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Research Paper :

Antioxidant activity in piper betel and nicotiania tabaum

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ABSTRACT

The aqueous extract of *Piper betel* L. and *Nicotiania tabaum* were studied for antioxidant activity on different *in vitro* models namely 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) assay, nitric oxide assay and trichloroacetic acid based reducing power method. Ascorbic acid was also evaluated for comparison. The extracts showed free radical scavenging property in the tested models. *Piper betel* L. showed 98.06% inhibition of DPPH at 1000 µg and its activity at 500 µg (*i.e.* 94.35%)was comparable to that of ascorbic acid at 30µg (93.58%). While the maximum percentage inhibition by *Piper betel* L. and *Nicotiania tabaum* in the nitric oxide model was found to be only 62.14% and 33.36%, respectively, the activity of 1024 µg of *Nicotiania tabaum* and 128µg of *Piper betel* L. of compares favorably with that of 30 µg ascorbic acid. *Piper betel* L. showed high reductive ability. This study demonstrates the higher anti oxidant activity is present in the leaves of *Piper betel* L. when compared to *Nicotiania tabaum.*

Key words : Antioxidant, *Piper betel* L. and Nicotiania tabaum, Free radicals, Reductive ability

The deep green heart shaped leaves of Betel vine are popularly known as "PAAN" in India. The scientific name of Betel vine is *Piper betel* L. It belongs to the family Piperaceae, the black pepper family. Significance of the leaves has been explained in relation to every sphere of human life including social, cultural, religious, and even day to day life (Guha, 2006)

The *Piper betel* plant is found widely growing in the tropical humid climate of South East Asia and its leaves, with a strong pungent and aromatic flavour are widely consumed as a mouth freshener. The leaves have digestive and pancreatic lipase stimulant activities. Gastro protective properties of leaf extract on experimentally induced gastric lesions is reported (Bhattacharya, 2007)

A material present in the betel leaf is Chavicol (Kochhar, 1999) Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils, and abscesses, conjunctivitis, Constipation, headache, hysteria, swelling of gums, rheumatism, abrasion, cuts, injuries, etc. (Guha, 2006) Chewing tobacco is smokeless tobacco, where ever smokeless tobacco products have been studied, they have found increase the risk of oral cancer. In India, known to increase of cancer belonging to upper aero- digestive tract. Also less fatal diseases such as recession of gums and oral mucosal lesions many of which are pre cancerous (Gupta, 2001)

Tobacco plant contains over 2200 compounds of which nitrogenous compounds comprise 30%. Nicotine is one important alkaloid contained in tobacco leaves. The primary commercial source of nicotine is by extraction from dried leaves of tobacco plant (*Nicotiania tabaum* and *Nicotiania rustica*) (Cooper, 2006). Reactive oxygen species are an entire class of highly reactive molecules derived from the metabolism of oxygen (Farrukh Aquil, 2006)

Reactive oxygen species are generated in plant cells during normal metabolic processes. The photosynthetic electron transport is the major source of active oxygen in the plant tissues having potential to generate singlet oxygen and super oxide (O_2-) that is the production of active oxygen (Arora, 2002)

Plants possess very efficient scavenging systems for reactive oxygen species that protects them from destructive oxidative reactions (Arora, 2002). The antioxidant defense system of the plant comprises a variety of antioxidant molecules and enzymes (Arora, 2002). There fore this study was entitled to accesses the Free radical scavenging activity of fresh betel leaves extracts and dried tobacco leaves extract was experimented for free radical scavenging activity, comparison of properties of betel leaves and dried tobacco leaves.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical grade and obtained from Ranbaxy Fine Chemicals and SD Fine Chem. Ltd., Mumbai, India. Ascorbic acid was obtained from Merck Ltd., Mumbai and 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) was obtained from Sigma chemicals, USA. The other chemicals used were N-(1-naphthyl) ethylene Diamine Dihydrochloride (NED), trichloro acetic acid (TCA), Sodium nitoprusside, sulphanilamide, O-phosphoric acid, sodium chloride

(NaCl), Ferrous sulphate ($FeSO_4$), Ferric Chloride ($FeCl_3$), disodium hydrogen-orthophosphate, potassium dihydrogen phosphate and potassium ferricyanide.

