NEGLECTED TROPICAL DISEASES AND PHYTOCHEMICALS IN DRUG DISCOVERY

EDITED BY
CHUKWUEBUKA EGBUNA | MUHAMMAD AKRAM
JONATHAN CHINENYE IFEMEJE

WILEY

Neglected Tropical Diseases and Phytochemicals in Drug Discovery

Neglected Tropical Diseases and Phytochemicals in Drug Discovery

Edited by

Chukwuebuka Egbuna
Chukwuemeka Odumegwu Ojukwu University,
Uli, Nigeria

Muhammad Akram
Government College University,
Faisalabad, Pakistan

Jonathan Chinenye Ifemeje
Chukwuemeka Odumegwu Ojukwu University,
Uli, Nigeria

WILEY

This edition first published 2022

Registered Office
John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

Editorial Office
111 River Street, Hoboken, NJ 07030, USA

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Library of Congress Cataloging-in-Publication Data

Names: Egbuna, Chukwuebuka, editor. | Akram, Muhammad, editor. | Ifemeje, Jonathan Chinenye, editor.
Title: Neglected tropical diseases and phytochemicals in drug discovery / edited by Chukwuebuka Egbuna, Muhammad Akram, Jonathan Chinenye Ifemeje.
Description: Hoboken, NJ : Wiley, 2022. | Includes index.
Identifiers: LCCN 2021032011 (print) | LCCN 2021032012 (ebook) | ISBN 9781119616603 (hardback) | ISBN 9781119617112 (adobe pdf) | ISBN 9781119617150 (epub)
Subjects: LCSH: Tropical medicine. | Phytochemicals–Therapeutic use. | Materia medica, Vegetable. | Drug development.
Classification: LCC RC961.N4383 2021 (print) | LCC RC961 (ebook) | DDC 616.9/883–dc23
LC record available at https://lccn.loc.gov/2021032011
LC ebook record available at https://lccn.loc.gov/2021032012

Cover Design: Wiley
Cover Images: Courtesy of Egbuna; gailhampshire/Berberis vulgaris berries/Flickr; Anna Frodesiak; Courtesy of Chukwuebuka Egbuna

Set in 9.5/12.5pt STIXTwoText by Straive, Pondicherry, India

10 9 8 7 6 5 4 3 2 1

Contents

8

Efficacy of Phytochemicals of Medicinal Plants for the Treatment of Human Echinococcosis

Echinococcal Disease, Hydatidosis, or Hydatid Disease Drug Discovery

Charles Oluwaseun Adetunji[1], Chukwuebuka Egbuna[2,3], Tolulope Olawumi Oladosun[4], Muhammad Akram[5], Olugbenga Samuel Micheal[6], Frances N. Olisaka[7], Phebean Ozolua[1], Juliana Bunmi Adetunji[8], Goddidit Esiro Enoyoze[9], and Olugbemi Tope Olaniyan[10]

[1] *Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo State University Uzairue, Iyamho, Nigeria*
[2] *Department of Biochemistry, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria*
[3] *Nutritional Biochemistry and Toxicology Unit, World Bank Africa Centre of Excellence, Centre for Public Health and Toxicological Research (PUTOR), University of Port-Harcourt, Port Harcourt, Nigeria*
[4] *Department of Microbiology, Federal University of Agriculture, Makurdi, Nigeria*
[5] *Department of Eastern Medicine and Surgery, Government College University Faisalabad, Faisalabad, Pakistan*
[6] *Cardiometabolic Research Unit, Department of Physiology, College of Health Sciences, Bowen University, Iwo, Nigeria*
[7] *Environmental and Public Health Microbiology Laboratory, Department of Biological Sciences, Faculty of Science, Benson Idahosa University, Benin City, Nigeria*
[8] *Nutritional and toxicological Research Laboratory, Department of Biochemistry Sciences, Osun State University, Osogbo, Nigeria*
[9] *Department of Biological Sciences, Plant Biology and Biotechnology Unit, Edo University Iyamho, Iyamho, Nigeria*
[10] *Laboratory for Reproductive Biology and Developmental Programming, Department of Physiology, Edo State University Uzairue, Iyamho, Nigeria*

List of Abbreviations

ADA	Adenosine deaminase
ADME	Absorption, distribution, metabolism, and elimination
ALP	Alkaline phosphatase
BLAST	Basic Local Alignment Search Tool
CD68	Cluster of differentiation 68

Neglected Tropical Diseases and Phytochemicals in Drug Discovery, First Edition.
Edited by Chukwuebuka Egbuna, Muhammad Akram, and Jonathan Chinenye Ifemeje.

DDBJ	Data Bank of Japan
DNA	Deoxyribonucleic acid
EBI	European Bioinformatics Institute
HCFA	Hydatid cyst fluid antigen
HFFs	Human foreskin fibroblasts
iNOS	Inducible nitric oxide synthase
NADH	Nicotinamide adenine dinucleotide
NCBI	National Centre for Biotechnology Information
NF-Kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
RH	Rat hepatoma
TNF-α	Tumor necrosis factor alpha

8.1 Introduction

It has been observed that several types of parasites have evolved during the evolution of mankind and they normally use human beings as their host organism. Moreover, most of these parasites constitute nuisance and cause high level of unpleasant or discomfort situations such as flea and lice infestation of the body which may seriously affect our health, especially the internal parasites. Most of the ectoparasites could be handled mechanically while removal of the internal parasites is complicated. Several medicinal plants have been utilized by mankind for the treatment of several illnesses and health disorders for several years. Most especially, the application of plant parts, secondary metabolites, and plant products as antiparasitic drugs. The infections by endoparasites can scarcely be inhibited by vaccination [1]. Moreover, several synthetic drugs have been produced for the management of endoparasites but there are still several challenges encountered in their utilization. Most of these synthetic drugs are not effective due to resistance coupled with the fact that the development of new and novel antiparasitic drugs has not been the top priority of most pharmaceutical industry because numerous diseases occur in most developing countries where a larger percentage of the population could not afford the exorbitant price of the antiparasitic drugs.

Therefore, in view of the aforementioned facts, an alternative to these is adequate research into the application of medicinal plants, their phytochemical components, or natural product derivatives [2–7]. Moreover, most of the new drugs that are derived from medicinal plants possess pharmacophores which implies they have functional groups with enhanced pharmacological activity obtained from natural products [8]. Also, there is a need to assess several traditional medicinal plants for the development of new drugs that could be utilized for the

treatment of these parasitic infections. Moreover, several interesting results have been documented from several results that have established the biological activities of these medicinal plants when tested in an *in vitro* assay against their several vectors or their actual causative parasites.

