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Evaluation of Antiurolithiatic Activity by In-Vitro Methods

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Disease, In-vitro, Kidney stone, Poly herbal formulation.

Abstract
In the present study, in vitro methods are used to evaluate of anti urolithiatic activity of polyherbal tablets. In in-vitro study there are several models includes Kramer and Tisdall'stitrimetry model, turbidimetric study by using simple NaCl solution, turbidimetric study by using artificial urine model, microscopic study, crystal inhibition on gel model, etc. Among these we have used Kramer and Tisdall'stitrimetry model, turbidimetric study by using simple NaCl solution, turbidimetric study by using artificial urine model, microscopic study.

1. Introduction

Kidney stone or calculi are commonly known for the urolithiasis. The stone may form at any level in the urinary tract, but most arise in the kidney. [1] Urinary calculus is very old condition and its history dates back ancient to the earliest period of civilization. [2] The calculi form in the kidney and bladder, when urinary constituents are getting precipitates.Usually oxalates and phosphatesare involved in the formation of kidney stones. Males after age of 30 years are more susceptible toform kidney stone. Regularly, it originates in the renal papillae and then passes into the renal pelvis where, their size may rise. When stones become disproportionately large to pass from ureter and barricade the outflow of urine it will cause kidney damage. Frequently in emergingrepublics and in children the stones produce in the bladder. [3]

When the level of abnormal constituents increases above the normal value, they become responsible for the kidney stone disease. Stones differ in shape, size, dimension, character, and chemical compositions. [4] Based on composition, kidney stones are commonly classified into five types as follows:

1. Calcium stones
   i. Calcium oxalate
   ii. Calcium phosphates
2. Struvite stones
3. Cystic stones
4. Uric acid stones
5. Drug induced stones [4][5]

To treat kidney disease there is need to remove the formed stone out of the body either by dissolving or by breaking it into small pieces and pass from urinary track through urine out of the body. None of the surgical treatment produces satisfactory result. So, we have decided to formulate a polyherbal formulation for assessment of the urolithiatic activity.

The wet granulation method was used for the formulation of polyherbal tablet. All tablets pass the weight variation test, hardness test, Friability test and disintegration test.

Plant material:

Plants were procured from local market of Pune city.

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Plant Common Name	Biological Source
Gokhru	*Tribulusterrestris*
Banana	*Musa balbisianacolla*
Paanfuti	*BryophyllumPinnata*
Guggul	Commiphorawightii

Extraction of Process of selected plants:

a) Extraction process of *Musa balbisianacolla*: The juice was extracted by pressing a pseudo stem using sugarcane press machine. The juice extracted was filtered to remove solid materials. The filtered juice was then freeze dried using a freeze dryer. The freeze dried extracts were then collected.

b) Extraction process of *Tribulusterestris*: The dried and matured fruits of *Tribuluterrestris* were obtained from local ares of Pune. Aqueous extract was prepared by using the dried and matured fruit of *Tribuluterrestris* was ground into fine powder and the extraction was carried out at temperature of 23.5 °C for a period of 19.50hours under constant stirring and solid to liquid ratio of 1 g /12mL of the solvent (water). Following this, the extract was filtered, and stored in air tight container.

c) **Extraction process *of BryophyllumPinnata*:** Fresh leaves of ***BryophyllumPinnata***were collected from the botanical garden of the Seth Govind Raghunath Sable College of Pharmacy, Saswad. The leaves were air dried, pulverized and extracted exhaustively in distilled water for 72 h by cold maceration. The filtrate was vanished to obtain the dry extract using a rotary evaporator.

d) Extraction process of *Commiphorawightii*: The stem barks of *Commiphorawightii*was collected from nearby area of SGRS college of Pharmacy, Saswad. The plant was washed, chopped in to small pieces and dried under shade then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use. Extraction of Plant Material of coarsely powdered plant material was extracted by Soxhlet extraction method using petroleum ether. All the extracts thus obtained were stored in air-tight bottles at 4ºC for further experiments. [6,7].

Preparation & Evaluation of Tablets: [8-9]

The polyherbal tablet was prepared by wet granulation method**.** All the ingredients weighed and mix them by doubling up method. Then add minute amount of water to the mixture to form dough. Then the formed dough pass through seive No. 44# to form grains. The formed granules are then dry and again pass through sieve No. 80#. The tablets are formed in the tablet punching machine. Evaluation of tablets was done by using the [7-8] The Preliminary studies of formulation like Angle of Repose (θ), Tapped density, Hausner's ratio, Percentage Compressibility, Weight variation, Hardness test Friability test, Disintegration test and Dissolution test.

