e-ISSN:2321-6182
p-ISSN:2347-2332

Research & Reviews: Journal of Pharmacognosy and Phytochemistry

# Chemical analysis and *in vitro* evaluation of antiurolithiatic activity of *Aerva lanata* (Linn.) Juss. Ex Schult. roots

KN Sunil Kumar[1]*, Suchitra Narayan Prabhu[1], B Ravishankar[1], Sahana[2], B Yashovarma[3]

[1]SDM Centre for Research in Ayurveda and Allied Sciences, Laxminarayana Nagar, Kuthpadi, Udupi–574 118, India

[2]Mangalore University Post Graduate Centre, Cauvery Campus, Madikeri, Kodagu-571 201, India

[3]SDM College (Autonomous), Ujire, Belthangadi Taluk, Dakshina Kannada-574 240, India

## Research Article

Received date: 17/06/2015
Accepted date: 28/06/2015
Published date: 05/07/2015

***For Correspondence**

Dr. K. N. Sunil Kumar, Senior Research Officer – Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Laxminarayana Nagar, Kuthpady, Udupi-574 118, India.

E-mail: sunilkumarnarayanan@gmail.com

Tel: +91-8050230864

**Keywords:** Pashanabheda, Calcium oxalate and phosphate nucleation, oxaluria

**ABSTRACT**

**Background:** *Aerva lanata* (Linn.) Juss. ex Schult. - Amaranthaceae is an herbaceous weed found throughout tropical India. The plant is known as pashanabheda/ gorakshaganja in Ayurvedic system of medicine. The roots are diuretic and used as cure for urinary troubles including stones. In the present manuscript, experimental evidences regarding antiurolithiatic activity of roots of *A. lanata* are reported in support of its use in lithiasis.

**Objective:** In the current study it attempted to evaluate the effectiveness of roots of *Aerva lanata* on renal calculus formation *In vitro.*

**Methods:** Investigation of antiurolithic activity was carried out by simultaneous flow static model (S.S.M). Dissolution of precipitate of calcium oxalate and calcium phosphate was determined by (S.S.M).

**Results:** One per cent aqueous extract preparation of *A. lanata* root was good at controlling calcium phosphate crystallization. Five per cent aqueous extract was good on calcium oxalate crystallization though it controls both type of crystal formation.

**Conclusions:** This attempt would provide encouragement for further research on novel drug development for prevention and treatment of urolithiasis.

## INTRODUCTION

*Aerva lanata* (Linn.) Juss. ex Schult. - Amaranthaceae is an erect to prostate herb, hoary- tomentose, dioecious, found throughout tropical India. It is a common weed in fields and waste places, and ascends up to an altitude of 900 m in the hills. It has a tap root which is cylindrical, branched, 7 to 12 cm long, 2 to 8 mm thick, straight or slightly twisted with fibrous lateral roots, pale yellowish brown externally, whitish internally, and with camphoraceous odor [1]. The plant is known as pashanabheda and gorakshaganja in Ayurvedic system of medicine. The whole plant is used in diabetes, for arresting bleeding in pregnancy [2], as demulcent and in lithiasis. Other properties ascribed for the plant are for uterus clearance after delivery [3]. The plant is also efficacious as diuretic [4] and dissolve kidney and gall bladder stones [5,6].

The plant was reported to contain sitosteryl palmitate [7]; hentriacontane, β- sitosterol, its D-glucoside, α-amyrin, and betulin [8]. Glycosides like kaempferol-3-galactoside and kaempferol-3-rhamnogalactoside along with alkaloids, saponins, sugars (fructose, rhamnose, galactose and sucrose) and minerals (Aftaq et al.,) [9] are also reported.

The root has diuretic, to cure diarrhoea, in urinary troubles [10] and as antirheumatic [11]. In the present manuscript,

e-ISSN:2321-6182
p-ISSN:2347-2332

phytochemical study and experimental evidences regarding antiurolithiatic activity of roots *A. lanata* are reported, the result being in support of its use in lithiasis.

