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Unveiling The Pharmacognostical, Macroscopical, Microscopical Features And Potent Antioxidant Activity Of Molluva Spicata Stem Extract

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ABSTRACT
The present study investigates the pharmacognostic, physicochemical, phytochemical, and antioxidant properties of the stem of Moullava spicata, a robust woody climber endemic to the Western Ghats. Macroscopical examination revealed characteristic features including recurved prickles, woody cylindrical stems, and nodal joints, while microscopical evaluation demonstrated a distinct circular outline with epidermis, cortex, well-developed vascular bundles, and large parenchymatous pith. Powder microscopy confirmed diagnostic elements such as xylem vessels and lignified fibers. Physicochemical constants were established, with total ash (7.40%), acid-insoluble ash (1.30%), water-soluble ash (2.70%), and loss on drying (6.66%), providing baseline parameters for quality control. Phytochemical screening of the alcoholic extract indicated the presence of alkaloids, glycosides, flavonoids, tannins, saponins, carbohydrates, proteins, and oils, while inorganic analysis revealed sodium, potassium, sulphates, phosphates, and chlorides. Antioxidant potential was evaluated using DPPH, ABTS, hydrogen peroxide scavenging, and reducing power assays. Results showed significant concentration-dependent activity, with inhibition values ranging from 25.45% to 83.54%, though lower than standard references. These findings suggest that Moullava spicata stem is a rich source of secondary metabolites and natural antioxidants, supporting its ethnomedicinal relevance and providing a foundation for further pharmacological and therapeutic exploration.

Keywords: *Moullava spicata, Pharmacognosy, Phytochemical screening, Antioxidant activity, Ash values, Western Ghats.*

1. INTRODUCTION

Medicinal plants have been an integral part of traditional systems of medicine worldwide, providing a rich source of therapeutic agents for the management of human ailments. They remain indispensable in drug discovery, particularly due to their diverse phytochemical constituents, which contribute to a broad spectrum of biological activities [1]. Among these, antioxidant-rich plants have garnered significant attention, as oxidative stress is implicated in the pathogenesis of several chronic diseases, including cancer, diabetes, cardiovascular disorders, and neurodegeneration. Pharmacognostical, macroscopical, and microscopical studies serve as foundational steps in authenticating medicinal plants, ensuring quality control, and establishing reliable pharmacological correlations with their bioactive properties [2-3].

Moullava spicata is a lesser-known but pharmacologically promising medicinal plant of the Fabaceae family. It is an endangered climbing shrub endemic to the Western Ghats of India and has been reported to possess a wide range of ethnomedicinal uses. Traditionally, the plant has been employed in the treatment of dermatological disorders, respiratory infections, wounds, and other inflammatory conditions [4-5]. These applications highlight its significance in indigenous systems of medicine, where it is valued for its therapeutic efficacy.

Phytochemical investigations of *M. spicata* have revealed that its various parts are rich in phenolics, flavonoids, tannins, steroids, terpenoids, and alkaloids, which are responsible for its antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities [6-8]. Phenolic compounds, in particular, play a pivotal role in neutralizing free radicals, thereby mitigating oxidative stress and preventing cellular damage. Reports also suggest that these phytochemicals contribute as reducing and stabilizing agents in green nanoparticle synthesis, underscoring the plant's dual relevance in both pharmacognosy and nanomedicine [9-10].

Several studies have documented the bioactivity of *M. spicata*. The leaf extract has demonstrated antioxidant activity through assays such as DPPH, ABTS, and FRAP, indicating strong radical scavenging potential [6-11]. Cytotoxic and genotoxic assessments revealed dose-dependent effects of the extract on yeast cell viability, erythrocyte hemolysis [12], and Allium cepa root growth, suggesting the presence of

bioactive molecules with therapeutic and toxicological significance [13-14]. Additionally, phytochemical screenings coupled with advanced analytical techniques like HPTLC, FTIR, NMR, and GC–MS have identified compounds such as lupeol, stigmasterol, bergenin, and friedelin, which are recognized for their pharmacological importance [15]. Furthermore, green synthesis approaches have shown that extracts of *M. spicata* can mediate the production of silver nanoparticles with enhanced antioxidant and anticancer properties, particularly against neuroblastoma cancer cells [16].

