



Effect of Essential Oils of *Thymus vulgris* on the Vitality of the Protoscolices of *Echinococcus granulosus*

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Abstract:

It was observed by dealing with the larval stage of the *Echinococcus granulosus* parasite, its high resistance and its ability to live for long periods in poor conditions outside the body of the intermediate host, as it was able to remain alive after several days at low temperatures as well as at relatively high temperatures. The study showed that the essential oil *Thymus vulgris* has an effective effect on the vitality of primary heads in the laboratory, results showed at concentrations of 10,000, 5000, and 2500 PPM. The percentage of deaths compared to the control increased to 100% immediately after treatment, while at a concentration of 1.250 PPM, the parasite was completely destroyed after an hour. Here we find that the first three concentrations have a very effective effect compared to the fourth concentration.

Keywords: *Thymus Vulgaris* , Essential Oil, *Echinococcus Granulosus*

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I. Introduction

Cystic echinococcosis (EC) is a zoonotic disease that affects both humans and animals and is caused by parasitic tapeworms. This parasite lives in the intestines of the definitive hosts in the adult stage, while in the intermediate hosts it is present in the larval form and is in the form of cysts in the organs Interior [1]. It is a zoonotic disease spread throughout the world caused by larvae of *Echinococcus granulosus*[2]. Humans can ingest *E. granulosus* eggs after touching contaminated materials with hands and mouth, such as soil or dog fur contaminated with the eggs, and by consuming contaminated food or water[3] Surgical treatment of hydatid cysts is the first line of treatment in humans, although the possibility of

leakage of hydatid cystic fluid leads to secondary hydatid formation [4].

This disease spreads in southeastern Europe, the Mediterranean countries, the Middle East, eastern Africa, Central Asia, northwestern China, and in some regions of South America, where the infection rate among humans reaches 50/100,000 people annually. research has tended towards the use of natural materials and compounds, especially medicinal plants, with curative efficacy for many diseases [5]. The disease in Iraq constitutes one of the major health problems, as it is considered one of the endemic diseases in the country and there are no organized national programs to control it [6].

The use of medicinal plants has developed, and with the development of science, scientists have manufactured these medicinal compounds from sources other than medicinal plants. At present, there are large numbers of manufactured medicines. Recently, attention has turned to reducing prepared medicines and moving to medicinal plants [7].

Medicinal plants produce many secondary metabolic compounds that have wide uses in medical treatments, including alkaloid compounds, phenolic compounds, terpene compounds, and others. Terpinat compound [8]

II. Materials and Methods

A. Sample collection and preparation

Samples of hydatid cysts were collected from infected sheep slaughtered in Karbala city/ Iraq “Fig.1” and transferred in a refrigerated container to the laboratory for isolation of germ membrane protoscolices “Fig.2” [9]



Fig 1 . Sheep's liver infected with hydatid cysts



Fig 2. Hydatid cyst germinal membrane

B. Collect the Protoscolices

The Smyth method [10] The method is done by sterilizing the outer surface of the affected organ with ethyl alcohol diluted to a concentration of 70% and at laboratory temperature. Then the fluid of the hydatid cyst was withdrawn using a sterile medical syringe of 5 ml with a 21g syringe. After that, an external incision was made of the cyst using clean, sterile scissors, and the cyst was washed from the inside using a Pasteur dropper. By withdrawing the germinal fluid and pumping it several times to obtain the largest number of heads that are attached to the germ layer, then all the liquid is withdrawn and placed inside a glass beaker or Petri dish, then the liquid is left for a period of time for all the heads to settle, after the heads are collected and deposited and the liquid is removed, then A little of what remains is placed in Ependorf tubes and then placed in a water bath to maintain a suitable temperature for the continuation of the life of the protozoans.

C. Appreciating the vitality of the Protoscolices

The vitality of the Protoscolices was estimated according to the method of Smyth and Barrett [11] The vitality of the protozoans was examined using the constant volume transfer method using a micropipette with a volume of 5 microlitres. 5 microliters were withdrawn from the protozoan suspension and placed on a glass slide. A similar volume of aqueous eosin dye at a concentration of 0.1% was added to it, mixed well and examined using an optical microscope under At 10X magnification, the dead heads were then calculated and were colored red as a result of the dye penetrating their walls, while the live heads were green in colour. The vitality rate of the heads was calculated for three replicates.

