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Review Article

In-vitro Evaluation of Macrotyloma Uniflorum In the Treatment Of Urolithiasis

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ABSTRACT

Urolithiasis a chronic disease and in medical terms refers to formation of stones in kidneys. A renal calculus or kidney stone is a solid aggregation formed in kidneys from various minerals present in the diet. The kidney stones differ in sizes, shape and types. Kidney stone formation is a complex process that involves nucleation, supersaturation, growth, aggregation and retention within the kidney. The treatment of removal of kidney stones includes Extra corporeal Shock Wave Lithotripsy which can also sometimes cause renal injury. Various plant species have been used for the treatment of kidney stones. *Macrotyloma uniflorum* (Horse gram) is a popular pulse of Uttarakhand, locally known as Gaheth. *M. uniflorum* seeds are rich in protein, consumed in majority by poorest section of the society and are cultivated in India since prehistoric times. *M. uniflorum* seeds have been used by people of Uttarakhand for the treatment of kidney stones though till date no scientific explanation regarding its mechanism of action is known. The leaves have been used as a source of animal feed and are not consumed by humans. In the present study we focus on the comparative anti-nephrolithiatic activity of seeds and leaves of *M. uniflorum* and also the different bioactive components that may be responsible for dissolution of experimental kidney stones.

INTRODUCTION

Horse gram (*Macrotyloma uniflorum* Lam. [Verdc.]) is considered as an unexplored and underutilized legume which is mostly cultivated in South Asian countries, Africa, Australia, and the West Indies. It is an ample source of proteins, minerals, vitamins, and has good tolerance to abiotic stresses such as drought and salinity. The presence of nonnutritive bioactive compounds

such as proteinase inhibitors, phenolic acid, and phytic acid has shown a significant effect on physiological and metabolic implications in human health. Horse gram is used as a therapeutic agent to treat common cold and fever, kidney stones, constipation, leucorrhea problems, diabetes, and coronary heart disease. Molecular docking studies have shown that DL-proline and geranyl geraniol phytoconstituents from horse

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gram sprouts and mixed sprouts, respectively, are novel antibiotic resistance breakers and can be recommended as a preventive measure for Shigellosis disease. Research progress is presented on the bioactive compounds of horse gram, its nutritional composition, molecular docking studies, and potential health benefits. This underutilized crop can be further explored as a source of nutraceutical and functional food. *Macrotyloma uniflorum* commonly known as horse gram is grown during late rainy season (from August to October) in drought-prone areas of South India. It can grow in regions with 90 cm of rainfall and as low as 40 cm without any irrigation. It can grow up to 30-40 cm in height on lateritic soils. Studies have shown that horse gram is well known for their drought tolerance, but does not tolerate frost and water logging. The plant grows as slender with cylindrical stems. Its weak stem makes it difficult to support their height and they grow as a bush in association with companion species. It has three stipulated leaflets (trifoliate) which are approximately 7-10 mm long. Each leaflet is 3.5-7.5 cm long and 2-4 cm broad, oval in shape with round base. Flowers are only 10-12 mm long, greenish yellow in color. Calyx has densely matted woolly hair-like structure with 2-3 mm long tube and lobes are 3-8 mm long. Attenuated ovary is covered with white mat-like structure. Each pod holds 6-7 seeds, each pods are 6-8 mm long and 4-5 mm broad, pale with small black dots. The consumable seeds are light red or often brown in color. They are ovoid in shape with 4-6 mm long and 3-5 mm broad. Small and Scattered black dots are present in the center portion of the seeds.

MATERIAL & METHOD

Extraction Of Plant Material:

Crude plant extract was made ready by means of Soxhlet extraction techniques. About 20 gm of powdered plant material was equally packed into a thimble and extracted with 250 ml of various

solvents one by one. Solvents used were methanol and ethanol as per increasing polarity. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor emerges as colourless. After that the extract was taken in a beaker and kept on a hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in the refrigerator at 4°C for their future use in phytochemical evaluation.

