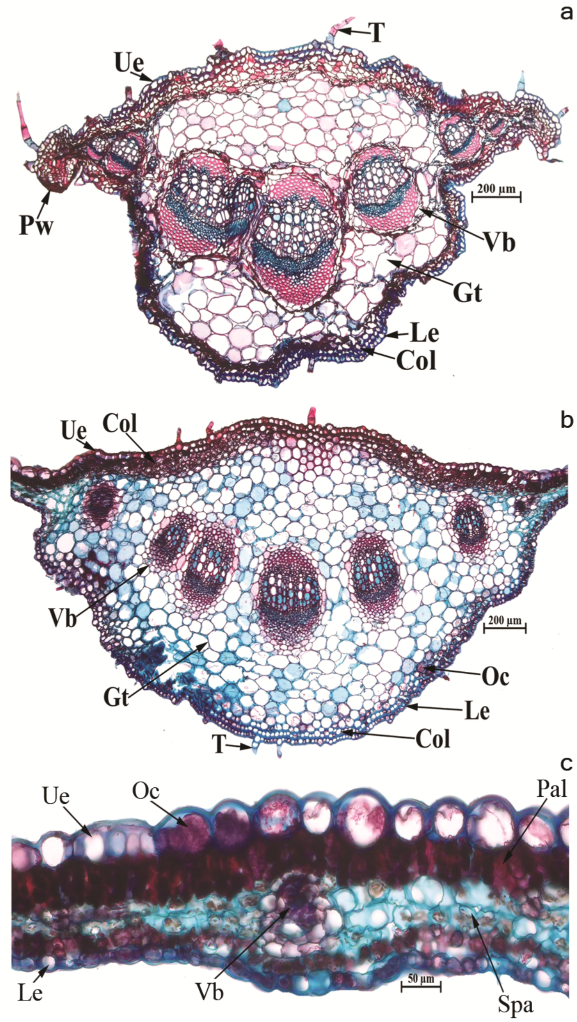


Fig. 3 — Detailed TS of *B. mollis* leaf. a) TS of petiole, b) TS of midrib, and c) TS of lamina. Col - collenchyma; Gt -ground tissue; Le - lower epidermis; Oc - oil cell; Pal - palisad parenchyma; Pw - projection wing; Spa - spongy parenchyma; T - trichome; Ue - upper epidermis; Vb - vascular bundle.

Table 2 — Quantitative microscopy of *B. mollis*

Parameters	Upper epidermis (/mm^2)	Lower epidermis(/mm^2)
Epidermal number	476 – 504	930 – 950
Stomatal Number	78 – 88	220 – 245
Stomatal index	14 – 15	19 – 21
Palisade ratio	10 – 14	
Vein islets	16 – 20	
Vein termination	11 – 14	

Powder microscopy

Green colour powder shows uniseriate, multicellular covering and multicellular head glandular trichomes; leaf upper and lower epidermis in surface view with anisocytic and anomocytic stomata; vessels with border pits, reticulate, spiral and

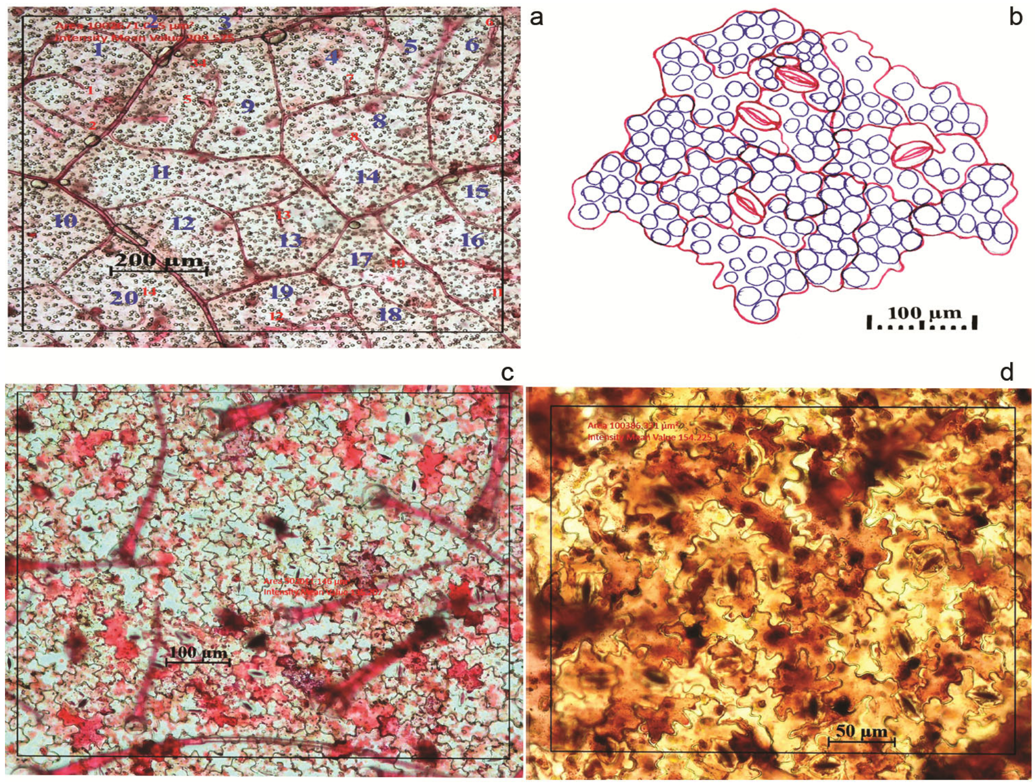


Fig. 4 — Quantitative microscopy of *B. mollis* leaf. a) Vein islet and Vein termination, b) Palisade ratio, c) Upper epidermis, and d) Lower epidermis.

annular thickenings; fragment of pitted parenchyma cells, lignified stone cells, thick-walled pericyclic fibres, thin-walled xylem fibre, sectional view of lamina with trichomes and rosette crystals of calcium oxalate (Fig. 5).