Despite these emerging reports, the pharmacognostical characterization of *M. spicata*, particularly its stem, remains inadequately explored. Pharmacognostical studies, including macroscopical and microscopical examinations, are critical for plant authentication, detection of adulterants, and establishing diagnostic characters that can guide pharmacological investigations. Such studies provide a reliable basis for standardization, ensuring reproducibility in therapeutic applications and industrial formulations. Detailed pharmacognostical profiling of the stem is especially relevant, as different plant parts may vary in their phytoconstituent distribution and biological activities. In addition, the antioxidant activity of *M. spicata* stem extracts has not been systematically correlated with its pharmacognostical features. Given the growing interest in natural antioxidants as alternatives to synthetic counterparts, establishing this correlation is vital for validating the plant's therapeutic claims and promoting its utilization in evidence-based medicine. Moreover, as an endangered species of the Western Ghats, scientific validation of its pharmacognostical identity and bioactivity can support conservation strategies and sustainable utilization practices.

The present study is therefore designed to unveil the pharmacognostical, macroscopical, and microscopical characteristics of *M. spicata* stem extract while concurrently evaluating its antioxidant potential. By integrating traditional knowledge with modern pharmacognostic tools, this work aims to contribute to the scientific understanding of M. spicata, paving the way for its inclusion in pharmacopoeial standards and potential therapeutic applications. Furthermore, recognizing its role in eco-friendly nanomedicine and biocompatible nanoparticle synthesis highlights the multifaceted relevance of this plant in contemporary research [9-10].

Thus, this investigation not only reinforces the ethnomedicinal value of *M. spicata* but also provides essential baseline data for its pharmacognostical authentication and antioxidant profiling, ultimately supporting its conservation, sustainable use, and potential integration into modern therapeutic practices.

2. Experimental Work

2.1. Materials

Fresh stems of *Moullava spicata* (Dalzell) Nicolson were collected in November from the vicinity of Koyna Wildlife Sanctuary in the Western Ghats, Maharashtra, India. The plant material was taxonomically authenticated by the Department of Botany, The New College, Kolhapur, and a voucher specimen was deposited in the departmental herbarium for future reference. The stems were thoroughly washed with distilled water to remove adhering debris and shade-dried at room temperature for 10–15 days. Dried stems were coarsely powdered using a mechanical grinder, passed through a sieve (60 mesh), and stored in airtight containers until further use.

All chemicals and solvents employed in the study were of analytical grade and procured from standard suppliers (Merck, HiMedia, India). For antioxidant assays, reagents such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric chloride ($FeCl_3$), potassium ferricyanide, trichloroacetic acid, Folin–Ciocalteu's reagent, gallic acid, and quercetin standards were used. Glassware was sterilized prior to use, and distilled water was utilized throughout the experimental procedures.

2.2. Pharmacognostical Evaluation

2.2.1. Macroscopic Examination:

The detail macroscopic characters of fresh plant was noted including special features such as colour, shape, size etc [17].

2.2.2. Microscopic Examination of *Moullava spicata*

A transverse section of the stem was prepared by slicing it across the cylindrical portion, perpendicular to its longitudinal axis. Fresh stem pieces measuring about 3–5 mm in diameter and 8–10 cm in length were selected for study. The samples were softened by boiling in water for a few minutes to facilitate sectioning. Thin sections were then obtained by free-hand cutting, stained with appropriate dyes, mounted on slides, and examined under a microscope for anatomical characterization.

2.2.3. Determination of Powdered Microscopy:

Powdered material of *Moullava spicata* stem was mounted on a clean glass slide for microscopic evaluation. The sample was treated with staining reagents such as phloroglucinol and concentrated hydrochloric acid to identify lignified elements, and dilute iodine solution to detect starch grains and other cellular inclusions. The prepared slides were kept aside briefly to allow staining to develop, and then examined under 10× magnification using a Motic digital photomicroscope. Diagnostic features such as the type of cells, nature of the cell wall, and presence of cellular contents were observed and compared with standard pharmacognostical characters reported for authentic drug material.

2.2.4. Drying of Plant Material

In the present study, the collected plant material was carefully sorted and thoroughly washed to remove adhering dust and debris. The material was then subjected to natural drying under shade conditions. For this purpose, the stems were spread in a thin layer and allowed to dry at ambient temperature for approximately two weeks, ensuring uniform drying and prevention of degradation of phytoconstituents.

