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Research Article

Studies on phytochemistry and bioefficacy of cultivars of *Piper betle* Linn.

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ABSTRACT

The plant, *Piper betle*. Linn is a creeper belonging to the Piperaceae family. The leaves are found to possess different medicinal properties and function as a stimulant. The taste is found to differ with different types of cultivars and the present study is conducted to know whether they differ in their phytochemical constitution and biological efficacies. Four different cultivars, i.e. Banarasi, Calcutta, Kammar and Kumbakonam of *Piper betle* were selected for this study and the methanolic extracts of the leaves were evaluated for antibacterial, anticandidal, antioxidant and larvicidal activities. Antibacterial and anticandidal efficacy was studied using Disc diffusion test. DPPH was used to detect antioxidant ability and the larvicidal potency was studied using the larvae of *Artemia salina*. Further, the aqueous extract of the leaves was screened for the presence of phytoconstituents. Tannins, flavonoids and terpenoids were detected in all the four cultivars however; Steroids are found to be absent. Among the cultivars, Kumbakonam variety has shown better antibacterial and anticandidal activity, Kammar has shown better antioxidant potency and the variety Calcutta has shown better larvicidal potency. The study aptly demonstrates the existence of difference in biopotency among the cultivars of *Piper betle* and proper selection of cultivar for specific usage in pharmaceutical industry is recommended.

Keywords: *Piper betle*; Cultivars; Antibacterial; Anticandidal; Larvicidal; Phytochemistry

INTRODUCTION

Piper betle is one of the popular plants which are integrated with cultural and traditional values in India. The plant is widely cultivated in different parts of India, China, Malaysia, Srilanka, Thailand (Parmer *et al*, 1997) and other Pacific Asian Nations. The plant belongs to the family Piperaceae. The leaves are used traditionally in treating hysteria, headache, swelling of gum etc., (Khanra, 1997). The leaves are also found to be nutritious and possess substantial vitamins and minerals (Guha and Jain, 1997). The essential oils of the leaves of betel are found to possess antibacterial and antifungal property (CSIR, 1969). The essential oil composition was found to differ according to the cultivars of betel. The difference in the composition of phytochemicals is attributed to the factors like variety, soil, climate etc., where the plant cultivars are grown (Sankar *et al*, 1996 and Ramalakshmi *et al*, 2002). In fact, the differences in medicinal and aromatic properties of cultivars are due to the presence of specified phytoconstituents

(Khanra, 1997). Samanta, (1994) and Guha, (1997) reported that there are about 100 varieties of betel vine in the world. There are many reports available on the antibacterial (Vani *et al*, 2011), anticandidal (Himratul-Aznita *et al*, 2011), antifungal (Srichana *et al*, 2009), antioxidant (Arambewela *et al*, 2006) and larvicidal (Wardhana *et al*, 2007) activities of the leaf extract of *Piper betle*. Most of the previous reports available are either based on the leaves of *Piper betle* as such or without mentioning the type of cultivar. Thus, in this present study an attempt was made to know the antibacterial, anticandidal, antioxidant and larvicidal activities of leaves of four different cultivars, i.e. Banarasi, Calcutta, Kammar and Kumbakonam of *Piper betle* procured from Chennai.

MATERIALS AND METHODS

Plant source

Four different cultivars of *Piper betle*, i.e. Banarasi, Calcutta, Kammar and Kumbakonam (Fig. 1) were procured from the local market of Chennai. The collected plant materials were identified and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Tambaram, Chennai. Healthy, uninfected and undamaged leaves only were used. The betel leaves were cleaned, washed and allowed to dry in shade. The dried leaves were pulverized using electric blender and stored for further usage.

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Figure 1: Leaves of different Cultivars of *Piper betle*

Phytochemical analysis

Aqueous extracts of the leaves were prepared by boiling the dry leaves in water at a ratio of 1:10 followed by filtration using Whatman No.1 filter paper. The extracts were used for detecting the presence of Phytochemicals like Tannins, Phlobatannins, Saponins, Flavonoids, Terpenoids, Cardiac glycosides and Steroids (Evans, 1996; Udayaprakash *et al.*, 2011 and 2013).

Preparation of plant extract

The methanolic extracts of betle leaves were prepared using cold-percolation method. To 15 g of each fresh leaf sample, 150 ml of Methanol was added and stirred in a temperature controlled shaker at 30 ± 2 °C. After 48 h the extracts were filtered and concentrated using rotary evaporator and stored for further usage. These extracts were reconstituted for evaluating antibacterial, anticandidal, antioxidant and larvicidal properties.

Antimicrobial studies

The standard cultures of *Staphylococcus aureus* (MTCC No. 9011) and *Streptococcus* sp. (MTCC No. 389) of bacteria and three cultures of *Candida*, i.e. *Candida albicans* (MTCC 3017), *Candida glabrata* (MTCC 3983) and *Candida* sp. (MTCC 8334) were procured from Microbial Type Cell Culture (MTCC), Chandigarh. Antibacterial and anticandidal studies were conducted using disc diffusion technique.

