RESULTS AND DISCUSSION

Urolithiasis is the formation of calculus in the urinary collecting system commonly arising in the kidneys. The major constituents of the stones are crystalline compounds produced due to the supersaturation of urine (Verma et al., 2015). Pathogenesis of kidney stones is multifactorial and intricate, and sex-derived differences are thought to influence its incidence (Atif et al., 2014).

The goal of surgical techniques is the removal of existing stones and medical treatment to prevent the recurrence of stones (Dodoala et al., 2010). The surgical methods available to treat kidney stones, namely Extracorporeal Shock Wave Lithotripsy, Percutaneous Nephrolithotomy, Uteroscopy and Nephrolithotomy, have serious side effects and do not always give satisfactory results. Medicinal plants have been used since time immemorial to treat various ailments. Phytotherapy is in great demand both in developed and developing countries because of their biological and medicinal activities, higher safety margin and lesser costs (Sunitha et al., 2012).

A weed, in general, is a plant that is considered as nuisance. The term ‘weed’ is normally applied to undesired plants in human controlled settings, especially farm, fields, gardens, lawn, park and other areas (Dhanam, 2014). The idea of using weeds for medicinal purpose is not new. Weeds have advantages over more desirable crop species because they often grow quickly, reproduce quickly, have short life span with multiple generations in the same growing season and produce seeds that persist in the soil seed bank for many years. The weeds continue to exist, because the environment is continually being disturbed to create open conditions for new generation, such as forest fires and human activities (Sankar and Satapathy, 2015).

Plants can produce many different types of secondary metabolites that subsequently are utilized by humans for their valuable characters in a diverse array of applications. Secondary metabolites include compounds produced in
stress. Currently, there is an increased interest in natural substances with medicinal value (Yamunadevi et al., 2011). However, the effectiveness and mechanism by which the weed plant works have not yet been fully understood (Rad et al., 2011).

The present study, therefore, focused towards “In vitro and In vivo Investigation of Antilithiatic and Antioxidant Activity of Aqueous Extract of Aerva lanata”. 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 selected plants with medicinal property

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 Aerva lanata extract

4.3.1. In vivo analysis in urine samples of experimental animals (rats)

4.3.1.1. Effect of Aerva lanata extract on volume and pH of urine

4.3.1.2. Effect of Aerva lanata extract on urinary calcium level

4.3.1.3. Effect of Aerva lanata extract on urinary oxalate level

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4.3.1.6. Effect of Aerva lanata extract on urinary creatinine level

4.3.1.7. Effect of Aerva lanata extract on urinary magnesium and citrate levels
4.3.2. **In vivo analysis in serum of experimental animals**

4.3.2.1. Effect of *Aerva lanata* extract on serum calcium, oxalate and phosphate levels

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4.3.3.2. Effect of *Aerva lanata* extract on the kidney weight

4.3.3.3. Effect of *Aerva lanata* extract on the levels of calcium and oxalate in kidney tissue

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

4.3.5. **Histopathological examination of the kidney tissues**

4.4. **Effect of *Aerva lanata* extract on lithiasis induced cultured kidney cells**

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

4.4.2. Morphological changes observed in NRK 52E cells

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

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4.5. **Antioxidant levels in *Aerva lanata***

4.5.1. Enzymic antioxidant activities in *Aerva lanata* flowers

4.5.2. Levels of non-enzymic antioxidants in *Aerva lanata* flowers

4.6. **Radical scavenging ability of *Aerva lanata* extract**

4.6.1. DPPH radical scavenging activity of *Aerva lanata* extract

4.6.2. ABTS radical scavenging activity of *Aerva lanata* extract

4.6.3. Hydrogen peroxide scavenging activity of *Aerva lanata* extract

4.6.4. Hydroxyl radical scavenging activity of *Aerva lanata* extract
4.7. Determination of phytochemical constituents of *Aerva lanata* extract

4.7.1. Preliminary qualitative phytochemical analysis

4.7.2. UV-visible absorption spectrum of the phytochemical fractions of *Aerva lanata* extract

4.7.3. HPTLC of *Aerva lanata* extract

4.7.4. HPLC analysis of *Aerva lanata* extract

4.7.5. FT-IR analysis of *Aerva lanata* extract

4.7.6. GC-MS analysis of *Aerva lanata* extract

4.7.7. TLC analysis of *Aerva lanata* extract

4.7.7. 1H NMR analysis of *Aerva lanata*

**PHASE I**

The *in vitro* antilithiatic potential of selected plant extracts, namely *Tribulus terrestris*, *Aerva lanata*, *Scoparia dulcis* and *Tridax procumbens*, were analyzed and the results obtained are presented and discussed below.

**4.1. Solvent Extraction**

Based on the information of the traditional use of the selected weeds, the leaves of *Tribulus terrestris*, *Scoparia dulcis* and *Tridax procumbens* and the flowers of *Aerva lanata* were used for the study. Chloroform, methanol and aqueous extracts were prepared by hot percolation method. It was observed that the per cent yield varied from 0.18-0.79% (Table 1).

The per cent yield was found to be more in the methanol and aqueous extracts, compared to the chloroform extract, in all the plants selected for the study. Maximum yield was observed in the aqueous fraction of *Aerva lanata* when compared to the extracts of the other plants.
Table 1

Extract Yield of selected weeds

<table>
<thead>
<tr>
<th>Weed</th>
<th>Solvents</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
<td>Methanol</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>0.26±0.20</td>
<td>0.70±0.06</td>
<td>0.76±0.04</td>
</tr>
<tr>
<td>Aerva lanata</td>
<td>0.18±0.32</td>
<td>0.72±0.25</td>
<td>0.79±0.8</td>
</tr>
<tr>
<td>Scoparia dulcis</td>
<td>0.30±0.10</td>
<td>0.60±0.05</td>
<td>0.71±0.10</td>
</tr>
<tr>
<td>Tridax procumbens</td>
<td>0.23±015</td>
<td>0.72±0.29</td>
<td>0.72±0.23</td>
</tr>
</tbody>
</table>

*The values are mean ± S.D. of triplicates*

4.2. Effect of *in vitro* antilithiatic potential of selected plants with medicinal properties

The initial step in understanding antilithiasis involves the study of the inhibitory capacity of the plant extracts *in vitro*, in the presence and the absence of inhibitors. The classical model for the study of oxalate crystallization was chosen for its simplicity and satisfactory reproducibility (Agarwal and Varma, 2014).

In the *in vitro* calcium oxalate assay, different solvent extracts of the selected plants at varying concentrations such as 50, 100, 200, 400, 800, 1600 and 3200µg/mL were used to assess their inhibitory potential against the CaOx crystal formation.

4.2.1 *In vitro* calcium oxalate assays

The crystallization of the calcium oxalate begins with increased urinary supersaturation, with the subsequent formation of the solid crystalline particles within the urinary tract. The stone-forming salts in supersaturated urinary solution coalesce into clusters, which then increase in size by the addition of new constituents. These crystals then grow and aggregate with other crystals in solution, and are ultimately retained and accumulated in the kidney (Kalpana *et al*., 2013).
4.2.1.1 Nucleation assay

Stone formation begins with the onset of tiny crystals in the kidney. Crystals are composed of substances such as calcium and oxalate that have been filtered into the urine. These salts bind to form the central core called the nuclei (Christi and Senthamarai, 2014).

The combination of the solutions of calcium chloride and sodium oxalate resulted in the formation of calcium oxalate crystals. The rate of nucleation was estimated by the comparison of induction time in the presence of the extract with that of the control. The absorbance was monitored at 620nm. Crystal nucleation and dissolution was measured in terms of turbidity. An increase in crystal dissolution increases the turbidity of the solution. The results as shown in Figures 9a-9d revealed that the turbidity of the solution in the presence of the plant extract increased in contrast to the control, indicating that oxalate crystallization was disturbed in the presence of the extract. The data represents that the inhibition of crystal formation was directly proportional to the increase in concentration of the plant extracts, with maximum activity observed at 1600µg/mL, beyond which, at 3200 µg/mL there was no further inhibition observed.

Among the four different weeds studied, the aqueous extract of Aerva lanata was able to render maximum inhibition against CaOx crystallization on comparison with the extracts of the other plants selected, as observed in the results.

The calcium oxalate crystals, under physiological condition, exhibit two different morphologies: Calcium Oxalate Monohydrate (COM) and tetragonal Calcium Oxalate Dihydrate (COD) (Wang et al., 2010). Kidney stones are mainly composed of COM crystals (Wesson and Ward, 2007).

The microscopic examination of the crystals (Plate 5) showed the presence of both polymorphs of COM and COD. A gradual reduction in the size of the crystals along with change in morphology (COM to COD) was observed with the addition of the different weed extracts. This ability of the extract to reduce the nucleation, increases the limit of oxalate in urine and prevents the precipitation of the CaOx.
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*.

**Figure 9**

**Effect of weed extracts on nucleation of CaOx Crystallization**

9a. *Tribulus terrestris*  

9b. *Aerva lanata*

9c. *Scoparia dulcis*  

9d. *Tridax procumbens*

The values are mean ± S.D. triplicates

The values are mean ± S.D. of triplicates
Plate 5

Calcium oxalate crystal nucleation morphology

(400X magnification)

Crystal Nucleation

(without extract)

Crystal Nucleation

(with extract)
Our results are in accordance with that of Pachana et al. (2010), who showed that there was reduction in the crystal count and crystal size on the addition of *Tribulus terrestris* extracts. Khare et al. (2014) showed that the aqueous extract of *Phyllanthus niruri* Linn. was potentially on par with Cystone, a standard drug in dissolving and inhibiting calcium oxalate crystals, showing the presence of antilithiatic compounds. The conversion of the spiked crystals to smooth round (COM to COD) crystal helps in the easy passage of the crystals in the urine, as pointed out by Parekh et al. (2007).

Agarwal and Varma (2014) proved that the ethanolic extract of *Ocimum gratissimum* Linn. was able to exert a concentration-dependent action against crystal formation. The microscopic studies showed that there was a decrease in the length and breadth of the crystals when compared to the control in the presence of the extract. Nucleation study by Bashir and Gilani (2009) showed that an aqueous-methanol extract of *Bergenia ligulata* rhizome caused a decrease in crystal count and modified calcium oxalate monohydrate crystal morphology.

The study done by Varghese et al. (2014) on calcium hydrogen phosphate dihydrate (brushite) crystals using an aqueous extract of *Aerva lanata* Linn. Juss. Ex Schult revealed a concentration-dependent decrease in crystal nucleation. The microscopic studies showed a conversion of star or spindle shaped crystals to smooth round or oval crystals in the presence of the aqueous extract. There was also a reduction in the size of the crystals. This might be due to the presence of some compounds in the plant extract that act like natural inhibitors for crystal nucleation.

The results of the nucleation assay indicate that the interference of the extracts with the crystal formation may be a therapeutic strategy to hamper stone formation. This ability of the extracts to reduce the nucleation and increases the oxalate excretion in urine, and prevent the precipitation of the CaOx crystal, indicate the presence of inhibitory compounds in the extracts.
4.2.1.2. Growth assay

The second stage of stone formation involves growth of additional layers of salt on to the nuclei. Growth of crystals results in a reduction in the potential energy of the atoms or molecules when they bind to each other (Aggarwal et al., 2010b).

The growth assay revealed that, in the presence of the plant extracts, there was a reduction in the crystal growth, which was observed by the increase in the absorbance at 214 nm (Figures 10a-10d).

The aqueous extract of Aerva lanata showed the maximum inhibition in comparison to all the weed extracts tested. Plate 6 represents that the number of crystals on treatment with the extracts were reduced in comparison to the control due to the inhibitory effect of the extracts. Reduction in the size of the crystal and growth was also recorded.

Antiurolithiatic activity of the methanol and ethyl acetate extracts of Solanum aguivi Lam. showed significant inhibitory effect against calcium oxalate precipitation (Mathew et al., 2014). The hydromethanolic extract of Centratherum anthelminticum inhibited the nucleation of calcium oxalate in solution. Lesser and smaller particles were formed with increasing concentrations of the extract. This proved that the extract had nucleation preventing agents. These limiting factors may be a reason for the prevention of crystal growth (Galani and Panchal, 2014a).

Ahmed et al. (2013) observed that the hydro alcoholic extract of Adiantum capillus-veneris Linn. reduced the number of COM crystals which are responsible for a higher risk for stone formation. Kalanchoe pinnata extract inhibited the growth of calcium oxalate monohydrate crystals in vitro in a dose-dependant manner (Phathak and Hendre, 2015).

Microscopic examinations revealed that the Beta vulgaris Linn. extracts inhibited the crystallization of calcium oxalate monohydrate and promoted calcium oxalate dihydrate formation in solution; further, there was a reduction in the size and number of the crystals (Saranya and Geetha, 2014).
Results and Discussion

Figure 10
Effect of weed extracts on growth of CaOx Crystallization

10a. *Tribulus terrestris*  
10b. *Aerva lanata*

![Graphs showing crystal dissolution for Tribulus terrestris and Aerva lanata](image1)

The values are mean ± triplicates

10c. *Scoparia dulcis*  
10d. *Tridax procumbens*

![Graphs showing crystal dissolution for Scoparia dulcis and Tridax procumbens](image2)

The values are mean ± S.D. of triplicates

*In vitro and in vivo* investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*
Plate 6

Calcium oxalate crystal growth morphology

(400X magnification)

Crystal Growth

(without extract)

Crystal Growth

(with extract)
Beghelia et al. (2009) have reported that the extracts of *Ammodaucus leucotrichus* inhibited calcium oxalate growth significantly by increasing more crystals in urine, thereby reducing supersaturation and the size of the particles. Quazi et al. (2014) reported that the alcoholic extract of *Clitoria ternatea* showed good inhibition against the growth of crystals.

The results of the present study indicated that the weed extracts were able to inhibit crystal growth, which might be due to the presence of phytoconstituents in the crude extract that may prevent the precipitation and formation of nuclei, thereby hindering with the growth of the crystals.

### 4.2.1.3. Aggregation assay

Aggregation is the clumping of small particles into larger ones. In this process, crystals in solution adhere together and form large particles. In various steps of stone formation, crystal aggregation is a more important step rather than nucleation and growth because aggregation occurs within a few seconds (Ratkalkar and Kleinman, 2011).

The results of the aggregation assay showed a similar trend to that of the nucleation and growth assays. Crystals treated with the weed extracts were less aggregated, while the rate of inhibition elevated with increase in concentration of the extracts. The results were supported by the spectroscopic analysis (Figures 11a-11d) as the absorbance was found to be increased in the extract treated groups when compared to the control due to disaggregation. This action of the extracts presumably helps in the easy expulsion of the crystals in the urine as the CaOx crystals are dispersed in the urine. In addition, altered crystal morphology from COM to COD as observed in Plate 7, also aids removal of stone from the kidney as it has lesser affinity towards kidney cells.

