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ORIGINAL RESEARCH ARTICLE

Phytochemicals Profile and Antioxidant Potency of *Hyptis Suaveolens* (L) Poit and *Hyptis Spicigera* Lam Traditionally Used for Malaria Control in Nigeria

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ABSTRACT

Biologically active compounds have always been of great interest to medical and public health parasitologists. The present study was carried out to determine the phytochemical constituents, the efficacy of extraction solvents, and the antioxidant activity of the leaf extracts of *Hyptis suaveolens* and *Hyptis spicigera*, two plants traditionally used for the direct control of malaria vector, *Anopheles gambiae* s.s., in Nigeria. *Hyptis suaveolens* and *Hyptis spicigera* plants were collected, identified, processed, and extracted according to established standard protocols. Aqueous and methanolic extracts of leaves and roots were subjected to qualitative and quantitative phytochemical screening using standard methods. Antioxidant potentials were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) bioassay. Key metabolites identified included alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, and saponins. The identified alkaloids, terpenoids, and flavonoids have been shown to have strong mosquitocidal and antiplasmodial activity, capable of controlling mosquitoes directly and malaria indirectly. Quantitative results from *H. suaveolens* aqueous leaf extract showed terpenoids (4.091 mg/100g), saponins (4.005 mg/100g), and flavonoids (3.882 mg/100g) as dominant compounds. Methanolic leaf extract recorded higher concentrations, with saponins (6.180 mg/100g) and terpenoids (5.351 mg/100g) being prominent. Similarly, *H. spicigera* aqueous leaf extract revealed cardiac glycosides (2.503 mg/100g) and saponins (2.140 mg/100g), while methanolic root extracts showed flavonoids (2.354 mg/100g) and terpenoids (2.774 mg/100g). The plant extract also showed strong antioxidant activity, as evidenced by its ability to scavenge free radicals. The analysis showed that methanolic leaf extracts exhibited 80.0% and 86.81% inhibition in *Hyptis suaveolens* and *Hyptis spicigera*, respectively. The IC_{50} values were 34.02 µg/ml and 22.05 µg/ml for *Hyptis suaveolens* and *Hyptis spicigera,* respectively; as such, *Hyptis spicigera* appeared more potent than *Hyptis suaveolens*. Comparatively, methanolic solvents yielded higher phytochemicals than aqueous solvents in both the leaf and the roots for the two plants under studied. Future research shall conduct larvicidal and adulticidal bioassays to test the mosquitocidal efficacy of the crude extract of the two *Hyptis* species studied.

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INTRODUCTION

For many years, medicinal plants have been used as phytomedicines to treat human illnesses and to repel mosquitoes that serve as vectors of malaria (Oscar *et al.,* 2020). Malaria, a disease majorly caused by *Plasmodium falciparum* and transmitted principally by infected female *Anopheles gambiae* s.s. (Hamza *et al.*, 2024), is a life-threatening mosquito-borne disease and remains a principal public health thread particularly in Sub-Saharan countries (World Health Organization (WHO), 2024). Control efforts, particularly against the principal malaria vector, *Anopheles gambiae s.l.* and *funestus* s.l. mosquitoes particularly in rural areas depends on the use of traditional plants (Saikia *et al.*, 2025). Medicinal plants are an indispensable foundation for modern pharmaceutical agents to eliminate insect vectors such as mosquitoes (Mozhiyarasi and Anuradha, 2016). The effectiveness of medicinal plants lies in the chemical constituents they contain as secondary metabolites (Usman *et al.*, 2025). For

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time immemorial, plants served as a rich source of biologically active compounds and are considered a potential source of therapeutic agents (Obi *et al.*, 2024).

Among the plants used, *Hyptis suaveolens* and *Hyptis spicigera* are the most acknowledged plants due to their traditional claimed efficacy. These two species of *Hyptis* are known for their rich phytochemical content, including alkaloids, flavonoids, and terpenoids, which are believed to possess potent biological activities, including mosquitocidal and antioxidant properties (Panwar, 2024). Despite their cosmopolitan traditional use (Worku et al., 2024), there is a scarcity of scientific data on their phytochemical profiles and antioxidant potential, particularly regarding different extraction solvents and their comparative efficacy. The present study aims to link this gap by conducting a solvent efficacy comparative study to ascertain the most efficacious solvents between methanol and water in terms of extracting more phytochemicals and antioxidant potency from the two *Hyptis* species. While both solvents are commonly used as extraction solvents (Royani et al., 2025), their relative efficacy in extracting bioactive compounds and antioxidants from these species collected in Nigeria remains untapped. This research is the first of its kind, focusing on the solvent efficacy of these two *Hyptis* species, offering novel insights into the most effective solvent for extracting the phytochemicals and antioxidants that contribute to their medicinal properties.

Hyptis suaveolens (L.) Poit., commonly known as "bush mint", belongs to the family Lamiaceae and is an aromatic, medium-sized perennial shrub with a brushy, erect growth habit and a sweet scent (Reddy, 2025). It has hairy stems, blue or purple flowers in axillary and terminal cymes, opposite leaves, and small black mucilaginous seeds (Shaikat, 2012). *Hyptis suaveolens* is widely distributed in tropical and subtropical countries and is readily available along roadsides throughout Nigeria (David et al., 2021), as well as in rocky areas and around grazing areas (Li *et al.*, 2020). The plant is commonly called "mosquito plant" or "Kafi Amarya Kanshi" in Hausa Land. The plant has been reported to have several pharmacological uses, including its ability to repel mosquitoes (Adda, 2011; Jesus *et al.*, 2013; Oscar *et al.*, 2020) and other insects that destroy stored grains (Abdulmalik, 2018). Phytochemically, *Hyptis suaveolens* is known to contain many bioactive compounds, including terpenoids, steroids, alkaloids, flavonoids, tannins, and essential oils, which are believed to underpin its medicinal properties (Panwar, 2024).

Hyptis spicigera Lam. belongs to the family Lamiaceae and is an erect, strong, aromatic, annual or perennial shrub growing up to 1 metre tall (Ngadvou *et al.*, 2023). It is commonly known as bush mint, and in Nigeria, it is known as Bunsurun fadama in Hausa, Ogunefon in Yoruba, and Ogwuawunta in Igbo (Idi and Muhammad, 2021). The plant is widely distributed in tropical and warm-temperate regions and is readily found in Nigeria around rice farms, cultivated rice areas, roadsides, and rocky areas (Masters, 2025). Morphologically, the plant has very tiny brown and black seeds clustered in groups within each flower, forming the inflorescence (Ladan *et al.*, 2014). It is known for its insecticidal and repellent activities against insects such as mosquitoes, and is also used to treat malaria (Atiko et al., 2016) and agricultural pests that attack economic crops (Baba *et al.*, 2012). *Hyptis spicigera* leaves have been used as a spray to protect crops and repel termites (Idi & Muhammad, 2021).

In addition to their mosquitocidal activity, *Hyptis suaveolens* and *Hyptis spicigera* possess significant antioxidant properties (Agarwal and Varma, 2016; Pritibha *et al.*, 2021), which enhance their value as multifunctional medicinal plants. Antioxidants are substances capable of preventing, removing, or delaying damage caused by oxidative stress, an action of free radicals in the human body that causes many life-threatening diseases (Dai *et al.*, 2025). Oxidative stress contributes to various pathological conditions in the human body (Chouikh *et al.*, 2025), and plants with strong radical scavenging activity are pivotal sources of natural therapeutic agents (Vaezi & Vaezi, 2025).

The study is based on the hypothesis that methanol, a polar solvent, will extract a greater variety and quantity of phytochemicals from both the roots and leaves of the two Hyptis plants and will extract more antioxidants than water, which may not extract some specific water-soluble compounds. This hypothesis will be tested by comparing the phytochemical contents obtained from the two plants and by further evaluating the antioxidant potency using conventional assays, such as the DPPH radical scavenging method.

This study aims to provide a deeper understanding of the phytochemicals and antioxidant potency of *H. suaveolens* and *H. spicigera*, contributing to the scientific validation of these traditionally used plants for mosquito control and informing the optimization of their extraction processes for potential therapeutic applications.

MATERIALS AND METHODS

Collection and identification of plant specimens

Fresh leaves (Plate 1) and roots of *Hyptis suaveolens* and *Hyptis spicigera* were collected within Federal University of Kashere (Latitude:9.914337°N Longtitude:11.003372°E) and from Bajoga (Latitude:10.863368°N, Longtitude:11.461678°E), respectively, all in Gombe State, Nigeria. The Plants were taxonomically identified and authenticated by Dr. Kolawole Opeyemi Saheed of the Department of Biological Sciences at the Federal University of Kashere, Gombe State. Specimen numbers were assigned by Mr. Umar Galadima (Head of the Biological Laboratory, FUK): *Hyptis suaveolens* (FUKH 105) and *Hyptis spicigera* (FUKH 106). The voucher specimen was deposited in the Federal University of Kashere herbarium for future reference.

