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Short Communication

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Phytochemical Screening and in Vitro Antibacterial and Antioxidant study of *Pergularia tomentosa* L. Collected from Southwestern Libya

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ABSTRACT

Libya is rich with wild range of medicinal plants, that represent a valuable source of bioactive compounds with potential therapeutic applications, where many species remain underexplored. *Pergularia tomentosa* L., a plant widely distributed in arid and semiarid regions of Libya, is subjected to investigation in this study for its phytochemical composition, antibacterial activity, and antioxidant potential. Whole plant material was extracted with Soxhlet method using three solvents of varying polarity (*n*hexane, chloroform, and methanol) in order to compare the biological effects of the resulting extracts. Antioxidant activity was evaluated through the DPPH radical scavenging assay relative to an appropriate reference standard. Antibacterial activity was assessed using the disc diffusion method against selected Grampositive and Gramnegative bacterial strains, using ciprofloxacin as a positive control and DMSO as negative control. Preliminary phytochemical screening revealed the presence of tannins, flavonoids, cardiac glycosides, steroids, and terpenoids. Notably, the plant was free of alkaloids, coumarins, and saponins. Biological assays showed that the nonpolar and semipolar extracts recorded the highest antibacterial activity against *Staphylococcus aureus*, whereas all extracts demonstrated a weak inhibitory effect on *Escherichia coli*. Conversely, the nonpolar extract exhibited the lowest antioxidant activity, while the semipolar and highly polar extracts demonstrated significant antioxidant potential. This study provides an initial framework for understanding the chemical profile and biological potential of the *Pergularia tomentosa* L. plant.

Key words- *Pergularia tomentosa* L.*; Libyan; plant; Acacus*; *phytochemicals*

INTRODUCTION

Herbal medicine is one of the oldest forms of healthcare known to man. About half of the drugs approved from 1998 to 2007 are based on natural source.[1] The thirteen natural product-related drugs were approved from 2005 to 2007. The medicinal value of plants is attributed to their rich chemical composition, which generates distinct physiological effects on biological systems. Among plant constituents, flavonoids, alkaloids, tannins, and phenolic compounds are recognized as the major bioactive substances responsible for therapeutic activity[1]. The global increase in antimicrobial resistance and the rising prevalence of oxidative stress-related diseases constitute major challenges to public health and modern medicine. Pathogenic bacteria resistant to conventional antibiotics have significantly reduced the effectiveness of current therapeutic agents, prompting an urgent need for alternative antimicrobial sources derived from natural products. In parallel, excessive generation of reactive oxygen species (ROS) disrupts cellular redox homeostasis, leading to oxidative damage of lipids, proteins, and DNA, and contributing to the development of chronic and degenerative diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions.[2,3] Medicinal and aromatic plants native to Libya were first briefly documented in a report by the United Nations Educational, Scientific and Cultural Organization, which provided morphological descriptions, medicinal properties, and the chemical composition of active substances in 93 wild poisonous plant species found in the country.[4] The Mediterranean climate of Libya supports the proliferation of a wide variety of plant species, many of which exhibit notable medicinal and antioxidant potential.[5] *Pergularia tomentosa* L. is classified within the family Asclepiadaceae and growing in the Saharan and sub-Saharan regions of North Africa, where it is reported in countries such as Algeria, Niger, and Egypt. The plant is a perennial herb exhibiting a climbing to partially erect growth form with 30 cm in height. Its stem is pale green to whitish in colour, extensively branched, and typically grows in an upright manner. The plant is characterized by the presence of a milky latex that can be readily extracted from its tissues.[6] The plant was used in folk medicine for diarrhea. The roots were used for the treatment of bronchitis, constipation, and skin diseases and leaves for bronchitis and tuberculosis.[7] *Pergularia tomentosa* L. has been found

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to contain many types of biologically active secondary compounds. Studies using phytochemical screening and chromatography on different parts of the plant have shown the presence of phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids, steroids, anthraquinones, quinones, and cardiac glycosides, with the leaves containing the greatest variety.[8] More advanced methods, such as HPLC and LC-MS, have identified specific compounds including gallic acid, ferulic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, catechin, naringenin, hesperetin, and kaempferol.[9] This study aims to examine *Pergularia tomentosa* L. (local name is Sellakha) collected from the Acacus Mountains in southwestern Libya and to compare its chemical composition with data reported from other regions where the plant naturally occurs. In addition, the study evaluates the antioxidant and antibacterial activities of its extracts to assess their potential as a new source of bioactive natural compounds.

