Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016j5-203

Preliminary phytochemical investigation and *in vitro* anthelmintic activities of *Maesa lanceolata* solvent extracts against *Lymnatis nilotica* (aquatic leech)

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ARTICLE INFO

Article history:
Received 19 Oct 2015
Received in revised form 4 Nov, 2nd revised form 13 Nov, 3rd revised form 19 Nov 2015
Accepted 5 Dec 2015
Available online 28 Dec 2015

Keywords:
In vitro anthelmintic activity
Maesa lanceolata
Aquatic leeches *(Lymnatis nilotica)*
Phytochemicals

ABSTRACT

Objective: To investigate phytochemicals constituents and check the anthelmintic activities of *Maesa lanceolata* (*M. lanceolata*) solvent extracts against aquatic leech.

Methods: Several phytochemicals were tested and screened from petroleum ether, chloroform and methanol extracts of *M. lanceolata* extracts and their anthelmintic activities were done based on the standard procedure against aquatic leech. Piperazine citrate (20 mg/mL) was used as a reference standard while distilled water was used as a control.

Results: Qualitative phytochemical analysis of different solvent (petroleum ether, chloroform and methanol) extracts of *M. lanceolata* revealed the presence of flavonoids and alkaloids in chloroform extracts and alkaloids in methanol extracts. In addition to the qualitative analysis of the plant extracts, the anthelmintic effects were also evaluated against aquatic leech (*Lymnatis nilotica*). Accordingly, various concentrations (10, 20, 40 and 60 mg/mL) of each solvent (petroleum ether, chloroform and methanol) extracts and 20 mg/mL of standard drug were prepared and tested against the selected leech. All the tested concentrations showed anthelmintic activities in a dose-dependent manner. From petroleum ether, chloroform and methanol extracts, chloroform extracts with 60 mg/mL concentration was effective with the paralytic time of (29.00 ± 1.06) min and death time of (65.00 ± 2.00) min in gradient extraction method. But in the case of extraction without gradient method, methanol extracts with 60 mg/mL concentration showed effective paralysis and death time (26.00 ± 1.73) and (56.00 ± 1.56) min, respectively, against the selected aquatic leech.

Conclusions: The present study revealed that *M. lanceolata* extracts have magic anthelmintic activities which are helpful to treat aquatic leeches (*Lymnatis nilotica*) which are associated with the nasal cavities of animals and human beings.

1. Introduction

Natural products are secondary metabolites (phytochemical) and important sources for biologically active compounds which have distinctive pharmacological effects and provide a rich source of anthelmintic drugs[1]. The origin of these effective drugs is found in the traditional medicine practices and several researchers have undertaken studies to evaluate the anthelmintic efficacy of the extracts[2]. *Maesa lanceolata* (*M. lanceolata*) is widely distributed in many parts of Africa especially in Ethiopia and the leaves of the plant are used traditionally to eradicate the aquatic leeches [*Lymnatis nilotica* (*L. nilotica*)] which are segmented worms under Phylum Annelida and Class Clitellata with distinctive properties from other annelids by the presence of anterior and posterior suckers, and constant number of body segments and body cavity largely filled with muscles and connective tissue[3]. They have strong muscular suckers. The anterior one surrounding the mouth contains saw-like teeth which are used to pierce the skin of the host and feed on blood of various animals to which they attach themselves at the nasal cavity[4-6]. The leeches get into the mouth when the animals drink the water with large number of them and attaches to the oropharyngeal by means of the powerful terminal sucker. They pierce the epidermis and suck blood by using this sucker[7]. Although detailed impact of the leeches on livestock has not yet been studied in Ethiopia, several externship students of Faculty of Veterinary Medicine, Debre Zeit, have reported it as one of the animal health problems in rural areas. In some highland areas where small streams and ponds are used for watering for livestock, it is common to find that cattle suffer from the effect of this parasite. A pharyngeal leech infestation of cattle is very common especially during the dry season[8].

