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PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF POLYHERBAL FORMULATION "RENOLITH" FOR RENAL STONE

MAYANK PANCHAL[1], DIPAK PATEL[1], BHAVIN VYAS[2]
SHRADDHA KACHHADIYA[2], DR. D.R. SHAH[2]

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1. Sahajanand Life Science Pvt Ltd, Sahajanand Estate, Surat.
2. Department of Pharmacology, Maliba Pharmacy College, Gopal Vidyanagar, Bardoli.

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Corresponding Author
Mr. Mayank Panchal

Abstract

Standardization of herbal formulations is essential in order to assess the quality of drugs, based 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 **RENOLITH** 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 renal stone. Specific plant extracts are used in the preparation of **RENOLITH** polyherbal formulation. **RENOLITH** Capsule has a good amount of herbal ingredients that possess antiurolithiatic activity. They were also screened for the evaluation of phytochemical parameters, presence of pathogens, heavy metals and their Quality Control Parameters.

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].

Urolithiasis is a major public health problem with serious complications. Urolithiasis has become a global problem in spite of advances in modern science. Patients with urolithiasis constitute an important part of everyday urological practice. The optical management of this disease requires knowledge of diagnostic procedures and treatment [6-9]. It leads to various complications like kidney failure etc. Owing to these complications, the researchers of different system of medicine are concentrating themselves for the development of the new urolithotriptic drugs. Different anti-urolithiasis drugs of main stream medicine have potent and effective action, but the long term use of these drugs results in development of various side effects. Therefore, there is a need to develop safe and effective drug for the management of urolithiasis. Thus, this study was planned to evaluate the efficacy of herbal coded formulation in the management of urolithiasis on scientific parameters. Herbal treatment could be an alternate and complimentary therapy that requires evidence based approach to eliminate urinary stones.

The polyherbal formulation **RENOLITH** containing ingredients such as herbal extracts of *Bergenia ligulata, Didymocarpus pedicellata, Dolichos biflorus, Tribulus terrestris, Boerhavia diffusa, Crateva nurvala.* This formulation is known to possess its antiurolithiatic 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 extract 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)[10]. 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 herbal extracts in accordance with the formula given in table 1. Each powder was weighed and thoroughly mixed together along with other excipients.

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

1. Description

Color, odour and taste were evaluated by using relevant senses[11].

2. Uniformity of weight

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

3. 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[12].

4. 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[12].

5. 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.

6. *Determination of Moisture content*

The test was performed using Karl Fischer instrument by SYSTRONICS.

7. 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[13,14].

8. 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[15-17].

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 [12].

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[18].

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[19].

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[20].

9. Thin Layer Chromatography

Sample solution: Residue in methanol

Development system: n-Butanol: Acetic acid: Water (40:10:50)

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

Detection: By Visible and UV Absorption Range 254nm.

The Extracts were spotted and chromatogram was developed and analyzed under visible as well as UV from 254 nm[21].

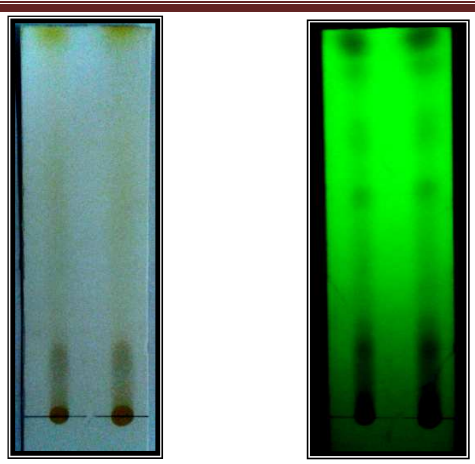
RESULTS AND DISCUSSION

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. 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 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.

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.



(λ=Visible light) (λ=UV: 254 nm)

Figure 1: Visualization of TLC plate

Table 1: Composition of Polyherbal formulation "RENOLITH"

Sr. No.	Biological name	Common name	Family	Quantity
1.	*Bergenia ligulata*	Paashaanbhed	Saxifragaceae	130 mg
2.	*Didymocarpus pedicellata*	Didymocarpus	Gesneriaceae	70 mg
3.	*Dolichos biflorus*	Kulathi	Fabaceae	70 mg
4.	*Tribulus terrestris*	Gokharu	Zygophyllaceae	70 mg
5.	*Boerrhavia diffusa*	Punarnava mool	Nyctaginaceae	60 mg
6.	*Crateva nurvala*	Three leaf caper	Capparaceae	50 mg

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	0.88 % w/w
10.	Total Bitters	5.30 % 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	NMT 10 PPM	Complies
2.	Arsenic	NMT 3 PPM	Complies
3.	Mercury	NMT 0.01 PPM	Complies
4.	Cadmium	NMT 0.25 PPM	Complies

NMT-Not more than

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 30 PPM	NMT 100CFU/GM
3.	*E. Coli*	Absent	Abesnt/gm
4.	*Salmonella*	Absent	Abesnt/10 gm
5.	*Pseudomonas*	Absent	Abesnt/gm
6.	*S. aureus*	Absent	Abesnt/gm

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