

Full Length Research Paper

Antiparasitic effects of *Allium paradoxum* as a conventional consumed vegetable

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Giardiasis is an important common intestinal infection; caused by the protozoan parasite *Giardia lamblia*. Numerous medications are available for the treatment of Giardiasis and can be prescribed by the health care provider. Metronidazole and other chemical drugs are used for Giardiasis. Considering the side effects of these drugs like other chemical agents, this prompted us to analysis an *in vitro* research into anti-giardial effect of *Allium paradoxum*, which has been used for centuries, as an herbal medicine, without harmful side effects. *Giardia* cysts were isolated from patients' fecal specimens obtained at Mazandaran hospitals, the cysts were isolated by sucrose 0.85 M solution from fecal specimens. The plant extracts were used at concentrations of 5, 10, 50 and 100 mg/mL throughout the experiments. The extracts at concentrations that ranged from 5 to 100 mg/mL, were incubated with several isolates of *G. lamblia* for 1, 5, 30, 60 and 180 min and then the viability were distinguished by eosin 0.01%. *A. paradoxum* extract at the concentration of 100 mg/ml for 3 h have acceptable anti-giardial activity. The anti-giardial effect of any concentrations of the chloroformic and hydroalcoholic extract of *A. paradoxum* was extremely significant as compared to the control groups at all exposure times ($p < 0.0001$). Thus, the results of present study have shown *A. paradoxum* extracts would be used as alternative treatment for Giardiasis.

Key words: *Allium paradoxum*, chloroformic extract, *Giardia lamblia*, hydroalcoholic extract.

INTRODUCTION

Allium spp. has been used as both food and medicine in many cultures for thousands of years (Ayaz et al., 2008; Masamha et al., 2010; Soffar and Mokhtar, 1991). It contains amino acids such as arginine, at least 33

organosulfur compounds such as alliin and allicin, eight minerals (germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc), enzymes such as allinase, and vitamins A, B1 and C. The physiological

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activity of *Allium sativum* is due to the allicin (diallyl thiosulphinate), which is one of the organosulfur compounds found in the bulb. It is responsible for the anti-microbial properties (Masamha et al., 2010; Thomson and Ali, 2003).

A recent review analyzed the active components of different herbs, including *Allium* that could be used as drug targets in parasitic diseases (Sakari et al., 2009). Antimicrobial properties of the *Allium* are attributed to allicin which is from Alliin (Mehrabi et al., 2011). The uses of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health have been widely observed, mostly because these products are safe and available at low cost (UNESCO, 1996). According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000).

Giardiasis is the intestinal infection resulting from infestation with the human parasite *Giardiasis lamblia*. Symptoms are highly variable but include persistent, usually short-term, diarrhea, epigastric pain, nausea, vomiting, weight loss, abdominal cramps, bloating and flatulence that may persist for weeks. Symptoms may be more severe in persons who are immune compromised (Ekanem and Brisibe, 2010; Roberts-Thompson et al., 1976). Metronidazole, Tinidazole, Furazolidone and Nitazoxanide are commonly available to treat *Giardiasis*. Undesirable side effects and failures in treatment are frequently reported due to use of these drugs (Johnson, 1993; Lemee et al., 2000; Llibre et al., 1989; Tracy and Webster, 1996; Voolmann and Boreham, 1993).

Recently, study show that garlic has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, hypoglycemic and antitumor activities. Lately, garlic has widely been used to treat intestinal parasites. The anti-helminthic effect of garlic has been a matter of interest of researchers. Their results showed that treatment with garlic evoked a significant reduction in the worm load (Edrisian et al., 2008; Johnson, 1993; Lemee et al., 2000; Topley et al., 2013). In addition, garlic has been used successfully in a single uncontrolled study in China, it was applied on 20 AIDS patients to treat *Cryptosporidium* (Johnson 1993). Moreover, garlic compounds were purified and tried as complementary medicine in the management of *Trichomonas vaginalis* (Sarkari et al., 2009; Singh and Singh 2008; Voolmann and Boreham, 1993). Thus, because many of the microorganisms susceptible to garlic extract are medically significant, garlic holds a promising position as a broad-spectrum therapeutic agent (Adetumbi and Lau, 1983).

Based on the need to develop new anti-giardial agents, trials were planned to test the effectiveness of traditional medicinal plants for treating *Giardiasis*.

Therefore, the present study aimed at investigating the

antiparasitic effectiveness of hydroalcoholic and chloroformic extracts of *A. paradoxum* (*Allium* spp) as a natural component in the prevention, as well as treatment of *Giardial* infections.

