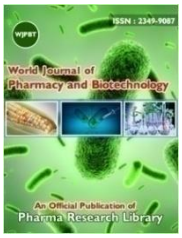

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Preparation, Standardization and *In Vitro* anti-arthritic evaluation of poly herbal formulation

Gollapalli Divya Mounika*, Y. Prapurna Chandra, C. Madhavi Latha
Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur (V&P), Muthukur (M), SPSR Nellore District – 524 346

A b s t r a c t
The present study is to formulate an ayurvedic capsule for treatment of arthritis. The formulated capsules are evaluated as per WHO guidelines and also performed pharmacological activity. The objectives of the study were Raw material analysis Studying the raw materials or ingredients of the formulation by carrying out the Preliminary raw materials analysis. Preformulation development: To formulate a capsule with six herbs by using preformulation studies. Standardization of the best batches: To standardise the physico-chemical parameters of the capsule. To analyse and quantify of the presence of phytoconstituents in the capsule. To analyse the fingerprint using HPTLC for the polyherbal formulation. Establishing the safety pertaining to heavy metals, pesticide residue and microbial load analysis. Stability studies: Establishing stability of the formulation under accelerated condition of Temperature and humidity as per ICH guidelines.
Keywords: WHO guidelines, ICH guidelines, HPTLC, preformulation studies

A r t i c l e I n f o

Corresponding Author
Gollapalli Divya Mounika
Department of Pharmacology,
Ratnam Institute of Pharmacy,
Pidathapolur (V&P), Muthukur (M), SPSR Nellore–524 346

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C o n t e n t s

1. Introduction

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage[1]. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction. Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is normal protective response to tissue injury caused by trauma, noxious chemical or microbial agent. It is body response to inactivate or destroy the invading organisms, to remove the irritant and set the stage for the tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrated cells[2]. It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 higher preponderancein female[3]. The most frequently affected joints are those of fingers and toes, wrists, knees, and ankles. Extra articular manifestations may occur and are frequently present in patients with severe disease which include ocular, pulmonary, hematologic, vascular, cardiac, neurologic and mucosal tissue.

StandardizationofHerbalDrugs[4]

"Standardization" of herbal drugs is not an easy task as numerous factors influence the bioefficacy and reproducible therapeutic effect. Inorder to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection, their extraction, purification process and rationalizing the combination in case of polyherbal drugs. Standardization

means adjusting the herbal drugs preparation to a define content of a constituent or a group of substances with known therapeutically activity respectively by adding excipients or by mixing herbal drugs o rherbal drug preparation. Evaluation of a drug means confirmation of its identity and determination of its quality and purity and detection of its nature ofadulteration.[5]

Quality Control of Herbal Medicine[6]

Quality can be defined as the status of a drug that is determined by identity, purity, content, and other chemical, physical and biological properties or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the validity of a manufactured product.

2. Materials & Methods

Plant Material: The plant materials used in the formulation were individually procured from the qualified vendors and they were authenticated by Dr. K. Madhavachetty, Asst. Professor, Dept. of Botany, S V University, Tirupati.

Preliminary Phytochemical Screening [7]

The crude raw material was subjected to preliminary phytochemical tests as perPhytochemical Methods.

Formulation Development Studies

Selection of Excipients[8]

For the formulation of capsules in addition to the active ingredients, excipients likediluents (filler), binder, disintegrating agent, lubricant and preservatives are required. The choiceof excipients was made keeping in mind the current Food and Drugs Administration (FDA) regulations.

Diluents:

Diluents/Fillers are added where the quantity of active ingredient is less (or) difficult to filling. Common tablet/capsule filler include Lactose, Dicalcium phosphate, Microcrystalline cellulose etc.

Lubricants:

They reduce friction during the filling process. In addition, they aid in preventing adherence of capsule material. Magnesium Stearate, Stearic acid, Hydrogenised vegetable oils and talc are commonly used lubricants.

Glidant:

It is used to improve flow of the powder materials by reducing the friction between theparticles. Themost effectiveglidants arethe Colloidalsilicondioxide, Talcand Starch.

Preservatives:

The preservatives are added to herbal formulation to prevent contamination, deterioration and spoilage by bacteria, fungal and other microorganisms. The most effective preservatives are the sodium methyl paraben, sodium propyl paraben, sodium benzoate and bronopol.

