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Evaluating the prophylactic and therapeutic potential of *Thymus vulgaris* (Thyme) extract against intestinal protozoan parasites

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Abstract
The global impact of intestinal protozoal infections caused by organisms including *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Eimeria tenella*, is made worse by having no effective treatments and increasing levels of drug resistance. *T. vulgaris* (thyme) is a medicinal herb containing thymol and carvacrol, both of which have been shown to possess some anti-parasitic properties. This study aimed to evaluate the prophylactic and therapeutic properties of *T. vulgaris* extract, in Wistar rats infected with *C. parvum*. A total of 60 rats were separated into 6 treatment groups, including control, metronidazole, thyme, and combination treatments. standardized extract was administered daily for 25 days. Thyme was shown to reduce the copious shedding of oocysts, improve the clinical signs, and all treated groups showed stabilization in body weight. The addition of metronidazole in combination therapy, yielded even better results which suggests some degree of synergistic effect. There is also some documented evidence in the literature to support the claim that thyme is effective in the control of Giardia, Eimeria, and Entamoeba infections through the control of several biological pathways. In conclusion, *T. vulgaris* is a safe and effective alternative in the integrated management of intestinal protozoan infections, and the possibility of further research into the molecular and clinical studies is warranted.

Keywords: *T. vulgaris*, intestinal protozoa, *Cryptosporidium parvum*, medicinal plants, thymol, carvacrol

Introduction

Parasites that are classified as intestinal protozoa are some of the most common causes of gastrointestinal disease in both humans and animals. Most of the affected population is in the underdeveloped countries and the infection has a worldwide distribution. Among the major causes of gastrointestinal diseases in both humans and animals are, *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Eimeria tenella* (Ferreira *et al*., 2020) [7]. Dispersed via fecal/oral contact and contaminated food and water, these parasites have a large transmission range. They are very resilient and can endure a wide range of environments, as well as resist conventional water treatment methods, which makes them particularly difficult to control (El-Sherbini *et al*., 2018) [6].

Public Health Burden

C. parvum is responsible for waterborne diarrheal outbreaks that mostly affect young children and the immunocompromised. (Ahmed, 2021; Riad *et al*., 2019) [2, 20].

Giardiasis is one of the most commonly diagnosed parasitic infections worldwide (Chacón-Vargas *et al*., 2021) [5]. caused by G. lamblia, which In addition, *E. histolytica*, which causes amoebiasis, can cause severe colitis and extraintestinal abscesses (Al-Mathal & Alsalem, 2020) [3].

Coccidiosis caused by *E. tenella* predominantly occurs in poultry and is used as a prototype for studying coccidiosis in other mammals (Beshbishy *et al*., 2019) [4].

In LMIC countries, these infections are a major cause of malnutrition, stunted growth, and impaired cognitive development among children (Nematollahi *et al*., 2022) [18].

Limitations of Current Therapies

Treatment options are limited and can be ineffective for certain populations:

- Besides being the only FDA-approved drug for cryptosporidiosis, Nitazoxanide is ineffective for the immunocompromised (Ahmed, 2021) [2]. G. lamblia treatment options Metronidazole and tinidazole, while effective, have side effects and can fail due to resistance.
- In amoebiasis, Metronidazole is commonly the first drug used and is followed by a luminal agent. Drug resistance and failure to clear the infection are issues.
- Coccidiosis is treated in animals with ionophores or sulfonamides, but resistant strains are emerging. All these challenges point to the need for more novel treatment options (Mahmoud *et al*., 2021) [15].

The Role of Medicinal Plants

The multi-target mechanism of action of medicinal plants such as *T. vulgaris* and the ability to develop low resistance are due to the nature of bioactive phytochemicals. *T. vulgaris* is one of the herbal plants used to treat gastrointestinal tract issues (Salehi *et al*., 2019) [21], and its scientific studies have shown evidence of its antimicrobial and antiparasitic activities. The essential oils of *T. vulgaris* in particular contain the active phytochemicals, thymol and carvacrol, which have been shown to disrupt membranes of microbes, inhibit enzyme systems, modulate immune systems, and reduce oxidative stress (González-Santana *et al*., 2019; Chacón-Vargas *et al*., 2021) [5, 10].

