

Pharmacognostic Characterization and Antioxidant Potential of *Abutilon crispum* (Malvaceae): An Underexplored Traditional Medicinal Plant

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ABSTRACT

Abutilon crispum (Family: Malvaceae) is a traditionally used yet underexplored medicinal plant known for its applications in managing respiratory ailments, wound healing, and inflammatory conditions. Despite its ethnomedicinal relevance, comprehensive scientific data on its pharmacognostic attributes and bioactive potential remain scarce. This study aimed to authenticate the plant material, investigate its anatomical characteristics, and evaluate the antioxidant activity of its ethanol extract.

Stems of *A. crispum* were collected from their natural habitat, taxonomically authenticated, and a voucher specimen (A260325272C) was deposited for future reference. The plant material was shade-dried, pulverized, and subjected to cold maceration using ethanol. The extract was concentrated under reduced pressure, yielding 23.18%, indicative of a high content of ethanol-soluble phytoconstituents such as flavonoids, phenolics, and glycosides.

Anatomical examination of transverse stem sections revealed typical dicotyledonous features, including a single-layered epidermis with multicellular trichomes, a collenchymatous cortex, open vascular bundles arranged in a ring, and a prominent parenchymatous pith—traits consistent with the Malvaceae family. The antioxidant potential of the ethanol extract was assessed using the DPPH radical scavenging assay, with ascorbic acid serving as the standard. The extract exhibited concentration-dependent free radical scavenging activity, achieving $56.95 \pm 1.29\%$ inhibition at $100 \mu\text{g/mL}$, compared to $90.15 \pm 1.51\%$ for ascorbic acid. The IC_{50} value of $74.04 \mu\text{g/mL}$ indicated moderate antioxidant efficacy.

These findings support the traditional use of *A. crispum* and underscore its potential as a natural source of antioxidants. Further phytochemical and pharmacological investigations are recommended to explore its therapeutic applications.

Keywords: *Abutilon crispum*, Phytochemistry, Traditional medicine, Biological activity, Antioxidant, Anti-inflammatory

I. INTRODUCTION

Medicinal plants have been a cornerstone of traditional healthcare systems for centuries, providing a rich repository of bioactive compounds that have shaped modern pharmacotherapy. It is estimated that over 80% of the world's population still relies on plant-based medicines for their primary healthcare needs, particularly in developing countries (WHO, 2022) [1]. The therapeutic efficacy of plants is largely attributed to their secondary metabolites, including phenolic compounds, flavonoids, alkaloids, terpenoids, tannins, and glycosides, which exhibit diverse pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective effects [2]. In recent years, there has been a resurgence of interest in exploring plant-based antioxidants as alternatives to synthetic compounds due to their safety, biocompatibility, and multi-targeted mechanisms of action.

Oxidative stress, arising from an imbalance between reactive oxygen species (ROS) and the antioxidant defense system, plays a critical role in the pathogenesis of various chronic and degenerative diseases, including diabetes mellitus, cardiovascular disorders, neurodegenerative conditions (e.g., Alzheimer's and Parkinson's diseases), and cancer [3, 4]. Free radicals such as superoxide anion (O_2^-), hydroxyl radicals ($\bullet\text{OH}$), and peroxy radicals initiate chain reactions that damage lipids, proteins, and nucleic acids, leading to cellular dysfunction and tissue injury [5]. Antioxidants act by scavenging free radicals, chelating transition metals, or inhibiting oxidative enzymes, thereby preventing or mitigating oxidative damage [6]. Consequently, evaluating the antioxidant potential of medicinal plants has become a primary focus in pharmacological research, particularly using in-vitro assays such as

the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, which is a rapid and reliable method to assess free radical scavenging capacity [7].

