2018 SCIENCELINE
Asian Journal of Medical and Pharmaceutical Researches
Asian J Med Pharm Res, 8(2): 05-14, June 25, 2018 ISSN 2322-4789

In-vitro Antihelmentic Evaluaion of Leaf Extract of *Bersama Abyssinica* (Mellanthaceae) on *Haemonchus Contortus*

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ORGINAL ARTICLE
PII: S2322-4789180002-8
Received: 15 April 2018
Accepted: 12 June 2018

ABSTRACT: *In-vitro* trial was conducted from November 2017 to April 2018 to determine anthelmintic effects of crude methanolic and ethanolic extracts of the leaf *Bersama abyssinica*. There was no significant ($P > 0.05$) variation between consecutive doses (50%, 90% and 95%) of methanolic plant extracts on egg hatch activity, whereas ethanolic extracts showen significant variation ($P < 0.05$). Methanolic extractions of *B. Abyssinia* were 0.15, 0.308 and 0.326mg/ml, while ethanolic extractions were 0.16, 0.352 and 0.385mg/ml respectively. Current findings, methanolic leaf extracts of the plant were more efficacious than ethanolic leaf extracts. The higher concentration methanolic extract caused significant egg hatching inhibition rate with 95.67%, which showed slightly lower effect as compared with that of Albendazole exposed control group (99.33%). Similarly, higher adult *H. contortus* mortality (76.6%) was observed for methanol extract at 8mg / ml concentrations while for ethanol, it was 60% at the same concentration. Therefore, the present study indicated that the leaf of *B. abyssinica* showed an effect on egg hatch activity and adult mortality. Hence, it can be concluded that leaf can be used as a potential alternative in the discovery of guide compounds that substitute commercially available anthelmintic effects. However, further *in-vivo* trial should be conducted.

Keywords: Anthelmintic, Bersama Abyssinica, Ethanolic Extract, Haemonchus Contortus, Methanol Extract

INTRODUCTION

Ethiopia constitutes the largest livestock and draft animal population in the African continent which is estimated to be about 30–40 million tropical livestock units (Lidya and Berihun, 2014). Despite the large livestock population of Ethiopia, economic benefits are still low due to more prevalence of diseases, poor production performance and poor management system (Tibbo et al., 2003).

Livestock diseases are the major cause of economic losses to the farmers in Ethiopia which is an important cause of reduced productivity of meat and milk as well as draft, hides and dung fuel. Of the diseases, endoparasitic diseases are one of the important limiting factors to the livestock production. They are responsible for the death of one-third of calves, lambs, and kids and considerable losses of parts of carcasses condemned during meat inspection (Sisay et al., 2012). They are recognized as a major constraint to both small and large-scale small ruminant production in developing countries (Kumsa and Wossene, 2006).

The abomasal nematode, *Haemonchus contortus*, which belongs to the family Trichostrongyloidea and genus *Haemonchus* (Khan, 2005) is a particularly important endoparasite and causes severe anemia and death in severely infected animal. The disease haemonchosis is especially prevalent in developing countries in association with poor management practices and inadequate control measures. Most of the parasite control programs are based upon a combination of chemotherapeutic control, grazing management, dietary management, biological control, vaccination and ethno veterinary treatment (Sisay et al., 2012).

Chemotherapeutic control practices (commercial anthelmintics) have been used for some decades throughout the world to minimize the losses caused by helminth infections. However, the threats of anthelmintic resistance for major anthelmintic drug classes, including (benzimidazoles, imidazothiazoles and macrocyclic lactones), risk of residue, availability and high cost especially to farmers of low income in developing countries, diverted the researchers' attention towards the development of alternate methods for the treatment of haemonchosis (Chartier et al., 2001).