3. Antiurolithiatic Study

The evaluation of anti urolithiatic activity was by using Kramer and Tisdall method and artificial urine method

a) Method for evaluation of anti-urolithiatic activity by Kramer and Tisdall method:

It is possible to investigate in-vitro antiurolithiatic action by creating calcium oxalate stones and observing the effect of polyherbal tablet. In the current investigation, in-vitro antiurolithiatic activity was assessed using the Kramer and Tisdall method. Accurate 1 mg of calcium oxalate and 10 mg of test and standards were weighed, placed in semi permeable membranes separately, and then properly tied after semi permeable membranes had been prepared. A conical flask containing 100 ml of 0.1M TRIS buffer was used to suspend the above prepared pouch of semi permeable membrane. For 7-8 hours, all of the conical flasks were left undisturbed at room temperature. The semi-permeable membrane's leftover contents were transferred into a test tube; 2 ml of 1N H2So4 was

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added and titrated with KMnO4 till colour change was obtained.
[10-14]

b) Artificial urine model:

I. Experimental procedure

Burns and Finlayson method was used to prepare an artificial urine sample. The effect of tablet on calcium oxalate crystallization was finding out by change in the turbidity due to the crystallization in artificial urine. These crystals are made on addition of 0.01M sodium oxalate into the artificial urine, every minute. The precipitation of calcium oxalate was calculated by the measurement of absorbance at 620 nm using UV/Vis spectrophotometer.

II. Study without inhibitor

0.5 ml of distilled water added with 1.5 ml of prepared artificial urine was transferred into cuvette and blank reading was taken. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume, and the measurement was immediately started for the duration of 10 min.

III. Study with inhibitor

0.5 ml of various conc. of drug solution (10%, 20%, up to 100%) and 1.5 ml of artificial urine was transferred in the cuvette. Aabsorbance without sodium oxalate was recorded and then 0.5 ml of 0.01M sodium oxalate solution was added. The absorbance was measured immediately for a period of 10 min. [15,16]

4. Results and Discussions

The polyherbal tablet formulation was evaluated with all standard tests and the tablet was passes all the IP standards tests.

I) Preliminary studies of formulation:

a) Pre-compression evaluation:

1. Angle of Repose (θ) was found to be 28.8°.

h = 2 cm, r = 3.62 cmθ = $\tan^{-1}\left(\frac{2}{3.71}\right)$= $\tan^{-1}(0.55)$= 28.8°

Angle of repose (θ) = 28.8°

As per the IP angle of repose between 25- 30 indicates good flow property of granules.

2. Bulk Densitywas found to be 0.31 gm/ml

Weight of the powder = 10 gm, Volume of the powder = 32 ml

LBD (Loose Bulk Density) = $\frac{10}{32}$ = 0.31 gm/ml

Bulk Density = 0.31 gm/ml

3. Tap Density was found to be 0.34 gm/ml

Weight of the powder = 10 gm, Tapped Volume of the powder = 30 ml

TBD (Tapped Bulk Density) = $\frac{10}{29}$ = 0.34 gm/ml

Tap Density = 0.34 gm/ml

4. Hausner's Ratio was found to be 1.25

Bulk Density = 0.31 gm/ml, Tap Density = 0.34 gm/ml

Hausner's ratio (H) = $\frac{TD}{BD} = \frac{0.34}{0.31}$ = 1.25

Hausner's Ratio = 1.09: According to IP lower Hausner's ratio (< 1.25) indicates better flow property than higher ones (> 1.25).

5. Percentage Compressibility was found to be 8.8 %

Percentage Compressibility = 8.8: As per the IP Carr's index between >10 indicates excellent flow of the granules.

b) Post- compression Evaluation:

1. Physicochemical analysis:

Table: Physicochemical Evaluation of Tablet

Parameter	**Observation**
Colour	Brown
Odour	Characteristic (mild)
Taste	Bitter
Solubility	Water soluble

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II) Quality control test:

1. Weight variation test:

Table: Weight Variation test of Tablets

Sr. No.	Wt. of each tablet in gm	Difference between Av. Wt. & Individual tablet Wt.	% Wt. Variation	Pass or not
1	0.244	-0.004	1.6	Pass
2	0.243	-0.003	1.2	Pass
3	0.244	-0.006	2.4	Pass
4	0.249	-0.001	0.4	Pass
5	0.248	-0.002	0.8	Pass
6	0.251	0.001	0.4	Pass
7	0.250	0.000	00	Pass
8	0.261	0.011	4.4	Pass
9	0.262	0.012	4.8	Pass
10	0.254	0.003	1.2	Pass
11	0.249	-0.001	0.4	Pass
12	0.259	0.008	3.2	Pass
13	0.262	0.012	4.8	Pass
14	0.248	-0.002	0.8	Pass
15	0.252	0.001	0.4	Pass
16	0.243	0.002	0.8	Pass
17	0.242	-0.008	3.2	Pass
18	0.258	0.008	3.2	Pass
19	0.243	-0.007	2.8	Pass
20	0.247	-0.003	1.2	Pass

Total weight of 20 tablets = 5.009 gm, Average weight of tablets = 0.250 gm = 250 mg

2. Hardness

Table: Hardness of tablets

Sr. No.	Hardness
1	66.8
2	61.1
3	62.6
4	58.9
5	53.7

Average Hardness = $\frac{303.1}{5}$ = 60.62 N

Hardness of Tablet = 60.62N = 6.14 kg/cm^2

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3. Friability

Initial weight of 20 tablets = 4.781 gm, Final weight of 20 tablets = 4.780 gm

$F = \frac{4.781 - 4.780}{4.781} \times 100 =$ **0.020 %**

4. Disintegration Time: Disintegration time of tablet was found to be 30 sec.

5. Dissolution time: Dissolution time of tablet was found to be 25 min.

In-vitro Models:

a) Titrimetry study:

Table: Data of Calcium oxalate dissolution by test and standard drug

Group	Volume of standard $KMnO_4$	Wt. of calcium estimated	Wt. of calcium reduced	% Dissolved
Control	0.001 ml of **$KMnO_4$**	0.001898 mg	00	00
Standard (Cystone)	0.15 ml**$KMnO_4$**	0.02747 mg	9.9725 mg	93.33%
Test Drug	0.1 ml**$KMnO_4$**	0.01890 mg	9.9811 mg	90.00%

*Corresponds to 10 mg

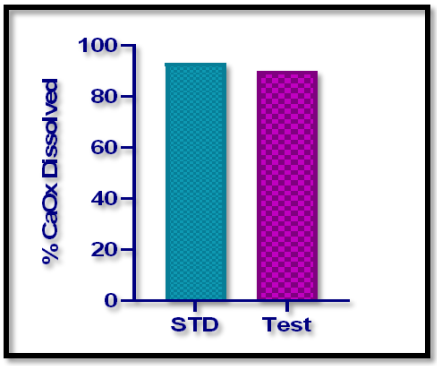

Figure: Comparison between %CaOx dissolved by standard & test drug

b) Artificial urine model:

Table: The maximum values of the variation of absorbance, and the turbidimetric slopes relating to the curves of crystallization without and with inhibitors (Cystone) in artificial urine

CI %	TS	I%	ΔD	R^2	CV %
00	-0.015	--	0.00212	0.860	11.86
10	-0.013	14.30	0.00146	0.888	7.83
20	-0.012	22.40	0.00123	0.954	8.41
30	-0.011	29.10	0.00117	0.830	7.04
40	-0.010	36.07	0.00098	0.866	5.66
50	-0.009	43.10	0.00064	0.891	5.23

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60	-0.008	52	0.00064	0.744	5.81
70	-0.008	52	0.00073	0.748	4.22
80	-0.007	58.14	0.0006	0.851	4.15
90	-0.006	65.27	0.00030	0.799	3.98
100	-0.006	65.27	0.00038	0.770	3.65

CI concentration of inhibitor, TS turbidimetric slope, R2 linear regression of the data, CV (%) coefficient of variation, ΔD variation of absorbance, I percentage of inhibition

Table: The maximum values of the variation of absorbance, and the turbidimetric slopes relating to the curves of crystallization without and with inhibitors in artificial urine

CI %	**TS**	**I%**	**ΔD**	**R^2**	**CV %**
00	-0.013	--	0.00209	0.856	11.83
10	-0.009	21.40	0.00143	0.816	6.77
20	-0.009	21.40	0.00113	0.946	5.49
30	-0.009	21.40	0.00135	0.840	7.59
40	-0.008	28.55	0.00121	0.790	6.00
50	-0.005	49	0.00046	0.912	6.66
60	-0.003	64.27	0.00021	0.965	4.17
70	-0.004	71.40	0.00018	0.943	4.23
80	-0.005	71.41	0.00021	0.937	4.51
90	-0.005	71.41	0.00014	0.950	3.60
100	-0.003	85.69	0.00003	0.798	3.06

CI concentration of inhibitor, TS turbidimetric slope, R2 linear regression of the data, CV (%) coefficient of variation, ΔD variation of absorbance, I percentage of inhibition

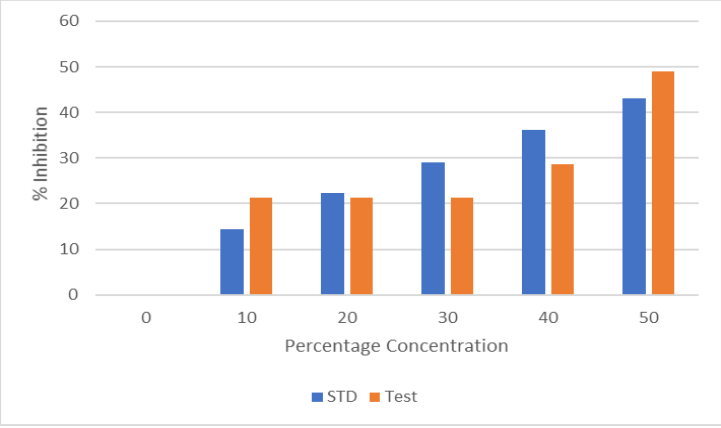

Figure: Percentage inhibition of CaOx by test & standard drug in artificial urine

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4. Conclusion

The artificial urine method and Kramer and Tisdall method egg permeability method were used for the evaluation anti urolithiatic activity of polyherbal formulation .The findings of the present study for the evaluation of anti-urolithiatic activity of polyherbal formulation (tablet) by in-vitro methods showed inhibition and reduction in the formation of crystals.The tablet was found to be effective for assessment of anti urolithiatic activity by in-vitro models.

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