Aqueous extract of *Trianthema decandra* exerted antiurolithiatic activity against crystal aggregation. The study showed that the crude extract may contain some phytochemicals which may be responsible for inhibiting heterogeneous nucleation and aggregation of calcium and oxalate (Kuncha et al., 2014).
Results and Discussion

Effect of weed extracts on aggregation of CaOx Crystallization

11a. Tribulus terrestris

The values are mean ± triplicates

11b. Aerva lanata

The values are mean ± triplicates

11c. Scoparia dulcis

The values are mean ± S.D. of triplicates

11d. Tridax procumbens

The values are mean ± S.D. of triplicates

In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of Aerva lanata
Plate 7

Calcium oxalate crystal aggregation morphology

(400X magnification)

Crystal Aggregation

(without extract)

Crystal Aggregation

(with extract)
Aggregation in the presence of hydroalcoholic extracts of *Adiantum capillus veneris* Linn. was lower than that in the control (without extract). The rate of inhibition increased with increase in concentration of the extract. COM crystals have a higher capacity to aggregate and adhere and it is the main form excreted by nephrolithiatic patients. It was observed that hydroalcoholic extract of *Adiantum capillus veneris* Linn. reduced mainly the amount of COM crystals (Ahmed *et al.*, 2013). The methanol extract of *Nymphaea odorata* could inhibit aggregation of calcium oxalate crystals (Deoda *et al.*, 2012). Patel *et al.* (2010b) revealed that the alcoholic extract of the plant *Pedalium murex* caused inhibition of crystal formation and aggregation as compared to its aqueous extract.

The treatment of the crystals with the extracts of the polyphyto formulations, which included extracts of 20 different medicinal plants, showed a dramatic reduction in the crystal size, while calcium level of the solution was increased proving the degeneration of the calcium-oxalate crystals (Bharadwaj *et al.*, 2013). The literatures quoted aided the observation that the plant extracts selected had good antilithiatic activity. The action may be due to the presence of organic inhibitory compounds that adsorb to the surface of the crystal, thereby inhibiting crystal nucleation, growth and aggregation (Basavaraj *et al.*, 2007).

The results of all the three assays performed, corresponding to the three phases of stone formation, revealed that all the four weed extracts could render inhibition against precipitation of stone forming constituents in all the three phases of stone formation.

However, the inhibitory potential observed in different solvents varied. As discussed earlier, the inhibitory potential might be due to the presence of phytochemical constituents in the weeds. These secondary metabolites would have eluted in the different solvent extracts based on the polarity. Further study needs to be conducted to understand the nature of the phytochemical constituents.

Among the weeds selected for the present study, *Aerva lanata* exhibited maximum inhibitory potential in all the three critical stages of stone formation *in vitro*, and, therefore, only *Aerva lanata* was chosen for further analysis of the study.
4.2.1.4. Dose Optimization

After choosing *Aerva lanata*, for an elaborate study to reiterate the inhibitory potential against crystal precipitation, it became imperative to select an appropriate concentration of the extract at which it exhibits maximum inhibitory effect against stone formation.

The *in vitro* calcium oxalate assay revealed a concentration-dependent inhibition against crystal nucleation, growth and aggregation. It was observed that maximum inhibition was noted in the aqueous extract at 1600µg/mL after which there were no significant changes in the extent of inhibition. The graphical representation of the results shows a plateau (Figures 12a-12c) after 1600 µg/mL.

**Figure 12**

Dose optimization

**12a. Nucleation assay**

**12b. Growth assay**

**12c. Aggregation assay**

*The values are mean ± S.D. of triplicates*
Hence 1600 µg/mL was used for further phase of the study. The dosage of the standard drug, Cystone was fixed as 12.7mg/kg body weight of experimental animals (as prescribed) (Kumara and Patki, 2011).

The results helped to affirm that the plant extracts possess certain compounds that act as anti-calcifying agents and prevent crystallization. These properties were further confirmed by the in vivo analyses to validate the study.

PHASE II

4.3. In vivo antilithiatic potential of Aerva lanata extract

Animal studies and clinical trials are the two forms of in vivo studies often employed over in vitro studies, as they are suited for observing the overall effects of an experiment on living subject. In vivo studies involving kidney stone research includes the induction of stone using ethylene glycol to study the efficacy of the drug in dissolving the stone. Ethylene glycol disturbs oxalate metabolism by way of increasing the substrate availability that increases the activity of oxalate synthesizing enzymes in the rat. The oxalate produced is not further metabolized and combines with calcium to form calcium oxalate stones (Sundararajan et al., 2006). Most of the data available on renal physiology are based on experiments in rats, as the animals most commonly used for the study of lithiasis (Butterweck and Khan, 2009).

The results of the in vitro analysis can be extrapolated to the in vivo assay. The calcium oxalate crystallization in vitro revealed that the aqueous extract of Aerva lanata had maximum inhibitory action against crystallization at a concentration of 1600µg/mL when compared to the other extracts. Hence, the aqueous extract of Aerva lanata, at a dose of 1600µg/mL was selected for further analysis.

The animals under study were fed with 75% ethylene glycol for the induction of the stones. Both preventive as well as curative properties of the aqueous extract of Aerva lanata were analyzed.

Calcium oxalate crystal deposition is indicated by increased excretion of stone forming constituents in the urine, such as calcium, oxalate, uric acid,
Results and Discussion

phosphorus and creatinine. For the present study, 24h urine sample on 0\textsuperscript{th}, 7\textsuperscript{th}, 14\textsuperscript{th}, 21\textsuperscript{st} and 28\textsuperscript{th} day of treatment from all the rats of different treatment groups were collected. This was used for carrying out estimations like calcium, oxalate, phosphorus, uric acid, creatinine and the pH change was also noted.

4.3.1. \textit{In vivo} analysis in urine samples of experimental animals

4.3.1.1. Effect of \textit{Aerva lanata} extract on volume and pH of urine

The effect of the aqueous extract on the volume of the urine excreted by the experimental animals is recorded in Table 2.

The results of the present study showed a significant decrease in the excretion of urine in the lithogen (ethylene glycol) treated rats. Nidus formation by the supersaturation of the urine with lithiasis promoting factors may cause obstruction of the outflow of urine in the urinary system. This leads to the decrease of glomerular filtration rate (GFR), resulting in reduced urine output in lithogenic (lithiatic group) rats (Kachchhi \textit{et al.}, 2012).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
\textbf{Treatment groups} & \multicolumn{5}{c|}{\textbf{Urine volume (mL/24h)}} \\
\hline
 & \textbf{0} & \textbf{7\textsuperscript{th}} & \textbf{14\textsuperscript{th}} & \textbf{21\textsuperscript{st}} & \textbf{28\textsuperscript{th}} \\
\hline
Control & 7.0±0.20 & 7.3±0.71\textsuperscript{a} & 7.4±0.23\textsuperscript{a} & 7.1±0.25\textsuperscript{a} & 7.2±0.07\textsuperscript{a} \\
Lithiatic Control & 7.4±0.32 & 6.4±0.21\textsuperscript{bc} & 5.0±0.12\textsuperscript{c} & 4.4±0.38\textsuperscript{c} & 3.5±0.04\textsuperscript{d} \\
Extract Control & 7.2±0.26 & 7.2±0.26\textsuperscript{a} & 7.3±0.26\textsuperscript{a} & 7.1±0.20\textsuperscript{a} & 7.4±0.51\textsuperscript{a} \\
Preventive & 7.1±0.35 & 6.0±0.38\textsuperscript{ab} & 6.3±0.45\textsuperscript{b} & 6.6±0.72\textsuperscript{a} & 6.7±0.36\textsuperscript{b} \\
Curative & 6.9±0.17 & 6.0±0.17\textsuperscript{c} & 5.3±0.15\textsuperscript{c} & 5.8±0.06\textsuperscript{b} & 6.2±0.12\textsuperscript{c} \\
Cystone & 7.4±0.14 & 5.0±0.06\textsuperscript{bc} & 5.0±0.31\textsuperscript{c} & 5.8±0.06\textsuperscript{b} & 6.5±0.15\textsuperscript{bc} \\
\hline
Tukey HSD at 5\% & NS & 0.9233 & 0.7577 & 0.9867 & 0.739 \\
\hline
\end{tabular}
\caption{Urine volume of experimental animals}
\end{table}

\textit{The values are mean ± S.D. of triplicates}

Groups with common superscripts do not differ significantly.
The treatment of the rats with the aqueous extract of *Aerva lanata* showed remarkable restoration in the urine volume in both the curative and preventive groups. An increase in urine output dilutes the concentration of crystal promoters, reducing the chances of precipitation of calcium and oxalate.

Our results followed a similar trend with Pareta *et al.* (2011b), who reported that treatment with ethylene glycol reduced the urine volume in the lithiatic group compared to the control group. Co-treatment with an aqueous extract of *Boerhaavia diffusa* increased the urine volume in a dose-dependant manner.

Elkhamisy (2015) revealed that pretreatment with the ethanol extract of *Petroselinum sativum* and *Curcuma longa* increased the urine output volume when compared to lithiasis-induced rats indicating the diuretic activity of the extracts. Chithra *et al.* (2011) showed that the alcoholic extract of *Boerhaavia diffusa* induced a diuretic effect on the lithiasis induced rats in the curative and preventive groups, thereby, increasing the volume of urine output.

Chavada *et al.* (2012) observed that there was a significant decrease in urine volume in ethylene glycol calculi-induced model control animals as compared to normal control animals. These changes were prevented by treatment with flavonoid fraction of *Citrus medica*.

Alteration in the urine volume was observed after the induction of lithiasis. On treatment with ethanol extract of *Hamelia patens*, the volume of urine excreted by the animals was improved when compared to the volume of the lithiatic control group. This reinforces that the plant has diuretic acitivity (Yitzhak *et al.*, 2014). Notable increase in the urinary output was observed in ethylene glycol treated rats. The diuretic effect of *Achyranthes aspera* Linn. extract increased the urine volume in the preventive and curative groups (Buela *et al.*, 2013).

Our observation was supported by the literatures, which shows that the aqueous extract of *Aerva lanata* has diuretic property, resulting in increased urine output. Increase in urine output, possibly dilutes the concentration of crystal promoters, resulting in continuous expulsion of calcium, oxalate and phosphate from the urinary system.
4.3.1.1 pH of urine

The changes in urine pH on administration of the extract and ethylene glycol are tabulated in Table 3.

Crystalluria is a pH dependent parameter. The urine pH was observed to be acidic in the lithiasis induced rats compared to the control group. The acidification of the urine favours the adhesion and retention of the calcium oxalate crystals in the renal tubules (Wagner and Mohebbi, 2010).

The urine pH in the present study was found to be in the range of 5.0-6.0 in the ethylene glycol treated group indicating that predominantly oxalate stones are formed. On administration of the aqueous extract, the pH was restored to its normal levels. This indicates the prevention of CaOx stone formation, which was evident by the marked increase in urinary excretion and decrease in renal deposition of calcium and oxalate.

### Table 3

**Urinary pH of experimental animals**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>24h Urine pH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7\textsuperscript{th}</td>
</tr>
<tr>
<td>Control</td>
<td>6.64±0.09</td>
<td>6.64±0.02\textsuperscript{a}</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>6.65±0.05</td>
<td>6.46±0.07\textsuperscript{b}</td>
</tr>
<tr>
<td>Extract Control</td>
<td>6.65±0.38</td>
<td>6.66±0.07\textsuperscript{a}</td>
</tr>
<tr>
<td>Preventive</td>
<td>6.63±0.22</td>
<td>6.50±0.08\textsuperscript{c}</td>
</tr>
<tr>
<td>Curative</td>
<td>6.64±0.10</td>
<td>6.42±0.03\textsuperscript{c}</td>
</tr>
<tr>
<td>Cystone</td>
<td>6.67±0.05</td>
<td>6.44±0.01\textsuperscript{b}</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>NS</td>
<td>0.1531</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates
Groups with common superscripts do not differ significantly
The types of stone formed can be predicted from the pH of the urine. Uric acid stones are likely to be formed at highly acidic pH (5.0 or below), calcium oxalate stones are formed at the pH of 5.0-6.5 and alkaline pH favors magnesium ammonium type of stones (Patel et al., 2010a).

Several studies have reported a similar trend with other plant extracts. An ethanol extract of Tridax procumbens reverted the pH of urine from a slightly acidic range (Sailaja et al., 2011). The methanolic extract of Withania somnifera exerted a diuretic action in the lithogen treated rats, which was observed as increase in urine volume and pH (Patel and Mandal, 2014). Betanabhatla et al. (2009) reported that treatment with Hibiscus sabdariffa Linn. extract restored the urine pH to normal range in the ethylene glycol treated rats.

Megarajanga Chooranam restored the urine pH to normal and increased the urine volume significantly [p<0.01] when compared to the control drug (Kanakavalli et al., 2013). An ethanolic extract of Portulaca oleracea Linn restored the urine pH and volume to normal levels when compared to the disease control rats (Kishore et al., 2013). Fenugreek seed extract exerted therapeutic action against ethylene glycol treated rats by restoring urinary pH and solute balance (Kapase et al., 2013).

Nirmaladevi et al. (2012) reported that treatment with standard (Cystone) and Hibiscus rosa-sinensis extract restored the pH to the normal range in lithiasis induced rats, suggesting that it prevents the precipitation of calcium oxalate.

In tune with these reports the results, of the present study showed that the action of aqueous extract of Aerva lanata induced an antilithiatic activity by increasing the pH thereby, preventing the stones from attaching to the surface of cells.

**4.3.1.2. Effect of Aerva lanata extract on urinary calcium level**

Table 4 depicts the concentration of calcium excreted in the urine. The administration of ethylene glycol leads to hyperoxaluria. The results showed an increase in calcium excretion in the urine of lithogen treated groups when compared to the control. Higher concentration of calcium salt leads to reduced activity of urinary inhibitors as it forms a complex with negatively charged inhibitors like citrate, resulting in the formation of stones.
On co-supplementation of ethylene glycol with the aqueous extract, there was a reduction in the calcium excretion in the curative group. This might be due to the prophylactic and diuretic action of the extract that probably hastens the process of crystal dissolution and increased expulsion in the urine.

Table 4

Levels of urinary calcium in experimental animals

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Urine calcium (mg/dL in 24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>1.25±0.03</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>1.23±0.01</td>
</tr>
<tr>
<td>Extract Control</td>
<td>1.28±0.02</td>
</tr>
<tr>
<td>Preventive</td>
<td>1.24±0.03</td>
</tr>
<tr>
<td>Curative</td>
<td>1.23±0.01</td>
</tr>
<tr>
<td>Cystone</td>
<td>1.22±0.03</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates

Groups with common superscripts do not differ significantly

The results of the preventive regime group was comparable with those of the standard drug (Cystone) treated group proving that the regular intake of the extract could readily prevent and protect humans from crystal formation during urolithiasis. It was also observed that the extract alone treated groups showed results similar to the control, indicating that the extract did not cause any toxic effects in the experimental rats.

