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Anti-Allergic Activity of Methanolic Extract of *Lagenaria Siceraria* Mol. Standley Leaves

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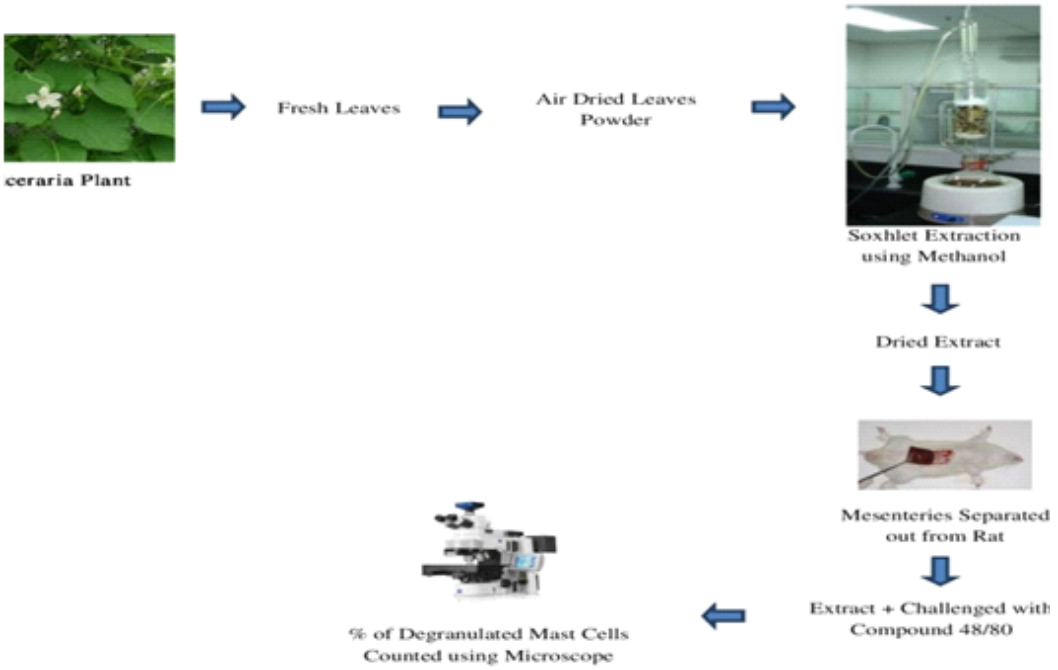
Graphical Abstract



Abstract

Background: *Lagenaria siceraria* Mol. Standley commonly known as bottle gourd is a climbing perennial herb cultivated as a vegetable in the tropical countries. *Lagenaria siceraria* leaves are used traditionally for cough, bronchitis, asthma and inflammation.

Purpose: On basis of ethano-medicinal uses, the anti-allergic activities of the methanolic extract of *Lagenaria siceraria* leaves was planned to investigate scientifically using animal models for allergic inflammation and anti-allergic activity.

Method: Leaves of *Lagenaria siceraria* were collected from Nirma Herbal Wealth, Nirma University, Ahmedabad, Gujarat, India and the sample was identified and authenticated by ethano- botanist. The methanolic extract of the leaves was prepared from 500 gm of leaves using soxhlet extraction; it yielded 16.70 % (w/w) of dry extract.

The Wistar rats were killed by exsanguinations. The pieces of mesenteries were spread and challenged with 1µg/ml of compound 48/80 for 10 min as negative control. Methanolic extract *L. siceraria* at different concentrations or standard ketotifen incubated mesenteries were challenged with 1µg/ml of compound 48/80 for 10 min. Pieces of mesentery were stained with 0.1% toludine blue solution and the % of degranulated mast cells were counted under microscope and the protection offered by methanolic extract / ketotifen of was calculated. In another model the Compound 48/80 (0.3µg in 0.05ml) were administered subcutaneously in the plantar region of the right hind paw of albino mice's.

Result: The methanolic extract of *L. siceraria* (LSM) showed significant dose dependent (10-30 µg/ml) anti allergic activity against compound 48/80 induced mast cell degranulation in rat mesenteries. LSM also showed significant anti-inflammatory effects at the dose of 100 mg/kg against compound 48/80 induced paw edema in mice.

Conclusion: The ethno medicinal uses of *L. siceraria* leaves were proved by us scientifically for treatment of allergic inflammation and anti-allergic activity using various animal models.

