4

RESULTS AND DISCUSSION

Urolithiasis is characterized by the formation of stones in the kidneys or urinary tracts. A large human population is suffering from urinary stone problem all over the globe. In addition, the recurrence rate is high, being more than 50%. The occurrence in hot arid zones and in certain areas is so alarming that they are known as 'Stone Belts'. The area of high incidence of urinary calculi includes British Islands, Scandinavian countries, central Europe, northern Australia, northern India, Pakistan and Mediterranean countries. The crystals of calcium oxalate are the primary constituents of majority of human kidney stones and they exist in the form of calcium oxalate monohydrate and calcium oxalate dihydrate (Chauhan *et al*., 2008).

The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes nucleation, crystal growth, crystal aggregation and crystal retention. The stone formation requires supersaturated urine, which depends on urinary pH, ionic strength and solute concentration. Various substances in the body have an effect on one or more of the above stone forming processes, thereby, influencing a person's ability to promote or prevent stone formation. Promoters of stone formation facilitate aggregation of stones while inhibitors prevent it (Aggarwal *et al*., 2014).

In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug use in clinical therapy. Endoscopic stone removal and ESWL are exorbitantly costly and recurrence is quite common with these procedures (Galani and Panchal, 2014). Thus development of a drug for the prevention of this disease or its recurrence would be of great interest.

The use of antilithiatic herbs and/or concoctions for treating renal stones has been practiced long before the use of modern medicine. The crystallization studies of calcium oxalate have been an interest to the researchers and urologist for many years. The clinical use of inhibitors to prevent the formation of CaOx stones has been limited to some

minerals. The medicinal plants contain chemical compounds, which themselves possess an inhibitory effect on crystallization of calcium oxalate.

Many Indian plants are found to be useful as antilithiatic agents, which are effective with least side effects besides being inexpensive. These plants are constantly being evaluated for possible antiurolithiatic effect in systematic manner (Segura *et al*., 1997). One such plant is banana, which is a most important fruit crop that grows all over the tropical regions of the world throughout the year and has a major commercial importance in many countries. India is the largest producer of banana with an annual production of 28.2 million tons, contributing about 24% of world banana production (http://agriexchange.apeda.gov.in/India%20 Production/ India _Productions .aspx?hscode=08030000).

Banana pseudostem and corm together constitute a major part of plant biomass, which are usually left in the plantation or incinerated and wasted. Pseudostem was found to contain 93.1% moisture whereas corm has a moisture per cent of 85.1. The ability of pseudostem for its antilithiatic activity has already been tested by Prasad *et al.* (1993); Prasobh and Revikumar (2011 and 2012); Saravanan and Aradhya (2011); Ponnambalam and Sellappan (2014). Till today no systematic study has been done with the corm of banana for its pharmacological effects. Hence it is much imperative and necessary to test the effect of corm for its antilithiatic ability, though it has been traditionally used in several Northeast states of India, especially in Arunachal Pradesh, where forest of banana exists. Thus, the present study was undertaken to investigate the inhibitory efficacy against mineralization of calcium oxalate, *in vitro* and *in vivo* conditions, using various solvent extracts of corm of different cultivars of banana genome and ploidy level (Plate 2). The phytochemical characterization of banana cultivars with maximum antilithiatic potential was also done to identify the biochemical nature of the compound responsible for crystal disaggregation.

Scientific investigation of antilithiatic role of banana corm could lead to develop potent, harmless and cost effective natural drugs for the treatment of kidney stone. The present study, therefore, was focused towards "**Evaluation of Antilithiatic Potential of Banana Cultivars of Different Genome and Ploidy"**. The results of the study are furnished and discussed under the following headings:

PHASE I

4.1. Solvent Extraction

4.2. Effect of *in vitro* antilithiatic potential of different banana corm

4.2.1. *In vitro* calcium oxalate crystallization assays

4.2.1.1. Nucleation assay

4.2.1.2. Growth assay

4.2.1.3. Aggregation assay

4.2.1.4. Dose optimization

PHASE II

4.3. *In vivo* antilithiatic potential of methanolic extract of Red banana corm

4.3.1. *In vivo* analysis in experimental animals

4.3.1.1. Effect of Red banana corm on volume of urine and pH

4.3.1.2. Effect of Red banana corm on calcium level

4.3.1.3. Effect of Red banana corm on oxalate level

4.3.1.4. Effect of Red banana corm on phosphate level

4.3.1.5. Effect of Red banana corm on uric acid level

4.3.1.6. Effect of Red banana corm on creatinine level

4.3.1.7. Effect of Red banana corm on magnesium and citrate levels

4.3.2. *In vivo* analysis in serum of experimental animals

4.3.2.1. Effect of Red banana corm on serum calcium, oxalate and phosphate levels

4.3.2.2. Effect of Red banana corm on serum uric acid and creatinine levels

4.3.3. *In vivo* analysis of body weight and kidney of experimental animals

4.3.2.1. Effect of Red banana corm on the body weight

4.3.2.2. Effect of Red banana corm on the kidney weight

4.3.2.3. Effect of Red banana corm on the levels of calcium and oxalate in kidney tissue

4.3.4. Activities of marker enzymes of serum, kidney and liver of experimental animals

4.3.5. Histopathological examination of the kidney tissues

4.4. Effect of Red banana corm on lithiasis induced NRK 52E cells

4.4.1. NRK 52E cell viability as assessed by MTT and SRB assay

4.4.2. Morphological changes of NRK 52E cells

4.4.3. Cytotoxicity of NRK 52E cells assessed by lactate dehydrogenase assay

PHASE III

4.5. Antioxidant levels in Red banana corm

4.5.1. Effect of Red banana corm on the activities of enzymic antioxidants

4.5.2. Effect of Red banana corm on the levels of non-enzymic antioxidants

4.6. Radical scavenging effects of Red Banana corm extract

4.6.1. DPPH radical scavenging activity of Red banana corm

4.6.2. ABTS radical scavenging activity of Red banana corm

4.6.3. Hydrogen peroxide scavenging activity of Red banana corm

4.6.4. Hydroxyl radical scavenging activity of Red banana corm

4.7. Characterization of phytochemical constituents of Red Banana corm

4.7.1. Preliminary qualitative phytochemical analysis

4.7.2. UV/visible absorption spectrum of the phytochemical fractions of Red banana corm

4.7.3. HPTLC of the methanolic extract of Red banana corm

4.7.4. HPLC analysis of methanolic extract of Red banana corm

4.7.5. FT- IR analysis of methanolic extract of Red banana corm

4.7.6. GC-MS analysis of methanolic extract of Red banana corm

4.7.7. ^{1}H NMR

PHASE I

In the first phase of the study, *in vitro* antilithiatic property of different banana cultivars were tested. The values obtained are presented and discussed below.

4.1. Solvent extraction

In the present study six different banana cultivars namely Poovan, Red banana, Neypoovan, Karpooravalli, Sannachenkadali and Bhimkol were chosen and extracted (individual extraction) using solvents of increasing polarity namely petroleum ether (Pet ether), benzene, chloroform, ethyl acetate, ethanol, methanol and water, and the yield was recorded (Table 1)

The aqueous extracts of all the cultivars showed a higher yield when compared to other solvent extracts used for the analysis, with the highest being recorded in the Red banana corm (1.27%) followed by aqueous extract of Karpooravalli, Poovan, Sannachenkadali, Neypoovan and Bhimkol.

Table 1

Yield per cent of banana cultivars

Banana cultivars	Solvents of increasing polarity						
	Pet ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Methanol	Water
	Extract yield (%)						
Poovan	0.47	0.46	0.49	0.52	0.68	0.71	1.10
Red banana	0.49	0.55	0.48	0.51	0.97	1.10	1.27
Neypoovan	0.51	0.44	0.56	0.43	0.81	0.84	0.99
Karpooravalli	0.54	0.39	0.47	0.89	0.93	0.99	1.21
Sannachenkadali	0.39	0.44	0.51	0.42	0.67	0.72	1.06
Bhimkol	0.37	0.41	0.49	0.58	0.83	0.89	0.93

The values are mean ± S.D. of triplicate

4.2. Effect of *in vitro* antilithiatic potential of different banana corm

4.2.1. *In vitro* calcium oxalate crystallization assay

From the epidemiological data, calcium oxalate is the most common component of the calculi in the urinary system. Calcium oxalate crystallization *in vitro* is usually carried out in the context of investigating urolithiasis. An initial step towards understanding the antilithiatic potential, the *in vitro* assays namely crystal nucleation, growth and aggregation were carried out in this study using different solvent extracts of banana cultivars of different genome and ploidy level. The concentrations used in the present study for assessing the antilithiatic property of banana varied from 50 – 3200µg, and the results obtained are presented as follows.

4.2.1.1. Nucleation assay

Nucleation is the establishment of the smallest unit of crystal formation in a solution and essential step in renal stone formation. There are two forms of nucleation: homogeneous and heterogeneous nucleation. Most renal calculi contain a mixture of more than one type of crystal responsible for the formation of most stones (Mathew *et al*., 2014). The results of crystal nucleation under *in vitro* conditions are presented in Figure 5.

CaOx calculi formed under *in vitro* conditions often results in decrease in the absorbance at 620nm. Addition of the corm extracts however, leads to the disruption of

calcium oxalate crystals, thereby causing an increase in the absorbance. Thus, the absorbance increases with increased dissolution of CaOx crystals.

From the graphical representation (Figure 5a-5f), it is evident that all the banana cultivars could inhibit the crystal formation at the initial stages (nucleation) of stone formation, in all the concentrations tested. Among the banana cultivars and solvent extracts tested, the highest dissolution of the crystals was recorded in Red banana corm extracts. The methanolic extract of Red banana corm was found to exhibit the highest crystal dissolution per cent when compared to other solvent extracts.

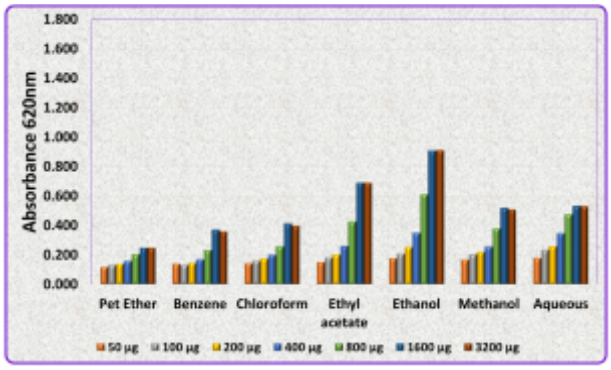
A microscopic observation (Plate 3) also confirmed a marked dissolution of crystals and a significant conversion of calcium oxalate monohydrate to calcium oxalate dihydrate in the extract treated group when compared to the control. COM, the pathogenic form, exhibited large faces, which gets attached to the renal surfaces. COM face exhibits the largest adhesion strength during flow in the renal tubules. Conversely, the protective COD crystals always exhibit extended faces and the exposed area of COD is minimal. Thus, COD, is nearly inaccessible for adhesion contacts, and displays the weakest adhesion strength. COD is less stable than COM, thereby reducing the tendency to form stones. COD microcrystals are present in voided urine of both healthy people and stone formers. COM is the most abundant phase; it is, however, seldom excreted by healthy individuals, and not by the stone formers. Based on the results of *in vitro* assays performed in the present study, a decrease in size of crystals and conversion of COM to COD in the extract treated groups were observed, indicating effective inhibition of stone formation. Thus, from the results of nucleation assay, it could be concluded that the methanolic extract of Red banana corm was the most effective in inhibiting the crystal nucleates.

The results of the present investigation were similar to the results of Pachana *et al.* (2010) and Parameshwar *et al*. (2001) who reported that the extract of *Tribulus terrestris* inhibits the nucleation of calcium oxalate crystals and decreases their size. It also promotes the formation of octahedral crystals, despite the presence of COM crystals.

Figure 5

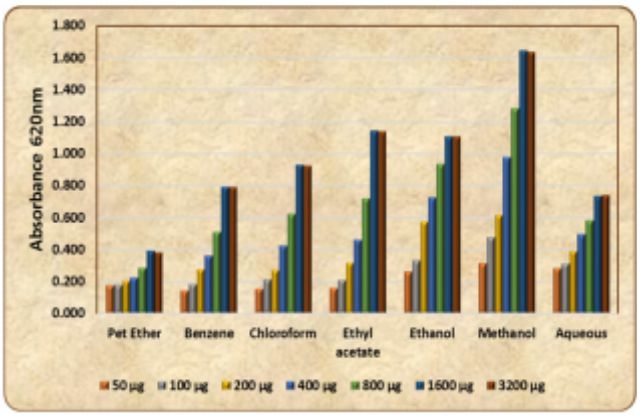
Banana corm extracts on calcium oxalate crystal nucleation

5a. Poovan



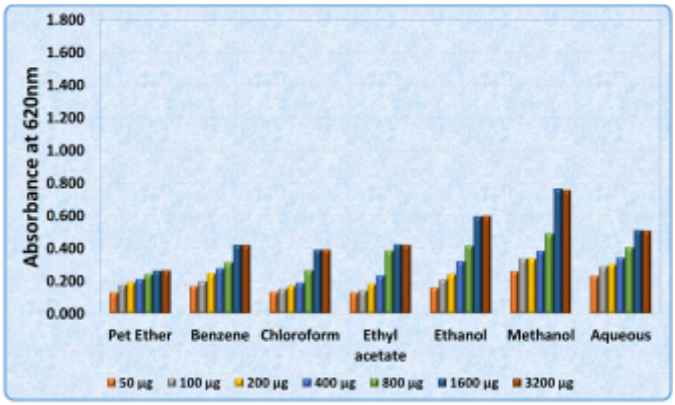
The values are mean ± S.D. of triplicate

5b. Red banana



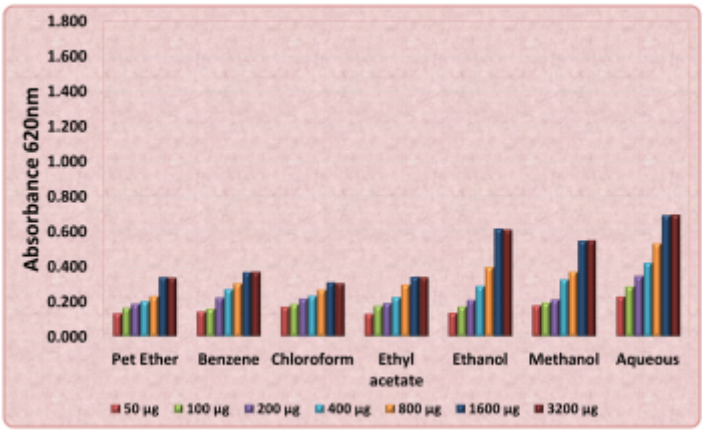
The values are mean ± S.D. of triplicate

5c. Neypoovan



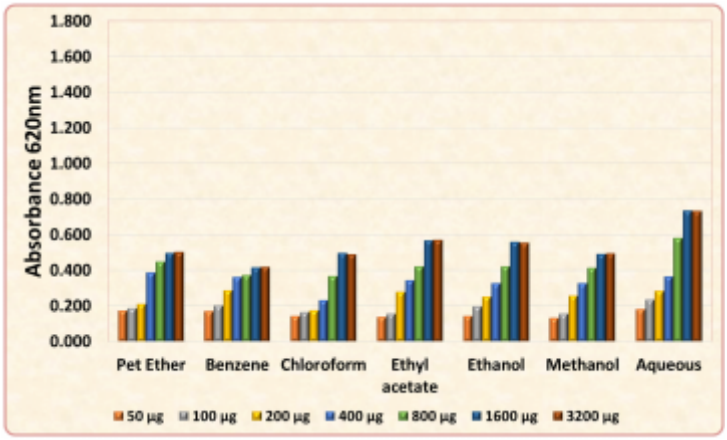
The values are mean ± S.D. of triplicate

5d. Karpooravalli



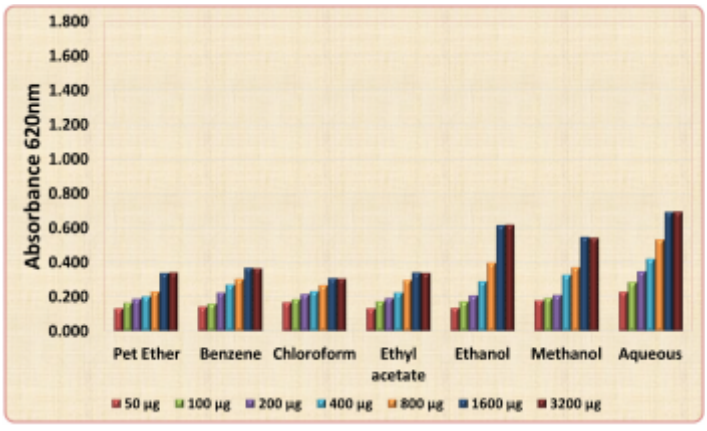
The values are mean ± S.D. of triplicate

5e. Sannachenkadali



The values are mean ± S.D. of triplicate

5f. Bhimkol

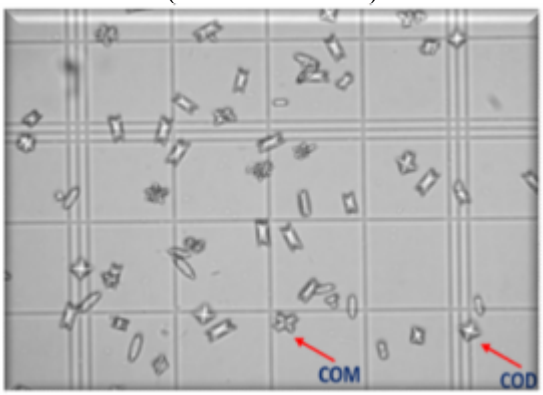


The values are mean ± S.D. of triplicate

Plate 3

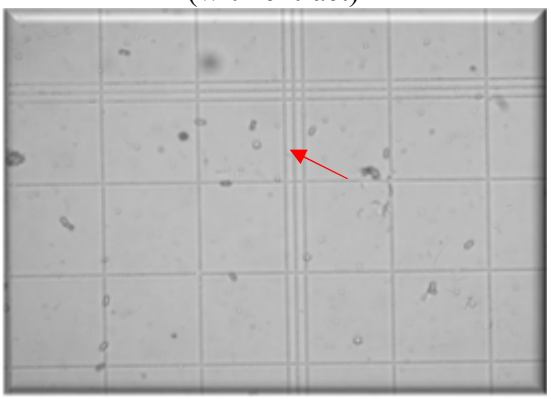
Calcium oxalate crystal nucleation morphology

Crystal Nucleation (without extract)

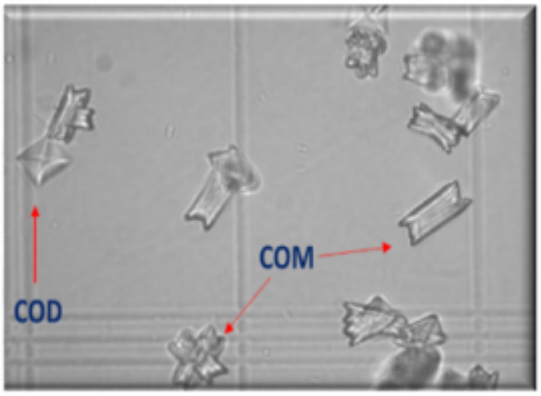


400X Magnification

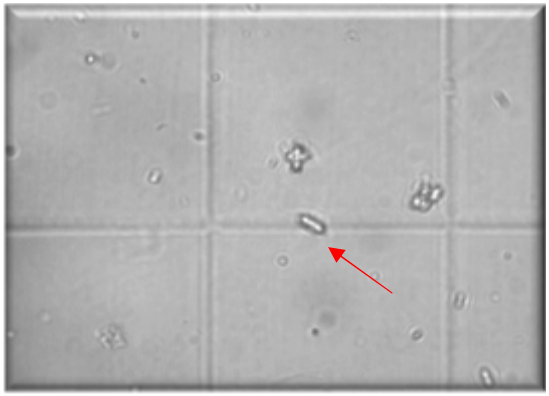
Crystal Nucleation (with extract)



400X Magnification



1000X Magnification



1000X Magnification

The antilithiatic effect of methanolic extract of *Coleus aromaticus benth* was studied by Khare *et al*. (2014a), and the results revealed that the maximum percentage inhibition of calcium oxalate nucleation was 62.48% in methanol extract, as compared to other solvent extracts tested. The aqueous extract of *Trianthema decandra* inhibited 54.87% calcium precipitation at the concentration of 1000µg/mL (Kuncha *et al*., 2014).

The inhibition against *in vitro* calcium oxalate crystal formation by *Ocimum gratissimum* L. extract revealed a concentration dependent inhibition and was found to be a potent and promising antiurolithiatic agent and is being used in traditional medicine (Agarwal and Varma, 2014).

Atmani and Khan (2000) have reported that an extract from the herb *Herniaria hirsuta* L., a plant that is traditionally being used in Morocco for the treatment of lithiasis, promoted the nucleation of calcium oxalate crystals, increasing their number but decreasing their size. The results of nucleation assay is supported by Suzuki *et al*. (1999), and showed Takusha (*Alismatis rhizome*), a Kampou medicine, had a strong inhibitory effect at all concentrations tested against crystal nucleation.

Solanum anguivi Lam. root extract in methanol inhibited the precipitation of calcium oxalate, while the same extract in ethyl acetate caused very little inhibition. Thus, the root extract of *Solanum anguivi* Lam. as a promising natural source, suitable for the formulation of drugs in pharmaceutical industry for the prevention and treatment of lithiatic diseases (Mathew *et al*., 2014)

The results of nucleation assay suggest that Red banana corm extract strongly suppressed the initiation step in CaOx crystal formation by inducing morphological changes, which favours COD crystal formation. Therefore, Red banana corm extract could be a potent herb for protection against lithiasis.

4.2.1.2. Growth assay

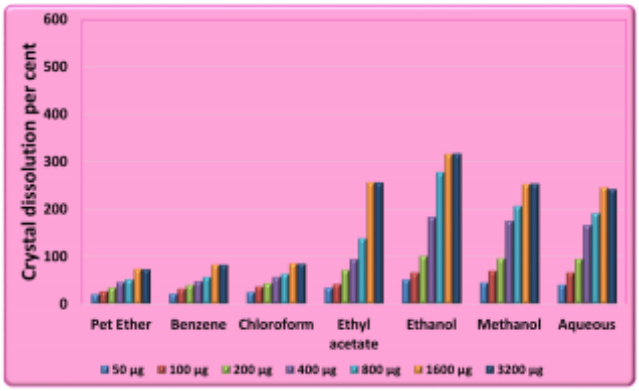
The next step in the stone formation is the crystal growth. Newly formed crystals may combine or grow to form a small, hard mass called stones. The extent to which the growth of calcium oxalate crystals as inhibited by the presence of different solvent extracts of banana corm was determined and presented in Figure 6a-6f. All the extracts could considerably inhibit the crystal growth. Among the various extracts tested, methanolic extract of Red banana corm showed higher percentage of inhibition. Increasing the concentration of methanolic extract of Red banana had increased the inhibitory effect.