Ethno veterinary medicines are used extensively and quite effectively for primary health care treatment to make

domestic animals productive and healthy. Over centuries, people have developed their own system, using age-old home remedies, surgical and manipulative techniques, husbandry strategies and associated magicoreligious practices. Ethno veterinary practices are often cheap, safe, long time tested and based on local resources and strengths. These can provide useful alternatives to modern animal health care systems (Phondani et al., 2010). However, the study of Ethiopian medicinal plants has not been realized as fully as that of other traditional communities elsewhere (Tilahun and Mirutse, 2007).

Therefore, to conduct the effectiveness of medicinal plants in the control of parasitic infection, the current study used *Bersama abyssinica* to assess its effect on *Haemonchus* contortus. *B. abyssinica* grows in several parts of Ethiopia and locally recognized as Azamer (Amharic) and Lolchissa (Oromeffa). This is a small, medium sized, evergreen African tree of the plant family Melianthaceae. The leaves are pinnately divided with a strongly winged rachis (common name winged *Bersama*) (Mathewos et al., 2015). *Bersama abyssinica* has been reported to possess a varied range of therapeutical and pharmacological applications due to the presence of bioactive compounds on the leaf, stem bark and root bark. The class of compounds identified includes terpenoids, vitamin, tannins, alkaloids, flavonoids, steroid, unsaturated and saturated fatty acids (Zakeya et al., 2013). In Ethiopian traditional medical practices, the leaves extract of *B. abyssinica* is administered orally for treating dysentery, tumor (Abate, 2000) stomach disorders such as abdominal pain, colic, diarrhea, cholera, intestinal worms, ameobiasis, rabies, syphilis, gonorrhoea and malaria are also treated with these decoctions (Mathewos et al., 2015).

A stem bark decoction is drunk to cure cancer and rheumatism. Apart from traditional medical practices, different studies were conducted on the effect of *B. abyssinica* leaf extract as antibacterial and antifungal agent but no anthelmintic effect of *B. abyssinica* has been assessed. This enhances the current study, interest in search for natural products with medicinal property as anthelmintic. Therefore, the main objectives of this study were to: Screen out the phytochemical compounds of the extracts of Bersama abyssinica. In addition to this, evaluating of anthelmintic effects of Bersama abyssinica on Haemonchus contortus

Ethno-veterinary medicine/ethno-botanical

For common diseases and chronic conditions (colds, skin diseases, worms, wounds, reproductive disorders, nutritional deficiencies, mild diarrhea and so on), Ethno-veterinary medicine has much to offer and should be strongly considered as an alternative or complement to modern treatments. This is especially true because some anthelmintic and other drugs have been overused, stimulating resistance among micro-organisms and leaving dangerous residues in meat, milk and ground water. Traditional medical consultancy including the consumption of the medicinal plants has a much lower cost than modern medical attention (Addis et al., 2001). Clearly, more research on the efficacy and economics of Ethno-veterinary medicine is needed if policy makers and development professionals are to begin using it on a regular basis.

Bersama abyssinica Fresen

Which is a plant, used in the current study, included under the Family of Melianthaceae and genus *bersama*, locally we call it **"**AZAMR**"** Amharic and **"**LOLCHISSA**"** Oromeffa (Mathewos et al., 2015). It is an Evergreen shrub distributed from Guinea Bissau through the coastal countries of West Africa except Benin, east to Eritrea and Ethiopia and south to Angola, Zambia, Zimbabwe and Mozambique. It is known as **"***winged bersama, bitter bark***"** in English and **"***Mwangwakwao, mtata***"** in Swedish. *Bersama abyssinica* grows in lowland bush savanna, gallery forests and Montana forests, from sea-level up to 2700 m altitude. It behaves as a pioneer species and is considered a weed in forest plantations (Buwa and Staden, 2006).

Bersama abyssinica F. is a small tree up to 12(–25) m tall; bark grey, brown or mottled, scaly. Leaves alternate, impair pinnately compound with up to 12 opposite pairs of leaflets, up to 1 m long; stipules 0.5–5 cm long; rachis usually with wide wings; leaflets nearly sessile, lanceolate to oblong or ovate-oblong, 3.5–22 cm × 1–8 cm, base cuneate to rounded, apex acuminate, margin entire to sharply and conspicuously toothed, glabrous to hairy, with 10–12 pairs of lateral veins. It has inflorescence an upright, dense, axillary raceme up to 35 cm long (Bene et al., 2014).