Ahmadi et al. (2012) reported that in both preventive and curative groups, treatment of rats with hydroalcoholic extract of Alcea rosea roots significantly
reduced calcium and oxalate excretion when compared to ethylene glycol treated group. Stone induction by ethylene glycol caused an increase in oxalate and calcium excretion in the urine. Calcium excretion was reduced by treatment with a methanol extract of *Launaea procumbens* in a dose-dependent manner (Makasana *et al.*, 2014)

Paula *et al.* (2012) evaluated the antilithiatic activity of the hydro alcoholic extract from *Costusigneus langesdorfii* leaves on urolithiasis in rats, the results of which showed that the extract significantly decreased the levels of uric acid, calcium and phosphate in the urine. Bayir *et al.* (2011) found that the aqueous extract of *Helichrysum plicatum* DC. flowers suppressed renal calcium accumulation and urinary CaOx levels in the litholitic (ethylene glycol) rats in a dose dependent manner.

Supplementation with *Triphala karpa* choornam significantly decreased the elevated levels of calcium in the urine of the curative and preventive groups (Tamilselvan *et al.*, 2013). Galani and Panchal (2014b) concluded that 28 days oral treatment with 70% methanolic extract of *Centratherum anthelminticum* seeds have potent antiurolithiatic activity against ethylene glycol induced nephrolithiasis in rats.

Similarly, an alcoholic extract of *Mimusops elengi* bark significantly (P<0.001) lowered the elevated levels of oxalate, calcium and phosphate in the urine and kidney when compared to calculi-induced animals (Ashok *et al.*, 2010). Treatment with ethanol extract of *Phyla nodiflora* Linn. caused a significant reduction in the urinary excretion of calcium, thus reducing the supersaturation of urine. This might be responsible for preventing and also dissolving preformed calcium oxalate stones (Dodoala *et al.*, 2010).

The present study showed that the aqueous extract of *Aerva lanata* flowers reduced the urinary concentration of calcium and also dissolved preformed crystals. This might be an indication of antilithiatic activity.
4.3.1.3. Effect of *Aerva lanata* extract on urinary oxalate level

The results of the oxalate assay showed a substantial increase in the levels of oxalate in the urine of the lithiasis induced group (Table 5). Ethylene glycol metabolizes to oxalate leading to the elevation of oxalate concentration. The increased oxalate precipitates to form calcium oxalate crystals, leading to crystalluria (Al-Jawad *et al*., 2012). The curative group effectively stabilized the oxalate excretion after the 14th day in the urine due to the regulatory action of the extract. This result was comparable to the standard drug treated group.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Urine oxalate (mg/dL in 24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.39±0.04</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>0.37±0.02</td>
</tr>
<tr>
<td>Extract Control</td>
<td>0.40±0.01</td>
</tr>
<tr>
<td>Preventive</td>
<td>0.41±0.02</td>
</tr>
<tr>
<td>Curative</td>
<td>0.38±0.10</td>
</tr>
<tr>
<td>Cystone</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>NS</td>
</tr>
</tbody>
</table>

*The values are mean ± S.D. of triplicates. Groups with common superscripts do not differ significantly.*

In the preventive group, the co-application of the aqueous extract with ethylene glycol showed that the extract was able to protect the cells before initiation of the disease. This observation shows that the plant extract could, not only dissolve existing crystals, but also prevent nuclei formation.
The administration of the aqueous extract of *Aerva lanata* showed a substantial decrease in the concentration of oxalate in the curative regimen. This can be attributed to the fact that the plant extract induces diuresis, leading to flushing out of the renal system and mechanical expulsion of the stone.

The hydroalcoholic extract of *Achyranthes indica* Linn. showed a dose-dependent action against crystalluria caused by an increase in oxalate concentration (Pareta *et al.*, 2011a). Our conclusions are comparable to that of Gilhotra and Christina (2011), who reported that *Rotula aquatica* Lour extract reduced the urinary calcium and oxalate excretion in the urolithiatic rats by minimizing the conditions favorable for crystal growth.

Supplementation with an ethanol extract of *Melia azedarach* restored urinary phosphate level, thereby reducing the risk of stone formation and lowering the levels of calcium as well as oxalate in urine of experimental rats (Dharmalingam *et al.*, 2014). Increased levels of calcium, oxalate, phosphate and uric acid in the urine and serum in the experimental rats were significantly reduced by the hydroalcoholic extract of *Nardostachys jatamansi* thereby preventing the formation of urinary stones (Vidhya *et al.*, 2013).

Our results agree with that of Sridhar *et al.* (2011) who reported that root extract of *Crataeva magna* altered the excretion of calcium and oxalate in urine and confirmed the stone inhibitory effect. Sachan (2012) showed that the *Moringa oleifera* Linn. ethanol extracts not only cured but also prevented the growth of urinary stones, and significantly reduced the level of lithiasis causing factors such as calcium, oxalate and phosphate. Ramachandran *et al.* (2011) reported that supplementation of aqueous and alcoholic extraction of poly-herbal constituents of *Aerva lanata*, *Dolichos biflorus* and *Musa* species effectively reduced the urinary oxalate excretion.

Our results are in agreement with Kumkum and Ranjana (2012) who have reported that supplementation with various extracts of *Hiptis suaveolens* (L.) significantly lowered the elevated levels of oxalate, calcium and phosphate in
Results and Discussion

urine and kidney. Our study is supported by the literatures quoted. The result showed that the plant has good action against hyperoxaluria. This might be due to the interference of the extract in the oxalate synthesis pathway.

4.3.1.4. Effect of *Aerva lanata* extract on urinary phosphate level

Table 6 shows the results of the phosphate assay. An elevated level of urinary phosphate was recorded in the calculi induced rats when compared to the control. Increased phosphate concentration provides a favourable environment suitable for calcium phosphate stone formation, which epitaxially confers calcium oxalate deposition.

**Table 6**

Levels of urinary phosphate in experimental animals

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Urine phosphate (mg/dL in 24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>3.74±0.09</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>3.79±0.02</td>
</tr>
<tr>
<td>Extract Control</td>
<td>3.77±0.04</td>
</tr>
<tr>
<td>Preventive</td>
<td>3.80±0.10</td>
</tr>
<tr>
<td>Curative</td>
<td>3.78±0.12</td>
</tr>
<tr>
<td>Cystone</td>
<td>3.76±0.14</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates
Groups with common superscripts do not differ significantly

The curative and preventive regimen showed restoration of phosphate levels close to normal range on treatment with the aqueous extract. The extract control was comparable to the control group, indicating the protective effect of the extract. The results were comparable with that of the standard drug treated groups. This points out the curative property of the aqueous extract of *Aerva lanata*. 
The results are in accordance with that of Sathya and Kokilavani (2012), who showed that the ethanolic root extract of *Saccharum spontaneum* effectively restored the phosphate levels in the lithiasis induced rats, thereby reducing the risk of stone formation. Kalpana *et al.* (2014) reported that *Tridax procumbens* effectively countered the action of ethylene glycol in lithiasis induced rats.

Treatment with aqueous extract of *Melia azadirachta* caused a significant reduction in the urinary excretion of phosphate in both the preventive and curative group animals, when compared to their respective controls. This explains the effect of the aqueous extract of *Melia azadirachta* in both preventing and also in dissolving the pre-formed stones (Hwisa *et al.*, 2014).

Our results showed similarity to the results obtained by Anbu *et al.* (2011) who showed that the treatment with ethylene glycol elevated the levels of phosphate in the calculi-induced rats. Treatment with ethyl acetate root extracts of *Ichnocarpus frutescens* restored phosphate levels thus reducing the risk of stone formation.

Agarwal and Varma (2012) reported that the extracts of *Hyptis suaveolens* (L.) Poit significantly lowered the elevated levels of oxalate, calcium and phosphate in the urine and kidney of the rats. On treatment with standard drug Cystone and *Achyranthes aspera* Linn. the phosphate excretion was restored to normal limits in the experimental rats (Awari *et al.*, 2009).

The results of the present assay proved that the *Aerva lanata* extract was effective in preventing phosphate accumulation, thereby reducing the risk of stone formation.

**4.3.1.5. Effect of *Aerva lanata* extract on urinary uric acid level**

The concentration of uric acid excretion in the test and control rats is tabulated in Table 7. It can be observed that the administration of ethylene glycol leads to increased excretion of the uric acid in the urine.

Hyperuricosuria, increased urinary uric acid excretion is a risk factor for calcium oxalate stones. The increase in uric acid in the urine may serve to salt out calcium oxalate, which is already supersaturated in the urine (Grover *et al.*, 2003).
Results and Discussion

Table 7
Levels of urinary uric acid in experimental animals

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Urine uric acid (mg/dL in 24h)</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.70±0.03</td>
<td>0.70±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>0.69±0.04</td>
<td>0.84±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract Control</td>
<td>0.71±0.07</td>
<td>0.75±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Preventive</td>
<td>0.68±0.02</td>
<td>0.77±0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curative</td>
<td>0.67±0.01</td>
<td>0.90±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cystone</td>
<td>0.69±0.07</td>
<td>0.85±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>NS</td>
<td>0.80</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates
Groups with common superscripts do not differ significantly

The administration of the aqueous extract of *Aerva lanata* leads to a reduction in the amount of uric acid excreted in the urine in the present study. The results also pointed out that the aqueous extract control group and preventive group were similar to that of the standard drug. The results clearly show that the extract action increases the levels of inhibitors preventing salting out of calcium oxalate by uric acid.

Chaitanya et al. (2010) reported that the aqueous and alcoholic extracts of *Macrotyloma uniflorum* restored the uric acid level in the lithiasis induced rats to its normal level. Kanakavalli et al. (2013) reported that the administration of Megarajanga Chooranam cured and prevented stone formation and restored all biochemical parameters namely, calcium, oxalate and uric acid.
Methanol and aqueous extracts of *Cassia fistula* restored the urine parameters creatinine, urea and uric acid, to their normal levels (Ramesh *et al*., 2010). Administration of 0.75 % (v/v) ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Stone forming promoters like oxalate, calcium, phosphate, uric acid, and urea excretion were grossly increased in calculi-induced animals. Both Cystone and flavonoid rich fraction of *Citrus medica* Linn. treatment significantly (P<0.05) lowered the elevated levels of these stone forming promoters in urine as compared to calculi-induced model control animals. This can be attributed to the diuretic action, decrease in promoters and increase in inhibitors level and antioxidant potential of the extract (Chavada *et al*., 2012).

The action of methanol extract of *Launea procumbens* restored the increased uric acid levels in the lithiatic rats to normal range (Makasan *et al*., 2014). The results obtained by Kumar *et al.* (2014) were similar to our observations, in that there was an increased oxalate, calcium, phosphate, creatinine and uric acid excretion in the litholitic rats. However, supplementation with ethanolic extract of *Coccinia indica* significantly prevented these changes in urinary oxalate, calcium, phosphate, creatinine and uric acid excretion dose-dependently.

The literature correlates with the results of the present study. The aqueous extract of *Aerva lanata* restored the ethylene glycol-mediated elevated levels of uric acid to its normal range. This points to the antilithiatic activity of the extract.

### 4.3.1.6. Effect of *Aerva lanata* extract on urinary creatinine level

Creatinine is a breakdown product of creatinine phosphate in muscle. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). If the filtering of the kidney is deficient, there will be a rise in blood creatinine level. Therefore creatinine levels in blood and urine may be used to calculate the creatinine clearance, which reflects the glomerular filtration rate (Khatib *et al*., 2010; Hariprasath *et al*., 2013).

The comparison of the results of the lithiatic group and the extract treated group (preventive, curative and standard drug) showed a significant restoration of the levels of creatinine elevated in the lithogen treated groups (Table 8).
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

Results and Discussion

Table 8

Levels of urinary creatinine in experimental animals

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Urine creatinine (mg/dL in 24h)</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>7.8±0.04</td>
<td>7.8±0.09c</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>7.8±0.22</td>
<td>10.3±0.70b</td>
</tr>
<tr>
<td>Extract Control</td>
<td>7.7±0.06</td>
<td>7.8±0.08c</td>
</tr>
<tr>
<td>Preventive</td>
<td>7.8±0.06</td>
<td>8.5±0.08c</td>
</tr>
<tr>
<td>Curative</td>
<td>7.9±0.11</td>
<td>11.17±0.90a</td>
</tr>
<tr>
<td>Cystone</td>
<td>7.9±0.17</td>
<td>11.2±0.13ab</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>NS</td>
<td>1.374</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates

Groups with common superscripts do not differ significantly

Our result coincides with that of Sathya and Kokilavani (2012a) who showed that ethanolic extract of *Saccharum spontaneum* Linn. prevented the impairment of renal function noticed by a decrease in the levels of nitrogenous substances in urolithiatic rats. Our results are in agreement with those of Paula et al. (2009) who reported that the extract of *Copaifera langsdorfi* leaves induced significant reduction in urine creatinine in urolithiasis induced rats. Ethylene glycol treatment raised the urinary calcium, phosphate, oxalate and protein levels significantly in the lithiatic group which was restored to normal levels on treatment with aqueous extract of *Adonis aestivalis* Linn. (Parameshwar et al., 2011).

Yitzhak et al. (2014) reported that the therapeutic effect of ethanol extract of *Hamelia patens* significantly reduced the levels of creatinine excreted in the urine of rats which was elevated due to the metabolic action of ethylene glycol.
The results obtained by Takawale et al. (2012) are similar to our observation. Their research showed that the addition of sodium oxalate lead to increased excretion of creatinine in the urine which was reversed in the presence of *Lagenaria siceraria* fruit powder.

The results of the present study showed that the aqueous extract reduced the level of creatinine excreted in the urine indicating the protective effect of the *Aerva lanata* by protecting the cells and minimizing the effect of tubular dysfunction.

### 4.3.1.7. Effect of *Aerva lanata* extract on urinary magnesium and citrate levels

Magnesium and citrate are inhibitors of crystallization. The results of the analysis showed a marked decrease in the concentration of magnesium and citrate in the presence of lithogenic agents (Table 9).

