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Research Article

ANTIOXIDANT ACTIVITIES OF TWO MEDICINAL VEGETABLES: *PARKIA JAVANICA* AND *PHLOGACANTHUS THYRSIFLORUS*

KHANGEMBAM VICTORIA CHANU[1]*, M. AYUB ALI[2], MEENA KATARIA[1]

[1]Division of Animal Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P-243122, India, [2]Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Aizawl, Mizoram-796014, India. *Email: drvictoriachanu@rediffmail.com

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ABSTRACT

Antioxidant activity of aqueous, ethanolic and methanolic extracts of two common vegetables of North-eastern states of India, *Parkia javanica* and *Phlogacanthus thyrsiflorus* have been estimated by three different methods: DPPH free radical scavenging, FRAP and Ascorbate-Iron (III) induced lipid peroxidation inhibition assay. The activity was expressed as both mg trolox and ascorbic acid equivalent (TE and AAE) per gram of extract for DPPH and FRAP assay. Methanolic extract of *P.thyrsiflorus* showed highest value of 61.07 and 77.29 mg TE/gm of extract in DPPH and FRAP assay respectively whereas its ethanolic extract caused maximum inhibition of lipid peroxidation. The extracts were also tested for its total phenol content (TPC) and correlated with antioxidant activity. In data analysis it was found that there was a positive correlation between TPC and antioxidant activity of the extracts (DPPH, R^2 = 0.6247; FRAP, R^2 = 0.785; Lipid peroxidation inhibition, R^2 = 0.3606). From this study it can be concluded that these two medicinal vegetables has high antioxidant property and should further be investigated for novel drug discovery.

Keywords: *Parkia javanica, Phlogacanthus thyrsiflorus*, Antioxidant activity, DPPH, FRAP, Total phenolic content, Lipid peroxidation,

INTRODUCTION

Antioxidant has become one of the most important topics of today's world because of its health benefits. These are a diverse group of chemicals which protect the body from oxidative damage induced by free radicals and reactive oxygen species by suppressing their formation[1]; acting as scavengers[2]; and acting as their substrate[3]. The best known natural antioxidants include hydrophilic compounds, such as vitamin C, thiols and flavonoids, as well as lipophilic compounds, such as Vitamin E, Vitamin A, Carotenoids and ubiquinols. Practically the natural antioxidants are obtained through ingestion of plant products such as fruits, vegetables, nuts, flours, vegetable oil, drinks and infusions, taken fresh or as processed foodstuffs[4]. There are reports that people who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases[5] and there is evidence that some types of vegetables, and fruits in general, protect against some cancers. Since fruits and vegetables happen to be good sources of antioxidants, this suggested that antioxidants might prevent some types of diseases. Today screening of natural products represents one of many approaches used to discover new drugs. Among various Indian medicinal plants reported, *Parkia javanica* and *Phlogacanthus thyrsiflorus* are two such medicinal plants which are consumed as vegetables.

P. javanica is a leguminous tree in the family of *Leguminosae* found in most of South East Asian countries. Various parts of the plant are edible right from the inflorescence and tender pods to mature seeds. The seeds are consumed as food either cooked or raw due to its high nutritional value. The bark and the pods of *P. javanica* are used by traditional healers of Manipur for treatment of various ailments such as diabetes[6], intestinal disorder, bleeding piles, diarrhea and dysentery[7].

P. thyrsiflorus is a gregarious shrub of Acanthaceae family. The inflorescence of the plant is consumed as vegetable and leaves are medicinal[8]. Both the leaves and flowers are cooked with fish and meat as a delicacy in Jaintia tribes of Meghalaya, North-east India[9]. It is an extremely popular medicinal plant and one of the highly preferred medicinal plants, by the traditional healers of Nepal[10]. The use of this plant in Assam for its anti-allergic effect was reported by Kalita and Bora[11] where the patients are given curry prepared from aerial portion of the plant, with rice once daily until cure. There are reports on the use of different parts of the plant as anti-septic, insecticide and in curing coughs and cold, chronic bronchitis, asthma and rheumatism[12]. According to Khanikar[13], flowers of *P. thyrsiflorus* are antidote to pox, used in jaundice, prevents skin diseases like sore, scabies etc. The leaves of *P. thyrsiflorus* are used against Gout, rheumatism in Chakma community of Bangladesh[14].

