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Extraction, Isolation Characterization and Antiasthamatic Activity of The Leaves of *Abelmoschus Esculenutus* (okra)

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ABSTRACT

Aims: The aim of study was to evaluate the scientific basis for the traditional use of Amebelmoschus esculentus leaves. Materials and Methods: In the present study, petroleum ether extract of Abelmoschus esculentus leaves was evaluated for preliminary phytochemical screening and antiasthmatic activity using histamine induced bronchospasm and histamine induced constriction on isolated goat tracheal chain at different dose levels. Student's t-Test and Dunett's test were used for statistical analysis. Results: The result of present investigation showed that the petroleum ether extract of Abelmoschus esculentus significantly ($P<0.001$) decreased the bronchospasm induced by histamine and It also decreased the histamine induce constriction on isolated goat trachea in dose-dependent manner. Phytochemical studies revealed the presence of flavonoids, and phenolic compounds in the extract. Conclusions: The present study concludes that the antiasthmatic activity of ethanolic extract of A.esculentus leaves may be due to the presence of flavonoids, Alkaloids glycosides, tannins, protein. Antiasthmatic action of the A. esculentus could be due to its antihistaminic, anticholinergic and mast-cell-stabilizing property.

Keywords : Antiasthmatic activity, Abelmoschus Esculentus, Pharmacological Screening , okra, Malvaceae

I. INTRODUCTION

Okra (Abelmoschus esculentus) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. In india, it ranks number one in its consumption but its original home is Ethiopia and Sudan, the north-eastern African countries. One of the first crops to be farmed, it is currently grown in many nations and can be found all over the world, from southern Europe

and America to Asia and Africa. It is a tropical to subtropical crop that is susceptible to forest conditions, low temperatures, water logging, and droughts. Its cultivation from various nations has given rise to several distinctive features that are unique to those nations. Although it is an oligopurpose crop, most people eat it for its soft green fruits, which may be prepared in a number of ways, as a vegetable. Vitamins, calcium, potassium, and other minerals abound in these fruits. The oil extracted from mature okra is considered to have exceptional nutritional content and is a good source of both oil and protein. Unsaturated fatty acids, such linoleic acid, which are vital for human nutrition, are abundant in okra seed oil. Crude fiber from its mature fruit and stems is utilized in the paper industry.

Okra takes 90–100 days to mature and is mostly reproduced by seeds. Typically, it is an annual plant. Its sturdy, tall stem varies in height from 0.5 to 4.0 meters and exhibits varied branching. The bloom is axillary and solitary, while the leaves are alternating and often have five lobed palmate leaves. Blooming never stops, yet it is heavily reliant on biotic and abiotic stress. Typically, the plant produces its first blossoms one to two months following seeding. After blossoming, the fruit is capable and expands swiftly. The greatest increase in fruit length, height and diameter occurs during 4th to 6th day after pollination. It is at this stage that fruit is most often plucked for consumption. The okra pods are harvested when immature and high in mucilage, but before becoming highly fibrous. Generally speaking, fruit production begins on the sixth day after fruit development and increases dramatically on the ninth day. Depending on the variety, the season, the moisture content and fertility of the soil, okra plants can continue to flower and produce fruit indefinitely. In fact, frequent harvesting encourages fruit to continue growing, to the point where in areas where growth is particularly rapid, it would be essential to pick every day. Although it is an oligo purpose crop, most people eat it for its soft green fruits, which may be prepared in a number of ways, as a vegetable. Vitamins, calcium, potassium, and other minerals abound in these fruits. The ripe okra seed has been found to have higher nutritional quality and is a strong source of protein and oil. Unsaturated fatty acids, such linoleic acid, which are vital for human nutrition, are abundant in okra seed oil. Crude fiber from its mature fruit and stems is utilized in the paper industry.

II. MATERIALS AND METHODS

Collection and Authentication Plant material:

The Abelmoschus esculentus leaves extract was used for experimental purpose. They were collected from Borgaon (meghe) Wardha. The fresh leaves of A.Esculentus were separated from the whole plant, sliced and dried under shade followed by an incubator for 2 day at 40◦c.

Drying and Size Reduction of Plant Material :

Abelmoschus esculentus leaves were dried in the laboratory under shade and then crushed in an electrical grinder then powdered, stored in a cool and dry place for further study.