Disc diffusion Method

Sterile petri dishes containing Mueller Hinton Agar and Sabourad dextrose agar were swabbed with the cultured bacterial and fungal species respectively. 15, 20 and 25 μ L of the plant extract was loaded onto sterile discs of 5 mm diameter. These discs, along with standard antibiotic discs were placed over the agar plates and incubated for 24 h for antibacterial study and 72 h for anticandidal study. Triplicates were maintained for each of the species. Zone of inhibition formed upon incubation of the plates were measured in mm and the mean value was recorded (Udayaprakash, *et al.*, 2012).

Antioxidant efficiency

The methanolic extracts of the leaves were studied for DPPH (2, 2 diphenyl-1-picryl hydrazyl) free radical scavenging activity. The extracts at various concentrations (100, 200, 300, 400 and 500 μ g/mL) were made up to 1 ml using methanol and 1 ml of 0.01mM DPPH was added to each of the tube. Similar solutions of DPPH in Butylated Hydroxyanisole (BHA) were used as reference while methanol was used as the blank. The solutions were incubated in dark for half an hour, after which the absorbance was read at 517 nm using UV-Visible Spectrophotometer (Cyberlab, USA). The percent inhibition was calculated using the formula:

$$\text{Effective concentration \%} = \frac{\text{Control Absorbance} - \text{Test Absorbance}}{\text{Control Absorbance}} \times 100$$

The concentration of the dry plant material (in μ g) per mL of the solvent (μ g/mL) that inhibits the formation of DPPH radicals by 50 % is considered as EC₅₀ (Udayaprakash *et al.*, 2014).

Larvicidal activity

The larvicidal activities of the leaves were evaluated using the eggs of *Artemia salina* procured from Philadelphia, USA. The eggs were allowed to hatch upon incubation for 48 h in a small water tank containing sea water. Required light was provided with Philips 40 Watts lamp for 12 hours cycle. The nauplii of *Artemia salina* were exposed to varying concentrations of the leaf extracts (62.5 mg/mL, 125 mg/mL, 250 mg/mL and 500 mg/mL) in different test tubes containing 10 mL of sea water and 20 larvae. The viability of the larva was recorded by 48 h time interval. The nauplii were considered dead when they were immobile and remained at the bottom of the test tube (Udayaprakash *et al.*, 2011). The percent mortality of the brine shrimp was calculated as hereunder.

$$\% \text{ Mortality} = \frac{\text{No. of Brine Shrimp dead}}{\text{No. of Brine Shrimp introduced}} \times 100$$

RESULTS

Phytochemistry

The study on the phytochemicals of four different cultivars of *Piper betle* showed that all the cultivars possess Tannins, Flavonoids and Terpenoids. However, steroids were not recorded in any of the cultivar studied for its phytochemical constituent. Phlobatannins were present only in Banarasi variety while Saponins and Cardiac glycosides were present only in two of the varieties studied. The detection of various phytochemicals is presented in Table 1.

Antimicrobial studies

The Kumbakonam and Banarasi cultivars have shown better antibacterial activity against the standard cultures of *Staphylococcus aureus* and *Streptococcus* sp. Calcutta pan and Kammar varieties have not shown any activity against *Streptococcus* sp. and *Staphylococ-*

Table 1: Presence of Phytochemicals in cultivars of *Piper betle* leaves

Cultivars	Tannins	Phloba Tannins	Saponins	Flavonoids	Terpenoids	Cardiac Glycosides	Steroids
Banarasi	+	+	+	+	+	+	-
Calcutta	+	-	-	+	+	-	-
Kammar	+	-	+	+	+	-	-
Kumbakonam	+	-	-	+	+	+	-

Table 2: Antibacterial and anticandidal activity of leaves of *Piper betle* cultivars

Microbial species	Cultivars of <i>Piper betle</i>											
	Banarasi			Calcutta			Kammar			Kumbakonam		
	15 μ L	20 μ L	25 μ L	15 μ L	20 μ L	25 μ L	15 μ L	20 μ L	25 μ L	15 μ L	20 μ L	25 μ L
<i>Staphylococcus aureus</i>	-	8	11	-	-	-	-	-	-	9	11	12
<i>Streptococcus</i> sp.	9	11	13	-	13	13	-	-	7	8	11	13
<i>Candida albicans</i>	-	11	12	-	-	-	-	-	-	7	11	12
<i>C. glabrata</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Candida</i> sp.	8	11	13	-	-	-	-	-	-	-	-	7