The genus *Abutilon* (Family: Malvaceae) comprises approximately 100 species distributed across tropical and subtropical regions. Species of this genus are well-known for their ethnomedicinal applications in treating fever, cough, wounds, inflammation, and gastrointestinal ailments [8]. Notably, *Abutilon indicum* (Indian mallow) [9] and *Abutilon hirtum* [10] have been extensively studied for their pharmacological activities, including antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and analgesic effects, which are attributed to the presence of bioactive compounds such as flavonoids, phenolics, alkaloids, and sterols [11, 12]. However, *Abutilon crispum* remains an underexplored species, despite its traditional use in certain rural and indigenous systems of medicine for managing respiratory conditions, wound healing, and inflammatory disorders. Given the chemical diversity reported in related species, *A. crispum* is likely to harbor similar bioactive constituents with therapeutic relevance.

Pharmacognostical studies, including anatomical and histological evaluations, form the foundation for the identification and standardization of medicinal plants. Misidentification or adulteration of herbal raw materials can compromise both efficacy and safety, underscoring the importance of such characterization [13]. Features such as epidermal trichomes, vascular bundle arrangements, collenchymatous cortex, and parenchymatous pith not only aid in plant identification but are also linked to the biosynthesis and storage of secondary metabolites. In this context, microscopic studies of *A. crispum* stems can provide vital diagnostic markers that ensure authenticity and help establish quality control parameters for pharmacological investigations.

The current study was designed to bridge the knowledge gap regarding *A. crispum* by undertaking a comprehensive evaluation of its stem. A standardized ethanol extraction protocol was employed, as ethanol is known to effectively extract a broad spectrum of phytoconstituents, including phenolics and flavonoids, without compromising their structural integrity. This work is among the first detailed studies on the pharmacognostical features and antioxidant potential of *A. crispum*. The findings are expected to provide a scientific basis for its traditional uses

and highlight its potential as a natural source of antioxidants.

II. METHODS AND MATERIALS

2.1 Collection, authentication, and preparation of *Abutilon crispum* extract

The fresh stems of *Abutilon crispum* were collected from their natural habitat, ensuring they were free from disease or damage. The plant material was authenticated by a Dr. P Radha, Research Office (Botany), Sci II i/c, Siddha Medicinal Plants Garden, CCRS, Ministry of Ayush, Tamilnadu, and a voucher specimen was deposited for future reference (A260325272C). The collected stems were washed to remove dust and shade-dried at room temperature (25–30°C) for 10–15 days. Once dried, the stems were cut into small pieces and ground into a coarse powder using a mechanical grinder, then sieved through a 40-mesh sieve. For extraction, 100 g of the powdered stem was soaked in 1 L of ethanol in a sealed glass container and left to macerate at room temperature for 72 hours with intermittent shaking. The extract was then filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40–50°C. The resulting semi-solid extract (EEAC) was further dried in a vacuum desiccator and stored in an airtight container at 4°C for future use. The percentage yield of the extract was calculated based on the initial weight of the plant material and the final weight of the dried extract [14].

2.2 Transverse section of *Abutilon crispum* stem

A representative stem segment was collected, rinsed with distilled water to remove contaminants, and fixed in a FAA solution (formalin: acetic acid: ethanol, 5:5:90) for 24 hours, after which it was gradually dehydrated in an ascending ethanol series (70%, 85%, 95%, and 100%) and cleared in xylene until the tissue became transparent; the sample was then embedded in melted paraffin wax, allowed to cool and solidify into a block, and sectioned into 10–12 µm thick transverse slices using a microtome, with the sections floated on a warm water bath to remove wrinkles and mounted on glass slides. The mounted sections were deparaffinized by immersing the slides in xylene and rehydrated through a descending ethanol series (100%, 95%, 85%, 70%) before rinsing in distilled water. The sections were subsequently stained with a suitable staining protocol to differentiate various anatomical

features. The prepared slides were then examined under a light microscope, and photomicrographs were captured for further analysis [15].

