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EVALUATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY OF *PIPER NIGRUM*

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ABSTRACT: *The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant Piper nigrum. Ethanolic extract showed its maximum efficiencies in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the Ethanolic seed extract of Piper nigrum was quite promising for further studies in this regard. In this study Neeri was used as standard drug.*

KEYWORDS: *In vitro antiurolithiatic activity, Ethanolic extract, urolithiasis, Piper nigrum, Neeri*

1. INTRODUCTION:

Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings¹. Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. The overuse of synthetic drugs results in higher incidence of adverse drug reactions has motivated humans to return to nature for safe remedies. Herbs and herbal drugs have created interest among the people by its clinically proven effects². Therefore, there is a compelling need for detailed scientific validation of all traditional medicinal plant drugs to establish their efficacy and safety in light of modern science.

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors³. Urolithiasis is considered as the third most common affliction of the urinary tract. It refers to the solid non-metallic minerals in the urinary tract. It is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidney. The formation of kidney stones involves several phytochemical events beginning with crystal nucleation, aggregation and end with retention within the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate stones representing up to 80% of the analyzed stones⁴. Calcium containing stones may be in the form of



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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%)⁵.

It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract diseases and among these kidney stones are common with an annual incidence of 0.5 -1.9%. About 12% of the population of India is expected to have urinary stones and out of that 50% of cases encounter loss of one or both 2 kidneys with or without renal damage up to some extent².

Stone disease is 2-3 times more common in males, than in females. It 5 has a reoccurrence rate of 70-81% in males and 47-60% in females⁵. In spite of substantial progress in pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Kidney dialysis, endoscopic stone removal and extra corporeal shock wave lithotripsy are prohibitively costly and reoccurrence is quite common with these procedures¹.

Data from in vitro and in vivo clinical trials revealed that phytotherapeutic agents could be useful as alternative therapy in the management of urolithiasis. Medicinal plants and their products are more useful, because they promote the repair mechanism in natural way¹. Pharmacological and phytochemical prospecting of medicinal plants based on traditional knowledge can lead to the discovery of new drug and development of pharmacologically important products for human health care⁶. Green medicines were safe and more dependable than the costly synthetic drugs, many of which have side effects⁷.

Whole Peppercorn of *Piper nigrum* or its active components are being used in different types of foods and as medicine. Pepper is used worldwide in different types of sauces and dishes like meat dishes. It contains major pungent alkaloid Piperine (1-peperoyl piperidine, Figure 1) which is known to possess many interesting pharmacological actions. It is widely used in different traditional systems of medicine like Ayurvedic and Unani System of medicines^{8[a,b]}. It is also known to enhance cognitive action and Fertility⁹

2. MATERIALS AND METHODS:

PLANT MATERIAL

The seeds of *Piper nigrum* was collected in the month of March 2018 from Sangareddy town, Sangareddy dist. of Telangana, India. The seeds were washed with tap water and dried under shade.



PREPARATION OF PLANT EXTRACT

The seeds 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 ethanol. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The extract was filtered, and evaporated to dryness using rotary evaporator. Further the dried extract was 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 ethanolic 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 extracts¹⁰.



3. RESULTS AND DISCUSSION:

Drug therapy has developed in response to population health care¹¹ 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 effects¹². 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 pathophysiology¹³. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials¹⁴. Plant based drug discovery programmes continue to provide an important source of new drug leads¹⁵. 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 women¹⁶.

This study evaluates the antiurolithiatic activity of Ethanolic extract of *Piper nigrum*. The highest percentage i.e.96.3% of calcium oxalate {CaOx} dissolution was observed in Ethanolic extract. Ethanolic extract of *Piper nigrum* was found to be more effective in dissolution of calcium oxalate than standard drug Neeri. From this study, it was observed that Ethanolic extract of *Piper nigrum* showed their highest dissolution of calcium oxalate. This in vitro study has given lead data and shown that Ethanolic extract is quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Piper nigrum* seeds extract.

	% of dissolution of calcium oxalate	
S.No	GROUPS	<i>Piper nigrum</i>
1.	Blank	0
2.	Positive Control	81
3.	Ethanolic extract	96.3

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

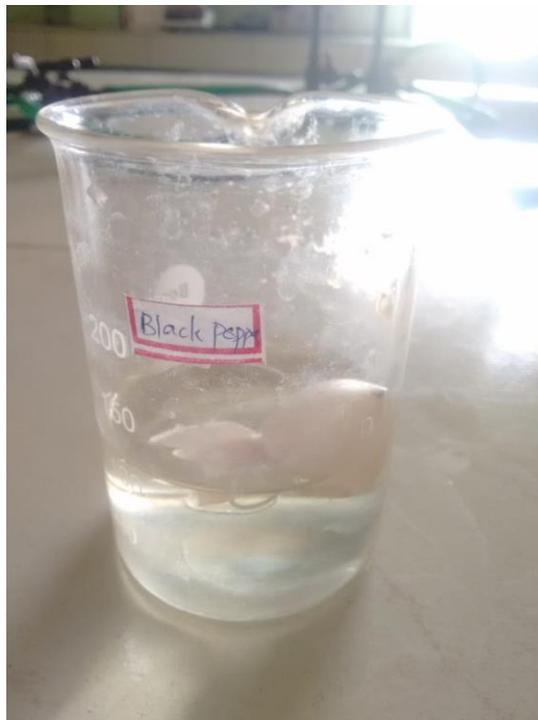
Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight.



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 *Piper nigrum* by using the standard drug, Neeri. The work was performed by using invitro antiurolithiatic model for calculating percentage dissolution of kidney stone. Ethanolic seed extracts of *Piper nigrum* shows highest dissolution than standard drug Neeri. This study has given primary evidence for *Piper nigrum* as the plant which possess antiurolithiatic property.

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