In vitro Anthelmintic Activity of Stem Extracts of *Piper betle* Linn Against *Pheritima posthuma*

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**ABSTRACT**

In this study the anthelmintic activity of ethanolic and aqueous extracts of stems of *Piper betle* Linn was performed. Indian adult earthworms were used for the assessment of anthelmintic activity. Albendazole (40 mg/ml) was used as standard and normal saline water was used as vehicle respectively. Observations were made for the time taken to paralysis and death. In ethanolic extract \([P \text{ (min)} = 1.15, D = 2.16]\), the activity was found to be more effective as compared to the standard drug Albendazole \([P \text{ (min)} = 2.34, D \text{ (min)} = 5.68]\) and aqueous extract \([P \text{ (min)} = 4.38, D \text{ (min)} = 7.16]\). The mode of action of Albendazole is to cause paralysis of worms and to expel them in the faeces. Albendazole causes degenerative alterations in the intestinal cells of the worm. Degenerative changes in organs like endoplasmic reticulum, the mitochondria results in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminthes. Due to diminished energy production, the parasite is immobilized and eventually dies. The ethanolic extract of stems of *Piper betle* Linn not only demonstrated anthelmintic property but they also caused death of the worms when compared with marketed standard preparation i.e. Albendazole (40mg/ml) and different concentrations of aqueous extract. It is concluded that stems of *Piper betle* Linn is potent anthelmintic. Since this is the preliminary work, separation of chemical constituents which are responsible for the activity may be done in the future.

**Key words:** Anthelmintic activity, *Piper betle* Linn, Stems extracts, *Pheritima posthuma*

**INTRODUCTION**

According to the World Health Organization two billion people are suffering from parasitic worm infections. Parasitic worms also infect living organisms and crops, affecting food production with a resultant economic impact. The morbidity due to parasitic diseases has been increasing in our population, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis, and schistosomiasis. Anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Therefore there is very much need of drug discovery programmes. There is thus a small repertoire of chemotherapeutic agents available for treatment (Table1). In some respects, this situation has been exacerbated by the remarkable success of ivermectin over the last twenty years, which has decreased motivation for anthelmintic drug discovery programmes. This prompts concern, as anthelmintic resistance has been widely reported in livestock and it may also only be a matter of time before this phenomenon occurs in parasites of humans. Anthelmintics are drugs that either kill or expel infesting helminthes (worms). Helminthiasis is a disease in which a part of the body is infected with worms such as pinworm, roundworm or tapeworm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs.

Broad spectrum anthelmintics are effective against parasitic flat worms and nematodes (e.g. Piperazine citrate, Diethylcarbamazine citrate, Benzimidazole, Albendazole, and Thiobendazole). However, the majority of drugs are more limited in their action e.g. Praziquantel, (a drug used in the treatment of schistosomiasis and thought to act by disrupting calcium homeostasis), has no activity against nematodes.

The Betle (*Piper betle* Linn), commonly known as paan is a vine belonging to the Piperaceae family. *Piperaceae* family...
belonging to superorder Nymphaeifloraea, order Piperales and
genus *Piper*. Genus *piper* comprises about 10 genera, 2000
species. The genus *Piper* (*Piperaceae*) is largely distributed in
tropical and subtropical regions of the world.6 Bete leaves
have been chewed along with the nut areca since very ancient
times. It can also be used as carminative, stomachic,
anthelmintic, tonic, and aphrodisiac. Leaves of the plant
*Piper betle* Linn are chewed by people at frequent intervals by
patients suffering from hookworms, immature worms
are thrown out with the frequent expectoration.7,8 *Piper
bete*/has light yellow aromatic essential oil, with sharp burning
taste. Leaf possesses activities like antidiabetic, antiulcer,
antipllatelet aggregation, antifertility, cardiotonic, antitumour,
antimutagenic, respiratory depressant.10–13

This became the basis of selection of this plant and particularly
the stems. This experiment present research work aims to
throw light upon the pharmacognostic, phytochemical and
pharmacological account of the stems of *Piper betle* Linn.

