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Research Article

PRELIMINARY PHYTOCHEMICAL TESTS, PHYSICOCHEMICAL ANALYSIS, *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *HYPTIS SUAVEOLANS* (L)

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ABSTRACT

The pharmacognostic features, basic phytochemical profiles, and pharmacological activities of stem extracts of *Hyptis suaveolans* (L) Poit are explored in the current study. Morphological studies were conducted to provide diagnostic characteristics that are useful in differentiating the material. Physicochemical analysis was performed. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids. The pharmacological work presented herein showed a variety of significant antimicrobial and antioxidant activities of *Hyptis suveolens* in folk medicine. The results of the plant provide useful insight for future studies to obtain herbal medicines based on *Hyptis suaveolens* extracts.

Keywords: Antimicrobial, Antioxidant, *Hyptis suaveolens*, Physicochemical Parameters, Phytochemical.

INTRODUCTION

Hyptis suaveolens is also known as Bush mint or wild spikenard is an aromatic shrub ingrained to the family Lamiaceae. This species is native to tropical areas in the Americas but has become very naturalised in Asia, Africa and Australia because of its adaptability and quick reproduction. It is known to have medicinal value because it was traditionally used to cure respiratory disorders, gastrointestinal conditions and skin infections. Some of the bioactive compounds present in the plant include the essential oils, which are attributed to its pharmacological properties, such as antimicrobial, anti-inflammatory and antioxidant properties. The broad distribution and conventional uses of *Hyptis suaveolens* indicate the possible importance of the plant in ethnomedicine and pharmacological studies (Mishra *et al*., 2021; Singh *et al*., 2005; Kamatou *et al*., 2010).

The family Lamiaceae, also known as Labiatae (the mint or deadnettle family), comprise a family of flowering plants. The family has a global distribution. The expanded Lamiaceae includes roughly 236 genera and is reported to have between 6,900 and 7,200 species, although the World checklist enumerates 7,534. The biggest genera include Salvia (900), Scutellaria (360), Stachys (300), Plectranthus (300), Hyptis (300), Teucrium (250), Vitex (250), Thymus (220), and Nepeta (200). Clerodendrum initially comprised over 400 species, but by 2010, it was reduced to approximately 150. All parts of the plants are fragrant and encompass numerous commonly utilized culinary herbs, including basil, mint, rosemary, sage, savory, marjoram, oregano, hyssop, thyme, lavender, and perilla. Certain plants include shrubs, trees like teak, or occasionally vines. Numerous family members are extensively grown, not just for their fragrant characteristics, but also for their simple cultivation: these species are some of the easiest to propagate using stem cuttings. In addition to those cultivated for their edible leaves, some are also grown for their ornamental foliage like coleus. Others are cultivated for food, but seeds are used rather than leaves, as seen with chia. The leaves grow oppositely, with each pair forming right angles to the last one (termed decussate) or in a whorled pattern. The stems are often square in cross section, though this is not true for all members of the family and can occasionally occur in other plant families.

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The flowers exhibit bilateral symmetry with 5 fused petals and 5 fused sepals. They are typically bisexual and verticillate (a floral arrangement resembling a whorl of blossoms but essentially made up of two dense clusters). The Lamiaceae is categorized into 7 subfamilies with 10 genera (Mishra *et al,* 2021; Ngozi *et al*., 2014).

MATERIALS AND METHODS

1, 1- diphenyl-2-picrylhydrazyl (Sigma Chemical Company, St. Louis, USA). All other chemicals and reagents used were of analytical grade. Methanol, 4% dimethyl sulphoxide (DMSO), Wagner's reagent, Mayer's reagent, Hager's reagent, Dragendorff's reagent, naphthol, Fehling's solution A and B, Barfoed's reagent, Sodium nitroprusside, chloroform, zinc dust, gelatine, sodium chloride, ammonium hydroxide.

Methods

The plant material was collected in December 2013 from the Andhra University campus, Andhra Pradesh, India, and authenticated by Dr. Venkaiah, taxonomist, Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. The Voucher specimens (PCR/SC-13-14) were deposited in the herbarium, College of Pharmaceutical Sciences, Andhra University. The freshly collected stem and root of the plant were shade-dried and powdered. The powdered materials were then subjected to the Soxhlet extraction process with methanol. This process enables us in obtaining the chemical constituents that are soluble in methanol, depending on the solubility.

