Isolation, structural elucidation and cytotoxicity evaluation of a new pentahydroxy-pimarane diterpenoid along with other chemical constituents from *Aerva lanata*

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*Aerva lanata* possesses various useful medicinal and pharmaceutical activities. Phytochemical investigation of the plant has now led to the isolation of a new 2α,3α,15,16,19-pentahydroxy pimar-8(14)-ene diterpenoid (1) together with 12 other known compounds identified as β-sitosterol (2), β-sitosterol-3-0-β-D-glucoside (3), canthin-6-one (4), 10-hydroxy canthin-6-one (aervine, 5), 10-methoxy canthin-6-one (methylaervine, 6), β-carboline-1-propionic acid (7), 1-O-β-D-glucopyranosyl-(2S,3R,8E)-2-[(20R)-2-hydroxy palmitoylamino]-8-octadecene-1,3-diol (8), 1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[(2′R)-2′-hydroxy tetracosanoylamino]-8(Z)-octadecene-1,3,4-triol (9), (2S,3S,4R,10E)-2-[(2′R)-2′-hydroxy tetracosanoylamino]-10-octadecene-1,3,4-triol (10), 6′-O-(4′-hydroxy-trans-cinnamoyl)-kaempferol-3-O-β-D-glucopyranoside (tribuloside, 11), 3-cinnamoyltribuloside (12) and sulfonoquinovosyldiacylglyceride (13). Among these, six compounds (8–13) are reported for the first time from this plant. Cytotoxicity evaluation of the compounds against five cancer cell lines (CHO, HepG2, HeLa, A-431 and MCF-7) shows promising IC50 values for compounds 4, 6 and 12.

**Keywords:** *Aerva lanata*; pimarane diterpenoid; 2D NMR (HSQC, HMBC, COSY, NOESY); cytotoxicity

1. **Introduction**

India is a well known source for medicinal plants and their extracts which are used in Ayurvedic, Siddha, and Unani systems of medicine for treating different types of diseases. One of them is *Aerva lanata* (L.) A. L. Juss. ex Schultes from the family Amaranthaceae locally known as ‘bui’ or ‘polpala’. It is an erect or prostrate under shrub with a long tap-root and many wolly-tomentose branches, found in the wild, with several names throughout India like Chaya (Bengali), Gorakhbuti (Hindi), Sirupulai (Tamil) and Bhadra (Sanskrit) (Khare 2007). It is also described in English as a stone breaking plant. *A. lanata* possesses medicinal and pharmaceutical importance (Ragavendran et al. 2012). A variety of pharmacological activities of this ethnomedicinally important plant has been reported as follows: Demulcent, antiinflammatory, expectorant, hepatoprotective and nephroprotective (Shirwaikar et al. 2004; Manokaran et al. 2008). Alcoholic extract of shoots of *A. lanata* has shown significant antidiabetic and antihyperglycaemic activities in rats (Deshmuk et al. 2008; Krishnan et al. 2009). Antimicrobial and cytotoxic (Chowdhury et al. 2002), diuretic and urolithiatic
Several types of phytochemical constituents of *A. lanata*, e.g. alkaloids, flavonoids, phenols, and tannins have been identified by qualitative phytochemical screening. The plant contains biologically active canthin-6-one alkaloids such as 10-methoxy-canthin-6-one, 10-hydroxycanthin-6-one and 10-O-β-D-glucopyranosylcanthin-6-one. It also contains alkaloids like β-carboline-1-propionic acid, 6-methoxy-β-carboline-1-propionic acid or ervolanine (Zapesochnaya, Kurkin, et al. 1991; Zapesochnaya, Pervykh, et al. 1991; Zapesochnaya et al. 1992).

It is a rich source of flavonoids such as kaempferol, quercetin, isorhamnetin and isorhamnetin 3-O-[4-p-coumaroyl-a-rhamnosyl(1→6) galactoside, the flavanone glucoside persinol, persinosides A and B, 5,4’-hydroxy-3,6,7-trimethoxyflavone, 5-hydroxy-3,4,6,7-tetramethoxyflavone, 5-hydroxy-2’,3’,5’,6,7-pentamethoxy flavone, 3,3’,5,7-tetrahydroxy-4’-methoxyflavone and apigenin 7-β-D-glucoside (Saleh et al. 1990; Pervykh et al. 1992; Ahmed et al. 2006).

