Anti-urolithiatic activity of hydrogenated naphthol isolated from *Aerva lanata* (L.) Juss. flower extract

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One new hydrogenated naphthol was isolated for the first time from methanolic extract of *Aerva lanata* (L.) Juss. flowers. The compound was elucidated as \( (2S.3R) \ 3-(3\text{-hydroxy}-3\text{-methyl \ pent}-4\text{-en-1-yl})-2, 5, 5, 8a \text{tetra-methyl-decahydronaphthalene-2-ol} \). The isolated compound (PC) was screened for anti-urolithiatic activity by ethylene glycol induced urolithiasis in rats. In the present study rats treated with the isolated compound (PC), reported for the first time from this taxon, reduced the deposition of calcium oxalate crystals by increasing their solubility and restoring the normal renal architecture.

**Keywords**: *Aerva lanata*, Hydrogenated naphthol, Urolithiasis, Ethylene glycol

**IPC Int. CL**: A61K 36/00, A01D 13/19, A01D 13/00, A01K 23/00

*Aerva lanata* (L.) Juss. (Family–Amaranthaceae) commonly known as *Gorakhganja* in Hindi is distributed throughout India in the wasteland. The dried flowers, which look like soft spikes, are marketed in the name of Buikallan or Boor. It has been recommended in the ancient literature of Ayurvedic system of medicine as an important ingredient of some formulation for the treatment of urolithiasis. A decoction of the flower is used for prophylaxis of urolithiasis¹⁻³. Traditionally, *A. lanata* has been documented as diuretics and demulcent. Diuretics action is said to be very effective in the treatment of urethral discharge and gonorrhea and also has value in urolithiasis and as anthelmintic. Leaves decoction is used as a gargle for the treatment of sore throats. Infusion is used to treat diarrhea, roots are used in snakebite, decoction of the flowers is said to cure stones in any part of the stomach and that of the root are diuretics. Various pharmacological investigation have also been carried out which includes, wound healing, anti-ulcer, hypoglycaemic⁴, anti-hyperlipidemic⁵, anti-inflammatory⁶, nephroprotective⁷, anti-oxidant⁸, anti-cancer⁹, anti-microbial¹⁰, hepatotoxicity¹¹, anti-diabetic¹²⁻¹³, antibacterial¹⁴⁻¹⁶, cardioprotective¹⁷, anti-fertility¹⁸ and immnomodulatory¹⁹ activities. The plant is astringent, bitter, cooling, emollient, vermifuge, diuretics, and lithotropic.²⁰ Flowers are used for removal of kidney stones.²¹ Therefore, the present investigation aimed to isolate an active component form the *Aerva lanata* flowers and to evaluate its anti-urolithiatic potential. Urolithiasis is a complex process result from a succession of the several physiochemical events includes super saturation, nucleation, growth, aggregation and retention within renal tubules. The types of stone formation correlate with the level of urinary super saturation. Calcium oxalate super saturation and crystallization are one of the major reasons in the kidney for the stone formation. The kidney plays a vital role for transient super saturation and excretion of millions of urinary crystals. In addition, fixed renal pain in the kidney radiates towards perineum and frequency of urination, oliguria, dribbling of urine and haematuria are common symptoms. To date, treatment of urolithiasis and its mechanistic approach is still remains a challenging and demanding task for researchers. Complete surgical removal coupled with herbal therapy may be used to avoid recurrence and prophylaxis against urolithiasis. Thus, the plant based

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therapy will expectantly lead to the development of effective targeted therapy in the future.

Experimental design

Materials
All chemicals used were of Analytical grade. Ethylene glycol was obtained from Sigma-Aldrich Chemicals Pvt. Limited, Hyderabad, India. Cystone was obtained from Himalaya Drug Company, Bangalore. Kits used for the estimation of calcium, oxalate, citrate, proteins, uric acid and phosphorus were purchased from Lab Care Diagnostic India Pvt. Limited, Bangalore.

