Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

STUDIES ON ANTIOXIDANT ACTIVITIES OF SIX CULTIVARS OF PIPER BETLE LINN

S. BHUVANESWARI[1], N. SRIPRIYA[1], S. DEEPA[2], N. K. UDAYA PRAKASH[1*]

[1]Research and Development, Marina Labs, 40, Anna Nedum Pathai, Choolaimedu, Chennai 600094, [2]Department of Science and Humanities, Vel Tech Dr. RR Dr. SR Technical University, 42, Avadi-Alamadhi Road, Avadi, Chennai 600062.

Email: nkudayaprakash@gmail.com

Received: 01 Aug 2014 Revised and Accepted: 10 Sep 2014

ABSTRACT

Objective: To study the existence of difference or similarity on total phenolic content, flavonoid content and antioxidant properties among different cultivars of *Piper betle* L.

Methods: The methanolic extracts of six different cultivars, i. e. Banarasi, Bangla, Calcutta, Kammar, Kumbakonam and Vellai were evaluated for total phenolic content (Folin-Ciocalteu method), total flavonoid content (Aluminium chloride method), total antioxidants (Phospho-molybdenum method) and other antioxidant properties (TBA assay, Ferric thiocyanate assay, FRAP assay and ABTS assay).

Results: Total phenolic content of the cultivars ranges from 12.5 to 13.9 mg TAE/g DW. Higher flavonoid content was observed in Kumbakonam (24.14 µg QE/g DW) while Bangla possessed the lowest (8.25 µg QE/g DW). The total antioxidants of Vellai accounted to 58.82 mg TAE/g DW while the lowest was recorded in Kumbakonam (39.34 mg TAE/g DW). Maximum % inhibition was observed in the cultivar Vellai for Ferric thiocyanate (FTC), Kumbakonam for Thiobarbituric acid (TBA) and Banarasi for Ferric Reducing Antioxidant Power **(**FRAP). However, no significant difference was exhibited among the cultivars for ABTS assay.

Conclusion: The study amply demonstrates the existence of difference in total phenolic content, total flavonoids and antioxidant property. Thus, proper selection of cultivar of *Piper betle* for specific use in the pharmaceutical industry is recommended.

Keywords: *Piper betle,* Cultivars, Total Phenol, Total flavonoid, FTC, FRAP, TBA, ABTS.

INTRODUCTION

The creeper, *Piper betle* Linn. belonging to the family Piperaceae is exploited for many reasons. The biological activities like antibacterial [1], leishmanicidal [2], anti-filarial [3], anti-fungal [4], antimalarial [5], larvicidal [6] and Antiproliferative [7] activities are already studied by different authors. The plant has also been exploited pharmaceutically. The leaves of the plant are used in treating bronchitis, dyspnea [8] and cough [9]. The plant is also considered as a cardio tonic [10]. Essential oils of *Piper betle* were used for the treatment of respiratory catarrhs [11], healing wounds and antiseptic [12]. Leaves of the plant are found to possess analgesic [13] and aphrodisiac [14] properties.

Oxidative stress plays a major role in human diseases which is due to free radicals and their activity [15]. Many diseases like cancer, diabetes, sclerosis, Alzheimer's and Parkinson's are also attributed to the presence or generation of free radicals [16]. It is a known fact that *Piper betle* has the biological benefits of inhibition of platelet aggregation [17], anti-diabetic [18], immunomodulatory [3], anti-hemolytic [19] and anti-allergic activities [20]. Some of these observed biological activities of *Piper betle* were attributed to the presence of high antioxidant activity [21]. Although many biological reports are available on *Piper betle,* they were mostly confined to the plant itself or for a single variety or cultivar [22]. Thus, in this study, an attempt was made to identify the better cultivar of *Piper betle* for best antioxidant potency. Six different cultivars, i. e. Banarasi, Bangla, Calcutta, Kammar, Kumbakonam and Vellai of *Piper betle* were analyzed for their total phenolic content, total flavonoid content, total antioxidant content and free radical scavenging ability.

MATERIALS AND METHODS

Plant source

Six different cultivars of *Piper betle*, i. e. Banarasi, Bangla, Calcutta, Kammar, Kumbakonam and Vellai (Fig. 1) were procured from the local markets of Chennai. Healthy, uninfected and undamaged leaves were used for the study. The leaves were cleaned, washed and allowed to dry in shade. The dried leaves were pulverized using electric blender and stored for further usage.