2.3 In-vitro DPPH inhibition assay

The DPPH radical scavenging activity of *Abutilon crispum* stem ethanol extract was evaluated using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. A stock solution of DPPH (0.1 mM) was prepared in methanol and stored in the dark at room temperature. Various concentrations of the extract (1, 10, 25, 50, and 100 µg/mL) were prepared in methanol. In a 96-well plate, 100 µL of the extract at different concentrations was mixed with 100 µL of DPPH solution and incubated in the dark at room temperature for 30 minutes. Ascorbic acid was used as the standard antioxidant, and a blank was prepared using methanol instead of the sample. The absorbance was measured at 517 nm using a microplate reader. The percentage inhibition of DPPH radicals was calculated using the formula:

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where A_{control} is the absorbance of the DPPH solution without the extract, and A_{sample} is the absorbance in the presence of the extract. The IC_{50} value, representing the concentration required to inhibit 50% of the DPPH radicals, was determined by plotting a graph of percentage inhibition against the logarithm of extract concentration [16].

III. RESULTS

3.1 Collection, authentication and extraction of *A. crispum* stem extract

The plant material was carefully sourced from its natural habitat, ensuring that the collected specimens were healthy, undamaged, and disease-free. The botanical identity of the plant was rigorously authenticated by an expert taxonomist, Dr. P. Radha, associated with the Siddha Medicinal Plants Garden, Central Council for Research in Siddha (CCRS), Ministry of Ayush, Tamil Nadu. The deposition of a voucher specimen (A260325272C) adds scientific validity to the study by ensuring future traceability and reproducibility. Once authenticated, the stems were shade-dried under ambient conditions (25–30°C) for 10–15 days to prevent degradation of thermolabile constituents, a crucial step in maintaining the chemical integrity of the plant material.

Post drying, the stems were pulverized into a coarse powder to increase the surface area for solvent interaction. The maceration process was carried out by soaking 100 grams of this powder in 1 liter of ethanol for 72 hours with periodic agitation. Maceration was intentionally chosen over more aggressive techniques to preserve delicate compounds that could degrade under high temperatures or mechanical stress. Ethanol was selected as the solvent due to its well-established ability to extract a wide polarity range of phytoconstituents while remaining safe for human use and compatible with traditional medicine systems like Siddha and Ayurveda. The intermittent shaking during maceration helped maintain dynamic equilibrium and promoted the dissolution of active compounds into the solvent. After extraction, the solution was filtered using Whatman No.1 filter paper, and the filtrate was concentrated using a rotary evaporator under reduced pressure at 40–50°C, an essential step to remove ethanol without degrading the extract. The final semi-solid residue was further dried in a vacuum desiccator to obtain a stable extract suitable for storage and future pharmacological investigations.

The calculated yield of 23.18% signifies an efficient extraction process and reflects the abundance of extractable metabolites in *Abutilon crispum* stem. This high extractive value suggests that the stem of *A. crispum* are rich in ethanol-soluble phytochemicals such as flavonoids, alkaloids, tannins, phenolics, and glycosides—compounds typically associated with a broad range of pharmacological activities. It ensures that a relatively small amount of raw plant material can produce sufficient extract for bioassays, standardization studies, and potential formulation development. Additionally, such a high extractive value reduces the environmental footprint and resource demand for scaling up the production. In the broader context, this outcome highlights *Abutilon crispum* as a promising source of bioactive compounds and supports its traditional use in ethnomedicine. The results validate the methodological rigor of the extraction protocol and provide a strong foundation for subsequent analyses, including phytochemical screening (to identify major constituents like flavonoids, saponins, sterols, etc.), biological activity testing (e.g., anti-inflammatory and analgesic assays), and chromatographic profiling. Furthermore, the success of this extraction process contributes to the standardization of *A. crispum* as a phytotherapeutic

agent, which is essential for its integration into evidence-based complementary medicine.