**Table 9**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Magnesium (mg/dL)</th>
<th>Citrate (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.12±0.02\text{a}</td>
<td>52.45±0.01\text{a}</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>0.93±0.12\text{d}</td>
<td>46.10±0.1\text{d}</td>
</tr>
<tr>
<td>Extract Control</td>
<td>3.16±0.06\text{a}</td>
<td>51.79±0.25\text{b}</td>
</tr>
<tr>
<td>Preventive</td>
<td>2.76±0.19\text{b}</td>
<td>49.68±0.24\text{c}</td>
</tr>
<tr>
<td>Curative</td>
<td>2.12±0.15\text{c}</td>
<td>48.38±0.21\text{c}</td>
</tr>
<tr>
<td>Cystone</td>
<td>2.17±0.11\text{c}</td>
<td>48.31±0.32\text{c}</td>
</tr>
<tr>
<td>Tukey’s HSD 5%</td>
<td>0.217</td>
<td>0.357</td>
</tr>
</tbody>
</table>

*The values are mean ± S.D. of triplicates*

*Groups with common superscripts do not differ significantly*
A decrease in the concentration of magnesium and citrate must be associated with the complex salt formation. Excess oxalate and calcium present in the supersaturated urine may lead to complex formation with magnesium and citrate respectively, thereby, nullifying the effect of the inhibitors (Ghaeni et al., 2014).

The aqueous extract treatment helped to restore the magnesium and citrate levels to the normal range. This might be an indication of recovery pointing out the antilithiatic activity of the Aerva lanata extract, where the extracts enhanced the level of the inhibitors and prevented nucleation and aggregation of the crystal promoters.

Our findings are in accordance with those of Sreelakshmi et al. (2014) where, decreased levels of magnesium were observed in lithiasis induced rats. Pretreatment with ethanol extract of Trianthema portulacastrum Linn. and ethanol extract of Gymnema sylvestre, restored the magnesium levels in a dose-dependent manner and thus reduced the growth of calcium oxalate crystals. Among the two extracts, Gymnema sylvestre treated animals showed significant increase in magnesium levels when compared to lithiatic control.

Parmar et al. (2012) studied that after the 28th day there was a reduction in the concentration of citrate and magnesium. The treatment with the standard and Swertia chirata stems restored the levels of the inhibitors to normal range. Jafar et al. (2011) reported that the treatment with aqueous extract of Petroselium sativum effectively restored the levels of magnesium in urine of the lithiasis induced rats.

Treatment with hydroalcoholic extract (400mg/kg) of Nardostachys jatamansi DC significantly lowered the decreased levels of magnesium in the ethylene glycol treated rats (Vidhya et al., 2013). Pinus eldarica Medw. fruits aqueous extract showed a beneficial effect on calcium oxalate deposition in the rat kidney. It restored the reduced levels of citrate to normal range (Hossein et al., 2010).

Treatment with ethylene glycol significantly lowered the levels of magnesium and citrate in the lithiatic group. Subsequent treatment with the standard drug Cystone and extract of Achyranthes aspera linn. enhanced the
excretion of magnesium and citrate in the urine indicating that the plant can successfully be used as antilithiatic agent (Buela et al., 2013).

Treatment with aqueous extract of *Crocus sativus*. resulted in restitution of the magnesium and citrate which was lowered by the action of ethylene glycol (Amin et al., 2015). The results of the current study showed that the *Aerva lanata* extract effectively increased the stone inhibitory factors in the urine, which may result in the prevention of stone formation.

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

4.3.2.1. Effect of *Aerva lanata* extract on serum calcium, oxalate and phosphate levels

The results of the serum analysis of calcium, oxalate and phosphate revealed that the litholitic effect of ethylene glycol on chronic administration resulted in the supersaturation of the serum with these crystal promoting factors (Table 10).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Calcium (mg/dL)</th>
<th>Oxalate (mg/dL)</th>
<th>Phosphate (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.68±0.15f</td>
<td>7.20±0.02e</td>
<td>0.54±0.02f</td>
<td>5.51±0.15d</td>
<td>1.48±0.06e</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>13.01±0.04a</td>
<td>14.91±0.08a</td>
<td>1.77±0.02a</td>
<td>9.89±0.04a</td>
<td>3.69±0.05a</td>
</tr>
<tr>
<td>Extract Control</td>
<td>7.86±0.07e</td>
<td>7.75±0.17d</td>
<td>0.58±0.03e</td>
<td>5.66±0.06d</td>
<td>1.52±0.03e</td>
</tr>
<tr>
<td>Preventive</td>
<td>9.09±0.10c</td>
<td>9.06±0.09c</td>
<td>0.71±0.02c</td>
<td>6.49±0.12c</td>
<td>2.05±0.06c</td>
</tr>
<tr>
<td>Curative</td>
<td>10.12±0.10b</td>
<td>10.09±0.16b</td>
<td>0.85±0.03b</td>
<td>7.72±0.19b</td>
<td>2.44±0.07b</td>
</tr>
<tr>
<td>Cystone</td>
<td>8.11±0.08d</td>
<td>9.10±0.08c</td>
<td>0.67±0.02d</td>
<td>6.36±0.05c</td>
<td>1.67±0.02d</td>
</tr>
</tbody>
</table>

Tukey’s HSD at 5%

|                | 0.191          | 0.205          | 0.046           | 0.248           | 0.111             |

The values are mean ± S.D. of triplicates
Groups with common superscripts do not differ significantly
Calcium, oxalate and phosphate deposition causes crystalluria, which, in turn, causes the obstruction of the renal system, leading to reduced glomerular filtration rate. The administration of the extract of *Aerva lanata* attenuates the action of ethylene glycol by reducing the concentration of crystal promoting factors. This effect may be due to factors present in the extract that form soluble complexes with promoters, leading to reduced precipitation.

Our observations are supported by the results of Goyal *et al.* (2014). They reported that a dose-dependent antilithiatic activity was exerted by the ethanolic extracts of *Tamarindus indica* against calcium, oxalate and phosphorus in the serum. It has been reported that an alcoholic extract of carum seeds (*Carum copticum*) significantly lowered the elevated serum levels of calcium, uric acid, oxalate, creatinin and phosphate in the lithiasis induced rats (Reddy *et al*., 2012).

Notable elevation in serum calcium, oxalates, phosphates and creatinine levels were observed in lithiatic control as compared to untreated control rats. Ethanolic extract of *Coccinia indica* exhibited a dose-dependent significant antilithiatic activity (Kumar *et al*., 2014). *Celosia argentea* effectively reduced the serum calcium, oxalate and phosphate, the extent of which was on par with the standard drug Cystone (Joshi *et al*., 2012).

### 4.3.2.2. Effect of *Aerva lanata* extract on serum uric acid and creatinine levels

A marked elevation in the serum levels of creatinine and uric acid was observed in the lithiatic groups (Table 10). On supplementing the aqueous extract, a noticeable drop in the levels of uric acid and creatinine were observed. Moreover, the curative regimen showed results close to that of the standard drug. The results indicate that the extract has certain factors that help to repair the damaged tissue due to crystalluria caused by reduction in excretion of crystal promoters.

The aqueous and alcoholic extracts of *Boerhaavia diffusa* roots showed a marked reduction in the concentration of the creatinine and uric acid elevated levels observed after treatment with ethylene glycol. The result points to the antilithiatic potential of the plant extract (Balaji *et al*., 2015). The treatment of lithiasis-induced rats with *Veronia cinerea* restored all the elevated biochemical parameters like creatinine, uric acid and blood urea nitrogen (Thamizhmozhi *et al*., 2015).
The ethanol extract of *Solanum virginianum* showed a therapeutic action against ethylene glycol treated group of rats by decreasing the elevated levels of creatinine and uric acid in the serum of the preventive and curative regimen (Chinnala *et al*., 2013). Ethyl acetate root extract of *Ichnocarpus frutescens* treatment significantly lowered the elevated levels of creatinine and uric acid as compared to the standard drug (Cystone treated) and calculi induced group of rats (Anbu *et al*., 2011).

Karadi *et al*. (2008) reported that the root bark of *Moringa oleifera* normalized the serum levels of urea, uric acid and creatinine in experimental rats. The results of the serum analysis supported the urine analysis results, which showed that *Aerva lanata* extract has good antilithiatic activity.

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

#### 4.3.2.1. Effect of *Aerva lanata* extract on the body weight

Considerable reduction in the body weight was observed in the calculi induced rats (Table 11). This condition may be due anorexia leading to the disturbances in the metabolism of the body and the absorption of nutrients (Tajammul *et al*., 2015).

#### Table 11

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Body weight</th>
<th>Kidney weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial – 0th Day (g)</td>
<td>Final – 28th Day (g)</td>
</tr>
<tr>
<td>Control</td>
<td>260.2±0.92&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>272.8±3.87&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>256.5±1.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>263.4±2.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract Control</td>
<td>261.7±2.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>274.5±2.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Preventive</td>
<td>261.7±2.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>275.1±3.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curative</td>
<td>263±2.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>275.9±1.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cystone</td>
<td>257.8±2.41&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>268.6±4.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>3.50</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*The values are mean ± S.D. of triplicates

Groups with common superscripts do not differ significantly*
It was observed that the body weight was restored to normal levels in the curative and preventive groups. This indicated the diuretic effect of the extract that helps to excrete the excess amount of crystal forming factors, which ultimately prevents aggregation and deposition of stones.

The methanolic extract of *Swertia chirata* and standard drug (Cystone) treatment resulted in an increase in the body weight of the litholitic rats. This shows that the plant extract has good diuretic effect leading to prevention of crystal formation and weight loss (Parmar et al., 2012). Similar results were observed in hydroalcoholic extract of *Bergenia ciliata* (Saha and Verma, 2011).

Treatment with lemon juice resulted in increasing the body weight of the ethylene glycol treated rats indicating the therapeutic action of the extract (Tauhami et al., 2007). The crude extracts of *Punica granatum* L. and *Syzygium cumini* L. effectively improved the health of ethylene glycol treated rats which was observed as increase in body weight (Tajammul et al., 2015). Our results are in accordance with those of Pareta et al., (2011b) who reported that the gross weight of the lithogen treated animals reduced in comparison to the control groups. This was reversed due to the diuretic effect of aqueous extract of *Boerhaavia diffusa*.

The results of the present study showed that the *Aerva lanata* extract has prophylactic action which plays a key role in the maintenance and improvement of health by increasing the body weights.

### 4.3.2.2. Effect of *Aerva lanata* extract on the kidney weight

The results of the kidney weight analysis are presented in Table 11. The ethylene glycol treated rats showed an increase in the kidney weight. However, addition of the aqueous extract of *Aerva lanata* showed a slight reduction in the weight of the kidneys. This may possibly be due to the flushing out of the crystals formed, by the diuretic effect of the weed extract.

Our results are supported by Tsai et al. (2008), who reported that the rats in the lithiatic group showed an elevation in the weight of the kidneys, which might be due to the precipitation of calcium, phosphorus and oxalate due to the supersaturation of the urine.
A dose-dependent reduction in the kidney weight was observed on the addition of ethanol extract of *Tridax procumbens*. This was substantiated by increased excretion of calcium in the urine (Sailaja *et al.*, 2011). Rad *et al.* (2011) reported that the antilithiatic activity of *Cynodon dactylon* reduced the kidney weight in ethylene glycol treated rats.

Marked renal damage was seen in ethylene glycol induced rats indicated by decreased GFR and significant kidney weight gain. Treatment with ethanolic extracts of *Solanum virginianum* plant extracts in both curative and preventive regimens caused diuresis along with loss of kidney weight, which may be due to the excretion of calcium and oxalate in the urine (Chinnala *et al.*, 2013). The findings of Mert *et al.* (2014) are in agreement with our study, who showed that the various extracts of *Viburnum opulus* were able to exert diuretic effect against lithiatic rats, thereby reducing the precipitation of calcium and oxalate in the kidneys.

There was a significant increase in the kidney weight of animals receiving 3% glycolic acid which was almost normalized in the oleanolic acid treated animals in a dose-dependent manner. Ethanol extract of *Lantana camara* treated animals also showed preventive effect against increase in kidney weight (Vyas and Argal, 2013). These reports give credence to the diuretic property of the *Aerva lanata* extract.

4.3.2.3. Effect of *Aerva lanata* extract on the levels of calcium and oxalate in kidney tissue

The results of the calcium and oxalate levels in kidney homogenate assay are tabulated in Table 12. The levels of calcium and oxalate were elevated in the kidney homogenate of the lithiatic rats. The protective effect of the *Aerva lanata* extract in the preventive and the curative groups resulted in reduced concentration of calcium and oxalate in the kidneys. This is possibly a result of increased diuresis, preventing the precipitation of the calcium and oxalate.
Results and Discussion

Table 12

Levels of calcium and oxalate in kidney tissue of experimental animals

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Calcium (mg/g tissue)</th>
<th>Oxalate (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.21±0.01</td>
<td>1.40±0.01</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>6.50±0.21</td>
<td>6.20±0.42</td>
</tr>
<tr>
<td>Extract Control</td>
<td>3.42±0.09</td>
<td>1.58±2.63</td>
</tr>
<tr>
<td>Preventive</td>
<td>4.54±0.16</td>
<td>3.07±0.08</td>
</tr>
<tr>
<td>Curative</td>
<td>5.50±0.40</td>
<td>3.99±0.05</td>
</tr>
<tr>
<td>Cystone</td>
<td>3.29±0.30</td>
<td>2.15±0.04</td>
</tr>
<tr>
<td>Tukey HSD at 5%</td>
<td>0.191</td>
<td>0.205</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates

Groups with common superscripts do not differ significantly

Maya and Pramod (2014) reported that the ethanolic leaf extracts of *Morus alba* L. significantly decreased the concentration of calcium and oxalate in the kidneys. The increased deposition of stone forming constituents in the kidneys of calculogenic rats were significantly reduced by curative and preventive treatment of ethanolic extract of *Annona reticulate* (Ramachandran et al., 2014). Ethanolic extracts of *Ageratum conyzoides* Linn. effectively reduced the excretion of calcium and oxalate in ethylene glycol treated rats (Muthukrishnan, 2014).

Jha et al. (2011) reported a significant reduction in the calcium and oxalate concentration in the kidney tissue due to the antilithiatic activity of *Musa paradisiaca* Linn. A significant decrease in the concentration of calcium in kidneys were observed on addition of NONI (*Morinda citrifolia*) a commercial formulation (Verma et al., 2009). Ethylene glycol feeding resulted in increased levels of calcium and oxalate in kidney, which was decreased after the treatment with methanol extract of *Hygrophila spinosa* (Ingale et al., 2012).
Supplementation with aqueous and alcohol extracts of *Jasminum auriculatum* flowers significantly lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney samples in curative regimens and preventive regimens (Bahuguna *et al.*, 2009). This observation was similar to results of the present study.

Thus, the results of the present study showed that the aqueous extract had good diuretic activity that helped in the expulsion of stone forming constituents and prevented precipitation in the kidneys.

