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Phytochemical screening, analgesic and antioxidant activity of methanol extract of *Abelmoschus esculentus*

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Abstract

The purpose of this study was to investigate the presence of major phytochemicals and the analgesic and antioxidant effects of methanol extract of the seeds of a plant *Abelmoschus esculentus*. Phytochemical analysis of the extract of *Abelmoschus esculentus* seeds indicated the presence of Alkaloid, Flavonoids and Tannin types of compounds. The analgesic activity of the samples was studied using acetic acid induced writhing model in mice. Significant analgesic effect was monitored ($p < 0.05$) as the oral extract dose of 500 mg/kg showed 55.64% inhibition of writhing movements compared to standard drug Diclofenac-Na which inhibited 78.14% writhing movement. The methanol extract of *Abelmoschus esculentus* seeds showed mild antioxidant activity. The IC_{50} of the plant extract was 499.17 μ g/ml, whereas IC_{50} of reference anti-oxidative agent Ascorbic acid was 16.40 μ g/ml.

Keywords: *Abelmoschus esculentus*, antioxidant, analgesic, phytochemical screening

1. Introduction

Plants and plant-derived sources provide us food, shelter and remedies for many years. Different chemical constituents contained in plant exhibit different activities for treating abnormal conditions of human or animals. Traditional medicine practitioners use different parts of plant having several chemical constituents. Large portion of current diseases are caused due to the 'oxidative stress' which results in enormous amount of free radicals, causing complications like tumor, atherosclerosis and cardiovascular illnesses [1]. Cells of the human body protect themselves from free radicals by catalysts such as ascorbic acid, tocopherol and glutathione. [2] However, these defensive systems are becoming imbalanced frequently and cell reinforcement supplements are required to counter oxidative harm, for this reason much consideration has been controlled towards the improvement of medicine with solid cell reinforcement properties [3, 4].

Currently used painkillers and anti-inflammatory drugs in most cases are either steroidal like corticosteroids or non-steroidal like NSAIDs. All of them cause more or less adverse effects such as renal failure, allergic reactions, hearing loss or affecting platelet function. On the contrary many plant derived medicines has been used from centuries without any serious adverse effects. Thus, large scale researches should be conducted to develop new pain management medicines with plant based origin [5, 6].

Abelmoschus esculentus is an important vegetable which is widely distributed from Africa to Asia. It plays an important role in the human diet by supplying carbohydrate, minerals and vitamins. K, Na, Mg and Ca were found to be the principle elements, with Fe, Zn, Mn and Ni also present. Its seeds could serve as alternate rich sources of protein, fat, fiber and sugar [7], [8]. The purpose of this study was to investigate the presence of phytochemical compounds and determine the analgesic and antioxidant activity of *Abelmoschus esculentus*.

2. Materials and Methods**2.1. Plant Materials**

Abelmoschus esculentus seeds were collected from Mohammadpur, Dhaka and authentically confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

2.2. Preparation of Plant Extract

The collected plant seeds were dried at room temperature (30 ± 3 °C), for 30 days to ensure the active constituents free from decomposition. The dried seeds were powdered in an electrical grinder after overnight drying in an oven below 50 °C. The powder was extracted with methanol at room temperature. The bottle was kept at room temperature and allowed to stand for 11 days with occasional shaking. When the solvent became concentrated, the liquid alcohol

contents were filtered through cotton & then through filter paper. Then, the solvent was allowed to evaporate using rotary evaporator at temperature 40-45 °C. Finally, a highly concentrated methanol crude extract was obtained.

2.3. Phytochemical Screening Methods

2.3.1. Test for Glycosides

2 ml solution of the extract was taken into a test tube. 1 ml mixture of Fehling solution was added into the test tube. The tube was placed in a water-bath at 60 °C. If brick red color forms that shows the presence of glycosides.

2.3.2 Test for Alkaloids

In testing for Alkaloids, about 0.5g of extract will be stirred with 5 ml of 1 percent aqueous hydrochloric acid on a water bath; 1 ml of the filtrate is to be treated with a few drops of mayer's reagent and a second 1 ml portion is to be treated the same way with Dragendorff's reagent. Presence of orange-red color indicates the presence of alkaloid.

2.3.3 Test for Flavonoids

A small quantity of test residue was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. If the pink, crimson or magenta color is developed within a minute or two that mean flavonoids are present.

2.3.4 Test for Tannins

About 5 g of each portion of plant extract will be stirred with 10 ml distilled water, filtered, and ferric chloride reagent will then be added to the filtrate. If dark green or deep blue color is obtained, it means tannins are present.

2.3.5 Test for Saponins

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth is obtained, it means saponins are present [9].

2.4 Analgesic property test

2.4.1 Drugs and Chemicals

Acetic acid was collected from laboratory of the University. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh.

2.4.2 Experimental Animals

Eight week-old Swiss albino mice were (50-52 g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25 °C, humidity 60-70%, 12 hours light: 12 hours dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

2.4.3 Experimental Protocols

For Analgesic test 9 mice were divided into three groups-

1. Control Group
2. Standard Group
3. Extract Group

For analgesic test all mice were divided into three groups (Control Group, Standard Group and Extract Group). Each group comprises 3 mice. Control group received 1% Tween 80 in water, Standard Group received Diclofenac sodium 10 mg/kg and Extract Group received 500 mg/kg extract.

