A STUDY ON ASSESSING THE ANTI-INFLAMMATORY EFFICIENCY OF *ABELMOSCHUS MOSCHATUS* (BHENDI)

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ABSTRACT

Steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used treatments in chronic inflammatory diseases. However, long-term use of NSAIDs leads to serious side effects. In this study we aimed to assess the anti-inflammatory potential of seed parts of *Abelmoschus moschatus.* Seeds of*A. moschatus*wassubjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 50% methanol(Extract I) and 50% ethanol (Extract II). The *in-vitro* anti-inflammatory activity of both aqueous methanolic and ethanolic seed extracts of *A. moschatus* was determined using albumin denaturation method. Results revealed that there was dose dependent inhibition (%) was observed in standard as well as Extract I and Extract II of *A. moschatus*. Furthermore, the inhibition (%) of Extract I and Extract II of *A. moschatus* at the concentration of 500 µg/mL was at par with that of standard drug i.e., Aspirin. The major phytochemicals found in Extract I and Extract II of *A. moschatus*were found to be flavonoids, glycosides, phenolic compounds, and tannins. Phenolic quantity was found to be highest in both Extract I (6.45 GAE) and Extract II (3.65 GAE) as compared to flavonoid and tannin quantities. In conclusion, results of the preset study demonstrated the anti-inflammatory potential of aqueous methanolic and ethanolic extracts seed extracts of *A. moschatus* and hence they could be exploredin the development natural anti-inflammatory drugs

Keywords: *Abelmoschus moschatus*, Anti-inflammatory potential, Extracts, Phenolic compounds, Flavonoids, Tannins

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INTRODUCTION

Inflammation is a complex process often associated with pain and involves occurrences such as increased vascular permeability, enhanced protein denaturation, and rearrangement of the membrane. When cells in the body are damaged by microbes, physical agents, or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured

area. Loss of function depends on the area and severity of the condition occurring. Since inflammation is one of the non-specific intelligences which is based on mechanisms of the body, a tissue response to an unintentional cut is similar to that of other forms of tissue damage caused by heat, radiation, bacterial, or viral.[1]

An organism or tissue elicits the inflammatory responses as a defensive mechanism; however, prolonged inflammation can result in undesired health effects as a result of interplaying various biomolecules that are secreted during the inflammation phase. Inflammation in many diseases has been documented including cancer.[2] Chronic pain induced by inflammatory processes is a major clinical problem worldwide, steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used treatments in these chronic pain states. NSAIDs, such as diclofenac, aspirin, and indometacin, block the biosynthesis pathway of prostaglandins by inhibiting the cyclooxygenase (COX) enzymes, producing anti-inflammatory, analgesic, and antipyretic effects.[3] Also, their long-term uses are associated with several serious adverse effects such as gastrointestinal disorders, water retention, renal failure, bronchospasm, and hypersensitivity reactions.[4] Hence, the discovery of new and safe analgesic and anti-inflammatory drug is needed.[5]

Screening of bioactive compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases. *Abelmoschus moschatus* Medik. is an aromatic medicinal plant (Figure 1) in the Malvaceae family, which is native to India. The plant is used in the treatment of various diseases as described in traditional and folk remedies. Every part of this medicinal plant is used in one or the other way.[6,7] Seeds are effective aphrodisiac and antispasmodic, and used in tonics. Also useful in treating intestinal disorders, urinary discharge, nervous disorders, hysteria, skin diseases etc.[8,9]

Figure. Showing *Abelmoschus moschatus* plant bearing pods

With this background, in the present study we aimed to assess the anti-inflammatory potential of seed parts of *A. moschatus.*

MATERIALS AND METHODS

Collection Seeds of *A. moschatus*

A. moschatus plants bearing pods were collected in and around district head quarter places of Karnataka. The seeds were removed, and then shade dried. The dried seeds of *A. moschatus* were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered seeds of *A. moschatus*were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of 50% methanol(Extract I) and 50% ethanol (Extract II). The extracts were concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the seed extracts of *A. moschatusviz.* Extract I and Extract II by using standard procedures to detect phytoconstituents as described by sofora,[10] Trease and Evans[11] and Harborne.[12]

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in both extracts of *A. moschatusviz.* Extract I and Extract II was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[13] The phenolic content of the extracts was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in methanol solution and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in both extracts of *A. moschatusviz.* Extract I and Extract II.[14] The flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mg QE/g).

Tannins

The tannin concentration was determined in both extracts of *A. moschatusviz.* Extract I and Extract II following a modified version of the vanillin-HCl method.[15]

Assay of *In-vitro* Anti-inflammatory Activity

The *in-vitro* anti-inflammatory activity of both extracts of *A. moschatusviz.* Extract I and Extract II was determined using albumin denaturation method. Control, Standard (Aspirin), and different concentrations of both extracts of *A. moschatusviz.* Extract I and Extract II(i.e., 50-500 µg/mL) were prepared as follows;

Control: 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and final volume was made up to 50 ml with double distilled water.

Standard (Aspirin): 2 ml of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations (50-500 µg/mL) of standard drug (Asprin) were taken and final volume was made up to 50 mL.

Extracts: 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations of both extracts of *A. moschatusviz.* Extract I and Extract II(i.e., 50-500 µg/mL) were taken and final volume was made up to 50 mL. The reaction mixtures of control, standard (Aspirin), and different concentrations of both extracts of *A. moschatusviz.* Extract I and Extract II (i.e., 50-500 µg/mL) were incubated at 37ºC for 15 minutes and heated at 70ºC for 5 minutes. After cooling the turbidity was measured at 660 nm. Percentage inhibition of albumin denaturation was calculated using the following formula;

Inhibition (%) = (1 − A2/A1) X 100

Where,

A1 = Absorption of the control sample; A2 = Absorption of the test sample

RESULTS

The major phytochemicals found in Extract Iand Extract IIof *A. moschatus*were found to be flavonoids, glycosides, phenolic compounds, and tannins. Whereas, phytochemicals alkaloids were found to be present only in Extract I. While, terpenoids were found to be presentonly in Extract II. Whereas saponins were found to be absent in both Extract I and Extract II (Table 1).

