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In-Vitro Study of Anthelmintic Activity of Polyherbal Extracts Against Pheretima Postuma

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ABSTRACT
Poly-herbal extracts of *Zingiber officinale* rhizome and *Punica granatum* peels were taken for *in-vitro* studies on the Anthelmintic activity against *Pheritima posthuma*. Equal amount of *Zingiber officinale* rhizome and *Punica granatum* peels mixed dried coarse powdered were extracted with Aqueous, Hydroalcohol 50:50 ratio, Hydroalcohol 20:80 ratio, Methanol and Ethyl acetate prepared by Maceration method for 72hrs and the same were evaluated for Anthelmintic activity with various concentrations such as 10,20,40,80,100 mg/ml. Albendazole concentrations 10,20,40,80, 100 mg/ml were used as a reference standard and distilled water was used as control. The results were expressed in paralysis and death time of the earthworms in minutes. The results obtained from the study indicate ethyl acetate was showing more significant Anthelmintic property compared to other extracts and also more effective than the standard. The results concluded that Polyhedral extracts of Ethyl acetate was more potent candidature as compared with standard drug albendazole.
KEYWORDS: Polyherbs, Maceration, Anthelmintic, paralysis time, Death time

I. INTRODUCTION

Helminthiasis is widespread worldwide (1/3rd of the world's population harbors them), but is more common in poorer personal and environmental hygiene developing countries. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes.

Several infestations are not infrequent in the same adult, GIT is the adobe of many helminths in the human body, but some also live in tissues or migrate their larvae to tissues. By depriving the host of nutrients, causing blood loss, damage to organs, intestinal or lympathetic obstruction, and secreting toxins they affect the host. Helminthiasis is seldom lethal, but it is an important cause of ill health.

It has been estimated that about half of the world's population suffers from *Helminthiasis* and the number is increasing day by day. It is not only limited to tropical and subtropical countries but is also to endemic in many regions because of poor sanitation, poor family hygiene, malnutrition and crowded living condition [1]. Potent anthelminitics are available today, and treatment is frequently done by using different types of drugs. However the high costs of modern anthelmintics have limited effective control of the parasites. In some cases, wide spread use of low quality anthelmintics are used for the development of resistance and hence causes reduction in use of anthelmintics [2]. Only few of plants are being used traditionally as anthelmintics e.g. *Aloe barberi, Trachyspermum ammi, Annona senegalensis* [3].

Anthelmintics are drugs that are used to treat infections with parasitic worms. This includes both flat worms, e.g., flukes and tapeworms and round worms, i.e., nematodes. Anthelmintics are medicines in which helminths are either destroyed (vermicide) or removed (vermifuge). Infections with helminths, or parasite worms, affect more than two billion people worldwide. They are of huge importance for human tropical medicine and for veterinary medicine. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections [4].

Despite the prevalence of parasitic worms, anthelmintic drug discovery is the poor relation of the pharmaceutical industry. The simple reason is that the nations which suffer most from these tropical diseases

have little money to invest in drug discovery or therapy. However, increasing problems of development of resistance in helminths [5,6] against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics [7,8]. A number of medicinal plants have been used to treat parasitic infections in man and animals [9,10]. So the present study revealed the comparative and poly herbal formulation of plant extract against the Anthelmintic activity.

II. MATERIAL AND METHODS

Methods of collection of punica granatum (pomegranate):-

The plant Punica granatum (pomegranate) was bought from the market of Paramathi Vellore, Namakkal district. 5kgs of pomegranate fruit was bought and peeled off. The peel was dried in shade for one week and it was powdered. Half kg of pomegranate peel powder was obtained.

Methods of collection of Zingiber officinale (ginger):-

The fresh rhizomes of Zingiber officinale (ginger) was bought from market of Paramathi Vellore, Namakkal district. 2kgs of ginger was bought and dried under shade for one week. It was powdered and half Kg ginger powder was obtained.

Methods of collection of Pheritoma posthuma (earthworm):

The appropriate time for the collection was found early in the morning in the summer, and noontime during the winter. Freshly collected alive worms were stored in the plastic bags, filled with suitable quantity of wet compost soil.

