

# Exploring the Phytochemical Properties of *Prosopis africana* Plants and Assessing their Antimicrobial Potential Against Selected Clinical Strains

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**Abstract.** Natural products continue to play a significant role in drug discovery and development, and plants are recognised as a reservoir of myriads of active antimicrobial natural products. *Prosopis africana* plants obtained from Bauchi were explored in the research to ascertain the bioactive components present in the plant parts. About one hundred (100 g) grams of ground samples of the root, stem bark, and leaves of the plant were extracted (maceration method) using n-hexane, diethyl ether, ethyl acetate, acetone, and methanol sequentially in order of increasing polarity. The percentage yields for the crude extracts of the root, stem bark, and leaves were 7.0, 6.0, and 4.0%, respectively, while those of acetone, ethyl acetate, diethyl ether, and hexane also showed significant variations. The presence of alkaloid, tannin, flavonoid, saponin, terpene, cardiac glycoside, starch, resin, phlobatannin, and phenol in the phytochemical test is an indicator of the great attributes of the plant's parts on microorganisms. This was further confirmed on strains of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albican* using agar healthy diffusion techniques. The antimicrobial results show a higher zone of inhibition at concentrations of 400 mg/ml compared to 200 mg/ml, 100 mg/mL, and 50 mg/ml of the extract concentration. It is therefore concluded that with the global quest to search for new antibacterial active components that arose as a result of multiple drug resistances, the plant parts (root, stem bark, and leaves) of *Prosopis africana* can provide an alternative source of such bioactive compounds, thereby mitigating the increase in drug resistance among species of bacteria.

**Keywords:** Phytochemical; Antimicrobial; Saponin; *Prosopis Africana*; Maceration.

## INTRODUCTION

Many plant species utilised in herbalism are referred to as "medicinal plants" (also known as "herbology" or "herbal medicine"). It is the application of plants as medicines and the research into these applications [1].

The English word "herb" originates from the Latin "herba" and ancient French "herbe." Today, it encompasses any plant part, including non-woody plants like fruits, seeds, stems, bark, flowers, leaves, stigmas, and roots. Previously, "herb" only referred to non-woody plants, excluding those from trees and shrubs. These medicinal

plants are also used as food, flavonoids, medicine, perfume, and for certain spiritual activities [2].

Due to Africa's incredible floral biodiversity, herbal health practitioners can access an outstanding natural pharmacy. This has led to prescription herbal medications to treat, manage, and/or control various human illnesses. The World Health Organisation consultative group describes a medicinal plant as such if one or more of its sections contain compounds that have therapeutic value or are potential building blocks for effective medications [3].

Centuries ago, well before recorded history, humans harnessed the power of plants for medici-

nal reasons. Historically, herbal remedies were extensively catalogued and utilised across diverse civilisations like China, Egypt, and Unani traditions. Evidence spanning over 4,000 years highlights the reliance on herbs by Unani Hakims, Indian Vaid, and various European and Mediterranean cultures for medicinal purposes. Indigenous groups in Rome, Egypt, Iran, Africa, and the Americas incorporated herbs into their healing practices. Moreover, distinct medical systems such as Chinese medicine, Ayurveda, and Unani evolved, emphasising the systematic integration of herbal treatments [2].

About 85% of rural residents, including the impoverished in urban areas, use medicinal plant products and minerals as primary and crucial healthcare resources in combating various physical health ailments. The significance and role of these traditional healthcare systems are expected to endure in the future, as they are culturally relevant, safer for health, and projected to remain cost-effective, mainly as modern healthcare services are limited and costly [4].

*Prosopis Africana* is a deciduous tree which grows slowly to reach a height of 10 m (32 ft) by 6 m (19 ft). Insects carry pollen to the flowers. Nitrogen can be fixed by it. It prefers well-drained soil and is suitable for light (sandy), medium (loamy), and heavy (clay) soils. Acid, neutral, and bare soils are ideal for growing plants; they can also be alkaline or acidic. It doesn't thrive in the shadows. It likes damp soil. Strong winds are acceptable for the plant, but exposure to the sea is not [5].

