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Research Article

COMPARATIVE STUDY OF BETEL LEAF EXTRACT AGAINST STANDARD ANTIBIOTICS AGAINST BACTERIA CAUSING DENTAL CARIES

Manabendra Nayak[1]., Rahul Nayak[2] and Lukpu Ngulom[3]

[1]Department of Medicine, Down town Hospital, Guwahati, Post Graduate Teacher of Medicine, National Board of Examination
[2,3]Department of Microbiology, Assam Down Town University, Guwahati

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ABSTRACT

Dental caries is a microbiological infection of the tooth which results in localized dissolution and destruction of the calcified tissue and it is one of the most common chronic infectious diseases in humans throughout the world. The leaves of *Piper betel* (locally known as Paan) have long been in use in the Indian local system of medicine for its antioxidant and antimicrobial properties. The purpose of the study was to determine the comparative activity of P. betel leaf extracts and standard antibiotics against the bacteria isolated from oral region (teeth).The teeth swabs were collected from patients suffering from dental caries. The bacteria present in the samples were isolated and identified by morphological and biochemical characteristics. Extracts preparation was carried out in different solvents (ethanol, methanol and aqueous). The isolated bacteria were subjected to antibacterial sensitivity test by agar well diffusion method against different concentrations of ethanol, methanol and aqueous extract. Standard antibiotics (tetracycline, streptomycin, ampicillin, pen-G, etc.) are used as positive control. All the extracts showed inhibitory effect against the tested bacteria. In conclusion it can be said betel leaves have potent antimicrobial effect on oral bacteria that lead to infection in mouth or normally present in mouth.

INTRODUCTION

Dental caries is recognized as one of the most infectious diseases worldwide. [1] The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by cariogenic bacteria. [2]Expanding resistance of bacterial pathogens to regularly utilize antibiotics has turned into general human concern. The spread of antibiotic resistance is causing fatalities, as well as a high financial inconvenience. In low economic nations, antibiotic resistance is considered to be more prevalent than in the developed countries. [3]

Piper betel L. (Piperaceae) leaf is widely used as a mouth freshener after meal. Betel leaf has been described from ancient times as an aromatic, stimulo-carminative, astringent and aphrodisiac. [4]Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, cuts and injuries. [5] The leaf has a significant antimicrobial activity against broad spectrum of micro-organisms. [6]Over 700 species of *Piper betel* has been distributed in both of the hemispheres of world. Of these, 30 species have been recorded in India, 18 in Srilanka and 3 are endemic. *Piper betel* is cultivated in India, Srilanka, Malaysia, Indonesia, Phillipine Islands and East Africa.[7] Betel leaf is mostly consumed in Asia, as betel quid or in paan, with or without tobacco, in an addictive psychological formation which may have an adverse health effects. The betel plant is evergreen perennial, with glossy heart-shaped leaves. Betel is notable for staining the teeth of regular users. The parts of piper betel utilized are leaves, roots, stems, stalks and fruits. Piper betel has light yellow aromatic essential oil with sharp burning taste. The leaves are pungent, bitter, sweetish, bitter and acrid in nature. [8]

***Vernacular Names*[9, 10]**

Table 1 Vernacular names of *Piper betel* L

English	Betel
Hindi	Paan
Tamil	Vetrilai
Malaysia	Sirihmelayu
Sanskrit	Nagavallari
Assamese	Paan

Corresponding author: **Manabendra Nayak*
Department of Medicine, Down town Hospital, Guwahati, Post Graduate Teacher of Medicine, National Board of Examination

The objective of this study includesisolating and identification of bacteria present in the oral region and to study the comparative antibacterial activity of betel leaf extract and standard antibiotics against the isolated bacteria.

METHOD

Clinical Samples

Teeth swabs were collected from patients suffering from dental caries using cotton swabs.The teeth swab collected in sterile container is brought to the laboratory and cultured. The teeth swab was inoculated in the nutrient agar plates directly by streaking back and forth. The plate was incubated overnight at 37^{o}C. The plate was looked for single bacterial colony and the single colony was again sub cultured by streaking method.

The isolated bacteria were characterized based on their colony morphology, shape, colour, staining and different biochemical tests. The different staining techniques performed for the characterization of the isolated bacteria includes Gram's staining, Capsule staining and Endospore staining. The different biochemical tests performed for the characterization of the isolated bacteria includes Indole test, Methyl-Red test, Citrate utilization test, Catalase test. The test organisms were identified by VITEK instrument at microbiology laboratory, down town hospital, Guwahati, Assam.

