[1]STUDY ON PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *PIPER BETLE* L. (BETEL VINE)

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Abstract

The present study was carried out to investigation of preliminary phytochemical constituents, antibacterial activity and antioxidant activity of *Piper betel* L. leaf (*Piperaceae*) commonly known as betel vine. The plant is locally known as Kun in Myanmar. Phytochemical investigation of *P. betle* leaf revealed the presence of alkaloids, glycoside, carbohydrate, amino acid, phenolic compound, flavonoid, steroid, terpenoid, saponin, tannin, starch, reducing sugar and organic acid. From the results of antibacterial activity assay, it was observed that ethyl acetate and ethanol extracts of *P. betle leaf* have high antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillius pumilus* and *E. coli*. Screening of antioxidant activity was done by DPPH free radical scavenging assay by using UV spectroscopic method. The IC_{50} values for the ethanol and ethyl acetate extracts were 54.61 µg/mL and 70.53 µg/mL, for watery extract was 105.80 µg/mL. The smaller the IC_{50} value the greater the antioxidant activity. Ethanol extract of *P. betle* showed the more effective results.

Keywords: *Piper betle* L., phytochemical, antibacterial activity, antioxidant activity

Introduction

Betel (*Piper betle* L.) is locally known as Kun and Betel vine in English. It belongs to the genus *Piper* of the *Piperaceae* family. Its heart-shaped leaves can grow up to the size of 18 cm in length and 12 cm in width. This plant is cultivated most parts of South India, Bengal, Sri Lanka, Myanmar and Thailand for its leaves. The *P. betle* leaves are the most important plant part and it possesses medicinal, religious and ceremonial value in Southeast Asia. In India, it is customary to serve *P. betle* leaves on various social, cultural and religious occasions and is also offered to guests as a mark of respect (Warrier *et al.,* 1995). Fresh *P. betle* leaves are chewed together with areca nut and slaked lime as natural tonic and breath refresher. Aqueous extracts of *P. betle* leaves have also been shown to reduce the adherence of early dental plaque bacteria (Razak *et al*., 2006). As well as use as a mouth freshener, the leaves are used for wound healing and digestive and pancreatic lipase stimulant activities in traditional medicine (Periyanayagam *et al*., 2012). A preliminary study has reported *P. betle* leaves extracts contain large numbers of bioactive molecule like polyphenols, alkaloids, steroids, saponins and tannins. Antioxidant, anti-bacterial and anti-fungal, antiinflammatory, anti-diabetic and radioprotective activities of *P. betle* leaves have also been reported (Chakraborty and Shah, 2011).

Scientific Classification

Family	:	Piperaceae
Genus	:	*Piper*
Species	:	*P.betle*
Botanical name	:	*Piper betle* L.
Common name	:	Betel vine
Myanmar name	:	Kun

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The photographs of *P. betle*. (Kun) plant and leaves are shown in Figure 1.

Figure 1 *P. betle* (Kun) plant and leaves

Phyto-constituents of *P. betle*

P. betle are one of the highly investigated plants and phytochemical studies show that *P. betle contains* a wide variety of biologically active compounds whose concentration depends on the variety of the plant, season and climate (Kumar, 1999). The specific strong pungent aromatic flavour in leaves is due to phenol and terpene like bodies. The quality of the leaf depends upon the phenolic content, i.e., more the phenolic content betters the leaf quality. Recently many researches work shows the *P. betle* leaves contains starch, diastases, sugars and an essential oil composing of safrole, allyl pyrocatechol monoacetate, eugenol, terpinen-4-ol, eugenyl acetate, etc. as the major components (Razak *et al.*, 2006). Phytochemical investigation on leaves revealed the presence of alkaloids, carbohydrate, amino acids, tannins and steroidal components. The middle part of the leaf contains largest quantity of Tannin. The terpenoids include 1, 8-cineole, cadinene, camphene, caryophyllene, limonene, pinene, Chavicol, ally pyrocatechol, carvacrol, safrole, eugenol and chavibetol are the major phenols found in betel leaf. Eugenol was identified as the antifungal principle in the oil. The fresh new leaves contain much more amount of essential oil diastase enzyme and sugar as compare to old leaves (Harborne, 1984).