The leaves cure various head conditions, including toothaches and headaches. Combining the leaves and bark treats rheumatism. The bark also treats fevers, caries, and skin conditions. Eye-wash is made from the bark. Diuretic roots are present. They treat bronchitis, dysentery, toothaches, and stomachaches [6].

*Prosopis africana* is the only species of its genus known to exist in Africa, and it grows wild throughout the Middle and Northern Belts of Nigeria. The stem bark has been utilised in traditional medicine to treat various conditions, including bronchitis, diarrhoea, fevers, caries, toothaches, and stomachaches. According to [7], the stem bark underwent chemical analysis, which led to the isolation and characterisation of component J29, a triterpene of the friedelane type. Using column chromatography, J29 was extracted from the ethyl acetate extract's chloroform fraction. The DEPT135 and J29 <sup>1</sup>H-NMR

<sup>13</sup>C-NMR spectra matched the distinctive information of the putative triterpene skeleton. Finally, the compound's identity as Friedelin was established through spectroscopic data, which included its IR spectra and 2D NMR. For the first time, Friedelin's chemistry has been linked to *Prosopis africana* [7].

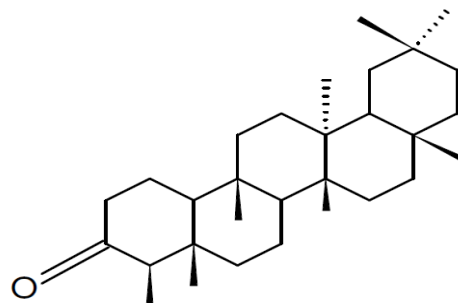


Figure 1 – Friedelin

Chromatographic methods were used to isolate two compounds (with antimicrobial and cytotoxic properties) from the aerial parts of *Prosopis africana* stem barks. These compounds were identified through spectroscopic techniques as 7, 3', 4'— trihydroxy-3-methoxy flavanone and dehydroabiatic acid [8].

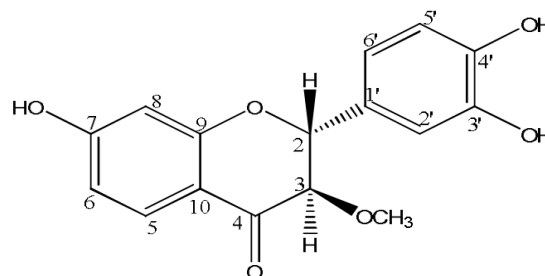


Figure 2 – 7,3',4'-trihydroxy-3-methoxy flavanone

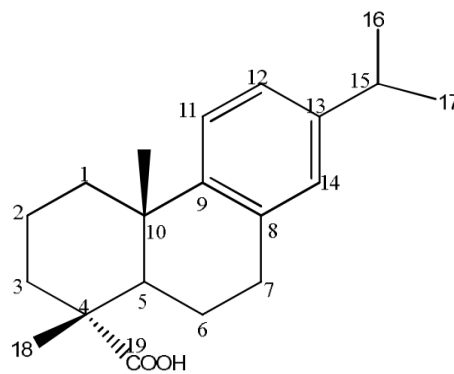


Figure 3 – Dehydroabiatic acid

*Prosopis africana*, a member of the Leguminosae family, is used in traditional medicine for various conditions such as diarrhoea, dysentery, malaria, male infertility, and a heart tonic. A study of [6] examined fatty alcohols' antibacterial and anti-tuberculosis properties. Heneicosanol (1), hexacosanol (2), nonacosanol (3),  $\beta$ -sitosterol (4), quercetin (5),  $\beta$ -sitosterol 3-O- $\beta$ -D-glucopyranoside (6), and quercitrin (7) were extracted from the leaves of *Prosopis africana*. Their structures were identified through spectroscopic analysis and comparison with existing literature data. For the first time, this plant's hexacosanol (2), nonacosanol (3), and heneicosanol (1) were extracted and showed antibacterial and antituberculosis properties. The study's findings indicate that compounds 1, 2, and 3 exhibited minimal bacterial concentrations (MBC) of 1.56  $\mu$ g/mL, 1.62  $\mu$ g/mL, and 0.12  $\mu$ g/mL against *M. tuberculosis* and *M. smegmatis*, respectively. Fatty alcohol research offers proof of action against clinical isolates of mycobacteria, strong anti-inflammatory and analgesic qualities, and a foundation for its potential application as a low-cost, high-potency natural anti-tuberculosis drug [6].

