



COMPARATIVE STUDY OF ETHANOLIC EXTRACT OF VARIETIES OF PIPER BETEL L.

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ABSTRACT

The leaves of *Piper betel* have been used in the Indian folk medicinal system for its antimicrobial, antioxidant, antihemolytic and antiallergic. In the present study, the comparative evaluation of the antibacterial activity of ethanol extracts of three varieties of *Piper betel* L. namely Kapoori, Bangla and Calcutta. Extracts of betel leaves prepared by using Soxhlet apparatus in 75% ethanol. 10 mg/ml concentration of extracts were tested against pathogenic bacteria such as *Klebsiella pneumoniae*, *Staphylococcus simiae* and *Proteus vulgaris*. The ethanolic extracts showed significant antibacterial activity against tested pathogens. Ethanol extracts of all three varieties of *Piper betel* showed a good zone of inhibition against *Klebsiella pneumoniae*. Extracts of *Piper betel* L. are effective against Gram-Positive as well as Gram-Negative bacteria.

KEYWORDS- Antibacterial activity, *Piper betel*, Ethanolic extract.

INTRODUCTION

In Ayurveda, Siddha, Unani and other folk medicinal systems medicinal plants have great importance. The last 2-3 decades widespread and pathogens continuously exposed to antibiotics is leading to the resistivity against standard antimicrobial agents like antibiotics, the value of medicinal plant increases day by day [1]. To overcome this resistivity problem is to use herbal medicine to treat infectious diseases. Medicinal constituents are derived from any part of the plant like leaves, seed, bark, roots etc.

Piper betel L. (Pan) belongs to the family Piperaceae, which is a deep green heart-shaped leaves, evergreen, dioecious, perennial creeper [2]. It is cultivated in India, Srilanka, Malaysia, Thailand and other South East Asia countries [3]. In the folk medicinal system *Piper betel* L. leaf is useful for the treatment of various diseases like acrid, healing, bad breath, conjunctivitis, constipation, headache, itches, stomachic, bronchitis, laxative, aphrodisiac, ringworm, swelling of gum [3-5] etc and betel leaf also possesses antibacterial, antifungal, antimalarial, anticancer, antioxidant, anti-allergic, antidiabetic and antihemolytic properties [6].

The Three cultivars of *Piper betel* L. taken for the experimental study were namely; Kapoori, Bangla and Calcutta. The varieties show many morphological similarities and dissimilarities, as described in [7]. Along with these morphological differences, they also differ in their smell, texture and taste. The present study evaluated the comparative antibacterial properties of ethanolic extracts of three varieties of *Piper betel* L. against pathogenic bacteria *Klebsiella pneumonia*, *Staphylococcus simiae* and *Proteus vulgaris*.

MATERIALS AND METHODS

Sample collection

Fresh, uninfected and undamaged leaves of *Piper betel* L. of all three varieties were purchased from the local market. Identification of the *Piper betel* L. leaves was confirmed by a taxonomist. The leaves were cleaned, washed and allowed to dry in shadow. The leaves were pulverized using an electric blender to dry the powder.

Bacterial strain and culture preparation

Three pathogenic bacterial cultures *Klebsiella pneumonia*, *Staphylococcus simiae* and *Proteus vulgaris* were used for the present study. Bacterial cultures were grown in nutrient broth at 37° C.

Preparation of plant extract by Soxhlet

The powdered of all three varieties were used for the preparation of extracts. Soxhlet apparatus was used for the extraction process. All these three extracts were prepared in 75% ethanol. After extraction the excess ethanol from the extracts was allowed to dry in a hot air oven at 40-50° C, so ethanol evaporated. Dried extracts were stored at 4° C in sterile cap bottles for further usage.

