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Research Article

"*IN-SILICO* STUDY FOR ANTIUROLITHIATIC ACTIVITIES OF COMPOUNDS EXTRACTED FROM HORSE GRAM (*MACROTYLOMA UNIFLORUM*) COLLECTED FROM UTTARAKHAND (INDIA) AS XANTHINE DEHYDROGENASE INHIBITOR BY MOLECULAR DOCKING"

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ABSTRACT

Horse Gram (*Macrotyloma uniflorum* (L)) traditionally known as antiurolithiatic legume in India, a representative of the family Leguminosae, is widely available in hills of Uttarakhand. It is used as antiurolithiatic, astringent, diuretic, antimicrobial, anti-inflammatory, hepatoprotective drug by Indian traditional system of medicines. It acts against oxidative stress, horse gram (HG) can be used as a comprehensive treatment in case of urolithiasis. Till date the molecular mechanism of antiurolithiatic activity of HG is not clearly known. LC-MS analysis of HG extract yielded more than thirty most important compounds beneficial to human health. Adenine phosphoribosyltransferase (APRTase) is an enzyme in purine salvage pathway which recycles a group of DNA building blocks (nucleotides), but Xanthinedehydrogenase (Xdh) degrades the APRTase and induce the kidney stone by formation of 2, 8-dihydroxyadenine (DHA). DHA is poorly soluble in urine and forms kidney stone. Molecular docking of compounds with Xdh has shown the significant reduction and change in properties of Xdh activity. Xdh was converted by binding of HG compounds and Xdh will not degrade the APRTase. This investigation proved that HG has potential to cure the kidney stone. The present investigation will be important information in the field of urolithiasis. This is the first hand report on *in-silico* analysis of antiurolithiatic activity of horse gram. The outcome of this research have tremendous scope as industrial insights and including contribution to research inputs for researchers working on the field of *in-vivo* and *in-vitro* studies.

INTRODUCTION

In India, *Macrotyloma uniflorum* is also known as Gahat, Muthira, Kulath or Kkulthi, Hurali. In an *in-vivo* study the effect of oral administration of aqueous and alcohol extracts of *Macrotyloma uniflorum* (*Fabaceae*) seeds on calcium oxalate urolithiasis has been studied *in vitro* (Sharma *et al*., 2019[a]). Ethylene glycol feeding resulted in hyperoxaluria as well as augmented renal flow of calcium and phosphate. Supplementation with aqueous and alcohol extract of *Macrotyloma uniflorum* seeds significantly reduced the elevated urinary oxalate showing a regulatory action on endogenous oxalate synthesis (Chaitanya *et al*., 2010). After the above study there is lake of mechanism of action and the chemical composition of horse gram. There is a need of molecular mechanism of antiurolothiais effect on kidney stones of horse gram extracts. The APRT gene provides instructions for making an enzyme called adenine phosphoribosyltransferase (APRTase). This enzyme is produced in all cells and is part of the purine salvage pathway, which recycles a group of DNA building blocks (nucleotides) called purines to make other molecules. APRTase found in humans on chromosome 16 (Valaperta *et al*., 2014). It is part of the Type I PRTase family and is involved in the nucleotide salvage pathway, which provides an alternative to nucleotide biosynthesis de novo in humans and most other animals (Silva *et al*., 2008). APRTase deficiency contributes to the formation of kidney stones (urolithiasis) and to potential kidney failure

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(Shi *et al*., 2001). In plants, as in other organisms, APRTase functions primarily for the synthesis of adenylate. It has the unique ability to metabolize cytokinins, a plant hormone that can exist as a base, nucleotide, or nucleoside into adenylate nucleotides (Allen *et al*., 2002). When APRTase has reduced or nonexistent activity, adenine accumulates from other pathways. It is degraded by xanthine dehydrogenase to 2, 8-dihydroxyadenine (DHA). Although DHA is protein-bound in plasma, it has poor solubility in urine and gradually precipitates in kidney tubules, leading to the formation of kidney stones (urolithiasis). If left untreated, the condition can eventually produce kidney failure (Shi *et al*., 2001). A diagnosis of APRTase deficiency can be made by analyzing kidney stones, measuring DHA concentrations in urine, or analyzing APRTase activity in erythrocytes. It is treatable with regular doses of allopurinol or febuxostat, which inhibit xanthine dehydrogenase activity to prevent the accumulation and precipitation of DHA (Edvardsson *et al*., 1993). Keeping in view of above facts the study was design to examine the role of compounds identify after the LC-MS analysis of Horse gram in urolithiasis collected from Kumaun region of Uttarakhand.