# MATERIALS AND METHODS

## Collection of plant material and extraction

Root of *Aerva lanata* was collected from SDM College of Ayurveda campus, Kuthpady, Udupi. The plant was identified and authenticated by referring to Flora of Udupi [12] with voucher specimen number 609/15050901 was deposited at Pharmacognosy department of SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpadi, Udupi. Shade dried plant material was stored in air tight container at 250°C for further study. Cold percolation method was followed for preparation of aqueous extract and phytochemical studies. The plant material weighing about 100 g was soaked with 2 litres of water and ethanol in a percolator for 24 hrs followed by filtration, the filtrates were poured individually to a pre-weighed china dish and concentrated to dryness over water bath and the dried residue was made moisture free in a desiccators. The extract thus prepared was used for phytochemical tests, HPTLC and *In-vitro* antiurolithiatic activity by simultaneous flow static model (S.S.M) [13].

## Physico-chemical examination

The powdered plant material was standardized as per Pharmacopoeial methods [14].

## Phytochemical screening

Total ethanol extract was tested for the presence of different phytoconstituents like alkaloid, steroid, flavonoid, tannin, glycoside etc. [15].

## HPTLC

**Sample preparation:** Dried plant root powder (1 g) was extracted with 10 mL of ethnol (90%) and filtered. The filtrates was made up to 10 mL and used for application (**Figure 1**).


**Figure 1.** Macroscopy dried root of *Aerva lanata.*

**Development and documentation:** Three, six and nine micro litres of the sample were applied on aluminium plate pre-coated with silica gel 60 F254 of 0.2 mm thickness (Merck, Germany) using CAMAG LINOMAT 5 applicator. The plate was developed in CAMAG glass twin trough chamber previously saturated with mobile phase toluene : ethyl acetate: formic acid (7.0:1.0:0.1). The plate was derivatized using vanillin- sulphuric acid (VS), and heated at 105 °C till the spots appeared [16,17]. The developed plates were visualized in CAMAG visualizing chamber and scanned in CAMAG Scanner 4 under 254, 366, 540 (pre-derivatisation) with the help of CAMAG WinCATS software. $R_f$ values and densitograms were recorded.

**Antiurolithiatic activity *In-vitro:*** Inhibition of calcium oxalate and calcium phosphate mineralization by aqueous extract of *Aerva lanata* was measured by simultaneous flow static model (S.S.M.). Inhibition of calcium oxalate and calcium phosphate mineralization, procedures were carried out in two sets; one served as blank set and other as experimental set, in blank set, 0.1 M sodium oxalate (10 ml), and 0.1 M calcium acetate (10 ml) were taken in two separate burettes where as in experimental set aqueous extract of the plant i.e. in the concentration of 1.0%, 5.0% (10 ml) were taken in the third burette. In both sets of experiments, chemicals were allowed to fall simultaneously, slowly and at steady pace into a 250 ml beaker. After 30 minutes, the mixtures were kept in hot water bath for 10 minutes, cooled to room temperature and collected into a pre-weighed centrifuge tube. Centrifugation of mixture was done at 3000 rpm for 15 minutes. Supernatant fluid was discarded and precipitate was obtained. All tubes with precipitate were dried in a hot air oven at 1200°C, cooled to room temperature and weighed. Similar process was repeated using 0.1 M sodium phosphate (25 ml) and 0.1 M calcium acetate (25 ml) for inhibition of calcium phosphate mineralization. The percentage inhibition of mineralization was calculated by the following formula [13].

$$\text{Inhibition}(\%) = \frac{(\text{Weight of ppt in blank set} - \text{Weight of ppt in experimental set}) \times 100}{\text{Weight of ppt in blank set}}$$

e-ISSN:2321-6182
p-ISSN:2347-2332

## RESULTS AND DISCUSSION

Number of medicinal plants such as Kanghi (Abuliton indicum), Chaya (*Aerva lanta*), Bishkapa or punarnava (*Boerhaavia diffusa*), Ajuba (*Bryophyllum pinnatum*), Gokhuru (*Tribulus terrestis*), Makka (*Zea mays*) etc shows antiurolithiatic activity.

