Growth and Characterization of Calcium Hydrogen Phosphate Dihydrate Urinary Stone and Inhibitory Effect of Juice And Seed of *Vitus vinifera*

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Abstract

Urolithiasis is a global problem afflicting human beings for several centuries. Kidney stone disease is a crystal concretion formed typically within the kidneys. It is a growing urological disorder of human health, affecting about 12% of the world population. Urinary stones have been found to contain calcium phosphate, calcium oxalate, uric acid and magnesium ammonium phosphate with apatite and struvites predominating. Calcium-containing stones are the most common variety of urinary stone, and it comprise about 75% of all urinary calculi, which are found in the form of pure calcium oxalate (50%), calcium phosphate (5%) or a mixture of both (45%). Urinary stones are characterized by its high recurrence rate, if patients are not treated appropriately. Among the treatments used are surgical removal, percutaneous techniques based on laproscopic and Extracorporeal Shock Wave Lithotripsy (ESWL) and drug treatment. Besides, these treatments cause undesirable side effects such as hemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation. Patients affected with kidney-stone forming are prone to its recurrence even after its surgical removal. From the above facts it is clear that there is a need to the study herbal plants for the treatment of urinary stones because of their efficacy, safety, lesser side effects and better compatibility with human body. Herbals presently available for the treatment of kidney stones are Vitus vinifera. To study the effects of aqueous extract of fruits and seeds of Vitus vinifera which are used as additives to induce the nucleation and growth of CHPD crystals by single diffusion gel growth technique under in vitro condition. Phytochemical compounds such as steroids, terpenoids, alkaloids, phlobatanin, flavonoids, saponins, tannins, coumarins, proteins, carbohydrates and glycosides were screened in aqueous extracts of Vitus vinifera. The fruits and seeds of Vitus vinifera extracts can promote the formation of hydroxyapatite crystals. crystals treat urinary stone by inhibiting the formation of CHPD crystals, a major component of calcium urinary stone under in vitro conditions. This research provides a multidisciplinary approach in characterizing urinary stone CHPD crystals grown in vitro and in vivo to help, formulate prevention or dissolution strategies in controlling calcium urinary stone growth. This research is also focused to find new alternative medicine Vitus vinifera for the treatment of calcium phosphate urinary stone. Therefore, the purpose of this research is to investigate the beneficial effects of *Vitus vinifera*at a different dose and single compound for the prevention of kidney stone formation.

Keywords: Extracorporeal Shock Wave Lithotripsy, *Vitus vinifera*, urinary stone, Urolithiasis.

Introduction

Kidney stones have been associated with an increased risk of chronic kidney diseases (K.Sigurjonsdottir et al.,2015), end-stage renal failure (Mikawlrawng K et al.,2014 and Z.M.El-Zoghby,J et al ., 2012), cardiovascular diseases (A. D. Rule et al.,2010 and E. M. Worcester and F. L. Coe,2008), diabetes, and hypertension (Taylor E. N. et al.,2005). It has

been suggested that kidney stone may be a systemic disorder linked to the metabolic syndrome. Nephrolithiasis is responsible for 2 to 3% of end-stage renal cases if it is associated with nephrocalcinosis (Courbebaisse M et al.,2017). The treatments cause undesirable side effects such as hemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation. The kidney stone forming patients are prone to its recurrence even after its surgical removal. Recurrence rates are close to 50%, and the cost of urolithiasis to individuals and society is high (Sutherland et al., 1985)

Treatment and removal of renal calculi is often dependent upon the composition and hardness of the stone, as well as stone size and placement in the urinary tract.Though the main components of renal stones tend to be calcium oxalate, calcium phosphate (hydroxylapatite), magnesium ammonium phosphate and uric acid. Intractable renal stones are treated through several different techniques, two of the more well-known methods being extracorporeal shockwave lithotripsy (ESWL) and surgical removal (Dietrich et al., 1990). Recent concerns as to the long term side-effects of lithotripsy and the damage caused to the renal system. Current research indicates that in addition to soft tissue damage, the development of brushite renal stones is directly related to treatment by ESWL, where HAP, COM, or UA stones are removed but brushite stones are formed in their place (Pearle et al., 1999). The ethanolic extract of Asparagus racemosus is more effective on lithiasis induced by 0.75% ethylene glycolated water to adult male albino wistar rats and also reduced the elevated level of calculogenic ions in urine and urinary concentration of magnesium . (Christina et al., 2005, Joseph et al., 2005 and Gambaro G et al.,2006). A reduced intake of calcium leads to an increased intestinal absorption of oxalate, which itself may account for an increased risk of stone formation. Calcium supplements may reduce oxalate absorption because the calcium binds dietary oxalate in the gut lumen. However, the benefit of taking calcium pills is controversial. Vitamin C has been implicated in stone formation because of in vivo conversion of ascorbic acid to oxalate. Therefore, a limitation of vitamin C supplementation is recommended (Park S and Pearle M.S, 2007)Urinary stone is characterized by high recurrence rate therefore requiring a preventive treatment by using medicinal plants or phytotheraphy. From the above facts it is clear that there is a need to study herbal plants for the treatment of urinary stones. Moreover, it is also beneficial for mankind for its efficacy, safety and quality (Terlecki et al., 2007, Yongtai et al., 2008). *V. vinifera* and its bioactive compounds have several pharmacological activities such as antioxidative, anti-inflammatory and antimicrobial activities, as well as in vitro activity against several can- cer cell lines and hepatoprotective and cardio protective effects. It seems that grape seed extract and its active components such as proanthocyanidins, resveratrol, and quercetin are potent antioxidants. The consumption of grapes and grape juice islikelytohave positive effects on human health and especially in postmenopausal women. These results suggest that grape seeds and their active components should be studied in more detail for development as agentsto assist in the treatment of cardiovascular, gastrointestinal and neurodegenerative diseases (MarjanNassiri et al., 2009)

Fruits of *Vitisvinifera* have been used for thousands of years because of their nutritional and medicinal benefits.They are rich in sugars, flavonoids, anthocyaninsandproanthocyanins, organic acids, tannin, mineral salts and vitamins. Grapes skin, especially from the red and black species is rich in resveratrol which is a derivative of stilben. Studies have shown that resveratrol is one of the strongest known natural antioxidants. It is found in a large quantity in

black grape juice, skinand seed (Ruaaazizjassim et al., 2010). Identification of medicinal plants for the treatment of kidney and urinary stones. Kidney stones are the third most common urinary tract problems after urinary tract infections and prostate pathology. Kidney stones may cause extreme pain and blockage of urine flow. Medicinal herbs are used in different cultures as a reliable source of natural remedies. This study determine to native medicinal plants used by traditional healers of Shiraz for the treatment of kidney stones. A total of 18 species belonging to 19 botanical families were recorded in study area. Species with the highest frequency of mentions were Alhagimaurorum (51.58%), Tribulusterrestris (51.58%), and Nigella sativa (48.14). The most frequently used plant parts were aerial parts (38%), leaf (33%) and fruits (17%). Decoction (68%) was the most frequently prescribed method of preparation. Most of the medicinal plants recommended by Shirazian herbalists have not been investigated in animal and humane models of renal stone which provides a new area of research (Mahmoud Bahmani et al., 2016). Kidney stone disease is a crystal concretion formed usually within the kidneys. It is an increasing urological disorder of human health, affecting about 12% of the world population. It has been associated with an increased risk of end-stage renal failure. The mechanism of stone formation is a complex process which results from several physicochemical events including super saturation, nucleation, growth, aggregation, and retention of urinary stone constituents with in tubular cells. These steps are modulated by an imbalance between factors that promote or inhibit urinary crystallization. It is also noted that cellular injury promotes retention of particles on renal papillary surfaces. The exposure of renal epithelial cells to oxalate causes a signaling cascade which leads to apoptosis by p38 mitogen-activated protein kinase pathways. Currently, there is no satisfactory drug to cure and/or prevent kidney stone recurrences. Thus, further understanding of the pathophysiology of kidney stone formation is a research area to manage urolithiasis using new drugs(TilahunAlelign et al., 2018)

Materials and methods

Collection of plant materials

The medicinal plant parts used in this study are fruits and seeds of *Vitus vinifera*collected from Trichy district, Tamilnadu, India. (december 2018) and identified at Rapinat herbarium, St. Joseph College, Tiruchirapalli, Tamil Nadu, India.

