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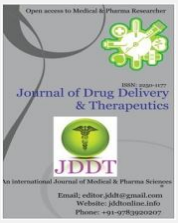

Open Access Research Article

ANTIOXIDANT ACTIVITY (PHENOL AND FLAVONOID CONTENT) OF THREE DIFFERENT CULTIVARS OF *PIPER BETLE* L. (PIPERACEAE)

Sapara Sekhar Harini[1], and Pagadala R Sougandhi[1], Tenkayala Shobha Rani[2]*, Kamalamma Ramalingam Gopinath[3]

[1] Research Scholars, Department of Chemistry, Dravidian University, Kuppam, Chittoor (DT), A.P., India

[2] Research Supervisor, Department of Chemistry, Dravidian University, Kuppam, Chittoor (DT), A.P., India

[3] Research Scholar, Research & Development Center, Bharathiar University, Coimbatore- 641 046, Tamil Nadu, India

ABSTRACT

In the present study, an attempt has been made for the estimation of total phenol and flavonoid content and their radical scavenging properties using *Piper betle* (L.) leaves. In that, Cultivars i.e., Nov Bangla (NB), Sirugamani-1 (SGM-1) and Halisar Sanchi (HS) were selected for this study. The total phenolic content was ranged from 95.04 to 127.33 mg/100g equivalent to gallic acid and flavonoids were ranged from 51.72 to 61.08 mg/ 100g equivalent to standards of Catechin. *In vitro* antioxidant activity was estimated using 1,1-diphenyl-2-picryl hydrazyl (DPPH), free radical scavenging activity, improved ABTS radical cation decolorization assay and ferric reducing antioxidant power (FRAP) assay. Among all the cultivars, the highest Phenol content (93.79%) was observed for Sirugamani-1 by DPPH method and highest Phenol content (96.12% & 6791.86 (µg/g) was obtained for Halisar Sanchi by ABTS assay and FRAP activity respectively. The study revealed that the leaves of *Piper betle* (L.) has higher amount of antioxidant activity and it could be used for any novel drug preparation.

Keywords: *Piper betle*, Cultivars, Methanol extract, phenol content, flavonoid and antioxidant.

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†Address for Correspondence:

Dr. T. Shobha Rani1*, Department of Chemistry, Dravidian University, Kuppam, Chittoor (DT), A.P., India.

1. INTRODUCTION

Betel vine (*Piper betle* L.), commonly called paan, and is the leaf of an evergreen, perennial vine of the Piperaceae family. Usually betel leaves are consumed after meals as a digestive stimulant and also mouth freshener. It possess a number of medicinal properties, including anti-inflammatory and oxidant activities. It was valued as a mild stimulant and also has its use in Ayurvedic medicine [1]. The plant originated in central and Eastern peninsular, Malaysia and distributed throughout east Africa and also the tropical regions of Asia. It was also cultivated as commercial crop and also was cultivated widely in many parts of India and Sri Lanka [2]. The use of betel leaf dated back as two thousand years ago [3]. Fresh leaves were chewed with betel nut, (*Areca catechu* -Arecaceae), and other adjuvants (betel liquid) in most parts of India [4, 5]. Due to their strong pungent aromatic flavor, betel leaves were used as a masticatory in Asia. As per earlier reports, the Betel leaves were contain an aromatic oil [6], minerals [7], glycosides [8], enzymes, vitamins, essential amino acids [9] and tannins [10].