**Traditional uses**
The leaves of *Piper betle* are pungent, bitter, sweetish, acrid,
heating, carminative, stomachic, anthelmintic, tonic,
aphrodisiac, laxative useful in vata, kapha foul smell in the
mouth, ozoena, bronchitis, elephantiasis of leg, improves
appetite should not be taken in eye diseases, leprosy, poisoning,
thirst, alcoholism, asthma, loss of consciousness. (Ayurveda)

The leaf has a sharp taste and good smell, improves taste and
appetite, tonic to the brain, heart, liver, strengthens the teeth,
lessens thirst, clear the throat vulnerary and styptic (unani).

**Chemical constituents**
Leaves of *Piper betle* Linn contain protein 3.1%, carbohydrate
6.9%, minerals 2.3%, and tannins 2%. It contains calcium,
phosphorus, iron, iodine and potassium is also present.

<table>
<thead>
<tr>
<th>Schistosomiasis (blood fluke)</th>
<th>Intestinal round worms</th>
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<tbody>
<tr>
<td>Antimonials</td>
<td>Piperazine</td>
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<tr>
<td>Metrifonate</td>
<td>Benzimidazoles</td>
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<tr>
<td>Oxamnique</td>
<td>Morantel</td>
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<tr>
<td>Praziquantel</td>
<td>Pyrantel</td>
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<tr>
<td>Cestodiasis (tape worm)</td>
<td>Avermectins and</td>
</tr>
<tr>
<td>Niclosamide</td>
<td>milbemycins</td>
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<tr>
<td>Benzimidazoles</td>
<td>Closantel (and</td>
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<tr>
<td>Praziquantel</td>
<td>halogenated salicylamides)</td>
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<td>Fasciolasis (liver fluke)</td>
<td>Emodepside</td>
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<tr>
<td>Praziquantel</td>
<td>Filariasis (tissue round</td>
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<tr>
<td>(and halogenated salicylamides)</td>
<td>worms)</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>Diethylcarbamazine</td>
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<tr>
<td>Closantel</td>
<td>Suramin</td>
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<tr>
<td>(and halogenated salicylamides)</td>
<td>Ivermectin</td>
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</tbody>
</table>

Vitamin B, vitamin C and vitamin A. Leaf contains bitter
compounds that are about 0.7 to 2.6%. It also contains an
aromatic compound and stable oils like phenol and terpene.
Besides this it contains eugenol, chavibetol and
hydroxychavicol.

The active ingredients of betle oil, which is obtained from
the leaves, are primarily a class of allylbenzene compounds.
Though particular emphasis has been placed on chavibetol
(betle-phenol; 3-hydroxy-4-methoxyallylbenzene), chavicol
(p-allyl-phenol; 4-allyl-phenol).15,16

**MATERIALS AND METHODS:**

Procurement and authentication of the crude drug
The stems of *Piper betle* Linn were collected in the month
of October from Baramati region, Maharashtra, India. Since
the plant consist of aromatic principles; they were then

![Figure 1: Anthelmintic activity of ethanolic extract of Piper betel stems. Results are expressed as Mean ± (n=6) from 6 observations.
Vehicle worms remain alive up to 24 hrs observations.](image)

![Figure 2: Anthelmintic activity of aqueous extract of Piper betel stems. Results are expressed as Mean ± (n=6) from 6 observations.
Vehicle worms remain alive up to 24 hrs observations.](image)
shade dried. Herbarium of the plant was prepared and it was authenticated by Agharkar Research Institute, Pune (SIOP/ authenticated/ 2010-11/3951).

Storage of crude drug material
The stems were powdered finely in the conventional mixer and stored in airtight self locking polybags. Since the *Piper betle* Linn consist of volatile oil and other active constituents.

Worms Collection
Indian adult earthworms i.e. *Pheritima posthuma* were used to study anthelmintic activity. The earthworms were collected from the water logged area of soil, Pune, washed with normal saline to remove all fecal matter. The earthworms of 5-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol.

Preparation of stem extracts
The stems of *Piper betle* Linn were shade dried. They were then milled using commercial laboratory blender. The powder of the stems of *Piper betle* Linn was defatted with petroleum ether. (40-50 degree) The ethanolic and aqueous extracts were successfully obtained from the defatted material by using soxhlet extraction method. All the extracts obtained were concentrated to dryness in vacuum at 40 °C and stored at 4°C in the refrigerator until further used. The extracts were subjected to phytochemical and pharmacological evaluation.