Histochemistry

Stem

Cutin present on epidermis; mucilage found in cortex; lignin observed in xylem; alkaloids and phenolic compounds detected in pericycle; oil globules found in epidermis and trichomes; resins not observed (Fig. 6.1).

Petiole

Cutin present in epidermis; mucilage found in cortex; lignin observed in xylem; alkaloids, phenolic compounds and oil globules detected in pericycle; resins absent in petiole (Fig. 6.2).

Midrib

Cutin present in epidermis; mucilage and oil globules found in ground tissue; lignin observed in xylem; alkaloids and phenolic compounds detected in pericycle; resins absent in petiole (Fig. 6.3).

Qualitative phytochemical analysis

Phytochemical analysis of the plant extract of *B. mollis* showed presence of phenols, tannins, flavonoids, saponins, terpenoids, fatty acids and alkaloids. Steroids and quinones are found to be absent (Table 3). The quantitative analysis revealed the concentrations of phytocompounds present in the extracts (Table 4). Alkaloids are found in more concentration (74.89 µg/mg) and glycosides detected with least concentration (12.13 µg/mg).

TLC/HPTLC

The TLC plate of methanol extracts, under UV 254 nm showed 9 spots. Under UV 366 nm 14 spots were seen. When viewed under UV 540 nm soon after dipping in VSR, 15 spots, the R_f Value indicate in Table 5. The HPTLC Densitometric scan at 254 nm showed 9 peaks, with R_f 0.79 was the major peak with the area of 22.32 % in the latter and 0.36 with 19.77% in the former (Fig. 7a). Densitometric scan at 366 nm showed 8 peaks respectively, spot with R_f 0.77 was the major peak with the area of 22.83% in the latter and 0.33 with 19.81% in the former (Fig. 7b). Densitometric scan at 520 nm (post derivatization

