Chapter

Natural Products as Therapeutic Option for Echinococcossis

Yaw Duah Boakye, Doreen Kwankyewaa Adjei, Kofi Oduro Yeboah, Daniel Obeng Mensah, Newman Osafo, Theresah Appiah Agana, Vivian Etsiapa Boamah and Christian Agyare

Abstract

Until the 1980s surgery remained the only treatment option for cystic echinococcosis, a neglected tropical disease caused by infection with tapeworms of the genus *Echinococcoccus*. Following the development of the benzmidazoles, there has been an increase in the use of chemotherapy over the years, especially as an adjunct to surgery or in the management of inoperable cysts. In spite of their usefulness, both surgery and chemotherapy are associated with significant limitations that warrants the search for or consideration of alternative treatment options such natural products. This chapter aims to discuss the scolicidal activity of different species of medicinal plants and their active metabolites in the treatment of echinococcosis. Excerpta Medica Database, Google Scholar, PubMed Central and Scopus were electronic databases used to retrieve the relevant literature. Medicinal plants used commonly and effectively against protoscoleces were *Zataria multiflora*, *Nigella sativa*, *Berberis vulgaris*, *Zingiber officinale*, and *Allium sativum*. Only *Z. multiflora* and *A. sativum* were shown to effective against *Echinococcus granulosus* protoscoleces *in vivo*. In addition, these natural products have not been associated with any significant adverse effect. In animal models Thus, natural products with demonstrated activity against *E. granulosus* may serve as alternative therapy in the management of echinococcosis.

Keywords: cystic echinococcosis, natural products, benzmidazoles, medicinal plants, toxicity

1. Introduction

Helminths are generally classified into two main phyla: Platyhelminthes (cestodes and trematodes) and Nematodes [1]. A third of the 3 billion people living in low socio-economic conditions in the developing countries of the Americas, Asia, and sub-Saharan Africa are infected with one or more helminthes. Helminthic parasitic infections are regarded as neglected tropical diseases because less than 1% of global research funding is allocated to these infections or diseases [2].

The etiological agent of cystic echinococcosis (CE)/hydatid disease, a neglected tropical disease with a global prevalence, is the cestode, *Echinococcus granulosus* sensu lato (s.l) (*E. granulosus*), a tapeworm of the family, Taenidae [3]. Globally, 1 to 3.6 million disability-adjusted life years (DALYs) are caused by human CE infections; with the majority of these cases living in low- and middle-income countries [4]. In China, South America, Europe, Australia, and Africa, CE raises a serious economic and public health concern. Moreover, infestations with CE result in great losses to the livestock industry (about $3 billion every year) through reduced milk supply, lower fertility, increased mortality, weight loss as well as morbidity and mortality in humans [1, 5].

Canids, such as dogs, wolves, foxes, and jackals serve as the infection's primary hosts in the home environment, with a wide range of other herbivores including sheep, goats, water buffalo, and cattle serving as intermediate hosts [5, 6]. Through the consumption of pasture grass contaminated with *E. granulosus* eggs released by infected dogs, intermediate hosts also get infected. The cycle is then completed when definitive hosts consume cysts (metacestodes) found in various organs (such as the liver, lungs, spleen, and heart) of infected intermediate hosts, notably sheep and goats. Ingestion of *E. granulosus* eggs accidentally from contaminated soil, water, and vegetables results in human infection. Humans are therefore regarded as the "accidental intermediate hosts". Humans typically develop fluid-filled hydatid cysts in the liver and lungs, with less frequency occurring in the abdominal cavity, muscle, heart, bone, and nervous system. Due to risky practices including sharing a home with unrestrained dogs, having no regulations governing the killing of animals, and living in unhygienic settings, socio-economic and cultural determinants have a significant influence in the transfer of illnesses to people [7].

Clinical signs only appear when the cyst puts pressure on the nearby tissues or organs or when they rupture, even though the infection may go years without showing any symptoms. Depending on the development and location of the cyst, the infection might constitute a major health risk to people. Ultrasound and, to a lesser extent, serology are the primary imaging methods used to diagnose CE [2, 3]. The size, location, and quantity of hydatid cysts determine the best treatment plan. Currently, anthelmintics, surgery, and percutaneous aspiration are the only treatments available for CE. The chemical medications used to treat human hydatid cysts are albendazole and mebendazole. In order to treat the disease, these medications are frequently used at high doses, which might have negative effects on the liver and other organs [1].

2. Diagnosis of cystic echinococcosis

Currently, diagnosis of CE is mostly performed by means of imaging techniques comprising magnetic resonance imaging (MRI), ultrasonography, computed tomography (CT) scan and/or conventional chest radiography [8]. These methods are indispensable, enabling the easy establishment of the specific stage of the hydatids and also the localization. For instance, the WHO Informal Working Group on Echinococcosis (WHO-IWGE) has issued ultrasonography standardized classification of stage-specific cystic images for the diagnosis and management of CE [9]. Although either of these imaging techniques are useful, MRI is preferred over CT due to better visualization of liquid areas within the matrix [10]. Tumors and infectious lesions are, however, considered for differential diagnosis [8].

As confirmatory test, serological analyses are used to support the findings of the imaging techniques. These tests may also be used as screening or for follow-up monitoring after CE diagnosis [11]. These serological methods are based on the detection of specific IgG antibodies produced against *E. granulosus*. Currently, the main immunological methods for the diagnosis of CE and follow-up in patients with the disease are enzyme-linked immunosorbent assays (ELISAs) and immunoblotting (IB). ELISAs are used as a screening test whereas IB is employed as a confirmatory test due to its higher specificity and sensitivity when compared to other assays [11]. Other serological methods that have been used in the diagnosis of human CE include immunofluorescence assay, indirect hemagglutination assay, immunochromatographic test and dot immunogold filtration assay. However, these tests are associated with lower sensitivity and specificity, thus are used less [12].

Given the pitfalls associated with radiological and immunological techniques, interest in the use of recombinant proteins and synthetic peptides have increased [9]. These molecular diagnosis or DNA-based analysis are very useful in the diagnosis of CE because they offer a wider and complete diagnostic picture of CE patients [8]. DNA probes for Southern hybridization tests and polymerase chain reactions are very helpful in confirming diagnosis of CE. Moreover, PCR has high sensitivity and specificity for the pathogen's DNA, thus allows for precise determination of infection status and identification of genus, species, and genotype [12]. As such, PCR is the foremost molecular analysis in the diagnosis of human CE.

3. Current treatment protocols

Treatment of CE depends on stage of the disease, size and location of the cyst, and complications that may be associated with the cysts. Currently, four treatment modalities are employed for the clinical management of CE. These modalities include surgery, chemotherapy with synthetic drugs and puncture aspiration injection and reaspiration [2]. However, for clinically silent and inactive cysts, active surveillance is the preferred intervention [13]. In this section, we focus on the strengths and limitations associated with current pharmacological and non-pharmacological management of CE.

3.1 Surgery

Until the 1980s surgery remained the only treatment option for CE [2]. Although other treatment modalities have been made available over the past few decades, surgery remains the treatment of choice for most cases of hydatid hepatic cysts [14]. Surgical intervention enables complete eradication of the parasite, treatment or prevention of complications, and avoidance of relapse. According to WHO-IWGE, treatment strategy of the disease must be based on the cyst stage. Accordingly, surgery is indicated in patients with cysts greater than 10 cm or with stages 2 or 3b CE that is with daughter cysts. Patients with other cysts that do not satisfy these criteria may also require immediate surgical treatment. These include infected cysts, superficial cysts with a higher risk of rupture and cysts communicating with the biliary tree [15].

Owing to its satisfactory outcomes, surgery is considered the preferred treatment modality for CE patients with large and complicated cysts [16]. Nonetheless, benzimidazole must be administered to sterilize cyst content prior to surgical treatment in order to prevent dissemination or anaphylaxis [14]. In addition, scolicidal solutions

must be used to eradicate protoscolices of the parasite that may be present within the content of the cyst. Such scolicidal solutions may include silver nitrate, hypertonic saline, povidone iodine, hydrogen peroxide and the anthelminthic albendazole which can be used alone or in combination.

In surgical management of CE, cysts that lie deep, in close proximity to large vessels, and contain multiple daughter cells or calcified cysts must be treated with open surgery [14]. In contrast, laparoscopic surgery is indicated for superficial cysts located on the anterior side of the liver. If open surgery is indicated, the operative site is scrupulously packed and a variety of conservative and radical operative techniques are employed [14].

3.1.1 Conservative operations

In conservative surgical procedures used in the management of hydatid cyst, only the parasitic cyst contents are removed. Pericystic membranes are retained and procedures such as capitonnage, omentoplasty and external drainage are used to manage the residual cavity. The modified Aydin technique has also been used in the management of giant pulmonary hydatid cyst. This technique is advantageous since it avoids major capitonnage complications [17]. In these conservative procedures, the cyst is exposed safely and the pericystic area and operating field are covered with scolicide-soaked pads. Thus, preventing the spillage of parasite-containing contents into surrounding tissues and peritoneal cavity. Subsequently, the cyst is punctured and as much fluid as possible is aspirated following which the scolicide is instilled into the cyst. This is to prevent dilution of the scolicidal agent after introduction into the cyst [18].