Soxhlet extraction

The dried powdered materials of the plant were extracted three times successively with methanol. The extracts thus obtained were concentrated under vacuum at a temperature of 43℃ by using a rotary evaporator, dried completely, weighed, and stored in a dessicator (De Castro and Priego-Capote, 2010).

PHYTOCHEMICAL ANALYSIS

The extract that was obtained was analyzed using known qualitative tests to identify the nature of chemical constituents that were present (Yadav and Agarwala, 2011).

Tests for alkaloids

Some 50 mg of solvent-free extract was stirred a small amount of dilute hydrochloric acid and filtered. A series of tests using different alkaloidal reagents were done with the filtrate as follows.

Mayer's test

Two drops of Mayer reagent were added to the sides of the test tube to a small amount of filtrate (of a few milliliters). In case the test is positive, it produces a white or creamy precipitate.

Wagner's test

A few drops of the reagent of Wagner were put there along with the sides of the test tube to a few milliliters of the filtrate. Beautiful reddish-brown precipitate is formed which confirms the test.

Hager's test

A little filtrate (1 or 2 ml) was added to 1 or 2 ml of Hager reagent. A yellow precipitate is a good sign of a positive test.

Dragendorff's test

1 or 2 ml of the reagent of Dragendorff was added to a few milliliters of filtrate. A positive test is represented by a fine reddish-brown precipitate.

Tests for carbohydrates

The extract was dissolved in 5 ml of distilled water and filtered with about 100mg of extract.

Molisch's test

Two drops of alcoholic solution of naphthol were added to 2 ml of filtrate. This mixture was shaken and 1 ml of concentrated sulphuric acid was put gradually along the sides of the test-tube, the test-tube was cooled in ice-water and left to stand. The presence of carbohydrates is indicated by a violet ring at the point of the joining of two liquids.

Fehling's test

1 ml filtrate was boiled on a water bath with 1 ml of Fehling solution A and B. Visualization of red precipitate is an indication of the presence of sugar.

Barfoed's test

1 ml of Barfoed reagent was added to 1 ml of the filtrate and allowed to boil on a water bath of boiling water, 2 minutes. The presence of sugar is denoted by red precipitate.

Benedict's test

0.5 ml of filtrate was added to 0.5 ml of Benedict reagent. The mixture was heated in a boiling water bath of 2 minutes. The precipitate is a brick red color which is the indication of the presence of sugar.

Tests for glycosides

In the identification of glycosides, 50 mg of extract was hydrolyzed using the concentrated hydrochloric acid during 2 hrs on a water bath, which was filtered and the filtrate subjected to the following tests.

Borntrager's test

A 3ml of chloroform was added, and mixed with 2 ml of filtrate hydrolysate, chloroform layer was separated and 10% ammonia solution added to it. Development of pink color is a sign of anthroquinone glycosides.

Legal's test

About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. The presence of a glycoside is indicated by a characteristic pink color.

Tests for saponins

Foam or froth test

A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two-centimeter layer of foam or froth, which is stable for 10 minutes, indicates the presence of saponins.

Tests for phytosterols and triterpenoids

Liebermann–burchard's test

The extract was dissolved in acetic anhydride, heated to boiling, cooled, and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink, or violet color at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

Salkowski test

A few drops of concentrated sulphuric acid were added to the chloroform extract, shaken on standing. Red colour in the lower layer indicates the presence of steroids, and golden yellow colour indicates the presence of triterpenoids.

Tests for phenolic compounds and tannins

Ferric chloride test

About 50 mg of extract was dissolved in distilled water, and to these few drops of neutral 5% ferric chloride solution were added. Formation of blue, green, and violet colors indicates the presence of phenolic compounds.

Gelatin test

A little quantity of extract was dissolved in distilled water, and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. The development of white precipitate indicates the presence of phenolic compounds.

Lead acetate test

A small quantity of extract was dissolved in distilled water, and to this, 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

Tests for flavonoids

Alkaline reagent test

Ammonium hydroxide solution (10 percent) was added to an aqueous solution of the extract - the characteristic of a yellow fluorescence was a sign of flavonoids.

