Anthelmintic Activity of Shell Extract of Selected Seeds

Moushumi Baidya*, J. Anbu

*[1]*Department of Pharmacology, Faculty of Pharmacy, Brainware University, Barasat, West Bengal, India.*

[2]*Department of Pharmacology, Faculty of Pharmacy, Ramaiah University, Bangalore, Karnataka, India.*

Correspondence
Email- baidyamoushumi@gmail.com

Abstract

Objective: The study was undertaken to evaluate the phytochemical study and anthelmintic activity of ethanolic extract of polyherbal seed shells (EEPSS) against Eisenia fatida.
Methods: The seed of Momordica charantia, Manilkara zapota, Emblica officinalis and Syzygium cumini collected from the local market, Bangalore, India. EEPSS was prepared from the dried seed shells of four different fruits using the solvent ethanol. Initially, EEPSS was screened for phytochemical constituents by standard protocol. Further, anthelmintic study was conducted against Eisenia fatida, collected from local Vermicomposting Farm, Bangalore.
Results: In the phytochemical screening, the EEPSS showed presence of glycosides, alkaloids, flavonoids, phenols, phytosterols and tannins. In the anthelmintic study, mortality was produced in earth worm populations by EEPSS.
Conclusion: The use of EEPSS as an anthelmintic was confirmed by using standard method against Eisenia fatida. The results indicated that the test drug has significant anthelmintic properties. Hence, it can be concluded that the EEPSS can be used as a novel drug for the treatment of worm infestations.

Keywords: *EEPSS, Phytochemical screening, Eisenia fatida, Helminthiasis*

INTRODUCTION

As per World Health Organization (WHO), higher than 80% of the world's population mostly relies on traditional medicine for their primary health care needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Different medicinal plants used for traditional medicine which contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A huge knowledge about plants to use them against different illnesses may be expected to have deposited in areas where the use of plants is still of great importance [1]. The plant which has the medicinal value lies in some chemical substances that produce a certain physiological action on the human body.

The most essential compound of plants is bioactive viz. alkaloids, flavonoids, tannins and phenolic compounds [2]. Medicinal plants

were used as traditional medicine is well known in rural areas of developing countries was claimed by traditional healers. Low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections in developing countries [4]. Based on ethnopharmacological information the phytochemical research is commonly considered an effective approach in the discovery of new anti-infective agents from higher plants [5].

Helminthiasis is a disease which is most prevalent and serious public health problems worldwide. With the increased world interconnection between the developing countries, helminthes infections are now prevalent throughout the world, affecting millions of human and animals. Parasitic diseases causing severe morbidities in many parts of the world include lymphatic filariasis, onchocerciasis, and schistosomiasis [6].

Children of all ages are very much prone to parasitic infection. In day care settings infants, toddlers and very young children are at risk for the parasitic disease called giardiasis that causes diarrhoea and spread through defiled feces. Preschool and young school age children are also prone to pinworm infection or enterobiasis. In childhood days, preschool and school-age children can easily be infested with head lice (pediculosis) or scrabies which are spread by close person-to-person contact [7].

In the United States, several parasitic diseases occur occasionally and more frequently in developing countries but their effectiveness have not been well studied. Due to worm infection, they include strongyloidiasis that is of particular danger for children with an impaired immune system. It was happened when larvae (immature worms) in soil contaminated with infected human feces come into contact and penetrate the skin.

They also include visceral toxocariasis, when children ingest soil which is contaminated with dog or cat feces containing the eggs of [3]. Traditional medicines are cheaper and more effective than modern medicine which cat or dog roundworms, and cutaneous larva migrans, spread when children walk barefoot on soil contaminated with cat or dog hookworm larvae that penetrate the skin.

The synthetic drugs are used to cure the disease but due to their toxicity and adverse effects use of herbal remedies predominates. The present study is mainly to focus on the process of extraction and evaluation of anthelmintic activity of EEPSS.

Literature survey data unfold that the seed shell extract used in this study were not yet to be screened for its anthelmintic activity. So the purpose of this work was to explore the anthelmintic properties of selected seed shell extract.