The driving force for crystallization is a reduction in the potential energy of the atoms or molecules when they form bond to each other. The crystal growth process starts with the nucleation stage when several atoms or molecules in a supersaturated liquid start to form clusters (Ashok Kumar *et al*., 2014). In the crystal growth experiments, the rate of crystallization is usually controlled by the number of crystals of calcium oxalate as a

Figure 6

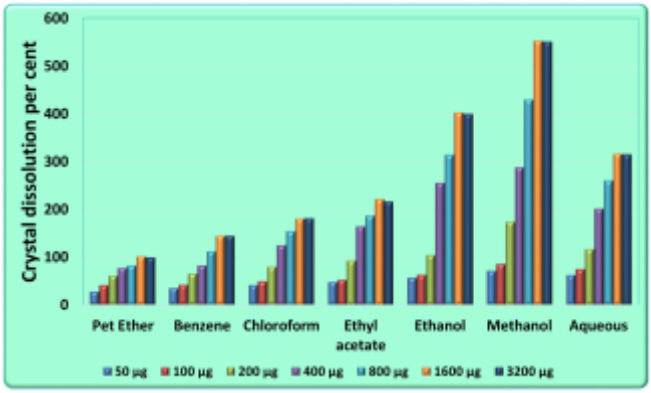
Banana corm extracts on calcium oxalate crystal growth

6a. Poovan



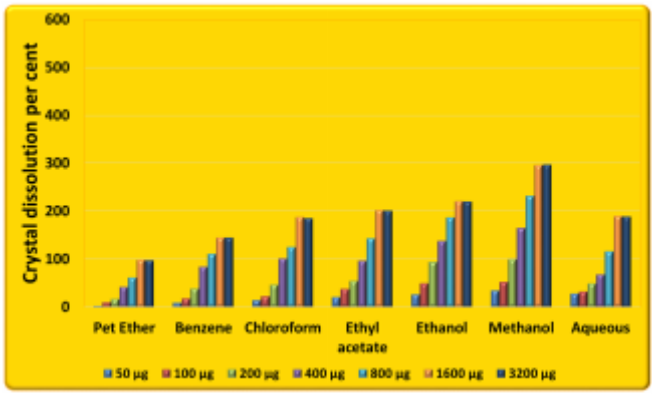
The values are mean ± S.D. of triplicate

6b. Red banana



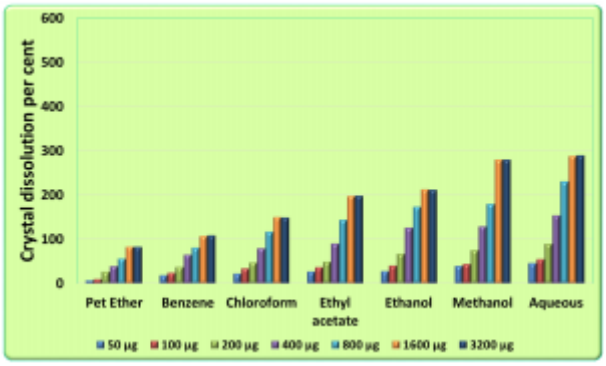
The values are mean ± S.D. of triplicate

6c. Neypoovan



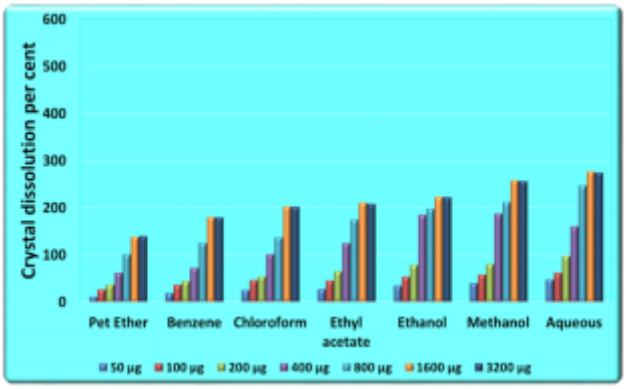
The values are mean ± S.D. of triplicate

6d. Karpooravalli



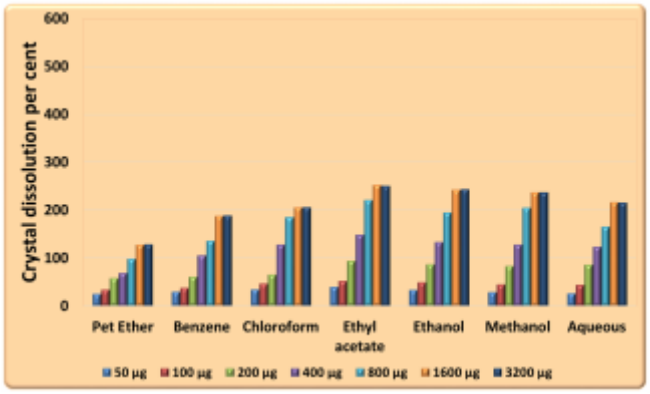
The values are mean ± S.D. of triplicate

6e. Sannachenkadali



The values are mean ± S.D. of triplicate

6f. Bhimkol



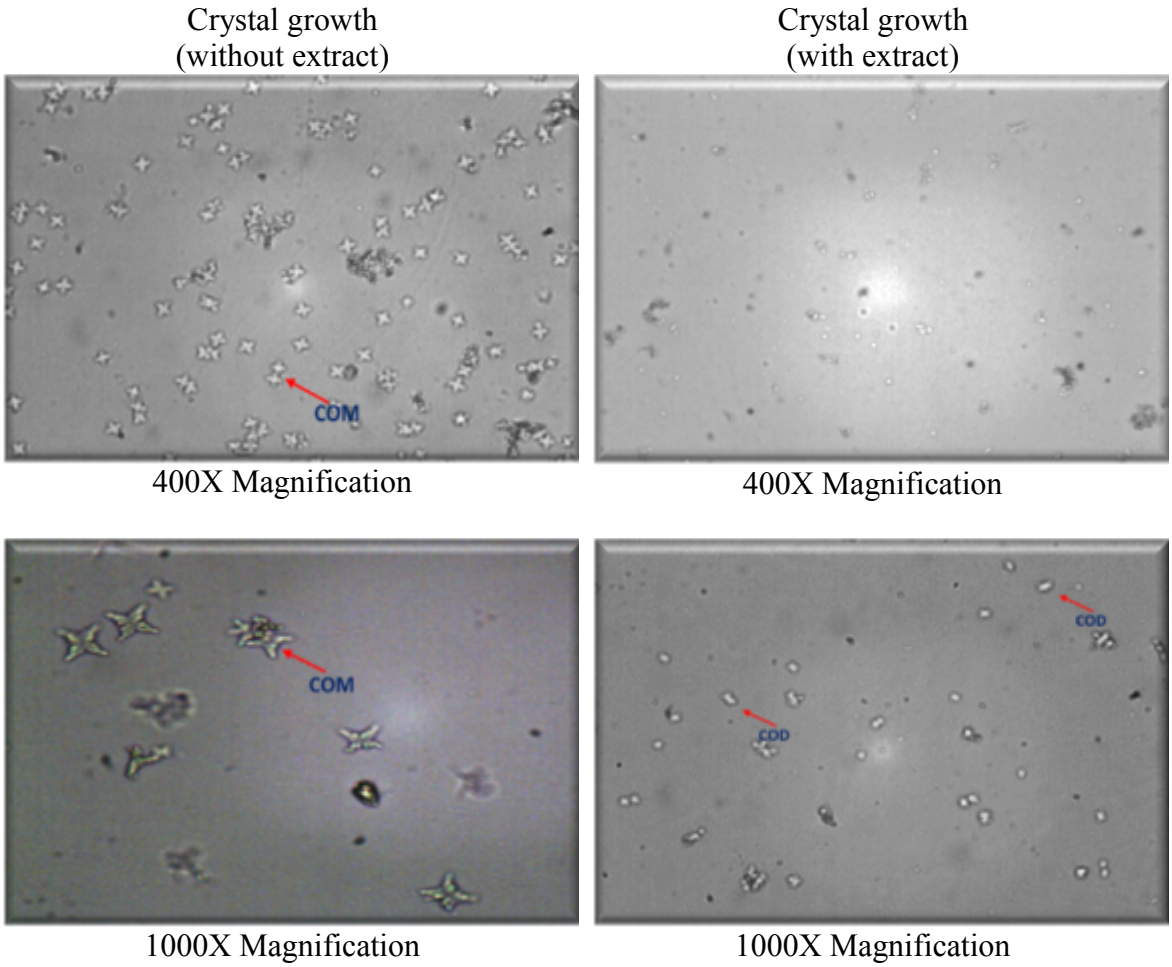
The values are mean ± S.D. of triplicate

function of time, following the introduction of seed crystals. As depicted in Plate 4, the extracts of banana corm of different cultivars reduced the rate of crystallization besides a significant change in the size of the crystals.

Alcoholic extract of leaves of *Clitoria ternatea* showed higher inhibition of calcium oxalate crystallization, *in vitro* (Quazi *et al*., 2014). Kumar and Mahapatra (2014) reported that glycolic acid treatment was helpful in urinary stone prophylaxis, and the crystal growth was reduced significantly. The aqueous extract of the leaves and root of *Beta vulgaris* L. were compared and the results revealed that the leaf aqueous extract exhibited better crystal growth than root aqueous extract (Saranya and Geetha, 2014).

Plate 4

Calcium oxalate crystal growth morphology



Methanolic, ethanolic and n-hexane whole plant extract of *Mucuna pruriens* were tested for its *in vitro* antilithiatic potential. The results of the study showed that the methanolic and ethanolic extracts of *Mucuna pruriens* dose-dependently inhibited the formation of calcium oxalate crystallization (Vamsi *et al*., 2014).

Barros *et al.* (2003), discovered that *Phyllanthus niruri* extract did not inhibit calcium oxalate nucleation, but inhibited crystal growth. *Ammi visnaga* is an excellent source of magnesium, seems to be the most important effect that the plant has acquired inhibitory effect against CaOx crystallization (Charafi *et al*., 2012). Thus it is evident that the methanolic extract of Red banana corm effectively reduced CaOx crystallization which might be due to the presence of diversified chemical constituents in the corm extract. These compounds might served as an inhibitor during the process of crystallization.

4.2.1.3. Aggregation assay

Stone crystals bind to one another through a process known as aggregation or agglomeration. Strong chemicals and electrical forces promote the aggregation process. Crystal aggregation is thought to have an important role in lithiasis since it is large enough to be retained in the collecting system (Gnessin *et al*., 2010). The extent of crystal dissolution ability of the banana cultivar corm extracts on the aggregation of calcium oxalate are presented in Figure 7a-7f.

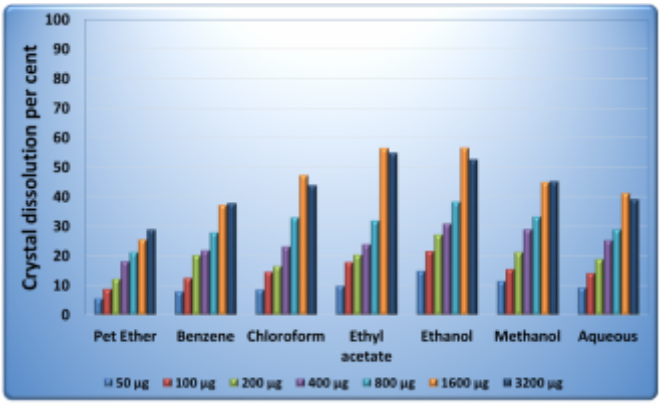
The addition of corm extracts reduced the crystal aggregation to a significant extent compared to the control (without extract), in all the cultivars tested. Among the cultivars tested, the methanolic extract of Red banana corm exhibited a high inhibitory potential. A considerable change in the crystal morphology was observed. The extract may contain substances that inhibit the agglomeration of particles and keep CaOx particles dispersed in solution and can be eliminated spontaneously in the urinary tract.

Microscopic view of calcium oxalate crystals showed that the crystal size was larger with more aggregation in the control (without extract) than the treated samples. The size of the crystal was also considerably reduced and very well dispersed in the presence of the corm extract (Plate 5). It is clearly evident from the results that the corm extract reduced the crystal aggregation to a greater extent due to the inhibitory nature of the components present in the banana corm, which could ultimately prevent the stone formation.

Figure 7

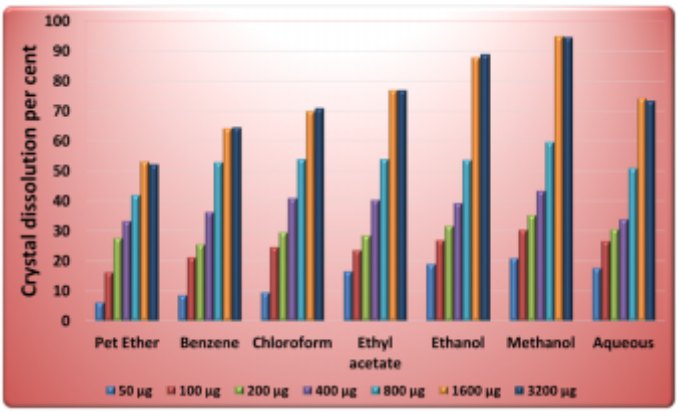
Banana corm extracts on calcium oxalate crystal aggregation

7a. Poovan



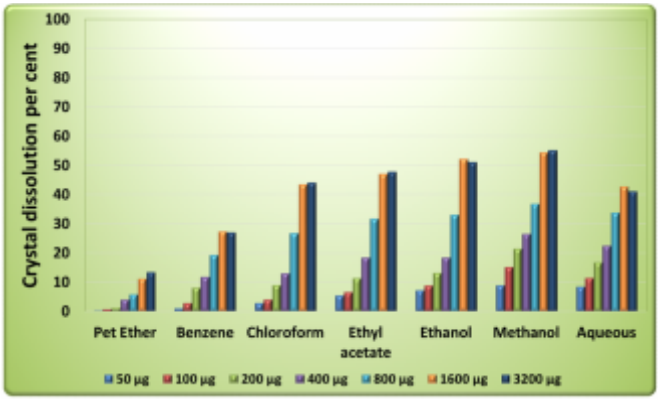
The values are mean ± S.D. of triplicate

7b. Red banana



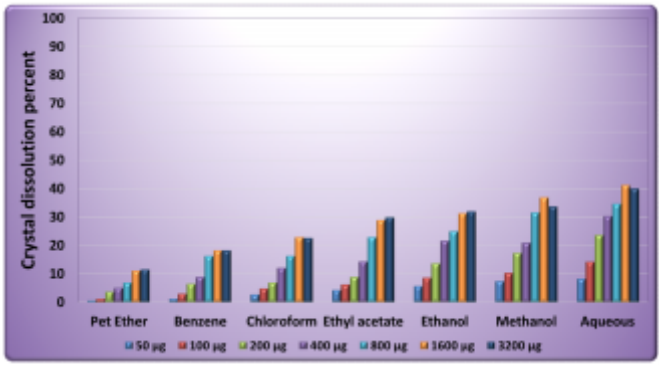
The values are mean ± S.D. of triplicate

7c. Neypoovan



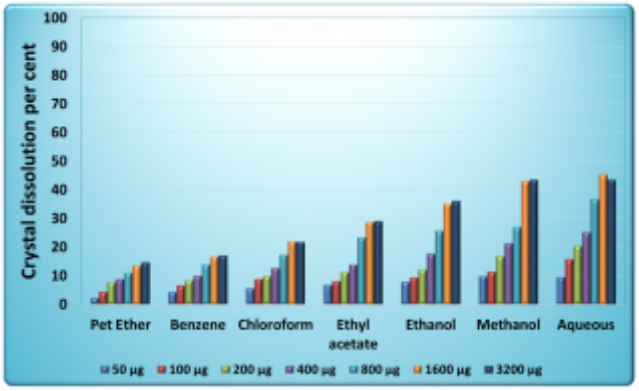
The values are mean ± S.D. of triplicate

7d. Karpooravalli



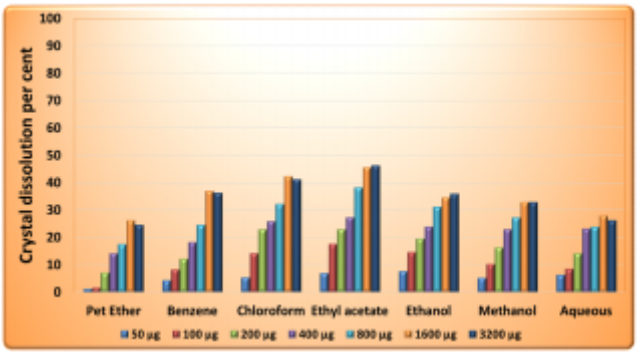
The values are mean ± S.D. of triplicate

7e. Sannachenkadali



The values are mean ± S.D. of triplicate

7f. Bhimkol



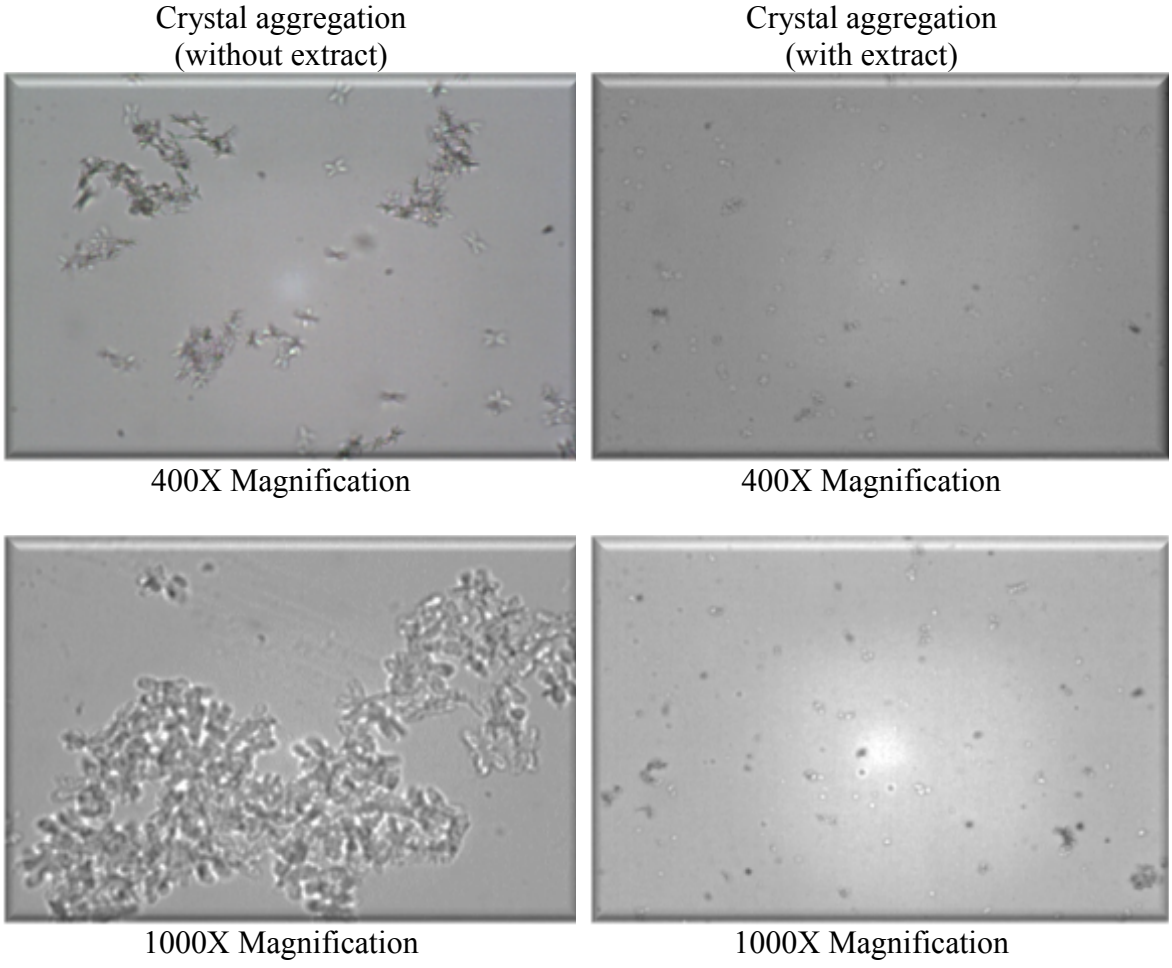
The values are mean ± S.D. of triplicate

The results obtained in the present study was comparable to that of several researchers who studied using various extracts of different medicinal plants. The methanolic extract of leaves of *Hyptis suaveolens* (L) Poit. showed inhibitory potential of calcium oxalate crystal aggregation (Agarwal and Varma, 2012).

Phyllanthus niruri Linn. leaf powder was serially extracted in petroleum ether, ethyl acetate, methanol and water. The results of this study revealed that the water extract could inhibit up to 53.09% aggregation of calcium oxalate, followed by methanol extract under *in vitro* conditions (Khare *et al*., 2014).

Plate 5

Calcium oxalate crystal aggregation morphology



The herbal tablet formulation of *Kalanchoe pinnata* and *Rotula aquatica* showed antilithiatic activity by inhibiting precipitation of calcium oxalate significantly

under *in vitro* conditions (Gilhotra *et al*., 2013). Patel *et al.* (2010a) demonstrated that the alcoholic extract of the plant *Pedalium murex* inhibited crystal formation and aggregation better than its aqueous extract.

Manjula *et al.* (2012) reported that COM and COD were frequently found in urinary calculi (stones). The inhibitory effect of aqueous stem extract of *Costus igneus* on the growth of COM crystals was studied. The results indicated that with an increase in the concentration of aqueous *C. igneus* extract, the mass of the formed crystals was gradually reduced. Nirmaladevi *et al*. (2012) reported that the extract of *Hibiscus rosa-sinesis* Linn. and *Aerva lanata* were found to inhibit crystal growth in all the three stages of stone formation.

A study by Grases and Costa-Bauza (1990), reported citrate inhibited aggregation, mucin enhanced the formation of COD crystals, whereas pentosan polsulphate had no perceptible effect on aggregation but favoured the formation of COD crystals. Effect of hydro-alcoholic extract of *Adiantum capillus-veneris* Linn. on calcium oxalate crystallization by *in vitro* study revealed that the nucleation was not inhibited but the crystal aggregation was significantly inhibited (Ahmed *et al*., 2013). *Plantago major* had a significant inhibitory effect on the size of calcium oxalate crystals as reported by Aziz *et al*. (2005).

Antinephrolithiatic activity of 70% methanolic extract of *Centratherum anthelminticum* seeds were evaluated *in vitro*, on nucleation and aggregation of calcium oxalate crystallization. The results of the study indicated that the seeds had higher capacity to inhibit the crystal formation and aggregation (Galani and Panchal, 2014).

Extracts of *Ammodaucus leucotrichus* and *Ajuga iva* were found to inhibit the nucleation, growth and aggregation phases of calcium oxalate crystallization, while *Erica multiflora* and *Globularia alypum* (flowers), inhibited nucleation and growth of the crystals but not their aggregation (Beghalia *et al*., 2008). The ethanol extract of *Hypoetes polythyrsa* Miq leaves have dissolved renal calculi significantly under *in vitro* conditions (Widana *et al*., 2012)

Using polarised light photography, the various phases of crystallization i.e. nucleation, growth and aggregation were demonstrated under *in vitro* conditions by

Beghalia *et al*. (2007). The results revealed that the *Tetraclinis articulate* inhibited both nucleation and growth phases with a maximum inhibitory effect, whereas in *Chamaerops humilis* the activity was higher in aggregation phase. It was concluded that the extracts of *Tetraclinis articulate* and *Chamaerops humilis* inhibited the growth of calcium oxalate monohydrate crystals *in vitro*. A similar result was observed in *Rieva hypocraterforis, Cynodon dactylone* and *Balanita aegypticae* under *in vitro* conditions by Patel *et al*. (2010).

The results of the *in vitro* assays in the present study clearly indicated that all the corm extracts readily prevented crystal nucleation, growth and aggregation. The methanolic extract of Red banana corm inhibited significant inhibitory potential against crystallization process. Therefore, the methanolic extract of Red banana corm was used for further study and the optimal dose to be used was also determined.

4.2.1.4. Dose optimization

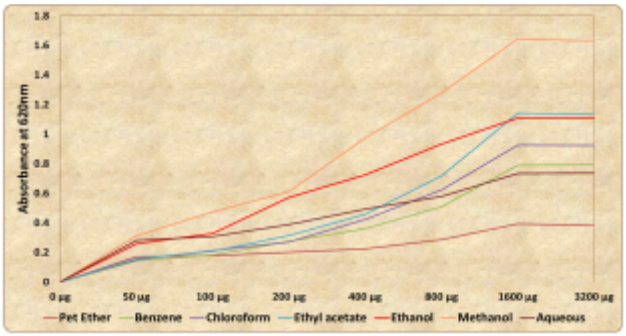
The results of all *in vitro* analysis revealed that the methanolic extract of Red banana corm exhibited maximum antilithiatic activity among all the cultivars and this extract was taken forward for the further studies. Once the extract with the maximum antilithiatic activity was identified, the minimum concentration at which this extract would evoke the maximum antilithiatic response was analyzed, for fixing optimal dose to be used further. Different concentration of the methanolic extract of Red banana corm at all the three stages of *in vitro* study was compared and the results obtained are depicted in Figure 8a-8c.

The values obtained showed that the extent of antilithiatic activity increased up to the dose of 1600µg and thereafter exhibited a plateau. This clearly indicated that 1600µg could be the optimal dose to be employed for further study. The dosage of standard drug Cystone was fixed as 12.7 mg/kg body weight of experimental animals (As prescribed).

Figure 8

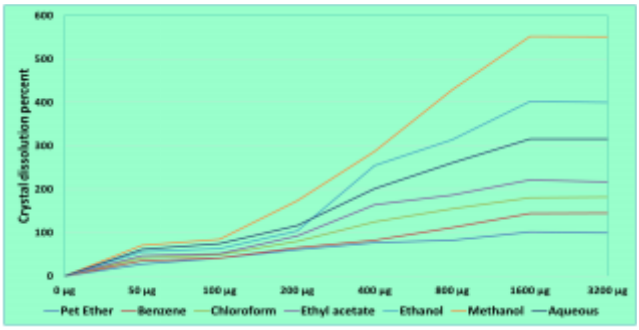
Dose optimization

8a. Nucleation assay



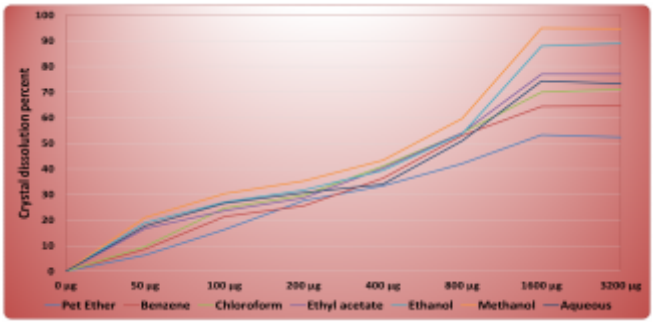
The values are mean ± S.D. of triplicate

8b. Growth assay



The values are mean ± S.D. of triplicate

8c. Aggregation assay



The values are mean ± S.D. of triplicate

PHASE II

To reiterate the results obtained under *in vitro* conditions, *in vivo* study was conducted using male Wistar rats. In order to understand in detail all the aspects of pathogenesis including the anatomical and physiological role of kidneys, animal models are frequently used. Most of the data available on renal physiology are based on experiments in rats, which are the most commonly used animals for the study of lithiasis.

4.3. *In vivo* antilithiatic potential of methanolic extract of Red banana corm

The study of urinary chemistry on stone forming minerals would provide a good indication of risks associated with stone formation. Urinary supersaturation of stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Though supersaturation of stone forming salts in urine is essential and abundance of these salts by itself may not always result in stone formation. Various substances in the body have an influence on one or more of the stone forming processes (nucleation, growth and aggregation and crystal retention), thereby influencing a person's ability to promote or prevent stone formation.