Shinoda test or magnesium -hydrochloric acid reduction

A small portion of extract was added to alcohol and a few pieces of magnesium turnings and conc. hydrochloric acid (drop wise) was added. The occurrence of any pink or crimson-red colour implies the existence of flavonol glycoside.

Zinc-hydrochloric acid reduction test

The alcoholic solution is added to pinch of zinc dust and few drops of concentrated hydrochloric acid - the colour is magenta after few minutes.

PHYSICOCHEMICAL PARAMETERS (Nenadis and Tsimidou, 2002; Sanmugarajah *et al.,* 2013).

Determination of total ash

The ground whole plant of *Hyptis suaveolens* in the form of powdered stems and roots that weighed approximately 20 grams were weighed in silica powder on a crucible that had already been ignited. It was burned by progressively raising the temperature to less than reddish-red (450^{o}C) without being allowed to reach carbon-free, then detach itself and weighed. The ash content has been determined. The experiment was repeated five times in order to achieve a constant weight.

Determination of water-soluble ash

Add 800 ml of water and boil the product to a boil until the solid dissolves. Cool the product at room temperature, then weigh it and divide the weight by that solvent mass (800 ml). The total ash was boiled using 25ml of water within 5 minutes and filtered using an ashless filter paper (Whatmann No. 41). Hot water was then used in washing. The silica crucible was ignited in the filter paper and cooled down and the insoluble matter that could not be dissolved in water was weighed. Water soluble ash was determined by dividing the total ash minus the water insoluble matter. Acid Insoluble Ash was determined by weighing the acid insoluble residue, which remained after weighing the acidic sample, then dividing it by the mass of the new solid sample.

Insoluble ash

The Acid Insoluble Ash was determined by weighing the acid insoluble residue that remained after weighing the

acidic sample, then dividing the mass of the new solid sample by the acid insoluble residue. The obtained ash was boiled 5 minutes with 10 percent w/v of dilute hydrochloric acid and then filtered using an ashless filter paper (Whatmann No. 41). Silica in the silica crucible was ignited, cooled and weighed acid acid-insoluble ash on the filter paper.

Loss on drying sum

In a bid to ascertain the loss on drying the following procedure was followed. The weighing bottle was dried on the drier and then weighed the powdered whole plant in the weight bottle in a weighing stopper weighing at least 15 g in the weighing register. The sample was then mixed carefully and left to dry in an oven at 100 º C to 105 º C by withdrawing the stopper. It was refrigerated, and reweighed. The loss in drying was calculated.

Determination of Alcohol Soluble Extractive

Maceration was in 5 grams of the powder in a closed flask with 100ml of the strength of alcohol required and was macerated over the 24 hours, shaking very often over 6 hours then leaving it to stand 18hrs. This was swished and precautions were taken to avoid the loss of alcohol and 25ml of the filtrate dried by evaporation at $105^{O}C$ in a shallow dish with a tared bottom and weighed. The alcohol soluble extractive proportion was determined.

Determination of water-Soluble extractive

A quantity of 5gms of the powder was dried in 50 ml of water at $80^{o}C$, and 2 gms of keiselghur were added to it, filtered, and 5ml of the filtrate was transferred to a tared evaporating dish, the evaporation was carried to a water bath and dried it half an hour, after which it was dried in a hot air oven two hours and weighed. The proportion of extractives that could be dissolved in water was determined.

Determination of 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity

A 0.004 percent solution of DPPH in methanol, 3 ml of aliquot and 0.1 ml of plant extract were combined at different concentrations. The solution was vigorously shaken and left to reach a steady state at room temperature (30 min) to measure the decolorization of DPPH by measuring the absorbance at 517 nm. 0.1 ml of the respective vehicle was used as a control in place of the plant extract/ascorbic acid. The percentage of inhibition activity was determined as [(A0-A1)/A0) x100]. A0 was the absorbance of the control and A1 was the absorbance of the plant extract/ ascorbic acid (Krimat *et al*., 2015)**.**

Techniques of measuring antimicrobial activity

Microbiological assay is a biological assay conducted using microorganisms such as bacteria, yeast, moulds etc. It is the quantification of the relative potency or activity of compounds, i.e., that quantity of test material needed to cause a desired effect on an appropriate organism under standard conditions (Dubale *et al.,* 2023; Prusti, 2008).