Methods
IR spectra were recorded on IR-prestige 21 spectrophotometer (Shimadzu, Japan). $^1$H and $^{13}$C-NMR spectra were carried on a Bruker Avance FT-NMR at 400 MHz ($^1$H) and 100 MHz ($^{13}$C), respectively. ESI-MS was carried out on Bruckner’s 10337 micrOTOF-Q II apparatus. Column chromatography (CC) was performed using silica gel. Single crystal X-ray data for the compound were collected at 100K on a Bruker KAPPA APEXII diffractometer.

Plant material and preparation of extract
The flowers of A. lanata were collected from Araku Valley, Visakhapatnam, Andhra Pradesh, India, and were identified by Dr N K Dhal, Senior Principal Scientist, Environment & Sustainability Department, Council of Scientific and Industrial Research-Institute of Minerals and Material Technology (IMMT), Bhubaneswar, Odisha, India and specimen voucher (12853/IMMT/01.02.2012) was deposited for future reference. The dried flowers were made free of dirt and ground to the powder using a commercial mill.

Extraction and isolation
The powdered dry flowers of A. lanata (1kg) were extracted 3 times with methanol (CH$_3$OH) (3L×3) at room temperature, for 3 days each. After evaporation of the solvent in vacuum, the combined crude extract of methanol (70 gm) was partitioned with ethyl acetate (EtOAc) (0.5 L×3) to afford 18 gm of extract. This was subjected to a Silica gel CC and eluted with chloroform (CHCl$_3$): ethyl acetate (EtOAc) with increasing polarity (9:1→1:1) to yield (1-32) fraction. The fraction (8→19) having the same RF combined to give a collective fraction. Then combined fraction is subjected for preparative separation (15 PSC plates 20x20 cm, RP-18 F254, 1mm, Merck KGaA, Darmstadt, Germany) using CHCl$_3$: CH$_3$OH: Acetone. This was dried in vacuum and crystallized using a mixture of chloroform and methanol.

Characterization of isolated compound (PC)
The isolated molecule was characterized and the structure was elucidated by using FTIR, $^1$H NMR, $^{13}$C-NMR, ESI-MS, XRD analysis.

Pharmacological activity

Animals

The study was carried out on healthy adult male Wistar albino rats (180-200 gm). Animals were kept in polyacrylic cages and maintained under standard humidity conditions (24±3 °C and humidity 60±5% with 12:12 light dark circle) and allowed to get acclimatized for one week. The animals were freely accessible to feed and water ad libitum. The animals are processed and the experimental protocol was in accord with the guideline of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA). The study was conducted according to guidelines of Institutional Animal Ethics Committee (IAEC), Albino Research and Training Institute, Hyderabad, India (1172/po/a/13CPCSEA/IAEC/Exp-42).

Acute toxicity study

To acclimatize the laboratory conditions the animals were randomly selected and placed in the cages for at least 5 days prior to dosing of the extract. The test substance was administered orally by using suitable intubation cannula. The animals were observed continuously for the first 2 hrs for any gross change in behavioral, neurological and autonomic profiles or any other symptoms of toxicity and mortality and intermittently for the next 6 hrs and then again after 24 hrs, 48 hrs and 72 hrs for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the in vivo experiment.

Ethylene Glycol induced Urolithiasis

All the animals were weighed and divided into 7 groups of 6 animals each. Group I served as control and received regular diet and drinking water ad libitum. Ethylene glycol (0.75% v/v) in drinking water was fed to Group II to Group V for induction of renal calculi till the 28th day. Group III and group IV
received as a curative regimen. Group III received isolated compound (PC: 10 mg/kg b.w) and group IV received isolated compound (PC: 20 mg/kg b.w) from 15th day till the 28th day. Group V received the standard antilithiatic drug, Cystone (750 mg/kg body weight) from 15th day till 28th days. PC and standard drug were given once daily by oral route.

**Histopathology**

Each kidney was fixed in 10% formalin solution for 48-55 hrs, dehydrated in a fused alcohol slides, embedded in Paraffin wax serially sectioned using microtones. The sections were stained in H & E (Haematoxylin and Eosin) staining and photographs were taken using a light microscope with a camera attachment (Figs. 1-4).

**Serum analysis**

All rats were sacrificed under thiopentone anesthesia (50 mg/kg I.P). Blood sample were collected by cardiac puncture and serum was separated by centrifugation at 10,000 rpm for 10 min. Calcium, citrate, oxalate, uric acid, phosphorus and protein levels were assessed by using auto-analyzer and specific kits. One kidney from one animal of each group was dissected and washed with tap water and preserved in 10% formalin solution for histopathological studies.