Preparation of aqueous extracts of seed

The seeds of *vitusvinifera* were air-dried at room temperature (37°C) for 2 weeks, after which they were ground to a uniform powder of 40 mesh size. The aqueous extracts were prepared by soaking 100 g of the dried powder plant materials in 1 L of aqueous by using a soxhlet extractor for 10 hr continuously. The extracts were filtered through whatman filter paper No. 42 (125mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure. The obtained residue 1.2 g (seeds) was used to prepare the series of (1%, 2%, 3%, 4% and 5%) aqueous supernatant concentrations for *in vitro* studies (table 1).

Preparation of aquevous extracts of juice

The fruits of *vitusvinifera*werecollected and the seeds are collected. It was ground to a uniform mixture.The aqueous extracts were prepared by soaking 100 ml of mixture in 1 L of aqueous by using a soxhlet extractor for 10 hr continuously. The extracts were filtered through whatman filter paper No. 42 (125mm).The filtered extract was concentrated. The obtained juice

used to prepare the series of (1%, 2%, 3%, 4% and 5%) aqueous supernatant concentrations for *in vitro* studies (table 2).

Phytochemical screening: VariousPhytochemical screenings such as Tannins, Phlobatannins, Saponins, Flavanoids, Steroids Terpenoids, Cardiac Glycosides, Leucoanthocyanin, Anthocyanins, Anthroquinone, Proteins, Coumarins, Glycosides, Phenols, Alkaloids Xanthoproteins, Emodin, Carbohydrates were performed separately in seed and juice extracts of *Vitus vinifera* using standard procedures **(Table 2)(Yadav M *et al*., 2014).**

Qualitative Analysis (Flavonoid, Tannin, Saponin, Alkaloid, Phenol, Terpenoid) were performed separately in Aqueous Extract of Grape Seed And Grape Juice of *Vitus Vinifera* using standard procedures

Screening Of Antibacterial Activities:

Test organism:

The extracts of medicinal plants were tested on the following selected pathogen which includes one gram positive bacteria strains *Staphylococcus aureus*(MTCC 25923) and one gram negative bacteria strains *Escherichia coli* (MTCC 25922).All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

Antibacterial activities of crude extract (disc diffusion method)

Antibacterial activity of crude extracts was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude extract at various concentrations of 50-250 mg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters.

Screening of Antifungal Activities:

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Inoculum

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal strains used

The clinical fungal test organisms used for study are *Candida albicans* ATCC 10231 and *Aspergillusflavus* ATCC 16404 were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Determination of antifungal activity

Antifungal activity of crude extracts was determined using the disc diffusion method Thepetridishes (diameter 60 mm) was prepared with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of crude extract at various concentrations of 50-250 mg/ml respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for

compound diffusion. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters.

***In-vitro* growth of urinary calculi**

Growth of CHPD crystals

Glass test tubes were used as crystallization apparatus and the single diffusion reaction technique was employed (Vimal*et al*., 2005). One of the reactants, 1 M Orthophosphoric acid, was mixed with Sodium metasilicate solution of density of 1.04g/cm^3 at pH9.4, so that the pH of the mixture was maintained at 6 and left undisturbed for 2-3 days. After gelation took place, the supernatant solution of 1 M Calcium chloride was gently poured onto the set gel in various test tubes. After adding supernatant solution on the set gel, the test tubes were capped with airtight stopples. The experiments were conducted at room temperature (37°C).

The nomenclature of different additive solution on the growth of crystals

An attempt was made to investigate the putative activity of the plant extract as inhibitors of CHPD crystal formation in gel method. The supernatant solutions as given in (Table 1) were added to the set gels and the results were noted. To study the effect of the aqueous extract of seed and juice of *Vitus vinifera* on the growth of CHPD crystals, a series of five different concentrations of 1, 2, 3, 4 and 5% of the plant extract and fractions isolated using column chromatography were added in equal amounts to the supernatant solution and the average weight of the grown crystals were measured.

Calculation of the percentage of inhibition (1%) was based on the formula:

1%= [(TSI-TAI)] /TSI) x 100

TSI represents the number of crystals without inhibitors and TAI,the number of crystals after addition of inhibitors (Beghalia*et al*., 2007).

Results and Discussions

Comparative qualitative analysis of aqueous extract of seed and juice of *Vitus vinifera*

The present study carried out on the extract of ***Vitus vinifera***revealed the presence of medicinal active constituents (Table.2 & Figure.2, 3). The phytochemical active compounds were qualitatively analyzed for seed and juice of ***Vitus vinifera***. Presence of different phytochemical compounds whereas tannins, flavonoids, phlobatannins, saponin, terpenoids, cardic glycosides, leucoanthocyanin, anthocyanins, anthraquinone, coumarin, glycosides, phenol, xanthoprotein, steroids, protein, emodine, alkaloid and carbohydrates were analysed in aqueous extract of seed and juice of ***Vitus vinifera***

The aqueous extract of seeds of ***Vitus vinifera*** indicated the presence of tannins, flavonoids, phlobatannins, saponin, terpenoids, cardic glycosides, anthocyanins, anthraquinone, coumarin, glycosides, phenol, xanthoprotein, emodine, steroids alkaloid and carbohydrates and absence of leucoanthocyanin and protein (Table.2& Figure2).

The aqueous extract of juice of ***Vitus vinifera*** indicated the presence of tannins, flavonoids, saponin, terpenoids, cardic glycosides, phenol, xanthoprotein, phlobatannins, steroids, leucoanthocyanin, alkaloids and absence of protein, anthocyanins, anthraquinone, coumarin, emodine, glycosides.(Table 2 & Figure 3).

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties.

Alkaloids are a class of naturally occurring organic compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. The terpenoids sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from terpenes. Most are multicyclic structures with oxygen-containing functional groups. About 60% of known natural products are terpenoids. Terpens are hydrocarbons. Flavonoids are widely distributed in plants, fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/ blue pigment. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentations.Glycoside is an organic compound, usually of plant origin, that is composed of a sugar portion linked to a non-sugar moiety. A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration.

A carbohydrate is a bio molecule consisting of carbon, hydrogen and oxygen atoms. The carbohydrates are hydrates of carbon structurally it is more accurate to view them as aldoses and ketoses. Phlobaphenes are reddish, alcohol- soluble and water- insoluble phenolic substances. They can be extracted from plants. Phlobaphens can be formed under action of acids or heating of condensed tannins or of the fraction of tannins called phlobatannins.

Tannins are generally defined as soluble, astringent complex phenolic substances of plant origin used in tanning of animal skins or precipitation of proteins. Tannins are chemically defined as phenylpropanoid compounds. Phenolic compounds are chemically defined as compounds containing hydroxylated aromatic rings.

