ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022

Intriguing Effect Of Pseudostem Exudate From *Musa Paradisiaca* (L.) Against Struvite Stone By Microscopic Analysis

Rajini R[1] and Dr. S.Sujatha[2]

[1]Research Scholar, Reg. No:18223082022001, Department of Biotechnology, Malankara Catholic College, Mariagiri-629153. Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, Tamilnadu, India.

[2]Research Guide, Department of Biotechnology, Malankara Catholic College, Mariagiri-629153. Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, Tamilnadu, India.

Corresponding Author: **Dr. S. Sujatha** Email: sujasukumar80@gmail.com

ABSTRACT

The current study was designed to evaluate the productive benefits of *Musa paradisiaca L.* pseudostem exudate against struvite stones. *Musa paradisiaca* Linn. is widely distributed throughout various tropical regions. Exudate (stem juice) of the pseudostem part of *M. paradisiaca* Linn.is highly beneficial and imperative naturally available medicine for human kidney. The present study was carried out to investigate the diminished effect of exudate on struvite stone. The *M. paradisiaca L.* Pseudostem Exudate reduced the crystal size and inhibited crystal growth. Because the experimental exudate possessed the biologically efficient secondary metabolites. Mainly, three different bioactive compounds were identified by GCMS technique such as Tricyclo[8.4.1.1(3,8)hexadeca-3,5,7,10,12,14-hexaene-2,9-dione,anti-, Olean-12-ene-3beta,28-diol and 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro. Besides the findings clearly showed that Olean-12-ene-3beta,28-diol biocompound contains medicinal property especially anti-urolithiatic effect against the struvite stone. Hence, the present research could be opined that *M. paradisiaca L. p*seudostem exudate might be used as healing agent for the treatment of urinary kidney stone disease.

Keywords: *Musa paradisiaca L.*, Pseudostem Exudate, GCMS, Struvite stones

INTRODUCTION

Banana an antique fruit crop known as “Apple of Paradise” plays an important and interesting roles in the history of human civilizations. All the parts of the plants are useful, even the hazardous waste pseudostem can also be used to make yarn, fabric, apparel as well as fertilizers, biochemicals, fish feed, paper, handicrafts, candy, pickles, etc. (Nuengchamnong *et al*., 2004; Mohiuddin *et al*., 2014). The parts of a banana plant including the pulp, leaves, and stem have a

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022**

pharmaceutical value in several countries Mohorcic *et al*., 2007). Exudates are mixtures of organic molecules oozed by plants, predominating, but not always as a result of the injury. Exudates contain high carbon and hydrogen atoms and are also commonly known as Bsap although the word Bsap is utilized to characterize any liquid that exodus inside plants. In contrast, the Bexudate^ word refers to any such compound when it is oozed out of the plant (Yadav *et al*., 2012). Decreasing the stones accumulation and dissolving the pre-formed stones was observed with the treatment by *Musa* stem juice (Vernwal *et al*., 2000). An important enzyme such as lignin peroxidase was included in *Musa paradisiaca* stem juice (Agarwal *et al*., 2009).Urolithiasis is a common urological disease. A large number of people are suffering from urinary stones problems. It is one of the oldest disease known to human beings. Urolithiasis are caused due to multi-factorial reasons. Renal colic presents as acute emergency in casualty with patients in severe pain (Shafi, *et al*., 2013). The formation of kidney stones is closely related to the crystallites in the urine (Daudona and Jungers, 2004; Verdesca *et al*., 2011; Ouyang *et al*., 2012). The agglomeration and growth of crystallites in urine are two main factors affecting the growth of urinary stone (Bareeva *et al*., 2008). Urine supersaturation leads to nucleation of insoluble salt (nanosized, generally <10 nm) and subsequent growth or aggregation of the crystal nucleus into crystallites of pathological size (microns or dozens of microns). After adhesion to the renal epithelial cells (Peng *et al*., 2012), urinary crystallites would gradually grow and finally form urinary stones (millimeter level, generally ranging from a few millimeters to a few centimeters) (He *et al*., 2010). Therefore, several studies believed that urolithiasis was more accurate to be predicted by crystalluria than 24 h urine volume or calcium excretion and even more accurate than urinary calcium and urinary oxalate concentration.