3. Physicochemical Parameters and its Evaluation

The World Health Organization (WHO) has prescribed quality control standards for herbal drugs to ensure their authenticity, safety, and efficacy, and these guidelines are equally applicable to *Moullava spicata* stem. The botanical parameters include sensory evaluation, removal of foreign matter, and microscopic authentication of diagnostic features. Physicochemical standards comprise chromatographic fingerprinting, determination of ash and extractive values, estimation of moisture content, volatile matter, and volatile oil content. Pharmacological parameters involve the evaluation of bitterness value, hemolytic property, astringent property, swelling index, and foaming index, which serve as functional indicators of phytoconstituents. Toxicological evaluation is equally important and requires assessment of heavy metals such as lead, mercury, cadmium, and arsenic, along with the detection of pesticide residues, microbial contamination, aflatoxins, and radioactive contamination. Adhering to these WHO guidelines for *Moullava spicata* stem is essential to establish its pharmacognostical identity, ensure quality assurance, and validate its use as a safe and effective herbal drug [18-23].

3.1. Determination of Moisture or Determination of Loss on drying (LOD):

Loss on drying (LOD) was determined to estimate the moisture and volatile matter content in the powdered stem of the selected plant material. About 1.5 g of accurately weighed stem powder was placed in a clean, dry porcelain dish and kept in a hot-air oven at 105 °C for 2 hours. The sample was then cooled in a desiccator and reweighed. The process of heating, cooling, and weighing was repeated until a constant weight was obtained. The percentage of loss on drying was calculated using the following formula:

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \times 100$$

3.2. Determination of Ash Values of a Crude Extract

3.2.1. Determination of Total Ash Value for *Moullava spicata* stem

The total ash value of the powdered drug was determined to assess the inorganic residue present after incineration. About 2 g of accurately weighed powdered sample of *Moullava spicata* stem was transferred into a previously ignited and weighed porcelain dish. The sample was first heated gently over a flame about 2 cm high with the dish positioned approximately 7 cm above the flame, and then heated strongly until all carbon was completely burnt off. The dish was cooled in a desiccator and weighed. The percentage of total ash was calculated with reference to the weight of the air-dried drug using the following formula:

$$\%\text{Total Ash Value} = \frac{\text{Weight of total Ash}}{\text{Weight of crude drug taken}} \times 100$$

3.2.2. Determination of Acid- Insoluble Ash Value for *Moullava spicata* stem

The acid-insoluble ash value of the powdered drug was determined to quantify the amount of siliceous matter present in the crude sample. Initially, total ash was prepared from 2 g of powdered *Moullava spicata* stem. The ash was then transferred into a 100 ml beaker using 25 mL of dilute hydrochloric acid and boiled for 5 minutes. The solution was filtered through ashless filter paper, and the residue was washed twice with distilled water. The filter paper containing the residue was dried, ignited until carbon was removed completely, cooled in a desiccator, and weighed. The percentage of acid-insoluble ash was calculated with reference to the weight of the air-dried drug using the formula:

$$\% \text{ Acid insoluble Ash Value} = \frac{\text{Weight of acid insoluble Ash}}{\text{Weight of crude drug taken}} \times 100$$

3.2.3. Determination of Water soluble Ash Value for *Moullava spicata* stem

The water-soluble ash value of the powdered drug was determined to estimate the amount of inorganic matter soluble in water. Initially, total ash was prepared from 2 g of powdered *Moullava spicata* stem. The ash was transferred into a 100 mL beaker with the aid of 25 mL of distilled water and boiled for 5 minutes. The solution was filtered through ashless filter paper, and the residue was washed twice with hot water. The filter paper along with the residue was ignited gently until vapors ceased and then strongly until complete carbon removal. The residue was cooled in a desiccator and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug using the following formula:

$$\% \text{ Water soluble Ash Value} = \frac{\text{Weight of total ash - Weight of water insoluble Ash}}{\text{Weight of crude drug taken}} \times 100$$

4. Soxhlet Extraction [24, 25]

The extraction of the stem material of *Moullava spicata* was carried out using a Soxhlet apparatus of one-liter capacity. The dried plant material was coarsely powdered using a mechanical grinder, and approximately **250 g** of the powdered stem was packed into a muslin thimble, with its diameter slightly smaller than the inner diameter of the Soxhlet chamber. Ethanol (95%) was used as the extraction solvent. Prior to initiating continuous extraction, the thimble containing plant material was wetted by allowing the solvent to siphon once without attaching the condenser. Porcelain pieces were added to the flask to prevent bumping during heating. The mouth of the Soxhlet extractor was fitted with a bulb-type condenser, and all joints were sealed with wax to avoid solvent loss. Extraction was carried out by maintaining the temperature close to the boiling point of ethanol. The cycle was continued for approximately **14 siphon cycles**, or until complete extraction was achieved.

