Research Article

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IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS ETHANOL EXTRACT OF *FLACOURTIA INDICA* MERR.

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ABSTRACT

The antioxidant activity of aqueous ethanol extracts of Vikankata (*Flacourtia indica* Merr.) stem bark was evaluated by various in vitro antioxidant assays such as evaluation of reducing power, DPPH radical scavenging activity, nitric oxide scavenging activity, superoxide anion scavenging activity. The results of the study show that the plants possess significant free radical scavenging properties and a clear correlation exists between the antioxidant activities.

Keywords: Vikankata, *Flacourtia indica*, reducing power, DPPH radical scavenging activity, nitric oxide scavenging activity, superoxide anion scavenging activity.

INTRODUCTION

The role of free radical reactions in disease pathology is well established. Suggesting that these reactions are necessary for normal metabolism but can be detrimental to health as well. The reactive oxygen species (ROS), super oxide ion, hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) produced during atmospheric oxygen metabolism are involved in various human diseases such as ischemic heart disease, Alzheimer's disease, aging, cancer, inflammation, diabetes, rheumatoid arthritis and atherosclerosis[1-5]. The antioxidant properties of phytoconstituents have a correlation with oxidative stress defense[6]. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals[7]. Numerous medicinal plants and their formulations are used as antioxidant in ethnomedicine practices as well as in traditional Indian medicines[8].

Vikankata (*Flacourtia indica* Merr. Syn. *F. ramontchi* L Herit.) is a member of the family Flacourtiaceae and a small deciduous, usually thorny tree or shrub, found in the sub-Himalayan tract and outer Himalayas up to 1220 m and also common throughout Indian deciduous forests [9]. This plant has been reported as an answer for the treatment of a variety of diseases and functional disorder. Fruits are used as digestive; remedy for hepatitis and splenomegaly. Barks are used as astringent and diuretics[10]. Significant progress has been made over the past years towards a much more comprehensive understanding of some of the important pharmacological components of *Flacourtia indica.* Isolatation of β-sitosterol (a well-known phytosterol), β-sitosterol-β-Dglucopyranoside, ramontoside, butyrolactone lignan disaccharide, flacourtin, coumarin such as scoparone and aesculetin has been described[11-13]. Fruits contain protein and sucrose; barks yield tannin and flacoutin, aphenolic glucoside ester[10]. In the recent years, the interest is centered on antioxidant derived from Ayurvedic medicines in view of their medicinal benefits[14]. In view of this, we selected Vikankata (*Flacourtia indica*) stem bark to assess the *in –vitro* antioxidant study.

MATERIALS AND METHODS

Material collection and identification

Vikankata (*Flacourtia indica* Merr.) stem bark was collected from the Narayanpur district, Chhattisgarh. Taxonomic identification of collected material was done in the Raw Materials Herbarium & Museum, Delhi (RHMD), National Institute of Science Communication and Information Resources (CSIR-NISCAIR), Dr. K. S. Krishnan Marg, New Delhi (Ref. No. NISCAIR/RHMD/CONSULT/2015/2866-59).

Instruments

Shimadzu UV-VIS Spectrophotometer (1240) was used for all spectrophotometric studies. Remi R24 centrifuge was used for centrifugation.

Chemicals and reagents

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Company, U.S.A. Folin-Ciocalteau reagent and NBT were obtained from Sisco Research Laboratories, Mumbai. Ascorbic acid and BHT were obtained from Himedia Lab. Ltd., Mumbai, India. All other chemicals were of Analytical Grade.

Preparation of the extract

The powdered plant material was extracted with Aqueous Ethanol (80%) using Soxhlet apparatus. The solvents were then removed under reduced pressure which obtained sticky residues. All the dried extracts were dissolved in 99% Ethanol and distilled water, respectively for various studies.

Estimation of Total Phenolic Compounds

The method of Naczk[15] was followed. 0.1ml of 10mg/ml aqueous solution of the extract was diluted with 46 ml of distilled water in an Erlen meyer flask. Afterwards 1 ml of Folin–Ciocalteu Reactive (FCR) was added into this mixture followed by addition of 3 ml of $Na_2 CO_3$ (2%) after 3 min. Subsequently mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760 nm. The concentration of total phenolic compounds was determined as micrograms of pyrocatechol equivalent by using the equation that was obtained from the standard pyrocatechol graph.

