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Screening for Anti-allergic and Anti-histaminic Activity of Extract of *Momordica dioica*, *Myrica esculenta* and *Euphorbia hirta* in Animal Models.

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ABSTRACT

In the present study, the effect of aqueous extract of *Momordica dioica,* aqueous extract of *Myrica esculenta* and ethanolic extract of *Euphorbia hirta* alone and in combination on histamine induced bronchospasm in guinea pig, milk induced leukocytosis and eosinophilia in mice and on antipruritic activity in mice was done. In the animal model of histamine induced bronchospasm, all plant showed significant protection against bronchospasm induced by histamine. In milk induced leukocytosis and eosinophilia model, all plants showed significant decrease in difference in no. of leucocytes and eosinophils count. In the antipruritic activity model of mice, all these plants showed significant reduction in pruritic activity. Thus these results suggest that MD, ME and EH may prove to be a potential therapeutic drug for treating allergic diseases and the result obtained in this study may be due to its antihistaminic, adaptogenic, antistress, and anti-inflammatory activities.

Keywords: Antiallergic**,** Momordica, Myrica, Euphorbia, leukocytosis, eosinophilia

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INTRODUCTION

Ayurveda remains one of the most ancient and yet living traditions that practised widely in India [1]. Atharvaveda, Charak Samhita and Sushrut Samhita are the main classics that give detailed description of over 700 herbs [2]. Indian health culture consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for a variety of chronic disease conditions [3].

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population [4]. Allergic diseases are responsible for significant morbidity, and have severe economic impact. Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases [5-6].

Intensive research during the last several decades has highlighted the role of lymphocytes, immunoglobulin's, mast cells and various autocoids in the etiopathogenesis of allergic conditions. In spite of the voluminous literature on the subject, the treatment of allergic diseases continues to be far from satisfactory. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations owing to low efficacy, associated adverse events, and compliance issues [7].

Currently antihistaminics like Chlorpheniramine, Promethazine, Cetirizine etc and Corticosteroids like Dexamethasone, Hydrocortisone etc are the mainstay of treatment in allergic reactions. But these agents are associated with limitations. Antihistaminics having common side effects like sedation, loss of appetite, nausea, vomiting, urinary retention etc and corticosteroids having side effects like cushing habitus, hyperglycemia, fragile skin, purple striae, delayed wound healing etc. Also certain antihistaminic agents need to be used cautiously while treating pregnant and lactating women because of their possible teratogenic effects [8].

With the advancement of ayurvedic tradition and its scientific exploration, several classes of plant species have been studied in order to evaluate their therapeutic potentials and to isolate the lead compounds. Medicinal plants are of great importance in providing healthcare to a large portion of the population in India. A number of plants are described in ayurveda for use in the treatment of allergic disorders, namely psoriasis, eczema, bronchial asthma etc [9].

Use of plant products is increasing in many scenario of the world for treatment of various diseases because these are natural, easily available, low cost and acceptable. There are many plants in ayurveda having antiallergic activity. Few of them are Inula racemosa, Myrica esculenta, Euphorbia hirta, Mentha spicata, Cynodon dactylon, Momordica dioica, Hedychinum spicatum, Indigofera dosua, Piper longum, Ocimum sanctum etc [10].

Study drugs

Momordica dioica commonly known as 'Kartoli' is a climbing creeper plant used both in the prevention and cure of various diseases. The whole plant is used for treatment of eye diseases, poisoning. Plant is claimed to be expectorant, analgesic. Root is used to stop bleeding from piles, as an expectorant and also in urinary and bowel complaints. Fruits, leaves and tuberous roots used in India as a folk remedy for diabetes. Roots also possess spermicidal activity and antihelminthic activity [11].

Myrica esculenta commonly known as 'Katphala' is an evergreen, sun-temperate tree growing to 12 meter height. The bark of Myrica esculenta is said to be useful in catarrhal fever, cough, throat infection, asthma, urinary discharges, bronchitis, anaemia, cholera, ulcers and in many other diseases. The fruits are used to heal ulcer [12].

