Research Article

Inhibitory effect of alcoholic extract of Tulsi (*Ocimum sanctum*) on calcium oxalate crystals: An *in-vitro* study

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Abstract

Objective: *Ociumum tenuiflorum* also known to the world as *O. sanctum*, Holy basil or Tulsi, is an aromatic plant show variety of pharmacological and therapeutical activities. In present study, ethanolic extract of *Ocimum sanctum* leaves was evaluated for inhibition of calcium oxalate crystals by *in vitro* methods. **Materials and methods:** The inhibitory activity of ethonolic extract of *Ocimum sanctum* leaves was seen to be similar to that of Cystone (a proprietary drug for kidney stone treatment). Fresh plant was collected and it was shade dried and then the leaves were powdered and extracted with alcohol with the help of soxhlet apparatus and the dried leaves were macerated with water for seven days for aqueous extract. The formation of calcium oxalate crystal (frequently found component of most urinary stones) can blocked *in-vitro* by ethonolic extract of *O. sanctum* and identified by titrimetric method. **Results:** Ethanolic extract of leaves of *Ocimum sanctum* shows effective calcium oxalate crystallization inhibition 77.18±1.15% *in vitro,* where as cystone, a drug with more potential for renal calculi showed calcium oxalate inhibition (92.34±0.96%) in terms of transformation of calcium oxalate precipitation. **Conclusion:** The result of current study shows that the ethonolic extract of *Ocimum sanctum* leaves showed similar activity to that of cystone in process of restricting the formation of calcium oxalate precipitate. The Alcoholic extract of *Ocimum sanctum* leaves could be further determined *in vivo* and then characterization of its active constituent could lead to a novel drug for patients with Urolithiasis.

Keywords: *Ocimum sanctum.* Ethanolic extract, cystone, urolithiasis, calcium oxalate crystal

Introduction

Natural products are those substances which are obtained from nature from natural source and which have very good biological and physiological properties that can be utilized in drug discovery and drug design. Herbal products play a very important role in the treatment of life-threatening conditions. These products can be obtained from extraction of any parts of plants, marine organism or from micro-organism fermentation. Substances which are obtained from plant source are getting very much attention from the scientist and researchers as they have number of applications. All these plants have medicinal properties which are the rich source of drugs used in nutraceuticals, medicine, modern medicines, folk medicines, food neutrinets, pharamaceutical exipients and chemical entities for synthetic drugs (Ncube et al., 2008). *Ociumum tenuiflorum* plant is also known as *Ocimum sanctum*, Holy basil or Tulsi is an aromatic plant with number of properties. It is native throughout the Eastern world and tropics and is widely cultivated throughout the world. The variety of *Ocimum tenuiflorum* used in Thai dishes is known as Thai Holy Basil (Staples and Kristiansen, 1999). Ocimum is a genus of which have around 35 species of aromatic annual and perennial shrubs and herbs. Some species includes *Ocimum basilicum* or Thai basil; *O. gratissimum* or African Basil, *O. campechianum* or Amazonian basil; *O. tenuiflorum* or *O. sanctum* or Tulsi, *O. sanctum* grow up to 60 cm high with purple or red sub quadrangular branches. The leaves are simple and hairy. Flowers are purple in color. Fruits are smooth in touch and not mucilaginous when get wet. It is produced by means of

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seeds. Seeds are planted directly in the ground. Young plants are transplanted to the field when they attain 8-10 cm height (Vijayalakshmi et al., 1997). Tulsi shows active action to reduce disease of the head and neck, swelling, pain, headache and skin disorders. Tulsi leaves have very potent for lung intestinal and cardiovascular diseases. Tulsi leaves are also very effective in blood sugar, blood cholesterol and reducing stress.