The scolicidal agent is allowed to remain in the cyst cavity for a period of 5–15 min after which it is aspirated, and the cyst is unroofed. In the case of hepatic hydatid cyst, cyst contents including germinative membrane and daughter cysts, are evacuated and the surgeon carefully explores the cavity for any gross communication with the biliary tract. At the same time, the surgeon explores the presence of any exogenous cyst that may be embedded in the wall [18]. Following this, external or internal drainage, capitonnage, omentoplasty, marsupialization, and introflexion can be used to manage the residual cavity [14]. These may give rise to the Mabit procedure where omentoplasty and external drainage is used to extract the parasite from the cavity, or Posadas procedure which employs capitonnage, i.e., the surgical closure of the cyst cavity via the application of sutures so as to cause approximation of the opposition surfaces. In all, conservative surgery is easy, safe, and rapid, but has significant limitations such as high morbidity and recurrence rates that sometimes necessitates the choice of radical operations [14].

3.1.2 Radical surgery

In recent times, the use of conservative surgical procedures has become more acceptable among surgeons [19]. However, invasive surgery is sometimes still needed to eradicate parasitic infection in patients with complicated hepatic cysts and also in patients who do not respond to anthelmintic therapy [16]. In contrast to conservative techniques, radical techniques used in hepatic infections can include cystectomy and may involve removal of the germinative layer by non-anatomical liver resection.

With the aim of eradication or elimination of local relapse or complications due to false orbiting, radical surgeries remove the cyst along with the pericystic membrane

and parasitic contents. The procedure may also involve liver resection if indicated [14]. In the treatment of hepatic cysts with radical surgery, procedures such as partial pericystectomy, subadventitial cystectomy, and hepatic resection may be used. Either procedure is associated with its own advantages and limitations. To illustrate, subadventitial cystectomy is not suitable for patients with cysts located near vital vessels of the liver or bile ducts. Hepatic resection on the other hand is time-intensive, nonetheless associated with a low rate of cyst recurrence. Although, recurrence rate is lower in subadventitial cystectomy and hepatic resection, the former is associated with less injury to healthy liver tissue than hepatic resection. In contrast to hepatic resection, pericystectomy and partial pericystectomy are easy to perform, less time-invasive and associated with little blood loss [14].

Regardless of the choice of procedure, depending on the cyst location, effectiveness and safety, radical surgery aims at a common goal, that is, the residual cavity must always be treated with excellent care [20]. This is crucial in preventing biliary leakage, biliary fistula, and abscess formation. Radical surgical approaches are associated with a low risk of postoperative complications, fewer relapse cases, long postoperative hospitalization, and low mortality rates; they are all operations with a high difficulty level mostly suitable for highly specialized liver surgeons. Owing to its low risk of postoperative complications, relapse and low mortality rates, radical surgery is considered superior to conservative surgery [21].

In spite of the low morbidity and mortality associated with radical surgeries, these procedures might not be applicable in all cases [22]. Thus, influencing the introduction of less harmful and more accurate treatment options such as chemotherapy.

3.2 Chemotherapy

According to the WHO and the World Organization for Animal Health's Manual on Echinococcosis in Humans and Animals, chemotherapy is indicated for inoperable cysts, cysts in multi organs, and for pre-emptive treatment of secondary echinococcosis. In contrast, the use of chemotherapy-alone is contraindicated in early and late pregnancy, and patients with inactive cysts or cysts with a greater risk of rupturing [23]. Although, chemotherapy has been indicated for inoperable cysts, evidence from several studies conducted over the past few decades, mainly case series, suggest that chemotherapy could be an alternative to surgery in patients with uncomplicated cysts [13]. This has resulted in an increased use of chemotherapy over the years.

Given the above, various factors need to be considered prior to the choice of anthelminthic therapy in the treatment of CE. When indicated, patients with inoperable cysts must undergo long-term treatment with benzimidazoles such as albendazole and mebendazole, or the pyrazinoisoquinoline praziquantel [24].

3.2.1 Mebendazole

Mebendazole, chemically known as methyl 5-benzoyl-1H-benzimidazole-2-yl-carbamate, is a broad spectrum anthelmintic used for the treatment of helminth infestations in both humans and animals. Since its development in the 1970s, mebendazole has been useful in the treatment of helminthiasis with varying causative organisms such as CE, ascariasis, trichuriasis and enterobiasis [25]. Recently, the use of mebendazole has largely been replaced with albendazole due to some advantages of the latter. For instance, the poor solubility of mebendazole limits its use in the treatment of CE and other tissue helminthiases. Consequently, the use of mebendazole in

hydatid cyst is obsolete, with albendazole being more preferred due to its better intestinal absorption and lower dosage [26].

3.2.2 Albendazole

Albendazole, a benzimidazole carbamic acid methyl ester, is a broad spectrum anthelmintic used for the treatment of various helminthiases. Since its introduction about four decades ago, the drug has been used for its vermicidal activity in infectious conditions such as CE, toxocariasis, taeniasis, gnathostomiasis, and cysticercosis [27]. By binding to intracellular microtubules, albendazole preferentially inhibits parasite's tubulin polymerization and prevents assembly of microtubules. Consequently, glucose uptake decreases resulting in the depletion of the parasite's glycogen stores [28]. This coupled with degenerative changes in the germinal cell mitochondria and endoplasmic reticulum, and increased lysosomal activity, albendazole decreases production of adenosine triphosphate and induces autolysis. Thus, reduces the survivability of the parasite.

In spite of its use in the medical treatment of CE, albendazole is also a useful adjunctive therapy to percutaneous treatment or surgery in preventing secondary CE. When used as an adjunct, albendazole is initiated at least 4–30 days before surgery, and continued for at least 1 month after surgery or percutaneous procedure [26]. Notwithstanding the usefulness of albendazole in the management of CE, studies have reported some adverse effects associated with its use. In one cohort study involving 35 children with abdominal CE, mild increase in the liver enzymes along with mild leukopenia were observed at daily doses of 10–15 mg/kg for 1 month [29]. Rarely, liver failure, hemolytic anemia and pancytopenia has been reported [25].

3.2.3 Praziquantel

Praziquantel is a broad spectrum anthelmintic that has been in use since 1980. The drug exhibits activity against various helminthic infections of human and veterinary origin. Although the exact mode of vermicidal action is uncertain, praziquantel is believed to cause rapid paralytic muscular contractions by increasing intracellular calcium influx and tegumental disruption. This paralytic action of the drug expels the worms from their primary habitat, after which they undergo degeneration due to tegumental disruption [30].

Although useful in the treatment of CE, praziquantel is not indicated as first-line option. The drug is nonetheless effective in perioperative treatment and in the treatment of bone or disseminated CE [31]. For instance, when used together with albendazole, praziquantel is effective in the preoperative treatment of intra-abdominal hydatidosis [26]. Unlike albendazole, the use of praziquantel is safe in pregnancy.

3.3 Challenges with current treatment protocols

Given the above, current surgical and chemotherapeutic interventions are essential therapeutic tools in the management of CE. However, these treatment strategies may be associated with some challenges that may limit their usefulness in the treatment of CE. For instance, surgical treatment of hepatic hydatid cysts may result in major complications such as cholestatic jaundice. Rupturing of cyst into the biliary tree adjacent structures, or the peritoneum during surgery may also result in secondary

infection, sepsis and anaphylaxis [14]. Postoperative hemorrhage, incisional fistulae, cholangitis, surgical site infection, pneumonia and pulmonary embolism are all major complications that have been reportedly observed following surgery. Moreover, spillage of cyst contents during removal and incomplete removal of the endocyst increases the risk of recurrence of the disease. The risk of local and secondary disease recurrence may also be increased by exophytic cyst development that surgeons fail to notice during surgical interventions [32].

Similarly, the use of benzimidazoles is also associated with significant drawbacks, albeit improves life-expectancy in patients with CE [24]. Specifically, the use of current chemotherapeutic agents can reduce cyst size but months of therapy may be required [23]. This may be explained in part by the poor oral absorption and the reduced oral bioavailability of these drugs. As a result, recent studies have suggested developing new formulations such as nanocrystals and liposome formulations to enhance oral absorption and bioavailability, and reduce duration of therapy [33, 34]. Not only is chemotherapy limited by its long course in the treatment of CE, but this treatment approach is also not effective against all stages of cyst development. Benzimidazoles may get diluted in large cysts with size greater than 10 cm, hence less effective against such large cysts. In addition, treatment failure and disease recurrence are more common when chemotherapeutic agents are used in treatment of CE involving multiple, or complicated cysts surrounded by thickened calcified tissue layers [10, 35].