Tannins and phenolic compounds are widely distributed secondary metabolites in plants and play a prominent role in general defense strategies of plant. Xanthoproteic comes from the Greek word xanthos, which means yellow. The intensity of the yellow colour deepens when the reaction occurs. Coumarin is a aromatic organic chemical compound in the benzopyrone chemical class. It is a natural substance found in many plants, and a colourless crystalline substance. Anthraquinone also called anthracenedione or dioxanthracene is a aromatic organic compound. It is a building block of many dyes and is used in bleaching pulp for paper making. Cardiac glycosides are a class of organic compounds that increase the output force of the heat and increase its rate of contractions by acting on the cellular sodium-potassium ATPase pump.

Quantitative analysis:

Quantitative anlaysis of important phytochemicals in the medicinal plant of *Vitus vinifera*contain these phytochemicals in varying amounts in the seed and juice. The phytochemical with the highest quantity was alkaloids followed by saponin, flavonoids, phenol, tannin and terpenoids respectively, as shown in (table 3).

Effect of *Vitus vinifera*on CHPD crystals of both samples

The effect of the methanol extract of seed and juice extract of *Vitus vinifera* on nucleation and crystallization characteristics of CHPD crystals is determined by measuring the weight of the formed crystals. The control using pure calcium chloride led to the nucleation of crystal growth

within 24 h of adding the supernatant solutions. The Liesegang ring was observed after 48 h of pouring the supernatant solution. The formation of Liesegang (5-10 rings) rings which have promoted crystals growth Figure (7, 11) However, at the same time the first few Liesegang rings started diffusion. The distance between two consecutive Liesegang rings was found to be increased towards bottom of the test tubes. The elongated broad needle shaped crystals were grown within the Liesegang ring as observed after 96 h. In the presence of aqueous extracts, nucleation was delayed and reduced masses of the crystals were observed after adding the supernatant solutions Figure (7, 11). The Liesegang rings formation was reduced after the addition of aqueous extract of seed of *Vitus vinifera*exhibited an inhibitive effect compared to control (pure calcium chloride), and a minimumapparent length of growing crystals was observed. Morphology of the harvested control CHPD and treated *Vitus vinifera*CHPD crystals as shown in Figure (7,11). With an increase in the concentration of aqueous extracts of juice from 1% to 5% (w/v), (Table 6).

Characterization of CHPD crystals

Grape seed

The FTIR spectra of CHPD crystals obtained in the presence and absence of the methanol extract of sample. In Fig.10 4a, the absorptions at 3009 cm^{-1} are due to intermolecular and weakly H bonded OH because of water of crystallization. The weak absorption at 2029 cm^{-1} is due to HPO_4^{2-}. The H-O-H bending gives rise to absorption at 1673 cm^{-1}. The absorption at 1114 $cm^{-1}$are due to P=O associated stretching vibrations. The P-O-P asymmetric stretching vibrations give rise to absorption at 988, 874 cm^{-1}. The absorption at 615 cm^{-1} is due to (H-O-) P=O. However, the strong absorption at 568 and 514 cm^{-1} are again due to acid phosphate. In (fig. 10 b), the absorptions at 3016 cm^{-1} are due to intermolecular and weakly H bonded OH because of water of crystallization. The week absorption at 2023 cm^{-1} is due to HPO_4^{2-}. The H-O-H bending give rise to absorption at 1411 cm^{-1}. Whereas, the absorption at 1117 cm^{-1} is due to P=O stretching vibrations. The P-O-P asymmetric stretching vibrations give rise to absorption at 989, 874 cm^{-1}. The absorption at 788 cm^{-1} is due to (H-O-)P=O. However, the strong absorption at 570 $cm^{-1}$are again due to acid phosphate. In (fig. 10c), the absorption at 3012 cm^{-1} is due to OH ions. The absorption at 1117 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 875 and 784 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 615, 570 and 517 $cm^{-1}$are again due to acid phosphate. In (fig. 10d), the absorption at 3012 cm^{-1} is due to OH ions. The absorption at 1112 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 873 and 769 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 603, 551 cm^{-1} are again due to acid phosphate. In (fig. 10e), the absorption at 3008 cm^{-1} is due to OH ions. The absorption at 1113 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 990, 773 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 608, 549 cm^{-1} are again due to acid phosphate. In (fig. 10f), the absorption at 3008 cm^{-1} is due to OH ions. The absorption at 1117 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 875 and 789 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 611, 568 cm^{-1} are again due to acid phosphate. In (fig. 10g), the absorption at 3008 cm^{-1} is due to OH ions. The absorption at 1117 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 875 and 789 cm^{-1} are due to P-O-P asymmetric stretching vibrations. In (fig. 10h), the absorption at 3002 cm^{-1} is due to OH ions. The absorption at 1115 cm^{-1} is due to

PO_4 stretching vibrations. Whereas, the absorption at 990, 872 and 782 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 573 $cm^{-1}$are again due to acid phosphate.

Characterization of CHPD

Grape juice

The FTIR spectra of CHPD crystals obtained in the presence and absence of the methanol extract of sample. In (Fig.14 4a) the absorptions at 3009 cm^{-1} are due to intermolecular and weakly H bonded OH because of water of crystallization. The weak absorption at 2029 cm^{-1} is due to HPO_4^{2-}. The H-O-H bending gives rise to absorption at 1673 cm^{-1}. The absorption at 1114 $cm^{-1}$are due to P=O associated stretching vibrations. The P-O-P asymmetric stretching vibrations give rise to absorption at 988, 874 cm^{-1}. The absorption at 615 cm^{-1} is due to (H-O-) P=O. However, the strong absorption at 568 and 514 cm^{-1} are again due to acid phosphate. In (fig. 14b), the absorptions at 3016 cm^{-1} are due to intermolecular and weakly H bonded OH because of water of crystallization. The week absorption at 2023 cm^{-1} is due to HPO_4^{2-}. The H-O-H bending give rise to absorption at 1411 cm^{-1}. Whereas, the absorption at 1117 cm^{-1} is due to P=O stretching vibrations. The P-O-P asymmetric stretching vibrations give rise to absorption at 989, 874 cm^{-1}. The absorption at 788 cm^{-1} is due to (H-O-)P=O. However, the strong absorption at 570 $cm^{-1}$are again due to acid phosphate. In (fig. 14c), the absorption at 3012 cm^{-1} is due to OH ions. The absorption at 1117 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 875 and 784 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 615, 570 and 517 $cm^{-1}$are again due to acid phosphate. In (fig. 14d), the absorption at 3009 cm^{-1} is due to OH ions. The absorption at 1112 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 873 and 769 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 603, 551 cm^{-1} are again due to acid phosphate. In (fig. 14e), the absorption at 3008 cm^{-1} is due to OH ions. The absorption at 1113 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 990, 773 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 608, 549 cm^{-1} are again due to acid phosphate. In (fig. 14f), the absorption at 3013 cm^{-1} is due to OH ions. The absorption at 1117 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 875 and 789 cm^{-1} are due to P-O-P asymmetric stretching vibrations. The absorption at 611, 568 cm^{-1} are again due to acid phosphate. In (fig. 14g), the absorption at 3012 cm^{-1} is due to OH ions. The absorption at 1117 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 989, 875 and 789 cm^{-1} are due to P-O-P asymmetric stretching vibrations. In (fig. 14h), the absorption at 3002 cm^{-1} is due to OH ions. The absorption at 1115 cm^{-1} is due to PO_4 stretching vibrations. Whereas, the absorption at 990, 872 and 782 cm^{-1} are due to P-O-P asymmetric stretching vibrations.The absorption at 573 cm^{-1} are again due to acid phosphate.