Urinary stones have been found to contain calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate or struvite crystals (Beghalia *et al*., 2008; Karki *et al*., 2022). Among the magnesium phosphates namely Ammonium Magnesium Phosphate Hexahydrate (AMPH) commonly known as Struvite. Magnesium Hydrogen Phosphate Trihydrate have also been reported to occur as constituents in renal calculi (Aggarwal *et al*., 2000) not only in adults but also in children (Griffith, 1978) Struvite calculi, found in 15– 20% of urinary calculi are mostly related to urinary tract infections with urolithic microorganisms in humans and animals (Hesse and Heimbach, 1999). Struvite is also known as triple phosphate stone, infection stone or urase stone. They are found more frequently in women and in persons older than 50 years (Ross *et al*., 1999) Urinary stones are characterized by high recurrence rate therefore requiring a preventive treatment by using the medicinal plants (Coe *et al*., 1992). Different sizes of urinary crystallites of struvite stone have been observed, such as crystallites with a size of 1 nm to 1000 nm (Gupta *et al*., 2016. Thus, the evaluations of the physicochemical properties of micro- and nanocrystallites in urines of struvite stone calculi patients and healthy controls may have potential clinical value because if the properties of urinary crystallites were reliable predictor of

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022

disease, such a method would represent a noninvasive method that could potentially by applied. controls to obtain differently sized (from nanometers to micrometers) urinary crystallites

Materials and Methods

All the chemicals, including analytical grade calcium oxalate and solvent were purchased from Sigma Aldrich.

Microscopic Observation:

The struvite crystals were analyzed using a Light Microscope (LM). For this analysis, samples were coated with a 5 nm thick glycerol in a sputter coater system. The conductive coating is important because it prevents the charging of the specimen surface.

Collection of plant material:

Musa paradisiaca pseudostem exudate was collected from Kuzhithurai region Kanyakumari District. Exudate was obtained from the pseudostem of the trees by making an incision on the barks with a sharp knife. The exudates were then sieved by 212 µm of sieve mesh and 500 ml was extracted and kept in a volumetric flask. Exudates (500 ml) were then separated in a beaker so as to observe any color change

Collection of urine sample: Twenty-four-hour urine specimens were collected from a healthy man, who had no history of kidney stone disease. The samples were refrigerated without preservative for the duration of the collection.

Study deprived of inhibitor (absence of plant extract):

A mixture of 150 ml of urine sample and 50 ml of 0.05 M calcium oxalate was taken in a beaker. After vigorous shaking, the solution was covered with a film and left undisturbed for 48 hrs. Crystals were centrifuged 3000 rpm for 30 min. The supernatant was discarded and the crystals were transferred to a specimen plate for SEM analysis. Study with inhibitor (with plant extract): A mixture of 150 mL of urine and 50 mL of 0.05 M calcium oxalate solution was taken in a beaker. After vigorous shaking, the solution was covered with a film and left undisturbed for 48 h. After 48 h, 50 mL of seed extract solution was added in the above mixture. The whole solution was covered with a film and left undisturbed for 24 h. Crystals were centrifuged 3000 rpm for 30 min. The supernatant was discarded and the crystals were transferred to a specimen plate for SEM analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out in a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min.

Other GC-MS conditions are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 16.2 psi; out time, 1.8 mm; and 1 µl injector in split mode with split ratio 1:50 with injection temperature of 300 °C. The column temperature started at 36 °C for 5 min and

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022

changed to 150 V at the rate of 4 °C/min. The temperature was raised to 250 °C at the rate of 20 °C/min and held for 5 min. The total elution was 47.5 min. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data.

Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *M. paradisiaca* L. Pseudostem Exudate fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NISTII).