Flowers bisexual or often functionally unisexual, zygomorphic, merous, scented; sepals 6 mm long, 2 anterior ones fused; petals 5, free, narrowly oblong, 10–20 mm long, white, yellowish or purple-pink, stamens, free or fused at base; ovary superior, densely hairy, 4–5-celled, style simple. Fruit a woody capsule 1–3 cm in diameter, 4–5-lobed, yellowish to reddish, 4–5-seeded. Its seed is up to 11 mm × 8 mm, bright red with cup-shaped yellow or orange aril (Tapondjou et al., 2006).

All parts of Bersama abyssinica are poisonous and have been implicated in killing humans and livestock. For

internal use the dosage is therefore critical. Bark, leaf and root decoctions are widely taken as a purgative to treat a range of stomach disorders, such as abdominal pain, colic, diarrhea, cholera, intestinal worms, ameobiasis and dysentery. Rabies, syphilis, gonorrhoea and malaria are also treated with these decoctions. A stem bark decoction is drunk to cure cancer and rheumatism (Mikkelsen and Seberg, 2001). Extracts of growing shoots are used for external treatment of burns, ulcers and to clean wounds (Bene et al., 2014). Root bark infusion is drunk, stem bark powder is sniffed, and leaf sap is applied as eye drops or leaf powder is sniffed to treat migraine, headache and colds. A root decoction is used to treat hemorrhoid and epilepsy. Shoots and leaves are pounded and used to control stalk borers in maize (Tapondjou et al., 2006).

Two hellebrigenin derivatives identified in an ethanol extract of the bark have shown inhibitory activity against human carcinoma of the naso-pharynx in cell cultures and methanolic leaf extract had an inhibitory effect on HIV-1 replication (Asres et al., 2001). Leaf extracts have cardiogenic, spasmolytic and hypoglycemic activities. Crude bark extracts slow down growth of *Bacillus cereus, Staphylococcus aureus, Shigella flexineri and Shigella dysenteria*, a root bark extract slows down that of *Bacillus subtilis* (Makonnen and Hagos, 1993).

Phytochemical screening of leaf extract of *Bersama abyssinica* revealed the presence of alkaloids, glycosides, flavonoids, steroids, phenols, tannins, triterpens, anthraquinones, polysterols, coumarins and of saponins. Which are the most important bioactive constituents of medicinal plants (Mathewos et al., 2015)?

MATERIALS AND METHODS

Study area

The study was conducted at University of Gondar, Tewodros campus, College of Veterinary Medicine and Animal Science (CVMAS) parasitology laboratory. The area is located in Amhara National Regional State, about 750 km Northwest of Addis Ababa, at an altitude of 2300 meters above sea level. It has mean annual rainfall of 1800 mm and a mean annual temperature of 20 degree centigrade (CACC, 2003).

Study design

In-vitro experimental study was conducted from November 2017 to April 2018 to investigate the anthelmintic activity of leaf of *B. abyssinica* against *Haemonchus contortus.*

Plant collection and identification

Fresh leaves of *B. abyssinica* were collected from October to November, 2017 at Tara Gedam, which is located in the Libo Kemkem District, South Gondar Zone in the Amhara Regional State, northwestern Ethiopia**.** Plants were identified and characterized by experienced botanists at the University of Gondar, College of Natural and Computation Science, department of Biology. Finally, voucher of the specimen was deposited in the University of Gondar, College of Natural and computational Science Herbarium.

