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IN-VITRO POTENTIAL OF *SPHATIKA* TABLET IN THE MANAGEMENT OF UROLITHIASIS (*MUTRAKRICHRA*)

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Abstract

The urinary system is mainly embedded of kidneys, ureters, urinary bladder, and urethra. Less water intake, electrolyte imbalance, some bacterial i.e. *Escherichia coli* & *streptococci*, viral and parasitic (*Dirofilaria immitis*) infections, autoimmune diseases might be act as causative factor which finally lead to the development of renal calculi. *Sphatika* (potash alum) is consider as *mutrakrichraghan dravya* which helps to break down the calculi and remove them through the urine. In the present work tablets of *Sphatika* were prepared by using direct compression technique. Crystal growth inhibition started at a concentration of 50 µg/ml but 650 µg/ml of drug showed maximum inhibition of 53.89%. The microbial load and presence of heavy metal in prepared *Sphatika* tablets was under the limits prescribed by The Ayurvedic Pharmacopoeia of India.

Keywords: *Mutravaha srotas*; Urinary disorders; Urinary system; *Sphatika*; *Fitkari*; *Mutrakrichra*; Urolithiasis.

Introduction

Water is an essential component liable for digestion, circulation, elimination, body temperature regulation (Brunton, Chabner, & Knollmann, 2011; Nilore, 1984). The urinary system pivotal function is to maintain the normal composition and volume of body fluid that can be executed by glomerular filtration, tubular reabsorption, tubular secretion of soluble and filterable components present in plasma (Satoskar, Rege, & Bhandarkar, 2015). The urinary system, the bowels, the skin and the lungs are four excretory system of the human body (Brunton *et al.*, 2011).

Urolithiasis is defined as the aggregation of urinary crystalloids (Balaji & Menon, 1997; Shanmugapriya & Kumar, 2017). It is concerned with a number of abnormalities associated with composition of urine which might be occur due to dietary indiscretions, physiological and metabolic disorders, or both (Baghel, Chopra, Bhatia, & Tamilvanan, 2018; Jung *et al.*, 2017; Vermeulen, Lyon, & Fried, 1965). The exact cause and mechanism of the stone formation in urinary system is still obscure. As far as the treatment is concerned the surgical and medical management of the disease which are in practice able to treat only some extent but they are imitated and associated with various complications (Pak *et al.*, 2004; Pearle, 2004; Taylor & Curhan, 2004).

The stone formation procedure relies upon urine volume, comprise calcium, phosphate, oxalate and sodium ions concentration (Mandel & Mandel, 1989). High ion levels, low urine volume, low pH, and low citrate level might be act as a precursor for the formation of kidney stone (Brunton *et al.*, 2011; Satoskar *et al.*, 2015).

Sphatika or Phitkari or *Kankshi* or Alum or Potash alum is a mineral origin drug of *Ayurvedic* medicine which have astringent, analgesic, haemostatic, desiccative, expulsive for foetus and placenta, antipyretic, detergent, corrosive, expectorant, emetic and irritant property (Chunekar & Pandey, 2004; Sharma, 2004; Sivananda, 2006; Tripathi, 1994; Vagbhata, 1961). It is a colourless, white transparent, odourless crystalline masses or a granular powder with a sweetish astringent taste contains Potassium, Aluminium, Hydrogen, Sulphur and Oxygen ($K_2SO_4Al_2(SO_4)_3.24H_2O$) (Roqaiya & Begum, 2015). When heated it melts and at about 200^0C and loses its water of crystallisation with the formation of the anhydrous salt. It is soluble as 1 part in 7.5 parts of water, 1 in 0.3 of boiling water, and 1 in 3 of glycerol (ALtaei & AI-Jubouri, 2005). Two types of *Sphatika* has been explained in the classics i.e. *Phataki* and *Phullika*. It is described under *Uparasa varga* in *Rasa ratna samuchaya* (Vagbhata, 1961), *Rasa Hridaya Tantra* (Govinda, 1998), *Rasendra Chudamani* (Vidhyalankar, 1932), *Rasa Prakasha sudhakara* (Siddhinandan, 2004).