Standardization was carried out as it is an important aspect in maintaining and assessing quality and safety of the crude drug. The herb was found to have no other parts as foreign matter. Only roots of the sample were selected for the study. Loss on drying reveals the moisture content, the sample has 5.09% of moisture; total ash is the indication of total inorganic content, 8.99% ash was detected in the sample; acid insoluble ash is the acid insoluble part of total ash, mainly silica, the sample showed 0.40% acid insoluble ash; water soluble ash is the water soluble part of total ash indicating inorganic content without water insoluble inorganic salts like silica, 4.68% was water soluble; water and alcohol soluble extractive is indicative of percentage active constituents soluble in water and ethanol, the values were 15.73 and 3.69% respectively. These physico – chemical standards would indicate the purity and authenticity of the leaves of *A. lanata* (**Table 1**).

**Table 1.** Physicochemical parameters of *Aerva lanata.*

| Parameter | Results n=3%w/w |
|---|---|
| Foreign matter | Nil |
| Loss On Drying | 5.09 ± 0.008 |
| Total ash | 8.99 ± 0.011 |
| Acid Insoluble Ash | 0.40 ± 0.013 |
| Water Soluble Ash | 4.68 ± 0.006 |
| Alcohol Soluble Extractive | 3.69 ± 0.015 |
| Water Soluble Extractive | 15.73 ± 0.013 |

Preliminary phytochemical screening revealed the presence of carbohydrates/glycosides, phenols, steroids, tannins. The phytochemical constituents present in the extract can be held responsible for different medicinal activities of the plant (**Table 2**).

**Table 2.** Phytochemical screening of leaf extract of *Aerva lanata.*

| Tests | Color if positive | Aerva lanata | Inference |
|---|---|---|---|
| **Alkaloids** | | | – |
| Dragendrof's test | Orange precipitate | Light brown solution | |
| Wagner's test | Red precipitate | Reddish brown solution | |
| Mayer's test | Dull white precipitate | Light brown solution | |
| Hager's test | Yellow precipitate | Light brown solution | |
| **Steroids** | | | + |
| Liebermann- buchard test | Bluish green | Bluish green | |
| Salkowski test | Bluish red to cherry red | Bluish red to cherry | |
| **Carbohydrate** | | | + |
| Molisch's test | Violet ring | Violet ring | |
| Fehling's test | Brick red precipitate | Brick red precipitate | |
| Benedicts test | Red precipitate | Red precipitate | |
| **Tannin** | | | + |
| With $FeCl_3$ | Dark blue or green or brown | Brown | |
| **Flavonoids** | | | – |
| Shinoda's test | Red to pink | Light brown solution | |
| Saponins | | | – |
| With $NaHCO_3$ | Stable froth | No stable froth | |
| **Triterpenoids** | | | – |
| Tin and thionyl chloride test | Pink | No color pink | |
| Coumarins | | | – |
| With 2 N NaOH | Dark yellow color | Light brown color | |
| **Phenols** | | | + |
| With alcoholic ferric chloride | Blue to blue black, brown | Brown color | |
| Carboxylic acid | | | – |
| With water and $NaHCO_3$ | Brisk effervescence | No brisk effervescence | |
| **Resin** | | | – |
| With aqueous acetone | Turbidity | No turbidity | |
| Quinone | | | – |
| 5% NaOH | Pink/purple/red | Brown color | |

HPTLC finger printing profiles of *Aerva lanata* under 254 nm showed the presence of 3 spots (all in green) at Rf of 0.43, 0.55 and 0.62. Under 366 nm there were 7 prominent spots (Fluoroscent) at Rf of 0.13, 0.43, 0.55, 0.62, 0.66, 0.85, 0.92 and

