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Food Chemistry 88 (2004) 219–224
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of *Piper betle* L. leaf extract in vitro

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Received 5 September 2003; received in revised form 8 January 2004; accepted 8 January 2004

Abstract

Piper betle leaves are used as masticatory in Asian countries. In the present study, antioxidant activities of aqueous extracts of three local varieties of *P. betle* leaves were evaluated by several in vitro systems, e.g. DPPH radical scavenging activity, superoxide radical scavenging activity in a riboflavin/light/NBT system, hydroxyl radical scavenging activity and inhibition of lipid peroxidation induced by $FeSO_4$ in egg yolk. Total antioxidant activity was measured by the reduction of Mo(VI) to Mo(V), by the extract, and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The extracts were found to have different levels of antioxidant activity in the systems tested. The data indicate that the antioxidant activities differed in varieties. The antioxidant activities of the three varieties are in the order Kauri > Ghanagete > Bagerhati. All three varieties of *P. betle* have more potential to prevent lipid peroxidation than does tea. Total antioxidant capacity (equivalent to gallic acid) of Kauri is also higher than tea. Total phenolic concentration, expressed as gallic acid equivalents showed correlation with the antioxidant activity, being highest in Kauri and lowest in Bagerhati.

Keywords: *Piper betle* leaf; Antioxidant activity

1. Introduction

The oxygen molecule is very stable in the ground state, but it is changed into O_2^-. (superoxide radical), H_2O_2 (hydrogen peroxide), $^{\cdot}OH$(hydroxyl radical) by environmental pollutants, radiolysis, UV and the reduction pathway to H_2O in living tissues. These oxygen radicals induce some oxidative damage to biomolecules, such as carbohydrates, proteins, lipids and DNA (Kellog & Fridovich, 1975; Lai & Piette, 1977; Wiseman & Halliwell, 1996), thus accelerating aging and illness (Ames, 1983). Among these oxygen radicals, $^{\cdot}OH$ is the most reactive and induces severe damage to the adjacent biomolecules (Gutteridge, 1984). The harmful action of the free radicals can, however, be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers (Gerber et al., 2002; Kris-Etherton et al., 2002; Serafini, Bellocco, Wolk, & Ekstrom, 2002) and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Di Matteo & Esposito, 2003), as well as inflammation and problems caused by cell and cutaneous aging (Ames, Shigrenaga, & Hagen, 1993).

Piper betle L. (Piperaceae) leaves have a strong pungent aromatic flavour and are widely used as masticatory in Asia. The leaves are credited with many properties (digestive, stimulant). Medicinally the leaves are useful in catarrhal and pulmonary affections (The Wealth of India, 1969). The phenolic constituent allylpyrocatechol from the leaves showed activity against obligate oral anaerobes responsible for halitosis (Ramji, Iyer, & Chandrasekaran, 2002). The leaf extract has significant stimulatory influence on pancreatic lipase activity in experimental rats (Prabhu, Platel, Saraswathi, & Srinivasan, 1995). The leaf extract inhibits radiation-induced lipid peroxidation. The extract also increased the activity of superoxide dismutase activity in a dose-dependent manner, indicating elevation of antioxidant status in Swiss albino mice (Choudhury & Kale, 2002). *P. betle* leaves also afforded a significant hepatoprotective effect and improved the tissue antioxidant status by

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doi:10.1016/j.foodchem.2004.01.036

increasing the levels of nonenzymatic antioxidants (reduced glutathione, vitamin C and vitamin E) and the activities of free radical-detoxifying enzymes in liver and kidney of ethanol-treated rats (Saravanan, Prakasam, Ramesh, & Pugalendi, 2002). *P. betle* leaf extract inhibited platelet aggregation via both antioxidative effects and effects on thromboxane B2 (TXB2) and prostaglandin-D2 (PGD2) production (Jeng et al., 2002). Piperbetol, methylpiperbetol, piperol A and piperol B, isolated from *P. betle*, are effective platelet activating factor (PAF) receptor antagonists in vitro (Zeng et al., 1997). In this paper, we report free radical scavenging effect and prevention of lipid peroxidation by three local varieties of *P. betle* leaf.