Cystic echinococcosis (CE) has been recognized as a zoonotic parasitic disease that could be caused by the larval stage of some small tapeworm of some animals. The incidence of CE could lead to death and high morbidity in livestock and human being [9]. Moreover, CE has been documented as one of the neglected tropical diseases by the World Health Organization [10]. The incidence of CE, hydatid cysts, matures in the internal organs, mainly in the lungs or liver of their intermediate hosts and also in humans such as unilocuslar fluid-filled bladder [11]. It has been observed that this infection tends to appear as asymptomatic at the outset for numerous years or permanently [12]. There may be a malfunction of several organs which may lead to the death or continued development of cysts without effectual treatment [13]. It has been highlighted that chemotherapy does have influence but may lead to complications. Hence, the application of an efficient protoscolicidal agent is important to reduce the challenges of intraoperative spillage of the cysts filling in the course of surgery and uninterruptedly reoccurrence of CE in almost 10% of the postoperative circumstances [14]. Also, different types of protoscolicidal agents have been documented to incapacitate the substances of the cysts, nevertheless generally are not nontoxic for the reason that might be linked to their high side effects comprising liver necrosis, methemoglobinemia, and sclerosing cholangitis [15].

In view of the aforementioned facts, this chapter intends to document the application of phytochemicals and medicinal plants that could be used for the management and treatment of *Echinococcus* infection.

The nucleotide sequencing of the mitochondrion in various species of *Echinococcus* is of immense diagnostic benefits as it is a veritable source of molecular markers, which in turn provides valuable information on its molecular epidemiology [16]. The disease phenotypes, host specificities, distribution, genetic variation, and evolution of these parasites are linkable to the differences occurring in specific regions of their corresponding mitochondrial genomes. Next-generation sequencing (NGS) technology has been of great advantage in solving a myriad of problems around genome sequencing. These analyses are relatively affordable, with high-throughput in mitochondrial genomic studies. There are basically six species of *Echinococcus*. Pathogenic types with global public health burden include *Echinococcus granulosus*, *Echinococcus multilocularis, Echinococcus oligarthrus,* and *Echinococcus vogeli*. Reports on newly identified species from rodents and lions from Africa namely *Echinococcus shiquicus* and *Echinococcus Felidis,* respectively, have been documented. By genotypic characterization of the mitochondrion, there exist not less than ten (10) strains of *E. granulosus* [17].

8.2 Molecular Analysis

Upon obtaining endocyst samples under aseptic conditions, they should be fixed in 95% ethanol, and the cyst fluids centrifuged at 500g for 30 minutes. The centrifuged sample should be stored at 20 °C in preparation for molecular analysis. A small portion of the endocyst (of about 11–16 mg) should be sampled. The ethanol used to fix it must then be removed, and this sample should be washed twice in phosphate-buffered saline (PBS) for 15 minutes. The sample could then be centrifuged at 3500g for five minutes and PBS removed in the process. The mitochondrial DNA could then be extracted from the sample using a designated DNA extraction kit following the manufacturer's instructions [18]. This could also be done traditionally using the glass bead method [19]. Mitochondrial genes – cytochrome c oxidase subunit 1 (*cox I*) and NADH dehydrogenase subunit 1 (*nd I*) – are the targets in this assay, they are to be amplified by polymerase chain reaction (PCR), with the specific primers (designed according to sequences obtained from the GenBank). DNA amplification should be performed in a 20 µl consisting of DNA constituting 1–10 ng, the premixed solution (0.2 mM dNTPs), 1 µM each of the primers, 1 × TBE buffer, and 0.5 U Taq polymerase enzyme. The PCR protocol ideal for the amplification of both genes (*cox I* and *nd I*) is two minutes (two minutes) at 95 °C, 45 seconds of 40 cycles at 95 °C, 45 seconds at 57 °C, 90 seconds at 72 °C, and finally 10 minutes at 72 °C [18]. The PCR products are then subjected to gel electrophoresis, purification, and sequencing. The nucleotides sequences obtained would then be analyzed with the Basic Local Alignment Search Tool (BLAST) and the databases from the National Centre for Biotechnology Information (NCBI), European Molecular Biology Laboratory – EBI (European Bioinformatics Institute), or DNA Data Bank of Japan (DDBJ).

8.3 Life Cycle of Echinococcosis

The two main species of *Echinococcus* responsible for echinococcosis with major public health concerns in human populations are *E. granulosus* and *E. multilocularis*. *E. granulosus* which is the causative agent of CE has canines such as dog as its definitive host, which houses the adult worms (Figure 8.1). In these hosts, they are not seen to cause infections in the organs. The adult worm lays eggs, shed in the feces of the definitive hosts, and are ingested by herbivores such as sheep while grazing, which serve as intermediate hosts. These eggs that are being ingested, hatch in the small intestine of the intermediate host into oncospheres (which are the embryonic forms of the worm usually enclosed in a spherical membrane having hooks). This oncosphere navigates through the intestinal wall, via the circulatory system to the liver predominantly, where it

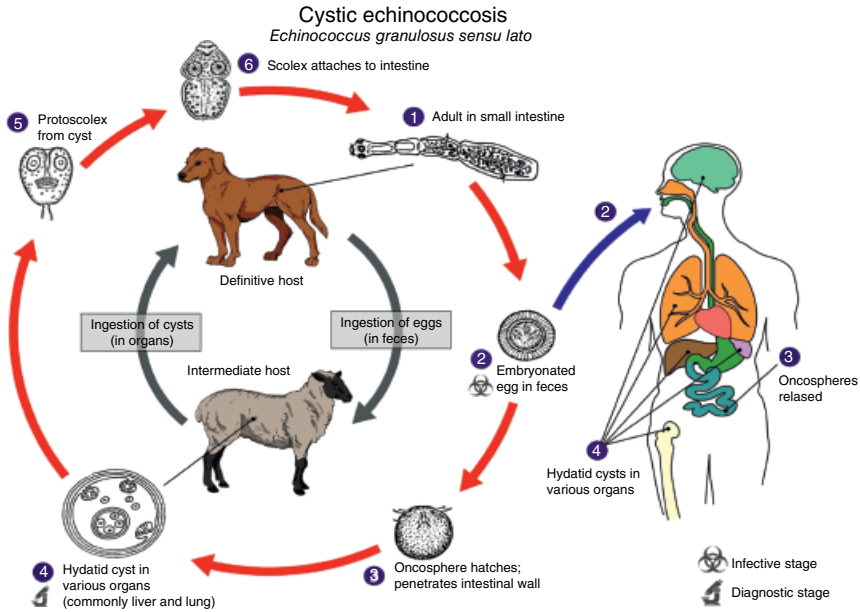

Figure 8.1 Life cycle of *Echinococcus granulosus*. *Source:* DPDx – Echinococcosis, Centers for Disease Control and Prevention, 16 September 2020.

develops into cyst. The cyst begins to enlarge and produces protoscoleces and daughter cysts within itself [17].

