ASIAN JOURNAL
ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 4, pp.059-064, November, 2011

RESEARCH ARTICLE

IN VITRO EVALUATION OF PHENOL, FLAVONOID AND ANTIOXIDANT PROPERTIES OF METHANOLIC EXTRACT OF *Phyllanthus fraternus* Webster

Das, R and Handique P J*

Department of Biotechnology, Gauhati University, Guwahati-781014, Assam, India

Received 25th May, 2011; Received in revised form; 18th June, 2011; Accepted 19th July, 2011; Published online 9th November, 2011

Antioxidant activity of fresh and dried *Phyllanthus fraternus* Webster (Euphorbiaceae) plant materials were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Antioxidant properties of methanolic extracts from shed dried plant showed strong free radical scavenging activity where inhibition (%) observed in aerial parts was 62.89 ± 0.027. The IC_{50} value of the aerial portions of the plant is 56.12 ± 0.050 where as catechin is 50.51 ± 0.022 at 20µg/ml. Total Phenol and Flavonoid of the methanolic extracts were estimated. Total phenolic content measured by Folin Ciocalteu reagent in terms of catechol equivalent content was 280mg ± 0.035. The total flavonoid content in the *Phyllanthus fraternus* was also determined by spectrophotometrically and calculated as quercetin equivalent content was 75.5±0.027. Plant phenolics are highly effective for free radical scavengers and exhibit strong antioxidant activity. Result suggests that the plant has great potential in the drug industry as functional drug ingredient.

Key words: *Phyllanthus fraternus*, Antioxidant, free radicals scavenging, Phenols, Flavonoids, 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

INTRODUCTION

Antioxidant research is an importance topic in the medical field as well as in the food industry. Recent research with importancet bioactive compounds in many plant and food materials have been received much attention. The oxidation induced by reactive oxygen species (ROS) can result in cell membrane disintegration, membrane damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular diseases (Liao and Yin, 2000). Free radicals and other reactive species present in the body can be generated both endogenously and exogenously. Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers and other degenerative diseases (Halliwell & Grootveld, 1987). Under normal circumstances, the free radicals generated in the body can be removed by the body's natural antioxidant defenses, e.g. glutathione peroxidase, catalase, and superoxide dismutase (Aruoma, 1994). Endogenously produced antioxidants are not enough to protect the cumulative effects of oxidative damage caused by ROS that remained in our system. Adequate antioxidants are required to inhibit the chain reaction of oxidation be supplied as natural or synthetic food additives. However, synthetic antioxidants have many side effects (Madsen and Bertelsen, 1995). Hence, plant based natural antioxidants have been supplied to our system

***Corresponding author:** pjhandique@rediffmail.com

through leafy vegetables, fruits, seeds, cereals and algae (Pokorny, 1991). The natural antioxidants are much safer and they also possess anti-viral, anti-inflimatory, anti-cancer, anti-mutagenic, anti-tumor and hepatoprotective properties.

Besides, phenolic compounds and flavonoids are also widely distributed in plants. Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, slikimate and phenylpropanoid pathways in plants (Randhir *et al.,* 2004). These compounds are one of the most widely occurring groups of photochemical of considerable physiological and morphological important in plants. Phenolics have been reported to have a capacity to scavenge free radicals. They are commonly found in both edible and non-edible plants and have multiple biological effects including antioxidant activity. The antioxidant activity of phenolics is mainly due to their radox properties (Rice *et al.*,1996, Kahkonen *et al.*,1999; Valenzuela *et al.*,2003, Hsu, 2006). Flavonoids are naturally occurring polyphenolic compounds with a C_6-C_3-C_6 backbone. This group of plant pigments which are found in fruits, vegetables, grains, herbs bark, roots, stems, flowers, tea and wine. Over 5000 different flavonoids have been identified in plant materials (Harborne *et al.*,2000, Humadi and Istudor, 2008). Polyphenolic compounds of flavonoids properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995).