Standardization of micro-organisms

Bacterial culture: 100 ml of sterile medium was inoculated with one loop-full of micro-organisms and incubated at 37c/24h. Fungal culture: 100 ml of sterile medium was inoculated and incubated with 48h at 27c. The 1 ml broth with the micro-organisms was added to 9 ml of peptone water, and 10-fold serial dilutions were spot on the sterile nutrient agar (SDA) plates and incubated at 37 C and 27 C, respectively, after 24 h/48 h incubation. Counts of colony forming units (CFU) were made, and counts of micro-organisms in 1 ml of stock culture were calculated (Indian Pharmacopoeia Commission. (2007).

Test and Standard solutions

Test compounds were introduced as a 5 and 10mg/ml stock solution in dimethylsulphoxide (DMSO) to dissolve the dried extracts. The reference standards (Rifampicin) were stocked as a solution of 0.6mg/ml in distilled water. The addition of 0.05 ml stock solution using a micropipette in every cup screened the antimicrobial activity.

Culture medium

The following media were used for the present antimicrobial studies.

Nutrient broth for bacteria

Beef extract (0.35%), Sodium chloride (0.5%), Peptone (0.5%). Ingredients, weighing 37 g, were dissolved in distilled water (1000 ml) PH was adjusted to 7.2-7.4 and sterilized by autoclaving at 15 lbs for 20 min was used to sterilize the media, water, etc. Glassware such as syringes, petridishes, pipettes, and empty test-tubes were sterilized in dry heat in an oven at 160 º C within one hour.

Nutrient agar for bacteria

Nutrient agar is prepared by dissolving peptone, beef extract, sodium chloride, and agar in distilled water, adjusting the pH to 7.2 ± 0.2, sterilizing at 121 °C for 15 minutes, pouring into sterile Petri plates, allowing to solidify, and then inoculating and incubating bacterial cultures at 37 °C for 18–24 hours for growth observation (Cappuccino & Sherman, 2019; Aneja, 2003).

Evaluation of antibacterial activity

Determination of zone of inhibition by cup plate method

The cylinder plate method of drug potency relies on the diameter of the zone of inhibition of microbial growth around cylinders (cups), where the different dilutions of the test substances are utilized.The agar medium containing the micro-organisms and 50mmol of the inoculums were spread with a sterile borer to prepare four cups of 5mm diameter. The spread plate method was used to spread these

cups on the agar plate. Micropipette was used to add accurately measured (50 µl) solution of each concentration and a reference to the cups. All plates were refrigerated at 2 to 8° C. 2 hours to effectively diffuse the test compounds and standards. They were then incubated at a temperature of 37°C during 24 hours. The fact that there was a definite zone of inhibition of any size around the cup reflected the presence of antibacterial activity. The solvent control was concurrently accomplished to determine the activity of dimethyl sulphoxide and water which were applied as a vehicle. There were three times of the experiments. The zone of inhibition was measured and the diameter taken as read. Four cups of 5 mm diameter were prepared on the agar medium that was sprayed with the micro-organisms and 50 uL of inoculums using a sterile borer. Plant extracts of *Hyptis suaveolens* contained in methanol (50 µg per cup), 100 µg per cup, 200 and 400 µg per cup) showed high antibacterial activity against the tested bacteria species (gram +ve and gram -ve) Rose, & Miller (1939).

RESULTS AND DISCUSSION

The results of extraction (Table 1), physicochemical parameters (Table 2) and phytochemical analysis were presented in, (Table 3). In the present study, antimicrobial screening was carried out using the cup plate method. In the cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a Petri dish or a plate to some extent so that the growth of the added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antimicrobial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader. The Methanolic extract of *Talinum portulacifolium* was screened for antimicrobial activity against a wide spectrum of micro-organisms, and the activity was compared with appropriate reference standards (Rifampicin for both gram-positive and gram-negative organisms). Microorganisms were grown in nutrient agar medium. Dimethyl sulphoxide (DMSO) and distilled water were used as the control and the drug vehicles for the plant extracts, and reference standards, respectively (Table 6).

Table 1. Details of the Extraction.

Plant material	Solvent used	Volume of the solvent	Weight of the extract
900 g	Methanol	3.6 L	34g

Table 2. Physicochemical Parameters.