MATERIALS AND METHODS

Giardia cysts purification

G. lamblia cysts were isolated from faeces of symptomatic and asymptomatic *Giardiasis* patients from some health centers in Mazandaran province, Iran. All samples were processed immediately after arrival. A highly purified cyst suspension was obtained by combining the sucrose flotation method with a simplified sucrose gradient method originally described by Sheffield and Bjorvatn (1977) and Roberts-Thompson et al. (1976). Samples were broken up in tap water and filtered through a 300 urn mesh sieve. 3 ml of the fecal suspension were layered on 3 ml of 0.85% sucrose and centrifuged at 600 g for 10 min at 4°C. The cysts at the sucrose-water interface were aspirated with a Pasteur pipette and washed with normal saline. Washed cysts were carefully added to the top of a discontinuous density gradient, consisting of two layers of 0.85 and 0.4 M sucrose. After centrifugation at 600 g for 10 min at 4°C, cysts concentrated at the 0.85-0.4 M sucrose interface were collected and washed again. Purified cysts were resuspended in normal saline and stored at 4°C.

Plant extracts preparation

The leaves of *A. paradoxum* are mainly consumed in northern Iran. Therefore, leaves were collected from Mazandaran forests (Savadkoh town) in autumn of 2010, and confirmed by Dr. Mohammad Azadbakht (Pharmacologist) and dried under shade, and powdered mechanically using a commercial electrical blender. Each part was extracted by percolation method using hydroalcohol concentration and half of each solution in ethanol and chloroform concentration. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal and the obtained filtrate was placed into a sterile glass container and stored at 4°C for further use.

Effect of *A. paradoxum* extracts on *G. lamblia* cysts

In the current study, four concentrations (1, 10, 50 and 100 mg/ ml) of the chloroformic and hydroalcoholic *A. paradoxum* extract were used for 5, 10, 30 and 60 min. To make the *A. paradoxum* extract solution at 1, 10, 50 and 100 mg/ml concentrations, 0.01, 0.1, 0.5 and 1 g of dried extract was dissolved in 10 ml of normal saline, respectively. Then 2 ml of each solution was placed in test tubes, and 10,000 washed cysts was added to these tubes. The contents of the tubes were gently mixed. The tubes were then incubated at 37°C for 5, 10, 30, 60 and 180 min. At the end of each incubation time, the upper phase was carefully removed so as not to interrupt the cysts. Two milliliters of 0.1% eosin stain was then added to the remaining settled cysts and mixed gently. The remaining pellet of cysts was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The percentages of dead cysts were determined by counting more than 500 cysts. No treated cysts were considered a control group in each experiment. The experiments were performed in triplicate.

Viability test

In the present study, eosin stain with the concentration of 0.01% (1 g



Figure 1. *G. lamblia* cysts were stained by eosin 0.01%, the no stained cysts were considered potentially viable, and the stained cysts recorded as dead.

Table 1. Percentage of mortality of chloroformic extract of the leaves of *A. paradoxum* on *G. lamblia* cysts *in vitro*.

Concentration/times	1 mg/ml (%)	10 mg/ml (%)	50 mg/ml (%)	100 mg/ml (%)	Control (%)
After 1 min	26	34	35	35	6
After 5 min	47	56	56	61	6
After 30 min	78	78	79	80	6
After 1 h	81	84	85	87	7
After 3 h	87	89	93	96	7

Table 2. Percentage of mortality of hydroalcoholic extract of the leaves of *A. paradoxum* on *G. lamblia* cysts *in vitro*.

Concentration /times	1 mg/ml (%)	10 mg/ml (%)	50 mg/ml (%)	100 mg/ml (%)	Control (%)
After 1 min	46	49	51	55	6
After 5 min	60	65	65	69	6
After 30 min	68	69	71	76	7
After 1 h	75	76	79	83	7
After 3 h	87	89	94	97	8

of eosin powder in 1000 ml distilled water) was used to check the viability of the cysts. After exposure to the stain, the cysts with no absorbed dye were considered potentially viable, while those with absorbed dye were recorded as dead (Figure 1).

Statistical analysis

The significance of differences was determined by analysis of variances (ANOVA) and Student's T-test considering a level of significance of 95% ($P < 0.05$), using Graph Pad Prism software (Graph Pad Software, La Jolla, CA, USA).