Natural antioxidants especially phenolics and flavonoids from fruits, vegetables, spices, herbs, tea and wine they already exploited commercially either as nutritional supplements (Schuler, 1990, Patel *et al.*,2010). Also many other plant species have been investigated in the search for novel antioxidants (Chu *et al.*,2000). Still there is a demand to find more information concerning the antioxidant potential of plant species as they safe and also bioactive. Thus interest in natural antioxidant, especially of plant origin has greatly increased in recent research (Jayaprakash and Rao, 2000). The plant *Phyllanthus fraternus* Webster, annual herb locally known as Bhuiamlakhi in Assamese, belong to the family Euphorbiaceae. The plant is grown in the tropical areas throughout the world including, China, Malaysia, Indonesia, Pakistan, Sri Lanka and in India, Uttar Pradesh, Bihar, Gujarat, Maharashtra, Kerala, West Bengal, entire north eastern part of India specially in Assam, Arunachal Pradesh and Manipur. The species have been widely used in folk medicine and in Ayurveda for the treatment of diarrhea, dysentery, dyspepsia and colic, dropsy, anti-diabetic, anti-malarial, jaundice, glactagogue, gonorrhea and diseases of urinogenital system (Grag 1969, Sharma 1986, (Unander *et al.*,1991). (Fig: 1, 2, 3 & 4)

MATERIALS AND METHODS

Chemical

Chemicals and reagents, including 2, 2-diphenyl-1-picrylhydrazyl (DPPH), catechins, quercetin, catechol, Folin Ciocalteu reagent were purchased from Sigma-Aldrich Co (USA), methanol (spectrograde). All other chemicals used in this study were of the reagent grade.

Plant Extract Preparation

The collected plant materials were dried in Greenhouse, crushed to powder using mortar and pestle and extracted by Soxhlet apparatus with the solvents methanol at 65^0C for 4 cycle and overnight stay. The plant extract was concentrated by Rotavapour and temperature set at 55^0C for the water bath at 115 voltage output. The extracts were kept at 4ºC until they were submitted to the antioxidant assay.

Free radical scavenging activity

Scavenging activity of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radicals of plant extracts or catechin were measured according to the method reported by Chang *et al.*,(2001) with minor modifications. Assays were performed in 3 mL reaction mixtures containing 2.0 mL of 0.1 mM DPPH-methanol solution,0.9 mL of 50 mM Tris-HCl buffer (pH 7.4), and 0.1 mL of methanol(as control) or plant extracts as test sample. After 30 min of incubation at room temperature, absorbance of the reaction mixtures were determined by Double Beam UV-VIS Spectrophotometer,

Fig. 1

Fig. 2

Fig. 3

Fig. 4.

Fig: 1. Naturally grown *Phyllanthus fraternus*, Fig: 2. Cultivated *Phyllanthus fraternus*
Fig: 3. Twig of the Plant and Fig: 4. Shed dried Plants

Spectrascan Uv-2600(Chemito) at 517 nm. The inhibitory effect of DPPH was calculated according to the following formula:

Inhibition (%) = [(Absorbance Control – Absorbance Sample)/Absorbance Control] × 100

IC_{50} of the extract was determined by the method described by Molyneux (2004).

Determination of Total Phenolic Content

The total phenolic content of the plant extract was determined spectrophotometrically using Folin Ciocalteu reagent according to method described by Sadasivan and Manikam (2003). The dilute methanolic extract (0.5ml of 1:10g ml^{-1}) was mixed with Folin Ciocalteu reagent (5ml; 1:10 diluted with distilled water) and aqueous sodium carbonate (Na_2 CO_3). The tubes containing reaction mixture was mixed thoroughly and boiled in a beaker for exactly 1minute and cooled at room temperature. After incubation of 30minutes the total phenols were determined by Double Beam UV-VIS Spectrophotometer, Spectrascan Uv-2600(Chemito) at 650nm. The total phenol content was expressed as mg catechol equivalent per100g of fresh materials.

Determination of Total Flavonoid Content

The total flavonoid content was determined by using Aluminium Chloride Colorimetric method with minor modification (McDonald *et al.*,2001, Chang *et al.*,2002, Pourmorad *et al.*,2006, Lim *et al.*,2008, Siddique *et al.*,2009, Doss *et al.*,2010). Each plant extract (0.5ml of 1.10g ml^{-1}) in methanol was separately mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 minute. The absorbance of reaction mixture was measured at 415nm with a Double Beam UV-VIS Spectrophotometer, Spectrascan Uv-2600 (Chemito). The calibration curve was plotted by preparing the quercetin solution at concentration 12.5 to 100g ml^{-1} in methanol.

Statistical Analysis

Measurements of absorbance were made in triplicate and the results presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Total Phenol and Flavonoid of the methanolic extracts

Bioactive components and antioxidant properties of *Phyllanthus fraternus* has been the area of research to justify the claims of traditional healers. The present study showed that the medicinal plants were the good source of antioxidant substances. The high potential of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups present in the plant cells (Sewa *et al.*,1999). It has been recognized that flavonoids show significant antioxidant action on human health and fitness. The flavonoids act through scavenging or chelating process (Kessler *et al.,* 2003). Plant phenolics are highly effective for free radical scavengers and exhibit strong antioxidant activity. Total phenolic content were measured by Folin Ciocalteu reagent in terms of catechol equivalent content was 280mg ± 0.035. The total flavonoid content in the *Phyllanthus fraternus* was also determined by spectrophotometrically and calculated as quercetin equivalent content was 75.5±0.027 (Table. 1).

Antioxidant Activity

Antioxidants are molecules, which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Free radicals are major factors leading to more than sixty different health problems including aging, cancer and atherosclerosis. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism that lead to degenerative diseases.

DPPH stable free radical method is an easy, rapid and sensitive way to analyze the antioxidant activity of plant extracts. Scavenging activity for free radicals of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant. Plant extracts from *Phyllanthus fraternus* medicinal plants for investigation of antioxidant activities. Catechin, a major phenolic constituent was employed as the reference compound in this experiment. Free radical scavenging activity of total crude extracts of *Phyllanthus fraternus* medicinal plants was quantitatively determined using a DPPH assay at

Table 1.Total phenolic content and total flavonoid content of methanolic extract from *Phyllanthus fraternus*

Plant Extract	Total phenolic (mg of catechol/g)	Total flavonoid (mg/g of sample)
Phyllanthus fraternus	280 ± 0.035	75.5 ± 0.027

Total phenols are expressed as catechol equivalent; Total flavonoid are expressed as mg of total flavonoid content/g of samples based on quercetin as standard values represent mean ± SD (n=3)

Table 2. Free Radical Scavenging activity of *Phyllanthus fraternus* extract are measured with DPPH.

Scientific Name	Common Name	Part Used	% of Inhibition ± SD
Phyllanthus fraternus	Bhuiamlakhi	Aerial Part	62.89 ± 0.027

Table 3. DPPH radical scavenging activity of *Phyllanthus fraternus* and Catechin

Sl no.	Concentration(µg/ml)	*Phyllanthus fraternus*	Catechin
1	10	48.97 ± 0.083	42.85 ± 0.031
2	20	56.12 ± 0.050[a]	50.51 ± 0.022[a]
3	30	78.57 ± 0.137[a]	56.12 ± 0.001[a]
4	40	80.10 ± 0.067[a]	58.67 ± 0.002[a]
5	50	82.14 ± 0.070[a]	60.71 ± 0.054[a]

Data reported as mean ± SD, where n=3; 50% and above of DPPH radical is considered as significant for scavenging activity[a].

517nm with Double Beam UV-VIS Spectrophotometer, Spectrascan Uv-2600 (Chemito). Antioxidant properties *Phyllanthus fraternus* exhibited strong activity on scavenging DPPH radicals in methanol extracts of Green House Shed-dried plant at 37^0C for 14 days and same plant was oven-dried at 65^0C (for 20 minutes) respectively i.e. Inhibition (%) 62.89 ± 0.027 (Table:2). The IC_{50} value of the methanolic extract of *Phyllanthus fraternus* was 56.12 ± 0.050^a where as catechin 50.51 ± 0.022^a at 20 µg/ml (Table: 3)

Conclusion

The result of methanolic extract of *Phyllanthus fraternus* showed strong antioxidant and free radical scavenging activity. It has been recognized that the total flavonoid and phenolic content enhance the free radical scavenging activity due to the presence of hydroxyl groups. Free radical mediated processes have been implicated in the pathogenesis of most of the diseases. It is well documented that free radicals take part in the pathogenesis of a large number of diseases (Gyamfi *et al.*,1999). The aerial parts of the *Phyllanthus fraternus* showed higher antioxidant activity than catechin in scavenging DPPH free radicals, which indicates that *Phyllanthus fraternus* extract has good potential as a source for natural antioxidants to prevent free radical mediated oxidative damage. It also suggests that it has great potential in the drug industry as functional drug ingredient.

ACKNOWLEDGMENT

The authors are very grateful to the UGC, North Eastern Regional Office, Guwahati, for granting the Award of Teacher Fellowship for this studies.

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