

A RESEARCH ANALYSIS ON PHYTOCHEMICAL AND THE ACTIVITIES OF ANTIBACTERIAL IN *PIPER BETEL* VARIETIES (KAARA VETRILAI AND VELLAI VETRILAI)

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ABSTRACT

Piper betel is a well-known ethno-botanical medicinal plant whose leaf is known to possess antiseptic, antileishmanian and antimicrobial properties. Considering these properties a preliminary study on the phytochemical present in *Piper betel* was carried to find the presence of any new antibacterial compounds. Two varieties (Kaaravettilai and Vellaivettilai) of *Piper betel* leaves were collected from the various local markets. Fresh and dry leaf sample were used for phytochemical analysis and antibacterial activity. Based on the qualitative analysis of *Piper betel* it was found to contain Tannins, Flavanoids, Alkaloids, Terpenoids, Saponins, Cardiac Glycosides and Glycosides. The extract was obtained from dried leaves using ethanol, ethyl acetate and chloroform and also aqueous extract was obtained using these crude extract an attempt was made to find out their antibacterial activity. The antibacterial activity studies showed the prominent zone of inhibition against *Bacillus subtilis* and *Escherichia coli*. Further, the isolation and identification of the extract using GC-MASS, HPTLC, NMR etc., will be carried out in future.

KEYWORDS: Physiology, biochemistry, Photochemistry and Histochemistry.

INTRODUCTION

MEDICINAL PLANTS

The term "medicinal plant" includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.

Piper betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for

its healing effects. This particular property has paved way for further experimental studies, which have established paan extract to have antimicrobial and anti leishmanian properties (Sarker *et al.*, 2008). *Piper* in the Piperaceae Family, order Piperales, commercially important because of *Piper nigrum*, the source of black and white pepper.

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The family comprises about 5 genera, of which 2 Piper (about 2,000 species) and Peperoni (about 1,600 species) are the most important. The plants grow as herbs, vines, shrubs, and trees and are widely distributed throughout the tropics and subtropics.

Phytochemical are plant or fruit derived chemical compounds. "Phytonutrients" refer to phytochemicals or compounds that come from edible plants. Phytochemical screening refers to the extraction, screening identification of medicinally active substances found in plants. Phytochemical studies and in vitro cytotoxicity screening of Piper betel leaf Chaurasiaet al.,(2010).

In our project two different extract of *piper betel* were checked against to different bacteria namely *Bacillus subtilis* and *Escherichia coli*. Two different extracts of Piper betel were checked against two different bacteria namely, *Bacillus subtilis* and *Escherichia coli*. Four different concentrations of plant extract were tested for antimicrobial activity using agar well diffusion method, standardized by National Committee for Clinical Laboratory Standards (2002) To find out the zone of inhibition.

MATERIALS AND METHODS

MACERATION

The collected leaves were allowed to dry for a week and then the dried leaves are finely powdered and were macerated using ethanol, ethyl acetate and chloroform in conical flask and it was placed in orbital shaker at room temperature for 24 hours. Then the extracts obtained were filtered using whattman filter paper to obtain ethanol, ethyl acetate and chloroform extract. The residue left was again subjected to second successive extraction with ethanol, ethyl acetate and chloroform following previously mentioned procedure to get the second ethanol, ethyl acetate and chloroform

extract. Then the both extract were condensed by evaporation to obtain thick viscous mass. This is called as the crude drug. Then the yield value was calculated for phytochemical test, Antibacterial activity

QUALITATIVE PHYTOCHEMICAL TEST

TEST FOR TANNIN

1 ml of plant extract and 2ml of 5% Ferric chloride gives greenish black which indicates the presence of Tannin.

TEST FOR SAPONIN

2ml of plant extract and 2ml of distilled water and shake 15mins forms 1cm foam layer which indicates the presence of Saponin.

TEST FOR FLAVANOIDS

2ml to 5 ml of Dilute Ammonium solution and plant extract gives yellow colour which indicates the presence of Flavanoids.