Preparation and processing of plant specimens

The fresh leaves and roots of *Hyptis spicigera* and *Hyptis suaveolens* were allowed to be shade-dried for 2 weeks until

constant weight was obtained. The plant samples were ground into fine powder using a mortar and pestle in accordance with the methods described by Pandey and Tripalhi (2014). The powdered leaves and roots of the two plants were kept in separate airtight Bama Bottles and left for further Investigation.

Plate 1: A: *Hyptis suaveolens* shrub collected from Federal University of Kashere Campus, Gombe State. B: *Hyptis spicigera* collected from Bajoga, Funakaye LGA, Gombe State.

Extraction of plant materials for analysis

Aqueous extraction

The hot-water extraction method, popularly called (decoction), was employed for the aqueous extraction as described in Pandey and Tripalhi (2014). 50 g of the powdered sample was soaked in 500 mL of distilled water and boiled for about 15 minutes. After boiling, the sample was double-filtered using cheesecloth, collected in a conical flask, and allowed to cool. The filtrate was dried in a hot-air oven at 70 ^{0}C.

Methanolic extraction

Methanolic extraction was done following the standard procedure (Hamza *et al.,* 2023). Fifty (50) grams of the powdered sample were soaked in 500 ml of absolute methanol and allowed to stand for 24 hours. The mixture was stirred occasionally. After 24 hours, the sample was double-filtered using cheesecloth and collected in a conical flask. The filtrate was dried in a hot-air oven at 45 ^{0}C to remove the solvent.

Phytochemical screening of aqueous and methanolic extracts of the plants

Qualitative phytochemical screening

The qualitative phytochemical screening was conducted at the Biochemistry Laboratory, Faculty of Science, Gombe State University, using standard methods outlined by Hamza *et al.* (2023) to identify the chemical constituents of the plant samples.

Test for alkaloid

A weighed amount (2g) of each powdered sample of the plants was transferred into a 250 ml beaker. Two hundred milliliters (200 ml) of 10% acetic acid was added, then covered and allowed to stand for 4 hours. Filtration was performed, and a water bath was used to adjust the concentration of the extracted content to one-quarter of the original volume. Dropwise addition of concentrated ammonium hydroxide to the extract was continued until the precipitate was complete. The entire solution was allowed to settle, and the precipitate was collected by filtration and weighed.

Test for flavonoid

The flavonoid content in the leaves and roots of Hyptis plants was determined using the aluminum chloride colorimetric method. One milliliter of each plant extract was mixed with 2 ml of methanol, 0.2 ml of 10 % aluminum trichloride (AlCl), 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water. The entire mixture was allowed to stand at room temperature for 30 minutes' after which the absorbance was measured at 420 nm. The total flavonoid content in each plant part was expressed in

terms of standardized quercetin equivalent (QE) per 100mg of each extracted compound.

Test for terpenoid

The Ferguson method was used to evaluate the total terpene content of the leaves and roots of the studied species. Ten grams of plant powder were taken separately and soaked for 24 hours. After filtration, the filtrate was extracted with petroleum ether, and the ether extract was treated as the total terpenoid.

Test for saponin

About 100 cm3 of 20% aqueous ethanol was added to a conical flask containing 20 g of the powdered samples. The mixture was properly shaken together, and then heated over a hot water o bath for 4 hours with continuous stirring at 55 ^{0}C. It was filtered, and the residue was re-extracted with 200 ml of 20 % aqueous ethanol. The combined extracts were then reduced to 40 ml and placed over a water bath at 90 ^{0}C after which the concentrate was transferred into a 250 ml separating funnel with the addition of 20 ml diethyl ether, and shaken vigorously. The aqueous layer of the solution was recovered while the ether layer was discarded. The purification process was then repeated, and 60 ml of n-butanol was added thereafter. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride, and the remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight, and the saponin content was calculated as a percentage at 380 nm.

Test for tannin

Half a gram of the dried, powdered leaf sample was weighed into a 50 ml plastic bottle, 50 ml of distilled water was added, and the mixture was shaken thoroughly for about 1 hour. The solution was filtered into a 50ml volumetric flask and made up to the mark. 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 M FeCl3 in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Salisu et al., 2025; Hamisu and Salisu, 2025; Isah et al., 2025; Usman et al., 2025).

Test for steroids

Leaf and root extracts (2 ml each) with 2 ml of chloroform and 2 ml of concentrated H_2SO_4 were added to the extract. The appearance of red color and yellowish-green fluorescence indicates the presence of steroids in the samples (Muhammad *et al.*, 2024).

Determination of cardiac glycoside

A five milliliter (5 mL) of each aqueous and methanolic extracts was treated with 2 mL of glacial acetic acid containing a drop of FeCl3 solution. This was underlayered with 1 mL of concentrated H_2SO_4. A reddish-brown ring at the interface indicated a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form gradually throughout the thin layer (Raad *et al.*, 2021).

Quantitative phytochemical screening

Quantitative phytochemical screening of the two plants was conducted according to the standard methods outlined by Hamza *et al.* (2023).

Preparation of fat free sample

Two grams of the sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours.

Alkaloid determination

A 5 g sample of the leaf and root was weighed into a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol was added, covered, and allowed to stand for 4 hours. This was filtered and the extract concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected, washed with dilute ammonium hydroxide, and then filtered. The residue was the alkaloid, which was dried and weighed.

Flavanoid determination

A 10g plant sample was repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred to a crucible, evaporated to dryness over a water bath, and weighed to constant weight.

Saponin determination

A 20 g plant sample was dispersed in 200 ml of 20% ethanol. The suspension was heated in a hot-water bath for 4 hours, with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 mL over a water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel, and 20 ml of diethyl ether was added, then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Then 60 ml of normal butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to constant weight. The saponin content was calculated in percentage.

Tannins determination

For the determination of tannins, 500 mg of each sample was weighed into a 50mL plastic bottle, 50mL of distilled water was added, the mixture was shaken for an hour, filtered into a 50mL volumetric flask, and adjusted to the appropriate level. The absorbance was measured at 120 mm within 10 minutes. After that, five milliliters of the filtrate were pipetted into a test-tube and mixed with

0.008M potassium ferrocyanide and two milliliters of 0.1M FeCl3 in 0.1M HCI (Namadina *et al.*, 2020).

Determination of total steroids:

Total steroid was determined using the method described by Tapas et al. (2008). A test extract of steroid solution (1 ml) was transferred into 10 ml volumetric flasks. Iron (III) chloride and sulphuric acid (4N, 2 ml) (0.5 % w/v, 2 ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5 % w/v, 0.5 ml). The mixture was heated in a water bath at 70±2 0C for 30 minutes with occasional shaking, then diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Determination of cardiac glycosides

One gram (1g) of the extracts was added to 10 mL of 70% alcohol and filtered. From the filtrate, 8 mL of the mixture was added to 8 mL of 12.5% lead acetate to precipitate resins, tannins, and pigments; the mixture was shaken well, made up to a volume of 100 mL with distilled water, and then filtered. The filtrate (50 mL) was transferred to another 100 mL volumetric flask, and 8 mL of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution was added. The mixture was then filtered through Whatman filter paper. 10 mL of Baljet's reagent was added to 10 mL of the purified filtrate, and 10 mL of a blank distilled water solution was also added to 10 mL of Baljet's Reagent. The two mixtures were left undisturbed for 1 hour (maximum coloring time), and the colour intensity was read at 495nm against a blank (20 mL distilled water) using a spectrophotometer.

Method Validation

To validate the method, the phytochemical bioassay was assessed for linearity and reproducibility. Linearity was determined by plotting the response of standard solutions of the phytochemical(s) in the range of 0.5 mg/100g to 10.0 mg/100g, yielding a calibration curve. Reproducibility was evaluated by performing triplicate analyses at three different concentrations, with relative standard deviations (RSD) of less than 5%, confirming the method's precision and consistency.

Determination of DPPH scavenging activity

Slight modifications were made to the standard method used for the determination of scavenging activity of DPPH free radical in the extract solution, as described in Hamza *et al.* (2023). Briefly, a 2.0 ml solution of the extract at different concentrations was diluted two-fold in methanol and mixed with 1.0 ml of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 minutes. Blank solutions were prepared for each test sample solution (2.0 ml) and for the negative control (1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol). L- ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV-26visible spectrophotometer. DPPH radical inhibition was calculated using the equation:

% Inhibition =

$$X = \frac{A\ Control - A\ Sample}{A\ Control} X100\%$$ (Salisu et al., 2024; Muhammad et al., 2024)

Where, AC [A control] is the absorbance of the control, and AS [A sample] is the absorbance of the tested sample. The IC represented the concentration of the extract that inhibited 50% of radical.