Euphorbia hirta commonly known as 'Dughdhika' is an herbaceous wild plant which grows in hotter parts of India. It is often used traditionally for female disorders, respiratory ailments (cough, coryza, bronchitis, and asthma), dysentery, jaundice, pimples, gonorrhoea, digestive problems, and tumours [13].

Hence, the present study was undertaken to evaluate the antiallergic and antihistaminic activity of these plants using different animal models. The activity was compared with standard drug Dexamethasone

and Chlorpheniramine maleate to find out the link between ayurveda and modern medicine and possibility of any future combinations of these drugs.

MATERIALS AND METHODS

Experimental animals

Swiss albino mice of either sex, weighing 20-25 gm and Guinea pigs (weighing 300 to 400 gm) of either sex were procured from central animal house approved by CPCSEA.. They were provided with normal diet and water ad libitum and provided with a standard light-dark cycle with illumination from 0700 to 1900 hr. The animals were housed under standard conditions of temperature (23 ± 2°C) and humidity (55 ± 15%).

All the experiments were performed between 10 a.m. and 4 p.m. in the central animal research laboratory of the institute.

Procurement of Plant Material

Aqueous extract of fruit pulp of Momordica dioica, aqueous extract of stem bark of Myrica esculenta, Ethanolic extract of aerial plant of Euphorbia hirta were obtained from *Ayurved Rasshala, Pune.* Solutions of all drugs were prepared by dissolving it in 0.9% normal saline. All the drugs were given by oral route.

Histamine induced bronchospasm [14-16]

Each animal of all the groups was placed in the histamine chamber and exposed to 1% Histamine dihydrochloride aerosol under a constant pressure of 40 mm Hg for the baseline readings. After exposure to the histamine aerosol, the animal showed signs of immediate immobilization. This was followed by shallow breathing symptoms, after which the animal collapsed, fell on its back and convulsed. The time taken by the animal to fall on its back after exposure to the Histamine aerosol was designated as the preconvulsive time. The preconvulsive time (PCT) for each animal in all the nine groups was noted. Once the animal fell on its back, it was immediately taken out of the chamber and exposed to fresh air where the animal returned back to normal. This time for PCT was considered as day 0 (baseline) values. On day 1, animals of control group were given Normal Saline (0.5ml), standard group animals were given Chlorpheniramine maleate (2mg/kg) orally and the animals of remaining group were received test drug orally. Two hour after the administration of drugs, each animal of all the groups was placed in the histamine chamber and exposed to 1% Histamine dihydrochloride aerosol and the preconvulsive time (PCT) was noted as described previously. This time for PCT was considered as day 1 values. The drug treatment was continued for 5 days in all the groups.

Milk induced leukocytosis and eosinophilia in mice [17-19]

Mice were randomly divided into nine groups containing six animals each. Leukocyte count and eosinophil count was done before the milk and drug administration. Blood was collected from retro-orbital sinus of the mouse through medial canthus approach. Microhematocrit tubes, 200ul pipettes or tube of similar diameter was used for collection of blood. Leukocyte count and eosinophil count was done by using Neubour's chamber. All aseptic precautions were maintained during whole procedure. After collection of the blood, animals of control group were given Normal Saline (0.5ml), standard group animals were given Dexamethasone (0.5mg/kg) orally and the animals of group 3 to 9 were received test drug orally. Eighteen hours after administration of test drug, sterile cow milk (boiled and cooled) was injected in each animal of all groups in a dose of 4 ml/kg subcutaneously over the shoulders. Again 24 hours after milk injection, blood was collected from retro-orbital sinus of the mouse, and leukocyte count and eosinophil count was measured as described previously. Difference in leukocyte and eosinophil count before and after administration of drug was determined.

Antipruritic activity in mice [20-22]

Mice were divided into nine groups each containing six animals. Before the experiments male swiss albino mice were acclimated in the acrylic cages for about 10 min. Control group animals were received Normal Saline (0.5ml) and standard group animals were received Chlorpheniramine maleate (0.3mg/kg)

subcutaneously into the loose skin over the neck and other groups were treated with the extract in graded doses orally. One hour after the administration of test drug, the rostral part of the skin on the backs of mice was clipped, 100 µL of 1% of compound 48/80 for each mouse was injected intradermally with the use of a 29-gauge needle. Immediately after the intradermal injection, the mice were put back into the same cages. The scratching behavior in mice is a repetitive fast up and down movement of the hind foot rubbing and scratching the side of the body, the neck or the face. Events of scratching behaviour on the whole body was counted for 20 minutes.

Statistical analysis

The results were expressed as Mean ± SEM from 6 animals. The analysis between two groups was done using the students unpaired and, Paired't' test (two-by-two t-test comparisons) with the help of GraphPad Prism 5 software. Bonferroni test was used for inter group comparison. Null hypothesis was rejected (statistically significant difference exists) if P value was < 0.05.

RESULTS

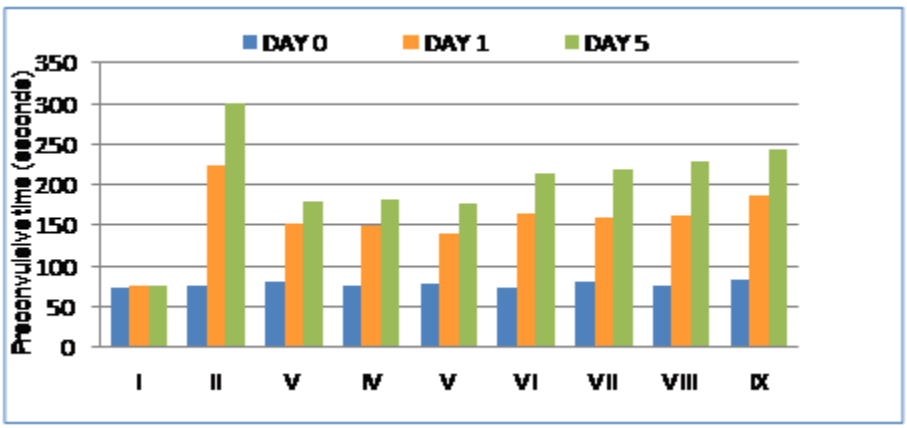
Effect of various drugs on PCT in Histamine induced bronchospasm in Guinea pigs

In each groups, the Preconvulsive time (PCT) in seconds on day 0 was compared with the same on days 1 & 5, for the respective drug groups. In Control group there was no significant increase in PCT on day 1 and day 5 as compared to baseline. In Chlorpheniramine group, the preconvulsive time was increased significantly (p <0.001) from 76.50±2.20 to 224±6.22 on day 1 and 300.17±8.76 on day 5. Each of the individual test groups showed a significant increase (p < 0.001) in the preconvulsive time on day 1 & day 5 as compared to baseline of the same group. Each of the combination test groups also showed significant increase (p<0.001) in the preconvulsive time of days 1 & 5, as compared to baseline, of that respective group.

Table 1: Effect of various drugs on PCT in Histamine induced bronchospasm in Guinea pigs

Group No.	Groups (n=6)	Preconvulsive time in seconds		
		(Day 0)	Day 1	Day 5
I	Control- Normal Saline (0.5ml)	74.17±2.18	76.67±2.95	76.17±2.33
II	Chlorpheniramine (2mg/kg)	76.50±2.20	224±6.22***	300.17±8.76***
III	Momordica dioica (50mg/kg)	80.83±1.87	152.67±5.55***	179.33±5.35***
IV	Myrica esculenta (150mg/kg)	75.17±2.76	148.67±5.48***	181.83±7.15***
V	Euphorbia hirta (100mg/kg)	77.50±4.01	139.50±3.47***	177.17±7.08***
VI	MD+ME (50mg/kg +150mg/kg)	73.17±3.34	164.17±6.94***	213.17±5.52***
VII	MD+EH (50mg/kg +100mg/kg)	79.83±3.09	160.33±5.58***	219.83±4.06***
VIII	ME +EH (150mg/kg +100mg/kg)	75.17±2.43	162.33±4.25***	227.83+3.59***
IX	MD+ME+EH(50mg/kg +150mg/kg +100mg/kg)	81.83±1.89	185.67±6.72***	242.83±3.91***

* <0.05, ** <0.01, *** <0.001 Comparison of PCT (in seconds) on Day 1 and Day 5 with Baseline.



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Effect of various drugs on Leukocyte count and eosinophilic count (/cmm) of mice in various groups before and after treatment

Subcutaneous injection of milk at dose of 4 ml/kg produced an increase in the leucocytes count and total eosinophil count after 24 hr of its administration. Animals treated with Dexamethasone (50 mg/kg, i.p.), has shown significant inhibition of milk-induced leucocytosis and eosinophilia as compared to positive control ($p< 0.01$). In the groups pretreated with aqueous extract of MD at the dose of 50mg/kg p.o ($p<0.05$), aqueous extract of ME 150mg/kg and ethanolic extract of EH at the dose of 100 mg/kg, showed significant inhibition of milk-induced leucocytosis and eosinophilia. The combination group also showed significant inhibition of milk-induced leucocytosis and eosinophilia.

Table 2: Leukocyte count (/cmm) of mice in various groups before and after treatment:

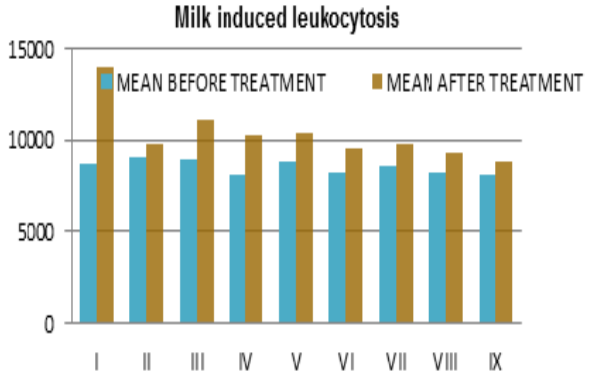
Group No.	Groups (n=6)	Leukocyte Count (/cmm)	
		Before treatment (Mean± SEM)	After treatment (Mean± SEM)
I	Control- Normal Saline (0.5ml)	8665± 283.95	13946.60± 244.06***
II	Dexamethasone (0.5mg/kg)	9125±144.91	9805±160.58
III	Momordica dioica (50mg/kg)	8946.60±57.95	11140±184.16***
IV	Myrica esculenta (150mg/kg)	8171.60±80.56	10278.33±301.62***
V	Euphorbia hirta (100mg/kg)	8808.30±87.88	10438.33±176***
VI	MD+ME (50mg/kg +150mg/kg)	8233.30±143.05	9566.60±207.12**
VII	MD+EH (50mg/kg +100mg/kg)	8551.66±189.22	9815±350.01**
VIII	ME +EH (150mg/kg +100mg/kg)	8220±202.37	9318.30±192.64*
IX	MD+ME+EH(50mg/kg +150mg/kg +100mg/kg)	8071.60±179.23	8883.30±150.41

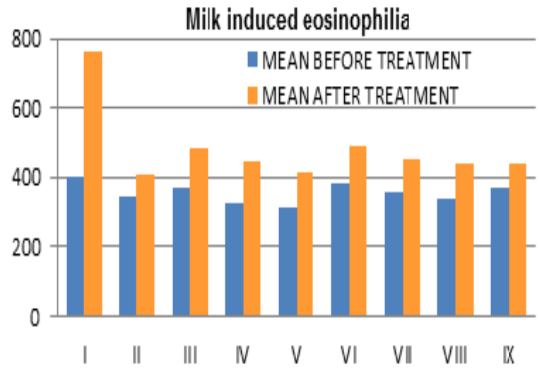
* <0.05, ** <0.01, *** <0.001 Comparison of leukocyte count before and after treatment.

Table No.3: Eosinophil count (/cmm) of mice in various groups before and after treatment:

Group No.	Groups (n=6)	Eosinophil Count (/cmm)	
		Before treatment (Mean± SEM)	After treatment (Mean± SEM)
I	Control- Normal Saline (0.5ml)	400.83±15.35	760±22.51***
II	Dexamethasone (0.5mg/kg)	347.50±30.71	406.67±24
III	Momordica dioica (50mg/kg)	371.67±16.26	481.67±13.52*
IV	Myrica esculenta (150mg/kg)	328.33±21.67	447.5±21.67**
V	Euphorbia hirta (100mg/kg)	315±16.68	411.67±14
VI	MD+ME (50mg/kg +150mg/kg)	382.50±18.79	488.33±12.22*
VII	MD+EH (50mg/kg +100mg/kg)	354.17±17.91	450±11.76
VIII	ME +EH (150mg/kg +100mg/kg)	336.67±21.24	437.50±20.56
IX	MD+ME+EH(50mg/kg +150mg/kg +100mg/kg)	369.17±22.89	441.67±20.56

* <0.05, ** <0.01, *** <0.001 Comparison of eosinophil count before and after treatment..





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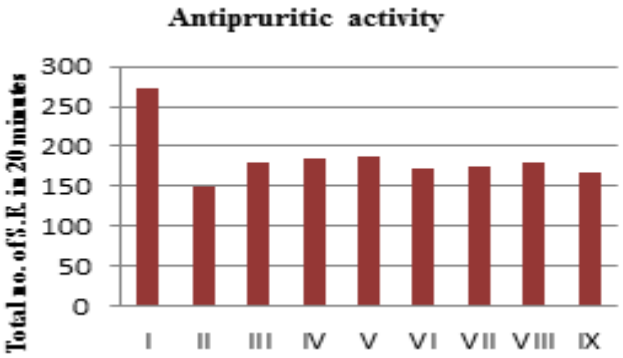
Effect of various study drugs on Compound 48/80-induced pruritic activity in mice

Subcutaneous injection of compound 48/80 elicited a significant scratching response in mice.The mean number of scratching events observed in control group was 272.83±6.27. However, in Chlorpheniramine treated group it was 148.83± 5.50, which is significantly lower ($p<0.01$) as compared to control group. The scratching events were significantly less ($p<0.01$) in case of the individual test groups, i.e. Momordica dioica (.179.83±5.92), Myrica esculenta (185.17 ± 4.93) and Euphorbia hirta (188.17 ± 5.95), as compared to the control group treated with Normal Saline. But it was significantly higher than Chlorpheniramine group. In case of the combination test groups, the scratching events was significantly less ($p<0.01$) for each of them as compared to the control group. However, there was no significant difference in scratching events when compared with Chlorpheniramine group except for group VIII (Myrica esculenta + Euphorbia hirta).

Table 4: Effect of various drugs on pruritic activity in mice

Group No.	Groups (n=6)	No of scratching events (Mean± SEM)
I	Control- Normal Saline (0.5ml)	272.83±6.27
II	Dexamethasone (0.5mg/kg)	148.83± 5.50**
III	Momordica dioica (50mg/kg)	179.83±5.92**
IV	Myrica esculenta (150mg/kg)	185.17±4.93**
V	Euphorbia hirta (100mg/kg)	188.17±5.95**
VI	MD+ME (50mg/kg +150mg/kg)	172±3.55**
VII	MD+EH (50mg/kg +100mg/kg)	174.17±7.16**
VIII	ME+EH(150mg/kg +100mg/kg)	178.83±7.11**
IX	MD+ME+EH(50mg/kg+150mg/kg +100mg/kg)	166.17±5.00**

* <0.05, ** <0.01, *** <0.001 Comparison of Group I Vs Group II, III,IV,V,VI,VII,VIII,IX



DISCUSSION

Allergy is one of the common diseases that affect mankind with diverse manifestations. The prevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population [23]. The etiology of allergen reactivity is based on IgE-medicated pathological processes in a variety of cell populations such as mast cells and basophils. Degranulation of mast cells and basophils with antigen cross-linked IgE releases histamine, prostaglandins, leukotrienes and cytokines affecting lymphocytes, macrophages, eosinophils and neutrophils. Finally cytokine-induced reaction causes tissue injury. Therefore antiallergic agents with additional anti-inflammatory actions may be more beneficial drugs for allergic diseases [24]. Modern approaches includes Mast cell stabilizers, Leukotriene receptor antagonists, Histamine receptor blockers, Methylxanthines and Corticosteroids but clinical evaluations of these drugs have shown incidences of relapse, side effects and drug interactions. For example leukotriene receptor antagonists like

Montelukast and Zafirlukast can cause headache and gastrointestinal disturbances [25]. Use of plant products is increasing in many segment of the population. At present, thousands of plant metabolites are being successfully used for the treatment of variety of diseases. According to an estimate, 80% of the world's population once relied upon plants for their medication. The use of the medicinal plants is increasing in many countries (e.g.India, China) where 35% of drugs contain natural products.

