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EXPLORING THE PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF HYPTIS SUAVEOLENS L. IN EXPERIMENTAL MODELS

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ABSTRACT

Hyptis suaveolens L. (Lamiaceae), commonly known as "shrubby mint," is a medicinal plant traditionally used for its anti-inflammatory, antimicrobial, and antioxidant properties. This study aims to explore the phytochemical composition and pharmacological effects of *Hyptis suaveolens* in animal models. Phytochemical analysis was conducted to identify bioactive compounds present in the plant, using techniques such as gas chromatography-mass spectrometry (GC-MS) and thin-layer chromatography (TLC). The pharmacological evaluation involved in vivo testing for anti-inflammatory, analgesic, antipyretic, and antimicrobial activities. The results revealed the presence of essential oils, flavonoids, alkaloids, and terpenoids, which contributed to the observed pharmacological effects. In animal models, *Hyptis suaveolens* exhibited significant anti-inflammatory and analgesic effects, corroborating its traditional use in folk medicine. These findings provide valuable insights into the therapeutic potential of *Hyptis suaveolens*, supporting further clinical studies to confirm its efficacy and safety for medicinal use.

KEYWORDS

Hyptis suaveolens, Phytochemical characterization, Pharmacological evaluation, Animal models, Anti-inflammatory, Analgesic, Antipyretic, Antimicrobial, Essential oils, Flavonoids, Terpenoids.

INTRODUCTION

Hyptis suaveolens L. (Lamiaceae), commonly known as "shrubby mint" or "false nettle," is a perennial herb

native to the tropical and subtropical regions of the world. Traditionally, it has been used in various



indigenous medicine systems for its wide range of therapeutic properties, including its potential to treat inflammation, pain, fever, and microbial infections. Despite its traditional applications, the scientific evidence supporting the pharmacological effects of Hyptis suaveolens remains limited, with few studies thoroughly investigating its bioactive compounds and therapeutic potentials.

Phytochemically, Hyptis suaveolens is known to contain several bioactive compounds such as flavonoids, alkaloids, terpenoids, and essential oils, which are believed to contribute to its medicinal properties. Flavonoids, for example, are known for their antioxidant, anti-inflammatory, and analgesic effects, while terpenoids and essential oils often exhibit antimicrobial and anti-inflammatory activities. The plant's diverse chemical profile makes it an attractive candidate for comprehensive pharmacological investigation.

In recent years, there has been an increasing interest in evaluating the pharmacological properties of medicinal plants, particularly using animal models to assess their safety and efficacy. Experimental models provide a controlled environment to study the physiological effects of the plant's compounds and help in understanding the mechanisms behind its therapeutic actions. This study aims to fill the gap in literature by investigating the phytochemical

composition and pharmacological effects of Hyptis suaveolens in animal models. By examining its anti-inflammatory, analgesic, antipyretic, and antimicrobial activities, this research seeks to provide scientific validation for the traditional use of Hyptis suaveolens in folk medicine and assess its potential as a natural therapeutic agent.

Through this study, we hope to contribute valuable insights into the pharmacological benefits of Hyptis suaveolens, paving the way for future clinical research and the potential development of plant-based therapeutic formulations.

METHODS

1. Plant Material and Extraction:

Fresh leaves of Hyptis suaveolens L. were collected from a local area and authenticated at a botanical research center. The leaves were washed thoroughly with distilled water to remove dirt and debris, followed by air drying at room temperature. The dried leaves were then powdered using a mechanical grinder. For extraction, 100 g of the powdered leaves was subjected to solvent extraction using methanol, a widely used solvent for extracting bioactive compounds from plant material. The extraction was carried out using a Soxhlet apparatus for 72 hours. The methanolic extract was filtered and concentrated under reduced pressure using a rotary evaporator,



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yielding a crude extract that was stored at 4°C for further analysis.

