

Chapter

Natural Products as Therapeutic Option for Echinococcosis

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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 *Echinococcus*. Following the development of the benzimidazoles, 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 scolicedal 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, benzimidazoles, 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 re-aspiration [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 anthelmintic 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 anthelmintic 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 helminthiasis. 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 vermicide 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 vermicide 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 scolical 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)	Sollicidal efficacy (%)	References
<i>Allium neeanum</i> (Reut)	Ethanolic	Leaves	Flavonoid	0.49	0.5	100	[37]
<i>Allium Sativum</i> (Garlic)	Ethanolic/ chloroform	Garlic cloves	Silver nitrate	200	15	17	[38]
<i>Allium sativum</i> (Garlic)	Methanolic	Garlic cloves	Mannitol	50	10	100	[39]
<i>A. sativum</i> (Garlic)	Chloroform extraction	Fresh garlic	N/A	200	1	100	[40]
Artemisia (Wormwood)	Methanolic	NA	N/A	100	15	97.24	[41]
<i>Artemisia sieberi</i> (Wormwood)	Hydrodistillation	Aerial parts	α -Thujone (31.5%)	0.005	120	99.30	[42]
<i>Artemisia sieberi</i> (Wormwood)	Aqueous	As a whole	N/A	50	20	100	[43]
<i>Atriplex halimus</i> (Orache)	Aqueous	Leaves	Phenolic and flavonoids	60	120	99.36	[44]
<i>Berberis vulgaris</i> (Barberry)	Aqueous	Fruit	N/A	4	30	100	[45]
<i>B. vulgaris</i> (Barberry)	Methanolic	Root	Berberine	2	10	100	[46]
<i>Blepharocalyx salicifolius</i> (Kunth)	Aqueous	Leaves	Gallic acid and rutin	200	5	100	[47]
<i>Bunium persicum</i> (Black Caraway)	Hydrodistillation	Seeds	g-terpinene (46.1%), cuminaldehyde (15.5%), r-cymene (6.7%), and limonene (5.9%)	0.0125	10	100	[48]
<i>Cannabis sativa</i> (Hemp)	N/A	Aerial parts	N/A	0.01	10	26.08	[49]
<i>Capparis Spinosa</i> (Caper)	Methanolic	Fruit	Flavonoids, tannins, glycosides and alkaloids	300	20	100	[50]
<i>Cassia fistula</i> (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
<i>Cinnamomum zeylanicum</i> (Cinnamon)	Hydrodistillation	Bark	Cinnamaldehyde (91.8%), metoxycinnamate (1.57%), and α -pinene (1.25%)	0.05	5	100	[52]
<i>Coriandrum sativum</i> (Coriander)	Hydrochloric acid + diethyl ether	Seeds	Phenols	750	10,080	100	[53]
<i>Corylus</i> spp.	Hydro-alcoholic	Seeds	N/A	50	20	98	[54]
<i>Cucurbitia moschata</i> (Pumpkin)	Hydroalcoholic	Seeds	N/A	1	60	16	[55]
<i>Curcuma longa</i> (Turmeric)	Ethanollic	As a whole	N/A	30	30	100	[56]
<i>Curcuma longa</i> (Turmeric)	Hydrodistillation	Rhizome	α -turmerone (27.1%), β -turmerone (21.8%), 1-phellandrene (8.8%), and ρ -cymene (5.4%)	0.1	5	100	[57]
<i>Curcuma zadoaria</i> (White turmeric)	Hydrodistillation	Rhizome	Pentadecane (29.6%), Delta-3-carene (14.7%), and Cis-cinnamic Acid (8.4%)	0.15	7	100	[58]
<i>Eucalyptus globules</i> (Bluegum)	Aqueous	Leaf	Eucalyptol (79.32%)	10	5760	94	[59]
<i>Eucalyptus globulus</i> (Bluegum)	NA	Leaves	Eucalyptol (79.32%)	5	3	100	[59]
<i>Ferula macrocolea</i> (Koma)	Hydrodistillation	Leaves	Terpinolene (77.72%), n-nonanal (4.47%), and linalool (4.35%)	0.3	10	100	[60]
<i>Lepidium sativum</i> (Garden cress)	Aqueous	Leaves	N/A	100	15	100	
<i>Mallotus philippinensis</i> (Kamala Tree)	Methanolic	Fruit	N/A	20	120	100	[61]
<i>Melaleuca alternifolia</i> (Tea tree)	N/A	Tree oil	Terpinen-4-ol (35.4%), α -terpinene (11%), γ -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