Material: Soxhlet extractor, heating mantle, Round bottomed flask, reflux condenser, stand, ethanol

METHOD:

Extraction:

It is dried powder material then obtained is seep with polar solvent(ethanol) for 24 hours in soxhlet extractor followed by polar solvent ethanol.

Procedure of extraction:

- 1)20gm powdered form of *Macrotyloma Uniflorum* is poured into Soxhlet extractor and its level is maintained. Cotton is used to cover connectors to prevent flow of fluids.
- 2)After setting Soxhlet extractor electricity is being supplied through heating mantle and temperature is maintained at 100°C Then Soxhlet extractor is being connected to colder to providing chilling.
- 3)This process is continued for 24 Hours. In such 24 hours process it involved step by step process in which firstly solid matrix is place in sox thimble, solvent is heated under reflux. Then condensation and extraction with fresh solvent.
- 4)Solute are transfer from extractor chamber into reservoir.
- 5)Continuous repetition of removal.
- 6)Exhaustive extraction is complete.

Phytochemical Screening and Quantitative Estimation of Phytoconstituents:

Preliminary phytochemical screening

Quantative test were perform by ethanolic extract of *Macrotyloma Uniflorum* to identify the various phytoconstituent.



The test and observation are given below

A) Test For Alkaloids: -

Dragendorffs test: -

Adding 1ml of Dragendorff's reagent to 2ml of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

Mayers test: -

2ml test solution and add into 0.1 ml mayers reagent. Formation of yellow precipitate is observed which indicate presense of alkaloids.

Hagers test: -

3ml of test solution and add in it 0.1 ml hagers reagent. formation of yellow ppt which indicate the presense of alkaloids.

B) Test for flavonoids: -

Lead acetate: -

Few ml of test solution add lead acetate solution and observed for colour ppt.

C) Test For Glycosides: -

Borntrager test: -

3ml extract + dilute H₂SO₄. boiled and filtrate to cold filtrate, added equal volume benzene or choloform shake well separate the organic solvent add ammonia, ammonical layered turned red.

D) Test For Carbohydrate: -

Molish test: -

Aqueous test solution alcoholic alpha naphthol + concentrated H₂SO₄. No purple violet ring at junction. Which indicate the absent of carbohydrate.

Fehlings test: -

Test solution, equal volume of fehling's A and fehling's B Reagent boil, formation of brick red ppt.

Bendicts test:

Few ml of test solution add few ml of Bendicts reagent formation of green colour ppt.

E) Test For Saponin

1)Foam Test:-

To 2ml of extract was treated with 8ml of water in test tube. The mixture was shaken vigorously and

observed for the formation of persistant foam for 5min that confirms the presence of saponin.

In vitro experiments:

Evaluation for anti-urolithiatic activity:

Step1: Preparation of experimental kidney stones (calcium oxalate stones)by homogeneous precipitation:

Exactly 1.47 g of calcium chloride dihydrate was dissolved in 100 ml distilled water and 1.34 g of Uric acid crystal was dissolved in 100 ml of 2 N sulfuric acid. Equimolar prepared solutions of calcium chloride dihydrate and sodium oxalate were allowed to react in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate precipitate was freed from traces of sulfuric acid by ammonia solutions, washed with distilled water, and dried at 60°C for 2 hour.

Step2: Preparation of semipermeable membrane from farm eggs;

The semi permeable membrane of eggs lies in between the outer calcified shell & the inner contents like albumin & yolk, shell was removed chemically by placing the eggs in 2M HCL for an overnight, which caused complete decalcification further, washed with distilled water,&carefully wioth a sharp pointer a hole is made on the top & the contents squeezed out completely from the decalcified egg.then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened conditioned for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7-7.4.

