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IN VITRO ANTILITHIATIC STUDIES ON *DOLICHOS BIFLORUS* LINN. (SEEDS) AND *PARMELIA PERLATA* ACH. (THALLUS)

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ABSTRACT

A study was undertaken to evaluate the *in vitro* antilithiatic activity of Soxhlet extract of *Dolichos biflorus* seeds and cold extract *of Parmelia perlata* thalus. The *in vitro* activity was determined by inhibition of calcium (titrimetric analysis) and Phosphate (colorimetric analysis) precipitation. Cystone (a marketed product) was used as reference drug for comparison. Extracts *of Dolichos biflorus* showed activity almost equivalent to cystone but extracts *of Parmelia perlata* were not as active as cystone. The combined effect was not as active as individual extracts.

Key words: Antilithiasis, *Dolichos biflorus*, *Parmelia perlata*, Cystone

INTRODUCTION

Lithiasis is the condition marked by formation of calculi, which is formed by deposition of various calcium and phosphorus salts and antilithiasis is prevention of the formation of urinary calculi. A kidney stone is a solid piece of material that forms from crystallization of excreted substances in the urine[1]. The stone may remain in the kidney or break loose and travel down the urinary tract. A small stone may pass all of the way out of the body, but a larger stone can be stuck in ureter, the bladder, or the urethra. This may block the flow of urine and cause great pain.

Dolichos biflorus is a common twining creeper, a branched sub-erect or trailing[2] annual with small trifoliate leaves, narrow flat curved pods 1.5-2 inches[3] long tipped with a

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persistent style. The pods contain 5-6 flattened, ellipsoid seeds 1/8-1/4 inch long. D. *biflorus* seeds are being used traditionally in many places of India for prophylaxis as well as treatment of urinary calculi.

Parmelia perlata is distributed throughout India, on the rocks of hills and sometime on tree trunks[4]. The vegetative body of this lichen (thallus) is flat, growing in rosettes or irregularly spreading over the substratum giving the appearance of a flower and hence, named a as stone flower. It has a darker lower side with rhizine (rootlets), which attach lichen to its substrate. The plant is astringent, bitter, acrid, cooling, emollient, anti-inflammatory, diuretic lithontriptic, digestive, carminative, cardiotonic, febrifuge and aphrodisiac. It is useful in inflammation, renal and vesicle calculi, skin disease, leprosy, gastropathy, dyspepsia, vomiting, diarrhoea, dysentery, cough, asthma and seminal weakness.

EXPERIMENTAL

Material and Methods

Plant material

Seeds of *Dolichos biflorus* were collected from Pulse Department of Birsa Agriculture University, Ranchi. *Parmelia perlata* was collected from the stem bark of Sal tree *Shorea robusta* from Birla Institute of Technology, Mesra, Ranchi, India and authenticated by Dr. M. P. Singh, Head, Department of Forest Science, Birsa Agriculture University, Ranchi, India. A voucher specimen (No. PG- MPH/17/04) was preserved in the Department for further references.

Chemicals used

Aqueous 50% sodium hydroxide, chloroform and methanol extracts of seeds of *Dolichos biflorus;* acetone and chloroform extracts of thallus of *Prarmelia perlata*[9]*;* aqueous extract of cystone (Himalaya Health Care Ltd.); buffer pH 7.4; 0.4 M hydrochloric acid, 25 mM $CaCl_2$. $2H_2O$, 25 mM Na_2HPO_4 $2H_2O$ and 25 mM $Na_2C_2O_4$.

Preparation of extracts

Dolichos biflorus: Extracts were prepared by exhaustive extraction of seeds in a Soxhlet apparatus with distilled water, 50% aqueous sodium hydroxide solution, chloroform and methanol, respectively filtered and concentrated in vacuum up to 100 mL.

Parmelia perlata: Extracts were prepared by placing lichen thalli in separate glass

tanks. Enough acetone or chloroform was added to saturate each tank, avoiding excess fluid. The tanks were closed and lichens were allowed to steep in acetone or chloroform for exhaustive extraction. After, the thalli were removed and extracts were concentrated in vacuum up to 100 mL. In both the procedures, 100 g of crude drug material was extracted with solvent and concentrated up to a final volume of 100 mL in order to yield the extracts of strength 1 g/mL with respect to crude drug.

Cystone: Aqueous extract was prepared by grinding a tablet to powder. This powder was mixed with 5 mL water and kept for 2-3 hrs and then centrifuged at 1000 rpm. The clear supernatant was used for the study.[5]

0.1 M TRIS Buffer (pH 7.4) [6]

Solution A was 0.4 M [48.4 g of TRIS (Trihydroxy methyl) amino methane per 1000 mL] and solution B was 0.4 M hydrochloric acid [33.6 mL of concentrated hydrochloric acid per 1000 mL]. A working solution was made up of 25 mL solution and 20.7 mL solution B made up to 100 mL. The pH was 7.4.

