

# A STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF PIPER BETEL VARIETIES (KAMAR AND KUMBAKONAM VETRILAI)

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## ABSTRACT

*Piper betel* is a well-known ethno-botanical medicinal plant whose leaf is known to possess antiseptic, antileshmanian and antimicrobial properties. Considering these properties a preliminary study on the phytochemicals present in *Piper betel* was carried to find the presence of any new antibacterial compounds. Two varieties (Kamar and Kumbakonamvetrilai) of *Piper betel* leaves were collected from various local markets. Fresh and dry leaf samples 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 extracts 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, Phytochemistry and Histochemistry.

## INTRODUCTION

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 that an extract to have

antimicrobial and anti leishmanian properties Sarker *et al.*, (2008). Piperaceae, the pepper family in the order Piperales, commercially important because of *Piper nigrum*, the source of black and white pepper. The family comprises about 5 genera, of which 2 *Piper* (about 2,000 species) and *Peperomia* (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.

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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 and identification of the medicinally active substances found in plants. Phytochemical studies and in vitro cytotoxicity screening of Piper betle leaf Chaurasia *et al.*, (2010).

In our project two different extracts of *Piper betel* were checked against two different bacteria namely *Bacillus subtilis* and *Escherichia coli*. Different concentrations of plant extraction were checked 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 EXTRACTION 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 and 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 Saponins.

### **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 $\beta$ -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  $\beta$ -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 glucosides.

### **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 CARBOHYDRATES:**

About 0.5g of each of the extract was mixed with molish reagent and then added sulphuric acid along the sides of the test tube 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*. Different concentrations of plant extraction were checked 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 37C 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 (1mg/ml). These plates were incubated at 37-48C 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 the phytochemical analysis we can conclude that *Piper betel* consists of Tannins, Saponins, Flavanoids, Cardiac glycoside, Acid, Steroids and Phenol. (Table 1) (Fig 2)

Antibacterial activity was obtained by determining the zone of inhibition around the well. (Table 2) (Fig 3)

#### **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 whatman 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, Terpenoids, Saponins, Cardiac glycosides, 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 Datta et 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, Saponins, Flavanoids, and Cardiac glycoside, Acid, Steroids and Phenol.

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

The leaf extract shows the gastro protective 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 OF PIPER BETEL VARIETIES (KAMARVETRILAI AND KUMBAKONAMVETRILAI)

Table I

CHEMICAL TEST	FRESH AND DRIED LEAF EXTRACT	
	(Var.)I	(Var.)II
TANNIN	+	+
SAPONIN	+	+
FLAVANOIDS	+	+
ANTHOCYANIN & β-		
CYANIN	-	-
QUINONE	-	-
GLYCOSIDE	-	-
CARDIAC GLYCOSIDE	-	+
ACID	+	+
STEROIDS	+	+

COUMARIN	-	-
PHENOL	+	+
TERPENOID	-	-
TRITERPENOID	-	-

Table II

ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACT ON LEAF OF <i>P. betel</i> L.(kamarvetrilai and kumbakonamvetrilai)								
S.NO	VARIETY	ORGANISM	EXTRACT	ZONE OF INHIBITION				
				100%	50%	25%	10%	CONTROL
1	I	<i>Bacillus subtilis</i>	CHLOROFORM	2.9	2.3	1.9	1.8	1.1
			ETHYL ACETATE	1.4	1.8	1.9	1.9	1.1
			ETHANOL	1.5	2.1	2.6	2.7	1.1
			AQUEOUS	1.6	2.1	2.4	1.8	1.1
		<i>Escherichia coli</i>	CHLOROFORM	1.6	2.1	2.3	1.6	1.1
			ETHYL ACETATE	2.7	2.8	2.4	1.7	1.1
			ETHANOL	1.5	1.9	2.3	2.5	1.1
			AQUEOUS	1.9	1.8	1.6	1.7	1.1
2	II	<i>Bacillus subtilis</i>	CHLOROFORM	2.4	2.5	1.5	2.1	1.1
			ETHYL ACETATE	2.3	2.2	1.9	1.9	1.1
			ETHANOL	2.4	1.9	1.4	1.5	1.1
			AQUEOUS	2.3	1.9	1.5	2.6	1.1
		<i>Escherichia coli</i>	CHLOROFORM	1.4	1.5	1.2	2.4	1.1
			ETHYL ACETATE	1.5	2.3	2.4	2.6	1.1
			ETHANOL	1.9	2.8	2.5	2.3	1.1
			AQUEOUS	1.6	2.3	2.6	1.9	1.1

**MORPHOLOGY OF PIPER BETEL VARIETIES**

**KAMAR VETRILAI KUMBAKONAM VETRILAI**



Leaves of *Piper betel* varieties

KAMAR VETRILAI

KUMBAKONAM VETRILAI



Fig 1.Extraction of *Piper betel* Varieties I and II (Kamarvetrilai and Kumbakonamvetrilai)



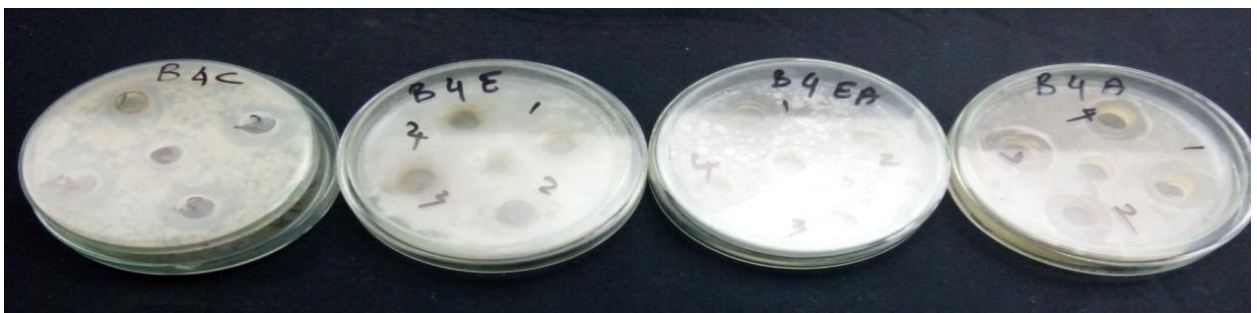
Figure 1.2.Filtrate of *Piper betel* Varieties I and II (Kamarvetrilai and Kumbakonamvetrilai)



Figure 2.Phytochemical Screening of *Piper betel* Varieties I and II (Kamar and Kumbakonamvetrilai)



Figure 3. Antibacterial Activity of *Piper betel* Varieties I and II (Kamar Vetrilai and Kumbakonam Vetrilai) against *Bacillus subtilis* and *Escherichia coli*





## AUTHOR'S CONTRIBUTION

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