Albeit the relevance of current treatment protocols cannot be overstated, these treatment approaches are associated with significant limitations that warrants the search for or consideration of alternative treatment options. These alternative options include natural products such as monoterpenes, taxanes, isoflavonoids and plant extracts which have been shown to be effective in the management of CE.

4. Natural products with reported activity against Echinococcus

For contemporary systems of herbal and natural drug development, medicinal plants with dependable therapeutic effects are valuable. The synthesis of more complicated semisynthetic chemical compounds can start with bioactive substances found in plants, which can also be used as a direct source of medicinal or bioactive chemicals [2]. Finally, plants can be utilized as bioactive markers for spectroscopic and chromatographic investigations together with the discovery of new compounds [36]. Isolated chemicals of medicinal plants can lead to the development of new medications. In this chapter, we discuss the medicinal plants, fungi, and isolated chemical compounds shown to have scolicidal activity against the protoscoleces of *E. granulosus*.

4.1 Medicinal plants with reported activity against Echinococcus

In all, 57 species were found to have been employed as echinococcicidal agents in the *in vitro* investigations as a result of our comprehensive review. The most popular extract for killing protoscoleces was *Zataria multiflora*, which was then followed by *Nigella sativa*, *Berberis vulgaris*, *Zingiber officinale*, and *Allium sativum* (**Table 1**).

The *in vitro* research made considerable use of leaves among herbs, methanolic extract among extraction, and herbs among plant forms. In the *in vitro* trials, it was discovered that plants like *Z. multiflora*, *Ferula assafoetida*, and *B. vulgaris* had a better efficiency. At a dosage of 1 mg/mL, *Z. multiflora* eliminated all scoleces in 5 min. At

Botanical name	Extraction method	Part used	Phytochemical component	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	References
Allium noeanum (Reut)	Ethanolic	Leaves	Flavonoid	0.49	0.5	100	[37]
Allium Sativum (Garlic)	Ethanolic/ chloroform	Garlic cloves	Silver nitrate	200	15	17	[38]
Allium sativum (Garlic)	Methanolic	Garlic cloves	Mannitol	50	10	100	[39]
A. sativum (Garlic)	Chloroform extraction	Fresh garlic	N/A	200	1	100	[40]
Artemisia (Wormwood)	Methanolic	NA	N/A	100	15	97.24	[41]
Artemisia sieberi (Wormwood)	Hydrodistillation	Aerial parts	α-Thujone (31.5%)	0.005	120	99.30	[42]
Artemisia sieberi (Wormwood)	Aqueous	As a whole	N/A	50	20	100	[43]
Atriplex halimus (Orache)	Aqueous	Leaves	Phenolic and flavonoids	60	120	99.36	[44]
Berberis vulgaris (Barberry)	Aqueous	Fruit	N/A	4	30	100	[45]
B. vulgaris (Barberry)	Methanolic	Root	Berberine	2	10	100	[46]
Blepharocalyx salicifolius (Kunth)	Aqueous	Leaves	Gallic acid and rutin	200	5	100	[47]
Bunium persicum (Black Caraway)	Hydrodistillation	Seeds	g-terpinene (46.1%), cuminaldehyde (15.5%), r-cymene (6.7%), and limonene (5.9%)	0.0125	10	100	[48]
Cannabis sativa (Hemp)	N/A	Aerial parts	N/A	0.01	10	26.08	[49]
Capparis Spinosa (Caper)	Methanolic	Fruit	Flavonoids, tannins, terpenoids, glycosides and alkaloids	300	20	100	[50]
Cassia fistula (Golden shower)	Ethanolic	Fruits	N/A	100	60	67.74	[51]

Botanical name	Extraction method	Part used	Phytochemical component	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	References
Cinnamomum zeylanicum (Cinnamon)	Hydrodistillation	Bark	Cinnamaldehyde (91.8%), metoxicinamate (1.57%), and _ pinene (1.25%)	0.05	5	100	[52]
Coriandrum sativum (Coriander)	Hydrochloric acid + diethyl ether	Seeds	Phenols	750	10,080	100	[53]
Corylus spp.	Hydro-alcoholic	Seeds	N/A	50	20	98	[54]
Cucurbita moschata (Pumpkin)	Hydroalcoholic	Seeds	N/A	1	60	16	[55]
Curcuma longa (Turmeric)	Ethanolic	As a whole	N/A	30	30	100	[56]
Curcuma longa (Turmeric)	Hydrodistillation	Rhizome	α-turmerone (27.1%), β-turmerone (21.8%), l-phellandrene (8.8%), and ρ-cymene (5.4%)	0.1	5	100	[57]
Curcuma zadoaria (White turmeric)	Hydrodistillation	Rhizome	Pentadecane (29.6%), Delta-3-carene (14.7%), and Cis-cinnamic Acid (8.4%)	0.15	7	100	[58]
Eucalyptus globules (Bluegum)	Aqueous	Leaf	Eucalyptol (79.32%)	10	5760	94	[59]
Eucalyptus globulus (Bluegum)	NA	Leaves	Eucalyptol (79.32%)	5	3	100	[59]
Ferula macrecolea (Koma)	Hydrodistillation	Leaves	Terpinolene (77.72%), n-nonanal (4.47%), and linalool (4.35%)	0.3	10	100	[60]
Lepidium sativum (Garden cress)	Aqueous	Leaves	N/A	100	15	100	
Mallotus philippinensis (Kamala Tree)	Methanolic	Fruit	N/A	20	120	100	[61]
Melaleuca alternifolia (Tea tree)	N/A	Tree oil	Terpinen-4-ol (35.4%), _-terpinene (11%), -terpinene (20.4%) and 1,8-cineole (3.4%)	20	5	90	[62]

Botanical name	Extraction method	Part used	Phytochemical component	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	References
Mentha species (Lamiaceae)	Methanolic	Aerial parts	Phenolic, flavonoid and flavonol	200	10	99.54	[62]
Myrtus communis (True myrtle)	Hydrodistillation	Leaves	α-pinene (24.7%), 1,8-cineole (19.6%), and linalool (12.6%)	0.1	5	100	[63]
Myrtus communis (true myrtle)	Methanolic	Leaves	N/A	100	20	100	[64]
Nectaroscordum tripedale (Sicilian Honey Garlic)	Ethanolic	Leaves	Terpenoids, flavonoids, tannins and fatty acids	50	10	100	[1]
Nigella sativa (Black Cumin)	Hydrodistillation	Seeds	Thymoquinone	10	10	100	[1]
Nigella sativa (Black Cumin)	Methanolic	Seeds	Thymoquinone	50	30	100	[1]
Ocimum bacilicum (Sweet basil)	Methanolic	Leaves	N/A	100	60	24.10	[65]
Olea europaea (Olive)	Aqueous	Leaves	N/A	1	120	96.7	[66]
Olea europaea (Olive)	Ethanolic	Leaves	N/A	150	25	100	[1]
Peganum harmala (Syrian rue)	Ethanolic	Seeds	N/A	62.5	2880	100	[67]
Pelargonium roseum	Hydrodistillation	Leaves	N/A	0.05	60	100	[68]
Pestalotiopsis spp.	Methanolic	Leaves, stems and roots	N/A	30	30	92	[1]
Piper longum (Long pepper)	Methanolic	Fruits	Phenolics, flavonoids, alkaloids, tannins, terpenoids, and glycoside	100	60	100	[1]
Pistacia khinjuk (Khiniuk)	Methanolic	Fruits	Terpenoids, flavonoids, and tannins	100	10	100	[1]

Botanical name	Extraction method	Part used	Phytochemical component	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	References
Poikilacanthus glandulosus (Ariza)	Ethanolic	Branches	Polyphenols and flavonoids	0.01	15	100	[68]
Punica granatum (Pomegranate)	Alcoholic	Stem and root	N/A	9	1440	100	[1]
Rhus coriaria (Sumac)	Methanolic	As a whole	N/A	30	20	98.89	[69]
Ruta graveolens (rue)	Methanolic	Aerial parts	Phenolic (25.53%), flavonoids (6.6%) and tannins (8.0%)	40	720	100	[1]
Salvadora persica (Miswak)	Ethanolic	Root	Indole alkaloids, flavonoids, tropaedoin, triterpenes, phytosterols, and isothiocyanates	50	10	100	[1]
Satureja hortensis (summer savory)	Aqueous	Aerial parts	Carvacrol and -terpinene	1	20	100	[70]
Satureja khuzistanica (Jamzad)	Hydrodistillation	Leaves and flowers	Carvacrol	5	60	100	[71]
Saussurea costus (Costus)	Ethanolic	Root	N/A	250	60	100	[1]
Sideritis perfoliate (Ironwort) 25 60,100	Methanolic	Leaves and flowers	Fumaric acid (260.13 mg/L), syringic acid (27.92 mg/L) and caffeic acid (26.84 mg/L), and a flavonoid, luteolin (11.23 mg/L)	25	60	100	[1]
Silybum marianum (Milk thistle)	Ethanolic	Seeds	Silydianin (14.41%), isosilybin A (10.50%), and silychristin (10.46%)	0.5	60	77	[1]
Taxus baccata (Common yew)	Hydroalcoholic	As a whole	Octane (13.36%), 4-methoxycarbonyl 3,5-diphenyl-1 (8.30%), and 9,12,15-octadecatrienoicacid (10.75%)	150	60	66.60	[72]
Teucrium polium (Felty germander)	Ethanolic	Flowers	N/A	100	50	100	[1]
Thymus vulgaris (Garden thyme)	Hydrodistillation	Leaves	Thymol	0.5	103,680	100	[73]