Fig. 1: Leaves of different cultivars of *Piper betle* L

Preparation of methanolic extract

The methanolic extracts of the cultivars were prepared by cold percolation method. The pulverized plant materials were mixed with methanol at a ratio of 1:10 and maintained in temperature controlled shaker for 48 h, at 30±2^{0}C. The crude extracts obtained upon filtering and concentrating, was reconstituted with the solvent for further analysis.

Determination of total phenolic content

The total phenolic content (TPC) of the cultivars of *Piper betle* was determined by the Folin-Ciocalteu method [23]. To 100 µL of the plant extract, 500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added and incubated for 6 min at room temperature. The final volume of the solution was made up to 3 mL after addition

of 1.25 mL of 7% sodium carbonate. The mixture was incubated for 90 min, followed by measuring the absorbance at 760 nm using UV-Visible spectrophotometer (Cyberlab, USA). The total phenolic content was expressed as mg TAE (Tannic acid equivalents) per g of the dry weight of the plant, using a standard plot of Tannic acid.

Determination of total flavonoid content

The total flavonoid content (TFC) of the plant was determined by the method adopted by Moussa et al [24]. Two hundred microlitre of the plant extract was taken in a test tube and the solvent was allowed to evaporate. The residue was mixed and shaken well with 5 mL of 0.1 M Aluminium chloride. Upon incubation of the solution for forty minutes at room temperature, the absorbance value was measured at 415 nm. A standard plot of Quercetin at varying concentrations was used to evaluate the total flavonoid content, expressed as µg QE (Quercetin Equivalent) per gram dry weight of the plant material.

Determination of total antioxidants

Phospho-molybdenum method was employed for the estimation of total antioxidant activity [25]. A reagent solution of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was used for the experiment.

The plant extract (0.5 mL) was mixed with 4.5 mL of the reagent solution and maintained in a boiling water bath at 95^0 C for 90 min. The absorbance value was measured at 695 nm upon cooling the solution at room temperature. The total content of antioxidants in the plant was expressed as mg TAE (Tannic acid equivalent) per g of the dry weight of the plant material.

Thiobarbituric acid assay

Two milliliters each of 20% trichloroacetic acid and 0.67% Thiobarbituric acid were mixed with 1 mL of 2.51% linoleic acid and 1 mL of plant extract. The solution was maintained in boiling water bath for 10 min. Upon cooling, the solution was centrifuged at 3000 rpm. The supernatant was passed through UV- visible spectrophotometer at 532 nm to measure the absorbance.

The percentage inhibition of the plant against the secondary products of lipid peroxidation was evaluated with reference to the standard solution of butylated hydroxyl toluene (BHT).

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}}\right) x\ 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

A mixture of plant extract (1 mL), phosphate buffer - 2.5 mL (of 0.2 M, pH 7) and 1% potassium ferricyanide (2.5 mL) was incubated at 50^0 C for 30 min. To the solution, 2.5 mL of 10% Trichloroacetic acid was added, mixed and centrifuged for 10 min at 6500 rpm. Distilled water of 2.5 mL and 0.5 mL of 0.1% $FeCl_3$ was added to 2.5 mL of the supernatant. The absorbance of the solution was measured at 700 nm. The reducing ability of the plant was evaluated in terms of percentage by relating the absorbance value of the plant and the standard, $FeSO_4$.

$$\% \text{ Reduction ability} = \left(\frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}}\right) x\ 100$$

Ferric Thiocyanate assay

The inhibitory effect of the plant against oxidation by peroxides was evaluated by Ferric Thiocyanate assay. 120 µL of 98% ethanol, 100 µL of 2.51% linoleic acid in ethanol and 9 mL of 40 mM phosphate buffer (pH 7) were successively added to 100 µL of the plant extract. The mixed solution was maintained in dark, at 40^0 C.

To 100 µL of the mixture, 9.7 mL of 75% ethanol, 100 µL of 30% ammonium thio cyanate and 100 µL of 20 mM $FeCl_3$ in 3.5% HCl were added. The absorbance of the solution was measured at 500 nm, after 3 min. The percentage of inhibition was calculated with Tannic acid as the standard, using the formula:

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}}\right) x\ 100$$

ABTS assay

A solution of 7 mM ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] and 2.45 mM potassium persulphate was incubated in dark for 12-16 h. This was followed by diluting the solution with ethanol till the absorbance reached 0.7±0.02 at 734 nm. One mL of the diluted solution was mixed with 100 µL of plant extract and the absorbance was evaluated at 734 nm after 6 min. The percentage reduction against ABTS was calculated with reference to the standard, Tannic acid.