Urolithiasis is a common disorder of the urinary tract, in this the formation of sediment in the urinary tract which contain one or more of the very poorly soluble crystalloids of urine. This disease is very vastly spreading in many country namely United States, South Africa, South Asia and in India. Approximately 2% of the total world population is suffering from renal stones disease with a male and female ratio of 2:1. This renal stones disease mostly occurs in the age after 30-40years. In renal stones the patient feels the immense pain (renal colic) as manifest by hematuria. These stones mostly effects the urinary tract and cause nephrolithiasis and inadequate microbial infections, urinary drainage, diet with excess oxalate and calcium, vitamins abnormalities i.e. excess of vitamin D, deficiency of vitamin A, metabolic diseases like hyperparathyroidism, intestinal dysfunction cystinuria, gout, (Tiseliu, 1998) and environmental factors like dry and hot climate condition (Ramesh et al., 2010). Urolithiasis is the condition of body in which accumulation of material mainly salt in any part of body in a form of stone. Oxalate is an end product of metabolic process and a major component of the mostly of renal stones. In this present study estimate the antiurolithiasis activity of ethanolic extract of *Ocimum sanctum* leaves.

Materials and methods

The plant material leaves of *Ocimum sanctum* were collected from Botanical garden of Guru Ramdas Khalsa Institute of Science and Technology, Pharmacy, Jabalpur MP, India during the month of March-April and plant was identified & confirmed with the herbarium of BSI. Eggs were obtained from local market. Alcohol, $KMnO_4$, Hydrochloric acid (HCl), calcium chloride dehydrates, sodium oxalate and Sulphuric acid (H_2SO_4) was purchased from Himedia, Mumbai, India. All other chemicals used were of analytical reagent grade were used as received.

Extraction

Fresh leaves of *O. sanctum* was collected and dried in shade and then the powdered the leaves and the sample of *O. sanctum* leaves was weighed on electronic balance. The powdered leaves were extracted with sufficient amount of alcohol by soxhlet apparatus (20 g of dried pulverized leaves were soaked with 200 ml of solvent for 72 h) and the solvent was decanted and the residue again soaked with the same solvent for 24 h. The final extract was collect and filtered and the solvent was evaporated by placing the extract on vacuum evaporator.

Preparation of experimental calcium oxalate crystal (kidney stones)

Equimolar solution of calcium chloride dehydrates and sodium oxalate was dissolved separately in 10 ml of distilled water, after formation of separate solution, the calcium chloride solution added drop wise into sodium oxalate solution with continuous stirring and formation of precipitate was obtained. The resulted precipitate was calcium oxalate. The precipitate was filter through filter paper and dried (Basavaraj et al., 2007; Patel et al., 2010; Bensatal and Ouahrani, 2008).

Preparation of semi permeable membrane from farm eggs

The semi permeable membrane obtained from eggs, lies between outer calcified shell and the inner content like albumin and yolk. The shell of eggs were removed and decalcify chemically by placing the egg in 2 M hydrochloric acid solution for an overnight, which caused decalcification of the outer calcified layer of egg. Further washed the egg with distilled water and carefully sharp pointer a hole is made on the top and the contents (yolk and albumin) squeezed out completely from the decalcified egg and washed thoroughly with the use of distilled water and placed it on PBS solution (pH 7.4), to provide moistened condition and then washed it with distilled water, store in refrigerator at PH of 7-7.4 (Bensatal and Ouahrani, 2008; Chaudhary et al., 2010; Chaudhary et al., 2009).

Estimation of calcium oxalate by titrimetric analysis method

For estimation of inhibition the amount of calcium oxalate crystal, the experiment was performed on three separate groups in which the first group contains 10 mg of the calcium oxalate and 100 mg of the *Ocimum sanctum* leaves extract and packed it together in semi permeable membrane by suturing after that it was placed on a conical flask containing 100ml of 0.1M of TRIS buffer solution. Second group served as positive control containing 10mg prescribed medicine (marketed formulation) and third group served as negative control (which contains 10 mg of calcium oxalate stone inside the membrane). All of the groups on the conical flask are placed on the incubator preheated upto 37°±0.5°C for 3 hours. For about 4-5 hours removed the content of semi permeable membrane of egg from all the three groups and placed it into a test tube. Added 5 ml of 1N H_2SO_4 and titrated with 0.9494N $KMnO_4$ solution till a light pink colour end point is obtained. The titration analysis was performed three times (n±3). One ml of 0.9494N of $KMnO_4$ is equivalent to 0.1898 mg of