Botanical name	Extraction method	Part used	Phytochemical component	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	References
Trachyspermum ammi (Ajowan)	Hydrodistillation	Fruits	Thymol	5	10	100	[74]
Zataria multiflora (Shirazi thyme)	Methanolic	Leaves	Carvacrol and thymol	25	1	100	[1]
Zataria multiflora (Shirazi thyme)	Diethyl ether	Essential oil	Thymol (66.9%), carvacrol (15.2%), carvone (7.3%), neo-dihydrocarveol (2%), and 1,8-Cineole (1.6%)	1	5	100	[1]
Zataria multiflora (Shirazi thyme)	Methanolic	Leaves	Carvacrol and thymol	10	10	100	[75]
Zataria multiflora (Shirazi thyme)	Hydrodistillation	Aerial parts	Thymol (41.8%), carvacrol (28.8%), and p-cymene (8.4%)	0.1	10	100	[1]
Zataria spp. (Satar)	Hydrodistillation	Leaves	Carvacrol and thymol	100	1	100	[1]
Zingiber officinale (Ginger)	Methanolic	Rhizome	N/A	100	30	100	[1]
Zingiber officinale (Ginger)	Methanolic	Root	N/A	100	40	100	[1]
Zingiber officinale (Ginger)	Aqueous	As a whole	[6]-gingerol	100	1440	100	[76]
Zingiber officinale (Ginger)	Ethanolic	Rhizomes sheets	N/A	200	30	100	[1]
Ziziphora tenuior (Mint)	Ethanolic	Shoots	Thymol	100	240	40.25	[77]

Table 1.
List of medicinal plants with in vitro activity against protoscoleces of Echinococcus.

dosages of 60 g/mL and 2 mg/mL for 10 min, *F. asafoetida* and *B. vulgaris* were shown to have 100% effectiveness [1, 2].

Two plant species, *Z. multiflora* and *A. sativum*, showed *in vivo* anti-echinococcal activity. In the *in vivo* studies for their validation against *E. granulosus* protoscoleces, the leaf extracts, peels and other parts were tested (**Table 2**) [1].

4.2 Fungi with reported activity against Echinococcus

Characteristic ultrastructural changes were observed when protoscoleces of *E. granulosus* was treated with extract of endophytic fungi *Eupenicillium* and *Pestalotiopsis* sp. isolated from *Azadirachta indica* and *Chaetomium* sp. *Piper longum* plants respectively. *Pestalotiopsis* sp. showed a promising scolicidal activity up to 97% mortality just within 30 min of incubation. In a study comparing commercial chitosan to fungal chitosan isolated from *Penicillium waksmanii* and *Penicillium citrinum*, it was observed that Fungal chitosan was the most bioactive type with higher degree of deacetylation showed stronger scolicidal activity *in vitro* [88].

4.3 Isolated compounds from natural products with reported activity against *E. granulosus*

A total of 8 active chemicals compounds extracted from various medicinal plant are reported to show activity against *E. granulosus*. They are, thymol, carvacrol, menthol, berberine, genistein, thymoquinone, ampelopsin, and gallic acid (**Figure 1**). In an *in vitro* study, thymol, berberine, and thymoquinone showed substantial *in vitro* scolicidal action at concentrations of 0.1, 0.5, and 1 mg/ml after exposure for 5, 10, and 1 min. *In vivo* tests with thymol and carvacrol also showed promising scolicidal efficacy [89, 90].

5. Toxicity and safety profile of the natural products with reported activity against *E. granulosus*

With increased advocacy for the use of natural products in the management of conditions such CE comes the heightened interest in the safety of these natural products. Obviously, alternatives to synthetic protoscolicidal agents are being sought not only because of associated reduced efficacy, increased recurrence rates and increased drug resistance, but also to the incidence of adverse effects [10]. Thus, successful integration of natural products in the treatment of CE will require the establishment of the toxic profile of these natural products. It is to be noted that, the idea that 'natural product' always implies 'safe' is deceptive since these products contain pharmacologically active compounds which may exert detrimental effects at high doses or in specific conditions [91].

Given the above, toxicity assessments have been conducted on some plants and active metabolites with reported activity against *E. granulosus*. For instance, *Z. multiflora* was associated with no significant toxicity in mice [92]. Similarly, essential oil obtained from *C. longa* was not shown in toxicological studies to exert any significant toxicity in NIH mice [93]. *In vivo* toxicity assessments of thymol in mice and golden hamsters also showed no overt toxicity or changes in serum biomarkers such as uric acid and bilirubin [94, 95]. Similarly, berberine at the tested clinical doses was not identified to exert cytotoxic and mutagenic effects [96]. Thymoquinone when

Botanical name (common name)	Extraction method	Part used	Phytochemical component	Experimental animal	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	Ref
Algerian propolis (Propolis)	Ethanolic	N/A	Polyphenol, flavonoid	Mice	25	10	100	[1]
Allium sativum (Garlic)	Methanolic	N/A	N/A	Mice	50	10	100	[78]
A. sativum (Garlic) Mice	Methanolic	Garlic cloves	1% Alliin	Mice	80	43,200	Significant	[79]
Annona squamosa (Sugar apple)	Alcoholic	Leaves	N/A	Rats	100	2880	100	[1]
Artemisia Herba-alba (Wormwood)	Ethanolic	Leaves and flowers	Alkaloids, phenols	Mice	0.28	1440	55.17	[1]
Nigella sativa (Black cumin)	Ionotropic gelation technique	Seed	N/A	Mice	1.14	86,400	100	[1]
Pistacia vera (Pistachio)	Hydrodistillation	Branch	Essential oil	Mice	200	10	100	[80]
Punica granatum (Pomegranate)	Aqueous	Peels	N/A	Mice	16	2880	100	[81]
P. granatum (Pomegranate)	Aqueous	N/A	Peel	Mice	0.65	86,400	66.7	[82]
Sophora moorcroftiana	N/A	seeds	N/A	Mice	0.25	60,480	76.1	[83]
Zataria multiflora	Essential oil and oleic acid	Essential oil	N/A	Mice	20	10	100	[84]
Zataria multiflora (Shirazi thyme)	Diethyl ether	Aerial parts	Gallic acid (1.1618 mg/g), catechin (2.808 mg/g), caffeic acid (5.531 mg/g), and quercetin (9.961 mg/g)	Mice	0.04	43,200	Significant	[85]

Botanical name (common name)	Extraction method	Part used	Phytochemical component	Experimental animal	Concentration (mg/mL)	Exposure time (min)	Scolicidal efficacy (%)	Ref
Zataria multiflora (Shirazi thyme)	Methanolic	Leaves	Thymol (66.9%), carvacrol (15.2%), and carvone (7.3%)	Mice	8	43,200	100	[86]
Zataria multiflora (Shirazi thyme)	Hydrodistillation	Essential oil	Thymol	Mice	2	10	100	[87]
Zingiber officinale (Ginger)	Ethanolic	As a whole	N/A	Mice	150	60	100	[1]

Table 2.
List of medicinal plants with in vivo activity against protoscoleces of Echinococcus granulosus.