As stated above *P. javanica* and *P. thyrsiflorus* have been reported to be used in various medicinal preparations but information on antioxidant activities of these plants are very scanty. So, this study has been undertaken to find out the antioxidant activities of different extracts of these two plants by DPPH (1,1-diphenyl -2-picrylhydrazyl) free radical scavenging, FRAP (Ferric Reducing Antioxidant Potential) and Ascorbate-Iron(III) induced lipid peroxidation inhibition assay and to correlate with their total phenol content. Outcome of this study will present information on antioxidant activities of the plant extracts ensuring easier selection of the extract with higher antioxidant activity for further exploration.

MATERIALS AND METHODS

Plant extracts

Seeds of *P. javanica* and leaves of *P. thyrsiflorus* were collected from Imphal, Manipur, India and were authenticated by a Senior lecturer, Department of Botany, Imphal College, Manipur University, Manipur.

Both seeds and leaves were air dried in shade and powdered. The powdered plant materials were then extracted separately with deionised water, ethanol and methanol in a soxhlet extraction apparatus. Evaporation of the solvent yielded the crude dry extract and the extracts were used for estimation of antioxidant activity and total phenol content.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8–tetramethyl-chromane-2-carboxylic acid (Trolox), Ascorbic acid and Thiobarbituric acid (TBA) were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate ($FeCl_3$. $6H_2O$), Folin-Ciocalteau Phenolic reagent, Sodium carbonate were obtained from Merck (Darmstall, Germany). Trichloroacetic acid (TCA) was obtained from Sisco Research Laboratories (SRL), Mumbai. All the chemicals used were of analytical grade.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and

Shui[15]. DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm. Eighty microlitres of extract (2 mg/ml) was added to 3 ml of DPPH solution and the decrease in absorbance was measured at different time intervals until the absorbance remained constant. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity and vice versa. A standard curve for calibration was constructed using trolox (250 – 1250 µg/ml) and the free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of extract. Another standard curve using ascorbic acid (176-704 µg/ml) was constructed and the results were expressed as mg ascorbic acid equivalent (AAE) per gram of extract. The experiment was carried out in triplicate and the results were expressed as mean values.

Ferric Reducing Antioxidant Potential (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain[16]. Briefly, 50µl of extract (2 mg/ml) was added to 3 ml of FRAP reagents (10 parts of 300 mM sodium acetate buffer of pH 3.6, 1 part of TPTZ and 1 part of 20 mM Ferric chloride solution). The reaction mixture was incubated at 37^{0}C for 30 min and the increase in absorbance was measured at 593 nm using spectroscan 2600 UV/Vis Spectrophotometer (Chemito). The standard curve was constructed using trolox (250 - 1000 µg/ml) and the results were expressed as mg Trolox equivalent (TE) per gram of extract. Another standard curve using ascorbic acid (176 - 704 µg/ml) was constructed and Ferric reducing antioxidant potential of the extracts was also expressed as mg ascorbic acid equivalent (AAE) per gram of extract. All the measurements were taken in triplicate and the mean values were calculated.

Ascorbate-Iron (III) Induced Lipid Peroxidation Inhibition

The assay was performed as described by Aruoma and coworkers[17] with slight modification. 100 mg of goat liver was homogenated with 10 ml of phosphate buffer saline (PBS, pH 7.4). 0.2 ml of liver homogenate was mixed with 0.1 ml of 1 mM $Fecl_3$ and 0.1 ml of extract (2 mg/ml). The peroxidation was initiated by adding 0.1 ml of 1 mM ascorbate. The mixture was incubated at 37^{o}C for 60 minutes. After incubation 1 ml of 10% trichloroacetic acid was added and mixture was centrifuged at 1800 rpm for 10 min. After centrifugation 1 ml of supernatant was collected and mixed with 1 ml of 0.67% thiobarbituric acid (TBA). The mixture was vortexed and heated in boiling water bath at 100^{o}C for 20 min. The mixture was then rapidly cooled and the extent of oxidation inhibition was estimated from the absorbance of the organic layer at 532 nm. A tube containing all the reaction mixture except the plant extract was used as control. The percent inhibition was calculated with the formula:

$$\text{Percent inhibition (\%)} = \frac{\text{(Abs control – Abs sample) X 100}}{\text{Abs control}}$$

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method[18]. One hundred microlitres of extract (2 mg/ml) were added to 1 ml of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 900 µl of sodium carbonate (7.5%) solution. After 1 hour incubation at room temperature, the absorbance was measured at 640 nm using spectroscan 2600 UV/Vis Spectrophotometer (Chemito). Different volume (20 -100 µl) of Gallic acid (100 µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE)/gm of extract. Triplicate measurements were taken and mean values calculated.

