Antioxidant activity of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori

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ABSTRACT

Introduction: Ingredients of Indian cuisine are well known for their antioxidant properties which can help prevent many diseases. The Betel (*Piper betel* L.) is the leaf of a vine belonging to the Piperaceae family; it is figured out both as a mild stimulant and for its medicinal properties. The betel plant originated from South and South East Asia. The leaves of *Piper betel* L. have been traditionally known for its various therapeutic uses. *Piper betel* L. Cv. Kapoori is a local cultivar obtained from Chintalapudi, Guntur district of Andhra Pradesh, India, but their antioxidant properties have not been investigated. **Methods:** Organic solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori was determined by DPPH free radical scavenging activity, reducing power by $FeCl_3$, nitric oxide free radical scavenging activity, super oxide scavenging activity.Also used specific standard for each test. **Results:** Solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori were compared with the light of respective standard drugs and the extracts were found to be equipotent with the standards in all the tested methods. **Conclusion:** Presence of phenols and phenolics (Chavicol, Chavibetol, Chavibetol acetate and Eugenol) in the Mokkathotapapada leaves of *Piper betel* L. may be credulous to be responsible for its antioxidant activity.

Keywords: Antioxidant; DPPH; $FeCl_3$; Nitric oxide; Superoxide and Phenolic compounds.

INTRODUCTION

Oxidative damage in the human body plays an important causal role in disease initiation and progression.[1] Damage from reactive oxygen species (ROS) including free radicals has been linked to some neurodegenerative disorders (Alzheimer's disease and Parkinson's)[2] and cancers.[3] ROS include free radicals such as superoxide anion radicals (O2•−), hydroxyl radicals (OH•) and non-free-radical species such as H_2O_2 and singled oxygen ($1O_2$). These molecules are exacerbating factors in cellular injury and aging process.[4,5] Recently, ROS have also been shown to play a critical role in the development of acute experimental gastric lesions. ROS also actuate some oxidative damage to biomolecules such as lipids, nucleic acids, proteins and carbohydrates. Wreckage of these biomolecules causes aging, cancer, and many other diseases.[6] As a result, ROS have been implicated in more than one hundred diseases, including malaria, heart disease, stroke, arteriosclerosis, diabetes, cancer and gastric ulcers.[7,8,9] The human body has inherent mechanisms to deflate free radical injury by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, and catalase. Exogenous free radical scavengers include vitamin E and vitamin C.[10,11] These vitamins protect the human body and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods.[12,13] Antioxidant defense systems, including antioxidant enzymes which are found in foods and drugs are crucial in the prevention of the toxic ROS effects.[14,15] Hence, the search for exogenous antioxidants is continued. The most commonly used antioxidants at present are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tert-butylhydroquinone.[16] However, BHA and BHT have been suspected of being

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responsible for liver damage and carcinogenesis.[17] Therefore, the development and utilization of more effective antioxidants is desired.[18] Much attention has been spotlighted on the antioxidative compounds present in plants because of safety concerns associated with synthetic antioxidants.[19]

The Betel (*Piper betel* L.) is the leaf of a vine belonging to the Piperaceae family; it is figured out both as a mild stimulant and for its medicinal properties (WHO). The betel plant is an evergreen perennial creeper, with glossy heart-shaped leaves and white catkin. The betel plant originated from South and South East Asia. The leaves of *Piper betel* L. have been traditionally known for its various therapeutic uses: stimulant, antiseptic, breath freshener,[20,21,22] antitussive, carminative, astringent (juice of leaves with oil), expectorant, sialagogue, stomachic, febrifuge and aphrodisiac, relieves gastrointestinal disorders, to help preserve the teeth and a prophylactic against stomach complaints, carminative, anti-platelet aggravating factory, anti-photosensitizer**,** hepatoprotective, anti-leishmaniasis, antihyperglycemic,[23] neuroprotective, antibacterial,[24] antifungal, antihistaminic and anti-ulcer. The present study focused on evaluation of in vitro antioxidant activity of solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori a local cultivar.

MATERIALS AND METHODS

Plant material collection

The Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori local cultivar were collected from Chintalapudi, Guntur district of Andhra Pradesh, India, in January 2011. A voucher specimen (ANU/PB/2011–001) was deposited at Department of Botany, Acharya Nagarjuna University, Nagarjuna nagar, Guntur, for future reference.

Preparation of the extract

The air dried plant material was milled to get a coarse powder, extracted separately by continuous hot percolation process using a soxhlet apparatus with different solvents such as ethanol, methanol, chloroform and petroleum ether. After extraction, the extracts were dried under reduced pressure at 40°C by using a rotary evaporator. The dried extracts were subjected to various chemical tests to detect the presence of different phyto constituents.[25]

Chemicals and instruments

DPPH (1,1-Diphenyl-2-picryl-hydrazil), 0.1 ml DMSO, Ascorbic acid, 1% Potassium ferricyanide, 10% Trichloro acetic acid, distilled water, 0.1% ferric chloride, Sodium nitro prusside, phosphate buffer, methanol, sulfanilic acid reagent (0.33% in 20% glacial acetic acid, naphthyl ethylene diamine dihydrochloride, 50 mM KH_2PO_4 -KOH pH 7.4, 1 mM EDTA. 100 mM hypoxanthine, xanthine oxidase, phosphate buffer, saline, NBT: 25 mg of nitro-blue tetrazolium was dissolved in 25 ml of Dimehtyl sulfoxide to give concentration of 1 mg/mL. All chemicals used were of analytical grade. Glassware, solvent extraction apparatus and UVvisible spectrophotometer.