2.4.4 Acetic acid-induced writhing test for Analgesic activity

The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Acetic acid was administered intraperitoneally. Test samples and vehicle were administered orally 30min before administration of acetic acid. Diclofenac-Na was administered 30 min before administration of acetic acid to standard group. The mice were observed for specific contraction of body referred to as "writhing" for the next 30 min [10, 11].

2.4.5 Statistical Analysis

All values were expressed as mean ± Standard Deviation (SD). Statistical comparison were performed by One-way analysis of variance (ANOVA), followed by using Dunnett test. Results were considered as significant when p values less than 0.05 ($p < 0.05$).

2.5 Antioxidant Property Test

2.5.1 Reagents

Methanol, DPPH (1, 1-diphenyl-2-picrylhydrazyl-hydrate)

2.5.2 Procedure

Stock solution of the plant extract was prepared in methanol from which a serial dilution was carried out to obtain concentration of 5µg/ml, 10µg/ml, 50µg/ml, 100µg/ml and 500µg/ml. Diluted solutions (2ml) were added to 2ml solution of DPPH then mixed and allowed to stand for 30 minutes for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then percent inhibitions were plotted against log concentration and from the graph IC₅₀ was calculated. The experiment was performed 3 times and average absorption was noted for each concentration [12-14].

3. Results and Discussion

3.1. Result of Phytochemical Screening

Table-1 shows the results of the phytochemical screening of the Methanolic Extract of *Abelmoschus esculentus*

Table 1: Results of chemical group tests

Tested groups	Methanolic Extract of <i>Abelmoschus esculentus</i>
Glycoside	-
Alkaloid	+
Saponin	-
Flavonoid	+
Tannin	+

Note: (+) Indicates the presence of the tested group, (-) Indicates the absence of the tested group. The tests identify the presence or absence of Alkaloids, Glycoside, Flavonoid, Saponin and Tannins in methanol extract of *Abelmoschus esculentus*

3.2. Result of Analgesic Test

Table 2 shows Analgesic effect of *Abelmoschus esculentus* extract on Acetic acid-induced writhing in mice.

Table 2: Effects of the methanol extract of *Abelmoschus esculentus* on Acetic acid-induced writhing in mice

Animal Group	Writhing Counting (Mean)	Percentage of Writhing Inhibition
Control Group	53.33 (±2.08)	-
Standard Group	11.66 (±1.52)	78.14
Extract group (500 mg/ kg)	23.66 (±1.53)	55.64

Values were expressed in mean value. Each group comprised 3 animals (n=3); $p < 0.05$ Dunnet test as compared to Control Group. Control Group animal received vehicle (1% Tween 80 in water), Standard Group received Diclofenac 10 mg/ kg body weight, Extract Group was treated with 500 mg/kg crude extract of *Abelmoschus esculentus*.

3.3. Result of Anti-oxidant Test

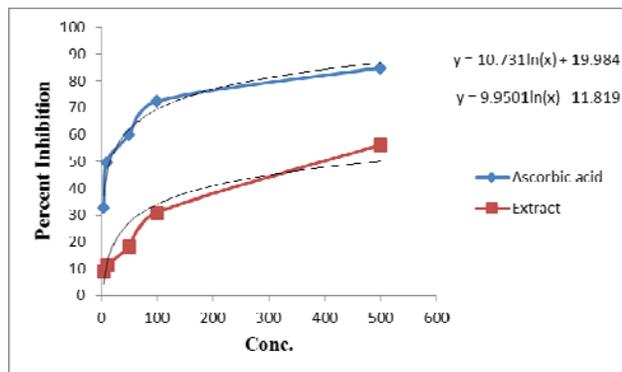


Fig 1: Antioxidant activity of Ascorbic acid and Plant Extract

Table 3: IC₅₀ Values of Ascorbic acid and Plant Extract

Sample	Equation	IC ₅₀ (µg/ ml)
Ascorbic acid	$Y=10.731 \ln(x) + 19.984$	16.407
Plant Extract	$Y=9.9501 \ln(x) - 11.819$	499.17

The antioxidant activity of the methanol extract of *Abelmoschus esculentus* is evaluated using DPPH free radical scavenging activity method. The methanol extract of *Abelmoschus esculentus* has minor antioxidant activity. The IC₅₀ of the extraction is 499.17 µg/ml, whereas IC₅₀ of Ascorbic Acid is 16.407 µg/ml.

4. Discussion

Phytochemical screening of *Abelmoschus esculentus* plant extract displayed that it contains Alkaloids, Flavonoid and Tannins. Glycosides and Saponins were absent in the extract. The analgesic tests displayed that it has good analgesic properties because the standard analgesic drug Diclofenac- Na inhibited 78.14% acetic acid induced writhing while the dose prepared by plant extract inhibited 55.64% acetic acid induced writhing.

The antioxidant test showed that the plant extract has mild antioxidant activity because the standard antioxidant agent ascorbic acid was found to have IC₅₀ of 16.407 µg/ml while the IC₅₀ of the plant extract is 499.17 µg/ml.

5. Conclusion

The results stated above showed that the methanol extract of *Abelmoschus esculentus* possessed good analgesic and mild antioxidant effects. It is evident that *Abelmoschus esculentus* has numerous beneficial properties, for that reason more researches should be conducted to determine the effects of this plant more accurately.

6. Conflict of interest

The authors declare that there is no conflict of interest about this article with any institution.

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