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

ALT and AST are enzyme markers that indicate liver and kidney tissue damage. The lithiatic group animals showed maximum damage in the kidneys (Table 13), evidenced by a reduction in the activities of ALT and AST in the liver and kidneys of the lithiatic group, whereas there was an increase in the ALT and AST activities in the serum.

#### Table 13

**Activities of marker enzymes in liver, kidney and serum**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Liver</th>
<th>Kidney</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST</td>
<td>ALT</td>
<td>AST</td>
</tr>
<tr>
<td>Control</td>
<td>18.9±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.5±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lithiatic control</td>
<td>12.9±0.61&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.0±0.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.2±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract control</td>
<td>18.4±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.8±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Preventive</td>
<td>16.2±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.8±0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Curative</td>
<td>14.2±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.8±1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.4±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cystone</td>
<td>17.0±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.6±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.4±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tukey’s HSD at 5%</td>
<td>0.991</td>
<td>1.125</td>
<td>0.223</td>
</tr>
</tbody>
</table>

The values are mean ± S.D. of triplicates

Groups with common superscripts do not differ significantly
Results and Discussion

The preventive and curative action of the extract significantly ameliorated the enzyme activities to normal range, this might be due to the cytoprotective action of the organic phytoconstituents in the *Aerva lanata* extract.

Treatment with *Cassia auriculata* leaf extract had a lipid-lowering effect in rats with experimentally induced liver damage. The observation pointed to the damage to the structural integrity caused by the calculi formation in the kidney and liver tissue. This may lead to the seepage of the enzymes located in the cytoplasm in to circulation, which in turn causes reduction of these enzymes in the kidney and liver with an increase in the serum (Senthilkumar *et al*., 2003).

The ethanolic fruit extracts of *Pedalium murex* Linn. showed significant improvement in the ALT, AST levels in the serum and tissue homogenate in the ethylene glycol induced renal damage (Mandavi *et al*., 2012). Significant increase in the biochemical parameters such as AST and ALT activities in the kidney homogenate indicated the induction of urolithiasis. Daily oral treatment with a hydroalcoholic extract of *Didymocarpus pedicellata*, ethyl acetate extract of *Taraxacum officinale*, methanolic extract of *Dendrophthoe elastic* and hydroalcoholic extract of *Citrus medica* Linn. reverted all the biochemical changes induced by calcium oxalate urolithiasis (Baheti and Kadam, 2013).

Shamina and Jishamol (2014) showed that the action of ethylene glycol decreased the activities of ALT and AST in the kidney due to calculi formation. The action of *Scoparia dulcis* aqueous extract helps in regaining the near normal values of these enzymes.

The therapeutic activity of the *Aclapha indica* extract restored ALT and AST enzyme activities to their normal range in the serum of the experimental animals (Sathya *et al*., 2011). The results demonstrated that the *Aerva lanata* extract prevented the cellular damage induced by crystal formation by preventing CaOx precipitation and increased diuresis.
4.3.5. Histopathological examination of the kidney tissues

The histopathological studies showed the deposition of crystals in the renal tubules (Table 14; Plate 8). In the ethylene glycol treated rat tissues, the histopathological architecture showed irregular calcium oxalate crystals in the lamina of the tubules, which caused dilation of proximal tubules along with interstitial inflammation. This damage induced as a result of crystal formation may result in necrosis. The addition of the aqueous extract of *Aerva lanata* conferred a protective effect on the tissue, causing the dissolution of the crystals formed. As a result, limited necrosis and tubular dilation were observed.

**Table 14**

**Histopathological architecture of the kidney tissue of male Wistar rats**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Histological features of kidney sections of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tubular congestion</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>Lithiatic Control</td>
<td>+ + + +</td>
</tr>
<tr>
<td>Extract Control</td>
<td>-</td>
</tr>
<tr>
<td>Preventive</td>
<td>+ +</td>
</tr>
<tr>
<td>Curative</td>
<td>+ +</td>
</tr>
<tr>
<td>Cystone</td>
<td>+ +</td>
</tr>
</tbody>
</table>

*Levels of abnormality:*

+ + + + Severe; + + + moderate; + + less; + very less; -- absence

The histopathological studies by Gandhi *et al.* (2013) showed ethylene glycol induced crystal deposition in the renal cells and most of the crystal deposition took place in the renal tubules. Administration of coconut water to urolithiatic animals prevented supersaturation of calcium oxalate and, thus, decreased their deposition in renal tubule.
Plate 8

Histopathology of kidney tissue sections of experimental animals

(100X magnification)

Control

Extract Control

Curative

Lithiatic Control

Preventive

Cystone

Arrows indicate deposition of crystals in the cells
Thangarathinam et al. (2013) reported that the treatment with polyherbal formulation with claims of diuretic effect reduced the damage caused by the crystal precipitation observed in the renal tubules. The histopathological study by Elkhamisy (2015) revealed that the ethanol extracts of *Petroselinum sativum* and *Curcuma longa* rendered therapeutic effect against crystal induced damage. It also revealed that the pre-treatment of the cells showed normal histological architecture of the renal glomeruli. Histopathological observations by Dharmalingam et al. (2014) reported the presence of glomerular atrophy and deposition of crystals in lithogen treated rats and attainment of normalcy of tubular epithelial cells and glomeruli when treated with an aqueous extract of *Melia azedarach* Linn leaves.

It is evident from the results of the histopathological studies that the aqueous extract of *Aerva lanata* contained constituents that inhibited crystal growth and aggregation. Administration of the aqueous extract of *Aerva lanata* may be a possible therapeutic strategy for the prevention of recurrent stone disease.

The results of the *in vivo* assays showed that the aqueous extract of *Aerva lanata* was able to prevent the supersaturation of crystal promoting factors in the urine, serum and the kidneys, thereby preventing crystal formation by increased urine output. Thus, the *in vivo* analyses established evidences for the use of *Aerva lanata* as a prophylatic agent. The antiurolithiatic effects may be mediated possibly through a combination of calcium oxalate crystal inhibition, diuretic and renal epithelial cell protective effects.

4.4. Effect of *Aerva lanata* extract on lithiasis induced cultured kidney cells

Cell lines have been widely used for research purposes and proved to be an excellent model for the study of biological mechanisms involved in various diseases (Louzada et al., 2012). The results of the research in cell lines are extrapolated to *in vivo* studies (van Staveren et al., 2009). Its importance as a model for drug testing and translation study have been recognized by many biomedical and pharmaceutical companies (Gazdar et al., 2010). Drug candidate and toxicity screening processes currently rely on results from early stage *in vitro* cell-based assays, which are expected to faithfully represent essential aspects of
Results and Discussion

In vivo pharmacology and toxicology (Ashtashkina et al., 2012). Therefore, it became imperative to study the potency of the aqueous extract of *Aerva lanata* flowers against oxalate induced injury using NRK 52E cell lines

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

The extent of cytoprotection exerted by the aqueous extract of *Aerva lanata* against oxalate-induced injury was studied by MTT and SRB assays. The results are represented in Figures 13 and 14 respectively. The study revealed that oxalate induced a significant injury to the cells, which was observed by a steep decrease in the viability when compared to the control. The viability of the cells increased on the administration of the aqueous extract of *Aerva lanata*. A similar trend was obtained for SRB assay, which gave proof to the cytoprotective effect of the *Aerva lanata* extract.

Several literatures have validated MTT and SRB assays as relevant tools for quantifying the extent of cell survival. MTT assay revealed that 1% Cystone, an ayurvedic formulation, induced effective cytoprotective action against COM crystal induced injury in NRK 52E cell lines (Vidyashankar et al., 2010).

Figure 13

Viability of NRK 52E cells on oxalate induced cell injury

The values are mean ± SD of triplicates

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
Results and Discussion

In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

4.4.1. Viability of NRK 52E cells on oxalate induced cell injury

The microscopic evaluation (Plates 9a-9d) showed that the cells treated with the COM crystals alone showed morphological changes. This might be due to the damage caused by the aggregation and adherence of the crystals on to the cell surface. The administration of the extract along with oxalate showed reduced damage compared to the oxalate alone treated group. It was also observed that the extract alone treated groups showed morphology similar to that of the control. This indicates that the plant extract, by itself, does not exert toxic action against the cells, indicating the protective effect of the *Aerva lanata* extract.

4.4.2. Morphological changes of NRK 52E cells

A dose-dependent cytoprotective effective was exerted by the aqueous extract of *Herniaria hirsute* against COM crystal adhesion in the renal culture cells (Atmani et al., 2004). Our results correlate with the literature that confirms the cytoprotective role of *Aerva lanata*.
Our results are in line with those of Khan et al. (2012) who reported that the pretreatment with the aqueous-ethanol extract of *Holarrhena antidysenterica* seeds significantly increased the survival rate and reduced the LDH release, a marker of cell membrane damage, in MDCK cells, when exposed to oxalate and COM crystals. This protective effect on the renal epithelial cells may be due its antioxidant activity.

Calcium oxalate crystals cause acute inflammation-mediated necrotic cell death in renal proximal tubular cells, but not in collecting tubule cells. The crystal-induced generation of reactive oxygen species by renal tubular cells is considered to be a general response to tissue damage (Schepers et al., 2005).
NRK52E cells express MCP-1 (Monocyte Chemotactic Protein). Expression and production are increased when these cells are exposed to high levels of oxalate and CaOx crystals, indicating possibility of this chemokine playing a significant role in the oxalate-induced inflammatory response of the kidneys. Stimulation of MCP-1 production is apparently accomplished by the generation of free radicals which were significantly reduced following treatments with catalase (Umekawa et al., 2002). The above studies showed that the plant has a good cytoprotective activity.

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

Lactate dehydrogenase is a stable cytosolic enzyme that is released when the cell is lysed or there is any injury on the cell membrane.

A significant increase in LDH release was seen when the NRK 52E cells were exposed to oxalate alone due to damage to the cell structure. When NRK 52E cells were treated with the weed extract, a decrease in per cent of LDH release was observed (Figure 15). This was due to the protective effect rendered by the extract during cell injury caused by the crystals formed.

**Figure 15**

*Cytotoxicity of NRK 52E cells as determined by lactate dehydrogenase assay*

*The values are mean ± S.D. of triplicates*
In a study with NRK 52E, *Tribulus terrestris* proved to have a protective effect towards the renal epithelial cells in a concentration-dependent manner (Aggarwal *et al.*, 2010a). Our results are in accordance with those of Tayal *et al.* (2012) who reported that there was a reduction in the release of LDH enzyme in the injured cells on treatment with *Terminalia chebula*. Vanachayangkul *et al.*, (2010) reported that the aqueous extract of *Ammi visnaga* fruits effectively prevented cell damage caused by oxalate which was supported by the reduction in LDH release on treatment with the extracts.

Thus, it can be concluded that the presence of phytocompounds in the *Aerva lanata* extract might help in dissolving the COM crystals as well as preventing the crystals from adhering to the cells, thereby reducing damage to the cells.

**PHASE III**

**4.5. Antioxidant levels in *Aerva lanata* flowers**

Based on the results obtained in Phase I and Phase II, it is evident that the aqueous extract of *Aerva lanata* was effective in prevention and treatment of kidney stones.

Free radicals are chemical species that contain one or more unpaired electrons, due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Free radicals can initiate the oxidation of biomolecules, such as proteins, lipids, amino acids and DNA which will lead to cell injury and can induce numerous diseases. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage; oxidative stress is the main cause of several diseases (Soni and Sosa, 2013).

Natural antioxidants, which are commonly present in medicinal plants, scavenge radicals and prevent oxidative damage in tissues and cells. The oxidant and antioxidant imbalance may be one of the causative reasons of stone deposition (Mayee and Thosar, 2011). The toxic effect of reactive oxygen and nitrogen species
in humans is balanced by the action of non-enzymic antioxidants, as well as by antioxidant enzymes. Such antioxidant defences are extremely important as they represent the direct removal of free radicals (prooxidants), thus providing maximal protection for biological sites (Rahman et al., 2012).

4.5.1. Enzymic antioxidant activities in Aerva lanata flowers

The enzymic antioxidants analyzed in the Aerva lanata flowers were superoxide dismutase, catalase, peroxidase, glutathione reductase, glutathione S-transferase and polyphenol oxidases. The activities obtained are presented in Table 15.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (U^1/g)</td>
<td>40.57±0.26</td>
</tr>
<tr>
<td>Catalase (U^2/g)</td>
<td>121.58±3.91</td>
</tr>
<tr>
<td>Peroxidase (U^3/g)</td>
<td>19.58±1.45</td>
</tr>
<tr>
<td>Glutathione S-transferase (U^4/g)</td>
<td>0.21±0.05</td>
</tr>
<tr>
<td>Catechol oxidase (Units^5 X 10^-3 / g)</td>
<td>0.47±0.04</td>
</tr>
<tr>
<td>Laccase (Units^5 X 10^-3 / g)</td>
<td>0.48±0.06</td>
</tr>
</tbody>
</table>

*The values are mean ± S.D. of triplicates*

1 Unit = Amount of enzyme that causes 50% reduction in NBT oxidation
2 Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units
3 Unit = Change in absorbance at 430 nm/minute
4 Unit = nmol of CDNB conjugated/minute
5 Unit = Amount of catechol oxidase/laccase enzyme which transforms 1 unit of dihydrophenol to quinine /minute
The results show that the *Aerva lanata* flowers possess considerable activities of all the enzymes analyzed. It is evident that the aqueous flower extracts are a good source of enzymic antioxidants. The levels of enzymic antioxidants indicated the role of the aqueous plant extract as an effective natural antioxidative agent thereby helping in attenuation of the oxidative damage caused by hyperoxaluria.

Significant activities of superoxide dismutase, peroxidase, polyphenol oxidase and catalase were reported in *Coleus forskohlii* by Khatun *et al.* (2011). Ashok *et al.* (2010) reported that the triterpenes, polyphenols and lupeol compounds present in the extract of *Mimusops elengi* may be responsible for the the protective effect exerted against oxidative stress in ethylene glycol induced rats.

The study of enzymic antioxidants in leaves of *Calotropis procera*, *Datura stramonium*, *Argemone mexicana*, *Withania somnifera* and *Solanum nigrum* indicated that among these medicinal plant leaves; the activities of enzymic antioxidants viz. catalase and glutathione reductase activities were highest in the leaves of *Withania somnifera* (Bind *et al.*, 2014).

The findings of Dhal *et al.* (2012) indicated that the non-enzymic antioxidants proved to be a better scavenger of free radical in comparison to enzymic antioxidants in *Curcuma zedoaria*, *Curcuma angustifolia* and *Curcuma caesia*. 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.