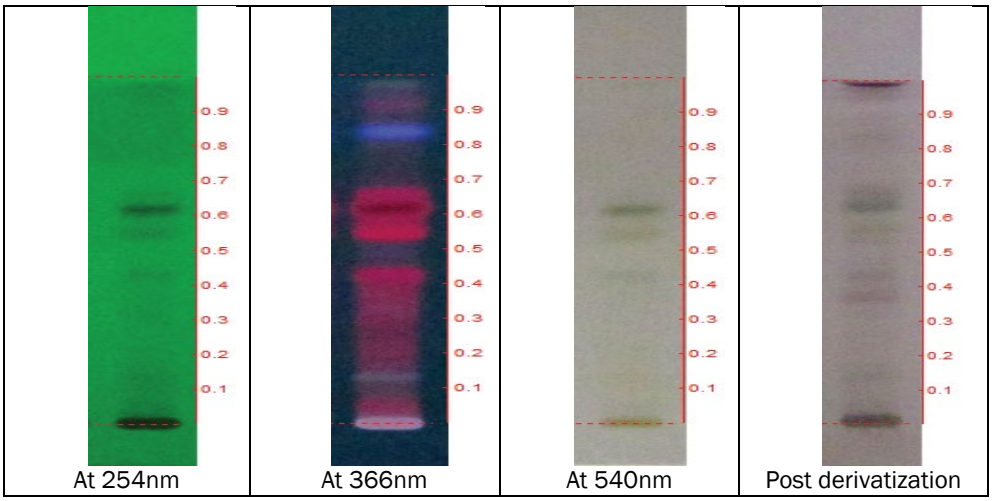
e-ISSN:2321-6182
p-ISSN:2347-2332

when scanned under white light 540 nm, 3 spots were present at Rf 0.43, 0.55, 0.62, following post derivatisation with vanillin sulphuric acid spots( in different colors) were evident at Rf 0.15, 0.38, 0.43, 0.55, 0.57, 0.64, 0.85. Among these the spots were common at $R_f$ of 0.43, 0.55 and 0.62 (except at post derivatisation) at different color intensities (**Table 3 and Figure 2**).

**Table 3.** $R_f$ values of ethanolic extract of *Aerva lanata* (9 µl).

| At 254 nm | At 366 nm | At 540 nm | Post derivatisation |
|---|---|---|---|
| - | 0.13(FL. pink) | - | - |
| - | - | - | 0.15(L. green) |
| - | - | - | 0.38(L. pink) |
| 0.43(L. green) | 0.43(FD. red) | 0.43(L. green) | 0.43(D. purple) |
| 0.55(L. green) | 0.55(FD. red) | 0.55(L. yellow) | 0.55(L. green) |
| - | - | - | 0.57(L. green) |
| 0.62(D. green) | 062(FD. red) | 0.62(D. green) | - |
| - | - | - | 0.64(D. green) |
| - | 0.66(FD. red) | - | - |
| - | 0.85(FD. blue) | - | 0.85(L. purple) |
| - | 0.92(FL. pink) | - | - |

L-light, D-Dark, F-Fluorescent




**Solvent system** - Toluene: Ethyl acetate: Formic acid (7.0:1.0:0.1)

**Figure 2.** TLC photodocumentation of ethanolic extract of *Aerva lanata* (9 µl).

$R_f$ values by densitometric scan of *Aerva lanata* showed 18 spots at 254 nm, 10 spots at 366 nm, and 5 spots at 540 nm respectively (**Table 4 and Figure 3**).

**Table 4.** $R_f$ values by peaks by densitometric scan of *Aerva lanata.*

| $R_f$ values | At 254 nm | At 366 nm | At 540 nm |
|---|---|---|---|
| 0.02 | 9.99 | 0.35 | - |
| 0.05 | 20.11 | - | 29.67 |
| 0.07 | - | 3.68 | - |
| 0.15 | 5.08 | 17.53 | - |
| 0.19 | 5.93 | - | 3.37 |
| 0.22 | 1.87 | - | - |
| 0.24 | - | 9.26 | - |
| 0.29 | 1.79 | - | - |
| 0.32 | 1.49 | - | - |
| 0.34 | - | 19.41 | - |
| 0.38 | 2.99 | - | - |
| 0.41 | 1.49 | - | - |
| 0.46 | - | 9.13 | - |
| 0.48 | 2.19 | - | - |
| 0.51 | - | - | 10.78 |
| 0.56 | 6.88 | 18.13 | - |
| 0.59 | 2.87 | - | - |

e-ISSN:2321-6182
p-ISSN:2347-2332

| | | | |
|---|---|---|---|
| 0.66 | 10.83 | - | 14.29 |
| 0.68 | - | 5.95 | - |
| 0.73 | 15.76 | 1.52 | 35.83 |
| 0.77 | - | - | 6.05 |
| 0.81 | 1.60 | 15.04 | - |
| 0.82 | 2.22 | - | - |
| 0.90 | 5.02 | - | - |
| 0.96 | 1.87 | - | - |