2. Material and methods

2.1. Samples

Piper betle leaves (local varieties Bagerhati, Ghanagete and Kauri) were collected from Howrah. The teas prepared from the dried leaves were made by boiling in distilled water for 5 min and were used for analysing antioxidant activity in vitro. Each experiment was repeated five times. For comparison tea leaves (CTC HGH) were used.

2.2. DPPH radical scavenging activity

The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Aqueous extract (0.1 ml) was added to 3 ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated as $[(A_o - A_e)/A_o] \times 100$ (A_o = Absorbance without extract; A_e = absorbance with extract).

2.3. Assay of superoxide radical (O_2^-) scavenging activity

The assay was based on the capacity of the extracts to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin–light–NBT system (Beauchamp & Fridovich, 1971). The method used by Martinez, Marcelo, Marco, and Moacyr (2001) for determination of superoxide dismutase was followed after modification. Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer, pH 7.8, 13 mM methionine, 2 μM riboflavin, 100 μM EDTA, NBT (75 μM) and 1 ml sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after a 10 min illumination from a fluorescent lamp. The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture were kept in the dark and served as blanks.

2.4. Assay of hydroxyl radical ($^{\cdot}OH$) scavenging activity

The assay was based on benzoic acid hydroxylation method, as described by Chung, Osawa, and Kawakishi (1997). In a screw-capped tube, 0.2 ml sodium benzoate (10 mM) and 0.2 ml of $FeSO_4$, $7H_2O$ (10 mM) and EDTA (10 mM) were added. Then the sample solution and a phosphate buffer (pH 7.4, 0.1 mol.) were added to give a total volume of 1.8 ml. Finally, 0.2 ml of an H_2O_2 solution (10 mM) was added. The reaction mixture was then incubated at 37 °C for 2 h. After this, the fluorescence was measured at 407 nm emission with excitation at 305 nm.

$$\text{OH-scavenging activity (\%)} = [1 - (\text{F.I.s} - \text{F.I.o})/(\text{F.I.c} - \text{F.I.o})] \times 100,$$

where F.I.o is fluorescence intensity at Ex 305 and Em 407 nm with no treatment, F.I.c is fluorescence intensity at Ex 305 and Em 407 nm of treated control, F.I.s is fluorescence intensity at Ex 305 and Em 407 nm of treated sample.

2.5. Lipid peroxidation assay

A modified thiobarbituric acid reactive species (TBARS) assay (Ohkowa, Ohisi, & Yagi, 1979) was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media (Ruberto, Baratta, Deans, & Dorman, 2000). Egg homogenate (0.5 ml of 10% v/v) and 0.1 ml of extract were added to a test tube and made up to 1 ml with distilled water; 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and the mixture incubated for 30 min. Then, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and then heated at 95 °C for 60 min. After cooling, 5.0 ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. Inhibition of lipid peroxidation (%) by the extract was calculated $[(1 - E/C) \times 100$ where C is the absorbance value of the fully oxidised control and E is the absorbance in presence of extract].

2.6. Determination of total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Prieto, Pineda, & Aguilar, 1999). 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM

sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and gallic acid.

2.7. Determination of total phenol content

Phenol was determined by Folin–Ciocalteau reagent in alkaline medium and was expressed as gallic acid equivalents.

3. Results and discussion

Taking 0% inhibition in the mixture without plant extract, regression equations were prepared from the concentrations of the extracts and percentage inhibition of free radical formation/prevention of lipid peroxidation in different systems of assay, e.g. DPPH assay, superoxide radical-scavenging assay, hydroxyl radical-scavenging assay and lipid peroxidation assay. IC_{50} values (concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%) were calculated from these regression equations. IC_{50} values of the three varieties of *P. betle* leaves were compared with the IC_{50} value of tea leaves in each system to assess and the antioxidant property of *P. betle* leaves (Fig. 1). IC_{50} value is inversely related to the activity.