RESULTS

Phytochemical Composition of *Hyptis suaveolens* and *Hyptis spicigera*

The qualitative and quantitative phytochemical analyses revealed the presence of seven major secondary metabolites, alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, and saponins, in both *Hyptis suaveolens* and *Hyptis spicigera*. All phytochemical classes were detected in aqueous and methanolic extracts of both leaves and roots, although their concentrations varied considerably depending on plant species, plant organ, and extraction solvent.

Table 1: Combined qualitative and quantitative phytochemical profile (mg/100 g) of aqueous and methanolic leaf and root extracts of *Hyptis suaveolens*

Phytochemical	Leaf (Aqueous)	Leaf (Methanolic)	Root (Aqueous)	Root (Methanolic)
Alkaloids	++ (2.228 ± 0.004)	++ (3.887 ± 0.002)	++ (3.727 ± 0.071)	++ (2.910 ± 0.001)
Flavonoids	++ (3.882 ± 0.006)	++ (4.262 ± 0.054)	++ (2.945 ± 0.073)	++ (3.330 ± 0.012)
Terpenoids	+++ (4.091 ± 0.005)	+++ (5.351 ± 0.023)	+++ (5.356 ± 0.061)	++ (4.706 ± 0.009)
Cardiac glycosides	+ (1.372 ± 0.006)	++ (2.716 ± 0.008)	+ (1.515 ± 0.006)	+ (1.744 ± 0.049)
Steroids	++ (2.445 ± 0.004)	++ (2.699 ± 0.016)	++ (2.333 ± 0.001)	++ (2.756 ± 0.019)
Tannins	+ (1.886 ± 0.005)	++ (3.247 ± 0.028)	+ (1.423 ± 0.003)	++ (2.838 ± 0.070)
Saponins	+++ (4.005 ± 0.001)	+++ (6.180 ± 0.087)	+++ (3.706 ± 0.006)	+++ (4.628 ± 0.056)

Key: Qualitative analysis: +++ = Strongly present, ++ = moderately present, + = weakly present, – = absent. Quantitative analysis: Values are expressed as Mean ± standard deviation (SD): n = 3

Table 1 presents the combined qualitative and quantitative phytochemical profile of aqueous and methanolic leaf and root extracts of *Hyptis suaveolens*. Terpenoids and saponins were the most abundant phytochemicals across all extract types. Methanolic leaf extracts recorded the highest concentrations of saponins (6.18 ± 0.09 mg/100 g), terpenoids (5.35 ± 0.02 mg/100 g), and flavonoids (4.26 ± 0.05 mg/100 g). In contrast, aqueous leaf extracts

showed lower concentrations of these metabolites, while root extracts generally contained moderate levels. Qualitative intensity scores (+++) further confirmed the dominance of terpenoids and saponins in *H. suaveolens*.

Table 2. Combined qualitative and quantitative phytochemical profile (mg/100 g) of aqueous and methanolic leaf and root extracts of *Hyptis spicigera*

Phytochemical	Leaf (Aqueous)	Leaf (Methanolic)	Root (Aqueous)	Root (Methanolic)
Alkaloids	+ (1.448 ± 0.007)	++ (3.002 ± 0.001)	+ (1.605 ± 0.079)	++ (1.710 ± 0.005)
Flavonoids	+ (1.773 ± 0.006)	+ (1.952 ± 0.063)	+ (1.943 ± 0.077)	++ (2.354 ± 0.009)
Terpenoids	++ (2.110 ± 0.002)	+++ (4.994 ± 0.017)	+ (0.866 ± 0.001)	++ (2.774 ± 0.002)
Cardiac glycosides	++ (2.503 ± 0.001)	++ (2.948 ± 0.071)	+ (1.113 ± 0.001)	+ (1.684 ± 0.022)
Steroids	+ (0.944 ± 0.064)	++ (1.438 ± 0.008)	+ (0.782 ± 0.001)	++ (1.158 ± 0.060)
Tannins	++ (2.001 ± 0.001)	+++ (4.332 ± 0.047)	++ (2.224 ± 0.002)	++ (3.580 ± 0.030)
Saponins	++ (2.140 ± 0.001)	+++ (5.053 ± 0.066)	++ (2.273 ± 0.006)	++ (3.167 ± 0.046)

Key: Qualitative analysis: +++ = Strongly present, ++ = moderately present, + = weakly present, – = absent. Quantitative analysis: Values are expressed as Mean ± standard deviation (SD): n = 3

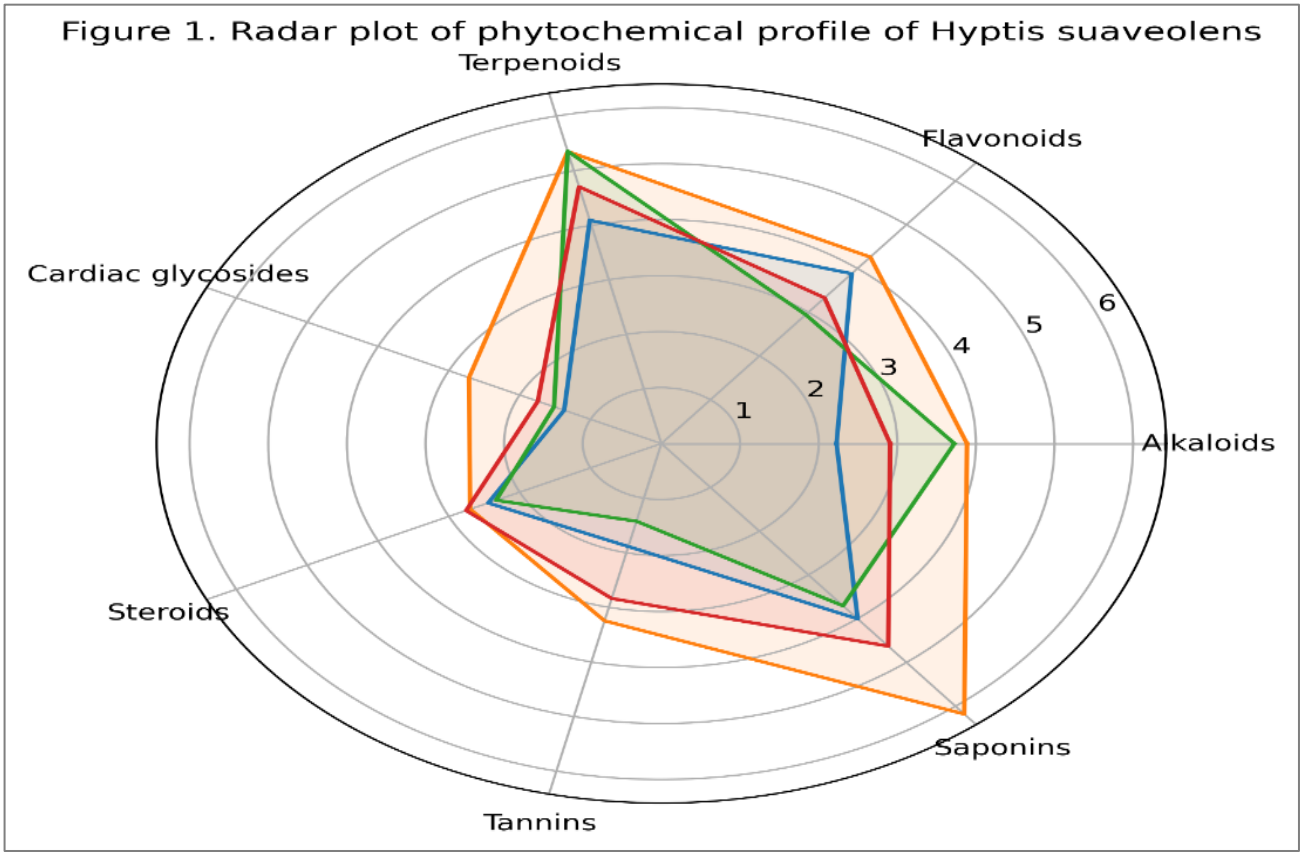

Figure 1: A radar plot showing the relative concentrations (mg/100 g) of major phytochemical classes in aqueous and methanolic leaf and root extracts of *Hyptis suaveolens*.