Table 3: Free radical scavenging effect of *Piper betle* cultivars

Cultivars	% Inhibition					EC ₅₀ (μ g/mL)
	100 μ g/mL	200 μ g/mL	300 μ g/mL	400 μ g/mL	500 μ g/mL	
Banarasi	10	20	40	80	90	325
Calcutta	10	20	30	30	50	500
Kammar	10	10	40	90	90	320
Kumbakonam	0	20	30	40	90	420

Table 4: Larvicidal activity of *Piper betle* cultivars

Cultivars	% Mortality			
	62.5 mg/mL	125 mg/mL	250 mg/mL	500 mg/mL
Banarasi	100	100	100	100
Calcutta	100	100	100	100
Kammar	85	90	95	100
Kumbakonam	65	70	95	95

cus aureus respectively. With regard to the anticandidal studies, none of the cultivars of *Piper betle* has potential activity against *Candida glabrata*. Kammar and Calcutta cultivars have not shown activity against the *Candida* species studied at any of the concentrations used in disc diffusion. The antibacterial and anticandidal potencies of the leaves of *Piper betle* evaluated by disc diffusion technique are presented in Table 2.

Antioxidant efficiency

Kammar variety was found to possess significant antioxidant activity in comparison with the other cultivars, with an EC₅₀ value of 320 μ g/mL followed by the cultivar, Banarasi. The least antioxidant potential was exhibited by the variety Calcutta. The percentage inhibition and EC₅₀ values of each variety studied are presented in Table 3.

Larvicidal activity

All the cultivars of *Piper betle* studied have shown potent activity against the brine shrimp larva, *Artemia*

salina. Banarasi and Calcutta cultivars have shown 100% mortality rate at the lowest concentration i.e. 62.5 mg/mL. The percentage mortality of methanolic extracts of the leaves of *Piper betle* against the larva is presented in Table 4.

DISCUSSION

The medicinal and pharmacognostic values of the plant *Piper betle* are well known through literature and review. Further, the importance on development of products like toothpastes and powders, perfumes, deodorants, soaps, facial creams and lotions, chocolates, incense sticks, tonics etc., on the utilization of *Piper betle* is raised (Guha, 2000). Due to the properties exhibited by the plant, the plant is rightly termed as green gold by Guha (2006).

In this study, there was no difference seen among the cultivars on the presence of tannins, flavonoids and terpenoids and on the absence of steroids. However, they are found to differ in the constituents of phlobatannins, where it was found to occur only in Banarasi

variety and Saponin, only in Banarasi and Kammar varieties and cardiac glycosides in Banarasi and Kumbakonam varieties. It is noticed that Banarasi is the only variety which showed the presence of more number of phytoconstituents when compared to other cultivars studied. The study conducted on the essential oil composition on Mitha, Bangla and Sanchi varieties have revealed that Mitha yielded more oil and Bangla variety is found to be better in quality (Guha, 2003). Similarly, Garg and Jain, (2006) have reported the difference in phytoconstituents of varieties is attributed to the factors like soil condition, climate and agronomic practice and majorly due to the cultivar (Garg and Jain, 1996). Variation in morphology among the cultivars of betel vine is reported (Pariari and Imam 2012; Khan *et al*, 2013). Differences in stomata and trichome characters in distinguishing two different varieties of *Piper betle* is reported by Dhongle and Kogje (2013). Bajpai *et al*, (2010) used DART MS to distinguish different cultivars of betel vine.

The present study revealed that the cultivars, Calcutta and Kammar have not shown any significant antibacterial or anticandidal activity. However, Banarasi and Kumbakonam cultivars have showed similar antibacterial activity and Banarasi showed better anticandidal activity when compared with all the cultivars studied. The difference in antimicrobial activity among the cultivars of betel is already reported (Agarwal *et al*, 2012). Anticandidal property of betel vine is studied by Himratul-Aznita *et al*, (2011).

Variation in antioxidant ability within the cultivars studied was also exhibited by the present study. Among the cultivars, Kammar and Banarasi have shown better EC₅₀ values when compared with other varieties. This is justified as they showed difference in their phytoconstituents. The antioxidant potency of betel leaf was already reported (Arambewela, 2006). Similarly, there is a difference existing between the cultivars related to their larvicidal potency. The present study revealed that the cultivars, Banarasi and Calcutta showed better larvicidal potency against *Artemia salina* when compared to others.

CONCLUSION

Through this study on cultivars of *Piper betle*, it is concluded that different cultivars have exhibited activities against different parameters studied. Though the antibacterial and anticandidal studies are not very significant, the Banarasi and Kumbakonam cultivars have shown similar antibacterial activity while better anticandidal activity was exhibited only by Banarasi. Kammar and Banarasi are potent antioxidants in comparison with the cultivars studied. Banarasi and Calcutta cultivars are potent against the larva of the brine shrimp, *Artemia salina*. The study amply demonstrates the existence of difference in biopotency among the cultivars of *Piper betle* and proper selection of cultivar

for specific usage in pharmaceutical industry is recommended.

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