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In vitro Antioxidant Activity of Methanolic and Aqueous Extract of *Flacourtia indica* Merr

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Abstract: The antioxidant activity of methanolic and aqueous extracts of *Flacourtia indica* leaves was evaluated by various *in vitro* antioxidant assays. IC_{50} values of methanolic and aqueous extracts were found to be 18 µg/ml and 26 µg/ml for DPPH and 62 µg/ml 75 µg/ml for nitric oxide scavenging activity. The extracts showed potent antioxidant activity in all models when compared with ascorbic acid having IC_{50} values 8.2µg/ml and 26 µg/ml for DPPH and nitric oxide scavenging activity respectively. Total antioxidant capacity of extract was found to be 260 µg/ml and 180 µg/ml ascorbic acid for methanolic and aqueous extracts respectively. The results indicate that both the extracts clearly have strong antioxidant effects. The freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids. The antioxidant activity may be attributed to flavonoids and phenolics present in methanolic and aqueous extracts of *Flacourtia indica*. Total phenolic content for methanolic and aqueous extracts were found to be 1.5 mg/g and 1.1 mg/g and total flavonoid content for methanolic and aqueous extracts were found to be 0.140 mg/g and 0.029 mg/g. Thus it could be concluded that the methanolic and aqueous extracts of *Flacourtia indica* possess significant antioxidant property.

Key words: Antioxidant · *Flacourtia indica* · Free radicals · DPPH.

INTRODUCTION

Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors [1]. *In vivo*, some of these ROS play a positive role such as energy production, phagocytosis, regulation of cell growth and intercellular signaling or synthesis of biologically important compounds [2]. However, ROS may also be very damaging; they can attack lipids in cell membranes and also attack DNA, inducing oxidations that cause membrane damage such as membrane lipid peroxidation and a decrease in membrane fluidity and also cause DNA mutation leading to cancer [3, 4]. A potent scavenger of these species may serve as a possible preventive intervention for free radical-mediated diseases [5]. Recent studies showed that a number of plant products including polyphenolic substances (e.g. flavonoids and tannins) and various plant or herb extracts exert antioxidant actions [6-10]. Antioxidants are added as redox syleavess possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition. In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or

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an electron to the free radical and receiving the excess energy possessed by the activated molecule [11]. It has been suggested that fruits, vegetables, natural plants contain a large variety of substance called phytochemicals which are present in plants and are the main source of antioxidant in the diet, which could decrease the potential stress caused by reactive oxygen species. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen etc [12]. The antioxidants can interfere with the oxidation process by reacting with free radicals [13]. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [14]. Antioxidants principles from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance [15].

Flacourtia indica Merr. (Family: Flacourtiaceae), commonly known as 'Baichi' or 'Katai', is an indigenous medicinal plant widely distributed in Bangladesh and India [16]. This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, diuretic, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera [16-17]. Previous phytochemical investigation on this plant resulted in the isolatation of β-sitosterol (a well-known phytosterol), β-sitosterol-β-D-glucopyranoside, ramontoside, butyrolactone lignan disaccharide [18] and flacourtin [19]. Recent report shows the presence of coumarin such as scoparone and aesculetin [20].

MATERIAL AND METHODS

Collection of Plant Materials: *Flacourtia indica* leaves were collected from the roadside location of the Sagar District, Sagar India. The plant was identified by Dr. A. K Singhai, Professor, Department of Pharmaceutical Sciences, Dr. H S Gour University, Sagar (M.P.) 470001. The leaves of the plant was collected in third week of December 2009 and preserved in herbarium of institution. The leaves were air dried under shade, powdered mechanically and stored in airtight containers.

Preparation of Extracts: Methanolic extract- About 500 gm of powder was extracted with methanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction the solvent was recovered by distillation and concentrated under vacuum.

Aqueous extract- About 500 gm of the powdered material was boiled with 2 L of distilled water for 30 min and filtered to obtain the aqueous extract. The extract was concentrated under reduced pressure and lyophilized. The freeze-dried material was weighed, dissolved in the water and used for this study.