Step 3: Estimation of calcium oxalate by titrimetry:

Weighed accurately 1 mg of the calcium oxalate and 10 mg of the extract/compound/ and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer one group served as negative control (contained only 1 mg of calcium oxalate) place the conical flask of all groups in an incubator, preheated to 37°C for 2



hours, for about 7-8 hours. Removed the contents of semipermeable membrane from each group into a test tube added 2 ml of 1N sulphuric acid and titrated with 0.9494 N KMnO_4 equivalents to 0.1898 mg of 4 calcium. The amount of undisclosed calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substances could dissolved.

Analytical Determination of Bioactive Compounds

UV-Visible Infrared Spectroscopy

UV-visible infrared (IR) spectroscopy is qualitative and an analytical screening approach together with chemometric pattern recognition for the identification of bioactive components from the plants and plant products [110]. Naturally found compounds and phenolic compounds such as tannins, phlobatannins, dyes, anthocyanins, and phenols form complexes with iron which can be easily detected. Typically, ultraviolet (UV) region range extends from wavelength 190 to 350 nm, visible range is 350 to 800 nm, and infrared range is from 800 to 2500 nm [111]. UV-visible spectroscopy mostly preferably used for the quantitative analysis of aromatic molecules, as they have strengthened chromophores in the range of UV [112]. This technique is cost-effective and not time consuming compared to other techniques for the determination of bioactive compounds

[113]. Antinutritional factor especially phytic acid is present in horse gram sprouts; IR and MS are used to characterize the bioactive compounds in the sprout extract

Thin Layer Chromatography

Thin layer chromatography (TLC) is an adsorption chromatography, which is mostly used to separate compounds with lower molecular weight. The separation of a compound occurs based on the interaction between adsorbent layers on the plate [89]. Using silica gel G254 the plate is transferred to an oven at 110 °C for 20 min for the activation. The extract dissolved in the respective solvent system is filtered and its aliquots (2 μl) are then applied on the activated silica gel plate with a standard sample. The plate is then placed in a developing chamber with the desired solvent system and allows running until reaching a height of approximately 10 cm from the point of application. The spots were detected using a spraying agent such as vanillin sulphuric acid or p-anisaldehyde reagent. Plates are then kept in an oven at 110 °C for 5-10 minutes for the color development and finally the R_f values were calculated. TLC method is used for the isolation and purification of bioactive compounds that are responsible for bioactivity.

Preparation of semipermeable membrane from farm eggs:



Estimation of calcium oxalate by titrimetry:



UV- visible Spectroscopy:



The *In vitro* Anti-Urolithiatic activity was performed by using extracts:

Sr.no	Burette Reading	In.ml
1	Blank	0.00
2	Ethanolic extract	12



Fig: (a) Before Titration

Fig: (b) After Titration

RESULT

The result expected from the experimental work suggests that this investigation would provide encouragement for further exploration into new drugs for the prevention and treatment of urolithiasis. The *In-vitro* evaluation of the selected plant extract against calcium oxalate crystal formation demonstrated promising inhibitory effects. The study successfully isolated and characterized key bioactive compounds from the plant, which showed significant reduction in the aggregation of calcium oxalate crystal. Overall, this study provides a strong scientific foundation for the potential use of the plant extract as a natural therapeutic agent for the prevention and management of calcium oxalate kidney stones.

SUMMARY & CONCLUSION

The result expected from the experimental work suggests that this investigation would provide encouragement for further exploration into new drugs for the prevention and treatment of urolithiasis. The present investigation provides useful information on antiurolithiatic activity of *Macrotyloma uniflorum*. The extract showed dissolution of stones (calcium oxalate). Further *in-vivo* pharmacological and clinical studies with suitable experimental models are required to understand the mechanism and the actual efficacy of the plant *Macrotyloma uniflorum* in treating urolithiasis.