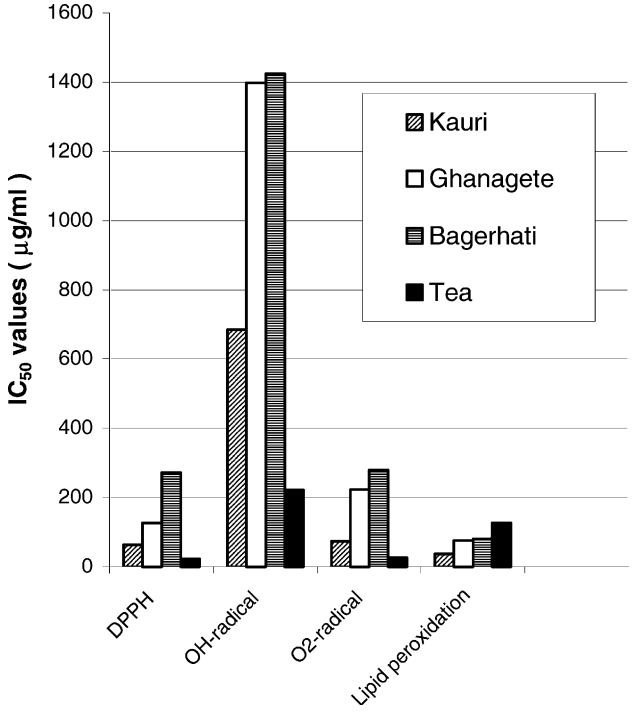

Fig. 1. Comparison of IC_{50} values.

Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralising its free radical character (Naik et al., 2003). The colour changes from purple to yellow and its absorbance at wavelength 517 decreases. Aqueous extract of *P. betle* leaf extract quenched DPPH free radical (Table 1) in a dose-dependent manner [$r = 0.9889$ ($p = 0.01$) for Kauri; $r = 0.9875$ ($p = 0.01$) for Ghanagete; $r = 0.9973$ ($p = 0.001$) for Bagerhati]. IC_{50} values were 62.6 µg/ml for Kauri, 126 µg/ml for Ghanagete and 271.5 µg/ml for Bagerhati. DPPH assay shows that, in this system, the radical-scavenging activities of the three varieties of *P. betle* leaves are in the order Kauri > Ghanagete > Bagerhati. The scavenging power of tea is about 2.75 times stronger than that of Kauri.

Hydroxyl radicals are generated by direct addition of iron(II) salts to a reaction mixture containing phosphate buffer (Gutteridge, 1984). Benzoate is hydroxylated to hydroxybenzoates. Benzoate is weakly fluorescent but, after monohydroxylation, forms highly fluorescent products (Gutteridge, 1987). Measurement of spectrofluorometric changes has been used to detect damage by hydroxyl radical. *P. betle* leaf extract was found to be a powerful scavenger of hydroxyl radicals (Table 2). There is a linear correlation between concentration of extract and $^{\cdot}OH$-scavenging activity [$r = 0.9232$ ($p = 0.01$) for Kauri; $r = 0.9884$ ($p = 0.001$) for Ghanagete; $r = 0.9886$ ($p = 0.001$) for Bagerhati]. IC_{50} values are 685 µg/ml for Kauri, 1397 µg/ml for Ghanagete and 1424 µg/ml for Bagerhati. Highest hydroxyl radical-scavenging activity was found in the variety Kauri and the scavenging power of this variety is about one third that of tea.

Photochemical reduction of flavins generates O_2^- which reduces NBT, resulting in the formation of blue formazan (Beauchamp & Fridovich, 1971). *P. betle* leaf extract inhibited the formation of the blue formazan (Table 3) and % inhibition is proportional to the concentration [$r = 0.9517$ ($p = 0.01$) for Kauri; $r = 0.9777$ ($p = 0.001$) for Ghanagete; $r = 0.9791$ ($p = 0.001$) for Bagerhati]. IC_{50} values were 73.8 µg/ml for Kauri and 224 µg/ml for Ghanagete and 279 for Bagerhati. In this system, also, Kauri has the highest activity which is about one third that of tea.