The aim of this study was to evaluate antiallergic and antihistaminic property of Momordica dioica, Myrica esculenta, Euphorbia hirta, individually as well as in combination and to compare their activity with standard anti-inflammatory and antiallergic drugs like Dexamethasone and Chlorpheniramine so as to answer the question-whether these agents have any role in allergic conditions.

Histamine when inhaled has been shown to induce bronchoconstriction by direct H1-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes In Histamine induced bronchospasm test, Momordica dioica has shown significant ($p<0.001$) increase in PCT on day 1 and on day 5 as compared to PCT on day 0, Myria esculenta treated group has shown significant ($p <0.001$) improvement in PCT on day 1 and day 5 as compared to baseline of same group and prior treatment of Euphorbia hirta (100 mg/kg, orally) protected the animals to a significant extent ($p <0.001$) from the development of asphyxia produced by histamine aerosol. This indicates that it has some protective role against bronchospasm induced by Histamine.

In the present study demonstrated that, after 24 hour of parental administration of milk (4 ml/kg, s.c.) to the vehicle treated group significantly increased the total leukocyte and eosinophil count, whereas, in the groups treated aqueous extract of *Momordica dioica,* aqueous extract of *Myrica esculenta* and ethanolic extract of *Euphorbia hirta* and in combination of these extracts, there was significant inhibition of milk induced leukocytosis and eosinophilia in mice. This probably indicates the adaptogenic activity of aqueous extract of *Momordica dioica,* aqueous extract of *Myrica esculenta* and ethanolic extract of *Euphorbia hirta.*

The pathological mechanisms in Type-I allergic conditions involve the degranulation of mast cells, followed by the release of mediators such as histamine, leukotrienes and prostaglandins from these cells . The degranulation of mast cells occurs in response to the immunological stimuli in which the antigen-antibody reaction on the cell surface predominates. In antipruritic activity model stimulation of mast cells with compound 48/80 initiates the activation of signal transduction pathway, leading to release of histamine, which is responsible for increase in pruritic behaviour in mice.

In the present study Momordica dioica, Myrica esculenta and Euphorbia hirta has shown statistically significant decrease ($p<0.01$) in scratching events as compared to control group, while it was slightly ($p<0.05$) higher than Chlorpheniramine group. This indicates that the plant has protective activity against pruritus behaviour in mice. While combination of all these three plants has shown statistically significant decrease ($p<0.01$) in scratching events as compared to control group, while it was nearly equal to ($p<0.05$) than Chlorpheniramine group.

CONCLUSION

The use of traditional medicine is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of innovative drugs. Plants extracts, however are some of the attractive sources of new drug and have shown to produce promising results in treatment of allergic inflammatory diseases such as asthma. Therefore this study was carried out for evaluation of antiallergic and antihistaminic activity of extract of Momordica dioica, Myrica esculenta and Euphorbia hirta by using following methods 1) Histamine induced bronchospasm in guinea pigs 2) Milk induced leukocytosis and eosinophilia in mice, 3)Antipruritic activity in mice .

All these plants individually as well as in combination showed significant protection of guinea pigs against histamine induced bronchospasm, significantly reduces the percentage of leukocytosis and eosinophilia after milk injection and significantly decreases the total number of scratching events in mice demonstrating that these plants have significant antiallergic and antihistaminic activity.

In conclusion, the drugs used in this study, i.e aqueous extract of fruit pulp of Momordica dioica; aqueous extract of stem bark of Myrica esculenta and ethanolic extract of aerial plant of Euphorbia hirta possess antiallergic and antihistaminic property when used individually. But their combination is superior to all. Therefore suitable combinations of all these drugs may be used in allergic disorders. However clinical studies are required for confirmation of antiallergic and antihistaminic potential of these plants in human volunteers and in different patients.

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