RESULTS AND DISCUSSION

Struvite urinary stones and crystals form readily in the urine of animals and humans that are infected with some of the pathogenic bacterial producing organisms. They are potentiated by alkaline urine and high magnesium excretion (high magnesium/plant-based diets) (Zhao *et al*., 2021). Struvite readily forms in alkaline conditions where its constituent ions are present. In nature, it forms primarily in areas associated with organic matter decomposition, including guano deposits, basaltic caves, and marshlands. Similar conditions are found when human bladders are infected by urease-producing bacteria, when wastewater is treated (Karki and Leslie, 2021). Largest size of crystals (without extract), while Fig. 1a showed comparatively less number and smaller size of crystals (with extract). There were less and smaller particles with increasing concentrations of extract as shown in various microscopic view (Fig. 2a-c). The extract had dissociated (Fig. 2a and b) and dissolved (Fig. 2c) the Struvite stone crystal growth. The extract causes fewer numbers of crystals in solution (Fig. 2a), thereby reduced super saturation and the size of the particles. This property of the extract is, therefore, advantageous in preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in urinary tract. Further, the *M. paradisiaca* L. Pseudostem exudate was considered to be diuretic effects may also reduce stone development when total fluid intake and output increased and such effects have been attributed to several herbal preparations. Very similar results to this study were obtained by Daudona and Jungers, (2004).

Fig. 1(a-b): SEM images of urine sample along with struvite stone crystals (without plant extract)

