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PHYTOCHEMICAL INVESTIGATION AND COMPARATIVE ANTHELMINTIC ACTIVITY OF BETWEEN METHANOL AND ACETONE EXTRACT OF *LIMONIA ACIDISSIMA* L (FRUIT PEEL)

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Abstract

Limonia acidissima L. is the gathering of Rutaceae (Citrus family) which has a spot with the monotypic assortment Limonia, limited to Southeast Asia. It is locally familiar as kath bel. It is used widely as a folk medicine for several severe diseases such as heartburn, fart, loose bowels, diarrhea and hemorrhoids. For anthelmintic activity, methanolic and acetonic fruit peels extracts of *Limonia acidissima* L. was investigated for their action against *Paramphistomum cervi*. Different doses of each extract were examined in the bioassay, which included assurance of time of paralysis and time of death of the worms. The reference standard albendazole showed paralysis time 13.4 minutes and death time 18.4 minutes. On the other hand, methanolic and acetonic extracts showed at doses 150 mg/ml significant effect which has paralysis time 19.6 and 24.4 minutes, death time 21.6 and 26.6 minutes; respectively.

Keywords: *Limonia acidissima*, *Fruit peels, Anthelmintic activity*, *Albendazole, Paramphistomum cervi*

Introduction

Intestinal parasite infections is one of the most challenging issues nowdays, especially in developing countries and the prime sufferer is the children who were living in inadequate sanitation and poor hygiene [1]. The WHO enumerates that a tremendous two billion people harbor parasitic worm infections [2]. Helminthiasis is a disorder that is a part of the body is infested with worms like as pinworm, roundworm [3]. Helminth parasite mainly exists in the human body in the intestinal tract, however, they are also found in tissues like larvae. Most causes of illness worms are chronic and debilitating in nature. They probably cause more morbidity and more economic as well as social deprivation among humans and animals [4]. The development of anthelmintic drug-resistance in helminthes in opposition to synthetic pills had been stated in variety of countries which offers a clear indication that manipulate packages based exclusively on their use are not sustainable. most of the bug infections are limited to tropical areas, but these can also arise in individuals who have visited such inflamed regions after which inflamed in temperate regions. Several anthelminthics, inclusive of praziquantel and albendazole, are contraindicated for certain agencies of patients, including pregnant or lactating wo. This has led to the boom in hobby of ethno clinical practices across the world for the use of medicinal plant life in remedy of helminthic sicknesses [5].Plants are the giant source of medicines because they produce wide range of bioactive molecules, most of which as chemical defense against predation or infection [6].*Limonia acidissima* L. is the group of Rutaceae (Citrus family) which has a place with the monotypic variety Limonia, restricted to India, Pakistan, Sri Lanka and Southeast Asia. It is otherwise called wood apple, elephant-apple, kath bel [7].This plant parts are utilized as a medication for the treatment of a few issue. Wood apple is an erect, moderate developing tree with a couple of upward-achieving branches twisting outward close to the summit. The bark is furrowed, fissured and textured. The deciduous, exchange leaves, 3 to 5 in long, dim green, weathered, frequently minutely toothed. Yellowish green blossoms, tinged with red, 1/2 in over, are borne in little, free, terminal or horizontal panicles.The tree is for the most part known for its hard woody natural product, size of a tennis ball, round to oval fit as a fiddle. The mash is dark colored, coarse, foul, resinous, astringent, corrosive or sweetish, with various little, white seeds dissipated through it [8][9]. Particularly it is utilized for treating heartburn, fart, loose bowels, diarrhea and hemorrhoids. The bark is bitten with that of Barringtonia and connected on venomous injuries [10]. The goal of the present study is to assess anthelmintic movement of methanolic and acetonic concentrate of *Limonia acidissima* L. just as to basis the utilization in helminthiasis in society medication.

Methods

Plant Materials

The peels part of the fruit of *Limonia acidissima* L. were collected from near Jahangirnagar University fields, Dhaka, Bangladesh. The identification of the plant material was confirmed by the experts of Bangladesh National Herbarium, Mirpur, Dhaka.

