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Anti-cryptosporidium Activity of Essential Oil: A Review

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Abstract

Plants and essential oils are used in traditional medicine against parasitic diseases. This review highlights their potential of essential oil as a source of new anti-parasitic compounds. In the present scenario of protozoal infections, new drugs are urgently needed to treat and control infections, which affect millions of people each year. In this review, we are focusing on articles related to antiprotozoal essential oils extracted from plants that have been published during the last years. Essential oils could be promising antiprotozoal agents, opening perspectives to the discovery of more effective drugs of vegetal origin for the treatment of diseases caused by protozoa.

Keywords

Essential oils, Parasite, Cryptosporidiosis

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1. History of *Cryptosporidium* Species

Cryptosporidiosis is a zoonotic protozoal diseases caused by coccidial species of the genus *cryptosporidium* and which is reported in more than 40 countries in the world [1]. In stool surveys of patients with gastroenteritis, the reported prevalence of *Cryptosporidium* is1-4% in Europe and North America and 1-37% in Africa, Asia, Australia, South and Central America [2, 3]. In most cases, *Cryptosporidium* infection resultsing as trointestinal problems such as severe diarrhea in both immuno-compromised and immuno-competent people. Among the five common *Cryptosporidium* species in humans, *Cryptosporidium parvum* and *Cryptosporidium hominis* are responsible for more than 90% of human cases of cryptosporidiosis [4].

Cryptosporidium is a protozoan parasite that causes cryptosporidiosis, a significant diarrheal illness that can occur in both healthy and immuno-compromised individuals. *Cryptosporidium* oocyst originate from a variety of sources including agricultural runoff (livestock), wild animals, domestic animals and human sewage or wastewater treatment plant (WWTP) effluent. The oocysts shed in feces and become waterborne, which can result in the contamination of food and water. The first *Cryptosporidium* species was described in 1907 by Edward Tyzzer. The parasite was found in the ventricular glands of mice and was named *Cryptosporidium muris*. In 1912, a smaller species found in the small intestine of mice was also described by Tyzzer and named *Cryptosporidium parvum*. Since then, *cryptosporidia* have been identified in all vertebrate classes. *Cryptosporidium* spp. was regarded as commensals until their association with diarrhea in young turkeys (*Cryptosporidium meleagridis*) in the 1950s. *Cryptosporidium parvum* was first recognized as an important pathogen in the 1970's, when it was linked to chronic diarrhea in an 8-month-old heifer [5]. The first cases of cryptosporidiosis in humans were reported in 1976 [6].

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However, the diagnosis of cryptosporidiosis in humans in 1976 and the subsequent connection of *Cryptosporidium* to epidemic waterborne disease have since fostered worldwide interest in the study of this microorganism. By the time the Centres for Disease Control and Prevention (CDC) implemented routine reporting of *Cryptosporidium* among AIDS patients in 1982, only 13 cases of human cryptosporidiosis had been documented [7]. Since 1982, more than 1,000 reports of human cryptosporidiosis have been documented in almost 100 countries, reaching all continents with the exception of Antarctica [8]. At the time of this writing, it is estimated that the annual number of cryptosporidiosis cases exceeds several million worldwide [9].

Cryptosporidium spp. cause significant diarrheal disease in humans and animals worldwide [10]. The genus *Cryptosporidium* has been classified in the phylum *Apicomplexa*, class Sporozoasida, subclass Coccidiasina, order Eucooccidiida, suborder Eimeriina, family Cryptospordiidae [11]. At least 22 species of *Cryptosporidium* have been named based on host occurrence, parasite morphology, host predilection and site of infection. However, only 13 species are considered valid by most investigators [5, 12]. *Cryptosporidium parvum*is the most commonly reported species in numerous species of mammals including humans.

Cryptosporidium spp. is transmitted via the fecal-oral route and has a direct life cycle, which means only a single host is required to complete the life cycle. The life cycle of most *Cryptosporidium* species is completed within the gastrointestinal tract (primarily small intestine and colon) of the host, with developmental stages being associated with the luminal surface of the mucosal epithelial cells. *Cryptosporidium* oocysts are spherical, or ovoid in appearance, and contain four parallel sporozoites surrounded by a smooth oocyst wall measuring only 3–6 μm in diameter. Thick-wall oocysts are excreted from the infected host in fecal material and represent the infective stage of the parasite. In the wall, a faint suture can be seen through which the sporozoites exit during excystation [13].

Cryptosporidium is excreted in the feces of an infected host in the form of an oocyst, which represents the only exogenous stage of the life cycle. The oocyst consists of four sporozoites housed within a sturdy, multi-layered wall that invade the epithelial cells and undergo asexual and sexual multiplication. The thick-walled oocyst is the environmentally resistant form of the parasite, resulting from the fertilization of macrogametes within the host, and it is appreciably resistant to natural decay in the environment as well as to most disinfection processes. The life cycle is repeated when sporulated oocysts excreted by an infected host are ingested by a new host and the sporozoites excyst within the new host's gastrointestinal tract. Depending on the parasite species, host and the host's immune-competency, the pre-patent period (time between infection and active oocyst shedding) ranges from 1 to 3 weeks, whereas the patent period (duration of oocyst shedding) can range from several days to months or years [14], demonstrating the potential of this infection to persist.