Inhibitors of stone formation include citrate, pyrophosphate, magnesium, and certain macromolecules like nephrocalcin, osteopontin, Tamm-Horsfall protein and high urine flow. Abnormalities in the structure and or/function have been detected in some of these proteins in stone former's urine. Substances that inhibit the nucleation, growth and aggregation of calcium oxalate crystals could play a role in protecting normal subjects from calcium stone formation, and abnormalities of these molecules might lead to stone formation (Worcester, 1994).

Ionic calcium, oxalate, sodium, low urine flow, low urine pH, uric acid, COM, protein aggregates and other crystals may provide analogous site for nucleation (Gupta *et al*., 2011). Thus, the analysis of such inhibitors and promoters in animal models would give useful information on aggregation and disaggregation of calcium oxalate crystals. Therefore, in the present study, these promoters of stone formation were tested for their presence in urine, serum and kidney. For this the animals were divided into six groups of 5 rats in each. The treatment groups consisted of control (untreated control), lithiatic control, extract control, preventive

regimen, curative regimen and standard drug (Cystone). This experimental protocol was approved by the Institute Animal Ethics Committee (Approval No. AUW.IAEC.2014.BC:03).

At the end of the study period, all the rats were subjected to mild anesthesia (diethylether) and the blood was collected by cardiac puncture. Serum was separated from the collected blood by centrifugation. The levels of calcium, oxalate, phosphate, uric acid, creatinine, ALT and AST were analyzed.

The experimental rats were killed by cervical dislocation, and the liver and kidney were dissected out. In liver, the marker enzymes such as ALT and AST levels were recorded, whereas in kidney, the levels of calcium, oxalate, phosphate, uric acid, creatinine, ALT and AST were estimated. The histopathological studies of kidney was also carried out.

The values obtained in all the biochemical analyses of urine, serum, liver and kidney are presented and discussed below.

4.3.1. *In vivo* analysis in experimental animals

For 24h urine collection, the animals were kept in metabolic cage and urine was collected on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of the study period. Animals had free access only to drinking water during urine collection period. The urine was analyzed for volume, pH, calcium, oxalate, phosphate, uric acid, creatinine, magnesium and citrate.

4.3.1.1. Effect of Red banana corm on volume of urine and pH

Urolithiasis was induced by administration of ethylene glycol (0.75%) into healthy male Wistar rats. For *in vivo* analysis, 24h urine samples were collected on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment from all the rats of different treatment groups and the samples were preserved and stored. pH and the urine volume were recorded immediately after collection. The collected urine was further analyzed for the presence of stone promoters like calcium, oxalate, phosphate, uric acid and creatinine. On 28^{th} day, the levels of inhibitors of stone formation namely magnesium and citrate were estimated.

The 24h urine volume recorded during the study period were set out in Table 2. From 7^{th} day to 28^{th} day of treatment, in lithiasis induced rats (lithiatic control), a drastic

decrease in urine volume (6.3mL (7^{th} day) – 3.5mL (28^{th} day)) was recorded when compared to control rats. A significant increase in urine volume was noticed in extract control group (6.9mL – 8.1mL) indicating the diuretic effect of banana corm juice. The volume of urine excretion was found to be significantly increased in the preventive and curative group rats upon supplementation of the corm extract indicating its antilithiatic potential.

The results were comparable to that of the standard drug treatment. This confirmed that the plant has good diuretic activities. Increased urine output might reduce the possibility of stone formation.

Table 2

Urine volume of experimental animals

Treatment groups (A)	Urine volume (mL/24h)				
	Days(B)				
	0	7^{th}	14^{th}	21^{st}	28^{th}
Control	7.0±0.200	7.3±0.709^{a}	7.4±0.231^{a}	7.1±0.252^{b}	7.2±0.067^{b}
Lithiatic Control	7.5±0.321	6.3±0.208^{ab}	5.0±0.121^{c}	4.4±0.379^{d}	3.5±0.035^{d}
Extract Control	6.9±0.173	7.3±0.252^{a}	7.6±0.265^{a}	7.8±0.115^{a}	8.1±0.252^{a}
Preventive	7.0±0.557	6.4±0.058^{ab}	6.4±0.058^{b}	6.5±0.153^{b}	6.6±0.058^{c}
Curative	7.0±0.321	6.2±0.252^{b}	5.0±0.153^{c}	5.6±0.208^{c}	6.3±0.058^{c}
Standard drug	7.1±0.306	6.1±0.058^{b}	5.0±0.306^{c}	5.8±0.058^{c}	6.5±0.153^{c}
Tukey HSD at 5%	NS	0.9803	0.5024	0.6029	0.5226
CD AT 1%	Treatments/(AxB) – 0.569 (Factor A – 0.255; Factor B – 0.232)				
CV	4.075				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

The fruits of *Viburnum opulus* L. was found to possess diuretic and antilithiatic effect when the extract was administered to the lithiasis induced rats and provided a scientific evidence for the traditional usage of *V. opulus* in Turkish folk medicine for easy passage of kidney stones in urine (Ilhan *et al*., 2014). The aqueous extract of the bark of *Raphanus sativus* was tested for its antiurolithiatic and diuretic activity. The urolithiasis

was experimentally induced by implantation of zinc disc in the urinary bladder of rats. This extract showed an increase in the 24 h urine volume as compared to the control (Vargas *et al*., 1999).

A similar observation was also recorded with hydroalcoholic leaf extract of *Copaifera langsdorffi* Desf. in urolithiasis induced male Wistar rats (Brancalion *et al*., 2012) and in the study conducted by Gindi *et al*. (2013) using the aqueous leaf extract of *Ageratum conyzoides*.

The pH of the urine was recorded immediately after urine collection and are presented in Table 3. In the present study a decrease from normal pH of 6.0 – 7.0 to 5.0 to 6.0 was observed in ethylene glycol induced urolithiatic model. Treatment with methanolic extract of Red banana corm reversed the acidic pH to neutral.

Table 3

Urinary pH of experimental animals

Treatment groups (A)	24h Urine pH				
	Days(B)				
	0	7th	14th	21st	28th
Control	6.99±0.090	6.93±0.021[a]	7.02±0.023[a]	7.00±0.047[a]	7.01±0.047[a]
Lithiatic Control	6.98±0.049	5.46±0.070[d]	5.43±0.042[d]	5.26±0.130[c]	5.09±0.076[c]
Extract Control	7.00±0.074	6.63±0.025[b]	6.65±0.006[b]	6.63±0.010[ab]	6.62±0.072[b]
Preventive	7.01±0.076	6.51±0.006[c]	6.53±0.015[b]	6.72±0.029[ab]	6.93±0.015[a]
Curative	7.00±0.075	5.44±0.044[c]	5.41±0.020[c]	6.43±0.101[b]	6.80±0.012[b]
Standard drug	7.01±0.031	5.39±0.040[d]	5.41±0.050[d]	6.79±0.032[ab]	6.95±0.055[a]
Tukey HSD at 5%	NS	0.1142	0.1399	0.4547	0.1357
CD AT 1%	Factor A – 0.052; Factor B – 0.047; Treatments/(AxB) – 0.116				
CV	0.823				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

Patel and Mandal (2014) have reported that the methanolic extract of *Withania somnifera* showed an increase in urine volume and pH, which is associated with its diuretic property and these results lends support to the results of the present study. A similar observation was also made by Mazdak *et al*. (2007) in *Raphanus sativus* on urinary pH.

Priya *et al.* (2010) also reported that in the starting days the pH value is ~ 7.4, up to 12th day it fluctuated in between 7.3 to 7.5 and at the 28th day the values were found between 7.1 to 7.3. Vyas and Argal., (2012) showed that the urinary pH remained the same in groups receiving ethylene glycol as compared with control group, whereas, group receiving the root extracts of *Lantana camara* was found to increase the urinary pH in a dose-dependent manner.

The type of stone formed can be predicted from the pH of the fasting urine. Crystalluria is pH dependent. Dissolution of calculi can be achieved by alteration in urinary pH (Patel and Patel, 2011). Uric acid type stones are likely to be formed if the pH is acidic (5.0 or below). If the pH lies between 5.0-6.5 it could be calcium oxalate type and in alkaline (7.2 or above) conditions it may be magnesium ammonium phosphate type. In the present study a decrease in pH from normal pH was observed with induction of calcium oxalate type of stones. Treatment with corm extract reversed the acidic pH to normal. This increase in urinary pH might be due to dissolving the complexes of calcium oxalate, which contributes to their significant antiurolithiatic activity, might be attributed by the diuretic property of Red banana corm,

4.3.1.2. Effect of Red banana corm on calcium level

Table 4 depicts the amount of calcium excreted in the various treatment groups of urolithiatic model. The result indicated that the level of calcium steeply increased in the urine sample of lithiasis induced rats. However, administration of the corm extract caused a decline in the level of calcium through urinary excretion. In addition, the corm extract alone treated group did not show any significant change in the level of calcium when compared to lithiatic control group.

The levels of calcium were found to be increased gradually in the preventive group (1.22 – 1.28 mg/dL), as compared to the curative group in which there was an increase in the calcium levels up to 14th day (3.49 mg/dL). These increased levels had further declined by the presence of corm extract in both the groups (preventive and curative). The levels of calcium in preventive group were comparable with that of control, whereas in curative group the levels were comparable to that of standard drug treated group. Thus the results revealed that the methanolic extract of Red banana

decreased urinary calcium excretion which was indicative of the preventive nature of the extract against the process of stone formation.

Ethylene glycol treatment raised the urinary calcium levels significantly in the lithiatic group. However, after the treatment with *Adonis aestivalis* Linn., the levels were found to be reduced (Parameshwar *et al*., 2011). Similarly, alcoholic extract of *Mimusops elengi* bark significantly lowered the elevated levels of calcium in the urine when compared to calculi-induced animals (Ashok *et al*., 2011). Hwisa *et al*. (2014) reported that aqueous extract of *Melia azadirachta* L. in rats, significantly reduced the stone forming constituent calcium in urine. Similar effect was observed in the study conducted by Laroubi *et al*. (2007) in *Trigonella foenum graecum* L.

Table 4

Level of urinary calcium in experimental animals

Treatment groups (A)	Urine calcium (mg/dL in 24h)				
	Days(B)				
	0	7th	14th	21st	28th
Control	1.25±0.025	1.19±0.044^{c}	1.26±0.036^{c}	1.24±0.035^{c}	1.25±0.021^{b}
Lithiatic Control	1.23±0.012	1.98±0.029^{a}	3.65±0.139^{a}	4.37±0.362^{a}	5.11±0.111^{a}
Extract Control	1.23±0.035	1.24±0.035^{c}	1.24±0.015^{c}	1.19±0.106^{c}	1.23±0.032^{b}
Preventive	1.22±0.114	1.55±0.051^{b}	1.62±0.020^{b}	1.59±0.065^{c}	1.28±0.244^{b}
Curative	1.24±0.015	1.98±0.036^{a}	3.49±0.142^{a}	2.57±0.125^{b}	1.48±0.046^{b}
Standard drug	1.22±0.025	1.98±0.137^{a}	3.60±0.097^{a}	2.47±0.140^{b}	1.40±0.015^{b}
Tukey HSD at 5%	NS	0.1832	0.2522	0.4646	0.3084
CD AT 1%	Factor A – 0.105; Factor B – 0.096; Treatments/(AxB) – 0.235				
CV	5.610				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

Betanabhatla *et al*. (2009) evaluated the antilithiatic activity of the ethanolic extract of *Hibiscus sabdariffa* in ethylene glycol induced rats. The majority of *in vivo* studies in rats had proven that certain plant extract or fruit juices decreased the excretion of urinary calcium and oxalate and showed a potential inhibitory effect on the development of urinary calculi (Butterweck and Khan, 2009). Similar results were

observed in *Ammannia baccifera* (Bhatjambol) and *Crateva nurvala* (Varun) (Dahanukar *et al*., 2000) and in *Pinus eldarica* Medw. aqueous fruits extract on lithiasis induced rats (Hosseinzadeh *et al*., 2010).

The decoction of *Rotula aquatica* Lour was tested for antilithiatic activity in male Wistar rats by feeding them 3% glycolic acid mixed feed for 45 days, which resulted in high urinary calcium and oxalate levels. Concurrent treatment with the decoction reduced calcium and oxalate ion concentration in urine, confirming the stone inhibitory effect (Christina *et al*., 2002; Mamta *et al*., 2010).

Treatment with methanolic extract of Red banana corm caused a significant reduction in urinary excretion of calcium in both preventive and curative group animals as compared to their respective controls. This explains the effect of methanolic extract of Red banana corm in both the groups and also in dissolving the pre-formed calcium oxalate crystals.

4.3.1.3. Effect of Red banana corm on oxalate level

Hyperoxaluria, is a higher risk factor in the pathogenesis of urolithiasis than hypercalciuria. Oxalate plays an important role in stone formation and has about 15 fold higher effect than urinary calcium (Borghi *et al*., 1996). Hyperoxaluria promotes calcium oxalate renal calculi formation (Robertson and Peacock, 1980) as urinary oxalic acid has the tendency to complex with calcium and form insoluble CaOx crystals in the kidney (Danpure and Purdue, 1995). Therefore, conditions that promote oxalate absorption from food or endogenous oxalate production can cause CaOx stone formation (von-Unruh *et al*., 2004).

The data on urinary excretion of oxalate in the rat urine are set out in Table 5. The results indicated that the incidence of hyperoxaluria was observed in lithiasis induced rats as indicated by the increased level of oxalate in rat urine. Significant changes were not observed in the extract control and untreated control.

Preventive group effectively reduced the level of oxalate in urine with the presence of Red banana corm extract. The excretion of oxalate in the curative group showed a drastic increase in oxalate level up to 14^{th} day and co-administration of corm extract of Red banana caused a significant reduction in the excretory levels of oxalate,

showing a regulatory action on endogenous oxalate synthesis. The results were comparable with that of the standard drug-cystone.

The fresh stem juice of banana cultivar 'Puttubale', was found to be effective in reducing the stone formation and also dissolving the pre-formed stones (Prasad *et al*., 1993; Swathi *et al*., 2011). *Musa balbisiana* (wild) stem extract elicit diuretic activity that might be the reason behind its urolithiatic activity, which was also proved in the study conducted by Ponnambalam and Sellappan (2014). Relative calcium oxalate supersaturation and the concentration ratio of calcium to oxalate are important determinants of crystal morphology (Carvalho and Vieira, 2004).

Table 5

Level of urinary oxalate in experimental animals

Treatment groups (A)	Urine oxalate (mg/dL in 24h)				
	Days(B)				
	0	7th	14th	21st	28th
Control	0.39±0.035	0.41±0.040^{c}	0.44±0.031^{bc}	0.39±0.061^{d}	0.38±0.046^{e}
Lithiatic Control	0.37±0.020	0.88±0.015^{a}	1.55±0.085^{a}	3.03±0.100^{a}	3.97±0.017^{a}
Extract Control	0.38±0.032	0.41±0.045^{c}	0.41±0.031^{c}	0.39±0.025^{d}	0.42±0.015^{e}
Preventive	0.36±0.030	0.51±0.045^{b}	0.58±0.012^{b}	0.59±0.025^{c}	0.53±0.020^{d}
Curative	0.39±0.038	0.89±0.025^{a}	1.61±0.096^{a}	1.25±0.002^{b}	0.76±0.032^{b}
Standard drug	0.37±0.046	0.90±0.019^{a}	1.51±0.017^{b}	1.15±0.042^{b}	0.65±0.021^{c}
Tukey HSD at 5%	NS	0.0931	0.1535	0.1450	0.0748
CD AT 1%	Factor A – 0.041; Factor B – 0.037; Treatments/(AxB) – 0.091				
CV	4.868				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

Bahuguna *et al*. (2009) reported that the aqueous and alcoholic extract of *Eleusine coracana* supplementation to male albino rats with hyperoxaluria, significantly reduced the elevated urinary oxalate levels in preventive and curative treatment group animals. Chaitanya *et al*. (2010) revealed that the aqueous and alcoholic extract of *Macrotyloma uniflorum* caused a reduction in the oxalate excretion. The aqueous extract of *Melia azedarach* Linn. was studied with ethylene glycol-induced lithiatic rats. The aqueous

extract of *M. azedarach* reduced urinary calcium, oxalate, phosphate and elevated urinary magnesium level and urinary volume (Christina *et al.,* 2006). Thus it is evident from the literature cited so far that the diuretic property of Red banana corm extract might be responsible for its strong antilithiatic activity.

4.3.1.4. Effect of Red banana corm on phosphate level

The data on urinary excretion of phosphate in all the experimental animals are tabulated in Table 6. There was a gradual increase in phosphate excretion in the preventive group but a sudden elevation was noted in the curative group. The elevated levels of phosphate as observed in lithiasis induced rats were found to be well counteracted by the presence of extracts. Red banana methanolic extract treatment caused a significant reduction in the level of phosphate after 14th day in curative group.

Table 6

Level of urinary phosphate in experimental animals

Treatment groups (A)	Urine phosphate (mg/dL in 24h)				
	Days(B)				
	0	7th	14th	21st	28th
Control	3.74±0.085	3.51±0.455^{c}	3.86±0.131^{c}	3.74±0.064^{e}	3.83±0.036^{c}
Lithiatic Control	3.79±0.023	4.77±0.683^{ab}	6.14±0.169^{a}	6.87±0.113^{a}	7.67±0.336^{a}
Extract Control	3.62±0.036	3.79±0.107^{c}	3.82±0.146^{c}	3.82±0.050^{de}	3.81±0.111^{c}
Preventive	3.64±0.127	4.02±0.106^{bc}	4.34±0.097^{b}	4.03±0.083^{d}	4.00±0.014^{c}
Curative	3.60±0.078	4.96±0.219^{ab}	6.25±0.089^{a}	5.51±0.178^{b}	4.88±0.028^{b}
Standard drug	3.76±0.141	5.16±0.134^{a}	6.30±0.035^{a}	5.04±0.053^{c}	4.50±0.075^{b}
Tukey HSD at 5%	NS	0.9771	0.3272	0.2759	0.4083
CD AT 1%	Factor A – 0.186; Factor B – 0.169; Treatments/(AxB) – 0.415				
CV	4.188				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

As supported by the literatures discussed here, *Tridax procumbens* has proved to be a good remedy for lithiasis, under *in vivo* conditions (Sailaja *et al*., 2011), *in vitro* conditions (Kalpana *et al*., 2014) A study conducted by Chitra *et al*. (2012) in lithiasis induced male albino Wistar rats by oral administration of ethylene glycol showed an

elevated levels of urinary concentration of crucial ions viz. calcium, phosphate, oxalate and uric acid. Such elevated levels of urinary stone forming constituents were reverted to normal by oral administration of alcoholic extract of *Boerhaavia diffusa* roots to lithiasis induced rats. The results of the present study are in accordance with the similar studies cited, indicating its stone dissolving property.

4.3.1.5. Effect of Red banana corm on uric acid level

Uric acid is known to promote calcium oxalate crystal growth (Soundararajan *et al.,* 2006). Hyperuricosuria with or without hypercalciuria amounted to about 23% of the possible cause of urolithiasis. Approximately three fourth of urolithiasis caused by hyperuricosuria was calcium oxalate stones and the rest was uric acid stones. Uric acid is one of the constituents of urinary stones itself, but has an activity of calcium oxalate stone formation. It has been postulated that the effect of uric acid on calcium oxalate crystallization was due to its ability to salt out calcium oxalate from solution (Grases *et al*., 2007). The amounts of uric acid excreted in urine of the rats in various treatment groups are depicted in Table 7.

The results revealed that the uric acid excretion increased in lithiasis induced rats, indicating the formation of stones. Control and extract treated groups showed no significant increase in uric acid levels, whereas the curative group rats showed an increase in uric acid levels up to 14^{th} day of ethylene glycol treatment. Subsequently treatment with methanolic extract of Red banana corm reduced the uric acid levels.

Chaitanya *et al.* (2010) reported that a reduction in the level of uric acid when administered with aqueous and alcoholic extracts of *Macrotyloma uniflorum,* to lithiasis induced rats. In India, 'Nayurivi' (Tamil name) *Achyranthes aspera* Linn. (Amaranthaceae family) is commonly used as a phytotherapeutic agent, which was evaluated for its antilithiasis (Pareta *et al*., 2011). The ethanolic extract of *A. aspera* reduced the uric acid and phosphate levels to a significant extent (Beula *et al*., 2013).

It is therefore, evident that the presence of corm extract caused cessation of stone formation due to the hinderance of 'salting out' by the lowered levels of uric acid, reiterating its antilithiatic potential.

Table 7

Level of urinary uric acid in experimental animals

Treatment groups (A)	Urine uric acid (mg/dL in 24h)				
	Days(B)				
	0	7th	14th	21st	28th
Control	0.70±0.031	0.70±0.042^{d}	0.71±0.049^{b}	0.74±0.012^{e}	0.68±0.025^{c}
Lithiatic Control	0.69±0.035	0.84±0.010^{a}	1.28±0.163^{a}	1.59±0.039^{a}	2.07±0.148^{a}
Extract Control	0.70±0.021	0.72±0.011^{cd}	0.68±0.013^{b}	0.68±0.010^{e}	0.70±0.015^{c}
Preventive	0.69±0.045	0.77±0.021^{bc}	0.82±0.013^{b}	0.86±0.026^{d}	0.85±0.038^{c}
Curative	0.71±0.015	0.82±0.011^{ab}	1.21±0.082^{a}	1.02±0.031^{b}	0.98±0.005^{b}
Standard drug	0.69±0.072	0.85±0.017^{a}	1.26±0.079^{a}	0.94±0.013^{c}	0.82±0.012^{bc}
Tukey HSD at 5%	NS	0.0590	0.2296	0.0668	0.1748
CD AT 1%	Factor A – 0.051; Factor B – 0.046; Treatments/(AxB) – 0.113				
CV	5.838				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

4.3.1.6. Effect of Red banana corm on creatinine level

The urinary creatinine levels in control and all treatment groups are presented in Table 8. An increase in creatinine level was observed in lithiasis induced animals when compared to control group. Administration of banana corm extract however, could render protection to kidney owing to the declined levels of creatinine in preventive and curative regimen groups. Kishore *et al.* (2013) indicates that hyperoxaluria promoted renal impairment, when compared to untreated control and other treatment groups in ethylene glycol induced nephrolithiasis.

The results of the present study are in agreement with Awari *et al*. (2009), who reported that the *Achyranthus aspera* Linn. treatment on lithiasis induced rats showed significant reduction in urine creatinine. Paula *et al*. (2012) reported that the extract from *Copaifera langsdorffi* leaves fed to urolithiasis induced rats revealed significant reduction in urine creatinine level. Administration of Megarajanga chooranam (a siddha medicine) to rats with ethylene glycol induced lithiasis, significantly reduced the growth of urinary stones, and besides restoring all the elevated biochemical parameters (calcium, oxalate,

phosphate, creatinine and uric acid) and it also restored the urinary pH to normal and increased the urine volume significantly as compared to control drug Cystone (Kanakavalli *et al*., 2013). A similar result was observed using root bark of *Moringa oleifera* Lam. as an antiurolithiatic agent (Karadi *et al*., 2008).

Table 8

Level of urinary creatinine in experimental animals

Treatment groups (A)	Urine creatinine (mg/dL in 24h)				
	Days(B)				
	0	7th	14th	21st	28th
Control	7.80±0.036	7.77±0.086^{c}	7.77±0.035^{d}	7.81±0.055^{e}	8.05±0.044^{d}
Lithiatic Control	7.82±0.216	10.3±0.702^{a}	20.3±0.972^{a}	24.7±0.601^{a}	29.0±0.312^{a}
Extract Control	7.88±0.025	7.83±0.180^{c}	7.80±0.068^{d}	7.91±0.176^{e}	8.13±0.083^{d}
Preventive	7.81±0.320	8.63±0.306^{b}	11.4±0.153^{c}	11.2±0.025^{d}	9.06±0.234^{c}
Curative	7.86±0.165	10.8±0.239^{a}	19.1±0.443^{ab}	14.5±0.546^{b}	10.6±0.217^{b}
Standard drug	7.85±0.170	11.2±0.131^{a}	18.8±0.615^{b}	12.9±0.604^{c}	9.08±0.238^{c}
Tukey HSD at 5%	NS	0.9370	1.3936	1.152	0.5657
CD AT 1%	Factor A – 0.334; Factor B – 0.334; Treatments/(AxB) – 0.747				
CV	3.014				

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

Thus, Red banana methanolic extract was found to decrease elevated levels of creatinine with a reduction in renal reimpairment and stone formation significantly.

4.3.1.7. Effect of Red banana corm on magnesium and citrate levels

Certain inorganic (E.g. citrate, magnesium) and organic (E.g. glycoaminoglycans, osteopontin – proteins) substances are known to inhibit stone formation. The levels of inhibitors namely magnesium and citrate in urine were analyzed, at the end of the study period (28th day), in all the treatment groups and presented in Table 9.

There was a significant rise in levels of magnesium and citrate after treatment with methanolic extract of Red banana corm, while it was found to be significantly lowered in ethylene glycol treated group. The level of magnesium and citrate however, were within normal limits in control and extract treated groups.

