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Antioxidant Potential from the Acetone Extract Stem and Leaves of Piper Betle

KEYWORDS: Piper betle, DPPH, Reducing power, Antioxidant

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ABSTRACT *Piper betle, commonly called betel leaf is the leaf of a vine belonging to the Piperaceae family, and has been recognised as one of the medicinal plants that has tremendous health benefits. Betel leaf is mostly consumed as betel quid or paan, with or without tobacco and plays a vital role in Indian tradition, customs and rituals. The medicinal properties of betel leaf are well known since time immemorial. According to traditional Ayurvedic medicine, chewing betel leaf is a remedy for bad breath. It acts as aphrodisiac and is known to kill or inhibit the growth of the deadly bacteria that cause typhoid, cholera, tuberculosis, etc. It is used as a stimulant and as an antiseptic.*

SUMMARY

The present study was undertaken to check theantioxidant activity from acetone extract of leaves and stem of P.betle andwas compared with DPPH (2,2- diphenyl picryl hydrazyl) and reducing power assay. Standard used in DPPH and reducing power are Quercetin and ascorbic acid respectively. By employing DPPH radical scavenging and reducing power assay it showed that the acetone extract of leaves showed more antioxidant activity than the stem extracts.

INTRODUCTION

Piper betle Linn (piperaceae)(Karpoori variety) commonly called as vetrilai in Tamil (Harborne, 1984; Lipschitz *et al.,*1943) is a dioecious perennial creeper plant which is distributed in west Bengal, kerala, Malaysia and parts of TamilNadu , India (Vogal, 1989; Becket*et al.,*1997). It has carminative, antibiotic, aphrodisiac, expectorant and immune boosting properties as well as anticancer properties. The leaf has been reported to contain methyl piper betlol, peperol-A, piperol-B and they have also been isolated. It also contains hydroxyl chavicol, eugenol piper betol. The betel oil contains carvacrol, allyl catechol, chavicol, chavibetol, cineole, estragol, p-cymene, caryophyllene, cadinene. The antispasmodic action of betel oil on involuntary muscle tissue, inhibiting excessive peristaltic movements of the intestines at moderate doses. The important ayuvedic formulations of *P.betle*plant are Lokantha Rasa, Puspadhava Rasal, Brhatsarwajwarahara, lanha, laghusutaseknara Rasa, Brhatvisamajwarantaka Rasa .

The consumption of antioxidant-rich foods help to neutralize the free radicals in the body, thus preventing or delaying the oxidative damage of lipids, proteins and nucleic acids (Lim *et al.,* 2007). It has been shown that the antioxidants could reduce mortality rate of cardiovascular disease (Devasagayam *et al.,* 2004; Agoramoorthy *et al.,* 2006) and protect against cancer and other chronic diseases (Anani *et al.,*2000).However, the plant antioxidant compounds that are responsible for reducing the risk of chronic diseases have not been identified (Saeed *et al.,* 2012).

Although the antioxidant defense systems includes both endogenously and exogenously derived compounds, dietary plants based antioxidant have recently received a great attention (Lipschitz *et al.,*1943).Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and a limited toxicity from medicinal plants. In this context, ethno pharmacology plays a significant part in the search for interesting and therapeutically useful plants. In the present study, *P. betle* (Husain *et al.,*1987) and its plant parts were screened to determine their free radical scavenging and antioxidant activities.

MATERIALS AND METHODS

Plant Materials

The plant material of *Piper betle*leaves and stem were collected from in and around Guindy. It was identified using standard books. The leaves and stem were shade dried and crushed into fine powder with electric blender. The powdered sample was sealed in polythene bags and was stored in desiccators until further uses.

Preparation of acetone extract

Dried and powdered betel leaves and stem (500 g) were extractedusing soxhlet with 100% acetone (1:5 W/V) for about 72 hours. The extracts was removed and it was concentrated to dryness in rotary vacuum evaporator below 50° C and stored until needed for the bioassays at -4 °C.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH free radical scavenging activity

The acetone extract of leaves and stem were subjected to *in vitro* antioxidant assay (Yen and Hsieh, 1997). For leaf acetone extracts reaction mixture consisted of 1 mL of 0.1 mM diphenyl-p-picrylhydrazyl radical (DPPH) in methanol and 1 mL of different concentrations (4 µg/mL, 5µg/mL, 6 µg/mL, 7 µg/mL, 8 µg/mL, 9 µg/mL and 10 µg/mL) were prepared. Acetone extracts of stem were prepared and diluted to concentrations (5 µg/mL, 6 µg /mL, 13 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL). 1.0 mL of DPPH and 1.0 mL ofmethanol were used as control. The reaction mix was left in the dark at room temperature for 30 minutes. The OD was measured using spectropho-

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tometer at 517 nm. Quercetin was used as standard. The inhibition percentage was calculated according to the formulae.

Inhibition percentage= A_C-A_S/A_C×100. Where, A_C- Absorbance of Control; A_S- Absorbance of Sample. The 50% inhibitory concentration (IC_{50}) values were calculated by plotting an x, y scatter trendline with regression equation.