Crude extraction

After collection and identification, the leaves of *Bersama abyssinica* were thoroughly washed, gently with tap water to remove dirt and soil. The procedure was conducted according to (Mathewos et al., 2015; Tadesse, 2005). The leaves of the plant were dried under shade and then, ground into a coarse size using electric mill. Then, the coarse powder of the plant was subjected to crude extraction. 200-300g of the dried and powdered plant material was macerated using ethanol and methanol for 2 days and carried out three times. Then, the suspension filtered through Whatmans filter paper No.1. The extracts were kept in a stoppered sample vial at 4^{o}C. Then, all extracts were subjected to volume reduction using a Rota vaporizer and then dried by using a lyophilizer, to remove its aqueous content. Finally, the dried extract was packed in a closed vessel and stored in at 4^{o}C until required for the experiment.

Phytochemical screening

Preliminary qualitative screenings for major secondary metabolites were conducted from methanolic and ethanolic leaf crude extracts of Bersama abyssinica. The methods used were chemical tests involving either of color changes, precipitations, or formation of foaming through reaction with different standard reagents.

Test for flavonoids. About 10ml of ethyl acetate was added to 0.25 g of the crude extract and heated on a water bath for 3 min. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonical layer indicated the presence of flavonoids (Ayoola et al., 2008).

Test for steroids. Mix about 0.5 g crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The

development of a greenish coloration indicated the presence of steroids (Wadood et al., 2013).

Test for alkaloids. About 0.25 g of the crude extract was stirred with 5 ml of 1% HCl on a steam bath. One milliliter of the filtrate was treated with a few drops of Mayer's reagent and another 1 ml was similarly treated with Dragendorff's reagent. Turbidity or precipitation with both reagents was taken as preliminary evidence for the presence of alkaloids (Ayoola et al., 2008).

Test for terpenoids (Salkowski test). About 0.25 g of each of the crude extract of *Bersama abyssinica* leaves was taken and 2 ml of chloroform was added. Then, 3 ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids (Wadood et al., 2013).

Test for cardiac glycosides. About 0.25 g of the crude extract was diluted in 5 ml water then 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under lied with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Ayoola et al., 2008).

Test for polyphones (Ferric Chloride test). About 5 ml of the crude extract was taken and 1 ml of FeCl3 (1%) and 1 ml K3 (Fe (CN) 6) (1%) were added. The appearance of fresh reddish blue color indicated the presence of polyphenols (Mathewos et al., 2015).

Test for tannins. About 0.25 g of each crude extract was boiled in 10 ml of water in a test tube and then filtered. The addition of a few drops of 0.1% ferric chloride to the filtrate resulting in blue, blue-black, green or blue-green coloration or precipitation was taken as evidence for the presence of tannins (Ayoola et al., 2008).

Test for Anthraquinones. About 0.5 g of sample of each plant extract was shaken with 5 ml of chloroform and filtered. A 10% ammonium hydroxide solution (5ml) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet color in the ammonical phase was taken as an indication of the presence of Anthraquinones (Aiyelaagbe and Osamudiamen, 2009).

Test for saponins. About 0.5g of extracts was diluted with 20ml distilled water and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins (Mathewos et al., 2015).

In-vitro experiments

Collection of eggs. Adult female *H. contortus* were obtained from the abomasum of sheep collected from local slaughter houses in Gondar town following the standard procedure as described by Rahman (1990). Immediately after slaughter, the abomasum was removed soon after evisceration and transported to the CVMAS parasitology laboratory. Then it was opened along the greater curvature and its contents emptied into a 4 liter plastic bucket containing 2 liters of water. The parasites were recovered by passing the content through a sieve of 100 µm diameter mesh and picked with wire loop. Female *H. contortus* was identified and separated from male and other parasites based on their morphological characteristics following the keys and description given by Taylor et al., (2007). The female parasites were triturated using mortar and pestle to liberate the eggs. The suspension was filtered and the filtrate was centrifuged for 2minutes at 1000 RPM. Most of the water was then decanted and the number of eggs per ml determined using McMaster slide and diluted to the required concentration for use in Egg Hatch Assay (EHA) (Jemal et al., 2011).