Table 9

Levels of urinary magnesium and citrate in experimental animals

Treatment groups	Magnesium	Citrate
	mg/dL	
Control	3.12±0.023[a]	52.45±0.007[a]
Lithiatic Control	0.93±0.121[d]	46.10±0.103[e]
Extract Control	3.04±0.104[a]	51.92±0.004[b]
Preventive	2.76±0.009[b]	49.61±0.127[c]
Curative	1.99±0.104[c]	48.11±0.025[d]
Standard drug	2.06±0.108[c]	48.28±0.121[d]
Tukey's HSD 5%	0.2427	0.2295

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

Magnesium can complex with oxalate and decrease supersaturation. Oral intake of magnesium would decrease the oxalate absorption and urinary excretion (Basavaraj *et al*., 2007). However, there is little evidence to recommend magnesium therapy in patients with urolithiasis. Sayana *et al*. (2014 and 2014a) showed that the ethanolic extract of leaves of *Cissampelos pareira* on lithiasis induced rats (0.75% ethylene glycol) decreased the urinary levels of calcium, uric acid and increased the levels of magnesium and this lends support to the results obtained in the present study.

Citrate is derived from endogenous oxidative metabolism. It is filtered freely through the glomerulus. Citrate in urine complex with calcium decreases the concentration of calcium oxalate. Fan *et al.* (1999) studied the crystallization of calcium oxalate in undiluted urine of healthy males and reported that free citrate and a calcium-citrate complex inhibited calcium oxalate crystallization. Inhibitory effect of lemon juice on *in vitro* crystallization of calcium oxalate was reported by Abdelkhalek *et al*. (2005). As revealed by the above study, ingestion of the lemon juice seems to dissipate an effect of great quantity of citrate molecules which in turn increases the excretion of oxalate and prevents CaOx precipitation (Touhami *et al*., 2007). The studies conducted by Kulaksizoglu *et al*. (2008), further substantiate the effectiveness of lemon juice to inhibit the rate of CaOx crystal nucleation and aggregation.

The results of the present study are in accordance with the Khan *et al*. (2012), who reported that aqueous-methanolic extract of *Holarrhena antidysenterica* has increased the levels of magnesium and citrate in rats.

Supersaturation, a step in the pathogenesis of urolithiasis, occurs when substances that make up the stone are found in high concentrations in urine, when urine volume decreases, and when urinary concentration of chemicals that inhibit stone formation decreases (Prstojevic *et al*., 2014). A similar condition was observed in the lithiasis induced rats. Red banana corm methanolic extract treatment, elevated the urinary crystallization inhibitors level, and thus, reduced the propensity to crystallize, thereby creating an ambience unfavorable for crystal precipitation in preventive, curative and standard drug treatment groups.

The methanolic extract of Red banana corm significantly reduced the renal content of stone forming constituents (oxalate, calcium and phosphate) in both preventive and curative regimens and increased the urine output by acting as a diuretic agent.

4.3.2. *In vivo* analysis in serum of experimental animals

At the end of the study period (28^{th} day), all the rats of different treatment groups were sacrificed by cervical decapitation. Serum was separated by centrifugation and analyzed for the levels of calcium, oxalate, phosphate, uric acid and creatinine. The results are presented in Table 10.

4.3.2.1. Effect of Red banana corm on serum calcium, oxalate and phosphate levels

Administration of ethylene glycol caused a marked elevation of calcium, oxalate and phosphate levels in the animals of lithiatic control group (till the end of study period) and in curative group (up to 14^{th} day). However, a significant reduction was observed in the animals treated with corm extract in preventive, curative and standard drug treated groups, when compared to control and extract treated groups.

Ahmed *et al*. (2013a) reported that hydro alcoholic extract of *Adiantum capillus veneris* Linn. was found to decrease the serum calcium, oxalate and phosphate levels in ethylene glycol administered rats. This extract is generally used by Unani physicians for its medicinal use in urolithiasis, was thus validated scientifically.

Celosia argentea seeds were scientifically evaluated to study antiurolithiatic activity in male Wistar rats, and the study showed a decrease in serum calcium, oxalate and phosphate, comparable with standard drug Cystone (Pranav *et al*., 2012).

Table 10

Levels of serum calcium, oxalate and phosphate in experimental animals

Treatment groups	Calcium	Oxalate	Phosphate	Uric acid	Creatinine
	mg/dL				
Control	7.67±0.148^{e}	7.20±0.025^{d}	5.51±0.155^{e}	1.47±0.057^{d}	0.54±0.017^{d}
Lithiatic Control	13.02±0.048^{a}	14.9±0.082^{a}	9.89±0.042^{a}	3.67±0.046^{a}	1.77±0.021^{a}
Extract Control	7.98±0.088^{d}	7.16±0.042^{d}	5.91±0.068^{d}	1.47±0.026^{d}	0.56±0.012^{d}
Preventive	8.32±0.015^{c}	8.43±0.033^{c}	6.20±0.073^{c}	1.60±0.017^{c}	0.65±0.008^{c}
Curative	8.83±0.069^{b}	9.12±0.025^{b}	6.81±0.039^{b}	2.05±0.052^{b}	0.73±0.013^{b}
Standard drug	8.11±0.079^{d}	9.07±0.078^{b}	6.35±0.057^{c}	1.66±0.022^{c}	0.67±0.017^{c}
Tukey's HSD at 5%	0.1903	0.119	0.1843	0.0897	0.0341

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

In contrast, the ethanolic extract of *Tamarindus indica* (at 250, 400 and 500 mg/kg body weight) exhibited a dose dependent antilithiatic activity on ethylene glycol treated mice. The serum calcium, oxalate, phosphate, uric acid and creatinine levels were found to be statistically not significant, also the antilithiatic effect of ethanol extract of *Tamarindus indica* was found less effective than the reference standard Cystone (Joseph *et al*., 2005; Goyal *et al*., 2014).

4.3.2.2. Effect of Red banana corm on serum uric acid and creatinine levels

The levels of serum uric acid and creatinine in male Wistar rats belonging to different treatment groups are furnished in Table 10. The levels of uric acid and creatinine in serum were significantly increased in lithiasis induced rats indicating renal dysfunction due to urolithiatic condition. The extract treated group showed a significant reduction when compared to lithiasis induced rats. The elevated levels of serum uric acid and creatinine were brought down by the presence of methanolic extract of Red banana

corm revealing its good preventive and curative property against lithiasis, and was comparable with that of standard drug Cystone.

In urolithiasis, the glomerular filtration rate decreases due to obstruction of the flow of urine by stones in urinary system. This causes impairment of renal functioning resulting in decreased excretion of waste products, particularly nitrogenous substances namely creatinine and uric acid with concurrent accumulation in blood (Kore *et al*., 2011). Coconut water was reported to have reduced the elevated levels of serum creatinine and uric acid in lithiasis induced rats. It also rebalances the serum urea and thus, unveils the potential effect of coconut water in maintaining renal functioning (Gandhi *et al*., 2013).

The effect of the ethanolic leaf extract of *Morus alba* L. against nephrolithiasis in Wistar rats, showed a high levels of serum calcium, oxalate, uric acid and creatinine in lithiasis induced rats compared to extract treated rats (Maya and Pramod, 2014). Findings of the present study are consistent with the antilithiasis studies of Khan *et al*. (2012) in *Holarrhena antidysentrica*; Krishna *et al*. (2010) in *Macrotyloma uniflorum*; Manjula *et al*. (2012) in *Costus igneus*; Prasobh and Revikumar (2012) in *Musa* tablet and Aggarwal *et al*. (2012) in *Achyranthes aspera* Linn.

Thus, the results of present study proved the potential of Red banana corm in regulating the renal functions and render a good prevention and protection against lithiasis.

4.3.3. *In vivo* analysis of body weight and kidney of experimental animals

4.3.3.1. Effect of Red banana corm on the body weight

Table 11 lists the change in body weight in all the treatment groups. In untreated control and extract control, the body weight was found to have increased significantly during the study period. All the animals treated with ethylene glycol exhibited increase in weight which was significantly lesser than the control. During the period of treatment, body weight of the rats treated with the extract (preventive and curative regimen) and standard drug showed a significant improvement.

Table 11

Body weight and kidney weight of experimental animals

Treatment groups	Body weight			Kidney weight	
	Initial – 0th Day (g)	Final – 28th Day (g)	Weight gain (%)	Absolute organ weight (g)	Kidney body weight ratio
Control	260±0.93^{ab}	280±1.29^{a}	7.6±0.472^{a}	1.59±0.017^{d}	0.567±0.006^{d}
Lithiatic Control	265±0.90^{a}	267±1.03^{c}	1.1±0.800^{c}	1.86±0.033^{a}	0.694±0.024^{a}
Extract Control	258±3.53^{abc}	277±4.72^{ab}	7.4±0.454^{a}	1.54±0.022^{e}	0.554±0.006^{d}
Preventive	258±4.87^{abc}	272±4.87^{bc}	5.2±0.488^{b}	1.71±0.021^{c}	0.631±0.007^{c}
Curative	256±0.66^{bc}	267±1.37^{c}	4.3±0.333^{b}	1.75±0.018^{b}	0.657±0.008^{b}
Standard drug	253±2.42^{c}	266±2.81^{c}	5.4±0.435^{b}	1.70±0.014^{c}	0.640±0.011^{c}
Tukey's HSD at 5%	6.2912	6.9181	1.7708	0.049	0.0156

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

Ahmed *et al*. (2013a) reported that the hydroalcoholic extract of *Adiantum capillus veneris* Linn. treatment in male Sprague Dawley rats, exhibited similar results as that of present study, in which an increase in body weight of the animals were observed. *Capaifera langsdorffii* Desf. commonly known as "Copaiba", tested for its antiurolithiatic activity revealed an increase in body weight when compared to lithiatic control (Paula *et al*., 2012).

Crataeva magna Lour. Bark, commonly known as 'Baruna', was investigated for urolithiasis in rats, and the results showed, an increase in body weight in extract treated group when compared to the ethylene glycol induced rats (Mekap *et al*., 2011). Ethanolic extract of rhizome of *Bergenia ligulate* at a dose of 10mg/kg body weight of the rat, protected it against deleterious effects of lithogenic treatment including weight loss (Aggarwal *et al*., 2014). In contrast, an insignificant difference in body weight with that of the control group was observed when the lithiasis induced rats were treated with *Ammi visnaga* seeds, while the kidney weight increased significantly (Khan *et al*., 2001). These results are in agreement with the present study and substantiate the protective effect of Red banana corm.

4.3.3.2. Effect of Red banana corm on the kidney weight

The kidney weight of untreated control and lithiasis induced rats were represented in Table 11. After treatment with methanolic extract of Red banana corm, the weight of kidneys were recorded and compared among the groups which was found to be in the range of 1.54 – 1.86g. There was a significant increase in kidney weight of animals in lithiatic control (1.86g), which was almost normalized in the extract treated groups i.e., in both preventive and curative groups. In lithiatic group animals, deposition of stone into kidney had resulted in the increase in weight of the kidney.

The results of the present investigation are in accordance with that of Rad *et al*. (2011) who investigated the antilithiatic property of roots of *Cynodon dactylon* in male Wistar rats and reported a similar effect on kidney weight. Traditionally, *Tridax procumbens* L. is being used for treating kidney stone disorders, which was scientifically validated *in vivo* by Sailaja *et al*. (2011) and the results are comparable with that of the present study.

Significant increase in kidney weight in urolithiatic rat might be due to calculogenesis, which is considered as a result of urinary supersaturation with respect to stone forming constituents, or water retention or may be due to inflammation of the nephrons epithelia mainly caused by calcium oxalate deposition. This significant increase in kidney weight was reported in many studies (Bashir and Gilani, 2009; Tsai *et al*., 2008; Mitra *et al*., 1998). Significant reduction in the kidney weight was observed in groups treated with methanol extract of Red banana corm, which in part could be due to the anti-inflammatory or diuretic property of the banana corm.

4.3.3.3. Effect of Red banana corm on the levels of calcium and oxalate in kidney tissue

Kidney tissues was assessed for calcium and oxalate levels, and the data are presented in Table 12. It has been reported that the kidneys are the major target organ for ethylene glycol toxicity and administration of ethylene glycol for a week resulted in significant urinary oxalate excretion and deposition of oxalate in kidney tissue (Sathish *et al*., 2010). Rats treated with ethylene glycol alone resulted in increased calcium and oxalate

content in kidney tissue, whereas negative control, extract control and standard drug treated animals showed normal levels. Administration of methanolic extract of Red banana corm significantly declined the calcium and oxalate accumulation. The results revealed the efficiency of the extract not only in preventing the formation of stones but also in dissolving pre-formed calcium oxalate calculi in kidney.

Table 12

Levels of calcium and oxalate in kidney tissue of experimental animals

Treatment groups	Calcium	Oxalate
	mg/ g tissue	
Control	3.23±0.024^{d}	1.40±0.010^{e}
Lithiatic Control	6.49±0.209^{a}	6.20±0.425^{a}
Extract Control	3.23±0.189^{d}	1.49±0.062^{e}
Preventive	4.18±0.071^{c}	2.88±0.089^{c}
Curative	4.93±0.126^{b}	3.37±0.092^{b}
Standard drug	3.27±0.303^{d}	2.15±0.046^{d}
Tukey's HSD at 5%	0.4028	0.4131

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

A similar result were observed by Manjula *et al*. (2012) who used *Costus igneus* stem extract and showed protective effect against urolithiasis in albino rats. The levels of calcium and oxalate in kidney tissue was found elevated in lithiatic control, whereas in extract administered group these levels were significantly low. The aqueous and alcoholic extract of *Aerva lanata*, *Dolichos biflour* and *Musa* (poly-herb extract) were evaluated for their antiurolithiatic activity in rats. The results showed that the poly-herb extract significantly lowered the calcium and oxalate levels in the kidney tissue of both curative and preventive group rats (Garimella *et al*., 2001; Ramachandran *et al*., 2011).

The effect of ethanol extract of dried roots of *Musa paradisiac* Linn. (Nendran-AAB) against ethylene glycol induced renal calculi in albino Wistar rats was evaluated. The results showed a significant decrease in the levels of calcium in the kidney tissues (Jha *et al*., 2011). Ethylene glycol induced rats, when administered with NONI a

commercial formulation (*Morinda citrifolia* fruit juice) showed a significant decrease in the level of calcium in the kidneys (Verma *et al*., 2009).

4.3.4. Activities of marker enzymes of serum, kidney and liver of experimental animals

Cell damage leads to the release of marker enzymes namely Alanine transaminase and Aspartate transaminase into blood circulation. Most of the urinary enzymes originating in the kidneys are localized to specific regions and cellular components of the nephron, and so, studies pertaining to these enzymes would show the exact pathological status of the kidney (Soundararajan *et al*., 2007). To ascertain the cell damage, the level of marker enzymes ALT and AST of serum, kidney and liver were analyzed. The activities of ALT and AST in serum, liver and kidney are presented in Table 13.

Table 13

Activities of marker enzymes of serum, liver and kidney

Treatment groups	Serum		Liver		Kidney	
	ALT	AST	ALT	AST	ALT	AST
	IU /g protein					
Control	33.45±0.001^{c}	30.27±0.054^{c}	27.49±0.457^{a}	18.93±0.351^{a}	9.73±0.068^{a}	9.53±0.068^{a}
Lithiatic control	59.43±0.015^{a}	62.75±0.111^{a}	17.08±0.605^{e}	12.94±0.613^{e}	3.70±0.057^{d}	6.24±0.057^{c}
Extract control	33.95±0.032^{c}	31.78±0.034^{bc}	26.75±0.361^{a}	18.29±0.121^{a}	9.57±0.079^{a}	9.43±0.079^{a}
Preventive	35.11±0.116^{bc}	33.13±0.068^{bc}	22.44±0.395^{d}	14.36±0.304^{d}	8.85±0.148^{b}	9.08±0.148^{c}
Curative	38.59±0.631^{b}	36.54±0.217^{b}	24.15±0.395^{c}	15.55±0.421^{c}	8.06±0.535^{c}	7.92±0.535^{b}
Standard drug	36.54±0.361^{bc}	35.99±0.101^{b}	25.64±0.632^{b}	17.02±0.015^{b}	9.30±0.075^{ab}	9.41±0.075^{a}
Tukey's HSD at 5%	4.051	5.178	1.073	0.8126	0.6831	0.5254

The values are mean ± S.D. of triplicate

Groups with common superscripts do not differ significantly

An evidence of hepatic injury is the leakage of cellular enzymes into the plasma. When the liver cell plasma membrane is damaged, a variety of enzymes, normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and the type of hepatocellular damage (Mitra *et al.*, 1998; Buwa *et al.*, 2001). The rise in ALT activity is almost always due to hepatocellular damage and is usually accompanied by a rise in AST (Ansari *et al*., 2003).

The activities of these enzymes have decreased significantly in kidney and liver tissues in lithiatic rats. Methanolic extract of Red banana corm brought the enzyme activity to near normal in preventive and curative groups, whereas the extract control animals did not show any significant changes. The activities of ALT and AST were significantly increased in serum of ethylene glycol induced urolithiatic rats compared with that of the control. The possible mechanism for this can be attributed to the damaged structural integrity of the renal and hepatic cells causing the enzymes located in the cytoplasm, being released into the circulation. If membrane of other organelles such as mitochondria is damaged, soluble enzymes such as compartmentalized AST would also be released. The release of these enzymes into circulation would indicate that both the plasma and organelle membranes are damaged. Due to such cellular damage, AST and ALT levels in kidney and liver tissues of the ethylene glycol induced urolithiatic rats would decrease (Senthilkumar *et al*., 2003).

Results of the present study are in accordance with the following studies. Similar observation was recorded by Shamina and Jishamol (2014 and 2014a), while evaluating antiurolithiatic effect of *Scoparia dulcis* against ethylene glycol induced urolithiasis in rats. The levels of kidney marker enzymes such as ACP, ALP, AST and ALT were decreased in ethylene glycol induced rats and the levels of these enzymes regained to near normal with *Scoparia dulcis* extract and *Pedalium murex* extract treatment (Mandavia *et al*., 2013).

The aqueous extract of *Petroselinum sativum* aerial parts and roots in all doses showed significant antiurolithiatic activity by restoring the level of marker enzymes of serum, kidney and liver (Jafar *et al*., 2012). Using *Hygrophila spinosa* T. Anders, *Plectranthus mollis* and *Hordeum vulgare* Linn. similar results were obtained by Singh *et al*., (2006), Baheti and Kadam, (2013) and Shah *et al*. (2012). In the present study, upon administration of methanolic extract of Red banana corm, the activities of these enzymes were found to restore to normal levels in the ethylene glycol induced urolithiatic rats, indicating its antiurolithiatic activity.

4.3.5. Histopathological examination of the kidney tissue

Histopathological examination (Plate 6) was carried out to confirm the above results. The architectural appearance of the kidneys of the rats in the control group showed a normal histological appearance with no calcium oxalate deposition, with normal glomeruli and tubules surrounded by the Bowman's capsule, proximal and distal convoluted tubules without any inflammatory changes and normal blood vessels was observed (Table 14). However, in lithiatic control, microscopic observation showed renal tubular damage consisting of tubular obstruction due to crystal formation, which were polymorphic and irregular in shape inside the tubules (3 or 4 large polygonal crystals in different segments of renal tubules). This caused dilation of the proximal tubules along with interstitial inflammation.

Table 14

Histopathological architecture of the kidney tissue of male Wistar rats

Treatment groups	Histological features of kidney sections of rats					
	Tubular congestion	Tubular cast	Epithelial disquamation	Glomerular congestion	Blood vessel congestion	Inflammatory cells
Control	-	-	-	-	-	-
Lithiatic Control	+ + + +	+ + +	+ + +	+ + + +	+ +	+ + +
Extract Control	-	-	-	-	-	-
Preventive	+ +	+	+	-	-	-
Curative	+ +	+	+	+ +	+	+
Standard drug	+ +	+	+	+ +		+

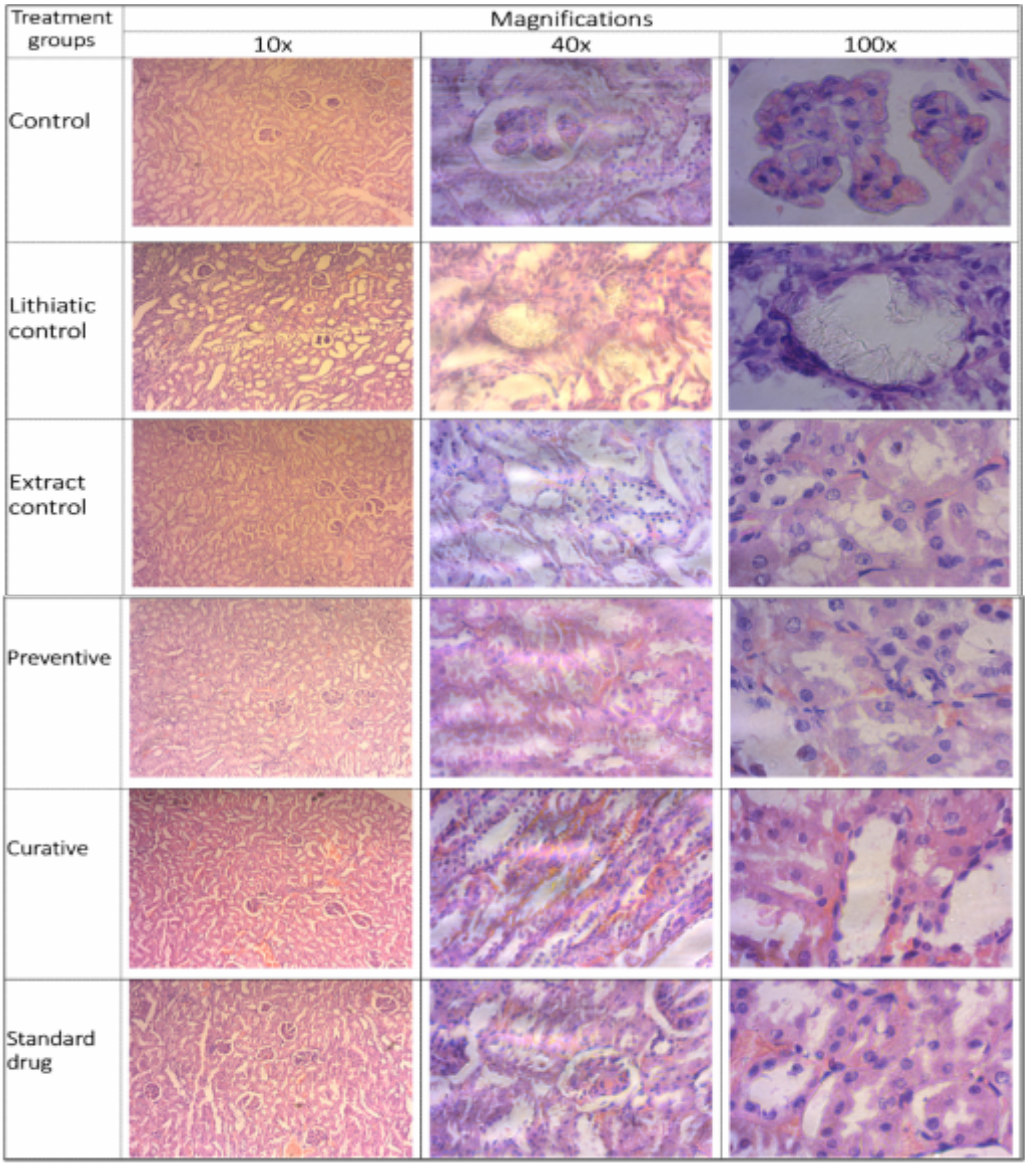
+ + + + Severe abnormality; + + + moderate; + + & + less; -- absence of abnormality

Administration of methanolic extract of Red banana corm to the preventive and curative groups, prevented supersaturation of calcium oxalate and thus decreased their deposition in renal tubules as observed in the photomicrograph. It is postulated that the extract inhibits the deposition of crystals in kidney tubules. Thus, the corm extract may interfere directly with the inhibition of crystal adhesion to the epithelium by blocking the attachment sites located either on the cell surface or on the surface of the crystals themselves.

It may be suggested that the extract contains substances that coat crystals, thereby blocking their adhesion to the cell surface and render its protection during all the stages of stone formation.

Plate 6

Histopathology of kidney tissue sections of experimental animals



Several studies have shown that, crystal formation results in cell damage and cell detachment from the basement membrane and the released degradation products further promote nucleation of crystals (Agarwal *et al*., 2012; Hackett *et al*., 1990). In another study by Atmani *et al*. (2009), it has been established that *Cynodon dactylon* extract has

beneficial effect in preventing and eliminating calcium oxalate deposition in the kidneys. The same results were reported with *Nigella sativa* seeds, in which its ethanolic extract reduced the number of calcium oxalate deposits in rats (Hadjzadeh *et al*., 2007). The hydroalcoholic extract of *Alcea rosea* root, at dose of 170 mg/ kg, to rats with ethylene glycol-induced lithiasis had a preventive effect on CaOx calculus formation in the rat kidney. The number of CaOx calculi is decreased in the *Alcea rosea* extract treated group and demonstrated a curative effect on the disruption of CaOx calculi formed in the kidney due to ethylene glycol consumption (Ahmadi, 2014). Similar histopathological changes were observed in *Bergenia ciliate* extract in the experimental animals (Saha and Verma, 2011).

Vyas and Argal (2012) reported that control group showed a normal glomeruli, proximal and distal collecting tubules and small blood vessels, whereas lithiatic rats showed marked vascular proliferation and thrombosed vessels, foci of mononuclear infiltration and necrosis of inflammatory cells in interstitium with small area of hemorrhage. This was reverted with the administration of ethanolic extract of roots of *Lantana camara* and oleanolic acid isolated from roots of *Lantana camara*. Similar effect was observed by Mayee and Thosar (2011).

Cellular damage was evident in the study conducted by Pranav *et al*. (2012) in male Wistar rats induced with ethylene glycol. However, treatment with *Celosia argentea* seeds showed significant reduction in crystal deposition and cell damage.