2. Materials and methods

2.1. Collection and extraction of the plant materials

The fresh leaves of *M. lanceolata* were collected from Awi Zone, Guangua Wereda, Amsti Kebele at Elala forest which was 220 km far from Bahir Dar City in Western Amhara region in December 2014. The collected fresh plant materials were washed under cold running tap water and gently brushed to remove soil and other debris. Then, it was dried in a shaded region and grinded for the extraction. One hundred fifty gram of the pulverized plant material was then soaked

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in 250 mL of the organic solvents (petroleum ether, chloroform and methanol) in a closed glass container for 48 h at a gradient manner. After 48 h in contact with the solvent, the residue of the plant material was separated from the solvent by decanting followed by a filtration using Whatman No. 1 filter paper[9,10]. At the end of the extraction, the resulting mixture containing the crude and the solvent was applied to Rotavapour to recover solvent from the crude extract. This procedure was also applied for the extraction of the plant materials by the solvents without gradient. Then, the extracts were stored in fridge until the *in vitro* anthelmintic activities and qualitative phytochemicals analysis were examined.

2.2. Phytochemical analysis of the plant extracts

The extracts obtained by petroleum ether, chloroform and methanol from the leaves of *M. lanceolata* were subjected to qualitative chemical investigation for the identification and analysis of different phytochemicals such as tannins, alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phlobatanins, proteins and carbohydrates. The phytochemical screening analysis of the extract was performed by using the standard procedures[11-17].

2.3. Tested organism and anthelmintic activities of the plant extracts

The tested organism, *L. nilotica* (aquatic leech) was collected from the ponds of Bimbila (local name) from Awi Zone, Guangua Wereda, Amsti Kebele during the seasons of December–February. Petroleum ether, chloroform and methanol extracts from the plant *i.e.* with gradient and without gradient extracts of *M. lanceolata* were tested for the anthelmintic activities against *L. nilotica* (aquatic leech) which was carried out as per the method of Ajaiyeoba *et al.*[17]. Four different concentrations of the tested sample (10, 20, 40 and 60 mg/mL in distilled water) of each extract was used to evaluate the time of paralysis and time of death of *L. nilotica*. Piperazine citrate (20 mg/mL) was taken as a standard reference drug and distilled water was used as a control. The anthelmintic assay of the plant extract was carried out by taking 38 *L. nilotica* (aquatic leeches with the same morphology) in each 100 mL beaker. There was 20 mL of the tested samples of the extract in each tested beaker, and the standard drug and the control were poured and then a leech was inserted into it in order to evaluate the tested sample's activity towards the selected leech. The time taken for paralysis and death of the individual leeches was carefully observed and recorded. All experiments were carried out in triplicate and the data were statistically expressed by mean ± SD of the three leeches in each group[18].

3. Results

The results of the phytochemical evaluation of the secondary metabolites and *in vitro* anthelmintic activities of solvent extracts of *M. lanceolata* are listed in the table below. Accordingly, this study has revealed the presence of active phytochemicals such as flavonoids and alkaloids in chloroform extracts and alkaloids in methanol extracts (Table 1). In addition to this, gradient and non-gradient extracts of the plant has shown the anthelmintic activities against aquatic leech (*L. nilotica*) (Tables 2 and 3).

Table 1

Preliminary phytochemical screening of *M. lanceolata* leave solvent extracts.

Phytochemical	Type of test	General confirmation test	Extracts		
			Petroleum ether	Chloroform	Methanol
Alkaloids	Mayer's test	Formation of creamy-white colored precipitate	-	+	+
	Wagner's test	Formation of reddish-brown precipitate	-	+	+
	Picric acid test	Formation of yellow precipitate	-	+	+
	Dragendorff's reagent	Formation of red precipitate	-	+	+
Flavonoids	Ammonium test	A yellow coloration at the ammonia layer	-	+	-
	Aluminum chloride test	Formation of yellow precipitate	-	+	-
Terpenoids	Chloroform and concentrated H_2SO_4 test	Formation of reddish brown color at the interface	-	-	-
Phlobatanins	Distilled water and HCl	Formation of red precipitate	-	-	-
Carbohydrates	Molisch reagent test	Formation of brown ring at the interface	-	-	-

-: Absence; +: Presence.

Table 2

In vitro anthelmintic activities of petroleum ether, chloroform and methanol gradient extracts.

