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Identifying the Mechanistic Role of Flavonoids Extracted from Abelmoschus Moschatus Seeds: Antioxidant Assay and it's In-Silico Analysis of Targeted Compounds

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Abstract

Introduction: Abelmoschus moschatus, known as musk mallow, is valued for its medicinal seeds with diuretic and antimicrobial properties. Flavonoids, abundant in plants, exhibit various medicinal benefits, including antioxidant and anti-inflammatory effects. This study aims to explore the antioxidant potential of A. moschatus flavonoids through assays and in-silico analysis.

Materials and methods: Prepare A. moschatus seeds by grinding them into a fine powder. Utilize ethanol or methanol for extraction, employing filtration and concentration steps. Conduct extraction with a suitable solvent like methanol using a Soxhlet extractor or ultrasonic bath. Concentrate the extract with a rotary evaporator. Generate diverse concentrations of the flavonoid extract and mix each with a2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution. Incubate in the dark, measuring absorbance at intervals. Compare results with a positive control, such as ascorbic acid, to assess antioxidant activity.

Results: In the DPPH assay, plant extract antioxidant activity increased with concentration, reaching 73.24% inhibition at 320ug, comparable to ascorbic acid. The lowest activity was at 20ug (12.34%). In the nitric oxide assay, the plant exhibited highest activity at 320ug (71.32%) and lowest at 20ug (14.23%). Molecular docking revealed apigenin's strong affinity to insulin signaling proteins, with notable binding energies and stabilizing hydrogen bonds.

Conclusion: Plant extract exhibited concentration-dependent antioxidant activity, with the highest inhibition observed at 320ug in both DPPH and nitric oxide radical scavenging assays. Molecular docking analysis revealed that apigenin demonstrated strong affinity and favorable binding energies with key regulatory proteins associated with insulin signaling. The study underscores the potential therapeutic relevance of the plant extract and apigenin in mitigating oxidative stress and regulating insulin signaling pathways.

Keywords: abelmoschus moschatus; anti-oxidant; radical scavenging; molecular docking

Introduction

Abelmoschus moschatus, commonly known as musk mallow or ambrette, is a flowering plant in the mallow family (Malvaceae). The seeds are considered the most medicinal part and are used for their diuretic, aphrodisiac, and digestive properties. They are also believed to have anti-inflammatory and antimicrobial effects. A.moschatus (family: Malvaceae) 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 [1]. A. 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, antiageing, antidepressant, anxiolytic, anticonvulsant, hypnotic, and muscle relaxant activity. In Indian indigenous system of medicine, seeds of A. moschatus are claimed to be useful for the renal calculi [2]. Flavonoids are phytochemical compounds present in many plants, fruits, vegetables, and leaves, with potential applications in medicinal chemistry. Flavonoids possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties [3]. They also have neuroprotective and cardio-protective effects. These biological activities depend upon the type of flavonoid, its (possible) mode of action, and its bioavailability. These cost-effective medicinal components have significant biological activities, and their effectiveness has been proved for a variety of diseases [4]. The most recent work is focused on their

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isolation, synthesis of their analogs, and their effects on human health using a variety of techniques and animal models. Thousands of flavonoids have been successfully isolated, and this number increases steadily. We have therefore made an effort to summarize the isolated flavonoids with useful activities in order to gain a better understanding of their effects on human health [5].

Flavonoids are secondary metabolites, which mainly consists of a benzopyrone ring bearing a phenolic or poly-phenolic groups at different positions. They are most commonly found in fruits, herbs, stems, cereals, nuts, vegetables, flowers and seeds. The presence of bioactive phytochemical constituents present in these different plants parts gives them their medicinal value and biological activities [6]. So far, over 10,000 flavonoid compounds have been isolated and identified. Most of the flavonoids are widely accepted as therapeutic agents. These are naturally synthesized through the phenylpropanoid pathway with bioactivity dependent on its absorption mechanism and bioavailability [7]. Antioxidants play a crucial role in neutralizing free radicals and reducing oxidative stress in the body. By measuring the antioxidant activity of a substance, researchers can understand its potential health benefits in preventing or mitigating diseases associated with oxidative damage, such as cancer, cardiovascular diseases, and neurodegenerative disorders [3]. Antioxidant assays allow for the comparison of different substances or compounds to determine their relative antioxidant capacities [8]. This helps in identifying the most potent antioxidants, which could be further studied or utilized for therapeutic purposes. The aim of our study is to identify the mechanistic role of flavonoids extracted from A. moschatus using anti-oxidant assays and in-silico studies.

