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PROXIMATE ANALYSIS, ANTIOXIDANT ACTIVITY OF *ABELMOSCHUS ESCULENTUS (L)* FLOWER EXTRACT

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ABSTRACT

Methanol extract of *Abelmoschus esculentus (L)* flower was investigated for proximate analysis and antioxidant activity tests. Proximate analysis of the flowers of *Abelmoschus esculantus.* (L) revealed to contain carbohydrate, protein, fat, fiber, ash and moisture content The moisture content, total ash, carbohydrate value were 7.27%, 14.55%, 47.69% respectively. For the evaluation of antioxidant activity, five complementary test systems namely 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power assay, total antioxidant capacity, Nitric oxide and $ABTS^{+}$ activity determination methods were used. Methanol extract revealed higher Nitric oxide scavenging activity (68.01 ± 0.02), DPPH (50.01 ± 0.02) and $ABTS^{+}$(48.01 ± 0.01 AAE/g) but lower reducing power activity than other extracts at 500µg/ml concentration.

KEYWORDS: *Abelmoschus esculentus (L),* Proximate analysis, Antioxidant activity.

INTRODUCTION

Almost 25 centuries ago, Hippocrates, the Father of Medicine, stated, "*Let food be thy medicine and let medicine be thy food".* Amidst ancient civilisations, India has been known to be rich repository of medicinal plants.[1] Today a number of chemicals obtained from plants are used as vital drugs in more countries in the world.[2] Nutrition is an important aspect of public health because it is linked to many significant diseases and health problems. The consumption of vegetable foods has been a public health issue; based on existing research, polyphones may be applicable to public health in primary and secondary

prevention, particularly concerning diseases associated with oxidative damage such as obesity, diabetes, cardiovascular disease, and cancers. Finding alternative and complementary ways to reduce the oxidative processes might have a beneficial interest in the context of developed countries.[3]

Antioxidants are our first line of defense is maintaining optimum health and well being. Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by acting as oxygen scavengers.[4] Antioxidants are described as a "substance that when present in low concentrations relative to the oxidisable substrate significantly delayed or reduced oxidation of the substrate".[5] The need for antioxidants becomes even more critical with increasing exposure to free radicals. Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems.

Phenolic compounds are the one that give vegetables fruits, grains, seeds, leaves, flowers and bark their colors. Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble compounds which give colour in fruits and vegetables.

Foods with a high flavonoid content include parsley, onions, blueberries and other berries, black tea, green tea and oolong tea, bananas, all citrus fruits, *Ginkgo biloba*, red wine, sea-buckthorns, and dark chocolate (with a cocoa content of 70% or greater).

Free radicals contribute to many different diseases. Free radicals are generated largely during the production of ATP in the mitochondria. The ultraviolet light that penetrate the skin and the air pollutant that is high in smog which we inhale generates free radicals too. Food, like lipid in the presence of (Fe3+, Fe2+) lead to the production of hydrogen peroxide from which further hydroxyl radicals are generated in a reaction that appear to depend on the presence of iron ions.[6] Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish. Estimates suggest 85% of American dietary intake of lycopene comes from tomatoes and tomato products.[7]

MATERIALS AND METHODS

Flower sample collection

The fresh flower samples were collected in the month of October, from in and around Nagapattinam, Nagapattinam district, Tamil Nadu, India. The flower samples were thoroughly washed under running tap water to remove adhering dust particles and blotted dry under shade for about two weeks, ground into milled powder and stored in an airtight container used for further investigations.

Preparation of extracts

The powdered *Abelmoschus esculentus* (L) flower samples (1000g) were weighed and mixed with 2000 ml of methanol. Then it is kept in an orbital shaker at 190-220 rpm for 48 hours. The supernatant was collected, filtered through Whatman No.1 filter paper and then concentrated by evaporating to dryness which gave a solid amorphous residue and it was dried thoroughly to remove the solvent used. The obtained dried extract was then accurately weighed, stored in small vials and used for the subsequent studies.

PROXIMATE ANALYSIS

The proximate analysis was done according to AOAC method 1990. The analysis carried out includes moisture content, fat content, crude protein, crude fibre, ash content, carbohydrates and energy.