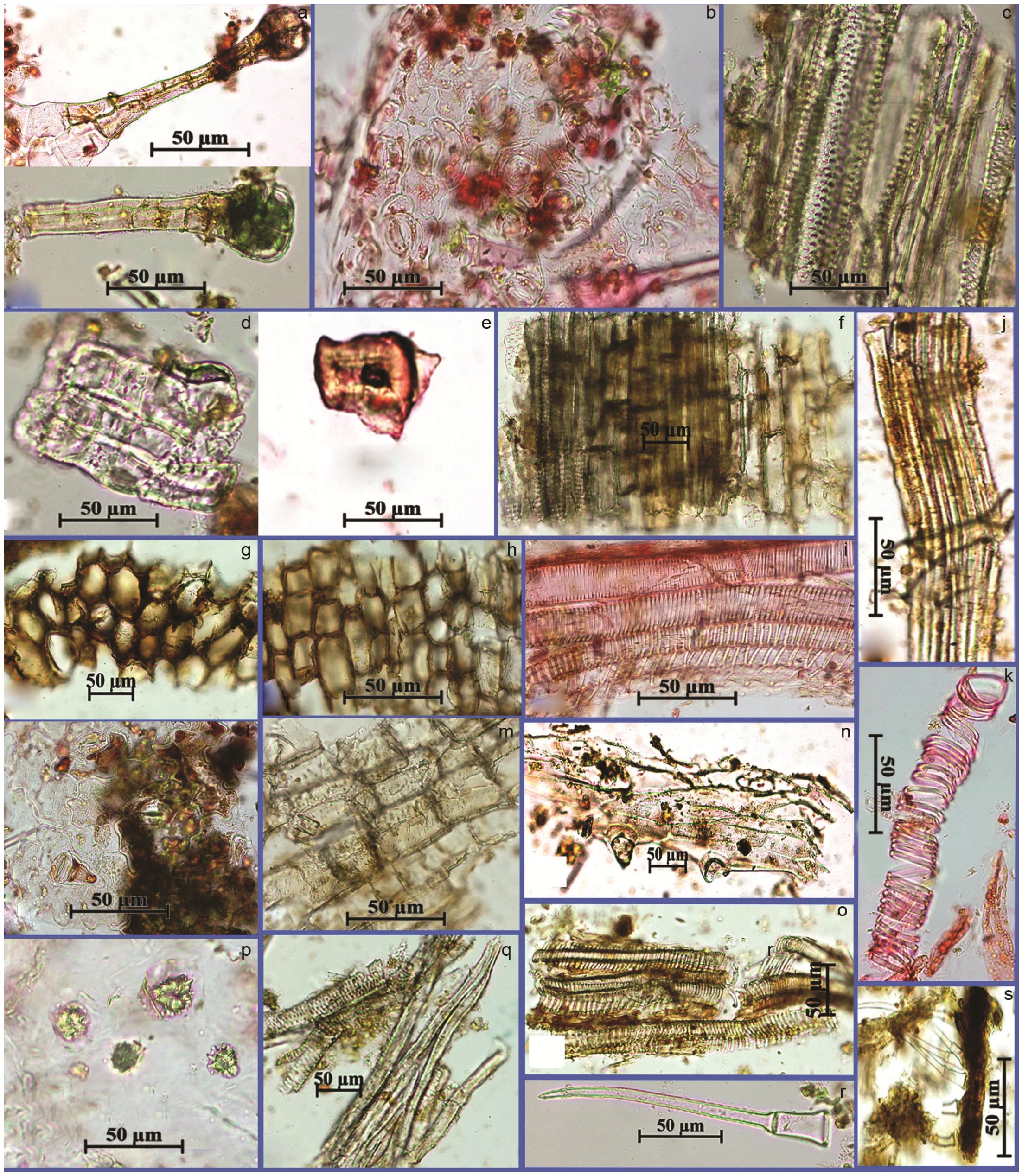


Fig. 5 — Powder microscopy of *B. mollis* aerial parts. a) multicellular head glandular trichomes, b) lower epidermis in surface view, c & f) tangential longitudinally cut stem vascular tissue shows border pitted vessels associated with other usual elements of xylem, d & e) lignified cells, g) parenchymatous cells, h) tangential longitudinally cut parenchyma tissue, i & o) tangential longitudinally cut petiole and midrib vascular tissue shows reticulated, spiral and annular thickening vessels, j) tangential longitudinally cut group of thick-walled pericyclic fibres, k) spiral vessels, l) upper epidermis in surface view, m) pitted parenchyma, n) stem epidermis with trichome in surface view, p) rosette crystals of calcium oxalate, q) thin-walled xylem fibre, r) covering trichome, and s) sectional view of lamina with trichomes.

with VSA) showed 12 peaks, spot R_f 0.35 were the major area 18.15% (Fig. 7c).

Effect of *B. mollis* on growth of chilli

Biometric parameters

The influence of *B. mollis* application on the growth attribute of the plant *viz*., plant height, internodal length and fruit yield per plant at different intervals along with other botanicals and chemical check are presented in Table 6.

Immediately after first spray at 30 days after planting (DAP), there was no significant differences observed in internodal length and plant height among the treatments. This suggests that the effect of *B.*

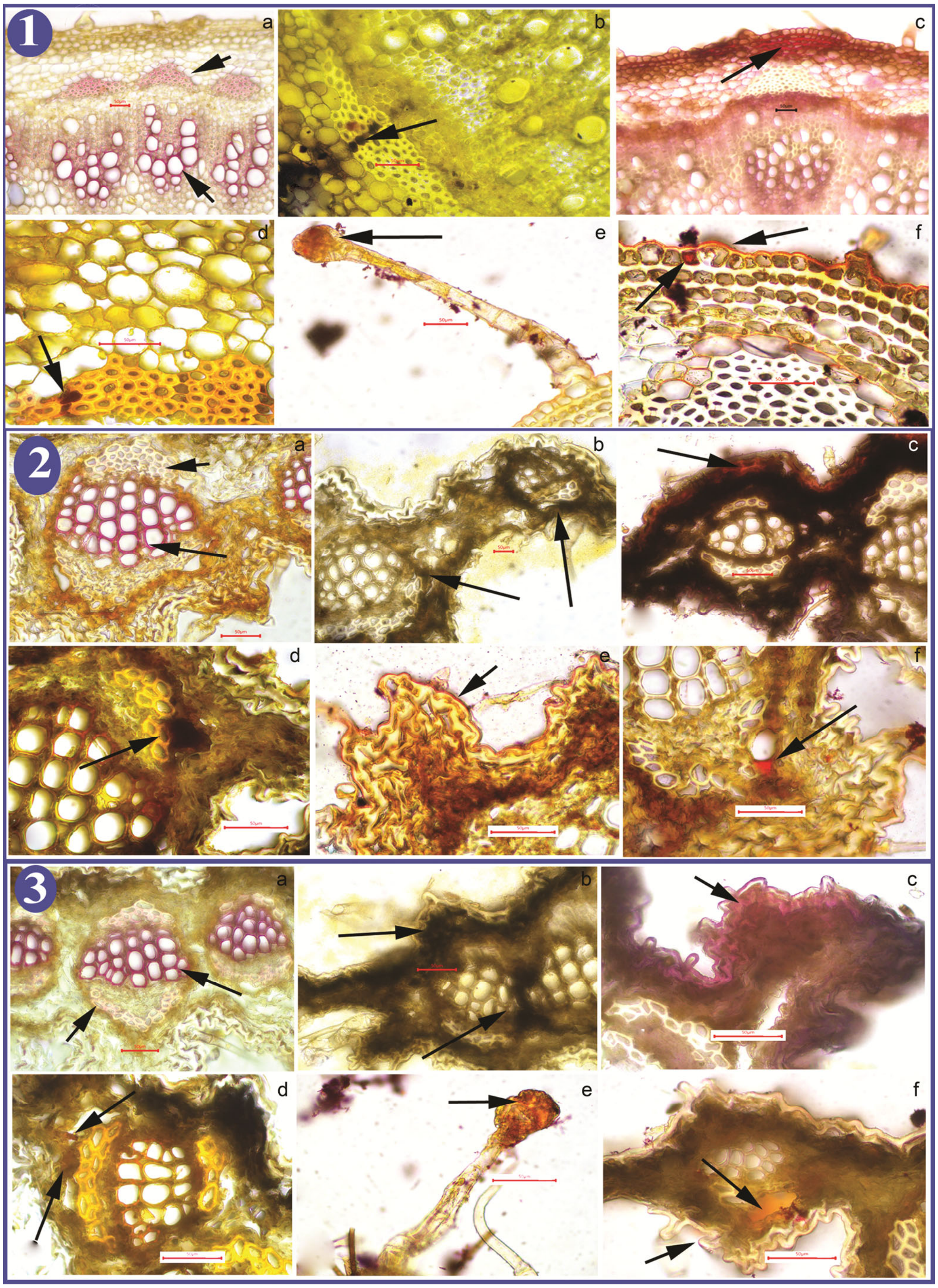


Fig. 6 — Histochemistry of *B. mollis*. 1. Stem: a) lignin, b) phenolic compounds, c) mucilage; d. alkaloids, e) oil globules, f) cutin and oil globules. 2. Petiole: a) lignin, b) phenolic compounds, c) mucilage, d) alkaloids, e) cutin, and f) oil globules. 3. Midrib: a) lignin, b) phenolic compounds, c) mucilage, d) alkaloids, e) oil globules, and f) thin cutin and oil content.

