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Antibacterial Activity Of *Piper Betel* Leaves

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Research Article

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Abstract

Aqueous and ethanol extract of the leaves of *Piper betel* L. were evaluated for antibacterial activity against three Gram positive, two Gram negative bacteria. Chloromphenicol was used as standards for antibacterial assay. The two extracts showed different degree of activity against the microorganisms investigated. The ethanol extract was considerably more effective than aqueous extract in inhibiting the investigated microbial strains.

Keywords: *Piper betel,* Antibacterial activity, Leaf extract, Medicinal plants.

Introduction

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [1]. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases [2].

Piper betel is one of the invaluable medicinal plants where its leaves have been used for many medicinal purposes. *Piper betel*, a member of the Piperaceae, which is a large plant family, is also known *Paan* in India and *Sirih* in Malaysia and Indonesia. The fresh leaves of betel leaves have been wrapped together with the areca nut, mineral slaked lime, catechu, flavouring substances and spices are chewed since the ancient time [3].

Betel leaf has been described from ancient time as an aromatic stimulo-carminative, astringent and aphrodisiac. The leaf produces and aromatic volatile oil contains a phenol called chavicol which has a powerful antiseptic properties. The alkaloid arakene has properties resembling cocaine in some respect. Pharmacological effects of betel chewing include abundant flow of saliva, temporary dulled of taste perception, stimulation of muscular and mental efficiency [4].

Scientific research on the leaf of this plant reveals that it possesses many beneficial bioactivities and its extract from betel leaves has a great potential to be used in developing commercial products. Due to the numerous benefits, betel vine is grown for its leaves. The best conditions for commercial betel vine cultivation are those of tropical rain forests, which provide cool shade, considerable humidity and an adequate supply of soil moisture like Indonesia, Malaysia, Philippines, Thailand, Cambodia, Vietnam and India [5].

In the present study, antibacterial activity of ethanol and water extract of Piper *betel leaves* was determined.

Methodology

The leaves of Piper betel were from wild source near to Penang Medical Hospital for experimental purpose. Chloramphenicol, was obtained from Sigma Aldrich Company. (St. Louis, USA). All solvents/chemicals used were of analytical grade and obtained from Hi-media. Clinical isolated Bacterial strains were obtained from Faculty of Biotechnology, AIMST University, Bedong, Kedah.

Preparation of extracts:

Apparently healthy *Piper betel leaves* were collected, washed thoroughly in tap water and dried in room temperature for 7 days. The dried 50 g leaves was powdered and soaked separately in 200 ml water by keeping it on shaker for 3 days. The extract was filtered through cheese cloth and reduced to 10% of its original volume. The ethanolic extract was prepared by using 50 g dried leaves powder in a soxhlet apparatus. The extract was filtered and concentrated under reduced pressure using rotary vacuum evaporator below 40°C. The concentrated extract was stored at 4°C till further use.

Phytochemical Screening:

Phytochemical components of the leaves of *P.betel* were screened by using the standard methods [6, 7]. The components analyzed were alkaloids, flavonoids, triterpenoids, antharacene glycosides, tannins, phenolics and saponins.

Inoculums: The test organisms, gram positive (Bacillus subtilis, Staphylococcus aureus and Micrococcus luteus) and gram

negative (Escherichia coli and *Pseudomonas Aeruginosa*) bacteria were obtained from culture repository of Faculty of Biotechnology, AIMST, Malaysia. The organisms were inoculated in to NB (Nutrient Broth) medium, (0.5% peptone, 0.5% Sodium Chloride, 0.15% yeast extract; pH 7.4) and incubated at 37°C for overnight. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10mM Sodium chloride, pH 7.4) and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 10^5 CFUml˙1 before use. [8}

Determination of antibacterial activity:

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the published procedure with slight modification [9]. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8mm diameter) were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) or standard antibiotic solution (positive control) viz., chloromphenicol (50 and 100 ug ml˙1) were also run parallel in the same plate [10]. The plates were incubated at 37°C for 18h and the antibacterial was assessed by measuring the diameter of the zone of inhibition. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drug, chloromphenicol.

Statistical analysis:

The resultant clear zones of around the discs were measured in mm. The antibacterial activity of *P.betel* leaf extracts was indicated by clear zones of growth inhibition. Data of three independent experiments were subjected to statistical analysis (Mean±SE), according to New Duncan's Multiple Range Test [11].