MATERIAL AND METHODS

Research Material and extract preparation: Cultivars of *M. uniflorum* were collected from local farmers from Kumaun region of Mornolla (Almora) at 2178m of Uttarakhand during the harvesting seasons. All chemicals used in the investigation were of molecular biology grade. Dried Seed material was crushed for preparation of Extracts (Figure 1), using mortar pestle, 20g material was used sufficient to fill the porous cellulose thimble (in a 25- x 80-mm) (Sharma *et al*., 2018). Once the process completed the alcohol and water was evaporated leaving a small yield of extracted seed material (about 2 to 3 ml) in the glass bottom flask.

Figure 1 Black and Red colored *M. uniflorum* collected from Kumaun of Uttarakhand (Sharma *et al*., 2018).

LC-MS analysis of extracts

All extracts were sent to APS Lab, Pune (Maharashtra) for analysis with the TOF/Q-TOF Mass Spectrometer (Component Model G6540B). Using standard protocols of LC-MS the bio-molecules such as carbohydrate, steroid, tannins, phenol, protein, amino acid, alkaloids, glycosides, flavonoids and saponins were identified.

Molecular docking studies

Identified compounds were studied by *in silico* technique using auto-mated docking software (Auto dock-vina). A protein sequence of Adenine phosphoribosyltransferase (APRTase) and Xanthine Oxidoreductase (Xdh) were downloaded from PDB (www.rcsb.org/pdb) which reportedly participate in kidney stone formation. Protein sequence of adenine phosphoribosyltransferase (APRTase) of *Homo sapiens* and the structure was downloaded from NCBI (Figure 2 and 3).

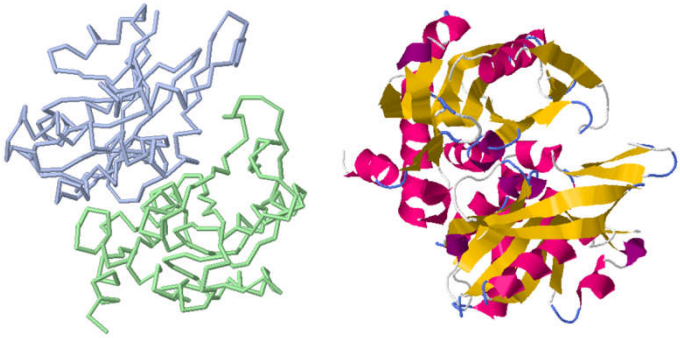


Figure 2 Structure of Adenine phosphoribosyltransferase (APRTase) of Homo sapiens constructed by Protein Structure Annotation Tool (PROSET) online software. Blue is Chain-A and Green is Chain-B.

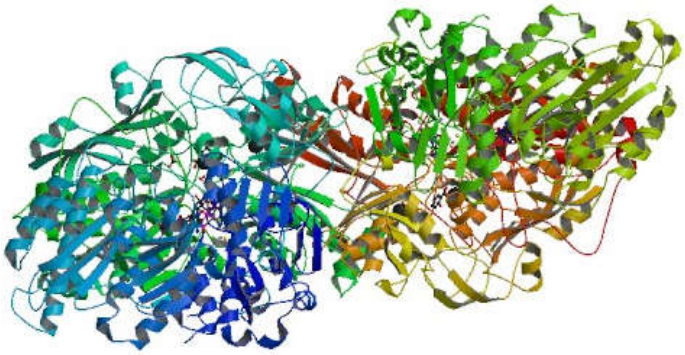


Figure 3 Ribbon Structure of Human xanthine dehydrogenase of *Homo sapiens* was downloaded from PDB (www.rcsb.org/pdb).