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022

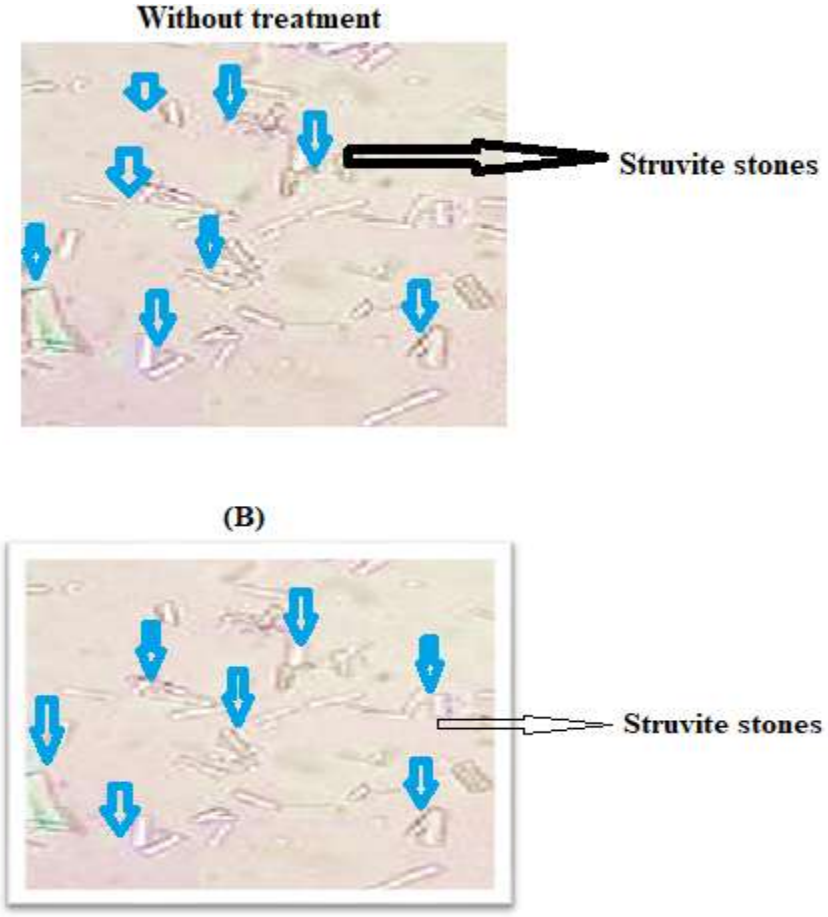
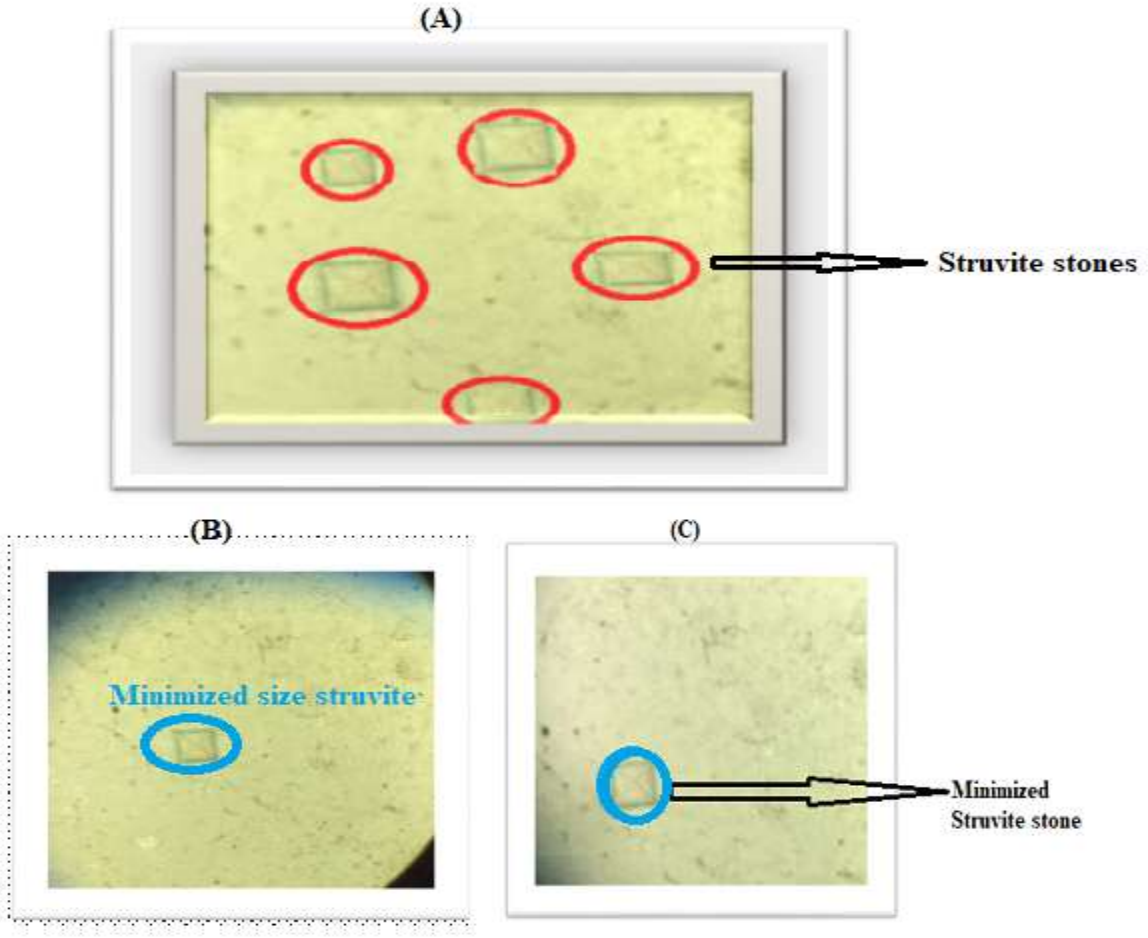


Fig. 2a,b,c). Microscopic images of urine sample with Exudate. Images clearly showed that, in the presence of *M. paradisiaca L.* Pseudostem Exudate the length and width of the struvite crystals were reduced,