3.2 Transverse section of *A. crispum* stems

Section 1



Figure 1: Transverse section of stem – 01

This image represents a complete transverse section of the stem, clearly exhibiting a ring of vascular bundles—a hallmark of dicot stems. The section demonstrates a well-organized tissue arrangement from the epidermis to the pith.

a) Epidermis

- A single-layered outer protective tissue composed of compact, rectangular cells.
- Covered by a cuticle and bearing multicellular trichomes—a diagnostic feature of *Abutilon crispum*.
- Trichomes provide protective functions and reduce water loss.

b) Cortex

- Beneath the epidermis lies the collenchymatous hypodermis, with cells showing unevenly thickened walls—providing mechanical strength to young tissues.
- The inner cortex consists of parenchymatous cells with large intercellular spaces aiding in storage and gaseous exchange.

c) Vascular Bundles

- Arranged in a distinct ring, each bundle is conjoint, collateral, and open.
- The phloem is oriented towards the outer side, consisting of sieve elements and companion cells.
- The xylem faces the center and is composed of tracheary elements—predominantly vessels and tracheids—facilitating conduction of water.

- A thin, cambial strip between xylem and phloem signifies the presence of secondary growth capacity.

d) Pith (Medulla)

- Located at the center, composed of large, thin-walled parenchyma cells.
- These cells may store metabolites and contribute to the turgor-driven rigidity of the herbaceous stem.

This section demonstrates a typical young dicot stem anatomy with primary growth dominance. The ring arrangement of vascular bundles with cambium indicates potential for secondary thickening, typical of maturing dicot stems. The multicellular trichomes and well-developed cortex reflect the plant's adaptive responses to stress and mechanical needs. This structure is especially relevant for taxonomical identification in pharmacognostical studies.

Section 2

This section provides a focused view of the central portion of the stem, particularly emphasizing the pith (medulla) and surrounding vascular bundles.

a) Pith (Central Parenchyma)

- Large, loosely packed parenchymatous cells with prominent intercellular spaces.
- Cells are nearly isodiametric, suggesting storage function and intracellular diffusion.
- A few regions may show lignified or thick-walled cells, hinting at the onset of maturation or mechanical reinforcement.

b) Vascular Bundle Details

- Xylem elements are rounded, some appearing circular in TS—indicating vessels.
- Phloem is compact and juxtaposed toward the cortex.
- The cambial ring may be indistinct in young stems but forms a continuous ring in older tissues.

c) Arrangement

- Bundles are organized in a ring, enclosing the central pith and maintaining radial symmetry.

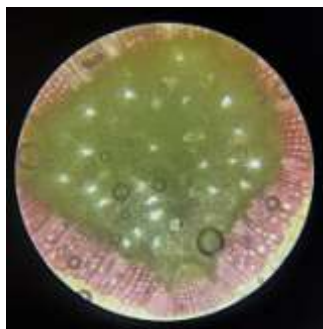


Figure 2: Transverse section of stem – 02

This image accentuates the conduction and storage tissues, pivotal to plant physiology. The large pith region facilitates the accumulation of metabolites, which is significant for medicinal plants like *Abutilon crispum*. The ring of vascular bundles with visible vessels supports the stem's structural and functional complexity. It also provides a basis for identifying developmental stages in tissue maturity.

Section 3

This high-magnification image zooms in on the outer stem region, offering clear visualization of epidermal, cortical, and vascular tissues.



Figure 3: Transverse section of stem – 03

a) Epidermis

- Outer single-cell layer with tightly packed cells, some of which bear unicellular and multicellular trichomes.
- Trichomes are distinctly visible—elongated and sometimes glandular.
- Covered by a cuticle, likely stained darker due to the histological stain uptake.

b) Cortex

- Collenchyma: Just beneath the epidermis, with thickened cell corners, offering flexibility and support.

- Parenchyma: Deeper into the cortex, with thin walls and intercellular spaces for storage.

c) Vascular Bundle Region

- The edge of the vascular ring can be seen, where xylem elements begin to lignify.
- Some vessel elements appear stained (possibly reddish or dark pink), which may indicate lignification or secondary wall deposition.

d) Pigmentation / Secondary Metabolite Indication

- The staining pattern in this section highlights the chemical nature of certain tissues—likely due to phenolics, lignin, or alkaloid presence.
- This can be related to the plant's defensive secondary metabolites, important for its medicinal role.