EXPERIMENTAL SECTION

Materials and methods:

Drugs and chemicals

Ethanol (Changshu Yangyuan Chemical), Albendazole (MAK Pharma).

Plant Material:

The fruits of Momordica charantia, Manilkara zapota, Emblica officinalis and Syzygium cumini were collected from the local market, Bangalore, India. The well ripened fruits were selected, seeds were isolated and kept for drying. The shells of seed were collected and powdered for further process of extraction.

Preparation of Ethanolic Extract

100 g (25g/each) mixture of four different seed shells was macerated with 400 ml of ethanol for 72 hours with intermittent shaking. Then it was filtered through Whatmann No.1 filter paper, dried until a constant dry weight of extract obtained and the residue was stored at 40 C for further screening of anthelmintic activity.

About Earth worm:

Indian earth worms, Eisenia fatida were collected from local Vermi composting Farm, Bangalore.

Table-1 Photochemical screening of EEPSS

Test	Result
1. Detection of glycosides a) Legal's Test	+(ve), glycosides present
2. Detection of flavonoids a) Lead acetate Test	+(ve), flavonoids present
3. Detection of alkaloids a) Hager's Tests	+(ve), alkaloids present
4. Detection of phenols a) Ferric Chloride Test	+(ve), phenols present
5. Detection of saponins a) Foam Test	-(ve), saponins absent
6. Detection of phytosterols a) Salkowski's Test	+(ve), phytosterols present
7. Detection of carbohydrates a) Fehling's Test	-(ve), carbohydrates absent
8. Detection of tannins a) Gelatin Test	+(ve),tannins present

Phytochemical screening

The EEPSS obtained by standard extraction procedure were evaluated for their anthelmintic activity as well as presence of various phytochemical constituents.

Anthelmintic activity

EEPSS was investigated for their anthelmintic activity against Eisenia fatida of each extract was tested in the bioassay to determine the of time of paralysis and time of death of the worms. Albendazole was included as standard reference and distilled water as control. The anthelmintic assay was carried as per the method of Ajaiyeoba et al., with minor modifications [8]. Due to worm's anatomical and physiological resemblance with the intestinal roundworm parasite of human beings, the assay was performed on adult Indian earthworm [9-12]. Earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro because of its easy availability [13-15].

Indian adult earthworms (Eisenia fatida) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The length and width of earthworms were 3-5 cm and 0.1-0.2 cm used for anthelmintic study. In the first set of experiment, six groups of six earthworms were released in to 50 ml of solutions of Albendazole, ethanolic extracts of seeds (25, 50, 100 and 200 mg/ml each) in distilled water. Albendazole was used as reference standard while distilled water as control. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could

be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Fig 1- Anthelmintic activity of EEPSS

i - Control, **ii** - Standard (100mg/ml), **ii**i - Standard (200mg/ml), **iv** - Test-I (12.5mg/ml), **v** - Test-II (25 mg/ml), **vi** - Test-III (50 mg/ml), **vii** - Test-IV (100 mg/ml), **viii** - Test-V (200 mg/ml)

Table-2 Anthelmintic activity of EEPSS

Extracts	**Concentration mg/ml**	***Eisenia fatida***	
		Time taken for Paralysis (min)	**Time taken for Death (min)**
Control	---	---	---
Alcoholic seed shells extract	12.5	377.16 ± 3.219	Not died
	25	308.83 ± 5.089	611.5 ± 3.677
	50	232.833 ± 5.57	490 ± 4.99
	100	86.66 ± 2.29	201.66 ± 3.116
	200	71.66 ± 2.17	160.83 ± 4.89
Albendazole	100	260 ± 3.612	Not died
	200	141.5 ± 6.054	Not died

RESULTS AND DISCUSSION

Phytochemical analysis of EEPSS of the screened plants were done for the presence or absence of active secondary metabolites or different constituents such as glycosides, alkaloids, flavonoids, phytosterols, carbohydrates, proteins, phenol's and saponins. Preliminary phytochemical screening of alcoholic extract revealed the presence of glycosides, alkaloids, flavonoids, phenols, phytosterols, tannins etc (table no. 1).