The results of the assays showed that the *Aerva lanata* flowers are a good source of antioxidants which could be responsible for quenching oxalate promoted free radicals for the induction of diuretic effect, thereby preventing deposition of crystal promoting factors.
4.5.2. Levels of non-enzymic antioxidants in *Aerva lanata* flowers

The levels of non-enzymic antioxidants, namely ascorbic acid, tocopherol, total carotenoids, lycopene, total phenols, reduced glutathione and flavonoids analyzed in the *Aerva lanata* flowers, are presented in Table 16.

**Table 16**

**Non-enzymic antioxidant levels in *Aerva lanata* flowers**

<table>
<thead>
<tr>
<th>Non-enzymes</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid (mg/g)</td>
<td>1.64±0.27</td>
</tr>
<tr>
<td>Tocopherol (μg/g)</td>
<td>3.49±0.47</td>
</tr>
<tr>
<td>Total carotenoids (mg/g)</td>
<td>23.2±5.27</td>
</tr>
<tr>
<td>Lycopene (mg/g)</td>
<td>6.54±0.25</td>
</tr>
<tr>
<td>Reduced glutathione (nmoles/g)</td>
<td>6.48±0.74</td>
</tr>
<tr>
<td>Total flavonoids (mg/g)</td>
<td>22.87±0.31</td>
</tr>
<tr>
<td>Total phenols (mg/g)</td>
<td>13.73±0.44</td>
</tr>
</tbody>
</table>

_The values are mean ± S.D. of triplicates_

The results revealed that the flowers of *Aerva lanata* exhibited appreciable amounts of all the non-enzymic antioxidants analyzed. This can be attributed to the redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

The levels of enzymic and non enzymic antioxidants analyzed in the leaves of *Clitora ternatea*, *Solanum nigrum* and *Aloe vera* showed that *Solanum nigrum* showed higher levels of enzymic and non enzymic antioxidants (Jayachithra and Krithiga, 2012). Mrudula et al. (2014) studied that the leaves of *Moringa oleifera* and *Centella asiatica* are good sources of non enzymic antioxidants. The radical scavenging activity of different extracts may be the contribution of the phytonutrients rendering antioxidant property.
The study of non-enzymic antioxidants in the leaves of *Calotropis procera*, *Datura stramonium*, *Argemone mexicana*, *Withania somnifera* and *Solanum nigrum* showed that the maximum levels of non enzymic antioxidant levels was recorded in *Argemone mexicana* (Bind *et al.*, 2014).

Dhal *et al.* (2012) showed that enzyme extracts of *Curcuma zedoaria* are a potential source of enzymic antioxidants that can be a good therapeutic agent in preventing or slowing down oxidative stress related diseases. The total antioxidant, free radical scavenging and reducing powers of *Amaranthus* species, *Centella asiatica*, *Murraya koenigii* and *Trigonella foenum graecum* were attributed to the presence of ascorbic acid, total carotenes, β-carotene and total polyphenol (Gupta and Prakash, 2009).

Thus, from the analysis, it is clear that the flowers of *Aerva lanata* are a rich source of enzymic and non-enzymic antioxidants. Oxidative stress is functional in urolithiasis as a result of decreased antioxidant status. This restricted antioxidative function may lead to damage to renal tubular cells causing increased crystal adherence and aggregation (Vasavidevi *et al.*, 2006). Hence the aqueous extract of *Aerva lanata* not only helps in restoring the antioxidant status in the system but also may prevent adhesion of crystals to the renal cells and their growth.

### 4.6. Radical scavenging effects of *Aerva lanata* extract

The radical scavenging effects of aqueous extract of *Aerva lanata* was determined *in vitro* against a team of radicals namely DPPH, ABTS, H₂O₂ and hydroxyl radicals.

#### 4.6.1. DPPH radical scavenging activity of *Aerva lanata* extract

The spectrophotometric analysis (Figure 16) revealed that the DPPH radicals were effectively scavenged by the presence of aqueous extract of *Aerva lanata*. 
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

**Results and Discussion**

**Figure 16**

**Radical scavenging effects of *Aerva lanata* extract**

![Graph showing radical scavenging effects](image)

*The values are mean ± S.D. of triplicates*

Khan *et al.* (2012) showed that the aqueous-ethanol extract of *Holarrhena antidysenterica* caused scavenging of DPPH radicals similar to the standard antioxidant BHT, which showed the presence of antioxidative activity in the plant extract. Risdian *et al.* (2011) showed that the DPPH radical scavenging potential of the ethanolic extract and different solvent fractions of the leaves of *Piper betle* varied significantly, which might be due to the presence of different phytoconstituents.

The methanolic extract of the aerial parts of *Sambucus ebulus*, *Lonicera tatarica* and *Viburnum opulus* have a role in the protection against oxidative stress, which was suggested to be due to the presence of total polyphenols and total flavonoid content of aerial parts (Bubulica *et al*., 2012).

The tannins extracted from the leaves, twigs and stem bark of *Canarium album* showed strong DPPH scavenging activity (Zhang and Lin, 2008). The fresh juices of orange and grape fruit showed increased DPPH radical scavenging ability compared to the concentrated form (Belaya *et al*., 2009). The DPPH radical scavenging potential of the methanol and aqueous extracts of *Punica granatum* peel showed that the methanolic extract was the most effective (Middha *et al*., 2013).

*Acokanthera oppositifolia* Lam. and *Adenia gumnifera* Harv. effectively scavenged DPPH radicals (Adedapo *et al*., 2008). Kumar *et al.* (2012a)
Results and Discussion

suggested that the aqueous and methanolic extracts of *Syagrus romanzoffiana* showed DPPH anion scavenging potential.

The result of the current analysis indicated that the *Aerva lanata* extract is a good source of antioxidants and has good radical scavenging ability.

4.6.2. ABTS radical scavenging activity of *Aerva lanata* extract

ABTS is a synthetic radical produced by a reaction. The ABTS radical scavenging test is widely used to determine the antioxidant activity of both hydrophilic and lipophilic compounds (Gao *et al*., 2007). The antioxidant activity of the extract was analyzed using another type of stable free radical, namely ABTS, and the results obtained are also shown in Figure 16. The results showed that the aqueous extract effectively scavenged the free radical ABTS.

Karamac (2010) revealed that the total phenolic contents of the isolated fractions from buckwheat seeds and the tannin fraction of the seeds showed strong scavenging activity against DPPH and ABTS. The methanolic extract of *Caesalpinia digyna* root exhibited strong ABTS scavenging effect (Srinivasan *et al*., 2007).

Gorinstein *et al.* (2008), reported that the methanol/water and methanol/chloroform extracts of cereals and pseudocereals showed a high percentage of ABTS inhibition in a dose-dependent manner. The ABTS scavenging activity of 95% ethanolic extract of *Agrimonia pilosa* was superior to butylated hydroxytoluene (BHT), which was suggested to be due to its high phenol and flavonoid contents (He *et al*., 2009).

Tayade *et al.* (2013) observed that the ABTS cation scavenging capacity of the methanolic and aqueous extracts of the roots of *Rhodiola imbricata* increased in a dose-dependent manner.

The above discussion shows that ABTS radical scavenging property is a very dependable measure of the antioxidant potential of the plant extract.

4.6.3. Hydrogen peroxide scavenging activity of *Aerva lanata* extract

Hydrogen peroxide is a highly reactive ubiquitous by-product of aerobic metabolism and plays an important role in the immune response and various
physiological processes. Hydrogen peroxide, in the presence of released iron, generates the highly reactive hydroxyl radical through Fenton reaction (Kalyanaraman, 2013).

Significant extent of H$_2$O$_2$ radical scavenging was elicited by the *Aerva lanata* extract (Figure 16). The assay revealed that the aqueous extract of *Aerva lanata* had good free radical quenching ability, thereby reducing the risk of oxidative stress.

Nishida *et al.* (2013) reported that the homoisoflavones isolated from the ethyl acetate extract prepared from the bulbs of *Scilla scilloides* Druce exhibited strong H$_2$O$_2$ scavenging activity. Several bioactive compounds isolated from plants have been identified as potent H$_2$O$_2$ scavengers, like glyceollins from soya bean (Kim *et al.*, 2010).

Raja and Pugalendi (2009) showed that the aqueous extract of *Melothria maderaspatana* was capable of scavenging H$_2$O$_2$ in a dose-dependent manner. The *in vitro* antioxidant activity of the ethyl acetate, ethanol, methanol and water extracts of the leaves of *Lagerstroemia speciosa* L. (Lythraceae) were studied by examining their free radical scavenging property for superoxide, hydroxyl ion scavenging, hydrogen peroxide, and by measuring lipid peroxidation (Priya *et al.*, 2008).

The aqueous extract of *Withania somnifera* roots exhibited better antioxidant activity against free radicals, including both the hydrogen peroxide and DPPH radicals (Pawar *et al.*, 2011).

Thus, the results of H$_2$O$_2$ analysis gives further assertion to the antioxidant status of the flowers of *Aerva lanata*.

### 4.6.4. Hydroxyl radical scavenging activity of *Aerva lanata* extract

Hydroxyl radical is the most reactive oxygen radical. It has a very short half-life (~10$^{-9}$ s) and reacts very rapidly with all types of molecules found in living cells (Powers and Malcolm, 2011).

The efficiency of aqueous extracts of *Aerva lanata* flowers to scavenge hydroxyl radicals in an *in vitro* system was performed and the results obtained
are depicted in Figure 17. It was observed that the extract was able to effectively scavenge radicals in comparison to the control.

The aqueous-methanol extract of *Bergenia ligulata* rhizome exhibits good antioxidant activity. It effectively quenched hydroxyl free radicals produced as a result of high level of oxalate (Bashir and Gilani, 2009).

**Figure 17**

Hydroxyl radical scavenging effect of *Aerva lanata* extract

![Graph showing hydroxyl radical scavenging effect](image)

*The values are mean ± S.D. of triplicates*

*The value of H$_2$O$_2$-treated group was fixed as 100 per cent and the relative values in percentage were calculated for the other groups*

Bokhari *et al.* (2013) showed that the aqueous fraction of the methanolic extract of *Galium aparine* L. strongly scavenged the DPPH, ABTS, hydroxyl, hydrogen peroxide and superoxide radicals. The methanolic and ethanolic extracts of *Rumex dentatus* exhibited their radical scavenging effect in a dose-dependent manner on superoxide anion radicals and hydroxyl radicals (Humeera *et al*., 2013).

The methanolic extract of *Cassia grandis* showed better hydroxyl radical scavenging effect compared to chloroform and petroleum ether extract (Meena *et al*., 2009). The ethyl acetate, ethanol, methanol and water extracts of the leaves of *Lagerstroemia speciosa* L. were found to possess high hydroxyl radical scavenging effect (Priya *et al*., 2008). Yin *et al.* (2008) explained that the methanol extract of *Cirsium japonicum* showed strong hydroxyl scavenging activity.
An overproduction of ROS and reduced cellular antioxidant status are due to the down regulation of expression of antioxidants (Adhikrao et al., 2008). The antioxidant analyses showed that the *Aerva lanata* has good antioxidant and radical quenching activity. The presence of natural antioxidants ameliorates the hyperoxaluria induced renal cell injury. The antioxidants may guard against reactive oxygen species (ROS) toxicities by scavenging reactive metabolites and converting them to less reactive molecules. Thus, the control of renal oxidative stress may prove to be effective therapy to prevent renal calculi deposition and increase diuresis.

The results of the present study shed light on the antilithiatic and antilithiatic potential of the aqueous extract of *Aerva lanata*. In order to identify the active compound responsible for the therapeutic action of the *Aerva lanata* flower extract further phytochemical analyses were performed.

### 4.7. Determination of phytochemical constituents of *Aerva lanata* extract

Phytochemicals are chemical compounds that occur naturally in plants and are responsible for the color and organoleptic properties, generally used to refer to chemicals that may have biological significance but are not established as essential nutrients (Ganatra et al., 2012). Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. The ingestion of natural antioxidants have been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing (Biradar and Rachetti, 2013).

The evidences provided by the above results showed that aqueous extract of *Aerva lanata* is a good source of antioxidants, and showed very good antilithiatic activity. These properties are obviously rendered by the chemical substances or the secondary metabolites present in the flowers of *Aerva lanata* that get extracted into aqueous medium. Therefore, it is essential to identify the active compounds that confer the protective effects of *Aerva lanata*. Hence, the
qualitative identification of the chemical nature of the active component present in the candidate plant was performed. This was followed by spectral and chromatographic studies such as UV absorption spectrum, HPTLC, HPLC, IR, GC-MS and NMR to identify the major components present in the crude extract of the sample.

4.7.1. Preliminary qualitative phytochemical analysis

The fresh flowers of *Aerva lanata* were subjected to phytochemical analysis to identify the presence of the major phytochemicals. The qualitative test showed the presence of alkaloids, phenols, flavonoids, steroids, terpenoids, tannins, saponins and phenols (Table 17). The presence of the various phytochemical constituents may directly or indirectly attribute to the biological activity of the extracts.

The study performed by Devi et al. (2012) on leaf extract of *Clerodendrum inerme* showed the presence of alkaloids, flavonoids, polyphenols and steroids which are responsible for rendering the antioxidant activity. Phytochemical analysis of various extracts of *Solanum aequipii* showed the presence of flavonoids, tannins, steroids, carbohydrates, alkaloids, glycosides, saponins, reducing sugars and amino acids. This study suggested that the extract of *Solanum anguivi* Lam. root possess significant urolithiasis activity which might be helpful in preventing or slowing the progress of diseases (Mathew et al., 2014). Ghongade (2013) reported that both the aqueous and ethanolic extracts of *Citrus karna* are good sources of phytochemicals.

The phytochemical analysis performed on crude extracts obtained from the fruit of *Morinda citrifolia* in different solvent extracts indicated the presence of a broad spectrum of secondary metabolites. Alkaloids, saponins and reducing sugars were predominantly found in all the three tested extracts followed by steroid, phenol, tannin and terpenoids (Nagalingam et al., 2012).
Results and Discussion

Table 17
Qualitative phytochemical analysis of *Aerva lanata* flowers

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Components</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Alkaloids</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mayer's test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Flavonoids</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aqueous NaOH test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Conc. Sulphuric acid test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Schinado’s test</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Sterols</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leiberman-Buchard test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Salkowski test</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Terpenoids</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leiberman-Buchard test</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Tannins</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Braemer’s test</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td><strong>Saponins</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Froth test</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td><strong>Phenols</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferrric Chloride test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td><strong>Carbohydrates</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molisch’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ indicates the presence of corresponding metabolites*

The methanol extracts of three medicinal plants namely *Leucas aspera*, *Dillenia indica* and *Enhydra fluctuans* confirmed the presence of biomolecules in the extract (Dutta, 2013). The stem, root and leaf extracts of *Centella asiatica* showed the presence of diverse groups of phytochemicals (Biradar and Rachetti, 2013).
Results and Discussion

Thus, the results of preliminary phytochemical assay is credited by the literatures cited above and revealed that the aqueous extract of *Aerva lanata* is a good carrier of therapeutically significant components like alkaloids, flavonoids, steroids, terpenoids, sterols, saponins, phenols, and carbohydrates which might be responsible for the strong antilithiatic activity. Thus can be used for treating diseases and can be used for medicinal preparations.