In-vitro antioxidant activity

DPPH (1,1-Diphenyl-2-picryl-hydrazil) free radical scavenging activity

The stable free radical scavenging effect of different solvent extracts of Mokkathotapapada leaves of *Piper betel* L.Cv. Kapoori were assessed by the discoloration of the respective extract solution of DPPH according to the Blois' method.[26] Test samples (20–100 µg/ml) were dissolved in 0.1 ml DMSO and then added to 0.1 ml of 0.1 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand for 10 minutes at room temperature (30°C) in the dark. The absorbance at 517 nm by DPPH was measured spectrophotometrically at 517 nm. Ascorbic acid was used as a positive control. The degree of discoloration indicates the scavenging potency of the extract. The potency to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = (A0 - A1/\ A0) \times 100$$

Wherein A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of solvent extract.[6,18]

Reducing power by $FeCl_3$

The assay system used to evaluate the reducing power potential of different solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori, a local cultivar is by using $FeCl_3$. Finally the absorbance of resulting solution was measured at 700 nm.

Nitric oxide radical inhibition assay

Sodium nitroprusside in aqueous solution at physiological pH automatically generate nitric oxide, which interacts with oxygen to produce nitrite ions. These ions can be estimated by the use of Griess Illosvoy reaction.[27]

The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

% antiradical activity = Control absorbance – Sample absorbance/Control absorbance × 100.

Scavenging of superoxide anion radical[28,29]

The superoxide anion radical was generated *in vitro* with hypoxanthine and xanthine oxidase. Decrease in the absorbance of reaction mixture indicates an increase in superoxide anion scavenging activity. The results are given as the percentage inhibition of NBT reduction rate with respect to the reaction mixture without solvent extract (saline only).

RESULTS

Since the DPPH test can accommodate a large number of samples in a short period of time and it is sensitive enough to detect natural compounds at low concentrations, it was used in the present study for primary screening of organic solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori for their free radical-scavenging activity. This assay furnishes information on the reactivity of test compounds with a stable free radical. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy (deep violet color). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes; resulting in stoichiometric decolorization with respect to the number of electrons taken up.[26] The solvent extracts exhibited DPPH free radical scavenging activity in a concentration-dependent manner (Table. 1).

Among the solvent extracts, ethanol extract exhibited very good activity followed by methanol, chloroform and petroleum ether extracts. Increasing concentrations of ethanol extracts were taken and the following percent inhibition was observed subsequently at 20 µg/ml (69.16 ± 1.25), 40 µg/ml (75.47 ± 2.91), 60 µg/ml (82.26 ± 1.32), 80 µg/ml (90.74 ± 1.99) and 100 µg/ml (94.61 ± 3.13) with Ascorbic acid equaling 100 µM (95.33 ± 1.19). Significant DPPH radical scavenging activity was evident at all tested concentrations of the different solvent extract.

For the measurement of the reductive ability, we investigated the Fe + 3®Fe + 2 transformations in the presence of Mokkathotapapada leaf extracts of *Piper betel* L. cv. Kapoori, a local cultivar using the method of Reducing power by $FeCl_3$.[30] The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The antioxidant activity, the reducing power of *Piper betel* L. Cv. Kapoori Mokkathotapapada leaf extracts increase with increasing concentration, as shown in Table.2. Increased absorbance of reaction mixture indicates increased reducing power.

Table 1 Measurement of DPPH free radical scavenging activity of solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori, a local cultivar

Test material concentration µg/ml	Percentage decrement of absorbance at 517 nm in different solvent extracts			
	Ethanol	Chloroform	Methanol	Petroleum ether
20	69.16 ± 1.25	47.58 ± 9.48	64.74 ± 1.99	44.67 ± 1.46
40	75.47 ± 2.91	54.39 ± 1.44	70.01 ± 2.37	47.25 ± 3.52
60	82.26 ± 1.32	68.24 ± 7.06	76.42 ± 1.25	60.43 ± 5.23
80	90.74 ± 1.99	72.67 ± 4.32	85.83 ± 2.89	66.52 ± 2.92
100	94.61 ± 3.13	76.23 ± 6.52	92.33 ± 2.23	72.02 ± 3.84
Std*	95.33 ± 1.19			

Std - Standard Ascorbic acid (100 µM). Each value represents the mean ± S.D. of four experiments, performed in duplicate.*

Table 2 Reducing power by $FeCl_3$ method for solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori, a local cultivar

