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EVALUATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY OF *MENTHA PIPERITA*

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ABSTRACT: *The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant Mentha piperita. Methanolic extract showed their maximum efficiencies in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the Methanolic leaf extracts of Mentha piperita were quite promising for further studies in this regard. In this study Neeri was used as standard drug.*

KEYWORDS: *In vitro antiurolithiatic activity, Methanolic extract, urolithiasis, Mentha piperita, Neeri*

1. INTRODUCTION:

Urolithiasis, formation of kidney stone presence of one or more calculi in any location within the urinary tract, is one of the oldest and wide spread diseases known to man¹. Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. Urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys².

Nephrolithiasis or renal stone disease remains a significant health problem in the adult population, with serious medical consequences, throughout a patient's lifetime. The worldwide incidence of urolithiasis is quite high, and more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate³. Kidney stone formation is a complex process which is the outcome of several physio-chemical events such as supersaturation, nucleation, crystal growth, aggregation, and retention⁴.

In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Endoscopic stone removal and extracorporeal shock wave lithotripsy are prohibitively costly and recurrence is quite common with these procedures⁵. Thus a drug for the prevention of this disease or its recurrence would be of great interest. Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological investigations on the medicinal plants used in traditional antiurolithic therapy have revealed their therapeutic potential in the in vitro models ~ 19 ~ Journal of Medicinal Plants Studies⁶⁻⁷.

Calcium containing stones may be in the form of pure calcium oxalate(50%) or calcium phosphate(5%) and a mixture of both(45%) followed by magnesium phosphate(15-20%),uric 5 acid(10%) and cystine(1%)⁸.



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Among the diversity of plants, *Mentha piperita* (Lamiaceae family) is one of the herbs most widely used worldwide, with a long history of safe use in medicinal preparations. Its leaf is used as a remedy for common cold, inflammation of the mouth, pharynx, liver, as well as disorders in the gastrointestinal tract such as nausea, vomiting, diarrhea, cramps, flatulence and dyspepsia. It is also used as antioxidant, antimicrobial, antiviral, antiinflammatory, and anti-carcinogenic⁹⁻¹⁷

2. MATERIALS AND METHODS:

PLANT MATERIAL

The leaves of *Mentha piperita* was collected in the month of march 2018 from Narsapur village, Medak dist. of Telangana, India. The plant was authenticated by D.Venkateshwara Rao, Deputy Director, Telangana. Forest Academy, Dullapally, Hyderabad, Rangareddy District. The leaves were washed with tap water and dried under shade.

PREPARATION OF PLANT EXTRACT

The leaves were shade dried and powdered. The crude plant extract was prepared by Soxhlet extraction method. 50g of powdered plant material was extracted with 500ml of Methanol. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The two extracts were filtered separately, and evaporated to dryness using rotary evaporator. Further the dried extracts were maintained in a refrigerator at 4°C for further antiurolithiatic activity.

CHEMICALS USED

Neeri, Sodium oxalate, Tris buffer, calcium chloride, Potassium permanganate (KMnO₄), Sulphuric acid (H₂SO₄).

INVESTIGATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY TEST BY TITRIMETRY

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H₂SO₄. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60°C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semi permeable membrane of egg as shown in the model designed given below. This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. First group served as blank containing only 1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the 10mg standard drug, i.e. Neeri. The 3rd group along with 1 mg of calcium oxalate



contain methanolic extract. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2 h. Remove the contents of semi permeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N KMnO₄ till a light pink colour end point obtained.

The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts¹⁸.

3. RESULTS AND DISCUSSION:

Drug therapy has developed in response to population health care¹⁹ needs. There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where the conventional medicine is devoid of satisfactory treatment. These are among the promising areas of research and development of medicines from the vast highly potential plant resources. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbens. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects²⁰. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of plant based therapy have been shown to be effective at different stages of stone pathophysiology²¹. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials²². Plant based drug discovery programmes continue to provide an important source of new drug leads²³. Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in Men, while phosphate stones formation is more in women²⁴.

This study evaluates the antiurolithiatic activity of Methanolic leaf extract of *Mentha piperita*. The highest percentage i.e. 98.1% of calcium oxalate {CaOx} dissolution was observed in Methanolic extract. Methanolic leaf extract of *Mentha piperita* was found to be more effective in dissolution of calcium oxalate than standard drug Neeri. From this study, it was observed that Methanolic extracts of *Mentha piperita* showed their highest dissolution of calcium oxalate. This study has given primary evidence for *Mentha piperita* as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that Methanolic leaf extract of *Mentha piperita* was quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Mentha piperita* leaves extract.

	% of dissolution of calcium oxalate	
S.No	GROUPS	<i>Mentha piperita</i>
1.	Blank	0
2.	Positive Control	81
3.	Methanolic extract	98.1

Figure 1: In vitro experimental model setup to evaluate antiurolithiatic activity.

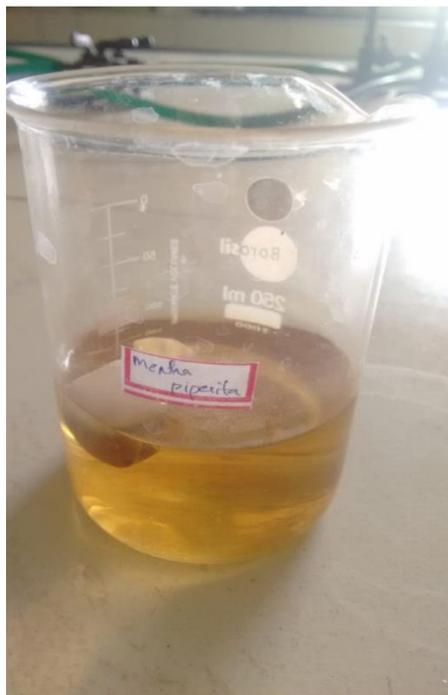
Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight.



Figure 1(b): Decalcified Eggs



Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer.



4. CONCLUSION:

In vitro urolithiasis has been performed on the selected plant *Mentha piperita* by using the standard drug, Neeri. The work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. Methanolic leaf extract of *Mentha piperita* shows highest dissolution than standard drug Neeri. This study has given primary evidence for *Mentha piperita* as the plant which possess antiurolithiatic property.

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