Original Article

EVALUATION OF ANTIUROLITHIATIC AVTIVITY OF SOME HERBS BY USING IN VITRO (TURBIDITY AND MICROSCOPIC) METHODS

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Abstract

With yearly incidence of 0.5% to 1.9%, kidney stones are one of the urinary tract conditions that are considered to impact at least 10% of the population in countries. 12% of Indians are estimated to have urinary stones and 50% of those cases result in the loss of one or both kidneys, as well as minor renal injury or loss. In the current investigation, a polyherbal formulation was made by choosing various herbs with strong antiurolithiatic properties. These plants have long been important in Indian culture. These plants are widely utilized throughout India and have been employed extensively in the ayurvedic medical system. The plant demonstrates a broad spectrum of pharmacological activities, including antibacterial, anti-helminthic, anti-pyretic, and many other effects.

Key words: Antiurolithiatic, Kidney stones, Calcium, Nephrolithiasis.

Introduction:

As from beginning of mankind's history, plants have offered food, the constituents for medicine, and a multitude of other basics for life[1]. Even current conventional medicine incorporates countless compounds that originate from plants as therapeutic agents. People are turning to environment for safe remedies as a result of the overuse of synthetic pharmaceuticals, which increases the incidence of serious drug reactions. Due to their professionally validated effects, herbs and herbal therapies have sparked interest among the public[2]. To prove the efficacy and safety of any and all indigenous herbal plant remedies in the modern context of science, there is a strong need for thorough scientific confirmation.

The combined effect of epidemiological, biochemical, and genetic risk factors results in kidney stone disease, which is a multifactorial condition[3]. The urinary tract condition known as urolithiasis is ranked third in terms of frequency. The solid non-metallic minerals in the urinary system are what is meant by this. A balance between promoters and inhibitors in the kidney results in a complicated process. Crystal nucleation, aggregation, and retention inside the urinary tract are the first three phytochemical processes that contribute to kidney stone formation. The most frequent kidney stone type, calcium oxalate stones, account for up to 80% of all examined stones [4]. Pure calcium oxalate (50%) or calcium phosphate (5%) or a combination of both (45%) may make up calcium-containing stones. Next come magnesium phosphate (15-20%), uric acid (10%), and cystine (1%)[5].

Kidney stones are among the urinary tract illnesses that are thought to affect at least 10% of the population in the industrialised world, with a yearly incidence of 0.5% to 1.9%. According to estimates, 12% of Indians have urinary stones, and in 50% of those cases, one or both kidneys are lost, along with or without minor renal injury[2].

Males are 2-3 times as likely than females to develop stone disease. It recurs at a rate of 70–81% in men and 47–60% in women5. Despite significant advancements in the pathogenesis and management of urolithiasis, no effective medication is currently being used in clinical therapy. Extracorporeal shock wave lithotripsy, endoscopic stone removal, and kidney dialysis are all prohibitively expensive, and recurrence is very typical with these operations[1].

According to data from clinical trials conducted in vivo and in vitro, phytotherapeutic substances may be effective as an alternative therapy for the treatment of urolithiasis. Due to the fact that they aid in the repair process in a natural way1, medicinal herbs and their products are more beneficial. Based on traditional knowledge, pharmacological and phytochemical prospecting of medicinal plants can result in the identification of new drugs and the development of pharmacologically significant items for human health care[6]. Compared to expensive synthetic pharmaceuticals, which can have negative effects, green treatments were safer and more trustworthy[7].

The chosen plant, *Tribulus terestris, Musa balbisianacolla, Bryophyllum Pinnata* and *Commiphora wightii* has long played a significant role in Indian folk medicine and culture. These plant has been used extensively in the Ayurvedic medical system and is used all over India. The plant exhibits a wide range of pharmacological properties, including anti-diabetic, anti-helminthic, anti-pyretic, anti-inflammatory, analgesic, and antibacterial properties [8,9].

MATERIALS AND METHODS

Extraction of Process of selected plants

1. Extraction procedure of *Musa balbisianacolla*:

The juice was extracted from a pseudo stem by pressing using a sugarcane press machine and this was done within 24 h after harvesting. 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[10].