4.7.2. UV-Visible absorption spectrum of the phytochemical fractions of *Aerva lanata* extract

From these results, it can be inferred that the active components in the flowers of *Aerva lanata* may be alkaloids, phenols, flavonoids, steroids, terpenoids, tannins, saponins and phenols. Hence, these phytochemical fractions were isolated and subjected to UV absorption which gave specific absorption spectra.

Several major and minor peaks were recorded by the alkaloid fraction in the flowers of *Aerva lanata*, beginning with a sharp peak at 225 nm, followed by another major peak at 200 nm. A few more well-defined peaks were noted at 240, 300, 320, 350 and 550 nm respectively (Figure 18).

**Figure 18**

UV-Visible absorption spectrum of the alkaloid fraction of *Aerva lanata* flowers

The UV-Visible absorption spectrum of the flavonoid fraction indicated well defined major peaks at 230, 250, 290, 320 and 340 nm. At 300 nm also a peak was observed, though not well defined. A few minor peaks were also noticed beginning with 200 nm as indicated in Figure 19.
Results and Discussion

In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

**Figure 19**

UV-Visible absorption spectrum of the flavonoid fraction of *Aerva lanata* flowers

![Flavonoid spectrum](image)

Figure 20 shows several major peaks of steroid fraction at 250, 275, 270 and 320 nm and 560 nm and a minor peak at 320 and 360 nm.

**Figure 20**

UV-Visible absorption spectrum of the steroid fraction of *Aerva lanata* flowers

![Steroid spectrum](image)

The UV-Visible absorption spectrum of the terpenoid fraction indicated well defined major peaks at 250, 290, 300, 320 and 340 nm. At 550 nm also a peak was observed. A few minor peaks were also noticed beginning at 210 and 220 nm as indicated in Figure 21.
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

**Figure 21**

UV-Visible absorption spectrum of the terpenoid fraction of *Aerva lanata* flowers

![UV-Visible absorption spectrum of the terpenoid fraction of *Aerva lanata* flowers](image)

The UV-Visible absorption pattern of tannins (Figure 22) showed well-defined peaks at nm ranging from 200-300. A minor peak was also recorded at 350 nm.

**Figure 22**

UV-Visible absorption spectrum of the tannin fraction of *Aerva lanata* flowers

![UV-Visible absorption spectrum of the tannin fraction of *Aerva lanata* flowers](image)

The UV-Visible absorption spectrum of the saponin fraction was determined and the spectrum is presented in Figure 23. At 200-325 nm saponins also exhibited peaks as in tannins.
Results and Discussion

Figure 23
UV-Visible absorption spectrum of the saponin fraction of
*Aerva lanata* flowers

![Image](image1.png)

Figure 24 shows the UV-Visible absorption spectrum of the phenolic fractions, which revealed major peaks at 200 and 230, 270-320 nm indicating the presence of major active principles present in the leaf extract. A single minor peak was also noted at 570nm.

**Figure 24**
UV-Visible absorption spectrum of the phenol fraction of
*Aerva lanata* flowers

![Image](image2.png)

Absorption spectroscopy is one of the most valuable analytical techniques. Its advantages include simplicity, speed, specificity and sensitivity (Parikh and
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata* (Karkhanis, 2011). The qualitative UV-visible spectral profile of *Stylosanthes fruticosa*, ethanol extract showed the peaks at 390 to 1100 nm and the profile showed the peaks 405, 534, 605 and 661 nm respectively (Sandosh *et al.*, 2013).

The UV-Visible spectral analysis of *Citrus reticulate* showed the peaks at 223.5nm, 258nm, 284nm, 303nm and 326.5nm respectively (Showmya *et al.*, 2014). The qualitative UV-visible spectral profile of *Vitex altissima* L. ethanolic extract was selected at wavelength from 400 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 422 and 664 nm (Sathish *et al.*, 2012).

The absorbance survey scan of the methanolic extract of *Rhinacanthus nasutus* leaves in the wavelength ranging from 190-1100nm revealed the presence of multiple components in the extract as evident by the presence of different peaks in the survey scan (Nirmaladevi *et al.*, 2010). The UV-visible absorption spectra of *Carpobrotus mellei* and *Carpobrotus quadrifidus* overlapped with that of catechin, which confirmed the presence of catechin (Maoela *et al.*, 2009).

Thus the literatures cited reiterates the results of the study. The absorption pattern showed that the *Aerva lanata* is a good source of major active principles responsible for the protective effect of the plant extract.

**4.7.3. HPTLC of *Aerva lanata* extract**

The aqueous extract of *Aerva lanata* was subjected to HPTLC analysis to determine the presence of alkaloids, phenols, flavonoids, saponins, steroids and tannins.

The alkaloid profile of the methanolic extract was done with the reference standard colchicine and the developed plate was sprayed with Dragendorff’s reagent. Coloured zone at day light mode present in the given standard and sample tracks were observed. It showed the presence of 4 different alkaloids visible at 254 and 366 nm. The \( R_f \) value of the standard was 0.41. The chromatogram confirmed the presence of alkaloids (Plate 10). Figure 25 and Table 18 represent the peak densitogram and peak table respectively.
The flavonoid profile of the aqueous extract was analyzed using quercetin as the standard which had an R\textsubscript{f} value of 0.94. Yellow and yellow green fluorescence zone at UV 366 nm was seen from the chromatogram, which confirmed the presence of 3 different flavonoids (Plate 11) shown in the peak densitogram (Figure 26) and Table 19.

The steroid profile of the aqueous extract was analyzed using solasodine as the standard. The presence of steroids was confirmed by the bands observed at 254 and 366 nm (Plate 12). The peak densitogram (Figure 27) and peak table (Table 20) confirmed the presence of steroids. The R\textsubscript{f} value of standard was 0.85.

The presence of terpenoid was observed as blue, yellowish brown coloured zones in the chromatogram after derivatization (Plate 13). This was further confirmed by the peak densitogram (Figure 28) and peak table (Table 21). This confirms the presence of terpenoids in the sample.

Using tannic acid as the standard, the tannin profile of the aqueous extract was analyzed by spraying with 5% ferric chloride. The bands observed confirmed the presence of tannins (Plate 14). The peak table (Table 22) and peak densitogram (Figure 29) supported the results. The R\textsubscript{f} value of the standard was 0.81.

Plate 15 confirmed the presence of saponins in the aqueous extract where saponin standard was used which showed band at R\textsubscript{f} value 0.93. Blue fluorescence and yellowish brown coloured zones in the UV range at 266nm and at 366nm were observed, which confirmed the presence of saponins in the samples. The peak densitogram (Figure 30) and the peak table (Table 23) represented the different saponins.

The phenolics present in the aqueous extract were analyzed using eugenol (R\textsubscript{f} value 0.80) as the reference standard. Zones at 254 and 366nm were present in the track, confirmed the presence of phenols in the aqueous extract (Plate 16). The peak densitogram (Figure 31) and peak table (Table 24) showed the presence of phenols.
Results and Discussion

In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

Plate 10

**HPTLC of alkaloids**

Before Derivatization

After Derivatization

254 nm

366 nm

Day Light

Day Light

Figure 25

HPTLC peak densitogram of alkaloids in *Aerva lanata* extract

Table 18

HPTLC peak Table for the alkaloids in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>$R_f$</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.38</td>
<td>Alkaloid 1</td>
</tr>
<tr>
<td>2</td>
<td>0.29</td>
<td>Alkaloid 2</td>
</tr>
<tr>
<td>STD</td>
<td>0.41</td>
<td>Colchicine standard</td>
</tr>
</tbody>
</table>
Results and Discussion

Plate 11

HPTLC of flavonoids

Before Derivatization

After Derivatization

Figure 26

HPTLC peak densitogram of flavonoids in *Aerva lanata* extract

Table 19

HPTLC peak table for the flavonoids in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.89</td>
<td>Flavonoids 1</td>
</tr>
<tr>
<td>3</td>
<td>0.78</td>
<td>Flavonoids 2</td>
</tr>
<tr>
<td>4</td>
<td>0.90</td>
<td>Flavonoids 3</td>
</tr>
<tr>
<td>STD</td>
<td>0.94</td>
<td>Quercetin standard</td>
</tr>
</tbody>
</table>
Plate 12

HPTLC of steroids

Before Derivatization  After Derivatization

254 nm  366 nm  Day Light  Day Light

Figure 27

HPTLC peak densitogram of steroids in *Aerva lanata* extract

Aerva lanata  Solasodine Standard

Table 20

HPTLC peak table for the steroids in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>R_f</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.80</td>
<td>Steroid 1</td>
</tr>
<tr>
<td>2</td>
<td>0.76</td>
<td>Steroid 2</td>
</tr>
<tr>
<td>3</td>
<td>0.69</td>
<td>Steroid 3</td>
</tr>
<tr>
<td>STD</td>
<td>0.85</td>
<td>Solasodine standard</td>
</tr>
</tbody>
</table>
Plate 13

HPTLC of terpenoids

<table>
<thead>
<tr>
<th>Before Derivatization</th>
<th>After Derivatization</th>
</tr>
</thead>
<tbody>
<tr>
<td>254 nm</td>
<td>Day Light</td>
</tr>
<tr>
<td>366 nm</td>
<td>Day Light</td>
</tr>
</tbody>
</table>

Figure 28

HPTLC peak densitogram of terpenoids in *Aerva lanata* extract

Table 21

HPTLC peak Table for the terpenoids in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>$R_f$</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.57</td>
<td>Terpenoid 1</td>
</tr>
<tr>
<td>2</td>
<td>0.60</td>
<td>Terpenoid 2</td>
</tr>
<tr>
<td>3</td>
<td>0.87</td>
<td>Terpenoid 3</td>
</tr>
<tr>
<td>STD</td>
<td>0.58</td>
<td>Artemisinin standard</td>
</tr>
</tbody>
</table>

In *vitro* and *in vivo* investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

**Plate 14**

**HPTLC of tannins**

**Before Derivatization**

254 nm

**After Derivatization**

366 nm

**Figure 29**

HPTLC peak densitogram of tannins in *Aerva lanata* extract

**Table 22**

HPTLC peak Table for the tannins in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>$R_f$</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.11</td>
<td>Tannin 1</td>
</tr>
<tr>
<td>2</td>
<td>0.76</td>
<td>Tannin 2</td>
</tr>
<tr>
<td>STD</td>
<td>0.81</td>
<td>Tannic acid standard</td>
</tr>
</tbody>
</table>
Plate 15

HPTLC of saponins

Before Derivatization

After Derivatization

Figure 30

HPTLC peak densitogram of saponins in *Aerva lanata* extract

Table 23

HPTLC peak Table for the saponins in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>(R_f)</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.93</td>
<td>Saponin 1</td>
</tr>
<tr>
<td>2</td>
<td>0.85</td>
<td>Saponin 2</td>
</tr>
<tr>
<td>3</td>
<td>0.48</td>
<td>Saponin 3</td>
</tr>
<tr>
<td>STD</td>
<td>0.93</td>
<td>Saponin standard</td>
</tr>
</tbody>
</table>
Results and Discussion

Plate 16

HPTLC of phenols

Before Derivatization

After Derivatization

Day Light

Day Light

254 nm

366 nm

Figure 31

HPTLC peak densitogram of phenols in *Aerva lanata* extract

Table 24

HPTLC peak Table for the phenols in *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>( R_f )</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.31</td>
<td>Phenol 1</td>
</tr>
<tr>
<td>3</td>
<td>0.78</td>
<td>Phenol 2</td>
</tr>
<tr>
<td>STD</td>
<td>0.80</td>
<td>Eugenol standard</td>
</tr>
</tbody>
</table>
HPTLC finger printing of acetone and methanol extract of greater cardamom fruit extracts separated 8 components and 11 components, respectively. It confirmed the presence of protocatechuic acid in both extracts. Acetone extract showed higher concentration of protocatechuic acid as compared to methanol extract (Shivanand and Mahalaxmi, 2010).

The results from HPTLC finger print for methanolic extract of *Wedelia chinensis* leaves showed the presence of alkaloid with R_f values (0.01 to 0.93). R_f value range of 0.97 confirmed the presence of flavonoids in the extracts. Phenol was confirmed with R_f value range 0.01 to 0.97. Tannin was confirmed in the extract by the R_f value range 0.01 to 0.94. These phytoconstituents in the methanolic extract had several visible colour spots on the plate (Banu and Nagarajan, 2014).

The presence of gallic acid in the methanolic extracts of leaf and flowers of *Saraca asoca* was confirmed with the help of HPTLC (Saha et al., 2012). The methanolic extract of stem, root, and seeds of *Aerva lanata* showed the presence of 27 different types of terpenoids. In general, degree of terpenoid diversity was observed in vegetative parts when compared to reproductive parts (Yamunadevi et al., 2011).

A densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile, which was used as a marker for quality evaluation and standardization of the drug of *Cardiospermum halicacabum* L. stem (Patil et al., 2011).

The investigation reports of the fruit extracts of *Physalis angulata* revealed that the fruits possessed numerous phytochemicals that are indirectly or directly attributed to the biological activity of the extracts (Porika et al., 2014). A distinct chemoprofile of all the fractions of *Terminalia chebula*, *Emblica officinalis* and *Piper nigrum* was obtained using HPTLC, which determined that the aqueous fraction showed the presence of a number of polyvalent phytoconstituents (Viswanath et al., 2011). The stem bark of *Catunaregam spinosa* possessed various phytoconstituents with different R_f values at various wavelengths which was obtained from the result of the HPTLC fingerprinting (Madhavan et al., 2011).
The saponin fraction of Achyranthes aspera L is a good diuretic agent (Dey, 2011). Chavada et al. (2011) reported that flavonoid fraction of Citrus medica unripe fruits may be responsible for increased diuresis and lowering the levels of stone forming constituents.

Thus, the present study correlates with the literatures which show that the aqueous extract of Aerva lanata is a good source of phyto constituents that helps in the antilithiatic activity of the extract. The extract might have rendered diuretic action and prevented crystal precipitation, due to the presence of these phytochemical constituents.

4.7.4. HPLC analysis of Aerva lanata extract

HPLC fingerprinting included recording of the chromatograms, retention time of individual peaks and the absorption spectra (recorded with a photodiode array detector) with different mobile phases.