The cysts are finally ingested by the primary (definitive) host through the consumption of infected organs from these herbivores [20]. The protoscoleces from the cysts escape, evaginate, and attach to the intestinal mucosa of the definitive host, where they develop into adults within 80 days, and hence the cycle is complete. Humans are usually considered to be a dead-end host and also an accidental intermediate host in the life cycle of *E. granulosus* [11]. The infection could be by direct contact with egg(s) from feces of the definitive host or contaminated water, food, and/or soil. These eggs mature into oncospheres which then pass through the small intestine to infect vital internal organs (such as the liver, bones, lungs, and rarely, kidneys, heart, and the brain) via the circulatory system. The cyst is usually round and its diameter in CE ranges between 1 and 20 cm, consisting of two layers of membranes – the inner membrane is nucleated, which is referred to as the germinal membrane, and the germinal layer contains clear yellowish fluid. The appearance of the scoleces in the hydatid fluid shares resemblance with sand grains and hence they are called hydatid sand, while the outer membrane is acellular, and appears laminated. The formation of a calcified fibrous capsule around the cyst is the immune response staged by the host to CE; this is the layer often captured mostly in imaging studies [21].

Unlike the CE, alveolar echinococcosis which is caused by *E. multilocularis* has a life cycle similar to that of *E. granulosus,* except that the intermediate hosts involved therein are rodents whereas the definitive hosts are foxes predominantly. The infection in humans results in the formation of hydatid cysts also, having close similarities with symptoms usually presented in human *E. granulosus* infections. Chamber formation in alveolar echinococcosis is multiple in nature as against the unilocular pattern of cyst formation in CE.

8.4 Previous Studies on the Positive Effects of Medicinal Plants and Phytochemicals

A lot of complications are still been recorded due to improper diagnosis and treatment of human cystic *Echinococcus*, regardless of the developments in therapeutic strategies and modern imaging procedures. It is important that this disease is detected early enough for proper management to prevent great numbers of motility and morbidity [22].

Gangwar et al. [23] demonstrated the *in vitro* efficiency of the fruit glandular hair extract of the plant *Mallotus philippinensis* for its protoscolicidal action against hydatid cysts of *E. granulosus* which were collected using aseptic techniques from the infected liver and lungs of cattle. Their studies showed a mortality rate of up to 99% after treatment for 60 minutes with a concentration of 20 mg/ml of the extract, while treatments with the same concentration for more than 120 minutes showed a 100% mortality rate, as well as the disintegration of the protoscoleces. The results obtained were compared with the recognized standard drug Praziquantel, an anticestodal drug. It was also observed that Praziquantel had a comparable scolicidal effect. Meanwhile, the fruit glandular hair extract of the plant *M. philippinensis* did so without the distressing side effects observed in patients treated with Praziquantel. In another study, Albani et al. [24] established the *in vitro* effect of thymol (extracted from the plants *Origanum vulgare* and *Thymus vulgare*) and terpene-containing essential oils extracted from *Mentha piperita*, *Mentha pulegium*, and *Rosmarinus officinalis* on the proliferation of *E. granulosus* larval cells. It was observed that the action of thymol or the other essential oils led to the seizure of cell division and also triggered a considerable decrease in the number of cells after the second day of incubation, with the highest inhibitory effect observed in the essential oil of *M. pulegium* (82%) after seven days. As a result of their findings, it was suggested that these plant extracts could be used as an antihelminthic in the treatment of Echinococcosis.

Almalki et al. [25] evaluated the *in vitro* scolicidal effects of various concentrations of the ethanolic extracts of *Curcuma longa* and *Zingiber officinale* on *Echinococcus protoscoleces*, isolated aseptically from sheep livers. They observed

that the *Z. officinale* extract showed the strongest scolicidal effect at a concentration of 30 mg/ml after 20 minutes (100%) while the maximum scolicidal effect of *C. longa* was 93.2% at a concentration of 50 mg/ml after 30 minutes. Their study concluded that the extracts from *Z. officinale* and *C. longa* could be used efficiently as a protoscolicidal agent against *E. granulosus* that causes hydatidosis, by inactivating the pathogens to prevent multiple secondary echinococcosis as a result of recurrence after surgical treatment of the initial infection. In an older study, Al-Mayah et al. [26] carried out a study to assess the *in vivo* effects of *Nigella sativa* aqueous seeds extract against metacestodes of *E. granulosus*. They studied the morphological and histopathological variations in cysts and the organs infected in white albino mice which were injected with protoscoleces of the parasite. They also observed the enzymic activities of adenosine deaminase (ADA) and alkaline phosphatase (ALP) in each mouse. Their studies showed that mice treated with the *N. sativa* seeds extracts had a lower number and diameter of cysts and a higher cyst decrease percentage than the control group which was infected. The authors reported deterioration and necrosis of cysts in histological sections of the liver from mice treated with various concentrations of the extract. An increase in ADA and a decrease in ALP activities were also observed in the treated mice. Their study revealed that a 25 mg concentration of the aqueous extract of *N. sativa* seed was significant in inhibiting the metacestodes of *E. granulosus*.

Verma et al. [27] investigated the anticestodal action of an endophytic fungi *Pestalotiopsis* sp. from Neem plant on the protoscoleces of hydatid cysts of *E. granulosus*. Various mortality rates were noticed at diverse concentrations with respect to the periods of exposure. *Pestalotiopsis* sp. (581 ± 15.0) proved a scolicidal activity of up to 97% mortality rate within 30 minutes of incubation. As compared with standard medications used, endophytic *Pestalotiopsis* sp. of Neem plant displayed meaningful anticestodal actions. Also, Labsi et al. [28] investigated the action of pomegranate (PGE) peel aqueous extract on the progress of secondary experimental echinococcosis on the viability of protoscoleces of *E. granulosus*, and the immunomodulatory characteristics of PGE. In their experiment, Swiss mice were injected intraperitoneally with viable protoscoleces of the helminth. Then, PGE was administered intragastrically daily during CE growth. The development of cysts and damage to the liver were examined macroscopically and histologically. Nitric oxide production (observed in patients infected with the parasite), TNF-α in plasma, and the hepatic expression of iNOS, NF-kB, and the marker CD68 were evaluated. After the treatment of the infected mice with PGE, a significant decrease in nitric oxide, TNF-α levels, iNOS, CD68, and NF-kB expression was recorded. This decrease was in a strong relationship with the inhibition of cyst development (63.08%). Hence, it was concluded that the use of PGE against the development of experimental echinococcosis had both anthelminthic and immunomodulatory results.