Table 2 summarizes the phytochemical composition of *Hyptis spicigera*. Similar to *H. suaveolens*, all seven phytochemical classes were present; however, *H. spicigera* exhibited comparatively higher tannin and saponin concentrations, particularly in methanolic leaf extracts. Methanolic leaf extracts showed the highest levels of saponins (5.05 ± 0.07 mg/100 g), tannins (4.33 ± 0.05 mg/100 g), and terpenoids (4.99 ± 0.02 mg/100 g). Root extracts contained lower concentrations overall, although flavonoids were relatively more prominent in roots than in leaves.

Solvent and Organ-Dependent Phytochemical Variation

Clear solvent- and organ-dependent variations in phytochemical profiles were observed in both species. Methanolic extracts consistently yielded higher concentrations of most phytochemicals compared with aqueous extracts, while leaf extracts showed greater accumulation than root extracts.

The radar plot for *Hyptis suaveolens* (Figure 1) illustrates these patterns by showing broader radial expansion for methanolic leaf extracts across most phytochemical axes. Saponins and terpenoids exhibited the greatest radial spread, indicating their dominance in the phytochemical profile of *H. suaveolens*. Aqueous extracts, particularly from roots, displayed narrower profiles, reflecting lower metabolite concentrations.

This figure illustrates the comparative distribution of alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, and saponins across different plant

organs and extraction solvents. Methanolic leaf extracts exhibit the widest radial expansion, indicating higher concentrations of most phytochemical classes, particularly saponins and terpenoids. Aqueous extracts, especially from roots, show comparatively lower phytochemical abundance. Values represent mean concentrations (mg/100 g).

Similarly, the radar plot for *Hyptis spicigera* (Figure 2) demonstrates pronounced solvent-dependent variation. Methanolic leaf extracts exhibited the widest radial expansion, especially for saponins, tannins, and terpenoids. Root extracts showed relatively narrower profiles, although flavonoid levels were higher in roots than in leaves, distinguishing *H. spicigera* from *H. suaveolens*.

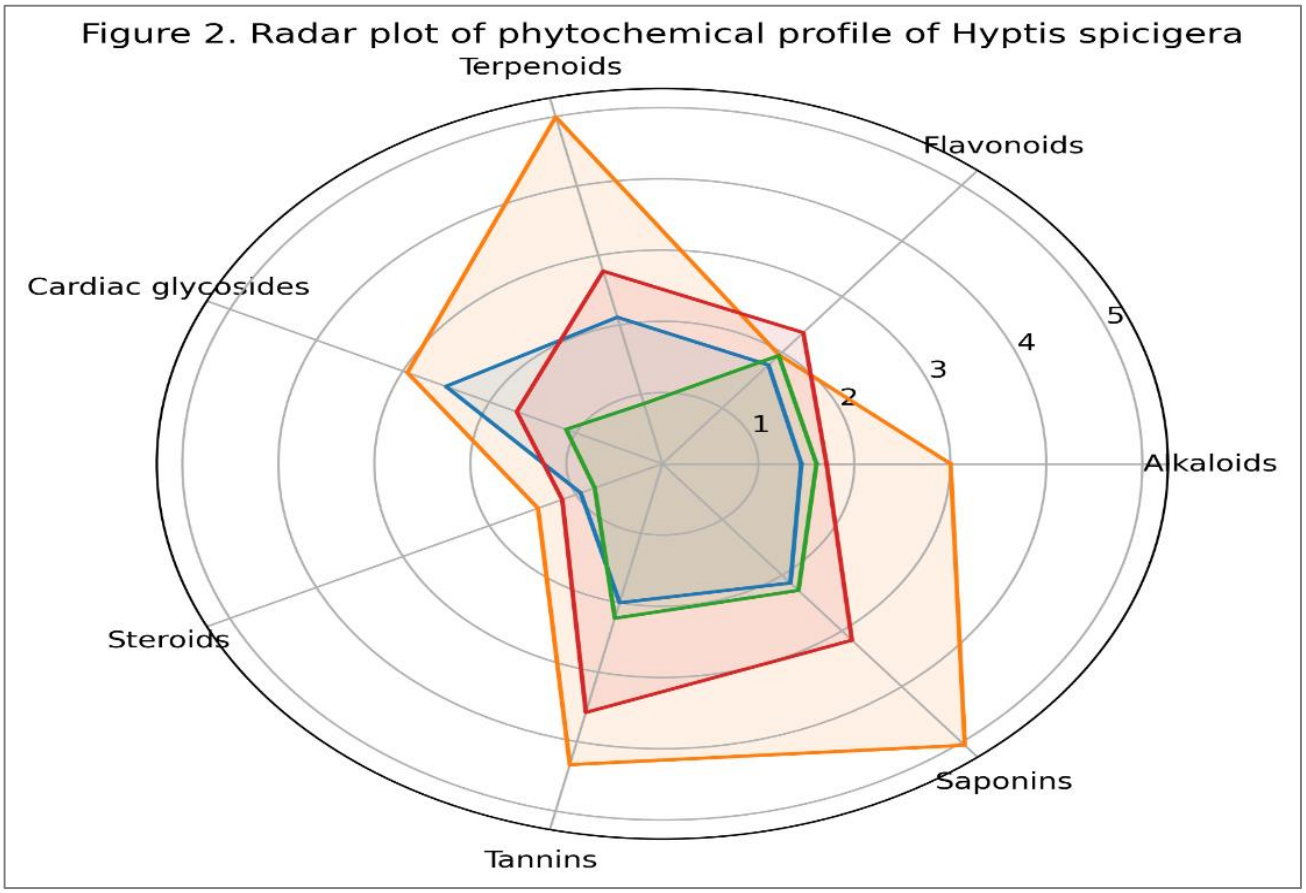

Figure 2: A radar plot illustrating solvent- and organ-dependent variation in phytochemical profiles of aqueous and methanolic leaf and root extracts of *Hyptis spicigera*.

The radar plot demonstrates marked variation in phytochemical composition influenced by the extraction solvent and plant organ. Methanolic leaf extracts display broader radial expansion for saponins, tannins, and terpenoids, reflecting higher accumulation of these metabolites. Root extracts show relatively higher flavonoid concentrations compared with leaves. Overall, methanolic extracts exhibit richer phytochemical profiles than aqueous extracts. Values represent mean concentrations (mg/100 g).

Comparative Total Phytochemical Yield

The cumulative phytochemical yield, calculated as the sum of quantified metabolites, is illustrated in Figure 3. Methanolic leaf extracts produced the highest total phytochemical yield in both species, followed by methanolic root extracts, aqueous leaf extracts, and aqueous root extracts.

Among the two species, the methanolic leaf extracts of *Hyptis suaveolens* exhibited the highest overall phytochemical yield, followed closely by *H. spicigera*. In contrast, aqueous root extracts of both species recorded the lowest yields. These findings highlight the superior extraction efficiency of methanol and the greater biosynthetic accumulation of secondary metabolites in leaves.

This figure presents the cumulative phytochemical yield calculated as the sum of all quantified metabolites. Methanolic leaf extracts of both species show the highest total phytochemical yield, followed by methanolic root extracts, aqueous leaf extracts, and aqueous root extracts. The chart highlights the superior extraction efficiency of methanol and the greater phytochemical accumulation in leaves. Bars represent mean values (mg/100 g).

Multivariate Clustering of Phytochemical Profiles

Heatmap visualization of quantitative phytochemical data (Figure 5) revealed distinct clustering patterns based on extraction solvent, plant organ, and species. Methanolic extracts clustered separately from aqueous extracts, reflecting higher concentrations and stronger co-expression of antioxidant-related metabolites.