Reducing power assay

The reducing power of crude acetone extracts of leaves and stem were determined by the method of Oyaizu (1986). Various concentrations of the plant extracts in 1.0 mL of solvent were mixed with phosphate buffer (2.5 mL) and potassium ferriccyanide (2.5mL) and incubated at 50°C for 20 minutes. Trichloroacetic acid (10%; w/v) 2.5 mL were added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared ferric chloride (0.1%; w/v) solution 0.5 mL. The Absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid with various concentrations was used as the standard reference. Increase in absorbance of the reaction mixture indicates the increase in reducing power.

RESULTS

DPPH radical scavenging activity from the acetone extract of stem and leaves of *Piper betle*have strong scavenging activity. Maximum inhibition activity for leaf extract was observed at 10µg/mL and for stem extract it showed 200µg/mL respectively. Antioxidant activity of leaf extract was comparable to quercetin standard. IC_{50} value of leaf extract less than quercetin, showed more activity.

In *P. betle* stem extracts, the IC_{50} value showed 21.23µg/mL. When compared with leaf and stem extracts, the IC_{50} value concentration of leaf is less than stem this shows the leaf is more potent than stemwhereas the standard (quercetin) showed IC_{50} of 8.65µg/mL respectively (Tables 1&1a); (Figures 1&1a)

Reducing power was assayed from the acetone extract of stem and leaves of *Piper betle* showed increasing activity of Fe^{3+}. Strong reducing powerwere observed in lower concentration of leaf than stem extracts. The concentration ranged from 64-1000 µg/mL. The acetone extract of leaves and stem of *Piper betle* was very potent and the power of the extract increased with quantity of sample. The concentration of extracts of leaves showed 0.631 at 1000µg/mL concentration whereas in stem, it showed 0.513 at 1000µg/mL concentration. The absorbance value of leaf extract is more than stem. When compared with standard ascorbic acid the leaf extract showed less activity.(Tables 2& 2a); (Figures 2& 2a)

DISCUSSION

DPPH free radical scavenging activity

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging (Ames *et al.*, 1993). Recently, phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C, E and carotenoids (Rice-Evans *et al.*,1995, Rice-Evans *et al.*, 1996).

Applications of antioxidants are increasing due to their multiple roles in minimising harmful effects of oxidative stress. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is routinely practiced for the assessment of antiradical properties of different compounds. A detailed literature survey had revealed use of different materials and methods for DPPH assay by different investigators resulted in variation in the values of reference standards and measured parameters of new antioxidants (Mishra *et al.*, 2012).

According to Hussain *et al.*,1987. DPPH radical scavenging activity from different extracts namely ethanol, chloroform, ethyl acetate of stem and leaves all the extracts investigated exhibited activity (more than 40%). In leaves (ethanol extract) of *Piper betle* displayed highest activities as removal of stable radical DPPH and lowest activity were found in chloroform extract of stem . According to Wahyu *et al.*,2011 DPPH scavenging activity (IC_{50}) value on *Piper betle, Catharanthus roseus, Dendrophtoe petandra, Curcuma manga* extracts showed 5.49µg/mL, 102.96 µg/mL, 4.74 µg/mL, 2.77 µg/mL. *P. betle* and *D.petandra* extracts are more active antioxidant compared to *C. roseus* and *C. manga* respectively.

In the present study the leaf extract showed maximum inhibition activity than stem at 10µg/mL concentration. In stem extracts the IC_{50} value showed 21.23µg/mL. when compared with two parts of the extract the IC_{50} value concentration of leaf is less than stem. (Table 1& Figure 1)whereas the standard has given IC_{50} 8.65µg/mL respectively (Table 1a& Figure1a).

Reducing power assay

Acetone extracts of leaf and stem of *P. betle* showed increasing reducing activity ofFe^{3+}strong reducing power were observed in leaf than stem extracts among various concentration (Table 2). The reducing power of any substance is dependent on the presence of reductants which show antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The presence of reductants or antioxidants in acetone extracts of leaf and stem caused the reduction of ferric (Fe^{3+})/ ferriccyanide complex, lused in this method to the ferrous form.

According to Bhuvaneswari *et al.,* 2014 from various cultivars of leaves of *P.betle* using methanol showed greater reduction of ferrous to ferric ions in the cultivarvellai. Similar observations were made by Kim *et al.,*2010 in the ethanol extracts of leaves at higher concentration.In the present study,the leaf extract at a concentration of1000µg/mL showed absorbance of 0.631whereas in stem extract at the same concentration of 1000µg/mL it showeda absorbance of 0.513 respectively.(Table 2) &(Figure 2).When compared with standard ascorbic acid the leaf and stem extracts showed less activity.(Table 2a) &(Figure 2a). However the leaf extract showed more activity than the stem extract.

