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Evaluating The Phytochemistry of Abelmoschus Moschatus Medik Seed Extract and Its Antioxidant Properties

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ABSTRACT

Abelmoschus moschatus, commonly known as musk mallow or Ambrette native plant growing in tropical regions of Asia, Africa, and South America. It is a traditional medicinal plant and its phytochemical studies showed that the plant is rich in phenolic compounds, flavonoids, terpenoids, carbohydrates, proteins, sterols, tannins, fixed oils, and fats notably present in its seeds and leaves. Seeds are also a rich source of various secondary metabolites including myricetin, hyperin, hibifolin, quercetin, isoquercetin, adenosine and stigmasterol. A. moschatus solvent extract contains higher level of polyphenols and flavonoids which are responsible for antioxidant and other cumulative activities, so it can be used as food and medicine mainly to improve insulin sensitivity. Phytochemistry of the plant have wide range of therapeutic properties, including antioxidant, antiproliferative, diuretic, antidiabetic, antimicrobial, antilithiatic, antidepressant, hepatoprotective, memory-enhancing, anti-ageing, anxiolytic, anticonvulsant, hypnotic, and muscle relaxant effects (Liu et al., 2010). The seeds of A. moschatus were collected in March, 2022 from the local fields of Nalgonda District, Telangana state. The collected seeds were washed; shade dried at room temperature (25-30°C) for 7-10 days and coarsely powdered using a mechanical grinder, sieved and used for extraction. The ethanolic extract of the seeds showed a variety of phytochemicals including alkaloids, flavonoids, tannins, glycosides, and saponins, confirming its effectiveness in extracting both moderately polar and polar constituents. The aqueous extract showed abundant polar phytoconstituents, including alkaloids, carbohydrates, tannins, and mucilage, highlighting water's ability to extract hydrophilic compounds. These findings validate the traditional uses of A. moschatus seeds and emphasize their potential as a natural source of antimicrobial and phytotherapeutic agents. Further in vivo and mechanistic studies are recommended to substantiate its therapeutic

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applications.

Keywords: Abelmoschus moschatus, Antioxidant, Flavonoids, Myricetin, Phytochemistry.

I. INTRODUCTION

Abelmoschus moschatus Medik., commonly called with various local names like wild okra, musk mallow or Ambrette, Latakasturi (Hindi), Kasturi Bende (Kannada/Telugu), and Kaattu Kasturi (Tamil/Malayalam) is tropical hibiscus-like plant belonging to the Malvaceae family. The plant is highly valued for its kidney-shaped seeds which emit a robust, natural musky aroma. Hence, the name given musk mallow. It is a fragrant and medicinal plant native to India, Africa, and South America valued for its rich content of dietary fibre and used in various indigenous medicines to treat various health issues. Since ancient times these medicinal practices were deeply rooted in local cultures and passed down through generations, habitually used when contemporary medical services are unreachable or in addition to conventional care and thus this herb playing a vital role in herbal medicine.

Plants produce various chemicals to defend themselves against pests and predators such as insects, fungi, and herbivorous animals. Many of these bioactive amalgams have been found to have therapeutic effects in humans. Currently, with an increased demand for herbal therapies, extensive research is proceeding to explore plant-based medicines (Ankit *et al.,* 2025). Within this context, *A. moschatus* is predominantly cultivated in tropical regions of Asia, Africa, and South America. Phytochemical evaluation of the plant material mainly seeds and leaves revealed that the plant is a rich source of phenolic compounds, flavonoids, quinines, alkaloids, sterols, tannins, terpenoids, myricetin, fixed oils, carbohydrates, proteins, and fats. Therapeutic investigations had confirmed a wide range of healing properties, including diuretic, antioxidant, antiproliferative, antimicrobial, antilithiatic, hepatoprotective, memory-enhancing, antidiabetic, anti-ageing, antidepressant, anxiolytic, anticonvulsant, hypnotic, and muscle relaxant effects (Liu *et al.,* 2010; Ahiakpa *et al.,* 2013; Mohite and Gurav, 2019).

A. moschatus is known for its aromatic compound and it has been used in Chinese traditional medicine as a remedy for depression and anxiety. The effect of decoction has been reported to exhibit hypotensive properties (Mohite and Gurav, 2020). The volatile compounds identified from *A. moschatus* seeds having an odour similar to the musk therefore used in perfume and cosmetics formulations (Pieroni and Ansari, 2005). Liu *et al.,* (2005) showed the hypoglycaemic activity of myricetin, a compound found in the seed extract, in diabetic rats. Maheshwari and Kumar, (2009) studied the antimicrobial activity of *A. moschatus* leaf extracts.

A. moschatus seed extract is a rich source of antioxidants -including flavonoids and phenolic compounds have potent scavenging activity against free radicals and reduce oxidative stress. It shows significant DPPH, hydrogen peroxide, lipid peroxidation, and superoxide radical scavenging activities. These antioxidant properties contribute to its reported anti-inflammatory, neuroprotective, and anti-aging effects (Sudhakar *et al.,* 2018). The aromatic seeds of this plant are aphrodisiac, ophthalmic, cardio tonic, antispasmodic and used in the treatment of intestinal complaints and check queasiness (Gul *et al.,* 2011).

