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EVALUATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY OF *DIGITARIA SANGUANALIS*

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ABSTRACT: *The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant Digitaria sanguinalis (crab grass). Butanolic extract showed its maximum efficiency in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the butanolic stem extracts of Digitaria sanguinalis were quite promising for further studies in this regard. In this study Neeri was used as standard drug. (22% unique).*

KEYWORDS: *In vitro antiurolithiatic activity, butanolic extract, Urolithiasis, Digitaria sanguinalis, Neeri.*

1. INTRODUCTION:

Plants are essential part of life on earth, they play an vital important role in the lives of animals and also in humans. Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of humanbeings¹. 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 in order to establish their efficacy and safety in modern science.

The knowledge of medicinal plants and their uses has become lost over the last few generations but it is making a great comeback. There are several different ways in which plant play their roles in sustenance of life on everyday basis, Food - Plants provide food to humans and animals. Plants prepare their own food themselves but plants are used by other living beings for food



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purpose. Water - Plants regulate the water cycle, they move water from soil to atmosphere and a process is called as transportation. Oxygen - Plants absorb carbon dioxide and release oxygen in the air that is used by all living beings for breathing. Medicines - Several plants are used for different medicinal purposes.

Kidney stone is a multi-factorial disorder resulting from combined influence of epidemiological, biochemical and genetic riskfactors³. Kidney stones are hard deposits of minerals and acid salts that stick together in concentrated urine, they can be painful when passing through the urinary tract but usually doesn't cause permanent damage. Urolithiasis is considered as the third most common disease of the urinary tract. It refers to the solid non-metallic minerals in the urinary system. It is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidney. The accumulation of kidney stones involves several phytochemical events beginning with crystal nucleation, aggregation and end with retention within the urinary system. Among several types of kidney stones, the most common are calcium oxalate stones representing up to 80% of the analyzed stones⁴. Calcium containing stones 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%)⁵.

It is estimated that at least 10% of the populace 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 two kidneys with or without renal damage up to some extent².

Stone disease is 2-3 times more prevalent in males, than in females. It 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 copacetic drug being utilized in clinical therapy. Kidney dialysis, endoscopic stone abstraction and extra corporeal shock wave lithotripsy are prohibitively costly and reoccurrence is quite prevalent 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 naturalway¹.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



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health care⁶. Green medicines were safe and more dependable than the costly synthetic drugs, many of which have side effects⁷.

The selected plant *Digitaria sanguinalis*, digitaria is a genus of plants in the grass family native to tropical and warm temperate regions. Common names include **crab grass**, **finger grass**, **fonio**. Though some *Digitaria* species are weeds, others have uses especially as food. The mostly notably those of fonio can be toasted and ground into a flour which can be used to make porridge or fermented to make beer. Fonio has decent nutrient qualities as a forage for cattle.

2. MATERIALS AND METHODS:

PLANT MATERIAL

The stems of *Digitaria sanguinalis* was collected in the month of may 2018 from Narsapur village, Medak dist. of Telangana, India. Using tap water the stems were washed and dried under shade.

PREPARATION OF PLANT EXTRACT

The leaves were dried in shade and powdered finely. Extraction of the crude plant extract was done using Soxhlet extraction method. Around 46g of powdered plant material was extracted with 430ml of butanol. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The crude 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 dihydrate 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 60⁰C. 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, 4th groups served as test group along with 1 mg of calcium oxalate contain butanolic extract. The conical flasks of all groups were kept in an incubator preheated to 37⁰C for 2 hours. 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 0.9494N 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 butanolic extract of *digitaria sanguanalis*. The highest percentage i.e. 98% of calcium oxalate {CaOx} dissolution was observed in butanolic extract which had a percentage dissolution. From this study, it was observed that butanolic extract of *digitaria sanguanalis* showed its highest dissolution of calcium oxalate. This study has given primary evidence for *digitaria sanguanalis* as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that butanolic extract is quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Digitaria sanguanalis* leaves extracts.

| S.No | % of dissolution of calcium oxalate | |
|------|-------------------------------------|------------------------------|
| | GROUPS | <i>Digitaria sanguanalis</i> |
| 1. | Blank | 0 |
| 2. | Positive Control | 81 |
| 3. | Butanolic extract | 98 |

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 Egg



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





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4. CONCLUSION:

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

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