Several other compounds like methyl grevillate, lupeol, lupeol acetate, benzoic acid, β-sitosteryl acetate and tannic acid are also found (Omoyeni & Adeyeye 2009).

Pimarane type diterpenoids are present in plants of the genus *Siegesbeckia* (Compositae) (Canonica et al. 1969; Liu & Röder 1991; Jiang et al. 1992; Xiang et al. 2004; Wang & Hu 2006). Currently, they attract considerable scientific and therapeutic interest because of various biological activities like anti-arthritic, cytotoxic (Isaka et al. 2011; Lu et al. 2012; Lu, Liu, et al. 2014; Lu, Qian, et al. 2014), anticarcinogenic (Severiano et al. 2010), antimicrobial (Porto, Furtado, et al. 2009; Porto, Rangel, et al. 2009), anti-inflammatory and analgesic (Wang et al. 2011), etc.

As a part of our investigation of phytochemicals from Indian Medicinal Plants, *A. lanata* was selected for the study as a potential source of medicinal and pharmaceutical ingredients. This investigation deals with the isolation and structure elucidation of a new 2α,3α,15,16,19-pentahydroxy-pimarane diterpenoid (1) and 12 other known compounds among which six are isolated for the first time from this plant.

### 2. Results and discussion

#### 2.1. Isolation and structural elucidation

The methanol extract of the aerial parts of *A. lanata* was subjected to silica gel (100–200 mesh) column chromatography to afford one new diterpenoid (1) and 12 other known compounds identified as β-sitosterol (2), β-sitosterol-3-O-β-D-glucoside (3), canthin-6-one (4), 10-hydroxycanthin-6-one (aervine) (5), 10-methoxycanthin-6-one (methylaervine) (6), β-carboline-1-propionic acid (7) (Goyal et al. 2011; Rajesh et al. 2011), 1-O-β-D-glucopyranosyl-(2S,3R,8E)-2-[(2’R)-2-hydroxypalmitoylamino]-8-octadecene-1,3-diol (8) (Ling et al. 2006, 1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[(2’R)-2’-hydroxytetracosanoyl-amino]-8(Z)-octadecene-1,3,4-triol (9) (Cateni et al. 2003), (2S,3S,4R, 10E)-2-[(2’R)-2’-hydroxytetracosanoylamino]-10-octadecene-1,3,4-triol (10) (Zha-Jun et al. 2003), 6’-O-(4’-hydroxy-trans-cinnamoyl)-kaempferol-3-O-β-D-glucopyranoside (tribuloside) (11) (Tsukamoto et al. 2004), 3-cinnamoyltribuloside (12) (Christopher et al. 2014) and sulfonoquinovosyldiacylglyceride (13) (Chatterjee et al. 2010; Bharitkar et al. 2014), respectively, by comparing their spectroscopic data with those reported in the literature (Figure 1). Compounds 8–13 are reported for the first time from *A. lanata*.

Compound 1 was obtained as white crystals. The positive ion HR-ESI-MS (m/z 377.2315, calcld for C_{20}H_{34}O_{5}Na, 377.2304) showed the molecular formula to be C_{20}H_{34}O_{5}, representing an unsaturation value of four. The IR spectrum of 1 showed absorptions at 3419 and 1658 cm⁻¹ corresponding to OH and C=–C functional groups. In the ¹H NMR spectrum signals for three
methyl groups located on quaternary carbons were observed as 3H singlets at δ 0.97, 1.23 and 1.65. Two pairs of oxygenated methylene protons [δ 4.21 (1H, bd, J = 13.8 Hz) and 3.85 (1H, m), 4.19 (1H, bd, J = 11.4 Hz) and 3.95 (1H, t, J = 9.6 Hz)] and three oxygenated methine protons [δ 3.87 (1H, m), 4.53 (1H, d, J = 11.4 Hz) and 4.68 (1H, s)] signals were observed. A trisubstituted olefin proton peak was observed at δ 5.71 (1H, s). The 13C NMR and DEPT spectra displaying 20 carbon signals indicated a diterpene skeleton consisting of three methyls (at δ 17.3, 23.5 and 24.1), seven methylene (two oxygenated, at δ 63.8 and 65.4), six methine (three oxygenated, at δ 66.9, 74.4, 80.1 and one olefinic, at δ 129.6), and four quaternary carbon atoms (one olefinic, at δ 137.7). Three methyl carbon signals were assigned to C-20, C-17 and C-18, respectively. Two oxygenated methylene carbon signals could be assigned to C-16 and C-19, respectively, whereas three oxygenated and one olefinic methine carbon signals were assigned to C-2, C-3, C-15 and C-14, respectively. The olefinic quaternary carbon signal at δ 137.7 represents the C-8 carbon.
The HMBC cross peaks (Table S1) between H-14 (δ 5.70) and C-7 (δ 36.9), C-8 (δ 137.7) and between C-14 (δ 129.6) and H-7 (δ 2.33) establish the attachment of B, C rings whereas cross peaks between H-9 (δ 1.96) and C-1 (δ 41.7), C-9 (δ 51.6) and H-1 (δ 2.16, 2.02) prove the attachment of A, B rings. The HMBC cross peaks of H-14 (δ 5.71) signal with peaks for C-15 (δ 80.1), C-13 (δ 38.8), and C-17 (δ 23.5), and of H-15 (δ 3.87) signal with peaks for C-14 (δ 129.6), C-13 (δ 38.8), C-17 (δ 23.5), and C-12 (δ 31.3) establish the attachment of the C-17 methyl group and the vicinal diol side chain at C-13 of ring C (Figure S1).

