

Research Article

Phytochemical Investigation and Screening of *In vitro* Anthelmintic Activity of *Plectranthus Amboinicus* Leaves Extracts

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ABSTRACT

Alcoholic and aqueous extracts from the leaves of *Plectranthus amboinicus* (Family- Lamiaceae) were investigated for their anthelmintic activity against adult Indian earthworms *Pheretima posthuma* and *Ascaridia galli*. Various concentrations (10-100 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control group. Both the extracts elicited significant anthelmintic activity at highest concentration of 100 mg/ml which was comparable to that of standard drug. The total phenolic and flavonoid contents were investigated in alcoholic and aqueous extracts of leaves of *Plectranthus amboinicus*.

Keywords: *Plectranthus amboinicus*, *Pheretima posthuma*, *Ascaridia galli*, Piperazine citrate, Anthelmintic, Phenolic content and Total flavonoid.

INTRODUCTION

Helmenthiasis is prevalent globally, but is more common in the developing countries with poorer personal and environmental hygiene. In the human body gastrointestinal tract is the abode of many helminthes, but some also live in tissue. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins¹.

Many humans harbor *Helminthes* (worms) of one species or another. In some cases infection results in discomfort and do not cause substantial ill health. The example being thread worm in children other worm infections, such as cytosomiasis (Bilharzias) and hook worm disease, can produce very serious morbidity². Infections with helminthes or parasitic worms, affect more than two billion people world wide³.

Helmenthiasis is among the most important animal diseases inflecting heavy production losses. The disease is highly prevalent particularly in third world countries⁴. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. The resulting economical damage demonstrated the urgent need for alternative method to reduce the worm burden in an animal. Plants have been used from ancient time to cure diseases of man and animals⁵.

Plectranthus amboinicus (Lour).Spreng (*Colens amboinicus* Lour) (*Colens aromaticus* Benth) belongs to family Lamiaceae (Tulsi-kulam). The plant is known in

different languages such as English-Country borage, Indian borage, Kannada-Karpurahalli, Sanskrit-Karpuravalli, Sugandhavalakam, Hindi-Patta ajavayan, Pathacur, Malyalam-Kannikkaurkka. It is distributed all over India⁶. The survey of literature reveals that the medicinal plant *Plectranthus amboinicus* leaves are used traditionally as carminative, digestive, expectorant, anthelmintic, diuretic and liver tonic. The leaves are also useful in dyspepsia, flatulence, and cholera especially in children, epilepsy, chronic asthma, hicough, bronchitis, renal and vesicle calculi, hepatopathy and malarial fever⁶.

The survey of the literature reveals that the leaves of *Plectranthus amboinicus* are found to be used traditionally for anthelmintic activity. However, the anthelemintic activity of leaves of *Plectranthus amboinicus* has not been scientifically investigated, hence the present study is undertaken for phytochemical investigations of leaves of *Plectranthus amboinicus* and to evaluate its traditionally claimed anthelmintic property.

MATERIAL AND METHODS

Plant material

The leaves of *Plectranthus amboinicus* were collected from Dhule, Maharashtra during May/June 2009. The plant was authenticated from Botanical Survey of India, Pune and a voucher specimen was deposited at the Department of Pharmacognosy, A.R.A. College of Pharmacy, Dhule. (The Voucher specimen no-

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Table 1. Analysis of shade dried powdered leaves of *Plectranthus amboinicus*

Sl. No.	Parameters	Observation
01	Ash values:	
	Total ash	12.5 % w/w
	Acid insoluble ash	0.9 % w/w
02	Water soluble ash	10.2 % w/w
	Extractive value:	
03	Alcohol soluble	1.6 % w/w
	Water soluble	2.0 % w/w
	Loss on drying	6.0 % w/w

BRKPA1). After authentication, leaves were subjected for the observation of macroscopic parameters viz. colour, odour, taste, ash value, extractive value and moisture content of leaves of *Plectranthus amboinicus*⁷.

