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In vitro antioxidant activity of *Abelmoschus moschatus*

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Abstract
Abelmoschus moschatus is a perennial plant of the tropics and subtropics which is cultivated in the tropical regions of Asia, Africa and South America for its seeds. The antioxidant activity of the roots of the plant was evaluated by three *in vitro* assay methods *viz.* DPPH free radical scavenging, ferric reducing antioxidant potential assay and total phenolic content determination. *The DPPH free radical scavenging activity, FRAP and total phenolic content estimated were respectively* 834.60±45.84 mg/ 100 g TE, 14.00±0.69 g TE/ 100 g and 117.54mg GAE/100 gm.

Keywords: *Abelmoschus moschatus*, DPPH, frap, total phenolic.

Introduction
Abelmoschus moschatus is a perennial plant of the tropics and subtropics which is found at the elevations upto 1,100 meters. It is cultivated in the tropical regions of Asia, Africa and South America for its seeds which are used mostly for the isolation of fragrance components (Rao *et al.*, 2005) [1]. In India, it is found wild all over the hilly regions of Deccan and Karnataka and also at the foothills of Himalayas (Khare, 2004) [2]. The young leaves, shoots and unripe seedpods are cooked and used as a vegetable. The seed is also used as a flavouring for liqueurs or to scent coffee. An essential oil is obtained from the plant and is used to flavour baked goods, ice cream, sweets and soft drinks. It is rich in a number of phenolic compounds, flavonoids, carbohydrates, proteins, steroids, tannins, fixed oil and fats. *Abelmoschus moschatus* has been extensively studied by various researchers for its biological activities and therapeutic potentials such as diuretic, antioxidant activity and free radical scavenging, antiproliferative, antimicrobial, antilithiatic, hepatoprotective, memory strengthening, antidiabetic, hemagglutinating, anti-ageing, antidepressant, anxiolytic, anticonvulsant, hypnotic and muscle relaxant activity (Pawar *et al.*, 2017) [3]. The seeds of this plant (powdered form in lukewarm milk) have been recommended for use in various traditional systems of medicine for the treatment of intestinal complains, constipation, dyspepsia and gonorrhea. The seeds are also used as stimulant, relaxant and also for casting out the poison of snakes. It also serve as cardiac tonic, aphrodisiac, diuretic and antispasmodic (Gul *et al.*, 2011) [4].

Reactive oxygen species (ROS) are produced inside the body as a byproduct of the reactions and also because of exogenous factors. ROS includes a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical (OH^-) etc. (Gul *et al.*, 2011) [4]. Inside the body, some of the ROS play a positive role in energy production, phagocytosis, cell growth regulation and inter-signal or the synthesis of biological important compounds (Pietta, 2000, Lallianchhunga *et al.*, 2016) [5,6]. However, when ROS exceed the antioxidant capacity of the biological systems, oxidative stress occurs which has been associated with artherosclerotic diseases, cancer, diabetes, arthritis, reperfusion damage and inflammation etc. (Rahman *et al.*, 2013) [7]. Antioxidants can scavenge free radicals produced due to environmental pollutants, radiations, chemicals, toxins, deep fried and spicy foods as well as physical stress, thereby preventing harmful effects on DNA, intracellular proteins, membrane lipids, and change in gene expression and can raise the level of endogenous antioxidant defense. There is dynamic balance between the amount of free radicals generated in the body and antioxidants against their deleterious effects (Finkel and Holbrook, 2000; Ali *et al.*, 2012) [8, 9]. However, the amounts of these protective antioxidant

principles present under the normal physiological conditions are sufficient only to cope with the physiological rate of free radicals, either from environment or produced within the body can tip the free radical (pro-oxidant) and anti-free radical (antioxidant) balance leading to oxidative stress; which may result in tissue injury and subsequent disease (Sies, 1991) [10]. In order to maintain the level of antioxidant in the body for healthy living, external supplementation is necessary (Ali *et al.*, 2012) [9]. Recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals. There is an increasing interest in natural antioxidants e.g. polyphenols present in medicinal and dietary plants which might help preventing oxidative damages ((Silva *et al.*, 2005; Lallianchhunga *et al.*, 2016) [11, 6]. Keeping in view of the above mentioned facts, the present investigation was conducted to evaluate the antioxidant activity of the *Abelmoschus moschatus* roots by three *in vitro* assay methods *viz*. DPPH free radical scavenging assay, FRAP assay and total phenol content assay.

Materials and Methods

Plant material

The fresh roots of *Abelmoschus moschatus* were carefully collected from Champhai district, Mizoram and identified by the Department of Botany, Mizoram University, Aizawl (No. MZU-FLM/BOT-HRB/01-19 dt. 10.01.2019).

The plant material was air dried in shade, protecting them from direct sunlight for a period of one week. On complete drying, the roots of the plant was ground to powder with the help of Willey / Laboratory Mill and sifted through sieve number 22. The dry powder of roots was subjected to Soxhlet extractor and rotary evaporator (Vinothapooshan and Sundar, 2010) [12].

A weighed quantity (500g) of the plant powders were then subjected to continuous hot extraction in Soxhlet apparatus with petroleum ether, methanol, chloroform. The extract was evaporated under pressure using a rotovac evaporator until all the solvent had been removed to give an extract sample. The yield of the extracts was recorded.

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), Gallic acid, Bovine brain extract (Sigma, B-3635) 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-Ciocalteu 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 (2001) [13]. DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm. 40 µl of extract 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 was prepared using trolox (250 -1250µg/ml) and the free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie and Strain (1999) [14]. Briefly, 50µl of extract 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 37^0C for 30 min and the increase in absorbance was measured at 593 nm using UV/Vis Spectrophotometer. The standard curve was prepared using trolox (250 -1000µg/ml) and the results were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method of Singleton and Rossi (1965) [15]. One hundred (100) microlitres of extract was added to 1ml 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 hr incubation at room temperature, the absorbance was measured at 640 nm using UV/Vis Spectrophotometer. Different volume (20-100µl) of Gallic acid (100µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE) /gm of dry leaves.

Results and Discussion

The antioxidant activity of the methanolic extract of the *Abelmoschus moschatus* roots was evaluated by three *in vitro* assay methods *viz*. DPPH free radical scavenging assay method, FRAP assay and Total phenolic content assay. The antioxidant activity of the extract based on DPPH free radical scavenging and FRAP assay are expressed as mg trolox equivalent (TE) whereas total phenolic content of the extract are expressed as mg gallic acid equivalent (GAE) per gram of the dry sample. In all the three methods of assay, the antioxidant content was high. The antioxidant content observed is given in Table1.

Table 1: Antioxidant content of *Abelmoschus moschatus* roots

Sl. No.	Methods of estimation	Antioxidant content/ 100 g of dry roots
01	DPPH free radical scavenging method	834.60±45.83mg TE
02	FRAP assay	14.00±0.69mg TE
03	Total phenolic content	117.54mg GAE

The DPPH free radical scavenging is one of the generally accepted mechanisms against lipid oxidation. Difference between DPPH free radical binding method and other method is the short run time allowing rapid determination of the radical scavenging. The effect of antioxidants on DPPH free radical scavenging was thought to be due to their hydrogen donating ability. The DPPH free radical scavenging activity of *Abelmoschus moschatus* roots in the present investigation was 834.60±45.84 mg/ 100 g TE. The presence of high antioxidant activity of *Abelmoschus moschatus* is also reported by other researchers (Pascal *et al.*, 2018, Pawar and Vyawahare, 2017, Gul *et al.*, 2011) [16, 3, 4].

The ferric reducing antioxidant potential assay is based on the reducing power of a compound (Antioxidant). It measures the reduction of Fe^{3+} (Ferric iron) to Fe^{2+} (Ferrous iron). As the ferric to ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that

of Fe^{3+}/ Fe^{2+}, the values in the FRAP assay expresses the corresponding concentration of electron donating antioxidants. The FRAP activity in the present investigation was 14.00±0.69 g TE/ 100 g dry roots. The high antioxidant activity of *Abelmoschus moschatus* in terms of ferric reducing/ antioxidant power (FRAP) is also reported by other researchers (Pascal *et al*., 2018, Gul *et al.*, 2011) [16, 4]. Pascal and coworkers (2018) [16]. Reported FRAP value of 6.38 mmol EAA/g of the fresh immature fruits of *Abelmoschus moschatus* while Gul and associates (2011) [4]. Reported that leave extracts of *Abelmoschus moschatus* exhibited considerably higher reducing power for Fe3+ than the seed extracts. The reducing ability of the leaf extracts was in the range of 3.02-6.28 mg/AAE/g dw. The FRAP values for the seed extracts were in the range of 0.38-0.54 mg AAE/g dw.

Phenolic compounds are herbal substances whose chemical structures may range from quite simple compounds to highly polymerized substances. The capacity of phenolics to scavenge free radicals may be due to many phenolic hydroxyl groups they possess. Phenols play an important role in antioxidant activity, because they transfer hydrogen to radicals and produce phenoxide radical, which is stabilized. Therefore, it is important to determine the total amount of phenolics to determination of antioxidant capacity of plants. The total phenolic content of *Abelmoschus moschatus* roots in the present investigation was 117.54mg GAE/100 g dry roots. The total phenolic content of the *Abelmoschus moschatus* in seeds and leaf and immature fruits were also evaluated by Gul *et al*. (2011) [4]. and Pascal *et al.* (2018) [16]. The total phenolic content in the seed extracts range from 1.56 to 3.74 mg GAE/g dw while in leave extracts the value ranges between 9.49 to 13.84 mg GAE/g dw (Gul *et al*., 2011). The total phenolic content in immature seeds was 28.655 mg/100 mg fresh immature fruits (Pascal *et al.*, 2018) [16].

Conclusion

Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or prevent the onset of degenerative diseases because of their redox properties, which allow them to act as hydrogen donors, reducing the agents, hydroxyl radicals (OH.) or superoxide radical (O2.) scavengers. Numerous studies have shown that *Abelmoschus moschatus* had a high phenolic content and antioxidant activity. In our study also, high content of phenolic compounds and antioxidant activity was observed.

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