Moazeni and Mohseni [29] investigated the *in vitro* scolicidal effect of methanolic extract of Sumac (*Rhus coriaria*). They aseptically obtained protoscoleces from sheep livers infected with hydatid cysts of the parasite. The protoscoleces were treated with several concentrations of the extract, leading to varying rates of inhibition. One hundred percent (100%) mortality rate was observed at a concentration of 50 mg/ml after 10 minutes of exposure. This *in vitro* study revealed that methanolic extracts of *R. coriaria* may be considered as an efficient natural scolicidal agent. While in a more recent study, Vakili et al. [30] analyzed the effects of the plant *Artemisia sieberi* on *E. granulosus* protoscoleces, collected from livestock, and the impact of three concentrations of aqueous extract of *A. sieberi* was assessed over three different periods of exposure. Their results revealed various mortality rates of the protoscoleces as a function of the periods of exposure and concentrations of the extract used. The highest mortality rate ($92.6 \pm 1.27\%$) was observed after treatment at a concentration of 75 mg/ml for 10 minutes. It was concluded that the aqueous extract *A. sieberi* had significant protoscolicidal activity and could be used as a natural agent against hydatid cyst protoscoleces which causes Echinococcosis.

Niazi et al. [31] carried out a study to assess the *in vitro* and *ex vivo* scolicidal effects of *Olea europaea* L. leaf extract on hydatid cyst protoscoleces, which were collected from the livers of infected sheep, after which they were treated with several concentrations of *O. europaea* L. extract *in vitro* and *ex vivo*. The mortality rate of protoscoleces was evaluated by the eosin exclusion test (0.1% eosin staining). The *in vitro* studies showed a mean mortality rate of 100% after 10 minutes of incubation with a concentration of 300 mg/ml of the *O. europaea* L. extract and a mean mortality rate of 100% after 20 minutes of incubation with a concentration of 150 mg/ml. After the inoculation of *O. europaea* L. extract directly into the hydatid cyst (*ex vivo*), the mean of the mortality of protoscoleces was 100% after 12 minutes of incubation with a concentration of 300 mg/ml or 25 minutes of incubation with a concentration 150 mg/ml of the extract. Based on their findings, it was concluded that the extract of the *O. europaea* L. had a substantial scolicidal effect on hydatid cyst protoscoleces.

Lv et al. [32] investigated the *in vitro* and *in vivo* efficiencies of chemotherapy against *E. granulosus* by albendazole liposome (L-ABZ), Huaier aqueous extract, and a Huaier aqueous extract/L-ABZ combination. Phosphate buffer was used for washing the protoscoleces which were collected. They observed that more than 95% of the *E. granulosus* protoscoleces parasites were still alive while more than 65% exhibited discrete movement with the L-ABZ, Huaier aqueous extract treatment. They observed a damaging effect of the cyst protoscoleces at 2 mg/ml Huaier aqueous extract and addition of 10 mg/ml L-ABZ. They concluded that Huaier aqueous extract had a significant reduction in viability of protoscoleces by 50% after incubation for 24 hours and there was no significance in the viable

protoscoleces in the mammalian cell line used. They established that Huaier could improve the role of L-ABZ in the treatment and management of CE.

Haghani et al. [33] studied the scolicidal action of methanolic extract of the plants *Ocimum bacilicum* and *Allium cepa*. Protoscoleces of *E. granulosus* were aseptically isolated from sheep livers containing hydatid cysts. The protoscoleces were exposed to the leaf extracts of the 2 plants at 2.5, 5, and 10% concentrations for a maximum of 60 minutes. The scolicidal action of the leaf extracts of the plants was not adequate. The highest concentration used (10% concentration) of *O. bacilicum* and *A. cepa* extracts after 60 minutes of exposure led to 24.1 and 16.8% of mortality of the proscoleces, respectively. It was observed that although these plants have high antibacterial activity, they did not have an equally high scolicidal effect and hence are not suitable to be used in surgical operations carried out to treat hydatid cysts. Yones et al. [34] investigated the *in vitro* action of alcoholic extracts of *Salvia officinalis*, *Thymus vulgaris*, and pure compounds thymol and menthol on *E. granulosus* protoscoleces. Various concentrations were used to test for the viability of protoscoleces within the first seven days post-treatment (PT). Total loss of viability of protoscoleces was observed with 500 μg/ml concentrations of *S. officinalis* and *T. vulgaris* extracts six and seven days PT, respectively. The pure compounds thymol and menthol exhibited strong effects with a concentration of 50 μg/ml two and five days PT, respectively. These results were similar to those shown by albendazole sulfoxide (800 μg/ml) and it was concluded that these plant extracts exhibited an even more effective scolicidal action than the synthetic albendazole sulfoxide.

Zibaei et al. [35] investigated the *in vitro* scolicidal action of hydroalcoholic extracts of *khuzestanica* leaves and aqueous extracts of *O. europaea* leaves on *E. granulosus* protoscoleces obtained from the liver of sheep diseased with the parasite. Different concentrations of the extracts were used over various exposure periods to check for the mortality rate of the proscoleces. A mortality rate of 96.7% was observed after 120 minutes of exposure to 0.01% of *O. europaea* extracts while 0.1% *Satureja khuzestanica* had very potent scolicidal effects, as a 100% scolicidal effect was observed. It was inferred that *S. khuzestanica* exhibited a stronger scolicidal effect than *O. europaea* against cystic *Echinococosis protoscoleces*. Rahimi-Esboei et al. [36] performed a study to assess the scolicidal activity of ultrasonic methanol extract of *Allium sativum* flower. Protoscoleces of the parasite were collected using aseptic techniques from sheep livers infected with hydatid cyst and were treated by various concentrations of the extract over different exposures periods. The highest mortality rate (98%) was observed when the protoscoleces were exposed to 100 mg/ml of the extract for a period of 180 minutes as confirmed by 0.1% eosin staining. This experiment revealed that ultrasonic *A. sativum* flower extract has a strong scolicidal effect and could be used in presurgical therapy to prevent a secondary recurrence of hydatid cyst after initial treatment.

Moazeni et al. [37] carried out an *in vivo* study on the preventive and therapeutic action of methanolic extracts of the plant *Zataria multiflora* on protoscoleces of *E. granulosus* which were isolated using aseptic techniques from the liver of the infected sheep. Mice were then infected intraperitoneally and after a period of eight months, they were treated with the extracts of *Z. multiflora* (8 g/l) for a period of 30 days. After the treatment period, the hydatid cysts were retrieved from the mice, weighed, and their sizes were measured. The results obtained were compared with those of Albendazole (ABZ), which was used on another class of mice among the originally infected lot. A significant decrease was recorded, and scanning electron microscopy was used to observe for damage to the germinal layer of the cysts at the ultrastructural level. A similar result was observed in a class of mice which was under prophylactic therapy of *Z. multiflora* extracts. These results led them to the conclusion that methanolic extracts of *Z. multiflora* could be used both in treatment and prevention of hydatidosis, though they pointed out the need for an evaluation of the viability of developing this natural agent into an efficient medication for the treatment of human hydatidosis.