Compared to the related pimarane diterpenoid darutigenol/kirenol/2β,3β,15,16-tetraydroxy-ent-pimar-8(14)-en (Herz et al. 1978; Jakupovic et al. 1987; Liu & Röder 1991) both C-2 and C-3 methylene peaks were replaced by downfield methine carbon peaks at δ 66.9, 74.4 in 1, suggesting that two OH groups were present at C-2 and C-3. This conclusion was supported by the downfield shift of the C-1 carbon (δ 41.7) as well as the C-4 carbon (δ 45.5) signals. HMBC cross-peaks of H-3 with C-2, C-4, C-18, and C-1 peaks, of H-1 with C-2 and C-3 peaks as well as the COSY relationship of H-3 signal with H-2 along with the C-3–OH proton (δ 5.72) supported the above assignments. The coupling constant of H-2 (J = 11.4 Hz) suggested it to be axial while H-3 must be equatorial to explain the absence of splitting. Furthermore, the absence of NOESY relationship between signals of H-5 and H-3 proved the equatorial orientation of H-3. Also the strong NOESY relationship in between H-2 and H-20 methyl proton suggested C-20 methyl to be axially-oriented. The NOESY correlation of H-11 (δ 1.58) with both H-20 methyl proton (δ 0.97) and H-17 methyl proton (δ 1.22) confirms the axial-orientation of C-17 methyl group (Figure S2).

The structure was ultimately confirmed by single crystal X-ray diffraction study of 1 where the β-orientation of H-2 and H-3 along with the C-19 oxygenated methylene and C-20, C-17 methyl were clearly visible (Figure S2).

2.2. Biological activities

Cytotoxicity evaluation of the isolated thirteen compounds against five cancer lines (CHO, HepG2, HeLa, A-431 and MCF-7) was performed via MTT assay using doxorubicin as standard.
Table 1. IC₅₀ values of the isolated compounds 1–13.

| Compounds | CHO  | HepG2 | HeLa  | A431  | MCF-7  |
|-----------|------|-------|-------|-------|--------|
| 1         | > 50 | > 50  | > 50  | > 50  | > 50   |
| 2         | ND   | ND    | ND    | ND    | ND     |
| 3         | ND   | ND    | 14.9  | 8.393 | 5.541  |
| 4         | 7.529| 4.551 | 8.393 | 5.541 |
| 5         | > 50 | > 50  | > 50  | > 50  | > 50   |
| 6         | 4.692| > 50  | 5.039 | 5.366 | 4.222  |
| 7         | > 50 | > 50  | > 50  | > 50  | > 50   |
| 8         | > 50 | 30.22 | > 50  | > 50  | > 50   |
| 9         | > 50 | > 50  | > 50  | > 50  | > 50   |
| 10        | > 50 | > 50  | > 50  | > 50  | > 50   |
| 11        | > 50 | > 50  | > 50  | > 50  | > 50   |
| 12        | > 50 | 12.5  | 35.29 | 11.84 | > 50   |
| 13        | > 50 | 45.6  | 44.53 | > 50  | > 50   |
| Doxorubicin| 0.45 | 0.50  | 0.35  | 0.65  | 0.40   |

*IC₅₀ (μM/mL)*

*Incubation: 72 h; values are average of three days assay, n = 2 each day.