**Statistical analysis**

Results were expressed as Mean ± S.E.M. The results among different groups were analysed by one-way ANOVA with posthoc. Statistical difference was considerate at p<0.05.

**Results**

**Characterization of isolated compound (PC)**

Colourless compound (yield: 0.80 gm) having melting point 110 °C was isolated from methanol extract of A. lanata is being reported for the first time from this taxon. The molecular formula for the compound (PC) was deduced to be C_{20}H_{36}O_2 based on 1H-NMR and 13C-NMR data and X-ray diffracted crystallographic structure analysis (represented below). The quasi-molecular on the peak was observed at M/Z (%) 331[M+Na]^+ 273.2625[M-2.OH]^+; 13C NMR (400MHz,CD_3OD,25 °C,TMS): 146.798, 111.781, 75.213, 74.456, 62.814, 57.604, 46.707, 43.310, 41.180, 40.595, 34.230, 33.977, 24.019, 22.029, 21.589, 20.721, 19.542, 16.131; δ111.781). The O-H asymmetric stretching frequency, as well as vinyl stretching, can be ascertained by the 3398.57cm^{-1} and 1674.21 cm^{-1} in FTIR.

**Yield** : 0.80 gm; Solubility-hot Methanol; ESI-MS(C_{20}H_{36}O_2): m/z^+(%) 331[M+Na]^+, 273.2625[M-2.OH]^+; 13C NMR (400MHz,CD_3OD,25 °C,TMS): 146.798, 111.781, 75.213, 74.456, 62.814, 57.604, 46.707, 43.310, 41.180, 40.595, 34.230, 33.977, 24.019, 22.029, 21.589, 20.721, 19.542, 16.131;

![Fig.1—Microscopic images of kidney sections from (a) vehicle control animals, (b) lithiatic group, (c) group treated with isolated compound (PC: 10 mg/kg b.w) and (d) group treated with isolated compound (PC: 20 mg/kg b.w).a1 to d1 reflect sections under polarized light microscope (100x) after Hematoxylin and Eosin staining and a2 to d2 reflect sections under polarized light microscope after Pizzolato's staining (40x), respectively. Lithiatic animals showed excessive accumulation of calcium oxalate crystals (CaOx) with marked histological changes including interstitial fibrosis (IF) with infiltration by eosino phils (E)](image-url)
$^1$H NMR (400MHz,CD$_3$OD,25°C,TMS): 5.97 (s, 1H), 5.93(d, $J$=3.99Hz, 1H), 5.90(s, 1H), 5.23(d,1H), 5.03(d, $J$ =5.035Hz,1H), 5.01 (d, $J$ =3.99 Hz, 1H), 1.2-1.8(m,CH$_2$,4H), 1.26(CH$_3$,6H), 1.14(CH$_2$,6H), 0.9(CH$_3$,6H), 0.84(CH$_3$,12H): FTIR: $\nu_{\text{max}}$(KBr)cm$^{-1}$: 3398.57 (O-H Stretching), 3321.12, 2978.09(C-H stretching (aromatic), 2839.22, 1724.36, 1674.21 (C-C stretching (Vinyl), 1458.18 (C-H bending (Alkane), 1369.46, 1296.16, 1219.01, 1080.14, 1033.85, 979.84 (=C-H bending), 920.40, 817.82, 713.66, 659.66, 574.79 (Table 1).

**X-ray Diffraeted Crystallography**

The X-ray diffracted crystallographic data revealed that the asymmetric unit contains one unit of the molecule. Both substituted cyclohexyl units are present in their chair form sharing the C4-C5 bond between them. The oxygen atoms (O1 and O2) exhibited strong Intra- and inter-molecular H-bonding between them. The non-bonded intra-molecular O1---(H---)O2 and intermolecular O1(---H)----O2 distances

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Table 1—$^1$H, $^{13}$C-NMR data of isolated compound (PC) at 400 MHz & 100MHz in CD$_3$OD at 25°C ($\delta$ in ppm; $J$ in Hz).

