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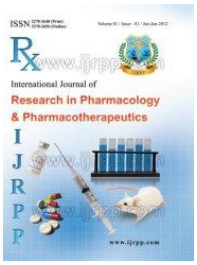

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Polyherbal Formulation Development for Anti-asthmatic activity

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ABSTRACT

The aim of the present work is to develop and standardize the herbal formulations for asthma using well documented herbs. Three herbs selected for this work namely *Tylophora indica, Tephrosia purpurea* and *Vitex negundo* based on the reported antiasthmatic, antihistaminic and anti-inflammatory activity. All the three plants were collected, authenticated and extracted with suitable solvents as per the literature. The ash values and extractive values were carried out for the selected plants and reported. Commercial extracts of the same plant were procured from Garlico Herbal Concentrate, MP, India with certificate of analysis for comparative studies. Two tablet formulations were prepared using in-house and commercial extracts and one capsule formulation was prepared by using commercial extracts. All the three formulations were subjected to various physical evaluations like friability, hardness, disintegration time and weight variation test and the results are complies as per the Pharmacopoeia standards. The anti-asthmatic activity was carried out for all the formulations and found that the all the formulations showed significant activity when compared to standard drug Chlorpheniramine maleate. Stability and clinical studies to be carried out in future to confirm the quality and efficacy of the product.

Keywords: In-house extracts, Commercial extracts and Anti-asthmatic activity.

INTRODUCTION

Over the past few years, however, the medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine in view of its lesser side effects compared to allopathic medicine in addition the necessity of meeting the requirements of medicine for an increasing human population[1]. The World Health Organization (WHO) has estimated the present demand for medicinal plants is approximately US $14 billion per year. The demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% annually. According to an estimate of WHO, the demand for medicinal plants is likely to increase more than US $5 trillion in 2050[2].

In present status various herbal formulations are available, but it is difficult to understand the theme of polyherbal preparations because of the more number of ingredients. It is very difficult to set the standards of these types of formulations. Keeping in view the growing popularity of herbals and more adverse effects of allopathic drugs, it is the need to formulate an herbal formulation.

On the basis of above evidence, the attempt was made to prepare a polyherbal formulation for asthma using well documented herbs namely; *Tylophora indica, Tephrosia purpurea, and Vitex negundo*[3,4&5]. The objective of the present work is to formulate and evaluate the tablet and capsule formulations for anti-asthmatic activity.

METHODOLOGY

Collection and Authentication of Plants

The plants *Tylophora indica Tephrosia purpurea* and *Vitex negundo* were collected from Erode and Madurai districts and authenticated from Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India.

Extraction of Herbs

The powdered aerial parts of all the collected herbs were extracted with 95% ethanol by soxhelation as per the literature and dried[6]. The dried extracts were used for the preparation of formulations. The commercial extract of *Tylophora indica*, *Tephrosia purpurea* and *Vitex negundo* were procured from Garlico. ltd, Mumbai for comparative studies.

Physiochemical Constant Determination

The physiochemical constants like ash values (total ash, acid insoluble ash and water soluble ash) and extractive values (alcohol soluble and water soluble extractives) were carried out and reported[7].

Formulation Development

Commercial extracts and In-house extracts of selected herbs used for the development of polyherbal tablet and capsule formulations. The formulations were prepared by non-aqueous wet granulation method using polyvinyl pyrrolidine (PVP) with isopropyl alcohol as granulating agent. The weighed quantities of ingredients were mixed thoroughly and the granulating liquid was added slowly till the powder become to a damp mass. This damp mass was passed through Sieve No.12 and dried in room temperature until IPA removed from the granules, then dried in an oven at 60ºc temperature. The dried granules were again passed through sieve No.16 to get uniform granules. The dried granules were then subjected to lubrication. Finally the tablets were compressed by using Cadmech 16 Station Rotary Compression Machine with average weight of 620 mg/tablet[8].