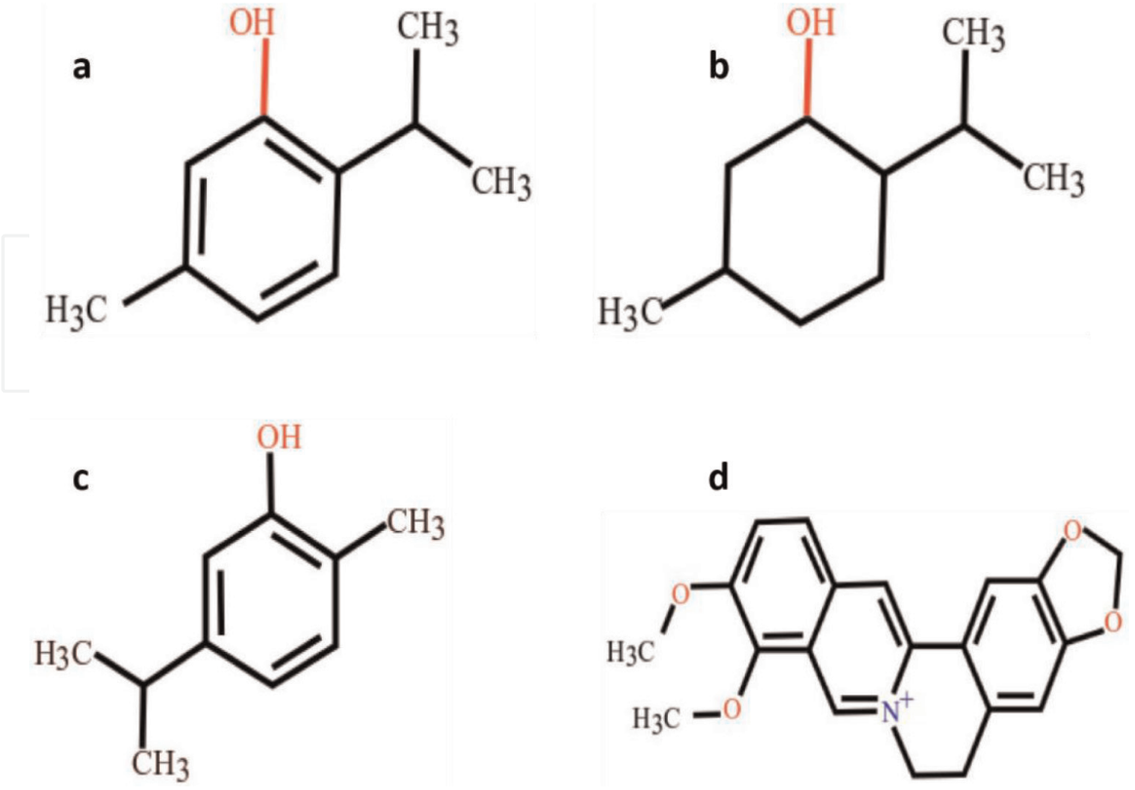

Figure 1.
Active chemicals compounds extracted from various medicinal plant are reported to be active against Echinococcus granulosus*. a: thymol; b: menthol; c: carvacrol; d: berberine.*

assessed for mortality and toxicity in mice, at doses of 0.1, 0.2, 0.3 mg/ml for a period of 3 months was proved to be safe [97].

Albeit toxicity data may not be available on all medicinal plants shown to be effective against *E. granulosus*, available evidence on numerous other plants shows that these natural products may be safely used in the treatment of CE [98, 99].

6. Discussion

Medicinal plants with dependable therapeutic effects are valuable sources of bioactive substances that can be developed into potential lead compounds in the development of drugs for treatment of CE, a neglected tropical disease [2]. Due to the rise in the emergence of resistant species associated with infectious diseases, developing novel and effective drugs is imperative for the continuous survival of the human race. Owing to this, there has been resurgence in the search of natural products that can serve as alternative synthetic agents in the management of diseases. These natural products contain a large variety of secondary metabolites that possess several biological effects including anthelminthic activity [100]. As such, large numbers of natural products have been screened particularly against *E. granulosus* protoscolices with the hope of identifying natural products with prominent scolicidal potential.

One such natural agent with prominent scolicidal activity is *Zataria multiflora*, the most reputable member of the family Lamiaceae [100]. It has been shown that, the essential oils of *Z. multiflora* exert powerful anti-hydatid effect even with short exposure times [101]. This remarkable activity of *Z. multiflora* essential oils has been

attributed to the presence of significant phenolic monoterpenes which contains a hydroxyl group and possess an innate hydrophobic nature. The presence of a hydroxyl group and the hydrophobic nature of phenolic compounds enable *Z. multiflora* essential oils to penetrate cell membranes resulting in the death of the helminth [102].

In eukaryotic cells, phenolic monoterpenoids primarily decrease the integrity of plasma and mitochondrial membranes, resulting in cell death. However, the exact mechanism of action of phenolic monoterpenes in protoscoleces has yet to be determined, albeit it has been shown to penetrate the cell membrane, damage the lipid bilayer and, alter cell permeability. This results in leakage of intracellular components, which lowers the membrane electric potential. This change in the plasma membrane electric potential probably causes leakage of ATP, proteins, potassium and calcium, resulting in membrane damage and cell death [103].

Other natural products such as the medicinal plants *Ferula assafoetida* and *A. sativum*, fungal chitosan, and extracts of endophytic fungi *Eupenicillium* and *Pestalotiopsis* sp. isolated from *Azadirachta indica* and *Piper longum*, respectively have also shown significant scolicidal activity. Essential oils obtained from *Ferula assafoetida* contain disulfide compounds which have been shown to be responsible for their scolicidal action [104].

In vitro and *in vivo* tests have both been used in investigating the pharmacokinetic characteristics and pharmacodynamic effects of target extracts, as well as host immune reaction to these natural products. However, majority of studies that evaluated the protoscolicidal activity of different medicinal plants during the past two decades have utilized *in vitro* assays. Only a few studies involved *in vivo* animal models [1]. More *in vivo* screening of these natural products with effect against CE are needed to develop a complete picture of their efficacy and toxicity in whole organisms. Considering currently available protoscolicidal agents are associated with serious adverse effects, close attention must be paid to the toxicity of these natural products in other to identify suitable alternatives to current management [10]. A good protoscolicidal agent is one that is steady in the cystic contents and possesses the least toxicity [105].

Moreover, owing to the multiplicity of active metabolites found in natural products, the risk of development of drug resistance may also be low. Data from toxicity studies available on these medicinal plants also shows the reduced risk of adverse effects associated with their use. However, additional studies will be desired to prove these outcomes by establishing the toxicity profile for all the other species of plants identified to possess activity against *E. granulosus.* In addition, the exact mechanism by which the extracts and their isolated compounds exert scolicidal activity, and their pharmacokinetic profiles must be well established. Knowledge obtained from these suggested studies should be well synthesized and used to appropriately design randomized controlled trials in human subjects in order to bridge the gap between the bench and the bedside for CE treatment.

7. Conclusion

Cystic echinococcosis is a public health menace, affecting humans and livestock worldwide. Although drug treatment is available for its management, in many cases, existing drugs are insufficiently efficacious, ineffective due to resistance, relative toxic or contraindicated in some populations. Thus, hindering global efforts to eliminate this neglected tropical disease. Interestingly, natural products have demonstrated

significant activity against *E. granulosus*, indicating their potential for use in the treatment of CE. Medicinal plants such as *Zataria multiflora* and *Allium sativum* have been shown to be effective in both *in vitro* and *in vivo* models. In light of the slow development of new anthelminthics over the past few decades due to lack of commercial attractiveness, natural products may serve as alternatives or adjuncts to current treatment approaches. Also, these medicinal plants are rich pools of active metabolites that may serve as drug leads in the development of scolicidal drugs.

Conflict of interest

The authors declare no conflict of interest." or delete this entire section.

Author details

Yaw Duah Boakye[1], Doreen Kwankyewaa Adjei[1], Kofi Oduro Yeboah[2], Daniel Obeng Mensah[3], Newman Osafo[2], Theresah Appiah Agana[1], Vivian Etsiapa Boamah[1] and Christian Agyare[1*]

1 Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

2 Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

3 Department of Pharmaceutical Microbiology, School of Pharmacy and Pharmaceutical Sciences, University of Cape Coast, Cape Coast, Ghana

*Address all correspondence to: cagyare.pharm@knust.edu.gh

References

[1] Alvi MA, Khan S, Ali RMA, Qamar W, Saqib M, Faridi NY, et al. Herbal medicines against hydatid disease: A systematic review (2000–2021). Life. 2022;**12**(5):676

[2] Ali R, Khan S, Khan M, Adnan M, Ali I, Khan TA, et al. A systematic review of medicinal plants used against *Echinococcus granulosus*. PLoS One. 2020; **15**(10):e0240456

[3] Piccoli L, Bazzocchi C, Brunetti E, Mihailescu P, Bandi C, Mastalier B, et al. Molecular characterization of *Echinococcus granulosus* in South-Eastern Romania: Evidence of G1-G3 and G6-G10 complexes in humans. Clinical Microbiology and Infection. 2013;**19**(6): 578-582

[4] Taghipour A, Ghaffarifar F, Horton J, Dalimi A, Sharifi Z. Silybum marianum ethanolic extract: in vitro effects on protoscolices of *Echinococcus granulosus* G1 strain with emphasis on other Iranian medicinal plants. Tropical Medicine and Health. 2021; **49**(1):71

[5] Bosco A, Alves LC, Cociancic P, Amadesi A, Pepe P, Morgoglione ME, et al. Epidemiology and spatial distribution of *Echinococcus granulosus* in sheep and goats slaughtered in a hyperendemic European Mediterranean area. Parasites & Vectors. 2021;**14**(1):421