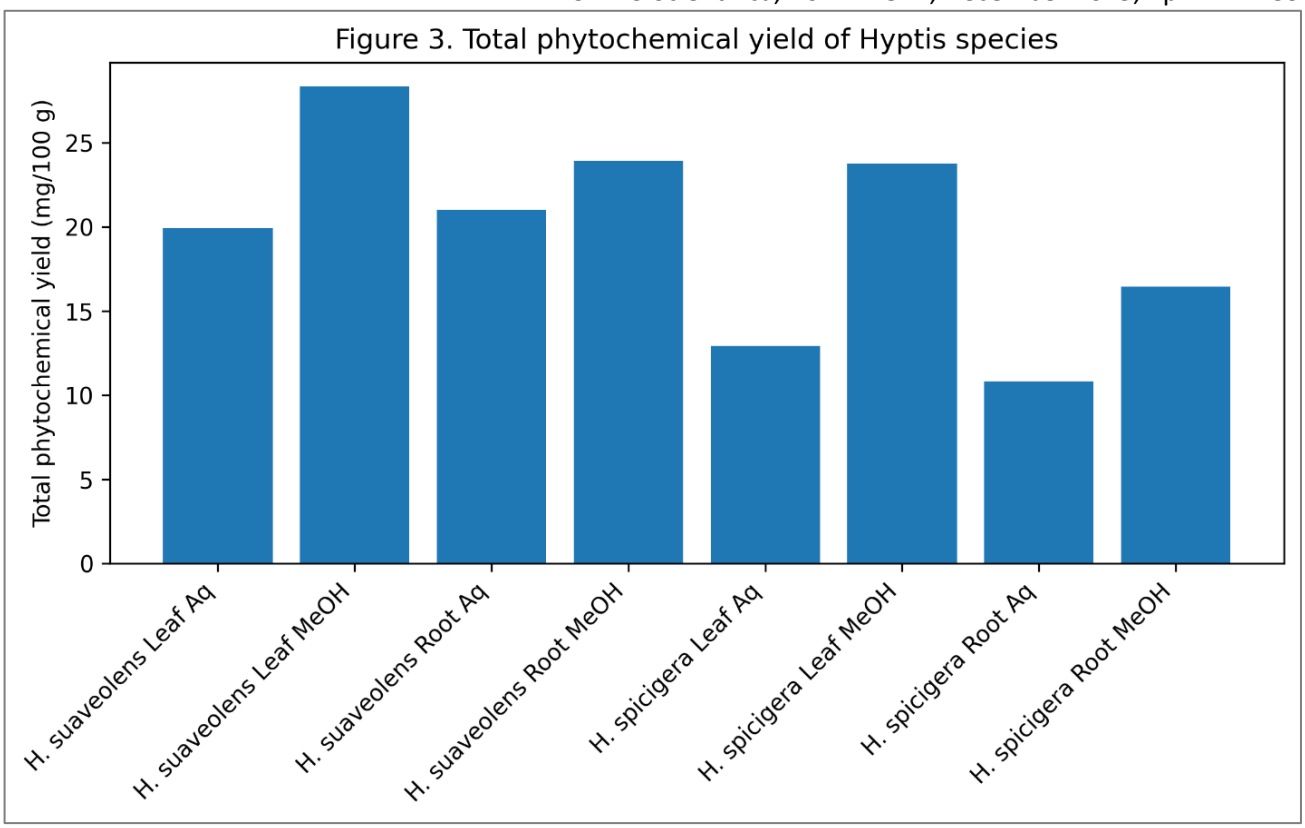

Figure 3: A bar chart comparing total phytochemical yield (Σ mg/100 g) obtained using aqueous and methanolic solvents from leaves and roots of *Hyptis suaveolens* and *Hyptis spicigera*.

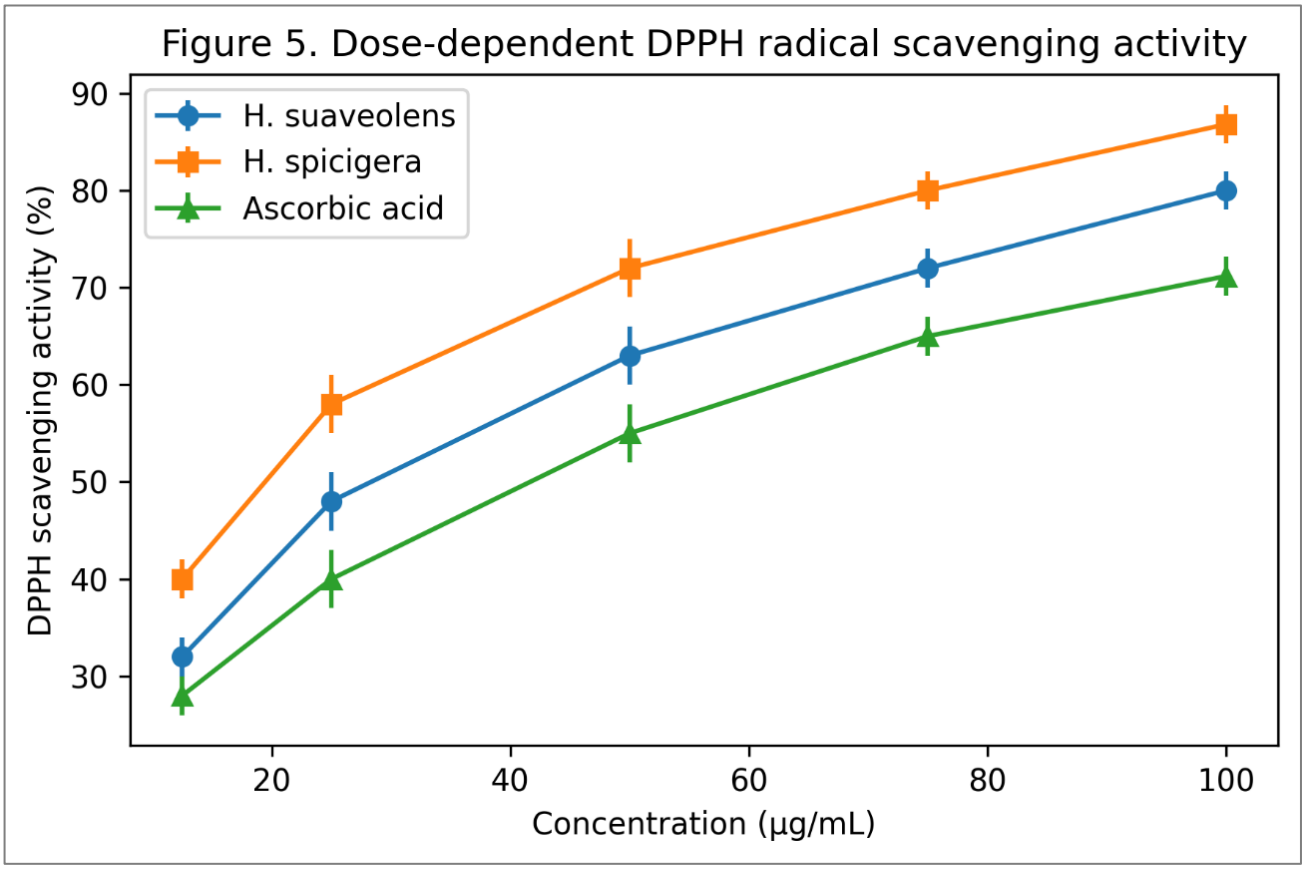

Figure 4: A dose-dependent DPPH radical scavenging activity curve of methanolic leaf extracts of Hyptis suaveolens and Hyptis spicigera compared with ascorbic acid.

Leaf extracts of both species formed a distinct cluster characterized by elevated saponins, terpenoids, and flavonoids. Species-level differentiation was also evident, with *Hyptis spicigera* clustering more closely with high tannin and saponin concentrations, while *H. suaveolens* showed a stronger association with terpenoid dominance.

