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PHYTOCHEMICAL SCREENING AND ANTHELMENTHIC ACTIVITY OF PIPER BETLE L. LEAVES AGAINST *PHERETIMA POSTHUMA*

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ABSTRACT

Herbs have played an important part in our development it provided us with food, medicine and cosmetics. Medicinal plants contain a variety of phytochemicals as well as minerals, vitamin and trace elements. Some of the phytochemicals are pharmacologically active and can exert at therapeutic action on the body. Piper betel is the leaf of a vine belonging to the Piperaceae family, which includes pepper and kava. P.betle is mostly originated in Asia. The roots, fruits of Piper battle has carminative, stimulant, antiseptic, and used in the treatment of Malaria. Betal leaf has antiseptic, analgesic, anti bacterial, anti-lactogogue (reducing Brest milk), antioxidant, antispasmodic, cardiotonic, expectorant, tonic and contraceptive property, it conclude that Ethanolic extract of P.betal leaves consists antihelminthic property against Pheretimaposthuma.

INTRODUCTION

Herbs have played an important part in our development. It provides us with food, medicine and cosmetics. It is strongly believe that, there is a remedy somewhere in nature for every illness. Today approximately 25% of all prescription drugs are derived from the trees, hubs or sherbs. Digitalis is extracted from the leaves of Foxglow, morphine and codine are derived from opium poppy, quinine from cinchona bark etc. The herbalist believes that the sum of the action of the whole plant is more balanced than that of any one of its main constituent. Medicinal plants contain a variety of phytochemicals as well as minerals, vitamins and trace elements some of phytochemicals are pharmacological active and can exert therapeutic action on the body. Helmenthiasis is also known as worm infection, is macroparasitic disease of humans and other animals in which a part of the body is infected with parasitic worms known

as helminthes. There are numerous species of these parasites which are broadly classified into tapeworms, flukes and roundworms. The list of Signs and symptoms mentioned in various sources for helminthiasis includes 28 symptoms and abdominal pain, diarrhea, fever, fatigue, enlarged liver, enlarged spleen, cough, eosinophilia, asymptomatic gastrointestinal inflammation, malabsorption, bowel obstruction, anemia, dehydration, bloody diarrhea, skin symptoms, chest pain, vomiting, constipation, weight loss, distended abdomen, itchy skin and symptoms, malaise, headache, itchy anus, neurological problems and irritability.

Antihelminthic: Antihelminthics are group of antiparasitic drugs that expel parasitic worms (helminthes) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may also be called vermifuges or vermicides. Antihelminthic are used to treat people who are infected by helminthes, a condition called helminthiasis. These drugs are also used to treat infected animals pills containing antihelminthic are used in mass deworming campaigns of school aged children in many developing countries.

The antihelminthic drugs can be isolated from two sources

- Synthetic source
- Natural source

Synthetic Source: Anthelmenthics are separated into classes on the basis of similar chemical structure and mode of action.

S. No	Class	Drugs
1	Benzimidazole	Thiabendazole fenbendazole Albendazole Oxfendazole
2	Nicotinic agonist imidazothiqzoles tetrahydro pyramidines	Levamisol Morantel Pyrantel
3	Microlytic lactones Avermectines Milbenmycines	Ivermectin Eprinomectin Doramectin Moxidectin
4	Piperazine	Piperazine

Naturalsource: With increasing level of antihelminthic resistance and a movement towards more sustainable farming practices, there is a renewed interest in natural natural dewormers. Many universities are conducting experiments to determine the efficacy of various natural

dewormers and other old time remedies. Numerous plants are being tested for their antihelminthic properties example pumpkin seed garlic.

S. No	Name of plant	Parts used	Species used
1	Ocimum sanctum	Essential oils	Caenorhabditis elegans
2	Melia azidarach	Drupes	Taeniasoliu, pheretimaposthum
3	Punicagranatum	Stem and root bark	Haemonchus contortus
4	Nigella sativa	Essential oil	Earth warm, tape warm and hook worm
5	Cucurbita maxima	Seeds	Ascaarislumbicoides
6	Cannabis sativa	Leaves	Fasciolipsisbuski
7	Caricapappaya	Seeds	Trematodes

The Betel (piperbetle) is the leaf of a vine belonging to the Piperaceae family which includes pepper and kava. The Betel plants are an evergreen perennial with glossy heart shaped leaves and white catkin. The betal plant originated in South and South East Asia.