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}}\right) x\ 100$$

RESULTS

The total phenolic content was found to be the highest in the Calcutta cultivar of *Piper betle* with 13.93±0.10 mg TAE/g DW, followed by the Vellai (13.50±0.07 mg TAE/g DW), while the lowest was in that of Kammar (12.54±0.79 mg TAE/g DW). The quantification of the phenols in each of the variety was based on the chromophores developed upon reduction of Folin-Ciocalteu reagent by the phenolate anion.

Higher flavonoid content was observed in the Kumbakonam cultivar (24.14±0.14 µg QE/g DW) while the Bangla cultivar possessed the lowest content of 8.25±0.10 µg QE/g DW. The total antioxidants of Vellai accounted to 58.82±0.39 mg TAE/g DW of the plant. The total phenolic content, flavonoid and total antioxidant activity recorded for different cultivars of *Piper betle* is presented in table 1.

Table 1: Total phenolic content, total flavonoids and total antioxidant activity of cultivars of *Piper betle*

Cultivar	Total Phenolic Content ± STD (mg TAE/g DW)	Total flavonoid Content ± STD (µg QE/g DW)	Total antioxidant activity ± STD (mg TAE/g DW)
Banarasi	12.61±0.04	11.23±0.12	45.03±0.40
Bangla	12.78±0.02	8.25±0.10	57.45±0.58
Kammar	12.54±0.79	11.74±0.14	40.84±0.49
Calcutta	13.93±0.10	15.76±0.11	43.71±0.34
Kumbakonam	13.42±0.03	24.14±0.14	39.34±0.79
Vellai	13.50±0.07	14.10±0.12	58.82±0.39

The ability of the cultivars of *Piper betle* L., against lipid peroxidation, evaluated using 2-Thio Barbituric Acid (TBA), revealed the efficiency of Kumbakonam cultivar in inhibiting the radicals at a percentage of 97.11 (Fig. 2). However, Kammar and Vellai cultivars showed poor inhibition capability. The cultivar Vellai showed greater reduction of ferrous ions to ferric ions, with a percentage of 98.04 and 90.20 as evaluated by FTC (Fig. 3) and FRAP (Fig. 4) assays respectively. With regard to the ABTS assay, all the cultivars were found to be highly potent inhibitors of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) with their inhibition closer to 98% (Fig. 5).

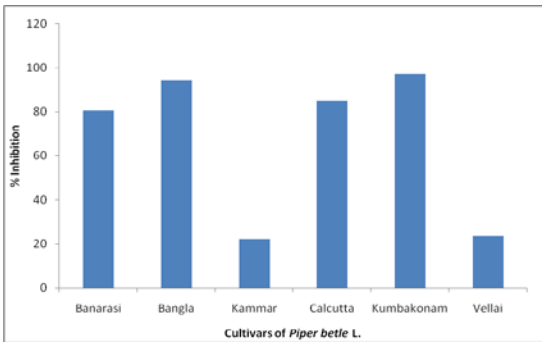

Fig. 2: Thio Barbituric Acid (TBA) Assay

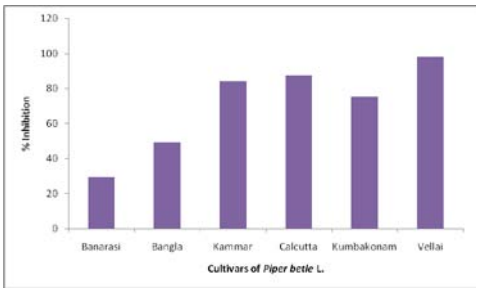

Fig. 3: Ferric Thiocyanate (FTC) assay

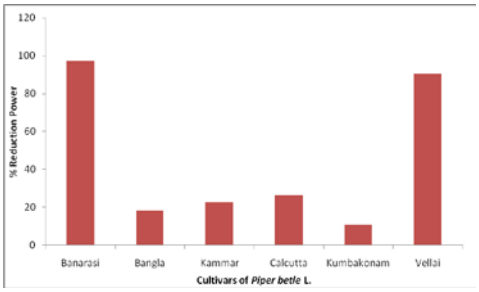

Fig. 4: Ferric Reducing Antioxidant Power (FRAP) Assay

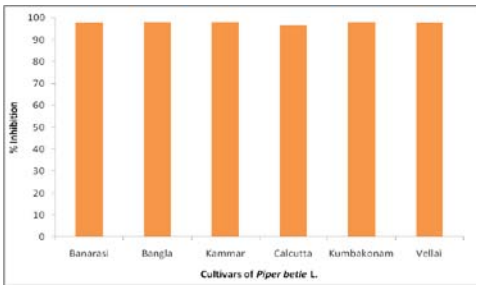

Fig. 5: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Assay

DISCUSSION

Constituents of plants play a key role in their biological activities, the concentration of which depends on the variety of the plant, season and climate [26]. The study has revealed the existence of difference in total phenolic content, flavonoid content and antioxidant activities. Among the cultivars studied, higher phenolic content was detected in the Calcutta cultivar while higher flavonoid and antioxidant content were detected in the cultivars Kumbakonam and Vellai respectively. Vellai, the cultivar with higher total antioxidant content, was found to be potent in reduction of ferrous ions to ferric ions as well. All the cultivars were potent suppressors of ABTS radical.