Calculation of binding free energy: Polarization and charge transfer play an important role in molecular interactions. The free-energy changes between adjacent steps were computed by using the Bennett acceptance ratio method (BAR) (Bennett, 1976).

Statistical analysis

Investigators computed the free energy caused by the induction between the ligand and its environment. The total statistical error in decoupling free energy is computed as the sum of the errors from individual steps. The polarization between ligand and surrounding atoms was turned off by scaling the damping coefficient from the original (Ren and Ponder, 2003).

RESULT AND DISCUSSION

The present study resulted that the *M. uniflorum* is of varied use and importance in nutrition and therapeutics, the finding of LC-MS analysis unzipped lot of information on the therapeutic compounds present in this less studied seed. The outcome of this investigation could be useful to understand the molecular mechanism of various compounds and nutraceutical value of *M. uniflorum*. Extracts of *M. uniflorum* demonstrated a broad spectrum of nutraceutical efficiency against many human metabolic disorders. To construct or design drugs against many human diseases or metabolic disorders this investigation along with the *In-silico* studies would be of significant use. This is first hand report with complete analysis by LC-MS of *M. uniflorum*, deciphering the HG research in respect to

nutraceuticals from high altitude of this Himalayan region. Kidney stone formation by the resulting of purine salvage pathway is representing in a flow chart (figure 4).

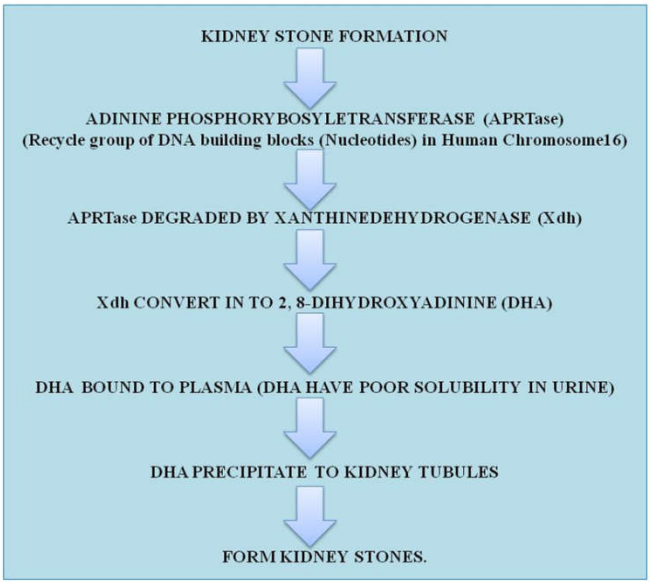


Figure 4 Flow chart indicated that how to stone formation in kidney in purine salvage pathway.

De novo synthesis deals with the synthesis of complex molecules from simple molecules such as sugars or amino acids, as opposed to recycling after partial squalor (Figure 5). In humans and other primates, uric acid is the end product of purine catabolism and is excreted in the urine. Birds, terrestrial reptiles, and many insects also excrete uric acid, but, in these organisms, uric acid represents the major nitrogen excretory compound, because, unlike mammals, they do not also produce urea. Instead, the catabolism of all nitrogenous compounds, including amino acids, is channeled into uric acid. This route of nitrogen catabolism allows these animals to conserve water by excreting crystals of uric acid in paste-like solid form (web.virginia.edu/Heidi/chapter27/chp27.htm).

