

EFFECT OF ESSENTIAL OILS OF EUCALYPTUS PLANT ON THE VITALITY OF THE PROTOSCOLICES OF ECHINOCOCCUS GRANULOSUS

Baneen Ali AL-Obaidi¹, Yarub Modhar AL-Qazwini²

^{1,2}Department of biology, College of education for pure Sciences, University of Kerbala, Kerbala, Iraq
m03151201@s.uokerbala.edu.iq yaarob.m@uokerbala.edu.iq

Abstract

The study showed that the high biological resistance of the Protoscolices of the Echinococcus granulosus against many aqueous extracts and chemical treatments lasted only a few minutes in front of the volatile oil of the Eucalyptus, as it took 15 minutes to destroy it at a concentration of 10.000 ppm, which was the highest concentration, and it took two hours at a concentration of 2.500 ppm, and this makes it a potential treatment and solution for this echinococcosis after being proven by clinical trials.

Keywords: Eucalyptus, Echinococcosis, essential oil, Echinococcus granulosus

1. INTRODUCTION

Echinococcus granulosus is a tapeworm that infects dogs. As for sheep, goats, and cows, it is an intermediate host. There are different strains of *E. granulosus* from G1 to G10, which are distributed throughout the world to infect many animals and humans. Tapeworms in dogs cause less ill effects. But it affects herbivores, specifically the liver, lungs, and spleen, which affects the economic value of meat [1]

This disease is widespread in southeastern Europe, the Mediterranean countries, the Middle East, East Africa, Central Asia, northwestern China, and in some regions of South America, where the human infection rate reaches 50/100,000 people per year [2]. 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 and monitor the disease [3].

Surgical treatment of a hydration cyst is the first line of treatment in humans, although the possibility of leakage of hydration cyst fluid leads to the formation of a secondary hydration cyst [4]

The use of medicinal plants began, and with the advancement of science, scientists manufactured these chemical compounds from various sources other than medicinal plants, and at the present time there are a large number of manufactured medicines, and attention has recently been directed to prepared medicines and the transition to medicine [5]

Many oils and their components have proven to be highly effective against parasites, especially protozoa. Therefore, they have been widely used in pharmacology due to their vital properties as an anti-spasmodic drug, as well as a general analgesic, anti-inflammatory and anti-varicose agent. Many essential oils have shown inhibitory activity against many microorganisms and parasites. [6]

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”



Fig 1 . Sheep's liver infected with hydatid cysts.

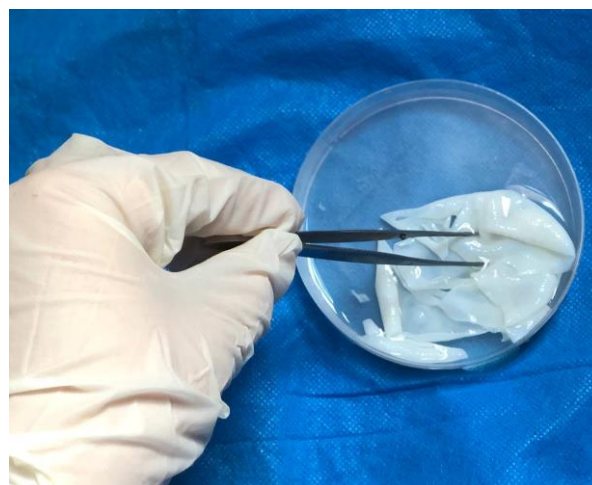


Fig 2. Hydatid cyst germinal membrane

B. Collect the Protoscolices

The Smyth method [7]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 Then an external incision was made of the cyst using clean, sterile scissors, and the cyst was washed. Enter 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 sedimented. After removing the supernatant liquid a little of what remains is placed in Eppendorf tubes and then placed in a water bath to maintain a suitable temperature for the continuation of the life of the primary heads.

C. Appreciating the vitality of the primary principals

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, then the dead heads were counted. They were colored red as a result of the dye penetrating their walls, while the live heads were green. (The vitality rate of the heads was calculated for three replicates [8]

D. Plant collection and preparation of essential oils

The essential oils were isolated from the Leaves of Eucalyptus. From dried parts 250g was mixed with D.W(1.2L) and boiled in Cleavenger. The plant material with D.W (1.2L) was boiled for 3h; the essential oil was kept at 4° C until used [9].