TEST FOR ANTHOCYANIN AND β -CYANIN

2ml of plant extract and 1 ml of 2N Sodium hydroxide heated at 100C /15mins gives yellow colour, which indicates the presence of Anthocyanin and β -Cyanin.

TEST FOR QUINONE

1ml of plant extract and 1ml of Conc.Sulphuric acid gives red colour which indicates the presence of Quinone.

TEST FOR GLYCOSIDE

2ml of plant extract and 3ml of chloroform and 10% of ammonium solution gives pink colour which indicates the presence of glycoside.

TEST FOR GLYCOSIDE

0.5ml of plant extract and 2ml of glacial acetic acid and drop of ferric chloride and

Conc.Sulphuric acid gives brown ring which indicates the presence of glycosides.

TEST FOR ACID

1ml of plant extract and sodium bicarbonate gives the brisk effervescence which indicates the presence of acid.

TEST FOR STERIODS

2ml of plant extract and 5ml of chloroform and 2ml of acetic acid and 2ml of Sulphuric acid gives violet to blue or green which indicates the presence of steroids

TEST FOR COUMARIN

1ml of plant extract and 10% sodium hydroxide gives yellow which indicates the presence of coumarin.

TEST FOR PHENOL

1ml of plant extract and 2ml of distilled water and few drops of 10% ferric chloride give green colour which indicates the presence of phenol

TEST FOR TERPENOID

0.5ml of plant extract and 2ml of chloroform and Conc.Sulphuric acid gives red brown which indicates the presence of terpenoid.

TEST FOR TRITERPENOID

1.5ml of plant extract and 1ml of acetic acid and a drop of sulphuric acid gives blue green which indicates the presence of triterpenoid.

TEST FOR CARBOHYRATES

About 0.5g of each of the extract was mixed with molish reagent and then added sulphuric acid along the sides of the testube form layers. Appearance of reddish violet ring the interference indicated the presence of carbohydrates.

ANTIBACTERIAL ACTIVITY

Two different extracts of Piper betel were checked against two different bacteria namely , Bacillus subtilis and Escherichia coli . Four different concentrations of plant extract were tested for antimicrobial activity using agar well diffusion method, standardized by National Committee for Clinical Laboratory Standards (2002). The microorganisms were inoculated in 100 ml flask containing nutrient broth. These flasks were incubated at 37°C for 24 hrs. Media was prepared using N-agar, test microorganisms were then spread over the solidified plates and wells were bored using sterile cup borer of 1mm diameter. These wells were then filled with different concentrations of plant extract. A bacterial positive control and antibiotic control were kept for comparative study. Antibiotic used was Gentamycin (1 mg/ml) 23. These plates were incubated at 37-48°C according to optimum temperature required for bacterial species. Antibacterial activity was obtained by determining the zone of inhibition around the well. In-vitro antibacterial activity of piper betel leaf extracts Bangash *et al.*, (2012) and Bhalerao *et al.*,(2013).

RESULTS

By this analysis we can conclude that *Piper betel* consists of Tannins, Anthraquinones, Flavanoids, Alkaloids, Saponins, Cardiac glycosides, Acid, Steroids and Phenol. (Table1)(Fig2)

Antibacterial activity was obtained by determining the zone of inhibition around the well (Table2) (Fig3)

DISCUSSION

The collected leaves were allowed to dry for a week and then the dried leaves are grind into coarse powder. The powder were macerated using ethanol, ethyl acetate and chloroform in

conical flask and it was placed in orbital shaker at room temperature for 24 hours.

Then the extracts obtained were filtered using whattman filter paper to obtain ethanol, ethyl acetate and chloroform extract. The residue left was again subjected to second successive extraction with ethanol, ethyl acetate and chloroform following previously mentioned procedure to get the second ethanol, ethyl acetate and chloroform extract. Crude drug was used for calculating phytochemical test, Antibacterial activity.

By this analysis we can conclude that *Piper betel* consists of Tannins, Anthraquinones, Flavanoids, Alkaloids, Saponins, Cardiac glycosides, reducing sugars.

Aqueous extract of the fresh *Piper betel* leaves on bacterial activity showed the effective inhibitory action against the microorganisms Shameem and Thirumal (2013) and Dattaet al., (2011).