II. Material and Methods:

The seeds of *A. moschatus* were collected in March, 2022 from the local fields of Nalgonda District, Telangana state.



Fig. a. *Abelmoschus moschatus* twig, b. Dried seeds

Drying and powdering of seeds:

The collected seeds were washed with normal tap water and after thoroughly washed with distilled water; the shade dried under shade at room temperature (25°C) for 7-10 days until dry enough and to prevent direct exposure of sunlight, which could degrade sensitive phytochemicals. The dried seeds were coarsely powdered using a mechanical grinder and sieved to get fine and uniform powder. This powder was used for extraction.

Extraction preparation:

100 grams of weighed dried seed's powdered was subjected to successive solvent extraction using a Soxhlet apparatus. The extraction was carried out with solvents of increasing polarity opening with petroleum ether (60-80°C) followed by ethanol and finally distilled water to ensure exhaustive extraction of phytochemical constituents. Each extraction was continued for 6-8 hours until the solvent in the siphon tube became colourless. The extracts obtained were concentrated under reduced pressure using a rotary vacuum evaporator and subsequently dried in a desiccator. The percentage yield of each extract was calculated, and the dried extracts were stored in airtight glass vials at 4°C for further phytochemical and pharmacological studies. The method was performed according to (Kokate *et al.,* 2014) with slight modifications.

III.Chemicals:

All the chemicals were purchased from Hi-Media and Merck, India. Analytical grade solvents were used in this study.

Assessment of total phenolic content:

The total amount of soluble phenolic content in seed extracts was determined according to the Folin-Ciocalteu method with a few modifications (Yang *et al.,* 2007). In brief, 10µL of extract solution was mixed with 100µL of Folin-Ciocalteu reagent. After 10 min of incubation, 300µL of 20% Na_2CO_3 solution was added and the final volume was adjusted to 1 mL by using distilled water. The mixture was incubated in dark for 2 hours and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer against blank samples. The total phenolic content present in the seed extract was measured as gallic acid equivalents (mg GAE)/gram of dry weight and the values were presented as means ± SEM.

DPPH radical scavenging activity:

The antioxidant property of the seed extracts was assessed on the basis of the radical scavenging effect according to the method of Braca *et al.,* (2002) using stable 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH solution (0.004% w/v) was prepared in 95% methanol and serial dilutions were carried out with the stock solutions (20 mg/mL) of the extracts. Different concentrations of extracts were mixed with DPPH solution (900 µL), incubated in dark for 30 min and then measured the absorbance at 517 nm.

Hydrogen peroxide scavenging activity:

Modified method of Long *et al.,* (1999) was used to determine the Hydrogen peroxide scavenging activity. Ferrous ion oxidation - xylenol orange (FOX) reagent was prepared by adding nine volumes of reagent 1 (4.4 mM butylated hydroxytoluene (BHT) in methanol) to one volume of reagent 2 (1 mM xylenol orange and 2.56 mM ammonium ferrous sulphate in 250 µM H_2SO_4). Seed extracts of various concentrations were incubated with 10 µL of 40 mM H_2O_2 for 10 min at room temperature in dark and 0.2 mL of FOX reagent was added and the final volume was made up to 1 mL with distilled water. The reaction mixture was vortexed and incubated at room temperature for 30 min. Violet colour indicates control reaction and discoloration indicates scavenging activity after the addition of seed extracts. The FOX reagent without extracts/H_2O_2 served as blank and with H_2O_2 served as control. The absorbance of the ferric-xylenol orange complex was measured at 560 nm.

Lipid peroxidation (LPO) scavenging activity:

Lipid peroxidation (LPO) scavenging activity states that the capacity of antioxidants to inhibit the oxidative degradation of lipids, a process often triggered by free radicals that damages cell membranes. This activity was measured by the inhibition of malondialdehyde (MDA) production, a key byproduct of lipid peroxidation. LPO was estimated by the method of Bhuyan *et al.,* (1981). The absorbance of the pink-coloured trimethine condensation product was measured at 533 nm on a spectrophotometer. The results were expressed in nanomole of TBARS/g tissue (TBARS - TBA-reactive substances).

Superoxide dismutase (SOD) scavenging activity:

Kakkar *et al.,* (1984) method was adopted with slight modifications to assess the superoxide dismutase scavenging activity of seed extract of *A. moschatus.* In brief, reaction mixture containing 156 µM nitroblue tetrazolium (NBT) dissolved in 50 mM phosphate buffer (pH 7.4), 468 µM nicotinamide adenine dinucleotide (NADH) and seed extract were mixed. In addition to 100 µL of 60 µM phenazine methosulfate (PMS) solution, the reaction was started and the final volume of the reaction was 3 ml. It was incubated at 25°C for 5 min and absorbance was read at 560 nm and NADH used as control samples.

Catalase scavenging activity: Catalase is a vital antioxidant enzyme that scavenges hydrogen peroxide in aerobic organisms by catalysing its decomposition into water and molecular oxygen. Catalase activity was assessed by measuring the rate of decomposition of H_2O_2. Disappearance of peroxide in the presence of enzyme source is the basis for assay which was measured spectrophotometrically at 505 nm. One unit decomposes one micromole of H_2O_2 per minute.

