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In vitro evaluation on antilithiatic activity on *Hyptis suaveolens* (L.) leaves

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SUMMARY

A study was undertaken to evaluate the in vitro antilithiatic activity of soxhlet extract of leaves of *Hyptis suaveolens* (L.) 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. Ethanolic Extract of *Hyptis suaveolens* (L.) showed activity almost equivalent to cystone.

Key words : Antilithiatic, *Hyptis suaveolens* (L.), Cystone

India is one of the richest floristic regions of the world and has been a source of plants and their product. The plant *Hyptis suaveolens* (L.) family – Lamiaceae commonly known as Wilayati tulsi is used to treat various diseases. The leaves have been reported to posses medicinal properties and are used in inflammatory condition (Harbone, 1988), wound healing (Shirwaikar *et al.,* 2003), antimalarial agent (Zieglar *et al.*, 2002), Antioxidant (Shirwaikar *et al.*,2003), protease inhibitors (Aguirre *et al.*, 2004), antiplasmodial agent (Chukwujekwu *et al.*, 2005), anticancer (Mabberly, 1990) and antifertility (in female) agent (Oliver, 1986) for the treatment of colics, stomach ache and fever. The essential oil of leaves shows antimicrobial activity (Asekun *et al.*, 1999). However, antilithiatic activity have not been reported for the leaves. Therefore efforts were devoted in this direction.

Lithiasis is the condition marked by formation of calculi, which is formed by deposition of various calcium, phosphorus salts and antilitiasis is prevention of the formation of urinary calculi. A kidney stone is a solid piece of material that forms from (Khan, 1991) crystallization of excreted substances in the urine. 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 may cause great pain.

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MATERIALS AND METHODS

Plant material:

The leaves of *Hyptis suaveolens* (L.) were collected from Bundelkhand region, Jhansi (U.P.) and authenticated by Dr. H.B.Singh, Head Raw material and museum, NISCARE, New Delhi.

Chemical used :

Aqueus, Ethanol, Petrolium ether and methanol extracts of leaves of *Hyptis suaveolens* (L.) aqueous extract of cystone (Himalaya Health care Ltd.). TRIS buffer pH 7.4, 0.4 M Hydrochloric acid, 25 mm $Cacl_{2..}H_2O$, 25 mm $Na_2HO_{4.}H_2O$, 25 mm $Na_2C_2O_{4.}$

Preparation of extracts :

Hyptis suaveolens (L.) :

Extracts were prepared by exhaustive extraction of leaves in a soxhlet apparatus with distilled water, Ethanol, Pet ether and methanol, respectively and filtered, concentrated in vaccum up to 100 ml.

Cystone :

Aqueus 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. (Jethi *et al.*, 1984).

0.1 M TRIS Buffer (pG 7.4):

Solution A was 0.4 M TRIS [45.4 g of TRIS (Trihydroxy methyl) amino methane per 1000 ml] : 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 A, 20.7 ml solution

B made up to 100 ml. The pH was 7.4 (Jethi *et al.*, 1984).

Calcium exalate:

Calcium oxalate precipitate was generated by mixing 1 ml of solution from tube having $CaCl_2$. $2H_2O$ and $Na_2HPO_4.2H_2O$ solution (Clark and Collip, 1925).

Calcium was estimated using titrimetry and phosphorus was estimated using colorimetric analysis. Appropriate standard curve were done with set of experiment. The amount pf precipitate of calcium and phosphate were determined in each of the sets by the methods of Clark and Collip (1925) and Fiske and Subbarrow (1925), respectively.

DATA analysis:

The data are presented as the mean ± S.E.M. of three different set of experiments. The statistical analysis was performed using the students t-test with $P<0.05$ being considered significant. Comparison was made between control and test of each group and between *Hyptis suaveolens* (L.) extrac and cystone groups.

RESULTS AND DISCUSSION

Results of present study clearly indicate the Cystone which is a prescribed treatment for urinary for renal calculi which showed a good inhibitory effect on the formation of the precipitate of calcium and phosphate (Table 1 and 2). Ethanol extract of *Hyptis suaveolens* (L.) was more active than the methanol, pet ether and distilled water extract of the same plant. Ethanolic extract of *Hyptis suaveolens* (L.) showed comparable activity to the marketed formulation in preventing the formation of calcium precipitate (Table 3 and 5 and Fig. 3).

Hyptis suaveolens (L.) leaves extract in Ethanol inhibited the precipitation of calcium and phosphate while the same in methanol, pet. Ether and aqueous caused very little inhibition. The result of the present study clearly showed the utility of *Hyptis suaveolens* (L.) in the treatment of renal and urinary calculi.

Table 1 : Per cent inhibition of calcium using different extracts of *Hyptis suaveolens* (L.)

Parameters	Ethanol extract (0.4 g/ml)		Methanol extract (0.6 g/ml)		Aqueous extract (0.7 g/ml)		Pet. Ether extract (0.9 g/ml)	
	Control	Test	Control	Test	Control	Test	Control	Test
% Inhibition	-	38.88	-	12.57	-	10.84	-	9.21

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

Table 2 : Per cent inhibition of phosphate using different extracts of *Hyptis suaveolens* (L.)