Number of researchers have been described that betel leaf has a widely spread in therapeutic applications. Most of the betel leaf extract got from various chemical substances such as ethanol, methanol and ethyl acetate which possess antimutagenic, anti carcinogenic, antiplaque, antidiabetic, anti-inflammatory and antibacterial activities [11-16]. Eugenol is one of the main chemical constituent of betel leaf and has been exposed to have anti-inflammatory properties in a number of animal studies [17]. Besides the eugenol, the betel leaves have other major constituents, hydroxychavicol, alpha-tocopherol, and allylpyro catechol have also play important role to enhance the levels of growth stimulating hormone (GSH) in mouse skin and liver [18, 19]. The anti-oxidant and anti-inflammatory properties of *Piper betle* have been attributed to various parts especially leaves. Generally leaves were having higher phenol and flavonoid content and have been found to have greater anti-oxidant, radical scavenging and anti-cancer activities [20, 21]. The aim of our study was to estimate the phenol and flavonoid content of three different cultivars of *Piper betle* and their potential radical scavenging properties of these three cultivars.

2. MATERIALS AND METHODS

2. 1. Plant Collection

Freshly harvested leaves from cultivars of Betel vine such as Nov Bangla (NB), Sirugamani-1 (SGM) and Halisar Sanchi (HS) were collected from Central Horticulture Experimental Station (CHES) Hirehalli, Tumakuru, Karnataka, India.

2.2. Chemicals

Chemicals and reagents, including Gallic acid, Catechin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), TPTZ (Tri pyridyl tri-azine) and Ferric chloride were purchased from Sigma Aldrich Chemical Co. Ltd., (MO, USA). Folin–Ciocalteu reagent, Sodium nitrite, Aluminum chloride, Sodium hydroxide, Sodium acetate and Glacial acetic acid were purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India.

2.3. Sample Preparation

The fresh and matured leaves were crushed using pestle and mortar. Further the samples were soaked and extracted (cold extract) with methanol. After that methanol was evaporated then semi solid samples were used for analyzing the experiments.

2.4. Determination of Total Phenolic Content

The 100 mg of extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water. 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 2 ml 20% of Na_2CO_3 solution was added[22]. Ultimately this solution was made up to 8 ml with triple distilled water followed by vigorous shaking and finally allowed to stand for 2 hours. After completion of this, the sample absorbance was taken at 765 nm. These data was used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

2.5. Determination of Total Flavonoid Content

The 0.3 ml of 5 % $NaNO_2$. was added to 1.0 ml of plant extract. This mixture is allowed to stand for 2 minutes and 0.3 ml of 10% $AlCl_3$ was added. 2 minutes later 3.4 ml of NaOH was added to mixture and allow it to stand for 30 minutes[23]. The flavonoids develop a brick red colour with $AlCl_3$ and $NaNO_2$ at alkaline pH. The absorbance of the complex is read at 510 nm. Later that sample absorbance was observed at 510 nm against blank. The Catechin is used as standard for this determination.

2.6. 1, 1-diphenyl -2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The antiradical efficiency was assessed by 1,1-diphenyl -2-picrylhydrazyl (DPPH) method as described with significant modification [24]. In this method, commercially available methanol soluble, and stable free radical DPPH was used. For the photometric assay, different volumes (250, 500, 750 and 1000 µg/ ml) of the plant extracts were taken in different test tubes. The plant extracts volume was adjusted to 1ml with respective volumes. The 3.5 ml of 0.1mM methanol solution of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was added to these tubes and shaken vigorously. The test tubes were allowed to stand for 30 min. at room temperature.

The control was prepared as above but without the test extract and methanol was used for the baseline correction. In its radical form, DPPH has an absorption band at 517 nm, which disappears upon reduction by an antioxidant compound or a radical species. The changes in the absorbance of the samples were monitored at 517 nm. The results were compared with the activity of ascorbic acid as standard. The percentage of DPPH discolouration of the samples was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{A 517 of Control} - \text{A517 of Sample}}{\text{A517 of Control}} \text{X } 100$$

The percentage of reduced DPPH was plotted against the concentration of each sample, and an IC_{50} value (the concentration required to scavenge 50% of the DPPH) was calculated.