mollis on the growth attributes of chilli plants did not show an immediate effect on the early growth stages of the plants.

However, the second spray at the vegetative stage (60 DAP), the effects of the treatments became evident. The treatment involving spraying at 30 DAP followed by subsequent applications at 60 and 90 DAP resulted in the highest recorded internodal length of 2.81 cm and plant height 41.6 cm. These measurements surpassed those of all other treatments, including both the chemical control and other botanical treatments. This indicates that the treatment regimen exerted a positive influence on the vegetative growth of the plants during this stage.

Table 3 — Qualitative phytochemical analysis of *B. mollis*

Test	*B. mollis*
Phenol	+
Tannin	+++
Flavonoid	+
Saponin	++
Terpenoids	+
Alkaloid	+
Glycoside	-
Steroid	-
Quinones	-
Fatty acid	+

+ positive; - negative

Table 4 — Quantitative phytochemical analysis of *B. mollis*

Name of test	Wavelength (nm)	Concentration (μg/mg)
Phenol	750	23.81
Tannin	700	58.49
Flavonoid	510	18.33
Terpenoids	548	47.55
Alkaloid	470	74.89
Glycoside	495	12.13

The cumulative effect of all three treatment applications, spanning from 30 to 90 DAP, further enhanced growth parameters. Specifically, the internodal length increased to 3.05 cm, and the plant height reached 71.53 cm. These results highlight the synergistic effect of multiple applications over the growth period, resulting in superior growth compared to single or fewer applications.

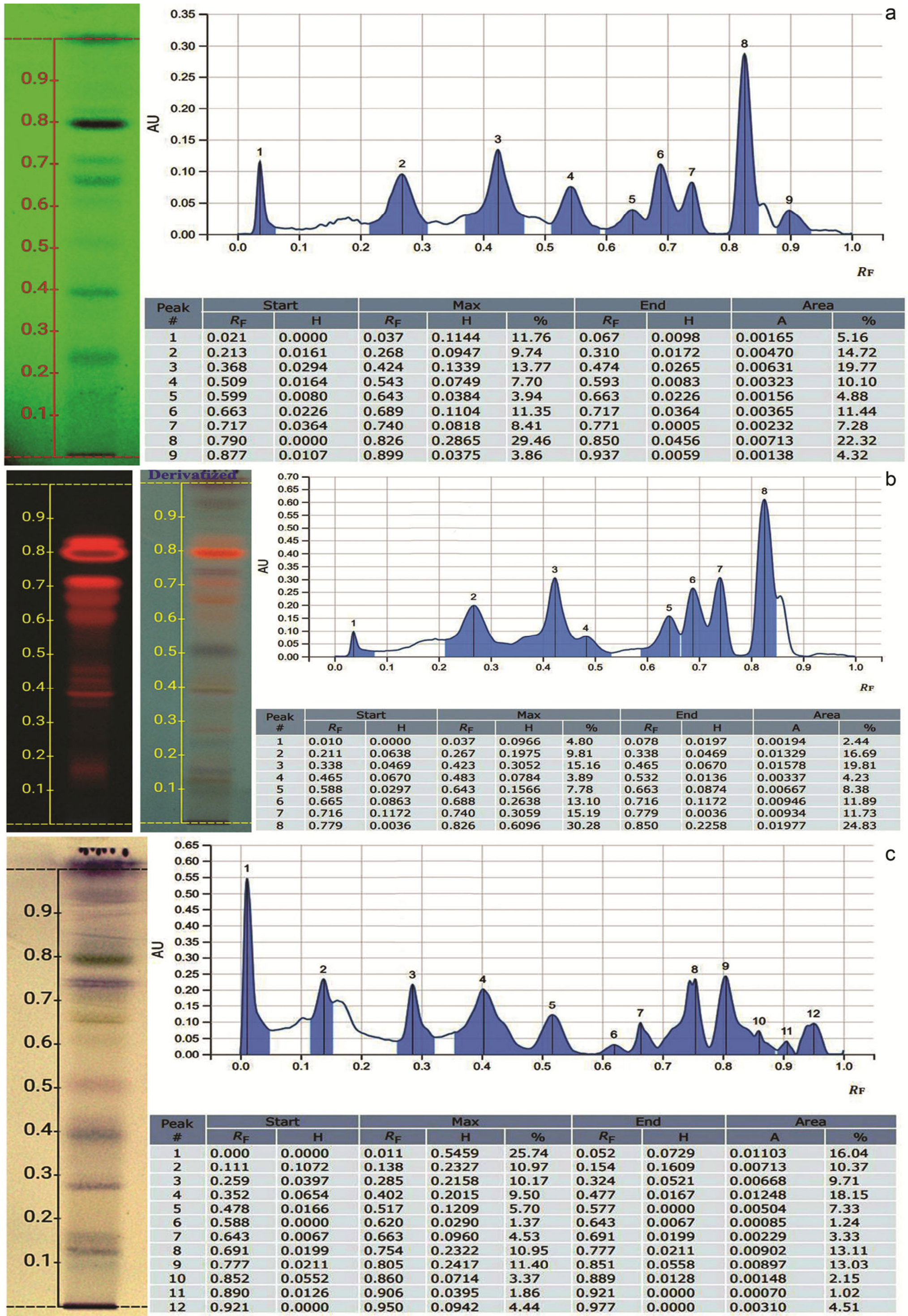
Evaluation of pesticidal efficacy of *B. mollis*