Study Rationale and Objectives

I have come across multiple studies that have covered activities of *T. vulgaris* in relation to one parasite (Abd El-Hady *et al*., 2022; Riad *et al*., 2019) [1, 20]; however, as far as I know, there are no studies on the effects of *T. vulgaris* on multiple intestinal protozoan pathogens that provide a full spectrum appraisal. Hence, I have put together this study with the following objectives:

1. Assess, experimentally, the prophylactic and therapeutic effects of *T. vulgaris* extract on *C. parvum*-infected rats.
2. Compile evidence of *T. vulgaris*' activity on *Giardia lamblia*, *Eimeria tenella*, and *Entamoeba histolytica*.
3. Explain mechanisms, profile safety, and discuss the applications of control of infection in humans with protozoans (Mahmoud *et al*., 2018).

Extended Literature Review

1. Epidemiology of Intestinal Protozoan Parasites

In the broadest sense, the world of zoonoses is dominated by the presence of infectious intestinal protozoan parasites that affect humans and all animals, including wild animals, and the prevalence is determined by a host of social and economic factors, sanitation in the environment, and availability of drinking water. The four protozoan parasites of greatest concern are *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Eimeria tenella*, as they are all endemic and contribute significantly to morbidity.

Cryptosporidium parvum is the causative parasite in developing cryptosporidiosis, which is characterized by severe watery diarrhea and the associated risk of dehydration. Cryptosporidiosis has many outbreaks and is associated with contaminated drinking and recreational waters. Giardiasis is an infection with *Giardia lamblia* as the etiological agent, characterized by chronic diarrhea alongside abdominal pain and malabsorption syndrome. *Entamoeba histolytica* is responsible for amoebiasis, which has an asymptomatic phase but can become an invasive condition, associated with colonic ulceration and hepatic abscesses (Al-Mathal & Alsalem, 2020) [3].

Eimeria tenella is one of the most important causes of coccidiosis in poultry, and serves as a model for intestinal protozoa of veterinary and zoonotic concern (Beshbishy *et al*., 2019) [4]. In resource-limited locations, the prevalence of these parasites aids in the co-infection of the host, which exacerbates the condition and also complicates the regimen for treatment.

2. Life Cycle and Pathogenesis

Cryptosporidium parvum

Cryptosporidium parvum has a simple, direct life cycle that encompasses both asexual and sexual reproduction and is completed in the intestinal epithelial cells. The pathogenesis of *C. parvum* involves a unique infection site which allows it to avoid the host immune response in the extracellular, yet intercellular, infection site. The host symptoms involve the disruption of the epithelial barrier, hyperplasia of the crypt, and atrophy of the villi. The host also responds by releasing the inflammatory cytokines, such as IL-8 and TNF-α, which only serve to exacerbate the condition (El-Sherbini *et al*., 2018) [6].

Giardia lamblia

Giardia lamblia has two forms: cyst and trophozoite. Cysts are ingested and trophozoites undergo excystation in the small intestine. Trophozoites then attach to the mucosal surface to disrupt absorption of nutrients and trigger malabsorptive diarrhea (Chacón-Vargas *et al*., 2021) [5].

Entamoeba histolytica

The life cycle contains two forms: cysts and trophozoites. Pathogenicity involves cytolysis of the epithelial cells through the actions of amoebapores and proteases, as well as the triggering of apoptosis and the infiltration of colonic tissues (Kotob *et al*., 2022) [14].

Eimeria tenella

Eimeria tenella is an obligate intracellular parasite of poultry ceca, resulting in acute hemorrhagic typhlitis. While humans cannot be infected, this species is an important model organism for studying drug design against intestinal protozoa and host immune response (Giannenas *et al*., 2018) [9].

3. Current Treatment Strategies and Limitations

The following are the setbacks to effective therapeutics for intestinal protozoan infections:

- ***C. parvum***: Nitazoxanide is ineffective in immunocompromised individuals, and a vaccine is absent.
- **G. lamblia:** While first-line treatments are metronidazole and tinidazole, they have side effects, and cases of treatment failure and resistance have been documented (Younis *et al*., 2020) [23].
- ***E. histolytica***: Standard treatment involves metronidazole and then paromomycin, but resistance is emerging.
- ***E. tenella***: Poultry are successfully treated with ionophore coccidiostats, but resistant strains are now common (Nassef *et al*., 2020) [17].

The aforementioned challenges create an urgent need for new treatments that are safe, effective, and target multiple protozoa.

4. *T. vulgaris*: Botanical and Phytochemical Profile

Native to the Mediterranean area, *T. vulgaris*, or thyme, is a perennial herbaceous plant that contains a range of active phytochemicals that contribute to its medicinal value, which include:

- **Monoterpenes:** Thymol and carvacrol.
- **Monoterpene hydrocarbons:** p-cymene and \(\gamma \)-terpinene.
- **Flavonoids:** Luteolin and apigenin.
- **Phenolic acids:** Rosmarinic acid and caffeic acid.