Egg hatch assay (EHA). Egg hatch assay (EHA) was conducted according to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles et al., 1992 and Tadesse et al., 2005). Methanolic and ethanolic leaf extracts of the plant *Bersama abyssinica* were used as the test treatment. Albendazole was used as positive control while untreated eggs in water were used as a negative control. About 100 eggs in 1ml of water were placed in each test tube. Ethanolic and Methanolic leaf extracts of *Bersama abyssinica* at concentrations of 2.0, 1.0, 0.5 and 0.25mg/ml was prepared together with water containing eggs. Albendazole was dissolved in Dimethyl sulfoxide (DMSO) and diluted at the concentrations of 0.005mg/ml with a tube containing 100eggs. All the test tubes were then covered and kept in an incubator at 27 ^{0}C for 48 hrs. The experiment was replicated three times for each concentration. Following incubation, add 50 µl of helminthological iodine to each test tube. This both fixes and stains the egg and larvae (Vercoe et al., 2010). Hatched

larva and unhatched eggs were then counted under dissecting microscope with 40X magnification.

Adult worm mortality assay

Adult *H. contortus* were collected from the abomasums of sheep slaughtered from local slaughter houses in Gondar town. Immediately after slaughter, the abomasums were collected and transported to the (CVMAS) parasitology laboratory. The parasites were then collected, washed and kept in phosphate buffered saline (PBS). The experiment was conducted according to (Eguale et al., 2007). Ten actively moving worms were placed in each Petri dish containing 8.0, 4.0, 2.0, and 1.0mg/ml of the Methanolic and Ethanolic leaf extracts of *Bersama abyssinica* diluted in PBS. PBS alone with ten actively moving parasites was used as the negative control group. Albendazole dissolved in DMSO at the concentrations of 0.5mg/ml with ten parasites was used as a positive control. Three replications per each treatment concentration were employed. After 12 hours, the plant extracts and albendazole were washed away and the parasites suspended in PBS for 30 minutes for possible recovery of the parasite motility. The number of motile (alive) and immotile (dead) worms were counted under dissecting microscope and recorded for each concentration. The dead worms were easily recognized by their straight flat appearance with no movements at the head and tail regions of the body.

Statistical analysis

The extract concentration required to inhibit 50% (ED_{50}), 90% (ED_{90}) and 95% (ED_{95}) egg hatching was calculated by logit probit analysis. Comparison of mean percentages of egg hatch inhibition and mortality of adult parasites at different concentrations with the control was performed by one-way ANOVA (Analysis of variance). All statistical analysis was performed by SPSS version 20.0 software package. The difference between the means was considered significant at $P < 0.05$. The value reported were Mean±SE.

RESULTS

Phytochemical screening

Phytochemical screening of the ethanol and methanol leaf extracts of B. Abyssinica revealed the presence of different secondary metabolites. The major secondary metabolites detected in both extract type were alkaloids, tannins and flavonoids but devoid of cardiac glycosides, Steroids and phenolic compounds whereas Anthraquinones, saponins and terpenoides were highly concentrated in methanolic extract as shown in Table 1.

Table 1. Phytochemical screening results for the ethanol and methanol leaf extracts of Bersama Abyssinica

Constituents	*Bersama abyssinica*	
	Methanol extract	**Ethanol extract**
Alkaloids	+++	+++
Anthraquinones	+++	++
Flavonoids	+++	+++
Glycoside	-	-
Saponins	+++	++
Steroids	-	-
Phenolic compounds	-	-
Tannins	++++	++++
Terpenoids	++++	++

