

BIOPROSPECTING *STACHYTARPHETA CAYENNENSIS*: EXTRACTION AND MOLECULAR INSIGHTS INTO ITS THERAPEUTIC POTENTIAL

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ABSTRACT

The water and ethanol extract of *Stachytarpheta cayennensis* leaves were studied for therapeutic activities. The present study aimed to establish a phytochemical profile of ethanol and water extract of *S. cayennensis*. The total phenolic content was determined using the Folin Ciocalteu method. The leaf extracts have been evaluated for antimicrobial activities against *Escherichia coli* using agar well diffusion method and anti-oxidant potential by DPPH method using ascorbic acid as standard. The phytochemical screening of both ethanol and water extract revealed the presence of carbohydrates proteins and amino acids. The plant ethanol extract showed bactericidal activities against *Escherichia coli*. DPPH assay on crude leaves extracts showed 88.08 percentage scavenging activity at 1000 µg/ml. The study revealed the ethanolic extract of the plant is good source of antioxidant. IR spectra of ethanol and water extracts revealed the presence of different types of functional groups such as alcohols phenol and amines.

KEYWORDS: DPPH, Folin Ciocalteu, agar well diffusion, IR spectra.

INTRODUCTION

Stachytarpheta cayennensis (L.C.Rich) Vahl. Family verbanaceae is found in several regions of the world including the Bahamas, Brazil, Ghana, Haiti, India, Jamaica, Cameroon, Malaysia, Mexico, West Indies and Nigeria as weeds (Schwontkowski, 1993). The extracts of the leaves are used ethno medicinally in many tropical countries including Nigeria as sedative, anxiolytic and antipsychotic remedies (Burkill, 1966; Akanmu et al, 2005). Also in Ghana, a West African nation like Nigeria, the extracts of the leaves of *Stachytarpheta cayennensis* (*S.cayennensis*) are employed in their traditional medicine for the management of mental illness (Burkill, 1966). It is an annual and sometimes perennial herb growing to the height of 60 to 150cm (Haselwood and Motter, 1966). Previous work on the leaves of *S.cayennensis* reported its anthelmintic (Alice et al, 1991), anti-inflammatory (Vela et al, 1997), anti-

nociceptive (Perido et al, 2006), anti-ulcerogenic (Almeida et al, 1995), antimalarial (Okonkon et al, 2008) and antidiarrhoea effects (Kvist et al, 2006). Our previous study reported the sleep modulation and toxicity of the leaves of this plant with a projection that the mechanism of its sedative activity and other effects would be investigated (Akanmu et al, 2005). The present study reports the qualitative and quantitative analysis of biological compounds and determination of antioxidant and antimicrobial activity of *S.cayennensis*.^[7,8]



Fig. 1: *Stachytarpheta cayennensis*.

MATERIALS AND METHODS

Collection of leaves: The leaves of *Stachytarpheta Cayennensis* were collected from Hoskote, Bangalore Rural District, Karnataka, India. The collected leaves material was immediately sprayed with ethanol to cease the enzymatic degradation of secondary metabolites. The leaves were shade dried and powdered inside the laboratory within 10-15 days at room temperature (28-30°C). The plant was identified by the Head of Department of Botany Dr. Jyothi S Uppar. Approximately 50 g of fresh leaves were collected and shade-dried. The dried leaves were finely powdered using a grinder, and 16 g of the powdered material was used for extraction with each solvent (ethanol and distilled water). The phytochemical screening is done by standard procedures.

