



## STANDARDIZATION AND EVALUATION OF POLY HERBAL EXTRACT FOR IN VITRO ANTI-UROLITHIATIC ACTIVITY

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### ABSTRACT

Standardization of poly-herbal extract is needed with a view to investigate of best drugs based on the concentration of their active principles, physical characters, chemical characters, Phyto-chemical parameters, standardization, In-vitro parameters. The present research study on standardization of poly-herbal extract is used to kidney stone diseases. The present research study have a look at became explores that Standardization and evaluation of poly herbal extract for *In-vitro* anti-urolithiatic activity. It was observed that the highest calcium oxalate crystals dissolution was observed in the poly herbal extract. It was observed that poly herbal extract as more efficient to dissolve calcium oxalate.

### INTRODUCTION

Urolithiasis is a problematic condition, especially with regards to its treatment, in all the systems of medical sciences. In the system of modern medicine, which is supposed to be the most advanced and highly scientific system, the problem of urolithiasis has no satisfactory answer. Despite modern techniques, the recurrence rate of Urolithiasis is approximately 50% within 5 years. The only rational treatment in this therapy is surgical removal or lithotripsy of the stone. But, this does not stop the formation of a subsequent stone. Thus for, there is no drug or therapy known that would dissolve or fragment the stone in the system by changing the lithogenic potential of a particular person.[1] Standardization of herbal extract is required in order to assess of quality

Drugs based on the concentration of their active principles, physical characters, chemical characters, Phyto-chemical parameters, standardization, In-vitro, In-vivo parameters. Plant material when used in large quantity may vary in its chemical content and therefore, in its therapeutic effect according to different types of cultivation, collection, harvesting, drying and storage on different seasons. In this condition the quality of herbal medicines may be fluctuate. The quality assessment of herbal formulations is of paramount importance in order to maintain their acceptance in modern system of medicine [2]. In this herbal extract consists of *Mimosa pudica* & *Musa sapientum*

***Mimosa pudica***

*Mimosa pudica* belongs to family Fabaceae. It is commonly known as touch me not. *Mimosa pudica* is native to the tropical Americas. It can also be found in Asian countries such as Singapore, Bangladesh, Thailand, India, Nepal, Indonesia, Taiwan, Malaysia, the Philippines, Vietnam, Cambodia, Laos, Japan, and Sri Lanka. *Mimosa pudica* is an herbaceous to woody plant, often spread on the ground but sometimes erect, reaching 20 to 50 cm, sometimes up to 1 m high. It is hairy to glabrous, with scattered curved spines. [3] The leaves are compound, alternate, on the top of a petiole, 2 to 6 cm long. 2 to 4 principal leaflets arranged in finger like shape, which are in turn composed of 10 to 28 pairs of linear to oblong divisions of the leaflets. The leaves are sensitive and close when touched. The flowers are grouped in pink balls at the end of a stalk at the base of the leaves. The fruits are agglomerated flat pods, composed of 3 to 4 articles and with long stiff hairs on the edge. [4]

#### ***Musa sapientum***

*Musa sapientum* belongs to family Musaceae. It is commonly known as Artipandu. Edible Bananas originated in the Indo-Malaysian region reaching to northern Australia. They were known in the Mediterranean region in the 3rd Century B.C and are believed to have been first carried to Europe in the 10th Century A.D. Early in the 16th Century, Portuguese mariners transported the plant from the West African coast to South America. It even spread into the Islands of the Pacific and to the West Coast of Africa as early as 200-300 BC. In different countries about 300 varieties of bananas are grown, of which a vast majority have been growing in Asian, Indo-Malaysian and Australian tropics and are now widely found throughout the tropical and subtropical countries. India, Philippines, China, Brazil, Indonesia, Mexico, Colombia, Thailand are the top banana producing countries. Bananas and Plantains are today grown in every humid tropical region and constitute the fourth largest fruit crop of the world, following the grape, citrus fruits and apple. [5]

#### **Material and method**

##### **Collection and authentication of crude drugs**

*Mimosa pudica* & *Musa sapientum* were procured from medicinal garden, Anantapur, India. And these were authenticated by Dr.B. Ravi Prasad, professor, Botany Department, The S.K. University, Anantapuram. The voucher specimens of the herbs have been deposited in the Pharmacy department.

#### **Glass wares and Chemicals**

For the entire test good quality glassware were used. The glass wares were washed with detergent first and rinsed with tap water and soaked in chromic acid solution. Chemicals and solvents of analytical grade were used.

**Preparation of Poly-herbal powder:** Poly-herbal powder was made by taking equal proportion of each powdered herbal drugs. All the procured and authenticated individual crude drug material was dried in shade and cleaned by hand sorting. The individual drugs are then pulverized and passed through mesh no.40.

**Organoleptic Evaluation:** Organoleptic evaluation means conclusions drawn from studies resulted due to impressions on organs of senses. It refers to evaluation of poly herbal extract by color, odour, taste, texture and touch [6].

**Physico-Chemical Evaluations:** Physico-chemical investigations were carried out including determination of extractive values like Water soluble extractive, Alcohol soluble extractive, Ether soluble extractive and Hydro-alcoholic soluble extractive values were determined. Then Ash values like Total ash, Water soluble ash and Acid insoluble ash was determined. In Physico-chemical evaluation determined the moisture content of the poly-herbal formulation by Loss on drying method at 105°C [7].

**Determination of Physical Characteristics of Poly-Herbal Formulation** Physical characteristics like bulk density, tapped density, angle of response, Hausner ratio and Carr's index were determined for poly-herbal formulation [8].