Results

Phytochemical screening of leaf extracts:

The preliminary phytochemical screening of the leaf extracts using ethanol and water extracts was reported. (Table.1). The positive result for the presence of alkaloids, tannins, phenolic substances observed in alcohol and water extracts. In water extract saponins and glycosides were present which were absent in the alcoholic extract.

Antibacterial activity of leaf extracts:

We used both alcohol and water for the extraction of active components from the leaves of *Piper betel* plant. The antibacterial activity of the leaf extracts was assessed using agar well diffusion method by measuring the diameter of growth inhibition zones with 50 and 100 ul of alcholoic and aqueous extracts (Table 2 and 3). The results showed that alcohol and water extracts possess antibacterial activity against the tested gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. At 50 ul concentration, the alcoholic extract showed pronounced inhibition against all the tested organisms, the maximum inhibition was observed on B.subitilis (13.2+0.22 mm), S.aureus (9.7+0.02 mm) and E.coli (8.9 +0.21 mm) and moderate inhibition was observed on Pseudomonas aeruginosa (7.2+0.42 mm). The water extract did not actively inhibit the growth of bacteria at 50 ul concentrations except B.subitilis (6.8+0.32 mm).

Table 1: Preliminary phytochemical analysis of leaf extracts of *P.betel*

S.No.	Name of the compound	Water extract	Alcoholic Extract
1	Alkaloids	+	+
2	Tannins	+	+
3	Anthraquinones	-	-
4	Glycosides	+	-
5	Reducing sugar	+	+
6	Saponins	+	-
7	Flavonoids	-	-
8	Phenolics	-	+
9	Phlobatannis	-	-
	Terpenoids	-	-

Key: +: Present -: Absent

The growth of B.subitilis was inhibited by alcohol and water extracts at 100ul concentration and maximum inhibition with alcoholic extract as 15.8+0.22 mm) which was slightly lower than the zone of inhibition caused by

Table 2. Antibacterial activity of leaf extracts (50 ug/ml) of *P.betel*

S.No	Solvent	Gram positive (+)			Gram negative (-)	
		S.aureus	B.subtilis	M.luteus	E.coli	P.aeruginosa
1.	Ethanol	9.7±0.02	13.2±0.22	5.4±0.31	8.9±0.21	7.2±0.42
2.	Water	5.4±0.01	6.8±0.32	3.5±0.27	-	-
3.	Chloromphenicol	15.6±0.23	18±0.12	9.8±0.14	18±0.01	11±0.02

the standard drug Chloromphenicol (16.4+ 0.12 mm). Similarly alcoholic extract showed produced maximum inhibition (18.0+0.18 mm) to the growth of S.aureus than chlorompenicol (17.4+0.33 mm) where as water extracts showed less inhibitory activity than chloromphenicol (12.3+0.22 mm).

Table 3. Antibacterial activity of leaf extracts (100ug/ml) of *P.betel*

S.No	Solvent	Gram positive (+)			Gram negative (-)	
		S.aureus	B.subtilis	M.luteus	E.coli	P.aeruginosa
1.	Ethanol	18.0±0.18	15.8.±0.22	5.0±0.11	11.0±0.12	6.7±0.10
2.	Water	12.3±0.22	4.9±0.02	4.2±0.13	8.5±0.10	7.2±0.31
3.	Chloromphenicol	17.4±0.33	16.4±0.12	8.0±0.10	15.4±0.11	10.8±0.03

Both the extracts have not shown any significant growth inhibition on *Micrococcus luteus*. The alcoholic extract showed more significant effect on all the organisms compared to the water extract except the Pseudomonas aeruginosa.

Discussion and Conclusion:

We used both aqueous and alcohol solvents for the extraction of active components from the leaves of *P.betel*.

The result of the study reveals that both the aqueous and alcoholic extracts were active against the strains of bacteria that are common cause of infections. The leaf extracts of *P.betel* shows significant antibacterial effect that may due to the presence of many potent compounds such as alkaloids, tannins, phenolic substances and glycosides etc. The antibacterial activity was expressed at varying degrees which was being both strain and dose dependant.

The essential oil isolated from the leaves of *P.betel* inhibited the growth of Staphylococcus aureus and Sterptococcus pyogenes.[12]. The present work shows that the compounds from leaf extracts of *P.betel* possess antibacterial activity and suggesting that the leaf extracts contains the effective active constituents responsible for eliminating the bacterial pathogens.

Finally it can be concluded that active chemical compounds present in *P.betel* should certainly find place in the treatment of various bacterial infections. The results of this study very encouraging and indicate that this herb should be studied more extensively to explore its potential in the treatment of many infectious diseases.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests