World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

PHARMACEUTICAL STANDARDIZATION OF ANTI EOSINOPHILIC AYURVEDIC FORMULATION - AQUEOUS EXTRACTS OF *HEDYCHIUM SPICATUM* [HAM.EX SMITH], *SASSUREA LAPPA* [C.B.CLARKE], *EMBLICA OFFICINALIS* [GAERTN] AND *CURCUMA LONGA* [LINN]

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Article Received on 06/01/2017 Article Revised on 28/02/2017 Article Accepted on 20/03/2017

ABSTRACT

Pharmaceutical standardisation of anti eosinophilic Ayurvedic formulation capsule is a quadric herbal aqueous extracts combination of Hedychium spicatum (Hamsmithex), Sassurea lappa (C.B.Clarke), Emblica officinalis (Gaertn) and Curcuma longa (Linn). in equal proportions and the ingredients were authenticity by the standard markers, HPTLC finger prints and analytical techniques as for the guidelines of Ayurvedic pharmacopeia of India and WHO. Physicochemical parameters of the formulation shows values of pH 4.53, loss on drying 6.88%, water soluble extractive 87.97%, alcoholic soluble extractive 40.15%, total ash 18.96%, acid. Insoluble ash 0.36%, heavy metal estimation of Lead <5ppm, Cadmium <1 ppm, Arsenic <2 ppm. Phytochemical compound quantification assessed for curcuminoids by spectrometric, tannis by volumetric and total alkaloids by gravimetric methods shows the values of 0.27%, 15.12% and 0.24% respectively. Pharmaceutical standardisation of capsule shows the average net content for capsule 608.60 mg, disintegration time 08min 03 sec, and dissolution of 73.65+0.24.It reveals the standardisation profile for the capsulation of Ayurvedic formulation was in conformity to the properties evaluated for clinical trial on tropical pulmonary eosinophilia in filaria.

KEYWORDS: Ayurveda, Aqueous extracts Herbal formulation, Pharmaceutical standardization.

INTORDUCTION

Ayurvedic medicine market is growing and sudden increase in herbal manufacture is due to least side effects and fear of increasing toxicity of the western medication. Traditional herbal medicine and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. However, one of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae, is extracted with boiling water during the decoction process. This may be the main reason why quality control of oriental herbal drugs is more difficult than that of western drug.[1] The World Health Assembly - in resolutions WHA31.33 (1978), WHA40.33 (1987) and WHA42.43 (1989) - has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards.[2] Analytical techniques like High Performance Thin Layer Chromatography (HPTLC) finger printing, physical analysis, pH, total ash, acid insoluble ash, bulk density, trapped density, heavy metals, and assay of marker compound by HPLC (High Performance Liquid Chromatography) method has a pivotal role in quality control and standardization.[3,4] Standardization and quality control of herbal as well as the Ayurvedic products is most essential for the acceptance on the modern parameters.[5, 6]

Standardisation is a system to ensure every pocket of medicine sold have correct amount and induce its therapeutic effect.[7] Ayurveda emphasizes the importance of standardization of medicinal herbs as well as the finished products on the basis of physical and

chemical parameters like the shape, texture, smell of the useful part. During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health.[8]

MATERIAL AND METHODS

The present work relates to standardization of parameters covering the processes of capsule preparation, qualitative and quantitative analysis of the major Phytochemical in a novel Ayurvedic formulation, which has to undergo clinical trial in Tropical pulmonary Eosinophilia with special reference to Filariasis. The physico, phyto chemical, organoleptic, HPTLC finger printing and spectrometric studies were conducted at Laila Impex, R andD division, Vijayawada. Pharmaceutical standardisation and capsulation carried out at IMIS Pharmaceuticals, Vijayawada. The Parameters tested for finished capsules were average content of filled drug, disintegration, dissolution and analysis of phytochemical contents percentage. (Table 1)

Table 1: Shows determination of proximate parameters of *Batch No: MIS_16 in c*apsule.