Aqueous and ethanol extract of *Piper betel* leaves on antibacterial activity using *Bacillus subtilis*, *E. coli* bacteria by agar well diffusion method reveals that both the aqueous and the alcoholic extracts be active besides the strains of bacteria which are common cause of infections Kavetiet al., (2011) and Chakraborty D and Shah B (2011).

CONCLUSION

The medicinal importance of the herb as discussed above evidently prove that betel leaf is one of the most promising commercial

botanical with earlier reported to possess a lot of therapeutic values. The leaf has the great potency to act as natural antioxidant.

Fresh leaves are taken and washed in tap water for few minutes to remove the dust particles. Then the washed leaves are taken and grinded using martin pestle. Then the extract was filtered and the filtrate was used for phytochemical tests.

By this analysis we can conclude that *Piper betel* consists of Tannins, Anthraquinones, Flavanoids, Alkaloids, Saponins, Cardiac glycosides, reducing sugars.

The *Piper betel* poses the antibacterial activity against various bacterial strains including *Bacillus subtilis*, *Escherichia coli*.

The leaf extract shows the gastroprotective activity by enhancing the mucus rather than decrease the acid production.

The future prospects of present research work including isolation and purification of the therapeutic antibacterial compounds from the active extract and there further pharmacological evaluation by several methods such as TLC, NMR, MS, GC-MS, HPLC.

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PHYTOCHEMICAL ANALYSIS ACTIVITY OF *Piper betel* varieties (Kaaravettilai and Vellaivettilai)

Table 1

FRESH AND DRIED LEAF EXTRACT		
CHEMICALS	VAR.I	VAR.II
TANNIN	+	+
SAPONIN	+	+
FLAVONIDS	-	+
ANTHOCYANIN & β - CYANIN	+	-
QUINONE	-	-
GYCOSIDE	-	-
CARDIAC GYCOSIDE	+	+
ACID	-	+
STEROIDS	+	+
COUMARI	+	-
PHENOL	+	+
TERPENOID	-	-
TRITERPENOID	-	-

Table 2

ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACT ON LEAF OF <i>P. betell.</i>								
S.NO	VARIETY	ORGANISM	EXTRACT	ZONE OF INHIBITION				
				100%	50%	25%	10%	CONTROL
1	I	<i>Bacillus subtilis</i>	CHLOROFORM	2.4	0.3	1.6	1.8	1.1
			ETHYL ACETATE	1.9	2.4	1.6	1.8	1.1
			ETHANOL	2.3	2.5	1.6	1.7	1.1
			AQUEOUS	2	2.2	1.7	1.6	1.1
		<i>Escherichia coli</i>	CHLOROFORM	1.8	1.9	1.5	1.4	1.1
			ETHYL ACETATE	2.4	1.9	2.6	2.2	1.1
			ETHANOL	2.2	1.5	1.3	2.7	1.1
			AQUEOUS	1.5	2.3	1.9	2.1	1.1
2	II	<i>Bacillus subtilis</i>	CHLOROFORM	2.1	2.5	1.5	1.8	1.1
			ETHYL ACETATE	1.9	1.9	1.9	1.9	1.1
			ETHANOL	2.3	2.3	2.3	2.3	1.1
			AQUEOUS	1.6	2.5	2.1	1.8	1.1
		<i>Escherichia coli</i>	CHLOROFORM	1.8	1.8	1.8	1.8	1.1
			ETHYL ACETATE	2.4	2.4	2.4	2.4	1.1
			ETHANOL	1.8	2.7	1.9	2.4	1.1
			AQUEOUS	2.1	2.3	2.5	1.7	1.1

MORPHOLOGY OF *PIPER BETEL* VARIETIES

KAARA VETRILAI VELLAI VETRILAI



Leaves of *Piper betel* varieties

KAARA VETRILAI VELLAI VETRILAI



Figure 1. Extraction of *Piper betel* varieties (Kaaravetrilai and Vellaivetrilai)