Materials:

- Methanol bought from lark chem.
- Ethyl acetate bought from Molychem.
- Dimethylsulfoxide bought from Molychem,
- Albendazole bought from Medihome Pharma.

Preparation of extracts

The collected Zingiber officinale rhizomes and punica granatum peels were shade dried completely. The rhizomes and peels was then coarsely powdered and mixed equally. The 100gm powder material extracted with Aqueous, Hydroalcohol (50:50), Hydroalcohol (20:80), Methanol, and Ethyl acetate. All the extracts were prepared by using Maceration method for 72hours. Then the filtered solution dried using water bath at 60-70^{0}c.

Preliminary phytochemical screening

The fractions of polyherbal extraction of *Zingiber officinale* and *Punica granatum* was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc. [11]

Evaluation of Anthelmintic activity

An indian earthworm 4 - 5 cm in length and 0.1 - 0.2 cm in width were used for the in-vitro anthelmintic bioassay. Because of easy availability, earthworms have been widely used for the initial evaluation of anthelmintic compounds in- vitro[12]. The anthelmintic assay was carried out as per the method of Ajaiyeoba et al[13]. The assay was performed in vitro using adult earthworm (pheritima posthuma) owing to its anatomical and physiological resemblance with the intestinal roundworms parasites of human being for preliminary evaluation anthelmintic activity [14-16]. The earthworms were divided into 6 groups containing six worms in each group.

Group I : Received 1% of v/v of DMSO (Dimethyl sulphoxide).
Group II: Received Albendazole at different concentration of 10-100mg/ml.
Group III : Received aqueous extract at different concentration of 10-100mg/ml
Group IV :Received Hydroalcohol (50:50) extract at different concentration of 10-100mg/ml (PHHYALE 50:50)
Group V: Received Hydroalcohol (20:80) extract at different concentration of 10-100mg/ml (PHHYALE 20:80)
Group VI : Received Methanol extract at different concentration of 10-100mg/ml (PHME)
Group VII : Received Ethyl acetate extract at different concentration of 10-100mg/ml (PHEAE)

20 ml of freshly prepared polyherbal formulation of different concentrations and different extracts were poured into petri-dishes. The worms were washing with saline and released into the petri-dish and the time taken for the worms to get paralysed and death was noted.

Observations were made for the time taken for paralysis were noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time of death of worms were recorded often ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0 C).

STATISTICAL ANALYSIS:

Experimental data are expressed as Mean+Standard Error of Mean (SEM). Standard analysis was performed by one way ANOVA followed by Dunnet's method of multiple comparisons was employed using GraphpadInstat 3.0 software. Data were considered significant at $p<0.05$, $p<0.01$, $p<0.001$.

III. RESULTS

Preliminary phytochemical screening

The preliminary phytochemical analysis of fractions of Polyherbal extract of *Zingiber Officinale* and *Punica Granatum* shows presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate.

Table 1: Phytochemical screening of Polyherbal extract of *Zingiber Officinale* and *Punica Granatum*

Extracts	Steroids	Alkaloids	Glycosides	Saponin	Flavonoid	Tannin	Carbohydrates	Phenolic compounds
Aqeuous	+	+	+	+	+	+	+	+
Hydroalcohol (50:50)	+	+	+	+	+	+	+	+
Hydroalcohol (20:80)	+	+	-	-	+	+	-	+
Methanol	+	+	-	-	+	+	-	+
Ethyl acetate	-	-	+	-	+	+	-	+

+ = Present, - = Absent

Anthelmintic activity

The results of anthelmintic activity are shown in Table and Figure shows the Ethyl acetate extract showed more significant paralysis as well as death time at all concentrations compared to standard. Aqueous extract & Hydroalcohol (50:50) extract has anthelmintic activity from 10,20,40,80 & 100 mg/ml shows effect but it is not significant when compared to standard drug albendazole. The Hydroalcohol (20:80) extract among all the concentrations 80 and 100 mg/ml revealed as like as standard drug. In Methanol extract of 20 mg/ml and 40 mg/ml revealed as like as standard and the 80mg/ml and 100 mg/ml exhibited the more potent anthelmintic activity when compared with Albendazole but the Ethyl acetate extract of 10,20,40,80, and 100 mg/ml exhibited the potent anthelmintic activity when compared with aqueous extract. Polyherbal EAE is showed more potent activity than the ME, HYALE (20:80) and Aqueous was less significant effect when compared to all other polyherbal extraction.