Materials and methods

Extraction of Flavonoids from A. moschatus Seeds Prepare A. moschatus seeds by grinding them into a fine powder. Utilize ethanol or methanol for extraction, employing filtration and concentration steps. Conduct extraction with a suitable solvent like methanol using a Soxhlet extractor or ultrasonic bath. Concentrate the extract with a rotary evaporator. Generate diverse concentrations of the flavonoid extract and mix each with a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution. Incubate in the dark, measuring absorbance at intervals. Compare results with a positive control, such as ascorbic acid, to assess antioxidant activity.

Antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity: Scavenging of DPPH radical was assessed by the method of Hatano et al. (1989). Briefly, DPPH solution (1.0 ml) was added to 1.0 ml of plant extracts at different concentrations (100, 200, 300, 400, and 500 µg/ml). The mixture was kept at room temperature for 50 min and the activity was measured at 517 nm. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as standard. The capability to scavenge the DPPH radical was calculated using the following formula: DPPH radicals scavenged (%) = (Control OD-Sample OD/Control OD) × 100.

Nitric Oxide (NO) Radical Scavenging Activity

Scavenging of NO radical was assayed by the method of Garrat (1964). Briefly, the reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml), and different concentrations (100, 200, 300, 400, and 500 µg/ml) of extracts of P. granatum (0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted out and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylenediamine dihydrochloride was added, mixed, and allowed to stand for 30 min at 25°C. A pink colored chromophore is formed in diffused light. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as standard. The activity was measured at 550 nm and the results were expressed as percentage of scavenging using the following formula: Nitric Oxide radical scavenged (%) = (Control OD − Sample OD/Control OD) × 100 H (0.1-0.5 mg/ml) is preincubated at 200C for 20mins. Then 20µl of 1% starch will be added and incubated at 370C for 30mins. 100µl of DNS colour reagent will be added and boiled for 10mins. Optical density will be measured at 540nm. Acarbose will be used as standard, % inhibition = (1-AS/AC) × 100.

In-Silico Analysis of Targeted Compounds

The research focused on examining how Apigenin (CID: 280443) interacts with various proteins linked to the Insulin signaling pathway. These proteins IL6, IR, and AS160 were sourced from the Protein Data Bank (https://www.pdb.org/pdb). Docking

computations employed the Lamarckian genetic algorithm (LGA) through 100 genetic algorithm cycles, using AUTODOCK software version 1.5.6. Visualization of the docking results was done via BIOVIA Discovery Studio software.

Results

Table 1

S.no	Phytocompounds	Presence/ Absence
1	Phenols	+++
2	Tannin	++
3	Saponins	-
4	Steroids	++

Phytochemical screening was done to find out which phytocompounds were present predominantly in the *A. moschatus* plant extract. The results showed that the plant extract is mainly composed of Phenol, followed by Tannin and Steroids which was present in a moderate amount. Whereas, Saponin's presence was not seen in the plant extract. Phenols may provide protection against DNA damage and cellular abnormalities that may result in the growth of cancer by aiding in the scavenging of ROS, and reducing oxidative stress.