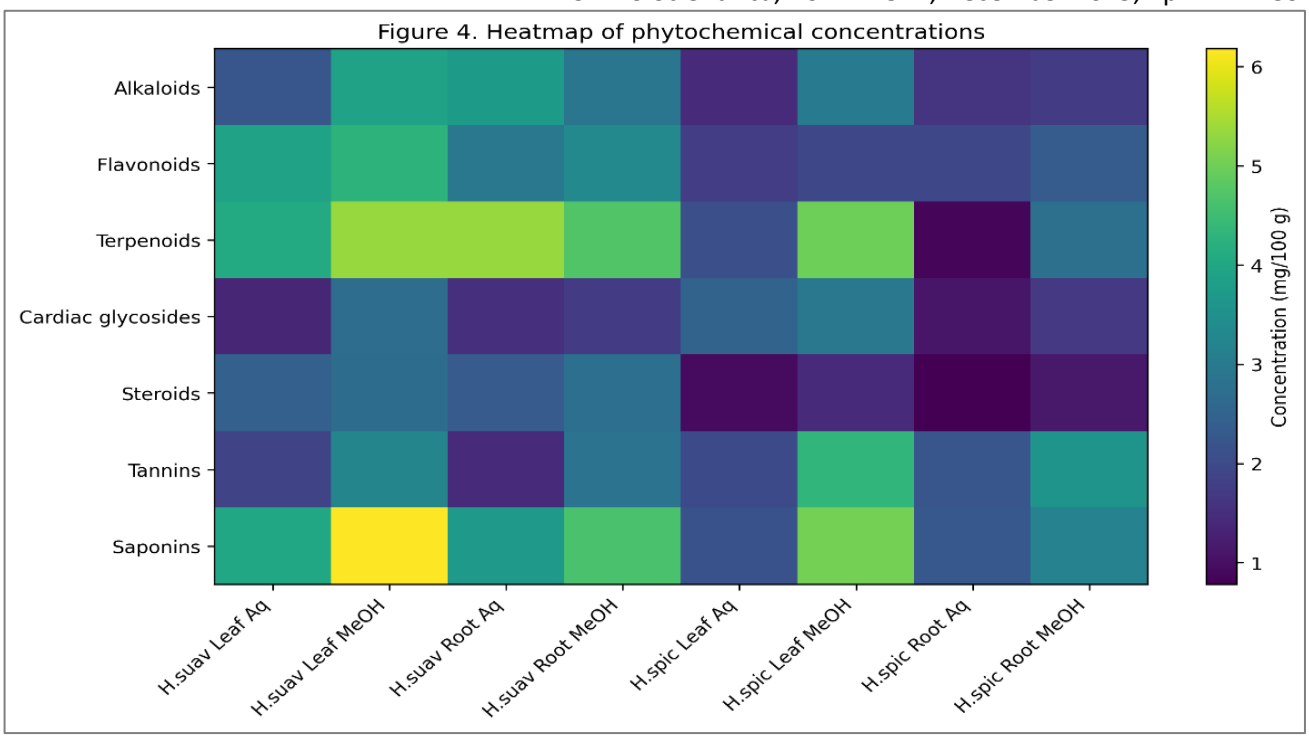

Figure 5: A heatmap visualization of quantitative phytochemical concentrations (mg/100 g) showing clustering patterns across species, plant organs, and extraction solvents.

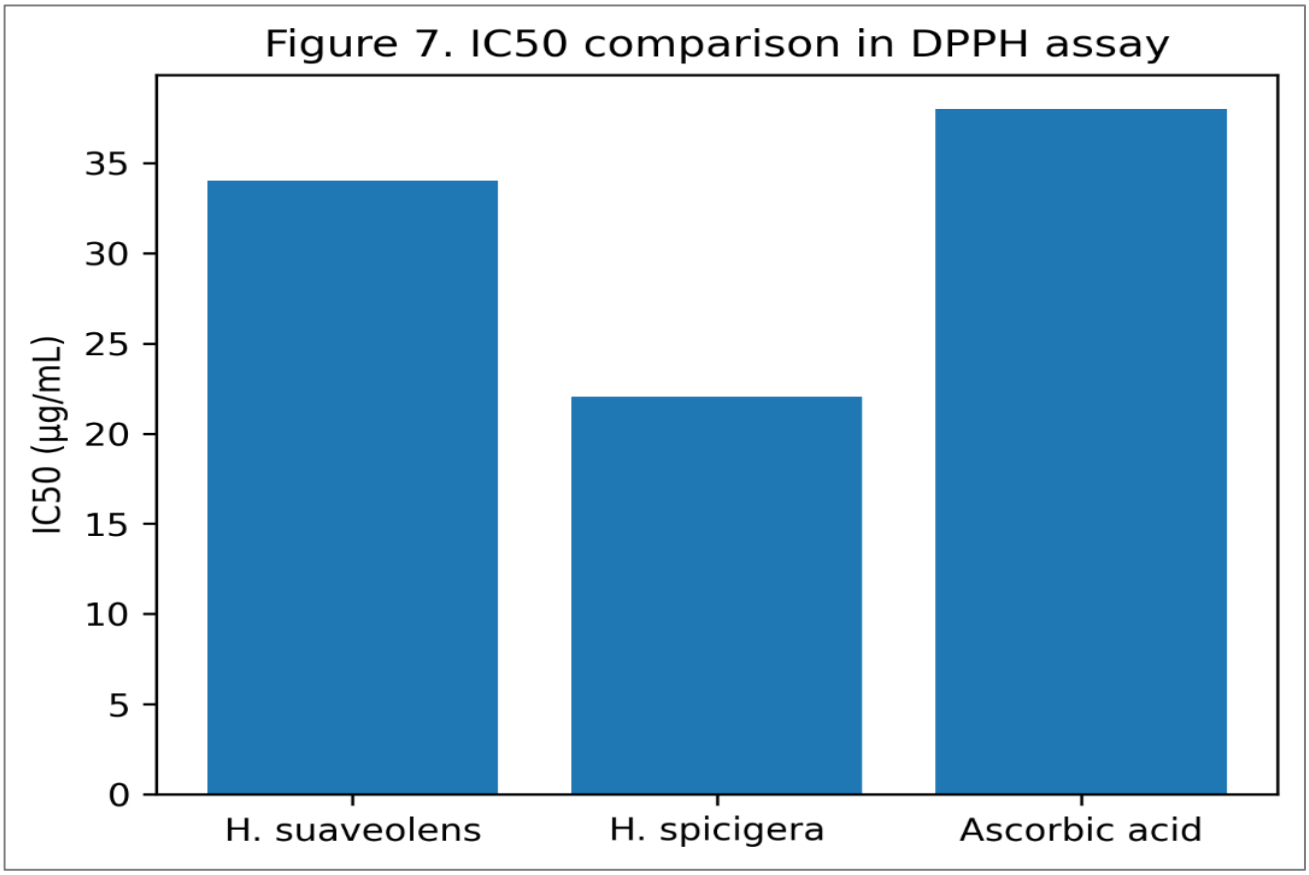

Figure 7: A comparison of IC_{50} values (µg/mL) of methanolic leaf extracts of *Hyptis suaveolens* and *Hyptis spicigera* relative to ascorbic acid in the DPPH assay.

These clustering patterns confirm the combined influence of species identity, plant organ, and solvent polarity on phytochemical composition.

The heatmap displays multivariate clustering of phytochemical concentrations across *Hyptis suaveolens* and *Hyptis spicigera*, plant organs (leaf and root), and extraction solvents (aqueous and methanolic).

Methanolic extracts form a distinct cluster characterized by higher concentrations of antioxidant-associated metabolites. Leaf extracts cluster separately from roots,

and species-level differences are evident: *H. spicigera* is associated with higher tannin and saponin levels, while *H. suaveolens* shows terpenoid dominance. Color intensity corresponds to metabolite concentration.

Table 3: IC_{50} values (µg/mL) of methanolic leaf extracts of *Hyptis* species and ascorbic acid in the DPPH assay

Sample	IC_{50} (µg/mL)
Hyptis suaveolens	34.02
Hyptis spicigera	22.05
Ascorbic acid	38.00*