Plant Sample

Betel leaves were collected from market of Guwahati. After collecting the leaves were washed and shade dried. After drying the leaves were grinded in mortal pestle till it become powdered form.

Extract Preparation

Water extract

20 gram of the powdered betel leaf sample was weigh and taken in 250 ml of distilled water and kept for 3 nights. The extract was then filteredusingWhattman no. 1 filter paper and allowed to evaporate in water bath at 50^{o} C. The dried extract is stored in refrigerator at 4^{o} C for further use.[11]

Methanol extract

20 gram of the powdered betel leaf sample was weigh and taken in 250 ml of 70% methanol and kept for 3 nights. The extract was then filteredusing Whattman no 1 filter paper and allowed to evaporate in water bath at 50^{o}C. The dried extract is stored in refrigeratorat 4^{o} C for further use.[11]

Ethanol extract

20 gram of the powdered betel leaf sample was weigh and taken in 250 ml of 70% ethanol and kept for 3 nights. The extract was then filtered using Whattman no 1 filter paper and allowed to evaporate in water bath at 50^{o} C. The dried extract is stored in refrigeratorat 4^{o} C for further use. [11]

Phytochemical Analysis

After the successful extraction of the plant, the resulted extracts are subjected to various phytochemical screening. Most of the plants contain medicinally active secondary metabolites. These metabolites are alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, trepenoids, flavonoidsproteins and phenolic compounds. So, all the extracts will be subjected to different chemical tests to detect the presence of constituent. [12]

Comparison of Isolates Against Extracts And Antibiotics

Different types of antibiotics used were penicillin, ampicillin, and tetracycline. Comparison between plant extracts and antibiotics was determined by agar well diffusion method against the bacteria isolated from the oral region (teeth). Pure isolate of each bacterium was sub-cultured in nutrient broth at 37°C for 24 hours. About 100 micro liter of each test bacterium was spread with the help of sterile spreader on to a sterile nutrient agar plate. The plate was allowed to dry. A well was made on the centre of the agar plate. The well was labeled and 50 micro liters vol. of the extract was poured in the well on the Muller Hinton agar plates. Standard antibiotics were used as standard compare to the extracts. The plates were then incubated at 37°C for 24 hours. After completion ofincubation period, zones of inhibition were measured and recorded to the nearest size in millimeters.

Preparation of Standard Concentration of The Extracts

1gm of each alcoholic extracts was dissolved in 1ml of Dimethyl sulphoxide (DMSO) and 1gm of aqueous extract was dissolved in distilled water. Thus, 1g/ml stock was obtained as a standard concentration of alcoholic extracts. Aqueous was sterilized using 0.40µm membrane filter.

Table 2 Phytochemical analysis of Extracts of P.betel leaf

Phytochemical tests		Ethanol extract	Methanol extract	Aqueous extract	Interference
Detection of alkaloids	Mayer's test	+ve	+ve	+ve	Alkaloids
	Wagner's test	+ve	+ve	+ve	
Detection of carbohydrates	Molish's test	-ve	-ve	-ve	Carbohydrates
	Barfoed's test	-ve	-ve	-ve	Carbohydrates
	Benedict's test	-ve	+ve	-ve	Reducing sugar
Detection of glycosides	Libermann's test	+ve	+ve	+ve	Steroids ring
	Salkowski's test	+ve	+ve	+ve	
Detection of saponins	Forth formation test	+ve	+ve	+ve	Saponins
Detection of tannins	Ferric chloride test	+ve	+ve	+ve	Tannins
	Gelatine test	+ve	+ve	+ve	
Detection of flavonoids	Ferric chloride test	+ve	+ve	+ve	Flavanoids
	NaOH test	+ve	+ve	+ve	
	Alkaline reagent test	+ve	-ve	-ve	
Detection of trepenoids	Ferric chloride test	+ve	+ve	+ve	Trepenoids
Detection of steriods	Carr-Price reagent	+ve	+ve	+ve	Steroids
Detection of protiens	Ninhydrin test	+ve	+ve	+ve	Amino acids
Detection of phenolic compounds	Ferric dhloride test	+ve	+ve	+ve	Phenolics

RESULTS

Staining characteristics of the isolates: Out of the 30 isolated bacteria 24 were gram positive and 6 gram negative, 21 bacteria possesses capsule and 20 bacteria contains endospore.

Biochemical test of the isolates: All the 30 bacterial isolates were Indole negative, 24 bacteria were MR positive, 27 utilized citrate and 20 bacteria were Catalase positive.