Egg yolk lipids undergo rapid non-enzymatic peroxidation when incubated in the presence of ferrous sulphate. Lipid peroxides are likely involved in numerous pathological events, including inflammation, metabolic disorders and cellular aging (Ames et al., 1993; Wiseman et al., 1996). The effects of *P.betle* leaf on non-enzymatic peroxidation are shown in Table 4. *P. betle* inhibited lipid peroxidation in a concentration-dependent manner [$r = 0.9836$ ($p = 0.001$) for Kauri; $r = 0.9860$ ($p = 0.01$) for Ghanagete; $r = 0.9596$ ($p = 0.01$) for Bagerhati]. IC_{50} values for the inhibition of lipid peroxidation were 14.8 µg/ml for Kauri, 77.2 µg/ml for Ghanagete and 81.5 µg/ml for Bagerhati. IC_{50} value of tea (CTC HGH) was

Table 1
DPPH radical scavenging activity of *P. betle* leaf

Sample	Concentration (μg/ml)	% Inhibition ± SD ($n = 5$)	Regression equation (r)[a]
Kauri	32.2	34.0 ± 1.35	$Y = 0.6986x + 6.2806$
	64.5	54.4 ± 1.62	($r = 0.9889$)
	96.7	77.0 ± 0.96	
	129	91.2 ± 2.37	
Ghanagete	64.5	30.3 ± 2.02	$Y = 0.3534x + 5.35$
	129	55.9 ± 2.98	($r = 0.9875$)
	193.5	79.0 ± 1.39	
	258	89.6 ± 1.97	
Bagerhati	64.5	14.01 ± 1.29	$Y = 0.178x + 1.6758$
	129	24.9 ± 3.63	($r = 0.9973$)
	193.5	37.5 ± 1.84	
	258	49.0 ± 5.35	
	322.5	56.9 ± 5.39	

[a] Correlation coefficient of dose response.

Table 2
Hydroxyl radical scavenging activity of *P. betle* leaf

Sample	Concentration (μg/ml)	% Inhibition ± SD ($n = 5$)	Regression equation (r)[a]
Kauri	250	38.0 ± 1.51	$Y = 0.0422x + 21.087$
	500	53.9 ± 1.11	($r = 0.9232$)
	1000	75.5 ± 1.49	
	1500	87.3 ± 1.03	
	2000	93.7 ± 1.23	
Ghanagete	500	20.4 ± 2.48	$Y = 0.0324x + 4.7278$
	1000	42.4 ± 2.08	($r = 0.9884$)
	1500	57.1 ± 4.56	
	2000	71.8 ± 3.17	
	2500	79.6 ± 3.37	
Bagerhati	500	21.2 ± 7.24	$Y = 0.0316x + 4.9879$
	1000	40.3 ± 10.89	($r = 0.9886$)
	1500	58.3 ± 7.78	
	2000	68.3 ± 7.31	
	2500	78.8 ± 6.69	

[a] Correlation coefficient of dose response.

Table 3
Superoxide radical scavenging activity of *P. betle* leaf

Sample	Concentration (μg/ml)	% Inhibition ± SD ($n = 5$)	Regression equation (r)[a]
Kauri	33.3	38.1 ± 3.76	$Y = 0.4827x + 14.362$
	66.6	57.0 ± 3.44	($r = 0.95169$)
	100	68.4 ± 2.23	
	133.3	76.9 ± 2.13	
	166.6	87.0 ± 2.22	
Ghanagete	66.6	15.1 ± 3.42	$Y = 0.204x + 4.3696$
	133	40.3 ± 3.73	($r = 0.97768$)
	200	48.0 ± 4.14	
	266.6	60.4 ± 2.20	
	333.3	66.5 ± 1.89	
Bagerhati	100	11. ± 5.30	$Y = 0.1749x + 1.1979$
	200	42.6 ± 6.09	($r = 0.9791$)
	300	60.3 ± 5.21	
	400	75.3 ± 2.04	
	500	80.4 ± 2.45	

[a] Correlation coefficient of dose response.