*Reference antioxidant (positive control

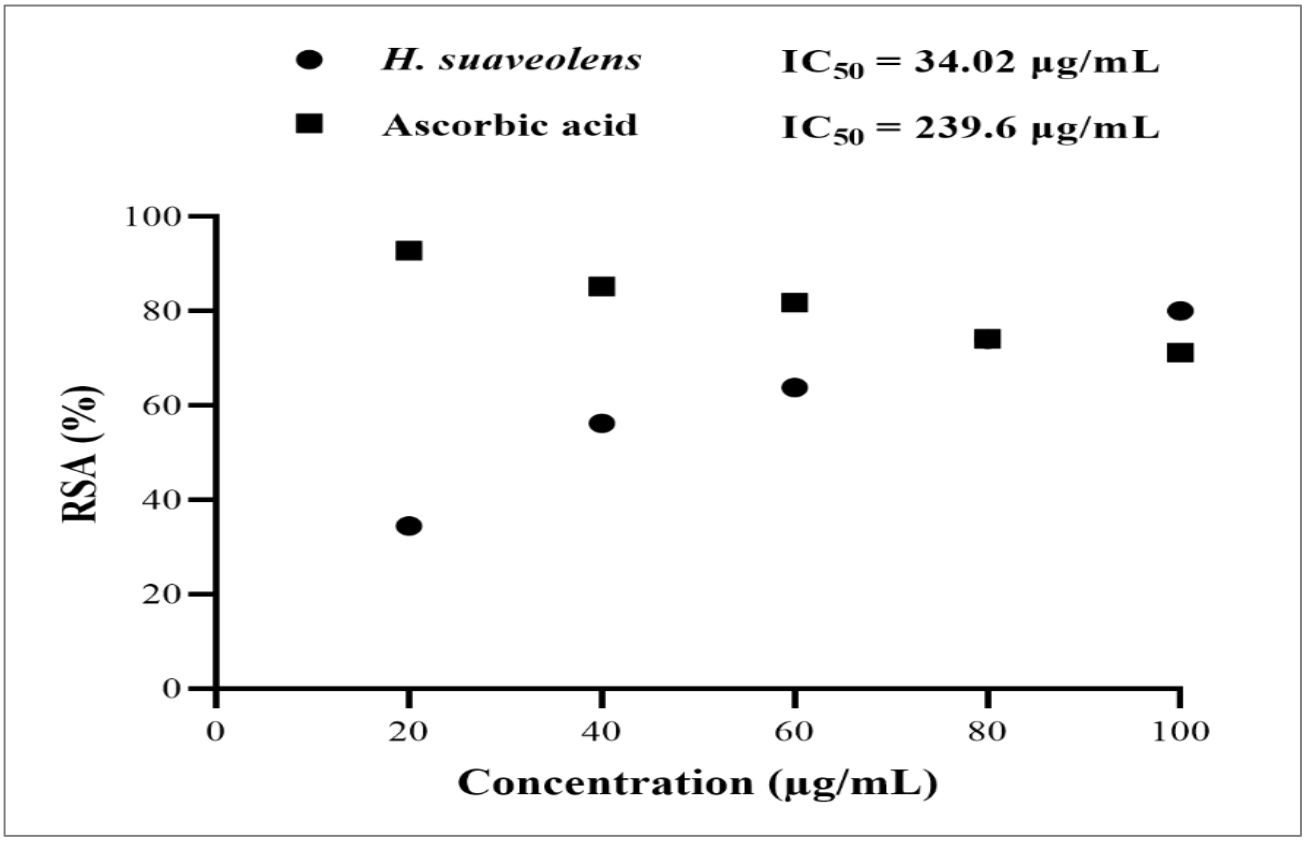

Figure 8. DPPH Radical Scavenging Activity (RSA) of Methanolic Leaf Extract of *Hyptis suaveolens* at Different Concentrations.

DPPH Radical Scavenging Activity

The antioxidant activity of methanolic leaf extracts was evaluated using the DPPH radical scavenging assay. Both *Hyptis suaveolens* and *Hyptis spicigera* extracts exhibited strong, concentration-dependent radical scavenging activity, as shown in Figure 4. This figure shows the percentage inhibition of DPPH radicals as a function of extract concentration. Both plant extracts exhibit concentration-dependent increases in radical scavenging activity. *Hyptis spicigera* consistently demonstrates higher inhibition across all tested concentrations, followed by *Hyptis suaveolens*, while ascorbic acid shows comparatively lower activity at higher doses. Values are expressed as mean ± standard deviation (SD).

At all tested concentrations, *H. spicigera* demonstrated a higher percentage of inhibition than *H. suaveolens*. At 100 µg/mL, *H. spicigera* achieved 86.81% inhibition, while *H. suaveolens* recorded 80.00% inhibition. Ascorbic acid, used as the reference antioxidant, showed lower inhibition (71.18%) at the same concentration. The dose–response curves indicate a steeper inhibition slope for *H. spicigera*, suggesting stronger radical scavenging efficiency.

IC_{50} Comparison of Antioxidant Potency

The IC_{50} values derived from the DPPH assay are presented in Table 3 and illustrated in Figure 7. *Hyptis spicigera* methanolic leaf extract recorded the lowest IC_{50} value (22.05 µg/mL), indicating the highest antioxidant potency. This was followed by *H. suaveolens* (34.02 µg/mL), while ascorbic acid exhibited a comparatively higher IC_{50} value.

This figure compares the concentration required to inhibit 50% of DPPH radicals. *Hyptis spicigera* exhibits the lowest IC_{50} value, indicating the highest antioxidant potency, followed by *Hyptis suaveolens*. Ascorbic acid shows a higher IC_{50} value, reflecting lower scavenging efficiency under the same experimental conditions.

The lower IC_{50} value of *H. spicigera* confirms its superior antioxidant capacity relative to *H. suaveolens* under the experimental conditions and supports the concentration-dependent trends observed in the DPPH scavenging assay.

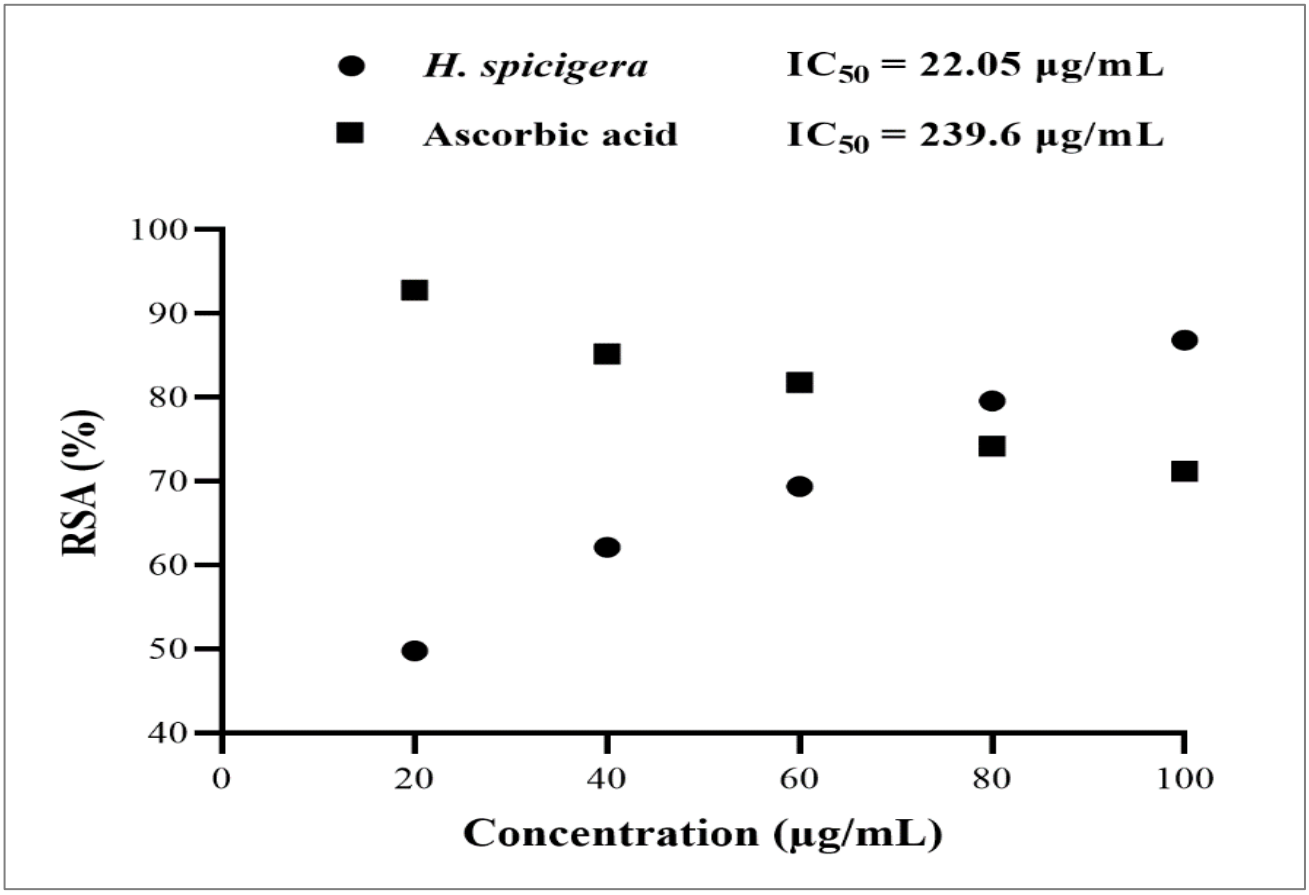

Figure 9. DPPH Radical Scavenging Activity (RSA) of Methanolic Leaf Extract of *Hyptis spicigera* at Different Concentrations.

