



Antibacterial Activity of Piper Betel Linn. Leaves

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Abstract:

Piper Betel Leaves is one of the invaluable medicine plants where its leaves have been for many medicinal purposes. Ethanol, Chloroform and Petroleum Ether Extract of the leaves of Piper betel L. were evaluated for antibacterial activity against three Gram positive, eight Gram negative. The Extraction of piper betel leaves taken by Soxhlet Assembly. The ethanol extract was considerably more effective than chloroform and petroleum extract inhibiting the investigated microbial strains.

Keywords: Piper betel, Antibacterial activity, leaves extract, Medicinal plants.

INTRODUCTION

Piper betel L. belongs to the family of Piperaceae, Its common name is betel in English, paan in India and Bangladesh, phlu in Thailand and Sirih in Bahasa Indonesian. Betel leaves is widely used as a mouth freshener after meal. This plant is extensively grown in Bangladesh, India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Leaves 10-20 cm long, broadly ovate, slightly cordate and often unequal at the base, shortly acuminate, glabrous, glaucous on both sides, bright green or yellowish, petiole stout 2.0-2.5 cm long. Male spikes cylindrical dense. Female spikes 2.5-5.0cm long, pendulous. Fruits rarely produced, often sunk in the fleshy spike, forming nodule-like structures (Sengupta R. and Banik J.K., 2013). The Betel (*Piper betel* L.) is a spice whose leaves have medicinal properties. The branches of the plant are swollen at the nodes. The plant has alternate, heart-shaped, smooth, shining and long-stalked leaves, with pointed apex. It has five to seven ribs arising from the base; minute flowers. Betel is a native of central and eastern Malaysia. It spread at a very early date throughout tropical Asia and later to Madagascar and East Africa. In India, it is widely cultivated in Tamil Nadu, Madhya Pradesh, West Bengal, Orissa, Maharashtra and Uttar Pradesh. Offering betel morsel (pan-supari) to guests in Indian subcontinent is a common courtesy.

Strong pungent aromatic flavored leaves are traditionally used for chewing in their natural raw condition along with many other ingredients like sliced areca nut, slaked lime, coriander, aniseed, clove, cardamom, sweetener, coconut scrapings, ashes of diamond, pearl, gold and silver (Ayurveda preparations), jelly, pepper mint, flavoring agent, fruit pulp etc. by the people. Indian system of medicine and health has adopted the use of betel leaves in various ways. In Indian folkloric medicine, betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for its healing effects. Essential oil extracted from

betel leaf may be used as an industrial raw material for manufacturing medicines, perfumes, mouth fresheners, tonics, food additives etc. The leaves have also been used in traditional medicine as carminative, stimulant, antiseptic, antifungal, and antibacterial agent. (M Mahfuzul Hoque *et al.*, 2011).

Plant Profile:

Taxonomical classification:

Kingdom	:	Plantae
Division	:	Magnoliphyta
Class	:	Magnolipsida
Order	:	Piperales
Family	:	Piperacene
Genus	:	<i>Piper</i>
Species	:	<i>betel</i>

Vernacular name:

Sanskrit: Tambool, Mukhbhushan, Varnalata

Hindi: Paan

English: Betel, Betel pepper, Betel vine

Telugu: Nagballi, Tamalapaku

Tamil: Vetrilai

Gujrati: Nagarbael

(Dwivedi V. *et al.*, 2014)

Nutritional composition of betel leaves

Approximately 85-90% of betel leaves is water, which means it has high moisture content and low calorie count. About 100 grams of betel leaves contain just 44 calories. It contains 0.4-1% fat and 3-3.5% proteins, which make it a low source of fat and moderate source of protein. Furthermore the betel leaves contain moderate amount of essential nutrient's such as iodine(3.4mcg/100grams), potassium (1.1-4.6%), vitamin A (1.9-2.9mg/100grams), vitamin B1(13-70mcg/100grams), vitamin B2(1.9-30mcg/100grams)and nicotinic acid (0.63-0.89mg/100grams). Besides these nutrients betel leaves contain essential oils and chemicals components such as a betel oil and chavicol, betelphenol, eugenol, terpene and campene. These chemical components possess medicinal properties and help in the treatment and management of various diseases and disorders.