IV. Discussion:

The present study mainly focussed on evaluation of phytochemistry of *Abelmoschus moschatus* seed extract and in vitro antioxidant properties of phytochemicals present in the seed extract. *A. moschatus* seeds medicinally most active part and is a rich source of bioactive volatile compounds including ambrettolide, farnesyl acetate, 2-methylbutyl-2-methylbutanoate. Seed components also include sterols, resins, and fatty

acids. In addition, the plant contains flavonoids (myricetin), glycosides, terpenoids, saponins, and sterols (beta-sitosterol), fixed oils, and resin which contribute to its antioxidant, antimicrobial, and medicinal properties (Ankit *et al.,* 2025). Earlier study reports (Ankit *et al.,* 2025) showed that the seed extract revealed presence of a variety phytochemicals. The petroleum ether extract contained steroids, sterols, and fixed oils, indicating the presence of non-polar compounds. The ethanolic extract demonstrated polar phyto-profile like alkaloids, flavonoids, tannins, glycosides, and saponins. The aqueous extract showed ample polar phytoconstituents, including alkaloids, carbohydrates, tannins, and mucilage.

Natural antioxidants widely present in fruits, vegetables and medicinal plants since they are effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants. The present study is a step towards the exploration of natural antioxidants from seed extracts of *A. moschatus* employing free radical scavenging assays in addition to anti-proliferative and antimicrobial activities (Ratnam *et al.,* 2006).

The DPPH system was an extensively studied system to examine the scavenging activities of numerous natural plant extracts including phenolic compounds, flavonoids or crude mixtures. The antioxidants' activity on DPPH may be due to their hydrogen donating ability (Prathapan *et al.,* 2010). The DPPH radical scavenging capabilities of the *A. moschatus* seed extract was observed in concentration dependent manner and the scavenging activity was significant. The extract had proton donating ability and it served as free radical inhibitors thus possibly working as primary antioxidants. The antioxidant activity of *A. moschatus* extracts in DPPH assay increased proportionally to the polyphenol content (Gul *et al.,* 2011) and similar results were observed in earlier reports where increased antioxidant activities showed linear relationship between DPPH values and total polyphenols (Malencic *et al.,* 2008; Chew *et al.,* 2008).

Hydrogen peroxide, an oxidant, is continuously generated in living cells as a result of general metabolic processes. The H_2O_2 detoxification is crucial for preventing the generation of extremely reactive oxygen species including hydroxyl free radicals. *A. moschatus* seed extract possesses an effective radical scavenging activity for H_2O_2 in a concentration gradient manner. Similar results were reported that scavenging activity of H_2O_2 by seed extracts which may be attributed to their phenolics, which can donate electrons to H_2O_2 to convert it into water (Bhaskar and Balakrishnan, 2009; Ebrahimzadeh *et al.,* 2009).

Lipid peroxidation is the cellular oxidative degradation of lipids, particularly polyunsaturated fatty acids (PUFAs), initiated by reactive oxygen species (ROS) or free radicals. It is a self-propagating chain reaction that produces lipid hydroperoxides and reactive aldehydes (e.g., MDA), causing membrane structural damage, cellular dysfunction, and, if severe, regulated cell death. Sudhakar *et al.,* (2017) studies showed the increased LPO levels in NaF received rats. Moreover, it was also reported the increased LPO levels in erythrocytes, liver, kidney tissues of rabbits exposed to fluoride. Present study reports showed that the *A. moschatus* seed extract reverses lipid peroxidation levels.

Superoxide dismutase (SOD) scavenging activity is the enzymatic process where SOD neutralizes toxic superoxide radicals ($O_2^{\bullet-}$) by catalyzing their conversion into oxygen and less harmful hydrogen peroxide (H_2O_2). This critical antioxidant defense mechanism protects cells and tissues from oxidative damage, with activity levels acting as a key marker for monitoring oxidative stress. *Abelmoschus moschatus* seed extract has been shown to reverse fluoride-induced oxidative stress, which includes restoring reduced superoxide dismutase (SOD) levels.

Catalase is an important antioxidant enzyme that significantly alleviate oxidative stress by degradation of hydrogen peroxide into water and oxygen. It is mainly located in peroxisomes. *Abelmoschus moschatus* seed extract has been shown to improved catalase activity.

V. Conclusion

Abelmoschus moschatus seeds' phytochemistry is rich in phenolic compounds, flavonoids, terpenoids, carbohydrates, proteins, sterols, tannins, fixed oils, and fats. In addition, seeds also rich in myricetin, hyperin, hibifolin, quercetin, isoquercetin, adenosine and stigmasterol like secondary metabolites. Presence of higher level of polyphenols and flavonoids were responsible for therapeutic properties including antioxidant, antiproliferative, diuretic, antidiabetic, antimicrobial, antilithiatic, antidepressant, hepatoprotective, memory-enhancing, anti-ageing, anxiolytic, anticonvulsant, hypnotic, and muscle relaxant effects.

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