RESULTS

The inhibitory capacity of the hydroalcoholic and

chloroformic extract of *A. paradoxum* were assayed using eosin 0.01%, as a result of which viable cysts of *G. lamblia* remained unstained whereas dead cells were light red in color (Rahimi-Esboei et al., 2013). The effects of (percentage of mortality) various concentrations of *A. paradoxum* hydroalcoholic and chloroformic extract against cysts of *G. lamblia* are shown in Tables 1 and 2.

Maximum effect of *A. paradoxum* chloroformic and hydroalcoholic extracts were observed at 97 and 96% of the cysts at the concentration 100 mg/ml after 180 min, respectively (Tables 1 and 2).

The anti-giardial effect of any concentrations of the chloroformic and hydroalcoholic extract of *A. paradoxum* was extremely significant as compared to the control groups at all exposure times ($p < 0.0001$).

DISCUSSION

For many years, medicinal plant as a result of anti-bacterial, antifungal, antiviral, antiprotozoal and anti-helminthic properties, has been used as effective therapeutic agent (Bingham et al., 1979). Unfortunately, there is a lack of interest of pharmaceutical companies in investing and developing these plants into drug and performing clinical trials. This study was designed to investigate anti-giardial effect of hydroalcoholic and chloroformic extracts of *A. paradoxum* *in vivo* and compare the mortality at a specified time (MICs) of these agents with control groups. Since *Allium* spp. is easily available throughout the nation and also is used in every house for cooking as a flavouring agent, the active compound from this can be prepared and used effectively for preventing the growth of the microbial pathogens.

Various experimental studies have investigated the anti-giardial effects of *Acanthus ebracteatus* Vahl (leaf, stem), *Alpinia galanga* (rhizome), *Barleria lupulina* Lindl (leaf), *B. lupulina* Lindl (stem), *Boesenbergia pandurata* (rhizome), *Coccinia grandis* (leaf), *Eclipta prostrata* (whole plant), *Gynura pseudochina* (leaf), *Murraya paniculata* (leaf), *Piper betle* (leaf), *Piper chaba* Hunter (Fruit), *Spilanthes acmella* (whole plant), *Zingiber zerumbet* (Rhizome) (Harris et al., 2001), *Rubus coriifolius*, *A. sativum*, *Artemisia ludoviciana* and *Thymus vulgaris* which showed moderate activity on both microorganisms with IC50 values ranging from 44.1 to 99.8 µg/ml for *G. lamblia* (Sawangjaroen et al., 2005). Alanis et al. (2003) examined the aerial parts of *R. coriifolius*, using *in vitro* assays for antiprotozoal activity against *Entamoeba histolytica* and *G. lamblia* (Alanis et al., 2003). Also concerning garlic (*A. sativum*), recent research demonstrated the anti-giardial activity of both whole raw garlic and some of its constituents (Fernando et al., 2006). Said et al. (2005) showed *in vitro* effect of ethanolic extract from *Artemisia ludoviciana* on *G. lamblia* trophozoites (Rahimi-Esboei et al., 2013). Recent studies have shown high activity of *Sambucus ebulus* (Fruit) methanolic extract on *G. lamblia* cyst and scolices of Hydatid cysts *in vitro* (Gholami et al., 2013; Rahimi-Esboei et al., 2013).

The family of *Allium* plants extracts were shown to decrease the oxygen uptake, reduce the growth of the organism, inhibit the synthesis of lipids, proteins and nucleic acids and damage membranes (28). Our results showed that as the concentration increased, the number of viable cysts decreased. Moreover, concentration being kept constant, the results of present study indicates that longer periods of exposure to the extracts, decreased the number of viable cysts. In addition, we showed that both extract of *A. paradoxum* revealed anti-giardial activity *in vitro* and significantly different from the control groups ($P < 0.001$), and both extracts after 180 min with nearly equal efficacy, however, after one minute, hydroalcoholic extracts proved to be more effective. It could be concluded that time is important condition for efficacy of

the drugs. Today, this new effective alternative treatment is extremely important, where species are becoming resistant and there is resurgence in the use of natural alternative therapies, instead of synthetic pharmaceuticals that often have severe side effects (Bingham et al., 1979). The necessity to search new, safe and effective agents for the treatment of *Giardiasis* is imperative due to the side effects and resistance of the reference drugs (Alizadeh et al., 2006; Calzada et al., 2006; Harris et al., 200).

It can be concluded that the hydroalcoholic and chloroformic extracts of *A. paradoxum* seems to be a noble anti-giardial candidate for *Giardiasis* treatment. However, some *in vivo* studies regarding its effects on animals and human should be investigated.

Conflict of Interests

The authors have not declared any conflict of interests.

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