[6] Heidari Z, Sharbatkhori M, Mobedi I, Mirhendi SH, Nikmanesh B, Sharifdini M, et al. *Echinococcus multilocularis* and *Echinococcus granulosus* in canines in North-Khorasan Province, northeastern Iran, identified using morphology and genetic characterization of mitochondrial DNA. Parasites & Vectors. 2019;**12**(1):606

[7] Calma CL, Neghina AM, Vlaicu B, Neghina R. Cystic echinococcosis in the human population of a western Romanian county, 2004-2010. Clinical Microbiology and Infection. 2011;**17**(11): 1731-1734

[8] Santucciu C, Bonelli P, Peruzzu A, Fancellu A, Marras V, Carta A, et al. Cystic echinococcosis: Clinical, immunological, and biomolecular evaluation of patients from Sardinia (Italy). Pathogens. 2020;**9**(11):907

[9] Manzano-Román R, Sánchez-Ovejero C, Hernández-González A, Casulli A, Siles-Lucas M. Serological diagnosis and follow-up of human cystic echinococcosis: A new hope for the future? BioMed Research International. 2015;**2015**:428205

[10] Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. Acta Tropica. 2010;**114**(1):1-16

[11] Siles-Lucas M, Casulli A, Conraths FJ, Müller N. Laboratory diagnosis of Echinococcus spp. in human patients and infected animals. Advances in Parasitology. 2017;**96**: 159-257

[12] Santucciu C, Masu G, Mura A, Peruzzu A, Piseddu T, Bonelli P, et al. Validation of a one-step PCR assay for the molecular identification of *Echinococcus granulosus* sensu stricto G1-G3 genotype. Molecular Biology Reports. 2019;**46**(2):1747-1755

[13] Stojkovic M, Zwahlen M, Teggi A, Vutova K, Cretu CM, Virdone R, et al. Treatment response of cystic echinococcosis to benzimidazoles: A

systematic review. PLoS Neglected Tropical Diseases. 2009;**3**(9):e524

[14] Angeliki V, Mariana P, Christina C, Dimitris Z. Surgical management of hydatid disease. In: Tonay I, editor. Echinococcosis. Rijeka: IntechOpen; 2017. p. Ch 3

[15] Rinaldi F, Brunetti E, Neumayr A, Maestri M, Goblirsch S, Tamarozzi F. Cystic echinococcosis of the liver: A primer for hepatologists. World Journal of Hepatology. 2014;**6**(5):293-305

[16] Al-Saeedi M, Ramouz A, Khajeh E, El Rafidi A, Ghamarnejad O, Shafiei S, et al. Endocystectomy as a conservative surgical treatment for hepatic cystic echinococcosis: A systematic review with single-arm meta-analysis. PLoS Neglected Tropical Diseases. 2021;**15**(5): e0009365

[17] Aydin Y, Ulas AB, Ince I, Kuran E, Keskin H, Kirimli SN, et al. Modified capitonnage technique for giant pulmonary hydatid cyst surgery. Interactive Cardiovascular and Thoracic Surgery. 2021;**33**(5):721-726

[18] Abdelraouf A, El-Aal AA, Shoeib EY, Attia SS, Hanafy NA, Hassani M, et al. Clinical and serological outcomes with different surgical approaches for human hepatic hydatidosis. Revista da Sociedade Brasileira de Medicina Tropical. 2015;**48**(5):587-593

[19] Patkowski W, Krasnodebski M, Grat M, Masior L, Krawczyk M. Surgical treatment of hepatic *Echinococcus granulosus*. Przeglad Gastroenterologiczny. 2017;**12**(3): 199-202

[20] Chen X, Chen X, Shao Y, Zhao J, Li H, Wen H. Clinical outcome and immune follow-up of different surgical approaches for human cyst hydatid disease in liver. The American Journal of Tropical Medicine and Hygiene. 2014; **91**(4):801-805

[21] Baraket O, Moussa M, Ayed K, Kort B, Bouchoucha S. Predictive factors of morbidity after surgical treatment of hydatid cyst of the liver. Arab Journal of Gastroenterology. 2014;**15**(3–4):119-122

[22] Deo KB, Kumar R, Tiwari G, Kumar H, Verma GR, Singh H. Surgical management of hepatic hydatid cysts - conservative versus radical surgery. HPB: The Official Journal of the International Hepato Pancreato Biliary Association. 2020;**22**(10):1457-1462

[23] Goussard P, Eber E, Mfingwana L, Nel P, Schubert P, Janson J, et al. Paediatric pulmonary echinococcosis: A neglected disease. Paediatric Respiratory Reviews. 2022;**43**:11-23

[24] Lundström-Stadelmann B, Rufener R, Ritler D, Zurbriggen R, Hemphill A. The importance of being parasiticidal … an update on drug development for the treatment of alveolar echinococcosis. Food and Waterborne Parasitology. 2019;**15**: e00040

[25] Hong ST. Albendazole and Praziquantel: Review and safety monitoring in Korea. Infection & Chemotherapy. 2018;**50**(1):1-10

[26] Nazligul Y, Kucukazman M, Akbulut S. Role of chemotherapeutic agents in the management of cystic echinococcosis. International Surgery. 2015;**100**(1):112-114

[27] Albonico M, Levecke B, LoVerde PT, Montresor A, Prichard R, Vercruysse J, et al. Monitoring the efficacy of drugs for neglected tropical diseases controlled by preventive chemotherapy. Journal

of Global Antimicrobial Resistance. 2015; **3**(4):229-236

[28] Verrest L, Dorlo TPC. Lack of clinical pharmacokinetic studies to optimize the treatment of neglected tropical diseases: A systematic review. Clinical Pharmacokinetics. 2017;**56**(6): 583-606

[29] Moroni S, Moscatelli G, Bournissen FG, Gonzalez N, Ballering G, Freilij H, et al. Abdominal cystic echinococcosis treated with Albendazole. A Pediatric Cohort Study. PLoS One. 2016;**11**(9):e0160472

[30] Chai JY. Praziquantel treatment in trematode and cestode infections: An update. Infection & Chemotherapy. 2013;**45**(1):32-43

[31] Nunnari G, Pinzone MR, Gruttadauria S, Celesia BM, Madeddu G, Malaguarnera G, et al. Hepatic echinococcosis: Clinical and therapeutic aspects. World Journal of Gastroenterology. 2012;**18**(13): 1448-1458

[32] Graeter T, Ehing F, Oeztuerk S, Mason RA, Haenle MM, Kratzer W, et al. Hepatobiliary complications of alveolar echinococcosis: A long-term follow-up study. World Journal of Gastroenterology. 2015;**21**(16): 4925-4932

[33] Li H, Song T, Shao Y, Aili T, Ahan A, Wen H. Comparative evaluation of liposomal Albendazole and tablet-Albendazole against hepatic cystic echinococcosis: A non-randomized clinical trial. Medicine (Baltimore). 2016;**95**(4):e2237

[34] Pensel P, Paredes A, Albani CM, Allemandi D, Sanchez Bruni S, Palma SD, et al. Albendazole nanocrystals in experimental alveolar echinococcosis: Enhanced chemoprophylactic and clinical efficacy in infected mice. Veterinary Parasitology. 2018;**251**:78-84

[35] Moro P, Schantz PM. Echinococcosis: A review. International Journal of Infectious Diseases. 2009;**13**(2):125-133

[36] Akshada Amit K, Rajendra Chandrashekar D, Chandrakant SM. Natural products in drug discovery. In: Shagufta P, Areej A-T, editors. Pharmacognosy. Rijeka: IntechOpen; 2019. p. Ch. 14

[37] Salemi Z, Goudarzi M, Hajihossein R, Noori M, Babaei S, Eslamirad Z. Evaluation of the apoptotic and scolicidal effects of crude and flavonoid extracts of allium noeanum on protoscolices and hydatid cyst wall. Jundishapur Journal of Natural Pharmaceutical Products. 2021;**16**(2): e96180

[38] Sadjjadi SM, Zoharizadeh MR, Panjeshahin MR. In vitro screening of different *Allium sativum* extracts on hydatid cysts protoscoleces. Journal of Investigative Surgery. 2008;**21**(6): 318-322

[39] Moazeni M, Nazer A. In vitro effectiveness of garlic (*Allium sativum*) extract on scolices of hydatid cyst. World Journal of Surgery. 2010;**34**(11): 2677-2681

[40] Barzin Z, Sadjjadi SM, Panjehshahin MR. Protoscolicidal effects of the garlic chloroformic extract on the protoscolices of hydatid cyst at a short exposure time, up to five minutes. Iranian Journal of Medical Sciences. 2019;**44**(1):28-34

[41] Faizi F, Parandin F, Mo ni S, Rezaee N, Sardar M, Roushan A, et al. Scolicidal effects of mixture of

Artemisia, Eucalyptus and ginger extracts on hydatid cyst Protoscolices. Journal of Mazandaran University of Medical Sciences. 2018; **27**(157):83-91