Statistical analysis

All data on antioxidant activity and total phenol content were the average of triplicate analysis and presented as mean±standard deviation (SD). Correlation analysis of antioxidant activity versus the total phenolic content was carried out using the correlation and regression programme in the Microsoft EXCEL program.

RESULTS AND DISCUSSION

The use of traditional medicine is widespread and plant still represents a large source of natural antioxidants that might serve as leads for the development of novel drugs[19]. The major antioxidants of vegetables are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids. Phenolic compounds, especially flavonoids, possess different biological activities, but the most important are antioxidant activity, capillary protective effect, and inhibitory effect elicited in various stages of tumor[20,21,22,23]. Because of the complex nature of phytochemicals in extracts, often a dozens of compounds with different functional groups, polarity and chemical behavior, could lead to scattered results, depending on the test employed. Therefore an approach with multiple assays for evaluating antioxidant activities of extracts would be more enlightening and even indispensable. In this study, three antioxidant activity assays, DPPH free radical scavenging activity, Ferric Reducing antioxidant potential and Ascorbate-Iron (III) Induced Lipid peroxidation inhibition assay were employed. The concentrations of total phenol of the extracts were also investigated and correlation between antioxidant activity and total phenol content was studied.

DPPH radical scavenging activity is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical DPPH-H. Substances which are able to donate hydrogen or an electron to DPPH, nitrogen centered free-radical, can be considered as antioxidants and therefore radical scavengers. The degree of discoloration of violet colour of DPPH, as it gets reduced, indicates the radical scavenging potential of the antioxidant[24]. The DPPH free radical scavenging activities of the extracts are presented in Table 1. Methanolic extract of *P.thyrsiflorus* had the highest DPPH radical scavenging activity of 61.07 mg TE/gm of extract and the lowest was observed in ethanolic extract of *P. javanica* with a value of 16.33 mg TE/gm of extract. As displayed in Table 2 the radical scavenging activity of the extracts as ascorbic acid equivalent varied from 20.42 mg AAE/gm ethanolic extract of *P. javanica* to 76.33 mg AAE/gm methanolic extract of *P. thyrsiflorus*. The free radical scavenging activity of the plants is in agreement with their widespread use in traditional medicine preparation. Since free radicals are highly toxic, can cause cellular damage, due to lipid peroxidation, causes serious derangements, such as ischemia-reperfusion injury, coronary arteriosclerosis, diabetes mellitus and neurodegenerative diseases. It is also associated with aging and carcinogenesis[25].

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant reaction[26]. Ferric reducing antioxidant assay depends upon the reduction of ferric-TPTZ (Fe (III)-TPTZ) to blue ferrous-TPTZ (Fe (II)-TPTZ). A higher absorbance indicates a higher ferric reducing power. Table 1 and 2 show difference in the antioxidant activity depending on the solvent used for extraction. The reducing potential of the *P. javanica* extracts showed a wide variation ranging from 15.11 mg TE and 8.24 mg AAE/gm methanolic extract to 48.79 mg TE and 26.6 mg AAE/gm of aqueous extract. Extracts of *P. thyrsiflorus* showed higher reducing power ranging from 52.96 mg TE and 28.88 mg AAE/gm of ethanolic extract to 77.29 mg TE and 41.81 mg AAE/gm of methanolic extract. The trend for ferric reducing potential of the extracts did not vary from their DPPH free radical scavenging activities with their Fe (III) reducing potential consistently higher than those obtained for DPPH assay. The probable reason for the lower DPPH radical scavenging activity of the extracts could be due to the presence of compounds not reactive towards DPPH. Antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently due to steric hindrance[27].