Effect of B. mollis on damage by sucking pest complex (leaf curl index)

During the vegetative and reproductive stages of the experiment, the effectiveness of the chemical treatment (Spiromesifen 22.9% SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha) in reducing damage by the sucking pest complex was evident, with leaf curl indices (LCI) measured at 0.6, 0.73, and 0.3 at 30, 60, and 90 days after planting (DAP), respectively. While this chemical treatment proved highly effective, attention was directed towards evaluating the efficacy of *B. mollis* as a botanical alternative.

Table 5 — TLC profile of *B. mollis*

UV 254 nm		UV 366 nm		UV 540 nm	
R_f	Colour	R_f	Colour	R_f	Colour
-	-	-	-	0.10	Gray
-	-	0.13	Red	0.13	Pink
0.15	Green	-	-	-	-
-	-	-	-	0.16	Gray
0.24	Green	0.24	Red	-	-
-	-	-	-	0.27	Pinkish blue
-	-	0.36	Pink	-	-
0.39	Green	0.39	Red	0.39	Pinkish blue
-	-	0.41	Red	-	-
-	-	-	-	0.42	Gray
-	-	0.43	Red	-	-
-	-	0.46	Red	-	-
-	-	-	-	0.50	Pink
0.51	Green	-	-	-	-
-	-	0.55	Red	-	-
-	-	0.61	Red	0.61	Gray
0.62	Green	-	-	-	-
0.66	Green	0.66	Red	0.66	Green
0.71	Dark Green	0.71	Red	0.71	Gray
-	-	-	-	0.74	Pink
-	-	-	-	0.79	Green
-	-	0.80	Red	-	-
0.82	Green	-	-	-	-
-	-	0.84	Red	0.84	Gray
-	-	-	-	0.89	Gray
-	-	-	-	0.94	Gray
-	-	0.95	Red	-	-
0.98	Green	-	-	-	-



Peak #	Start R_F	Start H	Max R_F	Max H	Max %	End R_F	End H	Area A	Area %
1	0.021	0.0000	0.037	0.1144	11.76	0.067	0.0098	0.00165	5.16
2	0.213	0.0161	0.268	0.0947	9.74	0.310	0.0172	0.00470	14.72
3	0.368	0.0294	0.424	0.1339	13.77	0.474	0.0265	0.00631	19.77
4	0.509	0.0164	0.543	0.0749	7.70	0.593	0.0083	0.00323	10.10
5	0.599	0.0080	0.643	0.0384	3.94	0.663	0.0226	0.00156	4.88
6	0.663	0.0226	0.689	0.1104	11.35	0.717	0.0364	0.00365	11.44
7	0.717	0.0364	0.740	0.0818	8.41	0.771	0.0005	0.00232	7.28
8	0.790	0.0000	0.826	0.2865	29.46	0.850	0.0456	0.00713	22.32
9	0.877	0.0107	0.899	0.0375	3.86	0.937	0.0059	0.00138	4.32

Peak #	Start R_F	Start H	Max R_F	Max H	Max %	End R_F	End H	Area A	Area %
1	0.010	0.0000	0.037	0.0966	4.80	0.078	0.0197	0.00194	2.44
2	0.211	0.0638	0.267	0.1975	9.81	0.338	0.0469	0.01329	16.69
3	0.338	0.0469	0.423	0.3052	15.16	0.465	0.0670	0.01578	19.81
4	0.465	0.0670	0.483	0.0784	3.89	0.532	0.0136	0.00337	4.23
5	0.588	0.0297	0.643	0.1566	7.78	0.663	0.0874	0.00667	8.38
6	0.665	0.0863	0.688	0.2638	13.10	0.716	0.1172	0.00946	11.89
7	0.716	0.1172	0.740	0.3059	15.19	0.779	0.0036	0.00934	11.73
8	0.779	0.0036	0.826	0.6096	30.28	0.850	0.2258	0.01977	24.83

Peak #	Start R_F	Start H	Max R_F	Max H	Max %	End R_F	End H	Area A	Area %
1	0.000	0.0000	0.011	0.5459	25.74	0.052	0.0729	0.01103	16.04
2	0.111	0.1072	0.138	0.2327	10.97	0.154	0.1609	0.00713	10.37
3	0.259	0.0397	0.285	0.2158	10.17	0.324	0.0521	0.00668	9.71
4	0.352	0.0654	0.402	0.2015	9.50	0.477	0.0167	0.01248	18.15
5	0.478	0.0166	0.517	0.1209	5.70	0.577	0.0000	0.00504	7.33
6	0.588	0.0000	0.620	0.0290	1.37	0.643	0.0067	0.00085	1.24
7	0.643	0.0067	0.663	0.0960	4.53	0.691	0.0199	0.00229	3.33
8	0.691	0.0199	0.754	0.2322	10.95	0.777	0.0211	0.00902	13.11
9	0.777	0.0211	0.805	0.2417	11.40	0.851	0.0558	0.00897	13.03
10	0.852	0.0552	0.860	0.0714	3.37	0.889	0.0128	0.00148	2.15
11	0.890	0.0126	0.906	0.0395	1.86	0.921	0.0000	0.00070	1.02
12	0.921	0.0000	0.950	0.0942	4.44	0.977	0.0000	0.00310	4.51

Fig. 7 — TLC plate and HPTLC densitogram of methanol extract of aerial parts of *B. mollis*. a) 254 nm, b) 366 nm, and c) 540 nm.