Similarly, it was reported that the essential oil composition of *Piper betle* of Sri Lankan variety is different from other countries [27]. Guha [28] studied the essential oil composition on Mitha, Bangla and Sanchi varieties, which revealed that Mitha yielded more oil and Bangla was better in quality. The difference in phytoconstituents [29], morphology [30], stomata [31] and the variation among antibacterial, antioxidant and larvicidal activities of cultivars [22] of *Piper betle* has already been reported. The results of the present study imply the potential use of different cultivars for the inhibition of free radicals in different pathways.

CONCLUSION

The study on total phenolic content, total flavonoid content and antioxidant content of six different cultivars of *Piper betle,* i. e. Banarasi, Bangla, Calcutta, Kammar, Kumbakonam and Vellai, revealed the existence of differences among them. The total phenolic content was found to be high in Calcutta, total flavonoids as well as better inhibition percentage of radicals in TBA assay in Kumbakonam cultivar. No significant differences existed among the cultivars of *Piper betle*, on total antioxidant activity. Greater reduction of ferrous ions to ferric ions was recorded with the cultivar Vellai evaluated by FTC and FRAP assays. With regard to the ABTS assay, all the cultivars were found to be highly potent inhibitors. The results of the present study imply the potential use of different cultivars for the inhibition of free radicals in different pathways. Proper selection of cultivar for specific use in the pharmaceutical industry is recommended.

CONFLICT OF INTEREST

No Conflict of Interest lies between Authors.

REFERENCES

1. Khan JA, Kumar N. Evaluation of antibacterial properties of extracts of *Piper betle* leaf. J Pharm Biomed Sci 2011;11(11):1-3.
2. 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. Parasitol Res 2008;102:1249-55.
3. Singh M, Shakya S, Soni VK, Dangi A, Kumar N, Bhattacharya SM. The n-hexane and chloroform fractions of *Piper betle* L. trigger different arms of immune responses in BALB/c mice and exhibit antifilarial activity against human lymphatic filaria brugia malayi. Int J Immunopharmacol 2009;9:716-28.
4. Himratul-Aznita WH, Mohd-Al-Faisal N, Fathilah AR. Determination of the percentage inhibition of diameter growth (PIDG) of *Piper betle* crude aqueous extract against oral *Candida* species. J Med Plants Res 2011;5(6):878-84.
5. Abdulelah HA, Zurainee MN, Hesham MA, Adel AA, Rohela Mahmud. Antimalarial activity of methanolic leaf extract of *piper betle* L. Molecules 2011;16:107-18.
6. Wardhana AH, Sujith Prasad, Kumarasinghe W, Arawwawala LDAM, Arambewela LSR. Larvicidal efficacy of essential oil of betel leaf (*Piper betle*) on the larvae of the old World screwworm fly, *Chrysomya bezziana in vitro.* Indian J Dermatol 2007;52(1):43-7.
7. Fathilah AR, Sujata R, Norhanom AW, Adenan MI. Antiproliferative activity of aqueous extract of *Piper betle* L. and *Psidium guajava* L. on KB and HeLa cell lines. J Med Plants Res 2010;4(11):987-90.
8. Rahman SA. Anti-ulcer effects of Piper betle, Solanum nigrum and Zingiber cassumunar on ulceration induced by selected ulcerogens in rats. Masters thesis, University Putra Malaysia; 2009. p. 4.
9. Agarwal T, Singh R, Shukla AD, Waris I, Gujrati A. Comparative analysis of antibacterial activity of four *Piper betle* varieties. Adv Appl Sci Res 2012;3(2):698-705.
10. Evans PH, Bowers WS, Funk EJ. Identification of Fungicidal and Nematocidal Components in the Leaves of *Piper betle* (Piperaceae). J Food Agric Chem 1984;32:1254-6.
11. Chandra T, Sadique J, Somasundaram S. Effect of Elipta alba on inflammation and liver injury. Fitoterapia 1987;58:23-31.
12. Ali SM, Mehta RK. Preliminary pharmacological and anthelmintic studies of the essential oil of *Piper betle*. Indian J Pharm 1970;32:132.
13. Ratnasooriya WD, Jayawardena KGI, Premkumara GAS. Antimotility effect of *Piper betle* (L) leaf extract on washed human spermatozoa. J Natl Sci Council Srilanka 1990;18:53-60.
14. Chatterjee A, Pakrashi SC. Treatise of Indian Medicinal Plants. CSIR Publication New Delhi; 1995. p. 26.
15. Udaya Prakash NK, Bhuvaneswari S, Sripriya N, Prameela L, Bhagya R, Radhika B, *et al*. Antioxidant activity of common plants of Northern Tamil Nadu, India. Int J Pharm Pharm Sci 2014;6(4):128-32.
16. Ozgen U, Mavi A, Terzi Z, Yildirim A, Coskun M. Houghton PJ. Antioxidant properties of some medicinal Lamiaceae species. Pharmacol Biol 2006;44:107-12.