Measured Parameter	Amount of powder taken (gm)	Amount of Powder obtained	Percentage
Loss on Drying	20gm	18.75gm	4%
Water soluble extractive value	5gm	4.94 gm	1.2%
Alcohol soluble extractive value	5gm	4.55gm	9%
Total Ash Content	15gm	14.4gm	4%
1. Water soluble Ash	2.6gm	0.6gm	1.76%
2. Acid insoluble Ash	2.6gm	0.97gm	5%

Table 3. Phytochemical analysis.

Name of the test	Methanolic extract
Phytosterol	+
Triterpenes	+
Glycosides	+
Saponins	-
Flavanoids	+
Taninis	-
Carbohydrates	-
Alkaloids	+

The methanolic extract of *Hyptis suaveolens* was found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC_{50} values for DPPH radical of methanolic extract of was found to be 323 µg. The mean IC_{50} value of ascorbic acid was found to be 41.89µg (Table 4 and 5) and Figure 1.

Table 4. Concentration-dependent percent inhibition of DPPH radical by methanolic extracts of *Hyptis suaveolens* and Ascorbic acid in vitro studies.

Concentration (µg/mL)	Ascorbic acid	Hexane extract
25	37.2 ± 0.88	12.27 ± 1.25***
50	52.37 ± 1.32	17.06 ± 2.41***
100	66.87 ± 1.43	25.33 ± 1.06***
200	78.83 ± 2.01	30.74 ± 1.55***
300	87.86 ± 0.89	40.00 ± 1.23***
400	99.44 ± 2.22	57.27 ± 0.98***

Table 5. IC_{50} of methanolic extract and Ascorbic acid on DPPH free radicles.

Name of the extract	50 % Inhibition (IC_{50}) (µg/mL)
Methanolic extract	323±4.02
Ascorbic acid	41.89±5.00

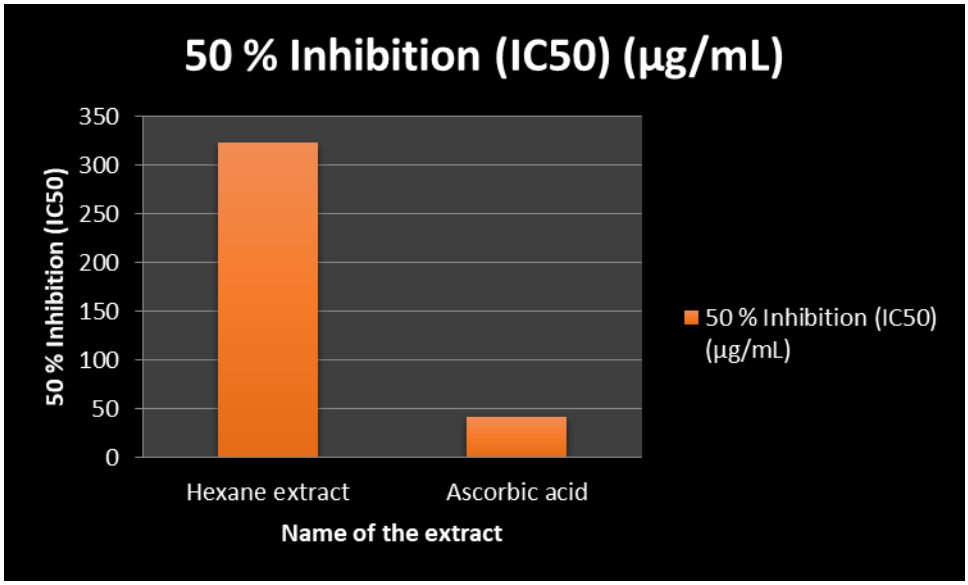

Figure 1. IC_{50} of methanolic extract and Ascorbic acid on DPPH free radicles.

Table 6. Antibacterial activity of *Hyptis suaveolens* stem extract.

Treatments	Dose (µg/cup)	Zone of growth inhibition				
		gram (+) ve		Gram (-)ve		
		B.s	S.a	E.c	P.a	P.a
Methanolic extract stem	50	10	18	09	07	07
	100	12	20	14	07	07
	200	17	25	18	10	10
	400	20	29	18	12	12
Rifampicin	50	24	22	19	24	24
DMSO		-	-	-	-	-
Water		-	-	-	-	-

S.a=*Staphylococcus aureaus*; E.c=*Escherichia coli*; P.a=*Pseudomonas aeruginosa*; B.s= *Bacillus substilis*.