Values in %area; highlighted values are compounds with same $R_f$.

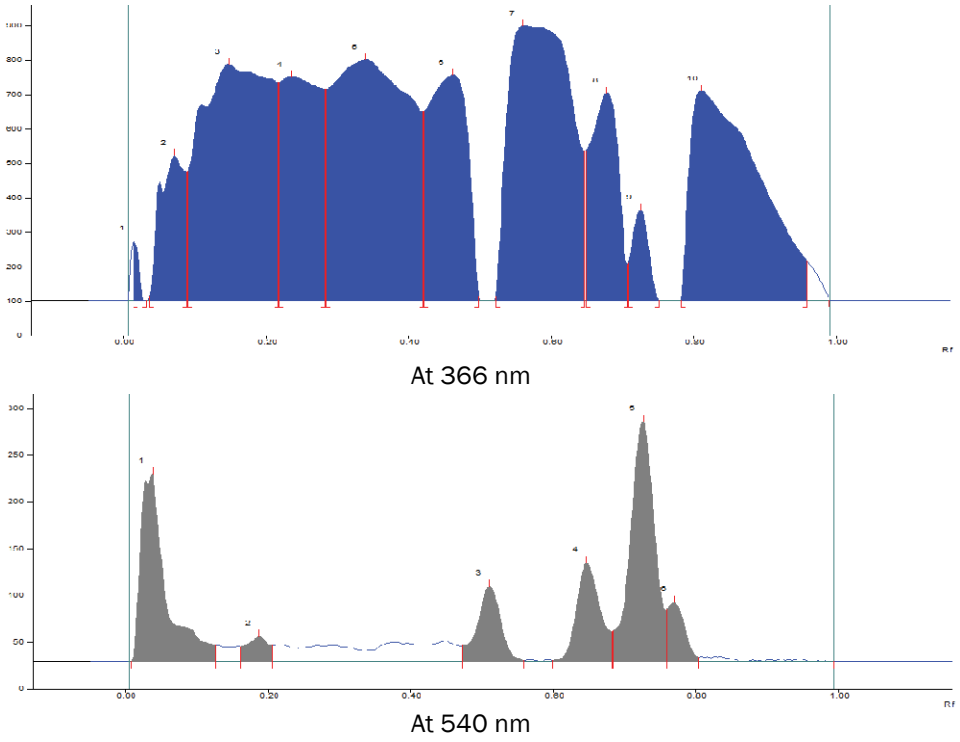



**Figure 3.** Densitometric scan of *Aerva lanata* (9 µl).

Urolithiasis is formation of one or more calculi in any location within the urinary tract; it is one of those oldest diseases known to mankind [18]. Cause is multifactorial and is related to dietary life style habits or practices [19]. It is common in age group of 20-40 in both sex but men are more prone than women [20]. Lithiasis is categorized into 2 types depending on its anatomical position; when formed in bladder, ureter or any part of urinary tract rather in kidneys as urolithiasis and on the other hand termed as nephorolithiasis when formed in kidneys [21]. Calcification from urine super saturation results in precipitation and subsequent crystallization depends on urinary pH, ionic strength, solute concentration and complexation/ Chelation [22] and is a multistep process which results with formation and ends in retention; crystal nucleation, crystal aggregation, crystal growth and its retention [23]. The precursor for oxalate crystal is oxalic acid/ oxalates biosynthesized from ascorbic acid, glycolates and glyoxylates. So for prevention of stone formation as precautionary measure the oxalate rich food should be avoided, such as spinach, nuts like almonds, wheat bran, strawberries, ascorbic acid rich foods, certain calcium supplements should be avoided as it has tendency to bind to oxalates inducing oxaluria, hypocalcaemia and increase risk of stone formation [24]. It is advisable to ingest large amount of fluid like water and barley and restrict potassium and protein intake [25].