A few studies on *in vitro* and *in vivo* antiurolithiatic property of *Asparagus racemosus* were conducted by Shashi *et al.* (2009) and Kumar *et al.* (2009). The ethanolic extracts significantly reduced the elevated levels of calculogenic ions and elevated the concentration of magnesium, one of the inhibitors of urinary crystallization. Reduction in the calcium (Titrimetric analysis) and phosphate (Colorimetric analysis) precipitation was also reported. The antiurolithiatic efficacy of *Plectranthus amboinicus* with special reference to calcium oxalate stones was subjected to detailed *in vivo* experimentation by Jose *et al.* (2005).

Mohamed *et al*. (2009), studied the inhibition of mineralization of urinary stone forming minerals by medicinal plants such as *Achyranthes aspera, Passiflora leschenaultia, Solena amplexicaulis*, *Scoparia dulcis* and *Aerva lanata*. As revealed in these studies, increased intake of fruit juice and seed extract of these plants would be helpful in urinary stone prophylaxis. *Costus spiralis* is extensively used in Brazilian folk medicine for expelling urinary stones. Aqueous extract of *C. spiralis* when used at a dose of 0.25g and 0.5g/kg/day for 4 weeks had significantly reduced the growth of calcium oxalate calculi in the urinary bladder of rats (Viel *et al.,* 1994).

The present data, based on the results of *in vivo* studies, entails that the administration of methanolic extract of Red banana corm on male Wistar rats with ethylene glycol, reduced and prevented the growth of stones, by establishing a balance between the urinary promoters and inhibitors. The outcome of the *in vivo* study supports the folk information regarding antilithiatic activity of the plant. The mechanism underlying this effect can apparently be related to increased diuresis and lowering of urinary concentration of stone forming constituents.

Considering the global scenario and global efforts to minimize animal sufferings, the present day biologists had thrown light on the usage of alternatives to model organisms (Forni, 2007). In this regard, it was chosen to test the effect of calcium oxalate cell injury on NRK 52E cell lines induced with lithiasis and its effect after treatment with methanolic extract of Red banana corm.

4.4. Effect of Red banana corm on lithiasis induced NRK 52E cells

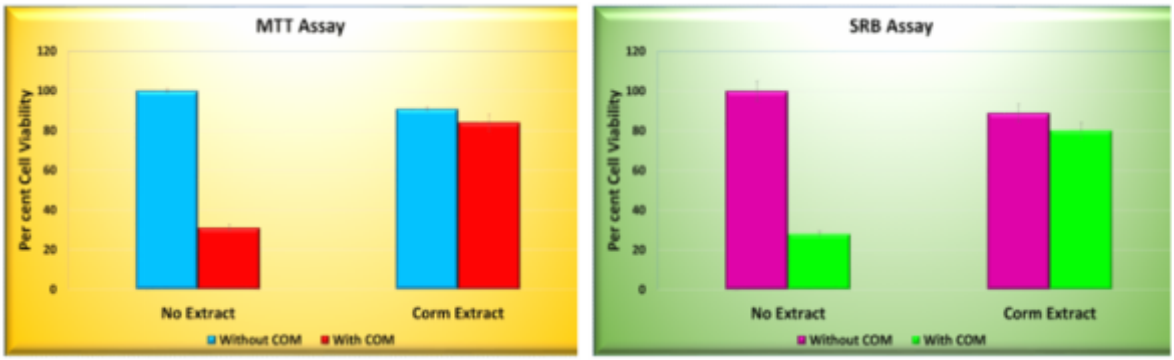
4.4.1. NRK 52E cell viability as assessed by MTT and SRB assay

Cell viability was assessed by MTT and SRB assays. Figure 9, illustrates the protective effect of the methanolic extract of Red banana corm towards the renal tubular epithelial cells as assessed by MTT assay. The oxalate induced a remarkable injury to the cells and this which could be ascertained by a decrease in viability from 100% in the control (untreated cells) to 59%. The injury due to oxalate however, was significantly reduced in those cells treated with the Red banana corm extract.

At the optimal concentration tested, the percentage viability of cells improved showing that the Red banana corm had an inhibitory activity towards the oxalate which caused injury to the renal cells. A similar trend was observed for SRB assay, which reiterated the protective effect of banana corm extract.

Figure 9

Viability of NRK 52E cells on oxalate induced cell injury



The values are mean ± SD of triplicate

The values of the untreated (negative) control group were fixed as 100% and the per cent viabilities in the other groups were calculated relative to this

Several reports have validated the MTT and SRB assays as relevant tools for quantifying the extent of cell survival. In a study conducted by Atmani *et al*. (2004), the adhesion of radioactive COM studied in the presence and absence of the aqueous extract of *Herniaria hirsute* in cultured renal cells revealed that the COM crystals binding to the cells were inhibited by the plant extract in a concentration dependent manner. Similar trend was observed in the present study also.

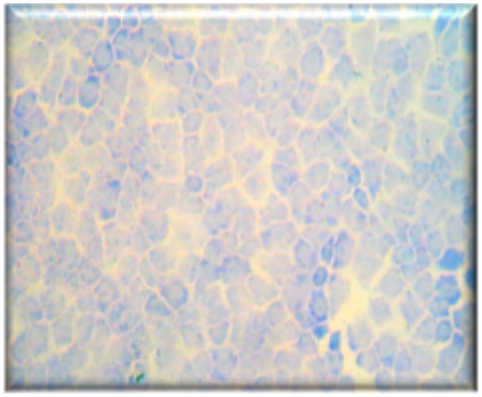
4.4.2. Morphological changes of NRK 52E cells

The characteristic morphological changes in renal epithelial cells were analyzed by Giemsa staining in the presence and absence of Red banana corm extract. The cells were viewed under a Phase contrast microscope and presented in Plate 7a - 7d. NRK 52E cells when treated with calcium oxalate caused a severe damage to the cell lines as observed by the appearance of deeply stained cells in the oxalate treated group, which was reversed by the administration of corm extract revealing the reversal of cell damage, thereby protecting kidney from oxalate exposure.

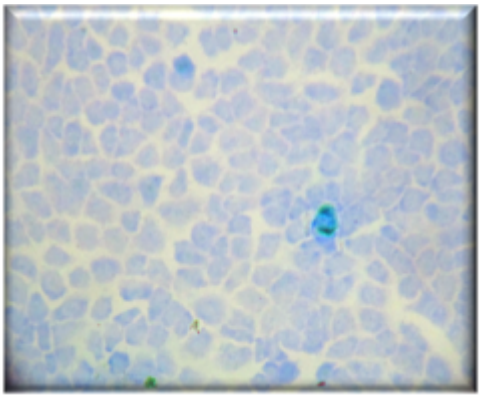
Plate 7

Morphological changes of NRK 52E cells stained with Giemsa staining

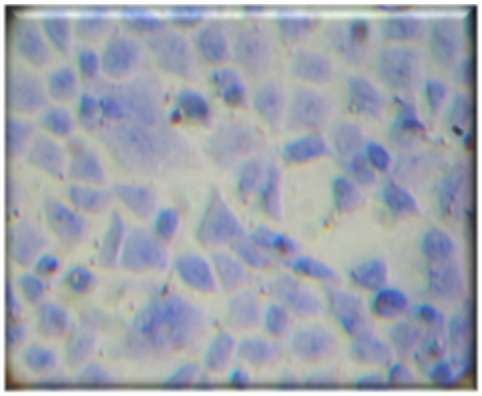
7a. Control



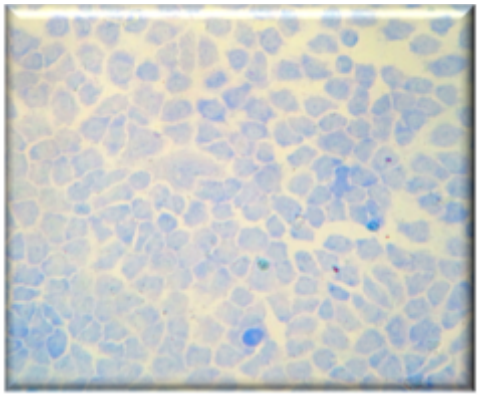
7b. Extract control



7c. Oxalate treated



7d. Oxalate with extract



In vitro effect of an aqueous extract of *Phyllanthus niruri* L., a plant used in Brazilian folk medicine for the treatment of urolithiasis, on a model of calcium oxalate crystal endocytosis by Madin-Darby canine kidney (MDCK) cells was investigated by Campos and Schor (1999). The extract exhibited potent and effective concentration-dependent inhibitory effect on the calcium oxalate crystal internalization.

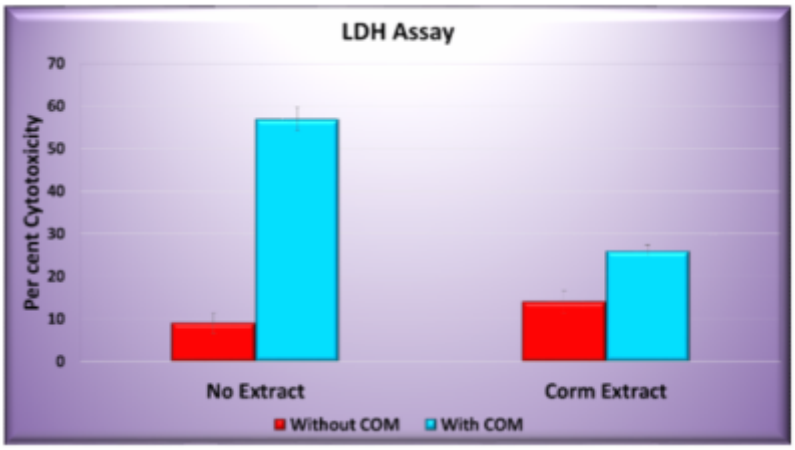
4.4.3. Cytotoxicity of NRK 52E cells as assessed by lactate dehydrogenase assay

The cytotoxicity was also quantified by monitoring the enzyme lactate dehydrogenase (LDH) leakage into the medium. Oxalate exposure in the form of COM crystals caused a steep rise in the extent of cell damage. LDH is a stable cytosolic

enzyme that is released when the cell is lysed or if there is any injury on the cell membrane. A significant increase in LDH release was seen when NRK 52E cells were exposed to oxalate alone. When NRK 52E cells were treated with the methanolic extract of Red banana corm along with oxalate, a reduction in oxalate-induced cell injury was observed, as assessed by decrease in LDH activity (Figure 10). Also, it was seen that the extract alone had no significant effect on the measure of cell injury in the absence of oxalate indicating its non-toxic nature.

Figure 10

Cytotoxicity of NRK 52E cells as determined by LDH release



The values are mean ± S.D. of triplicate

Beghalia *et al*. (2008a) have suggested in studies using certain Algerian medicinal plants that the herbal extract may contain substances that inhibit the growth of COM crystals. They further postulated that the plant extracts might contain substances that inhibit calcium oxalate crystal aggregation and also the binding of the crystals to the renal epithelial cell surface. This could explain a decrease in LDH release as seen in the cells treated with the plant extract as compared to those treated with oxalate alone.

The cytoprotective property of *Tribulus terrestris* tested using NRK 52E cells revealed low level of LDH leakage and increased cell viability (Aggarwal *et al*., 2010). Similar results were recorded in *Ammi visnaga* fruits (Vanachayangkul *et al*., 2010) and in *Phyllanthus niruri* leaves (Boim *et al*., 2010). The study conducted by Verkoelen *et al*. (2000) revealed that hyaluronan acted as binding molecule for COM crystals at the

surface of migrating and proliferating Madin-Darby canine kidney cells (MDCK). The effect of calcium on calcium oxalate monohydrate crystal-induced renal epithelial injury was studied by Khaskhali *et al*. (2009). In their study, human renal epithelial cell line, HK2, *in vitro* when exposed to CaOx monohydrate crystals at a concentration of 133 μg/cm^2 for 1, 3, 6 or 12h in the presence and absence of 5 and10 mM/L calcium ions, showed that the calcium and CaOx crystals affected the identical pathways as indicated by the LDH as marker of injury.

Renal epithelial cells when exposed to calcium oxalate crystals have confirmed the results obtained from animal model studies and have provided new insight into renal response to exposure of calcium oxalate crystals. Thus, the results of the present study with the cells cultured *in vitro* showed that the Red banana corm extract exhibited components that might have bound with excess oxalate thereby contributing antilithiatic effect towards the normal rat kidney cells and could be an effective alternate model for *in vivo* studies.

PHASE III

Based on the results of the Phase I and Phase II, it was evident that the methanolic extract of Red banana corm was found to be effective in the prevention and treatment of kidney stones.

Free radicals are constantly being generated *in vivo* for physiological purposes. They can be over produced under pathological conditions, causing oxidative stress. The body is endowed with both endogenous (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (vitamin C and E, carotene etc.) defense systems against free radicals generated within it. The beneficial effects of phytochemicals in this direction are associated with a number of biological activities including antioxidant and free radical scavenging properties (Oyebanji and Saba, 2011). Therefore, in the present study an attempt was taken to analyze the antioxidant status of Red banana corm extract.

4.5. Antioxidant levels in Red banana corm

The antioxidant content of Red banana corm was analyzed. Both enzymic and non-enzymic antioxidants were quantified and the values obtained are presented and discussed below.

4.5.1. Effect of Red banana corm on the activities of enzymic antioxidants

The antioxidants analyzed in the corm of Red banana were superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione S-transferase and polyphenol oxidase. The activities obtained are presented in Table 15.

Table 15

Enzymic antioxidant activities in Red banana corm

Enzymes	Activities
Superoxide dismutase (U/g)[1]	35.05 ± 0.865
Catalase (U/g)[2]	293.46 ± 2.215
Peroxidase (U/g)[3]	20.69 ± 0.327
Glutathione S-transferase (U/g)[4]	0.09 ± 0.015
Catechol oxidase(Units X 10^{-3}/ g)[5]	0.53 ± 0.021
Laccase (Units X 10^{-3} / g)[6]	0.45 ± 0.015

The values are mean ± S.D. of triplicate

[1] *1 Unit = Amount of enzyme that causes 50% reduction in NBT oxidation*

[2] *1 Unit = Amount of enzyme required to decrease the absorbance at 240nm by 0.05 units/minute*

[3] *1 Unit = Change in absorbance at 430 nm/minute*

[4] *1 Unit = nmoles of CDNB conjugated/minute*

[5] *1 Unit = Amount of catechol oxidase/laccase enzyme which transforms 1 unit of dihydrophenol to quinine /min*

The results revealed that the corm of Red banana had considerable activities of all the enzymic antioxidants studied. It is evident from the above tabulated values that the corm of Red banana is a good source of enzymic antioxidants. These results imply that the corm could serve as a potential candidature for the natural source of antioxidants. There are extensive literatures that correlated the antioxidant contents of the parts of plants with excellent medicinal properties.

Antioxidant activity of *Punica granatum* was attributed to the presence of several triterpenoids, phenolic compounds, saponins, flavonoids, tannins, alkaloids and steroids (Kaur and Saraf, 2012). The antioxidant status of different parts of *Coleus forskohlii*, including roots, stem, leaves and tubers were analyzed for enzymic antioxidant properties,

wherein significant activities of superoxide dismutase, peroxidases, polyphenol oxidase and catalase were reported (Khatun *et al*., 2011). The methanolic extract of *Cytisus scoparius* was found to be rich in SOD and CAT activity and ascorbic acid level besides controlling the lipid peroxidation to a great extent (Nirmal *et al*., 2008).

The aqueous extract of *Artemisia afra* Jacq. was found to be a good source of enzymic antioxidants, rich in GPx, GR and SOD (Afolayan and Sumonu, 2011). Nirmaladevi and Padma (2008) analyzed the fresh leaves of three under-exploited plants namely *Pergularia daemia*, *Rhinacanthus nasutus* and *Ruellia strepens* and found that *Rhinacanthus nasutus* was a potent source of both enzymic and non-enzymic antioxidants.

Administration of the flavonoid rich fraction of *Citrus medica* unripe fruits to rats with ethylene glycol induced lithiasis, prevented the growth of urinary stones, which could be due to decreased oxidative stress and diuretic effect of the extract (Chavada *et al*., 2012). It is evident that Red banana corm could serve as a rich source of antioxidants which in turn would be responsible for the diuretic effect of the corm extract, thus prevented the growth of urinary stones.

4.5.2. Effect of Red banana corm on the levels of non-enzymic antioxidants

Analyzing the levels of non-enzymic antioxidants has also been an integral part of several studies reporting the medicinal values of plants. The corm of Red banana was also found to contain considerable levels of non-enzymic antioxidants like ascorbate, tocopherol, total carotenoids, lycopene, reduced glutathione, total flavonoids and phenols (Table 16).

Kondakova *et al*. (2009) reported that the berry fruits namely *Vaccinium myrtillus, Rubus fruticosus, Ribes nigrum, Vaccinum corymbosum* and *Vitis vinifera* were found to be the rich source of antioxidants due to the presence of vitamin C and polyphenols such as flavonols, anthocyanins, phenolic acids and tannins. *Emblica officinalis* was reported to have antioxidant activity, which was attributed to the presence of vitamin C, tannins and flavonoids (Madhuri *et al*., 2011). Orčić *et al*. (2011) showed that a majority of the active principles present in *Umbilicaria cylindrical* expressed a very high antioxidative activity, which was attributed to flavonoids and phenolic acids.

Table 16

Non-enzymic antioxidant levels in Red banana corm

Non-enzymes	Levels
Ascorbic acid (mg/g)	1.35 ± 0.057
Tocopherol (μg/g)	5.13 ± 0.056
Total carotenoids (mg/g)	23.38 ± 1.191
Lycopene (mg/g)	12.42 ± 0.211
Reduced glutathione (nmoles/g)	153.2 ± 0.907
Total flavonoids (mg/g)	5.14 ± 0.128
Total phenols (mg/g)	12.97 ± 0.121

The values are mean ± S.D. of triplicate

Kumari and Achal (2008), reported that the non-enzymic antioxidants vitamins C, E and A were shown to be present in Oyster mushroom (*Pleurotus ostreatus*). Tiwari (2012) has brought to light that *Punica granatum* could be considered as a good source of antioxidant due to the abundance of vitamin C, B5 and polyphenols.

Vitamin C supplementation has been associated with decreased uric acid levels in the serum (promoter of kidney stone), decreased lipid peroxidation and enhancement of the activities of antioxidant enzymes in the kidney of albino rats (Olaiya *et al*., 2011).

The observations made in the present study showed that the corm of Red banana is a rich source of both enzymic and non-enzymic antioxidants, besides having medicinal properties against oxidant-induced disorders and diseases. Many studies on the urine of patients with CaOx stones revealed significant increase in the markers of oxidative damge, tubular injury and inflammation, indicating that renal impairment in these patients was most likely caused by reactive oxygen species (Aggarwal *et al*., 2013; Boonla *et al*., 2007; Mushtaq *et al*., 2007). Hence the Red banana corm extract with strong antioxidant activity was reason to impede calcium oxalate crystal growth and might inhibit stone recurrence.

Reactive oxygen species is an important contributor to renal injury and inflammation following exposure to oxalate or calcium oxalate crystals. They act in

synergy to enhance the risk of urinary stones and hence, in the present study Red banana corm was assessed for *in vitro* free radical scavenging effect.

4.6. Radical scavenging effect by Red banana corm extract

Knowing that the Red banana corm extract was rich in antioxidants, further analysis was carried out to assess the free radical scavenging activity. The methanolic extract was tested for their radical scavenging effects against a team of oxidant moieties that included the radicals DPPH, ABTS, H_2O_2 (non-radical), and $OH^{\bullet}$.

4.6.1. DPPH radical scavenging activity of Red banana corm

The per cent DPPH scavenging ability by the Red banana corm methanolic extract was carried out spectrophotometrically and the results are presented in Figure 11. It was observed that methanolic extract of Red banana corm effectively reduced the stable radical DPPH to a yellow-coloured compound diphenylpicryl hydrazine.

The results revealed that the corm extract could readily scavenge all the radicals tested. *Amorphophallus sp*. contains phytochemicals like polyphenols and flavonoid with antioxidative effect. Hexane extract and methanolic extract of *A. campanulatus* tuber were evaluated for *in vitro* antioxidant activities using DPPH, hydroxyl radical, reducing power and total antioxidant capacity assays. *In vitro* studies revealed that methanolic extract had higher antioxidant and radical scavenging activity than hexane extract, which might be attributed to its higher phenolic and flavonoid contents (Singh and Wadhwa, 2014).

Aydin *et al*. (2011) reported that the mature mulberry fruit extracts showed a concentration-dependent increase in the DPPH scavenging activity. *Ricconus communis* (root) tested for antioxidant activity using DPPH radical. The extracts with hexane and petroleum ether showed maximum antioxidant activity, among the four extracts namely hexane, petroleum ether, water and methanol.

Olorunnisola *et al*. (2012) observed that the extracts (ethanol and acetone) of leaves of *Hippobromus pauciflorus* when exposed to DPPH, significant scavenging activity with ethanolic extract was seen as compared to acetone extract. n-Hexane extract of *Mesua grandis* leaf was found to have high radical-scavenging activity using DPPH, when compared to other extracts namely dichloromethane, ethyl acetate and methanolic extract and hence a good source of antioxidant (Susanti *et al*., 2011).

Shad *et al*. (2012) reported that *Nilumbo micifera* ethanolic extract exhibited DPPH radical scavenging capacity and possessed good antioxidant properties. Viswanath *et al*. (2011) proved that the aqueous extracts of *Terminalia chebula*, *Emblica officinalis* and *Piper nigram*, had a direct correlation with the increase in polyphenol content and increased inhibition of DPPH radicals. The fruit extracts of *Zizyphus mauritiana* exhibited remarkable DPPH free radical scavenging ability at different concentrations analyzed (Bhuiyan *et al*., 2009).

In the present study, the observation that methanolic extract of Red banana corm can very effectively scavenge DPPH and showed the strong antioxidant activity of the corm, when extracted into methanol.

4.6.2. ABTS radical scavenging activity of Red banana corm

ABTS radical scavenging assay is a widely adopted parameter employed world over to establish the antioxidant activity of herbal components and extracts. ABTS is a synthetic radical produced by a reaction. The methanolic extract of Red banana corm was tested for its ability to scavenge the ABTS radical-cation and the results are presented in Figure 11. The methanolic extract exhibited higher ABTS scavenging effect.

The methanolic extract of *Allophylus africanus, Cardiospermum grandiflorum, Biligihia sapida, Bilighia unijugata, Deinbollia pinnata and Zanha golungensis* showed a dose-dependent ABTS scavenging activity (Sofidiya *et al*., 2012). Solarska *et al*. (2010) proved that carnitine is a good antioxidant, which was found to decolorize ABTS, and protect fluorescein against bleaching induced by AAPH-derived peroxyl radicals and peroxynitrite.

Florek *et al*. (2009) stated that effect of rutin on the total antioxidant status by ABTS radical-cation decolorization assay, in rats exposed to cigerrette smoke. Aqueous, methanol and chloroform extracts of *Majorana hortensis* leaves exhibited a dose dependent scavenging of ABTS radicals (Radha and Padma, 2012). The methanol extract of *Prosopsis cineraria* leaves showed maximum inhibitory activity against ABTS activity, when compared to its other solvents (Petroleum ether, benzene, chloroform, ethyl acetate and aqueous) extracts (Dharani *et al*., 2011).

Sahoo *et al*. (2010) demonstrated the potent ABTS scavenging activity of methanolic extract of *Alpinia malaccensis* leaves. Singh *et al*. (2012) reported that

methanol extract of *Stevia rebaudiana* leaves, roots, flower and stem had a dose dependent ABTS scavenging activity.

The radical scavenging properties of methanolic and aqueous extracts of the aerial parts of *Melissa officinalis* showed good ABTS scavenging and superoxide radicals (López *et al*., 2009; Koksal *et al*., 2011). Compounds isolated from *Diospyros lotus* were shown to have high antioxidant activity in different *in vitro* systems while detecting the free radical scavenging ability (Loizzo *et al*., 2009).

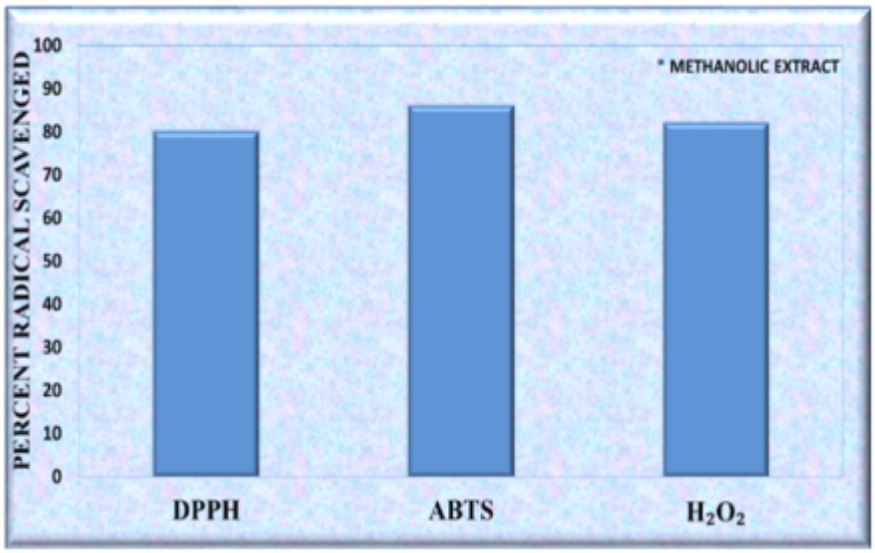
In the present study also, methanolic extract of Red banana corm extract possessed strong ABTS scavenging ability, which substantiates its antioxidant action.

