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IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF METHANOLIC EXTRACT OF *PIPER BETEL*

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ABSTRACT: The *Piper betel* is an evergreen and perennial creeper belonging to the Piperaceae family, with glossy heart-shaped leaves and white catkin. It is valued both as a mild stimulant and for its medicinal properties. The total phenolic content of methanolic leaves extract was estimated by Folin-Ciocalteu assay method, and was found to be 0.110 mg/CE/g (Catechine Equivalent per gram). However, antioxidant activity of methanolic leaves extract of *Piper betel* was determined by DPPH free radical scavenging method. The DPPH radical scavenging activity of methanolic extract of *Piper betel* was found to be highest at 100µl concentration which was 25.2%. Nevertheless, % DPPH scavenging activity of standard ascorbic acid at same concentration was found to be 40%. The % DPPH scavenging activity increases with the increasing concentration. The concentration of *Piper betel* needed for 50% inhibition (IC50) was found to be 282.31µg/ml whereas 369.19 µg/ml needed for ascorbic acid.

INTRODUCTION: In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethnopharmacological surveys conducted among herbal practitioners of traditional Arab medicine in Palestine and the Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials.

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The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins [1].

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [2].

Piper species, commonly used in diet and traditional medicine, were assessed for their antioxidant potential. The antioxidant and radical scavenging activities of black pepper (*Piper nigrum* Linn.) seeds have been well reported [3].

Both water extract and ethanol extract of black pepper exhibited strong antioxidant activity. Antimicrobial [4], larvicidal [5] and anti-cancer [6] activities of *Piper nigrum* Linn. have been reported.

The Betel (*Piper betel*) is the leaf of a vine belonging to the Piperaceae family, which includes pepper and Kava. It is valued both as a mild stimulant and for its medicinal properties. Betel leaf is mostly consumed in Asia and elsewhere in the world by some Asian emigrants, as betel quid or *paan*, with or without tobacco, in an addictive psycho-stimulating and euphoria-inducing formulation with adverse health effects [7].

The betel plant originated from South and South East Asia (India, Nepal, Bangladesh and Sri Lanka). In India, Burma, Nepal, Sri Lanka and other parts of South Asia, as well as Southeast Asia, the leaves of *Piper betel* are chewed together in a wrapped package along with the areca nut (also called as betel nut) and mineral slaked lime (calcium hydroxide). The areca nut contains alkaloid arecoline, which promotes salivation (the saliva stained red), and is itself a stimulant. This combination, known as a "betel quid", has been used for several thousand years.

Betel leaves are used as stimulant, an antiseptic and breath- freshener. The present investigations is undertaken to estimate the total phenolic content and antioxidant potential of *Piper betel* methanolic leaf extract though DPPH *in vitro* assay model.

MATERIALS AND METHODS:

Plant materials: The leaves of *Piper betel* were procured from local market, Sungai Petani, Kedah in the month of January, 2012.

Preparation of Methanolic Extract: The collected leaves were air-dried, pulverized in to a coarse powder by grinder and sieved. The dried powdered material was extracted with methanol in round bottom flask with Soxhlet apparatus connected to it for 1 hour. The extract was filtered and the solvent was removed by distillation under vacuum, extract is subjected to evaporate to dryness in china dish. After dryness, by adding corresponding solvent to it, it is used for further in-vitro studies.

Total Phenolic Content: The total phenolic content of leaves of plant *Piper betel* was determined with Folin–Ciocalteu reagent using the method developed by Harbertson and Spayd with slight modification [8].

An aliquot 1ml of extracts or standard solution of catechin (20, 30, 40, 50, 60, 70 mg/l) was added to 25ml volumetric flask, containing 9ml of distilled deionised water (dd H_2O). A reagent blank using dd H_2O was prepared. One millilitre of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5min 10 ml of Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (25ml) with dd H_2O and mixed. After incubation for 90min at room temperature, the absorbance against prepared reagent blank was determined at 765nm with an UV-spectrometer. Total phenolic content of leaves of plant Piper betel was expressed as mg Catechin equivalents (CE)/g.

DPPH Free Radical Scavenging Assay: The free radical scavenging activity of the fractions was measured in vitro by 1, 1- diphenyl- 2-picrylhydrazyl (DPPH) assay. About 0.3mM solution of DPPH in 100% methanol was prepared and 1ml of this solution was added to 3ml of the fraction dissolved in methanol at different concentrations. The mixture was shaken and allowed to was measured at 517nm using a shimadzu spectrometer. The percentage scavenging inhibition was determined and was compared with that of ascorbic acid (AA), which was used as the standard 64.

Preparation of extract: 10g of the dried powdered leaves from plants *Piper betel* were taken separately in a paper cone and placed into Soxhlet apparatus. 100ml of methanol a polar solvent was taken in the round bottom flak attached to the Soxhlet apparatus. A condenser was attached to this set up. The temperature was set in the range of 25^o - 30^oC. Methanol gets vaporized and rises up to the condenser where it condenser where it condenses back into liquid. This liquid falls back into the round bottom flask. This process was continued till all the compounds that can be extracted from the plant by methanol gets extracted and finally only clear liquid of methanol falling into the round bottom flask. The extract got from the above process was evaporated overnight stored in screw cap vials.

