

ANTIMICROBIAL AND ANTHELMINTIC ACTIVITY OF *PIPER BETLE* LEAVES

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ABSTRACT

The aim of present study is to a comparative evaluation of microbial properties of piper betle leaves against pathogenic microorganism such as bacteria and fungi in agar well diffusion method against some of the standard antibiotics available in the market. Ethanol extract of piper betle leaves were evaluated for Antimicrobial activity against pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans and Aspergillus floruss strains. Alloxan and Fluconazole were used as standards for antibacterial and antifungal assay respectively. Ethanol extract showed different degree of activity against antibacterial and antifungal investigation. Ethanolic extract of Piper betle leaves were

considerably more inhibitory action against fungi than bacterial strains. The study was also designed to evaluate to anthelmintic properties of ethanolic and aqueous extract of *Piper betle* Linn. All test sample taken at same concentration i.e. (20, 40, 60, 80, 100mg/ml) were tested on indian earth worms (*Pheretima posthuma*), paralysis time & death of time were consider as assessment of anthelmintic activity. It was noticed in this investigation that the time of paralysis & death of worms was the dose dependent.

KEYWORDS: *Piper betle* Linn., Antimicrobial activity, Anthelmintic, Ethanolic Extract.

INTRODUCTION

The *Piper betle* Linn. (Piperaceae) leaves locally known as paan, It is used in Indian medicine system due to its antioxidant and antimicrobial properties. Fresh leaves used as a post meal mouth freshener. It is cultivated in India, Sri Lanka, Malaysia etc. It is evergreen plant. It is used as an antiseptic, it apply on wound for healing purpose.^[1] Leaves are rich source of many nutrients like water, energy, Protein, fats, fiber, calcium, iron etc. Chief constituent of

leaves is the volatile oil. It is used as tonic for brain, heart and liver. It promote healthy teeth and skin. It is used as anthelmintic. It reduces the cough. It gives analgesic and cooling properties.^[2] Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, hysteria, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, ringworm, swelling of gum, rheumatism, abrasion, cuts and injuries etc as folk medicine while the root is known for its female contraceptive effects. The leaves are very nutritive and contain substantial amount of vitamins and minerals. The leaves also contain the enzymes like diastase and catalase besides a significant amount of all the essential amino acids except lysine, histidine and arginine, which are found only in traces.^[3] Anthelmintic drugs are used to treat infection with parasitic worms. It is important that anthelmintics are selectively toxic to the parasite and not the host. The mode of action of Albendazole is to cause paralysis of worms and expel them in the feaces.^[6]

MATERIAL AND METHOD

Sample Collection

Fresh leaves of Piper betel are collected from local place sangli. The characteristics of Piper betel leaves were identified and authenticated by Dr. Ms. M. V. Kale Vice-Principal, Jaysingpur college, Jaysingpur.

Preparation of Sample

The fresh leaves of Piper betel are washed with tap water. Leaves are dried under shade. Crushed in to fine powder in electric blender and packed in polythene bag until further process.

Preparation of ethanol extract

40gm of powder mixed with 150ml of ethanol and extracted by using soxlet apparatus for 04hrs. The filtrate was then evaporated at 60⁰c and stored at 40⁰c until further process.

Determination of Antimicrobial Activity^[3]

The antimicrobial activity was determined by Preparing plates of nutrient agar (NA) and sautrouds agar (SA). Spread bacteria on NA and fungus on SA. Prepare five wells on each plates. Dissolve given sample and standard in DMSO and prepare concentration (250, 150,50µg.) labelled each well with conc. and pour samples in each well. Almox 250 was used

as a std. for bacteria and fluconazole used for fungus. Incubates plates for 24hrs. Activity seen after 24hrs.

Phytochemical screening

In phytochemical screening assays with ethanol extract of Piper betel leaves found that, carbohydrate, protein, polyphenolic compounds, flavonoid, alkaloids and total antioxidant are present.^[3]

Preliminary Phytochemical Analysis^[5]