Extraction of Abelmoschus esculentus

The fresh leaves of A. esculentus were separated from the whole plant, sliced and dried under shade followed by an incubator for 2 day at 40◦c, crushed in an electrical grinder and then powdered. The powdered material (58 gm.) was subjected for extraction with various solvent in soxhlet apparatus for 24 hrs. Based on increasing polarity in order of petroleum ether (60- 80◦c). All the extracts were concentrated using rotary evaporator and the solvents were completely recovered in order to get dry leaves extracts, which were preserved in glass desiccators till further use. The quantitative yield of petroleum ether extract was 6.89 % w/w.The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

Figure 1: Soxhlet extraction of Plant Material

Isolation of compound by TLC

The extract was subjected to column chromatography containing silica gel of 60-120 mesh as the stationary phase in the glass column (100 X 3 cms). The isolation was carried out through isocratic elution method.

Stationary Phase: Silica gel

Mobile Phase*:* Abelmoschus esculentus leaves extract

Extract*:* extract of Abelmoschus esculentus leaves extract

Procedure

Preparation of Chromatographic plates

The required quantity of silica gel-G (e.g. 30 g) was weighed out and a suspension was prepared by shaking with sufficient amount of distilled water. The slurry was poured in to a TLC applicator, which was adjusted to 0.25mm thickness on flat glass plate (20 x 10 cm and 20x20 cm). The applicator was rolled over the glass plates set adjacent to each other to form uniform coated plates which were allowed to dry in air, followed by heating in an oven at 100 –105 ˚C for one hour, cooled and protected from moisture. The plates were stored in dry atmosphere. Whenever required the plates were activated by heating in hot air oven at 100 ˚C for 30 mins and used for thin layer chromatography.

Preparation of sample

The Petroleum ether extract was dissolved in suitable solvents (approx. 1.0 mg in 1.0 ml of solvent) in which the extract shows maximum solubility. The solution of extract was spotted on a TLC plate with the help of a narrow capillary tube 1 cm above the bottom of the plate. The spots were equally sized as far as possible and separated equidistant to each other and the edges of plate. The diameter of spots should not exceed 0.25cm.

Development of chromatogram:

The developing chamber was prepared by lining the inner wall of the chamber with filter paper and the developing solvent (the volume of developing solvent should be sufficient to cover 0.5 cm of the TLC plate) was poured in the chamber, the chamber was covered with a lid in order to provide chamber saturation. The chamber saturation avoids the edge effect and tailing effect. Immediately after chamber saturation (1hr approx.), the spotted silica gel plate was placed in the developing chamber with caution, the lid was closed and the solvent was allowed to run up to 3/4th of the chromatographic plate. The plate was then removed from the chamber and the level of solvent front on the plate was marked and allowed to dry in hot air. The spots on the plate are detected by placing the plate in a UV chamber, or an iodine chamber or by spraying chromogenic reagents.

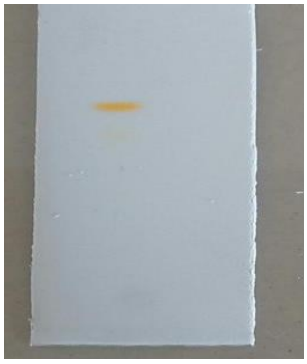

Fig. No. 2 -TLC of Isolated compound

Isolation of individual compound:

After development of chromatogram the plates were allowed to dry. Distance travelled by solute and solvent were marked and measured and their Rf values were calculated. Further from the data obtained from Rf values of each extract the individual spots were isolated from the TLC plates. After drying of the plates each individual spot was scrapped from the plate by using sharp pointer. Each spots were collected in different test tubes according to their Rf values and they were further analyzed for their identity by using analytical method such as GC-MS.

Characterization of Isolated compound by spectral data:

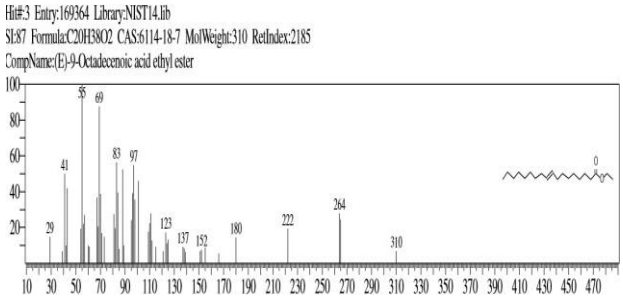

Fig.No 3 Mass spectrum of Isolated compound (E)-9-Octadecenoic acid

IR Spectrum

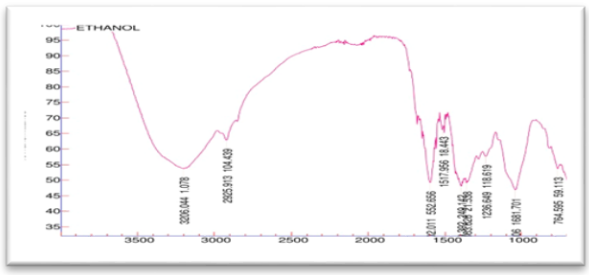

Fig.No 4 IR spectra of isolated compound of (E)- 9-Octadecenoic acid ethyl ester

The strong CO-O Stretching absorption (one or two) band is found from 1150 to 1250 cm^{-1}.AndC=C stretching absorption found from 1500 to 1600 cm^{-1}.

Compound of (E)- 9-Octadecenoicacidethyl ester

Experimental Animals

We employed 400–600 g guinea pigs of both sexes kept in conventional circumstances with 22 ± 2°C of temperature, 55 ± 5% of relative humidity, and 12 hours of light/dark cycles. They were given an ordinary pellet diet to eat and unlimited water. Following the guidelines provided by CPCSEA, the Ministry of Social Justice and Empowerment, Government of India, the Institutional Animal Ethical Committee approved the experimental methodology in Institute of pharmaceutical education and research wardha. (CPCSEA Protocol No. 379/01/ab).

In Vitro Antiasthamatic Activity

Evaluation of Anti-asthmatic activity

The Anti-asthmatic activity was evaluated using isolated goat tracheal chain preparation method:

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice cold oxygenated Kreb's solution.

Chemicals	Quantity(mM)
NaCl	115
KCl	4.7
CaCl2	2
NaHCO3	25
KH2PO4	1.2
MgCl2	1.2
Glucose	11

Table No : 5 Composition of Kreb'sSolution

Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution and maintaining at 37◦C, stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. Frontal tissue was allowed to equilibrate for 45 min under a load of 1000 mg. a dose response curve of for histamine was recorded at variant molar

concentrations by maintaining 15 min, time cycle. After obtaining dose response curve of histamine (30µg/ml) of trachea, the DB(10µg/ml) was added to reservoir and same dose of histamine were repeated. Graph of dose of histamine was plotted against petroleum ether extract 300 µg, 400 µg and 500 µg respectively .

In vivo Antiasthamatic Activity

Histamine induced bronchospasm in guinea pigs.

Histamine dihydrochloride aerosol (0.1% w/v) was given to two groups of six guinea pigs each, regardless of sex, in a histamine chamber. When animals were exposed to histamine aerosol, they developed increasing dyspnea. The endpoint, known as preconvulsion dyspnea (PCD), was measured as the interval between the aerosol exposure and the start of dyspnea that results in the initiation of convulsions. The animals were taken from the chamber and let outside as soon as PCD started. At this point, the PCD was set to day 0. For seven days, petroleum ether extract of *A. Esculentus* was administered once daily to both groups of guinea pigs at a dose of 100 mg/kg and 200 mg/kg, p.o respectively. On the 7 day 2 h after the last dose, the time for the onset of PCD was recorded as on day 0. Same procedure was followed in another set of animals (n = 6) for histamine induce bronchospasm study using 0.5% histamine. The percentage increased in time of PCD was calculated using following formula. Percentage increased in time of PCD = (1-T1 /T2) × 100 where T = time for PCD onset on day 0, T = time for PCD onset on day 7.

The percent protection offered by treatment was calculated by using the following formula Percentage Protection =

(1 –; T1/T2) × 100

Where,

T1 = mean of PCT before administration of test drugs.

T2 = mean of PCT after administration of test drugs at 1 hr, 4 hr and 24 hrs.

Statistical Analysis

The results of various studies were expressed as Mean ± SEM and analyzed using one-way ANOVA followed by Student's t-Test to find out the level of significance. Data were considered statistically significant at minimum level of P<0.001.

