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Phytochemical and *in vitro* Anthelmintic Activity of Aqueous Polyherbal Leaf Extract against Pheretima Postuma

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Abstract

The main aim and objective of the study was to evaluate the phytochemical screening and anthelmintic activity of aqueous polyherbal leaves extract (APLE) against Pheretima posthuma. The leaves of *Emblica Officinalis, Terminalia bellerica, Carica papaya, Citrus limon and Achras sapota* was identified and purchased from local market of Nuzvid. APLE was prepared from the dried leaves of five different plants using the solvent water. Initially, APLE was screened for phytochemical constituents by standard methods. Further, antihelmintic study was conducted against Pheretima posthuma, collected from local Vermicomposting Farm, Nuzvid. In the phytochemical screening, APLE showed presence of glycosides, alkaloids, flavonoids, phenols, phytosterols and tannins. In the anthelmintic study, mortality was produced in earth worm populations by APLE. The use of APLE as an anthelmintic was confirmed by using standard method against Pheretima posthuma. The results indicated that the test drug has significant anthelmintic properties. The data were found statistically significant by using one way ANOVA ($P< 0.0001$). Hence, it can be concluded that the APLE can be used as a novel drug for the treatment of worm infestations.

Keywords

Aqueous polyherbal leave extract (APLE), Phytochemical screening, *Pheretima posthuma*, Anthelmintic activity, Albendazole.

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INTRODUCTION:

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and has impact on global economic factor. The worms which cause helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintics. There are various types of worms such as hook worms, fluke worms, round worms, tape worms which causes helminthiasis. The names are given according to their shapes. The major organs which get affected in helminthiasis are stomach and intestine and major symptoms of sever helminthiasis include diarrhea, abdominal pain, general malaise, and impaired cognitive development. Chronic helminthiasis by hook worm led to intestinal bleeding and anemia. Pheretima is a genus of earthworms. *Pheretima posthuma* are long cylindrical shaped worms having length of 15-30cm. they are mostly found in moist soil and responsible

for vegetables and humus. Their life span is 3 to 10 years [1].

Emblica officinalis (Amla) is one of the most common medicinal herb used in ayurvedic proprietary medicines.

Emblica officinalis is also known as Phyllanthus emblica or Indian gooseberry, belonging to family Euphorbiaceae. E. officinalis has been used as medicine and nutritious tonic, possessing vital amino acids and vitamins. It is particularly a chief source of vitamin C and minerals compared to other citrus fruits. Although all parts of E. officinalis are used for medicinal purpose, the fruits are more widely used in rasayana, either alone or in combination with other traditional herbs for the treatment of many infectious and non-infectious diseases. [2].

Terminalia belerica Roxb. (Family *Combretaceae*) is a large deciduous tree with broadly elliptic leaves clustered at the ends of branches. It is wildly distributed throughout the world especially Indian subcontinent, Sri Lanka, Pakistan, Nepal, and Southeast Asia. *T. belerica* is used in traditional medicine due to the wide spectrum of pharmacological activities associated with the biologically active secondary metabolites present in this plant. Variety of phytochemicals are isolated from various parts of the plant which include alkaloid, coumarin, flavones, steroids, lignans, tannins, glycosides, terpenoid, saponin etc [3].

C. papaya L. belongs to the family Caricaceae and is commonly known as papaya, pawpaw, and kates. It is a perennial horticultural shrub originated from Mesoamerican Centre, Central America, and southern Mexico and is mainly cultivated in the tropical and subtropical regions of Brazil, Australia, Malaysia, China, India, Thailand, Myanmar, Philippines, and other adjoining. Papaya is not only cultivated for the ripe sweet fruit, even other parts of the plant such as seeds, leaves, roots, flowers, barks, and latex have been traditionally used worldwide for the preparation of various medicinal formulations . However, leaves have been emerged as one of the most useful parts with plethora of health-promoting compounds and activities [4].

Citrus limon (L.) Burm. f. is a tree with evergreen leaves and yellow edible fruits from the family Rutaceae. In some languages, C. limon is known as lemon (English), Zitrone (German), le citron (French), limón (Spanish), and níngméng (Chinese). The main raw material of C. limon is the fruit, particularly the essential oil and juice obtained from it. The C. limon fruit stands out as having well-known nutritional properties, but it is worth remarking that its valuable biological activities are underestimated in modern phytotherapy and cosmetology. C. limon fruit juice (lemon juice) has traditionally been used as a remedy for scurvy before the discovery of vitamin C. This common use of C. limon, known since ancient times, has nowadays been supported by numerous scientific studies. [5].