Antibacterial Activity

Antibacterial activity of ethanol extracts of three varieties of betel were carried out by well diffusion method [8]. Agar plates were prepared using sterile Muller Hinton agar. Overnight incubated bacterial suspensions (10⁶ CFU/ml) were spread on Muller Hinton agar plates (50µl inoculums/plate) and thereafter 5mm well were prepared using a sterile borer. The dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to prepare a final concentration of 10mg/ml. With a micropipette, 60µl of each extracts were added to each plate 1st, 2nd and 3rd well were ethanol extracts of Kapoori, Bangla and Culcutta respectively. Beside Plant extracts, four different standard available antibiotics (Ceftazidime, Tetracycline, Chloramphenicol and Ampicillin) were used to evaluate the study. The plates were incubated at 37° C for 24 hrs to observe results. The zone inhibition was measured in mm.

RESULTS AND DISCUSSION

The antibacterial activity of the plant extracts and antibiotics were observed. The zones of inhibition showed the tested antibiotics and plant extracts were observed and reported. Tested some bacteria are resistant and some susceptible to the antibiotics (Table 1 and Figure 1). Plant extracts of all three varieties showed good antibacterial activity against tested bacterial pathogens (Table 2 and Figure 2). The maximum zone of inhibition of Calcutta extracts observed while the minimum zone of inhibition of Kapoori leaves extracts observed against tested bacteria. As compared to Kapoori and Bangla extracts, Calcutta extracts showed maximum antibacterial activity. The maximum zone of inhibition of Calcutta extracts (21mm) observed against *Staphylococcus simiae*, followed by a zone of inhibition (20 mm) against *Klebsiella pneumonia* and *Proteus vulgaris* while Kapoori extracts showed less effective against tested bacteria as compared to

Bangla and Calcutta. Ethanol extracts of all three varieties of *Piper betel* showed a good zone of inhibition against *Klebshiella pneumonia*.

Table 1: Antibacterial Susceptibility Assay of Standard Antibiotics

Sr. no.	Antibiotics	Zone of inhibition (in mm)		
		<i>Klebshiella pneumonia</i>	<i>Staphylococcus simiae</i>	<i>Proteus vulgaris</i>
1.	Ceftazidime	08	09	0
2.	Chloramphenicol	26	28	22
3.	Tetracycline	23	30	29
4.	Ampicillin	19	29	0

Table 2: Antibacterial Susceptibility Assay of ethanol extracts of *Piper betel* L.

Sr. no.	Varity of <i>Piper betel</i> L.	Zone of inhibition (in mm)		
		<i>Klebshiella pneumoniae</i>	<i>Staphylococcus simiae</i>	<i>Proteus vulgaris</i>
1	Kapur	18	15	16
2	Bangla	18	16	16
3	Calcutta	20	21	20