Materials and Method

The *Sphatika* was collected from the local market of Jalandhar and its authentication was carried out by Herbal Health Research Consortium Pvt. Ltd., Amritsar.

PHYSICOCHEMICAL PARAMETERS

Determination of Foreign Matter (Lohar, 2007)

Drug sample (500 g) was taken and spread into tray. The unwanted material was separated out by visual inspection, using a magnifying lens. It was weighed and percentage of foreign matter was calculated.

Determination of Moisture Content (Loss on Drying at 105^0C) (Lohar, 2007)

Ten gm of the drug sample was taken and dried it at 105^0C for 5 hours in hot air oven and weighed after cooling in desiccator. It was then dried until the difference between two progressive readings was not more than 0.25 percent and computed the percentage of LOD.

Determination of Total Ash (Lohar, 2007)

Powdered 2 gm drug sample was incinerated in tarred silica crucible at 450^0C for 5 hrs in a muffle furnace until it turned white, indicating the absence of carbon. This was

cooled in a desiccator and weighed. The percentage of total ash was calculated with reference to the air-dried sample.

Determination of Acid Insoluble Ash (Lohar, 2007)

The acquired ash was boiled for 5 minutes with 25 ml of 6N HCl [hydrochloric acid], and filtered through ash less filter paper. The insoluble matter was washed with hot water until the filtrate became chlorine free, thenafter gathered the insoluble matter in a crucible. It was ignited to constant weight and then calculated the percentage.

Determination of Alcohol Soluble Extractive (Lohar, 2007)

Five gm of coarsely powdered sample drug was macerated with 100 ml of alcohol in a closed conical flask for 24 hours. Shaking was done frequently for 6 hours and then allowed to stand for 18 hours. It was filtered with taking precautions against loss of liquid. Twenty-five ml of filtrate was evaporated to dryness in a tarred flat evaporating dish, and dried at 105^0C to consistent weight and then weighed it. Calculated the percentages of alcohol soluble extractive with reference to air dried sample.

Determination of Water Soluble Extractive (Lohar, 2007)

The same procedure was followed as that of alcohol soluble extractive replacing alcohol with water.

pH of drug solution (Lohar, 2007)

The pH value of a 5% sample solution was noted down by using digital pH meter.

Refractive Index (Lohar, 2007)

The refractive index of a 5% sample solution was determined using Abbe refractometer.

PRE-COMPRESSION CHARACTERIZATION (Haritha, 2017; Lachman, Lieberman, & Kanig, 1986; Mannhold, Buschmann, & Holenz, 2019; Rowe, Sheskey, & Quinn, 2009)

Organoleptic characteristics

It included recording of organoleptic characteristics of the drug using descriptive terminologies since record of colour and odour of early batches is very useful in establishing appropriate specifications for production later on.

Density

Powder density may influence compressibility, sphericity, pellet porosity, and dissolution rate consequently.

Bulk density (BD)

Bulk density is a ratio of mass of powder to bulk volume of powder. The parameter was measured following standard procedure. The equation for determining bulk density is

$$BD\ (\rho b) = m/Vb, \quad ...(1)$$

where,

ρb = Bulk density, m = Mass of powder, vb = Bulk Volume

Tapped density (TD)

It is a measure used to describe void space of powder. The pre-weighed powder was filled in measuring cylinder. Then it was tapped in bulk density test apparatus. After 100 taps the volume was measured. The equation for determining tapped density is

$$TP\ (\rho t) = m/Vt, \quad ...(2)$$

where, ρt = Tapped density, m = Mass of powder, vt = Tapped volume

Carr's (Compressibility) Index (Ci)