The detailed visualization of the epidermal and cortical layers is crucial for understanding mechanical, protective, and physiological functions. The presence of glandular trichomes and lignified vessels correlates with the biosynthesis and storage of pharmacologically active compounds. This section is also significant for histochemical localization of active constituents and can aid in the microscopic authentication of herbal raw material.

The three transverse sections of *Abutilon crispum* stem collectively exhibit:

- Primary dicotstem characteristics with potential for secondary growth.
- Presence of anatomical markers like trichomes, collenchyma, open vascular bundles, and large pith, aligning with Malvaceae family traits.
- Tissue regions involved in mechanical support, transport, storage, and defense, all relevant to the plant's ethnopharmacological use.

3.3 In-vitro DPPH radical scavenging assay

The antioxidant potential of the ethanol extract of *A. crispum* was assessed using the DPPH radical scavenging assay, with ascorbic acid serving as the standard. The percentage inhibition of DPPH free radicals by various concentrations (1–100 µg/mL) of the extract and ascorbic acid is presented in Table 1. A dose-dependent increase in scavenging activity was observed for both the ethanol extract and ascorbic acid.

Table 1: In-vitro DPPH radical scavenging assay

Concentration (µg/ml)	Ethanol Extract (% Inhibition ± SD)	Ascorbic Acid (% Inhibition ± SD)
1	7.61 ± 0.45	31.07 ± 0.62
10	19.23 ± 0.68	40.61 ± 0.77
25	30.74 ± 0.91	58.43 ± 1.05
50	41.09 ± 1.12	76.91 ± 1.38
100	56.95 ± 1.29	90.15 ± 1.51