Key words: *Lagenaria siceraria,* mast cell stabilization, compound 48/80, paw edema.

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Introduction

Lagenaria siceraria Mol. Standley, belonging to Cucurbitaceae family, is a climbing herb commonly cultivated as a vegetable crop in India. *Lagenaria siceraria* is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health (Habib-ur-Rahman, A. S., 2003). *Lagenaria siceraria* fruit is traditionally used for its cardioprotective, cardiotonic, general tonic and aphrodisiac properties. It has been scientifically reported to have hepato protective, immunomodulatory, antihyperglycemic, antihyperlipidemic, analgesic and anti-

Inflammatory, antibacterial and diuretic properties (Meenal, S et al, 2010). *Lagenaria siceraria* leaves are used traditionally for cough, bronchitis, asthma, fever, inflammation and skin diseases (Sheth, A., 2005,Anonymous, 2002). Leaves of *Lagenaria siceraria* are taken as emetic in the form of leaf juice or decoction. This by adding sugar also used in Jaundice. Crushed leaves are used for baldness and applied on the head for the headache. Leaves are also used as alternative purgative (Chopra, B.N. and Chopra, I.C., 1992). These properties of *Lagenaria siceraria* fruit have been attributed to its saponins, carbohydrates and flavonoids. Bitter fruits yield 0.013% of a solid foam containing cucurbitacins B, D, G and H, mainly cucurbitacins- B. The leaves contain cucurbitacins B, D, and traces of E. The fruit juice contains beta-glycosidase (elasterase) (Khare, C.P., 2004). Various extracts of leaves are reported to possess anthelmintic, antimicrobial, analgesic and CNS depressant activity (Pawar, J., 2010 , Badmanaban, R., Patel, C.N., 2010). Based on ethanobotanical practice *Lagenaria siceraria* is a rich source of different chemical compounds with a variety of potential biological activities. Thus on basis of the ethano-medicinal uses, the anti-allergic activities of the *Lagenaria siceraria* leaves were investigated.

Materials and methods

Preparation of Extract

Leaves of *Lagenaria siceraria* were collected from Nirma Herbal Wealth, Ahmedabad, Gujarat, India and the sample was identified and authenticated by Dr. B.L. Punjani, Ethnobotanist, P.G. Centre in Botany, Smt. S.M. Panchal Science College, Talod, Gujarat, India. The voucher specimen (IPS/PCOG/MPH 10-11/505) has been preserved at Dept. of Pharmacognosy, Nirma University for future reference. The methanolic extract of the leaves was prepared from 500 gm of leaves using soxhlet extraction; it yielded 16.70 % (w/w) of dry extract.

Animals

Wistar rats (200-250 g) and albino mice Kasuali strain of either sex were procured from Torrent Research Centre, Ahmedabad. Animals were housed in the animal house of Nirma University, Ahmedabad under controlled conditions of temperature, light and humidity. Institutional Animal Ethics Committee approved the experimental protocol (IPS/PCOG/MPH10-11/2015).

Compound 48/80 Induced Paw Edema in Mice

Albino mice of either sex were divided into 5 groups (n=6). Compound 48/80 (0.3µg in 0.05ml) was administered subcutaneously in the plantar region of the right hind paw. The left hind paw received an equal volume of normal saline. The hind paw thickness was measured using a pocket thickness gauge (Mitutoyo Mfg. Co. Japan) after 30 minutes. The change in the paw thickness between compound 48/80 injected and saline injected paws reflected the edema formed in response to compound 48/80. The methanolic extract of *L. siceraria* was administered intraperitoneally at different doses, i.e. 50, 75 and 100 mg/kg, 30 minutes prior to the injection of compound 48/80. Ketotifen, 1 mg/kg i.p. was used as the reference standard. Drug effects were observed using the maximal edema response attained (Nair A.M, et al 1994).

Compound 48/80 Induced Mast Cell Degranulation Studies

Wistar rats were killed by exsanguinations. The piece of small intestine along with mesentery of rat was excised and connecting lobes of fat and blood vessels were rapidly dissected out. The pieces of mesenteries were spread in petri-dish containing Ringer Locke physiologic salt solution (Tripathi, R.M. et al 1979). Methanolic extract *L. siceraria* at different concentrations or standard ketotifen incubated mesenteries were challenged with 1µg/ml of compound 48/80 for 10 min (Geetha, V.S. et al, 1981). Pieces of mesentery were stained with 0.1% toludine blue solution containing 4% formaldehyde for 20-30 min and then treated with acetone and xylene and mounted on slides (Norton, S., 1954). The % of degranulated mast cells was counted under microscope and the protection offered by methanolic extract of drug was calculated.