## METHODS

**Sample collection and identification of the plants.** Fresh roots, stem bark and leaves of *Prosopis africana* were collected at Burshi Fulani, Sabon Laura and Gubi Dam, respectively, within the Bauchi metropolis. The plant samples were taken to Biological Science, Abubakar Tafawa Balewa University, Bauchi State, for proper identification. The samples were then air dried at room temperature to avoid destroying the active components by the sun's heat. The dried samples were then pounded using mortar and pestle into fine powder, which was kept at room temperature in an airtight container before analysis.

**Extraction.** About 100 g of each sample (stem bark, leaves, and roots) was extracted using a cold extraction method (maceration) with the following solvents: diethyl ether, ethylacetate, n-hexane, methanol, and acetone for four days. The extracts were then filtered and concentrated with a rotary evaporator.

### Phytochemical Screening of the Crude Extract

**Test for saponins.** The screening test for saponins in the sample was based on the saponins' capaci-

ty to froth in an aqueous solution and hemolyse red blood cells [9]. The methodology was applied. A test tube was filled with 5 ml of crude extract. Distilled water was measured using a 10 ml measuring cylinder, which was then added to the mixture and thoroughly agitated for approximately three minutes. The presence of continuous foam indicates saponins.

**Test for resin.** The crude extract (5 ml) was dissolved in distilled water and vigorously shaken until turbidity formed, indicating the presence of resins [10].

**Test for starch.** Using an analytical weighing balance, 0.1 g of iodine crystals and 0.1 g of potassium iodide were weighed. Five millilitres of distilled water were measured using a 10-millilitre measuring cylinder. The dissolved iodine crystals and potassium iodide were then mixed with two millilitres of the crude extract [10, 11] to describe the use of the Dragendorffs, Mayer's, and Wagner's tests to identify the presence of alkaloids in the crude extract.

The mixture was heated in a boiling water bath after adding 5 ml of 2 M HCl to 0.5 g of the crude extract. After filtering, the mixture was divided into three test tubes and utilised for the following test:

**Dragendorffs Test.** One millilitre of the filtrate was combined with a few drops of Dragendorff's reagent. The emergence of a deep brown colour indicates the presence of alkaloids.

**Mayer's Test.** One millilitre of the filtrate was mixed with a few drops of Mayer's reagent. A yellow colour change in the formation can detect alkaloids.

**Wagner's Test.** One millilitre of the filtrate was mixed with a few drops of Wagner's reagent. Alkaloids are present when a reddish-brown hue begins to appear.

**Test for phenols.** The Ferric chloride test involved dissolving 0.5 g of crude extract in 5 ml of distilled water, heating the mixture, and adding a few drops of 1% iron (III) chloride solution. A blue-black hue indicated the presence of a hydroxyl group attached to a phenol [10].

### Test for flavonoids

**Shinoda Test.** To an alcoholic solution of the sample (3 ml of ethanol and 0.5 g of crude extract), magnesium powder 0.1 g and four drops of concentrated HCl were added.

**10% NaOH Solution Test.** The crude extract (0.5 ml) was diluted in 5 ml of distilled water, followed by dropwise additions of NaOH to 20 ml of the solution. The presence of flavonoids was indicated by a yellow hue, which turned colourless upon adding HCl.

**Lead acetate.** The crude extract (0.5 g) was dissolved in 5 ml of distilled water, and then 1 ml of 1% lead acetate solution was added. The appearance of yellow colour indicates the presence of flavonoids [12].

**Test for terpenoids.** The detection of terpenoids in the crude extract was accomplished through Salkowski's test [13] and Harbone's test [10].

