Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Issue 1, 2012 ISSN - 0974-2441

Research Article

COMPARATIVE ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS OF *FLACOURTIA JANGOMAS* LOUR FRUITS

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Received: 17 September 2011, Revised and Accepted: 12 November 2011

ABSTRACT

Chloroform (CH) pet-ether (PE) and methanol (ME) extract of *Flacourtia jangomas* L., traditionally used in different ailments, were evaluated for antioxidant potential using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay, reducing power method, total antioxidant capacity, total phenol and total flavonoid content . The extract exhibited moderate to good anti oxidant activity when compared with ascorbic acid (ASA). The IC_{50} value of the Chloroform, Methanol and Pet ether extract were 523.15 µg mL^{-1}, 1623.87 µg mL^{-1} and 5811.35 µg mL^{-1} respectively while, the IC_{50} value of well known antioxidant Ascorbic Acid was 13.37 µg mL^{-1}. Among the extracts chloroform extract was found displaying strong DPPH radical scavenging action and this extract also exhibited highest phenol and flavanoid content as well as total anti oxidant capacity in comparison with pet ether and methanol extract. In case of reducing power these three extracts exhibited anti oxidant potential in a dose dependent fashion. Since reactive oxygen species are important contributors to serious disorder such as atherosclerosis, alcoholic liver cirrhosis and cancer, the antioxidant property of the extract of *F. jangomas*, as observed in the present study might be useful for the development of newer and more potent antioxidant.

Key words: *F. jangomas*, antioxidant, DPPH, Reducing power, ROS

INTRODUCTION

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen in several pathological situations. ROS produced *in vivo* include superoxide radical (O_2•-), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). H_2O_2 and O_2 can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical (•OH) [1]. ROS induces oxidation cause extensive damage to cells and tissues, during infections and various degenerative disorders such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer [2, 3, 4, 5]. Though our body possesses such defense mechanisms as enzymes and antioxidant nutrients that arrest the damaging properties of ROS [6, 7] but upon continuing exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body losing its capacity to control them, and cause irreversible oxidative damage [8]. That is why, antioxidants along with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases where oxidants or free radicals are implicated [9]. At present, it is criticized to have the possible toxicity of synthetic antioxidants, thus the frequent consumption of plant-derived phytochemicals from vegetables, fruits, tea, and herbs may contribute to shift the balance toward an appropriate antioxidant status [10]. Therefore, interest in natural antioxidant, mainly from plant origin, has greatly increased in recent years [11].

Plant Description

Flacourtia jangomas Lour (Family: Flacourtiaceae) known as locally Painnagola, Lukluki, Paniamra, Indian plum, coffee pulm that is widely distributed in Chittagong Hill Tracts, Cox's Bazar and Sylhet district of Bangladesh and south east Asia. The plant fruit having a remarkable reputation in the treatment of stomachic and digestive; allay thirst, useful in biliousness, fevers and relieves nausea. Leaves are diaphoretic, astringent and stomachic; good in diarrhoea, piles, weakness of limbs, bleeding gums, toothache and stomatitis; checks purging. Leaves and young shoots are prescribed in diarrhea. Leaves and young shoots are prescribed in diarrhoea. Decoction of the bark is useful in biliousness, bleeding gums and toothache [12].

MATERIALS AND METHODS

Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid) and ferric chloride were purchased from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine Chem.Ltd., Biosar, and Qualigens Fine chemicals, Mumbai, India respectively. Ammonium molybdate was obtained from Merck, Germany.

Sample preparation

Collection of plant material

The plant was collected from the Jahangirnagar University campus, Savar Dhaka-1342, Bangladesh, in March 2011 at the mature stage. Collected plant parts, after cutting into small pieces and were dried in shade at temperature between 21-30^{0}C for 15-20 days. The cutting pieces were pulverized by a mechanical grinder and passed through a 60 Mesh sieve to obtain fine powder and stored into an air-tight container.

Extraction of plant material

A portion of pulverized powder (1 kg) was extracted successively with petroleum ether, chloroform and methanol (2 L of each) by cold extraction process. The sample was stirred continuously and was kept for almost 72 hours in each portion. All the extracts were filtered off and evaporated to dryness (45^{o}C) under reduced pressure by rotary evaporator. The yields of the petroleum ether (PE), chloroform (CH) and methanol (ME) extracts were 2.1 g (0.21%), 3.7 g (0.37%) and 2.5 g (0.25%) respectively. Finally the extracts were defatted by refrigeration at 4° C temperature.

ANTIOXIDANT ACTIVITY TEST

Determination of free radical scavenging activity using DPPH assay

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by A Braca *et al* [13]. Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0] x100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were obtained.

Determination of reducing power capacity

The reducing power of *F. jangomus* extracts was determined according to the method of Oyaizu [14] where different concentration of *F. jangomus* extracts in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide

[K3Fe(CN)6] (2.5 ml, 1%). After that the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%) slightly added (2.5 ml) to the mixture and centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%). Finally, taken the absorbance at 700 nm where standard was Ascorbic acid and the Blank solution contained Phosphate buffer.

Determination of total antioxidant capacity

The total antioxidant activity of the extracts was evaluated according to the procedure of [15] by P Prieto *et al* the phosphomolybdenum method. The assay is based on the reduction of Mo (VI)–Mo (V) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A 0.3 ml of extract solution in various concentrations was mixed with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The reaction solution tubes were incubated at 95 °C for 90 min. After cooling to room temperature the absorbance of the solution was measured at 695 nm using a spectrophotometer (HACH 4000 DU UV–visible spectrophotometer) against blank. The number of equivalents of ascorbic acid expressed the activity of antioxidant.