4.6.3. Hydrogen peroxide scavenging activity of Red banana corm

The ability of methanolic extract of Red banana corm to scavenge hydrogen peroxide in an *in vitro* system was assessed in the present study and presented in Figure 11. The results revealed that, the methanolic extract of Red banana corm exhibited a strong scavenging effect against hydrogen peroxide.

Figure 11

DPPH, ABTS and H_2O_2 scavenging effects of Red banana corm extract



The values are mean ± S.D. of triplicate

Hydrogen peroxide is an endogenous radical produced by several endogenous processes, which can cross cell membrane rapidly. Once inside the cell, it can react with Fe^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Guguloth *et al*., 2011). Chang *et al*. (2010) reported in their study that nine natural flavonoids were found to be very potent antioxidants, which showed their ability to scavenge DPPH, superoxide and hydrogen peroxide radicals.

The leaves of *Azadirachta indica* A. (ethyl acetate and methanolic fraction) against various free radicals reduced $DPPH^{\bullet}$, ABTS, superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$) and nitric oxide radicals to non-radical forms as reported by Manikandan *et al*. (2009). A similar result was reported in *Baliospermum montanum* extract by Baburao *et al*. (2010). Kwape and Chaturvedi (2012) and Zachariah *et al*. (2012) proved the H_2O_2 scavenging activity of *Ziziphus mucronata* and *Mirabilis jalapa* extracts.

The results of the current studies are also in agreement with the study conducted by Nain *et al*. (2011) who reported H_2O_2 scavenging ability of methanol extract of *Jasminum humile* leaves. The methanolic extract of leaves of *Baccopa monnieri* also had significant H_2O_2 scavenging potential (Meena *et al*., 2012).

The above reports provide clear evidence to the fact that the H_2O_2 scavenging potential is indicative of the antioxidant effect of herbal preparations. In tune with this, the methanolic extract of Red banana corm effectively scavenged H_2O_2 generation and proved its strong antioxidant status in the present investigation.

4.6.4. Hydroxyl radical scavenging effect of Red banana corm

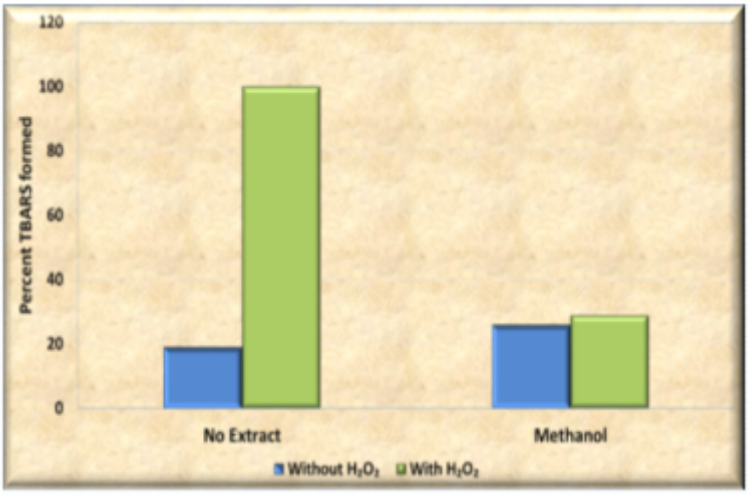
In free radical pathology, the hydroxyl radical is considered as a highly damaging species and extremely reactive free radical formed in biological systems and capable of damaging almost every molecule found in living cells (Radha, 2012). This radical has the capacity to join molecules in DNA and cause strand breakage which contributes to mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Thirunavukkarasu *et al*., 2011).

The hydroxyl radical has high reactivity and is short-lived. The extent of TBARS produced in the reaction is taken as a measure of hydroxyl radical production.

The inhibition of TBARS production is, thus, considered as a measure of hydroxyl radical scavenging efficiency. The exposure to H_2O_2 caused the maximum damage, which was very effectively reduced by the presence of the Red banana corm extract (Figure 12) in the present study.

Figure 12

Hydroxyl radical scavenging effects of Red banana corm extract



The values are mean ± S.D. of triplicate

The value of H_2O_2-treated group was fixed as 100 per cent and the relative values in percentage were calculated for the other groups

The whole plant methanolic extract of *Dolichos biflorus* contains large amount of phenolic compounds, which exhibit high antioxidant and free radical scavenging activities, by *in vitro* assays. This indicates that this plant extract is a better source of natural antioxidants, which might be helpful in preventing the progress of various oxidative stresses (Gowri *et al*., 2014).

The acetone extract of *Manilkara zapota* L., prevented the formation of deleterious radicals such as hydroxyl and hydroperoxides (Chanda and Nagani, 2010). Mulla *et al*. (2009) stated that *Alocasia indica* Linn. shown to effectively scavenge DPPH, ABTS, superoxide, nitric oxide and hydroxyl radicals.

An extract prepared from *Quercus salicina* Blume / *Quercus stenophylla* Makino could suppress renal cell injury induced by oxalate exposure by scavenging free radicals and suppressing the activation of NADPH oxidase (Moriyama *et al*., 2007). Jeong *et al*.

(2006) reported that epigallocatechin gallate from green tea also inhibited free radical production induced by oxalate.

The phenolics and flavonoids in *Caesalpinia crista* leaves scavenged hydroxyl radical to a full extent and was attributed to the antioxidant activity (Mandal *et al*., 2009). The methanolic extract of *Picrasma quassiades* (Yin *et al*., 2009), the ethanol extract of the leaves of *Stachytarpheta angustifolia* (Awah *et al*., 2010) and the aqueous extract of *Wagatea spicata* flowers (Samak *et al*., 2009) efficiently inhibited hydroxyl radicals.

Triphala, an ayurvedic commercial formulation, exhibited potent inhibition of hydroxyl radicals. The antioxidant activity of *Triphala* provide a mechanistic basis for relieving stress by way of combating oxidative damage (Shivaprasad *et al*., 2008). The antioxidant capacity of Apricot (*Prunus armeniaca* L.) has been attributed to the phenolic components present in it, which are effective scavengers of hydroxyl radicals (Jimenez *et al*., 2008). The extracts of *Boerhaavia diffusa* root possessed hydroxyl radical scavenging activity (Khalid *et al*. 2011). Liu *et al*. (2011) reported the antioxidant capacity of resveratrol from *Polygonum cuspidatum*, as reflected by hydroxyl scavenging, was shown in *in vivo* senescence-accelerated mice.

Free radical scavenging assays were performed to define the ability of the methanolic extracts of Red banana corm to quench an array of radicals generated after the induction of oxidative stress *in vitro*. The results revealed that the methanolic extract of Red banana corm was found to exhibit good radical quenching potential against all the radicals tested.

To conclude, there are several experiments to show the production of ROS and subsequent development of oxidative stress and associated cellular injury when renal cells are exposed to oxalate and calcium oxalate crystals. In the kidney of experimental animals, antioxidant treatments reduced the calcium oxalate crystal deposition (Doddola *et al*., 2010). Red banana corm with maximum antioxidant and radical scavenging activity can restore the SOD generation, CAT activity and glutathione peroxidase in renal cells and could reduce the risk of stone formation. Tocopherol can inhibit oxalate synthesis and enhance enzymatic and non-enzymatic antioxidant status in liver and kidney under lithogenic environment (Aggarwal *et al*., 2013). Thus, natural antioxidants strengthen the

endogenous antioxidant defences from ROS ravage and restore the optimal balance by neutralizing reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. Hence, antioxidants and free radical scavengers can provide superior renal protection in combination.

With the support of the above literature, it becomes clear that the methanolic extract of Red banana corm has very good hydroxyl radical scavenging activity, reiterating its strong antioxidant potential, which would be helpful to suppress the CaOx crystallization and thus renders protection during oxalate induced oxidative stress.

4.7. Characterization of phytochemical constituents of Red banana corm

Medicinal plants having an appreciable amount of antioxidant phytochemicals, have received growing attention as potential herbal agents. Epidemiological investigations have reported that antioxidants have a protective role in most of the health disorders (Khan *et al*., 2012). The results of the phytochemical analysis of *Verbena hastate* leaf have indicated the presence of alkaloids, flavonoids, saponins and glycosides in the methanolic and dichloromethane extracts. This finding supported its use as an effective antibacterial and cytotoxic agent (Edewor and Usman, 2012).

These properties are presumably rendered by the chemical substances or the secondary metabolites present in the extracts of different plant parts. In the present study, it becomes very much necessary to identify the active principle(s) providing the protective effects in Red banana corm that get extracted into methanol. Hence, this study emphasized on the qualitative identification of the chemical nature of the active components present in the candidate herb. This was followed by spectral studies such as UV absorption spectrum, HPTLC, HPLC, FTIR, GC-MS and ^{1}H-NMR to identify the major bio-active components present in the corm of Red banana that might be responsible for the antiurolithiatic activity.

4.7.1. Preliminary qualitative phytochemical analysis

The corm of Red banana was subjected to phytochemical analysis to identify the presence of major secondary metabolites. The qualitative tests performed showed the presence of alkaloids, flavonoids, sterols, terpenoids, tannins, saponins and phenols (Table 17).

From these results, it can be inferred that the active components in Red banana corm may be the following secondary metabolites which include alkaloids, flavonoids, sterols, terpenoids, tannins, saponins, phenols and carbohydrates. Hence these phytochemical fractions were isolated and subjected to UV absorption.

The qualitative analysis of Red banana corm revealed the presence of phytochemicals like alkaloids, flavonoids, sterols, terpenoids, tannins, saponins phenols and carbohydrates. The presence of above phytochemicals could have contributed to the antioxidant and therapeutic value of the plant. Several botanists and biologists have worked on various phytochemicals and many of the literature are available, as discussed below.

Table 17

Qualitative phytochemical analysis of Red banana corm

S.No.	Components	Result
1.	**Alkaloids**	
	Mayer's test	+
	Dragendroff's test	+
	Wagner's test	+
2.	**Flavonoids**	
	Aqueous NaOH test	+
	Conc. Sulphuric acid test	+
	Schinado's test	+
3.	**Sterols**	
	Leibermann-Buchard test	+
	Salkowski test	+
4.	**Terpenpoids**	
	Leibermann-Buchard test	+
5.	**Tannins**	
	Braemer's test	+
6.	**Saponins**	
	Froth test	+
	Haemolytic test	+
7.	**Phenols**	
	Ferrric Chloride test	+
	Lead acetate test	+
8.	**Carbohydrates**	
	Molisch's test	+
	Fehling's test	+

The aqueous extract of three medicinal plants, *Trigonella foemum-graecum*, *Echium amoemum* and *Tribulus terrestris*, showed presence of saponins, which act as surface active agent that have been used in pharmaceutical formulations for different purposes (Noudeh *et al*., 2011). The extract of root bark of *Delonix regia* revealed the presence of tannins, terpenoids, alkaloids, glycosides, carbohydrates and sterols suggesting that the root bark can be used for curing various ailments (Sama and Xavier, 2011). The phenols and flavonoids from the herbal extracts of *Oroxylum indicum* and *Tiliacora triandra* were shown to possess high antioxidant activity (Phadungkit *et al*., 2012).

Maoela *et al*. (2009) depicted that the South African *Carpobrotus* species contained hydrolysable tannins and various flavonoids like rutin and hyperoside, phytosterols and aromatic acids, which had a diverse range of pharmacological properties including antimicrobial and antioxidant activities. Vala *et al*. (2012) showed that the saponins, flavonoids and tannins from *Rhynchocorys elephas* exhibited antibacterial and mutagenic activity.

The leaves of *Senna alata* and *Cajanus cajan* showed the presence of alkaloids, tannins, saponins, steroids, terpenoids, flavonoids and cardiac glycosides (Uwangbaoje, 2012). Phenolic phytochemicals have antioxidant and antimicrobial activities (Arts and Hollman, 2005). Steroid, one of the phytochemical compounds have been found to possess numerous and diversified physiological functions and pharmacological properties (Singh and Kaushal, 2007).

Alkaloids are heterocyclic indole compounds which are shown to have antioxidant activity (Mallikharjuna *et al*., 2007). Herbs that have flavonoids and tannins as their components are reported to reduce disease risk and have therapeutic properties (Venkataswamy *et al*., 2010). Antiurolithiatic activity of Cerpegin alkaloid from *Ceropegia bulbosa* var. Lushii root was proved by Monika *et al*. (2012).

The literature cited so far added credit to the current findings on Red banana corm extract which carries a good source of pharmaceutically valuable components like alkaloids, flavonoids, sterols, terpenoids, tannins, saponins, phenols and carbohydrates in the qualitative screening performed. Thus, Red banana corm extract can very well be

considered for the formulation of many commercial pharmaceutical products for lithiasis and other several aliments in future.

4.7.2. UV/visible absorption spectrum of the phytochemical fractions of Red banana corm

The absorption spectrum of the different fractions of secondary metabolites namely, alkaloids, phenols, flavonoids, sterols and tannins of the Red banana corm were evaluated in the UV/visible range which gave specific absorption spectrum.

The alkaloid fraction of Red banana corm (Figure 13) showed several major and minor peaks, beginning with sharp peak at 195nm, followed by six major peaks between 225 – 350nm and a single major peak at 575nm.

Figure 14 shows the UV absorption spectrum of the flavonoid fraction, with well defined seven major peaks at 220, 250, 260, 275, 285, 325 and 340 nm corresponding to the presence of flavonoid compounds in the corm extract.

The UV absorption spectrum of the steroid fraction (Figure 15), possessed well defined seven major peaks at 220, 250, 270, 280, 300, 325, and 670 nm. This confirms the presence of useful steroids in the corm of Red banana.

Figure 16 showed the peaks obtained from the terpenoid fraction by UV/visible absorption spectrum. Several major peaks were observed between 190 – 350 nm (190, 220, 230, 245, 255, 270, 300, 310, 325 and 340 nm).

The UV/visible spectrum of tannin fraction was determined and the spectrum is presented in Figure 17. The peaks observed were seven major and one minor peak in the range of 200 – 325 nm (major peaks) and 340 nm (minor peak).

The saponin fraction of Red banana corm revealed the presence of six peaks, indicative of active principle constituent in the corm. Figure 18 showed the peaks at 225, 240, 275, 310, 340 and 660 nm.

Figure 19 showed the presence of two minor peaks of phenol fractions at 225 and 235 nm, followed by five major peaks at 265, 275, 285, 300 and 560 nm.

Figure 13

UV absorption spectrum of the alkaloid fraction of Red banana corm

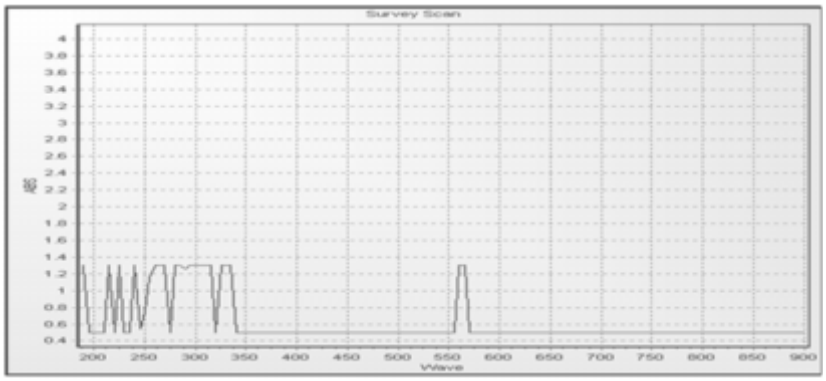


Figure 14

UV absorption spectrum of the flavonoid fraction of Red banana corm

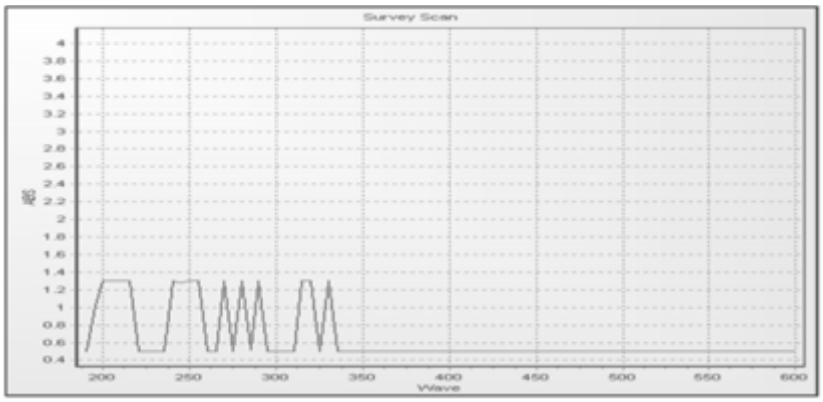


Figure 15

UV absorption spectrum of the steroid fraction of Red banana corm

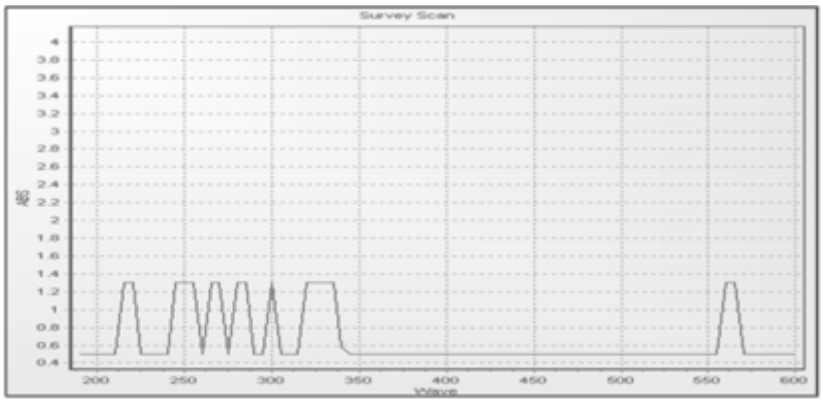


Figure 16

UV absorption spectrum of the terpenoid fraction of Red banana corm

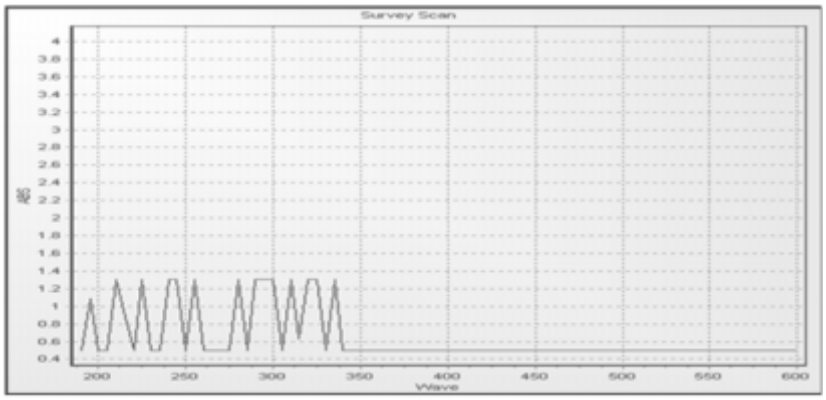


Figure 17

UV absorption spectrum of the tannin fraction of Red banana corm

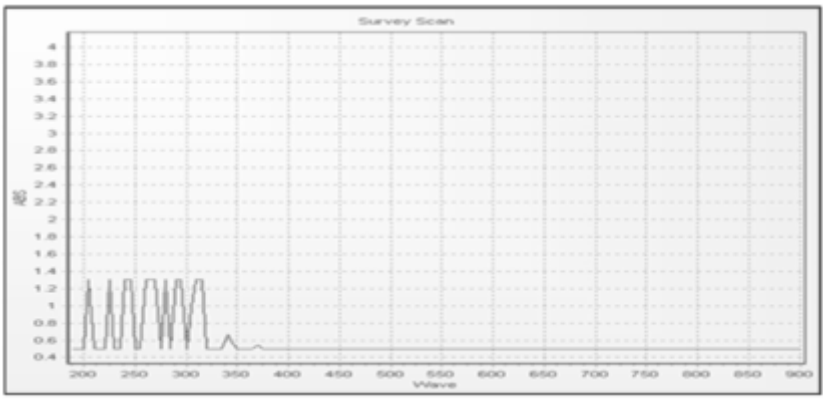


Figure 18

UV absorption spectrum of the saponin fraction of Red banana corm

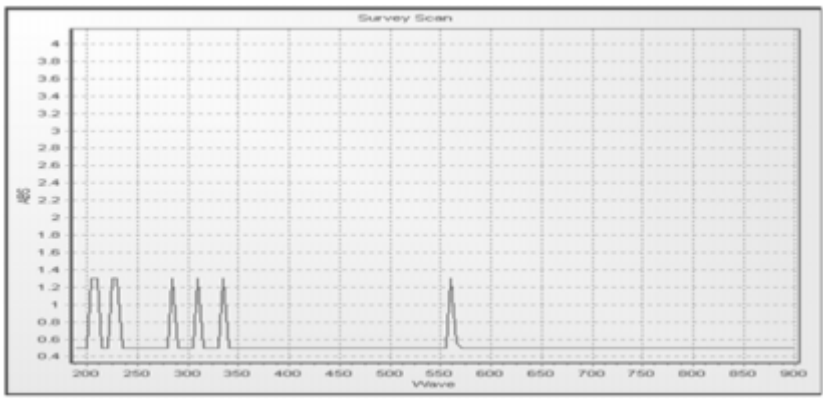
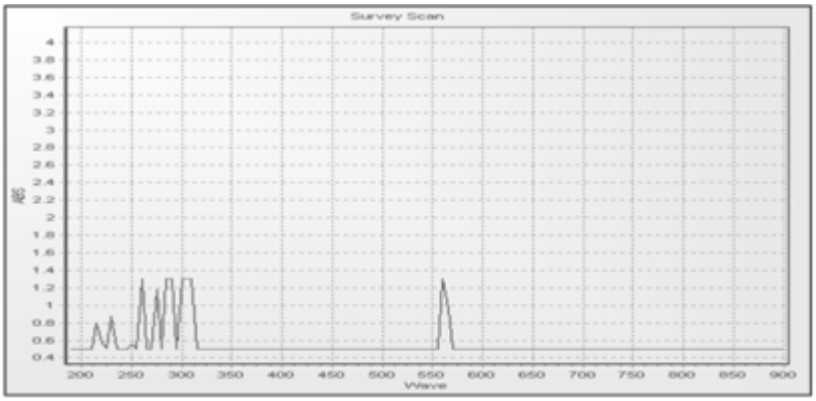


Figure 19

UV absorption spectrum of the phenol fraction of Red banana corm



Nirmaladevi *et al*. (2010), indicated the presence of multiple phytochemical components in the extract of *Rhinacanthus nasutus* leaves in the wavelength ranging from 190-1100nm in survey scan. A similar result was observed in *Majorana hortensis* leaf extract with major active principles in the survey scan (Radha, 2012). The alkaloids, phenolics and flavonoids are the major active principles responsible for the therapeutic potential of *Gouroupita guianesis* flowers, using the UV absorption spectrum (Kavitha, 2010).

In the present study, the absorption pattern of alkaloids, flavonoids, sterols, terpenoids, tannins, saponins and phenols confirmed that these may be the major active principles responsible for the therapeutic potential of Red banana corm and corroborates the finding of above mentioned literatures.

4.7.3. HPTLC of the methanolic extract of Red banana corm

The methanolic extract of Red banana corm was subjected to HPTLC analysis for the presence of alkaloids, flavonoids, sterols, terpenoids, tannins, saponins and phenols.

The alkaloid profile of the methanolic extract was done with the reference to standard colchicine and the plate was developed with Dragendroff's reagent. Orange-brown coloured zone at day light mode present in the given standard and sample tracks observed in the chromatogram after derivatization confirmed the presence of six alkaloids in the corm extract (Plate 8). The peak table (Table 18) and peak densitogram (Figure 20) were recorded.

The alkaloids against colchicine reference standard showed 10 peaks, in which peaks 2, 3, 5, 6, 7 and 9 corresponds to alkaloids. The R_f value of the standard was found to be 0.41.

The flavonoid profile of the methanolic extract of Red banana corm was analyzed using quercetin as the standard. Yellow and yellow green florescence zone at UV 366 nm was seen from the chromatogram, which confirmed the presence of flavonoids (Plate 9). There were four different flavonoids identified in the methanolic extract of Red banana corm as shown in the peak table (Table 19) and peak densitogram (Figure 21). The R_f value of standard was found to be 0.94.

The steroid profile of the methanolic extract of Red banana corm was analyzed using solasodine as the standard. Blue-violet coloured zone in the day light mode present in the given standard and sample tracks observed in the chromatogram after derivatization confirmed the presence of sterols in the Red banana corm extract (Plate 10). The peak table (Table 20) and the peak densitogram (Figure 22) confirmed the presence of five steroids. The R_f value of the standard was found to be 0.85.

Plate 11 confirmed the presence of terpenoids in the methanolic extract of Red banana corm where artemisinin standard was used. Blue, yellowish brown coloured zones in the visible light mode were present in the track observed from the chromatogram after derivatization, which confirmed the presence of terpenoids in the given samples. The peak table (Table 21) and the peak densitogram (Figure 23) represented 3 different terpenoids.

Using tannic acid as the reference standard, the tannin profile of the methanolic extract of Red banana was analyzed by spraying 5% ferric chloride. Bluish brown coloured zones in the day-light mode confirmed the presence of four tannins (Plate 12). The peak table (Table 22) and peak densitogram (Figure 24) showed the third tannin to be tannic acid.