**Salkowski's Test.** The crude extract (0.5 ml) was treated with chloroform (2 ml), and 3 ml of concentrated sulphuric acid was added in a slanted position. The appearance of a reddish-brown colour at the interface confirmed the presence of terpenoids.

**Harbone's Test.** The addition of 1 ml of acetic anhydride to the crude extract, followed by the addition of 1 ml of concentrated sulfuric acid in a diagonal position, resulted in the formation of a reddish-brown colouration at the interface, indicating the presence of Terpenoids.

**Test for cardiac glycoside.** The Keller-Killian test, utilised to detect cardiac glycoside, involved dissolving 0.5 g of the crude extract in 2 ml of glacial acetic acid with one drop of ferric chloride solution. Then, 1 ml of concentrated sulphuric acid was gently added to the solution along the side wall of the test tube. The presence of a brown ring at the interphase indicated the presence of cardiac glycoside.

**Check for the presence of steroids.** The presence of steroids can be detected by dissolving 0.5 ml of crude extract in 2 ml of chloroform, adding 0.5 ml of acetic anhydride solution, and then introducing two drops of sulfuric solid acid at a slant position. The formation of a pink hue, as described in [10], confirms the presence of steroids.

**To test for phlobatannins.** The crude extract measured at 0.5 mL was heated in a test tube in a water bath with 1% hydrochloric acid. Phlobatannins can be detected by the production of red residue [14].

**Preparation of the media.** After weighing 28 grammes of nutrient agar (NA), the material was put into a flask with one litre of distilled water. After heating the mixture on a hot plate until it

entirely dissolved, it was autoclave sterilised for 25 minutes at 121 °C, and then it was allowed to cool to 45°C (the temperature at which agar remains molten). After that, it was placed into petri dishes or other sterilised plates to gel or solidify.

#### **Inoculation and application of the extracts.**

A small amount (0.1 ml) of the liquid inoculum (containing test organisms) was spread onto the surface of agar plates using a spreading rod and a micropipette. Holes with an 8.0 mm diameter were created in the agar using a sterile cork borer. Drops of the extracts and control were placed in the holes to allow diffusion into the agar medium and were left at room temperature for an hour. Bacterial strains were then incubated for 24 hours at 37 °C, while fungal strains were incubated for 72 hours at 25 °C. The ability of the plant extracts to inhibit microorganisms was determined by measuring the inhibition zones in millimetres.

#### **Determination of minimum inhibitory concentration.**

The minimum indicator of turbidity in a test is the concentration at which no discernible turbidity is present. The dilution method determined the extracts' minimum inhibitory concentration values. The plant extract was prepared with different concentrations (400, 200, 100, and 50 mg/ml). Three test tubes, including control tubes, were labelled and filled with 0.5 ml of sterile water each. Then, 0.2 ml suspension of the test organisms was added to the respective test tubes using sterile Pasteur pipettes according to their labelling [15].

#### **Determination of minimum bactericidal concentration.**

The minimum bactericidal concentration (MBC) is determined by sub-culturing onto prepared nutrient agar plates and streaking with a sterile inoculating loop. This process, described by [15], follows serial dilution from the MIC tube. After 24 hours of incubation at 37 °C, tubes without microbial growth are sub-cultured onto new agar plates. Following another 24-hour incubation, plates with the lowest extract concentration and no bacterial growth are recorded as the MBC.

## **RESULTS AND DISCUSSION**

The percentage yield of the plant extract *Prosopis africana* root is the highest at 7% for methanol extract, followed by acetone at 5.0% and ethyl acetate at 3.0 %, as shown in Table 1. Hexane and

diethyl ether root extract had the lowest percentage. The leaf and stem bark extract gave a similar pattern, with methanol extract being the highest, followed by acetone, ethyl acetate, diethyl ether, and then hexane. These correspond to the report of [16]. Similarly, [17] reported the percentage yield of 2.5 % extract from 250 g of *Sterculia setigera* leaf sample.

Alkaloid, saponin, resin, starch, cardiac glycoside, steroid, phenol, terpene, tannin, flavonoid, and phlobatannin were all confirmed to be present based on the results. Alkaloid, saponin, resin, starch, cardiac glycoside, steroid, phenol, terpene, tannin, flavonoid, and phlobatannin were found in the phytochemical screening of *Prosopis africana* (root, stembark, and leaf) as seen in Ta-

ble 2. This finding was consistent with reports from [7] and [8].