Table 1: Photochemical screening of seed extracts of *A. moschatus*

Phytochemical Components	**Extract I**	**Extract II**
Alkaloids	+	-
Flavonoids	+	+
Glycosides	+	+
Saponins	-	-
Phenolic compounds	+	+
Tannins	+	+
Terpenoids	-	+

+: Present; -: Absent; Extract I: 50% Methanol extract; Extract II:50% Ethanol extract

The results of quantitative estimation of phytochemicals in seed extracts of *A. moschatus* (Extract I and Extract II) was represented in Table 2. Results revealed that the total phenolic quantity was found to be highest in both Extract I (6.45mg GAE/g) followed by total flavonoids (1.85mg QE/g), and quantities of tannins (1.24 mg GAE/g). Similarly in Extract II also the total phenolic quantity was found to be highest (3.65 mg GAE/g) followed by total flavonoids (0.82 mg QE/g), and quantities of tannins (0.54 mg GAE/g).

Table 2: Quantitative estimation of phytochemicals in seed extracts of *A. moschatus*

Phytochemical Components	**Extract I**	**Extract II**
Total phenolics	6.45 mg GAE/g	3.65mg GAE/g
Total flavonoids	1.85mg QE/g	0.82mg QE/g
Tannins	1.24mg GAE/g	0.54mg GAE/g

Values were expressed as mean; n=3; Extract I: 50% Methanol extract; Extract II: 50% Ethanol extract

The results of *in-vitro* anti-inflammatory activities of standard and extracts of *A. moschatus* was presented in Table 3. Results depicted that the mean inhibition (%) exhibited by standard was found to be 98.20, 128.54, 179.28, and 286.45 at the concentrations of 50 µg/mL, 100 µg/mL, 250

µg/mL, and 500 µg/mL respectively. The mean inhibition (%) exhibited by Extract I & Extract II of *A. moschatus* was found to be32.45 & 30.38, 79.28 & 69.62, 130.47 & 118.24, 262.11 & 249.58 at the concentrations of 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL respectively. These findings implied that there was dose dependent inhibition (%) was observed in standard as well as Extract I and Extract II of *A. moschatus*. Furthermore, the inhibition (%) of Extract I and Extract II of *A. moschatus* at the concentration of 500 µg/mL was at par with that of standard drug i.e., Aspirin.

Table 3: Effect of extracts of *A. moschatus* on *in-vitro* anti-inflammatory activity

Concentration (µg/mL)	**Standard**	**Extract I**	**Extract II**
50	98.20	32.45	30.38
100	128.54	79.28	69.62
250	179.28	130.47	118.24
500	286.45	262.11	249.58

Values were expressed as mean; n=3; Extract I: 50% Methanol extract; Extract II: 50% Ethanol extract

DISCUSSION

A number of factors, such as bacterial infection, chemical injury, and environmental pollution, can cause inflammation, which is a complicated process that can cause cell damage or death. The most widely used drugs in the world today are NSAIDs.[16,17] The most frequent inflammatory related complaints are pain and fever. The NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Market demand exists for orally active molecules that are more effective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms. Hence, in the current study we aimed for evaluation of the anti-inflammatory potential of aqueous methanolic and ethanolic seed extracts of *A. moschatus.*

Results of our study delineated that both aqueous methanolic and ethanolic seed extracts of *A. moschatus* demonstrated anti-inflammatory activity at par with standard drug Aspirin. Furthermore, both aqueous methanolic and ethanolic seed extracts of *A. moschatus* containstotal phenolic compounds, flavonoids, and tannins in considerable amounts.These findings were comparable with literature findings reports by various other research investigators.

Protein denaturation is one of the important known causes of certain anti-inflammatory diseases where electrostatic, hydrogen and disulphide bonding were altered in denaturation mechanism. Both the seed extracts were effective in inhibiting heat induced albumin denaturation at different concentrations and maximum inhibition of protein denaturation was observed with aqueous methanolic extract at 500µg/mlwhich was at par with that of standard drug i.e., Aspirin. These findings could be ascribed to phenolic compounds present in both aqueous methanolic and ethanolic extracts of *A. moschatus.* since these compounds have a capacity to inhibit either the production or the action of pro-inflammatory mediators resulting in anti-inflammatory capacity.[18]

Moreover, literature reports evidenced that flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages. Lipoxygenases are the key enzymes in the biosynthesis of leukotrienes which play an important role in several inflammatory diseases such as arthritis, asthma, cancer and allergic diseases. In this process, arachidonic acid gets converted to leukotrienes and prostaglandins through lipoxygenase and cyclooxygenase pathways

respectively.[19] In concurrence with literature findings, the anti-inflammatory activity exerted by both aqueous methanolic and ethanolic seed extracts of *A. moschatus* in our studycould also credited to the presence of flavonoid in the extracts.

CONCLUSION

In conclusion, results of the preset study evidenced the anti-inflammatory potential of aqueous methanolic and ethanolic extracts of *A. moschatus.* Furthermore, the polyphenolic compounds and flavonoids present in theaqueous methanolic and ethanolic extracts are majorly responsible for anti-inflammatory potential of aqueous methanolic and ethanolic seed extracts of *A. moschatus.* Hence, aqueous methanolic and ethanolic extracts of *A. moschatus* could explored in the development natural anti-inflammatory drugs.

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