III.RESULTS

Preliminary phytochemical screening

The petroleum ether extract of *Abelmoschus esculentus* showed the presence of flavonoids, Alkaloids glycosides, tannins, protein.The isolated goat tracheal chain preparation showed dose dependent significant (P<0.001) inhibition of the contraction of tracheal muscles induced by histamine as compared to control group shown in [Table 1]. Abelmoschus Esculentus significantly (P<0.001) increased the preconvulsive dyspnoea time following exposure to histamine and acetylcholine aerosols induced bronchospasm in guinea pigs shown in [Table 2].

Table 1 - Percent inhibition of Abelmoschus Esculentus on histamine induced contraction on isolated goat trachea.

Treatment	Conc. (µ/ml)	Peak height	% inhibition
Histamine	0.5	2.53 ± 0.10	-
Histamine + PEAE	300	1.78 ± 0.06*	29.64
Histamine + PEAE	400	1.50 ± 0.05*	40.71
Histamine + PEAE	500	0.90 ± 0.06*	64.42

EESL = Ethanolic extract of Abelmoschus esculentus, values are expressed as Mean ± SEM, n = 6, * P<0.001

as compared to positive control (histamine induced) group.

Table 2 - Effect of Petroleum Ether extract of Abelmoschus Esculentus (p.o., for 7 days) on histamine induced bronchoconstriction in guinea pigs

Groups	Dose	PCT before T1	PCT before T2	% protection
1	Control (saline)	1.526 ± 0.121	1.612 ± 0.112	5.34
2	1oo AE	1.214 ± 0.111	3.126 ± 0.0126	61.16
3	200 AE	1.253 ± 0.012	11.121 ± 0.121	88.73
4	Chlorphineramine Maleate	1.412 ± 0.110	15.145 ± 0.412	90.67

PEAE = Petroleum ether extract of Abelmoschus Esculentus, values are expressed as Mean ± SEM, n = 6, * P<0.001, as compared to control group.

IV. DISCUSSION

Exhaustive literature survey related that sufficient studies have been done on anti-oxidant, anti-microbial, anti-bacterial, anti-cancer, analgesic activity of *Abelmoschus esculentus* Linn plant. However there are no reports on anti-asthmatic activity .Therefore present study was undertaken to explore constituents from the extract and anti-asthmatic activity of plant *Abelmoschus esculentus* Linn. Belongs to the Malvaceae family plants are rich source of bioactive molecules, which have shown promising potential in the cure of variety of diseases as pure compound or in the form of extracts, for the treatment of various disease.

The present study deals with extraction, isolation, molecular characterization of secondary metabolites and pharmacological evaluation. Characterization of isolated compound was done by thin layer chromatography, GC-MS, NMR and FTIR. Constituent (E)-9- octadeconoic acid ethyl ester has been isolated from *Abelmoschus esculentus* Linn.

The plant *Ablemoschus esculantus* linn. Seems to be a promising candidate with respect to its anti-asthmatic activity and may be used as adjuvant to dietary therapy and drug treatment for controlling asthma. For evaluation of anti-asthmatic activity in-vitro goat tracheal chain model was used. Histamine contraction was significantly reduced by petroleum ether extract. On the other side ethanol extract shows satisfactory inhibited the concentration of histamine. Hence it is concluded that the *Abelmoschus esculentus* Linn is potential member for the activity. Therefore our future aim to explore the isolate molecules to a great detail for the derivatives and so on which might give a new line of research on plant derived drug development.

The relaxant effects of petroleum ether extract of *A. esculentus* on tracheal chains of guinea pigs might be produced by different mechanisms including stimulation of b-adrenergic receptors, inhibition of histamine H1 receptors, or an anticholinergic property of this plant. The relaxant effects of all concentrations of the extract of *A. esculentus* obtained were significantly lower than control group. These findings suggest probable b-adrenergic stimulatory, muscarinic and/or histamine H1 blocking properties of the plant extract.

V. CONCLUSION

Histamine contract the treachea-bronchial muscle of guinea pig, goat, horse, dog and man.Goat tracheal chain is easier to handle and to prepare it is also more sensitive than guinea pigtracheal chain. In present study Petroleum ether extract(300µg, 400µg,500 µg) of *Abelmoschus esculentus* showed significant dose dependant Antiasthmatic activity in goat tracheal chain model and Petroleum ether extract(100µg, 200µg) of *Abelmoschus esculentus* showed significant dose dependant Antiasthmatic activity histamine

induced bronchoconstriction in guinea pig. Thus *Abelmoschus esculentusLinn*. Can prevent there lease of inflammatory mediators or inflammation in asthma.

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