Conclusion:

Kidney stone disease is a common urinary stone disorder in humans and often causes serve pain, which may lead to emergent hospitalization, shock wave lithotripsy and surgery. Calcium oxalate is a major component if it is kidney stone. It forms upon the supersaturation of the urine with calcium and other salts especially oxalate. The size of the stone can increase and obstruct in the urinary system. Although the most effective treatment of kidney stone is extracorporeal shock wave lithotripsy, the side effects of this method are grave and can lead to recurrence of kidney stones. Therefore alternative treatments are of high interest means by using herbal medicines.

The use of herbal medicines is now wide spread for the treatment of various diseases and disorders. In this regards, this research work focuses on the most common and prevailing disorder, kidney stone and its treatment using herbal extracts from a traditionally used herbal plants *Vitus vinifera*(Seed and juice).Through our ongoing research in this area we found that this plant *Vitus vinifera*contain antiurolithiatic activity related compounds to solve the complicated problems of kidney stone was isolated, purified and characterized as discussed in chapters of this thesis.

Hence, the most common urinary stones CHPD crystals have been selected for *in-vitro* study to assess the inhibition effect of the seeds of *Vitus vinifera*by single diffusion gel growth technique. With an increase in the concentration of aqueous extracts of seed and juice of *Vitus vinifera* from 1% to 5% (w/v), the weight of the formed crystals were gradually reduced.

The formation of hydroxyapatite was observed in Brushite crystals formb due to inhibitory action by the aqueous extracts of *Vitus vinifera*under *in-vitro* conditions .This research provides a multidisciplinary approach in characterizing and controlling urinary stones; CHPD crystals grown *in vitro* and *in vivo* to help, formulate prevention or dissolution strategies in controlling calcium urinary stone growth. This research has also found new alternative herbal plant *Vitus vinifera* for the treatment of CHPD urinary stone.

Table 1: Supernatant solutions added separately to the set gels for CHPD crystals of seed and juice of *Vitus vinifera*

Crystals	**Supernatant Solutions (Groups and Treatments)**	**Compositions**
CHPD	I (control)	10 mL of 1M $CaCl_2$
	II(control+ distilledwater)	5 mL of 1M $CaCl_2$ + 5 mL of distilled water
	III(1%aqueous extract)	5 mL of 1M $CaCl_2$ + 5 mL of 1% of aqueous extract of *Vitus vinifera*
	IV(2%aqueous extract)	5 mL of 1M $CaCl_2$ + 5 mL of 2% of aqueous extract of *Vitus vinifera*
	V(3%aqueous extract)	5 mL of 1M $CaCl_2$ + 5 mL of 3%of aqueous extract of *Vitus vinifera*
	VI(4%aqueous extract)	5 mL of 1M $CaCl_2$ + 5 mL of 4% of aqueous extract of *Vitus vinifera*
	VII(5%aqueous extract)	5 mL of 1M $CaCl_2$ + 5 mL of 5% of aqueous extract of *Vitus vinifera*

Statistical analysis

The masses of the crystals (gm) are presented as the mean ±standard deviation for the control and treatment samples. One-way analysis of variance (ANOVA) followed by tukey's test for multiple comparisons were made between groups. Values of $p<0.05$ was considered to be significant.

TABLE 2: **COMPARITIVE AND QUALITATIVE ANALYSIS OF SEED AND JUICE EXTRACT OF *Vitus vinifera***

S.NO	PHYTOCHEMICAL ANALYSIS	Grape seed	Grape juice
1.	Tannins	+++	++
2.	Phlobatannin	+	+++
3.	Saponin	+++	++
4.	Flavonoid	++	+
5.	Steroids	+++	+
6.	Terpenoids	+++	+++
7.	Cardiac glycosides	+++	+
8.	Leucoanthocyanin	_	+++
9.	Anthocyanin	+++	++
10.	Anthraquinones	++	+
11.	Proteins	_	_
12.	Coumarin	++	_
13.	Glycosides	+++	_
14.	Phenol	+++	+++
15.	Xanthoprotein	+++	_
16.	Alkaloid	+++	++
17.	Emodin	++	_
18.	Carbohydrates	+++	+++

NOTE: (+++ strong, ++ mordant, +slightly, -absent)

TABLE.3: Quantitative analysis of aqueous extract of seeds and juice of *Vitus vinifera*

S.NO	PHYTOCHEMICAL CONSTITUENTS	*Vitus vinifera* (mg/g)	
		Seed extract	Juice extract
1.	Flavonoids	1.344	0.026

2.	Tannin	0.141	0.019
3.	Alkaloids	0.004	0.021
4.	Saponin	0.009	0.020
5.	Terpenoids	0.009	0.009
6.	Phenol	0.011	0.026

ANTIMICROBIAL ACTIVITY

Table 4: Antibacterial activity of sample. (figure 5)

Plant extracts	Concentrations (mg/ml)	Organisms/Zone of inhibition (mm)			
		Grape seed juice	Grape Juice	Grape seed	Grape
		Escherichia Coli	*Escherichia coli*	*Staphylocousaureus*	*Staphylocousaureus*
Extracts	50	0	0	0	0
	100	9	8	7	9
	150	10	8	8	10
	200	11	10	9	11
	250	12	11	10	12
Ethanol	10 µl/disc	0	0	0	0

Table 4 shows results of the antibacterial susceptibility test of the different plant extracts and against the test organisms. From the result, Sample 1extracts were the most effective and the highest activity was demonstrated against *Escherichia coli* (12 mm zone of inhibition) at 250 mg\ml, followed by the highest activity of other sample 2 plant extracts against *Escherichia coli* (11 mm zone of inhibition) at 250 mg\ml) and sample 5against *Staphylococcus aureus*(12 mm zone of inhibition) at 250 mg\ml.

Table 5: Antifungal activity of sample.(figure 6)

Plant extracts	Concentrations (mg/ml)	Organisms/Zone of inhibition (mm)			
		Grape seed juice	Grape juice	Grape seed	grape
		Candida albicans	*Candida albicans*	*Aspergillusflavus*	*Aspergillusflavus*
Extracts	50	0	0	0	0
	100	0	0	0	0
	150	9	0	0	0
	200	10	9	0	8
	250	12	11	9	10
Ethanol	10 µl/disc	0	0	0	0

Table 5 shows results of the antifungal susceptibility test of the different plant extracts and against the test organisms. From the result, Sample 1extracts were the most effective and the highest activity was demonstrated against *Candida albicans* (12 mm zone of inhibition) at 250 mg\ml, followed by the highest activity of other sample 2 plant extracts against *Candida albicans*(11 mm zone of inhibition) at 250 mg\ml). and sample 5against *Aspergillusflavus* (10 mm zone of inhibition) at 250 mg\ml.

Supplementary

The percentage of inhibition of CHPD crystals by medicinal plants are shown in (Table 6).