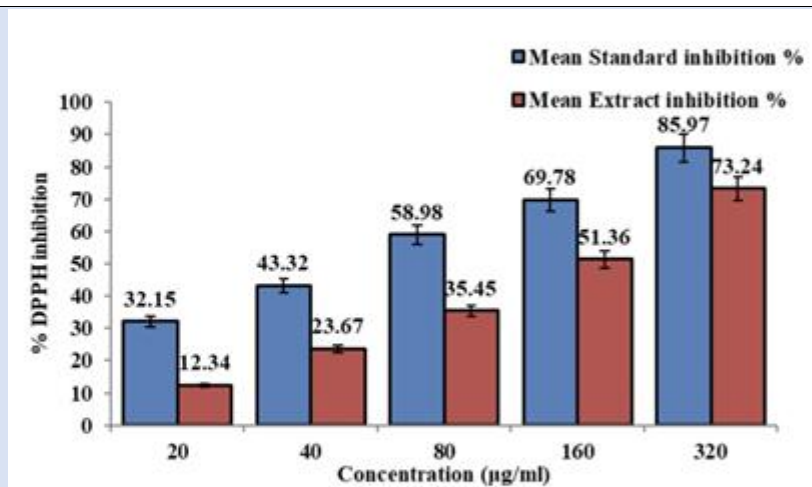

Figure 1: Anti- oxidant activity -DPPH Radical Scavenging assay. The above graph indicates the percentage of inhibition of *A. moschatus* plant extract.

DPPH assay was done to measure the anti-oxidant activity of the plant extract. The activity was measured at increasing concentration of 20ug, 40ug, 80ug, 160ug and 320ug and was compared to thestandard, ascorbic acid. It was observed that the anti- oxidant activity increased as the concentration increased. The highest activity measured was at 320ug that showed 73.24% inhibition which was almost as good as the standard. Lowest activity was measured at 20ug that showed 12.34% inhibition.

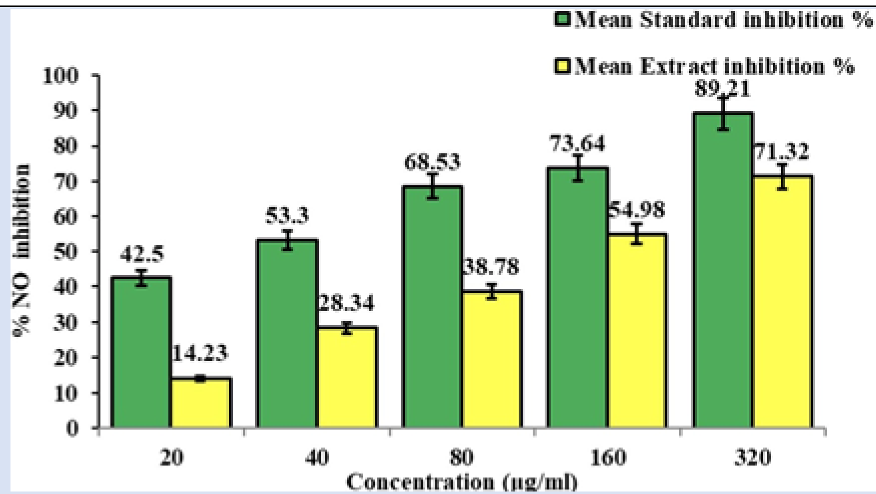

Figure 2: Nitric oxide radical scavenging activity. Percentage of inhibition of the plant extract is shown in the above graph.

Nitric oxide radical scavenging assay assay was also conducted to measure the antioxidant activity of the plant extract used. The results were further compared to the standard, ascorbic acid. The activity was measured at increasing concentration from 20ug, 40ug, 80ug, 160ug, 320ug. It was observed that the plant showed the highest antioxidant activity at 320ug that showed an inhibition of 71.32% while the lowest was measured at 20ug that showed 14.23%.

Table 2: Molecular docking analysis results using PyRx software and 3D structure visualized using Biovia Discovery Studio.

S.no	Drug	Protein	Bindingenergy	No. of 11 bonds involved	Amino acid residues
1	Apigenin (CID 280443)	IL-6	-6.4	2	LYS103, ARG63
2		IR	-7.1	4	ASN170, ASP159, SER161, TRP160
3		AS160	-6.6	1	HIS978

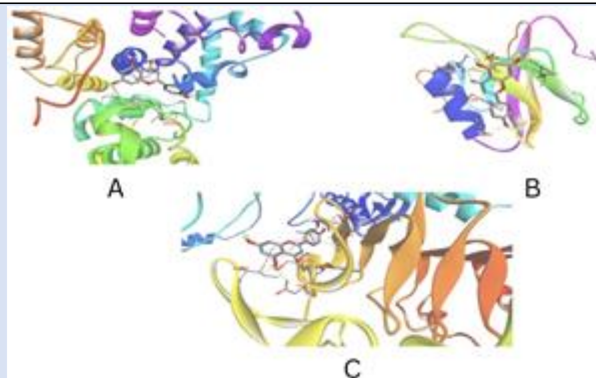

Figure 3: The above figure depicts the molecular docking results. A represents the protein IL6, B represents IR and C represents AS160.