Sr No	Test	Capsule- Extract
1	Size of the capsule	00 size : Hard gelatin capsules Yellow Brown color
2	Weight of active drugs	3.0 kg
3	Wt of 20 filled capsule	14.106
4	Wt of 20 empty capsules	1.934
5	Wt of net contents	12.172
6	Average net content for capsule mg	608.60
7	Disintegration time	08min 03 sec
8	Dissolution	73.65+0.24
9	Particle size through 40 mesh	100%
10	Loss on Drying	6.88%
11	Water soluble Extractive	87.97%
12	Alcoholic soluble Extractive	40.15%
13	pH	4.53
14	Total ash	18.96%
15	Acid insoluble ash	0.36%
16	Alkaloids(Gravimetric)	0.24% (NLT 0.2% on d/b)
17	Tannins (Volumetric method)	15.12%NLT10.0% on d/b
18	Curcumoids(Spectrophotometric)	0.27% NLT 0.2% on d/b

Collection of Plant Material

Rhizomes of *Heychium spicatu,*[9-12] roots of *Sassuea lappa,*[13-16] fruits of *Emblica officinalis*[17-20] and rhizomes *of Curcuma longa*[21-24] were purchased from the local market and identified by the taxonomist of the Rand D Division of the Laila Impex, Vijayawada and Assistant Director (Ay) in-charge, National Research Institute of Basic Ayurvedic Sciences, Pune.

Authentication: The fresh plant material collected was thoroughly cleaned and air-dried. The voucher specimens of the samples (No.3323/291, No.1 (Sassurea), RC/3321/463 and 3322/211) have been identified. Authenticity matched with the raw material specimen's in house museum and *Sassurea lappa* was identified by the microscopic and macroscopic characteristics mentioned in the Pharmacopeia of India and were deposited in the institute. The Water extracts of the *Hedychium spicatum* (DPB No: L10060516), *Sassurea lappa* (DP Batch Number: L 10060517), *Emblica officinalis* (DP B.NO: L 10060518), *Curcuma longa* (DP Batch Number: L 10060519) *and Formulation (Batch No: MIS_16) were* homogenized to fine powder and stored in air-tight bottles for further studies.

Pharmaceutical standardisation: Anti eosinophilic herbal formulation – capsulation. Water extracts of the *Hedychium spicatum* (DPB No: L10060516), *Sassurea lappa* (DP Batch Number: L 10060517), *Emblica officinalis* (DP B.NO: L 10060518), *Curcuma longa* (DP Batch Number: L 10060519) were obtained from Laila inpex Vijayawada and *Formulation (Batch No: MIS_16) were* homogenized to fine powder and used for pharmaceutical capsulation at IMIS pharmaceutical, Vijayawada.

Batch manufacturing details

1. Product : ANTI EOSINOPHILIC HERBAL FORMULATION
2. Batch no : 010912 3.Batch size: 6,000 no's
3. Mfg Date : 20th Jan -11
4. List of Raw materials

Weight of active drugs – 3.0 kg
De-mineralized water – 3 lts

Hard gelatin capsules (00 size) – Yellow Brown color – 6,000 no's

List of excipients

S.No	Name of excipients	Quantity(Kg)
1	Gum Acacia	0.0714 (2.38%)
2	Aerosil (Amorphous fumed Silica- Colloidal Silicon dioxide)	0.027 (0.92%)
3	Talc (Hydrous Magnesium Silicate- IP grade	0.055(1.83%)
4	Magnesium stearate	0.001 (0.37%)
5	Maize Starch	0.0825(2.75%)

Specification of raw materials

1. Chemiloids (Laila impex) data (CoA of all extracts)
2. COA of Hard gelatin capsules

Method of manufacturing

Stages of manufacturing

A. Formulating and mixing of raw active ingredients.
B. Development of pharmaceutical dosage form (capsules).

I. Dry mix II. Paste preparation
III. Wet mixing IV. Wet granulation
V. Drying VI. Dry granulation
VII. Drying VIII. Lubrication
IX. Capsule filling X. Polishing

1. Dry mix

Equipment required: Mass mixer
Time required: Two hours

a. Take the formulated mixture and pass it through mesh no 16# in the SS container.
b. Mix the above mixture thoroughly for uniformity in a mass mixer.

2. Paste preparation

Equipment required: Manual
Time required: one hour

a). Heat 4 liters of DM water in Stainless Steel vessel on gas flame up to boiling point.
b). Add Gum Acacia (2.38%) and Starch (2.75%) to the above boiling water.
c). Slowly heat the material with constant stirring till the material becomes paste.