2. Extraction procedure of *Tribulus terestris*:

The dried and matured fruits of *Tribulu terrestris* were obtained from local areas of Pune. Aqueous extract was prepared by using the dried and matured fruit of *Tribulu terrestris* was ground into fine powder and the extraction was carried out at temperature of 23.5 °C for a period of 19.50 hours under constant stirring. Following this, the extract was filtered, and stored in air tight container[11].

3. Extraction procedure *of Bryophyllum Pinnata*:

Fresh leaves of ***Bryophyllum Pinnata*** 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 by cold maceration. The filtrate was subsequently evaporated to obtain the dry extract using a rotary evaporator [12,13].

4. Extraction procedure of *Commiphora wightii*:

The stem barks of *Commiphora wightii* were collected from local market of Pune. 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[14].

Preparation of Tablet by Wet granulation method:

Weigh all the required ingredients and mix them by doubling up method. Then add little amount of water to the mixture to form a dough. Pass this dough through 44# mesh sieve to form granules. Dry the formed granules and again pass them through 80# mesh sieve. These granules are punched in tablet punching machine to form the tablets [15, 16, 17.]

Investigation of *in vitro* antiurolithiatic activity test by Turbidimetric and Microscopic study.

a) Turbidimetric study:

The Precipitation of calcium oxalate at 37°C and pH 6.5 has been studied by the measurement of turbidity at 620 nm. A spectrophotometer UV/Vis (JASCO V 630) was employed to measure the turbidity of the formation of calcium oxalate. Pure chemicals, including calcium chloride dehydrate ($CaCl_2$, $2H_2O$), sodium oxalate ($Na_2C_2O_4$) and sodium chloride (NaCl), are used for this study.The percentage of inhibition was calculated using the following formula:

$$I\ (\%) = \left[1 - \frac{Ti}{Tc}\right] \times 100$$

Where Ti is turbidimetric slope with inhibitor & Tc is turbidimetric slope without inhibitor[18 19].

b) Microscopic study

The photographs were taken using a microscope optic equipped with a digital camera and connected with a micro-computer. A drop of the mixture of crystallisable solution, or inhibiting solution is placed on the glass slide, which is immediately placed under the objective of the microscope [19, 20, 21].

Results:

The tablet was prepared by using wet granulation method and all the in process quality control tests were performed. According to pre-compression results, prepared granules have good flow property. All tablets passes the weight variation test. There hardness was found to be 6.14 kg/cm^2. Friability of tablets was found to be 0.020%i. e. within the official limit. Also tablet have disintegration time 30 sec. and dissolution time 25 min. Hence, we conclude that tablets pass the all IPQC tests

In-vitro Models:

a) Turbidimetric study:

Table No.1: The maximum values of the variation of absorbance, and the turbidimetric slopes relating to the curves of crystallization without and with inhibitors (Cystone)

CI %	**TS**	**I%**	**∆D**	**R^2**	**CV %**
00	0.017	00	0.00029	0.810	2.6
10	0.017	00	0.00292	0.735	6.88
20	0.015	13.5	0.00222	0.775	7.82
30	0.012	25	0.00193	0.770	6.23
40	0.011	31.25	0.00122	0.807	7.20

50	0.008	57.25	0.00058	0.745	6.29

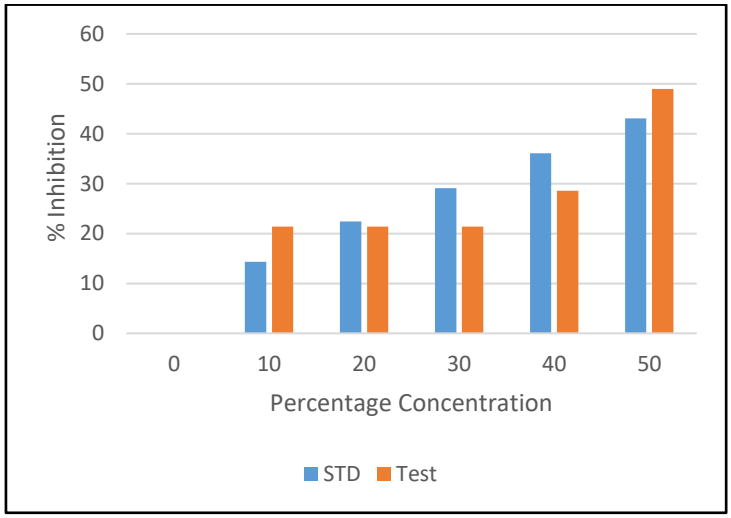
CI concentration of inhibitor, TS turbidimetric slope, R2 linear regression of the data, CV (%) coefficient of variation, ΔD variation of absorbance, I percentage of inhibition

