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Biochemical analysis of betel vine (*Piper betel*) leaves

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Abstract
In the present study, an attempt has been made for the estimation of chlorophyll, total phenol content and radical scavenging properties of *Piper betle* (L.) leaves. In that, chlorophyll was ranged from 3.80 to 4.00 (mg/l).The total phenolic content was ranged from 94.98 to 95.18 mg/100g equivalent to gallic acid and antioxidant activity was estimated using 1,1-diphenyl-2-picryl hydrazyl (DPPH), free radical scavenging activity, The study revealed that the leaves of *Piper betle* (L.) has higher amount of antioxidant activity. The presence of phenol and phenolic (Chavicol, Chavibetol, Chavibetol acetate and eugenol) in the *Piper betel* leaves may be credulous to be responsible for its antioxidant activity.

Keywords: *Piper betel* leaves

1. Introduction
The *Piper betel* leaf commonly known as 'paan' or 'Nagvalli' (family-*Piperaceae*) is an evergreen and perennial creeper significance of leaves has been explained in relationship to every sphere of human life including social, culture, religious and very much relevant even in modern days. Various properties of betel leaves include antioxidant, antifungal, antidiabetic, antimicrobial, anti-inflammatory, antifertility, antinacepative and radioprotective properties, (Sripradha, 2014) [18].
The particular properties of *Piper betel* leaves are antimicrobial and antileshmian properties. *Piper betel* leaves have long been studied for their diverse pharmacological actions (Sarkar *et al.*, 2008.) [14] *Piper betel* leaves also contain significant amount of antioxidants like hydroxychavicol, eugenol, ascorbic acid and ß carotene belonging to the propenylphenol group (Chakraborty and Shah, 2011) [3]. The presence of phenol and phenolic (Chavicol, Chavibetol, Chavibetol acetate and eugenol) in the *Piper betel* leaves may be credulous to be responsible for its antioxidant activity (Swapna *et al.*, 2012) [19].

2. Materials and Method
Fresh *Piper betel* leaves of Culcutta variety were procured from local market of Kolhapur city. Spectrophotometer Spectronic 20 Colorimeter (Geaesys 545TM Spectrophotometer, New York, USA) was used for analysis purpose. All the glasswares of Borosil make were used for analysis of product. All the chemicals used were Analytical M/S Reagent (AR) and Guaranteed Reagent (GR) grade for analytical work which was manufactured by Merk, India Ltd/ Glaxo India Ltd.

2.1 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 antioxidant and phenolic properties of *Piper betel* leaves.

2.2 DPPH Radical Scavenging activity assay
The antiradical efficiency was assessed by 1,1-diphenyl -2-picrylhydrazyl (DPPH) method as described with significant modification. (Harini *et al.*,2018) [6] In this method, commercially available methanol soluble, and stable free radical DPPH was used. For the photometric assay, different volumes (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 discoloration of the samples was calculated using the following formula:

$$\text{DPPH RSA} = \frac{\text{A517 of control} - \text{A517 of sample}}{\text{A517 of control}} \times 100$$

2.3 Determination of Total Phenolics Content
2.3.1 Reagents
A. Folin Ciocalteu reagent (0.2N) 2N Folin -Ciocalteu phenol reagent was diluted with distilled water in the ratio1:10
B. Sodium Carbonate solution 20% (v/v), add 20 mL of sodium carbonate solution to 80mL of distilled water and the volume made to 100 ml using volumetric flask.
C. Gallic acid stock solution (1 mg/ml)

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 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 were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid equivalent (GAE)/ml sample. (Harini *et al.*, 2018) [6].

2.4 Chlorophyll Analysis
One gram of leaf sample was finely cut and gently mixed with a clean pestle and mortar. To this homogenized leaf material, 20ml of 80% acetone and 0.5gm $MgCO_3$ powder was added. The materials were further grind gently. The sample was then put into a refrigerator at 4 °C for 4 hours. Thereafter, the sample was centrifuged at 500 rpm for 5 minutes. The supernatant was transferred to 100 ml volumetric flak. The final volume was made up to 100 ml with addition of 80% acetone.
The color absorbance of the solution was estimated by a spectrophotometer using 645 and 663nm wavelength against the solvent. Acetone (80%) was used as a blank. Leaf material – crushed using mortar and pestle added Acetone and $MgCO_3$ kept for 4 hours in freeze at 40 °C centrifuge at 500 rpm for 5 minutes then measured absorbance on spectrophotometer (Kamble *et al.*, 2015) [9].

Formula: Chl a= $11.75 \times A_{662.6} - 2.35 \times A_{645.6}$

Chl b=$18.61 \times A_{645.6} - 3.96 \times A_{662.6}$

Where, Ca and Cb are the chlorophyll a and chlorophyll b, A is absorbance.

2.5 Statistical Analysis
Data generated during the course of investigation were analyzed using completely randomized design (CRD) technique according to Snedecor and Cochran (1994) [16].