Vegetative stage (30 dap)

Initial observations of LCI did not reveal significant differences among treatments, with values ranging from 2.87 to 3.2, indicating consistent damage intensity before treatment application. Post-treatment assessment at 30 DAP showed that *B. mollis* extract at 5% concentration exhibited the lowest LCI among botanical extracts, recording 1.6. This result was comparable to *Andrographis paniculata* extract at 5% (LCI of 2) but significantly

Table 7 — Effect of *B.mollis* on leaf curl index at different stages

Treatments	Leaf curl index (LCI) at different days after planting (DAP)					
	30 DAP		60 DAP		90 DAP	
	Precount	7 DAS	Precount	7 DAS	Precount	7 DAS
Neem oil emulsion 2%	3.00	2.63[b]	3.10[b]	3.20[b]	3.50[a]	3.87[a]
Pongamia oil emulsion 2%	3.00	2.87[b]	3.20[ab]	3.10[b]	3.00[b]	3.30[b]
Andrographis extract 5%	2.87	2.00[c]	2.10[c]	2.00[c]	3.00[b]	2.30[c]
Blumea extract 5%	3.00	1.60[cd]	1.73[d]	1.20[d]	1.93[c]	0.87[e]
Wettable Sulfur $3g^{-1}$ + NO 2%	3.20	1.50[d]	1.60[d]	1.30[d]	1.60[d]	1.30[d]
Spiromesifen 22.9 % SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha	3.10	0.60[e]	1.20[e]	0.73[e]	1.00[e]	0.30[f]
Untreated control	3.20	3.43[a]	3.40[a]	3.73[a]	3.40[a]	4.00[a]
CD (0.05)	NS	0.454	0.298	0.452	0.259	0.354

DAP - Days after planting DAS - Days after spraying

Table 8 — Effect of *B. mollis* on Aphid (*Aphis gossypii* Glover) population

Treatments	Population of aphids/leaf at different growth stages					
	30 DAP		60 DAP		90 DAP	
	Precount	7 DAS	Precount	7 DAS	Precount	7 DAS
Neem oil emulsion 2%	29.87	24.63[b]	30.87[b]	26.77[b]	32.10[b]	32.40[c]
Pongamia oil emulsion 2%	29.73	24.40[b]	31.10[b]	27.63[b]	36.40[ab]	36.10[b]
Andrographis extract 5%	29.53	24.73[b]	32.07[b]	27.87[b]	36.97[a]	35.40[bc]
Blumea extract 5%	30.87	10.40[d]	19.85[c]	15.73[d]	16.40[d]	12.97[d]
Wettable Sulfur $3g^{-1}$ + NO 2%	29.50	16.63[c]	22.08[c]	19.87[c]	22.30[c]	11.87[d]
Spiromesifen 22.9 % SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha	31.10	4.50[e]	10.81[d]	3.97[e]	4.91[e]	1.40[e]
Untreated control	31.53	34.77[a]	37.73[a]	40.17[a]	37.40[a]	41.50[a]
CD (0.05)	**NS**	**3.914**	4.233	**3.742**	**4.337**	**3.566**

*Mean of 3 replications comprising 3 plants each DAS: Days After Spraying; DAP: Days after planting

lower than both the chemical check and wettable sulphur treatments.

Reproductive stage (60 and 90 DAP)

Spray applications were conducted at 60 and 90 DAP, with persistent pest load noted after the initial spraying. Notably, at 60 DAP, *B. mollis* demonstrated an LCI of 1.2, similar to wettable sulfur (1.3) and significantly lower compared to other treatments. By 90 DAP, *B. mollis* exhibited its effectiveness with continuous application with the second-best LCI of 0.87, following closely behind the chemical treatment (LCI of 0.3) significantly outperforming all other treatments. Wettable sulphur recorded a higher LCI of 1.3, indicating more substantial damage compared to *B. mollis*.

Effect of B. mollis on sucking pests of chilli

Population of the sucking pests *viz*., aphid (*Aphis gossypii* Glover) and yellow mites (*Polyphagotarsonemus latus* Banks) subsequent to the application of treatments undertaken at 30 DAP were recorded before treatment application and 7 days after treatment and are presented in Table 7.

Effect of B. mollis on aphid (Aphis gossypii Glover) population

During the vegetative and reproductive stages of the crop, the pre-count observations did not show significant differences in aphid populations, indicating uniformity across the treatments. At 30 days after planting (DAP), the chemical check using Spiromesifen 22.9% SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha recorded the lowest aphid population of 4.5 aphids/leaf at 7 days after treatment, significantly outperforming all other treatments. *B. mollis* treatment recorded 10.4 aphids/leaf, proving to be the second most effective treatment and superior to other botanicals such as Pongamia oil 2% (24.4 aphids/leaf), Neem oil 2% (24.63 aphids/leaf) and Andrographis extract 5% (24.73 aphids/leaf). One week after spraying at 60 DAP, *B. mollis* exhibited the lowest aphid population among botanical treatments, with 15.73 aphids/leaf. However, the chemical check maintained the lowest population at 3.97 aphids/leaf at 90 DAP. The aphid populations in plants treated with *B. mollis* and wettable sulfur were comparable, recording 12.97 and 11.87 aphids/leaf, respectively (Table 8).