Chemical Constituents:

Plant contains a terpinine, P-cymene, carvacrol, chavicol and its derivatives, allyl catechol, eugenol, estragol, oxalic acid, malic acid and amino acids. Leaves contain good amounts of vitamins particularly nicotinic acid, ascorbic acid and carotin. They also contain significant amounts of all essential amino acids except lycine, histidine and arginine. Large concentrations of asparagines are present while glycine and proline occur in good amount. Essential oil of leaf gives it the aromatic flavour. β -sitosterol is present in the root.

Table 1. Chemical composition of *Piper betel* Linn. leaves

Components	% of Components
Chavibetol	53.1
Chavibetol acetate	15.5
Caryophyllene	3.71
Allypyrocatechol diacetate	0.71
Campene	0.48
Chavibetol methyl ether	0.48
a-Pinene	0.32
f-Pinene	0.21
u-Limonene	0.21
Saprobe	0.11
1,8-Cineol	0.04
Allylpyrocatechol Monoacetate	0.23

(Sunil Kumar Shah *et al.*, 2016).

The use of betel leaf can be traced as far back as two thousand years. Betel leaves help to heal the following illnesses. Such as

- **Headache:** Betel leaf is a popular home remedy for headache. The betel leaf has analgesic and cooling properties. It can be applied with beneficial results over the painful area to relieve intense headache.
- **Scanty or Obstructed Urination:** Betel leaf juice is credited with diuretic properties. Its juice, mixed with dilute milk and sweetened slightly, helps in easing urination.
- **Weakness of Nerves:** Betel leaves play a vital role in the treatment of nervous pains, nervous exhaustion and debility. The juice of a few betel leaves, with a teaspoon of honey, will serve as a good tonic. A teaspoon of this can be taken twice a day.
- **Sore Throat:** Betel leaf is an excellent household remedy in the treatment of cough and sore throat. Local application of the leaves is effective in treating sore throat. The crushed fruit or berry should be mixed with honey and taken to relieve irritating cough. (Chopra R.N *et al.*, 1954).

AIM AND OBJECTIVES

The aim of this study is to evaluate the antibacterial activity of *Piper betel* L. used in traditional medicinal system for treatment and manifestations caused by opportunistic pathogens.

- Collection of *Piper betel* L. leaves.
- To prepare *Piper betel* L. leaves extract by soxlet.
- Phyto-chemical analysis of *Piper betel* L. leaves
- Antibacterial screening of *Piper betel* L. leaves extracts.

MATERIAL AND METHODS

The basic requirements for the study were as follows,

Plant Material

Healthy, uninfected and undamaged *Piper betel* (paan) leaves were purchased from local market. The leaves were cleaned, washed.

Bacteria

Eleven bacterial strains (3 Gram-positive and 8 Gram-negative) were used for the study.

Gram-positive bacteria

- *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*

Gram-negative bacteria

- *Escherichia coli*, *Pseudomonas putida*, *Salmonella typhi*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Klebsiella pneumoniae*, *Proteus mirabilis* (Fawad *et al.*, 2012).

Media

- Nutrient agar, Nutrient broth, Muller Hinton Agar (MHA).

Method

Preparation of leaf extract

The leaves were cleaned, washed and allowed to dry in shadow. Finely cut *Piper betel* L. leaves were taken for extraction. Antimicrobial metabolites from the dried leaves of *Piper betel* were extracted using various solvents such as Chloroform, Ethanol and Petroleum ether. All three mixtures were continuously extracted for about 4 hours. The extracts were then collected and concentrated under reduced pressure in a rotary vacuum evaporator until a semisolid substance was obtained. Extracts were stored in sterile cap bottles under refrigeration condition at 4^o C prior to use for subsequent assays.