Statistical Analysis

All values were expressed as mean ± S.E.M. The data were analyzed by using one-way ANOVA followed by Dunnet's test and P value less than 0.01 implied significance.

Results and Discussion

Compound 48/80 Induced Paw Edema in Mice

Methanolic extract of *L. siceraria* at the doses of 50, 75 and 100 mg/kg showed percent protection of 21.02%, 45.44%, 59.53% respectively against *Compound 48/80 induced paw edema* in mice compared to ketotifen which showed 83.06% inhibition (Table 1). Thus, LSM produced dose dependent inhibition of edema ($p<0.001$).

Compound 48/80 Induced Mast Cell Degranulation Studies

The control group showed 67.63% degranulation of mast cells. Methanolic extract of *L. siceraria* (LSM) showed significant ($p<0.001$) protection against degranulation of mast cells. The percent protection offered was found to be 72.16%, 80.22%,

Table 1: The Effect of LSM on Compound 48/80 Induced Paw Edema in Mice.

Treatment	Dose (mg/kg)	FootPad Thickness(mm)	Percent Inhibition
Control	-	[illegible]	-
LSM	50	38.51 ± 12.55	21.02 ± 0.98
LSM	75	22.60 ± 05.38	45.44 ± 2.43
LSM	100	19.73 ± 10.16***	59.53 ± 3.32
Ketotifen (Std. drug)	01	08.26 ± 01.35***	83.06 ± 2.78

85.40% at concentration of 10, 20 and 30 µg/ml respectively compared to ketotifen which showed 80.83% protection. The effect of LSM (Methanolic extract of *L. siceraria*) at the concentration of 20µg/ml showed effect comparable to ketotifen (Table 2).

Inflammation plays an important role in various diseases with high prevalence within populations such as rheumatoid arthritis, asthma and atherosclerosis. The most used test to study new anti-inflammatory agents evaluates the ability of a compound to reduce local edema induced in the rat/mice paw by injection of an irritant agent [13]. Edema formation in paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase blood flow (Ialenti, A. et al, 1995). Here compound 48/80 was used to induce inflammation in mice paw. LSM at the dose of 100 mg/kg showed significant inhibition of paw edema against compound 48/80 suggesting its use as an anti-inflammatory drug.

Allergy, especially immediate hypersensitivity such as asthma, allergic rhinoconjunctivitis, atopic dermatitis and eczema, is a principal health problem, and the prevalence of allergy has increased during the past two decades. Allergic inflammation associated with airway hyper sensitivity is the main feature of

Table 2: The Effect of LSM on Compound 48/80 Induced Mast Cell Degranulation in Rats.

Treatment	Conc (µg/ml)	% Degranulation	% Protection
Control (Compound 48/80)	-	67.63 ± 0.14	-
LSM 10	10	24.16 ± 1.16**	[illegible]
LSM 20	20	17.16 ± 0.75***	80.22 ± 0.41
LSM 30	30	12.66 ± 2.25***	85.40 ± 1.07
Ketotifen (Std. drug)	10	16.66 ± 1.81***	80.83 ± 0.52

allergic asthma. Mast cells play a key role in immediate-type allergic response through the release of a number of inflammatory mediators and cytokines [Kim E.K.., et al, 2004]. Mast cells on degranulation can produce a wide variety of inflammatory mediators such as leukotrienes, prostaglandins, proteases and several pro-inflammatory and chemotactic cytokines and interleukins of which histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity upon release (Kalesnikoff J. and Galli S.J. 2008,Petersen L.J.et al, 1996). Mast cell degranulation can be evoked by compound 48/80, which has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Ennis, M et al, 1980). LSM (10-30µg/ml) significantly and dose dependently protected the degranulation of mast cells by compound 48/80. The protective effect of LSM at the dose of 20µg/ml was comparable to ketotifen which is a potent mast cell stabilizer. Thus, from the present study it can be concluded that methanolic extract of leaves of *L. siceraria* can be useful in treatment of allergic inflammatory conditions.

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