Figure 1. *Piper betle* Leaf

Constituents of leaf: Leaf contains water (85 to 90%), proteins(3 to 3.5%), carbohydrates (0.5 -6.1 %), minerals (2.3 to 3.3 %), fat (0.4 to 1%), fibre (2.3%), Essential oil (0.08-0.2%), tannin (0.1-1.3%), alkaloid (arakene), vitamin C(0.005-0.01%), nicotinic acid(0.63-0.89mg/100 gm), vitamin-A(1.9-2.9mg/100gm), thiamine(10-17µg/100gm), riboflavin (1.9-30µg/100gm), calcium (0.2-0.5%), iron (0.0052-0.007), iodine (3.4 µg/100 gm), Phosphorus (0.052-0.6%),potassium(1.1-4.6%).

Traditional medicinal uses: The roots, leaves and fruits of Piper betle or Paan are carminative, stimulant, antiseptic and used for the treatment of Malaria. Betel leaves are antiseptic, analgesic, antibacterial, antilactagogue (reducing breast milk), antioxidant, antispasmodic, cardiotonic, carminative, expectorant, litholytic, tonic and contraceptive properties.

MATERIALS AND METHODS

Collection of plant material and preparation of extract: Fresh *P.betel* leaves were collected from the local market of Uppal. The leaves were first washed with running tap water to remove dirt and shade dried in a sun. The leaves are then milled into fine powder using laboratory grinder. Ethanol extracts were prepared by macerating 50 g of herb powder with 150 ml of 100% ethanol in schott's bottle wrapped in aluminium foil. The preparations were allowed to stand for a week at room temperature. The extract was filtered using WhatmanNo.1 membrane filter paper and dried under vacuum using rotary evaporator at 50^0C, 150rpm.The obtained crude extracts were stored at -20^0C until further use. The yield percentage of the extract was determined by using the equation.

Yield (%)= W2-W1/W0*100

Where

W2 =weight of the extract and container

W1= weight of the empty container

W0 = weight of the initial dried sample

Phytochemical screening: The extract was subjected to preliminary phytochemical screening for the presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Phenols, Tannins, Terpenes, Proteins, Amino acids and fats and oils.

Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

1. *Mayer's* test: Filtratewas treated with Mayer's reagent (potassium mercuric iodide).Formation of a yellow coloured precipitate indicates the presence of alkaloids.

2. Wagner's test: Filtratewas treated with wagner's reagent (iodine in potassium iodide).Formation of brown/reddish precipitate indicates the presence of alkaloids.

3. Dragendroff's test: Filtrate was treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

4. Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

1. Molish's test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

2. Benedict test: Filtrate treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. Fehling's test: Filtrate was hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolyzed with dilute HCl and then subjected to test for glycosides.

1. Modified Borntrager's test: Extracts were treated with ferric chloride solution and immersed in boiling water for about five minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides.

2. Legal's test: Extract were treated with Sodium nitro prusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of Saponins

1. Froth test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

2. Foam test: 0.5g of extract was shaken with 2 ml of water. If foam produced persists for 10 min it indicates the presence of saponins.

Detection of Phytosterols

1. Salkowski test: Extracts was treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

2. Liebermann Burchard's test: Extract was treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols

Ferric chloride test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

1. Alkaline reagent test

Extracts were treated with few drops of sodium hydroxide solution formation of intensive colour which becomes colourless on addition of dilute acid indicates the presence of flavonoids.

2. Lead acetate test

Extract was treated with few drops of lead acetate solution formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids

1. Xanthoproteic test: The extracts were treated with few drops of concentrated nitric acid formation of yellow colour indicates the presence of proteins.

2. Ninhydrin test: To the extract, 0.25% weight by volume in hydra in the agent was added and boiled for few minutes formation of blue colour indicates the presence of amino acid.

D**etection of diterpenes**

Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Anthelmintic Activity: Indian adult earthworms (*Pheretima posthumma*) were collected from moist soil of a farmhouse in Moinabad, Rangareddy district. The earth worms were maintained under normal vermicomposting medium with adequate supply of nourishment and water. Before the initiation of experiment, the earthworms were washed with normal saline. Adult Earth homes of approximately 8-10 cm in length and 0.2 to 0.3cm in width were used for the experiment. These worms were the selected model for antihelminthic activity due to

its anatomical and physiological resemblance with the intestinal round worm parasites of human beings.

Animal grouping

Group I: Control (20 ml of normal saline solution)

Group II: Standard (Piperazine 20mg/ml)

Group III: Leaves extract 50 mg/ml

Group IV: Leaves extract 100mg/ml

Group V: Leaves extract 150mg/ml

➢ **Preparation of normal saline solution:** 9 gm of sodium chloride was dissolved in 1000 ml of distilled water in a volumetric flask and volume is made up to the mark with distilled water**.**

➢ **Preparation of standard solution (20mg/ml):** The weighed amount of powdered Piperazine was dissolved in small amount of DMSO and then made up to 20 ml using normal saline solution.