Figure 1: Antibacterial Susceptibility Assay of Standard Antibiotics

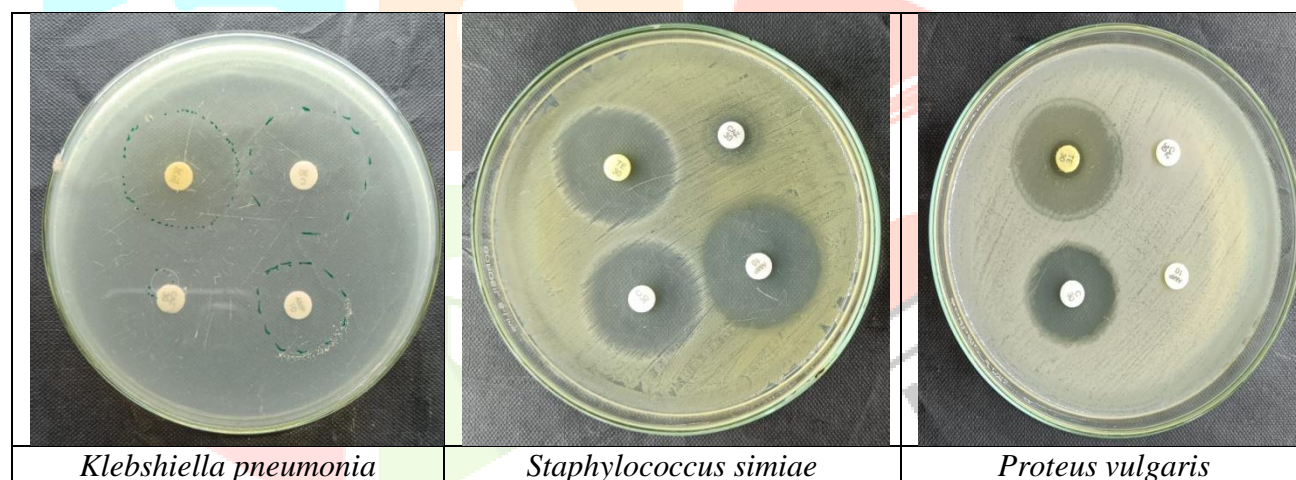
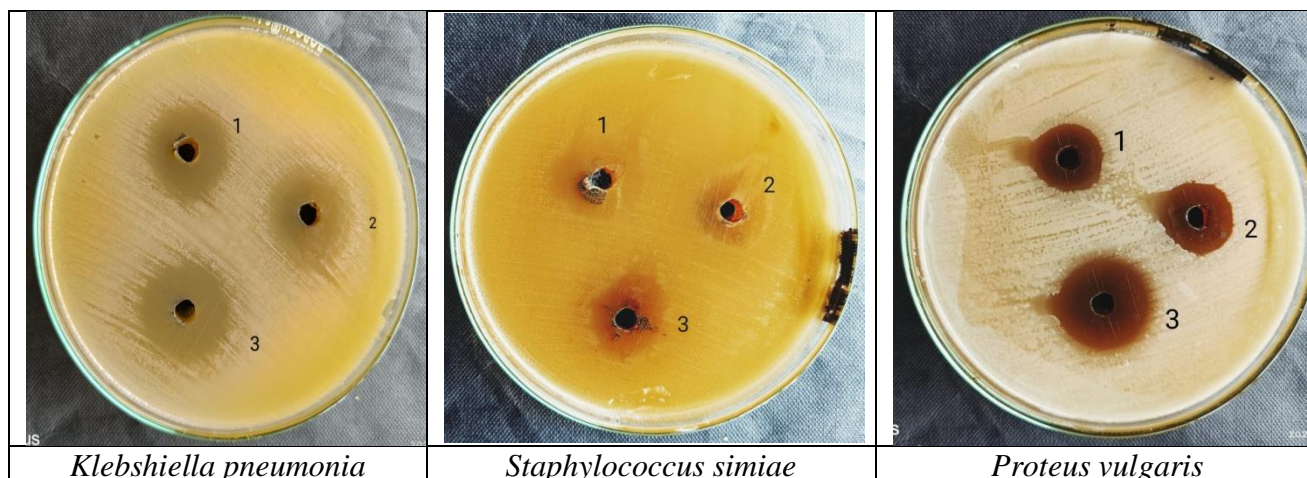


Figure 2: Antibacterial Susceptibility Assay of ethanol extracts of three varieties of *Piper betel* L.



The literature showed that aqueous and methanol extracts of *Piper betel* L. has antimicrobial activity against *Enterococcus faecalis*, *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Lactobacillus* sp. Maximum activity of methanol extracts (1000 µl) was observed (26 mm) against *Streptococcus mutans* [9]. In a study ethanolic extracts of *Piper betel* var. Jaleshwar (conc. 500 mg/ml) showed the maximum antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (1) which is compared with our study where the maximum inhibition (21 mm) of ethanol extracts Calcutta. [10] reported the ethanolic extracts of *Piper betel* has significant antibacterial activity against *Pseudomonas areuginosa*, *Proteus vulgaris*, *Staphylococcus aureus* and *Klebsiella pneumonia*.

In our study, extracts of *Piper betel* L. are effective against Gram-positive as well as Gram-negative bacteria.

CONCLUSION

From the present work, it can be concluded that the leaves of *Piper betel* L. of varieties Kapoori, Bangla and Calcutta are a very good source of herbal drugs. The further formulation prepared from purified herbal antibacterial compounds to treat diseases, may minimize the overuse of antibiotics. The future prospects of the present study are screening the bioactive compound from *Piper betel* L.

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