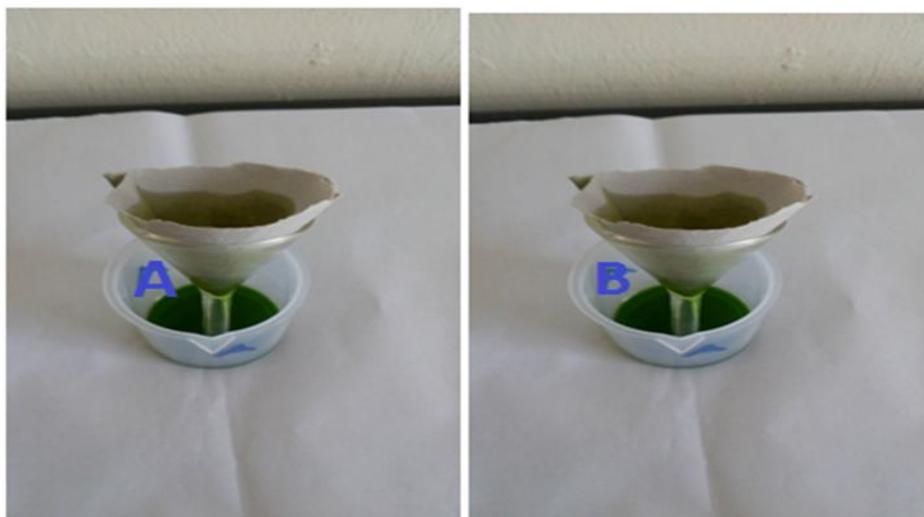


Figure 1.2. Filtrate of *Piper betel* varieties I and II (Kaaravetrilai and Vellaivetrilai)



Figure 2. Phytochemical screening of *Piper betel* varieties (Kaaravettilai and Vellaivettilai)



Figure 3. Antibacterial activity of *Piper betel* varieties (Kaaravettilai and Vellaivettilai) against *Bacillus subtilis* and *Escherichia coli*



AUTHOR'S CONTRIBUTION

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REFERENCE

1. Bangash FA, Hashmi AN, Mahboob A, Zahid M, Hamid B, Muhammad SA, Shah ZU, Afzaal H. In-vitro antibacterial activity of piper betel leaf extracts. *Journal of Applied Pharmacy*.2012;3(4): 639-646.
2. Bhalerao SA, Verma DR, Gavankar RV, Teli NC, Rane YY, Didwana VS, Trikannad A. Phytochemistry, pharmacological profile and therapeutic uses of Piper betle Linn- An overview. 2013; 1: 10-19.
3. Chakraborty D, Shah B. Antimicrobial, antioxidative and antihemolytic activity of Piper betel leaf extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2011; 3(3): 192- 199.
4. Chaurasia S, Kulkarni GT, Shetty LN. Phytochemical studies and in vitro cytotoxicity screening of Piper betle leaf

- (PBL) extract. Middle-East Journal of Scientific Research. 2010; 6(5):532- 536.
5. Datta A, Ghoshdastidar S, Singh M. Antimicrobial property of Piper betel leaf against clinical isolates of bacteria. International Journal of Pharma Sciences and Research. 2011; 2(3): 104-109.
 6. Deshpande SN, Kadam DG. GCMS analysis and antibacterial activity of Piper betle (Linn) leaves against Streptococcus mutans. Asian J Pharm Clin Res. 2013; 6: 99-101.
 7. Kaveti B, Tan L, Sarnnia TS, Baig M. Antibacterial Activity of Piper Betel Leaves. International Journal of Pharmacy Teaching & Practices. 2011; 2(3): 129-132.
 8. Sarkar A, Sen R, Saha P, Ganguly S, Mandal G, Chatterjee M. An ethanolic extract of leaves of Piper betle (Paan) Linn mediates its antileishmanial activity via apoptosis. Parasitology research. 2008; 102(6):1249.
 9. Shameem PM, Thirumal M, Srilekha A, Ushajain D, Kumar VV, Kumar SN, Rishi IA, Nayak CN, Chandy V. A preliminary antimicrobial screening on leaves of Piper betle Linn. Contemp. Invest. Obs. Pharm. 2013; 2:22-26.