Compounds 4, 6 and 12 are quite promising as indicated from their IC₅₀ values and further evaluation is in progress (Table 1).

3. Experimental

3.1. General experimental procedure

Melting points were determined in capillaries and are uncorrected. IR spectra were recorded as KBr pellets using a JASCO 410 FTIR spectrometer (Tokyo, Japan). The NMR spectra were recorded using a Bruker 600 DPX spectrometer (Coventry, UK) operating at 600 MHz for ¹H and 150 MHz for ¹³C in Py-d₅ with TMS as internal standard and the chemical shifts are reported in δ units. Mass spectra (positive mode) were obtained on a LC-ESI-Q-TOF micro mass spectrometer in the electro spray ionization mode. All other solvents and chromatographic absorbents were procured from E. Merck (Darmstadt, Germany) and SRL (Mumbai, India) Ltd. unless otherwise indicated. Thin layer chromatography was performed on pre-coated silica gel 60 F₂₅₄ aluminum sheets (E. Merck, Darmstadt, Germany) using various solvent systems (1%, 5% and 10% MeOH in CHCl₃) and spots were developed using UV irradiation, iodine and Liebermann–Burchard reagent.

3.2. Plant material

Mature whole aerial part of A. lanata was collected from the Muzaffarpur district, Bihar, India during November 2012 and identified by Botanical Survey of India, Howrah, West Bengal. A voucher specimen (No.YB-2) was deposited in the Chemistry department, Indian Institute of Chemical Biology, Kolkata.

3.3. Extraction fractionation and isolation

The whole aerial parts of A. lanata were cut into small pieces and air dried at room temperature (24–27°C). The dried plant material (2.4 kg) was defatted with petroleum ether (60–80°C) for 24 h and extracted with methanol (15 L × 3) for 48 h each time at ambient temperature. The extract was filtered and the solvent was dried under vacuum at 40–45°C to afford 285 g of crude extract (yield 11.9%). It was partitioned between water-saturated n-BuOH and water. The organic layer was further washed with water for complete removal of inorganic impurities,
free sugars and other water-soluble residues and then evaporated to dryness under reduced pressure using a rotary evaporator to yield a dark brown residue (217 g).

The extract was chromatographed on a silica gel (60–120 mesh) column. Graded elution was carried out with chloroform followed by various mixtures of CHCl₃–MeOH (19:1, 9:1, 85:15, 4:1 and 3:1). A total of 165 fractions (200 mL each) were collected and fractions giving similar spots on TLC were combined. The fraction eluted with chloroform was subjected to rechromatography on silica gel (100–200 mesh) to obtain four compounds; compound-2 (65 mg), compound-4 (25 mg), compound-5 (43 mg) and compound-6 (55 mg). Fraction eluted with chloroform: methanol (19:1) when subjected to rechromatography on silica gel (100–200 mesh) yielded four compounds: compound-3 (115 mg), compound-8 (95 mg), compound-9 (58 mg) and compound-10 (40 mg). Fraction eluted with chloroform:methanol (9:1) after rechromatography on silica gel (100–200 mesh) furnished four compounds: a novel compound-1 (8.5 mg), compound-7 (130 mg), compound-11 (88 mg) and compound-12 (17 mg). Fraction eluted with chloroform: methanol (85:15) similarly provided compound-13 (105 mg). All compounds were characterized by the application of various spectroscopic analyses like IR, ESI-MS, ¹H NMR, ¹³C NMR with DEPT 90 and 135, 2D NMR (COSY, NOESY, HSQC and HMBC).

3.3.1. Spectroscopic data of compound 1 [7-(1,2-Dihydroxy-ethyl)-1-hydroxymethyl-1,4a,7-trimethyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-phenanthrene-2,3-diol]