Table 2: In vitro anthelmintic activity of Different extracts of Polyherbal Formulation

Groups	Treatment	Concentration/ml	Paralysis time in Minutes	Death time in minutes
Group I	Control	20 ml	0	0
Group II	Albendazole	10mg	52.6 ± 0.50^{a}	77.40 ± 0.74^{a}
		20mg	43.4 ± 0.67^{a}	62.00 ± 0.83^{a}
		40mg	22.2 ± 0.37^{a}	31.40 ± 0.50^{a}
		80mg	17.1 ± 0.37^{a}	26.20 ± 0.91^{a}
		100mg	11.3 ± 0.71^{a}	20.90 ± 0.46^{a}
Group III	Aqueous Extraction	10mg	163.5 ± 3.31^{d}	176.9 ± 3.39^{d}
		20mg	151.2 ± 1.87	176.8 ± 0.76^{d}
		40mg	120.9 ± 2.21^{d}	138.3 ± 1.86^{d}
		80mg	111.7 ± 2.63^{d}	137.2 ± 3.40^{d}
		100mg	99.2 ± 3.24^{d}	123.2 ± 0.86^{d}
Group IV	Hydro-alhocolic Extraction (50:50)	10mg	151.08 ± 3.63^{d}	171.1 ± 2.26^{d}
		20mg	135.9 ± 3.21^{d}	164.1 ± 1.54^{d}
		40mg	90.9 ± 1.81^{d}	125.3 ± 8.94^{d}
		80mg	60.6 ± 2.18^{c}	92.60 ± 3.05^{c}
		100mg	31.5 ± 2.79^{c}	59.50 ± 2.89^{c}
Group V	Hydro-alcoholic Extraction(20:80)	10mg	97 ± 9.73^{c}	159.9 ± 11.46^{c}
		20mg	55.5 ± 1.92^{c}	139.8 ± 0.91^{c}
		40mg	49.05 ± 0.40^{c}	90.9 ± 1.81^{c}
		80mg	21.8 ± 2.46^{a}	37.75 ± 1.82^{a}
		100mg	12.5 ± 0.77^{a}	30.3 ± 1.95^{a}
Group VI	Methanolic Extraction	10mg	90.5 ± 21.05^{c}	142.1 ± 13.19^{c}
		20mg	47 ± 3.61^{a}	133.1 ± 12.41^{c}

		40mg	44±1.47[c]	119±5.81[c]
		80mg	14.6±1.53[b]	34.7±1.97[a]
		100mg	8.01±0.52[b]	21.7±1.05[a]
Group VII	Ethyl acetate Extraction	10mg	23.2±0.45[b]	41.2±1.99[b]
		20mg	14.21±0.48[b]	31.07±1.88[b]
		40mg	14.03±0.40[b]	23.3±1.06[b]
		80mg	7.31±0.19[b]	14.4±0.56[b]
		100mg	6.5±0.50[b]	11.4±0.60[b]

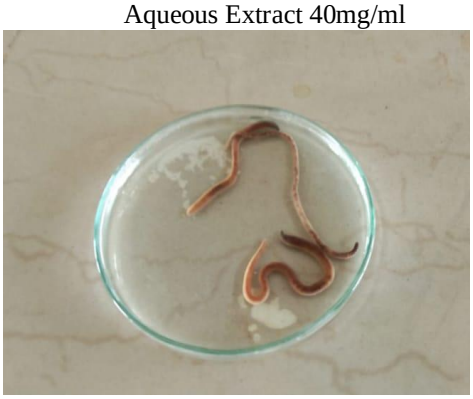
All values are expressed as mean ± S.E.M.; (n=6) animals in each group. 'a' compared to b, c & d. b=*P<0.5, c=**P<0.01 d=***P<0.001. Mean bearing same superscripts do not differ significantly. Mean bearing different superscript differ significantly.