IN VITRO ANTIOXIDANT ACTIVITY

Total antioxidant capacity

The total antioxidant capacity was determined by Kumaran and Karunakaran.[8] 0.3 ml of the extract (10 mg/ml) was mixed with 3ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in Eppendoff tube. The tubes were capped and incubated in a thermal block at 95°C for 90 minutes. After 90 minutes, the mixture was cooled to room temperature; the absorbance was measured at 695 nm against reagent blank. Methanol (0.3 ml) in the place of extract is used as the blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

DPPH Scavenging Activity

The free radical scavenging capacity of the methanol extract of was determined by using DPPH.[9] DPPH (200 µm) solution was prepared in 95% methanol. Various concentration of

flower extract (50 to 250µg/ml) was taken in five test tubes. Then 0.5 ml of freshly prepared DPPH solution was incubated with test drug and after 15 minutes, absorbance was taken as 517nm using spectrophotometer. Standard ascorbic acid was used as reference.

Reducing Power Assay

The Reducing Power of the methanol extract of was determined.[10] 1 ml of varying concentration (1-5 mg/ml) of extract was mixed with 2.5 ml phosphate buffer and 2.5 ml of Potassium ferric cyanide. The mixture was incubated at 50° C for 20 minutes. Aliquots of 2.5 ml of Trichloro acetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with equal volume of distilled water to this 0.5 ml of freshly prepared ferric chloride solution was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

NITRIC OXIDE SCAVENGING ACTIVITY[11]

The nitric oxide scavenging activity of the methanol extract of was determined.[11] Sodium Nitroprusside (5 mµl) in standard Phosphate buffer solution was incubated with different concentrations (50-250 µg/ml) of the extract and made upto 5 ml with Phosphate buffer (0.025 m, pH 7.4) and tubes were incubated at 25˚C for 3 hours. Control tube without the flower extract but with equivalent amount of buffer was maintained in an identical manner. After 3 hours 0.5 ml of the incubated solution was removed and diluted with 0.5 ml of Griess reagent (1% Sulphanilic acid, 5% Phosphoric acid and 0.1% Napthyl ethylene diaminedihydrochloride). The absorbance of the chromophoric formed during diazotization of nitrite ions with Sulphanilic acid and it subsequent coupling with napthyl ethylene diamine was read at 546 nm.

$ABTS^+$ RADICAL SCAVENGING ACTIVITY[12]

The $ABTS^+$ radical scavenging activity of the methanol extract of was determined.[12] Varying concentration(100 µg -500 µg) of flower extract and different concentration(50-250 µg/ml) of standard ascorbic acid solution were taken in a series of test tubes. 0.3 ml ABTS solution was added and the volume was made up to 2.5 ml with phosphate buffer. To the control 0.3 ml of ABTS solution and 2.2 ml of Phosphate buffer. The solution was read immediately at 734 nm.

RESULTS

Table 1: Proximate Analysis of *Abelmoschus Esculentus*.(l) flowers.

S.NO	PARAMETERS	RESULTS (g / 100 g)
1.	Protein	4.03
2.	Fat	1.92
3.	Fiber	24.54
4.	Carbohydrate	47.69
5.	Ash	14.55
6.	Moisture	7.27
7.	Energy(Kcal)	224.16

Table 2: Evaluation of antioxidant activity by DPPH assay.

S.No	Concentration (µg/ml)	Methanolic Extract % of Inhibition
1	50	20.02 ± 0.02
2	100	25.01 ± 0.02
3	150	30.03 ± 0.03
4	200	40.02 ± 0.01
5	250	50.01 ± 0.02

Assay was performed in triplicates. Values are expressed as mean ± SD

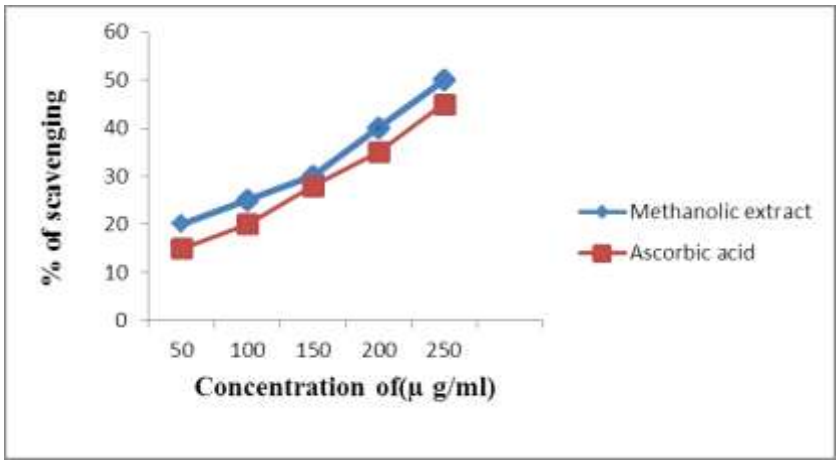

Fig 1: Evaluation of antioxidant activity by DPPH assay.