Lipid peroxidation is one of the major causes of deterioration in foods that results in the formation of potentially toxic compounds. In human, lipid oxidation is also thought to induce physiological obstruction, causing aging of the cells and carcinogenesis[28]. Ascorbate-Iron Induced Lipid Peroxidation Inhibition assay of the plant extracts was performed using goat liver homogenate. The

percent inhibition caused by the extracts ranged from 6.09 by ethanolic extract of *P. javanica* to 81.28 by methanolic extract of *P. thyrsiflorus* (Table 1). Medicinal properties of *P. javanica* and *P. thyrsiflorus* and their use in various medicinal preparations[7,14] may be because of its high antioxidant properties. *P. thyrsiflorus* in combination with other plants, is also used for treatment of cancer in Mizoram[29]. The use of this plant in treatment of cancer can be linked with its high antioxidant properties since free radicals are associated with carcinogenesis and the physiological role of antioxidant compound is to scavenge for free radicals[25, 30].

Table 1: Antioxidant activity of the plant extract estimated by DPPH radical–scavenging assay, ferric reducing antioxidant potential (FRAP) assay, total phenolic content determination and Ascorbate iron induced lipid peroxidation

Name of the plant	Types of extract	DPPH assay(mg of Trolox equivalent / gm)[a]	FRAP assay(mg of Trolox equivalent / gm)[b]	Ascorbate-Iron Induced Lipid Peroxidation (% inhibition)[c]	Total Phenolic contents (mg of GAE/ gm)[d]
	Aqueous	37.93±0.31	48.79±0.25	42.91±0.35	51.09±0.78
Parkia javanica	Ethanolic	16.33±0.12	23.12±0.25	6.09±0.50	22.53±0.20
	Methanolic	22.13±0.50	15.11±0.34	35.77±1.41	7.11±0.20
	Aqueous	44.4±1.39	58.9±0.35	76.74±0.0	46.02±0.47
Phlogacanthus thyrsiflorus	Ethanolic	42.53±0.31	52.96±0.69	81.28 ±0.1	37.34±0.54
	Methanolic	61.07±0.61	77.29±0.51	79.13±0.20	48.75±1.43

[a,b,c,d] Mean of three determinations ± S.D. (standard deviation)

Table 2: DPPH free radical scavenging activity and Ferric reducing antioxidant potential of the extracts with ascorbic acid as standard

Name of the plant	Types of extract	Antioxidant activity	
		DPPH assay (mg of ascorbic acid equivalent / gm)[a]	FRAP assay (mg of ascorbic acid equivalent / gm)[b]
Parkia javanica	Aqueous	47.42±0.38	26.6±0.14
	Ethanolic	20.42±0.14	12.61±0.14
	Methanolic	27.67±0.63	8.24±0.19
Phlogacanthus	Aqueous	55.58±1.66	32.12±0.19
thyrsiflorus	Ethanolic	53.17±0.38	28.88±0.38
	Methanolic	76.33±0.76	41.81±0.68

[a,b]Mean of three determinations ± S.D. (standard deviation)

The antioxidant activity of plants is mainly contributed by the active compounds present in them. Phenolics are one of the antioxidants commonly found in most plants. Generally, extracts that contain a high amount of polyphenols also exhibit high antioxidant activity[27]. Good correlation co-efficients for total phenol content and antioxidant activities of methanolic extract have also been reported for the plant *Lygodium flexuosum* [31]. As shown in Table 1 aqueous extract of *P. javanica* revealed maximum total phenol content of 51.09 mg of GAE/gm of extract. *P. thyrsiflorus* showed higher antioxidant activity in all the assays but it had a slightly lower total phenol content ranging from 37.34 to 48.75 mg GAE/gm of ethanolic and methanolic extract respectively as compared to aqueous extract of *P. javanica*. However, there are reports of good correlation between antioxidant activity and total phenolic compounds in some Chinese medicinal plants[27]. The correlation between antioxidant activity and TPC was investigated by using scatterplots of Microsoft EXCEL program. It was found that TPC was positively correlated with DPPH free scavenging activity (R^2 = 0.6247) (Fig. 1) and Ferric Reducing antioxidant potential (R^2 = 0.785) (Fig. 2). However, the ability of the extracts to inhibit Ascrbate-Iron (III) induced lipid peroxidation showed a very low correlation (R^2 = 0.3606) with total phenol content (Fig. 3). This might be due to the presence of other compounds showing activity indicating that the antioxidant activities of plant extracts are not limited to only phenolics. The activity may also come from the other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins[32].