According to [6], *Prosopis africana* stem bark's alkaloids are responsible for its analgesic properties and capacity to treat fever and toothaches. The *Prosopis africana* plant is utilised as an anti-cancer and wound-healing agent due to the saponins' demonstrated hypolipidemic and anti-cancer properties [18].

The plant's chemical components may affect its medicinal efficacy. For instance, phenolbauannins, secondary metabolites with well-known antibacterial qualities, can help heal burns and wounds [19, 20].

Table 1 – Crude extract percentage yield for *Prosopis africana*

Sample	Weight of Sample (g)	Solvent used and percentage recoveries (%)				
		Hexane	Diethyl ether	Ethyl acetate	Acetone	Methanol
Prosopis Africana						
Root	100	1.0	2.0	3.0	5.0	7.0
Stembark	100	0.4	1.0	2.0	4.0	6.0
Leaves	60	3.0	3.0	1.7	3.2	4.0

Table 2 – Phytochemical Screening of *Prosopis Africana*

Sample	Solvent Extract Type	Saponin	Tannin	Flavonoid	Mayers Alkaloid	Wanger's Alkaloid	Steroid	Terpenes	Starch	Resin	Cardiac glycoside	Phtobatanin	Phenol
Prosopis Africana root	Diethyl ether	+	+	+	+	+	+	+	-	+	+	+	+
	Ethyl acetate	+	+	+	+	+	-	+	-	+	+	-	+
	Acetone	+	+	-	+	+	-	-	+	+	+	+	+
	Methanol	+	+	+	+	+	+	-	+	+	+	+	+
Prosopis Africana stembark	Diethyl ether	+	+	+	+	+	+	+	-	+	+	+	+
	Ethyl acetate	+	+	+	+	+	+	+	+	-	+	+	+
	Acetone	+	+	-	+	+	+	+	-	+	+	+	+
	Methanol	+	+	-	+	+	-	+	+	+	+	+	+
Prosopis Africana leaves	Diethyl ether	+	+	+	+	+	+	+	-	+	+	+	+
	Ethyl acetate	+	+	+	+	+	-	+	+	+	+	+	+
	Acetone	+	+	-	+	+	-	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+	+	+	+	+	+	+

Notes: "+" – Present; "-" – Absent.

Table 3 – Antimicrobial Activity of *Prosopis africana* (PA) Root

Extract	Conc. In mg/ml	Zones of inhibition in mm				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Fungi <i>Candida albican</i>
PA (roots) Diethyl ether	400	17.50	15.00	16.00	15.00	14.00
	200	13.00	12.10	15.00	13.00	11.00
	100	10.00	09.00	11.00	12.00	09.00
	50	06.50	07.00	06.00	04.00	00.00

Extract	Conc. In mg/ml	Zones of inhibition in mm				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Fungi <i>Candida albican</i>
PA (roots) Ethyl acetate	400	10.0	07.0	06.0	09.0	14.0
	200	08.0	05.0	05.0	04.0	08.0
	100	06.0	04.0	04.0	02.0	06.0
	50	06.0	02.0	02.0	02.0	00.0
PA (roots) acetone	400	16.10	18.0	16.50	16.0	18.60
	200	12.00	10.0	13.20	12.0	10.50
	100	8.00	8.0	09.00	12.0	3.50
	50	6.50	6.0	04.00	10.0	6.00
PA (roots) methanol	400	18.50	22.0	20.00	19.00	21.50
	200	14.00	16.0	17.50	14.00	16.00
	100	10.50	12.0	09.00	10.00	12.60
	50	7.00	08.0	07.00	07.00	8.50

Table 4 – Antimicrobial Activity of *Prosopis africana* (PA) Stem bark (SB)