Phytochemical screening
Freshly prepared extracts were subjected to phytochemical screening tests for various constituents like proteins, fats, carbohydrates, alkaloids, steroids, and flavanoids by standard reagents. 

Experimental Animals
Indian adult earthworms (*Pheritima posthuma*) were collected from moist soil and washed with normal saline to remove all fecal matter was used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1 - 0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal Roundworm parasites of human beings.

Drugs
Standard drug: Albendazole (40mg/ml) (Bendex Suspension, Protec division of Cipla Ltd.)

Chemicals:-Ethanol A.R (Thomas baker chemicals Pvt Ltd), Distilled water, Dimethyl sulfoxide (Thomas baker chemicals Pvt Ltd), Vehicle used: Saline water.

Assessment of Anthelmintic activity
The assay was performed on adult Errieten earthworm *Pheritima posthuma*, due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being.[9] All animals were divided into six group containing six earthworms in each. Ethanolic extract of *Piper betle* Linn was dissolved in minimum amount of dimethyl sulfoxide while aqueous extract in water. All the earthworms were washed in a normal saline solution and then earthworms from each group were released into 10 ml of respective formulations as follows: Vehicle (5%DMSO in normal saline), Albendazole (40mg/ml), Ethanolic extract (10-50mg/ml) and aqueous extract(10 -50mg/ml).[18]

Observations were made for the time taken to paralysis (was said to occur when the worm did not revive even in normal saline solution) and death (was concluded when the worms lost their motility even when vigorously shaken or dipped).

RESULTS
Preliminary phytochemical analysis showed the presence of flavanoids, tannins and steroids like phytoconstituents in the ethanolic extracts and aqueous extract respectively. (Table 2)[21,22] Ethanol and aqueous extracts were used to evaluate anthelmintic activity has shown dose dependant activity. The Mean ± S.D. values (statistical analysis) were calculated for each extracts. The result of anthelmintic activity on earthworm *Pheritima posthuma* was given in Table 3 and Table 4, reveals that, the different concentration used for both aqueous and alcoholic extracts has shown paralysis and death of earthworms and it was compared in the same concentration with albendazole as reference drug. Indian adult earthworms were used for the assessment of anthelmintic activity as experimental animals. Albendazole (40 mg/ml) was used as standard and normal saline water was used as vehicle respectively. Observations were made for the time taken to paralysis and death.[23] Paralysis was said to occur when the worm did not revive even in normal saline solution and death was concluded when the worms lost their motility even when vigorously shaken or dipped.

<table>
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<tr>
<th>Table 2: Preliminary Phytochemical Investigations of Stem Extracts</th>
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<tr>
<td><strong>Piper betle Stem Extract</strong></td>
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<tr>
<td>Ethanolic extract</td>
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<td>Aqueous Extract</td>
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The data revealed that the ethanolic extract showed remarkable anthelmintic activity as compared to the standard drug and aqueous extract. The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms and to expel them in the faeces. The ethanolic extract of stems of *Piper betle* Linn not only demonstrated anthelmintic property but they also caused death of the worms when compared with marketed standard preparation i.e. Albendazole (40mg/ml) and different concentrations of aqueous extract. Phytochemical analysis of extracts of *Piper betle* Linn revealed the presence of tannins and flavonoids in ethanolic extract. Tannins were shown to produce anthelmintic activities chemically tannins are polyphenolic compounds.[30] Some synthetic phenolic anthelmintic e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation.[31] Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite[32,33]

CONCLUSION

It is concluded based on the findings of the present study that the stems of *Piper betle* Linn are potent anthelmintic. Phytochemical screening states the presence of tannins and flavonoids in stem extracts; therefore anthelmintic activity may be due to tannins or flavonoids. However, dose and the form in which they can be used, require standardization. Further research efforts are required for...
the depilation of chemical constituent and study of the individual constituents which all are responsible for this activity.

REFERENCES