White crystal compound; mp 266–268°C; IR (KBr, ν max cm⁻¹): 3419, 3264, 2945, 1657, 1626, 1451, 1412, 1293, 1137, 1079, 1036; ¹H NMR (Py d₅, 600 MHz): δ 6.05–5.96 (3H, m, –OH), 5.72 (1H, m, –OH), 5.71 (1H, s), 5.65 (1H, m, –OH), 4.68 (1H, s), 4.51 (1H, d, J = 11.4 Hz), 4.21 (1H, bd, J = 13.8 Hz), 4.19 (1H, bd, J = 11.4 Hz), 3.95 (1H, t, J = 9.6 Hz), 3.87 (1H, m), 3.85 (1H, m), 2.33 (1H, dd, J = 3, 8.4 Hz), 2.16 (1H, m), 2.12 (1H, m), 2.02 (1H, m), 1.99 (1H, m), 1.96 (1H, m), 1.78 (1H, m), 1.73 (1H, m), 1.71 (1H, m), 1.65 (3H, s), 1.58 (1H, m), 1.54 (1H, m), 1.46 (1H, qd, J = 4.2, 13.2 Hz), 1.23 (3H, s), 0.97 (3H, s); ¹³C NMR (Py d₅, 150 MHz): δ 137.7 (C), 129.6 (–CH), 80.1 (–CH), 74.4 (–CH), 66.9 (–CH₂), 65.4 (–CH₂), 63.8 (–CH₂), 51.6 (–CH₂), 49.3 (–CH₂), 45.5 (–C), 41.7 (–CH₂), 39.5 (–C), 38.8 (–C), 36.9 (–CH₂), 31.3 (–CH₂), 24.1 (–CH₃), 23.5 (–CH₃), 22.7 (–CH₂), 19.5 (–CH₂), 17.3 (–CH₃); HR-ESI-MS (m/z 377.2315) for [M + Na]⁺ (calcd. 377.2304 for C₂₀H₃₄O₅Na).

3.4. Crystal data for 1

X-ray data were collected at room temperature with Mo Kα radiation (graphite monochromator λ = 0.7107 Å) on a Huber four circle diffractometer (type 512) equipped with a Bruker-APEX area detector. Data reduction and absorption correction were carried out using SAINT and SADABS.

Structural solution and refinement (programs SHELXT and SHELXL) ran routinely. C₂₀H₃₄O₅, Mr = 388.49, white rectangular shaped crystals were grown from chloroform–methanol. Space group ‘monoclinic’ ‘P2₁’. Lattice constants (Å): a = 11.7030 (11), b = 6.2643 (6), c = 13.8473 (12); α = 90, β = 92.432 (6), γ = 90, cell volume V = 1014.25 (16) Å³, formula units/cell Z = 2, number of independent reflections (N_ref) 4183, after convergence R₁ = 0.0757 (3145), wR₂ = 0.2519 (4183). Crystal packing cell was supplied in the supplementary material (Figure S3).

4. Conclusion

In conclusion, a new 2α, 3α,15,16,19-pentahydroxy pimar-8(14)-ene diterpenoid (1) in addition to 12 other known compounds (2–13) were isolated and characterized using through
spectrometric analysis (IR, 1D and 2D NMR, Mass) and comparison of the literature data for the known compounds. Among these 6 compounds (8–13) are reported for the first time from this plant. Cytotoxicity evaluation of the isolated thirteen compounds against five cancer cell lines were performed which shows promising IC$_{50}$ values for compound 4, 6 and 12.

**Supplementary data**

$^1$H, $^{13}$C NMR, IR and HRMS data along with spectrometric copy of compound 1 associated with this article can be found in the online version. Crystallographic data in CIF format are available free of charge via the Internet at CCDC 1014285. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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**References**

Ahmed E, Imran M, Malik A, Ashraf M. 2006. Antioxidant activity with flavonoidal constituents from *Aerva persica*. Arch Pharm Res. 29:343–347.

Anantha D, Kumar TI, Kumar MS, Reddy AM, Mukharjee NSV, Rao AL. 2010. In vitro anti helmentic activity of aqueous and alcoholic extracts of *Aerva lanata* seeds and leaves. J Pharm Sci Res. 2:317–321.

Bharitkar YP, Bathini S, Ojha D, Ghosh S, Mukherjee H, Kuots K, Chattopadhyay D, Mondal NB. 2014. Antibacterial and antiviral evaluation of sulfonoquinovosyldiacylglyceride (SQDG): a glycolipid isolated from *Azadirachta indica* leaves. Lett Appl Microbiol. 58:184–189.

Canonica L, Rindone B, Sostatico C, Han KD, Kim JH. 1969. A new diterpenoid with pimarane skeleton. Tetrahedron Lett. 10:4801–4804.