QUALITATIVE ANALYSIS

Done by standard procedures.^[1,2,3,5]

QUANTITATIVE ANALYSIS

Determination of total phenol content

Total phenolic content was estimated by Folin Ciocalteu's method. 1ml of aliquots and standard gallic acid was positioned into the test tubes, 5 ml of distilled water and 5ml of Folin Ciocalteu reagent was mixed and shaken well. After 5 min 1.5 ml of 20% sodium carbonate was added and volume was made up to 10 ml with distilled water. It was allowed to incubate for 45 min at room temperature. Intense blue colour was developed, absorbance was measured at 750 nm using spectrophotometer. Gallic acid is used as standard. The equivalent concentration of Gallic acid (C₁) was determined from the calibration curve based on the sample absorbance. Use the formula: $C = C_1 \times VmC = mC_1 \times V$ Where: C = Total phenolic content (mg/g, expressed as Gallic Acid Equivalent, GAE). C₁ = Concentration of Gallic acid obtained from calibration curve (mg/ml). V = Volume of extract (ml). m = Weight of extract (g).^[8]

ANTIOXIDANT ACTIVITY

Freshly prepared the DPPH solution (100µM) in 80% methanol was taken in test tubes and test compounds with different concentrations (31.25, 62.5, 125, 250, 500,

1000µg/ml) were added in 1:1 ratio to all test tubes to achieve the final volume of 1ml. Incubate the Test Tubes for 30mins at room temperature in the absence of light by covering with aluminium foil. Each and every reaction mixtures keep in separate tubes to keep them as a blank correction. Ascorbic acid with different concentrations was used as standard and 80% Methanol as a Blank. Control sample was prepared containing the same volume without any sample (DPPH alone). Read the absorbance on a Plate absorbance reader at 517nm. The % radical scavenging activity of the given compound was calculated using the following formula: % DPPH Radical Scavenging Activity = $[(\text{Abs of Control} - \text{Abs of Sample}) / \text{Abs of Control}] \times 100$. IC₅₀ value was calculated from the linear or logarithmic regression equation ($Y = mx + C$) derived from % DPPH inhibition graph. In the equation, Y=50, and m and C values were derived from the DPPH inhibition graph.^[1,4,5,12,13,14]

IR SPECTROSCOPY

Procedure: IR spectroscopy is used to identify functional groups in molecules by seeing how they absorb infrared light. The liquid sample of the extract is placed between the sodium chloride plates, while the solid sample is finely ground with dry KBr and compressed into a transparent pellet. The spectrometer is switched on and background spectrum is recorded. The prepared sample is then placed in the sample holder and the spectrum is scanned over the range of 4000-400 cm⁻¹. Based on the bands obtained the functional groups present in the extract can be detected.

ANTIMICROBIAL ACTIVITY

Bacterial Culture and cell suspension Preparation

Luria Bertani (LB) broth (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Distilled water 1000mL) 30mL was prepared in 4 Erlenmeyer flasks by adding Tryptone 0.3g, Sodium chloride 0.3g, Yeast extract 0.18g, Distilled water 30mL and autoclaved at 121°C for 15 minutes. Later, *Escherichia coli* was inoculated respectively in 30mL of sterilized LB broth and incubated at 37° C for 24h. Bacterial Culture preparation Cultured organism (*Escherichia coli*, were centrifuged at 6000rpm for 10

minutes respectively, supernatant was discarded and the pellets were dissolved in 1% (%) Sodium chloride and adjusted to absorbance 1.000 at 600nm under UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer).

Sample preparation

The sample 10mg (*S.cayennensis* extract) is was dissolved in 1mL. Dimethyl sulfoxide (DMSO) respectively. Different aliquots of the sample and control was prepared by pipetting 10 μ L (100 μ g), 20 μ L (200 μ g), 30 μ L (300 μ g) and 40 μ L (400 μ g) and the final volume was made upto 50 μ L by adding DMSO. Media preparation for MIC Luria Bertani (LB) agar media (Tryptone 10g, Sodium chloride 10g, Yeast extract 6g, Agar 20g, Distilled water 1000ml) 500mL was prepared by adding Tryptone 5g, Sodium chloride 5g, Yeast extract 3g, Agar 10g, Distilled water 500mL in Erlenmeyer flask and autoclaved at 121°C for 15 mins. 29 Plating for MIC against organisms.^[1,5,9]

RESULTS AND DISCUSSIONS

Table 1: Phytochemical screening of ethanol and water extracts.