The saponin profile of the methanolic extract of Red banana corm was analyzed using saponin as the standard. Blue florescence and yellowish brown coloured zone at UV 366 nm and day light respectively, was seen from the chromatogram, which confirmed the presence of saponins (Plate 13). There were four different saponins identified in the methanolic extract of Red banana corm as shown in the peak table (Table 23) and peak densitogram (Figure 25). The R_f value of standard was found to be 0.93.

The phenolics present in the methanolic extract of Red banana corm were analyzed using quercetin as the reference standard. Blue colored zones after derivatization with 25% aqueous Folin-Ciocalteau reagent which confirmed the presence of phenolics in Red banana corm (Plate 14). The peak table (Table 24) and peak denstiogram (Figure 26) showed the presence of four phenols.

Paramasivam *et al*. (2008) showed that the HPTLC analysis of different parts of *Curcuma longa* was found to contain alkaloids. The aqueous fraction of *Terminalia chebula, Emblica officinalis* and *Piper nigrum* showed the presence of number of polyvalent phytoconstitutents, in the chemoprofile obtained using HPTLC (Viswanath *et al*. 2011). The methanolic extract of *Solena amplexicaulis* tuber showed the presence of 5 alkaloids, 6 flavonoids, 2 glycosides, 10 saponins and 7 terpenoids in the HPTLC fingerprint (Karthika *et al*., 2012).

Albizia amara methanolic extract showed the presence of alkaloids in HPTLC (Rajkumar and Sinha, 2010). The HPTLC fingerprint of stem bark of *Catumarefgam spinosa* possessed various phyto-constituents with different R_f values at various wavelengths (Madhavan *et al*., 2011). The methanolic extract of stem, leaves, root, flower and seeds of *Aerva lanata* revealed the presence of 30 different types of steroids with 30 different R_f values (Yamunadevi *et al*., 2011). Rathi *et al*. (2011) showed the presence of alkaloids, flavonoids, phenolics, steroids, tannins and terpenoids in the ethanol extract of *Spermacoce hispida* by HPTLC analysis. *Emilia sonchifolia* showed the presence of terpenoids in HPTLC analysis (Sophia *et al*., 2011).

A characteristic fingerprint profile was obtained by densitometric HPTLC analysis, which was used as a marker for quality evaluation and standardization of the drug of *Cardiospermum halicacabum* L. stem (Patil *et al*., 2011). Rahman *et al*. (2002) and Prakash *et al*. (2008) showed the HPTLC fingerprint of bacoside A from *Bacopa monnieri*. A comparative HPTLC fingerprint of *Citrullus colocynth* and *Sonchus oleraceus* revealed that the former contained more quercetin than the later.

Plate 8

HPTLC of alkaloids

Before Derivatization **After Derivatization**

254 nm 366 nm DAY LIGHT DAY LIGHT

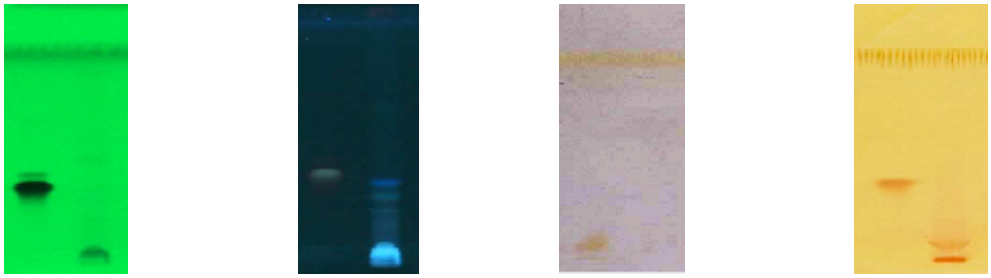
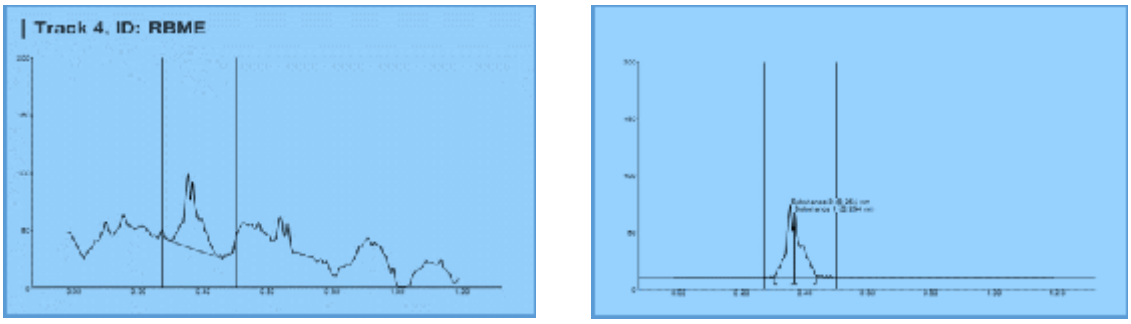


Figure 20

HPTLC peak densitogram of alkaloids in Red banana corm



Red Banana Colchicine Standard

Table 18

HPTLC peak table for the alkaloids in Red banana corm

Peak	R_f	Assigned substance
1	0.04	Unknown
2	0.11	Alkaloid 1
3	0.20	Alkaloid 2
4	0.26	Unknown
5	0.31	Alkaloid 3
6	0.34	Alkaloid 4
7	0.40	Alkaloid 5
8	0.63	Unknown
9	0.72	Alkaloid 6
10	0.90	Unknown
STD	0.41	Colchicine standard

Plate 9

HPTLC of flavonoids

Before Derivatization **After Derivatization**

254 nm 366 nm DAY LIGHT 254 nm

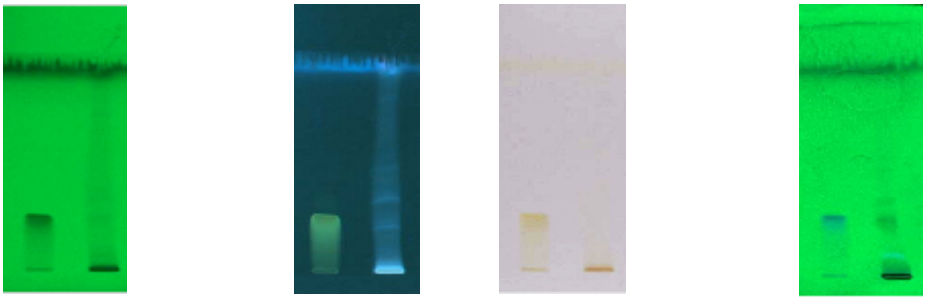
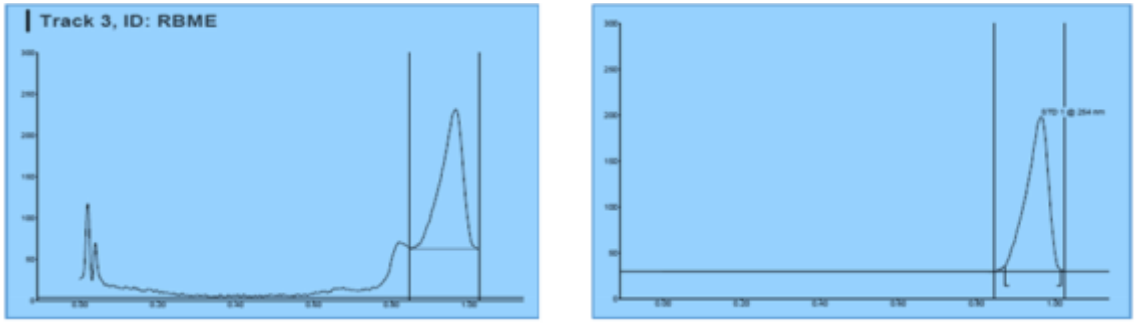


Figure 21

HPTLC peak densitogram of flavonoids in Red banana corm



Red Banana Quercetin Standard

Table 19

HPTLC peak table for the flavonoids in Red banana corm

Peak	R_f	Assigned substance
1	0.12	Unknown
2	0.15	Flavonoids 1
3	0.80	Flavonoids 2
4	0.95	Flavonoids 3
STD	0.94	Quercetin standard

Plate 10

HPTLC of steroids

Before Derivatization **After Derivatization**

254 nm 366 nm DAY LIGHT DAY LIGHT

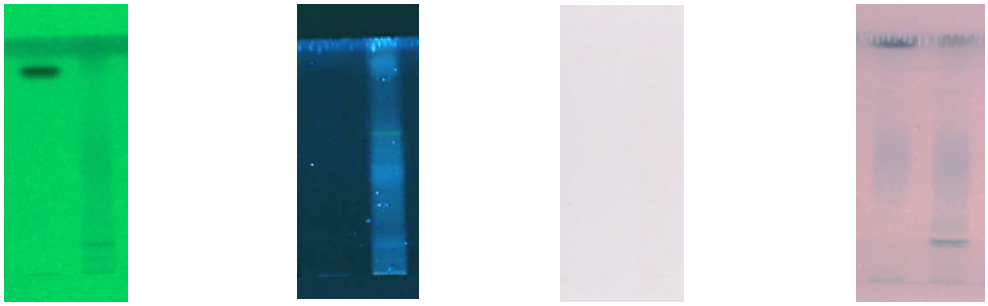
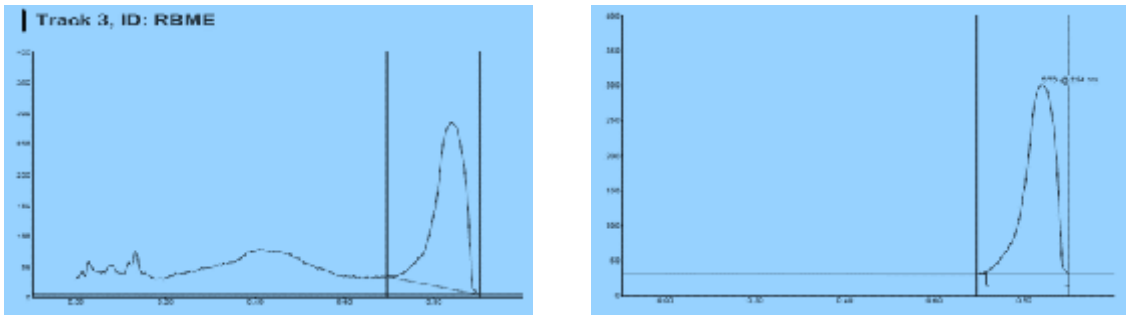


Figure 22

HPTLC peak densitogram of steroids in Red banana corm



Red Banana Solasodine Standard

Table 20

HPTLC peak table for the steroids in Red banana corm

Peak	R_f	Assigned substance
1	0.12	Unknown
2	0.14	Steroid 1
3	0.16	Steroid 2
4	0.18	Steroid 3
5	0.40	Steroid 4
6	0.84	Steroid 5
STD	0.85	Solasodine standard

Plate 11

HPTLC of terpenoids

Before Derivatization **After Derivatization**

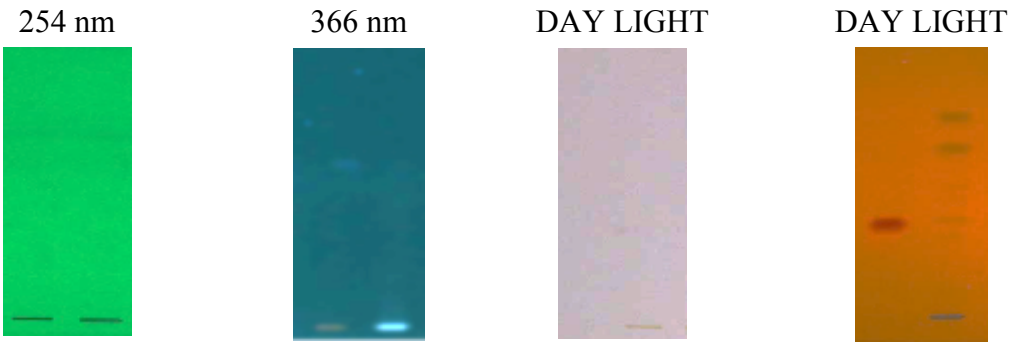
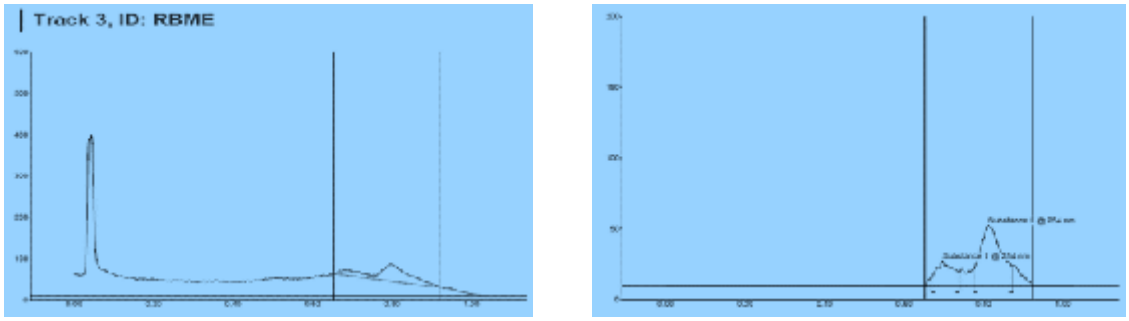


Figure 23

HPTLC peak densitogram of terpenoids in Red banana corm



Red Banana Artemisinin Standard

Table 21

HPTLC peak table for the terpenoids in Red banana corm

Peak	R_f	Assigned substance
1	0.14	Terpenoid 1
2	0.52	Unknown
3	0.69	Terpenoid 2
4	0.80	Terpenoid 3
STD	0.72	Artemisinin standard
STD	0.81	Artemisinin standard

Plate 12

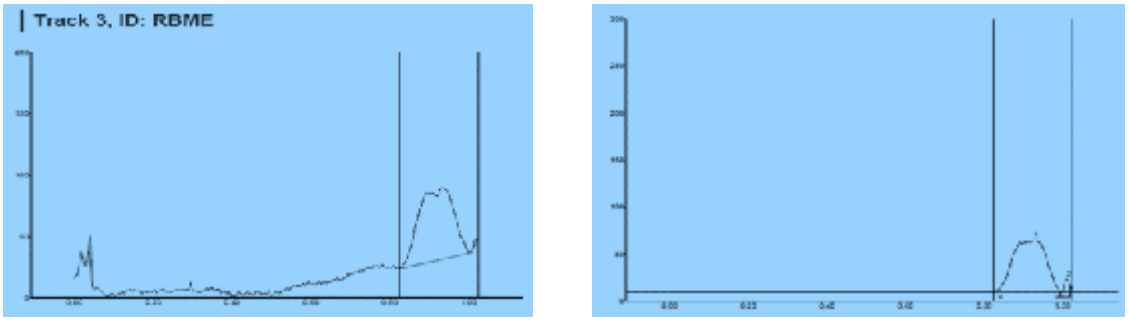
HPTLC of tannins

Before Derivatization **After Derivatization**

254 nm **366 nm** **DAY LIGHT** **DAY LIGHT**

Figure 24

HPTLC peak densitogram of tannins in Red banana corm



Red Banana Tannic Acid Standard

Table 22

HPTLC peak table for the tannins in Red banana corm

Peak	R_f	Assigned substance
1	0.11	Unknown
2	0.13	Tannin 1
3	0.30	Unknown
4	0.61	Unknown
5	0.90	Tannin 2
6	0.94	Tannin 3
7	0.98	Tannin 4
STD	0.95	Tannic acid standard

Plate 13

HPTLC of saponins

Before Derivatization **After Derivatization**

254 nm 366 nm DAY LIGHT DAY LIGHT

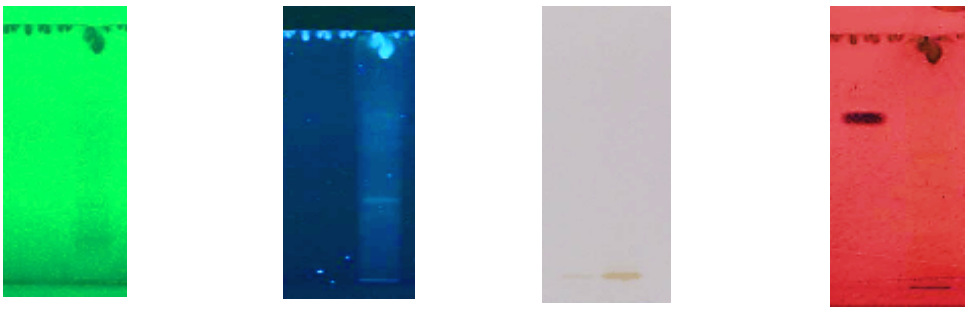
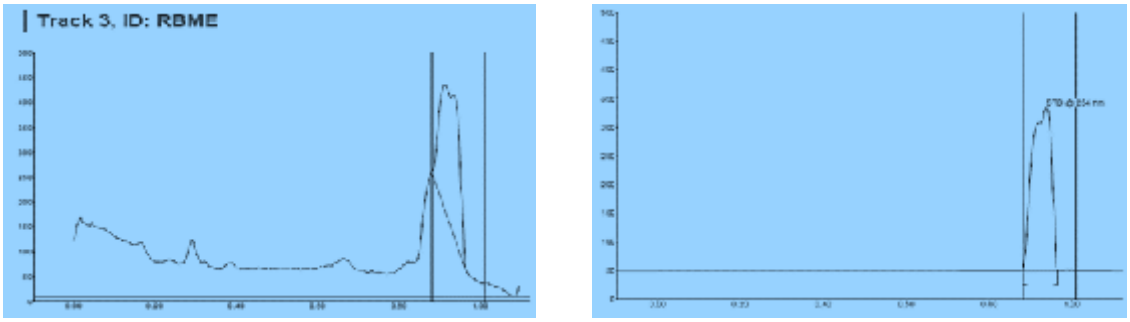


Figure 25

HPTLC peak densitogram of saponins in Red banana corm



Red Banana Saponin Standard

Table 23

HPTLC peak table for the saponins in Red banana corm

Peak	R_f	Assigned substance
1	0.12	Unknown
2	0.14	Unknown
3	0.19	Saponin 1
4	0.30	Unknown
5	0.33	Saponin 2
6	0.64	Unknown
7	0.85	Saponin 3
8	0.90	Saponin 4
STD	0.93	Saponin standard

Plate 14

HPTLC of phenols

Before Derivatization **After Derivatization**

254 nm 366 nm DAY LIGHT DAY LIGHT

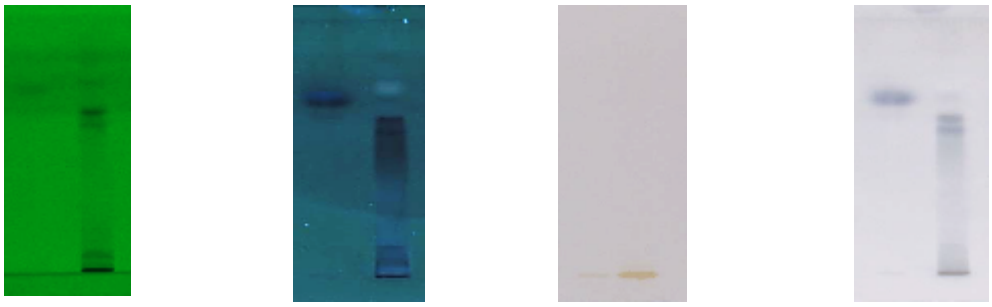
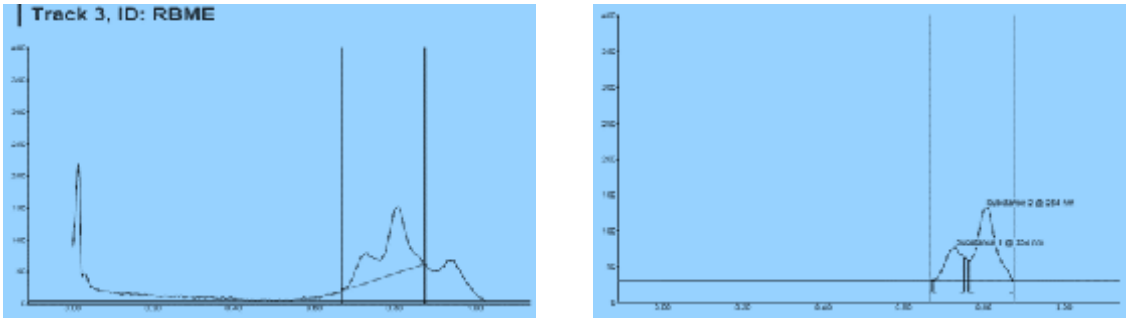


Figure 26

HPTLC peak densitogram of phenols in Red banana corm



Red Banana Eugenol Standard

Table 24

HPTLC peak table for the phenols in Red banana corm

Peak	R_f	Assigned substance
1	0.11	Phenol 1
2	0.69	Unknown
3	0.74	Phenol 2
4	0.81	Phenol 3
5	0.94	Phenol 4
STD	0.71	Eugenol standard
STD	0.80	Eugenol standard

Soundararajan *et al*. (2006), had reported the dissolution of calcium oxalate crystals due to the effect of flavonoids (Kaemepferol-3-rhamnoside and kaempferol-3-rhamnogalactoside), triterpenes (betulin) and tannins. It is also reported that saponin rich fractions of other plants like, *Herniaria hirsuta* act as a great inhibitor of calcium stone formation under *in vitro* and *in vivo model* studies (Fouada *et al*., 2006).

Lupeol (a phytosterol) has been found efficient in reducing the risk of stone formation in animals by preventing crystal-induced tissue damage and dilution of urinary stone-forming constituents (Malini *et al*., 2000). Researchers like Hariharan and Rangaswami (1970) and Ali (1993**),** conducted **c**hemical investigations on the seeds of *Achyranthes aspera* and identified saponins A (D-Glucuronic Acid) and saponins B (β -D-galactopyranosyl ester of D-Glucuronic Acid).

The chemical fingerprint through HPTLC analysis for various secondary metabolites of methanolic extract of Red banana corm revealed the presence of 6 alkaloids, 3 flavonoids, 5 steroids, 3 terpenoids, 4 tannic acids, 4 saponins and 4 phenolics.

It is quite evident from the above supporting literatures that the presence of these secondary metabolites in Red banana corm extract would certainly render a good protection and prevention against crystal-induced kidney damage. It acts as a diuretic agent as well as a potent inhibitor of calcium stone formation in kidney. Further studies need to be conducted to understand the actual mechanism of action of these metabolites rendering therapeutic property against lithiasis. The raw material (corm) availability is about 25 tons per hectare even by a moderate estimate. Thus Red banana corm alone or in combination with other medicinal plants appears optimistic for the formulation of products in pharmaceutical field in future as an alternative to allopathic treatments.

4.7.4. HPLC analysis of the methanolic extract of Red banana corm

The HPLC analysis of the methanolic extract of Red banana corm was carried out using C18 reverse phase column (Shimadzu equipped with UV detector). The results are presented in Figure 27. The HPLC spectrum showed 8 peaks (5 major and 3 minor) in the methanolic extract of Red banana corm. The retention time of the major and minor peaks with the peak area of all the peaks are represented in Table 25.

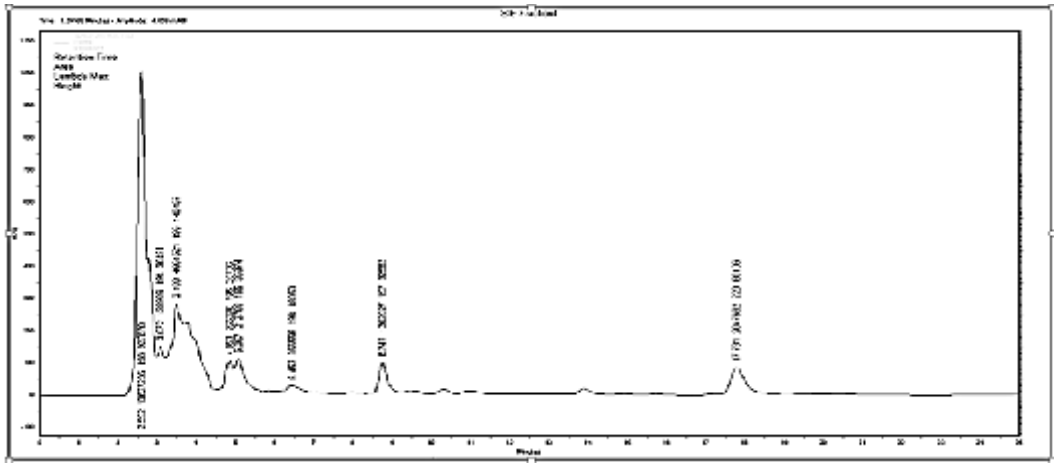
Table 25

HPLC peak table of the methanolic extract of Red banana corm

Scanning at 254 nm (UV short range)	
Retention Time	**Peak Area**
2.592	13627236
3.488	4664521
4.853	265396
5.067	212788
6.453	355556
8.747	1302027
17.781	2047682
42.635	2237048

Figure 27

HPLC analysis of the methanolic extract of Red banana corm



HPLC is a popular method for the analysis of herbal medicines which is not limited by the volatility or stability of the sample. It is essential to provide optimal separation conditions such as the different compositions of the mobile phase, their pH adjustment, pump pressures etc. Thus a good experimental design is needed for good separation (www.pharmainfo.net/reviews/chromagraphic-fingerprint-analysis-herbal-medicines-quality-control-tool).

The methanolic extract of *Majorana hortensis* subjected to HPTLC revealed 5 peaks indicative of 5 major phytochemical components (Radha, 2012). Commercial sample of the powder of *Hoodia gordonii*, confirmed the presence of active P57 glycosides, through qualitative analysis by HPLC (Pereira *et al*., 2011). Different types of alkaloids present in *Atropa belladonna* and *Atropa acuminata* were determined by HPLC (Ashtiania and Sefidkonb, 2011).