Concentration µg/ml	Absorbance at 700nm in reaction mixtures				
	Ethanol	Chloroform	Methanol	Petroleum ether	Std*
10	0.4403 ± 0.23	0.4023 ± 0.35	0.4213 ± 0.12	0.3868 ± 0.54	0.5071 ± 0.24
20	0.4831 ± 0.42	0.4465 ± 0.87	0.4632 ± 0.63	0.4223 ± 0.22	0.5823 ± 0.31
30	0.5570 ± 0.12	0.5160 ± 0.24	0.5315 ± 0.32	0.4943 ± 0.12	0.6068 ± 0.28
40	0.8087 ± 0.78	0.7478 ± 0.68	0.7856 ± 0.24	0.7078 ± 0.85	0.7535 ± 0.35
50	0.8092 ± 0.65	0.7393 ± 0.54	0.7889 ± 0.28	0.7154 ± 0.64	0.8562 ± 0.29

Std - Standard, Butylated hydroxytoluene (BHT). Each value represents the mean ± S.D. of four experiments, performed in duplicate.*

Table 3 In vitro antioxidant activity of solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori, a local cultivar against Nitric oxide method

Test material	IC_{50} values ± S.E.* (µg/ml)
Ethanol extract	424.64 ± 9.48
Chloroform extract	418.54 ± 6.42
Methanol extract	422.85 ± 8.79
Petroleum ether extract	374.34 ± 5.62
Std*	472.94 ± 10.99

Std - Standard, Rutin. *. Average of 10 determinations.*

Among the solvent extracts and standard tested for antioxidant activity using the nitric oxide radical inhibition method, the ethanol extract showed better activity followed by methanol, chloroform and petroleum ether extracts. The ethanol extract showed the IC_{50} value of 424.64 ± 9.48 µg/ml, where as the standard, Rutin showed 472.94 ± 10.99 µg/ml (Table 3).

The solvent extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori scavenges superoxide radical and thus inhibits NBT reduction rate. Table 4 illustrates increase scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori extracts.

DISCUSSION

Chronic damage associated with the development of aging can generate destructive oxidants and oxygen free radicals, which are very toxic to tissues resulting in tissue necrosis and the cellular damage. Cellular mechanisms and external factors involved in the production of oxidative stress include the inflammatory response, free radical leak from mitochondria, auto-oxidation of catecholamines, xanthine oxidase activation, prooxidant activities of toxins such as CCl_4 and exposure to ionizing radiation.[31] Aerobic cells are endowed with extensive antioxidant defense mechanisms including both low molecular weight scavengers, such as cysteine, reduced glutathione, ascorbic acid and enzymatic systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Red) and glucose-6-phosphate dehydrogenase (G6PD), which counteract the damaging effects of reactive oxygen species.[32] However, when the balance between these reactive species and antioxidants are altered, a state of oxidative stress results, possibly leading to permanent cellular damage. Our results demonstrate that MSF exhibited an interesting antioxidant activity in cell-free systems. It was able to quench the synthetic DPPH radical and exhibited a SOD-like effect,inhibiting O2•− formation in a dose-dependent manner.[31] The results obtained showed that the solvent extracts exhibited DPPH free radical scavenging activity in a concentration-dependent manner. The IC_{50} values observed were found to be slightly lower than that of standard rutin in nitric oxide radical inhibition assay. The results clearly indicated that both ethanol and methanol extracts were found to be more effective in scavenging the DPPH free radical when compared to the nitric oxide radical. All the experimental results demonstrated that the solvent extracts were effective in inhibiting the oxidative process at different concentrations.

CONCLUSION

On the basis of these experiments, it could be concluded that the ethanolic and methanolic extracts of Mokkathotapapada leaves of *Piper betel* Linn. Cv. Kapoori can act as potent primary and secondary antioxidants compared to that of chloroform and petroleum ether extracts. It scavenges free radicals and therefore inhibits the lipid per-oxidation and may have beneficial effect on prevention of diseases, where reactive oxygen species are involved. Antioxidant properties of its phenols and phenolic compounds can be at the origin of these effects, but further in vivo experiments are planned to verify the relationship between chemical composition and antioxidant activity.

Table 4 Super oxide anion radical scavenging activity of extracts of Mokkathotapapada leaves of *Piper betel* L. Cv. Kapoori, a local cultivar

Concentration µg/ml	Absorbance at 560 nm in reaction mixtures				
	Ethanol	Chloroform	Methanol	Petroleum ether	Std*
10	29.01 ± 3.16	25.32 ± 5.62	27.59 ± 4.86	23.51 ± 6.43	35.72 ± 7.43
20	51.77 ± 7.70	46.76 ± 3.54	49.50 ± 5.60	42.61 ± 6.57	49.44 ± 4.05
30	58.01 ± 5.50	54.81 ± 7.31	57.83 ± 6.34	50.67 ± 4.82	57.12 ± 12.5
40	61.50 ± 4.72	57.51 ± 6.42	60.24 ± 4.26	52.46 ± 5.21	69.28 ± 4.96
50	67.24 ± 3.57	63.15 ± 7.81	65.26 ± 3.48	60.54 ± 4.71	78.82 ± 9.89

Std - Standard Ascorbic acid. Each value represents the mean ± S.D. of four experiments, performed in duplicate.*

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