Achrus sapota L. is an important tropical fruit also grown in subtropical region of the world. *Sapota (Achras sapota L.)* belongs to the family Sapotaceae [6]. Sapota (*Achras sapota* L.) commonly known as chiku is mainly cultivated in India for its fruit value, while in South-East Mexico, Guatemala and other countries it is commercially grown for the production of chickle which is a gum like substance obtained from latex and is mainly used for preparation of chewing gum [7].

MATERIALS AND METHODS:

Collection of Plant Material:

The leaves are collected from the medicinal garden of Sri Siddhartha Pharmacy College,

Preparation of Extract:

The dried polyherbal leaves was collected, then dried leaves were powdered to get a coarse powder. The dried powder leaves were taken in beaker and add 1000 ml of distilled water. Then it was kept for maceration for 3 days. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used [8].

Preliminary Phytochemical Screening:

The preliminary phytochemical investigation was carried out with aqueous extract of polyherbal leaves for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods [9].

Test Organism:

Indian adult earthworms (*Pheretima posthuma)* were used during the experiment. The earthworms were collected from a local supplier. Worms were washed with normal saline to remove all fecal matter .The earthworms of 8-10 centimeter (cm) in length and 0.2 -0.5 cm width were used for all the experiment protocol. Ready availability, anatomical and physiological resemblance of (*Pheretima posthuma)* made it to be used initially for *in-vitro* evaluation of anthelmintic activity. Time for paralysis was noted either when any movement could not be observed except when the worms where shaken vigorously. Death was included when the worms lost their motility followed by white secretions and fading away of their body colour [10].

Evaluation of Anthelmintic activity:

The anthelmintic activity was evaluated on adult Indian earthworm. The earthworms were randomly

chosen and divided into five groups having five earthworms in each as follows:

Group I: Control Group

Group II: Standard Group – Albendazole [11] 50, 100, 200,300, 400mg/ml

Group III: Test-I -Aqueous extract of polyherbal leaves 50,100, 200,300,400mg/ml

Observations were made for the time taken by worms to paralyze and death was observed. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Death was ascertained by applying external stimuli which stimulate and induce movements in worms as well as fade of the body color was noted.

STATISTICAL ANALYSIS:

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between haloperidol group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 7.0.

RESULTS AND DISCUSSION:

In this study, we found that aqueous polyherbal leaves extract possess the following chemical constituents (Table 1).

Table 1: Phytochemical screening of APLE

Phytochemical constituents	Aqueous polyherbal leave extract [APLE]
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Phenols	+
Saponins	+
Terpenoids	+
Steroides	+
Tannins	+
Amino acids	–
Glycosides	+
Fixed oils and fatty acids	+

+ indicate the compulsory present and – indicate the absent.

Antihelmintic activity:

The aqueous polyherbal leaves extract produced a significant antihelmintic activity in dose dependent manner as shown in below table.

Table 2: Anthelmintic activity of aqueous polyherbal leaves extract (APLE) & Standard drug on earth worm

Groups	Concentration	Time Taken in minutes	
		Paralysis (P)	Death (D)
Control		-	-
Standard (Albendazole)	**50mg/ml**	261±0.97	283±1.30
	100mg/ml	217± 0.81	239±0.51
	200 mg/ml	133±0.73	147±0.91
	300 mg/ml	86±0.83	111±0.63
	400 mg/ml	72±1.07	82±1.12
Test-I [APLE]	**50 mg/ml**	190 ±0.51	215±0.20
	100 mg/ml	155±0.24	173±1.03
	200 mg/ml	115±0.24	127±0.83
	300 mg/ml	44±0.63	94±1.50
	400 mg/ml	36±0.58	71±0.97