A stone mainly comprises of calcium oxalates [26] and trace amount of calcium phosphates and calcium carbonates [27]. Stones bigger than 5 mm which fails to pass through requires medical intervention, involving extracorporeal shock wave lithotripsy (ESWL), Ureteroscopy (USP), percutaneous nephrolithotom y(PNL) [28] but unfortunately propensity of reoccurrence is not altered and is still about 50% [29]. ESWL has side effect like renal damage, it also results in induced hypertension or renal impairment [30]. In fact there is still no treatment for the prevention of reoccurrence of stones.

As there is a urgent need for the prevention of urinary caliculi formation the investigation carried out in-vitro for calcium oxalate and calcium phosphate mineralization satisfies the need for its potential activity. One % aq. extract of *Aerva lanata* was effective in controlling calcium phosphate mineralization where as 5% aq. extract was found to inhibit calcium oxalate and calcium phosphate mineralization. One % aq. extract was effective in controlling calcium phosphate mineralization to an extent of 68.22% in comparison with calcium oxalate mineralization restricting it to only 36.44%.

On the contrary 5% aq. extract was effective in controlling both types of crystal mineralization; however it had an upper

hand in controlling calcium oxalate than calcium phosphate. Five %aq. extract of *A. lanata* showed maximum activity in inhibiting calcium oxalate and calcium phosphate mineralization to percentage of 68.53% and 58.05% respectively (**Table 5**).

**Table 5.** Inhibition% of crystals by water extract of *Aerva lanata.*

| Concentration (%) | Calcium oxalate (%) | Calcium phosphate (%) |
|---|---|---|
| 1 | 36.44 | 68.22 |
| 5 | 68.53 | 58.05 |

The extract had an appreciable potential in controlling calcium oxalate and calcium phosphate mineralization, this property of the extract supports for the claim that chances of crystal formation is reduced thereby preventing its aggregation and subsequent retention in the urinary tract.

## CONCLUSION

The present investigation provides useful information on antiurolithiatic activity of aq. extract of *Aerva lanata* in controlling calcium oxalate and calcium phosphate mineralization thus this study has high significance in the prevention of urolithiasis. Further studies should be done to understand pharmacological action and its possible mechanism through elaborate preclinical experimentation and clinical trials in preventing urolithiasis in susceptible population.

## ACKNOWLEDGEMENT

Authors are grateful to revered President, Dr. D. Veerendra Heggade, SDM Educational Society for the encouragement.

