Research Article

Phytochemical Screening and Standardization of Polyherbal Formulation "RIPARE" for Arthritis

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ABSTRACT

Standardization of herbal formulations is essential in order to assess the quality of drugs, base on the concentration of their active principles, physical and chemical standards. Standardization of the poly herbal formulation is possible by following modern scientific quality control procedure both for raw material and the finished product. The phytochemical constituents found to be present in the raw material used for the preparation of "RIPARE" possibly facilitate the desirable therapeutic efficacy of standardized medicinal formulation as a whole, and also could help in knowing the underlying mechanisms of the pharmacological action. The article reports on standardization of polyherbal formulation used to heal the arthritis. Specific plant extracts are used in the preparation of "RIPARE" polyherbal formulation. "RIPARE" Capsule has a good amount of herbal ingredients that possess antiarthritic activity. They were also screened for the evaluation of phytochemical parameters, presence of pathogens, heavy metals and their quality control parameters.

Keywords: Standardization, polyherbal, antiarthritic, RIPARE

Received 07 May 2013 Received in revised form 14 May 2013 Accepted 19 May 2013

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INTRODUCTION

In herbal medicine plant based formulations are used to alleviate the diseases but, the most important challenges faced by these formulations arise because of their lack of complete evaluation. So evaluation is necessary to ensure quality and purity of the herbal product. It is very important to establish a system of evaluation for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous (1).

These days, world is witnessing medicine going back to nature - a shift in global trend from synthetic to natural medicine. Medicinal herbs have been known for centuries and are highly valued all over the world as a rich source of therapeutic agents for prevention of diseases and ailments. India is perhaps the largest producer of medicinal herbs and is rightfully called the "Botanical Garden of the World". India also has a very unique position in the world, where a number of recognized indigenous systems of medicine viz. Ayurveda, Siddha, Unani, Homeopathy, Yoga and Naturopathy are practiced even today for health maintenance (2).

In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition. Therefore, quality control of herbal medicines offers a host of problems. To solve this problem, first and foremost task is the selection of the right kind of plant material which is therapeutically efficacious (3).

The process of evaluating the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observation is called standardization (4). Standardization is an

essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principle. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation of its quality, safety and efficacy (5).

Polyherbal formulations RIPARE possess arthritic healing activity. Arthritis represents a group of debilitating diseases of the joints, bones, tendons, muscles and eventually organs. The two most common types are osteoarthritis (OA) and rheumatoid arthritis (RA), traditionally defined as age related "wear-and-tear" arthritis and "auto-immune" arthritis, respectively. However, inflammatory response has been identified to be a common mediator in both types of arthritis (6, 7).

Osteoarthritis is the most important of the rheumatic diseases and is responsible for a huge burden of pain and disability (8)**.** Osteoarthritis is characterized by both degeneration of articular cartilage and simultaneous proliferation of new bone, cartilage, and connective tissue (9). Pain on walking, stiffness of the joint and difficulty with steps and stairs are the major symptoms. The physical signs depend on the distribution and severity of the osteoarthritis within the joint. Wasting of the quadriceps muscle, bony swelling, and tenderness on and around the joint line, painful limitation of full flexion and course crepitus are the usual signs. A wide variety of treatments are available for those who suffer from osteoarthritis of the knee and self management, weight reduction, hydrotherapy, footwear and walking aids, other rehabilitation measures, physical therapy, systemic drug therapy, intra-articular drug therapy and surgery (10-12).

Typically arthritis is a common inflammatory joint disease characterized by inflammation of the synovial membrane, pain and restricted joint movement (13). Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact (14). The prevalence of arthritis is approximately in the West (15). The prevalence of RA in India subcontinentis 1.5-2 percent of population. The epidemiological ratio of arthritis in female: male is 3:1 and the prevalence is 1% of the world population. Adjuvant induced arthritis (AIA) in rats; a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joints resembles RA in humans (16).

The polyherbal formulation "RIPARE" containing ingredients such as *Boswellia serrata, Commiphora mukul, Cissus quadrangularis, Vitex negundo, Centella asiatica, Tinospora cordifolia, Curcuma longa, Euphorbia hirta* and *Piper nigrum.* This formulation is known to possess antiarthritic activity. The formulation was evaluated for its physico-chemical study such as ash value, extractive value, behavior of powder with different reagents, phytochemical and antimicrobial study. Fresh formulation prepared in the laboratory was tested for the presence of above mentioned parameters.

MATERIAL AND METHODOLOGY

Collection and authentification of plant extracts:

Plant extracts were collected from herbal suppliers and authentication was checked and confirmed. The raw materials were preliminary identified by the Ayurvedic parameters such as Varna (color), Gandha (odour), Ruchi (taste), Aakruti (shape) and Parimana (size) (17). Six ingredients involved in the polyherbal formulation with various morphological plant parts were separately shade dried and powdered passed through a 30 mesh sieve. Polyherbal formulation was prepared by mixing the specific quantities of the individual drugs in

accordance with the formula given in (**Table 1**). Each powder was weighed and thoroughly mixed together along with other excipients.