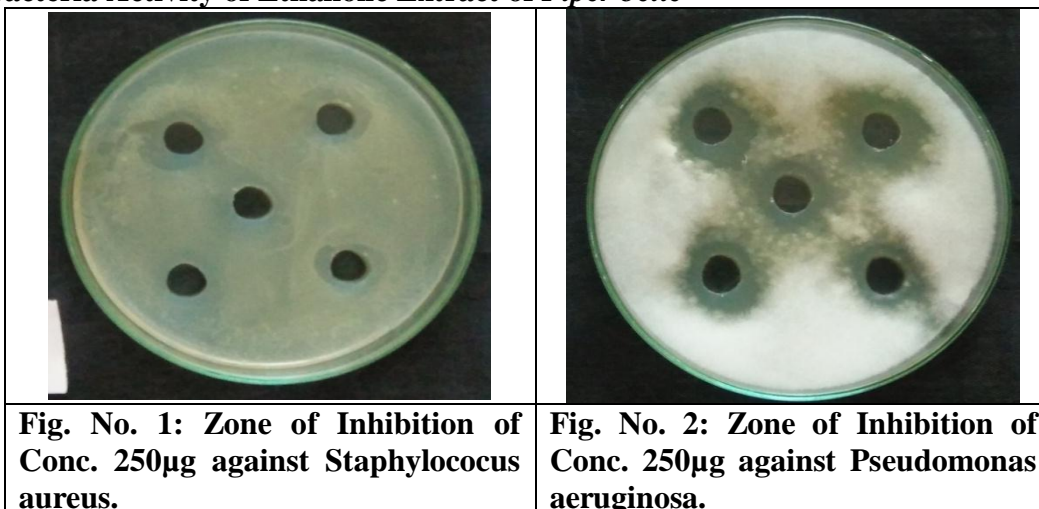
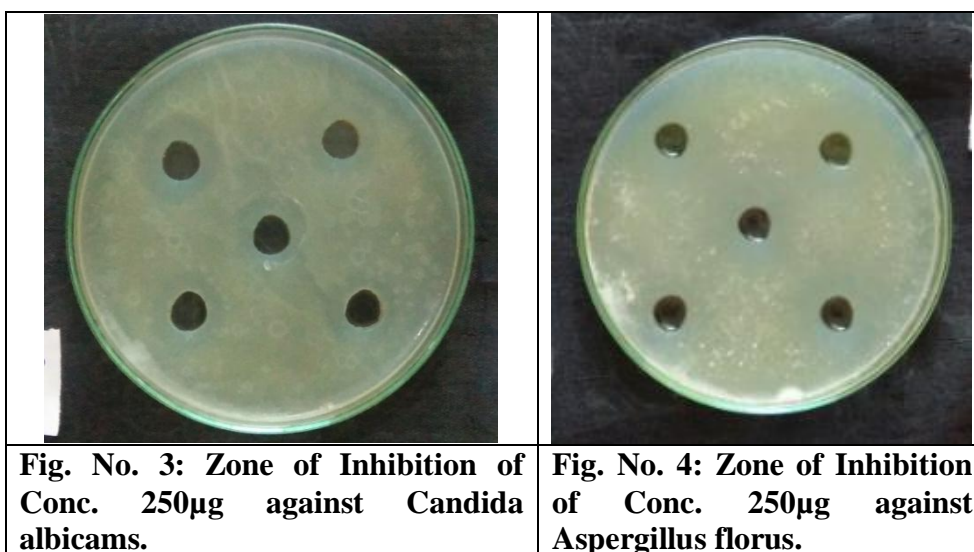
Table No: 1

| Sr. No. | Chemical Classes | Ethanol extract of <i>Piper betle</i> leaves |
|---------|------------------|----------------------------------------------|
| 1. | Flavonoids | Positive |
| 2. | Tannin | Positive |
| 3. | Saponins | Positive |
| 4. | Alkaloid | Positive |
| 5. | Glycoside | Negative |
| 6. | Carbohydrate | Positive |

Antibacterial And antifungal Activity of *Piper betle*

Table No. 2

| Sr. No. | Classes of strains | Conc. of in μg | Zone of Inhibition(mm) | |
|---------|------------------------|---------------------------|------------------------|--------------------------|
| | | | By Extract | By Almox/ Fluconazole |
| 1 | Staphylococcus aureus | 50 | 17.05 | 16.05 |
| | | 150 | 19.05 | |
| | | 250 | 21.00 | |
| 2 | Pseudomonas aeruginosa | 50 | 17.05 | 17.05 |
| | | 150 | 16.00 | |
| | | 250 | 17.00 | |
| 3 | Candida albicans | 50 | 10.05 | 12.00 |
| | | 150 | 11.05 | |
| | | 250 | 12.05 | |
| 4 | Aspergillus floruss | 50 | 11.00 | 13.00 |
| | | 150 | 12.05 | |
| | | 250 | 13.05 | |