Experimental set up[6]

The experiment consisted of the following tubes for control and test, 1 mL each of 25 mM $CaCl_2.2H_2O$, 25 mM $Na_2HPO_4.2H_2O$ or 25 mM $Na_2C_2O_4$.Then two test tubes were taken for each set in triplicate, in each test tube 2 mL of Tris-HCl buffer and 1 mL of 105 mM NaCl was added. The pH was maintained as 7.4. Then 2 mL of extract and vehicle under investigation was added. The tubes were incubated at 37^0C for 4 hrs. The precipitate of calcium phosphate and calcium oxalate were generated as follows --

Calcium oxalate[7]

Mixing 1 mL solution from tube having $CaCl_2.2H_2O$ and $Na_2C_2CO_4$ solution generated calcium oxalate precipitate.

Calcium phosphate[8]

Mixing 1 ml solution of from the tube having $CaCl_2.2H_2O$ and $Na_2HPO_4.2H_2O$ solution generated calcium phosphate.

Calcium was estimated using titerimetry and phosphorus was estimated using colorimetric analysis. Appropriate standard curves were done with each set of experiment. The amount of precipitate of calcium and phosphate were determined in each of the sets by the methods of Clark and Collip and Fiske and Subbarrow, respectively.

Data analysis

The data are presented as the mean ± SEM of three different sets of experiments. The statistical analysis was performed using the student's t- test with $p < 0.05$ being considered significant. Comparison was made between control and test of each group and between *Dolichos biflorus* extract, *Parmelia perlata* extract and cystone groups.

RESULTS AND DISCUSSION

Results of present study clearly indicate the potential of aqueous extract and sodium hydroxide extract of seeds of *Dolichos biflorus* as therapy for lithiasis. The acetone extract of *Parmelia perlata* was more active than the chloroform extract of the same plant. The combination of the two plants was not as active as the individual extract.

Aqueous extract and sodium hydroxide extract of seeds of *Dolichos biflorus* and acetone extract of *Parmelia perlata* showed comparable activity to the marketed formulation in preventing the formation of calcium precipitate and aqueous extract of *Dolichos biflorus* shows comparable activity to the marketed formulation in terms of inhibiting the formation of phosphate precipitate.

On deproteinization of the aqueous extract of the *Dolichos biflorus* with saturated ammonium sulphate the inhibitory activity increased slightly. The detannated aqueous extract of *Dolichos biflorus* showed in terms of inhibiting the formation of calcium and phosphate precipitate with respect to whole extract *of Dolichos biflorus.*

Cystone, which is a prescribed treatment for urinary renal calculi showed a good inhibitory effect on the formation of the precipitate of calcium and phosphate.

The D. *biflorus* seed extract in distilled water and aqueous sodium hydroxide strongly inhibited the precipitation of calcium and phosphate while the same in methanol and chloroform caused very little inhibition suggesting that methanol and chloroform do not extract inhibitory activity from the intact seeds. The result of this study clearly showed the utility *of Dolichos biflorus* in the treatment of renal and urinary calculi. It can be inferred that Dolichos *biflorus* is more active than *Parmelia perlata.* It is evident from present investigation that D. *biflorus* seeds contain water soluble substances, which under physiological condition of the reaction system, inhibited calcium and phosphate ions precipitation.There results conclude that these inhibitors of crystallization were heat stable, polar, non, tannin and likely to be non-protein in nature.

Table 1. Percent inhibition of phosphate using different extracts of *Dolichos biflorus*

Parameter	Aqueous sodium hydroxide extract (0.2 g / mL)		Aqueous extract (0.5 g/mL)		Methanolic extract (0.7 g /mL)		Chloroform extract (0.8 g/mL)	
	Control	Test	Control	Test	Control	Test	Control	Test
% Inhibition	-	38.09	-	41.49	-	13.96	-	9.84

$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, Statistically analyzed by student's t test

Table 2. Percent inhibition of calcium using different extracts of *Parmelia perlata*

Parameter	Acetone extract (0.3 g/mL)		Chloroform extract (0.7 g/mL)	
	Control	Test	Control	Test
% Inhibition	-	36.88	-	10.57

$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, statistically analyzed by student's t test

Table 3. Percent inhibition of calcium using combined extracts of *Dolichos biflorus and Parmelia perlata*

Parameter	Aqueous extract of DB (0.5 g / mL) and acetone extract of PP (0.3 g/mL).		Aqueous sodium hydroxide extract of DB (0.2 g/mL) and acetone extract of PP (0.3 g/mL)		Chloroform extract of DB (0.8 g / mL) and chloroform extract of PP (0.7 g/mL)		Aqueous extract of cystone (Standard) 2 mL of 1 tablet /5 mL	
	Control	Test	Control	Test	Control	Test	Control	Test
% Inhibition	-	24.28	-	22.88	-	2.18	-	36.36