Plant Material, the leaves of Betel and Tobacco leaves were purchased from the local market in May 2007 and authenticated by a botanist by comparision with the preserved samples in the herbarium of PVP arts and Science College, Loni.

Plant extracts:

The plant parts were shade dried, powdered and extracted individually with distilled water by continuous boiling, using Soxhlet apparatus. The extracts were filtered and concentrated in vacuo and kept in a vacuum desicator for complete removal of the water. Aqueous extract of *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) were obtained in the yield of 9.5 %(w/w) and 14.4% (w/w), respectively.

DPPH radical scavenging activity (Shirwaikar, 2004):

DPPH scavenging activity was measured by the spectrophotometric method (Cotelle,1996) To 3 ml of an Methanolic solution of DPPH (200 µM), 0.05 ml of test extracts/ascorbic acid (30 µg) dissolved in ethanol were added. Test extracts were prepared in different concentrations (10-1000µg). The solutions were incubated at 37^{0}C for 30 min, absorbance measured at 517 nm using spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with the extract) using the formula

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Nitric oxide scavenging activity (Sreejayan, 1997):

Nitric oxide radicals were generated from sodium nitroprusside solution at physiological pH. 1 ml of sodium nitroprusside (10mM) was mixed with 1 ml of the test extracts / ascorbic acid (30 µg) in phosphate buffer (pH 7.4). The test extracts were prepared in different concentrations (10-1000µg). The mixture was incubated at 25^{0}C for 150 mins. To 1.5 ml of the incubated solution, 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition calculated.

Reductive ability (Jay Prakash, 2001):

Reducing power of the test extracts was determined based on the ability of antioxidants to form coloured complex with potassium ferricyanide, TCA and $FeCl_3$. 1 ml of the test extracts (10-1000 µg) / ascorbic acid (30 g) in ethanol were mixed with 2.5 ml potassium

Ferricyanide (1%) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50^0 C for 20 min. 2.5 ml TCA (10%) were added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml water and 0.5 ml $FeCl_3$ (0.1%). Absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

Piper betel L. (PB) and *Nicotiania tabaum* (NT) in graded concentrations were tested for their antioxidant activity in three different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in the models studied. The maximum percentage inhibition of DPPH by *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) was 98.06 %and 58.43%, respectively at 1000 mg concentration (Table 1). Standard ascorbic acid showed 93.58% inhibition of the DPPH radical at 20 µg. In the nitric oxide model, the maximum

Table 1 : DPPH Assay: Free radical scavenging activity of *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) leaves

Sr. No.	Concentration (µg)	Percentage inhibition	
		Piper betel L.	*Nicotiania tabaum* (NT)
1.	10	13.01±0.042	2.47±0.20
2.	16	13.52±0.013	3.20±0.10
3.	32	24.92±0.012	5.17±0.01
4.	62	44.64±0.42	9.66±0.08
5.	125	64.23±0.62	14.33±0.36
6.	250	84.42±0.063	29.51±0.66
7.	500	94.35±0.064	44.91±0.11
8.	1000	98.06±0.051	58.43±0.44

% inhibition of DPPH due to ascorbic acid (30µg) is 93.58
Values are mean of triplicate determinations (Mean ± SEM)

percentage of inhibition of nitric oxide radicals of *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) was 62.14% and 33.36%, respectively (Table 2). However, ascorbic acid at 30 µg caused only32.88% inhibition which is similar to the inhibition of 128 µg of *Piper betel* L. (PB) and 1024 µg of *Nicotiania tabaum* (NT).The reducing power of *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) was also dependent on the concentration as shown in Table 3. The maximum absorbance of *Piper betel* L. (PB) at 500 µg compares favorably with ascorbic acid. On a comparative basis *Piper betel* L. (PB) was better at quenching DPPH, nitric oxide radicals and also the

Table 2 : Nitric oxide scavenging activity of *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) leaves