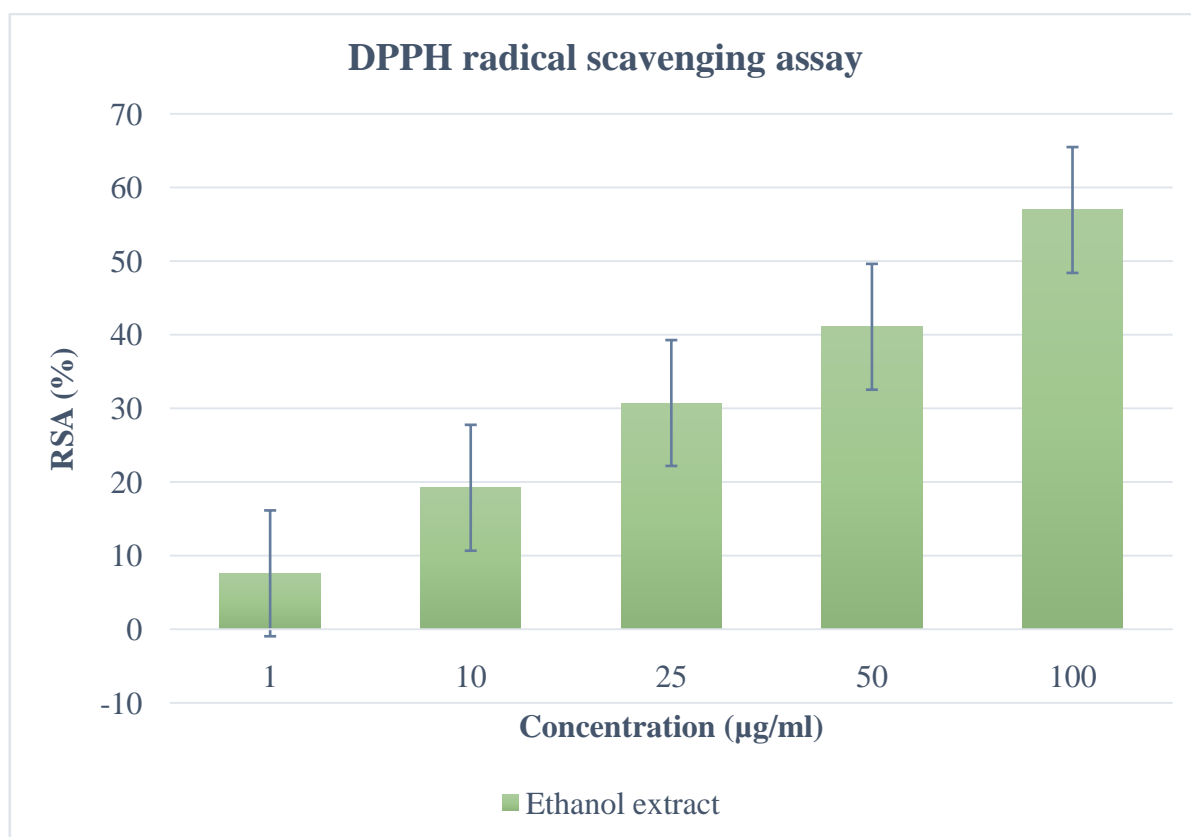


Figure 4: DPPH radical scavenging assay of EEAC

At 1 µg/mL, the ethanol extract exhibited 7.61 ± 0.45% inhibition, which increased progressively to 56.95 ± 1.29% at 100 µg/mL. In comparison, ascorbic acid demonstrated a

significantly higher antioxidant effect, with 31.07 ± 0.62% inhibition at 1 µg/mL and reaching 90.15 ± 1.51% at 100 µg/mL.