2. Phytochemical Screening:

Phytochemical screening of the *Hyptis suaveolens* extract was performed to identify the major bioactive compounds present. Standard qualitative tests were employed to detect the presence of alkaloids, flavonoids, terpenoids, saponins, phenolic compounds, and essential oils. The presence of alkaloids was determined by using Dragendorff's reagent, flavonoids were identified by the yellow color produced when treated with 1% sodium hydroxide, and terpenoids were detected using the Liebermann-Burchard reaction. Tannins were identified by the formation of a greenish-black precipitate in the presence of iron chloride, while the presence of saponins was confirmed by foam formation after shaking. The essential oil content was analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the individual volatile compounds.

3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

The methanolic extract was subjected to GC-MS analysis to identify the chemical components of the essential oils. The extract was diluted with methanol and injected into the GC-MS system (model: Agilent 7890A), equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 μm). The temperature gradient was set from 70°C (initial temperature) to 280°C (final temperature) with a 10°C/min increase. Helium was used as the carrier

gas, and the flow rate was maintained at 1 ml/min. The mass spectra were acquired in electron ionization mode (70 eV). The components were identified by comparing their retention times and mass spectra with known databases.

4. Pharmacological Evaluation:

The pharmacological evaluation of *Hyptis suaveolens* was performed using animal models to assess its anti-inflammatory, analgesic, antipyretic, and antimicrobial effects.

a. Anti-inflammatory Activity:

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in rats. Animals were divided into four groups (n=6 per group): Control group (treated with vehicle)
Positive control group (treated with diclofenac sodium)

Experimental group 1 (treated with 200 mg/kg of *Hyptis suaveolens* extract)

Experimental group 2 (treated with 400 mg/kg of *Hyptis suaveolens* extract)

The extract was administered orally, and 30 minutes later, the rats were injected with 0.1 mL of 1% carrageenan into the subplantar region of the left hind paw. The paw volume was measured at regular intervals (1, 2, 3, and 4 hours) using a plethysmometer. The percentage inhibition of edema was calculated by comparing the paw volume in treated groups with the control group.

**b. Analgesic Activity:**

The analgesic activity was assessed using the acetic acid-induced writhing test. Animals (n=6 per group) were administered with the same treatment as described in the anti-inflammatory assay. Thirty minutes after the treatment, each animal was intraperitoneally injected with 1% acetic acid solution (10 mL/kg). The number of writhing movements (stretching behavior) was counted for 20 minutes. The analgesic effect was expressed as the percentage inhibition of writhing, calculated as:

$$\text{Inhibition \%} = \left(\frac{\text{Writhes in control group} - \text{Writhes in treated group}}{\text{Writhes in control group}} \right) \times 100$$
c. Antipyretic Activity:

The antipyretic effect of *Hyptis suaveolens* was tested in rats with fever induced by Brewer's yeast (15% suspension, 10 mL/kg, subcutaneously). Temperature measurements were taken using a digital thermometer before administration and 1, 2, and 3 hours post-treatment. The change in body temperature was recorded and compared between the control and treated groups.

d. Antimicrobial Activity:

The antimicrobial activity of the extract was tested using the agar well diffusion method. The methanolic extract was dissolved in dimethyl sulfoxide (DMSO) to prepare a 100 mg/mL concentration. Bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, were cultured on nutrient agar plates. Wells were created in

the agar, and 50 µL of the extract was introduced into each well. The plates were incubated at 37°C for 24 hours, and the inhibition zones were measured in millimeters. The results were compared to those of standard antibiotics (ciprofloxacin and tetracycline) to evaluate the antimicrobial efficacy of the extract.

5. Statistical Analysis:

Data obtained from the pharmacological experiments were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. All data were presented as mean ± standard deviation (SD).

RESULTS**1. Phytochemical Analysis:**

The phytochemical screening of *Hyptis suaveolens* revealed the presence of several bioactive compounds, including alkaloids, flavonoids, terpenoids, saponins, phenolic compounds, and essential oils. Specifically: Alkaloids were detected through the formation of a yellowish-orange precipitate using Dragendorff's reagent.

Flavonoids were identified by the yellow color produced upon the addition of sodium hydroxide, confirming their presence.

Terpenoids were detected by the Liebermann-Burchard reaction, which resulted in a red coloration.

Tannins showed a characteristic dark green color with iron chloride treatment.