2.7. Ferric Reducing Antioxidant Potential (FRAP) Activity

Total antioxidants were estimated using Ferric Reducing Antioxidant Potential (FRAP) method as described [25] with trivial modifications. Acetate buffer, Tri pyridyl triazine (TPTZ) and Ferric chloride in 10:1:1 ratio was freshly prepared before use. The different solvent extracts (0.2 ml) of the sample was taken into the test tubes and 1.8 ml of working FRAP reagent was added. Then the tubes were kept in dark for incubation at room temperature for 30 minutes. The developed colour was read at 593 nm by U. V. spectrophotometer. Different concentrations of ascorbic acid (1 µg-10 µg) were prepared and Optical Density was read at 593 nm. The standard graph (Concentration Vs Optical Density) was drawn using ascorbic acid as standard. The

concentration of samples was calculated based on the standard curve µg/g equivalent to ascorbic acid.

2.8. 2-2[1]-azino -bis (3-ethyl Benzo) thiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) Assay

Initially, the ABTS was dissolved in water with 7 mM concentration and ethanol was added (Stock solution). To the ABTS stock solution, the 2.45 mM potassium persulfate is also added then ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting the contents and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Since ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS. The radical was stable in this form for more than two days when stored in the dark at room temperature.

For the study of phenolic compounds and food extracts, the $ABTS^{\bullet+}$ solution was diluted with ethanol and for plasma antioxidants with PBS, pH 7.4, to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30°C. After addition of 1.0 ml of diluted $ABTS^{\bullet+}$solution (A734nm) to 10 ml of antioxidant compounds or Trolox standards (final concentration 0–15 mM) in ethanol the absorbance reading was taken at 30°C exactly 1 min. after initial mixing and up to 6 min. Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times (in triplicates), on each occasion and at each separate concentration of the standard and samples. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a functio3+n of concentration of antioxidants and of Trolox for the standard reference data. The concentration-response curve for 5 sequentially and separately prepared stock standards of Trolox [26].

2.9. Statistical Analysis

All the above method activities and assays were performed in triplicates and the results were represented by their mean ± standard deviation (SD). The data was analyzed by a one-way analysis of variance (ANOVA) was used to evaluate significance of any differences between the betel vine samples.

3. RESULTS AND DISCUSSION

Generally plants that have significant therapeutic properties which have been found to be rich in phenolics, and with high antioxidant properties [27]. In the present study, cultivars of Nov Bangla produced high phenolic content i.e., 127.33 ± 0.62 (mg/100 g) equiv. to Gallic acid among other cultivars of Halisar Sanchi (122.78 ± 0.11) and Sirugamani-1 (95.04 ± 0.05). The total phenol content and the flavonoid content of the betel leaves have been identified as chevibetol and allyl pyrocatechol. In the same way, the total flavonoid content of the three cultivars were estimated and results showed that flavonoid content (mg/100g) equiv. to Catechin was present in Nov Bangla (61.08 ± 2.96) cultivar among other cultivars of Halisar Sanchi (54.87 ± 2.89) and SGM-1 (51.72 ± 1.11) cultivars which were presented in Table 1.

Table 1: The Total Phenol and Flavonoid Content values of Cultivars of *Piper betle*

Sample	Phenol content (mg/100 g) equiv. to Gallic acid	Flavonoid content (mg/100 g) equiv. to Catechin
Nov Bangla	127.33 ± 0.62	61.08 ± 2.96
SGM-1	95.04 ± 0.05	51.72 ± 1.11
Halisar Sanchi	122.78 ± 0.11	54.87 ± 2.89