Sl. No	Phytochemicals tests	Water extract	Ethanol extract
1	Alkaloids	+	+
2	Polyphenols	+	+
3	Tannins	-	-
4	Glycosides	+	+
5	carbohydrates	+	+
6	Saponins	+	-
7	Flavonoids	+	+
8	Steroids	-	+
9	Terpenoids	-	+
10	Proteins and amino acids	+	+

ANTIOXIDANT ACTIVITY

Table 3: DPPH Radical Scavenging Activity (Ethanol extracts).

Sl no	Tested sample	Conc of sample in μ g/ml	Percentage Inhibition	IC ₅₀ (μ g/ml)
1	Ascorbic acid	1.56	34.70	2.35
		3.125	59.87	
		6.25	81.79	
		12.50	92.86	
		25	96.11	
		50	98.12	
		100	99.62	
2	<i>S.cayennensis</i> Ethanol extract	62.5	21.97	247.70
		125	29.44	
		250	52.01	
		500	67.14	
		1000	88.08	

The antioxidant activity determined by free radical scavenging, DPPH assay using gallic acid as standard. Ethanol extract showed 88.08% scavenging activity at 1000 μ g/ml.

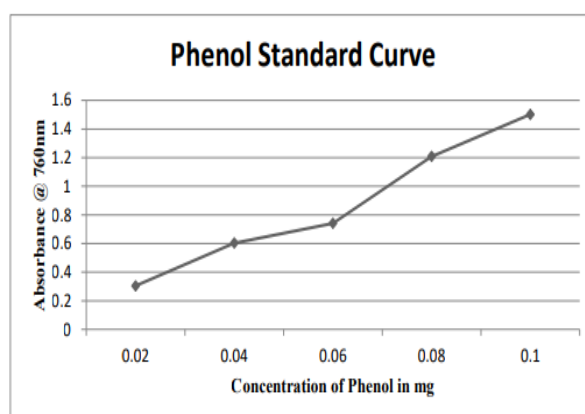
(+) = presence & (-) = absence

Phytochemical screening revealed the presence of alkaloids, steroids, polyphenols, glycosides, flavonoids, terpenoids, carbohydrates, Proteins and amino acids. However, and saponins tannins were absent in ethanol extract. Water extract revealed the presence of alkaloids, Polyphenols, flavonoids, glycosides, carbohydrates, Proteins and amino acids. However, and tannins, Steroids and terpenoids were absent in ethanol extract.

QUANTITATIVE ESTIMATION

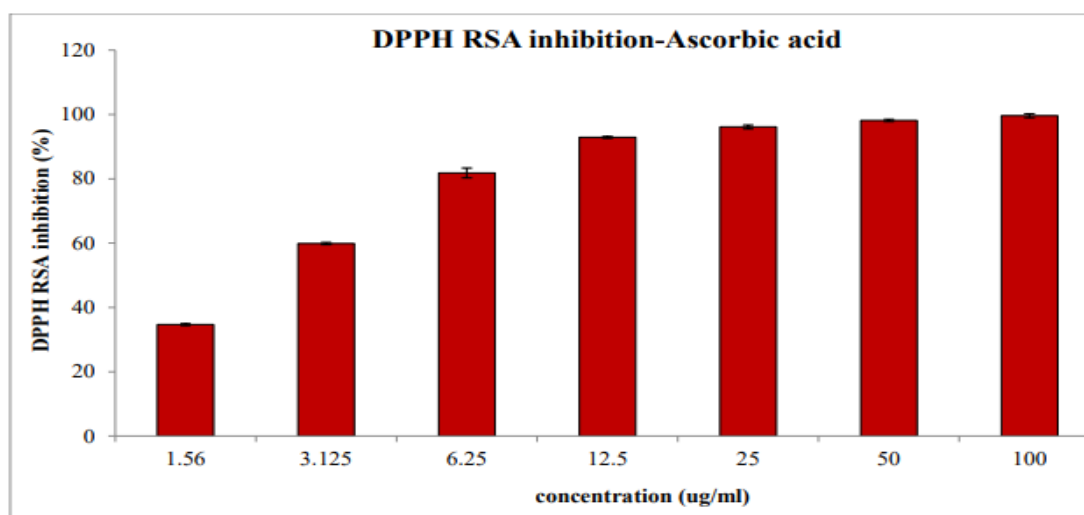
Table 2: The total phenolic content.

SL.NO	EXTRACT	RESULTS (%)
1	ETHANOL	0.15
2	WATER	0.15

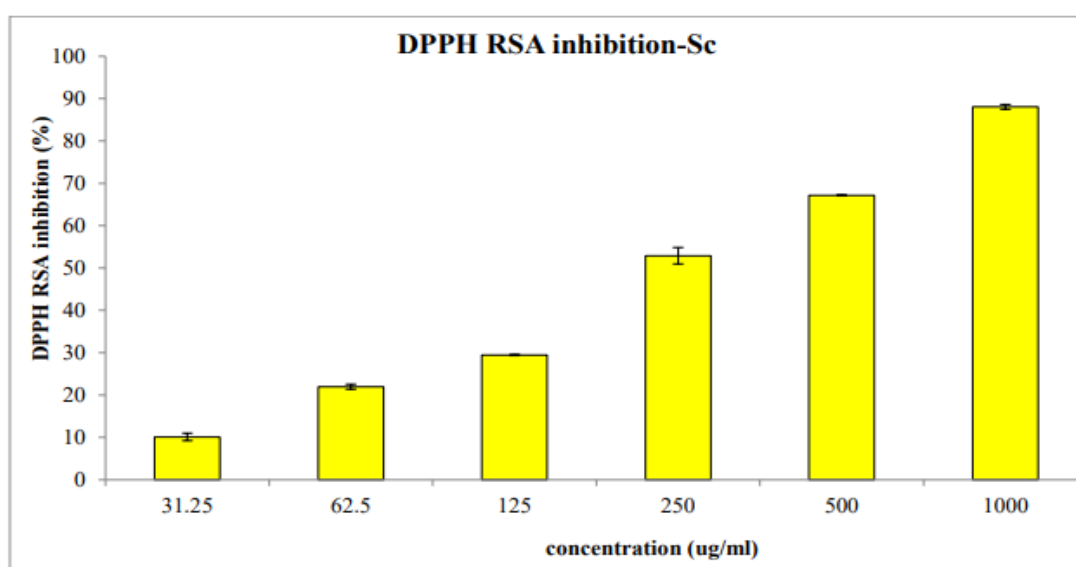


Graph 1: Phenol standard curve for the estimation of total phenol content.

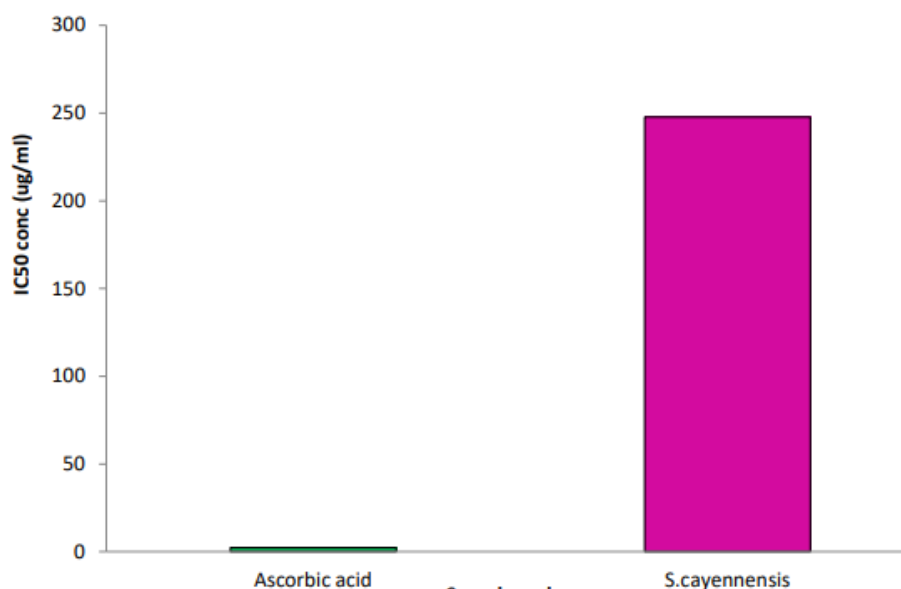
The total phenolic content in the water and ethanol extract of *Stachytarpheta cayennensis* leaves was found 0.15% (W/W).



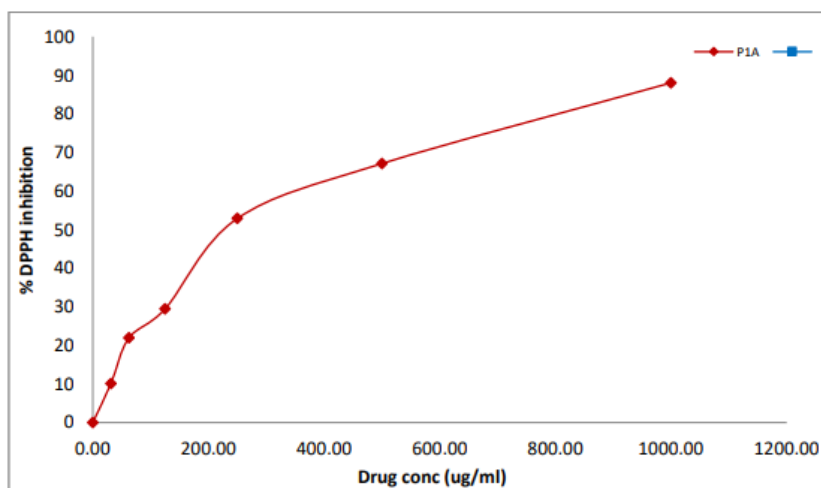
Graph 2: DPPH RSA inhibitory effect of Ascorbic acid with different concentrations.



Graph 3: DPPH RSA inhibitory effect of *S.cayennensis* with different concentrations.



Graph 4: IC₅₀ value of *S.cayennensis* extract and ascorbic acid by DPPH assay.



Graph 5: DPPH Radical Scavenging Activity of Ethanol extracts of *S.cayannensis*.

Table 4: Antimicrobial activity of ethanol and water extracts.

Sample	Test strain	Zone of inhibition
Ethanol	<i>Escherichia coli</i>	observed
Water	<i>Escherichia coli</i>	not observed

The present study involved the extraction and analysis of ethanol and water extracts of the plant which exhibited antibacterial activity against *Escherichia coli*. The

antibacterial properties may be attributed to the presence of phytochemicals.