EXCIPIENTS USED:

Diluents / Fillers: Starch, Lactose, Magnesium Stearate.
Glidants: Talc, Colloidal Silicon Dioxide, Starch
Preservatives: Sodiummethylparaben, Sodiumbenzoate

GRANULATION[9]

Granulation is defined as a process of size enlargement, widely used in pharmaceutical, food, chemical, agriculture, animal feed and other industries in which powder particles are madet of or mlarger, multiparticle entities called granules. Pharmaceutical granules typically have a Size range between 0.2-0.4mm, depending on their subsequent use. It is one of the most important unit operations for powder handling in pharmaceutical industry. The appearance, elegance and ease of filling of capsules are dependent on variety of variables like materials used, processing techniques and equipment used for ultimate quality of granules produced.

Table.1 Formula for granule preparation

Active Ingredients	Quantity Mg/Capsule
Asparagus racemosus	20mg
Bacopa monnieri	1.5mg
Lippia nodiflora	110mg
Oldenlandiaheyneii	100mg
Allium sativam	15mg
Smilax zeylanica	100mg

Preparation of granules:

Step1: All the individual herb plant materials were cleaned and then washed with demineralized water for 3-4 times and dry with shade.

Step2: Each dried plant material was pulverized and passed through sieve 30 slotted stainless steel mesh.

Step 3: Plant ingredients weighed individually and pulverized into a moderately fine powder in a stainless steel pulveriser then mixed along with the binder and other excipients. To get uniform mixing.

Step4: After, QA approval the granules were filledin "0"sizeredcapsulewithaveragecontentweight at 520mg.

Trial batches: (Size:500Capsules)

Various trial batches (size: 500 capsules) were formulated by varying the composition of the excipients proportions. Based on the various excipients proportions five trial batches given in Table. Trial batches (Size: 500capsules)

Capsule Filling:

- The formulated granules were filled in"0"size capsules to an average net content weight of 520mg.
- The capsules were then dedusted, transferred into polybags, labelled and the samples were evaluated as per the testing requirements.
- After approval from QAD the capsules were packed as per the packing instructions.
- From the final trial, samples were taken for accelerated stability studies as per the Testing requirements.

Standardization of Finished Product[10,11]

The developed capsules were subjected to following studies for their standardization:

- Evaluation of capsules
- Physicochemical parameters
- Phytochemical studies
- Heavy metal analysis
- Microbial load analysis

Evaluation of Capsules

- Description

- Uniformity of weight
- Disintegration test
- Moisture content

Chromatographic Finger Printing of Herbal Products[13]

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. Recently it has become one of the most powerful tools for quality control of herbal medicines. The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents observed by various chromatography techniques such as HPLC, TLC, HPTLC, GC, capillary electrophoresis.

HPTLC Finger printing of polyherbal capsules:

Chromatographic conditions:

Instrument : CAMAG HPTLC
HPTLC Applicator: CAMAG LINOMAT IV
HPTLC Scanner: CAMAG TLC SCANNER II
Method: as per AHRF Method
Mobile phase: Ethyl acetate: Hexane (6:4)
Stationary phase: HPTLC Silica MERCK 60 F254
Sample dilution: 10 mg of sample dissolved in 1 ml of ethyl acetate
Vol. of sample loaded: 20 µl
Lambda max: 254 nm
Detection: Rf value was calculated, peak area of each band was detected.

In-Vitro Antiarthritic Activity[14, 15, 16]

Inhibition of Proteinase Enzyme Activity

Principle: Neutrophils are known to be a rich source of proteinase. They carry many neutral serine Proteinase in their lysosomal granules. It was previously reported that leucocyte proteinase play an important role in the development of tissue damage during inflammatory reactions. Trypsin is a serine protease found in the digestive system of many vertebrates, where it hydrolyses proteins. In this assay trypsin is used as protease enzyme and casein is used as substrate.

Procedure

The reaction mixture (2.0 ml) contains 0.06 mg trypsin, 1.0 ml of 25mM Tris-HCl buffer (pH7.4) and 1.0ml Aqueous solution of test sample (100,200,400,800mcg/ml) and were incubated at 37ºC for 5 minutes. Then 1.0ml of 0.8% (w/v) Casein was added and incubated for 20 minutes. 2.0 ml of 70% (v/v) Perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Optical density of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated using the following formula.

$$\text{Percentage inhibition} = \frac{(\text{O.D. of control} - \text{O.D. of sample})}{\text{O.D. of control}} \times 100$$

3. Results and Discussion

Preliminary quality control of the raw materials

The raw materials were sampled, authenticated and studied for their compliance to Preliminary qualitative standards as established by Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India and other standard references. For those raw materials, for which no official standard were available, in house (IH) standards were created and results were compared with them. The raw materials were divided into two category viz., Crude herbal drugs and Extracts of the crude drugs. Then, they were analyzed forth equalitative and quantitative standards. The results were compared with the standard references and observed for its compliance. Those materials which met the standards were taken for the formulation and development. All the tests were carried out as per the test methods detailed in the materials and methods part previously.