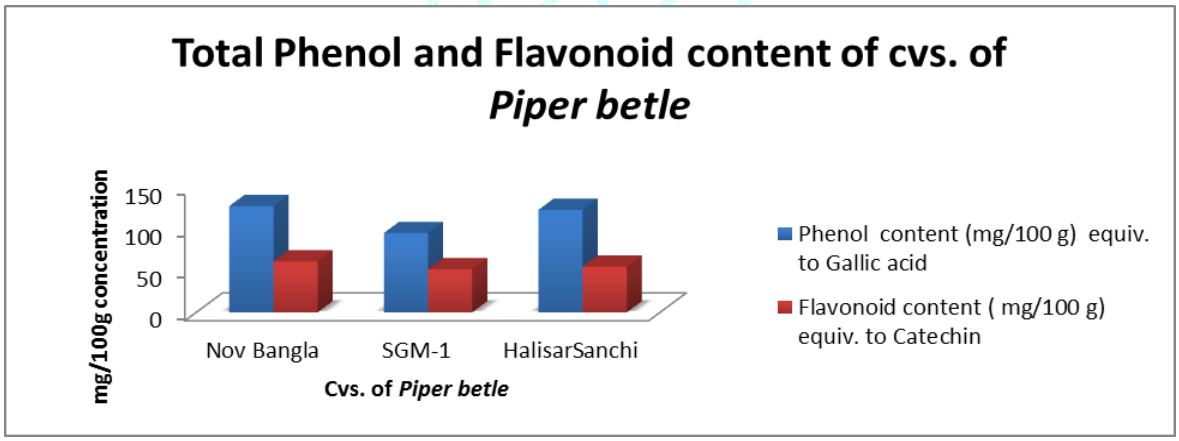

Figure 1: Graphical Representation of Total Phenol and Flavonoid Contents of Cultivars of *Piper betle*

Different species of betel leaves such as female (Bangalore local) type, male (Madras) type and Meetha (sweet) type have been investigated for their anti-oxidant activity by FRAP (Ferric Reducing Anti-Oxidant Potential) assay and radical scavenging activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The results were shown significant antioxidant activity in various extracts[20]. In the current findings, The DPPH antioxidant activity was higher in Sirugamani-1 i.e., 96.63 ± 0.54 cultivar among other cultivars of Halisar Sanchi (93.79 ± 0.20) and Nov Bangla (65 ± 0.20) which were presented in Table 2.

Table 2: The Radical Scavenging Activity Values by DPPH assay of Cultivars of *Piper betle*

Samples/ Conc.µg/ml	% of DPPH inhibition			
	250	500	750	1000
Nov Bangla	24.05 ± 0.28	50.95 ± 0.28	62.97 ± 0.1	65 ± 0.20
SGM-1	83.64 ± 0.27	88.92 ± 0.66	92.69 ± 0.35	96.63 ± 0.54
Halisar Sanchi	84.84 ± 0.27	89.94 ± 0.33	92.86 ± 0.28	93.79 ± 0.20