The solvent was then recovered by distillation, and the concentrated extract was filtered through Whatman No. 42 filter paper. The filtrate was further concentrated on a heating mantle, and the dried extract was weighed and stored in airtight containers until further use.

5. Preliminary Phytochemical Screening [26, 27]

The ethanolic extract of *Moullava spicata* stem was subjected to preliminary phytochemical investigation to identify the major classes of secondary metabolites. Standard qualitative chemical tests revealed the presence of alkaloids, glycosides (anthraquinone, cardiac, and saponin types), flavonoids, tannins, phenolic compounds, carbohydrates, proteins, and fixed oils. Solubility studies further demonstrated that the extract was completely soluble and stable in polar solvents such as ethanol and methanol, while showing partial solubility in water and organic solvents like acetonitrile. These findings confirm that the stem extract of *Moullava spicata* is a rich source of bioactive phytoconstituents, many of which are known to exhibit potent pharmacological activities.

5.1. Inorganic Constituent Analysis

Inorganic elements present in the ash of *Moullava spicata* stem were evaluated through standard qualitative tests. The analysis confirmed the presence of calcium, sodium, potassium, iron, sulphates, phosphates, and chlorides in detectable amounts. Sodium and potassium were further confirmed through flame tests, while calcium and iron were identified by precipitation reactions with ammonium oxalate and potassium ferrocyanide, respectively. These essential mineral constituents contribute to the nutritional and therapeutic potential of the plant.

5.2. Antioxidant Activity [28-30]

Antioxidants are compounds that inhibit or delay oxidative processes mediated by reactive oxygen species (ROS). The antioxidant activity of *Moullava spicata* stem extract was evaluated using four in vitro assays: DPPH radical scavenging, ABTS radical cation decolorization, hydrogen peroxide scavenging, and reducing power assay.

6. DPPH Radical Scavenging Assay

The free radical scavenging activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) was evaluated following the method of Mensor et al. (2001). Briefly, 20 µL of the extract at different concentrations was mixed with 0.5 mL of 0.3 mM DPPH solution in methanol and incubated for 30 min at room temperature. The decrease in absorbance was recorded at 518 nm using a UV–Visible spectrophotometer. Methanol served as the blank and DPPH without extract as control. The percentage radical scavenging activity was

calculated from the reduction in absorbance compared with the control.

$$\text{Scavenging activity \%} = \frac{\text{A518 (sample) - A518 (blank)}}{100} \text{ X } 100$$

6.1. ABTS Radical Cation Scavenging Assay

The ABTS assay was performed as described by Re et al. (1999). ABTS radical cation (ABTS•+) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating in the dark for 16 h. The radical solution was diluted to obtain an absorbance of 1.0 at 734 nm. To this solution, different concentrations of extract were added, and absorbance was measured after 6 min. Ascorbic acid was used as the reference standard. The scavenging potential was expressed as ascorbic acid equivalent antioxidant capacity (AaEAC).

$$\text{\% scavenging of hydrogen peroxide} = \frac{(A_0 - A_1)}{A0} \text{ X } 100$$

Where, A_0 - Absorbance of control, A_1 - Absorbance in the presence of plant extract

6.2. Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity was assessed by the method of Ruch et al. (1989). A 40 mM solution of H_2O_2 was prepared in 0.1 M phosphate buffer (pH 7.4). Extracts at concentrations ranging from 10–50 µg/mL were mixed with 0.6 mL of H_2O_2 solution, and the final volume was adjusted to 3 mL with buffer. After incubation, absorbance was recorded at 230 nm. Ascorbic acid was used as standard. The percentage scavenging activity was calculated relative to control.

$$\text{\% Loss on drying} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \text{ X } 100$$

6.3. Reducing Power Assay

The reducing power of the extract was determined by the method of *Vijayalakshmi et al. (2016).* Different concentrations of extract were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min, followed by addition of 2.5 mL trichloroacetic acid (10%) and centrifugation. The upper layer was mixed with distilled water and 0.5 mL ferric chloride (0.1%), and absorbance was recorded at 700 nm. Increased absorbance indicated greater reducing power, and results were compared with ascorbic acid as standard.

$$\text{\% Increase in reducing power} = \frac{A_{test}}{A_{blank}} \text{-1 X } 100$$

7. RESULT AND DISCUSSION

7.1. Macroscopical Characteristics

Moullava spicata is a robust woody climber endemic to the Western Ghats with prickles recurving along its branches. The stem is firmly woody; young stems are covered with soft hairs and armed with thorn-like recurved prickles, especially along the main rachis and nodes. The inner texture becomes harder and more fibrous as the stem matures. The colour externally tends toward olive-green or brownish on older parts, while the inner wood is lighter, often pale yellowish or whitish when split. Although literature does not provide explicit details about taste or odour of the stem, the plant (leaves, aerial parts) is noted for its aromatic pungent smell and bitter taste in its traditional use. Flowering stems also bear dense spikes/racemes with colourful inflorescences, but strictly for stem macroscopy, the presence of recurved prickles, woody texture, cylindrical contour, and nodal joints are diagnostic.