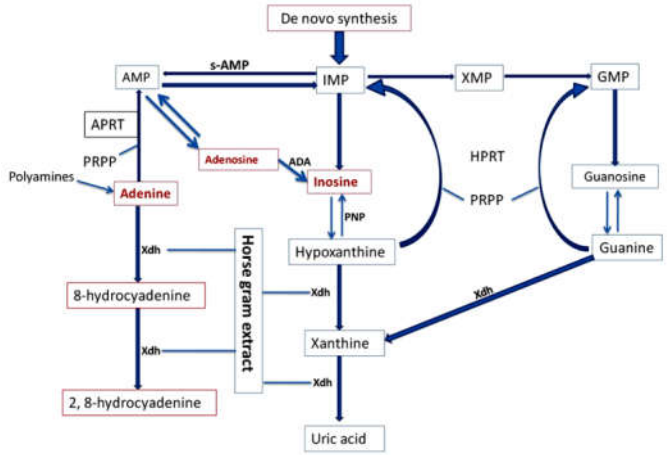


Figure 5 Adenine metabolism pathways and the role of adenine phosphoribosyltransferase (APRT) **(Guillaume *et al.*, 2012).** In humans, adenine cannot be converted to adenosine as hypoxanthine to inosine; the only alternative pathway in APRT deficiency is oxidation of adenine to 2,8-hydroxyadenine by xanthine dehydrogenase (XDH). Horse gram extract acts by inhibiting XDH, thus preventing 2,8-dihydroxyadenine synthesis. ADA, adenosine deaminase; AMP, adenosine monophosphate; GMP, guanosine monophosphate; HPRT, hypoxanthine phosphoribosyltransferase; IMP, inosine monophosphate; PNP, purine nucleoside phosphorylase; PRPP, 5-phosphoribosyl-1-pyrophosphate.

After LC-MS analysis many compound were identified in horse gram extract (**Table 1**) and all examines compound were important for human nutraceuticals.

Table 1 Major components after LC-MS analysis and their metabolic function. (Molecular mass and structure were constructed with the help of https://www.webqc.org/ mmcalc.php) (Sharma *et al.*, 2019[b]).

S. No.	Name of components	Molecular Formula	Molecular weight (g/mol)
1.	(2E)-3-(4-Methoxyphenyl)-2-propenyl 6-O-a-L-arabinopyranosyl-b-D-glucopyranoside	C21 H30 O11	458.1787
2.	[6]-Gingerol	C17 H26 O4	294.1828
3.	D-erythro-Dihydrosphingosine	C18 H39 N O2	301.2988
4.	Methyl gallate	C8 H8 O5	184.0356
5.	Resveratrol	C14 H12 O3	228.0788
6.	Rutaecarpine	C18 H13 N3 O	287.1061
7.	Syringic acid hexoside	C15 H20 O10	360.1037
8.	Tyramine	C8 H11 N O	137.0842
9.	Asperuloside	C18 H22 O11	414.1141
10.	Ginkgolide A	C20 H24 O9	408.1414
11.	Cerulenin	C12 H17 N O3	223.1208
12.	Melatonin	C13 H16 N2 O2	232.1211
13.	Pelargonidin chloride	C15 H11 O5	271.0598
14.	Pilocarpine	C11 H16 N2 O2	208.1224
15.	Prostaglandin E1	C20 H34 O5	354.2382
16.	Sinapic acid	C11 H12 O5	224.0679
17.	Swertiamarin	C16 H22 O10	374.1217
18.	Uridine	C9 H12 N2 O6	244.0712
19.	Dimethylarginine	C8 H18 N4 O2	202.1422
20.	2'-Hydroxy-5'-methyl-4-methoxychalcone	C17 H16 O3	268.3071
21.	Linolenic acid	C18 H32 O2	268.1089
22.	Shanzhiside	C16 H24 O11	392.1321
23.	3'-O-Acetylhamaudol	C17 H18 O6	318.1083
24.	Abscisic acid	C15 H20 O4	264.1343
25.	Calciferol	C28 H44 O	396.3394
26.	Oleuryopein-aglycone	C19 H22 O7	362.1348
27.	Proline-betaxanthin	C14 H16 N2 O6	308.1014
28.	Thymol	C10 H14 O	150.1039
29.	(-)-Riboflavin	C17 H20 N4 O6	376.1375
30.	Sweroside	C16 H22 O9	358.125