Extract	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
Distilled water	-	-	-
Piperazine citrate	20	59.00 ± 1.61	88.00 ± 1.00
Petroleum ether	60	64.00 ± 1.73	105.00 ± 1.35
	40	73.00 ± 1.65	113.00 ± 1.85
	20	83.00 ± 1.61	122.00 ± 2.00
	10	93.00 ± 1.00	133.00 ± 1.85
Chloroform	60	29.00 ± 1.06	65.00 ± 2.00
	40	37.00 ± 1.65	78.00 ± 1.45
	20	45.00 ± 1.00	85.00 ± 1.53
	10	51.00 ± 1.65	91.00 ± 1.55
Methanol	60	41.00 ± 1.00	81.00 ± 1.15
	40	48.00 ± 1.00	88.00 ± 1.85
	20	57.00 ± 1.00	97.00 ± 2.00
	10	63.00 ± 1.00	102.00 ± 1.00

The data are statistically expressed as mean ± SD; -: No anthelmintic activity.

Table 3

In vitro anthelmintic activities of petroleum ether, chloroform and methanol without gradient extracts.

Extract	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
Distilled water	-	-	-
Piperazine citrate	20	59.00 ± 1.61	88.00 ± 1.00
Petroleum ether	60	64.00 ± 1.73	105.00 ± 1.23
	40	73.00 ± 1.65	113.00 ± 1.25
	20	83.00 ± 1.61	122.00 ± 2.00
	10	93.00 ± 1.00	133.00 ± 1.86
Chloroform	60	32.00 ± 1.54	70.00 ± 2.15
	40	36.00 ± 1.00	82.00 ± 1.34
	20	39.00 ± 1.65	89.00 ± 1.73
	10	63.00 ± 1.00	103.00 ± 1.00
Methanol	60	26.00 ± 1.73	56.00 ± 1.56
	40	36.00 ± 1.46	73.00 ± 1.08
	20	45.00 ± 1.00	85.00 ± 0.77
	10	51.00 ± 1.65	91.00 ± 1.65

The data are statistically expressed as mean ± SD; -: No anthelmintic activity.

4. Discussion

The preliminary phytochemical screening (Table 1) of the different solvent extracts of *M. lanceolata* leaves revealed that flavonoids and alkaloids were present from chloroform extracts, and alkaloids were present from methanol extracts.

As it is clearly recorded in Tables 2 and 3, the solvent (petroleum ether, chloroform and methanol) extracts of *M. lanceolata* have shown the anthelmintic activities against aquatic leeches (*L. nilotica*) for both gradient and non-gradient extracts. But the higher concentrations of the plant extracts have shown paralytic effect much earlier than the lowest concentrations and the time taken to death was shorter for all leeches. Even though all the plant extracts showed anthelmintic activities to the selected leeches, the petroleum ether extract shown less activity than those of the chloroform and methanol extracts. This might be mainly due to the less polarity of compounds extracted by petroleum ether than those by chloroform and methanol. Moreover, the extract might luck some functional groups which are responsible for the anthelmintic activity.

Up on the comparison of the anthelmintic activities of chloroform and methanol extracts (both gradient and non-gradient extracts), chloroform extract has shown the highest anthelmintic activity (less time taken for paralysis and death of the leeches, Table 2) than that of methanol extract in gradient method, but in the case of non-gradient method the methanol extract has shown the highest anthelmintic activity (less time taken for paralysis and death of the leech, Table 3). The variation of the anthelmintic activities of extracts obtained by these two methods of extraction might be due to the synergetic effects of the components *i.e.* the components extracted by methanol need the combination with the components extracted by chloroform in order to show the anthelmintic activity towards aquatic leeches.

Evaluation of the anthelmintic activity of different concentrations of the plant extracts was compared with the reference standard piperazine citrate (Tables 2 and 3). Accordingly, all concentrations of chloroform gradient extracts (*i.e.* 20, 40 and 60 mg/mL) have shown the paralysis time and death time [(45.00 ± 1.00), (37.00 ± 1.65), (29.00 ± 1.06), and (85.00 ± 1.53) (78.00 ± 1.53), (65.00 ± 2.00) min, respectively] which is less than that of the standard drug [(59.00 ± 1.61) and (88.00 ±1.00) min for paralysis and death of the aquatic leech]. But 10 mg/mL showed less time for paralysis time [(51.00 ± 1.65) min] which is less than that of the standard drug [(59.00 ± 1.61) min] and death time [(91.00 ± 1.55) min].

Generally, *M. lanceolata* extracts have magic anthelmintic activities which are helpful to eradicate the aquatic leeches (*L. nilotica*) which are associated with the nasal cavities of animals and human beings as it is being frequently used by farmers traditionally.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors of this project paper are pleased to express the deepest gratitude to the Research and Community Service, Bahir Dar University, College of Sciences for the financial support.

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