7.2. Microscopical Characteristics

Single layer of rectangular epidermis cells layer. Many layers of thin walled cellulosic parenchyma below the epidermis are observed. Large thin walled, lignified big polygonal parenchyma is seen at the centre called pith. As shown in **Figure 1**.

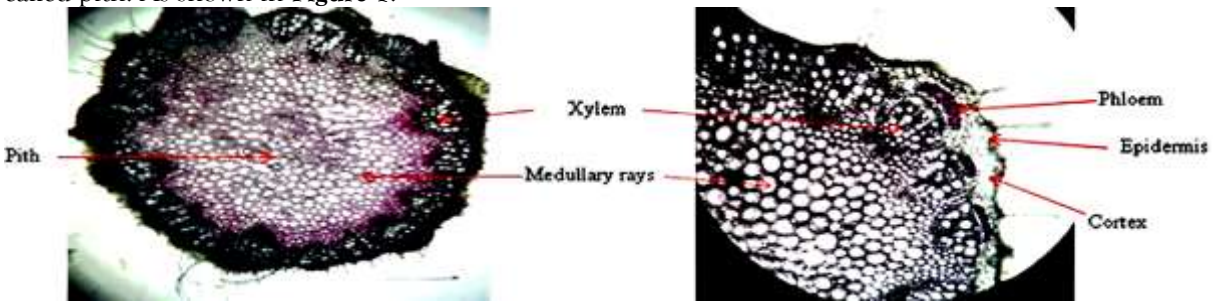

Figure 1, T.S of *Moullava spicata* Stem

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7.3. Transverse Section of Stem

The T.S. of *Moullava spicata* stem revealed a circular outline with distinct anatomical features. The outermost single-layered epidermis was covered with a thin cuticle. Beneath the epidermis, collenchymatous cells were present, followed by a broad cortex composed of parenchymatous cells. The vascular bundles were arranged in a ring, conjoint, collateral, and open type, with well-developed xylem and phloem tissues. Xylem vessels were lignified and showed spiral to reticulate thickening, while phloem consisted of sieve tubes and companion cells. Medullary rays were distinct and uniseriate, traversing the xylem towards the pith. The central pith region was large, composed of thin-walled parenchymatous cells **(Figure 2)**.

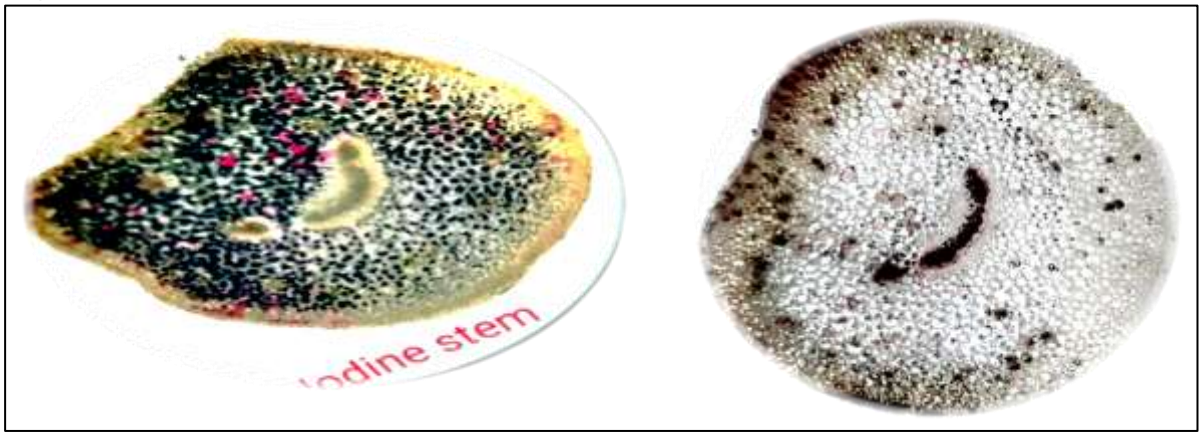

Figure 2: Transvers Section of steam

7.4. Powdered Characteristics

Powdered drug examined under compound microscope showed presence of xylem vessels, lignified fibre which was indicates confirmation of *Moullava spicata* plant **(Figure 3)**.