DPPH Radical Scavenging Activity: DPPH scavenging activity of the plant extract was carried out. DPPH chemical is brought from Sigma Aldrich, USA.

The stock solution was prepared by dissolving plant extract with methanol at concentration of 1mg/ml. From this stock solution different concentrations were prepared 20, 40, 60, 80, 100 mg/ml. About 0.3mM solution of DPPH in 100% methanol was prepared and 2.5ml of this solution was added in different concentrations of plant extract which was prepared from stock solution.

The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting was measured at 517nm UV-Visible spectrophotometer. Blank was prepared without the addition of DPPH. Absorbance of plant extract is compared with standard solution concentrations which is ascorbic acid.

Percentage of DPPH Scavenging Activity determined as follows:

$$\% \text{ DPPH radical scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

RESULTS AND DISCUSSION:

Total Phenolic Content: The phenolic content of leaf extract of *Piper betel* was measured by Folin-Ciocalteu assay method (**table 1**). The absorbance of sample was measured at 765nm using a UV-Spectrophotometer and was to be 1.894 Abs (**fig. 1**). The phenolic content calculated from calibration curve of catechin was found to be 0.110 mg CE/g.

TABLE 1: ABSORBANCE OF CATECHIN AT 765 NM

Conc. (ug/ml)	Absorbance at 765 nm	Mean absorbance ± SD
20	0.122	0.122±0.002
	0.121	
	0.124	
30	0.262	0.263± 0.002
	0.263	
	0.265	
40	0.535	0.538±0.003
	0.538	
	0.541	
50	0. 748	0.751±0.002
	0.750	
	0.752	
60	0.972	0.972±0.001
	0.974	
	0.971	
70	1.101	1.102±0.001
	1.102	
	1.104	

TABLE 2: ABSORBANCE OF SAMPLE AT 765 NM

Absorbance	Mean
1.892	1.894±0.002
1.894	
1.896	

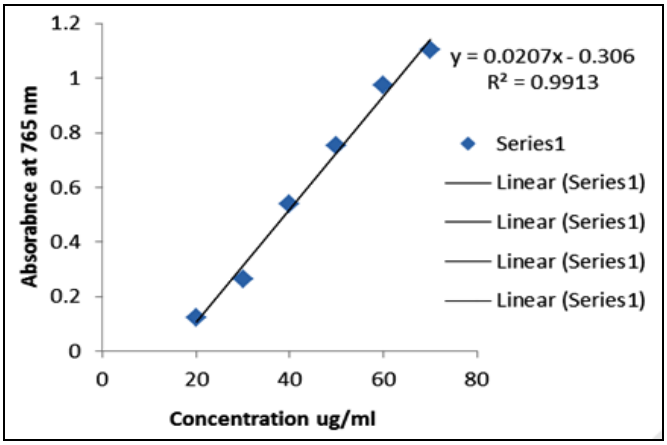

FIG. 1: CALIBRATION CURVE OF CATECHIN STANDARD

Antioxidant Activity: The %DPPH radical scavenging activity is presented in **table 2**. The DPPH radical scavenging activity of methanolic extract of Piper *betel* was found to be highest at 100µl concentration which was 25.2%. Nevertheless, %DPPH scavenging activity of standard ascorbic acid at same concentration was found to be 40.0% (**fig. 4**).

The % DPPH scavenging activity increases with the increasing concentration. The concentration of *Piper betel* needed for 50% inhibition (IC_{50}) was found to be 282.31µg/ml whereas 369.19 µg/ml needed for ascorbic acid.

TABLE 2: ANITOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF PIPER BETEL

Conc. ug/ml	Absorbance at 518 nm		%DPPH Scavenging activity		Mean %DPPH Scavenging activity		IC_{50} Value (ug/ml)	
	Sample	Standard	Sample	Standard	Sample	Standard	Sample	Standard
20	0.645 0.646 0.648	0.415 0.416 0.412	13.9 13.8 13.6	36.9 36.8 37.5	13.94±0.15	37±10.21		
40	0.632 0.635 0.634	0.401 0.403 0.402	15.2 14.9 15.0	38.3 38.1 38.0	14.9±0.15	38.1±0.15		
60	0.611 0.610 0.612	0.398 0.400 0.401	17.3 17.4 17.2	38.6 38.4 38.3	17.4±0.10	38.4±0.15	282.31	369.19
80	0.578 0.575 0.576	0.387 0.386 0.384	20.6 20.9 20.8	39.5 39.4 39.6	20.9±0.15	39.5±0.15		
100	0.534 0.532 0.531	0.383 0.384 0.381	25.0 25.2 25.3	40.1 40.0 40.3	25.2±0.15	40±0.15		