From the anthelmintic activity study, the alcoholic extract at a dose of 100 mg/ml has significant anthelmintic activity where as 50mg/ml has showed moderate activity.

From the results shown in table no. 2, the predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis.

Albendazole is a benzimidazole carbamate drug. Benzimidazoles selectively bind to nematode ß-tubulin, inhibiting polymerization, thus preventing the formation of microtubules and so stopping cell division. Impaired uptake

of glucose, leading to depletion of glycogen, and reduced stores of ATP has also been noted.

The alcoholic seed extract demonstrated paralysis as well as death of worms in a less time as compared to albendazole especially at higher concentration of 200 mg/ml. Phytochemical analysis revealed presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity [16]. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [17].

CONCLUSION & FUTURE DIRECTIONS

In conclusion, the use of EEPSS as an anthelmintic has been confirmed as it displayed activity against the worms used in this study. The results suggest that ethanol extract of seeds possess anthelmintic properties which can be used as new drug for therapy of worm infestation. Further studies required to establish the mechanisms of action.

ACKNOWLEDGEMENT

Authors are grateful to Dr. V. Madhavan, Dean, Faculty of Pharmacy, M.S.R.U.A.S., Bangalore, for providing all the facilities to carry out this research work.

References

[1] Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. J Pharmaceutical Biology, 1999; 37: 80-91.

[2] Sandhu DS, Heinrich M. The use of health foods, spices and other botanicals in the Sikh community in London, J Phytotherapy Research, 2005; 19 (7): 633-642.

[3] Gupta MP, Solis PN, Calderon AI, Guionneau-Sinclair F, Correa M, Galdames C, et al. Medical ethnobotany of the Teribes of Bocas del Toro, Panama, J of Ethnopharmacology, 2005; 96: 389-401.

[4] Rojas JJ, Ochoa VJ, Ocampo SA, Muñoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine, A possible alternative in the treatment of non-nosocomial infections, BMC Compelmentary and Alternative Medicine, 2006;6: 2.

[5] Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L, Antibacterial screening of some Peruvian medicinal plants used in Callería District, J of Ethnopharmacology, 2005; 99: 309.

[6] Stoll N.R, This wormy world. J. Parasitol,1999; 85: 392.

[7]www.cdc.gov/parasites/children.htmlviewed on 21th august 2016.

[8] E.O. Ajaiyeoba, P.A. Onocha & O.T. Olarenwaju. In vitro Anthelmintic Properties of Buchholzia coriaceae and Gynandropsis gynandra Extracts, J Pharmaceutical Biology, 2001; 39 (3), 217-220

[9] Vidyarthi RD, A Text Book of Zoology, fourteenth ed., S. Chand and Co, New Delhi, 1967.

[10] Thorn GW, Adams RD, Braunwald E, Isselbacher KJ, Petersdorf RG, Harrison's Principles of Internal Medicine, McGraw Hill Co, New York, 1977.

[11] Vigar Z, Atlas of Medical Parasitology, second ed., P.G. Publishing House, Singapore, 1984.

[12] Chatterjee KD, Parasitology, Protozoology and Helminthology, sixth ed., Guha Ray Sree Saraswaty Press Ltd, Calcutta, 1967.

[13] Sollmann T, Anthelmintics. Their efficiency as tested on earthworms, J Pharmcol Exp Ther, 1918;12: 129-170.

[14] Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB. Evaluation of Evolvulus alsinoids Linn, For anthelmintic and antimicrobial activities, J Nat Rem, 2002; 2 :182-185.

[15] Szewezuk VD, Mongelli ER, Pomilio AB, Antiparasitic activity of Melia azadirach growing in Argentina, Molecular Med Chem, 2003;1: 54-57.

[16] Bate-Smith EC, The phenolic constituent of plants and their taxonomic significance, dicotyledons. J Linn Soc Bot, 1962; 58: 95.

[17] Martin RJ, Mode of action of anthelmintic drugs, Vet J., 1997; 154: 11-34.