Table 3: Evaluation of antioxidant activity by Nitric oxide scavenging activity.

S. no	Concentration (µg/ml)	Methanolic Extract % of Inhibition
1	50	15.02 ± 0.02
2	100	32.01 ± 0.02
3	150	42.03 ± 0.03
4	200	55.02 ± 0.01
5	250	68.01 ± 0.02

Assay was performed in triplicates. Values are expressed as mean ± SD.

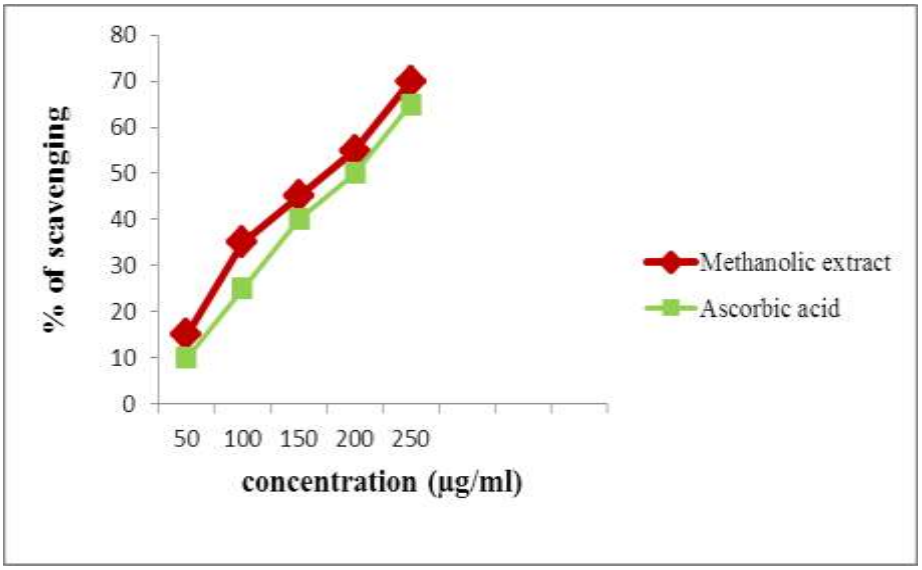

Fig 2: Evaluation of antioxidant activity by Nitric oxide scavenging activity.

Table 4: Evaluation of antioxidant activity by ABTS$^+$ radical scavenging activity

S. No	Concentration (µg/ml)	Methanolic Extract % of Inhibition
1	100	17.02 ± 0.01
2	200	25.01 ± 0.03
3	300	33.01 ± 0.03
4	400	45.03 ± 0.02
5	500	48.01 ± 0.01

Assay was performed in triplicates. Values are expressed as mean ± SD

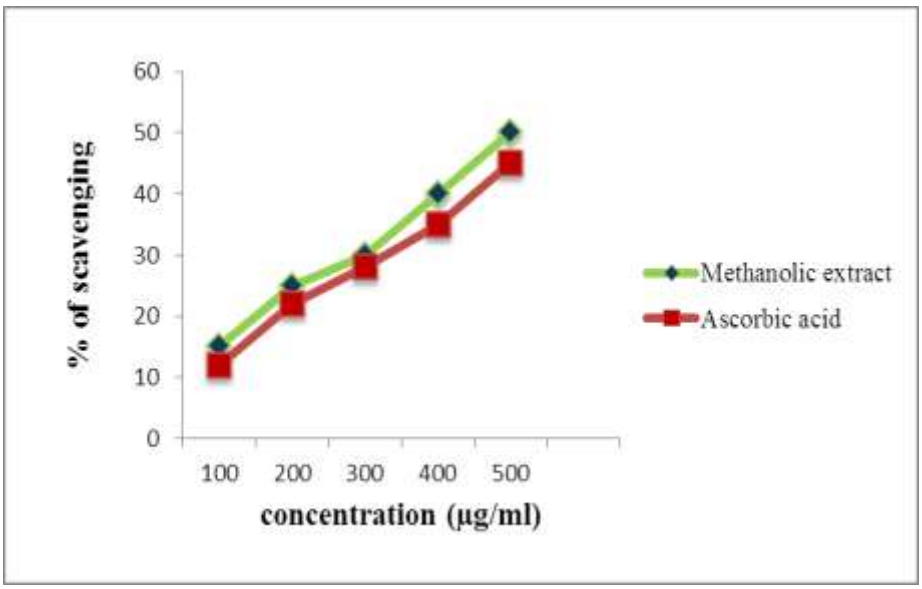

Fig 3: Evaluation of antioxidant activity by ABTS$^+$ radical scavenging activity.