DISCUSSION

The results of this study have demonstrated the phytochemical profiles of the two plants traditionally used for mosquito control and documented their antioxidant potency. Moreover, the study is the first of its kind to report the comparative extraction efficacy of the two solvents, methanol and water, which are commonly employed separately to extract the embedded therapeutic and pharmacological secrets behind plants' medicinal properties using Nigerian *Hyptis* species. The results not only reported the solvent effects but also reconfirmed the traditional potential of these two *Hyptis* species against mosquitoes. The current study reported the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, and saponins in both the aqueous and methanolic leaf and root extracts of *Hyptis suaveolens*, qualitatively and quantitatively. These secondary metabolites, as chemical substances, are known for their mosquitocidal and repellent activities, as reported (Hamza *et al.,* 2023; Magaji *et al.*, 2023). Other studies have reported similar bioactive compounds in the leaves and roots of *Hyptis suaveolens* (Hamza *et al.,* 2023; Aliyu *et al.*, 2022; Jeeva *et al.*, 2019; Magaji *et al.*, 2023; Moola, 2023; Namadina *et al.*, 2025; Usman *et al.*, 2025). The presence of these phytochemicals, particularly Alkaloids, flavonoids, and terpenoids, has substantiated the rationale for the cosmopolitan use of *Hyptis suaveolens* leaves for mosquito control. Once the leaves are in the room, hang around the room, or their powder is burned in the room, the smoke from the leaves can instantly repel mosquitoes. These bioactive compounds have been reported to possess mosquitocidal activity, qualifying the plants for traditional use in repelling mosquitoes (Aliyu et al., 2022; Pukuma et al., 2023; Moola, 2023; Namadina *et al.,* 2025). The findings in this current study tally with the report of Gaikwad *et al.* (2023) and Namadina *et al.* (2025), who likewise documented the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, saponins, phenols, carbohydrates, and triterpenes in a methanolic leaf extract of *Hyptis suaveolens* in a study conducted in Kano State, Nigeria. The study by Namadina et al. (2025) did not reveal the presence of terpenoids, as reported in this study, but it did reveal the presence of carbohydrates, phenols, and triterpenes, which are not reported in this study. The study conducted in Edo State, Nigeria, by Akharaiyi *et al.* (2023) reported phytochemicals similar to those found in the methanolic leaf extract of Hyptis suaveolens, except that saponin was not detected in the aqueous extract. In a study conducted in India to explore the phytochemical properties of *Hyptis suaveolens*, Panwar (2024) reported the presence of only essential oils, alkaloids, flavonoids, and terpenoids in the methanolic leaf extract of *Hyptis suaveolens*. This finding slightly varies from the methanolic leaf extract of Hyptis suaveolens documented in this study, which reported additional steroids, saponins, tannins, and cardiac glycosides. The current study disagrees with the findings of Changela

(2022), conducted in western India, which reported only flavonoids and tannins in the leaf extract of *Hyptis suaveolens*. The variation may be attributed to differences in the solvents used for extraction: the current study uses aqueous and methanol, whereas the former uses acetone.

Methanolic solvents yielded higher concentrations of the phytochemicals than aqueous solvents, as revealed in this study. Higher contents of saponin, followed by terpenoids, flavonoids, and alkaloids, were revealed in the leaves of *Hyptis suaveolens*, while the roots had the highest content of terpenoids, followed by saponin, flavonoids, and alkaloids. The leaf has the higher content of terpenoids, flavonoids, and alkaloids. These findings agree with the report by Yadav and Mishra (2022) in India, which reported higher levels of flavonoids and alkaloids in the ethanolic leaf extract of *Hyptis suaveolens*. Other studies also show that the leaves of *Hyptis suaveolens* have higher levels of these bioactive compounds (Dipak Koche *et al.*, 2010). This implies that the leaf of *Hyptis suaveolens* is richer in phytochemicals than the root. This further substantiated the rationale for using the leaf for mosquito control in traditional folklore rather than the root.

Furthermore, aqueous and methanolic leaf and root extracts of *Hyptis spicigera* reveal the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins, and saponins as found in *Hyptis suaveolens*. The current finding implied that the two plants have similar bioactive compounds. This substantiated the common use of the two plants in traditional folklore for both direct mosquito control and indirect malaria control. This is in line with the findings of Adamu (2020), who reported the presence of flavonoids, alkaloids, cardiac glycosides, tannins, and saponins in the aqueous leaf extract of *Hyptis spicigera* in a study conducted in Kaduna State. Similarly, the current study aligns with the report of Ladan et al. (2014), which reported the presence of similar phytochemicals, the absence of tannins in the methanolic leaf extract of *Hyptis spicigera*, and the addition of vitamins, carbohydrates, and coumarins, which are not found in the current study. The results differ from those of Ngadvou et al. (2023), who reported only tannins and terpenoids in the methanolic leaf extract of *Hyptis spicigera*. Other studies reporting similar phytochemicals include Adamu et al. (2021); Adamu et al. (2020); Idi and Muhammad (2021); and Ngadvou *et al.* (2023).

Higher contents of saponins, followed by tannins, terpenoids, and alkaloids, were revealed in the leaves of *Hyptis suaveolens*, while the roots had higher contents of tannins, followed by saponins, terpenoids, and flavonoids. Among the principal bioactive compounds, the methanolic leaf extract had the highest content of terpenoids and alkaloids than the root, but the flavonoid content was higher in the root than in in the leaf. The current study aligns with the findings of Ladan et al. (2014), who reported higher levels of terpenoids, saponins, and alkaloids in the methanolic leaf extract of *Hyptis spicigera*. Adamu *et al.* (2021) reported higher and lower levels of flavonoids and tannins in the aqueous leaf extracts of *Hyptis spicigera* and linked their presence to an important biological roles.

The current study revealed that methanol, as a polar solvent, is superior to water for extraction. It has been reported that the quantity of phytochemicals extracted from plants depends on the solvents used in the extraction process (Dirar *et al.*, 2019). In this study, qualitatively, the two solvents extracted alkaloids, flavonoids, terpenoids, cardiac glycosides, steroids, tannins and saponins and this tally with the findings of Chapeta *et al.* (2024), who reported phenols, flavonoids, tannins, steroids, cardiac glycosides, and saponins as the phytochemicals extracted by methanol and aqueous solvents in a study conducted at Malawi to determine the effect of solvents extraction on phytoconstituents from two medicinal plants. In this study, methanol extracted higher concentrations of secondary metabolites than aqueous solvents. These differences can be associated with the polarity, solubility, and cell-penetration potency of methanol solvents compared to aqueous solvents. Methanol has a moderate polarity, enabling it to dissolve both polar and semi-polar compounds.

Furthermore, the ability of methanol to diffuse deeper into plant tissues due to its low viscosity, smaller molecular size, and capacity to effectively disrupt cell membranes further substantiates the extraction superiority of methanol over aqueous. In this study, methanol extracts yielded high levels of cardiac glycosides and tannins from the leaves of *Hyptis suaveolens*. This study is in line with the study by Johnson *et al.* (2015), who likewise reported high concentrations of cardiac glycosides and tannins extracted with methanol in a study conducted in Lagos, Northwestern Nigeria. This contradicts the report of Chapeta et al. (2024), who documented the superiority of aqueous solvents for extracting cardiac glycosides and tannins over methanolic solvents. Furthermore, methanol extraction yielded significantly higher concentrations of antioxidant-rich secondary metabolites (alkaloids, flavonoids, terpenoids, tannins, and saponins) than aqueous solvents across all plant parts. This suggests that methanol is a superior solvent for extracting secondary metabolites compared to water. This observation is in agreement with previous studies (Altemimi et al., 2017; Muhammad et al., 2023; and Chapeta *et al.*, 2024), which indicated that methanol have high efficiency in the extraction of antioxidants than aqueous solvents.

In this study, it was observed that both the leaves of *Hyptis suaveolens* and *Hyptis spicigera* exhibit strong antioxidant activity, indicating the presence of high levels of antioxidant compounds in the leaf extracts of the two plants. Although the two plants showed strong antioxidant activity, the leaf extract of *Hyptis spicigera* is stronger and more potent in scavenging free radicals. Oxidative stress is linked with many deplorable human diseases and results from insufficient ability of the biological system to counteract excessive free radicals. Plants with radical-scavenging activity can neutralize these

free radicals. This result suggested that the leaf extract of *Hyptis spicigera* can scavenge DPPH free radical and counteract oxidative stress more than that of *Hyptis suaveolens*. These results agree with the findings of Agbafor *et al.* (2015) and Mishra *et al.* (2025). This higher antioxidant potency, as observed, corresponds to the presence of flavonoids, terpenoids, tannins, and alkaloids, as detected in the methanolic leaf extracts of the two plants. These secondary metabolites have been reported as the rationale behind the radical scavenging activity of the two plants under study (Chigor, 2018; Reddy, 2025). The long-lasting use of *Hyptis suaveolens* and *Hyptis spicigera* as traditional agents for the direct control of mosquitoes and the indirect control of malaria is due to these secondary metabolites, which have been reported to possess potent mosquitocidal activity. The two plants, on the other hand, can be used as antioxidants, with *Hyptis spicigera* as the most potent.

CONCLUSION

In this study, the phytochemical profile, the comparative effects of extraction solvents on the secondary metabolite content, and the radical-scavenging potency of *Hyptis suaveolens* and *Hyptis spicigera* were assessed. The two plants are rich reservoirs of secondary metabolites and exhibit radical-scavenging activity, as revealed by the bioassay. The study further revealed the superiority of methanol as an extraction solvent compared to water. Limitations of the study include the inability to use the plant extract to conduct larvicidal, adulticidal, and antiplasmodial bioassays to directly test the efficacy of the extracts, even though the sole aim was to unravel the underpinning secrets behind their cosmopolitan traditional usage for mosquito control and indirect control of malaria, a public health emergency in Sub-Saharan Africa. To improve their use, future research should include further optimization and bioassays.

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