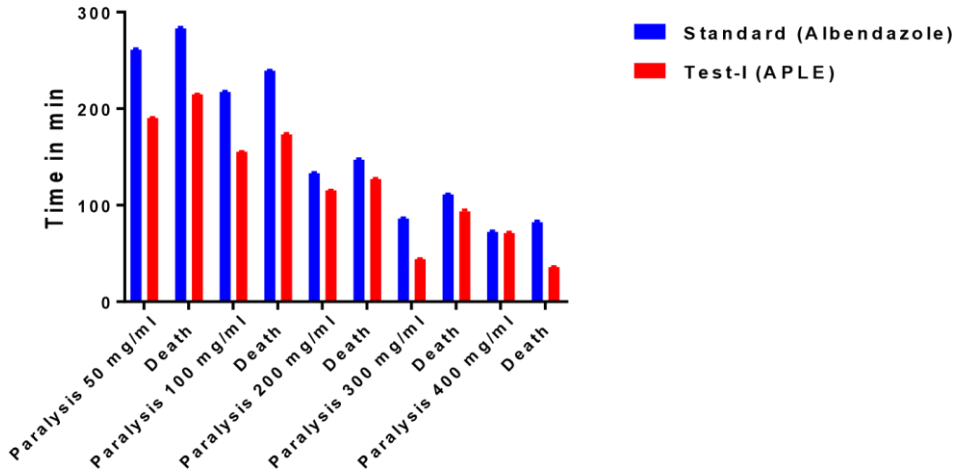

Figure 1: Anthelmintic activity of standard, APLE, Values are expressed as Mean ± SEM, P < 0.0001.

Figure 2: Standard (Albendazole) 50,100,200,300,400mg/ml- Paralysis

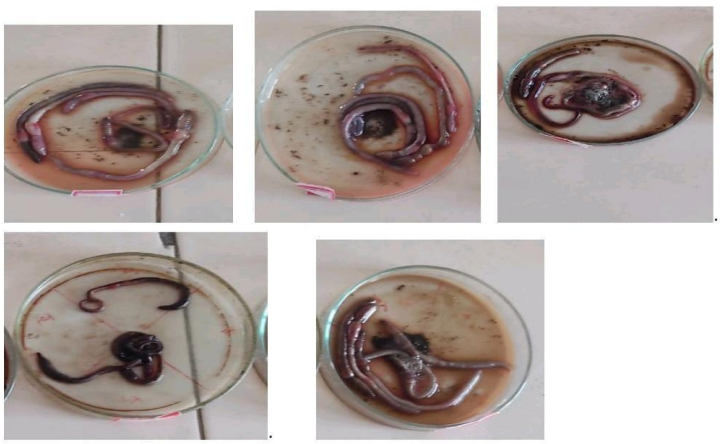

Figure 3: Standard (Albendazole) - 50,100,200,300,400mg/ml- Death

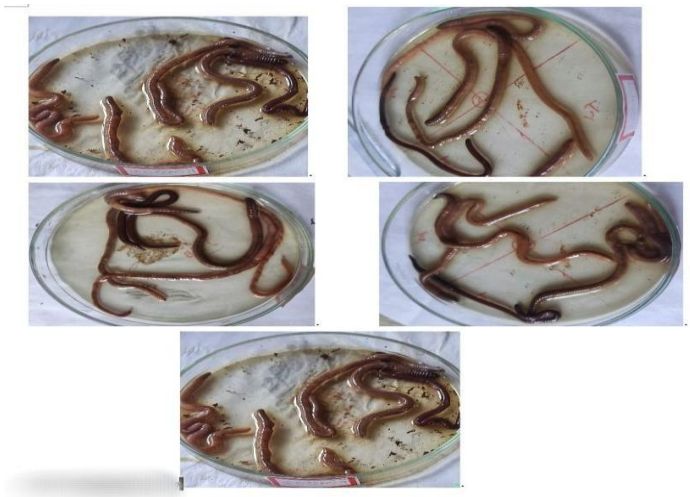

Figure 4: Test I (APLE) -50,100,200,300,400mg/ml-Paralysis

Figure 5: Test I (APLE) -50,100,200,300,400mg/ml- Death

CONCLUSION:

In the present investigation, aqueous polyherbal leaves extract possess the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, phenols, terpenoids, steroids, glycosides and fixed oils & fats. Tannins are chemically polyphenolic compound and where shown to produce anthelmintic activities and reported the effect of tannin can bind to free proteins in gastro intestinal tract of host animal or glycoproteins on the cuticle of parasite and may cause death. These facts suggest that tannins present in the aqueous polyherbal leaves extract showed the anthelmintic effect by above mentioned mechanisms. From the result shown in table-2 aqueous polyherbal leaves extract showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death. From the anthelmintic activity study, the aqueous polyherbal leaves extract at a dose of 400 mg/ml has significant anthelmintic activity where as 50mg/ml has showed moderate activity. The aqueous polyherbal leaves extract at normal concentration i.e. 50 mg/ml to higher concentration i.e. 400mg/ml showed good anthelmintic activity and this is compared with effect produced by reference standard drug albendazole. The aqueous polyherbal leaves extract demonstrated paralysis as well as death of worms in a less time as compared to albendazole especially at higher concentration of 400 mg/ml. The study finally concluded aqueous polyherbal leaves extract showed marked and potent anthelmintic activity than the standard drug albendazole.

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