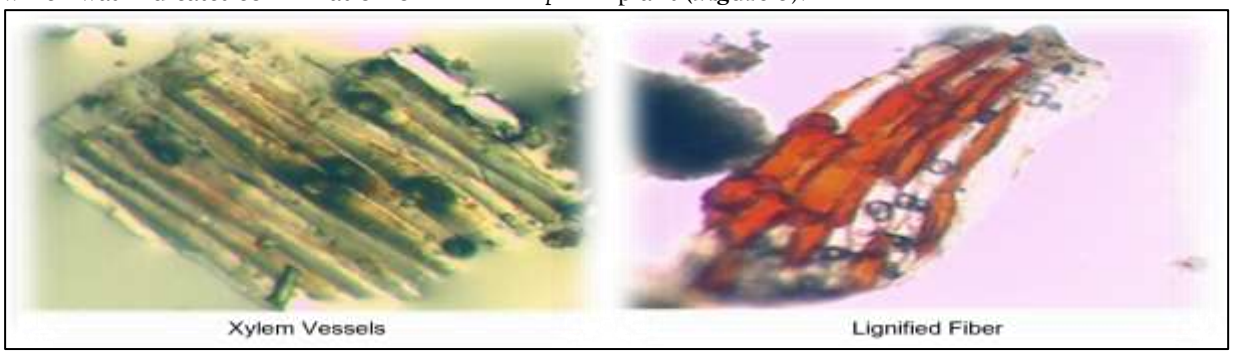

Figure 3, Powdered characteristics of *Moullava spicata*

7.4.1. Determination of Moisture or Determination of Loss on drying (lod):

The powdered drug weighed 1.5 g initially, which was reduced to 1.4202 g after drying. The weight loss observed was 0.10 g, corresponding to a 6.6601% loss on drying. This value indicates the presence of moisture and volatile matter within acceptable limits for crude drug material.

7.5. Determination of Ash Values of a Crude Extract

7.5.1. Determination of Total Ash Value for *Moullava spicata* stem

The total ash value of the powdered drug was determined by incineration of 2 g of *Moullava spicata* stem. After complete combustion of the organic matter, the weight of ash obtained was 0.1482 g. This corresponded to a total ash value of 7.402 % with reference to the air-dried drug. The result reflects the amount of inorganic matter present in the sample, which may include physiological ash derived from the plant tissue as well as non-physiological ash such as adhering soil or other extraneous material.

7.5.2. Determination of Acid- Insoluble Ash Value for *Moullava spicata* stem

The determination of acid-insoluble ash value revealed that out of the 2 g powdered stem sample, the total ash obtained weighed 0.10 g, of which the residue insoluble in dilute hydrochloric acid measured 0.027 g. This corresponded to an acid-insoluble ash value of 1.3021 % with reference to the air-dried sample. The relatively low value indicates minimal presence of siliceous matter, such as sand or other extraneous inorganic impurities, in *Moullava spicata* stem.

7.5.3. Determination of Water soluble Ash Value for *Moullava spicata* stem

In the present study, the total ash obtained from 2 g of powdered *Moullava spicata* stem weighed 0.10 g, of which the water-insoluble fraction accounted for 0.047 g. The water-soluble fraction was therefore calculated as 0.055 g, corresponding to a water-soluble ash value of 2.7021 % with reference to the air-dried drug. This value reflects the presence of water-soluble inorganic salts, which may be attributed to naturally occurring minerals within the plant tissue.

7.6. Results for Qualitative Chemical Investigation of alcoholic extract of *Moullava spicata* stem

The qualitative phytochemical analysis of the alcoholic extract of *Moullava spicata* stem demonstrated the presence of a broad spectrum of secondary metabolites. Alkaloids were confirmed by Mayer's, Dragendorff's, Wagner's, and Hager's tests, each yielding characteristic precipitates. Glycosides were also present, with anthraquinone glycosides showing positive results in both Borntrager's and modified Borntrager's tests, while cardiac glycosides were indicated by positive responses in Legal's and Keller–Killiani tests. Persistent foam formation confirmed the presence of saponin glycosides. Proteins were detected through Biuret and precipitation tests, while flavonoids were verified by alkaline reagent and zinc hydrochloride tests. Tests for fats and oils indicated solubility in organic solvents and a red coloration with tincture alkali. Carbohydrates were strongly confirmed by Molisch, Fehling's, and Benedict's tests, producing characteristic colour reactions. Tannins and phenolic compounds were indicated by ferric chloride, gelatin, and acetic acid tests, all giving positive results. These findings reveal that *Moullava spicata* stem extract is rich in phytoconstituents such as alkaloids, glycosides, saponins, flavonoids, tannins, proteins, carbohydrates, and oils, supporting its potential therapeutic value.