3. Result and discussion
3.1 Total Phenolic Content (TPC)

Table 1: Total Phenolic Content of Betel vine (*Piper betel)* Leaves

Replication	Total Phenolic Content mg GAE/100 gm
I	95.07
II	94.98
III	95.18
Mean	95.07
SE	0.05

Table 1 revealed that the average TPC in *Piper betel* leaves used in the investigation was 95.07 mg GAE/100gm. Shiban *et al.* (2012) [15] reported that plant phytochemicals (e.g. phenolics) compounds from plants exhibit various physiological properties, such as anti-allergenic, anti-inflammatory, anti- microbial and antioxidant. Total phenol investigated in betel leaf constituents, eugenol, hydroxychavicol and alpha-tocopherol, was reported by Rintu *et al.* (2015) [13]. Harini *et al.* (2018) [6] estimated the betel vine plants have significant therapeutic properties and found rich in phenolics and antioxidant componants.
They were stuidied cultivars of Nov Bangla produced high phenolic content i.e., 127.33 ± 0.62 (mg/100 g) equiv. to Gallic acid. Kamble (2015) [9] reported chlorophyll content in different leaves Mango, Guava, Neem and Ashoka as 6.48, 12.54, 36.18 and 6.48 mg/l respectively. Also, Guha (2006) [4] reported that chlorophyll content in *Piper betel* leaves 0.01 to 0.25 %.

3.2 Antioxidant Property of *Piper betel* Leaves

Table 2: DPPH Radical Scavenging Assay

Replication	Samples Conc. µg/ml		
	500	750	1000
I	45.21	58.56	63.18
II	44.18	57.34	62.91
III	46.06	58.03	61.85
Mean	45.15	57.97	62.64
SE	0.54	0.35	0.4

The result generated are reported in table 2 and fig.1 plant extract sample concentration for 1000 was 62.64 µg/ml. Harini *et al.* (2018) [6] reported *Piper betel* cultivars Nov Bangla produced high antioxidant content i.e., plant extract 1000 sample concentration was 65± 0.20.
Betel leaves are potential non-toxic natural antioxidant (Arambewela *et al.* 2006) [1]. Antioxidant capability was assayed by superoxide radical scavenging activity and free radical scavenging activity, using DPPH, a stable free radical was studied by (Venugopalan *et al.* 2008) [20].
Blois (1958) [2] reported the DPPH free radical scavenging activity of a compound indicates its hydrogen-donating tendency. Heo *et al.* (2005) [7] reported the high correlation between DPPH radical scavenging activities and total polyphenolics.
Similarly, antioxidant activity of plant extracts is also correlated with their reducing powers observed by Pin-Der-Duh, (1998) [12]. Gulcin *et al.* (2004) [5] reported, reducing

power of aqueous extract was seen to increase with enzymatic treatment. Which may influence the antioxidant activity.

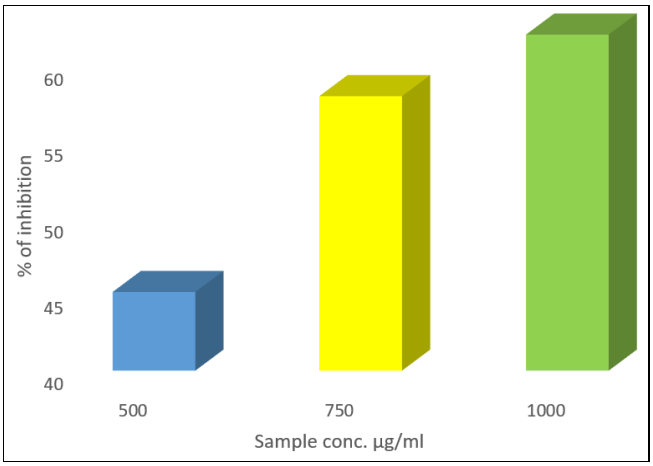

Fig 1: DPPH radical scavenging assay of *Piper betel* leaves

3.3 Chlorophyll Content

Table 3: Chlorophyll Content of Betel Vine (*Piper betel)* Leaves

Replication	Chlorophyll (mg/l)
I	4.00
II	3.90
III	3.80
Mean	3.90
SE	0.05

Table 3 revealed that the average chlorophyll content of *Piper betel* leaves used for the investigation was 3.90 mg/l. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants was estimated by (Mirza *et al.*, 2013 and Srichaikul *et al.*, 2011) [10, 17]. Green plants have different characters because of the presence of various pigments like chlorophyll, carotenoid, other pigments and water content which together constitute the spectral characters of a plant body was studied by Philip and Shirly (1978) [11] and Jan-Chang Chen, (2007) [8]. Kamble *et al.*, (2015) [9] analysed chlorophyll content of different leaves and reported that chlorophyll a concentration was higher than that of chlorophyll b.

4. Conclusion

The importance of the betel leaf as discussed above prove that leaf has a great potency to act as natural antioxidant also leaf possess the broad spectrum chlorophyll and total phenolic activity.

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