In order to confirm the nature of the active components observed, spectral analysis (FT-IR, GC-MS and ^{1}H-NMR) were carried out.

4.7.5. FT-IR analysis of the methanolic extract of Red banana corm

Fourier Transform Infra-Red (FT-IR) spectroscopy is a chemical analytical technique which measures infrared intensity versus wavelength (wave number) of light. It detects the vibration characteristics of chemical functional groups in a sample (Kumar and Yadav, 2009). Spectral analysis provides molecular level information of functional groups, bonding types and molecular conformations. Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. These bands are relatively narrow, easy to resolve, and sensitive to molecular structure, conformation and environment (Prasad *et al*., 2011).

The methanolic extract of Red banana corm was analyzed for the IR spectrum using FT-IR spectrophotometer using KBr pellet method (Figure 28). It exhibited bands at 3322.53 cm^{-1} which is the characteristic of –OH stretching and imitates the presence of –OH group. The band at 2941.57 cm^{-1}, 2830.66 cm^{-1} and 2521.07 cm^{-1} reveals the -C-H stretching vibrations of alkanes. Presence of -C≡C- strong stretching vibrations of alkynes at frequency of 3322.53 cm^{-1} and 2239.77cm^{-1}. A peak at 1442.82 cm^{-1}, 1022.32 cm^{-1} and 652.93 cm^{-1} indicate strong =C-H bending vibrations. A detailed characteristic IR absorptions are presented in Table 26.

Figure 28

FT-IR spectrum of the methanolic extract of Red banana corm

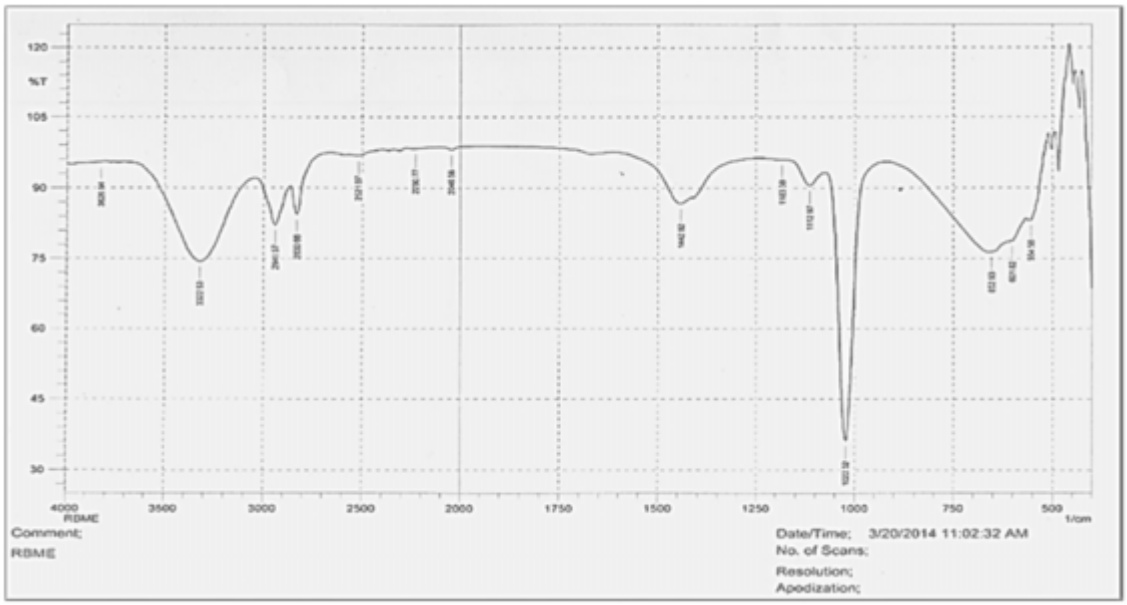


Table 26

IR absorption of methanolic extract of Red banana corm

Frequency (cm^{-1})	Bond	Functional group
3322.53	O-H stretch (m) -C≡C-H: C-H stretch (n,s)	Phenols
2941.57	C–H stretch (m)	Alkanes
2830.66	C–H stretch (m)	Alkanes
2230.77	–C≡C– stretch (w)	Alkynes
1442.82	C–H bend (m)	Alkynes
1183.38	C–O stretch (s)	Phenols
1022.32	=C–H bend (s)	Alkenes
652.93	=C–H bend (s) –C≡C–H: C–H bend (b, s)	Alkenes Alkynes

m=medium, w=weak, s=strong, n=narrow, b=broad

The methanolic extract of *Bacopa monnieri* showed the presence of –NH, functional group and carbonyl group in its FT-IR spectrum (Radha, 2010). The presence of several functional groups was found in the extract of *Acorus calamus* Linn. when exposed to FT-IR analysis (Paphonngam *et al*., 2011). Yamunadevi *et al*. (2012) studied the functional groups present in the crude powder of *Aerva lanata* stem, leaves, root and flower through FT-IR spectroscopy and confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, esters, ethers, alkyl halides and aliphatic amines in flower extract. Compared to flower extract, in roots all the above functional groups except phenol and amides, in leaves all the functional groups except ketones and primary amines and in stem all the functional groups except alkenes and aldehydes were present.

Similar results of FT-IR spectra were obtained by Nirmaladevi *et al*. (2010) in the analysis of methanolic extract of the leaves of *Rhinacanthus nasutus*. The methanolic leaf extract of *Mentha piperita* FT-IR results indicated the molecular configuration of different functional groups (Pramila *et al*., 2012). FT-IR results also confirmed the presence of compounds with functional groups such as NH, OH, C-H, C=N and chloride in *Morinda tinctoria* and NH, OH, C-H and C=N in *Nerium indicum* (Sreena, 2013). The IR spectrum of root extracts of *Pseudarthria viscida* subjected to FT-IR analysis confirmed the presence of polyphenols (Rajan and Muthukrishnan, 2013).

Cacumen platycladi aqueous leaf extract was found to contain C=O and C-O groups in abundance when subjected to IR spectroscopy (Zhan *et al*., 2011). The IR spectrum of *Majorana hortensis* leaf extract was indicative of the presence of several carboxyl, hydroxyl and ester groups, which attributed to the presence of phenols and saponins (Radha, 2012). There are several phytochemicals which could be accountable for the antiurolithiatic effect. According to Arafat *et al*. (2008), flavonoids and triterpenes have a key role in preventing urolithiaisis. It is also believed that saponins and tannins act as antiurolithiatic phytoconsituents (Doddola *et al*., 2008). Thus the results reveal that banana corm extract is a good source of various phytochemicals. As indicated by the results of IR spectrum, it is proved that the antilithiatic potential of Red banana corm might be due to the presence of secondary metabolites such as polyphenols, flavanoids, tannins and saponins.

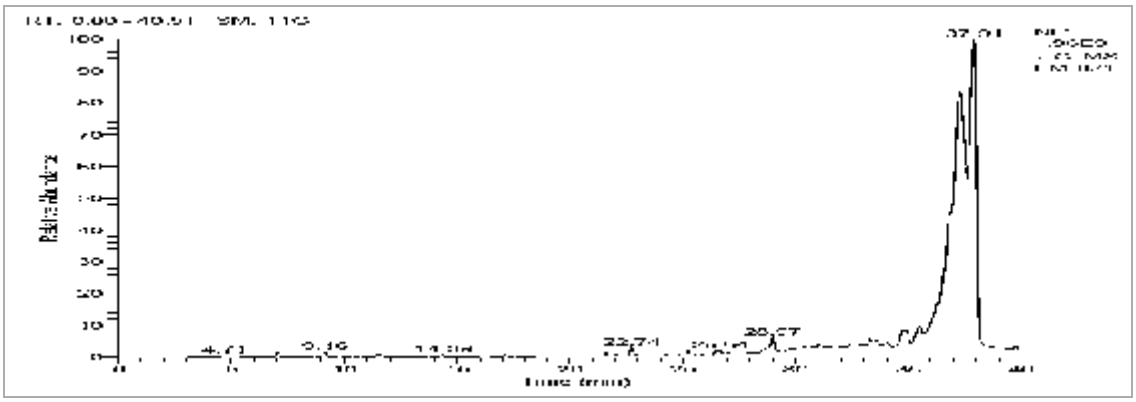
4.7.6. GC-MS analysis of the methanolic extract of Red banana corm

GC-MS analysis of the methanolic extract of Red banana corm was carried out to identify the nature of the components present. This would lay foundation for further research and development of medicinal products (Ze-kun and Chen-Haixia, 2012). The GC-MS results showed the presence of four major and three minor components at retention times 22.71, 26.43, 28.97, 34.77, 35.50, 36.25 and 37.96 (Figure 29).

In mass spectrum of the GC peak at retention time 37.96, molecular ion (M^+) peaks were observed at m/z 386.3, 368.3, 301.3, 275.3, 255.2, 231.2, 213.2, 161.1, 147.1, 133.1, 119.1, 91.0 and 67.0 (Figure 30). Characteristic M-18 peak was observed at m/z 368.3 and at m/z 213.2, indicating the presence of hydroxyl groups. M-14 peak observed at m/z 147.1, 133.1 and 119.1, is the characteristics of the presence of double bonds. The presence of M-28 peak at m/z 91.0 in the spectrum indicated that the compound may contain carbonyl group.

Figure 29

GC-MS profile of the methanolic extract of Red banana corm



The mass spectrum of peak at retention time 36.25, showed M^+ peak at m/z 386.3, other characteristic peaks were observed at m/z 368.3, 353.3, 316.8, 301.3, 275.3, 255.3, 213.2, 173.1, 145.1 121.1 105.0 and 69.1 (Figure 31). M-18 peak at 368 was observed suggesting the presence of hydroxyl group. The presence of M-28 peak at m/z 145 in the spectrum indicated that the compound may contain carbonyl group.

The mass fragmentation pattern of peak at retention time 35.50, displayed M^+ peaks at m/z 386.3, 371.3, 353.3, 301.3, 275.3, 255.3, 231.2, 213.2, 161.1, 135.1, 107.1 and 91.0 (Figure 32). A characteristics M-18 peak at m/z 353 and 213, indicating the

presence of hydroxyl group. The presence of M-28 peak at m/z 107 in the spectrum indicated that the compound may contain carbonyl group.

The mass spectrum of peak at retention time 34.77 registered M^+ peak at m/z 386.3, 368.3, 353.3, 327.2, 302.3, 255.2, 231.2, 213.2, 199.1, 161.1, 145.1, 105.1, 91.1, 79.1 and 67.1 (Figure 33). M-14 peak was observed at m/z 199 and 91, which is characteristic of the presence of double bonds. Also, two M-18 peak were observed at m/z 368 and 213 suggesting the presence of a hydroxyl group in the compound.

Figure 30

Peak fragmentation pattern of GC-MS spectrum (37.96)

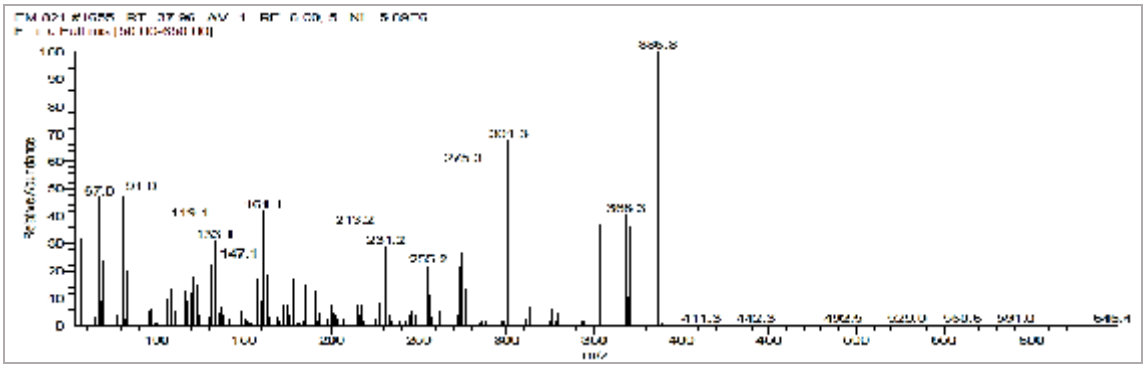
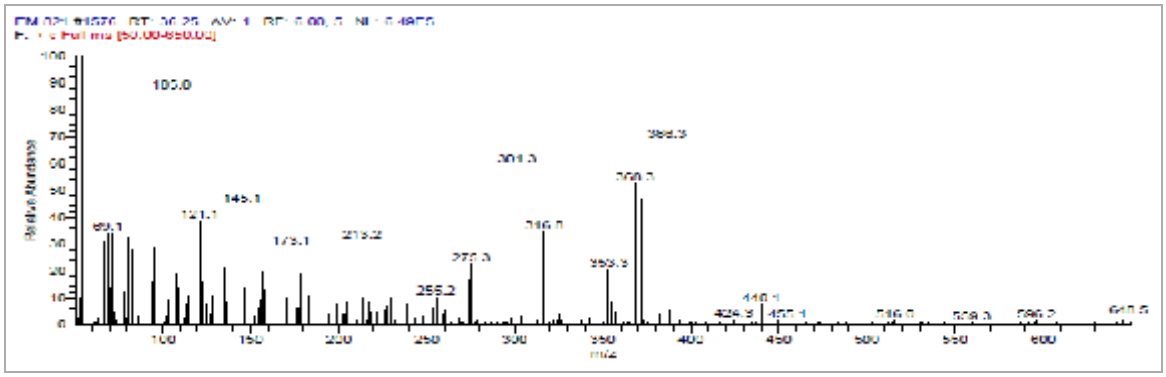


Figure 31

Peak fragmentation pattern of GC-MS spectrum (36.25)



The mass fragmentation pattern of peak at retention time 28.97 displayed a molecular peak at m/z at 450.5, 421.4, 407.5, 379.4, 337.4 309.3, 281.3, 267.3, 239.3, 225.3, 197.2, 183.2, 169.2, 155.2, 127.1, 113.1, 98.1 and 86.1 (Figure 34). Seven M-14 peak at m/z 421, 267, 225, 183, 169, 155 and 113, indicated the presence of double

bonds. The spectrum also displayed six M-28 peaks at m/z 379, 309, 281, 239, 197 and 127, characteristic of carbonyl group.

Figure 32

Peak fragmentation pattern of GC-MS spectrum (35.50)

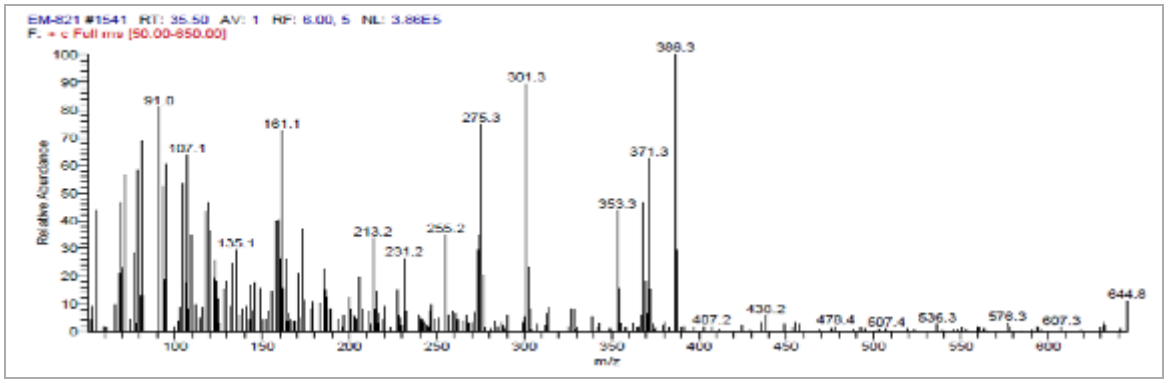


Figure 33

Peak fragmentation pattern of GC-MS spectrum (34.77)

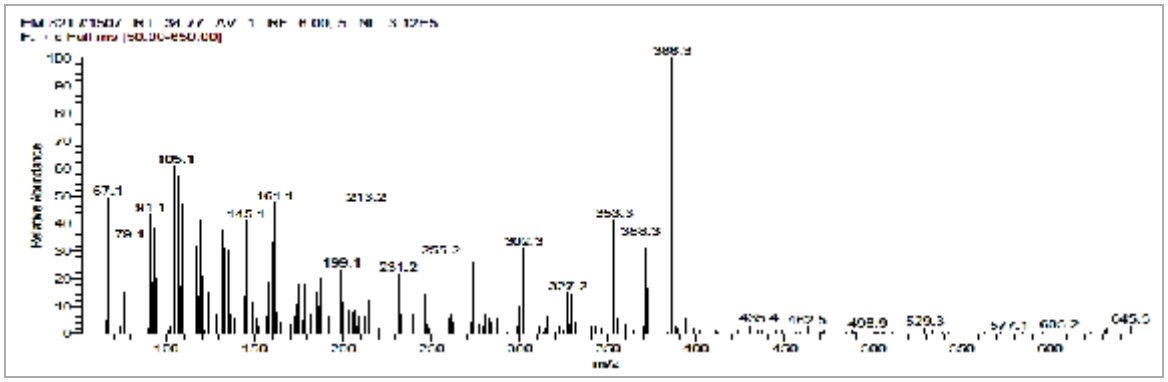
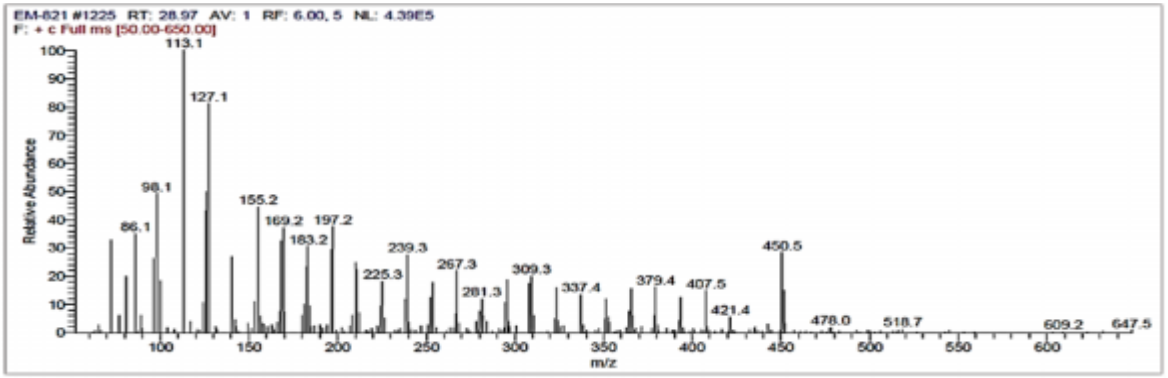


Figure 34

Peak fragmentation pattern of GC-MS spectrum (28.97)

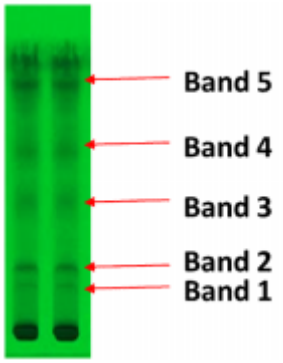


Ethanolic extract of *Aloe vera* identified 26 bioactive phytochemical compounds using GC-MS technique based on the peak area, molecular weight and molecular formula (Arunkumar and Muthuselvam, 2009). *Euclayptus globolus* extract and *Oleo europaea* extract was composed of several phytochemical compounds and *Thymus vulgaris* plant extract was mainly composed of thymol, carvacrol and –cymene, as analyzed by GC-MS (Al-Rahman *et al*., 2011).

The GC-MS technique has become an established platform for metabolite profiling in both plant and non-plant species by several other studies. Padma *et al*. (2007) reported that the *Majorana hortensis* leaf extract exhibited the presence of several carbonyl groups and esters. Twenty peaks indicating the presence of 20 phytochemical constituents which were characterized and identified in GC-MS chromatogram of ethanolic extract of *Mussaenda frondosa* leaves (Gopalakrishnan and Vadivel, 2011). The unsaponifiable fraction of petroleum ether fraction of *Tridax procumbens* revealed the presence of campesterol, stigmasterol and β–sitosterol by GC-MS analysis (Gadre and Gabbe, 1993).

Plate 15

TLC profile of methanolic extract of Red banana corm



The ethanolic extract of leaves of *Elaeocarpus serratus* by GC-MS analysis revealed the presence of thirty bioactive components (Geetha *et al*., 2013). Yang *et al.* (2013) have identified five monoterpenes and eleven sesquiterpenes in the extract of *Gossypium hirsutum* using GC-MS.

GC-MS profile of Red banana corm showed the presence of several functional groups including carbonyl groups and hydroxyl groups, which attributed to the phytochemicals present in the extract responsible for its therapeutic activity.

In the present study the methanolic extract of Red banana corm was subjected to thin layer chromatography in which five major bands were observed (Plate 15), and were scrapped out of the plate. This was dissolved in methanol and centrifuged, the supernatant was decanted.

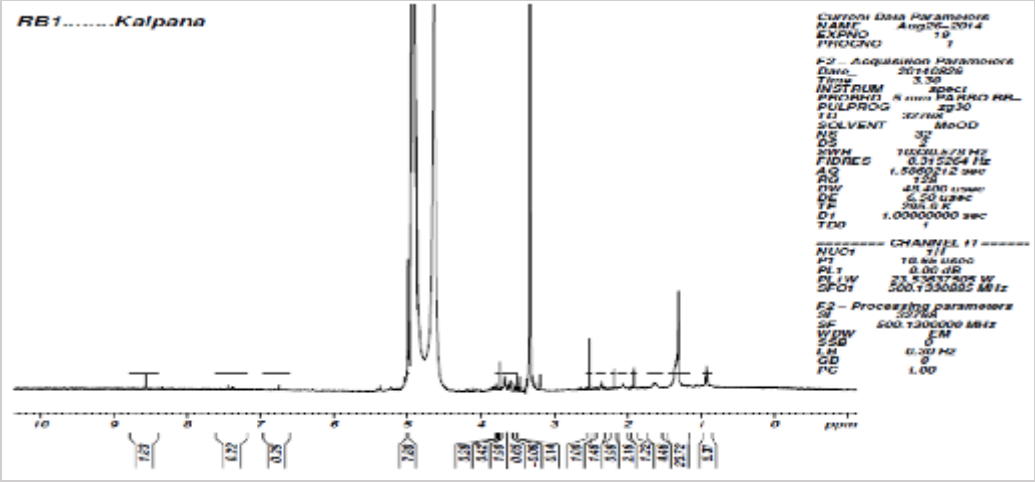
Based on the TLC profile, similar bands were pooled and evaporated to dryness and refrigerated. All the five major bands were assessed for its *in vitro* antilithiatic property. From the results the two bands (Band 2 and 5) were found to exhibit maximum inhibitory potential against stone formation under *in vitro* conditions. These two bands were subjected to NMR spectroscopy and the results were presented and discussed below.

Nuclear Magnetic Resonance

The ^{1}H-NMR spectra of the Band 2 and Band 5 of methanolic extract of Red banana corm are shown in Figure 35 and 36. The results of ^{1}H NMR reiterates the presence of secondary metabolites that might have contributed for its antilithiatic property.

Figure 35

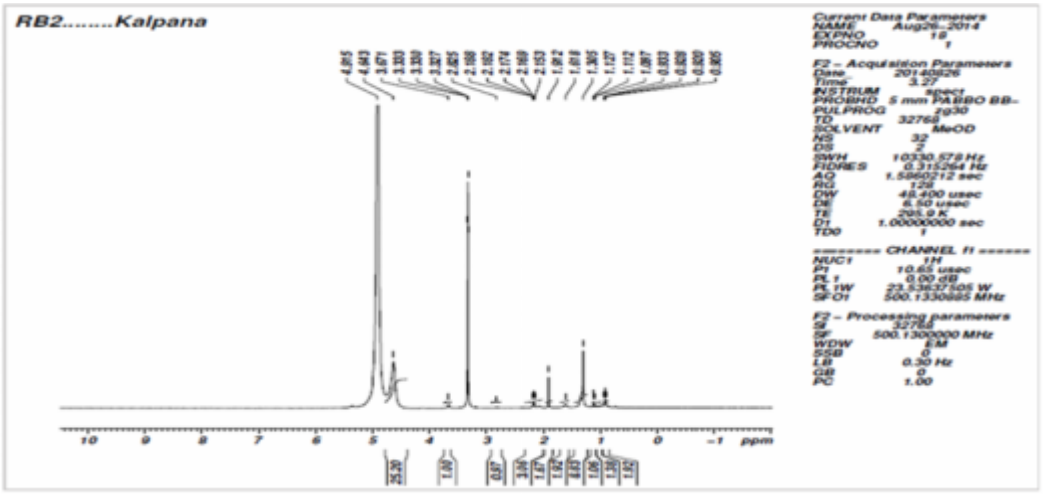
^{1}H NMR spectrum of methanolic extract of Red banana corm (Band 2)



The research outcome of the present study, thus, highlights the antioxidant, radical quenching, biomolecule-protective and antilithiatic properties of the methanolic extract of Red banana corm. The study also showed that the extract of Red banana corm is rich in several major types of biologically active phytoconstituents.

Figure 36

^{1}H NMR spectrum of methanolic extract of Red banana corm (Band 5)



In conclusion, the statistical analysis of these data and emerging results clearly supported the rejection of null hypothesis and the acceptance of the alternate hypothesis, which stated that the corm of different banana cultivars contained components that exhibit strong antilithiatic activity.

The findings of the present study are summarized and the conclusion drawn from the observations are presented in the next chapter.