7.7. Detection of Inorganic Constituents of alcoholic extract of *Moullava spicata*

The inorganic qualitative analysis of the alcoholic extract of *Moullava spicata* stem revealed the presence of several essential mineral constituents. Sodium was confirmed by the appearance of a characteristic golden-yellow flame, while potassium was detected by both the yellow precipitate with sodium cobalt nitrite and violet coloration in the flame test. Sulphates were indicated by the formation of a white precipitate with lead acetate, soluble in sodium hydroxide, whereas phosphates were confirmed by the development of a yellow crystalline precipitate with ammonium molybdate. Chlorides were detected by both silver nitrate and lead acetate tests, producing white precipitates that dissolved in dilute ammonia and hot water, respectively. On the other hand, calcium and iron were not detected in the stem extract, as indicated by the absence of characteristic precipitates or colour reactions. These results highlight that *Moullava spicata* stem contains appreciable amounts of sodium, potassium, sulphates, phosphates, and chlorides, which may contribute to its therapeutic potential and biological activity.

7.8. Results of Antioxidant Activity

7.8.1. DPPH (2, 2-diphenyl-2-picryl hydrazyl hydrate) Spectrophotometric Assay:

The antioxidant activity of *Moullava spicata* stem extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) spectrophotometric assay. The extract exhibited a concentration-dependent free radical scavenging activity. At a lower concentration of 10 µg/mL, the stem extract showed 71.05 ± 2.45% inhibition, compared to 92.25 ± 2.54% for the standard. At a higher concentration of 50 µg/mL, the extract demonstrated enhanced activity with 83.54 ± 1.56% inhibition (**Table 1**), while the standard showed 98.05 ± 1.56% inhibition. These results indicate that the *Moullava spicata* stem extract possesses significant antioxidant potential, with efficacy increasing at higher concentrations, though slightly lower than that of the reference standard.

Table 1: DPPH radical scavenging activity of *Moullava spicata* stem extract

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Concentrations (µg/ml)	**Standard % inhibition**	***Moullava spicata* stem extract % inhibition**
10	92.25±2.54	71.05±2.45
20	94.45±1.89	74.25±1.02
30	95.2±2.56	78.54±2.65

40	97.85±1.89	81.25±2.65
50	98.05±1.56	83.54±1.56

7.8.2. ABTS Scavenging Effects

The ABTS radical scavenging activity of *Moullava spicata* stem extract and its nanoparticle formulations was evaluated at 200 µg/mL. The stem extract exhibited 75.21 ± 0.75% inhibition, whereas the standard showed 94.71 ± 0.16% inhibition. These results demonstrate that *Moullava spicata* stem extract possesses significant ABTS radical scavenging activity, indicating its potential as a natural antioxidant, although its activity was slightly lower than that of the reference standard.

7.8.3. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of *Moullava spicata* stem extract was evaluated at concentrations of 10 µg/mL and 50 µg/mL. At the lower concentration, the extract exhibited 48.51 ± 1.54% inhibition, while the standard showed 62.54 ± 0.61% inhibition. At the higher concentration, the extract demonstrated enhanced scavenging activity with 62.79 ± 1.30% inhibition compared to 105.54 ± 1.25% for the standard **(Table 2)**. These results indicate that the stem extract possesses concentration-dependent hydrogen peroxide scavenging activity, reflecting its potential as a natural antioxidant, though its efficacy was lower than that of the reference standard.