TABLE: 6 INHIBITION OF CRYSTAL GROWTH

Crystals	Supernatant Solutions (Groups and Treatments)	Weight of crystal		Percentage of inhibition of crystal	
		juice extract	seed extract	Juice Extract	seed extract
CHPD	I	0.084	0.084	0%	0
	II	0.062	0.062	26.19 %	26.19 %
	III	0.045	0.050	46.42 %	40.47 %
	IV	0.030	0.042	64.28 %	50.00 %
	V	0.028	0.031	66.66 %	63.09 %
	VI	0.021	0.028	75.00 %	66.66 %
	VII	0.009	0.019	89.28 %	77.38 %

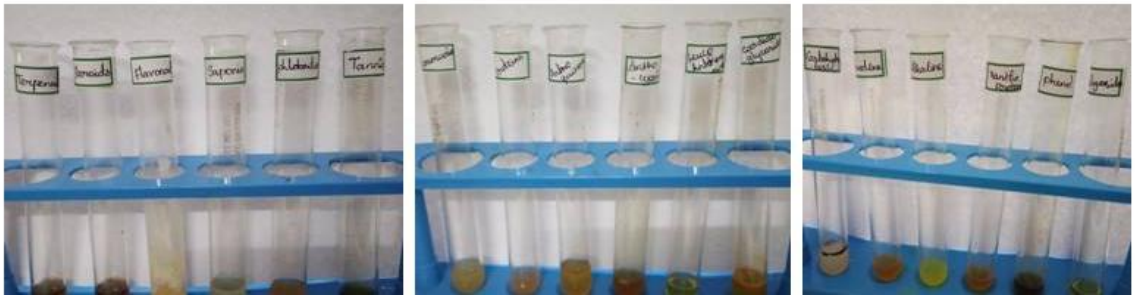


Figure.2: Qualitative analysis of aqueous extract of seeds of *Vitus vinifera*

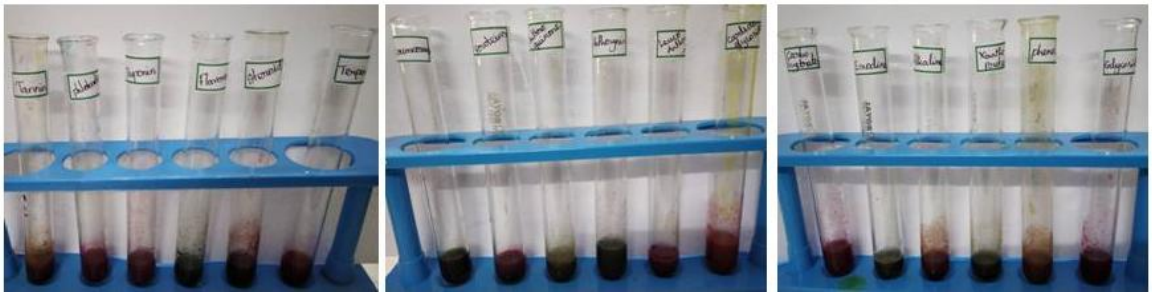


Figure.3: Qualitative analysis of aqueous extract of juice of *Vitus vinifera*

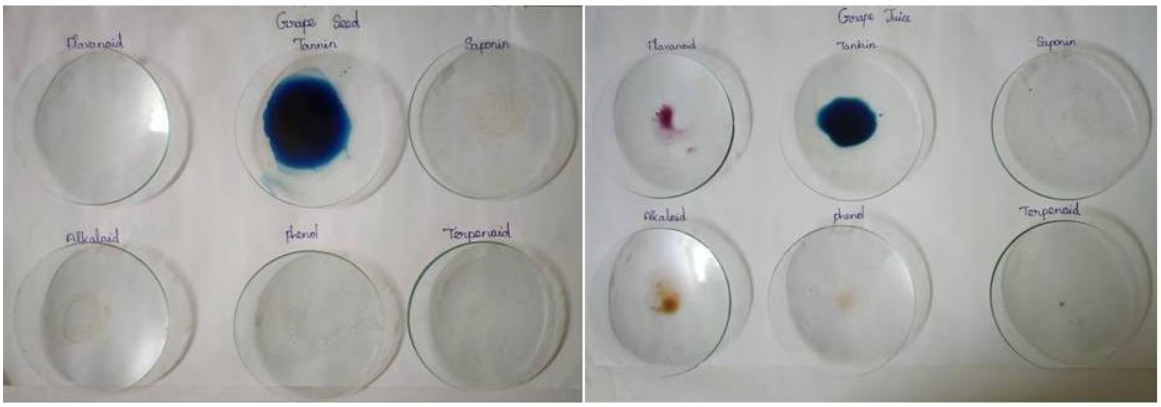
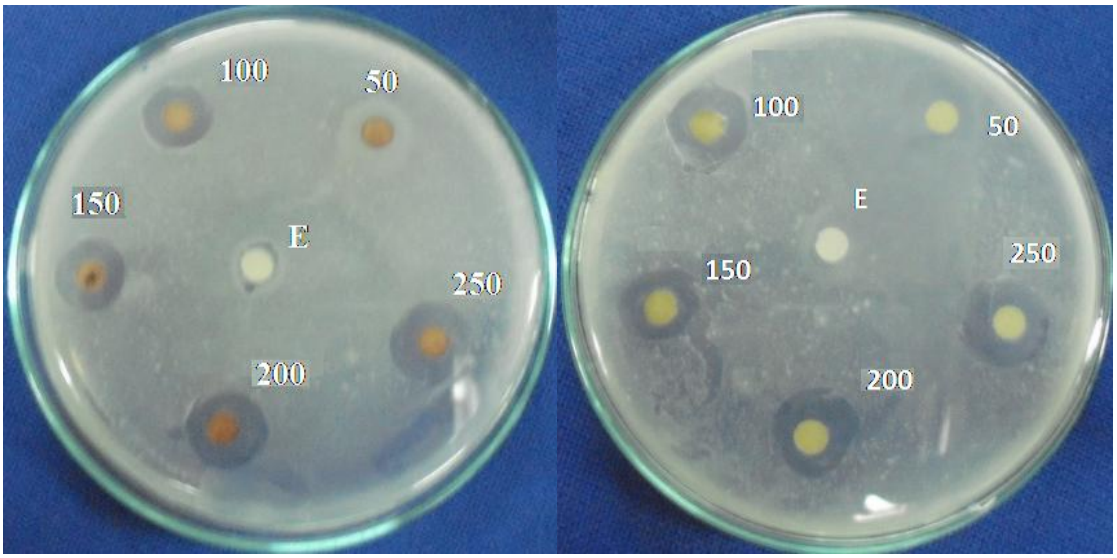


Figure 4: Quantitative analysis of aqueous juice and seed extract of *Vitus vinifera*

