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COMBINATION OF ESSENTIOL OIL EXTRACTS FOR ENHANCED ANTHELMINTIC ACTIVITY OF OREGANO AND MINT PLANT

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ABSTRACT

This study investigates the synergistic effects of oregano (Origanum vulgare) and mint (Mentha piperita) essential oils in enhancing anthelmintic activity. The essential oils were extracted using steam distillation to identify the bioactive compounds. The anthelmintic activity of individual oils and their combinations was assessed using in vivo assays on earthworms. Results showed that both oregano and mint essential oils exhibited significant anthelmintic activity, with oregano oil demonstrating stronger effects. However, when combined, the essential oils exhibited a notable synergistic effect, enhancing their overall anthelmintic activity compared to individual oils. The combination of carvacrol and menthol was identified as a key factor in this enhanced effect. This study suggests that the combination of oregano and mint essential oils may offer a promising natural alternative for controlling helminthic infections, with potential applications in both veterinary and human medicine.

KEYWORDS: Oregano, Mint, Essential oils, Synergistic effect, Helminthic infections, Natural alternative medicine, Veterinary and human applications.

INTRODUCTION

Helminthic infections represent a significant global health challenge, affecting both humans and animals, and are associated with a wide range of adverse consequences including nutritional deficiencies, impaired growth, reduced productivity, and the onset of various

diseases. Conventional synthetic anthelmintic drugs, while effective, are increasingly constrained by issues such as the development of drug resistance, undesirable side effects, and high economic costs. These limitations have driven growing scientific interest toward plant-derived natural products as safer, sustainable, and eco-friendly alternatives.

Among medicinal plants, oregano (*Origanum vulgare*) and mint (*Mentha piperita*) are well-recognized for their rich phytochemical profiles and diverse pharmacological properties, including antimicrobial, antioxidant, and therapeutic activities. Their essential oils are particularly noteworthy: oregano oil contains carvacrol, and mint oil contains menthol—both compounds with reported anthelmintic potential. Despite these promising findings, research exploring the combined or synergistic effects of these two essential oils remains limited.[1-5]

Synergistic interactions between plant extracts can enhance therapeutic efficacy, lower the required dosage, and potentially delay or prevent the emergence of resistance. In this context, the present study aims to systematically evaluate the anthelmintic activity of oregano and mint essential oils, both individually and in combination, to determine whether their synergistic use provides superior worm-killing efficacy compared to their independent applications.[6-8]

Parasitic worm–induced diseases are caused by helminths (parasitic worms) that infect humans and animals

Common parasitic worms (helminths)

Nematodes (roundworms) – e.g., Ascaris lumbricoides, Enterobius vermicularis, Wuchereria bancrofti.

Figure:1(roundworms)

Figure:2(flukes)

Trematodes (flukes) – e.g., Schistosoma spp.

Cestodes (tapeworms)

Figure. 3: (tapeworms).

Transmission Routes

Helminths spread in different ways:Oral (fecal–oral route),Eating food/water contaminated with worm eggs or larvae.

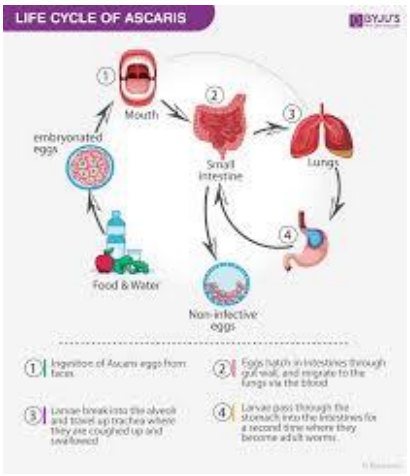

Fig: 4 (fecal–oral route)

Skin penetration: Larvae in contaminated soil or water penetrate the skin (e.g., hookworm, schistosomes).

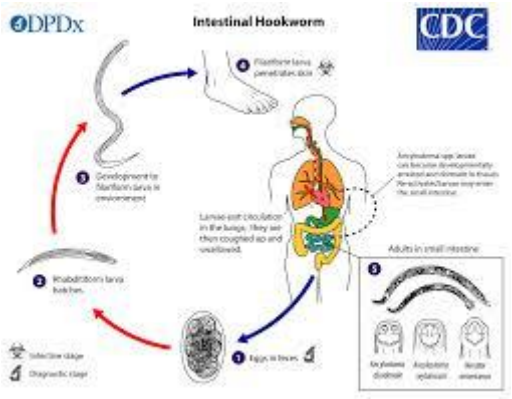

Fig. 5: (Larvae in contaminated soil or water penetrate the skin)

Vector-borne: Biting insects transmit larvae (e.g., mosquitoes for filariasis).