III. RESULTS AND DISCUSSION

Three concentrations of volatile oil of Eucalyptus were used and the highest concentration was 10,000 ppm, followed by 5,000 and then 2,500. Percentages were calculated by counting live and dead heads in a volume of 5 µl of sample and for three replicates, dead pods stain red when treated with 0.1 % eosin is aqueous Fig.3” , due to the penetration of the dye into their membranes, while the live pods remain green “Fig.3”.

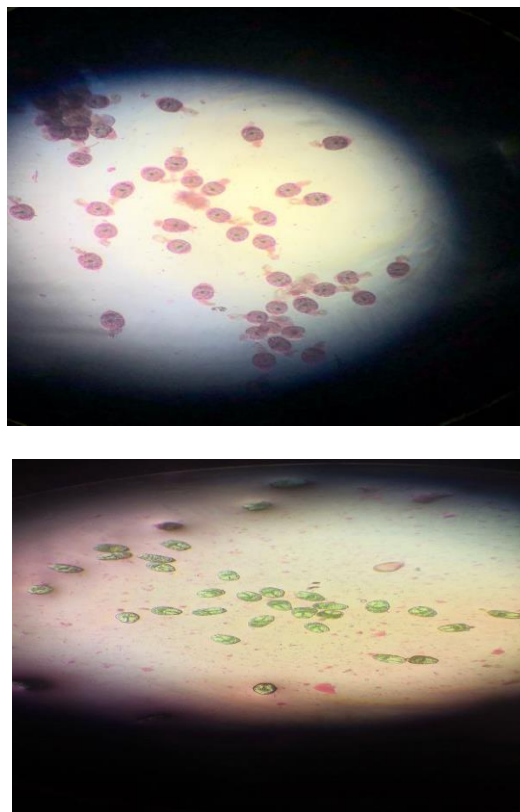


Fig 3. Microscopic view showing the viable bright (green) and dead protoscolices (red).

The concentration of 10,000 ppm had a highly significant effect on the vitality of the primary coliforms, as it killed 100% when the treatment was added directly, and this period increases with decreasing concentration, as it took 30 minutes at a concentration of 5000, while it took 5 hours at a concentration of 2500 (Figure 4). Note that the percentage of live protozoa in the untreated sample remained constant when counted after 10 hours

The concentration	Percentage of loss for each time period						
	Immediately after the transaction	After 15 minutes	After 30 minutes	After 1 hour	After 2 hours	After 3 hours	After 4 hours
10000	100	100	100	100	100	100	100
5000	0.00	46.20	100	100	100	100	100
2500	0.00	11.30	16.31	20.50	23.30	27.50	38.60
1250	0.00	11.30	13.30	15.70	27.70	35.50	40.30
Mortality rate for each time period	40.00	53.76	65.92	67.24	70.20	72.60	75.70
L.S.D	The concentration			Time period			
	0.2629			0.3899			

Figure 4. Effect of Eucalyptus essential oil concentrations on vitality of Protoscolices at 37°C.

The vital activity of the volatile oil resulting from the active components present in a combination such as β-damascone, damascenone, and β-ionone, which is obtained from the decomposition of carotene [10]. in addition to multiple minerals such as phosphorus, calcium, sodium, potassium, magnesium, iron, manganese, boron and zinc, as well as

phenolic compounds that are present in large quantities, as it shows a wide range of pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, anti-cancer, free radical removers and antidepressants [11]. as well as many other components that have the ability to penetrate the cell membranes of the parasite and make changes in its physiological state, which leads to its death, especially in high concentrations. Since penetration of eosin dye is a physical process, so any physiological defect that occurs for whatever reason, the permeability increases allowing the dye to enter, while the live protuberances continue to show their usual green color [12]. The lethal effect of some essential oils results from the effect it causes on the mitochondrial membrane. The use of TMRE dye, which accumulates on the mitochondria of healthy cells, showed the extent of the deterioration of their membrane after treatment with essential oils [13]. Eucalyptus oil, specifically Eucalyptus globulus essential oil, has been shown to be highly effective against cysts. Studies have shown that eucalyptus essential oil has powerful effects in killing the protozoa of hydrocysts, leading to a high mortality rate [14]. In addition, eucalyptus oil has been found to reduce the effectiveness of Echinococcus granulosus, indicating its effectiveness in combating hydatidiform disease [15]. Moreover, eucalyptus oil showed promising antiparasitic activity against Giardia lamblia cysts and Entamoeba histolytica trophozoites, indicating its potential as an antiprotozoal drug [16].