[42] Hosseini MJ, Yousefi MR, Abouhosseini M. Comparison of the effect of Artemisia Sieberi essential oil and Albendazole drug on Protoscolices of hydatid cyst under in vitro conditions. Babol-Jbums. 2017;**19**(12):63-68

[43] Vakili Z, Radfar MH, Bakhshaei F, Sakhaee E. In vitro effects of *Artemisia sieberi* on *Echinococcus granulosus* protoscolices. Experimental Parasitology. 2019;**197**:65-67

[44] Bouaziz S, Amri M, Taibi N, Zeghir-Bouteldja R, Benkhaled A, Mezioug D, et al. Protoscolicidal activity of Atriplex halimus leaves extract against *Echinococcus granulosus* protoscoleces. Experimental Parasitology. 2021;**229**: 108155

[45] Rouhani S, Salehi N, Kamalinejad M, Zayeri F. Efficacy of Berberis vulgaris aqueous extract on viability of *Echinococcus granulosus* protoscolices. Journal of Investigative Surgery. 2013; **26**(6):347-351

[46] Mahmoudvand H, Saedi Dezaki E, Sharififar F, Ezatpour B, Jahanbakhsh S, Fasihi HM. Protoscolecidal effect of *Berberis vulgaris* root extract and its main compound, berberine in cystic echinococcosis. Iranian Journal of Parasitology. 2014;**9**(4):503-510

[47] Noal CB, Monteiro DU, Brum TF, Emmanouilidis J, Zanette RA, Morel AF, et al. In vitro effects of *Blepharocalyx salicifolius* (H.B.K.) O. berg on the viability of *Echinococcus ortleppi* protoscoleces. Revista do Instituto de Medicina Tropical de São Paulo. 2017;**59**: e42

[48] Mahmoudvand H, Tavakoli Oliaei R, Mirbadie SR, Kheirandish F, Tavakoli Kareshk A, Ezatpour B, et al. Efficacy and safety of Bunium Persicum (Boiss) to inactivate protoscoleces during hydatid cyst operations. Surgical Infections. 2016;**17**(6):713-719

[49] Youssefi AR, Youssefi MR, Abouhosseini TM. Comparison of the in vitro effect of *Cannabis sativa* essential oil with Albendazole on Protoscolices of hydatid cyst. Journal of Gorgan University of Medical Sciences. 2020; **21**(4):107-113

[50] Mahmoudvand H, Khalaf AK, Beyranvand M. In vitro and ex vivo evaluation of *Capparis spinosa* extract to inactivate Protoscoleces during hydatid cyst surgery. Current Drug Discovery Technologies. 2021;**18**(5): e18082020185049

[51] Sarvestani A, Karimian A, Mohammadi R, Cheraghipour K, Zivdri M, Nourmohammadi M, et al. Scolicidal effects of *Cassia fistula* and *Urtica dioica* extracts on protoscoleces of hydatid cysts. Journal of Parasitic Diseases. 2021;**45**(1):59-64

[52] Mahmoudvand H, Mahmoudvand H, Oliaee RT, Kareshk AT, Mirbadie SR, Aflatoonian MR. In vitro protoscolicidal effects of *Cinnamomum zeylanicum* essential oil and its toxicity in mice. Pharmacognosy Magazine. 2017;**13** (Suppl 3):S652-S6S7

[53] Dawwas A. Investigation of biochemical effect of phenols extract isolated from *Coriandrum sativum* seeds against *Echinococcus granulosus* parasite in vitro. University of Thi-Qar Journal of Science. 2019;**1**(1):2-9

[54] Eskandarian AA. Scolicidal effects of squash (Corylus spp) seeds, hazel (Curcurbia spp) nut and garlic (*Allium*

sativum) extracts on hydatid cyst protoscolices. Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences. 2012;**17**(11):1011-1014

[55] Hesari Z, Sharifdini M, Sharifi-Yazdi MK, Ghafari S, Ghasemi S, Mahmoudi S, et al. In vitro effects of pumpkin (*Cucurbita moschata*) seed extracts on *Echinococcus granulosus* Protoscoleces. Iranian Journal of Parasitology. 2020; **15**(1):76-83

[56] Almalki E, Al-Shaebi EM, Al-Quarishy S, El-Matbouli M, Abdel-Baki AS. In vitro effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on Echinococcus protoscoleces. Saudi Journal of Biological Sciences. 2017; **24**(1):90-94

[57] Mahmoudvand H, Pakravanan M, Aflatoonian MR, Khalaf AK, Niazi M, Mirbadie SR, et al. Efficacy and safety of *Curcuma longa* essential oil to inactivate hydatid cyst dprotoscoleces. BMC Complementary and Alternative Medicine. 2019;**19**(1):187

[58] Mahmoudvand H, Pakravanan M, Kheirandish F, Jahanbakhsh S, Sepahvand M, Niazi M, et al. Efficacy and safety *Curcuma zadoari*a L. to inactivate the hydatid cyst Protoscoleces. Current Clinical Pharmacology. 2020; **15**(1):64-71

[59] Moazeni M, Hosseini SV, Al-Qanbar MH, Alavi AM, Khazraei H. In vitro evaluation of the protoscolicidal effect of *Eucalyptus globulus* essential oil on protoscolices of hydatid cyst compared with hypertonic saline, povidone iodine and silver nitrate. Journal of Visceral Surgery. 2019;**156**(4):291-295

[60] Alyousif MS, Al-Abodi HR, Almohammed H, Alanazi AD, Mahmoudvand H, Shalamzari MH, et al. Chemical composition, apoptotic activity, and antiparasitic effects of *Ferula macrecolea* essential oil against *Echinococcus granulosus* Protoscoleces. Molecules. 2021;**26**(4):888

[61] Gangwar M, Verma VC, Singh TD, Singh SK, Goel RK, Nath G. In-vitro scolicidal activity of Mallotus philippinensis (Lam.) Muell Arg. fruit glandular hair extract against hydatid cyst *Echinococcus granulosus*. Asian Pacific Journal of Tropical Medicine. 2013;**6**(8):595-601

[62] Monteiro DU, Azevedo MI, Weiblen C, Botton SDEA, Funk NL, Silva CDEBDA, et al. In vitro and ex vivo activity of *Melaleuca alternifolia* against protoscoleces of *Echinococcus ortleppi*. Parasitology. 2017;**144**(2):214-219

[63] Mahmoudvand H, Fallahi S, Mahmoudvand H, Shakibaie M, Harandi MF, Dezaki ES. Efficacy of *Myrtus communis* L. to inactivate the hydatid cyst Protoscoleces. Journal of Investigative Surgery. 2016;**29**(3): 137-143

[64] Amiri K, Nasibi S, Mehrabani M, Nematollahi MH, Harandi MF. In vitro evaluation on the scolicidal effect of *Myrtus communis* L. and *Tripleurospermum disciforme* L. methanolic extracts. Experimental Parasitology. 2019;**199**:111-115

[65] Haghani A, Roozitalab A, Safi SN. Low scolicidal effect of *Ocimum bacilicum* and *Allium cepa* on protoccoleces of hydatid cyst: An in vitro study. Comparative Clinical Pathology. 2013;**23**:847-853

[66] Zibaei M, Sarlak A, Delfan B, Ezatpour B, Azargoon A. Scolicidal effects of *Olea europaea* and *Satureja khuzestanica* extracts on protoscolices of

hydatid cysts. The Korean Journal of Parasitology. 2012;**50**(1):53-56

[67] Hammoshi MH, Shareef AY, Younis GTH. Effect of ethanolic extract and crude alkaloides of *Peganum harmala* seeds on the viability of *Echinococcus granulosus* protoscolices in vitro. Rafidain Journal of Science. 2005;**16**(9):1-8

[68] Tabari MA, Youssefi MR, Nasiri M, Hamidi M, Kiani K, Alian Samakkhah S, et al. Towards green drugs against cestodes: Effectiveness of *Pelargonium roseum* and *Ferula gummosa* essential oils and their main component on *Echinococcus granulosus* protoscoleces. Veterinary Parasitology. 2019;**266**:84-87

[69] Moazeni M, Mohseni M. Sumac (*Rhus coriaria* L.): Scolicidal activity on hydatid cyst Protoscolices. Surgical Science. 2012;**03**:452-456

[70] Abdel-Baki AA, Almalki E, Mansour L, Al-Quarishy S. In vitro Scolicidal effects of *Salvadora persica* root extract against protoscolices of *Echinococcus granulosus*. The Korean Journal of Parasitology. 2016;**54**(1):61-66

[71] Moazeni M, Saharkhiz MJ, Hoseini AA, Alavi AM. In vitro scolicidal effect of *Satureja khuzistanica* (Jamzad) essential oil. Asian Pacific Journal of Tropical Biomedicine. 2012;**2**(8):616-620

[72] Norouzi R, Hejazy M, Azizi D, Ataei A, Effect of Taxus baccata L. Extract on hydatid cyst protoscolices in vitro. Archives of Razi Institute. 2021; **75**(4):473-480

