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Research Article

INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION IN-VITRO BY VARIOUS EXTRACTS OF HYPTIS SUAVEOLENS (L.) POIT.

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ABSTRACT
Hyptis suaveolens (L) Poit. commonly known as Vilayati tulsi, belongs to the Mint family Lamiaceae. The inhibition of in-vitro calcium-oxalate crystal (a major component of most urinary stones) formation by various extracts of Hyptis was investigated by titrimetric method. The inhibitor potency of alcohol extracts of *Hyptis suaveolens* (L.) Poit was found to be comparable to that of cystone (a proprietary drug for dissolving kidney stones). Thus alcohol extract could be further analyzed in vivo and further characterization of its active compound could lead to the discovery of a new candidate drug for the patients with urolithiasis.

KEYWORDS: Vilayati tulsi, Cystone, Lamiaceae, Urolithiasis.

INTRODUCTION

Lithiasis is the formation of calculi or stone which is a concretion of material mainly mineral salts in any part of the body. Antilithics are agents that prevent the formation or promote the dissolution of formed calculi.

Lithiasis mostly occurs in any part of the whole urinary tract which includes the ureter, urinary bladder and kidney as well as it also occurs in the gallbladder. Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. Reference to stone formation is made in the early Sanskrit documents in India between 3000 and 2000 BC.[1] In the writings of Charaka, Sushruta and Vagbhatta who lived in 2nd, 5th and 7th century AD, description of the disease and indications for treatment has been found. They recognized four types of stones. As early as 23-79 AD Pliny prescribed infusions of *Paeonia officinalis*, Mentha spp and *Cicer arietinum* to dissolve stones in bladder and kidney. Hindu writings also contain many prescriptions for dissolving the stone. Pashanbheda is a drug mentioned in the Ayurvedic system of medicine which is used as a diuretic and lithotriptic. Many plants having the property of breaking and disintegrating stones have been referred to as pashanbheda. Several proprietary composite herbal drugs have been introduced for dissolving kidney stones like Cystone (Himalaya Drug Co.Bombay) and Calcury (Charak Pharmaceuticals, Bombay).[2] The incidence of Kidney stones has increased in western societies in the last five decades, in association with economic development.[3] The recent treatment procedures for treating stones like surgical removal, extracorporeal shock wave lithotripsy are very costly for common man as well as recurrence of stone and a number of side effects are quite common with these procedures.[1] Hence search for new antilithiatic drugs from natural sources has assumed greater importance as herbal alternatives are cost effective along with this they confer least side effects.

Hyptis suaveolens (L) Poit. commonly known as Vilayati tulsi belongs to the family Lamiaceae or the Mint family. It is a shrubby, scented plant with tetragonal hispid stems. Leaves opposite, petiolate, ovate, cordate, denticulate or serrate. Flowers small, blue or purple in colour, the fruiting calyx campanulate and ribbed with five aristate teeth. Corolla two lipped. Anthers purple in colour, filaments with whitish hairs. Fruit of four dry ovoid nutlets with small basal scar. (Fig.I-II)[4]

The plant has been considered as a weed, distributed throughout the tropics and subtropics. Common in open uncultivated areas, rocky dry substratum, on roadsides, waste grounds. Flowering and fruiting occurs during October till January.[4]

Almost all parts of this plant are being used in traditional medicine as well as it has immense ethnomedicinal importance for treating various diseases. And the lot of research work done on this plant has also revealed various other activities in this plant. (Table.I)

Oxalate, a metabolic end product and a major constituent of the majority of renal stones, has been shown to be toxic to renal epithelial cells of cortical origin. It leads to the disruption of normal activities of renal epithelial cells. Calcium containing stones are the most commonly occurring ones to an extent 75-90%, followed by magnesium ammonium phosphate (struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%. Calcium oxalate (CaOx) stones are found in two different varieties[33]. Most calculi in the urinary system arise from a common component of urine eg calcium oxalate (CaOx), representing up to 80% of analyzed stone.[34]

Hyptis suaveolens (L) Poit. has been found to have useful effect in case of urinary tract infection[35] as well as it is diuretic and used in kidney disorders[36]. Shashi A. *et.al* has earlier evaluated antilithiatic effect of its leaves[37]. Thus the present study has been undertaken to re-investigate the inhibition efficiency of various extracts of *Hyptis suaveolens* (L) Poit. growing in Bhopal district on the mineralization of calcium oxalate, *in-vitro*.