DPPH Radical Scavenging Activity*:* The antiradical activity for the plant extracts was assessed on the basis of the radical-scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical [21]. The concentration of DPPH was kept at 300 µM in MeOH. The extracts were dissolved in MeOH. 10 µl of each extract solution was allowed to react with 200 µl DPPH at 37°C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as reference.

Nitric Oxide Scavenging Activity: [22] Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction [23]. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \times 100]$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract/Standard.

Total Antioxidant Capacity: Total antioxidant capacity was measured according to the method reported by Prieto *et al*. with slight modifications [24]. In brief, 100 µg of extract and 100 µg of ascorbic acid (as standard) were taken in 0.1 ml of alcohol, combined separately in an eppendroff tube with 1.9 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1.9 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in µg per ml of extract.

Total Phenolic Content: [25-26]- Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent using gallic acid as a standard phenolic compound. 1.0 ml of extract solution containing 1.0 mg extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as gallic acid equivalents in mg/g of dry extract.

Total Flavonoid Content: [25] Aluminum chloride colourimetric method was used for determination of flavonoids. To the 10 ml volumetric flask 2 ml of water and 1 ml of plant extract (1 mg/ml) was added. After 5 min 3 ml of 5 % sodium nitrite and 0.3 ml of 10 % aluminum chloride was added. After 6 min, 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 ml with water. Absorbance was measured at 510 nm. The percentage of total flavonoids were calculated from calibration curve of quercetin (10-250 µg) plotted by using the same procedure and total flavonoids was expressed as quercetin equivalents in milligrams per gram sample.

Statistical Analysis: Linear regression analysis was used to calculate IC_{50}.

RESULTS

Several concentrations of methanolic and aqueous extracts of *Flacourtia indica* were tested for antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by test compound in concentration dependent manner up to given concentration in all the models. The maximum inhibitory concentration in all models *viz*. DPPH, reducing power and nitric oxide were found to be respectively at 1 mg/ml concentration. Total antioxidant capacity of extract was found to be 260 µg/ml and 180 µg/ml ascorbic acid for methanolic and aqueous extracts respectively.

% scavenging was calculated by using the formula

$$\% \text{ scavenging} = (A_{control} - A_{sample})/A_{control} \times 100$$

On a comparative basis, extracts showed better activity in quenching DPPH with IC_{50} value 18 µg/ml and 26 µg/ml for methanolic and aqueous extracts (Figure 1). The extract also showed encouraging response in quenching nitric oxide radicals with IC_{50} values 62 µg/ml and 75 µg/ml for methanolic and aqueous extracts respectively (Figure 2). Total phenolic content for methanolic and aqueous extracts were found to be 1.5 mg/g and 1.1 mg/g respectively compared with gallic acid (R squared value - 0.9953) and total flavonoid content for methanolic and aqueous extracts were found to be 0.140 mg/g and 0.029 mg/g respectively compared with quercetin (R squared value - 0.997).

DISCUSSION

DPPH Radical Scavenging Activity: The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 490 nm and also for visible deep purple color [27]. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extracts and ascorbic acid is shown in Figure 1.