[73] Pensel PE, Maggiore MA, Gende LB, Eguaras MJ, Denegri MG, Elissondo MC. Efficacy of essential oils of *Thymus vulgaris* and *Origanum vulgare* on *Echinococcus granulosus*. Interdisciplinary Perspectives on Infectious Diseases. 2014;**2014**:693289

[74] Moazeni M, Saharkhiz MJ, Hosseini AA. In vitro lethal effect of ajowan (*Trachyspermum ammi* L.) essential oil on hydatid cyst protoscoleces. Veterinary Parasitology. 2012;**187**(1–2):203-208

[75] Jahanbakhsh S, Azadpour M, Tavakoli Kareshk A, Keyhani A, Mahmoudvand H. *Zataria multiflora* Bioss: Lethal effects of methanolic extract against protoscoleces of *Echinococcus granulosus*. Journal of Parasitic Diseases. 2016;**40**(4):1289-1292

[76] Amri M, Touil-Boukoffa C. In vitro anti-hydatic and immunomodulatory effects of ginger and [6]-gingerol. Asian Pacific Journal of Tropical Medicine. 2016;**9**(8):749-756

[77] Shahnazi M, Azadmehr A, Aghaei H, Hajiaghaee R, Oladnabidozin M, Norian R, et al. Hydatid cyst killing mechanism of *Ziziphora tenuior* by inducing apoptosis via mitochondrial intrinsic pathway. Research Journal of Pharmacognosy. 2020;**7**(1):17-22

[78] Shirgholami Z, Borji H, Mohebalian H, Heidarpour M. Effects of *Allium sativum* on IFN-γ and IL4 concentrations in mice with cystic echinococcosis. Experimental Parasitology. 2021;**220**:108042

[79] Haji Mohammadi KH, Heidarpour M, Borji H. In vivo therapeutic efficacy of the *Allium sativum* ME in experimentally *Echinococcus granulosus* infected mice. Comparative Immunology, Microbiology and Infectious Diseases. 2018;**60**:23-27

[80] Mahmoudvand H, Kheirandish F, Dezaki ES, Shamsaddini S, Harandi MF. Chemical composition, efficacy and safety of *Pistacia vera* (var. Fandoghi) to inactivate protoscoleces during hydatid

cyst surgery. Biomedicine & Pharmacotherapy. 2016;**82**:393-398

[81] Labsi M, Khelifi L, Mezioug D, Soufli I, Touil-Boukoffa C. Antihydatic and immunomodulatory effects of *Punica granatum* peel aqueous extract in a murine model of echinococcosis. Asian Pacific Journal of Tropical Medicine. 2016;**9**(3):211-220

[82] Labsi M, Soufli I, Khelifi L, Amir ZC, Touil-Boukoffa C. A preventive effect of the combination of albendazole and pomegranate peel aqueous extract treatment in cystic echinococcosis mice model: An alternative approach. Acta Tropica. 2019;**197**:105050

[83] Zhang G, Wang J, Luo Y, Yuan M, Gao Q, Gao H, et al. In vivo evaluation of the efficacy of Sophora moorcroftiana alkaloids in combination with Bacillus Calmette-Guérin (BCG) treatment for cystic echinococcosis in mice. Journal of Helminthology. 2018;**92**(6):681-686

[84] Karimi Yazdi M, Haniloo A, Ghaffari A, Torabi N. Antiparasitic effects of *Zataria multiflora* essential oil nano-emulsion on larval stages of *Echinococcus granulosus*. Journal of Parasitic Diseases. 2020;**44**(2):429-435

[85] Moazeni M, Larki S, Saharkhiz MJ, Oryan A, Ansary Lari M, Mootabi AA. In vivo study of the efficacy of the aromatic water of *Zataria multiflora* on hydatid cysts. Antimicrobial Agents and Chemotherapy. 2014;**58**(10):6003-6008

[86] Moazeni M, Larki S, Oryan A, Saharkhiz MJ. Preventive and therapeutic effects of *Zataria multiflora* methanolic extract on hydatid cyst: An in vivo study. Veterinary Parasitology. 2014;**205**(1–2):107-112

[87] Moazeni M, Borji H, Saboor Darbandi M, Saharkhiz MJ. In vitro and in vivo antihydatid activity of a nano emulsion of *Zataria multiflora* essential oil. Research in Veterinary Science. 2017; **114**:308-312

[88] Rahimi-Esboei B, Fakhar M, Chabra A, Hosseini M. In vitro treatments of *Echinococcus granulosus* with fungal chitosan, as a novel biomolecule. Asian Pacific Journal of Tropical Biomedicine. 2013;**3**(10): 811-815

[89] Yones DA, Taher GA, Ibraheim ZZ. In vitro effects of some herbs used in Egyptian traditional medicine on viability of protoscolices of hydatid cysts. The Korean Journal of Parasitology. 2011;**49**(3):255-263

[90] Elissondo MC, Ceballos L, Alvarez L, Sánchez Bruni S, Lanusse C, Denegri G. Flubendazole and ivermectin in vitro combination therapy produces a marked effect on *Echinococcus granulosus* protoscoleces and metacestodes. Parasitology Research. 2009;**105**(3): 835-842

[91] Tariq A, Sadia S, Pan K, Ullah I, Mussarat S, Sun F, et al. A systematic review on ethnomedicines of anti-cancer plants. Phytotherapy Research. 2017; **31**(2):202-264

[92] Mahmoudvand H, Tavakoli Kareshk A, Nabi Moradi M, Monzote Fidalgo L, Mirbadie SR, Niazi M, et al. Efficacy and safety of *Zataria multiflora* Boiss essential oil against acute toxoplasmosis in mice. Iranian Journal of Parasitology. 2020;**15**(1):22-30

[93] Mahmoudvand H, Pakravanan M, Aflatoonian MR, Khalaf AK, Niazi M, Mirbadie SR, et al. Efficacy and safety of *Curcuma longa* essential oil to inactivate hydatid cyst protoscoleces. BMC Complementary and Alternative Medicine. 2019;**19**(1):187

[94] Robledo S, Osorio E, Munoz D, Jaramillo LM, Restrepo A, Arango G, et al. In vitro and in vivo cytotoxicities and antileishmanial activities of thymol and hemisynthetic derivatives. Antimicrobial Agents and Chemotherapy. 2005;**49**(4):1652-1655

[95] Maggiore M, Pensel PE, Denegri G, Elissondo MC. Chemoprophylactic and therapeutic efficacy of thymol in murine cystic echinococcosis. Parasitology International. 2015;**64**(5): 435-440

[96] Lin CC, Ng LT, Hsu FF, Shieh DE, Chiang LC. Cytotoxic effects of *Coptis chinensis* and *Epimedium sagittatum* extracts and their major constituents (berberine, coptisine and icariin) on hepatoma and leukaemia cell growth. Clinical and Experimental Pharmacology and Physiology. 2004;**31**(1–2):65-69

[97] Badary OA, Al-Shabanah OA, Nagi MN, Al-Bekairi AM, Elmazar M. Acute and subchronic toxicity of thymoquinone in mice. Drug Development Research. 1998;**44**(2–3): 56-61

[98] Kaushik ML, Jalalpure SS. Effect of Curcuma zedoaria Rosc root extracts on behavioral and radiology changes in arthritic rats. Journal of Advanced Pharmaceutical Technology & Research. 2011;**2**(3):170-176

[99] Mahmoudvand H, Mahmoudvand H, Oliaee R, Kareshk A, Mirbadie S, Aflatoonian M. *In vitro* protoscolicidal effects of *Cinnamomum zeylanicum* essential oil and its toxicity in mice. Pharmacognosy Magazine. 2017; **13**(51):652-657

[100] Kohansal MH, Nourian A, Rahimi MT, Daryani A, Spotin A, Ahmadpour E. Natural products applied against hydatid cyst protoscolices: A review of past to present. Acta Tropica. 2017;**176**:385-394

[101] Moazeni M, Larki S, Pirmoradi G, Rahdar M. Scolicidal effect of the aromatic water of *Zataria multiflora*: An in vitro study. Comparative Clinical Pathology. 2015;**24**(5):1057-1062

[102] Dorman HJ, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of Applied Microbiology. 2000;**88**(2):308-316

[103] Deb DD, Parimala G, Saravana Devi S, Chakraborty T. Effect of thymol on peripheral blood mononuclear cell PBMC and acute promyelotic cancer cell line HL-60. Chemico-Biological Interactions. 2011;**193**(1):97-106

[104] Bagheri SM, Sahebkar A, Gohari AR, Saeidnia S, Malmir M, Iranshahi M. Evaluation of cytotoxicity and anticonvulsant activity of some Iranian medicinal Ferula species. Pharmaceutical Biology. 2010;**48**(3): 242-246

[105] Swamy MK, Akhtar MS, Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: An updated review. Evidence-Based Complementary and Alternative Medicine. 2016;**2016**:3012462