Materials and Methods

Collection of Sample

In the present research, the *P. betle* leaves were collected from Kun Gyan Kone Township, Yangon Region. The leaves were washed and air dried for two weeks. Then, the dried leaves were powdered in a grinding machine. The powdered sample was stored in air-tight bottle, so as to prevent moisture changes and contamination.

Preliminary Phytochemical Investigation

The *P. betle* leaves were screened for the presence of various bioactive principles. Phytochemical investigations were performed to know the different types of chemical constituents such as alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, cyanogenic glycosides, starch, reducing sugars, organic acids and quinones according to the appropriate reported procedure ((Harborne, 1984). After treating the test solution with specific reagents, the tests were detected by virtual observation of colour change or precipitate formation.

Screening of Antibacterial Activity of Various Extracts from *P. Betle* L.

The antibacterial activities of different crude extracts such as petroleum ether, ethyl acetate, ethanol and water extracts were determined against five bacteria such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* species by employing agar well diffusion method at Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar (Mar Mar Nyein *et al.*, 1991).

Antioxidant Activity Assay

Screening of antioxidant activity of the crude extracts (aqueous and ethanol) of *P. betle* leaves were carried out by DPPH free radical scavenging assay using UV spectroscopic method (Brand-Williams *et al*., 1995). Ascorbic acid was used as a standard. Absorbance measurements were done in triplicate for each sample solution. Absorbance values obtained were used to calculate % inhibition, 50 % inhibitory concentration (IC_{50}) and standard deviation. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Results and Discussion

Preliminary Phytochemical Investigation

Phytochemical investigations were carried out on the petroleum ether, ethyl acetate, ethanol and water extracts of *P. betle* leaves sample using standard procedures to identify the constituents. The preliminary phytochemical investigation of *P. betle* leaves was carried out to view the different types of chemical constituents present in the leaf according to the procedures. From results, it was observed that alkaloids, glycosides, carbohydrates, α-amino acids, phenolic compounds, flavonoids, steroids, terpenoids, saponins, tannins, starch, reducing sugars and organic acids were present in the leaves of *P. betle* (Table 1). The phytochemical compounds such as flavonoids, tannin, phenolic compounds and saponins have potentially significant application against bacteria. Many phytochemicals are antioxidants such as flavonoids, polyphenols and have been found to possess different pharmacological properties. The *P. betle* leaf has biologically active principles.

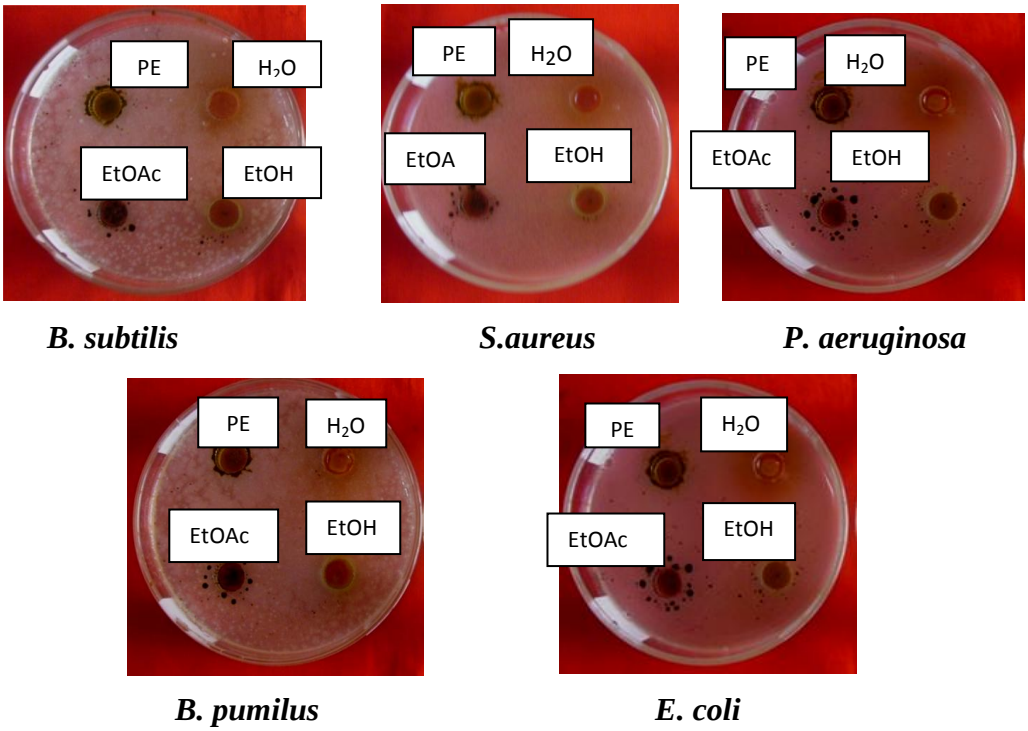
Screening of Antibacterial Activity of Crude Extracts from *Piper betle L.*