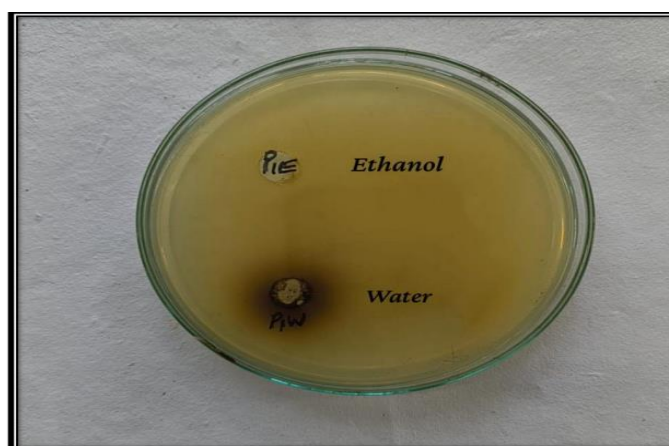
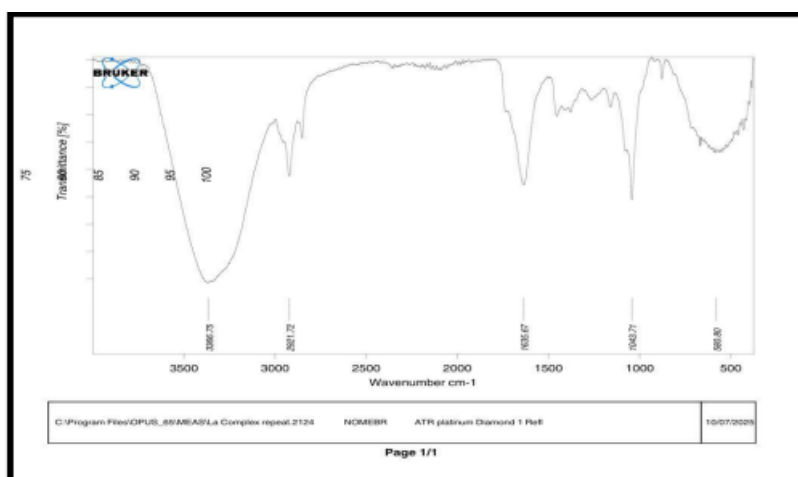


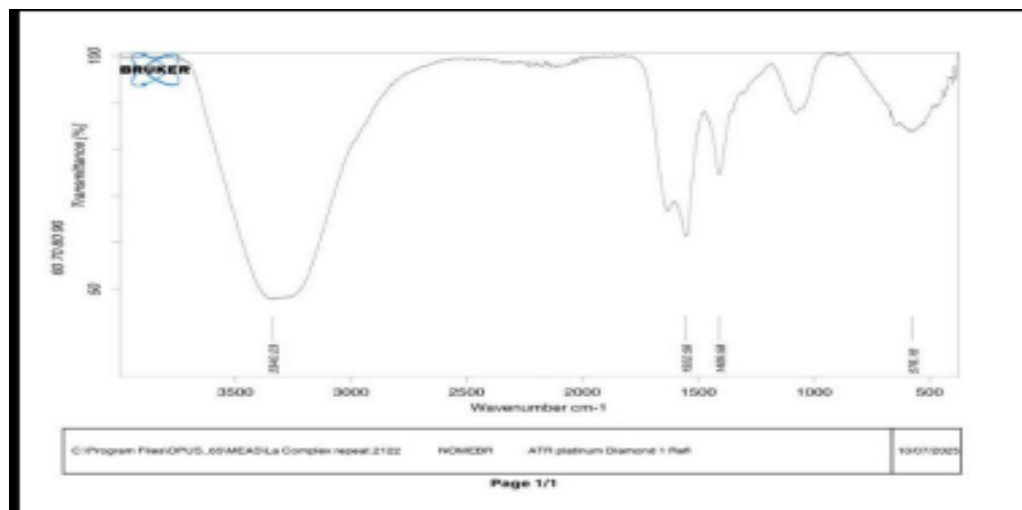
Fig. 2: Zone of inhibition of ethanol and water extract of *S.cayannensis* against *E.coli*.



Graph 6: IR spectra of ethanol extract of *S.cayannensis*.