Absorbance of control at 518 nm was 0.784

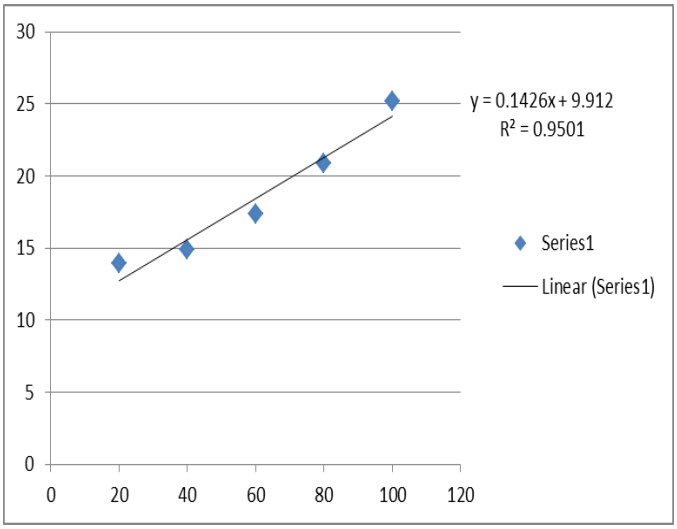

FIG. 2: CALIBRATION CURVE OF SAMPLE

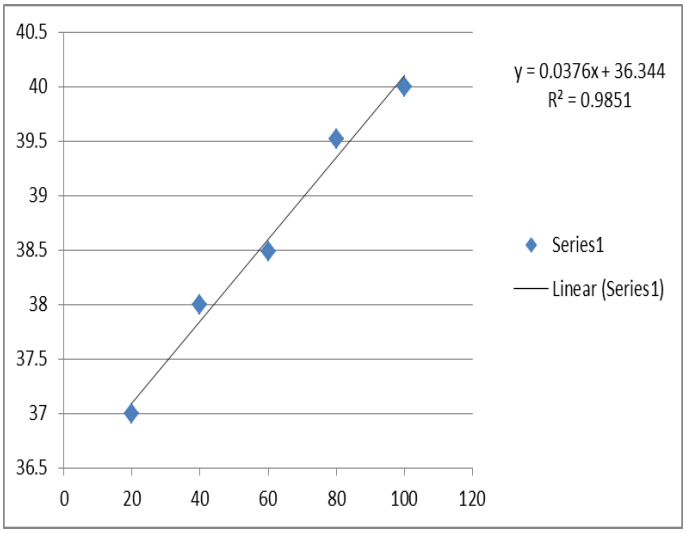

FIG. 3: CALIBRATION CURVE OF STANDARD ASCORBIC ACID

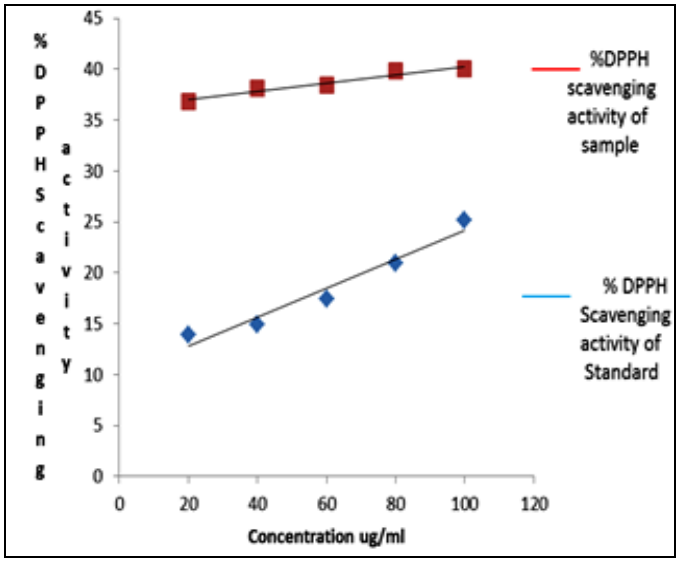

FIG. 4: %DPPH SCAVENGING ACTIVITY OF SAMPLE AND STANDARD

CONCLUSION: In the present investigational studies on important medicinal plant *Piper betel*, it is revealed that the phenolic content of leaf extract of *Piper betel* was measured by Folin- Ciocalteu assay method. The absorbance of sample was measured at 765nm using a UV Spectrophotometer and was to be 1.894 Abs.

The phenolic content calculated from calibration curve of catechin was found to be 0.110 mg CE/g. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, as well as ability to modify the gene expressions.

The DPPH radical scavenging activity of methanolic extract of *Piper betel* was found to be highest at 100µl concentration which was 25.2%. Nevertheless, % DPPH scavenging activity of standard ascorbic acid at same concentration was found to be 40%. The % DPPH scavenging activity increases with the increasing concentration. The concentration of *Piper betel* needed for 50% inhibition (IC_{50}) was found to be 282.31µg/ml whereas 369.19 µg/ml needed for ascorbic acid. DPPH radical scavenging activity of *Piper betel* suggested that it may be used as antioxidant.

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