COMPARATIVE STUDY OF MAST CELL STABILIZING EFFECT OF EXTRACT OF SELECTED PLANTS WITH PROVEN H1 ANTAGONIST ACTIVITY

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ABSTRACT
Mast cells play a key role in allergy and inflammation which have profound patho-physiological role in asthma. In the present study a comparative effect of mast cell stabilizing activity of different plant extracts *Momordica dioica* fruit pulp (family - Cucurbitaceae), *Cuminum cyminum* seeds (family - Apiaceae), *Piper nigrum* seeds (family - Piperaceae), *Boerhaavia diffusa* roots (family - Nyctaginaceae.), *Withania somnifera* roots (family - Solanaceae), *Mangifera indica* leaves (Anacardiaceae), *Plantago ovata* (Plantaginaceae) was carried out. For this purpose plant extracts were subjected to the preliminary phytochemical analysis and mast cell stabilizing action by assessing the prevention of degranulation of mast cells by known degranulating compound 48/80. The result showed a higher stabilization potential in ethanolic extract of *Piper nigrum* seeds which showed 55.17±1.56, 64.00±1.18 and 70.50±0.96 percentage inhibition of mast cell degranulation. Minimal activity was observed in the ethanolic extract of *Plantago ovata* with percentage inhibition of 21.17±1.54, 22.17±2.21, 24.33±2.20 at three dose levels. Other plant extracts showed intermediate activity. Further investigation of the 5-Lipooxygenase activity, leukotriene antagonist activity and anti-inflammatory activity along with the findings of the present work would give us more clear understanding of the mechanism by which the above extracts exert beneficial effect in the management of asthma.

KEY WORDS: Mast cell, Inflammation, degranulation, compound 48/80.
INTRODUCTION
Asthma is a chronic airway inflammatory disorder with inflammation due to complex interactions between inflammatory cells, mediators, and airway cells. This is characterized by airway hyper reactivity to a variety of non-specific stimuli, leading to a variable degree of airway obstruction, some of which may become irreversible over many years.\(^1\) The mechanism of the inflammatory response resulting in asthma is complex and involves numerous cell types, including mast cells.\(^2\) Mast cell activation causes process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines,\(^3, 4\) on activation, mast cells immediately released the preformed and the de novo synthesized mediators such as histamine, proteases, leukotrienes, prostaglandins, and cytokines.\(^5\) As a consequence, the acute reactions such as vasodilation, increased vascular permeability, and bronchoconstriction were induced. In addition, allergic responses also trigger the influx and activation of a variety of inflammatory cells including eosinophills and lymphocytes.\(^6\) Therefore, mast cell stabilization is a key factor in controlling the occurrence of asthma.

Various systems of medicine such as Ayurveda, Unani, Siddha and Chinese employed plant based drugs for the treatment of diseases which dates back to 5000 B.C. Because of the low incidence of adverse reactions and cost effectiveness, many countries have now shifted their focus to plant based medicines.\(^7\) It is the need of the hour to ensure the perseverance of the knowledge base of traditional medicines and employ them in the management of ailments.\(^8\) The formidable challenges facing the traditional medicine are developing standards and guidelines maximizing the economic potential to ensure safe and efficacious provision of medicines WHO (2002). It is reported in various literature that the different parts of plants *Momordica dioica* fruit pulp (family - Cucurbitaceae), *Cuminum cyminum* seeds (family - Apiaceae), *Piper nigrum* seeds (family - Piperaceae), *Boerhaavia diffusa* roots (family - Nyctaginaceae.), *Withania somnifera* roots (family - Solanaceae), *Mangifera indica* leaves (Anacardiaceae), *Plantago ovata* (Plantaginaceae) seeds are traditionally used in the management of asthma.\(^9,10,11,12,13,14,15,16\) While there are various models to assess anti-asthamatic activity, in the present work we have screened the extract of the above plants by investigating their mast cell stabilizing effect.
MATERIALS AND METHODS

Chemicals
The Compound 48/80 was purchased from Sigma Aldrich, Bangalore, India and preserved in deep freeze until use. The standard protective drug disodium chromoglycate was procured from Yarrow chemicals and stored as per storage requirements. All the other chemicals were of analytical grade.

Plant material and preparation of extract
Momordica dioica fruit pulp (family - Cucurbitaceae), Cuminum cyminum seeds (family-Apiaceae), Piper nigrum seeds (family - Piperaceae), Boerhaavia diffusa roots (family - Nyctaginaceae.), Withania somnifera roots (family - Solanaceae), Mangifera indica leaves (Anacardiaceae), Plantago ovata (Plantaginaceae) seeds were collected from different regions of Mangalore, Karnataka, India. The plants were collected in the month of May and June and were authenticated by Professor Krishnakumar G, Head of Applied Botany, Mangalore University, Konaje, Mangalore, India. The reference voucher number MU/AB/July, 2014 was assigned to the plant samples. The extraction procedure was carried out by the method of Handa et al., (2008).\textsuperscript{17} Approximately 500 g of plant materials were extracted separately in a soxhlet apparatus using 95 % ethanol. The solvent from the total extract was removed by means of rotary flash evaporator, later made to a syrupy consistency by evaporating the excess of solvent on a water bath. These extracts were then suspended in 0.6% Sodium CMC to get concentrations of 100 μg/ml, 200 μg/ml, 400μg/ml for screening.