**Bulk Density** The term bulk density refers to a indicating a packing of particles or granules. The volume of packing can be determined in an apparatus consisting of graduated cylinder mounted on mechanical tapping device that has a specially cut rotating can. 100gm of

weighed poly-herbal powder was taken and carefully added to cylinder with the aid of a funnel. The initial volume was noted and sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

**Angle of Repose** Angle of repose has been used as an indirect method quantifying powder flow ability, because of its relationship with inter-particle cohesion. The fixed funnel and the free standing cone method employs a method that is secured with its tip at a given height, above the glass paper that is placed on a flat horizontal surface. Powder was carefully poured through the funnel until the apex of the conical pile just touched the tip of funnel.

**Hausner Ratio:** Hausner ratio is related to inter-particle friction and as such can be used to predict the powder flow properties.

**Carr's Index** Carr's index is another indirect method of measuring the powder flow from bulk density.

**Preparation of Poly-herbal extracts (PHE):** Poly-herbal powder becomes prepared by using soxhlet extraction approach using ethanol and a decoction comes to be prepared. Decoction turned into filtered via clear out fabric to gain ethanol extract, focused under vacuum the usage of rotatory evaporator at the way to do away with the ethanol content material and gain the extract in dry stable form.[9]

**Preliminary Phytochemical Analysis:** The poly-herbal extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present in the plant drugs. In this preliminary phytochemical analysis various tests like i.e. Test for alkaloids, test for glycosides, test for carbohydrates, Test for steroids, Test for flavonoids, Test for terpenoids, and Test for proteins [10].

**Investigation of In Vitro Antiurolithiatic Activity by Titrimetry:** The experimental kidney stones of Calcium Oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N  $H_2SO_4$ . Both were

allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of  $H_2SO_4$  by ammonia solution, washed with distilled water and dried at 600C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semi permeable membrane of egg. This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. Group-I served as blank containing only 1 mg of calcium oxalate. Group-II served as positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, i.e. Neeri. Group-III along with 1 mg of calcium oxalate contain methanol extract. The conical flasks of all groups were kept in an incubator preheated to 370C for 2 h. Remove the contents of semi permeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N  $KMnO_4$  till a light pink colour end point obtained. The amount of remaining un-dissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts [11].

## RESULT AND DISCUSSION

In this standardization procedure poly-herbal extract were tested for relevant organoleptic, physicochemical evaluation, and determination of physical characteristics like bulk density, tapped density, angle of repose and Hausner's ratio and carr's index, preliminary phytochemical analysis, The organoleptic characteristics are given in the [Table 1]. The physicochemical characteristics are determined and given in the [Table 2]. The physical characteristics of poly-herbal powder was determined and given in the [Table 3]. Percentage yield of poly-herbal extract was determined and given in the [Table 4]. The preliminary phytochemical analysis was conducted for poly-herbal extract and identified different active constituents which are responsible for treating Kidney stone disease given in the [Table 5]. The percentage dissolution of calcium oxalate by Polyherbal extract was evaluated and

given in the [Table 6]. In these Physico-chemical characteristics like extractive values

indicates the presence of acids, sugar and inorganic compounds.

Table 1. Organoleptic characteristics of Poly-herbal powder

Organoleptic characters	Polyherbal powder
<b>Nature</b>	Coarse powder
<b>Colour</b>	Light yellow
<b>Odour</b>	Characterstics
<b>Taste</b>	Characterstic

Table 2. Physicochemical parameters of Poly-herbal powder

Parameters	PHP (% w/w)
<b>Ash values</b>	
Total ash	3.3
Water soluble ash	1.22
Acid insoluble ash	0.81
<b>Extractive valve</b>	
Water soluble	5.5
Alcohol soluble	6.7
<b>Loss on drying</b>	3.5

Table 3. Physical characters of Poly-herbal powder

S.No.	Parameters	PHP
1	Bulk density	0.43 gm/cc
2.	Tapped density	0.77 gm/cc
3.	Houcner's ratio	0.79
4	Carr's idex	17.30%
5	Angle of Repose	41°.1'

Table 4. Percentage yield of Poly-herbal extract

Extract	Yield %w/w
PHE	16.2

Table 5. Preliminary phytochemical analysis of Poly-herbal extract

Phytoconstituents	PHE
Flavanoids	+
Alkaloids	+
Glycosides	+
Tannins	+
Saponins	+
Steroids	+
Carbohydrates	+
Aminoacids	+

Table 6: Percentage dissolution of calcium oxalate by Poly-herbal extract.

Groups	Groups	% Dissolution of (CaOx)
I	Blank	0
II	Standard	80
III	Poly-herbal extract	85

Less or more extractive values indicate addition of exhausted material, adulteration or

incorrect processing during drying, storage or formulating. The ash values are like total ash

indicates amount of minerals and earth materials present in the plant material. The deterioration time of poly-herbal extract depends up on the amount of the water present in the plant material. If the water content is high the extract easily undergoes to deteriorate due to microbial attacks. Tapped density gives information on consolidation of a powder. The Hausner and Carr's Index both measures the flow properties of the powders. The smaller the Carr's Index the better the flow properties. In the present study, Titrimetric method was used to assess the antiurolithiatic activity of Poly-herbal extract. This study, it was observed that Poly-herbal extract showed antiurolithiatic activity more than that of the standard. This study has given primary evidence for the Poly-herbal extract which possess lithotriptic property. This in vitro study has given lead data and shown that Poly-herbal extract is quite promising for further studies in this regard.

## CONCLUSION

The present research study on standardization and evaluation of poly-herbal extract is used to kidney stone diseases. In this herbal extract consists of *Mimosa pudica* and *Musa sapientum* shows significant effect based on the literature. In the present work, the dissolution of calcium oxalate crystals by poly-herbal extract was studied by using the standard drug neeri. The work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. This study has given primary evidence poly-herbal extract which possess antiurolithiatic property.

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