Cateni F, Zilic J, Falsone G, Hollan F, Frausin F, Scarvia V. 2003. Preliminary biological assay on cerebroside mixture from *Euphorbea nicaensis* Aff. Isolation and structure determination of five glucocerebrosides. IL Farmaco. 58: 890–817.

Chatterjee R, Singh O, Pachuau L, Malik SP, Paul M, Bhadra K, Paul S, Kumar GS, Mondal NB, Banerjee S. 2010. Identification of a sulfonoquinovosyl-diacylglyceride from *Azadirachta indica* and studies on its cytotoxic activity and DNA binding properties. Bioorg Med Chem Lett. 20:6699–6702.

Chowdhury D, Sayeed A, Islam A, Bhuiyan MSA, Khan GRMAM. 2002. Antimicrobial activity and cytotoxicity of *Aerva lanata*. Fitoterapia. 73:92–94.

Christopher R, Nyandoro SS, Chacha M, de Koning CB. 2014. A new cinnamoylglycoflavonoid, antimycobacterial and antioxidative constituents from *Heritiera littoralis* leaf extracts. Nat Prod Res. 28:351–358.

Deshmuk T, Yadav BV, Badole SL, Bodhankar SL, Dhaneshwar SR. 2008. Antihyperglycaemic activity of alcoholic extract of *Aerva lanata* (L.) A.I Juss. Ex J.A. Schultes leaves in alloxan induced diabetic mice. J Appl Biomed. 6:81–87.

Goyal M, Pareek A, Nagori BP, Sasmal D. 2011. *Aerva lanata*: a review on phytochemistry and pharmacological aspects. Pharmacogn Rev. 5:195–198.

Herz W, Bhat SV, Murari R. 1978. The diterpene darutigenol from *Palafoxia arida*. Phytochemistry. 17:1060–1061.

Isaka M, Palasar S, Prathomphai W, Laksancharoen P. 2011. Pimarane diterpenes from the endophytic fungus *Eutypella* sp. BCC 13199. Chem Pharm Bull. 59:1157–1159.
Jakupovic J, Castro V, Bohlmann F. 1987. Millerenolides, sesquiterpene lactones from *Milleria quinqueflora*. *Phytochemistry*. 26: 2011–2017.

Jiang X, Yunbao M, Yunlong X. 1992. Diterpenoids from *Siegesbeckia pubescens*. *Phytochemistry*. 31:917–921.

Joanofare J, Vamsadhara C. 2003. Evaluation of anti diarrhoeal activity of Aerva species. *Nat Prod Sci*. 9:177–179.

Khare CP, ed. 2007. *Indian medicinal plants. An illustrated dictionary*. Berlin/Heidelberg: Springer-Verlag. ISBN: 978-0-387-70637-5.

Krishnan GA, Rai VK, Nandy BC, Meena KC, Dey S, Tyagi PK, Tyagi LK. 2009. Hypoglycemic and antihyperlipidaemic effect of ethanolic extract of aerial parts of Aerva lanata Linn. in normal and alloxan induced diabetic rats. *Int J Pharm Sci Drug*. 1:191–194.

Lu Y, Xiao J, Xiao J, Wu HZ, Chen YY. 2014. Kirenol, a compound from *Herba siegesbeckiae*, induces apoptosis in human chronic myeloid leukemia K562 cells. *Pharmacazie*. 69:148–153.

Lu Y, Xiao J, Wu ZW, Wang ZM, Hu J, Fu HZ, Chen YY, Qian RJ. 2012. Kirenol exerts a potent anti-arthritic effect in collagen-induced arthritis by modifying the T cells balance. *Phytopharmacology*. 19:882–889.

Manokaran S, Jaswant A, Sengottuvelu S, Nandhakumar J, Duraisamy R, Karthikeyan D, Mallegaswari R. 2008. Hepatoprotective activity of Aerva lanata Linn. against paracetamol induced hepatotoxicity in rats. *Res J Pharm Tech*. 1:398–400.

Omoyeni OA, Adeyeye EL. 2009. Chemical composition, calcium, zinc and phytate inter relationships in Aerva lanata (Linn.) Juss. ex schult leaves. *Orient J Chem*. 25:485–488.

Pervykh LN, Karasartov BS, Zapsochynaya GG. 1992. A study of the herb Aerva lanata IV Flavonoid glycosides. *Chem Nat Compd*. 28:509–510.