3. Wet mixing

Equipment required: Mass mixer/ Manual Time required: 30 minutes
a). Add contents of stage 1 (dry mix) to the paste of stage 2 and mix uniformly in a SS vessel.
b). Make the material into two wet lumps.
Note: commonly mass mixer is used for wet mixing. But in this formulation mass mixer was not utilized as the material was sticky and forming lumps.

4. Wet granulation

Equipment required: Multi mill (SOP and and Equip. Specification)
Time required: 30 Minutes (multi mill) and 30 minutes manually
a). Fit the mesh no. 6 # in the multi mill.
b). Add the contents of stage 3 (wet lumps) to the multi mill.
c). Collect the granules in a SS container.
d). Pass the above granules manually through the mesh no 10#.

5. Drying

Equipment required: Multi mill (SOP and and Equip. Specification)
Time required: Two hours (multi mill) and 3 ½ hours manually

a) Evenly spread the granules of stage 4 in the ss trays
b) Place the above trays in the tray dryer
c) Dry the granules at 45^0 C FOR three hours.

6. Dry granulation

Equipment required: Manual
Time required: One hour manually

a) Pass the granules of stage 5 manually through mesh no 16#
b) Mix the granules for uniformity

7. Drying

Equipment required: Manual Time required: 3 ½ hours manually
a). Evenly spread the granules of stage 6 in SS trays.
b). Place the above trays in the tray dryer.
c). Dry the granules at 45^0 C FOR three hours.

8. Lubrication

Equipment required: Mass mixer Time required: ½ hours manually

a) Separately pass the magnesium stearate ,talc and aerosol through the mesh no 16#
b) Pass the granules of stage 7 (both part A and B) through mesh no 16 #
c) Take the above granules and add aerosol, talc and magnesium steerages one after and another and mix through mass mixer for uniformity.

9. Capsule filling

Equip required: Semi automatic capsule filling machine
Time required: 3 hours manually
a). Take 3.5 kg of uniform blended granules of above stage and fill the capsules through the capsule feeding trays.

10. Polishing

Equipment required: Manual Time required: 30 minutes manually.
a). Take 5 ml of liquid paraffin and over the smooth and clean towel/cloth.
b). Take approximately 1000 of filled capsules of stage 9 and polish by rubbing on the cloth.

Physico-chemical studies: Physico-chemical phytochemical, organoleptic, microbial parameters of the formulation studied at laboratories of Laila Impex, Vijayawada. Physicochemical parameter of the formulation was determined as per guidelines of W.H.O. [3,4] Total Ash value, loss on drying, water soluble ash, acid insoluble ash, heavy metals, alcohol soluble extractive and water soluble extract values were determined.[3,4]

Microbial screening

Microbial screening carried out for the safe use of the individual plant extract as well as the mixed formulation and checked for prescribed limits of total aerobic count, total yeast and mould count[3,4] High performance Thin Layer Chromatography for identification of formulation extract dry powder evaluated the chromoplate in 254 UV nm.[5] (Table 2) and (Fig.1).