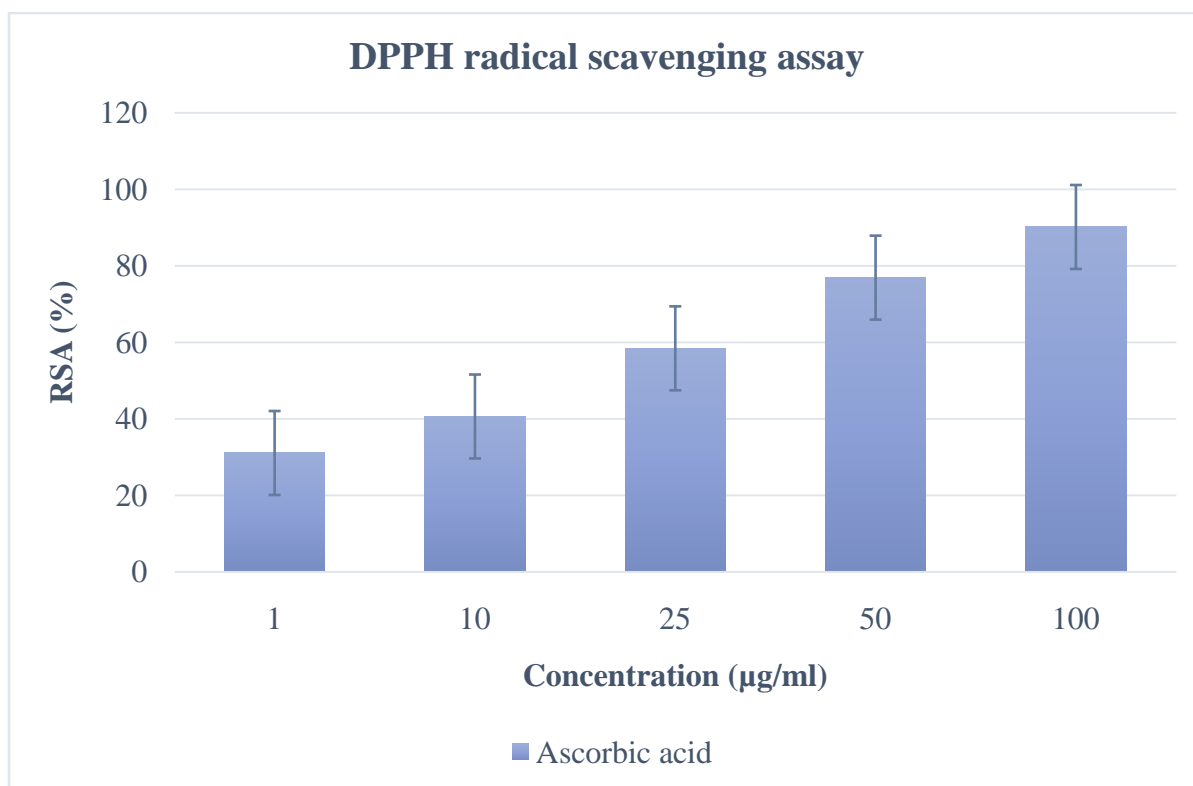


Figure 51: DPPH radical scavenging assay of ascorbic acid

The half-maximal inhibitory concentration (IC_{50}) values were calculated and found to be 74.04 µg/mL for the ethanol extract of *A. crispum*, indicating moderate antioxidant activity, whereas ascorbic acid showed a much lower IC_{50} value of 17.43 µg/mL, confirming its strong antioxidant potential.

The results from the DPPH assay indicate that the ethanol extract of *A. crispum* exhibits concentration-dependent free radical scavenging activity. This suggests the presence of bioactive compounds in the extract that are capable of donating hydrogen atoms or electrons to stabilize DPPH free radicals, a characteristic feature of antioxidant agents.

The mechanism of DPPH inhibition is based on the reduction of the DPPH radical (a stable free radical with a deep violet color) in the presence of an antioxidant. Upon interaction with antioxidant compounds, the DPPH radical accepts an electron or hydrogen atom and gets reduced to a yellow-colored diphenylpicrylhydrazine, resulting in a decrease in absorbance. The degree of discoloration reflects the scavenging potential of the test sample.

The moderate IC_{50} value of the ethanol extract (74.04 µg/mL) compared to ascorbic acid

(17.43 µg/mL) implies that while the extract is not as potent as the standard, it still possesses promising antioxidant constituents. This activity could be attributed to the presence of flavonoids, phenolic compounds, and other phytochemicals commonly reported in *A. crispum*. These compounds are known to act as effective free radical scavengers due to their structural capacity to donate electrons or hydrogen atoms and to delocalize unpaired electrons.

The observed antioxidant potential of *A. crispum* supports its traditional use and suggests its possible therapeutic application in preventing oxidative stress-related disorders such as cardiovascular diseases, neurodegenerative conditions, and inflammation.

IV. DISCUSSION

The present study comprehensively evaluated the extraction yield, anatomical characteristics, and antioxidant potential of *Abutilon crispum* (*A. crispum*), a plant widely recognized in traditional medicinal systems such as Siddha and Ayurveda. The ethanol extract of the stem displayed a 23.18% extraction yield, which is considerably high and indicative of the plant's rich phytochemical profile. Ethanol, a solvent of

intermediate polarity, is particularly effective at extracting diverse bioactive compounds such as phenolics, flavonoids, glycosides, and tannins, which are reported to contribute to antioxidant and anti-inflammatory activities. Similar studies on species within the Malvaceae family have shown ethanol extracts to yield bioactive fractions with strong pharmacological properties due to the solvent's ability to dissolve both hydrophilic and lipophilic constituents [17]. The extraction process followed here, involving shade drying and cold maceration, was specifically designed to preserve thermolabile compounds. This aligns with pharmacognostic best practices, ensuring chemical integrity and reproducibility of the extract.