Table I. Percentage yield of of different plants extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>Colour</th>
<th>Consistency</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boerhaavia diffusa</td>
<td>Dark brown</td>
<td>Semi-solid</td>
<td>1.32</td>
</tr>
<tr>
<td>Cuminum cyminum</td>
<td>Dark green</td>
<td>Semi-solid, pasty</td>
<td>10.50</td>
</tr>
<tr>
<td>Piper nigrum</td>
<td>Dark green</td>
<td>Semi-solid</td>
<td>3.02</td>
</tr>
<tr>
<td>Magnifera indica</td>
<td>Dark green</td>
<td>Viscous, Semi solid</td>
<td>20.82</td>
</tr>
<tr>
<td>Momordica dioica</td>
<td>Dark brown</td>
<td>Semi-solid</td>
<td>2.34</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Amber coloured, Brown</td>
<td>Resinous</td>
<td>11.62</td>
</tr>
<tr>
<td>Plantago ovate</td>
<td>Black</td>
<td>Semi solid</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Qualitative Phytochemical Estimations
The crude 95% ethanolic extract of plant materials was examined by standard methods and preliminary study was carried out by chemical test Kokate et al. (1988).\textsuperscript{18}
Test for Alkaloids- Dragendroff’s test: To 1 ml of Dragendroff’s reagent was added to different plant extracts. Formation of orange or orange red precipitate indicates the presence of alkaloids.

Hager’s test: To 1 ml of different plant extract, few drops of Hager’s reagent were added. Formation of yellow precipitate indicates the presence of alkaloids.

Wagner’s test: To 1 ml of different plant extract Wagner’s reagent was added. Yellow or brown precipitation indicates the presence of alkaloids.

Mayer’s test: 1 ml of Mayer’s reagent was added to different plant extract. Formation of pale yellow precipitate indicates the presence of alkaloids.

Test for Carbohydrates- Molisch’s test: Plant extracts were mixed with few drops of molish reagent and concentrated sulphuric acid was added through the side of the test tube. Formation of red-violet ring at junction indicates the presence of carbohydrates.

Benedict’s test: To 0.5 ml of different plant extract, few drops of Benedict’s solution was added and heated. Formation of brick red color indicates the presence of carbohydrates.

Fehling’s test: 1 ml of Fehling’s solution A and Fehling’s solution B were mixed with 2 ml of different plant extracts and heated. Formation of red color indicates the presence of reducing sugar.

Test for Flavonoids- Shinoda test: To 0.5 ml of different plant extract, few magnesium turnings and few drops of dilute hydrochloric acid were added and heated. Formation of pink or reddish brown indicates the presence of flavonoids.

Test for anthraquinone glycosides- Borntrager’s test: Different plant extracts were boiled with ferric chloride solution for about 5 min. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. Formation of rose pink or cherry red colour in the ammonical layer indicates the presence of anthraquinone glycosides.

Test for Steroids and triterpenoids- Liebermann-Burchard’s test: Different plant extracts was dissolved in acetic anhydride and heated. The mixture was cooled and 1 ml of
concentrated sulphuric acid was added along the sides of the test tube. Formation of green color indicates the presence of steroids.

**Salkowsk reaction:** Different extract were treated with few drops of concentrated sulphuric acid. Formation of red and yellow color indicates the presence of steroids and triterpenoids respectively.

**Test for resins:** 1 ml of different plant extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicates the presence of resins.

**Test for saponins:** 5 ml of different plant extract was taken in test tubes; a drop of sodium bicarbonate was added. The test tube was shaken well and left for 3 minutes. Formation of stable froth indicates the presence of saponins.

**Test for tannins:** Add 2 ml of lead acetate solution to different plant extracts. Formation of white cloudy precipitate indicates the presence of tannins.

**Experimental design**
Male albino rats of *Wistar* strain (180-220 g) were obtained from central animal house KSHEMA, Deralakatte, Mangalore. The animal experiments were executed after obtaining Institutional Animal Ethical clearance in accordance with the guidelines of the committee for the purpose of control and super vision of experiments on animals (CPCSEA) formed by the government of India (approval number; KSHEMA/IAEC/17/2014). Rats were housed in polypropylene cages under an ambient temperature of 25 ± 2°C, Relative humidity 50 ± 60% with dark and light cycle 12h/12h. Animal were maintained on a standard pellet diet and water *ad libitum*. The animals were allowed for acclimatization for a period of seven days before initiation of the experiments.