IV. CONCLUSION AND FUTURE

From this we conclude that the essential oil of the *Eucalyptus* plant has an effective effect on the vitality of primary shoots and its effect is stronger than the effect of some chemical drugs and plant extracts. Therefore, we call for repeating the clinical trial and determining 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 aspect of patients suffering from hydatid cysts involves the use of biologically active components of *Eucalyptus* plants to control the growth of the biota of the parasite *Echinococcus granulosus* in the laboratory.

References

1. Wang, H., Yu, Q., Wang, M., Hou, J., Wang, M., Kang, X., ... & Zhang, C. (2023). Hepatic macrophages play critical roles in the establishment and growth of hydatid cysts in the liver during *Echinococcus granulosus sensu stricto* infection. *PLoS Neglected Tropical Diseases*, 17(11), e0011746.
2. 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 .
3. Athmar, K. A. A. & Ban-Abbas, A. M. (2014). Immunization mice with DNA from protoscolices of human hydatid cyst. *Immunological study. International Journal Advanced Biology Research* . 4(1): 89- 95.
4. R. Morar, and C. Feldman, "Pulmonary echinococcosis." *European Respiratory Journal* 21.6 (2003): 1069-1077.
5. 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.
6. Andrade Neto, V. V., Cunha Junior, E. F., Faioes, V. D. S., Martins, T. P., Silva, R. L., Leon, L. L., & Santos, E. C. T. (2018). Leishmaniasis treatment: update of possibilities for drug repurposing.
7. McManus, D. P. (2013). Current status of the genetics and molecular taxonomy of *Echinococcus* species. *Parasitology*, 140(13), 1617-1623.
8. Landa-Garacia J I, Alonso E, Gonzalez-Uriarte J and RoderiguesRomano D (1997) Evaluation of scolicidal agents in experimentalhydatid disease model. *Eur. Sur. Res.* 29, 202-208.
9. 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.
10. F. C. Huang, G. Horváth, P. Molnár, E. Turcsi, J. Deli, J. Schrader, ... and W. Schwab, "Substrate promiscuity of RdCCD1, a carotenoid cleavage oxygenase from *Rosa damascena*." *Phytochemistry* 70.4 (2009): 457-464.
11. F. C. Huang, G. Horváth, P. Molnár, E. Turcsi, J. Deli, J. Schrader, ... and W. Schwab, "Substrate promiscuity of RdCCD1, a carotenoid cleavage oxygenase from *Rosa damascena*." *Phytochemistry* 70.4 (2009): 457-464.
12. T. Hongratanaworakit, "Relaxing effect of rose oil on humans." *Natural product communications* 4.2 (2009): 1934578X0900400226.
13. A. B. Dehkordi, B. Sanei, M. Yousefi, S. M. Sharafi, F. Safarnezhad, R. Jafari, and H. Y. Darani "Albendazole and treatment of hydatid cyst: review of the literature." *Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders)* 19.2 (2019): 101-104.
14. Ahmad, A. A., Maurice, M. N., Monib, M. E. S. M., Soliman, M., Al-Thagfan, S. S., & Huseein, E. A. M. (2023). Eugenol essential oil and nanoemulsion as antihydatic agents with antifibrotic and immunomodulatory effects in cystic echinococcosis. *Tropical Medicine and Infectious Disease*, 8(5), 253.
15. Maurice, M. N., Huseein, E. A. M., Monib, M. E. S. M., Alsharif, F. M., Namazi, N. I., & Ahmad, A. A. (2021). Evaluation of the scolicidal activities of eugenol essential oil and its nanoemulsion against protoscolices of hydatid cysts. *Plos one*, 16(11), e0259290.
16. Hosseini, S. V., Al-Qanbar, M. H., Khazraei, H., Khodaei, S., Mokhtari, M., & Iranpour, P. (2020). Evaluation the effects of Eucalyptus essential oil and hypertonic saline as scolicidal agents in induction of sclerosing cholangitis in rabbits. *Advanced Biomedical Research*, 9(1), 9.