calcium oxalate. To determine the amount of inhibition of calcium oxalate crystal/stone, the amount of undissolved calcium oxalate crystal was then subtracted from the amount of calcium oxalate stone used in the experiment in the beginning (Chauhan and Joshi, 2008; Khan et al., 2012; Kore et al., 2011; Patel et al., 2010; Chaudhary et al., 2010; Chaudhary et al., 2009).

Results and discussion

Some chemicals e.g. uric acid, calcium, oxalate, levels in the urine may increases during stone formation (Kumar et al.,1991). There are many factors which play important role in the kidney stones formation, including inadequate water intake and dehydration, slow urinary flow and reduced urine volume. In normal and routine life, we do not take sufficient water, our food that is too rich in calories and table salt, but have deficiencies in fiber and alkali. All these conditions leads to the urinary stones formation.

Table 1. Percentage inhibition of calcium oxalate crystal

Groups	% inhibition
First (Alcoholic extract of *Ocimum sanctum* leaves: Calcium oxalate crystal) 10:100	77.18±1.15%
Second (positive control) (Cystone: Calcium oxalate crystal) 10:100	92.34±0.96%
Third (negative control) (consist only 10 mg Calcium oxalate crystal)	0.0%

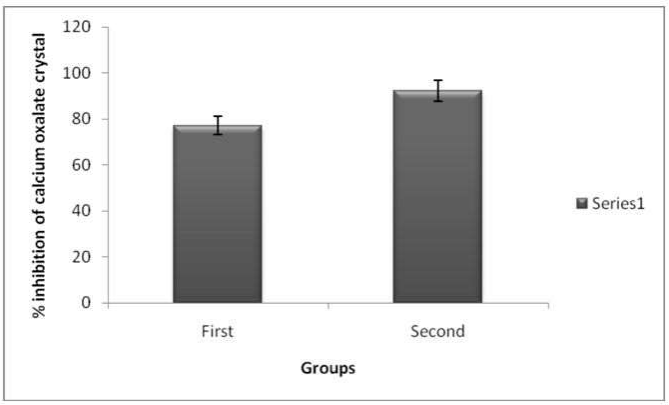

Figure 1. Percentage inhibition of calcium oxalate crystal by groups, First (Alcoholic extract of *Ocimum sanctum* leaves: Calcium oxalate crystal) and Second (positive control) (Cystone: Calcium oxalate crystal).

The phytochemical screening of ethanol extract showed the presence of glycosides, flavonoids, tannins and amino acids. In a study on some Ethnomedicinal plants from Talaja taluka of Bhavnagar district, Gujarat, Bhatt et al. (2002), had reported some plants (*Corchorus depressus, Solanum surattense, crataeva nurvala, Pedalium murex, Ascarantha longifolia, Ocimum sanctum* and *Hackelochola granularis*) which are useful for calculi cure. On the basis of ethnomedicinal studies *Ocimum sanctum* leaves were selected for in vitro studies. In the present study alcoholic extract of *Ocimum sanctum* leaves showed calcium oxalate crystallization inhibition 77.18±1.15% in case of first group whereas the Cystone, a prescribed medical formulation for inhibition of renal calculi showed highest inhibition (92.34±0.96%) in terms of formation of calcium oxalate precipitation. According to the result of the study it was found that the alcoholic extract of *Ocimum sanctum* leaves shows significant urolithic activity.

Conclusion

The present study shows that ethanolic extract of leaves of *Ocimum sanctum* have a very potent action against calcium stone inhibition activity as compared to various other marketed products. The ethonolic extract of *Ocimum sanctum* leaves can be further estimated *in-vivo* and then characterization of active constituents could used to prepare new formulations patients with Urolithiasis.

Declaration of interest

The authors report no conflicts of interest.

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