Table 1: Composition of Polyherbal formulation "RIPARE"

Sr. No.	Biological name	Standardized Common name	Family	Quantity
1.	*Boswellia serrata*	Indian frankincense	Burseraceae	125 mg
2.	*Commiphora mukul*	Guggul	Burseraceae	105 mg
3.	*Cissus quadrangularis*	Winged treebine	Vitaceae	105 mg
4.	*Vitex negundo*	Chinese chaste tree	Verbenaceae	95 mg
5.	*Centella asiatica*	Gotu kola	Umbelliferae	80 mg
6.	*Tinospora cordifolia*	Indian tinospora	Meninspermaceae	75 mg
7.	*Curcuma longa*	Turmeric	Zingiberaceae	55 mg
8.	*Euphorbia hirta*	Pill bearing spurge	Euphorbiaceae	50 mg
9.	*Piper nigrum*	Pepper	Piperaceae	10 mg

Evaluation of Quality Control Parameters for Finished Product (Capsule):

Description

Color, odour and taste were evaluated by using relevant senses. (18).

Uniformity of weight

Test for uniformity of weight was performed as per Indian pharmacopoeia, 2007.

Determination of pH

The pH value of a solution was determined potentiometrically by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. 1 g capsule powder was taken and dissolved in 100 ml demineralized water. The electrodes were immersed in the solution and the pH was measured (19).

Disintegration test for capsule

Disintegration test was performed using the digital microprocessor based disintegration test apparatus by ELECTROLAB. One capsule was introduced into each tube and added a disc to each tube. The assembly was suspended in the water in a 1000 ml beaker. The volume of water was such that the wire mesh at its highest point is at least 25 mm below the surface of the water, and at its lower point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained the temperature at 370 ± 20C. Noted down the time require to all capsules to disintegrate and pass through wire mesh (19).

Physicochemical parameters

The physicochemical parameters like ash value (total ash and acid insoluble ash) and extractive matter (alcohol soluble extractive and water soluble extractive) were determined as per WHO guideline, 2002

Determination of Moisture content

The test was performed using Karl Fischer instrument by SYSTRONICS.

Fluorescence analysis

Capsules preparation was studied for any color changes under ordinary light and UV light. Samples were studied as such, after treating with 50% HCl and 50% NaOH and the results were tabulated (20, 21).

Phytochemical evaluation

Qualitative chemical tests were carried out on powder of capsule for presence/absence of various phytoconstituents like alkaloids, glycosides, Saponins, tannins carbohydrates etc (22-24).

Estimation of Total Alkaloids:

Accurately weighed 3gm of the sample was taken. To the sample 5 ml of ammonia was added & shaken for some time then mixture of 75 ml of ether and 25 ml of alcohol was added. This solution was shaken continuously for 1 hour. The solution was filtered in the separator through cotton plug. The residue was washed from conical flask with mixture of 75 ml of ether and 25 ml of alcohol. The total solution was extracted with 25ml dilute sulphuric acid. Then extracted with mixture of 25 ml dilute sulphuric acid, 55 ml distilled water and 20

ml ethanol. Entire acid layer was collected and washed with 25 ml of chloroform. Acid layer was collected and made it alkaline with ammonia. It was extracted with chloroform (25x4). Water washing given to chloroform (20 ml). The chloroform layer was filtered in a weighed beaker and evaporates to dryness (19).

Estimation of Total Saponins:

20 g of the capsule powder were weighed and 100 ml of 20 % aqueous ethanol was added. Then the sample was heated over a hot water bath for 4 hours with continuous stirring at about 55º C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extract was reduced to 40 ml over water bath at about 90º C. The concentrate was treated with 20 ml of diethyl ether and the aqueous layer was recovered while the ether layer was discarded. This process of purification was repeated three times and then 60 ml of n-Butanol was added and extracted. The n-Butanol extract obtained was then washed two times with 10 ml of 5% aqueous Sodium chloride. The remaining solution was heated in a water bath for evaporating the solvent. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage (25).

Estimation of Total Tannins:

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrometrically. All determinations were performed in triplicate. The Folin-Ciocalteu reagent being sensitive to reducing compounds including polyphenols is producing a blue color upon reaction which is measured spectrophotometrically (26).

Estimation of Total Bitters

Weigh accurately 2 gm Sample. Extract with 50ml × 5 portion of methanol and filter through a suitable filter paper. Combine the methanol fraction and evaporate to dryness and dissolve the residue in water. Extract the aqueous extract repeatedly with 25, 20, 15, 15 and 10 ml ethyl acetate; Collect the ethyl acetate to beaker, evaporate, dry, weigh and calculate the bitters (27).

TLC analysis

Sample solution: Residue in methanol

Development system: Petroleum ether: Ethyl acetate: Methanol (72:24:4)

Stationary Phase: Silica gel 60 F254 TLC plate of 0.2mm thickness.

Detection: By visible and UV Absorption Range from 254nm.