Compressibility is indirectly related to the relative flow rate, cohesiveness and particle size distribution of the powder. Tapped density (ρt) and bulk density (ρb) of powder material was used to measure compressibility of a powder material. The equation for determining Carr's index is

$$CI\ (\%) = (\rho t - \rho b)/\rho t * 100, \quad ...(3)$$

Where, ρb = Bulk density, ρt = Tapped density

Angle of Repose

Angle of repose is the maximum angle possible between the surface of a pile of powder and the horizontal plane. The angle of repose of powder blend was determined by "fixed funnel and free-standing cone method". The accurately weighed powder blend was taken in the funnel and tip of funnel was blocked by thumb initially. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the powder blend (fixed at approximately 2 cm from plane to tip of funnel). The powder blend was allowed to flow through the funnel freely on to the surface. It is used to describe flow ability of the powder material. Angle of Repose was determined by

$$\theta = \tan\text{-}1\ (h/r), \quad ...(4)$$

Where, θ = Max. angle between pile of powder and horizontal plane, h = Height of pile of powder, r = Radius of the base of conical pile

Hausner's Ratio (Hr)

It is the ratio of bulk volume to tapped volume or tapped density to bulk density. It is a measure of compressibility of powder. Tapped density (ρt) and bulk density (ρb) of powder material were used to measure Hausner's Ratio.

Preparation of *Sphatika* tablet (Lachman *et al.*, 1986)

Tablets were prepared by using direct compression technique.

Post-compression Parameters (Haritha, 2017; Lachman *et al.*, 1986; Mannhold *et al.*, 2019; Rowe *et al.*, 2009)

Shape and Appearance

Shape and appearance of prepared tablets were observed by visual inspection.

Diameter and Thickness

Dimension of the tablets was measured by using a calibrated dial caliper. Five tablets were picked out randomly and their diameter and thickness were measured individually.

Hardness

The prepared tablets were subjected to hardness test. It was carried out by using Monsanto hardness tester and the observation were expressed in kg/cm^2.

Friability (F)

The friability was determined using Roche friabilator and expressed in percentage (%). Twenty tablets from batch were weighed separately ($W_{initial}$) and placed in the

friabilator, which was then operated for 100 revolutions at 25 rpm. The tablets were reweighed (W_{final}) and the percentage friability was calculated for each batch by using the following formula –

$$F = (W_{initial} - W_{final}) / W_{initial} \times 100. \quad ...(5)$$

Weight Variation Test

The weight variation test was done by taking 20 tablets randomly and weighing them accurately. The composite weight divided by 20 provided an average weight of a tablet. The deviation from average weight was determined.

Disintegration Time

Six tablets were placed individually in each tube of disintegration test apparatus and discs were placed. Disintegration time was measured in distilled water at 37± 2°C. The tablets were considered as completely disintegrated when all particles passed through the wire mesh.

Stability studies of optimized formulation (Haritha, 2017; Lachman *et al.*, 1986; Mannhold *et al.*, 2019; Rowe *et al.*, 2009)

Stability of pharmaceutical product may be defined as the capability of a particular formulation, in a specific container/package, to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life. Stability study was carried out for 6 months at accelerated storage conditions (40 ± 2^0C / 75% RH ± 5%) following ICH guidelines.

Antiurolithic Activity (Ahmed, Hasan, & Mahmood, 2016; Shah *et al.*, 2014)

The antiurolithiatic effect of *Sphatika* Tablet on calcium oxalate crystallization was determined by the time course measurement of turbidity changes owing to the crystallization in artificial urine on adding 0.01M sodium oxalate solution. The precipitation of calcium oxalate was measured in terms of turbidity using UV spectrophotometer (620 nm).

Synthesis of Calcium Oxalate crystals

The inhibitory effect of aqueous extracts on calcium oxalate crystallization was observed in the form of turbidity due to the crystal nucleation and aggregation while adding 0.01M sodium oxalate to artificial urine. It was observed with the help of UV spectrophotometer that calcium oxalate was precipitated at pH 6.8, temperature 37^0C and wavelength 620 nm.