Table 4
Prevention of lipid peroxidation by *P. betle* leaf

Sample	Concentration (μg/ml)	% Inhibition ± SD ($n = 5$)	Regression equation (r)[a]
Kauri	3.75	13.3 ± 0.87	$Y = 3.3386x + 0.7201$
	7.5	23.6 ± 7.55	($r = 0.9836$)
	15.0	48.8 ± 13.44	
	22.5	88.9 ± 1.42	
	30.0	92.6 ± 1.75	
Ghanagete	30	16.3 ± 4.45	$Y = 0.6185x + 2.2401$
	60	43.4 ± 3.23	($r = 0.9860$)
	90	66.3 ± 0.79	
	150	89.2 ± 2.45	
Bagerhati	37.5	24.3 ± 3.64	$Y = 0.5013x + 9.1465$
	75	58.4 ± 2.60	($r = 0.9596$)
	112.5	77.9 ± 3.12	
	150	85.3 ± 0.96	
	187.5	91.1 ± 1.00	

[a] Correlation coefficient of dose response.

Table 5
Total antioxidant capacity of *P. betle*

Material	Equivalent to ascorbic acid (mg)/mg plant material	Equivalent to gallic acid (mg)/mg plant material
Kauri	0.192	0.50
Ghanagete	0.076	0.021
Bagerhati	0.083	0.022
Tea	0.443	0.115

127 μg/ml. So aqueous extracts of the three varieties of *P. betle* leaf prevent lipid peroxidation more than this tea sample. The variety Kauri has the highest activity, which was about 8.6 times more than that of tea. The results suggest that consumption of *P. betle* leaf may afford a cytoprotective effect.

Total antioxidant capacity of *P. betle* leaf is expressed as the number of equivalents of ascorbic acid and gallic acid (Table 5). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (Prieto et al., 1999). Kauri variety had a higher capacity than the other two varieties. Total antioxidant capacity (equivalents of gallic acid) of Kauri was higher than that of tea.

The results from various free radical-scavenging system revealed that the three local varieties of *P. betle* had significant antioxidant activity. The extracts were found to have different levels of antioxidant activity in the systems tested. The antioxidant activities of the three varieties were in the order Kauri > Ghanagete > Bagerhati. Total phenol content, expressed as gallic acid equivalents was 0.96 mg/mg plant material in Kauri, 0.46 mg/mg plant material in Ghanagete and 0.084 mg/mg plant material in Bagerhati. Total phenolic concentration showed close correlation with the antioxidant activity, being highest in Kauri and lowest in Bagerhati. Plant phenolics present in fruit and vegetables have received considerable attention because of their potential antioxidant activity (Lopez-Velez, Martinez_Martinez, & Del Valle-Ribes, 2003). Natural polyphenols have chain-breaking antioxidant activities and are believed to prevent many degenerative diseases, including cancer and atherosclerosis (Roginsky, 2003). More detailed chemical and pharmacological understanding of *P. betle* leaves is necessary.

Acknowledgements

The authors gratefully acknowledge the financial support of the University Grants Commission, New Delhi. The authors are also grateful to Prof. D. J. Chattopadhyay, University of Calcutta for the tea leaves (CTC HGH) collected from Nilgiri and to Dr. B. Mitra for *P. betle* leaves.

References

Ames, B. N. (1983). Dietary carcinogens and anticarcinogens – oxygen radicals and degenerative diseases. *Science, 221*, 1256–1264.

Ames, S. N., Shigrenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and degenerative diseases of aging. *Proceedings of the National Academy of Sciences of the United States of America, 90*, 7915–7922.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*, 276–287.

Braca, A., Tommasi, Nunziatina De, Bari, Lorenzo Di, Pizza, Cosimo, Politi, Mateo, & Morelli, Ivano (2001). Antioxidant principles from *Bauhinia terapotensis*. *Journal of Natural Products, 64*, 892–895.

Choudhury, D., & Kale, R. K. (2002). Antioxidant and non-toxic properties of *Piper betle* leaf extract: In vitro and in vivo studies. *Phytotherapy Research, 16*, 461–466.

Chung, S. K., Osawa, T., & Kawakishi, S. (1997). Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). *Bioscience Biotechnology and Biochemistry, 61*, 118–123.