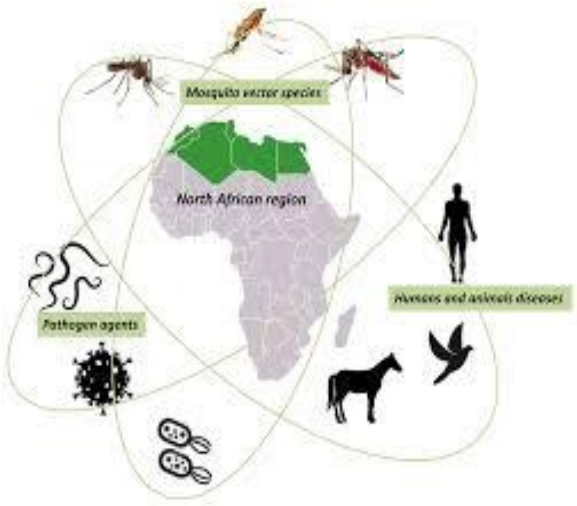

Figure. 6: (Biting insects transmit larvae).

Undercooked meat: Eating infected pork, beef, or fish (e.g., tapeworms).

Figure. 7: (Eating infected pork, beef, or fish).

The standard drug for most parasitic worm infections in humans(Albendazole)

Mechanism of Action

Binds to β-tubulin in parasitic worms.Inhibits microtubule polymerization → disrupts glucose uptake → depletes glycogen stores.Results in immobilization and death of the parasite.

Dosage Forms

Tablets (chewable / conventional)Oral suspension.

Figure. 8:(Albendazole).

Dose

For intestinal worms: 400 mg single dose (adults & children >2 years). For hydatid disease: 400 mg twice daily for 28 days, repeated after intervals. For neurocysticercosis: 15 mg/kg/day in 2 divided doses (max 800 mg/day).

Side Effects

Gastrointestinal: nausea, abdominal pain, Headache, dizziness Reversible alopecia (long-term use), Elevated liver enzymes.

Rare: bone marrow suppression.

Common: Nausea, Vomiting, Abdominal Pain, Diarrhea, Headache, Dizziness, Fever.

Less Common: Elevated liver enzymes (hepatotoxicity), Reversible hair loss (alopecia), Fatigue, Rash or itching.

Rare/Serious: Bone marrow suppression (leukopenia, pancytopenia)Severe liver injury, Aplastic anemia, Anaphylaxis (severe allergic reaction).

Plant Profile – Oregano (Origanum vulgare).

Botanical Name: Origanum vulgare.

Figure. 9: Origanum Vulgare.

Family: Lamiaceae.

Common Names: Oregano, Wild marjoram.

Morphology: Perennial herb with aromatic leaves.

Height: 20–80 cm.

Leaves: Ovate, opposite, green, with fine hairs.

Flowers: Pink to purple, small, clustered.

Distribution: Native to Mediterranean regions, widely cultivated worldwide Major Chemical Constituents: Carvacrol (phenolic monoterpenoid – main bioactive)Thymol, p-cymene,γ-terpinene.[9-12]

Pharmacological Activities: Antimicrobial, Antifungal, Antioxidant, Anti-inflammatory, Anthelmintic.

Anthelmintic Activity

Mechanism

Carvacrol disrupts parasite cell membranes, causing leakage of cellular contents and death.

Effective against both gastrointestinal nematodes and earthworms in in vitro and in vivo studies.

Shows paralysis and death of worms like some synthetic drugs.

Plant Profile – Mint (Mentha piperita)

Botanical Name: Origanum vulgare.

Figure. 10: *Origanum vulgare.*

Family: Lamiaceae.

Common Names: Peppermint, Pudina.

Morphology: Perennial aromatic herb.

Height: 30–90 cm.

Leaves: Dark green, lanceolate, serrated margins, aromatic when crushed.

Stems: Smooth or slightly hairy, square in cross-section.

Flowers: Purple to pink, arranged in terminal spikes.

Distribution: Native to Europe; cultivated in many temperate and subtropical regions worldwide.

Major Chemical Constituents: Menthol (primary bioactive compound)Menthone, Menthyl acetate,1,8-cineole, Limonene.

Pharmacological Activities: Antimicrobial, Analgesic, Anti-inflammatory, Antispasmodic, Anthelmintic.

Anthelmintic Activity: The ability of a substance, often a drug or natural compound, kill or expel parasitic wormsfrom host.

Mechanism

Menthol interferes with neuromuscular activity of helminths, causing paralysis and death.

Effective against nematodes, cestodes, and trematodes in various in vitro and in vivo studies.[13-15]

MATERIALS AND MEHODOLOGY

Plant Materials

Fresh leaves of *Origanum vulgare* (oregano) and *Mentha piperita* (mint) were procured from a local herbal market and the institutional medicinal garden. The plant specimens were authenticated by a qualified botanist, and voucher specimens were deposited for reference.