Identification of the test organisms

The test organisms were identified by VITEK instrument at microbiology laboratory, down town hospital. Predominantly three organisms were identified to be *Staphylococcus xylosus*, *Klebsiella pneumonia* and *Bacillus sp.*

Phytochemical analysis of Extracts of P. betel leaf

Comparison of Antibacterial Activity of Extracts and Antibiotics against the Isolated Bacteria Antibacterial susceptibility test was performed against the bacteria isolated from oral region (teeth).Standard antibiotics such as ampicillin, penicillin, tetracycline, streptomycin, etc. was used as positive control.

Comparison of Ethanol Extract and Antibiotics against the Isolates

Comparative studies of ethanol extract and antibiotics against *Staphylococcus xylosus* showed that the ethanol extract showed a zone of 25mm, tetracycline was effective with a zone of 20mm, followed by ampicillin with a zone of 13mm and penicillin 11mm.

Comparative studies of ethanol extract and antibiotic against *Klebsiella pneumonia* showed that the ethanol extract was effective with a zone of 26mm. Tetracycline was found to be sensitive with a zone of 24mm followed by penicillin with a zone of 11mm. Ampicillin showed no zone.

The ethanol extract was effective against *Bacillus species* and showed a zone of 30mm, tetracycline was found to be effective with a zone of 25mm. Ampicillin and penicillin show resistant against *Bacillus sp.*

Zone of inhibition (mm) on comparison on activity of ethanol extract and antibiotics

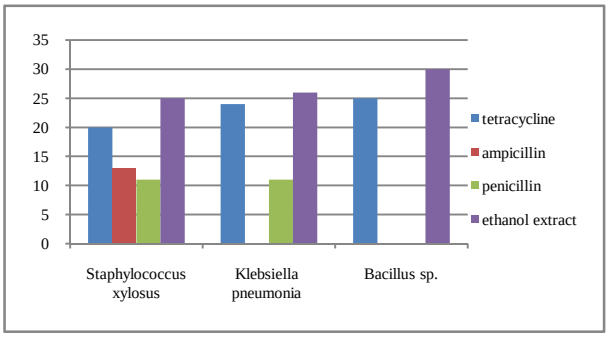


Figure 2 Comparative activities between ethanol extract and antibiotics against isolates

Comparison of Antibacterial Activity of Methanol Extracts and Antibiotics against The Isolates

In the comparative studies of methanol extract and antibiotics showed that the methanol extract was effective against *Staphylococcus xylosus* and showed a zone of 25mm. The antibiotics were found to be sensitive against the bacteria. Tetracycline showed the widest zone with 19mm, followed by ampicillin with 15mm and penicillin with 14mm.

Comparative studies of methanol extract and antibiotics against *Klebsiella pneumonia* showed that the extract was effective and showed a zone of 25mm. Tetracycline was effective and showed a zone of 16mm. Ampicillin and penicillin had shown no zone of inhibition against *Klebsiella pneumonia.*

Comparative studies of methanol extract and antibiotics against *Bacillus species* showed that methanol extract was effective and showed a zone of inhibition of 23mm. tetracycline was effective with a zone of 20mm. Ampicillin and penicillin had shown no zone of inhibition against *Bacillus species.*

(A)Staphylococcus xylosus ***(b) Klebsiella pneumonia*** ***(c) Bacillus sp.***

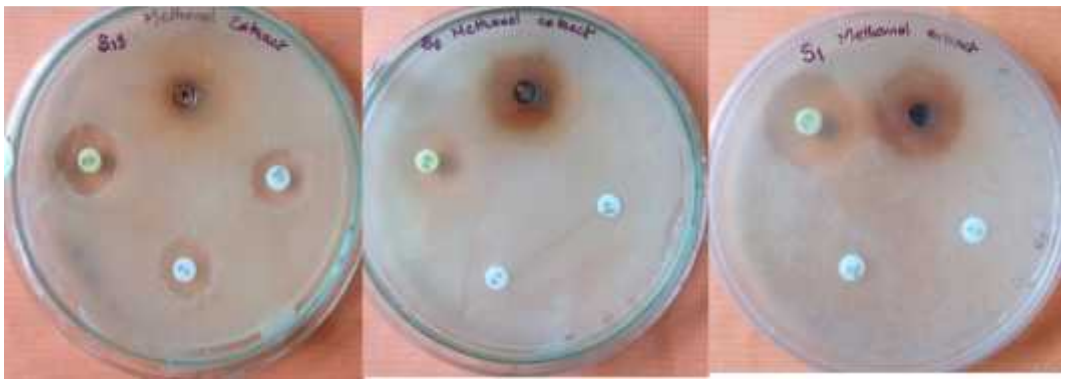
Figure 1 (a-c) Bacterial strains demonstrating susceptibility to *Piper betel* extracts.