8.4.1 *In vitro* and *in vivo* Effect of Phytochemicals Against *Echinococcus* Infection

The *in vitro* and *in vivo* activities of carvacrol against the metacestodes of *E. granulosus* were investigated [38]. For the *in vitro* assay, Carvacrol was dissolved in dimethyl sulphoxide (DMSO) at concentrations of 10, 5, and 1 μg/ml, while for the *in vivo*, 2.5 mg/ml ABZ was dissolved in deionized water and mixed vigorously for 12 hours, while carvacrol at 4 mg/ml drug concentration was dissolved in olive oil. Then the mixture, after shaking, was given intragastrically to the mice. The authors observed that 10 μg/ml of carvacrol had the greatest protoscolicidal effect. After six days post-incubation (p.i), viability was lowered to $17.62 \pm 13.93\%$ and after 60 days, it was further lowered to 0% while the 5 and 1 μg/ml concentrations of carvacrol, later triggered a protoscolicidal effect, lowering viability of protoscoleces after 60 days p.i to 16.17 ± 1.26 and $31.24 \pm 10.52\%$, respectively, showing a reduction in cyst weight. Also, upon treatment of the germinal layer of cyst with carvacrol, there was a loss of the multicellular structure features, suggesting damage of the protoscoleces. They therefore established that carvacrol could be used as a substitute for the treatment of Human Echinococcosis.

Elissondo et al. [39] reported the *in vitro* efficiency of thymol against the protoscoleces of *E. granulosus*. Protoscoleces obtained aseptically from cyst collected from naturally infected cattle were cultured in medium 199 adding 100 IU penicillin, 100 μg/ml streptomycin, and 4 mg/ml glucose. Thymol at a drug concentration of 10 mg/ml was dissolved in DMSO and added to the medium bringing it to a final concentration of 10, 5, and 1 μg/ml with a control having protoscoleces

incubated with 10 µl DMSO in culture medium. The authors noted that the highest protoscolicidal effect was observed with thymol at 10 µg/ml, with viability lowered after 12 days of incubation to 53.5 ± 11.9% and further reduced to 0% in 80 days. Meanwhile, at 5 and 1 µg/ml concentrations of thymol, there was an after-protoscolicidal effect, thereby lowering of protoscoleces viability after 42 days of incubation to near 50%. They observed that the thymol-treated protoscoleces showed ultrastructural damage at the tegument of the parasite. The authors concluded that thymol is effective in the treatment against *E. granulosus* protoscoleces.

Echinococcus multilocularis, E. granulosus, and *Taenia solium* are known to be responsible for the most serious and life-threatening helminth infections. Hemphill et al. [40] carried a review on the use of the laboratory models of *E. multilocularis* and *E. granulosus* in the quest for new drugs that could be adopted for chemotherapy of echinococcosis. Their study revealed that the *E. multilocularis* model, unlike other cestode models, fulfilled the conditions that would permit for intensified drug screening processes as a result of its rapid growth and proliferation *in vitro*, easy access to comprehensive genomic information and EST databases, among other advantages.

Küster et al. [41] evaluated the *in vitro* action of amino ozonides against the metacestode of *E. multilocularis*. Metacestodes were dissected from mice which have been infected experimentally and were incubated in 20 mg/l each of levofloxacin and ciprofloxacin and PBS overnight. The metacestodes were collected after culturing for about two months. All neutral and acidic ozonides were screened before their use against *E. multilocularis,* while nitazoxanide and artesunate were used as positive control. They observed that all neutral and acidic ozonides tested had no effect on *E. multilocularis* metacestodes and was toxic at high concentrations in human foreskin fibroblasts (HFFs) and rat hepatoma (RH) cells. They also revealed that the primary amines with an aminopropylether substructure were most effective (with variance of c. 75–100%) in metacestode damage as shown by high levels of phosphoglucose isomerase (PGI) which is a main constituent of the metacestode vesicle fluid. They concluded that ozonides induced significant metabolic damage in metacestodes after exposure but was less effective than Nitazoxanide.

Gonźalez-Sapienza et al. [42] carried out a study to improve the already existing immunodiagnostic techniques used to determine the presence of hydatid infection in a patient. *E. granulosus* Antigen B which possesses the highest diagnostic value among other peptide antigens (such as Ag5) was studied further and a 38-mer peptide (p176) was delineated from the parent protein. The peptide, p176, was observed to have a higher diagnostic sensitivity (80%) and specificity (94%) when tested against the same panel of sera. It was suggested that p176 could be used more efficiently in the laboratory diagnosis of hydatidosis.

Barbieri et al. [43] compared a synthetic peptide (GU4) collected from Antigen-B with crude hydatid cyst fluid antigen (HCFA), immunopurified AgB, antigen 5 (Ag5), peptides 65 (AgB mimotope), and 89–122 (Ag5 mimotope) for efficiency in diagnosis of cystic hydatidosis. From their study, it was observed that AgB showed a higher diagnostic efficiency (82%) than Ag5 (74%) and HCFA (71%). It was also observed that peptides 89–122 and AgB when combined, exhibited a higher sensitivity (82%) as opposed to the sensitivity of AgB alone with specificity equal to that of AgB (86%). It was suggested that hydatid serology might be more efficient by combining several defined antigens during the diagnosis of cystic hydatidosis.

Horton [44] investigated the use of ABZ in the chemotherapy of echinococcosis infections. 253 patients with *E. granulosus* cysts were treated with ABZ, of which 79.5% were either cured or had improved. His findings provided adequate proof that ABZ was effective and could be used as maintenance therapy for inoperable cases.

Moreno et al. [45] experimented on the *in vivo* combined use of Praziquantel and ABZ in the treatment of hydatid disease. It was observed that the combination was highly effective (100% efficiency) as a type of prophylactic therapy in the prevention of the development of hydatid cysts as a result of the propagation of protoscoleces during surgical procedures. This combination, however, was unsuccessful for the treatment of experimental hydatidosis, as there was no substantial difference between the treated and control mouse groups. The combination of Praziquantel and ABZ was therefore suggested for implementation in chemoprophylaxis against human hydatidosis.

Wang et al. [46] studied the use of recombinant *E. granulosus* ferritin (rEgferritin, cloned from *E. granulosus* and expressed in *Escherichia coli*) as a vaccine to confer immunity on mice, which were exposed intraperitoneally to brood capsules collected aseptically from fertile *E. granulosus* cysts from the livers of diseased humans. The vaccinated and exposed mice displayed significant protective efficiency of up to 85.6%. Thus, they inferred that rEgferritin could be a potential candidate as an efficient vaccine to confer immunity to echinococcosis.

Küster et al. [47] evaluated the *in vitro* and *in vivo* efficiency of mefloquine, a synthetic quinine analog, against *E. multilocularis* metacestodes responsible for alveolar echinococcosis. The parasites were cultured *In vitro* in the presence of 24 μM mefloquine for 10 days after which a parasiticidal dose-dependent effect of the drug was observed and confirmed by murine bioassays. *in vivo,* mice were infected with *E. multilocularis* and 25 mg/kg of mefloquine was administered twice a week for eight weeks intraperitoneally. The results observed were similar to the decline seen with ABZ (200 mg/kg/day) applied orally. On the other hand, when mefloquine was administered orally, it was unsuccessful in attaining any decrease in parasite weight. In summary, they observed that mefloquine was an effective parasiticide that could be used in the treatment of alveolar echinococcosis.