Preparation of artificial urine

The artificial urine was prepared by following the reported method of Finlayson *et al.*, 1978, at a constant temperature of 37^0C in capped bottle. A reported formula was used for making artificial urine. All the chemical reagents (sodium chloride 105.5 mmol/liter, sodium phosphate 32.3 mmol/liter, sodium citrate 3.21 mmol/liter, magnesium sulfate 3.85 mmol/liter, sodium sulfate 16.95 mmol/liter, potassium chloride 63.7 mmol/liter, calcium chloride 4.5 mmol/liter, sodium oxalate 0.32 mmol/liter, ammonium hydroxide 17.9 mmol/liter and ammonium chloride 0.0028 mmol/liter) were dissolved in deionized water and the pH was adjusted to 6.

Observation without the addition of prepared dosage from

One ml of artificial urine and 0.5 ml distilled water were transferred into the cell and blank reading was taken on a spectrophotometer. Then 0.5 ml of 0.01M sodium oxalate was added and readings were taken after a time period of 10 minutes.

Observation in the presence of *Sphatika* tablet

Different concentrations of *Sphatika* tablet i.e. 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 and 650 µg/ml were tested for calcium oxalate crystallization inhibition. Half ml of each concentration was added to 1 ml of artificial urine and blank reading was taken through UV spectrophotometer at 620 nm. Then half ml of 0.01 M sodium oxalate was further added and the measurement was done after a period of 10 minutes. Three replicates were run for each experiment.

Microscopic examination

All the above mentioned sample concentrations were studied under a trinocular microscope (45X) for the appearance of calcium oxalate crystals and the pictures were taken using digital camera.

Heavy metals determination (Lohar, 2007)

Atomic absorption spectrophotometer was used in the determination of heavy metal elements i.e. Lead, Mercury, Arsenic and Cadmium.

Microbial load (Lohar, 2007)

The presence of microbial load was carried out as per the method described in The Ayurvedic Pharmacopoeia of India.

Results and Discussion

Physicochemical properties

The various physicochemical parameters i.e. foreign matter, loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, and water-soluble extractive were determined as per the standard procedures mentioned in API and findings are tabulated below in Table 1.

Table 1: Physiochemical parameters for *Sphatika*

Sr. No.	Parameters	Observation (Mean ± SD)	
		***Sphatika* (BP)**	***Sphatika* (AP)**
1	Foreign matter (% W/W)	Nil	Nil
2	Loss on drying	2.3 ±0.31	2.6 ±0.15
3	Total Ash (% W/W)	42.3 ±0.20	41.3 ±0.76
4	Acid-insoluble ash (% W/W)	18.2 ±0.21	17.8 ±0.35
5	Alcohol-soluble extractive (% V/W)	45.8 ±0.93	48.8 ±0.81
6	Water-soluble extractive (% V/W)	98.2 ±1.10	95.2 ±1.27

All values are expressed as mean (±) n=3, BP= Before purification and AP=After purification

Evaluation parameters of powder blend of tablet

Powder blend was evaluated for the following pre-compression parameters i.e. bulk density, tapped density, Carr's compressibility index, Hausner's ratio, and angle of repose. Ten grams of sample was taken for the studies. The results are given in Table 2.

Table 2: Powder flow properties

Sr. No.	Parameters	*Sphatika* (Zero day)*	*Sphatika* (6 Months) *	Interpretation
1	Bulk density (g/cm^3)	0.310 ±0.12	0.312 ±0.22	Fair
2	Tapped density (g/cm^3)	0.408 ±0.10	0.411 ±0.14	Fair
3	Compressibility index	24.0 ±0.13	24.06 ±0.15	Passable
4	Hausner's ratio	1.32 ±0.12	1.34 ±0.17	Poor
5	Angle of repose	36.12 ±0.27	36.23 ±0.22	Fair

*±(n=3)

Post-compression evaluation parameters for tablets

Prepared tablets (weighing 300 mg) were evaluated for the post-compression parameters i.e. shape, diameter, thickness, hardness, friability, weight variation test, disintegration time, and percentage drug release. The observation are tabulated in Table 3, 4.