MATERIALS AND METHOD

Plant Collection and Identification

The aerial part of *Hyptis suaveolens* (L) Poit. was collected from road side area of Kolar road, Bhopal, Madhya Pradesh, India, during the month of October 2011 and plant was identified with the help of regional Floras (4) and taxonomists and finally confirmed with the herbarium of Botanical Survey of India (BSI), Allahabad, voucher specimen No.1234.128.605 BSI/CRC/Allahabad.

Extraction

Fresh plant, after collection was shade dried, and then the powered leaves was extracted with alcohol by Soxhlet apparatus while aqueous extract was obtained by percolation for 24 hours.

Experimental Work

Preparation of reagents and solution

All the chemicals used were of AR grade. Crystalloid forming solutions, *viz.*, solution of calcium acetate and sodium oxalate (for calcium oxalate) and the inhibitor solution i.e solution of various extracts of leaves of *Hyptis suaveolens* (L) Poit. were prepared in distilled water.

Aqueous extract of cystone (a marketed herbal formulation for urolithiasis) was prepared by grinding a tablet to powder. This powder was mixed with 50mL water and kept for 2–3 h and then centrifuged at 1000 rpm in a centrifuging machine (Remi equipments, Bombay). The clear supernatant was used for the study. The extracts of the plants were compared with the aqueous extract of cystone for their antilithiatic activity.[38]

Antilithic activity in different extracts of leaves of *Hyptis suaveolens* (L.) Poit. was investigated as per the method of N. A. M. Farook et al. with minor modifications.[39]

The whole amount of inhibitor solution (50 mL) was placed in the beaker in the beginning itself and the two salt forming solutions were allowed to run into it drop wise through burettes. Thus, a reservoir of inhibitor was created into which the salt forming solutions ran down. At the end the mixture was boiled on a heating mantle (Elite scientific instruments co.) for 10 min., cooled to room temperature and the precipitate was collected into a pre-weighed centrifuge tube by centrifuging (Remi equipments, Bombay) small volumes at a time and rejecting the supernatant liquid. Next, the tube with the precipitate was dried in a hot air oven (Ambassador), cooled to room temperature and weighed till constant weight using a weighing balance (Sansui electronics). Weight of the precipitate was determined. Simultaneous blank experiments with water in place of inhibitor were also carried out for evaluating the inhibition efficiency of inhibitors compared to water. All the experiments were conducted at room temperature. Data were expressed as mean values of three independent experiments as Mean ±STDEV.

Percentage efficiency of inhibitor was calculated using the following formula.

$$\text{Percentage Inhibition} = \frac{\text{Weight of precipitate in blank set- weight of precipitate in experimental set}}{\text{Weight of precipitate in blank set}} \times 100$$

RESULT

Large number of plants and plant products are used as ethnomedicine to treat urolithiasis as a prophylactic or curative agent. Methanolic extract of leaves of *Hyptis suaveolens* (L.) Poit. showed higher calcium oxalate crystallization inhibition (73.88±1.2 %) in-vitro as compared to ethanol (60.27 ±2.09 % inhibition) and water extracts (53.88 ± 4.19 % inihition). While cystone a prescribed medicine for renal calculi showed highest inhibition (90.55±1.27 %) in terms of the formation of calcium oxalate precipitation. (Figure:III)

DISSCUSSION

The result of present study clearly indicates that methanol extract of leaves of *Hyptis suaveolens* (L) Poit. showed comparable activity to that of cystone in terms of inhibiting the formation of calcium oxalate precipitate. The reduction of stone forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, thus methanolic extract of leaves of *Hyptis suaveolens* could be further analyzed in vivo and further characterization of its active compound could lead to a new candidate drug for the patients with urolithiasis.

Thus this study provides a basis for utility of *Hyptis suaveolens* (L) Poit. in the treatment of renal and urinary calculi and it is in accordance with earlier study by Shashi et.al and put forth the possibility of use of *Hyptis* leaves as the therapeutic agent to treat urolithiasis. Literature search has shown that no such work on antilithic activity of *Hyptis suaveolens* (L) Poit. has been done in Bhopal district. Thus to the best of our knowledge this is the first report on potent antilithic efficiency of *Hyptis suaveolens* (L) Poit. found growing in Bhopal district.

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TABLE I. VARIOUS TRADITIONAL, ETHNOMEDICINAL AND OTHER REPORTED USES OF *HYPTIS SUAVEOLENS* (L.) POIT.