Spicher et al. [48] demonstrated the *in vitro* and *in vivo* treatment of *E. multilocularis* and *E. granulosus* larval stages with the antimalarials and artesunate. While 6 weeks intragastric treatment of mice infected with metacestodes of the parasites had no influence, *in vitro* treatment of the larval stages of the parasites with the antimalarial drugs dihydroartemisinin (DHA) and artesunate showed promising results. Scanning and transmission electron microscopy revealed that treatment with artesunate and DHA led to intense tissue modifications and loss of the multicellular structure of the germinal layer. They also observed that the artesunate-albendazole and dihydroartemisinin-albendazole combination treatments led to lower average parasite weight values compared with ABZ treatments alone, though the differences were just short of statistical significance.

Mohammadnejad et al. [49] investigated the *in vitro* efficiency of the antihelminthic drugs artemether, artemisinine, ABZ, and their combinations against protoscoleces of *E. granulosus* which were aseptically collected from the livers of sheep infected with hydatid cysts of the parasite and then treated with various concentrations of the drugs. The protoscolicidal action of artemether and its combinations was observed to be significantly stronger than the other combinations. The maximum protoscolicidal action was observed with 200 µg/ml of artemether four days PT, while ABZ killed protoscoleces seven days PT. Artemisinin exhibited favorable results against evaginated protoscoleces nine days PT. It was observed that the drug combination with the greatest efficiency was artemether and artemisinin. The desirable action of artemether and the surprising result displayed by artemisinin portrays them as potential scolicidal agents that could be used in the treatment of human hydatidosis.

Pensel et al. [50], in an *in vitro* study, evaluated the efficiency of 5-fluorouracil (5-FU) and paclitaxel (PTX) against *E. granulus* larvae and cells, collected from the liver and lungs of diseased cattle. The results showed that 5-FU inhibited the protoscoleces in a time and dose-dependent manner and was stronger in effect than PTX. Studies found that 5-FU led to total loss of viability after 24 days. It was concluded that both 5-FU and PTX would produce desirable results at realizable concentrations to hinder the persistence of protoscoleces and metacestodes of the parasite.

Spicher et al. [48] performed an *in vitro* and *in vivo* experiment with Artemisinin and Artemisinin derivatives for the treatment of the protoscoleces and metacestodes of *Echinococcus*. They observed that exposure of *E. multilocularis* metacestodes *in vitro* to artesunate and DHA caused an obvious tissue variation and damage of the typical multicellular structure of the germinal layer. Also, artesunate-albendazole and DHA-albendazole treatments showed low mean parasite weights than were found with only the ABZ treatment. They concluded that combination treatment with ABZ led to a lower parasite weight than when ABZ is taken alone for the treatment of protoscoleces and metacestodes of *Echinococcus*.

8.5 Synthetic Drugs Previously Used for Management of Echinococcosis Disease

Antiparasitic drug therapy employed in the treatment of echinococcosis includes the use of benzimidazoles, ABZ, and mebendazole (MBZ) exclusively for use in humans which are licensed having gone through empirical tests in detecting their efficacy and safety for use. The pharmacokinetics and pharmacodynamics of the drugs are also very important points to note in the therapeutic administration of these drugs. Pharmacokinetics which is concerned with the dose–concentration of the therapeutic agent and pharmacodynamics which relates to the concentration-effect will come into play by evaluating the vital processes of Absorption, Distribution, Metabolism, and Elimination (ADME) – Pharmacokinetics and the pharmacodynamics concepts of maximum response and sensitivity are involved in the determination of the magnitude of the effect the drug exerts at specific concentrations. Search into novel sources of therapy such as synthetic molecules with proven effects in the treatment of other kinds of infections such as parasitic, mycotic, and bacterial infections are also being tested for their possible adoption for treatment of echinococcosis [51]. Below is an appraisal of each of the existing antiparasitic drugs listed above being used for the treatment of echinococcosis:

In cases where a surgical operation is not possible, treatment is usually achieved through administering benzimidazole for a prolonged duration. It was first discovered as an antiparasitic drug against parasitic worms in the gastrointestinal tract of domestic animals and farm animals. Today, they are widely in use in both human and veterinary medicine. Their activities extend beyond anthelminthic capabilities to anti-tumorigenic, antibacterial, anti-inflammatory, antimycotic, antioxidant, and antidiabetic potentialities [52]. Benzimidazoles interfere with the assembly of the worm's cytoskeletal protein tubulin into polymers of microtubule and hence subsequent inhibition of microtubule-mediated metabolic cellular processes, such as cell division. It disrupts the mechanisms involved in the uptake of glucose by the developing larva of the parasite which is necessary for survival and metamorphosis, thereby causing a drastic reduction in the level of glycogen available in the larva and the collapse of organelles such as mitochondria and the endoplasmic reticulum [53]. BMZ has been reported to exert inhibitory effects on *Echinococcus* sp. rather than the destruction of parasites [54].

Studies revealed that the age of patients to which BMZ is administered also determines the therapeutic effect. It was discovered that it was more effective in children and adolescents rather than adult patients. The target organ also is perceived to contribute to the outcome of treatment. MBZ and ABZ are derivatives of BMZ. MBZ was reported with high efficacy, with proven broad-spectrum

activity. Its biocidal effect on *E. granulosus* has been documented. However, a very small fraction is always absorbed at the intestinal level due to its insolubility and thereby passed to the liver for metabolism.

8.6 Conclusion and Future Prospects

Echinococcosis is a parasitic infection which manifests in two distinct forms in humans. The first being CE which is also known as hydatidosis while the second is alveolar echinococcosis. Both are caused by the tapeworms *E. granulosus* and *E. multilocularis*, respectively. The devastating effects of these parasites to humans in endemic areas have caused severe losses and poor livelihood. Efforts to treat the infection are complemented by traditional knowledge of herbs. However, due to the shift in attention to other diseases affecting people in the developed world, research for drugs in the area of echinococcosis is limited, hence, leaving the affected populace to rely on hearsay herbs or old drugs which often come with severe side effects. To this end, it is recommended that research be redirected to investigating medicinal plants, their phytochemical components or derivatives as potential source of new drugs for the treatment of echinococcosis.

References

1 Van Wyk, B.E. and Wink, M. (2004). *Medicinal Plants of the World: An Illustrated Scientific Guide to Important Medicinal Plants and Their Uses*. Portland, OR, USA: Timber Press.

2 Adetunji, C.O., Olaleye, O.O., Adetunji, J.B. et al. (2011a). Studies on the antimicrobial properties and phytochemical screening of methanolic extracts of *Bambusa vulgaris* leaf. *International Journal of Biochemistry* 3 (1): 1–8.