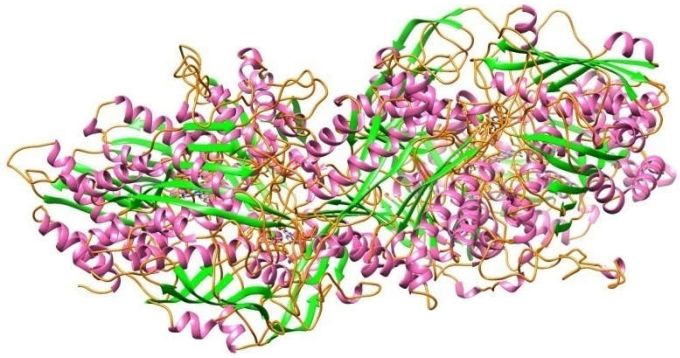
Polarization and charge transfer play a significant role in molecular interactions. After molecular docking Rutaecarpine (-9.7 Kcal/mol) was observed high binding free energy and Tyramine (-5.9 Kcal/mol) observed less (Table 2).

Table 2 Binding free energy (Kcal/mol) of horse gram derived compounds obtained trough LC-MS and molecular docking against Xanthinedehydrogenase (Xdh) and number of Hydrogen bands with interacting amino acid residue.

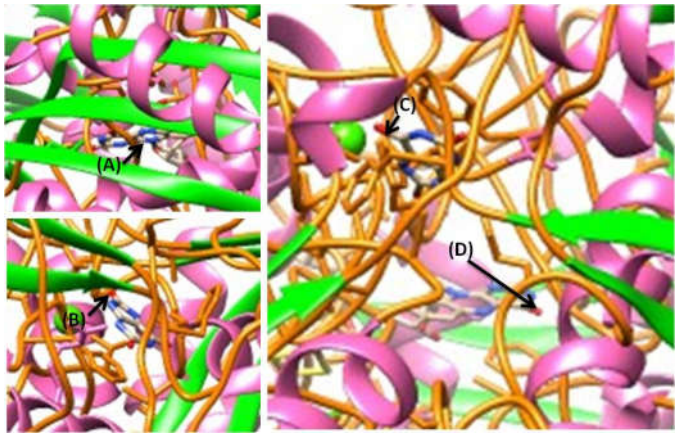
S. No.	Natural compounds Vs Xdh	Binding free energy (Kcal/mol)
1.	Rutaecarpine	-9.7
2.	Sweroside	-9.6
3.	Calciferol	-9.0
4.	Ginkgolide_A	-8.9
5.	Asperuloside	-8.7
6.	2-Hydroxy_5__methyl_4_methoxychalcone	-8.2
7.	Oleuryopein_aglycone	-8.2
8.	Swertiamarin	-8.2
9.	Uridine	-8.1
10.	(-)-Riboflavin	-8.0
11.	Pelargonidin_chloride	-8.0
12.	Resveratrol	-8.0
13.	Proline_betaxanthin	-7.6

14.	Abscisic_acid	-7.5
15.	Shanzhiside	-7.4
16.	6__Gingerol	-7.3
17.	Sinapic_acid	-7.1
18.	D_erythro_Dihydrosphingosine	-6.8
19.	Methyl_gallate	-6.8
20.	Melatonin	-6.7
21.	Dimethylarginine	-6.6
22.	Linolenic_acid	-6.6
23.	Prostaglandin_E1	-6.5
24.	Thymol	-6.5
25.	Cerulenin	-6.1
26.	Pilocarpine	-5.9
27.	Tyramine	-5.9

Determining the binding sites establish in protein structure is an important task. Many efforts have been made to develop some tools that can successfully discover the cavities for prediction of binding affinity and scoring with ligand (s) through molecular docking. Some important compounds were bound on protein structure (Figure 6) and it may change their functions and may be prevent DHA formation. This study clearly indicated that compounds of horse gram able to prevent kidney stone formation after DHA precipitation in kidney.



(1)



(2)

Figure 6 (1):3D structure of Xdh protein structure and binding site with natural compounds. (2): Compound bind on the Xdh (A; B; C and D are bound ligands).