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022



Plant extracts may contain substances that inhibit the growth of struvite crystals. The current study clearly showed that microscopic observation revealed that the extract visibly reduced the crystal size with significant decrease in number of the experimental stone of struvite (magnesium ammonium phosphate) is a phosphate mineral crystals. These results indicate that extract of *M.paradisiaca* is highly responsible for the inhibition of struvite growth. This could be beneficial in the prevention of stone formation. Not only will the formed minerals be smaller, but the consequence that these crystals might have a lower probability of aggregation shows that the crystals formed could more easily be removed by naturalpotential bioactive compounds from the experimental sample of *M. paradisiaca* L. Pseudostem Exudate. Medicinal plants are used for the prevention of kidney stones from a long time in different countries (Imam and Akter, (2011; Tsamo *et al.*, 2015). The struvite stone preventive efficiency mainly depends with the phytochemicals or bioactive compounds present in the experimental sample of *M. paradisiaca L.* Pseudostem Exudate. Apart from the present study showed that important bioactive compound such as Olean-12-ene-3beta, 28-diol indicated that antiurolithiatic effect

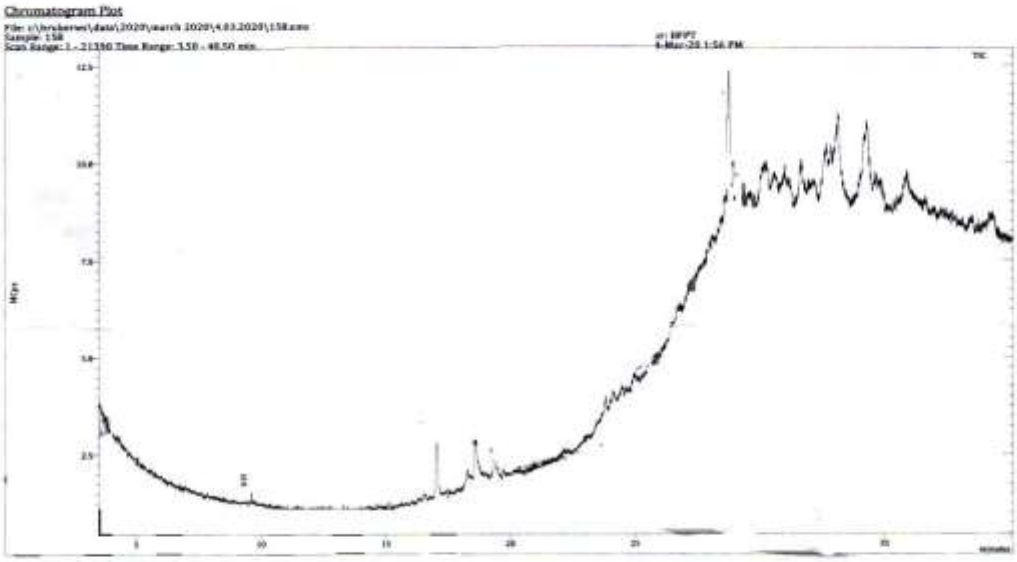
ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022

against the experimental styuvite kidney stone. From the GCMS result totally, three compounds were identified from the GC-MS study (Table 1 and Fig. 3). Furthermore, GC-MS study revealed the presence of various phytochemicals like Alkaloid, Cardiac glycoside, Flavonoid, Phlobotannins, Anthraquinone etc., (Table-1) may possesses a protective effect against development of calcium stones in the kidneys. This is precisly valuable for kidney stone affected patients. Since studies suggest that the biochemical pathways of a stone former will always promote stone formation but the presence of growth modifiers such as phytochemicals found in seeds of *M. paradisiaca L.* Pseudostem Exudate could be act as a natural potenial medicines as well as drug particularly struvite stone

Fig. 3:- GCMS Analysis of *M. paradisiaca L.* Pseudostem Exudate .

GC- MS/MS Chromatogram



ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022

Table-1: Bioactive compounds elluucidated from p*seudostem* Exudate of *M. paradisiaca* L

Sl. No	RT(min)	Name of the Compound	Molecular Formula	Molecular weight	Peak Area %
1	9.61	2H-Pyran,2-(7-heptadecynyloxy) tetrahydro-	$C_{22}H_{40}O_2$	336	7.08
2	18.62	Tricyclo[8.4.1.1(3,8)] hexadeca-3,5,7,10,12,14-hexaene-2,9-dione, anti-	$C_{16}H_{12}O_2$	236	72.45
3	19,35	Olean-12-ene-3,28-diol, (3β)-	$C_{30}H_{50}O_2$	442	20.48