Chemicals and Reagents

The chemicals and reagents used included distilled water, normal saline solution, and analytical-grade solvents for cleaning and extraction. Albendazole was employed as the reference standard drug for comparative evaluation.

Equipment

Essential oil extraction was carried out using a Clevenger-type steam distillation apparatus. Other laboratory equipment utilized included standard glassware (beakers, conical flasks, and measuring cylinders), Petri dishes, forceps, an analytical balance, a stopwatch/timer, and a compound microscope for observation of test organisms.

Test Organisms

Adult earthworms (*Pheretima posthuma*), collected from moist soil beds, were used as the experimental model for anthelmintic activity studies, owing to their close physiological resemblance to intestinal roundworms of humans.

Essential Oil Extraction

Freshly collected leaves of oregano and mint were thoroughly washed with distilled water, shade-dried at room temperature, and subjected to steam distillation using the Clevenger apparatus. The extracted essential oils were collected, dried over anhydrous sodium sulfate to remove residual moisture, and stored in amber-colored airtight glass vials at 4 °C until further use.

Preparation of Test Solutions

Essential oils of oregano and mint were diluted in a suitable emulsifying agent to obtain various test concentrations. For combination studies, oregano and mint oils were mixed in equal proportions to prepare blended test solutions.

Figure. 11: (oregano)

Figure. 12:(mint)

Essential Oil Extraction

The leaves were washed, and subjected to steam distillation and Oils were collected, and stored in airtight amber bottle.

Figure. 13: Steam Distillation.

Preparation of Plant Material

Fresh leaves of *Origanum vulgare* (oregano) and *Mentha piperita* (mint) were collected, thoroughly washed with clean distilled water to remove surface contaminants, and coarsely chopped to increase surface area and facilitate efficient extraction of essential oils.

Steam Distillation Setup

A Clevenger-type steam distillation apparatus was assembled, consisting of a round-bottom flask serving as the boiler, connected to a condenser through a delivery tube. The condenser outlet was attached to a Clevenger trap for the collection and separation of essential oils. The plant material was placed in the distillation flask, and distilled water was added to cover approximately half of the plant material volume.

Distillation Procedure

The flask was gently heated to generate steam, which permeated the plant material and volatilized the essential oil constituents. The resulting vapor mixture of water and oil passed through the condenser, where it was cooled and converted into liquid form.

Oil–Water Separation

The condensed mixture collected in the Clevenger trap, where phase separation occurred based on density differences. The essential oil layer was separated from the aqueous distillate.

Drying and Storage of Essential Oils

Residual moisture in the extracted oils was removed using anhydrous sodium sulfate. The dried oils were stored in amber-colored airtight glass bottles at 4 °C to prevent degradation from light, heat, or oxidation.

Qualitative Analysis of Essential Oils

Solubility Test

Essential oils were tested for solubility characteristics. They were found to be insoluble in water but miscible with organic solvents such as ethanol. Observation: Oils separated from the aqueous layer and floated or sank depending on density.

Spot/Stain Test

A drop of essential oil was placed on filter paper. The formation of a permanent translucent spot confirmed the lipidic nature of the oil.

Odor Evaluation

- *Origanum vulgare*: Exhibited a strong, warm, phenolic odor characteristic of carvacrol.
- *Mentha piperita*: Produced a cool, refreshing odor attributable to menthol.

Color Reaction Tests

- Vanillin–HCl Test: Phenolic oils produced a reddish-brown coloration.
- Ferric Chloride Test: Presence of phenolic compounds was indicated by violet or greenish coloration.
- Sudan III Test: An orange-red coloration confirmed the lipid nature of the essential oils.

Collection of Test Organisms

Adult earthworms (Pheretima posthuma) were used as experimental models due to their anatomical and physiological similarity to intestinal roundworms of humans.

Figure. 14: earthworms.

Preparation of Test Solutions

1. Essential Oils: The essential oils of *Origanum vulgare* (oregano) and *Mentha piperita* (mint) were extracted by steam distillation and used for assay.
2. Dilution: The oils were diluted using an appropriate emulsifying agent (e.g., Tween 80) to obtain the required concentrations (prepared freshly before each assay).
3. Combination Solution: For synergistic evaluation, oregano and mint essential oils were mixed in a 1:1 ratio (v/v) and subsequently diluted to the desired concentrations.
4. Reference Drug: Albendazole, prepared in normal saline, was used as the standard reference anthelmintic drug.
5. Control: Normal saline with emulsifier (without essential oils) was used as the negative control.