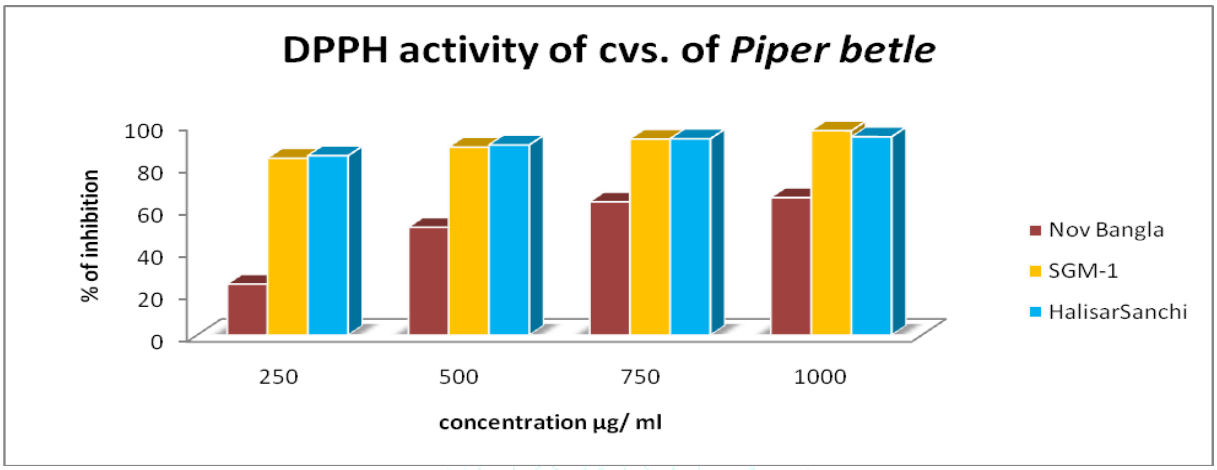

Figure 2: Graphical Representation of Radical Scavenging Activity by DPPH assay of Cultivars of *Piper betle*

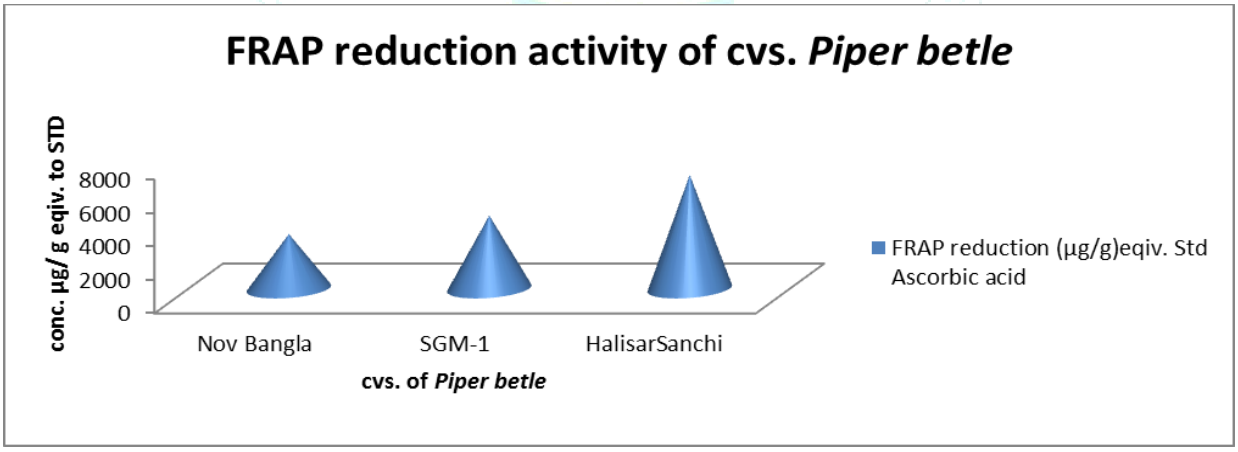

Figure 3: Graphical Representation of FRAP Reduction Activity of Cultivars of *Piper betle*

The FRAP reduction assay was showed that the reduction (µg/g) equiv. Std ascorbic acid was higher in Halisar Sanchi cultivars 6791.86 ± 96.31 followed by Sirugamani-1(SGM-1) (4358.97 ± 74.67) and Nov Bangla (3254.90 ± 171.97) cultivars. It was interesting to note that Nov Bangla showed higher phenolic content but DPPH and FRAP activities was less in this Nov bangla cultivar (Table 3).

Table 3: The FRAP Reduction Assay of Cultivars of *Piper betle*

Samples/ Conc.@ 1000 µg/ ml	FRAP reduction (µg/g) equiv. Std Ascorbic acid
Nov Bangla	3254.90 ± 171.97
SGM-1	4358.97 ± 74.67
Halisar Sanchi	6791.86 ± 96.31

High content of various phenolic and non-phenolic compounds and other uncharacterized moieties may contribute to its use, not only as a highly nutritive, edible plant part, but also as a nutraceutical substance, to be used prophylactically as well as therapeutically in oxidative inflammatory diseases.This correlation has been confirmed with the antioxidant activity being detected in the extract of betel leaf [28]. In contrast the

present study showed Sirugamani-1 (SGM-1) has less phenolic content compared to Nov Bangla and Halisar Sanchi cultivars but produced the highest antioxidant activity. So the study also revealed not only the phenols and other phytochemicals constituents also may contribute the antioxidant activity of *Piper betle*. But the ABTS activity was measured above 90% in all cultivars shows that the antioxidant activity could chemical depended manner which were presented in Table 4.