➢ **Preparation of extract solution:** The required amount of ethanol leaves extract were weighed for 50 mg/ml, 100 mg/ ml and 150mg/ml solution and were dissolved separately in small amount of DMSO and then the volume was made up to 20 ml using normal saline solution separately.

The Anthelmintic activity Procedure

The Anthelmintic activity on leaves extract of *P.betle* was evaluated on adult Indian earthworms *Pheretimaposthuma*. 20 ml of sample solution containing three different concentrations of ethanol extract (50,100 and 150 mg/ml) and standard Piperazine solution (20mg/ml) were prepared and kept in separate the petriplates. Approximately equal size of the earth worms were released in each group. Observations were made for the time taken for paralysis and death of individual worms. Paralysis is said to occur when the worms do not revive even in normal saline and death was concluded when the worms lose their motality and do not revive in warm water and with fading of colour. 20 ml of normal saline was used as control group. Time was noted in minutes for all worms individually.

Fig. 2: Earth worms in control group.

Fig. 3: Earth worm in standard Piperazine.

Fig. 4: Earth worm in leaves extract of piper betal.

RESULTS AND DISCUSSION

Leaves extract of Piper betal is selected for the study about five 50 g of leaves are powdered and extracted with ethanol used in maceration extraction procedure the nature of extracts and their extractive values are as follows.

S. No	Extract	Colour	Yield(%w/w)
1	EEHF	Dark green semisolid mass	18.7

Phytochemical investigation: The preliminary phytochemical analysis of Ethanol leaves extract of Piper battle revealed the presence of alkaloids.

S. No	Test	Ethanol leaves extract
1	Carbohydrates	+
2	Alkaloids	+
3	Glycosides	+
4	Saponins	-
5	Phytosterols	-
6	Phenols	+
7	Tannins	-
8	Flavanoids	-
9	Proteins and aminoacids	+
10	Terpenes	-

Anthelmintic activity Results: In vitro study, it is found that ethanol leaves extract of Piper betle extract anthelmintic activity against Indian adult earthworm *pheretima posthuma* in the concentration range of 50 to 150 mg/ml and showed increase in activity with increase in concentration (dose-dependent).As showing in table and chart the extract showed significantly more activity with 150 mg/ml with paralytic time of 18.08 minutes and death time of 20.34 minutes when compared to standard with paralytic time 37.33 minute and death time of 61.33 minute. Ethanol leaves extract of 50 mg/ml and 100 mg/ml also showed anthelmintic activity with the paralysis time 57.33 and 51.07 min respectively and the death time of 73.07 and 64. 12 minutes respectively but to a lesser extent when compared to standard piperazine.

Table: paralysis and death time of leaves extract of piper betel against Pheretimaposthuma

S. No	Group	Paralysis time (min)	Death time (min)
1	I	-	-
2	II	3733±4.37	61.33±1.67
3	III	57.33±11.74	73.07±1.35
4	IV	51.07±3.75	64.12±1.25
5	V	18.08±0.27	20.34±0.00

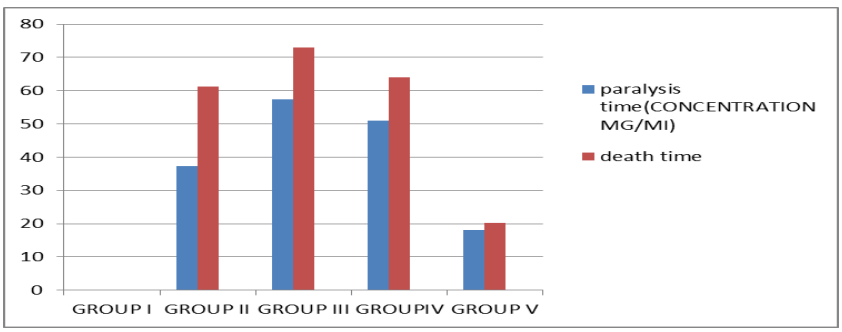

Chart showing paralysis time and death time of leaves extract of piper betal against *Phereitimaposthuma*

CONCLUSION

The present study showed that Piper betel leaves ethanol extract is found to exhibit a significant anthelmintic activity against *Peretima posthuma.* Therefore, the active constitutes present in P.betle or leaves responsible for anthelmintic activity.

SUMMARY

Piper betel is an ever green panel with glossy heart shaped leaves and white catkin.It is revealed that this plant have anthelmintic activity but so far no work is carried out using 100% ethanol solvent for leaves of piper betle for carrying anthelmintic activity.

In-vitro anthelmintic activity carried out parts of an hour is carried out against Indian adult earth worms pheretimaposthuma using ethanol leaves extract and of Piper betle showed significantly good activity, when compared to standard and increase its activity with increase in concentration.

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