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Table 1. Comparative analysis of DPPH antioxidant activity from acetone extracts of stem and leaves of *P. betle*

Sample	Concentration of extract µg/mL	Inhibition percentage (IC_{50})	IC_{50} value (µg/mL)
Leaf	4	41.15±2.88	4.31
	5	57.99±4.05	
	6	65.45±4.58	
	7	96.37±1.20	
	8	97.01±6.79	
	9	99.78±6.98	
	10	99.82±6.98	
Stem	5	15.65±1.09	21.23
	6	20.12±1.40	
	13	48.45±3.39	
	25	56.86±3.98	
	50	64.72±4.53	
	100	78.43±5.49	
	200	89.06±6.23	

Table 1a. DPPH antioxidant activity using the Standard (Quercetin)

Sample	Concentration of extract µg/mL	Inhibition percentage (%)	IC_{50} value (µg/mL)
Quercetin	3	35.32±2.47	8.65
	6	43.26±3.02	
	9	52.56±3.67	
	12	65.45±4.58	
	15	68.66±4.80	
	18	79.86±5.59	
	21	93.98±6.29	

Table 2.Comparative analysis of Fe^{3+} reducing power activity from acetone extracts of stem and leaves of *P. betle*

S.NO	Concentration of extract µg/mL	Reducing Power	
		Leaf	Stem
1.	64	0.246±0.017	0.042±0.016
2.	125	0.268±0.018	0.258±0.018
3.	250	0.364±0.025	0.361±0.021
4.	500	0.432±0.030	0.382±0.026
5.	1000	0.631±0.044	0.513±0.035

Table 2a.Fe^{3+} reducing power activityusing the standard (Ascorbic acid)

Sample	Concentration of extract µg/mL	Absorbance at 700 nm
Ascorbic acid	64	0.328±0.022
	125	0.480± 0.033
	250	0.530±0.037
	500	0.620±0.043
	1000	0.780±0.054

Figure 1. Comparative analysis of DPPH antioxidant activity from acetone extracts of stem and leaves of *P. betle*

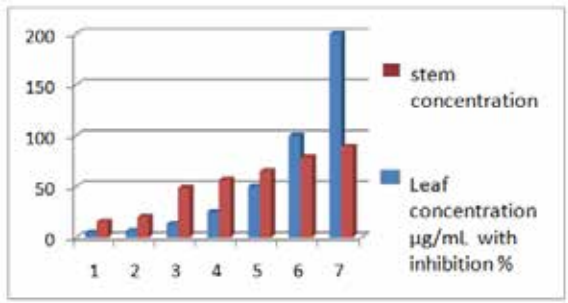


Figure 1a. DPPH antioxidant activity using the Standard (Quercetin)

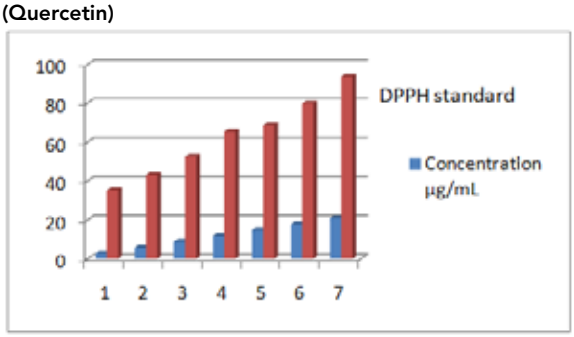


Figure 2. Comparative analysis of Fe^{3+} reducing power activity from acetone extracts of stem and leaves of *P. betle*

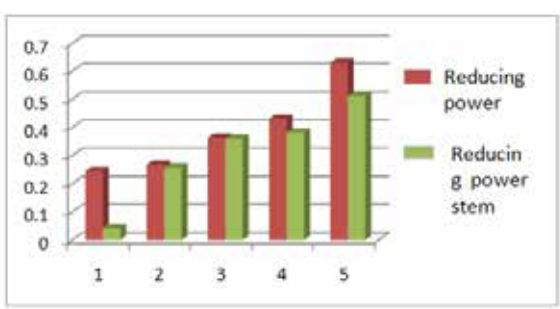
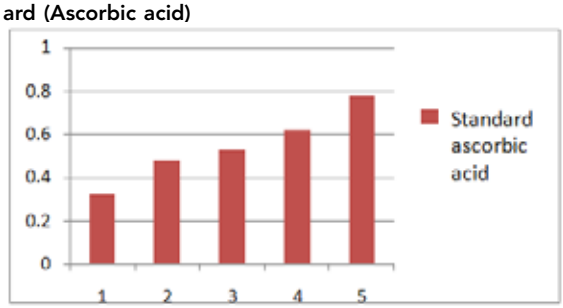


Figure 2a.Fe^{3+} reducing power activity using the standard (Ascorbic acid)



CONCLUSION

P. betle is a tropical creeper distributed in parts of Tamil-Nadu. It has a prominent role in ancient medicine such as siddha and Ayurveda. It is mixed with the people of South India in various rituals and customs such as marriages and other ceremonies. So the actual role of *P.betle* had to be studied so as to bring back the traditional use of

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this herb. In the present study the leaf and stem extracts showed potent antioxidant activity comparable with that of control of which the leaf extracts showed better results at low concentration.

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