Table 3: Post-compression evaluation parameters for tablets

Sr.No	Parameters	*Sphatika tablet*
1	Shape	Round
2	Diameter (mm)	6.07 ± 0.03
3	Thickness (mm)	1.13 ± 0.03
4	Hardness (kg/cm^2)	5 ± 1
5	Friability (%W/W)	0.90 ± 0.04
6	Weight variation	1.7 ± 0.32
7	Disintegration time (minutes)	4 ± 1

All values are expressed as mean (±) n=3

Table 4: Post-compression evaluation parameters for tablets [After 6 month, 40^{o}C ± 2^{o}C/ 75% RH ± 5%]

Sr.No	Parameters	*Sphatika tablet* (after 6 months)
1	Shape	Round
2	Diameter (mm)	6.06 ± 0.09
3	Thickness (mm)	1.12 ± 0.02
4	Hardness (kg/cm^2)	4 ± 1
5	Friability (%W/W)	0.96 ± 0.24
6	Weight variation	1.3 ± 0.12
7	Disintegration time (minutes)	3 ± 1

***In-vitro* study**

The prepared sample of *Sphatika* started showing inhibition of crystal growth from zero minute. With the passage of time the percentage (%) inhibition also changed. Percentage of inhibitions was calculated as

Percentage of inhibition

= (1-OD (experimental)/OD(control)X100 ...(6)

A concentration of 650 µg/ml of drug showed approx. 53.89 % calcium oxalate (CaOx) crystal inhibition and exhibited concentration dependant inhibition. The observations are tabulated in Table 5 and (Figure 1-14).

Table 5: *In-vitro* inhibitory activity of CaOx crystals growth by UV spectrophotometer at 620nm

Sr. No.	Drug Conc. (µg/ml)	Absorbance (UV spectrophotometer)	Percentage inhibition*
1	50	0.288	2.38
2	100	0.276	6.45
3	150	0.269	8.82
4	200	0.255	13.56
5	250	0.239	18.99
6	300	0.220	25.43
7	350	0.215	27.12
8	400	0.212	28.14
9	450	0.203	31.19
10	500	0.195	33.9
11	550	0.176	40.34
12	600	0.150	49
13	650	0.136	53.89

***** Control sample Absorbance without drug 0.295

Microscopic Study

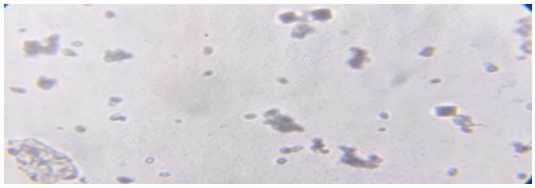

Fig. 1: Crystal growth (Control)