Porto TS, Furtado NAJC, Heleno VCG, Martins CHG, Da Costa FB, Severiano ME, Silva AN, Veneziani RCS, Ambrosio SR. 2009. Antimicrobial ent-pimarane diterpenes from *Viguiera arenaria* against Gram-positive bacteria. *Fitoterapia*. 80:432–436.

Porto TS, Rangel R, Furtado NAJC, De Carvalho TC, Martins CHG, Veneziani RCS, Da Costa FB, Vinholis AHC, Cunha WR, Vello VCG, Ambrosio SR. 2009. Pimarane-type diterpenes: antimicrobial activity against oral pathogens. *Molecules*. 14:191–199.

Ravagnandan P, Arul RC, Sophia D, Sterlin T, Gopalakrishna VK. 2012. Elemental analysis of *A. lanata* (L.) by EDX method. *Int Res J Pharm*. 3:218–220.

Rajesh R, Chitra K, Paarakh PM. 2011. *Aerva lanata* (Linn.) Juss Ex Schult. – an overview. *Indian J Nat Prod Resour*. 2:5–9.

Saleh NAM, Mansour RMA, Markham KR. 1990. An acetylated isorhamnetin glycoside from *Aerva javanica*. *Phytochemistry*. 29:1344–1345.

Savadi R, Alagawadi K. 2009. Antifertility activity of Ethanolic extracts of plumbago indica and Aerva lanata on albino rats. *Int J Green Pharm*. 3:230–233.

Severiano ME, Simao MR, Porto TS, Martins CHG, Veneziani RCS, Furtado NAJC, Arakawa NS, Said S, De Oliveira DCR, Cunha WR, et al. 2010. Anticariogenic Properties of ent-pimarane diterpenes obtained by microbial transformation. *Molecules*. 15:8553–8566.

Shirwaikar A, Issac D, Malini S. 2004. Effect of Aerva lanata on cisplatin and gentamicin models of acute renal failure. *J Ethnopharmacol*. 90:81–86.

Soundararajan P, Mahesh R, Ramesh T, Begum VH. 2006. Effect of Aerva lanata on calcium oxalate urolithiasis in rats. *Indian J Exp Biol*. 44:981–986.

Soundararajan P, Mahesh R, Ramesh T, Begum VH. 2007. Hypolipidemic activity of Aerva lanata on ethylene glycol induced calcium oxalate urolithiasis in rats. *Pharmacol Online*. 1:557–563.

Tsukamoto S, Tomise K, Aburatani M, Onuki H, Hirotta H, Ishihara-jima E, Ohta T. 2004. Isolation of cytochrome P450 inhibitors from strawberry fruit, *Fragaria ananassa*. *J Nat Prod*. 67:1839–1841.

Vetrivelvan T, Jegadeesan M, Senthil PM, Murali NP, Sasikumar K. 2000. Diuretic and antiinflammatory activities of Aerva lanata in rats. *Indian J Pharm Sci*. 62:300–302.

Wang JP, Zhou YM, Ye YJ, Shang XM, Cai YL, Xiong CM, Wu YX, Xu HX. 2011. Topical anti-inflammatory and analgesic activity of kirenol isolated from Siegesbeckia orientalis. *J Ethnopharmacol*. 137:1089–1094.

Wang LL, Hu LH. 2006. Chemical constituents of Siegesbeckia orientalis L. *J Integr Plant Biol*. 48:991–995.

Xiang Y, Zhang H, Fan CQ, Yue JM. 2004. Novel diterpenoids and diterpenoid glycosides from Siegesbeckia orientalis. *J Nat Prod*. 67:1517–1521.

Zapsochynaya GG, Pervykh LN, Kurkin VA. 1991. A study of the herb Aerva lanata. III. Alkaloids. *Chem Nat Compd*. 27:336–340.
Zapesochnaya GG, Kurkin VA, Okhanov VV, Perzykh LN, Miroshnilov AI. 1991. Structure of the alkaloids of *Aerva lanata*. Chem Nat Compd. 27:725–728.

Zapesochnaya GG, Kurkin VA, Okhanov VV, Miroshnikov AI. 1992. Canthin-6-one and β-carboline alkaloids from *Aerva lanata*. Planta Med. 58:192–196.

Zha-Jun Z, Han-Dong S, Hou-Ming W, Jian-Min Y. 2003. Chemical components from the fungus *Engleromyces goetzei*. Acta Bot Sin. 45:248–252.