3 Adetunji, C.O., Arowora, K.A., Afolayan, S.S. et al. (2011b). Evaluation of antibacterial activity of leaf extract of Chromolaena odorata. *Science Focus* 16 (1): 1–6.

4 Adetunji, C.O., Olaleye, O.O., Umanah, J.T. et al. (2011c). *In vitro* antibacterial properties and preliminary phytochemical of *Kigelia africana*. *Journal of Research in Physical Sciences* 7 (1): 8–11.

5 Adetunji, C.O., Kolawole, O.M., Afolayan, S.S. et al. (2011d). Preliminary phytochemical and antibacterial properties of *Pseudocedrela kotschyi*: a potential medicinal plant. *Journal of Research in Bioscience. African Journal of Bioscience* 4 (1): 47–50.

6 Adetunji, C.O. and Olaleye, O.O. (2011). Phytochemical screening and antimicrobial activity of the plant extracts of *Vitellaria paradoxa* against selected microbes. *Journal of Research in Biosciences* 7 (1): 64–69.

7 Adetunji, C.O., Olatunji, O.M., Ogunkunle, A.T.J. et al. (2014). Antimicrobial activity of ethanolic extract of *Helianthus annuus* stem. *Sikkim Manipal University Medical Journal* 1 (1): 79–88.

8 Newman, D.J. and Cragg, G.M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products* 70: 461–477.

9 Neumayr, A., Tamarozzi, F., Goblirsch, S. et al. (2013). Spinal cystic echinococcosis – a systematic analysis and review of the literature: part 2. Treatment, follow-up and outcome. *PLoS Neglected Tropical Diseases* 7: e2458. https://doi.org/10.1371/journal.pntd.0002458.

10 WHO (2012). *Accelerating work to overcome the global impact of neglected tropical diseases: a roadmap for implementation: executive summary*. World Health Organisation pp 22. WHO/HTM/NTD/2012.1. http://www.who.int/neglected_diseases/NTD_RoadMap_2012_Fullversion.pdf.

11 Thompson, R.C. and McManus, D.P. (2002). Towards a taxonomic revision of the genus *Echinococcus*. *Trends in Parasitology* 18: 452–457.

12 Liu, C., Zhang, H., Lei, W. et al. (2014). An alternative mebendazole formulation for cystic echinococcosis: the treatment efficacy, pharmacokinetics and safety in mice. *Parasites & Vectors* 7: 589–598.

13 Adas, G., Arikan, S., Kemik, O. et al. (2009). Use of albendazole sulfoxide, albendazole sulfone and combined solutions as scolicidal agents on hydatid cysts (*in vitro* study). *World Journal of Gastroenterology* 15: 112–116.

14 Junghanss, T., da Silva, A.M., Horton, J. et al. (2008). Clinical management of cystic echinococcosis: state of the art, problems, and perspectives. *American Journal of Tropical Medicine and Hygiene* 79: 301–311.

15 Gholami, S.H., Rahimi-Esboei, B., Ebrahimzadeh, M.A., and Pourhajibagher, M. (2013). *In vitro* effect of *Sambucus ebuluson* scolices of hydatid cysts. *European Review for Medical and Pharmacological Sciences* 17: 1760–1765.

16 Jex, A.R., Littlewood, D.T., and Gasser, R.B. (2015). Sequencing and annotation of mitochondrial genomes from individual parasitic helminths. In: *Parasite Genomics Protocols, Methods in Molecular Biology*, vol. 1201 (ed. C. Peacock), 51. New York: © Springer Science+Business Media https://doi.org/10.1007/978-1-4939-1438-8_2.

17 Bhutani, N. and Kajal, P. (2018). Hepatic echinococcosis: a review. *Annals of Medicine and Surgery* 36: 99–105.

18 Piccoli, L., Bazzocchi, C., Brunetti, E. et al. (2012). Molecular characterization of Echinococcus granulosus in south-eastern Romania: evidence of G1–G3 and G6–G10 complexes in humans. *Clinical Microbiology and Infection* 19: 578–582.

19 Ibrahim, E.A.A., Yara, M.A., and Moustafa, A.A. (2016). Prevalence and molecular characterization of hydatid cyst isolates from cattle in Egypt. *Asian Journal of Animal and Veterinary Advances* 11: 794–804.

20 Lissandrin, R., Tamarozzi, F., Piccoli, L. et al. (2016). Factors influencing the serological response in hepatic *Echinococcus granulosus* infection. *American Journal of Tropical Medicine and Hygiene* 94: 166–171. https://doi.org/10.4269/ajtmh.15-0219.

21 Gottstein, B. (2000). Hydatid disease, major tropical syndromes by body system. In: *Systemic Infections*. Chap 2000: 169 section 6.

22 Siracusano, A., Teggi, A., and Ortona, E. (2009). Human cystic echinococcosis: old problems and new perspectives. *Interdisciplinary Perspectives on Infectious Diseases* 2009: 474368. https://doi.org/10.1155/2009/474368 7 pages.

23 Gangwar, M., Verma, V.C., Singh, T.D. et al. (2013). 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* 6 (8): 595–601.

24 Albani, C.M., Denegri, G.M., and Elissondo, M.C. (2014). Effect of different terpene-containing essential oils on the proliferation of *Echinococcus granulosus* larval cells. *Interdisciplinary Perspectives on Infectious Diseases* 2014: 746931. https://doi.org/10.1155/2014/746931.

25 Almalki, E., Al-Shaebi, E.M., Al-Quarishy, S. et al. (2017). *In vitro* effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on *Echinococcus protoscoleces*. *Saudi Journal of Biological Sciences* 24: 90–94.

26 Al-Mayah, K.S., Al-Bashir, N.M., and Al-Azzawi, B.M. (2012). *In vivo* efficacy of *Nigella Sativa* aqueous seed extract against metacestode of *Echinococcus granulosus*. *Medical Journal of Babylon* 9 (1): 140–151.

27 Verma, V.C., Gangwar, M., Yashpal, M., and Nath, G. (2013). Anticestodal activity of endophytic *Pestalotiopsis* sp. on protoscoleces of hydatid cyst *Echinococcus granulosus*. *BioMed Research International* 2013: 308515. https://doi.org/10.1155/2013/308515, 11 pages.

28 Labsi, M., Khelifi, L., Mezioug, D. et al. (2016). Antihydatic and immunomodulatory effects of *Punica granatum* peel aqueous extract in a murine model of echinococcosis. *Asian Pacific Journal of Tropical Medicine* 9 (3): 211–220.

29 Moazeni, M. and Mohseni, M. (2012). Sumac (*Rhus coriaria* L.): scolicidal activity on hydatid cyst protoscolices. *Surgical Science* 3: 452–456.

30 Vakili, Z., Radfar, M.H., Bakhshaei, F., and Sakhaee, E. (2019). *In vitro* effects of *Artemisia sieberi* on *Echinococcus granulosus* protoscolices. *Experimental Parasitology* 197: 65–67. https://doi.org/10.1016/j.exppara.2018.10.011.