Parameters	Ethanol extract (0.3 g/ml)		Methanol extract (0.5 g/ml)		Aqueous extract (0.7 g/ml)		Pet. Ether extract (0.9 g/ml)	
	Control	Test	Control	Test	Control	Test	Control	Test
% Inhibition	-	41.70	-	11.52	-	10.23	-	9.81

[a]P<0.05, [b]P<0.01, [c]P<0.001, *P>0.05, Statistically analyzed by student's t test

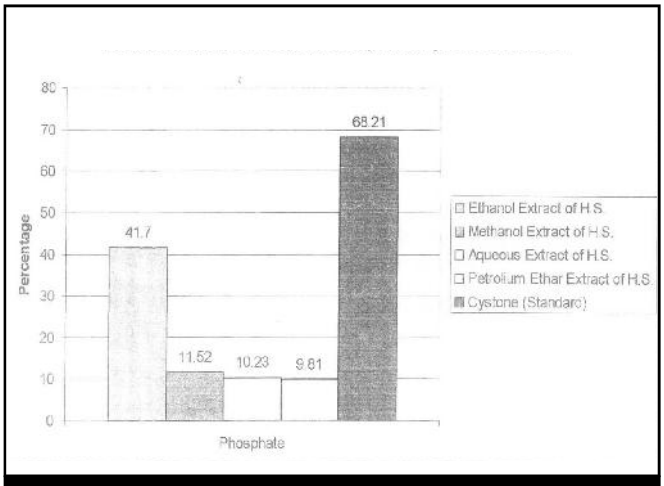

Fig. 1 : Per cent inhibition of Calcium precipitation by different extracts

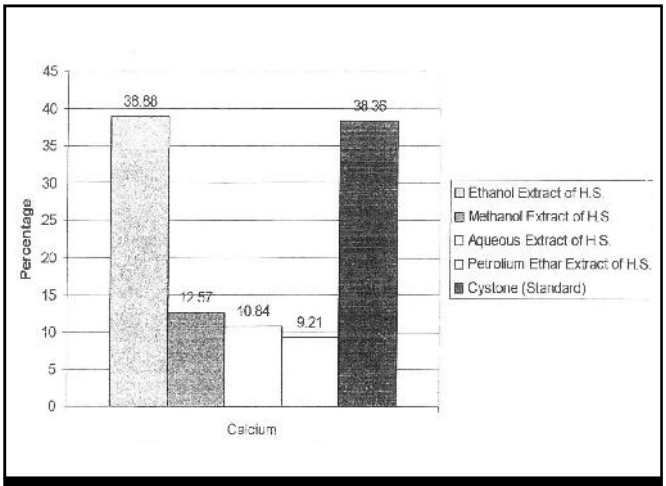

Fig. 2 : Per cent inhibition of phosphate precipitation by different extracts

Table 3 : Per cent inhibition of calcium using aqueous extracts of Cystone

Parameters	Aqueous extract of Cystone (Standard) 2ml of 1 tablet/5ml.	
	Control	Test
% inhibition	-	38.36

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

Table 4 : Per cent inhibition of phosphate using aqueous extracts of Cystone

Parameters	Aqueous extract of Cystone (Standard) 2ml of 1 tablet/5ml.	
	Control	Test
% inhibition	-	38.36

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

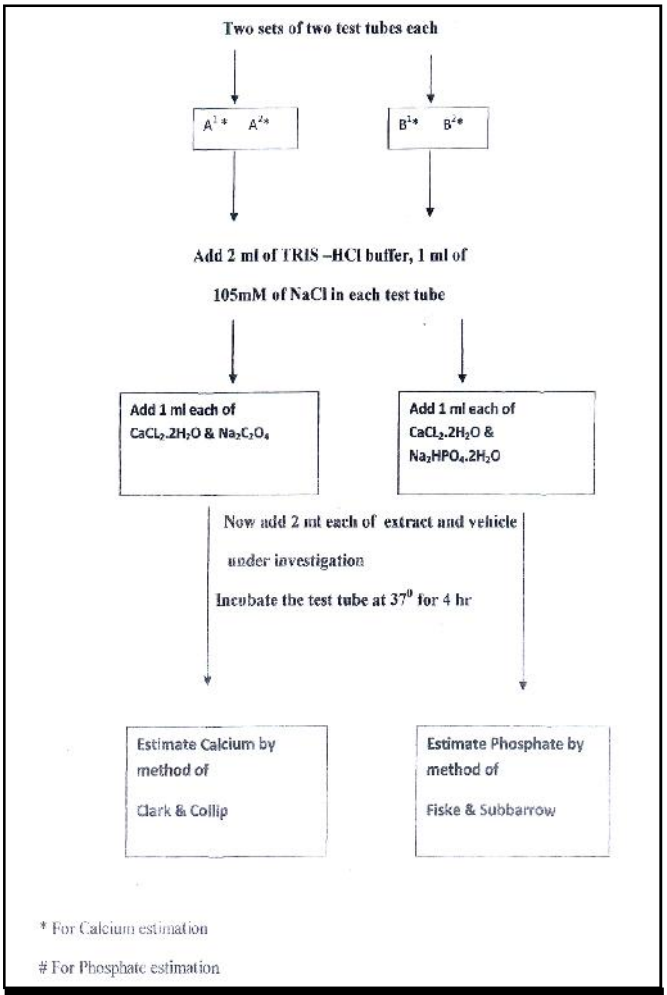

Fig. 3 : Schematic figure of estimation of calcium and phosphate

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