All thirty selected horse gram identified compounds and available reference inhibitors were docked with Xdh to evaluate the binding affinity between them. Rutaecarpine showed binding energy-9.7 kcal/mol with Xdh (Figure 7); and rest of the molecules showed binding energy ranging from -9.7 to −5.9 kcal/mol. It may be considered for further investigations and development of future natural inhibitors of Xdh for prevention and treatment of urolithiasis.

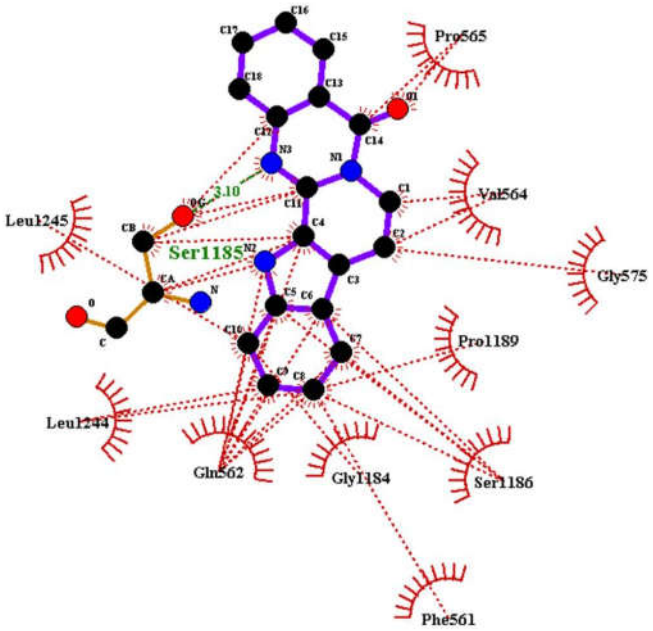


Figure 7 Protein-ligand interaction analysis: Xdh docked complex structure generated through hydrogen bond interaction with amino acid residues. Whereas amino acid residue involved in interaction through hydrophobic bonding are showing as arcs (red.)

The protein and ligand complexes were generated and visualized to investigate the interacting amino acid residues forming hydrogen and hydrophobic bonding among ligand and receptor. The top identified molecule Rutaecarpine formed three hydrogen bonds with Xdh amino acid residues Leu1244, Glu562, Gly1184, Phe561, Ser1186, Pro1189, Gly575, Val1564 and Pro565 whereas the amino acid residues such as Ser1185 also interacted through hydrophobic bonding (Figure 7).

Since earliest times, medicinal plants have wide acceptance due to a large number of reward such as slighter toxic effects, secure, effectual, despicable, less chances of recurrence of disease, easily available in rural areas. There is no proper medicine in allopathy for the treatment of urolithiasis, and allopathy drugs are having side effects. Furthermore the surgical treatment is another alternative but it has the more chances of reappearance. So, *M. uniflorum* is considered suitable for the treatment of kidney stones. The present review containing information of kidney stones and *M. uniflorum* used as antiurolithiasis agents, it will help in guiding the researcher to identify new source of drugs for this ever prevailing human ailment to overcome the various disadvantages faced by the wide range of population now-a-days and get relieve from the urolithiasis.

The findings of this study prove significant role of compounds present in horse gram in various human physiological disorders and summarizes a range of medicinal properties and nutritional efficiency of HG used to treat various diseases. HG was commonly used by farmer community and stumpy profits group people due to its unacceptable taste and flavor in earlier days (Vandarkuzhali and Sangeetha, 2015).

CONCLUSION

The collected horse gram seed sample from hills of Uttarakhand showing promising and surprising results in antilithiasis. The complete chemical composition was analyzed first time with LC MS and identified compound examine their nutraceutical properties in human health. Till date the molecular mechanism of antiurolithiatic activity of HG is not clearly known. The molecular docking analysis is clearly showing that the maximum compound functionally active to interrupt the formation of DHA during purine salvage pathway prevent the kidney stone construction in kidney. The present investigation is a leading for formulation of herbal drug to cure kidney stone. Further investigation may be lead to *in-vivo* examination the effect of dissolution of kidney stones by treatment of horse gram extract.

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