++++: highly concentrated, +++: concentrated, ++: moderate, −: absent

Egg Hatch Assay

Table1 and 2 show the summary of mean percentage inhibition of egg hatching post exposure to eggs of *H. contortus* to various concentrations of plant extracts. Both methanolic and ethanolic leaf extracts of the plant *B. abyssinica* exhibited good activities against the eggs of *H. contortus* which induced egg hatching inhibition in a dose-dependent manner. From the result, as the concentration of extracts increase the inhibition rate also increases. However, there is no significant ($P > 0.05$) variation between consecutive doses of metanolic plant extracts whereas ethanolic extracts show significant variation ($P < 0.05$) with in tested concentrations. Methanol extracts at different concentration showed a significantly lower inhibition rate as compared to 0.005mg/ml of albendazole concentration. Similarly, 2mg/ml of plant extract was shown significantly higher inhibition effect as compared with 0.25 mg/ml methanol concentration. In the current finding methanolic leaf extracts of the plant were more efficacious than ethanolic leaf extracts.

The methanolic extract of *B. abyssinica* caused maximum egg hatching inhibition at higher concentration (2mg/ml) with 95.67% inhibition slightly lower effect as compared with that of the albendazole exposed control group (99.33%), but ethanolic extract exhibited lower egg hatching activity when compared with methanolic extract at the same concentration (89%). All the concentrations showed higher significant variation ($P < 0.05$) as compared with the negative control.

The effective doses required to induce 50%, 90% and 95% (ED_{50}, ED_{90} and ED_{95}) inhibition of egg hatching was calculated by logit probit analysis. Of investigated

methanolic extract of *B.abyssinica* induced 50%, 90% and 95% inhibition of egg hatching at lower concentration, i.e., 0.15, 0.308 and 0.326mg/ml, respectively. Ethanolic extracts have also performed the next inhibition of egg hatching at concentration of 0.16, 0.352 and 0.385mg/ml, respectively.

Table 1. Mean percentage inhibition of egg hatching of *Haemonchus contortus* after 48 hours exposure to different concentration of Methanolic plant extracts (mg/ml) and Albendazole (mg/ml).

Concentration	*Bersama abyssinica* Methanol Extract
2mg/ml	95.67±0.88[a]
1mg/ml	93.33±0.66[ab]
0.5mg/ml	92.33±0.33[abc]
0.25mg/ml	90.67±0.66[bcd]
Alb0.005mg/ml	99.33±0.66[e]

The same subscript letters indicate that there is no significant variation in the mean percentage of egg hatching inhibition rate across the column.

Table 2. Mean percentage inhibition of egg hatching of *Haemonchus contortus* after 48 hours exposure to different concentration of Ethanolic plant extracts (mg/ml) and Albendazole (mg/ml).

Concentration	Extract types Ethanol
2mg/ml	89.00±0.57[a]
1mg/ml	82.67±0.67[b]
0.5mg/ml	77.67±0.45[c]
0.25mg/ml	71.00±0.57[d]
Alb0.005mg/ml	99.66±0.33[e]

The same subscript letters indicated that there is no significant variation in the mean percentage of egg hatching inhibition rate across the column.

Effects on adult parasites

Both methanolic and ethanolic leaf extracts of the plant *B.abyssinica* showed inhibitory effect on the survival of *Haemonchus contortus* in a dose dependent manner. There is no significant variation ($P > 0.05$) in both extract types of consecutive (tested) concentrations. But the higher concentration (8mg/ml) has a significant variation ($P < 0.05$) as compared with tested concentrations. The highest adult *H. contortus* mortality observed for methanol extract was (76.6%) with concentration of (8mg/ml) while the least mortality observed were (40%) at the concentration of (1mg/ml). In both extracts the control group (albendazole) has statistically significant ($P < 0.05$) value with the rest concentrations However, in most of the concentrations, like that of inhibition of egg hatching, methanolic extracts are more effective than ethanolic counterparts, i.e. ethanolic extract requires a maximum of 8mg/ml, to induce 60% of adult mortality.