Phytochemical Screening

For phytochemical analysis, extract were prepared by using successive solvent extraction method of dried powder of leaves of *P. betel* L. These extracts were screened for the presence of various phytochemical constituents such as of alkaloids, steroids/triterpenoids, saponins, polyphenols, tannins, flavonoids, quinone, monoterpenes, and sesquiterpenes in the leaves of *P. betel* L.

Antibacterial activity

The antibacterial activity of *Piper betel* leaf extract was determined by Agar Well Diffusion method against selected bacteria strains. Muller Hinton Agar was used for antibacterial activity. Pure isolate of each bacterium was sub-cultured in nutrient broth at 37°C for 18 hours. About 100µl of each test bacterium was spread with the help of sterile spreader on to a sterile Muller Hinton Agar (MHA) plates. The plates were allowed to dry and then a sterile metal borer of diameter 8mm was used to form wells in each of the agar plates. Each of the three wells was labeled for three different extract solutions. The extract was dissolved in DMSO 0.01% concentrations. Subsequently, 50µl volume of each extract was poured in relatively labeled well on muller hinton agar plates. Levofloxacin, used as a positive control, in the centre of all the muller hinton

agar plates. The plates were allowed to stand for 30 minutes for diffusion to take place and then incubated at 37°C for 24 hours. After completion of incubation period, zones of inhibition were measured.

Photoplate 1



Collection of *Piper betel* L. leaves



Dried *Piper betel* L. leaves



Powder of *Piper betel* L. leaves



Extraction of *Piper betel* L. leaves by Soxhlet assembly

RESULTS AND DISCUSSION**Phytochemical Screening**

Table 1 Explains the preliminary screening of secondary metabolites obtained in *piper betel* L. extract. It was concluded that ethanol extract had high concentrations of sterols, and, phenols and flavonoids, as well as tannins. Similarly O. Sita Kumari and Nirmala Babu Rao (2015); Sapana Saini *et al.*, (2016) secondary metabolites were obtained in ethanol extract. phytochemical analysis of betel leaves showed that alkaloids, tannins, glycosides, reducing sugars, and saponins were found in the petroleum ether extract of betel leaves. Total content of phenol, flavonoid, and tannin in water, ethanol, ethyl acetate, acetone, and chlorofam extracts of betel leaves. similarly Sapana Saini *et al.*, (2016), phenol, flavonoid, alkaloids, tannins, glycosides, reducing sugars, and saponins phytochemicals were found in the petroleum ether, water, chloroform, petroleum ether extracts of *Piper betel* L. leaves.

Table 1: Phytochemicals present in different extract

Phytochemicals	Petroleum ether (40-60 °C) extract	Chloroform extract	Ethanol extract
Carbohydrates	- ve	- ve	+ ve
Proteins	- ve	- ve	+ ve
Fats and oils	- ve	- ve	- ve
Phytosterols	- ve	- ve	+ ve
Glycosides	- ve	- ve	- ve
Saponins	- ve	- ve	- ve
Flavonoids	+ ve	- ve	+ ve
Alkaloids	+ ve	- ve	+ ve
Tannis and phenol	+ ve	- ve	+ ve

+ indicates presence,-indicates absence (O. Sita Kumari and Nirmala Babu Rao 2015; Sapana Saini *et al.*, 2016)

Antibacterial activity

Following the extraction of dried leaves of *Piper betel* L. plant using Ethanol, Chloroform and Petroleum ether by soxhlet method, the antimicrobial activity of the extracts was determined by agar-well diffusion method. The table 2 shows the antimicrobial activity of the *Piper betel* leaf extracts on the selected Gram-positive and Gram-negative bacterial strains. The extracts were effective against both Gram-positive and Gram-negative bacteria.

Table 2 : Zones of Inhibition (mm) of Ethanol, Chloroform and Petroleum ether extracts on Bacterial Species.