MATERIALS AND METHODS

Plant materials:

Collection and Identification

Pergularia tomentosa L. was collected from the Acacus Mountains, southwestern Libya (26 January 2025), the plant was identified and authenticated by Prof. Mohamed Abuhadra at the Herbarium, Faculty of Science, University of Tripoli, where A voucher specimen number of 68951 was given to the plant.

Drying and Processing

The collected plant material was shade dried at room temperature. The dried sample was ground into a fine powder using an industrial mill. The resulting powder (73.8 g) was stored in airtight containers for further analysis.

Extraction

A sequential Soxhlet extraction was performed on the powdered plant material (58.3 g) using 250 mL each of n-hexane, chloroform, and methanol (Analytical Grade). Each extraction stage lasted approximately 6-8 h until the siphoning solvent was colourless. The resulting extracts were filtered (Whatman No. 1) and concentrated to dryness using a rotary evaporator under reduced pressure at a temperature below 40 °C. The crude extracts were transferred to dried, pre-weighed glass vials, and stored at °4 C in airtight containers until further analysis.[10]

Phytochemical screening:

Preliminary phytochemical screening of the plant extracts was conducted using standard qualitative tests to identify major classes of secondary metabolites.[11] Cardiac glycosides and anthraquinone glycosides were detected using the Keller–Killiani and Borntrager's tests, respectively. Steroids and terpenoids were identified using the chloroform–concentrated sulfuric acid test and the Salkowski test, respectively, while alkaloids were screened using Mayer's reagent. Tannins were assessed by the ferric chloride test, and saponins were evaluated using the foam test. Coumarins were identified employing the UV test, and flavonoids were screened using the sodium hydroxide (NaOH) reaction. The development of characteristic colour changes or stable froth was considered indicative of a positive result.

Antioxidant Activity (DPPH Assay)

Antioxidant activity of the plant extracts was assessed using a thinlayer chromatography (TLC)–DPPH assay. The three extracts and ascorbic acid (positive control) were applied to silica gel TLC plates and airdried at room temperature, followed by spraying with a 0.15% methanolic solution of 2,2diphenyl1picrylhydrazyl (DPPH). The appearance of yellow zones against a violet background was interpreted as evidence of free radical scavenging activity.[12]

Disc diffusion Antibacterial method

Escherichia coli (Gramnegative) and *Staphylococcus aureus* (Grampositive) were used as test organisms for antibacterial evaluation. Each strain was cultured on nutrient agar and incubated at 37 °C for 24 h to obtain fresh colonies, after which bacterial suspensions were prepared in sterile 0.85% saline and standardized to a 0.5 McFarland turbidity. Antibacterial activity of the plant extracts was assessed using the disc diffusion method on Mueller–Hinton agar; standardized suspensions were evenly swabbed onto the agar surface, and sterile 6 mm filter paper discs soaked with 20 µL of each extract were placed on the inoculated plates. Ciprofloxacin (5 µg) was used as the positive control, while 10% DMSO was the negative control.[13]

RESULTS

The percentage of yield was calculated for each residue (Table 1), Among the different extracts obtained, the *n*-hexane extract produced the lowest amount of yield. In comparison, the methanol extract gave the highest yield, while the chloroform extract showed a moderate yield

Table 1: The yields of plant extractions

	n-Hexane extract	Chloroform extract	Methanol extract
Weight (g)	0.963	1.098	2.766
% of Yield	3.52%	4.01%	10.10%

Preliminary phytochemical screening of the *Pergularia tomentosa* L. extract was carried out using standard qualitative tests to detect selected secondary metabolites. The Keller–Killiani test showed the formation of a brownish ring at the junction of the two layers, indicating the presence of cardiac glycosides. The ferric chloride/sodium chloride

test produced a yellow colour, indicating the presence of flavonoids, while the ferric chloride test gave a dark green colour, confirming the presence of tannins.

The foam test resulted in unstable froth measuring approximately 2.5 cm in height that persisted for 15 seconds, which indicated the absence of saponins, steroids were detected in the plant extract using the chloroform/sulfuric acid test, and terpenoids were identified using the Salkowski test.

The Borntrager's test for anthraquinone glycosides showed no colour change and was therefore recorded as negative. Similarly, the plant extract produced negative results for alkaloids and coumarins when tested using Mayer's reagent and UV identification tests, respectively.

Table 2: The preliminary phytochemical screening results for *Pergularia tomentosa* L.