Description: $\sim 3600\text{--}3200\text{ cm}^{-1}$ O–H (alcohols/phenols), N–H (amines/amides) Broad, strong peaks here suggest OH or NH groups $\sim 3000\text{--}2850\text{ cm}^{-1}$ C–H (alkanes) Typical for sp^3 C–H stretching $\sim 1300\text{--}1000\text{ cm}^{-1}$ C–O or C–N Stretching of ethers, alcohols,

or amines $\sim 1680\text{--}1620\text{ cm}^{-1}$ (sat) or $1650\text{--}1600\text{ cm}^{-1}$ (conj) Alkenes C=C stretch $\sim 500\text{--}600\text{ cm}^{-1}$ Usually associated with metal–oxygen (M–O) or metal–nitrogen (M–N) stretching modes.



Graph 7: IR spectra of water extract of *S.cayennensis*.

Description: $\sim 3400\text{ cm}^{-1}$ – Broad peak likely corresponds to O–H stretching (from water or hydroxyl groups) or N–H stretching if the complex has amine/amide ligands. Broadness suggests hydrogen bonding, which is common in coordination compounds. $\sim 1400\text{ cm}^{-1}$ often due to C–H bending (methyl or methylene). May also indicate carboxylate COO^- symmetric stretching if present $\sim 1570\text{--}1490\text{ cm}^{-1}$ Nitro Compounds NO_2 stretch $\sim 500\text{--}600\text{ cm}^{-1}$ Usually associated with metal–oxygen (M–O) or metal–nitrogen (M–N) stretching modes.

CONCLUSION

The present study on *Stachytarpheta cayennensis* has provided valuable insights into its phytochemical profile, biological activity, and potential applications in medicinal chemistry. The study aimed at the extraction, qualitative and quantitative analysis, antimicrobial screening, antioxidant activity evaluation, and IR spectroscopic characterization of the bioactive constituents from the plant.

Through Soxhlet extraction using ethanol and water as solvents, the phytochemical screening confirmed the presence of carbohydrates and phenolic compounds, whereas proteins and amino acids were not detected. This indicates that the plant contains important secondary metabolites that may contribute to its medicinal value. Ethanol proved to be a more efficient solvent compared to water in extracting bioactive molecules, as evident from the subsequent biological assays.

The antimicrobial susceptibility testing (AST) performed against *Escherichia coli* revealed that the ethanol extract of *S. cayennensis* exhibited a clear zone of inhibition,

while the water extract showed no such activity. This demonstrates the selective antimicrobial potential of ethanol-soluble phytochemicals and further supports the ethno medicinal use of the plant for treating infections and gastrointestinal disorders. The results also highlight that solvent choice plays a critical role in determining the effectiveness of plant extracts in antimicrobial studies.

In addition to antimicrobial potential, the antioxidant properties of *S. cayennensis* were assessed using the DPPH radical scavenging assay. The ethanol extract exhibited an IC_{50} value of $247.7\text{ }\mu\text{g/mL}$, indicating moderate antioxidant capacity. Although this was weaker compared to the standard antioxidant ascorbic acid ($\text{IC}_{50} = 2.35\text{ }\mu\text{g/mL}$), the results still demonstrate that the plant extract possesses considerable free radical scavenging ability. Antioxidants play a crucial role in preventing oxidative stress-related diseases such as cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions. Thus, the antioxidant activity of *S. cayennensis* provides a scientific basis for its traditional use in the treatment of inflammatory and metabolic disorders.

Spectroscopic analysis using IR further confirmed the presence of functional groups such as hydroxyl (–OH), carbonyl (C=O), alkene (C=C), and possible amine or nitro groups.

However, it is important to note that this study focused primarily on preliminary screening and in vitro assays. While the antimicrobial and antioxidant activities were clearly demonstrated, further work is needed to isolate and characterize the specific active compounds responsible for these effects. Advanced techniques such as HPLC, GC-MS, and NMR spectroscopy can be

employed in future research to identify, purify, and determine the structures of bioactive constituents. Additionally, toxicity studies and in vivo experiments will be essential to establish the safety and efficacy of these compounds for potential therapeutic use.

Conflict of Interest

None.

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