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H-NMR</th>
<th>$^{13}$C-NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_a$(1)</td>
<td>5.97, 5.95</td>
<td>146.798</td>
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<tr>
<td>H(2)</td>
<td>5.9(d, 1H)</td>
<td>111.781</td>
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<tr>
<td>H$_b$(1)</td>
<td>5.23(d, 1H)</td>
<td>75.213</td>
</tr>
<tr>
<td>H(7)</td>
<td>5.18(d, $J$=3.99Hz,1H)</td>
<td>74.456</td>
</tr>
<tr>
<td>H(11)</td>
<td>5.03, 5.01</td>
<td>62.814</td>
</tr>
<tr>
<td>H$_a$&amp;H$_b$(10), H$_a$&amp;H$_b$(6)</td>
<td>1.2-1.8(m,CH$_2$,4H)</td>
<td>57.604</td>
</tr>
<tr>
<td>H$_a$&amp;H$_b$(5), H$_a$&amp;H$_b$(9)</td>
<td>1.26 (CH$_2$, 4H)</td>
<td>46.707</td>
</tr>
<tr>
<td>H$_a$&amp;H$_b$(13), H$_a$&amp;H$_b$(14), H$_a$&amp;H$_b$(15)</td>
<td>1.14 (CH$_2$, 6H)</td>
<td>43.310</td>
</tr>
<tr>
<td>H$_a$&amp;H$_b$(12)</td>
<td>0.9 (CH$_3$, 6H)</td>
<td>41.180</td>
</tr>
<tr>
<td>H$_a$&amp;H$_b$(20), H$_a$&amp;H$_b$(4), H$_a$&amp;H$_b$(8)</td>
<td>0.84 (CH$_3$, 9H)</td>
<td>40.595</td>
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<tr>
<td></td>
<td></td>
<td>34.230</td>
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<td></td>
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<td>33.977</td>
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<td>24.019</td>
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<td>20.721</td>
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<td>19.542</td>
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<td>16.131</td>
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</tbody>
</table>
were found to be 2.702 Å and 2.823 Å, respectively. The packing diagram indicated an α-helical expansion of the framework along H-bonding interactions.

**Crystallographic data:** \(\text{C}_{20}\text{H}_{36}\text{O}_{2}\); \(M_w = 308.49\); needle-shaped; white, orthorhombic, space group \(\text{P } 2_1 \text{ } 2_1 \text{ } 2_1\), \(a = 6.3507(3) \text{ Å}, \ b = 13.2317(5) \text{ Å}, \ c = 22.9991(9) \text{ Å}, \ \alpha = \beta = \gamma = 90.00, \ U = 1932.63(14)\text{Å}^3, \ T = 296(2) \text{ K}, \ Z = 4, \ \check{\Delta}(\text{Mo K}\alpha) = 0.066 \text{ mm}^{-1}, \ F(000) = 688, \ \rho_{\text{calc}} = 1.060 \text{ mg/m}^3, \ 3805\) reflection data with 199 parameters, 2339 \([I \geq \sigma(I)]\) unique reflections used in calculations. The final \(R_1 = 0.0612, \ wR_2 = 0.1752, \ S = 1.047\) (Fig. 5).

**Anti-urolithiasis and Histopathology**

Liver and kidney act as main sites of endogenous oxalate synthesis. Calcium and oxalate excretion were significant increases in ethylene glycol induced urolithiatic rats when compared with normal control rats (Table 2). Isolated compound (PC) therapy reverted the above changes to normal values. Serum phosphorus, uric acid and protein levels are significantly elevated in urolithiatic treated rats as compared with control group. PC therapy reduced the phosphorus, uric acid and protein to the normal values (Table 2).

The study of the kidney by light microscopy, in the normal control group, showed the normal structure of kidney. Diseases control group shows damage to the medulla, glomeruli, tubules and interstitial spaces. The damage was found to be almost recovered in standard and test groups. The histopathological observation of kidney showed the normal structure and architectural intactness without any apparent damage in the control group and PC treated and drug control rats. Kidneys of ethylene glycol induced urolithiatic rats showed polymorphic irregular crystals accumulated in the tubules that caused dilation of the proximal tubules and led to the dilation of the tubular axis of the tubules along with interstitial inflammations (Figs. 1-4).