Anthelmintic Assay

1. Test Organism Selection: Adult earthworms (*Pheretima posthuma*) of approximately equal size (6–8 cm in length) were collected from moist soil beds and acclimatized under laboratory conditions prior to experimentation.
2. Grouping of Worms: Worms were divided into experimental groups, with each group consisting of 6 worms (n = 6) for statistical reliability.
3. Treatment Solutions: Each group of worms was placed in Petri dishes containing:

- Different concentrations of oregano oil solution
- Different concentrations of mint oil solution

- A 1:1 combination solution of oregano and mint oils
- Albendazole solution (standard control)
- Saline with emulsifier (negative control)

4. Observation Parameters: Two endpoints were recorded:
   - Paralysis time: The time at which worms lost spontaneous movement and did not revive upon gentle mechanical stimulation.
   - Death time: The time at which worms showed no movement even after exposure to warm water (50 °C).
5. Data Recording: All times were recorded in minutes using a stopwatch, and mean values ± standard deviation were calculated for each group.
6. Statistical Analysis: Data were analyzed using one-way ANOVA followed by post hoc tests to determine significant differences between treated and control groups ($p < 0.05$ considered significant).

Figuer. 15: blank-saline, sandard, test.

In Normal Saline

Trial	Treatment	conc.	Worm ID	Paralysis(mins)	Death(mins)
1.	Saline	0.9%	1	-(no paralysis)	-(alive)
2.	Saline	0.9%	2	-(no paralysis)	-(alive)
3.	Saline	0.9%	3	-(no paralysis)	-(alive)

In Origanum vulgare oil extract

Trial	Treatment	conc.	Worm ID	Paralysis (mins)	Death (mins)
1.	Oregano oil	10 mg/ml	1	300	480
2.	Oregano oil	10 mg/ml	2	260	420
3.	Oregano oil	10 mg/ml	3	250	420
MEAN				270	440

In Mentha piperita oil extract

Trial	Treatment	conc.	Worm ID	Paralysis(min)	Death(min)
1.	Mint oil	10 mg/ml	1	240	480
2.	Mint oil	10 mg/ml	2	250	430
3.	Mint oil	10 mg/ml	3	240	480
Mean				243	463

In combination (Oregano:Mint)(1:1)

Trial	Treatment	conc.	Worm ID	Paralysis(min)	Death(min)
1.	Combo(1:1)	2% v/v	1	2 min	4 min
2.	Combo(1:1)	2% v/v	2	2mins 45 sec	3 mins 45 sec
3.	Combo(1:1)	2% v/v	3	2 mins 30 sec	4 min
MEAN				2 min 25 sec	3 min 55 sec

In Albendazole solution

Trial	Treatment	conc.	Worm ID	Paralysis(min)	Death (min)
1.	Albendazole	10 mg/ml	1	15 min	30 min
2.	Albendazole	10 mg/ml	2	15 min	28 min
3.	Albendazole	10 mg/ml	3	17 min	35 min
Mean				16 min 6 sec	31 min

6. Data Analysis

Results were compared between individual oils, their combination, and saline, the standard drug to evaluate potency and synergistic effects. Protocol (for each trial set).

S.no	Treatment	Concentration	Mean Paralysis Time(min)	Mene Death Time(min)
1.	Normal Saline(0.9%)	0.9%	no paralyse	no death
2.	Albendazole	10mg/ml	15 mins 66sec	31 mins
3.	Origanum vulgare	2%v/v	270 mins	440 mins
4.	Mentha piperita	2%v/v	243 mins	463 mins
5.	Combination (1:1)	2%v/v	2 mins 25 sec	3 mins 81 sec

Number of worms per group

3 adult earthworms (Pheretima posthuma) per treatment.

Replicates

3 independent trials (perform the whole experiment three times on separate days or with fresh worms).

Volume in Petri dish

10 ml of test solution.

Testing

Place worms immediately into petri dish, start stopwatch at time 0. continuously watch and record the time of events (or check every 1–2 min until events occur).

Temperature

room temperature (note exact temperature, e.g., 25 ± 2°C).

OBSERVATION

Paralysis time = time (2 mins 25 sec) at which the worm shows no movement even after gentle stimulation (touch with forceps) and cannot restore normal movement.

Death time = time (3 mins 81 sec) when the worm shows no movement and does not respond to vigorous stimulation AND body shows complete immobility and loss of turgor (confirm by transferring to distilled water — no revival).

CONCLUSION

The present study demonstrates that both oregano (Origanum vulgare) and mint (Mentha piperita) essential oils possess significant anthelmintic activity, with oregano oil showing stronger individual effects. Importantly, their combination produced a synergistic enhancement in activity, likely due to the complementary action of carvacrol and menthol. These findings indicate that the combined use of oregano and mint essential oils may serve as an effective, natural, and eco-friendly alternative for controlling helminthic infections in both human and veterinary applications.

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