Table 1. Composition of tablet formulation

S.No	Contents	For 1 tablet (mg)	For 250 tablets (gm)
1	*T.asthmatica*	100	25
2	*T.purpurea*	100	25
3	*V.negundo*	50	12.5
4	Starch	260	65
5	Microcrystalline cellulose	40	10
6	Polyvinyl pyrrolidine	10	2.5
7	Sodium benzoate	0.5	0.125
8	Isopropyl alcohol	q.s	q.s
9	Primolose	10	2.5
10	Aerosil	10	2.5
11	Sodium starch glycolate	10	2.5
12	Starch	14.5	3.625
13	Talc	10	2.5
14	Magnesium stereate	5	1.25
	Total weight	**620 mg**	**155 gm**

Pre-formulation study

The overall objective of pre-formulation testing is to generate information useful to the formulator in developing stable and bioavailability dosage form, which can be mass-produced obviously, the type of information needed

depend on the dosage form to be developed. In the present work the pre-formulation studies viz: angle of repose, bulk density, fines and loss on drying were carried out[9].

Capsule formulation

The same tablet granulation method was adopted for the preparation of capsule using the ingredients listed in the table 2. The granules were filled into the capsule shell by hand filling method with the average weight of 260 mg/capsule[8].

Table 2. Composition of capsule formulation

S.No	Contents	For one capsule (mg)	For 250 capsule (gm)
1	*T.asthmatica*	100	25
2	*T.purpurea*	100	25
3	*V.negundo*	50	12.5
4	Polyvinyl pyrrolidine	5	1.25
5	Isopropyl alcohol	q.s.	q.s
6	Talc	5	1.25
	Total weight	**260 mg**	**65 gm**

Evaluation of Formulations

The prepared tablet and capsule formulations were subjected to various physical evaluations like weight variation, hardness, friability test and disintegration time as per the procedure given in Indian Pharmacopoeia and reported[10].

Pharmacological Study

All the three formulations were subjected into antihistaminic activity against vehicle control where the only vehicle has given and positive control where the chlophenaramine at 2 mg/kg dose level has given to guinea pigs[11].

Histamine Induced Bronchospasm

Male guinea pigs were fasted overnight with water ad libitum before the experiment. The apparatus used was an air tight perplex box of 30 x 40 x 50 cm with a sliding door in the front and a small slit on the back of the apparatus for the passage of aerosol. On day 1 of the experiment the overnight fasted g.pigs were placed in a experimental chamber and histamine acid phosphate (0.25 %w/v) aerosol was exposed to the guinea pigs. The aerosol was generated by an ultrasonic nebulizer [OMRON CX3 (NE-C16/EN2)], which had an aerodynamic mass diameter of 0.5-5 micro meter. About 0.25 ml solution was nebulized per min with a pressure of 30-6 psi.

The preconvolsive time (PCT) was determined from the time of exposure to the onset of dysponea. As soon as dysponea was noted, the animal was removed from the chamber and exposed to fresh air. The animals were recuripated for a period of 3-4 days. On day 3 or 4 the overnight fated guinea pigs were administered with various concentrations of test doses. One hour later the guinea pigs were placed in the experimental chamber and histamine acid phosphate (0.25 % w/v) aerosol was exposed to the guinea pigs and the PCT was noted (this constitute PCT2).

Animals which stood with the bronchospamogenic aerosol for 15 mins were considered as completely protected. The increase in latency to the appearance of PCD was calculated substracting PCT2 with PCT1.

Five groups were selected with four animals each.

Group . I - Vehicle control
Group. II - Positive control (Chlorpheniramine malate)
Group. III - Test drug, Tablet.A
Group. IV - Test drug, Tablet.B
Group. V - Test drug, Capsule

RESULTS AND DISCUSSION

Physiochemical Constant Determination

Ash values

The ash value viz total ash, acid insoluble and water soluble ash were performed for all the selected plant drugs and the results are reported in Table 3. The *Tylophora asthmatica* plant showed more total ash, acid insoluble ash value, water soluble ash value than *Tephrosia purpurea* and *Vitex negundo*.

Table 3. Ash values of selected medicinal plants

S.No	Parameters	*Tylophora asthmatica*	*Tephrosia purpurea*	*Vitex negundo*
1	Total ash value (%w/w)	25.40	05.90	06.50
2	Acid insoluble ash value (%w/w)	12.60	00.50	00.30
3	Water soluble ash value (%w/w)	12.20	05.30	02.30

Extractive values

The water soluble and alcohol soluble extractives were performed for all the plant drugs and results are tabulated in Table 4. *Vitex negundo* showed more alcohol and water soluble extractive values than the *Tylophora asthmatica* and *Tephrosia purpurea*.

Table 4. Extractive values of selected medicinal plants.