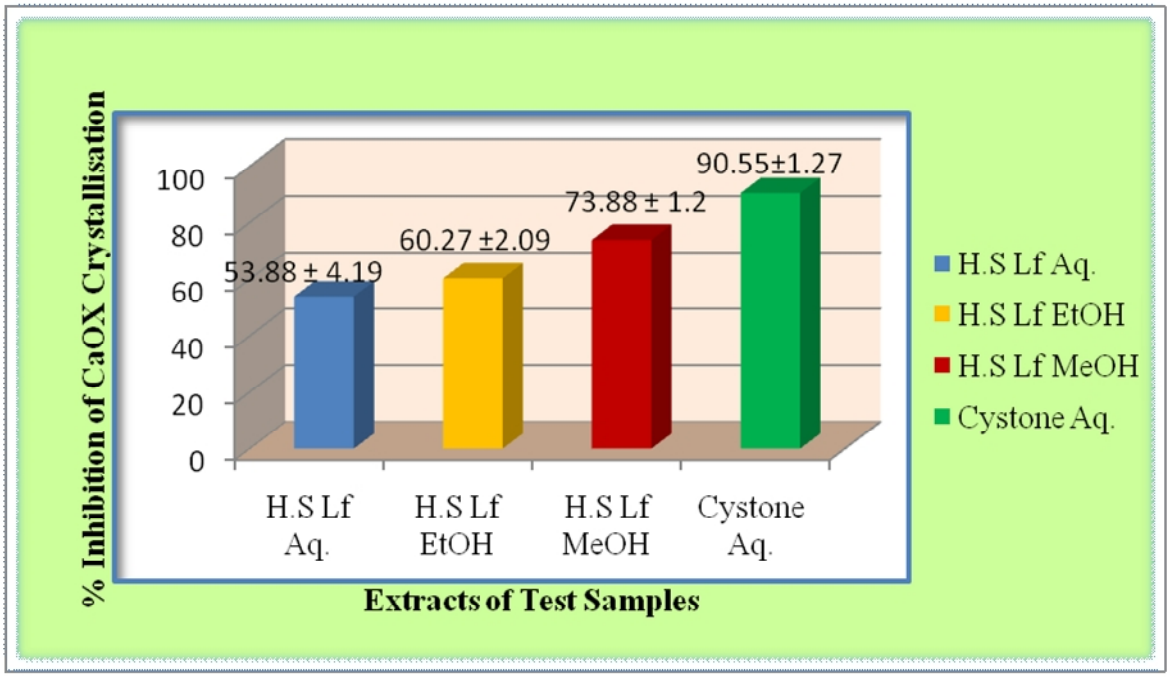
S.no	Plant name	Type of use	Plant part	Reported activity
1.	*Hyptis suaveolens* (L.) Poit.	Traditional uses	Leaves	stimulant, carminative, sudorific, galactogogue, cure parasitic cutaneous diseases.[5]
			Essential oil of leaves	antifungal, antibacterial, anticonvulsant activity.[5]
			Plant	respiratory track infections, colds, pain, fever, cramps.[5]
2.		Ethnomedicinal uses	Plant	antispasmodic, anti-rheumatic[30/29], used in case of snake bite fever, constipation [34/30] scorpion bite and skin infection
			Seeds	dysuria and urinary complaints[36/31] , nervous and visceral disorders, stomach ache [37/32] and in rheumatism
3.		Other uses	Plant	anthelmintic[5],anti-inflammatory[6], antimalarial[7,8,9,10],antimicrobial[11,12], antiplasmodic[13],antitumor ,antioxidant[15/14,16/15,17/16,18/17] anticancer[19/18,20/19] antiulcer[21/20]gastrointestinal disorders [22/21] hepatoprotective[23/22] hypoglycemic[24/23] pesticidal[25/24,26/25]wound-healing[27/26] analgesic[28/27] immunomodulatory [29/28].



Figure I. A twig of *hyptis suaveolens* (l.) poit.



Figure II. A flowering twig of *hyptis suaveolens* (l.) poit.



Values are expressed as Mean ± STDEV of three observations.
Abbreviations used: H.S- Hyptis suaveolens, Lf- Leaf, Aq- Aqueous, EtOH- Ethanolic, MeOH-Methanolic

Figure III: % inhibition of calcium oxalate crystallization in-vitro by various extracts of leaves of *hyptis suaveolens* (l.) poit. and cystone aqueous extract.

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