Extract	Conc. In mg/ml	Zones of inhibition in mm				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Fungi <i>Candida albican</i>
PA (Stembark) Diethyl ether	400	14.50	13.00	13.50	12.50	17.50
	200	13.00	11.00	10.00	10.00	16.00
	100	04.00	09.00	10.00	09.00	12.50
	50	02.10	04.00	04.00	06.00	69.00
PA (Stembark) Ethyl acetate	400	10.50	13.00	10.50	12.00	16.50
	200	09.00	11.50	08.00	09.00	15.00
	100	08.10	07.00	07.50	05.00	12.00
	50	02.00	04.00	02.00	03.00	06.00
PA (Stembark) acetone	400	16.00	21.0	18.00	21.0	18.0
	200	12.00	16.5	13.50	17.2	12.0
	100	10.50	13.2	10.00	11.0	08.0
	50	05.00	09.0	09.00	67.0	06.0
PA (Stembark) methanol	400	18.00	19.0	17.00	17.5	19.2
	200	15.50	15.0	12.00	11.0	16.0
	100	13.25	11.0	09.00	08.0	11.0
	50	7.00	09.0	03.00	07.0	08.0

Table 5 – Antimicrobial Activity of *Prosopis africana* (PA) Leaf

Extract	Conc. In mg/ml	Zones of inhibition in mm				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Fungi <i>Candida albican</i>
PA Leaf Diethyl ether	400	08.10	09.10	10.50	10.00	12.10
	200	04.10	06.50	09.00	06.00	10.50
	100	02.00	04.50	04.00	03.00	08.00
	50	00.00	02.00	02.00	02.00	02.00
PA leaf Ethyl acetate	400	10.20	11.50	13.60	09.50	10.00
	200	06.10	10.00	10.50	07.00	09.00
	100	04.50	09.00	09.00	04.00	04.10
	50	02.10	02.00	06.00	00.00	02.10
PA leaf acetone	400	16.00	15.00	19.50	16.00	18.00
	200	12.10	12.50	14.00	14.00	14.00

Extract	Conc. In mg/ml	Zones of inhibition in mm				
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	Fungi <i>Candida albican</i>
	100	08.00	09.50	12.10	06.00	12.00
	50	04.00	06.00	10.00	02.00	06.00
PA leaf Methanol	400	15.00	17.40	18.50	15.30	19.5
	200	12.00	12.30	16.00	10.00	16.0
	100	9.00	08.00	14.00	08.00	12.6
	50	6.00	06.00	02.00	0.00	8.5

The roots stem, bark, and leaves of *Prosopis africana* underwent a maceration process using n-hexane, diethyl ether, ethyl acetate, acetone, and methanol in increasing polarity over 72 hours. Phytochemical screening confirmed the presence of alkaloids, saponins, resins, starch, cardiac glycosides, steroids, phenols, terpenes, tannins, flavonoids, and phlobatannins. Antibacterial properties against *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* were evaluated using clinical isolates. Methanol extract yielded the highest percentage, followed by hexane, acetone, ethyl acetate, and diethyl ether. The antimicrobial result demonstrates broad-spectrum activity in all the isolates as well.

Additionally, the results indicate that the 400 mg/ml extract concentration has a higher zone of inhibition value than the 50 mg/ml concentration, which is why the 400 mg/ml extract dosage is favoured, as shown in Table 3. The presence of secondary metabolites demonstrated the therapeutic value of *Prosopis africana*'s stems, roots, and leaves, which may account for some of the plant's reported antibacterial action. This clarifies why it has been used traditionally in health care and is the initial stage for discovering new drugs.

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## CONCLUSIONS

In conclusion, the research demonstrated significant photochemical diversity and potent antimicrobial activities in the roots, stem bark, and leaves of *Prosopis africana*. These findings underscore the medicinal potential of this plant species and provide valuable insights for further research in natural product discovery and drug development. Despite the limitations of our study, including instability of light and difficulty in securing the samples, the results warrant continued investigation into the therapeutic applications of *Prosopis africana*. Overall, our findings contribute to the growing body of knowledge on the bioactivity of medicinal plants and highlight the importance of preserving biodiversity for sustainable healthcare solutions.

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## Conflict of Interests

The researchers of this great work declare no contradictory interest.

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