Insilco analysis

Our study delves into molecular docking analysis to explore how apigenin binds to key regulatory proteins associated with insulin signaling. IL6, IR, and AS160 were the proteins scrutinized. The resulting binding energies, detailed in, highlighted the strong affinity of apigenin, demonstrating a notably high binding energy of IL6 (-6.4 kcal/mol), IR (-7.1 kcal/mol), and AS160 (-6.6 kcal/mol). Other proteins also exhibited favourable binding energies. Furthermore, hydrogen bonds played a significant role in stabilizing these interactions. The visual representation in Figure elucidated the 3D and 2D interactions between curcumin and the selected proteins, providing insight

into specific binding sites and structural conformations.

Discussion

Flavonoids are widely recognized for their antioxidative properties, playing a pivotal role in scavenging free radicals and preventing oxidative stress. To delve into the outcomes of our antioxidant assays, particularly employing methodologies like DPPH or ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), it is essential to emphasize the dose-dependent responses observed in the flavonoids extracted from *A. moschatus* seeds [9]. The noteworthy scavenging effects observed suggest a potential capacity of these compounds to neutralize free radicals, thereby indicating their ability to alleviate oxidative damage. We discussed the possibility of the flavonoids acting through a hydrogen atom transfer mechanism. This involves the donation of a hydrogen atom to a free radical, stabilizing it and preventing further damage. We also discussed the interaction between flavonoids and specific molecular targets implicated in oxidative stress. Strong binding affinities and the potential formation of stable complexes were highlighted [10].

In a previous study it was found that *A. moschatus* contains considerable number of total polyphenols and flavanoids and exhibited good antioxidant activity by effectively scavenging various free radicals. In addition, it has been demonstrated that *A. moschatus* is a potential antiproliferative and antimicrobial agent [11]. The antioxidant and biological activities might be due to the synergistic actions of bioactive compounds present in them. However, it is still unclear which components are playing vital roles for these activities. Therefore, further studies are still needed to elucidate mechanistic way how the plant contributes to these properties. Phytochemical investigation is also proposed to isolate the active fraction and eventually the pure compound(s) from this plant.

In another study, three chloroplast genomes of *A. moschatus*, *A. manihot* and *A. sagittifolius* were sequenced and annotated in the present study, and compared with the chloroplast genomes of *A. esculentus* and related species in Malvaceae. The results revealed the gene number and order, amino acid frequency, and codon usage were similar in *Abelmoschus*. However, the differences were also found in IR boundaries, intron-containing genes and the number of repeat sequences and SNPs. *Abelmoschus* species also showed relatively independent IR boundary traits compared with related species in Malvaceae, and identified thirty mutational hotpots might be useful for developing molecular markers and resolving taxonomic discrepancies and biogeographical origin both at genus *Abelmoschus* and family Malvaceae levels [12]. The study focused on DPPH and nitric oxide assays, providing insights into radical scavenging but not capturing the full spectrum of antioxidant mechanisms. The in-silico analysis provides valuable insights into molecular interactions but lacks confirmation through in vitro or in vivo studies, limiting the clinical relevance of the findings.

Conclusion

Plant extract exhibited concentration-dependent antioxidant activity, with the highest inhibition observed at 320ug in both DPPH and nitric oxide radical scavenging assays. Molecular docking analysis revealed that apigenin demonstrated strong affinity and favorable binding energies with key regulatory proteins associated with insulin signaling. The study underscores the potential therapeutic relevance of the plant extract and apigenin in mitigating oxidative stress and regulating insulin signaling pathways.

Declarations

Acknowledgement

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Conflict of Interest

The authors would like to declare no conflict of interest in the present study.

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