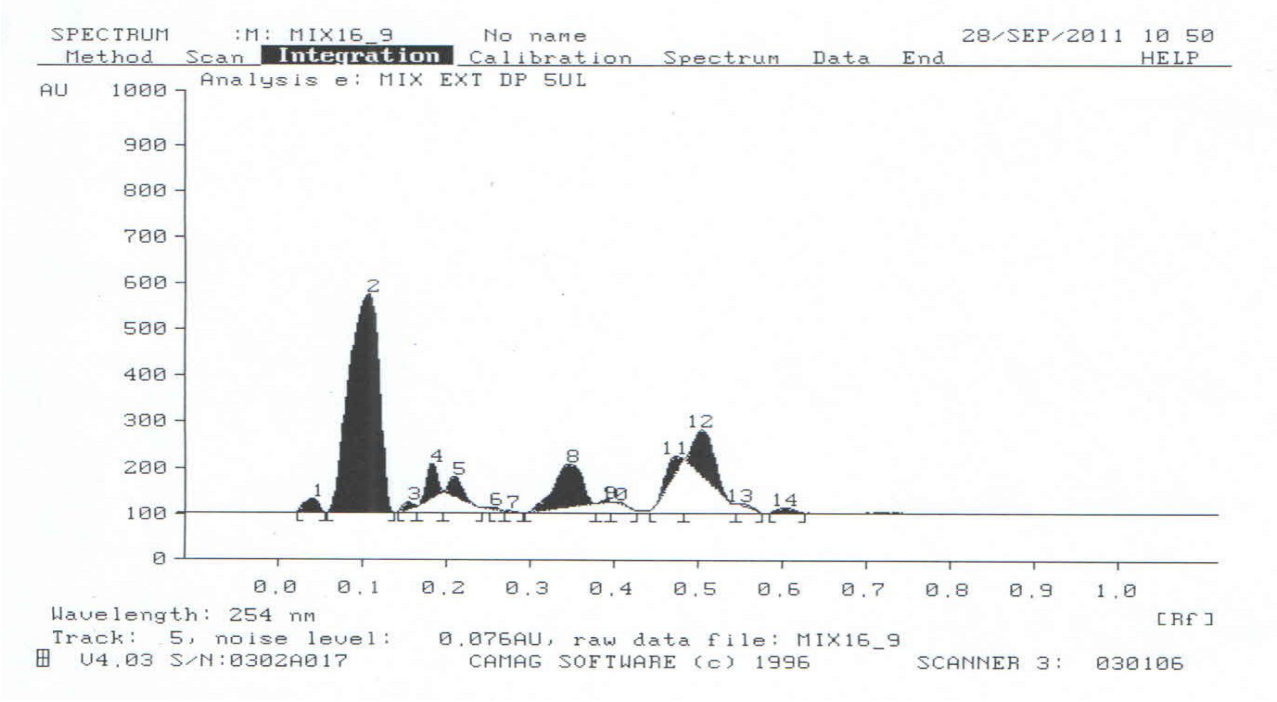

Fig. 1: High Performance Thin Layer Chromatography (MIS_16) at 5 UL and 254 nm.

Table 2: High Performance Thin Layer Chromatography (MIS_16) at 5 UL and 254 nm.

Peak	Start		Max			End		Area	
#	*Rf*	H	*Rf*	H	%	*Rf*	H	F	%
1	0.22	0.0	0.04	30.4	3.37	0.06	0.0	494.6	2.06
2	0.06	0.0	0.11	464.6	51.47	0.14	0.0	15497.0	64.60
3	0.14	0.0	0.16	17.5	1.94	0.17	0.0	171.8	0.72
4	0.17	0.0	0.19	75.2	8.33	0.20	0.0	892.1	3.72
5	0.20	0.0	0.21	45.0	4.99	0.24	0.0	648.4	2.70
6	0.25	0.0	0.26	5.5	0.61	0.27	0.0	52.3	0.22
7	0.27	0.0	0.28	1.9	0.21	0.30	0.0	14.7	0.06
8	0.30	0.0	0.35	91.3	10.12	0.38	0.0	2907.4	12.12
9	0.38	0.0	0.39	5.4	0.60	0.40	0.0	45.8	0.19
10	0.40	0.0	0.41	4.7	0.52	0.43	0.0	44.1	0.18
11	0.45	0.0	0.47	41.6	4.61	0.49	0.0	613.3	2.56
12	0.49	0.0	0.51	100.8	11.17	0.55	0.0	2279.5	9.50
13	0.55	0.0	0.56	7.1	0.78	0.58	0.0	101.5	0.42
14	0.59	0.0	0.61	11.5	1.27	0.63	0.0	225.7	0.94

CONCLUSION

The present study is focussed on the pharmaceutical standardisation of the Anti Eosinophilic capsulation. The results of physicochemical, analytical, spectrometric and pharmaceutical parameters shows that extract formulation and finished product were good in quality. Sample of raw material was examined for probable adulterants of similar morphological characters which were found to be absent and authenticity by the taxonomist. Pharmaceutical standardisation parameters also reveal the good standard as for the Good manufacturing practices and the product is safe to conduct clinical trial for anti eosiphil effect in Tropical Pulmonary Eosinophilia.

ACKNOWLEDGEMENT

The authors are grateful to the Director General CCRAS, New Delhi; Assistant Director, RARISD - Vijayawada, Shri G.Ganga Raju,Chairman, G.Rama Raju Managing Director Laila Impex - Vijayawada for their help and Guidance in the study. The study has been affiliated to Dr. N.T.R. University of Health Sciences as PhD studies.

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