Absorbance = 0.001 X Pyrocatechol (µg) + 0.0033

Evaluation of reducing power

The reducing power of the extract was determined according to the method of Oyanzu[16]. The plant extract (50-500µg) in 1 ml of distilled water was mixed in to the mixture of 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% Potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. Following incubation 2.5 ml of 10% Trichloroacetic acid was added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated by the method of Nagai[17]. The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of 99% ethanol and 0.3 ml of extract solution of concentrations varying from 50 µg to 500 µg/ml. The solutions were rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid (1 mM) was used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. The percent (%) radical scavenging was calculated by the following equation.

% DPPH radical-scavenging = $[(A_c - A_s) / A_c] * 1oo.$
Where Ac = Absorbance of control at 517 nm and As = Absorbance of sample at 517 nm.

Nitric oxide Scavenging Activity

The method of Sreejayan[18] was followed. For the experiment, Sodium nitropruside (10 mM) in phosphate buffered saline was mixed with different concentrations of the extract dissolved in Methanol and incubated at room temperature for 2½ h. The same reaction without the sample but equivalent amount of Methanol served as control. After incubation period 0.5 ml of Griess reagent was added. Absorbance of the chromophore formed was measured at 546nm. Ascorbic acid was used as positive control.

The procedure is based on the principle that Sodium nitropruside solution spontaneously generates Nitric oxides which reacts with oxygen to produce nitric ions that can be estimated using Greiss reagent. Scavengers of nitric oxides compete with oxygen leading to reduce production of nitric ions.

Superoxide anion scavenging activity

The assay for super oxide ion scavenging activity was performed as per standard procedure Beuchamp[19] was followed. The reaction mixture contained 50 mM phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA and 0.1mg/ml of NBT (nitro blue tetrazolium) all added in chronological sequence. Reaction was started by illuminating the reaction mixture containing different concentrations of the sample extract for 90 seconds and then measuring the absorbance at 590 nm. Ascorbic acid was taken as the positive standard.

RESULTS

Amount of total phenolic compounds: As shown in Table 1 the plant extract has good number of phenolic compounds.

Reducing power: The reducing powers determined by the present assay depend on the redox potentials of the compounds present in the extract of the plant characterized by the complexity of their constituents. As shown in Table2, reducing power of the extract was shown. BHT was taken as a positive control. All the results are comparable with the standard.

DPPH radical scavenging activity: As shown in Table 3, DPPH decolorization was increased by the plant extract in a concentration dependent manner. The extract at a concentration of 500 µg was able to scavenge 51.33% of DPPH free radical. All the results are comparable with Ascorbic acid used as standard.

Nitric oxide Scavenging Activity: The percent inhibition of NO-scavenging as shown in Table 4, proves the extract of the plant inhibits the reaction in a concentration dependent manner. At a concentration of 50µg/ml the extract was able to produce 60% inhibition. All the results are comparable with Ascorbic acid which was able to scavenge 77.33% NO- free radical at the same level of concentration.

Assay for Super oxide anion Scavenging Activity: We studied the extract of the plant for their ability to scavenge super oxide ion. The extract was able to scavenge the super oxide ion up to 52% at the concentration of 37.5µg/ml. The Ascorbic acid was used as standard which was able to scavenge up to 58% at the same concentration. All the results are comparable with the standard.

Table 1: Total Phenolic content of Aqueous Ethanol extracts of *Flacourtia indica*

Extracts	Pyrocatechole Equivalents
Aqueous Ethanol extract	31.9±3.00

n=3, values are Mean ± S.E.M

Table 2: Reducing activity of Aqueous Ethanol extracts of *Flacourtia indica*

Concentration (µg/ml)	Absorbance	
	Extract	BHT
50	0.138±0.0006	0.264±0.019
100	0.276±0.0008	0.340±0.019
250	0.302±0.001	0.621±0.022
500	0.361±0.002	0.717±0.010

n=3, values are Mean ± S.E.M

Table 3: DPPH-Radical scavenging activity of Aqueous Ethanol extracts of *Flacourtia indica*