<i>Mentha species</i> (Lamiaceae)	Methanolic	Aerial parts	Phenolic, flavonoid and flavonol	200	10	99.54	[62]
<i>Myrtus communis</i> (True myrtle)	Hydrodistillation	Leaves	α -pinene (24.7%), 1,8-cineole (19.6%), and linalool (12.6%)	0.1	5	100	[63]
<i>Myrtus communis</i> (true myrtle)	Methanolic	Leaves	N/A	100	20	100	[64]
<i>Nectaroscordum tripedale</i> (Sicilian Honey Garlic)	Ethanollic	Leaves	Terpenoids, flavonoids, tannins and fatty acids	50	10	100	[1]
<i>Nigella sativa</i> (Black Cumin)	Hydrodistillation	Seeds	Thymoquinone	10	10	100	[1]
<i>Nigella sativa</i> (Black Cumin)	Methanolic	Seeds	Thymoquinone	50	30	100	[1]
<i>Ocimum basilicum</i> (Sweet basil)	Methanolic	Leaves	N/A	100	60	24.10	[65]
<i>Olea europaea</i> (Olive)	Aqueous	Leaves	N/A	1	120	96.7	[66]
<i>Olea europaea</i> (Olive)	Ethanollic	Leaves	N/A	150	25	100	[1]
<i>Peganum harmala</i> (Syrian rue)	Ethanollic	Seeds	N/A	62.5	2880	100	[67]
<i>Pelargonium roseum</i>	Hydrodistillation	Leaves	N/A	0.05	60	100	[68]
<i>Petalotopsis</i> spp.	Methanolic	Leaves, stems and roots	N/A	30	30	92	[1]
<i>Piper longum</i> (Long pepper)	Methanolic	Fruits	Phenolics, flavonoids, alkaloids, tannins, terpenoids, and glycoside	100	60	100	[1]
<i>Pistacia khinjuk</i> (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
<i>Poikilacanthus glandulosus</i> (Ariza)	Ethanolic	Branches	Polyphenols and flavonoids	0.01	15	100	[68]
<i>Punica granatum</i> (Pomegranate)	Alcoholic	Stem and root	N/A	9	1440	100	[1]
<i>Rhus coriaria</i> (Sumac)	Methanolic	As a whole	N/A	30	20	98.89	[69]
<i>Ruta graveolens</i> (rue)	Methanolic	Aerial parts	Phenolic (25.53%), flavonoids (6.6%) and tannins (8.0%)	40	720	100	[1]
<i>Salvadora persica</i> (Miswak)	Ethanolic	Root	Indole alkaloids, flavonoids, tropaedoin, triterpenes, phytosterols, and isothiocyanates	50	10	100	[1]
<i>Satureja hortensis</i> (summer savory)	Aqueous	Aerial parts	Carvacrol and -terpinene	1	20	100	[70]
<i>Satureja khuzistanica</i> (Jamzad)	Hydrodistillation	Leaves and flowers	Carvacrol	5	60	100	[71]
<i>Saussurea costus</i> (Costus)	Ethanolic	Root	N/A	250	60	100	[1]
<i>Sideritis perfoliate</i> (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]
<i>Silybum marianum</i> (Milk thistle)	Ethanolic	Seeds	Silydianin (14.41%), isosilybin A (10.50%), and silychristin (10.46%)	0.5	60	77	[1]
<i>Taxus baccata</i> (Common yew)	Hydroalcoholic	As a whole	Octane (13.36%), 4-methoxycarbonyl 3,5-diphenyl-1 (8.30%), and 9,12,15-octadecatrienotacid (10.75%)	150	60	66.60	[72]
<i>Teucrium polium</i> (Fely germander)	Ethanolic	Flowers	N/A	100	50	100	[1]
<i>Thymus vulgaris</i> (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
<i>Trachyspermum ammi</i> (Ajowan)	Hydrodistillation	Fruits	Thymol	5	10	100	[74]
<i>Zataria multiflora</i> (Shirazi thyme)	Methanolic	Leaves	Carvacrol and thymol	25	1	100	[1]
<i>Zataria multiflora</i> (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]
<i>Zataria multiflora</i> (Shirazi thyme)	Methanolic	Leaves	Carvacrol and thymol	10	10	100	[75]
<i>Zataria multiflora</i> (Shirazi thyme)	Hydrodistillation	Aerial parts	Thymol (41.8%), carvacrol (28.8%), and p-cymene (8.4%)	0.1	10	100	[1]
<i>Zataria spp.</i> (Satar)	Hydrodistillation	Leaves	Carvacrol and thymol	100	1	100	[1]
<i>Zingiber officinale</i> (Ginger)	Methanolic	Rhizome	N/A	100	30	100	[1]
<i>Zingiber officinale</i> (Ginger)	Methanolic	Root	N/A	100	40	100	[1]
<i>Zingiber officinale</i> (Ginger)	Aqueous	As a whole	[6]-gingerol	100	1440	100	[76]
<i>Zingiber officinale</i> (Ginger)	Ethanollic	Rhizomes sheets	N/A	200	30	100	[1]
<i>Ziziphora tenuior</i> (Mint)	Ethanollic	Shoots	Thymol	100	240	40.25	[77]