In vitro screening of antibacterial activity of various extracts such as petroleum ether, ethyl acetate, ethanol and watery extracts from *P. betle* leaves were done by agar well diffusion method (Figure 2). In this investigation, the extracts were tested against five bacteria such as *B. subtilis, B. pumilus, S. aureus, P. aeruginosa* and *E. coli.* All extracts of *P. betle* were active on tested bacteria. The petroleum ether extract was found low activity on the all organisms and watery extract was found to be low activity aganist *B. subtilis, B. pumilus, S. aureus* and *E. coli* species. Ethyl acetate extract show the highest activities on *P. aeruginosa* and *E. coli*. But ethyl acetate extract showed the medium activities on others microorganisms. In ethanol extract, exception of *P. aeruginosa* showed the highest activities on tested bacteria (Table 2).

Table 1 Results of Phytochemical Investigation on *P. betle* Leaf

Sr No.	Chemical Constituents	Test Reagent	Observation	Inference
1	Alkaloids	(i) Dragendorff's	Orange ppt.	+
		(ii) Mayer's	White ppt.	+
2	α-Amino acids	Ninhydrin	Purple colour solution	+
3	Carbohydrates	Molisch's	Violet ring	+
4	Cyonogenic Glycoside	Sodium picrate	No change	-
5	Flavonoids	Shinoda's	Pink solution	+
6	Glycosides	10 % lead acetate	Brown ppt.	+
7	Organic acids	Bromocresol green	Yellow solution	+
8	Phenolic compound	5 % ferric chloride	Brown ppt.	+
9	Quinones	Hydrochloric acid	Yellow colour solution	+
10	Reducing sugars	Benedict's	Light green ppt.	-
11	Saponins	Foam test	Marked frothing	+
12	Starch	Iodine	Blue colour solution	+
13	Steriods	Liberman Burchard	Greenish yellow solution	+
14	Tannins	Ferrous sulphate	Green ppt	+
15	Terpenoids	Liberman Burchard	Pink ppt.	+

(+) = presence (-) = not detected



B. subtilis ***S.aureus*** ***P. aeruginosa***

B. pumilus ***E. coli***

Figure 2 Inhibition zones indicating the antibacterial activities of various crude extracts of *P. betle* against tested bacteria

Table 2 Inhibition Zone Diameters of *P. betle* Leaf Extracts

Microbial Strains	Inhibition zone diameters of different extracts (mm)			
	PE	EtOAc	EtOH	H_2O
B. subtilis	14(+)	19 (++)	22 (+++)	13 (+)
S. aureus	13 (+)	19 (++)	20 (+++)	14 (+)
P. aeruginosa	13 (+)	20 (+++)	17 (++)	16 (++)
B. pumilus	12 (+)	19 (++)	20 (+++)	14 (+)
E. coli	13 (+)	20 (+++)	24 (+++)	14 (+)

(+) = 10-14 mm (low activity), (++) =15-19 mm (moderate activity)
(+++) = 20 mm and above (high activity), (−) = no zone of inhibition
Agar well diameter = 8 mm