Table 5: Evaluation of antioxidant activity by reducing power assay.

S. NO	Concentration (µg/ml)	Methanolic extract % of inhibition
1	50	15.02 ± 0.02
2	100	20.03 ± 0.01
3	150	25.01 ± 0.02
4	200	36.03 ± 0.02
5	250	45.01 ± 0.02

Assay was performed in triplicates. Values are epressed as mean ± SD

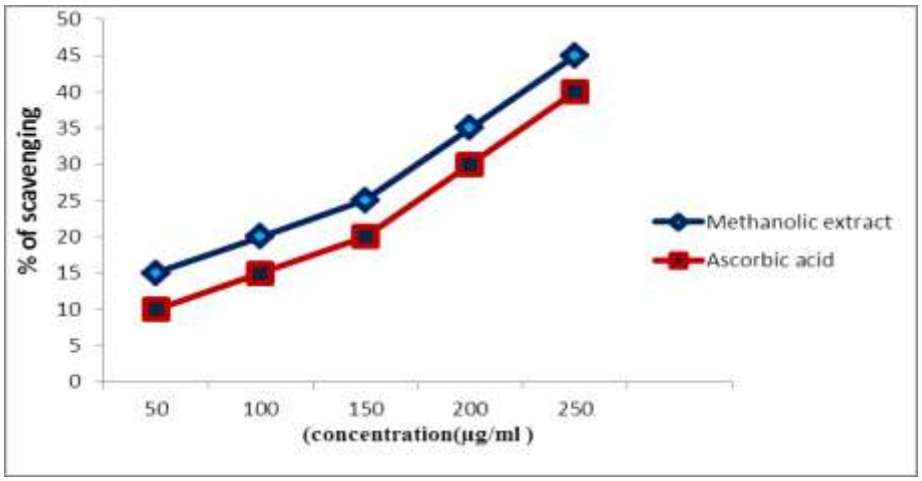

Fig 4: **Evaluation of antioxidant activity by reducing power assay.**

DISCUSSION

The powdered flower of *Abelmoschus esculentus*.(L) was subjected to evaluate its moisture content, total ash, carbohydrate, fiber, fat and protein extractive value. The air dried sample contains 7.27% moisture. The low moisture content of the flower would hinder the growth of microorganism and storage life would be high.[13] The total ash (14.55%) indicates that the flower is comparatively rich in fiber elements. The extractive values for carbohydrate, fat, protein and fiber were 47.69%, 1.92%, 4.03% and 24.54% respectively (Table 1).

The free radical scavenging activity of *Abelmoschus esculentus*.(L) was accessed by the DPPH assay. The DPPH values were increased in a dose dependent manner. DPPH is frequently used to determine radical scavenging activity of natural compounds as it is a stable free radical The present study illustrated that DPPH free radical scavenging activity of *Abelmoschus esculentus*.(L) increased with the increase of concentration of the extract. In case of reducing power assay, with the increase of concentration, the absorbance and of the extract and standards increased gradually. Recently, it has been reported that there is a direct correlation between antioxidant capacities and reducing power of certain plant extracts.

Nitric oxide is a potent pleiotropic inhibitor of physiological process such as smooth muscle relaxation, neural signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities.[25] In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions.[26] In the present investigation, flower extract of *Abelmoschus esculentus*.(L) exhibited outstanding scavenging effects on DPPH, ABTS, nitric oxide and H_2O_2 radicals. It was observed that the flower extract contained high level of phenolic and flavonoid content that might have accounted for the strong activity observed against the free radicals. Results revealed that flowers of *Abelmoschus esculentus*.(L) have many phytochemical constituents which may be responsible for many pharmacological activities. 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.[24] The reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The study revealed that the presence of reductones might contribute significantly to the reductive capability of the flower extracts. Never the less, based on the above presented results, flower extract of *Abelmoschus esculentus*.(L) could be investigated as a possible new source of natural antioxidants in the food, nutraceuticals and cosmetic industry.

CONCLUSION

It can be concluded that *Abelmoschus esculentus*.(L) flower contains nutrients and antioxidants and the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

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