31 Niazia, M., Sakib, M., Sepahvanda, M. et al. (2019). *In vitro* and *ex vivo* scolicidal effects of *Olea europaea* L. to inactivate the protoscolecs during hydatid cyst surgery. *Annals of Medicine and Surgery* 42: 7–10.

32 Lv, H., Jiang, Y., Liao, M. et al. (2013). *In vitro* and *in vivo* treatments of *Echinococcus granulosus* with Huaier aqueous extract and albendazole liposome. *Parasitology Research* 112: 193–198.

33 Haghani, A., Roozitalab, A., and Safi, S.N. (2014). Low scolicidal effect of *Ocimum bacilicum* and *Allium cepa* on protocoleces of hydatid cyst: an *in vitro* study. *Comparative Clinical Pathology* 23: 847–853. https://doi.org/10.1007/s00580-013-1701-0.

34 Yones, D.A., Taher, G.A., and Ibraheim, Z.Z. (2011). *In vitro* effects of some herbs used in egyptian traditional medicine on viability of protoscolices of hydatid cysts. *Korean Journal of Parasitology* 49 (3): 255–263. https://doi.org/10.3347/kjp.

35 Zibaei, M., Sarlak, A., Delfan, B. et al. (2012). Scolicidal effects of *Olea europaea* and *Satureja khuzestanica* extracts on protoscolices of hydatid cysts. *Korean Journal of Parasitology* 50 (1): 53–56. https://doi.org/10.3347/kjp.2012.50.1.53.

36 Rahimi-Esboei, B., Ebrahimzadeh, M.A., Fathi, H., and Anzahaei, F.R. (2016). Scolicidal effect of *Allium sativum* flowers on hydatid cyst protoscolices. *European Review for Medical and Pharmacological Sciences* 20: 129–132.

37 Moazeni, M., Larki, S., Oryan, A., and Saharkhiz, M.J. (2014). Preventive and therapeutic effects of *Zataria multiflora* methanolic extract on hydatid cyst: an *in vivo* study. *Veterinary Parasitology* 205 (1–2): 107–112. https://doi.org/10.1016/j.vetpar.2014.07.006.

38 Fabbri, J., Maggiore, M.A., Pensel, P.E. et al. (2016). *in vitro* and *In vivo* efficacy of carvacrol against *Echinococcus granulosus*. *Acta Tropica* 164: 272–279. https://doi.org/10.1016/j.actatropica.2016.09.001.

39 Elissondo, M.C., Albani, C.M., Gende, L. et al. (2008). Efficacy of thymol against *Echinococcus granulosus* protoscoleces. *Parasitology International* 57: 185–190.

40 (a) Hemphill, A., Stadelmann, B., Scholl, S. et al. (2010). *Echinococcus metacestode* as laboratory models for the screening of drugs against cestodes and trematodes. *Parasitology* 137: 569–587. (b) McManus, D.P., Gray, D.J., Zhang, W., and Yang, Y. (2012). Diagnosis, treatment and management of echinococcosis. *British Medical Journal* 344: e3866. https://doi.org/10.1136/bmj.e3866.

41 Küster, T., Kriegel, N., Stadelmann, B. et al. (2014). Amino ozonides exhibit *in vitro* activity against *Echinococcus multilocularis* metacestodes. *International Journal of Antimicrobial Agents* 43: 40–46.

42 Gonzalez-Sapienza, G., Lorenzo, C., and Nieto, A. (2000). Improved immunodiagnosis of cystic hydatid disease by using a synthetic peptide with higher diagnostic value than that of its parent protein, *Echinococcus granulosus* antigen B. *Journal of Clinical Microbiology* 38 (11): 3979–3983.

43 Barbieri, M., Fernandez, V., Gonzalez, G. et al. (1988). Diagnostic evaluation of a synthetic peptide derived from a novel antigen B subunit as related to other available peptides and native antigens used for serology of cystic hydatidosis. *Parasite Immunology* 20: 51–61.

44 Horton, R.J. (1989). Chemotherapy of echinococcus infection in man with albendazole. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 83: 97–102.

45 Moreno, M.J., Urrea-Paris, M.A., Casado, N., and Rodriguez-Caabiero, F. (2001). Praziquantel and albendazole in the combined treatment of experimental hydatid disease. *Parasitology Research* 87: 235–238.

46 Wang, Y., Li, Z., Li, Z. et al. (2009). Recombinant ferritin protects mice against challenge with *Echinococcus granulosus*. *Acta Parasitologica* 54 (4): 335–340.

47 Küster, T., Stadelmann, B., Hermann, C. et al. (2011). *In vitro* and *in vivo* efficacies of mefloquine-based treatment against alveolar echinococcosis. *Antimicrobial Agents and Chemotherapy* 55 (2): 713–721.

48 Spicher, M., Roethlisberger, C., Lany, C. et al. (2008). *In vitro* and *in vivo* treatments of echinococcus protoscoleces and metacestodes with artemisinin and artemisinin derivatives. *Antimicrobial Agents and Chemotherapy* 52 (9): 3447–3450. https://doi.org/10.1128/AAC.00553-08.

49 Mohammadnejad, F., Ghaffarifar, F., Dalimi, A., and Hassan, Z.M. (2016). *In vitro* effects of artemether, artemisinine, albendazole, and their combinations on *Echinococcus granolosus* protoscoleces. *Jundishapur Journal of Natural Pharmaceutical Products* 11 (1): e30565. https://doi.org/10.17795/jjnpp-30565.

50 Pensela, P.E., Albania, C., Gambo, G.U. et al. (2014). *In vitro* effect of 5-fluorouracil and paclitaxel on *Echinococcus granulosus* larvae and cells. *Acta Tropica* 140: 1–9.

51 Siles-Lucas, M., Casulli, A., Cirilli, R., and Carmena, D. (2018). Progress in the pharmacological treatment of human cystic and alveolar echinococcosis: compounds and therapeutic targets. *PLoS Neglected Tropical Diseases* 12 (4): e0006422. https://doi.org/10.1371/journal.pntd.0006422.

52 Keri, R.S., Hiremathad, A., Budagumpi, S., and Nagaraja, B.M. (2015). Comprehensive review in current developments of benzimidazole – based medicinal chemistry. *Chemical Biology & Drug Design* 86: 19–65. https://doi.org/10.1111/cbdd.12462.

53 Wu, X.W., Chen, X.L., Zhang, S.J. et al. (2011). Pericyst may be a new pharmacological and therapeutic target for hydatid disease. *China Medical Journal* 124: 2857–2862.

54 Hemphill, A., Stadelmann, B., Rufener, R. et al. (2014). Treatment of echinococcosis: albendazole and mebendazole – what else? *Parasite* 21: 70. https://doi.org/10.1051/parasite/2014073.