Table.2. The chemical tests for various Phytoconstituents in the raw materials were carried out and the results were tabulated

S.No	Chemical constituent	*Asparagusra cemosus*	*Bacopam onneri*	*Alliums ativum*	*Oldenlandi aheyneii*	*Lippiani diflora*	*Smilaxzeyl anica*
1	Carbohydrates	_	_	_	_	+	_
2	Alkaloids	+	+	_	+	+	+
3	Steroids	+	+	+	_	+	+
4	Glycosides	_	_	_	+	+	_
5	Flavanoids	+	+	+	+	+	+
6	Tannins	_	_	_	_	+	+
7	Phenolic compounds	_	_	+	_	+	+
8	Proteins	_	_	_	_	_	+
9	Terpenoids	_	+	+	+	+	+
10	Fats and oils	_	_	_	+	_	_
11	Gums and Mucilage	+	_	_	_	_	_

\+ present, -absent

Preformulation and Formulation Development Studies: Totally five trials of formulation were carried out using different choices of excipients. Considering different facets of manufacturing problems as well as quality defects in mind. All there sultant formulations were evaluated for their flow property, uniformity of filling, uniformity of weight, moisture content and disintegration time.

Table.3. Evaluation of trial batches

Parameters	Trial1	Trial2	Trial3	Trial4	Trial5
Bulk density(g/cm^2)	0.34±0.026	0.40±0.01	0.41±0.015	0.33±0.04	0.35±0.04
Tapped density(g/cm^2)	0.57±0.025	0.54±0.03	0.61±0.02	0.53±0.03	0.57±0.02
Compressibility index (%w/w/)	40.36±1.12	26.6±2.08	32±2.64	34.6±2.08	33±1.5
Housner's ratio	1.4±0.36	1.41±0.01	1.43±0.02	1.53±0.04	1.45±0.03
Angle of repose(degrees)	34.9±3.35	35.3±3.78	42±1.0	35±1.0	27.6±0.57

Table.4. Organoleptic characters of capsules

S.No	Parameters	Observations
1	Nature	Powder
2	Color	Lightbrown
3	Odour	Slightaromatic
4	Taste	Characteristic

Table.5. Uniformity weight of the capsule

S.NO	Average weight/capsule(mg)	IP specification(mg)
1	515	**±7.5%**
2	510	
3	506	
Mean ± S.D	510.3±4.5	

Table.6. Disintegration time

S.NO	Disintegration time(min)	IP specification (min)
1	10.21	NMT30 Minutes
2	10	
3	11	
Mean±S.D	10'9±0.5	

Table.7. Determination of Moisture Content Loss on drying

S.NO	LOD% w/w	IP specification
1	2.1	NMT5%w/w
2	1.0	
3	2.2	
Mean ± S.D	2.1±0.1	

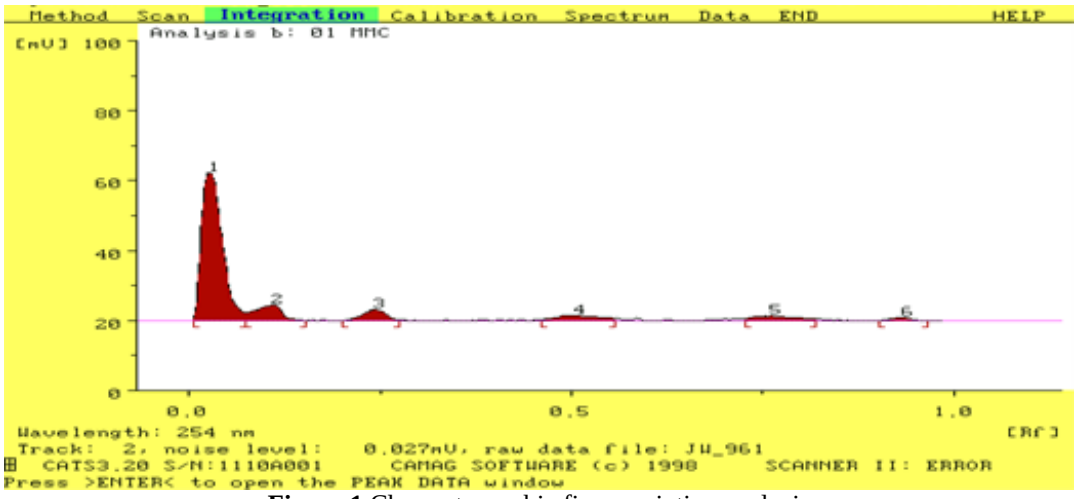

Figure.1. Chromatographic finger printing analysis

Table.8. R_f value of the capsules (Chromatogrphic finger printing)