Table 3 Zone of inhibition (mm) on comparison on activity of ethanol extract and antibiotics

Test organisms	Zone of in inhibition of antibiotics (in mm)			Zone of in inhibition of extract (in mm) (50µl)
	Tet	Amp	P	
Staphylococcus xylosus	20	13	11	25
Klebsiella pneumonia	24	0	11	26
Bacillus species	25	0	0	30

Table 4 Zone of inhibition (mm) on comparison on activity of methanol extract and antibiotics

Test organisms	Zone of in inhibition of antibiotics (in mm)			Zone of inhibition of extract (in mm) (50µl)
	Tet	Amp	P	
Staphylococcus xylosus	19	15	14	25
Klebsiella pneumonia	16	0	0	25
Bacillus species	20	0	0	23



(a) Staphylococcus xylosus *(b) Klebsiella pneumonia* *(c) Bacillus species*

Figure 3 (a-c) Bacterial strains demonstrating susceptibility to *Piper betel* extracts.

Zone of inhibition (mm) on comparison on activity of methanol extract and antibiotics

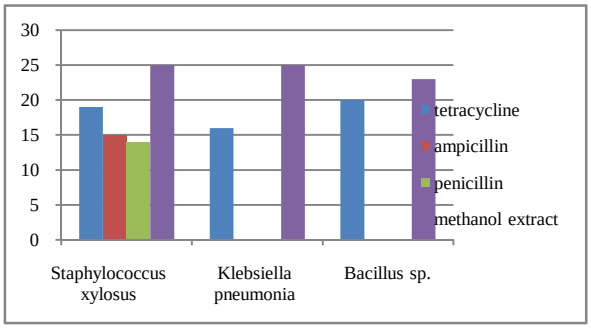


Figure 4 Comparative activities between methanol extract and antibiotics against isolates.

Comparison of Antibacterial Activity of Aqueous Extract And Antibiotics Against The Isolates

Comparative studies of aqueous extract and antibiotics against *Staphylococcus xylosus* showed that the aqueous extract was effective and had shown a zone of clearance of 19mm. Tetracycline was effective against the bacteria and showed a zone of 16mm. Ampicillin was effective and showed a zone of 13mm. Penicillin had shown no zone of inhibition against *Staphylococcus xylosus.*

Comparative studies against *Klebsiella pneumonia* showed that the extract was effective and showed a zone of clearance of 23mm. Tetracycline was effective against the bacteria with a zone of inhibition of 16mm while the other two positive controls showed no zone of inhibition against *Klebsiella pneumonia.*

Comparative studies of aqueous extract and antibiotics against *Bacillus species* showed that the extract was effective with a zone of clearance of 22mm. Tetracycline was effective against the bacteria with a zone of inhibition of 16mm while the other two controls had shown no zone of inhibition against *Bacillus species.*

Zone of inhibition (mm) on comparison on activity of aqueous extract and antibiotics

Table 5 Zone of inhibition (mm) on comparison on activity of aqueous extract and antibiotics

Test organisms	Zone of in inhibition of antibiotics (in mm)			Zone of in inhibition of extract (in mm) (50µl)
	Tet	Amp	P	
Staphylococcus xylosus	16	13	0	19
Klebsiella pneumonia	16	0	0	23
Bacillus species	16	0	0	22

DISCUSSION

Medicinal plants have been used to treat a variety of disorders including inflammatory conditions, infections with microorganisms, cancer, allergy and other disorders. Herbal medicines are a valuable and readily available resource for primary health care and complementary health system.

(a) Staphylococcus xylosus *(b) Klebsiella pneumonia* *(c) Bacillus sp.*

Figure 5 (a-c) Bacterial strains demonstrating susceptibility to *Piper betel* extracts.

They can be the best alternative for the available antibiotics against which the pathogens are adapting resistance. Phytochemicals are the bioactive component present in most of the plants. Plants show antibacterial activity because of the presence of different phytochemical compounds.

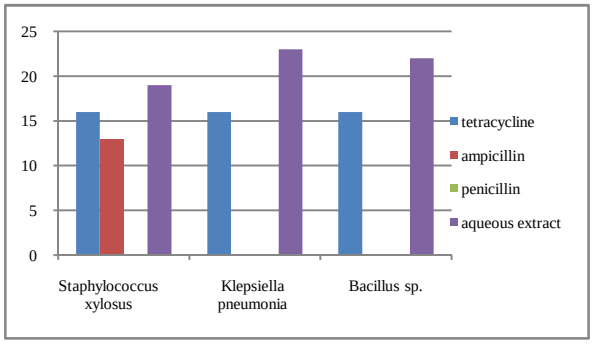


Figure 6 Comparative activities between of aqueous extract and antibiotics against isolates.