Figure 5: Antibacterial activity

Grape seed *Escherichia coli* **Grape juice *Escherichia coli***



Grape seed *Staphylococcus aureus*Grape juice *Staphylococcus aureus*

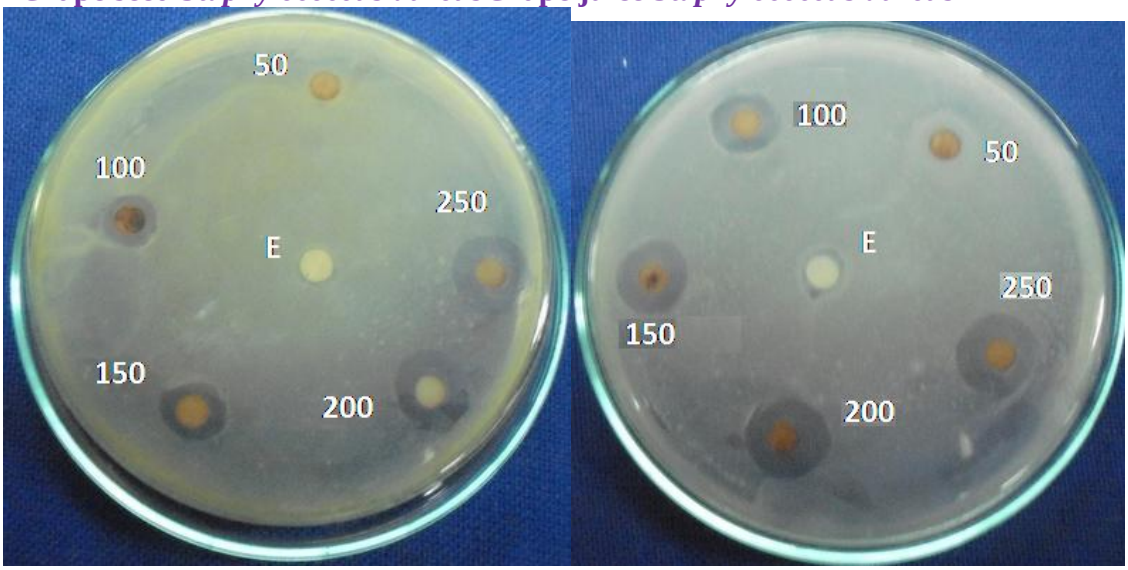
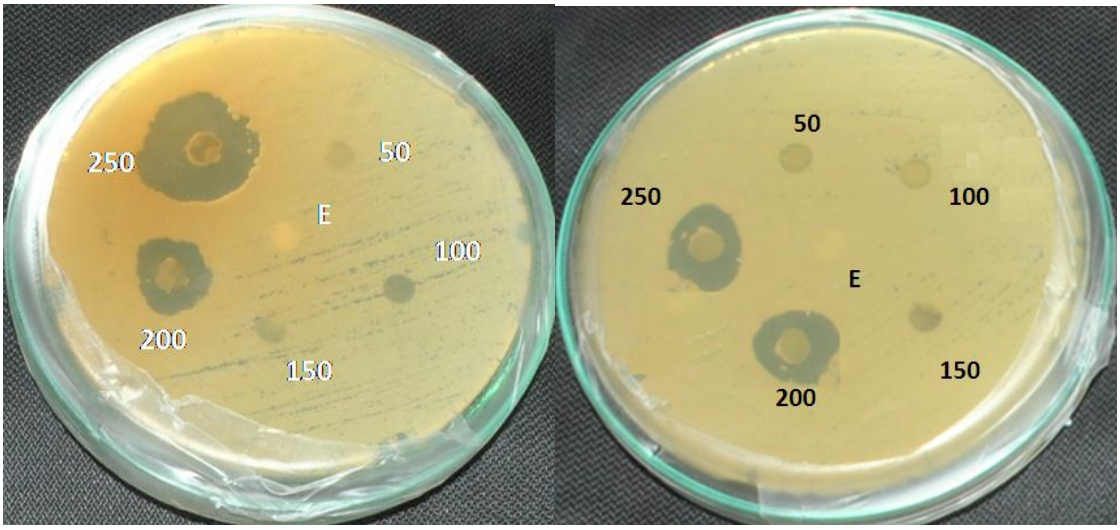
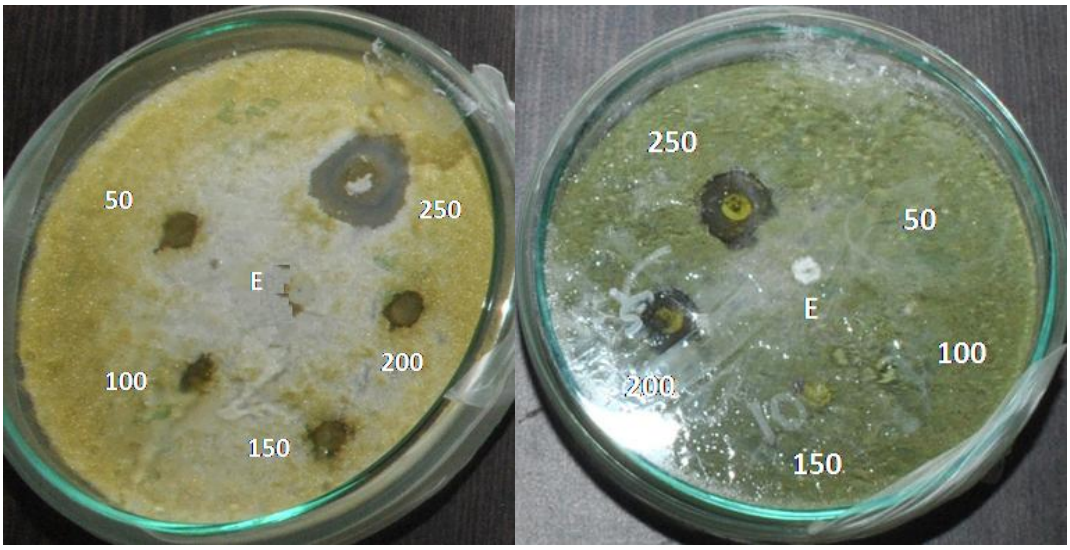


Figure 6: Antifungal activity

Grape seed *Candida albicans*Grape juice *Candida albicans*



Grape seed *AspergillusflavusGrape* juice *Aspergillusflavus*



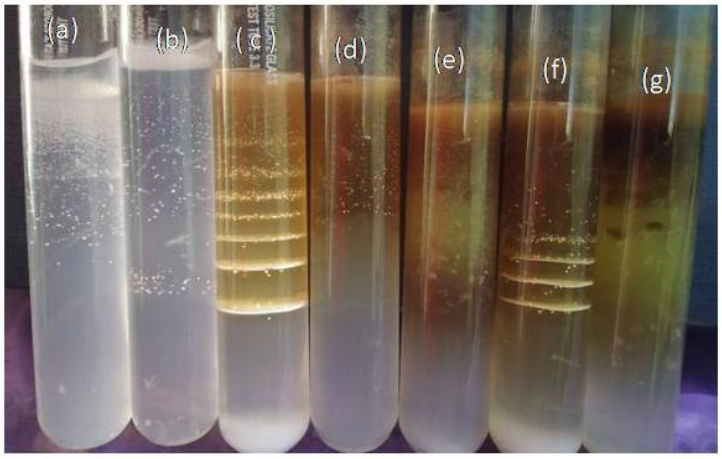


Figure 7: The effect of seeds of *Vitus vinifera*son CHPD crystals in the gel method (a) without any additive (b) with the distilled water (c) with the 1%extract (d) with the 2% extract (e) with the 3% extract (f) with the 4% extract (g) with the 5% extract after 7 day

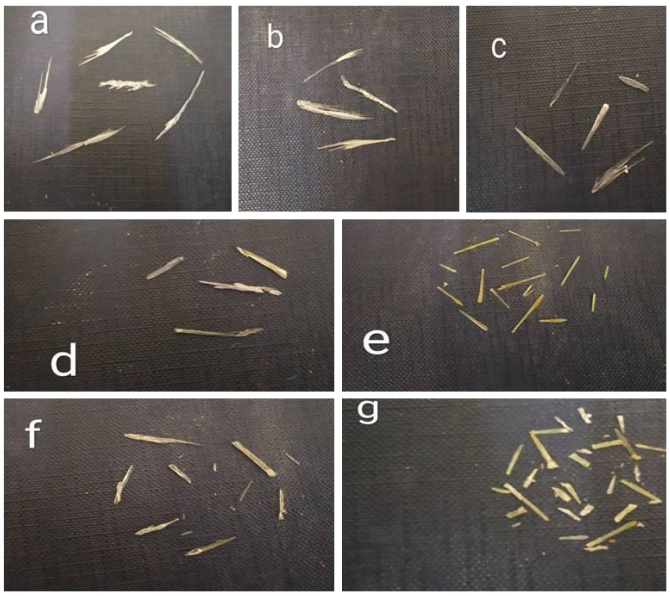


Figure 8: Morphology of harvested CHPD crystals: (a) Pure Control, (b) with distilled water, (c) with 1extracts% (d) with 2% extracts, (e) with 3% extracts, (f) with 4% extracts, (g) with 5% extracts