S.No	Rf	Height	Area	Lambdamax(nm)
1	0.02	42.4	878.1	222
2	0.10	4.6	125.6	203
3	0.24	3.2	76.3	205
4	0.50	1.6	67.0	237
5	0.76	1.4	59.6	400
6	0.93	0.9	18.6	200

Table.9. *In-vitro* antioxidant and anti-arthritic activity of polyherbal capsule formulation

Assay	Percentage inhibition at different concentrations				
	100µg	**200µg**	**400µg**	**800µg**	**200µg(std)**
Protein denaturation	45.32±0.42	51.23±0.12	65.84±0.84	74.92±0.43	78.34±0.56
Inhibition of protenase Enzyme	54.23±0.22	59.21±0.45	69.43±0.67	76.43±0.47	80.32±0.34
Membrane stabilization	43.23±0.49	49.39±0.34	58.30±0,40	68.32±0.11	72.49±0.19
Reducing power assay	42.48±0.05	52.24±0.84	65.43±0.43	71.53±0.32	72.43±0.56
Nitric oxide scavenging Assay	40.53±0.47	51.38±0.48	69..85±0.36	74.27±1.38	75.94±0.75

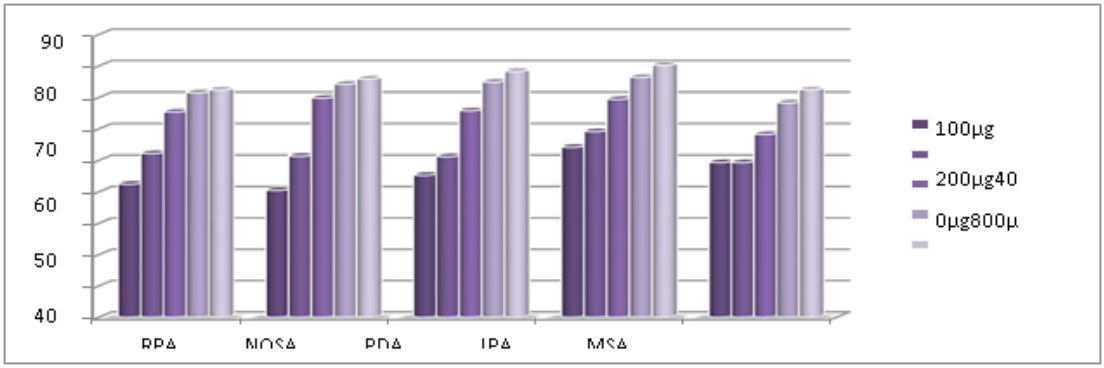

Figure.2. Graphical representation of anti-oxidant and anti-arthritic activity of poly herbal formulation of the capsules

4. Conclusion

The present study was attempt to development, standardization and pharmacological activity of anti-arthritic polyherbal formulation. Based on the extensive review of literature, six active ingredients were selected for the formulation of polyherbal capsules to treat rheumatoid arthritis. The raw materials which are procruded from TTK Healthcare Ltd are subjected to various raw material analysis for their identy, quality and purity. The materials which complied with the specification were taken for further studies. Preformulation studies such as bulk density, tapped density, compressibility index, housner's ratio and angle of repose were done for the all raw materials. From the preformulation studies best batches was selected to formulate capsules. The formulated capsules were standardized as per WHO guidelines using various parameters and include quantification of phytoconstituent, HPTLC, Heavy metals. Stability studies of the capsules are carried out as per ICH guidelines. The antioxidant activity of capsules showed the reducing power assay and nitric oxidescavenging assay was maximum at the concentration 800µg (71.53%, 74.27%) which was less than the standard drug, ascorbic acid 200µg (72.43%,75.94%). Future studies can be directed towards the exact mechanism of action responsible for this antiarthritic activity and further the study can be extended for clinical trials also.

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