Table 2: Total phenolic content of the alcohol and aqueous leaf extracts of *Plectranthus amboinicus*

Sr. no	<i>Plectranthus amboinicus</i>	
	Alcohol extract (Mg/g of extract)	Aqueous extract (Mg/g of extract)
01	216 ± 5.1	210 ± 2.2

Preparation of extracts

In the present study, the shade dried leaves were powdered and about 180-200 gm of powdered material was subjected to exhaustive continuous hot extraction with alcohol 90% in succession using Soxhlet apparatus. After the effective extraction, the solvent was distilled off. The extract was then concentrated on water bath and finally reduced to dryness. Maceration process was used for aqueous extraction whereas chloroform water (I.P 1996) was used as solvent for maceration. After drying, the extract was weighed and yield was recorded⁸.

Table 3: Total flavonoid content of the alcohol and aqueous leaf extracts of *Plectranthus amboinicus*

Sr. no	<i>Plectranthus amboinicus</i>	
	Alcohol extract (Mg/g of extract)	Aqueous extract (Mg/g of extract)
01	35 ± 2.8	59 ± 3.0

Phytochemical investigation

The alcohol and aqueous extract of leaves of *Plectranthus amboinicus* were subjected to qualitative chemical investigation such as test for alkaloids, glycosides, flavonoids, tannins, steroids etc⁷.

Determination of Phenolic content⁹

The alcohol and aqueous extracts of each plant material (100 µl) were mixed with 0.2 ml Folin-Ciocalteu reagent, 2 ml of water, and 1 ml of 15 % Sodium carbonate, and absorbance of the mixture was measured at 765 nm after 2 h at room temperature. The mean of the three readings was used and the total phenolic content was expressed in milligram of gallic acid

equivalents/1 g extract. The coefficient of determination was $r^2 = 0.9958$.

Determination of Total Flavonoids¹⁰

The flavones and flavonols in alcohol and aqueous extracts of each plant material were expressed as quercetine equivalent. Quercetine was used to make the calibration curve (0.04, 0.02, 0.0025 and 0.00125 mg/ml) in 80 % ethanol (v/v). The standard solutions or extracts (0.5 ml) were mixed with 1.5 ml 95 % ethanol (v/v), 0.1 ml 10 % aluminium chloride (w/v), 0.1 ml of 1 mol/L Sodium chloride was substituted by same volume of distilled water in blank. The absorbance of reaction mixture was measured at 415 nm. The mean of the three readings was used and the total flavonoid content is expressed in milligram of quercetine equivalents/1 g extract. The coefficient of determination was $r^2 = 0.9961$.

Evaluation of In Vitro Anthelmintic Activity

Animal selection

Pheretima posthuma (Annelida), commonly known as earthworm collected from the water logged areas and *Ascaridia galli* (nematode) worms were obtained from freshly slaughtered fowls (*Gallus gallus*). Both the worm types were identified at P.G.Department of Zoology, S.S.V.P.S College, Dhule.

The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Because of easy availability, earthworms have been used widely for initial evaluation of anthelmintic compounds *invitro*^{11, 12, 13, 14}. *Ascaridia galli* worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic drug was advocated earlier^{15, 16, 17}.

Method of screening

Fifty milliliter of formulation containing three different concentrations, each of crude alcoholic and aqueous extract (10, 50 and 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both types of worms. Time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as control¹⁸.

STATISTICAL ANALYSIS

Results were reported as Mean ± S.D. for determination of significant intergroup difference each parameter was analysed separately and one-way analysis of variance (ANOVA) was carried out¹⁹.

RESULTS

Pharmacognostical Investigation

The pharmacognostic study revealed that the plant is a stout monoecious leafy shrub, 0.9 to 2 m high. The plant is a large succulent aromatic perennial herb with hispidly

Table 4: Anthelmintic activity of alcohol and aqueous extracts of *Plectranthus amboinicus* leaves.