Gas chromatography-mass spectroscopy profiling of aqueous fraction of *M. paradisiaca L. Pseudostem* Exudate. Total of 3 compounds were identified from the GC-MS analysis. It can exhibiting various phytochemical activities. The chromatogram is presented in Fig. 3. While the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), and concentration (%) are presented in Table 1. The following bioactive compounds were present in the GC-MS analysis carried on *Pseudostem Exudate* such as named as Tricyclo[8.4.1.1(3,8)]hexadeca-3,5,7,10,12,14-hexaene-2,9-dione, anti- and second bioactive compound named as Olean-12-ene-3beta,28-diol finally third compound as 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro, Similar result has been opined by (Chabuck,*et al*., 2013)

CONCLUSION

It is concluded that Microscopic observation is a necessary tool to identify and study several renal structures and their behavior as a consequence of the calculi removal, so that it is possible to supply important information for the surgical ways of intervention in order to prevent a deterioration. The *M. paradisiaca L.* Pseudostem Exudate diminished the crystal size and inhibited crystal accumulation. Hence the exudate contains proper quantity of several phytochemicals particularly this bioactive compounds named as Olean-12-ene-3beta,28-diol act as a better potential natural drug (without synthetic substance) against the kidney stone diseases. Hence, the present research could be advised that *M. paradisiaca L.* Pseudostem Exudate can be used as therapeutic agent for the treatment of urinary calculi or its anticipation.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022**

REFERENCES

Agarwal, P. K., Singh, A., Gaurav, K., Goel, S., Khanna, H. D., & Goel, R. K. (2009). Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* Var. *Paradisiaca*) in rats.

Aggarwal S, Tandon CD, Forouzanveh M, Simgla SK, Kiran R, Jethi RK. Role of biomolecules from human renal stone matrix on COM crystal growth. *Mol Cell Bio chem*. 2000; 210:109-19.

Bareeva, I. I. Turkovsky, L. M. Molodkina, B. A. Paramonov, K. P. Pomorsky, and A. B. Samatigo, "UHF-dielectrometry of the urine in prognosis of aggregation stability," Bulletin of Experimental Biology and Medicine, vol. 145, no. 4, pp. 433–435, 2008.

Beghalia M, Ghalem S, Allali H, Belouatek A, Marouf A. 2008.Inhibition of calcium oxalate monohydrate crystal growth using Algerian medicinal plants. J Med Plants Res.; 2(3):066-70

Chabuck, Z. A. G., Al-Charrakh, A. H., Hindi, N. K. K., & Hindi, S. K. K. (2013). Antimicrobial effect of aqueous banana peel extract, Iraq. Iraqi Journal of Pharmaceutical Sciences, 1, 73–75**.**

Coe FL, Parks JH, Asplin JR. The pathogenesis and treatment of kidney stones. New Engl J Med. 1992; 327:1141

Daudon, C. Hennequin, G. Boujelben, B. Lacour, and P. Jungers, "Serial crystalluria determination and the risk of recurrence in calcium stone formers," Kidney International, vol. 67, no. 5, pp. 1934–1943, 2005.

Daudona, M. and P. Jungers, "Clinical value of crystalluria and quantitative morphoconstitutional analysis of urinary calculi," Nephron: Physiology, vol. 98, no. 2, pp. p31–p36, 2004.

Griffith D.P. Struvite stones. Kidney Intl. 1978; 13:372- 382

Gupta, G. Gill, and R. Mahajan, "Possible role of elevated serum testosterone in pathogenesis of renal stone formation," International Journal of Applied and Basic Medical Research, vol. 6, no. 4, pp. 241– 244, 2016.

H. Shafi, Z. Shahandeh, B. Heidari. "Bacteriological study and structural composition of staghorn stones removed by the anatrophic nephrolithotomic procedure," *Saudi Journal of Kidney Diseases and Transplantation,* vol. 24, no. 2, pp. 418–423, 2013.