The present study is aimed at the evaluation of the antibacterial activity of *Piper betel* leaf extracts in comparison to standard antibiotics against the bacteria isolated from the oral region (teeth). 30 samples of teeth swabs were collected by direct swabbing of the teeth of the patient suffering from dental caries. Isolation was done by swabbing the teeth swab on the nutrient agar plate and colonies were obtained. The bacteria present in the samples were identified by morphological and different biochemical tests.

In this present study, various phytochemical tests were performed for the *Piper betel* leaf extracts. Phytochemical analysis of *P. betel* leaf showed the presence of alkaloids, flavanoids, tannins, saponins, glycosides, protein and carbohydrates. Extracts were prepared from dried leaves of *Piper betel* plant using Ethanol, methanol and water. The antimicrobial activity of the extracts was determined by agar-well diffusion method. Ethanol, methanol and aqueous extracts of *P. betel* leaf were taken for the antibacterial studies against the bacterial isolated from the oral region (teeth). The test organisms were identified by VITEK instrument at microbiology laboratory, down town hospital. The organisms were identified to be *Staphylococcus xylosus, Klebsiella pneumonia* and *Bacillus sp.*

In the present study, the P. betel leaf extracts (ethanol, methanol and aqueous) showed activity against the isolated bacteria.

In the present study, the ethanol extract (50 µl concentration) showed zone of inhibition with a diameter of 25mm in case of *Staphylococcus xylosus*, 26mm in case of *Klebsiella pneumoniae* and 30 mm in case of *Bacillus species.* The antibiotic tetracycline showed a zone of inhibition of 20 mm in case of *Staphylococcus xylosus*, 24mm in case of *Klebsiella pneumoniae* and 25 mm in case of *Bacillus species.* Ampicillin showed a zone of inhibition of 13 mm in case of *Staphylococcus xylosus* and showed resistant against *Klebsiella pneumoniae* and *Bacillus species*. Penicillin showed a zone of inhibition of 11 mm in case of *Staphylococcus xylosus*11 mm in case of *Klebsiella pneumoniae* and resistant against *Bacillus species*(shown in table 3).

In the present study, the methanol extract (50 µl concentration) showed zone of inhibition with a diameter of 25mm in case of *Staphylococcus xylosus*, 25mm in case of *Klebsiella pneumoniae* and 23 mm in case of *Bacillus species.* The antibiotic tetracycline showed a zone of inhibition of 19 mm in case of *Staphylococcus xylosus*, 16 mm in case of *Klebsiella pneumoniae* and 20 mm in case of *Bacillus species.* Ampicillin showed a zone of inhibition of 15 mm in case of *Staphylococcus xylosus and* showed resistant against *Klebsiella pneumoniae* and *Bacillus species*. Penicillin showed a zone of inhibition of 14 mm in case of *Staphylococcus xylosus and* showed resistant against *Klebsiella pneumoniae* and *Bacillus species* (shown in table 4).

In the present study, the aqueous extract (50 µl concentration) showed zone of inhibition with a diameter of 19 mm in case of *Staphylococcus xylosus*, 23 mm in case of *Klebsiella pneumoniae* and 22 mm in case of *Bacillus species.* The antibiotic tetracycline showed a zone of inhibition of 16 mm in case of *Staphylococcus xylosus*, 16 mm in case of *Klebsiella pneumoniae* and 16 mm in case of *Bacillus species.* Ampicillin showed a zone of inhibition of 13 mm in case of *Staphylococcus xylosus and* showed resistant against *Klebsiella pneumoniae* and *Bacillus species*. Penicillin was found resistant to *Staphylococcus xylosus, Klebsiella pneumoniae* and*Bacillus species* (shown in table 5).

In this study, it shows that the Piper betel leaf extracts successfully inhibited the growth of bacteria isolated from oral region (teeth) in comparison to the standard antibiotics used.

CONCLUSION

The plant sample was collected from the local market, sixmile, Guwahati. The plant extracts were assayed for phytochemical compounds and antibacterial activity. Antibacterial activity was screened against the bacteria isolated from oral region (teeth). Plant leaves were used for extraction of antibacterial metabolites using ethanol, methanol and water. All the extracts showed effect against the isolated bacteria. The standard antibiotics used were less effective in comparison to the plant extract. So from the present study it can be concluded that *Piper betel* leaf can be a tool for fighting against the oral pathogenic bacteria which are gaining resistance to the drugs available in the market.

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