CONCLUSION

Pharmacognostic, preliminary phytochemical, and powder analysis that features loss on drying, total ash content, acid-soluble, and water-soluble extractive values investigations carried out on the stem of *Hyptis suaveolens*. The sequential extraction procedure that yields 34 g of methanolic extract and their qualitative phytochemical evaluation. Pharmacognostic and phytochemical evaluation of the test extract of *Hyptis suaveolens* was conducted according to established protocols. Hyptis suaveolens extracts were revealed to contain carbohydrates, alkaloids, flavonoids, terpenoids, and sterols. The findings were presented in the Tables. The findings of the antioxidant activity research indicate that the methanolic extract of *Hyptis suaveolens* exhibits considerable antioxidant activity. The findings of the anti-microbial research indicate that the methanolic extract demonstrated notable anti-bacterial effects. The methanolic extract of *Hyptis suaveolens* shows reduced anti-bacterial activity against all tested pathogenic bacterial species. *Hyptis suaveolens* is a traditional medicinalplant with widespread use in phytopharmaceutical formulations for therapeutic purposes, owing to its numerous pharmacological properties as supported by literature and the current study.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Aneja, K. R. (2003). *Experiments in microbiology, plant pathology and biotechnology* (4th ed.). New Age International Publishers.

Cappuccino, J. G., & Sherman, N. (2019). *Microbiology: A laboratory manual* (12th ed.). Pearson Education.

Krimat, S., Dob, T., Toumi, M., Kesouri, A., & Noasri, A. (2015). Assessment of phytochemicals, antioxidant, antimicrobial and cytotoxic properties of *Salvia chudaei* Batt. et Trab. endemic medicinal plant from Algeria. *Journal of Materials and Environmental Science, 6*(1), 70–78.

Rose, S. B., & Miller, R. E. (1939). Studies with the agar cup-plate method: I. A standardized agar cup-plate technique. *Journal of Bacteriology, 38*(5), 525–537.

De Castro, M. L., & Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of Chromatography A, 1217*(16), 2383–2389. https://doi.org/10.1016/j.chroma.2009.11.027

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology, 15*, 51–62. https://doi.org/10.2147/JEP.S428591

Indian Pharmacopoeia Commission. (2007). *Indian Pharmacopoeia*. Ministry of Health and Family Welfare, Government of India.

World Health Organization. (1998). *Quality control methods for medicinal plant materials*. Geneva: Author.

Kamatou, G. P., Viljoen, A. M., & Steenkamp, P. (2010). Antioxidant, anti-inflammatory activities and HPLC analysis of South African *Salvia* species. *Food Chemistry, 119*(2), 684–688.

Mishra, P., Sohrab, S., & Mishra, S. K. (2021). A review on the phytochemical and pharmacological properties of *Hyptis suaveolens* (L.) Poit. *Future Journal of Pharmaceutical Sciences, 7*(1), 65. https://doi.org/10.1186/s43094-021-00207-8

Nenadis, N., & Tsimidou, M. (2002). Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH•) tests. *Journal of the American Oil Chemists' Society, 79*(12), 1191–1195. https://doi.org/10.1007/s11746-002-0625-9

Ngozi, L. U., Ugochukwu, N., Ifeoma, P. U., Charity, E. A., & Chinyelu, I. E. (2014). The efficacy of *Hyptis suaveolens*: A review of its nutritional and medicinal applications. *[Journal information not provided]*.

Prusti, A. (2008). Antibacterial activity of some Indian medicinal plants. *Ethnobotanical Leaflets, 2008*(1), 27–33.

Sanmugarajah, V., Thabrew, I., & Sivapalan, S. R. (2013). Phyto, physicochemical standardization of medicinal plant *Enicostemma littorale* Blume. *IOSR Journal of Pharmacy, 3*(2), 52–58.

Singh, S., Malhotra, M., & Majumdar, D. K. (2005). Antibacterial activity of *Hyptis suaveolens*. *Indian Journal of Pharmaceutical Sciences, 67*(6), 826–828.

Yadav, R. N., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology, 3*(12), 10–14.