Contaminated water represents the major source of *Cryptosporidium* infections for humans. Several water borne out breaks of cryptosporidiosis has been reported implicating contaminated drinking water and recreational water [15]. The most severe and largest human water borne outbreak occurred in Milwaukee in 1993, where more than 400,000 people were infected [16]. Cryptosporidiosis in humans typically manifests itself as a self-limiting disease with a median duration of 9–15 days, resulting in total recovery in healthy individuals. The major symptom is watery diarrhea associated with abdominal cramps, anorexia, weight loss,nausea, vomiting, fatigue and low-grade fever [16]. Symptoms are similar in children and adults, although cryptosporidiosis acquired during infancy may have permanent effectson growth and development [17]. However, it is in the immuno-compromised host (due to a variety of causes including butnot limited to HIV infection and AIDS, drugs, organ transplantation,cancer chemotherapy, etc. [18] that the infections are most chronic and debilitating. Patients can have chronic diarrhea that can last for more than 2 months, shed oocysts in stool during the entire period, contributing to severe dehydration, weight loss and malnutrition, extended hospitalizations, and mortality [18]. Thus, the severity and duration of illness depends on the host's immune status. The groups implicated with higher risks of infection include children and staff in daycare centers, farmers and animal handlers, and health careworkers. Travelers are at risk when they travel from developedto developing countries with high prevalence of the disease.

As reports of human and animal infections increased, interest in *Cryptosporidium* was heightened in the veterinary field, not only because animals were seen as a source of infection for humans, but also because the organism could cause economic losses in production animals and was proving to be very difficult to control.

Cryptosporidiosis in ruminant species is typically symptomatic in the young. Among cattle, calves are susceptible to infection shortly after birth and remain so for several months [19]. Infection in dairy calves is most often detected (via fecal oocyst shedding) between 8 and 15 days of age, whereas infection in beef calves most often occurs between1 and 2 months of age [20]. Infection in lambs and goat kids is more common in animals under 1 month old [21]. Infection can be spread animal-to-animal by the fecal-

oralroute, usually when animals are housed together in an overcrowded environment, but contamination of udders and water is another common source of transmission in livestock.

2. Detection and Diagnosis

Methods for detection of *Cryptosporidium* infection can be detected in several ways. A common method is microscopy of fecal samples, which can be mounted on slides either directly or after flotation or sedimentation and gradient techniques, which are used to remove fecal debris and concentrate oocysts. This facilitates detection of infection in animals shedding lower numbers of oocysts, thus increasing the sensitivity of analysis. Different techniques for microscope visualization also exist. Oocysts can be detected without staining, using phase contrast microscopy, but using a stain facilitates detection. Staining methods were then developed to detect and identify the oocysts directly from stool samples. Modified Ziehl Neelsen stainis traditionally used to most reliably and specifically detect the presence of cryptosporidial oocysts where oocysts appear purple on a blue background, is commonly used. Immuno-fluorescence staining with monoclonal antibodies against oocyst wall antigens produces bright green oocysts at epi-fluorescence microscopy [22].

Other methods, such as histology of intestines, can be used to detect the different intracellular parasite stages in deceased animals. Today molecular analysis is used to identify different species of *cryptosporidia*. The first method was described in 1991 [23], and in 1995 a molecular method to distinguish between genotype i (anthroponotic or human adapted) and genotype ii (zoonotic) of *Cryptosporidium parvum* was published [24]. In 2002, genotype i was upgraded to a separate species, namely *Cryptosporidium hominis* [13]. It has now been shown that there are several species morphologically similar to *Cryptosporidium parvum*, and today this species is mainly considered to infect cattle and humans. Molecular analysis is vital to determine species when oocyst morphology is compatible with several species.

Cryptosporidiosis is transmitted via the oral–fecal route either by ingestion of contaminated water or food or by direct person-to-person (anthroponotic) or animal to-person (zoonotic) contact [4, 25, 26]. The infectious dose depends on the infecting strain, with as few as 10 oocysts sufficient to cause infection. Cryptosporidiosis is largely a water-borne infection and Cryptosporidium spp. has been the causative agents of numerous outbreaks of waterborne illness worldwide [26]. Oocysts, the resilient, infectious stage of the parasite are resistant to chlorination and can survive in water, for prolonged periods of time. Cryptosporidium spp. is one of the commonest causes of water-borne outbreaks of diarrheal disease in industrialized countries [26].

Outbreaks of cryptosporidiosis should be investigated quickly and the source isolated or quarantined to prevent further infection. Babies, toddlers, and diarrheic individuals must limit their contact with recreational water or use only pools made specifically for them. Train pets to defecate outside the house and bath them regularly, especially if there is any children teething and crawling around in the house. These measures may seem trivial at first glance, but they are in reality very important in minimizing the risk of getting cryptosporidiosis, particularly in those with underdeveloped or immuno-compromised immune system like children, the sick, and the elderly [27].