Test Substance	Concentration (mg/ml)	Time taken for paralysis (P) and death (D) of worms in min.			
		<i>Pheretima posthuma</i>		<i>Ascaridia galli</i>	
		P	D	P	D
Control	-	-	-	-	-
Alcoholic extract	10	23 ± 0.1	63 ± 0.4	16 ± 0.6	45 ± 0.1
	50	16 ± 0.4	43 ± 0.6	08 ± 0.8	33 ± 0.5
	100	10 ± 0.2**	28 ± 0.8**	28 ± 0.8**	29 ± 0.6**
Aqueous extract	10	25 ± 0.1	66 ± 0.3	17 ± 0.2	48 ± 0.6
	50	18 ± 0.7	48 ± 0.2	10 ± 0.6	36 ± 0.9
	100	09 ± 0.8**	30 ± 0.1**	06 ± 0.6**	27 ± 0.2**
Piperazine extract	10	21 ± 0.2	59 ± 0.6	12 ± 0.01	41 ± 0.4

Results are expressed as mean ± SEM from six observations, **Significant value. **P<0.01, *P<0.5 considered as significant value.

villous or tomentose fleshy stem. Leaves are simple, opposite, broadly ovate, crenate, fleshy and very aromatic. Leaves are sinuate-toothed, lower large, 15-30 cm long, oblong-ovate or rounded or palmately 3-5 lobed, upper small 5-8 long, lanceolate, petioles with a pair of stipular glands. Leaves are sinuate-toothed, the upper small lanceolate, penninervial, the lower large, reaching 15-30 cm long and often as broad, ovate. The physical evaluation of dried powdered leaves was determined and reported (Table- 1).

Phytochemical Investigations:

The Alcoholic extract showed the presence of steroids, tannins, alkaloids and flavonoids. The aqueous extract showed the presence of tannins, flavonoids, proteins, amino acids, and glycosides. Shinoda test was used for identification of flavonoids, Ferric chloride test was used for identification of tannins, Xanthoproteic test used for identification of proteins and Brontrager test was used for identification of glycosides²⁰.

The aqueous and alcoholic extract has shown presence of total phenols and flavonoid content and is expressed in milligram of Gallic acid and Quercetin equivalents/1 g extract (Table 2 & 3).

Pharmacological Screening

The alcoholic extract and aqueous extract of leaves of *Plectranthus amboinicus* elicited a significant anthelmintic activity at higher concentrations. Both the extracts exhibited anthelmintic activity in dose dependant manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for both type of worms. The alcoholic extract of *Plectranthus amboinicus* caused paralysis in 10 min and death in 28 min, whereas aqueous extract showed death in 28 min, while aqueous extract showed P and D in 9 and 30 min. respectively against the earthworm *P. posthuma*. The reference drug Piperazine citrate showed the same effect at 21 min and 59 min respectively.

The alcoholic and aqueous extract has showed significant anthelmintic activity against *Ascaridia galli* worms. The alcoholic extract caused paralysis in 5 min,

death in 29 min and aqueous extract exhibited P and D in 6 and 27 min, respectively, at higher concentration of 10 mg/ml. Piperazine citrate showed the same activity at 12 and 41 min. The anthelmintic activity of both the extracts of leaves of *Plectranthus amboinicus* has elicited significant activity comparable to that of standard drug Piperazine citrate. (Table- 4)

DISCUSSION

The Piperazine citrate, by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis²¹. The leaves extract of *Plectranthus amboinicus* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts revealed presence of tannins as one of the chemical constituents. Tannins were shown to produce anthelmintic activity²².

Tannins are polyphenolic compounds²³. Some synthetic phenolic anthelmintics eg niclosamide, oxycylozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation²⁴. It is possible that tannins present in the extracts of *Plectranthus amboinicus* produced similar effects.

Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal²⁵ or glycoprotein on the cuticle of the parasite²⁶ and cause death.

Hence it can be concluded that the leaves of *Plectranthus amboinicus* particularly alcoholic and aqueous extract possess good anthelmintic activity.

However, this claims demands further study of isolation of individual components and observing their effect in the treatment of helmenthis infections.

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