The anatomical analysis of the stem provided critical diagnostic markers that support the correct identification of *A. crispum*. Pharmacognostical studies play a crucial role in the standardization of herbal raw materials, especially in ethnomedicine, where adulteration or misidentification of plant material is common. The presence of multicellular trichomes, collenchymatous cortex, open vascular bundles, and a large parenchymatous pith are all hallmark features of the Malvaceae family [18]. Trichomes, apart from their taxonomic value, are known to serve as sites for the biosynthesis and secretion of secondary metabolites, including phenolic compounds and terpenoids, which are often linked to antioxidant and antimicrobial activity. The clear visualization of lignified xylem vessels and cuticle layers further suggests that the stem tissues are adapted for both mechanical support and the synthesis of protective compounds. These structural attributes reinforce the pharmacological potential of *A. crispum* and support its use in traditional medicine.

The DPPH radical scavenging assay confirmed the antioxidant potential of the ethanol extract of *A. crispum* (EEAC). A concentration-dependent scavenging activity was observed, with the extract achieving $56.95 \pm 1.29\%$ inhibition at $100 \mu\text{g/mL}$, compared to $90.15 \pm 1.51\%$ for ascorbic acid at the same concentration. Although the extract exhibited moderate activity ($\text{IC}_{50} = 74.04 \mu\text{g/mL}$) compared to the potent standard ($\text{IC}_{50} = 17.43 \mu\text{g/mL}$), the results suggest that *A. crispum* contains active compounds capable of neutralizing free radicals. Free radicals, particularly reactive oxygen species (ROS), play a central role in oxidative stress-related pathologies such as cardiovascular diseases, neurodegeneration, and chronic inflammation [19]. Antioxidants such as

phenolics and flavonoids are known to donate hydrogen atoms or electrons to stabilize free radicals, and the observed activity of EEAC may be attributed to such mechanisms.

Previous studies have reported the presence of bioactive constituents like alkaloids, sterols, tannins, and flavonoids in *Abutilon* species, all of which are documented for their antioxidant and anti-inflammatory properties [20]. Flavonoids such as quercetin, kaempferol, and their glycosides, commonly found in Malvaceae, are effective free radical scavengers due to their ability to delocalize unpaired electrons via conjugated aromatic systems [21]. Similarly, phenolic acids are known to reduce oxidative stress by chelating metal ions and inhibiting lipid peroxidation. The moderate IC_{50} observed for EEAC in this study suggests that while the extract may not be as potent as pure ascorbic acid, it contains a complex mixture of synergistic compounds that collectively exhibit antioxidant activity. Importantly, crude plant extracts often demonstrate lower activity than pure standards due to the presence of inactive or antagonistic components; however, their multifaceted nature offers a broader range of biological effects [22].

The findings of this study are consistent with previous research on related *Abutilon* species. For example, ethanol extracts of *Abutilon indicum* and *Abutilon hirtum* have been reported to show comparable antioxidant activity with IC_{50} values ranging between 10 and $60 \mu\text{g/mL}$ and 0.50 and 0.72 mg/ml , supporting the notion that *Abutilon* species are rich sources of natural antioxidants [23, 24]. Furthermore, the antioxidant activity observed here provides a scientific basis for the ethnomedicinal use of *A. crispum* in managing conditions associated with oxidative stress, such as inflammation, pain, and degenerative disorders.

The correlation between anatomical traits and phytochemical activity is particularly noteworthy. The presence of trichomes and collenchymatous tissues suggests an evolutionary adaptation to stress conditions, often linked to the accumulation of phenolic and flavonoid compounds [25]. This structural-phytochemical relationship is well-documented in medicinal plants and underscores the importance of combining pharmacognostical studies with phytochemical and bioactivity assays.

V. CONCLUSION

The current findings highlight *Abutilon crispum* as a promising source of natural

antioxidants. The high extraction yield, coupled with the moderate free radical scavenging activity, suggests that the stem extract could serve as a potential therapeutic agent for oxidative stress-related disorders. Future work should focus on phytochemical profiling (e.g., HPLC, LC-MS) to identify and quantify the specific bioactive compounds, as well as in vitro and in vivo studies to explore additional pharmacological activities such as anti-inflammatory, analgesic, or antimicrobial effects. Standardization of the extract and formulation studies will further enhance its potential integration into evidence-based herbal therapeutics.

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