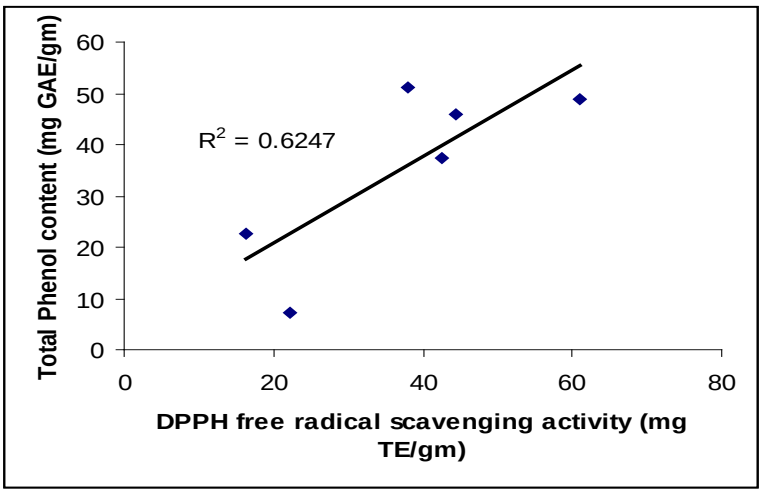

Fig. 1: Relation of DPPH free radical scavenging activity of the extracts (mg TE/gm) with Total Phenol content (mg GAE/gm).

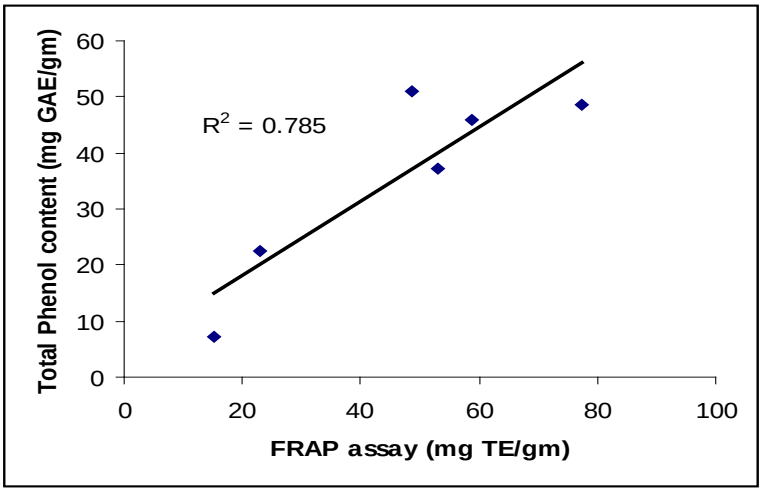

Fig. 2: Relation of Ferric Reducing Antioxidant Potential of the extracts (mg TE/gm) with Total Phenol content (mg GAE/gm).

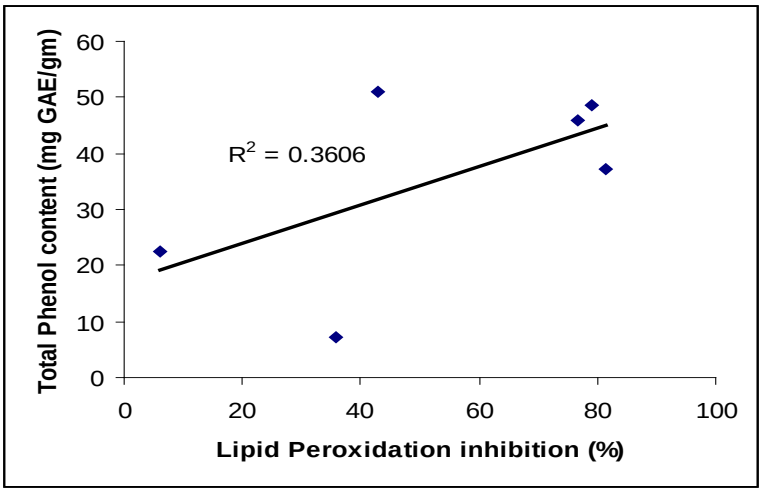

Fig. 3: Relation of percent inhibition of Ascorbate-Iron(III) induced Lipid Peroxidation by the extracts with total phenol content (mg GAE/gm).

CONCLUSIONS

P. javanica and *P. thyrsiflorus* are two very common vegetables of north-eastern states of India which are used in various traditional medicines. In this study antioxidant activity of different extracts of the two plants were determined by three different methods and correlated with their total phenol content. The present study showed high antioxidant properties with positive correlation to TPC. So these two plants can be considered as a good source of natural antioxidants and should be further analyzed for their chemical and biological properties.

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