17. Jeng JH, Chen SY, Liao CH, Tung YY, Lin BR, Hahn LJ, *et al*. Modulation of platelet aggregation by areca nut and betel leaf ingredients: roles of reactive oxygen species and cyclooxygenase. Free Radical Biol Med 2002;32:860–71.
18. Arambewela LSR, Arawwawala LDAM, Ratnasooriya WD. Antidiabetic activities of aqueous and ethanolic extracts of *Piper betle* leaves in rats. J Ethnopharmacol 2005;102:239–45.
19. Devjani C, Shah B. Antimicrobial, anti oxidative and anti hemolytic activity of *Piper betel* leaf extracts, Int J Pharm Pharm Sci 2011;3(3):192-9.
20. Wirotesangthong M, Inagaki N, Tanaka H, Thanakijcharoenpath W, Nagai H. Inhibitory effects of *Piper betle* on production of allergic mediators by bone marrow-derived mast cells and lung epithelial cells. Int J Immunopharmacol 2008;8:453–7.
21. Dasgupta N, De B. Antioxidant activity of *Piper betle* L. leaf extract *in vitro*. Food Chem 2004;88:219–24.
22. Smila KH, Priyanka JD, Srinithya B, Sripriya N, Udaya Prakash NK. Studies on phytochemistry and bioefficacy of cultivars of *Piper betle* Linn. Int J Res Pharm Sci 2014;5(2):1-5.
23. Deepa S, Ramesh Kannan P, Kanth SV, Raghava Rao J, Chandrasekaran B. Antioxidant and cytotoxic effects of methanolic extract of *Salicornia brachiata* L. in HepG2 cells. Int J Res Pharm Sci 2013;4(4):512-7.
24. Moussa AM, Emam AM, Diab YM, Mahmoud ME, Mahmoud AS. Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats. Int Food Res J 2011;18:535-42.
25. Chunpeng W, Yanying Y, Shouran Z, Wei L, Shuge T, Shuwen C. Antioxidant activity and free radical-scavenging capacity of *Gynuradi varicata* leaf extracts at different temperatures. Pharmacognosy Magazine 2011;7(25):40-5.
26. Satish AB, Deepa RV, Rohan VG, Nikhil CT, Yatin YR, Vinodkumar SD, Ashwin T. Phytochemistry, pharmacological profile and therapeutic uses of *piper betle* linn–an overview. Res Rev: J Pharmacognosy Phytochem 2013;1(2):10-9.
27. Kumaratunga KGA. Gas chromatographical and antimicrobial studies on *Alpinia calcarata* and *Piper betle* from Sri Lanka M Phil Thesis, University of Kelaniya, Sri Lanka; 2003. p. 57–91.
28. Guha P. Extraction of essential oil from *betel* leaves grown in and around Midnapur District, In: Annual Report of All India Coordinated Research Project on Post Harvest Technology (ICAR). IIT, Kharagpur (Ed.); 2003. p. 15-23.
29. Garg SC, Jain R. Chavicol rich essential oil of *Piper betle* L, Cutivar Sagar Bangla. Eur Cosmetics 1996;5:27-8.
30. Pariari A, Imam MN. Evaluation of betelvine (*Piper betle L*,) cultivars in the Gangetic alluvial plains of W. Bengal, India. J Spices Aromatic Crops 2012;21:1-8.
31. Dhongle SS, Kogje KK. Comparative pharmacognostic study of leaves of two varieties of *piper betle* l. namely calcutta, bangla and assam kapoori. Int J Herbal Med 2013;1(3):115-9.