Table 2: Hydrogen peroxide scavenging activity of *Moullava spicata* stem extract

Concentrations	**Standard % inhibition**	***Moullava spicata* stem extract % inhibition**
10	62.54±0.61	48.51±1.54
20	78.54±1.65	50.54±0.89
30	88.75±2.02	55.57±1.55
40	95.45±1.32	60.55±2.01
50	105.54±1.25	62.79±1.30

7.8.4. Reducing Power Assay

The reducing power of *Moullava spicata* stem extract was evaluated at concentrations ranging from 10 to 50 µg/mL. The extract exhibited a concentration-dependent increase in reducing activity, with % inhibition values of 25.45 ± 2.32, 29.45 ± 0.95, 36.54 ± 2.15, 41.45 ± 2.15, and 47.54 ± 1.13 at 10, 20, 30, 40, and 50 µg/mL, respectively **(Table 3)**. In comparison, the standard showed higher reducing activity at the same concentrations, ranging from 62.52 ± 1.25 to 73.69 ± 2.03% inhibition. These results indicate that *Moullava spicata* stem extract possesses notable reducing power, which increases with concentration, although it remains lower than that of the reference standard.

Table 3: Reducing power assay activity of *Moullava spicata* stem extract

Concentrations	**Standard % inhibition**	**Plant extract % inhibition**
10	62.52±1.25	25.45±2.32

20	64.02±2.35	29.45±0.95
30	69.42±1.23	36.54±2.15
40	70.37±1.54	41.45±2.15
50	73.69±2.03	47.54±1.13

8. DISCUSSION

The pharmacognostic and phytochemical evaluation of *Moullava spicata* stem provides significant insights into its identity, purity, and therapeutic potential. The macroscopical characteristics, such as its woody texture, cylindrical stem with recurved prickles, and distinct nodal joints, are diagnostic features that aid in proper identification. Microscopical examination revealed a well-differentiated anatomy with a single-layered epidermis, collenchymatous cortex, distinct vascular bundles, and a parenchymatous pith, all of which align with structural features typical of climbing legumes.

The presence of lignified xylem vessels and fibers observed in powdered microscopy further confirms the plant's authenticity. The physicochemical constants, including moisture content (6.66%), total ash (7.40%), acid-insoluble ash (1.30%), and water-soluble ash (2.70%), fall within acceptable ranges for crude drugs, ensuring minimal contamination and a reliable inorganic profile. These values establish baseline parameters for quality control. Phytochemical screening of the alcoholic extract demonstrated the presence of a wide array of bioactive compounds such as alkaloids, glycosides, flavonoids, tannins, saponins, carbohydrates, proteins, and oils. The coexistence of these secondary metabolites supports the traditional use of *Moullava spicata* in folk medicine, as many of these groups are known for antioxidant, antimicrobial, and anti-inflammatory properties. The detection of essential inorganic constituents like sodium, potassium, sulphates, phosphates, and chlorides suggests additional physiological significance, although calcium and iron were absent. Antioxidant assays revealed that the stem extract possesses notable free radical scavenging activity, though consistently lower than standard references.

In the DPPH assay, the extract exhibited 71.05% to 83.54% inhibition across tested concentrations, reflecting strong electron-donating ability. Similarly, in the ABTS assay, the extract achieved 75.21% inhibition, supporting its radical quenching potential. Hydrogen peroxide scavenging activity demonstrated moderate efficacy, with 48.51% at 10 µg/ml and 62.79% at 50 µg/ml, again showing a concentration-dependent response. The reducing power assay confirmed a gradual increase in activity with concentration, though comparatively less than the standard. Overall, these findings establish that *Moullava spicata* stem is a promising source of bioactive phytoconstituents with significant antioxidant potential. The combined pharmacognostic, physicochemical, phytochemical, and antioxidant profiles provide a strong foundation for future investigations into its therapeutic applications and possible development into standardized herbal formulations.

9. CONCLUSION

The present study provides comprehensive pharmacognostic, physicochemical, phytochemical, and antioxidant evaluations of *Moullava spicata* stem. Macroscopical and microscopical features such as recurved prickles, woody cylindrical stems, distinct vascular bundles, and parenchymatous pith serve as reliable diagnostic markers. Physicochemical constants including total ash, acid-insoluble ash, water-soluble ash, and loss on drying establish baseline parameters for quality control and purity assessment. Phytochemical screening confirmed the presence of diverse bioactive secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, saponins, and carbohydrates, along with essential inorganic constituents like sodium, potassium, sulphates, phosphates, and chlorides. Antioxidant assays, including DPPH, ABTS, hydrogen peroxide scavenging, and reducing power tests, revealed significant concentration-dependent free radical scavenging activity, though comparatively lower than standard references. Collectively, these findings highlight the therapeutic potential of *Moullava spicata* stem as a natural source of antioxidants and bioactive compounds, providing a scientific basis for its traditional use and supporting further pharmacological and clinical investigations.

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