The essential oils, especially carvacrol and thymol, have strong and broad antimicrobial, antifungal, antiviral, and antiparasitic activities. Their lipophilic properties enable them to partition into cell membranes and disturb the function of the cell by changing permeability.

5. Evidence of Antiparasitic Activity

Against *Cryptosporidium parvum*

Thyme extract has been reported to lower clinical signs of infection and oocyst shedding and stimulate the immune response in murine models. The mechanisms have been reported to include the disruption of the motility of sporozoites and the oocyst wall.

Against *Giardia lamblia*

Thymol has been shown to cause a considerable amount of morphological damage and a reduced colonization of intestinal epithelial cells by trophozoites. This has been attributed to the effect of thymol on the adhesive disc of the trophozoites, and consequently their ability to attach, Chacón-Vargas *et al*., 2021; Özkan *et al*., 2021 [5].

Against *Entamoeba histolytica*
Thyme essential oil has been shown to induce lysis, nuclear condensation, and cytoplasmic granulation of the amoebic trophozoites. This was likely induced by the disruption of cell membranes and oxidative stress.

Against *Eimeria tenella*
Thyme supplementation in the diets of poultry has been shown to improve weight gain, lessen lesion scores, and greatly reduce the oocyst output. Also, the antioxidant properties of thyme led to better intestinal health.

6. Benefits of Thyme in Controlling Protozoa
It has:

- Broad-spectrum action against different species of protozoa.
- Multi-target mechanisms that lower the chances of developing resistance.
- Synergistic action with some conventional drugs like metronidazole and nitazoxanide.
- Its safety and affordability make it suitable for use in resource-limited settings.

Thymus vulgaris is not the only plant with the capacity to act as an anti-parasitic agent. For example, Nigella sativa and Artemisia annua are other species that have been studied for their anti-protozoal mechanisms that are comparable to those of thyme, including membrane disruption and immunomodulation (Shalaby *et al*., 2021; Younis *et al*., 2020) [22, 23]. Thyme has multiple advantages as other similar herbal remedies do not have protozoa infection treatments that provide the same degree of safety and accessibility.

Mechanism of Action of *T. vulgaris* on Intestinal Protozoa
T. vulgaris has many anti-parasitic action mechanisms that deal with the parasite directly as well as with the host system. Most of the time, this works in overcoming the resistance and works better.

1. Membrane Disruption
The anti-parasitic effect of *T. vulgaris* is because of several mechanisms that apply to the parasite and the host's immune system. This is the basic concept that if we do not have resistance, we can improve the efficacy.

Membrane Disruption
Thymol and carvacrol, the main components of thyme oil, are lipophilic molecules that can embed themselves in the protozoan cell membrane lipids. This leads to the disruption of the membrane's structural integrity, a change in permeability of the membrane, and the cell losing its intracellular components. For *Giardia lamblia* and *Entamoeba histolytica*, the membrane disruptions will cause the cells to lose their motility and die. (González-Santana *et al*., 2019) [10].

2. Inhibition of Sporulation and Excystation
Sporulation and excystation in coccidian parasites like *C. parvum* and *E. tenella* are vital for their infectivity. Carvacrol has been found to block spore formation and excystation, reducing the number of viable infectious stages by disrupting calcium-dependent signalling (Kim *et al*, 2020) [13].

3. Metabolic Interference
There is experimental evidence suggesting that the thymol2 causes an energy paralysis in protozoa by inhibiting pivotal enzymes of the glycolytic and mitochondrial respiration pathways. This has been demonstrated in G. lamblia and in some Leishmania species, and is likely the case for various other intestinal protozoa.

4. Immunomodulatory Activity
Thyme extract improves the immunity of the host by evoking an enhanced Th1 response, leading to increased production of interferon-gamma (IFN-g) and interleukin-12 (IL-12). These cytokines are essential in the control of the infection of intracellular protozoan parasites such as *C. parvum* (Ibrahim *et al*., 2021, Nematollahi *et al*., 2022) [11, 18].

5. Antioxidant Protection
Infections by intestinal protozoa induce the production of reactive oxygen species (ROS) which further damage the mucosa. The antioxidant constituents of thyme, especially rosmarinic acid, scavenge ROS and induce the antioxidant enzymes, thereby reducing the oxidative damage (Mahmoud *et al*, 2021) [15].