Determination of total flavonoid contents

To determine the flavonoid content of the different extracts of *F. jangomus*, Kumaran and Karunakaran method was applied [16] where quercetin is used as standard. In this procedure, 1 mg of plant extracts in methanol was mixed with 1 ml of aluminium trichloride in Ethanol (20 mg/ml) and added a drop of acetic acid. Then diluted up to 25 ml with ethanol and measured the absorbance at 415 nm after 40 min. The absorption of blank samples and standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions.

Determination of total phenolic content

Folin-Ciocalteu method was followed to determine the total phenolic content of *F. jangomus* extracts, Folin-Ciocalteu oxidized the extracts whereas sodium carbonate neutralized it [17] formed blue color solution and the absorbance were measured at 760 nm after 60 min by using gallic acid (GA) as standard. Total Phenolic content was expressed as mg GA equivalent/gm of extract.

RESULTS

DPPH radical scavenging activity

The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. In DPPH radical scavenging assay of different extracts of *F. jangomus* is showed in concentration dependent scavenging of DPPH radical as was with the reference ascorbic acid (Figure-1). The IC_{50} value of the Chloroform, Methanol and Pet ether extracts were 523.15 µg mL^{-1}, 1623.87 µg mL^{-1} and 5811.35 µg mL^{-1} respectively while, the IC_{50} value of well known antioxidant Ascorbic Acid was 13.37 µg mL^{-1}. Scavenging of DPPH radical was found to rise with increasing concentration of the extracts.

Reducing power capacity assay

Figure-2: shows the reductive capabilities of the *F. jangomus* plant extracts compared to Ascorbic acid that was determined by using the potassium ferricyanide reduction method. The reducing power of the extracts was moderately strong while increasing dose it shows little increment. However, Chloroform extract shown the highest reducing power, then it was Methanol and Pet ether extracts.

Total antioxidant capacity, total phenol and total flavonoid contents

The total antioxidant capacity, total phenol and total flavonoid content of *F. jangomus* plant extracts were expressed in ascorbic acid, gallic acid and quercetin equivalents respectively and are represented in Table 1. The content of phenolics in the extracts under this investigation correlates with the antioxidant activity; being highest in Ethyl Acetate extract (32.62 mg/g GAE), Methanolic extract showed moderate results (15.79 mg/g GAE). Among the selected plant extracts the highest amount of flavonoid was found in methanolic extract (12.53 mg/g quercetin equivalent) while Water and Methanolic fraction exhibited the most prominent and similar result for total antioxidant capacity and shown in Table 1.

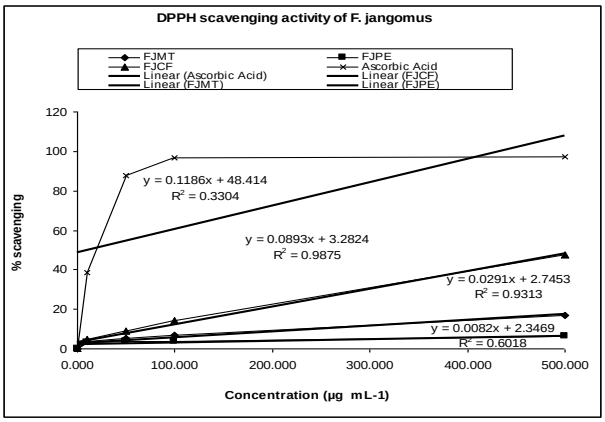

Fig 1: DPPH radical scavenging activity of different extract of *F. jangomus*

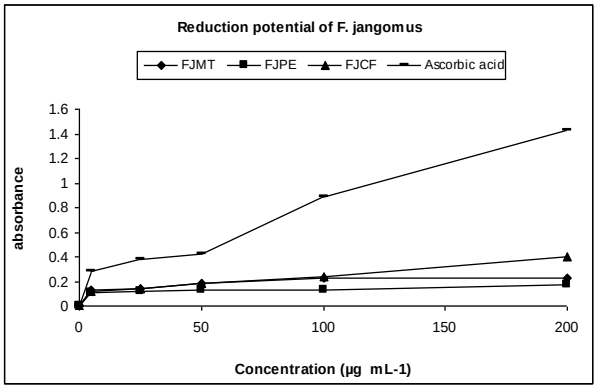

Fig 2: Reducing power of the crude plant extracts of *F. jangomus* and ascorbic acid.

Table 1: Total antioxidant capacity, total phenol and total flavonoid contents *of F. jangomus.*

Extracts	Total antioxidant (in mg/g, ascorbic acid equivalents)	Total phenol (in mg/g , Gallic acid equivalents)	Total flavonoid (in mg/g, quercetin Equivalents)
FJCF	84.534	25.54	30.82
FJPE	67.674	76.27	24.69
FJMT	54.883	35.14	22.65

Here, FJCF, FJPE and FJMT denote for Chloroform, Pet ether and Methanol extracts of *F. jangomus* respectively.

DISCUSSION

Different studies show that different types of polyphenolic compounds (flavonoids, phenolic acids) found in plants have multiple biological effects, including antioxidant activity [18] and present studies indicate the presence of polyphenolic compounds in different extracts of *F. jangomus.* Though, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes [19]. In DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Chloroform extract of *F. jangomus* showed good DPPH scavenging activity where ascorbic acid was chosen as the reference antioxidant for this test. The reducing properties are generally associated with the presence of

reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [20].

CONCLUSION

From the experiment, it can be implicit by considering the results that the plant extract of *F. jangomus* possess good antioxidant potential which correlates traditional use in various diseases conditions as well as with various established research reports. However, the study conducted is only a preliminary and there are plenty of scopes for further thorough investigation for better understanding of its exact pharmacological activities, mechanism of action as well as the active compound(s) responsible for these actions.

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