The HPLC analysis of the aqueous extract was carried out using C18 reverse phase column (Shimadzu equipped with PDA detector). The results obtained are presented in Figure 32. The HPLC profile showed 9 peaks in the aqueous extract. The retention time of the major and minor peaks along with the peak area of all the 9 peaks are represented in Table 25.

Lai et al. (2008) identified the phytoconstituents present in thirteen samples of Cordyceps sinensis and its similar products with the help of HPLC. Ashtiania and Sefidkonb (2011) showed that Atropa belladonna and Atropa acuminatac contained different types of alkaloids as determined from the HPLC spectrum.

Phenolic compounds were detected at 260 nm from Calendula officinalis flowers. The separated compounds were identified by comparing their retention time (Rt) and UV spectra with those of standards. Compounds were quantified using a standard calibration curve. The compounds were identified by comparing with standards of each identified compound using Rt, the absorbance spectrum profile, and also by running the samples after the addition of pure standards (Butnariu and Coradini, 2012).
Results and Discussion

In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

Figure 32

HPLC analysis of the *Aerva lanata* extract

![HPLC analysis](image)

Table 25

HPLC peak table of the *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.016</td>
<td>7895924</td>
</tr>
<tr>
<td>2.453</td>
<td>1480413</td>
</tr>
<tr>
<td>3.072</td>
<td>1627651</td>
</tr>
<tr>
<td>3.381</td>
<td>620436</td>
</tr>
<tr>
<td>4.416</td>
<td>849203</td>
</tr>
<tr>
<td>6.048</td>
<td>1183326</td>
</tr>
<tr>
<td>9.109</td>
<td>627123</td>
</tr>
<tr>
<td>14.837</td>
<td>2431928</td>
</tr>
<tr>
<td>19.691</td>
<td>146215</td>
</tr>
</tbody>
</table>
The HPLC and FTIR of aqueous extract of *Terminalia chebula* confirmed the presence of phytocomponents in extracts (Rathinamoorthi and Thilagavathi, 2014). HPLC analysis of the methanol extract of *Ixora coccinea* showed several peaks. One among them was quercetin, a flavonoid which has various biological actions (Sumathy *et al.*, 2011).

The compositional fingerprint analysis of neotropical blueberries showed that the extracts contained flavonoids and cinnamic acid derivatives, some of which were identified and quantititated (Dastmalchi *et al.*, 2011). The HPLC analysis of *Ficus carica* Linn. ethanolic extract was carried out which exhibited 7 prominent peaks, as well as the presence of quercetin was also confirmed (Kannur and Khandelwal, 2014). The HPLC results of the present study reiterate the results of HPTLC and other phytochemical analysis which showed the presence of various phytochemical compounds based on the peaks obtained in the spectrum. Further, to identify the nature of the active compounds responsible for the therapeutic action FTIR, GC-MS and NMR were carried out.

### 4.7.5. FT-IR analysis of *Aerva lanata* extract

FT-IR is used for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

The aqueous extract of *Aerva lanata* was analyzed for the IR spectrum in FT-IR spectrophotometer using KBr pellet method. It exhibited bands at 3327 cm\(^{-1}\) characteristic of \(-\text{C} \equiv \text{C} - \text{H}\): C–H stretch (n,s) O–H stretch, H–bonded (s,b) N–H stretch (m) along with bands at 2137.22 cm\(^{-1}\) characteristics of \(-\text{C} \equiv \text{C} - \text{stretch}\) (w). A band at 597.96 and 560.35 cm\(^{-1}\) indicates the presence of C–Cl stretch (m) C–Br stretch (m). The study showed the presence of phenols, alkynes, alkenes and alkanes. A detailed characteristic IR absorption is presented in Figure 33 Table 26.
**Results and Discussion**

In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

---

**Figure 33**

FT-IR spectrum of the *Aerva lanata* extract

---

**Table 26**

IR absorption of *Aerva lanata* extract

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3327.35</td>
<td>O-H stretch (m)</td>
<td>Phenols</td>
</tr>
<tr>
<td></td>
<td>-C≡C-H: C-H stretch (n,s)</td>
<td></td>
</tr>
<tr>
<td>2941.57</td>
<td>C–H stretch (m)</td>
<td>Alkanes</td>
</tr>
<tr>
<td>2830.66</td>
<td>C–H stretch (m)</td>
<td>Alkanes</td>
</tr>
<tr>
<td>2230.77</td>
<td>–C≡C– stretch (w)</td>
<td>Alkynes</td>
</tr>
<tr>
<td>1442.82</td>
<td>C–H bend (m)</td>
<td>Alkynes</td>
</tr>
<tr>
<td>1183.38</td>
<td>C–O stretch (s)</td>
<td>Phenols</td>
</tr>
<tr>
<td>1022.32</td>
<td>=C–H bend (s)</td>
<td>Alkenes</td>
</tr>
<tr>
<td>652.93</td>
<td>=C–H bend (s)</td>
<td>Alkenes</td>
</tr>
<tr>
<td></td>
<td>–C≡C–H: C–H bend (b, s)</td>
<td>Alkynes</td>
</tr>
</tbody>
</table>

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

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*In vitro and in vivo* investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*
The FTIR spectrum is used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups of *Hybanthus enneaspermus* confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in ethanol extract (Anand and Gokulakrishnan, 2012).

The FTIR spectrum of the methanol extract of *Citrus reticulate* confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines (Shoumya et al., 2014). The FTIR analysis of various extracts of *Peristrophe bicalyculata* (Retz.) Nees. showed the presence of alkanes, alkynes, carboxylic acid, sulphur compounds, amides and halogens. The results of the study showed that *P. bicalyculata* may be rich sources of phytoconstituents (Janakiraman et al., 2011).

The IR spectrum of *Marjorana hortensis* leaf extract was indicative of the presence of several carboxyl, hydroxyl and ester groups, which may be attributed to the presence of phenols and saponins (Radha, 2012). The aqueous extract of *Cacumen platycladi* leaf when subjected to IR spectroscopy was found to contain C=O and C–O groups in abundance which was useful for capping nanoparticles (Zhan et al., 2011).

The result of the FTIR analysis of *Citrus karna* showed the presence of various secondary metabolites identified through the presence of various functional groups (Ghonghade, 2013). Nithyadevi et al. (2014) performed the phytochemical and FTIR analysis on different extracts of *Terminalia bellerica*. The FT-IR analysis has revealed the presence of phenols, alcohol, amines and carboxylic acid as functional groups in *Terminalia bellerica*.

Thus, the literatures cited support the findings of the study and confirmed the antilithiatic potential of the aqueous extract of *Aerva lanata* which is attributed to the various phytochemicals present in the extract.
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4.7.6. GC-MS analysis of Aerva lanata extract

Gas chromatography coupled with mass spectrometry (GC-MS) is a fast and accurate method widely applied in diagnostics, functional genomics and for screening purposes. The spectral profile of GC-MS confirmed the presence of 6 major components with the retention time 4.73, 7.04, 11.48, 25.21, 33.51, 35.49 respectively (Figure 34). This gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in Aerva lanata. The individual fragmentation patterns of the components are illustrated.

Figure 34

GC-MS profile of the Aerva lanata extract

Figure 35 shows the mass spectrum peak at retention time 35.49 which showed molecular ion (M⁺) peaks at 423.1, 404.2, 369.2, 351.2, 332.1, 305.1, 289.1, 252.2, 223.1, 207.1, 153.1, 115, 82 and 67.1 respectively. M 27 peak at m/z 305 corresponds to nitrogen group and M 16 peak at m/z 3289 and 207 corresponds to methyl substitutes.
Results and Discussion

Figure 35

Peak fragmentation pattern of GC-MS spectrum (35.49)

Figure 36 shows the mass spectrum peak at retention time 33.54 which showed molecular ion (M+ ) peaks at 417.2, 402.2, 361.2, 331.1, 253.1, 208.2, 165.1, 150.1, 115.1 and 79.1 respectively. M 44 peak at m/z 208 and 165 corresponds to COOH groups.

Figure 36

Peak fragmentation pattern of GC-MS spectrum (33.51)

Figure 37 shows the mass spectrum peak at retention time 22.21 which showed molecular ion (M+ ) peaks at 296.3, 264.3, 222.3, 180.2, 166.2, 137.1, 96.1 and 69.1 respectively. M 14 peak at m/z 166.2 showed the presence of double bonds.
Results and Discussion

**Figure 37**

Peak fragmentation pattern of GC-MS spectrum (25.21)

Figure 38 shows the mass spectrum peak at retention time 11.48 which showed molecular ion (M⁺) peaks at 415.1, 401, 385, 355.1, 341.1, 327, 281, 267, 221.1, 207, 191, 162.6, 147.1, 131.1, 87.1 and 73 respectively. M14 peak at m/z 401, 341.1, 267 showed the presence of double bonds in the extract. M16 peak at m/z 131 indicates the presence of methyl substitutes.

**Figure 38**

Peak fragmentation pattern of GC-MS spectrum (11.48)

Figure 39 shows the mass spectrum peak at retention time 7.02 which showed molecular ion (M⁺) peaks at 453.3, 429.1, 397.1, 367.1, 341.1, 325, 295, 267, 223, 207, 163.1, 147.1 and 133 respectively. M16 peak at m/z at 325 indicates the presence of methyl substitutes.
Results and Discussion

Figure 39

Peak fragmentation pattern of GC-MS spectrum (7.04)

Figure 40 shows the mass spectrum peak at retention time 4.73 which showed molecular ion (M⁺) peaks at 358.1, 325, 295.1, 251, 237, 205, 162.1, 133.1 and 98.3 respectively. M14 peak at m/z at 237 indicates the presence of double bond in the extract.

Figure 40

Peak fragmentation pattern of GC-MS spectrum (4.73)

Sathish et al. (2012) studied the GC-MS profile of *Vitex altissima*. These mass spectra revealed the presence of phenols, alkaloids, terpenes and alcohols in the extract which was identified using the NIST data library. The study suggests that ethanolic extract is a potent therapeutic agent.
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The analysis of plant extracts by GC/MS revealed that *Thymus vulgaris* extract is mainly composed of thymol, carvacrol and p-cimene. *Olea europaea* extract was composed of anthracenedione, terbutaline and propiolic acid while eucalyptol, globulol and silane were the principal components of *Eucalyptus globolus* extract (Al-Rahman et al., 2011).

Arunkumar and Muthuselvam (2009) identified 26 bioactive phytochemical compounds in the ethanolic extract of *Aloe vera* using the GC-MS technique. They also expressed that the identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula.

The GC-MS analysis of *Elaeocarpus serratus* showed the presence of thirty bioactive components (Geetha et al., 2013). The GC-MS investigation by Kalimuthu and Prabakaran (2013) showed the existence of various compounds with different chemical structures in *Ceropegia pusilla*. The GC-MS analysis of the ethanolic flower extract of *Calotropis gigantean* Linn. revealed the presence of 14 major compounds (Dhivya and Manimegalai, 2013). The GC-MS analysis of the concentrated ethanol extract and methanol extract of rhizomes of *Nervilia aragoana* Gaud. showed the presence of many compounds which have diverse use (Thomas et al., 2013).

The literatures discussed supports results of the present study. The presence of the bioactive compounds in the aqueous extract of *Aerva lanata* lends credence to its use as a therapeutic agent.

**TLC profile of aqueous extract**

The present study revealed that the aqueous extract of *Aerva lanata* is a good source of phytocompounds responsible for the antilithiatic activity of the plant. In order to find the structure of the active component responsible for the therapeutic action, the aqueous extract was subjected to TLC separation.

The TLC analysis (Plate 17) showed two distinct bands that were scraped out and dissolved in methanol and subjected to $^1$H NMR study.
4.7.7. \(^1\)H NMR of *Aerva lanata*

A nuclear magnetic resonance spectrum gives the largest amount of information about the structure of a compound. In NMR Spectroscopic method, a substance is placed in a strong magnetic field that affects the spin of the atomic nuclei. A radio wave passes through the substance, and reorients these nuclei. When the wave is turned off, the nuclei release a pulse of energy that provides data on the molecular structure of the substance and that can be transformed into an image by computer techniques (Patra *et al.*, 2012).

The \(^1\)H NMR spectra of the Band 1 and 2 (Figure 43 and 44) of the aqueous extract of *Aerva lanata* supports the previous phytochemical analyses. The study shows that the plant extract is a good source of secondary metabolites and hence can be used for therapeutic formulations.

The \(^1\)H NMR analysis of *Zehneria scabra* reveals the presence of a bioactive principle, gypenoside (Anand *et al.*, 2011). NMR structural analysis of leaf of *Dillenia indica* Linn indicated the presence of triterpenoids and flavonoids (Muhit *et al.*, 2010). The proton NMR spectral data of chloroform extract of *Ocimum gratissimum* L. suggested the presence of benzoid group, methoxy group and an amide group (Karpagam, 2012).
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In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata*

Figure 41

$^1$H NMR spectrum of *Aerva lanata* (Band 1)

Figure 42

$^1$H NMR spectrum of *Aerva lanata* (Band 2)

Both the *in vitro* and *in vivo* analyses performed clearly evidenced the antilithiatic property of all the weed extracts used in the study namely *Tribulus terrestris*, *Aerva lanata*, *Scoparia dulcis* and *Tridax procumbens*. Among the weeds and their extracts of differing polarity selected, aqueous extract of *Aerva lanata* showed maximum inhibitory potential against all the critical stages of stone
Results and Discussion

formation (nucleation, growth and aggregation) indicating its therapeutic efficacy and diuretic property against all the stages in kidney stone formation. The results depicted from preventive and curative regimen rats confirmed that *Aerva lanata* extract could readily prevent the stone forming constituents such as calcium and oxalate from precipitation and also the extract has the ability to dissolve preformed crystals, indicating the preventive and protective property of *Aerva lanata* extract against lithiasis. These were supported by the analyses performed using NRK 52E cell lines.

The present study, also validates the strong antioxidant effects of the aqueous extract of *Aerva lanata*. The *Aerva lanata* flowers showed high levels of antioxidants (both enzymic and non-enzymic). The aqueous extract was very effective in scavenging a spectrum of oxidants and free radicals.

The above phytochemical analyses suggest that the aqueous extract of *Aerva lanata* is an effective therapeutic agent, rendered by the phytochemicals like alkaloids, flavonoids, steroids, terpenoids, tannic acids, phenols and saponins present in the extract. It paves the way for the development of several treatment regimens based on this extract. Further research is necessary to identify and purify the predominant active compounds presumably responsible for therapeutic action. More spectral studies need to be conducted in order to determine and characterize the principle components present in the *Aerva lanata* extracts.

Thus, the present study gives substantial proof to support the alternate hypothesis which stated that the aqueous extract of *Aerva lanata* contained phytoconstituents that exhibited strong antilithiatic and antioxidative activity.

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