Drying and grinding

The collected fruit peels were separated from undesirable materials. Then these were dried in for one week in the sunlight and these were cutting into small pieces. The fruit peels were converted into coarse powder by using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of methanol and acetone extracts

At first, two clean flat flat-bottomed glass containers was taken and added about 400 and 450gm of powdered sample into the container, respectively. Then 1500 ml of 90% methanol and 1800ml acetone were added into the two containers as well as soaked the powder into the methanol and acetone, respectively. Afterwards, containers were sealed with their contents and kept for a period of 10 days accompanying occasional shaking and stirring. After that, the coarse parts of the fruits were separated from the mixture by using white cotton. Then the liquid portion was also filtered three times with the help of white cotton. Then again, these were filtered through whatman filter paper. Then the filtrates were kept in Rotary

evaporator machine which separates solvent and desirable crude extracts was obtained.

Experimental worms

Live parasites *Paramphistomum cervi* (Trematoda) were collected from freshly slaughtered cattle at local abattoirs and identified by experts. After cleaning, parasites were stored in 0.9% phosphatebuffered saline (PBS) of pH 7.54 prepared with 8.01g NaCl, 0.20 g KCl, 1.78 g Na2HPO4 and 0.27 g KH2PO4 in 1 liter of distilled water at 37±1°C.

Phytochemical screening

Phytochemical screening of *Limonia acidissima* L. was carried out to identify the functional groups as described. [11-13**].**

Anthelmintic Activity

Preparation of sample

To prepare the suspension of methanolic and acetonic fruit peels extract of *Limonia acidissima* L. the concentrations of 50, 100 and 150mg/ml; respectively. 0.5, 1, 1.5 gm of extract were taken and triturated with Tween 80 as a suspending agent and final volume was made to 50 ml for respective concentration with PBS. For the preparation of standard albendazole at concentrations of 20 mg/ml; 200 mg of albendazole powder were taken and triturated with of Tween 80as a suspending agent and final volume was made to 10 ml for respective concentration with PBS[14][15].The anthelmintic activity of gynura procumbens was evaluated according to the method of Kratika et al 2010 [16]. The animals were divided into eight groups containing five earthworms. 10ml of control (Distilled water), standard (Albendazole) and both extracts of each concentration were taken in different petri dishes. Experimental five animal parasites of both types were taken in each different petri dish. The concentration of standard and extract were Standard Albendazole 20 mg/ml, both extracts 50, 100 and 150mg/ml. I recorded the time of paralysis when motion was not observed unless shaken violently. The death time wasrecorded after evaluating that the parasites did not move when shaken vigorously dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity is expressed as the time required for paralysis and death of parasites as compared to control [17].

Results

Result of phytochemical investigation

Result of Anthelmintic Potency

Discussion

Anthelmintic Potency

As appeared in Table 2, the methanolic and acetonic fruit peels extract of *Limonia acidissima* L. showed anthelmintic properties. The concentrates indicated fixation related anthelmintic exercises with every one of the worms utilized in the examination, with 150mg/ml giving a most brief time of paralysis (P) and death (D) for all the worm types. Death time of standard Albendazole is 13.4 min (20mg/ml). The outcomes from the table demonstrated that the methanolic extract showed a higher movement than the acetonic extract for all the worm types utilized. The preliminary phytochemical analysis of the extracts has shown the presence of phenolics, like tannins and flavonoids [18]. The capacity of most worm expellers like albendazole citrate is to cause loss of motion of worms with the end goal that they are ousted in the feaces of man and creatures. The concentrates not just shown this property, they additionally caused passing of the worms, particularly at 100 and 150mg/ml.

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Table 1: Result of phytochemical investigation.

Tested groups	Methanol extract	Acetone extract
Tannins	+	+
Phenols	-	+
Flavonoids	+	-
Saponins	-	+
Terpenoids	+	+
Gum	+	+
Alkaloids	+	+
Glycosides	+	+

(+) Indicates presence, (-) Indicates absence.

Table 2: Anthelmintic activity of methanolic and acetonic fruit peels extract of *Limonia acidissima* L.

Treatment	Concentration(mg/ml)	*Paramphistomum cervi*	
		Paralysis	Death
Control (Distilled water)	-	-	-
Standard (Albendazole)	20 mg/ml	13.4±1.52	18.4±1.67
Methanolic extract (ME)	50 mg/ml	25.6±2.97	47.8±3.70
	100 mg/ml	22.2±3.56	30.6±3.65
	150 mg/ml	19.6±1.81	21.6±2.30
Acetonic extract (AC)	50 mg/ml	32.6±3.65	58.2±4.97
	100 mg/ml	25.8±1.30	35.6±3.05
	150 mg/ml	24.4±1.14	26.6±2.07

All Values represent Mean± SD; n=5 in each group.

Figure 1: Anthelmintic activity of *Limonia acidissima* L. fruit peels extracts on *Paramphistomum cervi.*