Antioxidant Activity of Extracts of *P. betle*

The crude extract of watery, ethanol and ethyl acetate extracts were taken for screening of antioxidant activity by determination of DPPH free radical scavenging property by using UV spectroscopic method. The percent oxidative inhibition values of crude extracts were measured at different concentrations. From these experimental results, it was found that as the concentrations were increased, the absorbance values were decreased, i.e., increase in concentration and increase in radical scavenging activity of crude extracts expressed in term of % inhibition (Table 3). IC_{50} values in µg/mL were calculated by linear regression equation. DPPH free radical scavenging activity of extracts of *P. betle* was clearly indicated that ethanol extract of the leaves of *P. betle* had the higher activity than that of watery and ethyl acetate extracts. The IC_{50} value for the ethanol extract was 54.61 µg/mL, those for watery and ethyl acetate extracts were respectively 105.8 µg/mL and 70.53 µg/mL, and that of standard ascorbic acid was 35.81 µg/mL (Table 4). The smaller the IC_{50} value the greater the antioxidant activity. Ethanol extract of *P. betle has* higher activity because the IC_{50} value of ethanol extract was much lesser than that of watery and ethyl acetate extracts.

Table 3 Percent Radical Scavenging Activity (% RSA) of Extracts of *P. betle* Leaf and Standard Ascorbic Acid

Concentration (µg/mL)	% RSA			
	H_2O extract	EtOH extract	EtOAc extract	Ascorbic acid
12.5	17.20	28.46	28.89	37.85
25	29.22	39.82	40.58	43.57
50	41.88	48.93	45.05	57.85
100	48.70	59.64	56.51	62.85
200	71.10	74.06	71.49	76.42

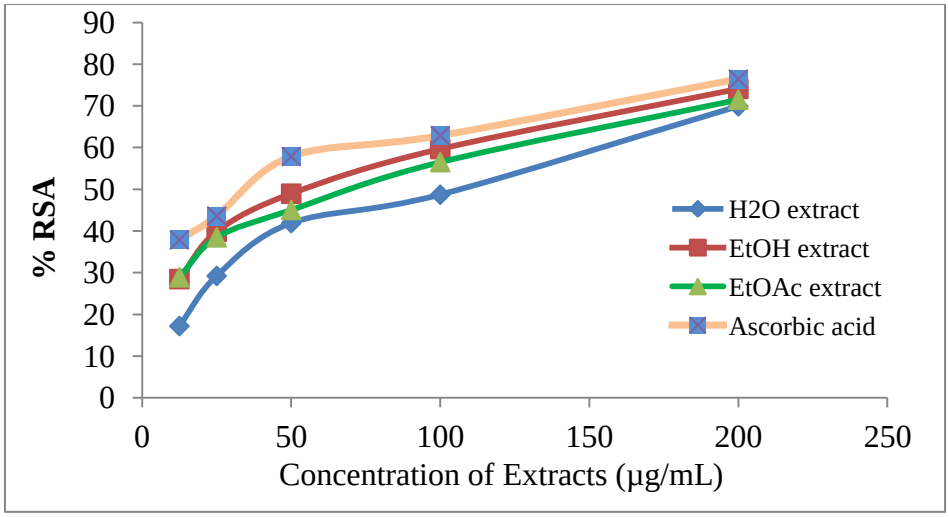


Table 4 IC_{50} Values of Extracts of *P. betle* Leaf and Standard Ascorbic Acid

Sample	IC_{50} (µg mL^{-1})
EtOH extract	54.61
EtOAc extract	70.53
H_2O extract	105.80
Ascorbic Acid	35.81

Conclusion

Herbal medicines are valuable and readily available resources for primary health care. *P. betle* leaves are also used as traditional medicine around the world. According to this research, *P. betle* leaves contain various bioactive compound. Based on this study, various extracts of leaves exhibited antibacterial activity against various gram positive and gram-negative pathogens. In this study, antibacterial activity and antioxidant activity of ethanol extracts are likely to show good relationship. Ethanol extract showed the highest activities on tested microorganisms and DPPH radical scavenging activity with the IC_{50} value of 54.61 µg/mL compared to other extracts of P. *betle* leaf in this study.

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