No.	Phytochemical group	Test used	Result
1	Cardiac glycosides	Keller–Killiani	+ve
2	Flavonoids	NaOH	+ve
3	Tannins	$FeCl_3$	+ve
4	Saponins	Foam test	−ve
5	Steroids	Chloroform\Sulfuric acid	+ve
6	Alkaloids	Mayer›s Reagent test	-ve
7	Coumarine	UV test	-ve
8	Terpenoids	Salkowski test	+ve
9	Anthraquinone glycosides	Borntragers test	-ve

Antioxidant activity

The antioxidant activity of *Pergularia tomentosa* L. extracts was assessed using the DPPH radical scavenging assay. The *n*-hexane extract showed nonsignificant activity, as slight change in the violet colour of the DPPH reagent was observed. In contrast, both chloroform and methanol extracts caused a clear discolouration of the DPPH solution from violet to yellow, indicating effective free radical scavenging activity. The intensity of the colour change was comparable to that produced by the ascorbic acid positive control, suggesting that these extracts possess a significant antioxidant potential.

Table 3: The summarized the antioxidant activity results of *Pergularia tomentosa* L. extracts

Extract	Observation (DPPH reaction)	Antioxidant Activity
n-Hexane extract	Slight change in colour	Nonsignificant activity
Chloroform extract	Violet → yellow colour change	Significant activity
Methanol extract	Violet → yellow colour change	Significant activity
Ascorbic acid (Positive control)	Violet → yellow colour change	Strong activity

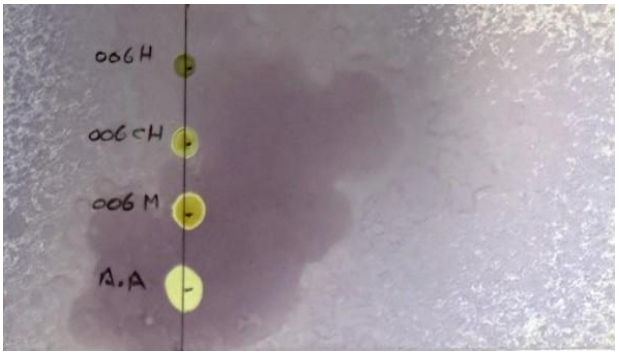

Figure 1: Antioxidant results for the three plant extracts.

Antibacterial activity

No antibacterial effect of *Pergularia tomentosa* L. extracts was observed against *Escherichia coli*, as the inhibition zones for all extracts were limited to the disc diameter (6 mm), which was identical to the negative control. In contrast, the extracts showed measurable antibacterial activity against *Staphylococcus aureus*. The diameters of inhibition zones against this strain ranged from 8 mm to 16 mm. Among the tested extracts, the *n*-hexane and chloroform extracts exhibited the highest antibacterial activity (16 mm), with an effect comparable to that of the positive control (24 mm). Overall, the results demonstrate that *Pergularia tomentosa* L. extracts possess antibacterial activity selectively against Grampositive bacteria.

The inhibition zone diameters produced by *Pergularia tomentosa* L. extracts against the tested bacterial strains are presented in Table 4.

Table 3: The inhibition zone diameters of *P. tomentosa L.* extracts against *E. coli* and *S. aureus.*

No.	Material	E. coli	S. aureus
1	*Pergularia tomentosa L.* n-hexane extract	6mm	16mm
2	*Pergularia tomentosa L.* chloroform extract	6mm	16mm

No.	Material	E. coli	S. aureus
3	*Pergularia tomentosa L.* methanol extract	6mm	8mm
4	Ciprofloxacin -positive control	26mm	24mm
5	DMSO- negative control	6mm	6mm

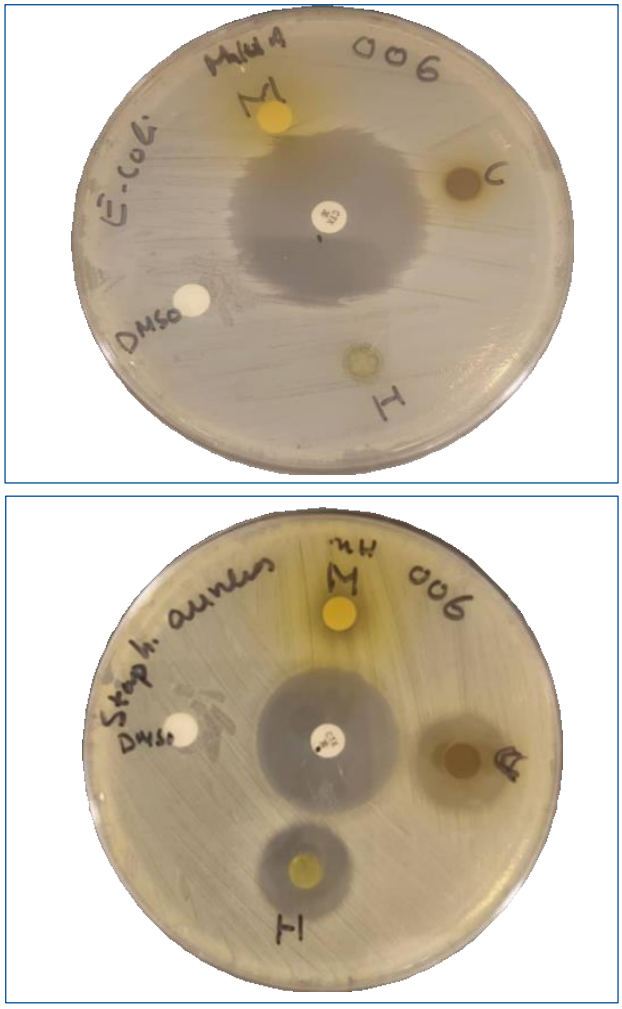

Figure 2: Antibacterial results for the three plant extracts.

DISCUSSION

The preliminary phytochemical screening of *Pergularia tomentosa* L. confirmed the presence of cardiac glycosides, flavonoids, tannins, steroids, and terpenoids, supporting previous reports that identify these metabolites as major constituents of the species. The positive Keller–Killiani reaction is consistent with the well-documented occurrence of cardenolides in *P. tomentosa*, which are considered responsible for its cardiotonic and cytotoxic activities. Similarly, the detection of flavonoids and tannins aligns with earlier investigations reporting high phenolic content in the aerial parts and leaves, contributing to the plant's antioxidant and antimicrobial properties.[14]

The presence of steroids and terpenoids further corroborates previous phytochemical studies that reported triterpenoid and sterol-related compounds in *P. tomentosa* L.[14] In contrast, the absence of saponins, anthraquinone glycosides, alkaloids, and coumarins in the present study differs from some published findings. These variations may be attributed to differences in extraction solvents, plant parts analyzed, geographical origin, or sensitivity of the qualitative tests employed.[15] Overall, the phytochemical profile observed in this study reinforces the medicinal potential of *P. tomentosa* L. and highlights the influence of methodological factors on reported phytochemical outcomes.

The antioxidant activity of *Pergularia tomentosa* L. extracts evaluated by the DPPH radical scavenging assay showed clear solvent dependent variation. The non-significant activity of the *n*-hexane extract suggests that nonpolar constituents contribute minimally to the antioxidant potential of the plant, whereas the considerable activity observed for the chloroform and methanol extracts indicates that antioxidant compounds are predominantly semipolar to polar in nature. The strong discolouration of the DPPH solution from violet to yellow produced by the chloroform and methanol extracts, comparable to that of ascorbic acid, confirms effective free radical scavenging capacity. This observation is consistent with previously reported antioxidant activity of *P. tomentosa* L. extracts assessed using the DPPH assay, where polar extracts exhibited significantly higher activity than nonpolar fractions.[16,17]

The observed antioxidant activity can be attributed to the presence of phenolic constituents, particularly flavonoids and tannins, which were detected in the phytochemical screening of the extracts. Previous studies have demonstrated a strong correlation between total phenolic and flavonoid contents and antioxidant activity in *P. tomentosa* L., highlighting these compounds as key contributors to its free radical scavenging properties.[7] Collectively, the present findings are compatible with the literature and support the potential of *Pergularia tomentosa* L. as a promising natural source of antioxidant agents.

The antibacterial activity of *Pergularia tomentosa* L. extracts showed clear selectivity toward Grampositive bacteria. No inhibitory effect was observed against *Escherichia coli*, which may be attributed to the protective outer membrane of Gramnegative bacteria that restricts the penetration of many plantderived compounds.[14] Similar resistance of *E. coli* to *P. tomentosa* extracts has been reported in previous studies.

In contrast, measurable antibacterial activity was observed against *Staphylococcus aureus*, with inhibition zones ranging from 8 mm to 16 mm. The highest activity was exhibited by the *n*-hexane and chloroform extracts, indicating that lipophilic and moderate polar constituents play an important role in the antibacterial effect. This finding is consistent with earlier reports demonstrating

stronger susceptibility of Grampositive bacteria, particularly *S. aureus*, to *P. tomentosa* L. extracts.[18,19] Based on these findings, these results suggest that *Pergularia tomentosa* L. possesses selective antibacterial potential primarily against Grampositive bacteria, likely due to the presence of bioactive metabolites such as steroids, terpenoids, and cardiac glycosides.

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