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

dosages of 60 g/mL and 2 mg/mL for 10 min, *F. asafetida* 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 scolical 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 scolical 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* scolical 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 scolical 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 protoscolical 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
<i>Algerian propolis</i> (Propolis)	Ethanolic	N/A	Polyphenol, flavonoid	Mice	25	10	100	[1]
<i>Allium sativum</i> (Garlic)	Methanolic	N/A	N/A	Mice	50	10	100	[78]
<i>A. sativum</i> (Garlic) Mice	Methanolic	Garlic cloves	1% Alliin	Mice	80	43,200	Significant	[79]
<i>Annona squamosa</i> (Sugar apple)	Alcoholic	Leaves	N/A	Rats	100	2880	100	[1]
<i>Artemisia Herba- alba</i> (Wormwood)	Ethanolic	Leaves and flowers	Alkaloids, phenols	Mice	0.28	1440	55.17	[1]
<i>Nigella sativa</i> (Black cumin)	Ionotropic gelation technique	Seed	N/A	Mice	1.14	86,400	100	[1]
<i>Pistacia vera</i> (Pistachio)	Hydrodistillation	Branch	Essential oil	Mice	200	10	100	[80]
<i>Punica granatum</i> (Pomegranate)	Aqueous	Peels	N/A	Mice	16	2880	100	[81]
<i>P. granatum</i> (Pomegranate)	Aqueous	N/A	Peel	Mice	0.65	86,400	66.7	[82]
<i>Sophora moercroftiana</i>	N/A	seeds	N/A	Mice	0.25	60,480	76.1	[83]
<i>Zataria multiflora</i>	Essential oil and oleic acid	Essential oil	N/A	Mice	20	10	100	[84]
<i>Zataria multiflora</i> (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
<i>Zataria multiflora</i> (Shirazi thyme)	Methanolic	Leaves	Thymol (66.9%), carvacrol (15.2%), and carvone (7.3%)	Mice	8	43,200	100	[86]
<i>Zataria multiflora</i> (Shirazi thyme)	Hydrodistillation	Essential oil	Thymol	Mice	2	10	100	[87]
<i>Zingiber officinale</i> (Ginger)	Ethanolic	As a whole	N/A	Mice	150	60	100	[1]

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

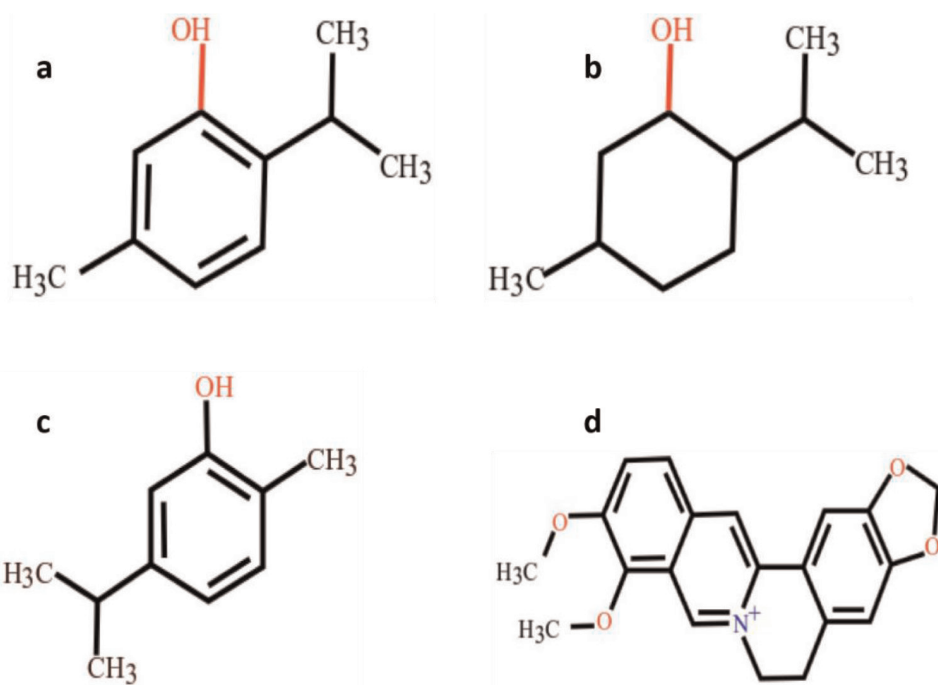


Figure 1.

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 bio-active 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 anthelmintic 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 protoscolecids 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 order 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 anthelmintics 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.

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