He, S.-P. Deng, and J.-M. Ouyang, "Morphology, particle size distribution, aggregation, and crystal phase of nano crystallites in the urine of healthy persons and lithogenic patients," IEEE Transactions on Nanobioscience, vol. 9, no. 2, pp. 156–163, 2010.

Hesse A, Heimbach D. Causes of phosphate stone formation and the importance of metaphylaxis by urinary acidification: a review. World J Urol. 1999; 17:308

Imam, M. Z., & Akter, S. (2011). Musa paradisiaca L. and Musa sapientum L: a phytochemical and pharmacological review. Journal of Applied Pharmaceutical Science, 1, 14–20.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper, **UGC CARE Listed (Group -I) Journal Volume 11, Iss 8 , 2022**

Karki, Niraj; Leslie, Stephen W. (2022), "Struvite and Triple Phosphate Renal Calculi", *StatPearls*, Treasure Island (FL): StatPearls Publishing, 01-16

M. Ouyang, Z.-Y. Xia, G.-N. Zhang, and H.-Q. Chen, "The nanocrystallites in urine and its relationship with formation of kidney stones," Reviews in Inorganic Chemistry, vol. 32, no. 2–4, pp. 101–110, 2012.

Mohiuddin AKM, Manas Kanti Saha, Md. Sanower Hossian and Aysha Ferdoushi. Usefulness of Banana (*Musa paradisiaca*) Wastes in Manufacturing of Bio-products: A Review. A Scientific Journal of Krishi Foundation, 2014; 12(1):148-158.

Mohorcic, M., Friedrich, J., Renimel, I., Andre, P., Mandin, D., & Chaumont, J. P. (2007). Production of melanin bleaching enzyme of fungal origin and its application in cosmetics. Biotechnology and Bioprocess Engineering, 12, 200–206.

Nuengchamnong N, Lokkembol AH, IngKanian H. Separation and detection of the antioxidant flavonoids, rutin and quercetin, using HPLC coupled on-line with colorimetric detection of antioxidant activity. Naresuan University Journal, 2004; 12(2):25-37.

Peng, J.-M. Ouyang, X.-Q. Yao, and R.-E. Yang, "Interaction between sub-micron COD crystals and African green monkey renal epithelial cell," International Journal of Nanomedicine, vol. 7, no. 8, pp. 4727–4737, 2012.

Ross SJ, Osborne CA, Lulich JP, Polzin DJ, Ulrich LK, Koehler LA Canine and feline nephrolithiasis. Epidemiology, detection, and management. Vet Clin North Am (Small Anim. Pract. 1999; 29:231

Tsamo, C. V. P., Herent, M., Tomekpe, K., Emaga, T. H., QuetinLeclercq, J., Rogez, H., Larondelle, Y., & Andre, C. (2015). Phenolic profiling in the pulp and peel of nine plantain cultivars (*Musa* sp.). Food Chemistry, 15, 197–204.

Verdesca, G. B. Fogazzi, G. Garigali, P. Messa, and M. Daudon, "Crystalluria: prevalence, different types of crystals and the role of infrared spectroscopy," *Clinical Chemistry and Laboratory Medicine*, vol. 49, no. 3, pp. 515–520, 2011.

Vernwal, S. K., Yadav, R. S., & Yadav, K. D. (2000). *Musa paradisiaca* stem juice as a source of peroxidase and ligninperoxidase. Indian Journal of Experimental Biology, 38,1036–1040.

Yadav, P., Singh, V. K., Yadav, M., Singh, S. K., Yadava, S., & Yadav, K. D. (2012). Purification and characterization of Mnperoxidase from *Musa paradisiaca* (banana) stem juice. Indian Journal of Biochemistry & Biophysics, 49, 42–48.

Zhao, Tian-Lei; Li, Han; Jiang, Hao-Fan; Yao, Qi-Zhi; Huang, Ying; Zhou, Gen-Tao (December 2021). *"Morphogenesis and evolution mechanisms of bacterially-induced struvite"*. Scientific Reports. **11** (1): 170.