No	Bacterial Strain	Positive control Levofloxacin	Ethanol extract	Chloroform extract	Petroleum ether extract
1	<i>Staphylococcus aureus</i>	16	18	10	4
2	<i>Bacillus subtilis</i>	25	25	20	25
3	<i>Micrococcus luteus</i>	21	13	15	12
4	<i>Pseudomonas putida</i>	8	26	15	20
5	<i>Klebsiella pneumonia</i>	8	33	23	33
6	<i>Escherichia coli</i>	7	12	8	4
7	<i>Shigella flexneri</i>	19	18	9	8
8	<i>Pseudomonas aeruginosa</i>	29	20	20	18
9	<i>Salmonella typhi</i>	20	23	13	17
10	<i>Proteus mirabilis</i>	33	20	15	16
11	<i>Vibrio cholera</i>	65	58	44	45

(Fawad Ali Bangash *et al.*, 2012)

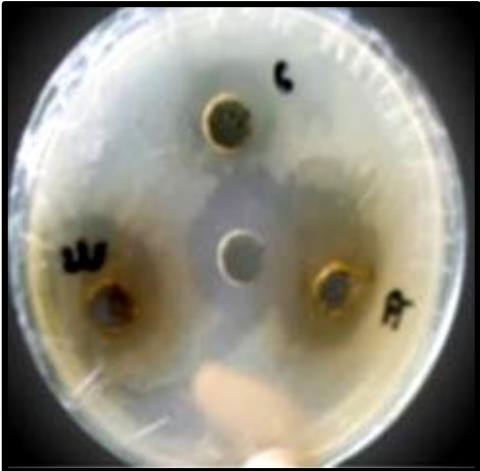
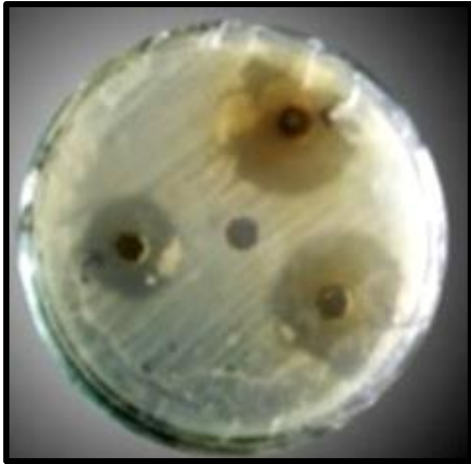

The ethanolic extract turned out to be most effective for its antibacterial activity against all eleven selected bacterial strains. The Widest zone of inhibition with a diameter of 58mm was observed in the case of *Vibrio cholerae*, followed by *Klebsiella pneumoniae* with the zone of inhibition extending up to 33mm. Amongst the Gram-negative bacteria, the extract showed higher activity against *Pseudomonas putida*, *Escherichia coli* and *Salmonella typhi* with a diameter of zone of inhibition 26mm, 12mm and 23mm respectively. Similarly, Arif Budiman *et al.*, 2018 studied the antibacterial activity of ethanol extract of *Piper betel* L. against *Staphylococcus aureus* and *Propionibacterium acne*. Ethanol extract showed activity against *Staphylococcus aureus* and *Propionibacterium acne* with the zone of inhibition 18.5mm and 16mm respectively.

The Chloroform extract was less effective for antibacterial activity as compared to Ethanolic extract. Similarly, widest zone of inhibition with a diameter of 44mm was observed in case of *Vibrio cholerae*, followed by *Klebsiella pneumoniae* with zone of inhibition extending up to 23 mm. Zones of inhibition produced by chloroform extract for Gram-negative *Pseudomonas putida* and *Escherichia coli* were also observed to be wider than the positive control levofloxacin

The Petroleum ether extract was least effective among all three extracts for its antibacterial activity. Like other two extracts, petroleum ether extract came up with widest zone of inhibition with a diameter of 45mm was observed in case of *Vibrio cholerae*, followed by *Klebsiella pneumonia* with zone of inhibition extending up to 33mm. Only one Gram-positive bacteria *Bacillus subtilis* showed comparable results with levofloxacin (Fig 1).