Table No.2: The maximum values of the variation of absorbance, and the turbidimetric slopes relating to the curves of crystallization without and with inhibitors

CI %	**TS**	**I%**	**ΔD**	**R^2**	**CV %**
00	0.016	00	0.00028	0.807	2.5
10	0.014	12.5	0.00195	0.926	7.37
20	0.012	25	0.00167	0.810	7.86
30	0.008	50	0.00087	0.754	6.62
40	0.008	50	0.00081	0.722	4.94
50	0.006	62.5	0.00049	0.668	5.04

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 No.1: Percentage inhibition of crystallization with inhibitor



a) Microscopic study:

Figures 2 and 3 correspond to the tries with and without inhibitor to determine the calcium oxalate crystallization inhibition

Figure No.2: without inhibitor

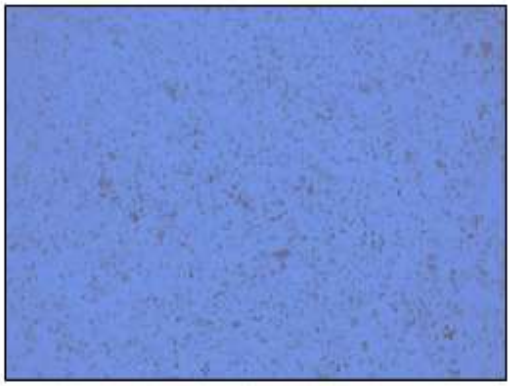
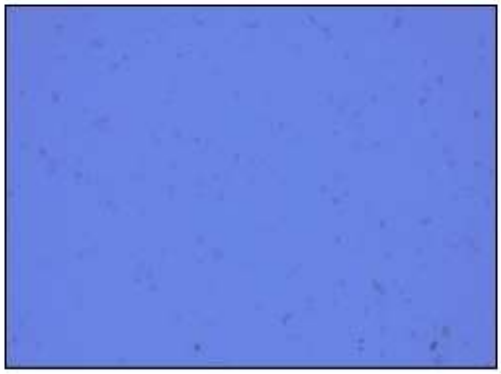


Figure No.3: with inhibitor



Discussion:

To treat kidney stone 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. In case of smaller stones, they may get dissolved or forced to pass out through urinary track by drinking lots of water and for reduction of pain administering painkiller. But in case of larger stones they need the proper therapy or sometimes surgery. None of the surgical treatment produces

Satisfactory result. Hence in this present study for assessment of the urolithiatic activity a new polyherbal formulation was prepared.

In present study for formulation of polyherbal formulation extracts of all selected herbs has done by using specific method of extraction. In case of *Musa balbisianacolla* juice was extracted from a pseudo stem by pressing using a sugarcane press machine. The aqueous extract of *Tribulu terrestris* was prepared by using the dried and matured fruit. The fresh leaves which were air dried, pulverized and extracted by cold maceration. Extraction of *Commiphora wightii* of coarsely powdered plant material was successively extracted by Soxhlet extraction method.

Turbidometric Study:

In the present study 10%, 20%, 30%, 40% & 50% concentration of inhibitor solution was treated with stone forming solution and analyze under UV at 620 nm to determine the inhibition of calcium oxalate crystallization. At 10% concentration the inhibition of calcium oxalate by test drug was found to be 12.5% and by the standard (cystone) was found to be 00. At 20% concentration it was 25 % by test drug and 13.5% by standard drug. Similarly at 30% concentration, it was 50% by test drug & 25% by standard. At 40% and 50% concentration, it was found to be 50% by test drug & 31.25% by standard, 62.5% by the test drug and 57.25% by standard respectively (Table No.1 & 2, and Fig. No.1).

b) Microscopic study:

The inhibition of calcium oxalate crystals were determined by observing the solutions both with and without inhibitor under the motic image microscope. The Fig No.2 shows the presence of calcium oxalate crystals without inhibitor solution and Fig. No.3 shows the inhibited calcium oxalate crystals with inhibitor solution.

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