The Extracts were spotted and chromatogram was developed and analyzed under visible and under UV 254 nm (28).

RESULTS AND DISCUSSION

Polyherbal formulation was subjected for various evaluation parameters with the analytical techniques. Polyherbal formulation composed of six ingredients, belonging to different families, different morphological plant parts and different phytoconstituents.

Macroscopic evaluation shows that it is having light brown color, characteristic odour, bitter taste and granular in appearance. Fluorescence analysis showed that formulation has light brown fluorescence in day light and greenish brown fluorescence in UV light. The results of fluorescent studies of the po wdered plant material using different chemi cal reagents were studied. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material (29). If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by reagents hence some crude d rugs are often assessed qualitatively in this way and it is an important parameter of Pha rmacognostical evaluation (30). Capsule passed the test of uniformity of weight. All capsules disintegrated within 12-16 minutes. Moisture content of capsule was <6%w/w which indicates that there is less

chances of microbial growth and capsule will not become soft. Results of ash values are within the limit and show that there are fewer impurities in the capsule powder. Water soluble extractive value indicates that capsule powder has good water solubility. Phytochemical evaluation shows presence of many constituents in the capsule. Capsule powder contain considerable amount of Alkaloids and Saponins, tannins and bitters in it. Data of TLC profile indicates that capsule powder was from genuine plant or part of the plant with the presence of six spots. Capsule passed the limit for heavy metals and microbial contamination. It also passed the pesticides residual analysis and synthetic steroids.

Table 2: Organoleptic properties

Sr. No.	Parameters	Observation
1.	Color	Brown
2.	Odour	Characteristic
3.	Nature	Fine powder
4.	Taste	Bitter

Table 3: Quality tests for the finished product

Sr. No.	Parameters	Values
1.	Average weight of capsules (mg)	545.85±4.34
2.	Disintegration time (Min)	15.08±1.04`
3.	Weight variation	10.921±0.39
4.	Moisture content	3.46% w/w
5.	pH	6.45±0.67
6.	Total Ash	3.26% w/w
7.	Acid insoluble Ash	1.1% w/w
8.	Water soluble extractive	91.66 % w/w
9.	Total Alkaloids	1.41 % w/w
10.	Total Tannins	8.17 % w/w

Table 4: Quality tests for the finished product

Sr. No.	Parameters	Observation
1.	Triterpenoids	+
2.	Flavones	-
3.	Alkaloids	+
4.	Carbohydrates	+
5.	Glycosides	+
6.	Phenols	+
7.	Proteins	-
8.	Resins	-
9.	Saponins	+
10.	Tannins	+
11.	Steroids	-
12.	Bitters	+

+ indicates presence and – indicates absence

Table 5: Fluorescence analysis

Sr. No.	Powdered drug	Day/visible light	UV visible light
1.	Formulation as such	Light Brown	Greenish Brown
2.	Formulation+50% HCl	Dark Brown	Green
3.	Formulation+50%NaOH	Yellowish Brown	Yellowish Green

Table 6: Heavy Metal Analysis

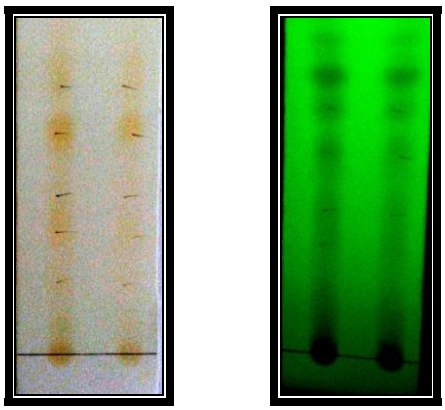
Sr. No.	Parameters	Limits	Results
1.	Lead	Not more than 10 PPM	Complies
2.	Arsenic	Not more than 3 PPM	Complies
3.	Mercury	Not more than 0.01 PPM	Complies
4.	Cadmium	Not more than 0.25 PPM	Complies

Table 7: Microbial Load Analysis

Sr. No.	Parameters	Results	Limits as per WHO
1.	Total bacterial count	NMT 50 PPM	NMT 1000CFU/GM
2.	Total fungal count	NMT 20 PPM	NMT 100CFU/GM
3.	*E. Coli*	Absent	Absent/gm
4.	*Salmonella*	Absent	Absent/10 gm
5.	*Pseudomonas*	Absent	Absent/gm
6.	*S. aureus*	Absent	Absent/gm

TLC Analysis

Fig. 1: Visualization of TLC Plate



(λ=Visible light) **(λ=UV: 254 nm)**

CONCLUSION

Data suggested that capsule were consistent with various identity, quality, and purity parameters such as organoleptic parameters, physiochemical parameters, TLC profile, heavy metal analysis and microbial analysis. Selected polyherbal capsule have passed through all the WHO parameters which were tested. So it can be concluded that use of capsule was safer and ready to use.

ACKNOWLEDGEMENT

The research was supported by grants from Sahajanand Life Sciences Pvt Ltd. So, authors are thankful to this organization.

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