Table 4: The ABTS inhibition Activity of Cultivars of *Piper betle*

Samples/ Conc. µg/ ml	% of ABTS inhibition			
	250	500	750	1000
Nov Bangla	76.74 ±1.26	79.26 ±1.45	83.33 ± 1.98	91.61 ± 0.91
SGM-1	63.03 ± 1.08	79.14 ± 2.0	84.17 ± 1.57	92.93 ± 1.10
Halisar Sanchi	72.18 ± 2.34	84.05 ± 1.81	95.68 ± 1.08	96.16 ± 0.55

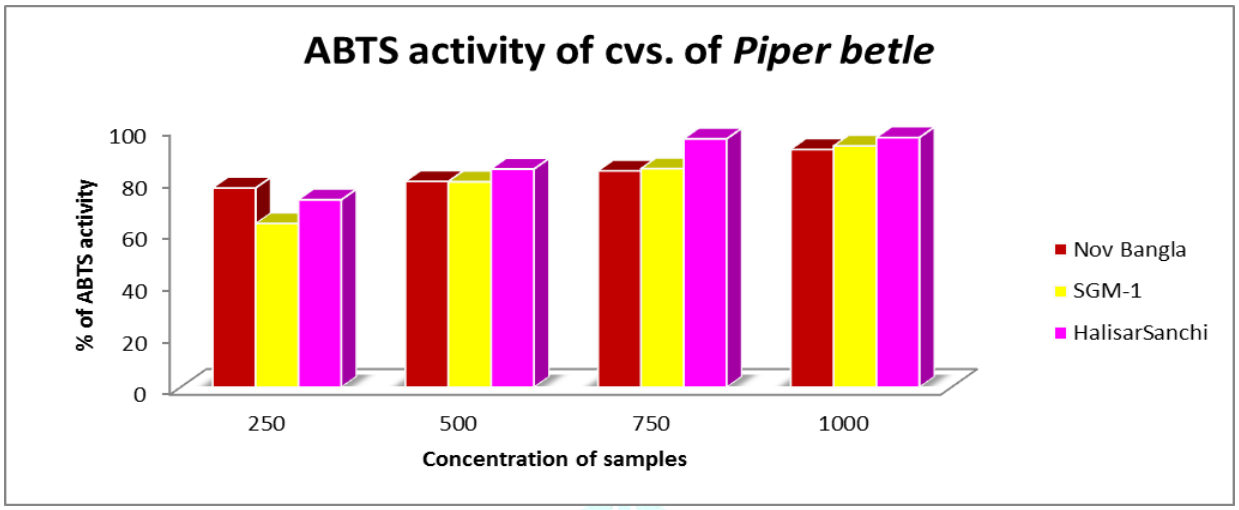

Figure 4: Graphical Representation of ABTS inhibition Activity of Cultivars of *Piper betle*

4. CONCLUSIONS

The phenolic, flavonoid and antioxidant content of betel cultivars are reported as per DPPH method, FRAP method and ABTS assay. The total phenolic content was assessed using Folin-Ciocateau method using Gallic acid as standard. Inhibition of DPPH radical scavenging activity is observed in three varieties of betel cultivars where maximum inhibition is seen in SMG-(96.63+0.54) where as minimum inhibition was observed in Nov Bangla (65+0.20). The highest flavonoid content was seen in Nov bangla (61.08+2.96) whereas lowest was seen in SGM-1(51.72+2.96) equivalent to Catechin. This paper results may lead to several findings and therapeutically useful as drugs.

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