Table 9 — Effect of *B.mollis* on yellow mite (*Polyphagotarsonemus latus* Banks) population

Treatments	Population of mite leaf					
	30 DAP		60 DAP		90 DAP	
	Precount	7 DAS	Precount	7 DAS	Precount	7 DAS
Neem oil emulsion 2%	28.53	25.07^{a}	28.53^{a}	23.83^{b}	24.63^{b}	22.87^{b}
Pongamia oil emulsion 2%	28.97	26.30^{a}	30.27^{a}	23.40^{b}	19.53^{c}	17.07^{c}
Andrographis extract 5%	27.30	23.53^{ab}	29.97^{a}	24.33^{b}	23.27b^{c}	20.17^{bc}
Blumea extract 5%	28.87	11.67^{c}	22.73^{b}	17.97^{c}	24.87b^{c}	7.40^{d}
Wettable Sulfur 3g^{-1} + NO 2%	28.53	19.93^{b}	22.40^{b}	16.33^{c}	21.20b^{c}	6.07^{d}
Spiromesifen 22.9 % SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha	29.73	3.53^{d}	7.88^{c}	4.87^{d}	6.67^{d}	1.40^{e}
Untreated control	27.97	28.27^{a}	29.63^{a}	32.30^{a}	29.87^{a}	31.60^{a}
CD (0.05)	**NS**	**4.859**	**3.583**	**3.085**	**4.896**	**4.170**

*Mean of 3 replications comprising 3 plants each DAS: Days After Spraying; DAP : Days after planting

Effect of B. mollis on yellow mite (Polyphagotarsonemus latus Banks) population

The pre-count mite population remained consistent during the vegetative stage and did not show significant differences among treatments. At 30 days after planting (DAP), the chemical check using Spiromesifen 22.9% SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha recorded the lowest mite population of 3.53 mites/leaf. Among all other treatments, *B. mollis* extract at 5% concentration showed the lowest population with 11.67 mites/leaf, significantly different from all other treatments. Seven days after spraying at 60 DAP, the mite populations in plants treated with *B. mollis* (17.97 mites/leaf) and wettable sulfur (16.33 mites/leaf) were comparable. All other botanical treatments were less effective than *B. mollis* and recorded higher mite populations. By 90 days after planting (DAP), a similar trend was observed, where the chemical check registered significantly lowest mite population (1.4 mites/leaf). This was followed by Wettable sulfur and *B. mollis*, with 1.4, 6.07 and 7.4 mites/leaf, respectively. Spiromesifen + Thiamethoxam was significantly superior, while *B. mollis* and wettable sulfur treatments were comparable (Table 9).

Discussion

The taxonomic identification of the largest genus *Blumea* is found to be difficult due to lack of diagnostic characters between about 100 species. Many new species are being added to this genus even though there is a limited availability of herbarium specimens, less data on deep details of morphological and anatomical features. This approach of taxonomic treatment has a great impact on *Blumea* classification leading to controversy. Pharmacognostic studies of plants will help identification and characterisation at species level. Many of the *Blumea* species are morphologically similar and cannot identify in their dried form. Since the herbs collected for preparation of traditional medicines are mostly in dried form the authentication of this plant is very difficult. The pharmacognostic studies of *B. mollis* can act as valid scientific data for its identification process.

In pharmacognosy studies, microscopic analysis of drugs plays an important role in authentication. Data collected by anatomical studies, powder microscopy and histochemical tests can be used significantly for the exact authentication process of herbal drugs. Systematic analysis of anatomical features at cellular level, quantitative and qualitative macro and microscopic characters will assist even the differentiation of closely related species and strengthen the taxonomic identification between market samples. The qualitative phytochemistry and TLC studies are necessary for herbal drug standardisation along with microscopic identification. Pharmacopoeias on herbal drugs emphasize the use of TLC for the identification of raw drugs procured from the market before using for formulations[26].

The present study has unveiled the diagnostic characters of *B. mollis* aerial parts such as uniseriate multicellular covering trichomes, multicellular glandular trichomes, anisocytic and anomocytic stomata with stomatal index 14 - 15 in upper epidermis and 19 - 21 in lower epidermis and palisade ratio 10 - 14, lignified stone cells, rosette crystals, etc. The histochemical studies confirmed the presence of mucilage, alkaloids, lignin, phenolic compounds and oil globules. This is the first comprehensive pharmacognostic study of *B. mollis* stem and leaf even though few studies reported earlier like Prasanth *et al.*[27] reported the characteristic features of *B. mollis* leaves. The quantitative parameters recorded in their study can be correlated to the present study with slight variations.

Compared to their study the morpho anatomical features and powder microscopic analysis in this study revealed more diagnostic features.

The phytochemical and TLC studies of *B. mollis* aerial parts showed similar results as that of previous studies. The presence of alkaloids, phenolics and tannins, saponins, etc., confirmed by Sreelekha, *et al.*[28]. The other compounds such as flavonoids, steroids, terpenes were recorded by Jyothilakshmi *et al.*[29] is similar to the findings of present study. The HPTLC profiling of *B. mollis* showed 3 highest peak area at 254 nm such as 14.72, 19.77, and 22.32% with R_f values 0.268, 0.424, and 0.826, respectively; 3 highest peak area at 366 nm such as 16.69% with R_f value 0.267, 19.81% with R_f value 0.423 and 24.83% with R_f value 0.826 and two highest peak areas at 540 nm, 16.04 and 18.15% with R_f values 0.011 and 0.754, respectively.