D. Plant collection and preparation of essential oils

The plant samples included the aerial parts, leaves and stems of the Thymus tree that were purchased from the local market. After collection, the plants were cleaned, then washed with tap water and dried at room temperature, then placed in tightly sealed plastic containers away from moisture until use [12]. The volatile oils of these plants were

isolated from dried thyme leaves, using the steam distillation method, whereby 250 grams of the dried parts of the two plants were taken separately and then boiled with steam-distilled water in a volume of 1.2 liters for 3 hours in a steam distillation device. After isolating the essential oils of each plant, they were kept at Temperature 4°C until used [13].

III. Results and Discussion

Four concentrations of volatile *Thymus vulgaris* were used, with the highest concentration being 10,000 ppm, followed by 5,000, then 2,500 and 1,250. Percentages were calculated by counting live and dead protoscolices within 5 microns of the sample, where dead protoscolices stain red

when treated with 0.1% aqueous eosin due to dye permeation through their membranes, while live protoscolices stay green.

The essential oil of the thyme plant has an effective effect on the vitality of primary shoots. The table shows the results at concentrations of 10,000, 5000, and 2500 PPM. The percentage of deaths compared to the control increased to 100% immediately after treatment, while the PPM concentration was 1.250(Fig. 3). The time of complete destruction of the parasite was at the thirty-minute mark. Here we find that the first three concentrations have a very effective effect compared to the fourth concentration and the control sample in which the primary heads remained alive for up to 72 hours.

<u>Concentration</u>	Percentage of loss for each time period				Mortality rate per concentration
	Immediately after the transaction	after 15 minutes	after 15 minutes	After 1 hour	
10000	100	/	/	/	/
5000	100	/	/	/	/
2500	100	/	/	/	/
1250	0.00	24.60	67.30	100	47.97
Mortality rate for each time period	80.00	84.92	93.46	100	/
<u>L.S.D</u>	<u>Concentration</u>		<u>Time period</u>		<u>Interference</u>
	0.2609		0.2334		0.5218

Fig 3. Effect of Melaleuca alternifolia oil concentrations on vitality of Protoscolices at 37°C.

The oils have been studied Volatile extracts from plants are widely used for their diverse biological activities on different systems. These oils exhibit a wide range of effects, including anti-parasitic, anti-fungal, anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory, anti-cancer, anti-aging, and neuroprotective properties [14]. . Furthermore, volatile compounds from certain oils such as citronellal and 1,8-cineole have significant acaricidal effects on engorged tick larvae and females, surpassing insecticides in effectiveness [15]. In addition, after studying the volatile variants for their insecticidal properties, which showed promising results in combating spoilage such as the

greater wax moth, *Galleria mellonella*, especially when combined with Gamma [17]. Thyme oil, specifically from *Thymus capitatus*, has shown significant effects on *Echinococcus* spp. Research has shown that the F2 and F4 fractions of *T. capitatus* essential oil contain potent anticoccidial compounds, with higher toxicity against metacysts than to mammalian cells [18]. In addition, thyme essential oil showed strong antimicrobial activity against various bacteria, although it was not completely effective in eliminating *E. coli* from minimally processed watercress [19]. Since eosin dye penetration is a physical process, if for any

reason a physiological abnormality occurs, the permeability will increase thus

allowing the dye to enter, while the live primary grafts continue to show their natural green color [20].

IV. Conclusion:

We conclude from this that the essential oil of the *Thymus vulgaris* has an effective effect on the vitality of the protozoa, and its effect is stronger than some chemical drugs and plant extracts. Therefore, we call for a clinical trial to be repeated and to see the extent of its therapeutic effect on echinococcosis.

V. Applications:

The application of research in the field of human health and the effect of extracts on the therapeutic side of patients with hydatid cysts involves the use of bioactive components from plants *Thymus vulgaris* to control the growth of bioheads of the parasite *Echinococcus granulosus* In Vitro.

References:

1. Center for Food Security and Public Health (CFSPH) . (2020) . *Echinococcosis* . page 1-14
2. Wang, Y., Zhang, J., Wang, X., Ahmed, H., Shen, Y., & Cao, J. (2023). Molecular Epidemiology and the Control and Prevention of Cystic *Echinococcosis* in China: What is Known from Current Research. *Zoonoses*, 3(1).
3. Tamarozzi, F. ; Mariconti, M. ; Neumayr, A. & Brunetti, E. (2016). The intermediate host immune response in cystic *echinococcosis*. *Parasite Immunology* . 38(3): 170-181.
4. Chai, J. Y., Jung, B. K., & Hong, S. J. (2021). Albendazole and mebendazole as anti-parasitic and anti-cancer agents: an update. *The Korean Journal of Parasitology*, 59(3), 189.
5. Brunetti, E. ; Kem, P. & Vuitton, D.A. (2010) . Expert consensus for the diagnosis and treatment of cystic and alveolar *echinococcosis* in humans . *Acta Tropica* .114(1):1-16 .
6. Athmar, K. A. A. & Ban-Abbas, A. M. (2014). Immunization mice with DNA from protoscolices of human hydatid cyst. Immunological study. *International Journal Advaced Biology Research* . 4(1): 89- 95.
7. Tan, T. Y. C., Lee, J. C., Yusof, N. A. M., Teh, B. P., & Mohamed, A. F. S. (2020). Malaysian herbal monograph development and challenges. *Journal of Herbal Medicine*, 23, 100380.
8. Bhalshing, S.R . and Maheshwar ,V.L.(1998) . Plant culture a potential source of medicinal compound .*J. Sci . Ind. Res.*, 57 : 703-708
9. Craig, P. S., Hegglin, D., Lightowers, M. W., Torgerson, P. R., & Wang, Q. (2017). *Echinococcosis: control and prevention*. *Advances in parasitology*, 96, 55-158.
10. McManus, D. P. (2013). Current status of the genetics and molecular taxonomy of *Echinococcus* species. *Parasitology*, 140(13), 1617-1623.
11. Kern, P., Da Silva, A. M., Akhan, O., Müllhaupt, B., Vizcaychipi, K. A., Budke, C., & Vuitton, D. A. (2017). The *echinococcoses*: diagnosis, clinical management and burden of disease. *Advances in parasitology*, 96, 259-369.
12. Abed, I. J.; Ahmed S. M. and AL-Shimmary, H. (2021). Rosemary volatile oil as a preservative agent in some canned meat foods, *Iraqi Journal of Agricultural Sciences*, 52(1):155-162..
13. Khalaf, A. N. Abed, I. J. (2021). Evaluating the in vitro Cytotoxicity of *Thymus vulgaris* Essential Oil on MCF-7 and HeLa Cancer Cell Lines, *Iraqi Journal of Science*, Vol. 62, No. 9, pp: 2862-2871.
14. Sattayakhom, A., Wichit, S., & Koomhin, P. (2023). The effects of essential oils on the nervous system: a scoping review. *Molecules*, 28(9), 3771.
15. Rodrigues, L., Giglioti, R., Gomes, A. C. P., Katiki, L. M., Otsuk, I. P., da Silva Matos, R., ... & Veríssimo, C. J. (2020). In Vitro Effect of Volatile Substances from Eucalyptus Oils on

- Rhipicephalus microplus. *Revista Brasileira de Farmacognosia*, 30(5), 737-742.
16. Mohamed, H. F., EL-Naggar, S. E. S., Ibrahim, A. A., Elbarky, N. M., & Salama, M. S. M. (2020). Effect of volatile oils and/or gamma irradiation on the 4th instar larvae of *Galleria mellonella*. *Journal of Nuclear Technology in Applied Science*, 8(1), 15-28.
17. Sattayakhom, A., Wichit, S., & Koomhin, P. (2023). The effects of essential oils on the nervous system: a scoping review. *Molecules*, 28(9), 3771.
18. Amani, Hizem., Britta, Lundström-Stadelmann., Selim, M'rad., Sawssen, Souiai., Hichem, Ben, Jannet., Guido, Flamini., Roberta, Ascrizzi., Kamel, Ghedira., Hamouda, Babba., Andrew, Hemphill. (2019). Activity of *Thymus capitatus* essential oil components against in vitro cultured *Echinococcus multilocularis* metacestodes and germinal layer cells.. *Parasitology*, doi: 10.1017/S0031182019000295
19. Roseli, Jacobi, Veloso., Nei, Fronza., Álvaro, Vargas, Junior., Vânia, Silva, Carvalho., Miriam, Fumiko, Fujinawa., Sheila, Mello, da, Silveira. (2019). Potential of thyme essential oil on arugula sanitization. *Ciencia E Agrotecnologia*, doi: 10.1590/1413-7054201943006819.
20. Rottini, M. M., Amaral, A. C. F., Ferreira, J. L. P., Oliveira, E. S. C., Silva, J. R. D. A., Taniwaki, N. N., ... & Calabrese, K. D. S. (2019). *Endlicheria bracteolata* (Meisn.) essential oil as a weapon against *Leishmania amazonensis*: In vitro assay. *Molecules*, 24(14), 2525.