In this investigation, it was observed that *Piper betel* L. leaf extracts successfully inhibited growth of both groups of bacterial strains. It has been reported that the major constituents of *Piper betel* L. leaf are phenolic in nature that may be responsible for inhibiting bacterial growth. *Piper betel* L. leaf also possesses some volatile oils. As phenolic groups are slightly acidic in nature and also possess slight polarity, maximum yield and maximum activity was observed for ethanolic extracts. Ethanol is a slightly polar organic solvent that sufficiently dissolves slightly polar organic compounds. Whereas the other two solvents are non-polar in nature, thus their activity was relatively less. Similarly Arif Budiman *et al.*, 2018; Fawad Ali Bangash *et al.*, 2012 observed that the ethanol extracts of *Piper betel* L. were found more effective against both Gram-positive and negative bacteria as compared to chloroform, petroleum ether, water and others extracts.

Photoplate 2

	
<i>Bacillus subtilis</i>	<i>Klebsiella pneumonia</i>
	
<i>Vibrio cholera</i>	

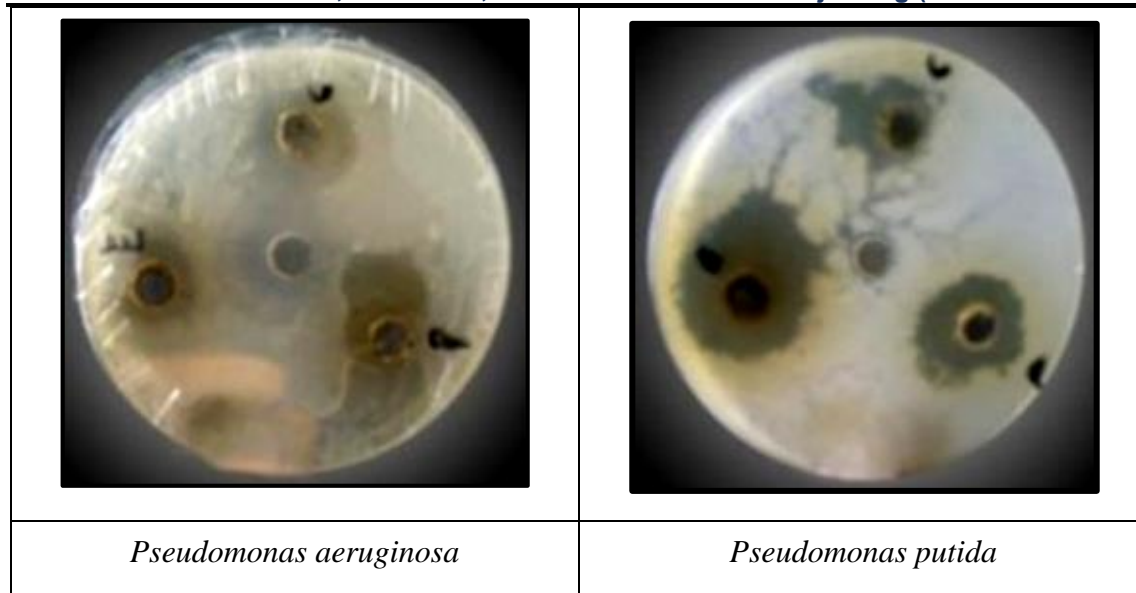


Fig. Bacterial strains demonstrating susceptibility of *Piper betel* L. extracts (Fawad Ali Bangash *et al.*, 2012)

CONCLUSION

From this review that betel leaf contains a number of phytoconstituents which reveals its uses for various therapeutic purposes. The Plant or its individual parts can be used for the treatment of various disorders in human beings such as diabetes, fungal infection, microbial infection, inflammation, antihistaminic, antiulcer, local anesthetic etc.

The betel leaves really as a cheap, natural and easily available. Still, so much work is required with the betel leaf to investigate the mechanism of actions with other therapeutic activities.

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