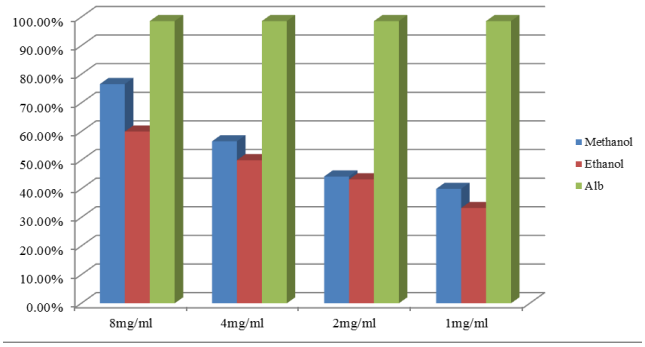

Figure 1. Percentage of mortality of adult *H. contortus* after 12 hours of exposure to methanolic and ethanolic leaf extract of *B.abyssinica* and albendazole

DISCUSSION

The use of nematicidal bio-products obtained from plants is an alternative control method that could partially replace the use of chemical anthelmintic drugs against sheep parasites. A plant material evaluated in the current study was identified from various areas to serve as anthelmintic agents by traditional healers or farmers in different parts of Africa. Except for this literature survey indicated, no prior scientific evaluation was conducted for *B.abyssinica* as anthelmintic effect.

There were only putative reports of the traditional use of this plant for deworming purposes. To support these reports invitro methanolic and ethanolic leaf extracts of the plant *B.abyssinica* was conducted and exhibited good activities against egg hatching and adult motility of *Haemonchus contortus* in a dose-dependent manner, which indicate the fact that increasing the dose of the plant extracts increases the proportion of the chemical ingredient(s) with pharmaceutical value in the crude plant extract.

The current study of qualitative Phytochemical screening of *B.abyssinica* leaf revealed secondary metabolites like alkaloids, flavonoids, saponins, Tannins and terpenoids that agree with earlier work of Mathewos et al. (2015) with good anthelmintic activity. As Debella (2002) finding, these classes of plant secondary metabolites is considered as the sources of chemical components responsible for wide therapeutic activities of several medicinal plants. However, steroids, phenolic compounds and glycosides are not found in the current study. It might be decomposed before crude extraction is started or during draying.

In the current experimental study, methanolic leaf extracts of the plant *B.abyssinica* were more efficacious than ethanolic leaf counterparts that might be due to the

presence of more pronounced tannins, antraquinononones, terpeinods and saponins, in methanolic extract which have a great anthelmintic activity against *H. contortus*. This finding agrees with the finding of Dereje (2008) which indicated that the probable reason for variation might be due to difference in the proportion (concentration) of the active components responsible for anthelmintic activity resulting from the difference in solubility either in ethanol or methanol. The other possible reason for the better performance of methanol extracts compared to ethanolic extracts, on the adult mortality could be due to easy transcuticular absorption of the methanol extracts into the body of the parasite more than the ethanolic extract which is stated by (Sisay et al., 2012*)*.

The present plant extract revealed the presence of concentrated terpenoids in the methanolic extract of leaf; it might be to stop the *H. contortus* adult motility and this finding is agree with in Maciel et al. (2006), Camurc¸a-Vasconcelos et al. (2007) and Molan et al. (2003) which indicated that some terpenoids reduced the mobility and the consequent migration ability of ovine nematode parasites, usually act by binding to surface molecules (proteins or sterols) inducing inhibition of the protein expression, and/or lyses of the cell.

The current phytochemical screening of the medicinal plant *B.abyssinica* revealed secondary metabolites like alkaloids and flavonoids. These classes of plant secondary metabolites might be good anthelmintic activity and the sources of chemicals responsible for wide therapeutic activities of several medicinal plants which in line with the report of Debella (2002). As Roy et al. (2010) finding the interference of development of helminth in general and paralysis on adult *H. contortus* in particular by alkaloids through acting on central nervous system and may suppress the transfer of sucrose, alkaloids act as antioxidant, capable of reducing the nitrate generation which can interfere in local homeostasis, thereby interrupts the development of parasites.