$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, Statistically analyzed by student's t- test

Table 4. Percent inhibition of calcium using different extracts of *Dolichos biflorus*

Parameter	Aqueous sodium hydroxide extract (0.2 g / mL)		Aqueous extract (0.4 g/mL)		Methanolic extract (0.7 g /mL)		Chloroform extract (0.8 g/mL)	
	Control	Test	Control	Test	Control	Test	Control	Test
% Inhibition	-	45.58	-	67.16	-	41.11	-	15.08

[a]p < 0.05, [b]p < 0.01, [c]p < 0.01, *p > 0.05, Statistically analyzed by student's t test

Calculation of all test absorbance is against 0.856 absorbance of standard at 680 nm.

Table 5. Percent inhibition of calcium using different extracts of *Parmelia perlata*

Parameter	Acetone extract (0.3 g/mL)		Chloroform extract (0.8 g/mL)	
	Control	Test	Control	Test
% Inhibition	-	41.70	-	8.52

[a]p < 0.05, [b]p < 0.001, [c]p < 0.001, *p > 0.05, statistically analyzed by student's t test

Table 6. Percent inhibition of calcium using combined extracts of *Dolichos biflorus* and *Parmelia perlata*

Parameter	Aqueous extract of DB (0.4 g / mL) and acetone extract of PP (0.3 g/mL).		Aqueous sodium hydroxide extract of DB (0.4 g/mL) and acetone extract of PP (0.3 g/mL)		Chloroform extract of DB (0.8 g / mL) and chloroform extract of PP (0.7 g/mL)		Aqueous extract of cystone (Standard) 2 mL of 1 tablet /5 mL.	
	Control	Test	Control	Test	Control	Test	Control	Test
% Inhibition	-	32.19	-	24.58	-	5.02	-	68.21

[a]p < 0.05, [b]p < 0.01, [c]p < 0.001, Statistically analyzed by student's t- test

Table 7. Percent inhibition of calcium using detannated and deproteinized extract of *D. biflorus*

Parameter	Detannated extract		Deproteinized extract	
	Control	Test	Control	Test
% Inhibition	-	45.057	-	46.867

Table 8. Percent inhibition of phosphate using detannated and deproteinized extract of D. *biflorus*

Parameter	Detannated extract		Deproteinized extract	
	Control	Test	Control	Test
% Inhibition	-	69.34	-	68.75

$^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, Statistically analyzed by student's t- test