**In vitro mesenteric mast cell degranulation**
The plant extracts were suspended in 0.6% Sodium CMC to get concentrations of 100 μg/ml, 200 μg/ml and 400 μg/ml for evaluation of mast cell stabilization effect. The overnight fasted rats were sacrificed by cervical dislocation. The abdomen was cut open to expose the intestine and the small pieces of the mesentery were cut and placed in a petri dish containing Ringer Locke solution (NaCl 154 mM, KCl 5.6 mM, CaCl₂ 2.2 mM, NaHCO₃ 6.0 mM and dextrose 5.5 mM) at 37°C and then subjected to the following treatment schedules.
**Petri dish I:** Vehicle control (Ringer Locke solution only)

**Petri dish II:** Positive control (0.8μg/ml of C.48/80 only)

**Petri dish III:** Standard (disodium chromoglycylate-DSCG, 1 mg/ml)

**Petri dish IV:** 100μg/ml of plant extract.

**Petri dish V:** 200μg/ml of plant extract.

**Petri dish VI:** 400μg/ml of plant extract.

Each petri dish was incubated for 15 min at 37° C and later compound 48/80 at 0.8μg/ml was added to each petri dish except vehicle control and again incubated for 10 min at 37° C. After incubation, all pieces were immersed in 4% formaldehyde solution containing 0.1 % toluidine blue for 20-30 min and then were treated with acetone and then xylene for 5 min and mounted on slides. The stained mesentery pieces were focused through a digital light microscope at 100x magnification. Minimum 100 mast cells were counted and percentage of intact and fragmented mast cells was determined. Each cell was considered either fragmented or not fragmented, and percentage protection from degranulation of mast cells was calculated using the following formula (Norton et al., 1954).[^19]

\[
\text{Percentage of intact mast cells} = \frac{\text{Total no of mast cells} - \text{total no of degranulated cells}}{\text{Total no of mast cells}} \times 100
\]

**RESULTS**

**Qualitative phytochemical estimations**

The plant extracts were examined for preliminary phytochemical screening through different standards showing the presence of alkaloids, carbohydrates, steroids, flavonoids, saponins, steroids, tannins, and triterpenoids in the phytochemical screening, on the basis of number of secondary metabolites (Table II).

### Table II. Phytochemical analysis of ethanolic extract of different plants

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Test</th>
<th>EBD</th>
<th>EMD</th>
<th>ECC</th>
<th>EPN</th>
<th>EMI</th>
<th>EWS</th>
<th>EPO</th>
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<tbody>
<tr>
<td>1.</td>
<td><strong>Test for alkaloids</strong></td>
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<tr>
<td></td>
<td>Dragendorff’s test</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>Hager’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>Mayer’s test</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>2.</td>
<td><strong>Test for carbohydrates</strong></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Benedict’s test</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Fehling’s test</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
<td>Molisch’s test</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>3.</td>
<td><strong>Test for flavanoids</strong></td>
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</tbody>
</table>

[^19]: Norton et al., 1954
<table>
<thead>
<tr>
<th>Test for</th>
<th>Shinoda’s test</th>
<th>Anthracene test (Modified borntraggers test)</th>
<th>Triterpenoids and steroids</th>
<th>Liebermann – Burchard test</th>
<th>Salkowski reaction</th>
<th>Test for resins</th>
<th>Test for saponins</th>
<th>Test for tannins</th>
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</thead>
<tbody>
<tr>
<td>4.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Test for glycosides</td>
<td>Anthracene test (Modified borntraggers test)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>-</td>
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<td>-</td>
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<td>7.</td>
<td>+</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>9.</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
</tr>
</tbody>
</table>

+: present, -: absent

Momordica dioica fruit pulp (EMD), Cuminum cyminum seeds (ECC), Piper nigrum seeds (EPN), Boerhaavia diffusa roots (EBD.), Withania somnifera roots (EWS), Mangifera indica leaves (EMI), Plantago ovata (EPO)

**In vitro mesenteric mast cell degranulation**

The result showed a higher stabilization potential was exhibited by ethanolic extract of *Piper nigrum* seeds which showed 55.17±1.56, 64.00±1.18 and 70.50±0.96 percentage inhibition of mast cell degranulation (Figure 1). Minimal activity was observed in the ethanolic extract of *Plantago ovata* with percentage inhibition of 21.17±1.54, 22.17±2.21, and 24.33±2.20 at three dose levels (Figure 2). Other plant extracts showed intermediate activity (Figure 3-7).