According to the current finding, the presence of mainly tannins in both methanolic and ethanolic extract of *B.abyssinica*, might be responsible for the observed anthelmintic activity that agree with Patel et al. (2010) through hindering energy generation of worms by uncoupling oxidative phosphorylation or they bind to free protein of the GIT tract of the host animal or glycoprotein on the cuticles of the worm which leads to death. Athanasiadou et al. (2001) revealed that plants with higher content of condensed tannin have shown anthelmintic effect on different gastrointestinal nematode parasites of sheep this is in agreement with the current finding. Brunet et al. (2011) also described the direct effect of tannins that comes from their affinity to bind to proteins of the parasite, causing changes in its cuticle architecture as well as degeneration of the musculature and intestinal cells. These injuries can reduce the motility of the *H. contortus* due to the metabolic alterations arising from structural breakage of the cuticle. The parasite's nutrition can be affected as a result of changes in the front end, and the release of eggs by females can also be impaired by destructuring of their reproductive appendage. The earlier work of Hoste et al. (2012) indicates another effect caused by tannins occurs due to the interaction of these metabolites and the sheath of L3 larvae, preventing their exsheathment (shedding of the retained sheath of the third laval stage of nematodes before the parasitic stage of its existence can begin) and hence impairing penetration in the host's gastrointestinal tract. In parallel, tannins can interfere in the nutrition of adult *H. contortus*.

The current finding of both alcoholic extract of *B.abyssinica* exhibited good activities against eggs of *H. contortus* which induced egg hatching inhibition in a dose-dependent manner which in line with the finding of Magdeleine et al. (2002) alcoholic extracts of plant leaf may contain a large spectrum of compounds that might have a multiple target activity on egg hatching that impedes the hatching of larvae from the eggs after contact with it. This can be achieved by interrupting the blastular development of the nematode embryo, blockage of the enzymatic pathways or interference in the action of enzymes associated with hatching.

The current finding, revealed that like that of adult motility test methanolic extract have good egg hatching inhibition activity than ethanolic extract, the possible reason of this might be the presence of concentrated saponins in methanolic extract this agrees with Camurac et al. (2007); Eguale et al. (2007) which reported that saponins, which are secondary metabolites potentially present in the methanolic extract of plants, these molecules are known to stop *H. contortus* egg from hatching through diffusion to egg shells.

Molan et al. (2003) described tannins might inactivate enzymes responsible for the egg hatching process in addition to adult mortality. These finding strengths the current egg hatching inhibition assay on *H. contortus*. Generally the effectiveness of the current medicinal plant *B.abyssinica* against adult motility and egg hatch assay might be due to synergistic effects of alkaloids, saponins, tannins, flavonoids, antraquinononones, and terpenoids with other bioactive principles which were not addressed but

contribute their own share for the anthelmintic activities of the crude extract.

CONCLUSION AND RECOMMENDATIONS

A holistic approach supported by the participation of specialists from a large range of scientific fields is highly desirable to help small farmers to maintain nematode parasite infections in sheep by relying on natural resources. The findings from the current study revealed that *B. abyssinica* leaf has promising *in vitro* anthelmintic activity against eggs and adult *H. Contortus*, which supports the traditional use of these plants as anthelmintics. Both methanolic and ethanolic leaf extracts from *B. abyssinica* have shown good activities against the eggs of *H. contortus* and adult motility, which induced in a dose-dependent manner. Inhibition of eggs from hatching is important in reducing pasture contamination, thereby helping in the overall helminth control programme. In this trial, methanolic leaf extracts of the plant *B. abyssinica* has better efficacy as compared with ethanolic extracts. Thus, it could be used as potential alternative in the discovery of guide compounds that substitute commercially available anthelmintic.

DECLARATIONS

Acknowledgements

The authors' heartfelt thanks will also go to University of the Gondar, research and community service V/President office, collage veterinary medicine and animal science for finance and recourse supporting.

Authors' Contributions

All authors contributed equally to this work.

Conflict of interest

The authors declare that they have no conflict of interest.

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