The occurrence of diverse phytochemical compounds gives the bioactive potential of the traditional plant *B. mollis*. Since only few bioactivity studies have been reported so far, this study can promote the exploration of therapeutic value of this medicinal plant. Presence of phenol and tannin is found to be the key compounds for antioxidant, anti-inflammatory, cytotoxic and larvicidal activities.

The pesticidal efficacy of *B. mollis* proved in the present study clearly depicts the reduced damage symptom due to the sucking pests and lower population dynamics of pests *viz, Aphis gossipii* and *Polyphagotarsonemus latus* which throws a light towards the pesticidal efficacy of *B. mollis* in chilli crop. The trial consisted of commonly used botanicals like Neem, *Pongamia*, *Andrographis*, *Blumea* and chemical check. Among the botanical pesticide, *B. mollis* extract at 5% was proved to be a potential candidate with a lower LCI of 1.6 and was comparable with that of *Andrographis paniculata* leaf extract 5% demonstrating its superior efficacy which is comparable to conventional botanicals like Neem oil and pongamia oil. This initial efficacy suggests the potential of *B. mollis* as a botanical alternative, warranting further investigation into its efficacy over time. After imposing a second round of application of treatments at 60 DAP, *B. mollis* maintained its superior performance among botanicals and the result was comparable with that of wettable sulphur, a chemical alternative against mites emphasising the efficacy of botanicals through repeated application. Pesticidal efficacy of botanical pesticides needs repeated application in the field to achieve better result[30]. The efficacy of wettable sulphur in controlling sucking pest complex including aphids and mites was already reported and it is widely recommended as an acaricide in vegetable cultivation[31]. Nevertheless, botanical pesticides are found to be less effective than chemical insecticides, they seem to be safe and cheaper than chemical insecticides[32]. Similar trends were observed with chemical check in the present study, where in the chemical check, Spiromesifen 22.9% SC 96g ai/ha + Thiamethoxam 25 WG 50g ai/ha registered superior efficacy in all the evaluation parameters regarding pesticidal property viz., leaf curl symptom, population of pest dynamics etc. Spiromesifen, a contact insecticide cum acaricide, has a mode of action in which inhibits lipid biosynthesis, especially triglycerides and free fatty acids belonging to the titronic acid class of compounds[33]. The combination chemical tried viz., Thiamethoxam, a N-nitroguanidine group of neonicotinoid having systemic action which affects the insect central nervous system via competitive modulation[34].

Though inferior to spiromesifen + thiamethoxam, the pesticidal efficacy of *B. mollis* extract with repeated application was found to be on par with wettable sulphur, which is a commonly used safe acaricide cum fungicide in crop protection. Botanical pesticides are known for its pesticidal efficacy only by way of repeated application in the field due its fast degrading nature[30]. The pesticidal property of *B. mollis* that observed in the present study was supported by the findings of Baskar *et al.*[35] against *Helicoverpa armigera* and the mosquitocidal property against *Culex quinquefasciatus*[36]. Asteraceae members were reported to have anti insecticidal properties[37,38].

The results highlighted the repeated application of *B. mollis* at fortnightly intervals in order to achieve a comparable result as that of wettable sulphur for managing the sucking pest complex; aphid and mite population in chilli. Hence it could be integrated effectively into pest management programs, either as a standalone treatment or in rotation with other botanical or chemical controls. The effectiveness of *B. mollis* extract against pests may be influenced by factors such as concentration, formulation type, time of application and the active ingredient composition. Further research could explore the identification of active compound responsible for pesticidal property, formulation type and application strategies to enhance its efficacy under field conditions. *B. mollis* offers a potent botanical candidate for farmers seeking alternatives to synthetic pesticides for integrated pest management. Another *B. mollis* essential oil sample from India was found to be devoid of 2,5-dimethoxy-pcymene; the major components were linalool (19.4%), γ-elemene (12.2%), copaene (10.9%), estragole (10.8%), allo-ocimene (10.0%), γ-terpinene (8.3%), and allo-aromadendrene (Senthil Kumar *et al.*). This latter sample was, however, found to be larvicidal against mosquitoes (*Culex quinquefasciatus*)[4]. Future studies should focus on elucidating the mode of action of *B. mollis* against pests, its impact on non-target organisms and evaluating its performance across different crops under different agroecological conditions.

Conclusion

The present study has focused on the comprehensive pharmacognostic and phytochemical characterizations of the traditional medicinal plant *B. mollis*. The plant is traditionally used in treatment of various ailments. The pharmacognostic study revealed the morpho-anatomical features of the aerial parts of *B. mollis* along with detailed phytochemical profiling leading to the exploration of pharmacological activities of the plant. This work will be a distinctive pharmacognostic profile of *B. mollis*. The pesticidal efficacy evaluation of *B. mollis* showed it as a promising botanical alternative for integrated pest management strategies when compared to the commonly used chemicals recommended for controlling the sucking pests of chilli available in the market such as Spiromesifen 22.9% SC 96 g, Thiamethoxam 25 WG 50 g ai/ha and wettable Sulfur $3g^{-1}$ and available botanicals like Neem oil and pongamia oil. Aerial parts of *B. mollis* including leaf, stem and flowers possess pesticidal activity. The phytochemicals like Terpenes, flavonoids, alkaloids and polyphenols compounds detected in phytochemical analysis can be responsible for the potential pesticidal activity.

Conflict of interest

The authors declared no conflict of interest.

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