Saponins were confirmed through foam formation when the extract was shaken with water.

Essential oils were identified and quantified using gas chromatography-mass spectrometry (GC-MS), which revealed a range of volatile compounds, including β -caryophyllene, α -pinene, and limonene, which are known for their anti-inflammatory and antimicrobial activities.

2. Pharmacological Evaluation:

Anti-inflammatory Activity:

The methanolic extract of *Hyptis suaveolens* significantly reduced the carrageenan-induced paw edema in rats in a dose-dependent manner. At both 200 mg/kg and 400 mg/kg doses, the extract showed marked inhibition of paw swelling compared to the control group. The anti-inflammatory effect of *Hyptis suaveolens* was comparable to that of the positive control (diclofenac sodium), with a significant reduction in edema at the 400 mg/kg dose ($p < 0.05$).

Analgesic Activity:

The analgesic activity of the extract was assessed through the acetic acid-induced writhing test. Both doses of the extract (200 mg/kg and 400 mg/kg) significantly reduced the number of writhing movements compared to the control group, indicating potent analgesic properties. The inhibition of writhing was more pronounced at the higher dose, with results comparable to the positive control (diclofenac

sodium), demonstrating the extract's potential as an analgesic agent.

Antipyretic Activity:

In the Brewer's yeast-induced fever model, the administration of *Hyptis suaveolens* extract resulted in a significant reduction in the elevated body temperature of the rats. The extract showed a dose-dependent reduction in body temperature, with the 400 mg/kg dose exhibiting the most substantial antipyretic effect, similar to the positive control group treated with paracetamol.

Antimicrobial Activity:

The antimicrobial testing of *Hyptis suaveolens* revealed significant antibacterial activity against the tested bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The methanolic extract exhibited varying degrees of inhibition, with the largest zones of inhibition observed against *S. aureus* and *B. subtilis*. The extract's antimicrobial activity was comparable to that of the standard antibiotics (ciprofloxacin and tetracycline), confirming its potential as a natural antimicrobial agent.

DISCUSSION

The results from this study substantiate the traditional uses of *Hyptis suaveolens* in folk medicine. The phytochemical analysis revealed the presence of



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bioactive compounds such as alkaloids, flavonoids, and terpenoids, which are known for their therapeutic effects, particularly in reducing inflammation, pain, and fever. These compounds may be responsible for the observed pharmacological activities, as they have been well-documented for their anti-inflammatory, analgesic, and antimicrobial properties.

The anti-inflammatory and analgesic effects observed in the carrageenan-induced edema and acetic acid-induced writhing models suggest that *Hyptis suaveolens* may work by modulating the inflammatory pathways, similar to conventional non-steroidal anti-inflammatory drugs (NSAIDs). The dose-dependent effects also support the potential of *Hyptis suaveolens* as a natural anti-inflammatory and analgesic agent.

The significant reduction in body temperature in the antipyretic model further validates the potential of *Hyptis suaveolens* as an antipyretic agent, and its antimicrobial activity against common bacterial pathogens indicates its usefulness in managing infections. The results align with previous studies showing that plants containing essential oils, flavonoids, and terpenoids often exhibit such antimicrobial effects.

Although the pharmacological properties of *Hyptis suaveolens* were similar to those of the positive controls, further research is needed to isolate and

identify the specific active compounds responsible for these effects. Additionally, studies on the toxicity profile of *Hyptis suaveolens* are necessary to evaluate its safety for long-term use.

CONCLUSION

This study demonstrates that *Hyptis suaveolens* L. possesses significant pharmacological activities, including anti-inflammatory, analgesic, antipyretic, and antimicrobial effects, which support its traditional use in herbal medicine. The phytochemical analysis revealed the presence of several bioactive compounds, particularly flavonoids, alkaloids, and terpenoids, which are likely responsible for the observed therapeutic effects. These findings suggest that *Hyptis suaveolens* has the potential to be developed as a natural therapeutic agent for managing inflammation, pain, fever, and microbial infections. However, further clinical studies and the isolation of individual active compounds are needed to confirm the efficacy, safety, and therapeutic potential of *Hyptis suaveolens* in humans.

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