Sr. No.	Concentration (µg)	Percentage inhibition	
		Piper betel L.	*Nicotiania tabaum* (NT)
1.	08	7.42±0.022	3.61±0.016
2.	16	9.71±0.062	5.92±0.032
3.	32	11.33±0.084	6.83±0.041
4.	64	28.76±0.045	10.66±0.072
5.	128	33.63±0.021	11.76±0.78
6.	256	44.14±0.068	15.20±0.032
7.	512	53.84±0.044	23.02±0.007
8.	1024	62.14±0.037	33.36±0.043

% inhibition of Nitric oxide due to ascorbic acid (30µg) is 32 .88
Values are mean of triplicate determinations (Mean ± SEM)

Table 3 : Reducing power/reducing ability of *Piper betel* L. (PB) and *Nicotiania tabaum* (NT) leaves

Sr. No.	Concentration (µg)	Percentage inhibition	
		Piper betel L.	*Nicotiania tabaum* (NT)
1.	10	0.176±0.0002	0.118±0.0001
2.	16	0.186±0.0005	0.123±0.0004
3.	32	0.286±0.0003	0.135±0.0002
4.	62	0.358±0.0009	0.143±0.0001
5.	125	0.468±0.0003	0.154±0.0003
6.	250	0.578±0.0002	0.165±0.0003
7.	500	0.678±0.0005	0.175±0.0006
8.	1000	0.762±0.0004	0.188±0.0002

% inhibition of reducing power/reducing ability due to ascorbic acid (30µg) is 0.602
Values are mean of triplicate determinations (Mean ± SEM)

reductive ability was far greater better than *Nicotiania tabaum* (NT).

DPPH is relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichometrically depending on the number of electrons taken up (Blois, 2001). From the results it may be postulated that both the plant extracts have hydrogen donors thus scavenging the free radical DPPH. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, alzheimer's, and arthritis (Sainani, 1997). Oxygen reacts with the excess Nitric Oxide to generate nitrite and peroxy nitrite anions, which act as free radicals. In the present study the nitrite produced by incubation of solutions of sodium nitoprusside in standard phosphate buffer at 25^{0}C was reduced by *Piper betel* L. (PB) and *Nicotiania tabaum* (NT). This may be due to the antioxidant priciples in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that *Piper betel* L. (PB) have caused a greater inhibition than ascorbic acid which has shown only 62.14% inhibition of NO. It is known that ascorbic acid acts as a pro oxidant *in vitro* in the presence of transition metal ions such as iron (Parthasarathy, 1999).This could explain its major antioxidant effect in this method. This effect is however unlikely to be important in vivo where metal ions are sequestered and other reductants are present. An increase in absorbance in the reducing power method implies that extracts are capable of donating hydrogen atoms in a dose dependent manner. In the reducing power, Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim, 2001). The reducing power was determined according to the method of (Jay Prakash, 2001). In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in the reductive ability.

The extract of *Piper betel* L. (PB) exhibited a good reducing power at 0.762 to 0.176 mg ml^{-1} which was compared with *Nicotiania tabaum* (NT). Because the reductive ability of the extract was significantly compared and the extract showed reductive potential and could serve as electron donor, terminating the radical chain reaction.

It is evident from the results that *Piper betel* L. (PB) has a higher antioxidant potential than *Nicotiania tabaum* (NT). Several flavonoids apart from chlorogenic acid have been reported from *Piper betel* L. (PB). These could be the antioxidant principles mediating the anti inflammatory activity was not reported earlier for this herb. The results thus support the folklore claim of the usefulness of the leaves in auspicious conditions. The radical scavenging potential of *Piper betel* L. is explained by the presence of chavicol compound in the leaves. It also correlates with the anti mutagenic activity reported earlier, as chemicals that scavenge free radicals prevent DNA strand breaks(Kochhar, 1999). Demonstration of the antioxidant potential of the leaves, especially in view of the presence of a rich spectrum of bio active molecules

of therapeutic significance, makes us to realize that bio activity of fractionation phytomolecules from the leaves of *Piper betel* L. should be analyzed.

Conclusion:

This study enlightens upon that betel leaves have free radical scavenging activity as compared to tobacco leaves. It was observed that the antioxidant activity of Betel leaves was masked by the free radicals generated due to tobacco extract.

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