S.No	Parameters	*Tylophora asthmatica*	*Tephrosia purpurea*	*Vitex negundo*
1	Alcohol soluble extractive value (%w/w)	04.20	05.50	07.50
2	Water soluble extractive value (%w/w)	13.50	10.80	15.00

Extraction of Herbs

The extraction was carried out by soxhelation method. The yield of individual extract as follows.

The percentage yield of individual plant extract is as follows;

Tylophora indica - 15.6% W/W
Tephrosia purpurea - 11.2% W/W
Vitex negundo - 13.5% W/W

Tylophora indica showed more percentage yield than *Tephrosia purpurea* and *Vitex negundo*.

Formulation Development

Pre-formulation studies

The granules for tablet and capsule formulations were prepared by non-aqueous wet granulation method using polyvinyl pyrrolidine (PVP) with isopropyl alcohol as granulating agent. Then the granules were subjected to various pre-formulation studies like angle of repose, bulk density, moisture content, fineness ratio and the results are complying with pharmacopoeia standards. The results suggested that the granules were of good physical properties like flow and cohesion. The results are tabulated in table 5 & 6.

Table 5. Pre-formulation studies of granules used for tablet preparation.

S.No	Parameters	Granules. A	Granules. B
1	Angle of repose (°)	26.67±1.30	25.51±1.20
2	Loose bulk density (g/cm^3)	0.458±0.035	0.439±0.041
3	Tapped bulk density(g/cm^3)	0.538±0.023	0.553±0.019
4	Loss on drying %w/w	03.20	02.50
5	Fineness %w/w	12.20	11.60

A. Granules prepared by in-house extract
B. Granules prepared by commercial extract

Table 6. Pre-formulation studies of granules used for capsule preparation.

S.No	Parameters	Values obtained
1	Angle of repose (°)	23.57±1.50
2	Loose bulk density (g/cm^3)	0.428±0.035
3	Tapped bulk density(g/cm^3)	0.518±0.013
4	Loss on drying %	2.5
5	Fineness %	13.5

Tablet formulation

The granules were compressed into tablets with average weight of 620 mg per tablet. Tablet A is the formulation prepared by using in-house extracts and Tablet B is the formulation prepared by using commercial extracts.

Tablet A

Tablet B

Capsules

Evaluation of Formulations

Physical evaluation

The physical parameters like average weight, hardness, friability test and disintegration time were carried out for tablet and capsules formulations. All the parameters were complies with Indian Pharmacopoeia standards. All the formulated tablets and capsules were found to have good physicochemical properties and the results are reported in Table 7 & 8.

Table 7. Physical evaluation of tablet formulations.

S.No	Parameters	Tablet A	Tablet B
1	Average weight(gms)	620 ± 2.24	620 ± 1.56
2	Hardness(Kg/m^2)	3.0 ± 0.98	3.5 ± 0.92
3	Friability (%)	00.65	00.75
4	Disintegration time (mins)	09’.20’’- 09’.30’’	10’.10’’- 10’.20’’

Table 8. Physical evaluation of capsule formulations.

S.No	Parameters	Capsule
1	Average weight (mg)	260 ± 1.16
2	Disintegration times (mins)	07'.28''- 09'.32''

Pharmacological Evaluation

Histamine Induced Bronchospasm

The results of antihistaminic activity reveals that vehicle treated group on spasmogen exposure showed exposition time (20.5 ± 5.6) where as standard group showed significant increase in exposition time (249.0±3.5; P<0.001). Tablet A showed better activity when compared to Tablet B and Capsule. All the three formulations showed statistically significant increase in exposition time on spasmogen exposure (187.3±3.3, 159.3±2.6 & 173.1±3.2) when compared to standard group. The results are tabulated in table 9.

Table 9. Results of antihistaminic activity

Treatment	No of animals	Dose mg/kg	Increase in mean exposition time (sec) on spasmogen exposure
Control	4	-	20.5 ± 5.6
Positive control	4	2	249.0±3.5*
Tablet A	4	300	187.3±3.3*
Tablet B	4	300	159.3±2.6*
Capsule	4	300	173.1±3.2*

Values expressed as Mean ± SEM, n=5. Compared all the treatment groups with the positive control using student t-test. *P < 0.05.

CONCLUSION

The results of all the formulations were complies with quality standards. All the three formulations showed significant anti-asthmatic activity. Clinical and stability studies to be carried out to confirm the stability and efficacy of the products in future.

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