Antibacteria Activity of Ethanolic Extract of *Piper betle***Fig. No. 1: Zone of Inhibition of Conc. 250µg against Staphylococcus aureus.****Fig. No. 2: Zone of Inhibition of Conc. 250µg against Pseudomonas aeruginosa.****Antifungal Activity of Ethanolic Extract of *Piper betle*****Fig. No. 3: Zone of Inhibition of Conc. 250µg against Candida albicans.****Fig. No. 4: Zone of Inhibition of Conc. 250µg against Aspergillus floridus.****Anthelmintic Activity of Ethanolic & Aqueous Extract of *Piper betle*****Table No. 2**

| Test Drug | Conc. (mg/ml) | Time of Paralysis (min.) | Time of Death (min.) |
|-----------------------------------------------|---------------|--------------------------|----------------------|
| Ethanolic Extract of <i>Piper betel</i> Linn. | 20 | 39±03.60 | 41.66±04.16 |
| | 40 | 34±02.06 | 36±02.06 |
| | 60 | 28±02.00 | 31.33±01.15 |
| | 80 | 22.33±2.08 | 26.66±01.52 |
| | 100 | 19±01.00 | 21±01.00 |
| Aqueous Extract of <i>piper betel</i> | 20 | 47±02.00 | 50.66±02.00 |
| | 40 | 42±02.00 | 43.33±01.52 |
| | 60 | 37±02.00 | 39.33±01.52 |
| | 80 | 31.66±01.52 | 34.33±01.52 |
| | 100 | 25±02.00 | 27.33±02.51 |



Fig. No. 5: Effect of Ethanolic Extract of Piper Betle leaf.

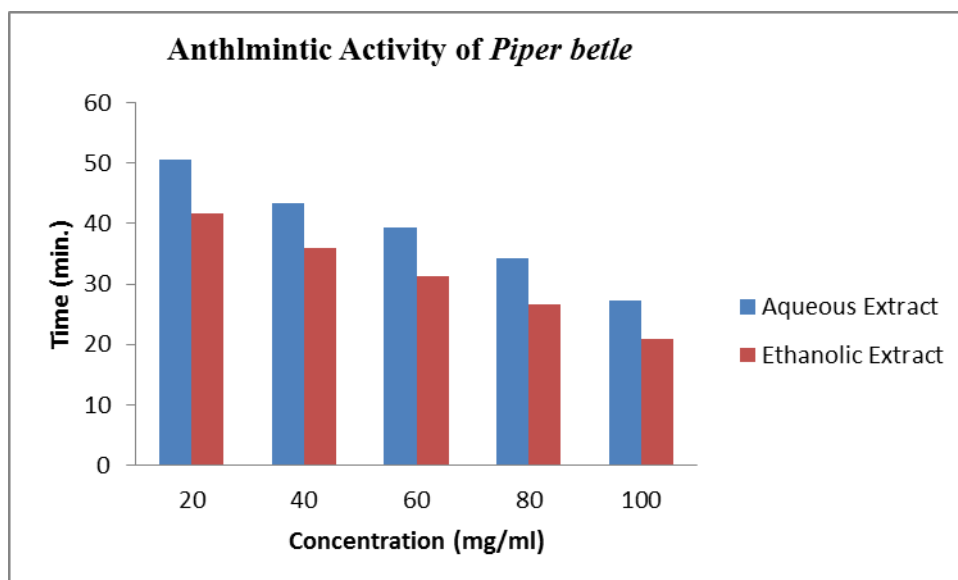


Fig. No. 3: Anthelmintic Activity of Ethanolic & Aqueous Extract of *Piper betle*.

RESULT AND DISCUSSION

The present study of Antimicrobial activity of ethanolic extract *Piper. betle* inhibited the growth of all strain i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus floruss*. The effect may due to the presence of many potent compounds such as alkaloids, tannins, phenolic substances and glycosides etc. In case of anthelmintic the *Piper betle* extract showing dose dependent response i.e. from loss of motility to death of worms. In case of anthelmintic activity test sample of *Piper betle* leaf 100mg/ml conc. showed Paralysis at 19mins. and death occurred within 21mins. So these all finding shown that test sample showed significant anthelmintic activity in a dose dependent manner. Ethanolic test sample shown faster action than aqueous test sample.

CONCLUSION

This research is concluded that Ethanolic extract Piper betle were more active against the strains of bacteria than fungi. The ethanol extract of *Piper betle* of conc. 250 µg showed the good Zone of Inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. And ethanolic extract of crude Betle leaf is having more potent activity against pheritima posthuma worms.

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