Figure 1: Anthelmintic activity of different extracts of poly-herbal formulation

Aqueous Extract 10mg/ml

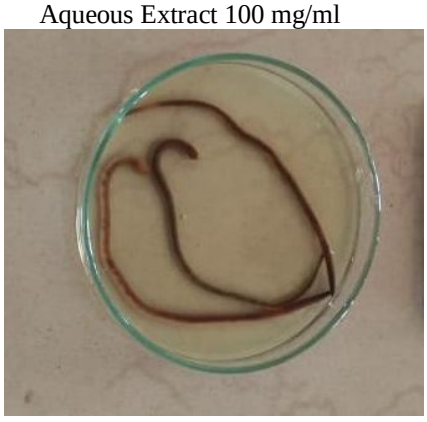
Aqueous Extract 20mg/ml

Aqueous Extract 40mg/ml



Aqueous Extract 80mg/ml

Aqueous Extract 100 mg/ml



Hydroalcoholic Extract (50:50) 10mg/ml

Hydroalcoholic Extract (50:50) 20mg/ml

Hydroalcoholic Extract (50:50) 40mg/ml

Hydroalcoholic Extract (50:50) 80mg/ml

Hydroalcoholic Extract (50:50) 100 mg/ml

Hydroalcoholic Extract (20:80) 10mg/ml

Hydroalcoholic Extract (20:80) 20mg/ml

Hydroalcoholic Extract (20:80) 40mg/ml

Hydroalcoholic Extract (20:80) 80mg/ml

Hydroalcoholic Extract (20:80) 100mg/ml

Methnol Extract 10mg/ml

Methnol Extract 20mg/ml

Methnol Extract 40mg/ml

Methnol Extract 80mg/ml

Methnol Extract 100mg/ml

Ethylacetate extract 10mg/ml

Ethylacetate extract 20mg/ml

Ethylacetate extract 40mg/ml

Ethylacetate extract 80mg/ml

Ethylacetate extract 100mg/ml

Albendazole 10mg/ml

Albendazole 20mg/ml

Albendazole 40mg/ml

Albendazole 80mg/ml

Albendazole 100mg/ml

IV. DISCUSSION

Preliminary phytochemical screening of polyherbal extract revealed the presence of steroids while, methanol, ethyl acetate, hydro alcohol and aqueous extracts showed the presence of glycosides, alkaloids, flavanoids, tannins and phenolic compounds. The predominant effect of albendazole on primary action is binding to beta tubulin and thus inhibition of microtubule polymerisation. More specific to parasites beta tubulin than that of host. Immobilisation and death of parasites occur slowly and they produce many biochemical changes in susceptible nematodes inhibition of mytochondrial fumarate reductase, reduced glucose transport and uncoupling of oxidative phosphorylation. The Methanol, Ethyl acetate, Hydro alcohol and Aqueous extracts of polyherbal formulation demonstrated paralysis as well as death of worms in a less time as compared to albendazole especially at higher concentration of 100 mg/ml. Phytochemical analysis of the crude extracts revealed presence of flavanoids, tannins and phenolic compounds as one of the chemical constituent. Earlier studies proved that the polyphenolic compounds show anthelmintic activity [17] and some synthetic phenolic

anthelmintics example. Niclosamide, Oxyclozanide and Bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[18]. Polyphenols from bryophytes were shown to a anthelmintic activity against *Nippostrongylus brasiliensis*[19]. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal [20] or glycoprotein on the cuticle of the parasite [21] and cause death.

So the above point supported the anthelmintic activity of Methanol, Ethyl acetate, Hydro alcohol and aqueous extracts of polyherbal formulation might be due to phytoconstituents like flavanoids, tannins and phenolic compound. The mechanism of ME and EAE interfere with polymerisation of tubulin and energy production in helminthiasis.

V. CONCLUSION

The anthelmintic/wormicidal activity of various Polyherbal extracts of of *Zingiber Officinale* and *Punica Granatum* suggests that it is effective against parasitic infections of humans mainly poly-herbal ethylacetate extract is significant. Further, study necessary toidentify and isolate the possible active phytoconstituents responsible for the anthelmintic activity.

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