



## Anthelmintic activity of *Piper betle* on *Pheretima posthuma*

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### Abstract

The study was conducted to investigate the anthelmintic activities of *Piper betle* leaves. The extract was subjected to assay for anthelmintic activity with the determination of paralysis time and death time using earthworm (*Pheretima posthuma*) at four different concentrations. In this study the anthelmintic activity of extract of leaves of *Piper betle* Linn was performed. Indian adult earthworms were used for the assessment of anthelmintic activity. Piperazine citrate (10 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.

**Keywords:** *Piper betle*, anthelmintic, extract and medicinal plants, *Pheretima posthuma*

### Introduction

The leaves of *Piper betle* Linn have long been use in the Indian local system of medicine. In ancient India, betel leaves are considered auspicious and are still extensively used during religious functions in Asia. It is generally found in hot and moist climatic condition. In India it is found in Bihar, Bengal, Orissa, South India and Karnataka. The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves and white catkin <sup>[1]</sup>. There are various types of leaves, the most popular being: Calcutta, Banarasi, Magahi, etc. In Bangladesh Dinajpur, Rangpur, Chittagong, Faridpur, Jessore, Narayanganj, Barisal and Sylhet are the areas producing the most betel. The harvested leaves are used both for domestic consumption and for export to Middle East, to European countries, USA, UK, Pakistan, and Myanmar. Paan is one of the major economic sources of rural Bangladesh. The best betel leaf is the "Magadhi" variety (literally from the Magadha region) grown near Patna in Bihar, India. In Kerala, the famous variety of betel leaf is from Venmony near Chengannur and it is called "Venmony Vettilla". Betel leaf cultivated in Tirur in Kerala, Hinjilicut in Odisha are of fine quality. Betel leaves exported from Tirur are famous in Pakistan as "Tirur Pan". *Piper betle* is one of the invaluable medicinal plants where its leaves have been used for many medicinal purposes. *Piper betle*, a member of the Piperaceae, which is a large plant family, is also known Paan in India and Sirih in Malaysia and Indonesia, show in (figure 1). The fresh leaves of betel leaves have been wrapped together with the areca nut, mineral slaked lime, catechu, flavoring substances and spices are chewed since the ancient time <sup>[2]</sup>. The whole betel plant had some very bad press due to reports associating the usage of the herb with mouth cancer. It also helps in reducing difficulty in breathing for people suffering from asthma. Apply some mustard oil to the leaves of the betel plant, warm it and then keep it on the chest to bring relief from asthma <sup>[3]</sup>. A preliminary study has reported *Piper betle* leaves extract contains large numbers of bioactive molecules. *Piper betle* contains a wide variety of biologically active compounds whose concentration depends on the variety of the plant, season and climate. Pharmacological Profile has shown antiplatelet, anti-

inflammatory effects as well as immuno modulatory, gastro protective and antidiabetic activity. Paan has been referred to in Saktatantra as one of the means of achieving siddhi. It was believed that without betel chewing and offering pan to Guru no siddhi can be gained. Tambool has also been referred to as facilitating the sadhak in chewing dharma, yasha aisvarya, Srivairagya and mukti. It was reported that fresh leaves contains: moisture 85.4, protein 3.1, fat 0.8, carbohydrate 6.1, fibre 2.3, calcium 230 mg, phosphorous 40 mg, iron 7 mg, ionisable iron 3.5 mg, iodine 3.4 µ. They have a high content of potassium nitrate (0.26 - 0.42 %). The sugars identified in betel leaves include glucose, fructose, maltose and sucrose. The average content of free reducing sugars in different types of betel leaves varies from 0.38 - 1.46 %. It also contains the enzyme like diastase and catalase. *Piper betle* leaves are earlier reported to possess anticancer potential. Hence, the aqueous extract of the leaves was subjected to cytotoxicity studies on Hep-2 cell line using Micro culture Tetrazolium and Sulphorhodamine β assays (Chaurasia, Sundeep *et al.*). *Piper betle* leaf oil can be used as an industrial raw material for manufacturing medicines, perfumes, food additives etc. The leaves are nutritive and contain anti carcinogens showing promise for manufacturing of a blood cancer drug (Sengupta).

### Scientific classification

**Synonyms:** Chavica Beta. Artanthe Hixagona

**Kingdom:** Plantae

**Order:** Piperales

**Family:** Piperaceae

**Genus:** *Piper*

**Species:** *betle*

**Division:** Magnoliophyta

**Taste:** Pungent tasting and warming.

### Vernacular names

**Sanskrit:** Tambool, Mukhbhushan, Varnalata,

**Hindi:** Paan leaf,

**English:** Betel, Betel pepper, Betel-vine,

**Telugu:** Nagballi, Tamalapaku,

**Tamil:** Vetrilai,

**Gujarati:** Nagarbael,

**Bengali:** Paan, Paana, Tambulaballi (plant), Parnakari (leaf),  
**Assamese:** Paan, Paana,  
**Kannada:** Eleballi, Panu, Vileyadele,  
**Gujarati:** Paan, Tanbola,  
**Malayalam:** Vettilla,  
**Nepalese:** Naagavalli (plant), Paan (leaf),  
**Indonesia:** Bakik serasa, Daun sirih, Sirih, Serasa, Seveh, Seureuh,  
**German:** Betel pfeffer, Betel-Pfeffer,  
**Chinese:** Ju jiang, Tu bi ba, Tu Wei teng, Wei zi, Wei ye, Da geng teng, Ch'ing Chu.