Concentration (µg/ml)	Absorbance	
	Ethanol Extract	Ascorbic acid
50	13.33±0.33	64.33±3.53
100	23.66±0.66	78.33±1.76
250	39.33±0.88	89.33±2.60
500	51.33±1.45	95.66±1.20

n=3, values are Mean ± S.E.M

Table 4: Nitric oxide scavenging activity of Aqueous Ethanol extracts of *Flacourtia indica*

Concentration (µg/ml)	% of inhibition	
	Ethanol Extract	Ascorbic acid
4.17	19±1.73	27.66±1.765
8.33	27±1.00	40.33±1.454
16.67	36.33±2.40	51.33±2.029
33.33	49.66±2.73	66.33±2.188
50	60±2.51	77.33±1.858

n=3, values are Mean±SEM

Table 5: Superoxide anion scavenging activity of Aqueous Ethanol extracts of *Flacourtia indica*

Concentration (µg/ml)	% of inhibition	
	Ethanol Extract	Ascorbic acid
12.5	15.66±0.88	21.66±2.029
18.75	20.66±1.45	26.33±0.882
25	30.33±1.45	32±1.734
37.5	52±1.529	58±1.734

n=3, values are Mean±SEM

DISCUSSION

In the present study, the extract of the plant showed antioxidant activity. In spite oxygen is essential for life its transformation to reactive oxygen species (ROS) may provoke uncontrolled reactions. The free radicals are involved in the etiology of several degenerative diseases and various inflammatory diseases[20]. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and some other mechanism[21]. The extract which is showing significant antioxidant activity might be helpful in slowing the progress of various oxidative stress related diseases.

Our experimentation further support to the local use of the plant as anti-inflammatory and in the treatment of rheumatoid arthritis because the involvement of ROS as mediators of tissue damage in patients with rheumatoid arthritis[22-24].

Phenols are very important plant constituents because of their free radical scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans[25]. In the extract a very good amount of pyrocatechol equivalent was detected.

The reducing property of the extract, compared with BHT is shown. The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of the samples increased with increased amount of concentration. All of the samples showed higher activity than control and these differences were statistically very significant.

The antioxidant activity of the extract was determined by DPPH radical scavenging ability. This method is based on the reduction of DPPH, a stable free radical. Because of the odd electron of DPPH it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple color). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor that is a free radical scavenging antioxidant the absorption strength decreases and the resulting decolorization is stiochiometric with respect to the number of electrons captured[26]. This reaction has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the anti-oxidative activity of foods and plant extracts[27-30]. The extract showed to possess DPPH radical scavenging ability as compared to the standard.

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions which act as free radicals[31]. In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions. All the results were concentration dependent and comparable with Ascorbic acid.

Super oxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reaction such as auto oxidation by catecholamines. In the present study, the *in-vitro* super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitroblue tetrazoline) system reduction. The super oxide radical reduces NBT to a blue colored formazone that can be measured at 560-nm[32]. The effect of the extract and Ascorbic acid is shown. All the results were statistically significant and were comparable with Ascorbic acid. The probable mechanism of scavenging the super oxide anions may be due to the inhibitory effect of the sample towards generation of super oxide in the in vitro reaction mixture. Therefore, the herbal formulations based on the plant extract can be used for the prevention and treatment of oxidative stress related disorders such as cancer, vascular disease and rheumatism.

REFERENCES

1. Freeman BA. Free radicals in molecular biology, aging, and disease, Raven Press: New York; 1984, 43-52,
2. Harman D. Free radical theory of aging: Current status. In: Zs-Nagy I, ed. Lipofuscin-1987: State of the art. Amsterdam: Elsevier Science Publishers; 1988: 3–21.
3. Singh A. CRC handbook of free radicals and antioxidants in biomedicine, CRC press Inc.: Florida; 1989, 17-25.
4. Squadrito GL, Pryor WA. Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. Free Radic Biol Med 1998; 25:392-403.
5. Pryor WA. Oxidation and atherosclerosis. Free Radic. Biol. Med. 2000; 28: 1681- 1682.

6. Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 2004; 84: 551-562.
7. Osawa T, Kavakishi S, Namiki M. Antimutagenesis and anticarcinogenesis mechanisms II, Plenum: New York; 1990, 139–153.
8. Babu B H, Shylesh B S and Padikkala J. "Antioxidant and hepatoprotective effect of Alanthus icici focus", Fitoterapia 2001; 72:272-277.
9. Anonymous. The Ayurvedic Pharmacopoeia of India, Part I, Vol. IV, Govt. of India, M.H & F.W, Dept.of ISM & H; 2001:136-137.
10. Chatterjee A, Satyesh CP. The Treatise on Indian Medicinal Plants. New Delhi: National Institute of Science Communication; Vol. II, p. 144.
11. Nazneen M, Mazid MA, Kundu JK, Bachar SC, Rashid MA and Datta BK. Protective effects of Flacourtia indica aerial parts extracts against paracetamol-induced hepatotoxiciy in rats. J Biol Sci. 2002; 11(2):183-187.
12. Satyanarayana V, Kurupadanam GL and Srimanaraya G. A butyrolactone lignin disaccharide from Flacourtia ramontchi. Phytochemistry 1991; 30:1026-1029.
13. Bhaumik PK, Guha KP, Biswas GK and Mukherjee B. Flacourtin, a phenolic glucoside ester from Flacourtia indica. Phytochemistry 1987;26:3090-3091.
14. Rout OP, Acharya R and Mishra SK. In-vitro antioxidant potentials in leaves of coleus aromaticus Benth and rhizomes of Zingiber zerumbet (L.) SM. Journal of Applied Pharmaceutical Science 2011; 01 (08):194-198.
15. Naczk M. and Shahidi F. The effort of methanol-ammonia-water/hexane treatment on the content of phenolic acids of canola, Food Chem. 1989; 31:159–164.
16. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of browning products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 1986; 44:307–315.
17. Nagai T, Inoue R, Inoue H, Suzuki N. Preparations and antioxidant properties of water extract of propolis. Food Chem 2003; 80:29-33.
18. Sreejayan, Rao MNA. Nitric oxide scavenging by curcuminoids.. J .Pharm. Pharmacol. 1997; 49:105-107.
19. Beuchamp C, Fridovich I. Superoxide dismutase: Improvedassays and an assay applicable to acrylamide gels.. Anal Biochem. 1971; 44: 276-277.
20. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. U.S.A 1993; 90: 7915-7922.
21. Gillman B, Papachristodouluo DK, Thomas JH. Wills' Biochemical Basis of Medicine, Butterworth-Heinemann: Oxford 1977; 334.
22. Cimen MY, Cimen OB, Kacmaz M, Ozturk HS, Yorgancioglu R, Durak I. Oxidant/antioxidant status of the erythrocytes. Clin Rheumatol. 2000;19: 275-277.
23. Thabrew, MI, Senaratna L, Samarawickrema N, Munasinghe C. Antioxidant potential of two polyherbal preparations used in Ayurveda for the treatment of rheumatoid arthritis. J Ethnopharmacol 2001;76:285–291
24. Taysi S, Polat F, Gul M, Sari RA, Bakan E. Lipid peroxidation, some extracellular antioxidants and antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatol Int 2002; 21: 200-204
25. Tsao R, Akhtar MH. Nutraceuticals and functional foods: Current trend in Phytochemical antioxidant research. J. Food Agri. Environ. 2005; 3(1): 10-17.
26. Blois MS. Antioxidant Determinations by the use of a Stable Free Radicals. Nature 1958; 181: 1199-1200.
27. Ekanayake P, Lee YD, Lee J. Antioxidant Activity of Flesh and Skin of Eptatretus Burgeri (Hag Fish) and Enedrias Nebulosus (White Spotted Eel). Food Sci Technol Int 2004; 10: 171-177.
28. Zhou K, Su L, Yu L. J. Phytochemicals and antioxidant properties in wheat bran. Agric. Food Chem. 2004; 52: 6108-6114.
29. Panovska TK, Kulenova S, Stefova M. In vitro antioxidant activity of some Teucrium species (Lamiaceae). Acta Pharm. 2005; 55: 207-214.
30. Rodríguez R, Jaramillo S, Rodriguez G, Espejo JA, Guillén R, Fernández-Bolaños J, Heredia A, Jiménez A. J. Agric. Food Chem. 2005; 53: 5212-5217.
31. Marletta MA. Nitric oxide: biosynthesis and biological significance, Trends. Biol. Sci.1989; 14:488-492.
32. Seth UK, Dadkar NK, Kamat UG. Selected Topics in Experimental Pharmacology, Kothari Book Depot: Bomba; 1972: 126.

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