![Figure 1. Effect of ethanolic extract of Piper nigrum seeds on Mast cell Stabilization activity on rat mesentery tissue](image-url)
Figure 2. Effect of Ethanolic extract of Plantago ovata seeds on Mast cell stabilization activity on rat mesentery tissue

Figure 3. Effect of ethanolic extract of *Boerhaavia diffusa* roots on Mast cell Stabilization activity on rat mesentery tissue

Figure 4. Effect of ethanolic extract of Momordica dioica fruit pulp on Mast cell Stabilization activity on rat mesentery tissue
Figure 5. Effect of Ethanolic extract of Mangifera indica leaves on Mast cell Stabilization activity on rat mesentery tissue

Figure 6. Effect of Ethanolic extract of Cuminum cyminum seeds on Mast cell Stabilization activity on rat mesentery tissue

Figure 7. Effect of Ethanolic extract of Withania somnifera roots on Mast cell stabilization activity on rat mesentery tissue
DISCUSSION

The present work has been taken up with an objective of screening a large number of plants which have been reported to possess anti-histaminic, anti-allergic and immunomodulatory activity. Several approaches have been tried for the prevention and cure of asthma, which affects nearly 25% of the population. The environmental pollution and rapid industrialization have contributed to a dramatic increase in the incidence of asthma in the recent times.\(^{20}\) It has been clearly established that the airway inflammation is the main cause of asthma, which leads to bronchoconstriction and mucus secretion.\(^{21, 22}\) With this understanding, various approaches for the management of asthma have been investigated. Steroidal anti-inflammatory drugs, bronchodilators, \(\beta\)-adrenergic stimulants, leukotriene antagonists, phosphor diesterase inhibitors, anti-\(\text{IgE}\) antibodies, monoclonal antibodies, 5- lipoxygenase inhibitors and mast cell stabilizers have been used in the prevention and cure of asthma.\(^{23}\)

In the present study, we have concentrated on the screening of several plants for their ability to prevent the degranulation of mast cell and release of mediators of inflammation such as histamine, tryptase, chymase, heparin, leukotrienes, interleukins, nitric oxide etc. Our findings show that the efficacy of the plant extracts in asthma may be partially due to the mast cell stabilizing effect. Based on the literature survey, we have selected seven plants which are employed in the treatment of allergy and respiratory disorders in the traditional or folklore medicine. The selected plants are *Momordica dioica* fruit pulp (family - Cucurbitaceae), *Cuminum cyminum* seeds (family - Apiaceae), *Piper nigrum* seeds (family - Piperaceae), *Boerhaavia diffusa* roots (family - Nyctaginaceae), *Withania somnifera* roots (family - Solanaceae), *Mangifera indica* leaves (Anacardiaceae), *Plantago ovata* seeds (Plantaginaceae).

The study revealed that the ethanolic extracts of all except the seeds, of *Plantago ovata* seeds showed positive results for the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, glycosides and tannins. All the plants showed significant activity. The highest activity was exhibited by the ethanolic extract of *Piper nigrum* and almost insignificant activity in the ethanolic extract of *Plantago ovata*. The presence of triterpenoids and flavonoids which have proven histamine release inhibitory action might have contributed to the stabilization of mast cells.\(^{24}\) For the same reason, *Plantago ovata* was least effective. Moreover, the scavenging of free radicals by the phyto-constituents in the above plants also contributes to the Stabilization of mast cells.\(^{25}\) Allergen-activated and recruited inflammatory cells such as
eosinophils, macrophages, monocytes and neutrophils generate more ROS in the asthmatic condition.\[26\] In addition to the cells, the mediators like lipid mediators, chemokines, adhesion molecules, and eosinophil granule proteins are potential stimuli of ROS production, so released in the process of inflammation also add to the oxidative stress in the individuals.\[27\] The ROS can perturb the airways and can worsen the pathophysiological changes which are associated with the asthma. ROS also cause the release of histamine from the mast cells and cause mucus secretion from airway epithelial cells.\[28\] ROS are also involved in production of a number of inflammatory mediators, most notably eicosanoids, by activating phospholipase A2 (PLA2).\[29\] The plants have their own strategy to detoxify the free radicals which may be done by direct enzymatic breakdown of oxidant radicals using SOD, catalase, ascorbate peroxidase, peroxidase, glutathione reductase and monodehydro ascorbate reductase which convert oxygen radicals to reduced products.\[30\] On the basis of the present finding it is concluded that extract of *Piper nigrum* seeds showed the highest percentage of inhibition compared to the other extracts and can be useful for the disorders associated with the mediators of inflammation released from the mast cells. However, further studies on other experimental models are needed to support the hypothesis. A detailed study needs to be conducted to evaluate the phytoconstituent responsible to produce above result and their clinical efficacy in the treatment of asthmatic patients.

**Conflict of interest**

We authors declare no conflict of interest.

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