### Chemical constituents

Betel leaves contain tannins, sugar and diastases and an essential oil. The essential oil is a light yellow liquid of aromatic odour and sharp burning in taste. It contains a phenol called chavicol which has powerful antiseptic properties. The alkaloid arakene in it has properties resembling cocaine in some respects. An analysis of the betel leaf shows it to consist of moisture 85.4 per cent, protein 3.1 per cent, fat 0.8 per cent, minerals 2.3 per cent, fibre 2.3 per cent and carbohydrates 6.1 per cent per 100 grams. Its minerals and vitamin contents are calcium, carotene, thiamine, riboflavin, niacin and vitamin C. Its calorific value is 44 [4].

### Plant description

A perennial root climber with semi woody stems, leaves, simple, alternate, ovate, cordate, acuminate or acute, entire and bright green. Male spikes are dense and cylindrical. Female spikes are pendulous. Roots arise from each node and that aid in fixing the plant in host tree.

### Image of piper betel leaves



Fig 1: Leaves of piper betel

### Material and method

#### Procurement of Plant Material

The leaves of Piper betel have been collected from the Indol region of Jalgaon District [Maharashtra]. The plant is authenticated by Dr. Santosh K. Tayade, [Head of Dept. of Botany] P.S.G.V.P.M's, Art's, Science and Commerce Collage, Shahada, Dist- Nandurbar (M.S.). After authentication, leaves of plant were collected, powdered and dried under the shade for a period of 7 - 8 days and then pulverised in mechanical grinder to obtained coarse powder. That powder was stored in airtight container.

### Worms collection

Earthworms were collected from the water logged area of soil along Lonkheda road, Shahada. The average size of earthworm was 6 – 8 cm.

### Preparation of extract

The coarse leaves powdered material was soaked in distilled water and acetone as 70:30 solutions by Maceration technique for continuous 72 hrs and then strained and the concentrated solution was evaporated on water bath until concentrated semisolid thick mass is left.

### Drugs and chemicals

1. Piperazine citrate
2. Saline solution

### Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*, [5]. The assay was performed *in vitro* using adult earthworm [*Pheretima posthuma*] owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being for preliminary evaluation anthelmintic activity [6, 7].

Test sample of the extract was prepared at concentration of 25, 50, 75 & 100 mg/ml in distilled water two worm i.e. *Pheretima posthuma* approximately equal size [ same type ] were place in each 9 cm Petri plate /dish contain 25 ml of above solution of extract. Piperazine citrate [10 mg/ml] was used as reference standard and saline solution as control. All the test solution and standard drug solution were prepared freshly before start in the experiment. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water [50°C]. All the results were expressed as a mean  $\pm$  SEM of two worms in each group.

Table 1: Anthelmintic Activity of Leaves of Piper betel Extract on Indian Earthworm [*Pheretima posthuma*]

Sr. No.	Extract	Conc. [mg/ml]	Indian Earthworm [ <i>Pheretima posthuma</i> ]	
			Time of paralysis in min.	Time of death in min.
1.	Aqueous	25	26.80 $\pm$ 1.30	100.52 $\pm$ 3.0
		50	13.44 $\pm$ 1.55	40.21 $\pm$ 2.50
		75	2.64 $\pm$ 1.25	11.63 $\pm$ 1.50
		100	1.29 $\pm$ 0.55	13.66 $\pm$ 1.0
2.	Standard	10	8.55 $\pm$ 0.65	13.05 $\pm$ 1.35
3.	Saline	-	-	-



Fig 3: Earthworms in Different Conc. of Solution

## Result and Discussion

From the observation made, higher concentration of extract produced paralytic effect much earlier and the time of death was shorter for all worms. Both the aqueous extract show anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 100 mg/ml concentration, for worms. Evaluation of anthelmintic activity was compared with reference standard Piperazine citrate. From the above result, it is concluded that Piper betel by tirable traditionally to treat intestinal worm infection, show significant anthelmintic activity. 25 mg/ml exert paralytic effect in  $26.80 \pm 1.30$  and death time  $100.52 \pm 3.0$ . 50 mg/ml exert paralytic effect in  $13.44 \pm 1.55$  and death time  $40.21 \pm 2.50$ . 75 mg/ml exert paralytic effect in  $2.64 \pm 1.25$  and death time  $11.63 \pm 1.50$ . 100 mg/ml exert paralytic effect in  $1.29 \pm 0.55$  and death time  $13.66 \pm 1.0$ . The experimental evidence obtained in the laboratory model could provide a rational for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

## Conclusion

From the above results, it is concluded that the aqueous extract of *Piper betel* show potent anthelmintic activity to standard anthelmintic drug. Further studies using in vivo model are required to carried out and establish the effectiveness and pharmacological rational for the use of *Piper betel* as an anthelmintic drug. The drug can be further explored for the isolation and characterization of the active constituents responsible for anthelmintic activity. The paralytic effect and the death time is more effective in 100 mg/ml.

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