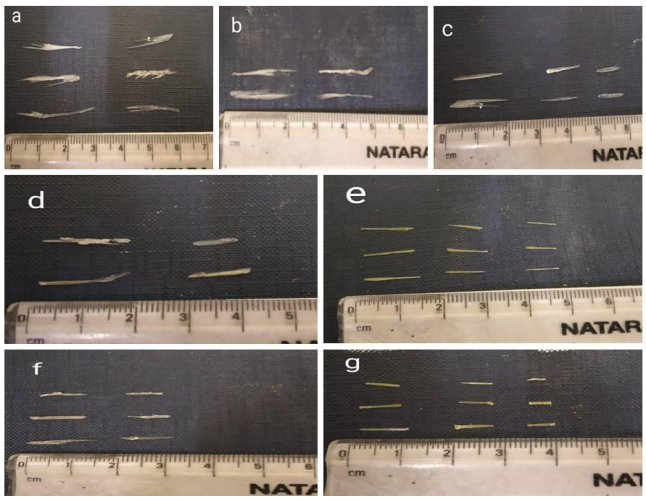


Figure 9: The measurement of CHPD crystals obtained from seeds of *Vitus vinifera*in the gel method (a) without any additive (b) with the distilled water (c) with the methanol with the 1% extract (d) with the 2% extract (e) with the 3% extract (f) with the 4% extract (g) with the 5% extract after 7 days.

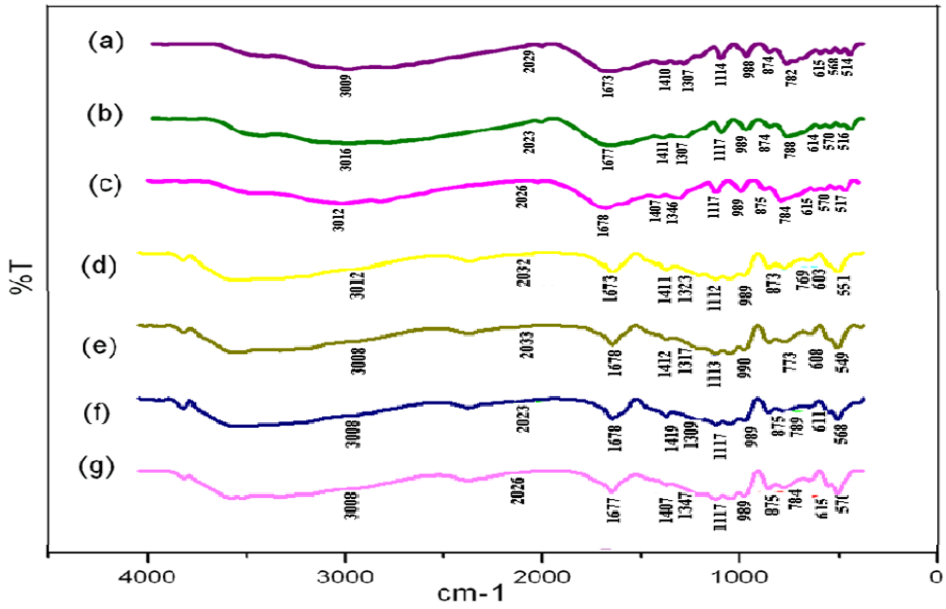


Figure 10: The FTIR spectra of CHPD in the gel method (a) without any additive (b) with the distilled water (c) with the 1% of methanol extract (d) with the 2% of methanol extract (e) with the 3% of methanol extract (f) with the 4% of methanol extract (g) with the 5% of methanol extract.

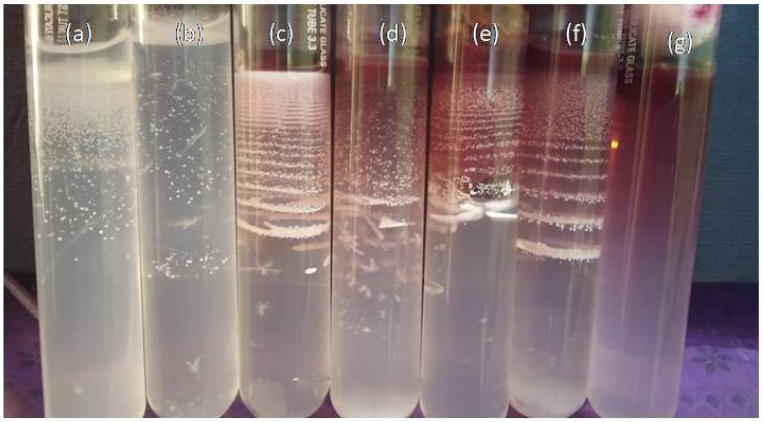


Figure 11: The effect of juice of *Vitus vinifera*son CHPD crystals in the gel method (a) without any additive (b) with the distilled water (c) with the 1%extract (d) with the 2% extract (e) with the 3% extract (f) with the 4% extract (g) wit the 5% extract after 7 day

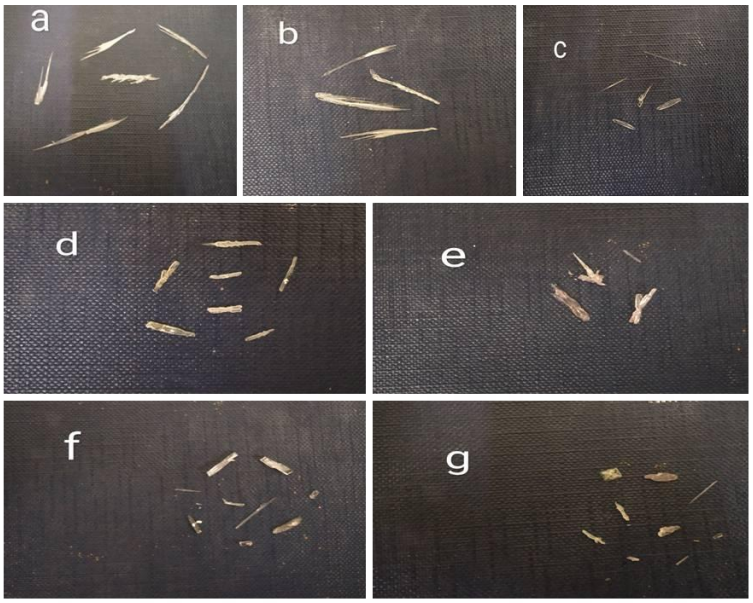


Figure 12: Morphology of harvested CHPD crystals: (a) Pure Control, (b) with distilled water, (c) with 1extracts% (d) with 2% extracts, (e) with 3% extracts, (f) with 4% extracts, (g) with 5% extracts

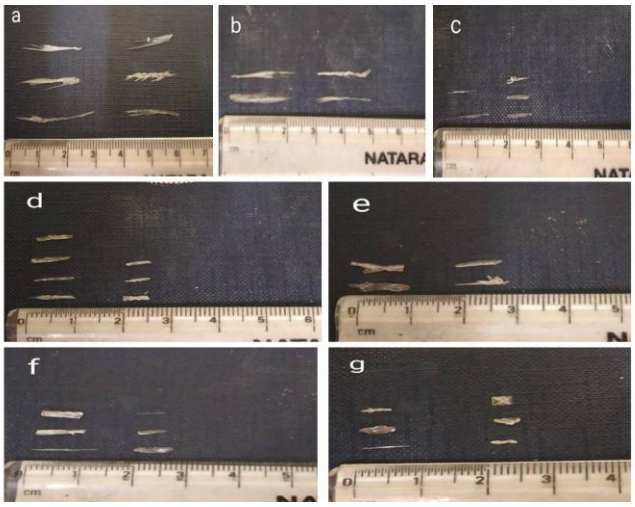


Figure 13: The measurement of CHPD crystals obtained from juice of *Vitus vinifera*in the gel method (a) without any additive (b) with the distilled water (c) with the methanol with the 1% extract (d) with the 2% extract (e) with the 3% extract (f) with the 4% extract (g) with the 5% extract after 7 days.