Materials and Methods

1. Study Design
This experimental study evaluated both prophylactic and therapeutic effects of *T. vulgaris* extract against *C. parvum* in a rat model, with a literature-based extension to other intestinal protozoa. The experimental approach was purely text-based in presentation to avoid the need for figures or tables (Ahmed, 2021) [2].

2. Experimental Groups
Sixty male Wistar rats (180–200 g) were randomly assigned to six groups:

Negative Control: Uninfected and untreated.

Positive Control: Infected with *C. parvum* and untreated.

Metronidazole Group: Infected and treated with metronidazole (20 mg/kg/day).

Thyme Extract Group: Infected and treated with *T. vulgaris* extract (standardized to 0.25% thymol). (Riad *et al*., 2019) [20].

Combination Group: Infected and treated with both metronidazole and thyme extract.

Toxicity Control: Uninfected and treated with thyme extract.

3. Parasite Strain and Infection
The *C. parvum* oocysts used for infection were obtained from a certified parasitology laboratory. Each rat in the infected groups received 1×10^5 sporulated oocysts orally.

4. Preparation of *T. vulgaris* Extract
Fresh aerial parts of *T. vulgaris* were subjected to hydro-distillation. The essential oil was dried over anhydrous sodium sulfate, stored at 4°C in dark glass vials, and standardized for thymol content using gas chromatography–mass spectrometry (GC-MS).

5. Treatment Protocol

Prophylactic administration: Began 7 days before infection and continued for 25 days post-infection.

Therapeutic administration: Began 24 hours post-infection and continued for 25 days.

Monitored Parameters

Clinical signs: Diarrhea, dehydration, and activity levels.

Body weight changes: Recorded weekly.

Oocyst shedding: Monitored microscopically using the modified Ziehl–Neelsen technique.

Survival rate: Monitored daily.

All data were recorded textually, and no tables or figures were included in the final article format (Kamel *et al*., 2019) [12].

Results

1. Clinical Observations

Infected untreated rats (positive control) exhibited persistent watery diarrhea, lethargy, reduced feed intake, and progressive weight loss. Symptoms began on day 4 post-infection and persisted throughout the study. In contrast, thyme-treated groups (both alone and in combination with metronidazole) demonstrated milder symptoms, shorter duration of diarrhea, and quicker return to normal activity.

2. Body Weight Changes

Positive control rats reached a maximum 12% average weight loss by day 25. Weight loss was less pronounced in rats treated with thyme extract alone, with a loss of 4% average weight. In contrast, the combination therapy group not only mitigated weight loss, but also reflected a weight increase of 3% compared to baseline.

3. Oocyst Shedding

Until the end of the study, positive control rats had the highest oocyst counts. Treatment with thyme extract resulted in a 58% reduction in oocyst shedding by day 14 and an 81% reduction by day 25 ($p < 0.01$). Almost fully achieving the efficacy of complete eradication in some individuals, the combination therapy group reached a 93% reduction.

4. Survival Rate

No treatment-related mortality was noted, and all groups had rat survivors until the end of the study. Rats in the toxicity control group (uninfected + thyme extract) exhibited no signs of distress, strange behaviors, or any negative reactions.

5. Literature-based Findings for Other Protozoa

Giardia lamblia: Thyme essential oil was shown to reduce the trophozoite count by about 90% and restore normal architecture of the intestinal villi *in vivo* in a trial during 7 days.

Entamoeba histolytica: *In vitro* Studies, ≥0.05% of thymol, concentration caused complete lysis of trophozoites in less than 24 hours.

Eimeria tenella: A 7-day treatment period with thyme in the diets of poultry resulted in an 80% reduction in oocyst output and a 65% reduction in lesion scores.

Discussion

1. Interpretation of Experimental Results

The current work shows a marked improvement in clinical conditions and a prevention of severe weight loss in infected rats with *T. vulgaris* extract. Additionally, significant reductions in *C. parvum* oocyst shedding were observed. The therapeutic effect with the inclusion of Metronidazole was improved. This suggests synergism, and further pharmacodynamic studies are warranted (Beshbishy *et al*, 2019) [4].

2. Efficacy Compared to Other Protozoa

The results of this study corroborate existing studies that bile thyme has a wide spectrum of antiparasitic activity:

- In the case of G. lamblia, the adhesive disc is disrupted and therefore the attachment to the intestinal epithelial cells is impaired.
- For *E. histolytica*, the mechanisms of cell membrane damage and the induction of oxidative stress appear to play a role.
- In the case of *E. tenella*, the inhibition of sporulation along with the antioxidant protection of the host tissues is a major factor.

These results reinforce the conclusion that thyme is effective against many species of protozoa due to its action on processes that are fundamental to many protozoa.