**Discussion**

The isolated hydrogenated naphthol compound (PC) in *Aerva lanata* (L.) Juss. contains polar hydroxyl groups and might be covalently bonded with calcium oxalate crystal which in turns lead to excretion of metabolites. Isolated compound (PC) from *A. lanata* dissolves the calcium oxalate stone and restored the renal structure in ethylene glycol induced urolithiatic rats. Uric acid is known to promote calcium oxalate crystal growth. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggest its primary role in stone formation. In the present study, higher concentration of uric acid was observed in ethylene glycol induced urolithiatic rats. PC treatment restored the uric acid level to normal thus reducing the risk of stone formation. A gradual increase in serum phosphorus level was observed in ethylene glycol induced urolithiatic rats. PC therapy maintained the uric acid level to normal thus reducing the risk of stone formation. A gradual increase in serum phosphorus level was observed in ethylene glycol induced urolithiatic rats increased serum phosphorus level along with oxalate seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which induce calcium oxalate deposition. In PC therapy phosphorus level was maintained to normal and reduced the risk of stone formation. In the present study, serum protein level was raised in ethylene glycol induced urolithiatic rats and PC therapy minimizes the serum protein level and thus might have prevented the formation of crystal nucleation. These results are in close agreement with the previous works carried out

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**Table 2—Effect of isolated compound (Pc) & standard drug (Cyst One) on serum parameters in control & experimental animals**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Calcium levels</th>
<th>Oxalate levels</th>
<th>Citrate levels</th>
<th>Uric acid levels</th>
<th>Phosphorus levels</th>
<th>Protein levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.69±0.02</td>
<td>0.51±0.04</td>
<td>2.00±0.03</td>
<td>1.06±0.02</td>
<td>5.50±0.06</td>
<td>2.19±0.04</td>
</tr>
<tr>
<td>Urolithiatic</td>
<td>2.26±0.03</td>
<td>2.90±0.04</td>
<td>0.81±0.03</td>
<td>1.40±0.05</td>
<td>7.41±0.08</td>
<td>3.18±0.03</td>
</tr>
<tr>
<td>PC (10mg/Kg)</td>
<td>1.15±0.03</td>
<td>1.02±0.02</td>
<td>1.21±0.03</td>
<td>2.13±0.03</td>
<td>6.01±0.04</td>
<td>2.79±0.04</td>
</tr>
<tr>
<td>PC (20mg/Kg)</td>
<td>0.74±0.04</td>
<td>0.62±0.05</td>
<td>2.10±0.03</td>
<td>1.07±0.02</td>
<td>5.55±0.04</td>
<td>2.21±0.06</td>
</tr>
<tr>
<td>Cystone (Standard)</td>
<td>0.71±0.02</td>
<td>0.51±0.05</td>
<td>2.08±0.03</td>
<td>1.07±0.03</td>
<td>5.54±0.06</td>
<td>2.18±0.03</td>
</tr>
</tbody>
</table>
by Nirmaladevi et al. (2013) and Laxmi et al. (2015) on aqueous extract of A. lanata. Ethylene glycol induced urolithiatic rats treated with PC had increases the solubility of calcium oxalate crystal deposit and restored the normal renal architecture. In earlier report it was found that A. lanata aqueous suspension restored the magnesium excretion and thus could reduced the growth of calcium oxalate crystals in ethylene glycol induced urolithic rats. In the present study rats treated with the isolated compound (PC), reported for the first time from this taxon, reduced the deposition of calcium oxalate crystals by increasing their solubility and restoring the normal renal architecture.

**Conclusion**

The present study concludes that the administration of isolated compound (PC) from flowers of A. lanata to ethylene glycol induced urolithiasis rats, reduced and prevented the formation and growth of urinary stones, supporting the folklore claim regarding anti-urolithiasis activity of the plant. Ethylene glycol induced urolithiatic rats treated with PC had increases the solubility of calcium oxalate crystal deposit and restored the normal renal architecture. This recovery effect was due to PC and it concludes that isolated compound (PC) is having anti-urolithiasis activity and it can serve as a lead compound for the treatment of urolithiasis.

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**References**