REFERENCE

1. Kumar DS, Prashanthi G, Avasarala H, Banji D (2013) Antihypercholesterolemic effect



2. Asha KI, Latha M, Abraham Z, Jayan PK, Nair MC, Mishra SK (2006) Genetic resources. In: Kumar D (ed) Horse gram in India. Scientific Publisher, Jodhpur, pp 11-28
3. Kadam SS, Salunkhe DK, Maga JA (1985) Nutritional composition, processing, and utilization of horse gram and moth bean. *Crit Rev Food Sci Nutr* 22(1):1-26 Bioactive Compounds of Horse Gram (*Macrotyloma uniflorum* Lam. [Verde 31
4. Wong PYY, Kitts DD (2006) Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. *Food Chem* 97:505- 515
5. Fuller DQ, Murphy C (2018) The origins and early dispersal of horse gram (*Macrotyloma uniflorum*), a major crop of ancient India. *Genet Resour Crop Evol* 65(1):285-305
6. Neelam DA (2007) Identification and quantification of nutraceuticals from Bengal gram and horse gram seed coat. Dissertation for Bachelor of Technology. Department of Biotechnology, Sathyabama University Chennai (India), India
7. Kingwell-Banham E, Fuller DQ (2014) Horse gram: origins and development. *Encyclopedia of global archaeology*. Springer, New York
8. Prodanov M, Sierra I, Vidal-Valverde C (1997) Effect of germination on the thiamine, riboflavin and niacin contents in legumes. *Z Lebensm Unters Forsch* 205:48-52. <https://doi.org/10.1007/s002170050122>
9. Ramteke V, Kurrey VK, Panigrahi TK, Yadav P (2016) Horse gram (Kulthi): pulse of rural peoples in Chhattisgarh. *Innov Farm* 1(4):205-208
10. Chakravarty S, Ray S, Talapatra SN (2019) Antibacterial phytochemicals in *Macrotyloma uniflorum* (Lam.) Verdc. on DNA-gyrase: an in silico study. *Res J Life Sci Bioinfo Pharma Chem Sci* 5(2):221-235
11. Bhardwaj J, Yadav SK (2015) Drought stress tolerant horse gram for sustainable agriculture. *Sustainable agriculture reviews*. Springer, Cham, pp 293-328
12. Garc'a-Granero JJ, Lancelotti C, Marco M, Ajithprasad P (2016) Millets and herders: the origins of plant cultivation in semiarid North Gujarat (India). *Curr Anthropol* 57(2):149-173
13. Blumenthal MJ, Staples LB (1993) Origin, evaluation and use of *Macrotyloma* as forage. *Trop Grassl* 27:16-29
14. Kharkwal MC, Gupta VP (2003) Advances in arid legumes research. Published for Indian Arid Legumes Society, Jodhpur
15. Brown C (2007) Ultraviolet, visible, and near-infrared spectrophotometers. *Appl Spectrosc Rev* 35(3):151-173. <https://doi.org/10.1081/ASR-100101223>
16. Mohamed VS, Wang CS, Thiruvengadam M, Jayabalan N (2004) In vitro plant regeneration via somatic embryogenesis through cell suspension cultures of horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). *In Vitro Cell Dev Biol Plant* 40:284-289
17. Kumar D (2006) Horse gram research: an introduction. In: Kumar D (ed) Horse gram in India. Scientific Publishers, Jodhpur, pp 1-10
18. Bhartiya A, Aditya JP, Pal RS, Bajeli J (2017) Agromorphological, nutritional and antioxidant properties in horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] germplasm collection from diverse altitudinal range of North Western Himalayan hills of India. *Vegetos* 30:1. <https://doi.org/10.4172/2229-4473.1000215>
19. Gupta A, Bhartiya A, Singh G, Mahajan V, Bhatt JC (2010) Altitudinal diversity in horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] land races collected from hill region. *Plant Genet Resour Charact Util* 8(3):214-216



20. Sadawarte SK, Pawar VS, Sawate AR, Thorat PP, Shere PD, Surendar J (2018) Effect of germination on vitamin and mineral content of horse gram and green gram malt. IJCS 6 (3):1761-1764

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