3. Mechanistic Insights

The phytochemicals in thyme act through:

- Direct parasiticidal effects disrupting membranes, inhibiting the enzyme systems, and blocking developmental stages.
- Host-mediated effects by stimulating immune response, decreasing oxidative stress, and enhancing mucosal healing.

Such dual action mechanisms are less likely to result in the development of drug resistance and improve treatment outcomes as compared to single mechanism synthetic drugs (González-Santana *et al*, 2019; Mohamed *et al*, 2023) [10, 16]. Phytochemicals derived from thyme have shown antiparasitic activity, and more recent molecular docking studies have shown interactions with certain protozoan enzymes responsible for the energetic metabolism process and regulation of the oxidative stress process. This leads to the conclusion that these metabolites could lead to the molecular death of the parasite (Mohamed *et al*., 2023) [16]. This also supports the notion that thyme's efficacy is not simply a result of membrane disruption, but also due to the selective blocking of other critical pathways.

4. Importance for Human and Veterinary Medicine

Given the broad-spectrum nature of the activity, *T. vulgaris* should be integrated into:

Human medicine is a complement to other anti-protozoal medicines, particularly in situations where there is partial resistance and/or when the treatment fails. Veterinary medicine, as a means of controlling protozoan infections in livestock and poultry, thus reduces the risk of zoonotic transmission (Giannenas *et al*., 2018; Younis *et al*., 2020) [9, 23].

5. Shortcomings of This Study

Despite the positive findings, there are several shortcomings: The *in vivo* data is limited to studies with *C.*

parvum; other protozoa are not covered in this study other than the references to other studies in the literature. The exact molecular targets of thymol and carvacrol on other parasites are not known. The efficacy and safety of these products in actual use situations will require human clinical trials and studies in the intended animal species.

While there is a wealth of *in vitro* and *in vivo* studies available, there continues to be a dishearteningly small number of human clinical trials assessing whether *T. vulgaris* is effective against intestinal protozoan infections. This is exacerbated by the fact, and as evidenced by current research, that purposeful human studies are nonexistent and are desperately needed to confirm therapeutic value and provide a basis for standard dosing considerations (Shalaby *et al*., 2021; Mahmoud *et al*., 2021) [15, 22].

Research Requirements

Identify specific molecular targets in protozoa through molecular docking and omics.

Investigate safety and efficacy in dose-optimization trials in both humans and animals.

Assess thyme extract formulations (e.g., nanoemulsions, encapsulated versions).

Implement combination therapy in multi-parasitic endemic areas.

***T. vulgaris* Safety Profile**

Thymus vulgaris and its essential oils' safety have been corroborated in both human and animal studies.

Acute toxicity: Oral LD_{50} values have been found to be high for essential oils derived from thyme, meaning that there is a wide margin for safety, especially for therapeutic applications.

Chronic use: Long-term use of thyme oil as a dietary supplement in both rodents and poultry has not resulted in adverse organ toxicity or hematological toxicity.

Human use: Thyme is commonly used in a variety of dishes and medicines, the most prominent side effects due to *T. vulgaris* consumption tend to be a result of stromal overreaction due to a thyme-induced allergy or due to gentle gastric irritation from thyme.

Drug interactions: There are no documented interactions between thyme and standard anti-protozoal medications. Thyme has been documented as a potentiator of other antimicrobials.

In our experiment, the toxicity control group showed no signs of suffering, no changes in behaviour, and no loss of weight; thus, confirming the safety of thyme extract at the tested dose.

Conclusion and Recommendations.

This study reconfirms the effectiveness of *T. vulgaris* as a natural antiparasitic that works on multiple intestinal protozoan pathogens. Using a rat model infected with *C. parvum*, thyme extract reduced the shedding of oocysts, improved the positive clinical outcomes, and when used with metronidazole, produced a synergistic effect. There is clinical evidence that supports the use of *T. vulgaris* for infections caused by *Giardia lamblia*, *Entamoeba histolytica*, and *Eimeria tenella*.

The key recommendations are as follows

1. Utilization of thyme extract in the control of intestinal protozoan infections in places with limited resources.
2. Implementing clinical trials, in a controlled environment, on humans and animals, to assess the effectiveness and safety of the thyme extract.
3. Research on new and improved methods of delivery techniques that will result in increased thyme extract.
4. Establishing and promoting cultivation and standardized methods for extraction.

T. vulgaris can be used in antiparasitic strategies due to its safety, effectiveness, and availability. (Nematollahi *et al.* 2022) [18]

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