To date, there is no established specific treatment of patients with cryptosporidiosis. One drug, nitazoxanide, however, has been approved by the FDA for treatment of immuno-competent patients. For patients with compromised immune systems, such as HIV/AIDS patients, anti-retroviral therapy, which has been shown to reduce oocyst excretion, is recommended. Because diarrhea results in the rapid loss of fluids, it is also recommended that patients drink plenty of fluids to prevent dehydration. Anti-diarrheal medicine may help slow down fluid loss [28].

3. Essential Oils as Anti-cryptosporidiosis Activities

Garlic (*Allium sativum* L.) has been shown to have multiple beneficial effects such as antibacterial [29], antihelmintic [30], anti-coccidiosis [31] and anti-cryptosporidiosis [19] activities. Garlic contains many active chemical constituents such as; amino acids as arginine, organ sulphate compounds as aliin and allicin, enzymes as allinase, minerals and vitamins A, B1 and C. The physiological activity of dietary garlic is attributed to allicin (Diallylthiosulphinate), which is one of the organ sulphate compounds found in the bulb [32]. The major components for the essential garlic oils were sulfur compounds with diallyltrisulfide, diallyl disulfide and methyl allyltrisulfide [33].

Many drugs have been subjected to screen tests for their efficacy against *Cryptosporidium* infection in mammals [34]. The emergence of parasites resistant to current chemotherapies highlights the importance of plant essential oils as novel anti parasitic agents and the essential oils, or their active component, have activity against parasites reside in the intestine such as Cryptosporidium, Coccidian and nematodes [35]. Onion (*Allium cepa*) and cinnamon (*Cinnamomum zeylanicum*) oils being anti Cryptosporidiosis [36, 37]. Abd El-Aziz et al. [38] demonstrated that both of

onion (*A. cepa*) and cinnamon (*C. zeylanicum*) oils show anti- protozoal activity in a murine model of *Cryptosporidium parvum* infection. Both oils had effect on oocysts shedding, although there was no complete elimination of the parasite these may be due to the anti oxidant action of *A. cepa* and *C. zeylanicum* which help elimination of parasite [39, 40].

Moreover the anti-parasitic properties and the medicinal effect of *A. cepa* oil might be attributed to the presence flavonoids and sulphoid compound which had offer protection against cellular damage [41]. It offers direct chemo protective roles in animal cells and helps reduce oxidative stress and may also initiate the animal cells to produce their own chemical oxidative defense mechanisms [42, 43]. The anti-parasitic effect of the cinnamon may be due to its phenolic compounds which were deemed responsible for its anti-protozoal effect [44].

Abu-El Ezz et al. [37] found that both of onion and cinnamon oils had induced a significant reduction in oocysts count of *Cryptosporidium parvum* starting from the 3 day post-treatment till scarification of mice on day 17th post treatment. As infection with *Cryptosporidium* was highly related with the state of immunity of the host and was self-limiting in stimulating the immune system of the body rendering the intestinal cells less susceptible to infection with *Cryptosporidium* and consequently, leading to a sharp reduction in oocysts count. Furthermore, these herbal oils proved to have improved the appearance of villi of ileum, where the parasite colonized. The villi in treated mice retained their normal appearance, while, those in non-treated mice suffered from apparent shortening, atrophy and desquamation of most of villi. These results agreed with Harp *et al*. [45] who they indicated that plant oils might compete for or block receptor sites on the surface of ileum, thus, leading to reduction in *Cryptosporidium parvum* colonization. This study is evaluates the effectiveness of onion (*A. cepaa)* and cinnamon *(C. zeylanicum*) oils against experimentally *Cryptosporidium parvum in* mice as first time in Egypt and it point out to that administration of these oils proved too had an efficient therapeutic effect on the opportunistic zoonotic *C. parvu*m and such result could be adapted in similar infections in animal or even man. This is proven by Ranasinghe and Galappaththy [46] and Gaur et al. [47].

Finally, Cryptosporidium sp. could infect its host by three main routes. The first is through contamination of raw food ingredients from farms or abattoirs. The second is through contaminated water whether from unprocessed sources (rivers, wells) or from water treatment plants. The third is transferred from infected host such as unhygienic food handlers, pets, or pests like flies and cockroaches [27].

Taking preventive measures is the best way to manage Cryptosporidium sp. infection. Raw vegetables and food must be washed thoroughly before consumption. Clean tongs or utensils should be always used at a salad bar to prevent contamination from handlers' or patrons' hands. Water, especially untreated surface water, should be boiled before drinking to kill any oocysts in it. Water treatment plants have to be monitored regularly to prevent defective treatment and subsequent outbreak to houses receiving the water supply. Hands must be washed after contact with uncooked meat, soil, or animals before eating or touching around the mouth area. Pastures need to be fenced properly and the water bodies inside pastures (ponds or lakes) must never mix with the municipal water sources or reservoirs in order to prevent cross-contamination.

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