## REFERENCES

1. Anonymous. Quality standards of Indian medicinal plants. Indian council of medical research New Delhi: 2005; 3: 10-12.
2. Yoganarasimhan, SN, Bhat, AV, Togunashi, VS. Medicinal plants from Mysore district Karnataka. Ind. drug pharmaceut. Ind. 1979; 14: 7-22.
3. John D. One hundred useful raw drugs of Kani tribes of Trivandrum forest division, Kerala, India. Int J Crude drug res. 1984; 22: 17-39.
4. Raj KS, Patel MR. Some medicinal plants of Cambay and its immediate vicinity and their uses in Indian indigenous system of medicine. Ind. drugs. 1978; 15: 145-152.
5. Vedavathy S, Rao KN. Nephroprotectors-folk medicine of Rayalseema- Andhra Pradesh. Ancient Sci. Life. 1990; 9: 164-167.
6. Sudhakar A, Chetty KM. Medicinal importance of some angiospermic weeds used by rural people of Chitoor district of Andhra Pradesh, India. Fitoterapia. 1998; 69: 390-400.
7. Aiyar VN, Narayanan V, Seshadri TR, Vydeeswaran S. Chemical components of some Indian medicinal plants. Ind J Chem. 1973; 11: 89-90.
8. Chandra S, Sastry MS. Chemical constituents of *Aerva lanata*. Fitotherapia. 1990; 61: 188.
9. Afaq SH, Tajuddin, Afridi R. Bisehri booti (*Aerva lanata* Juss.): some lesser known uses and Pharmacognosy. Ethnobotany. 1991; 3: 37-40.
10. Hemadri K, Raj PV, Rao SS, Sharma CRR. Folklore claims from Andhra Pradesh-I. J. Sci. Res. Plant Med. 1980; 1: 37-49.
11. Kakrani HKN, Saluja AK. Traditional treatment through herbs in Kutch district, Gujrat state, India. Part II. Analgesic, anti-inflammatory, antirheumatic, antiarthritic plants. Fitoterapia. 1994; 65: 427-430.
12. Bhat GK. Flora of Udupi. Udupi Manipal press ltd. 2003
13. Farook NA, Dameem GA, Alhaji NM, Sathiya R, Muniyandi J, et al. Inhibition of Mineralization of Urinary Stone Forming Minerals by Hills Area Fruit. E-J Chem. 2004; 1: 137-41.
14. Quality control methods for medicinal plant materials, WHO, Geneva, 1998. 16-20, 25-80.
15. Dey P, Mukherjee M, Bhakta T, Ghosh TK. Preliminary Phytochemical Studies of Leaf Extracts of Molineria Recurvata J. Chem. Pharm. Res. 2012; 4, 7: 3727-3730.
16. Stahl E. Thin layer Chromatography: a laboratory hand book. Berlin Heidelberg: Springer-Verlag; 1969.
17. Wagner H, Bladt S. Plant Drug Analysis. 2nd ed. Berlin Hiedelber: Springer-Verlag; 1996.
18. Prasad KVSRG, Sujatha D, Bharti K. Herbal drugs in urolithiasis: a review. Pharmacog Rev. 2007; 1(1):175e178.
19. Grover PK, Kim DS, Ryall RL. The effect of seed crystals of hydroxyapatite and brushite on the crystallization of calcium oxalate in undiluted human urine in vitro: implications for urinary stone pathogenesis. Mol. Med. 2002; 8(4). 2000e2009.

20. Worcester EM, Coe FL. Nephrolithiasis. Prim Care. 2008; 35: 369-371.

21. Colella J, Kochis E, Galli B, Munver R. Urolithiasis/nephrolithiasis: what's it all about? Urol Nurs. 2005; 25(6):427-448, 475, 449.

22. Basavaraj DR, Biyani CS, Browning AJ, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAUEBU Update Ser. 2007; 5: 12-36.

23. Aggarwal KP, Narula S, Kakkar M, Tandon C. Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. Bio Med Res Int. 2013.

24. Massey LK, Roman-Smith H, Sutton RA. Effect of dietary oxalate and calcium on urinary oxalate and risk of formation of calcium oxalate kidney stones. J Am Diet Assoc. 1993; 93(8):901-906.

25. Finkielstein VA, Goldfarb DS. Strategies for preventing calcium oxalate stones. CMAJ. 2006; 174(10):1407-1409.

26. Durgawale P, Shariff A, Hendre A, Patil S, Sontakke A. Chemical analysis of stones and its significance in urolithiasis. Biomed. Res. 2010; 21(3):305-310.

27. Noonan SC, Savage GP. Oxalate content of foods and its effect on humans. Asia Pacific J. Clin. Nutr.1998; 8(1): 64-74.

28. Coll DM, Varanelli MJ, Smith RC. Relationship of spontaneous passage of ureteral calculi to stone size and location as revealed by unenhanced helical CT. Am. J. Roentgenol. 2002; 178: 101-103.

29. Aboumarzouk OM, Kata SG, Keeley FX, Nabi G. Extracorporeal shock wave lithotripsy (ESWL) versus ureteroscopic management for ureteric calculi. Cochrane Database Syst. Rev. 2007.

30. Tombolini P, Ruoppolo M, Bellorofonte C, Zaatar C, Follini M. Lithotripsy in the treatment of urinary lithiasis. J. Nephrol. 2000; 13(3):S71-S82.