Fig. 2: Crystal inhibition at 50 µg/ml

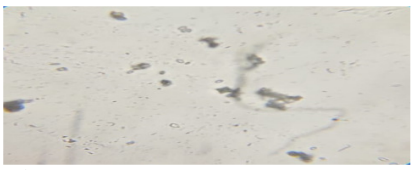

Fig. 3: Crystal inhibition at 100 µg/ml

Fig. 4: Crystal inhibition at 150 µg/ml

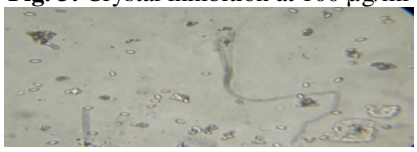

Fig. 5: Crystal inhibition at 200 µg/ml

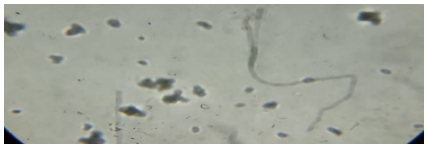

Fig. 6: Crystal inhibition at 250 µg/ml

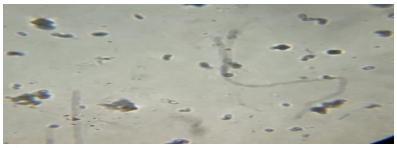

Fig. 7: Crystal inhibition at 300 µg/ml

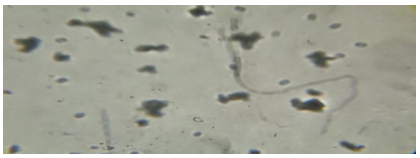

Fig. 8: Crystal inhibition at 350 µg/ml

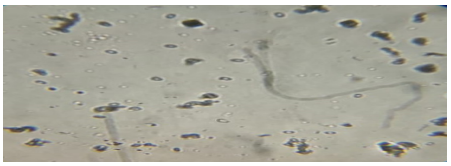

Fig. 9: Crystal inhibition at 400 µg/ml

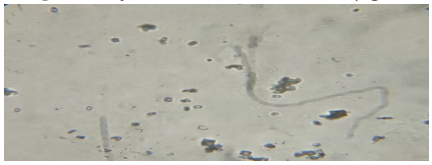

Fig. 10: Crystal inhibition at 450 µg/ml

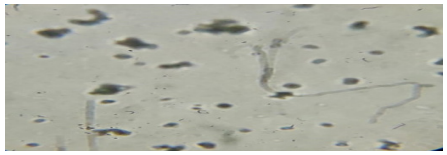

Fig. 11: Crystal inhibition at 500 µg/ml

Fig. 12: Crystal inhibition at 550 µg/ml

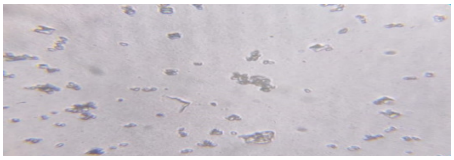

Fig. 13: Crystal inhibition at 600 µg/ml

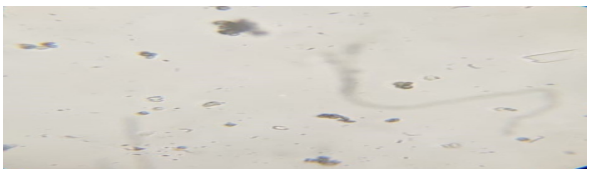

Fig. 14: Crystal inhibition at 650 µg/ml

Heavy metals determination

The determination of heavy metals in the prepared *Sphatika* tablets was carried out using atomic absorption spectroscopy and results are tabulated in table 6.

Table 6: Heavy metal concentrations in *Sphatika*

Sr. No.	Metals	Lead	Mercury	Arsenic	Cadmium
1	Observed values	Not detected	0.22 ppm	2.8 ppm	Not detected
2	Limit as per API	10 ppm	1 ppm	3 ppm	0.3 ppm

Microbial Load

The presence of microbial load was carried out as per the method described in The Ayurvedic Pharmacopoeia of India in table 7.

Table 7: Observations of Microbial load

Microbial analysis	Total bacterial count	Total yeast and mould	*E. coli*	*S. spp.*	*S. aureus*	*P. aeruginosa*
Limit as per API	NMT 10^5 CFU/ml	NMT 10^3 CFU/ml	Absent	Absent	Absent	Absent
Observed values (*Sphatika*)	1200 CFU/ml	Nil	Nil	Absent	Absent	Absent

Conclusion

This work involved evaluation and assessment of *In-vitro* potential of prepared *Sphatika* (potash alum) tablets against urolithiasis (*Mutrakrichra*). The drug sample was subjected to physicochemical evaluation parameters like foreign matter, total ash, moisture content, alcohol, water-soluble extractives and the results were found satisfactory. The *Sphatika* and excipients were thoroughly mixed and subjected to preformulation studies. Carr's index, Hausner's ratio and angle of repose were found to be satisfactory. The compressed tablets were evaluated for post-compression parameters like hardness, friability, weight variation, and disintegration time and were able to comply with the pharmacopoeial standards. Crystal growth inhibition started at a concentration of 50 µg/ml but 650 µg/ml concentration of drug showed maximum inhibition of 53.89 %. The microbial load and presence of heavy metal in prepared *Sphatika* tablets was under the limits prescribed by The Ayurvedic Pharmacopoeia of India. Tablets were stable over a period of 6 months when exposed to accelerated stability studies. It can be concluded that prepared tablet dosage form of *sphatika* was found to be effective in the management of urolithiasis (*Mutrakricchra*) by in-vitro technique.

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