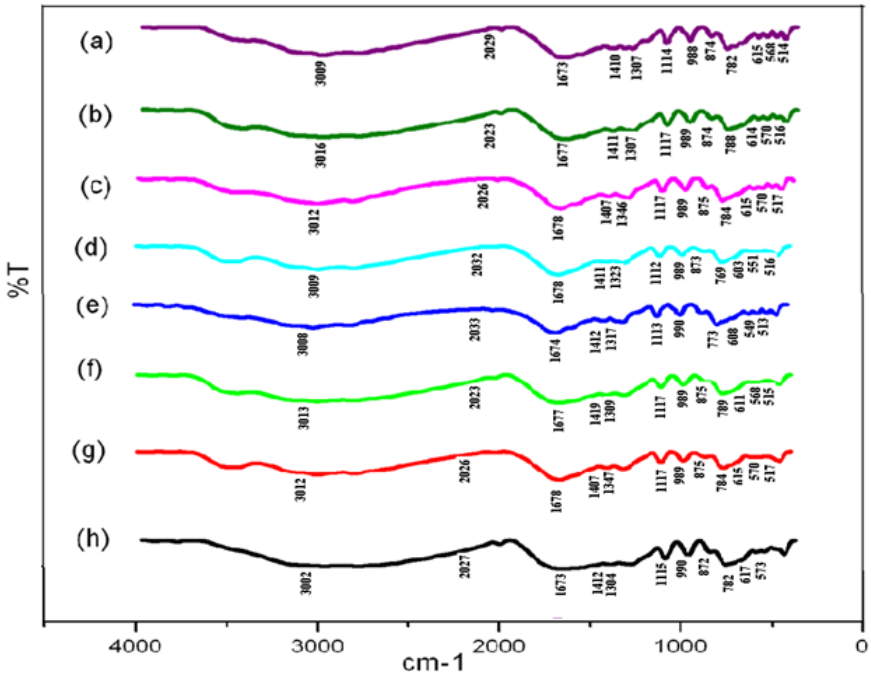


Figure 14: The FTIR spectra of CHPD in the gel method (a) without any additive (b) with the distilled water (c) with the 1% of methanol extract (d) with the 2% of methanol extract (e) with the 3% of methanol extract (f) with the 4% of methanol extract (g) with the 5% of methanol extract.

References

1. Becker K, Jablonowski H, Haussinger D (1996). Sulfadiazine-associated nephrotoxicity in patients with the acquired immunodeficiency syndrome. *Med*. 75:185–194.
2. Christina AJ, Ashok K, Packialakshmi M, Tobin GC, Preethi J and Murugesh N (2005). Antilithiatic effect of *Asparagus racemosus*Willd on ethylene glycol-induced lithiasis in male albino Wistar rats. Methods. Exp. Clin. Pharmacol., Vol.27, pp.633- 638.
3. Christina AJ, HajaNajumadeen NA, Vimal Kumar S, Manikandan N, Tobin GC, Venkataraman S and Murugesh N(2006). Antilithiatic Effect of *Meliaazedarach*on Ethylene Glycol-Induced Nephrolithiasis in Rats. Pharmaceutical Biology., Vol.44, pp.480- 485.
4. Coe F.L., Evan A., Worcester E.,"Kidneystonedisease," Journal of Clinical Investigation, vol. 115, no. 10, pp. 2598– 2608, 2005.
5. Courbebaisse M, Prot-Bertoye C, Bertocchio J "Nephrolithiasis of adult: from mechanisms to preventive medical treatment," Revue MedicaleInternationale, vol. 38, no. 1, pp. 44–52, 2017.
6. Dietrich BL, Blaschke R, Schmidt W (1990). Results of 5035 stone analysis. A contribution to epidemiology of urinary stone disease. Scan. J. Urol. Nephrol. Vol. 24, pp. 205-210.
7. Facino MR, Carini M, Aldini G, Bombardelli G, Morazzoni P, Morelli R; Free radicals scavenging action and antienzyme activities of procyanidines from Vitisvinifera A

mechanism for their capillary protective action, Arzneimittelforschung, 44, 592- 601 (1994).
8. Gambaro G, Valente ML, Zanetti E, Barbera MD, Prete DD, Angelo AD, Trevisan A. Mild Tubular Damage Induces Calcium oxalate crystalluria in a model of subtle Hyperoxaluria: Evidence that a second hit is necessary for renal lithogenesis. J Am Soc Nep. 2006,17, 2213-2219.
9. Joseph KC,.Bharat B,.Parek H, and.JoshiM.J,"Inhibition of growth of urinary type calcium hydrogen phosphate dihydrate crystals by tartaric acid and tamarind," Current Science, vol. 88, pp. 1232–1238, 2005.
10. MarjanNassiri-AslandHosseinHosseinzadeh; Re- view of the Pharmacological Effects of *Vitisvinifera*(Grape) and its Bio active Compounds, Phytother.Res., 23, 1197-1204, (2009).
11. Mikawlrawng K, Kumar S, and. Vandana R, "Current scenario of urolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (North East India): a review,"InternationalJournalofHerbalMedicine,vol.2,no.1, pp. 1–12, 2014.
12. Park S and Pearle MS, "Pathophysiology and management of calcium stones," Urologic Clinics of North America, vol. 34, no. 3, pp. 323–334, 2007.
13. Pearle, M.S. Roehrborn, C.G. Pak, C.Y.C. 1999. Meta-analysis of randomized trialsfor medical
14. Ruaaazizjassim, Denisamihele, Elenadogaru;study regarding the influence of *vitisvinifera* fruit (mus-cat of hamburg species) on some biochemical parameters, farmacia, 58(3), 332-340 (2010).
15. Rule AD, Roger VL, Melton LJ (2010). Kidney stones associate with increased risk for myocardial infarction, Journal of the American Society of Nephrology, 21:1641–1644,
16. Sigurjonsdottir VK, Runolfsdottir HL, Indridason OS., "Impact of nephrolithiasis on kidney function," BMC Nephrology, vol. 16, no. 1, p. 149, 2015.
17. Sutherland, J.W. Parks, J.H. Coe, F.L. 1985. Recurrence after a single renal stone in a community practice. Miner electron metabol., Vol. 11(4), pp. 267.
18. Taylor E. N., Stampfer M. J., and Curhan G. C., (2005.)"Obesity, weight gain and the risk of kidney stones," Journal of the American Medical Association, vol. 293, no. 4, pp. 455–462.
19. Terlecki, R.P. Triest, J.A. 2007. A contemporary evaluation of the auditory hazard of Extracorporeal shockwave lithotripsy. Urology., Vol. 70(5), pp.898-899.
20. TilahunAlelign , and BeyenePetros, Kidney Stone Disease., 2018.
21. Worcester E. M. and Coe F. L.,(2008.) "Nephrolithiasis," Primary Care, vol. 35, no. 2, pp. 369–391.
22. Yongtai, Z. Jiansheng, T. Nianping, F. and Xiangdong, H. 2008. Crystal growth of calcium oxalate induced by the extracts of *Semen Plantaginis* and *Folium Pyrrosiae*. Cryst. Res. Technol., Vol.9, pp.931-934.
23. Zoghby ZMEJ, Lieske C, Foleyetal R.N..,"Urolithiasis and the risk of ESRD," Clinical Journal of the American Society of Nephrology, vol. 7, no. 9, pp. 1409–1415, 2012.