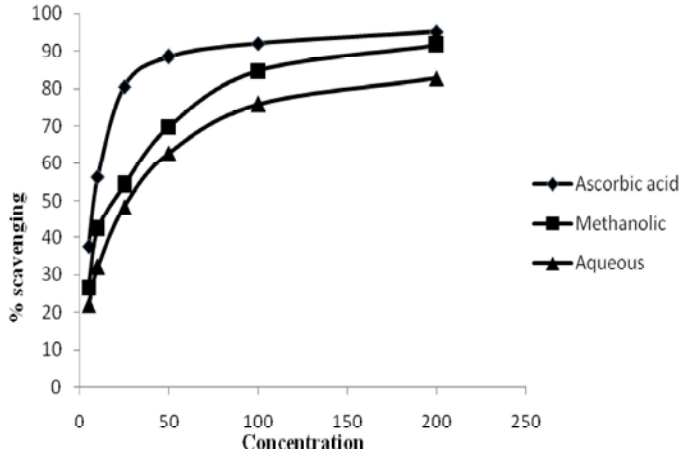

Fig. 1: DPPH scavenging activity of *Flacourtia indica* Merr

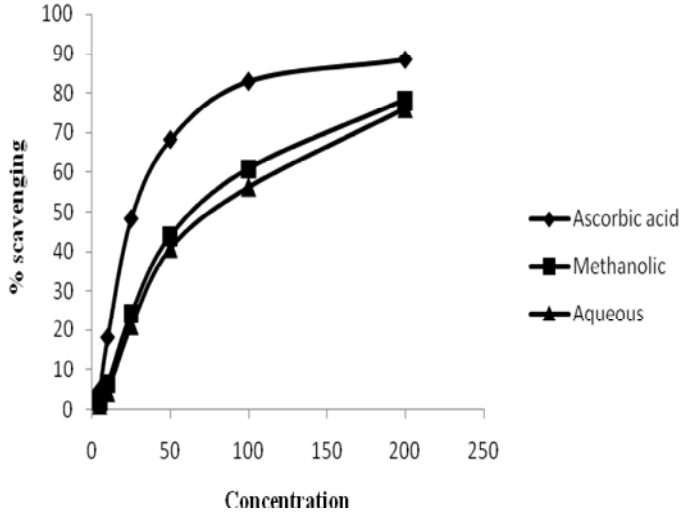

Fig. 2: Nitric oxide scavenging activity of *Flacourtia indica* Merr

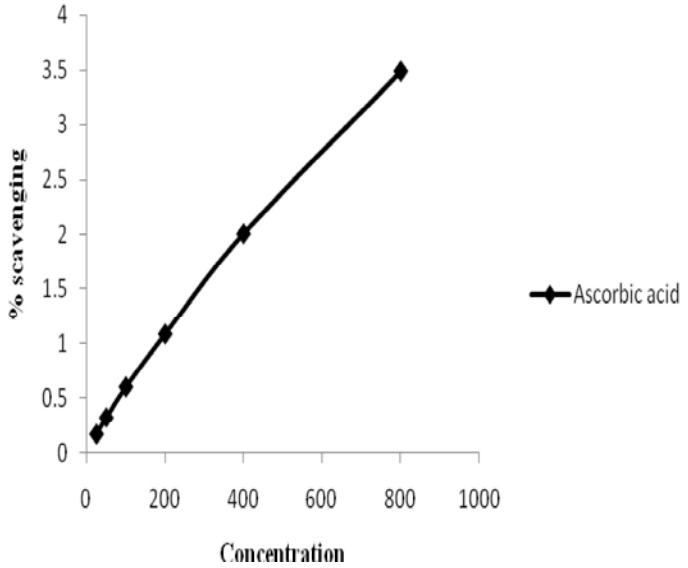

Fig. 3: Total Antioxidant activity of *Flacourtia indica* Merr

Nitric Oxide Scavenging Activity: Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by *Flacourtia indica* extracts. Figure 2 illustrates the percentage inhibition of nitric oxide generation by extract.

Total Antioxidant Capacity: The total antioxidant capacity of the extract was determined, based on the formation of phosphomolybdenum complex which was measured spectrophotometrically at 695 nm [28].

Total Phenolic Content: Phenolics present in leaves have received considerable attention because of their potential antioxidant activities [29]. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin- Ciocalteu reagent [30]. However, it should be noted that some chemical group of amino acids, proteins, organic acids, sugars and aromatic amines could react with the reagent. In this investigation, *Flacourtia indica* leaves was dried before extraction while ascorbic acid was lost during drying process and amino acids, proteins and sugars can be removed from the extraction solvents. Thus, interference from ascorbic acid or other compounds like amino acids, proteins and sugars should be very little [31].

Total Flavonoid Contents: Flavonoids are one of the most diverse and widespread group of natural compounds, are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties [31].

CONCLUSION

Reactive Oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process. Thus antioxidants which can scavenge ROS are expected to improve these disorders. The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. The results obtained in the present study indicate that *Flacourtia indica* leaves extract exhibit potent free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that *Flacourtia indica* leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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