Di Matteo, V., & Esposito, E. (2003). Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Current Drug Target CNS Neurological Disorders, 2*, 95–107.

Gerber, M., Boutron-Ruault, M. C., Hercberg, S., Riboli, E., Scalbert, A., & Siess, M. H. (2002). Food and cancer: State of the art about the protective effect of fruits and vegetables. *Bulletin du Cancer, 89*, 293–312.

Gutteridge, M. C. (1984). Reactivity of hydroxyl and hydroxyl radicals discriminated by release of thiobarbituric acid-reactive material from deoxy sugars, nucleosides and benzoate. *Biochemical Journal, 224*, 761–767.

Gutteridge, M. C. (1987). Ferrous salt promoted damage to deoxyribose and benzoate. *Biochemical Journal, 243*, 709–714.

Jeng, J. H., Chen, S. Y., Liao, C. H., Tung, Y. Y., Lin, B. R., Hahn, L. J., & Chang, M. C. (2002). Modulation of platelet aggregation by areca nut and betle leaf ingredients: Roles of reactive oxygen species and cyclogenase. *Free Radical Biology and Medicine, 32*, 860–871.

Kellog, E. W., & Fridovich, I. (1975). Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. *Journal of Biological Chemistry, 250*, 8812–8817.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E., & Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine, 113*(Suppl 9B), 71S–88S.

Lai, C. S., & Piette, L. H. (1977). Hydroxyl radical production involved in lipid peroxidation of rat liver microsomes. *Biochemical and Biophysical Research Communications, 78*, 51–59.

Lopez-Velez, M., Martinez_Martinez, F., & Del Valle-Ribes, C. (2003). The study of phenolic compounds as natural antioxidants in wine. *Critical Reviews Food Science and Nutrition, 43*, 233–244.

Martinez, A. C., Marcelo, E. L., Marco, A. O., & Moacyr, M. (2001). Differential responses of superoxide dismutase in freezing resistant *Solanum curtibolum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. *Plant Science, 160*, 505–515.

Naik, G. H., Priyadarsini, K. I., Satav, J. G., Banavalikar, M. M., Sohoni, P. P., Biyani, M. K., & Mohan, H. (2003). Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry, 63*, 97–104.

Ohkowa, M., Ohisi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351–358.

Prabhu, M. S., Platel, K., Saraswathi, G., & Srinivasan, K. (1995). Effect of orally administered betel leaf (*Piper betle* Linn.) on digestive enzymes of pancreas and intestinal mucosa and on bile production in rats. *Indian Journal of Experimental Biology, 33*, 752–756.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry, 269*, 337–341.

Ramji, N., Iyer, R., & Chandrasekaran, S. (2002). Phenolic antibacterials from *Piper betle* in the prevention of halitosis. *Journal of Ethnopharmacology, 83*, 149–152.

Roginsky, V. (2003). Chain breaking antioxidant activity of natural polyphenols as determined during the chain oxidation of methyl linoleate in Triton X-100 micelles. *Archives of Biochemistry and Biophysics, 414*, 261–270.

Ruberto, G., Baratta, M. T., Deans, S. G., & Dorman, H. J. D. (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Medica, 66*, 687–693.

Saravanan, R., Prakasam, A., Ramesh, B., & Pugalendi, K. V. (2002). Influence of *Piper betle* on hepatic marker enzymes and tissue antioxidant status in ethanol-treated Wister rats. *Journal of Medicinal Food, 5*, 197–204.

Serafini, M., Bellocco, R., Wolk, A., & Ekstrom, A. M. (2002). Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology, 123*, 985–991.

The Wealth of India (1969). *A dictionary of Indian raw materials and industrial products* (Vol VIII). New Delhi: Publications and Information Directorate, CSIR.

Wiseman, H., & Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. *Biochemical Journal, 313*, 17–29.

Zeng, H. W., Jiang, Y. Y., Cai, D. G., Bian, J., Long, K., & Chen, Z. L. (1997). Piperbetol, metylpiperbetol, piperol A and piperol B: A new series of specific PAF receptor antagonists from *Piper betle*. *Planta Medica, 63*, 296–298.