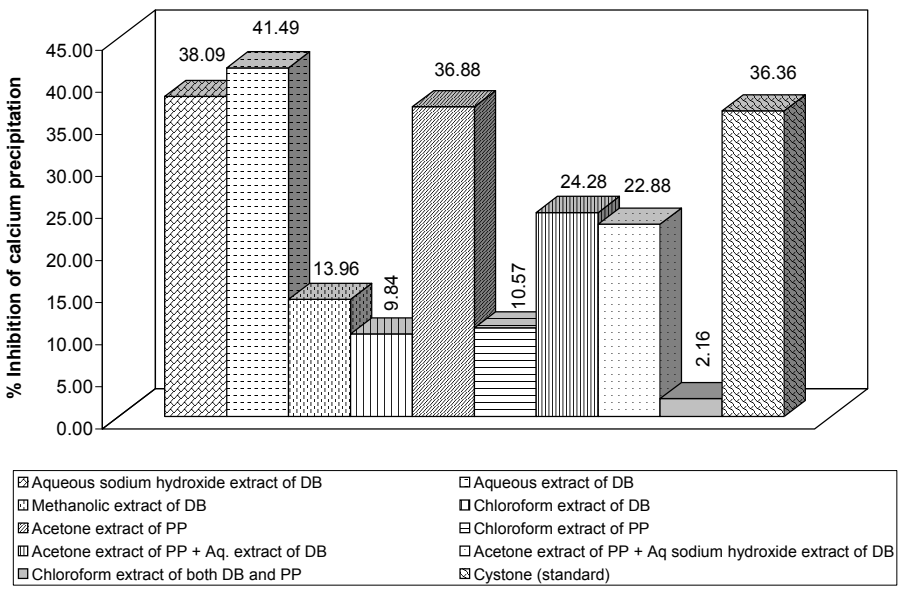

Fig. 1: Percent inhibition of calcium using different extracts

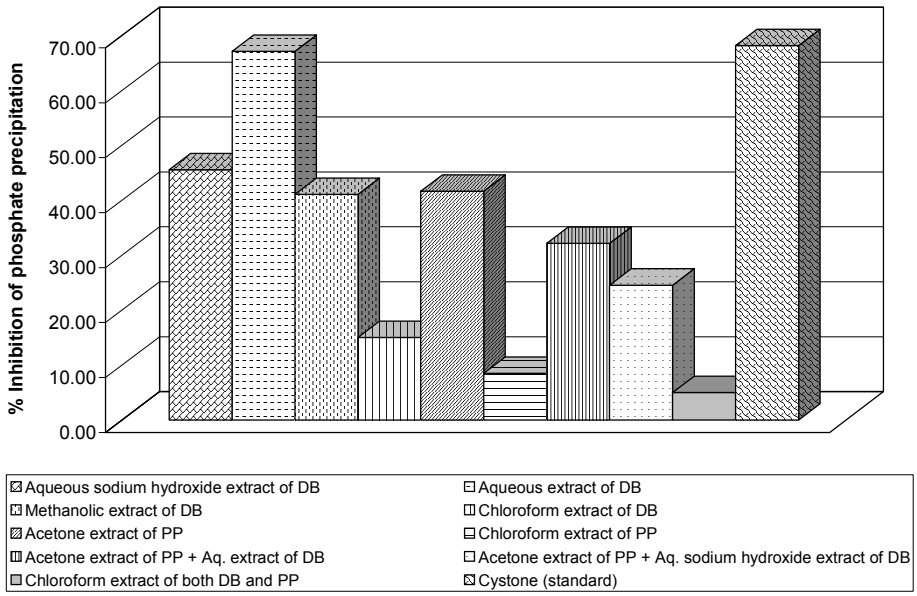

Fig. 2: Percent inhibition of phosphate using different extracts

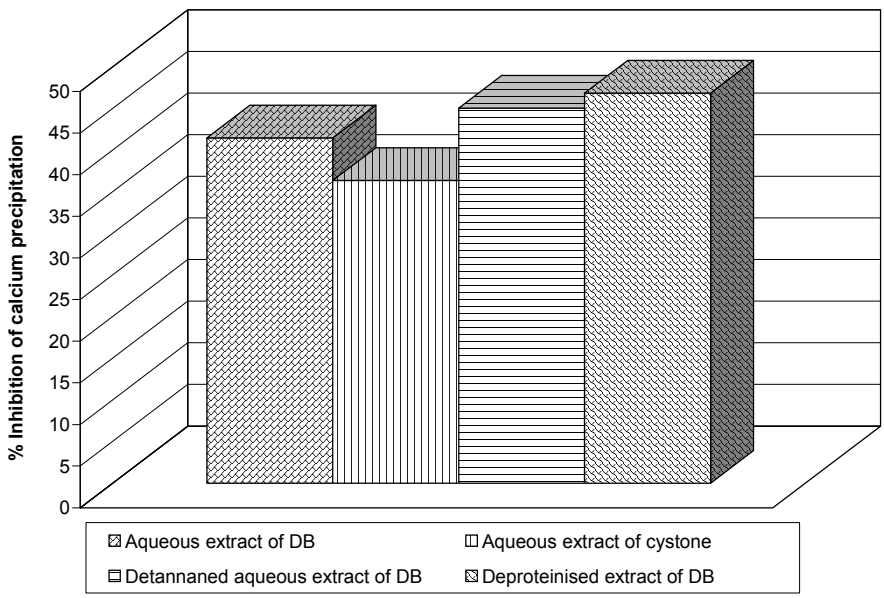

Fig. 3: Percentage inhibition of calcium by different extract of *D. biflorus*

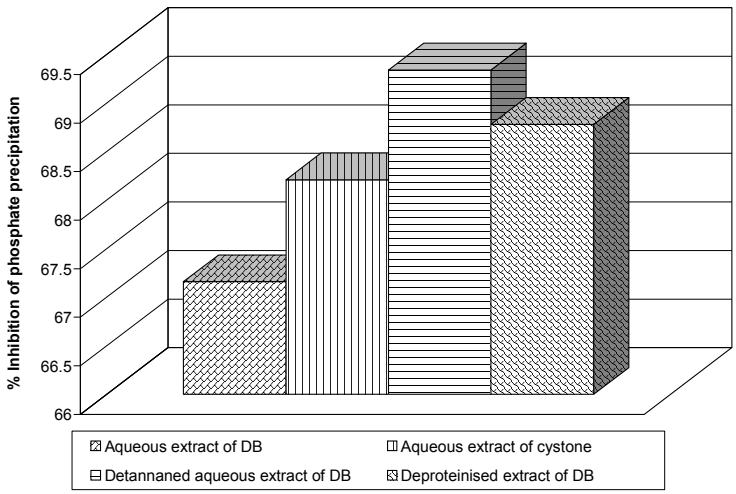

Fig. 4: Percentage inhibition of phosphate by different extract of D. biflorus

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