

**ANTINOCICEPTIVE ACTIVITY OF STIGMOSTEROL-3-GLYCERYL-2-LINOLEIATE, CAMPESTEROL AND DAUCOSTEROL ISOLATED FROM *AERVA LANATA* LINN. AERIAL PARTS**HEMALATHA KAMURTHY<sup>1\*</sup>, SUMALATHA. Ch<sup>2</sup>, SUNANDAN RAO. N<sup>2</sup>, MUVVLA SUDHAKAR<sup>3</sup>.Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secundrabad-14, Hyderabad, Andhra Pradesh, India,<sup>2</sup>Lalitha College of Pharmacy, Ghatkeser, Hyderabad, Andhra Pradesh, India, Email: kamurthy18@gmail.com

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**ABSTRACT**

Objective: To evaluate the antinociceptive activity for stigmosterol-3-glycerol-2'-linoleate, campesterol and daucosterol isolated compounds from aerial parts of *Aerva lanata*. Methods: Antinociceptive activity was evaluated for all the isolated compounds using acetic acid induced writhing method and tail immersion method in mice at a dose of 100 and 200 mg/kg b.w. Results: The results indicated stigmosterol-3-glycerol-2'-linoleate, campesterol and daucosterol ( $P < 0.01$ ) compounds from methanolic extract of *A. lanata* at a dose level of 100 and 200 mg/kg p.o., showed a significant effect in acetic acid-induced writhing method (peripheral analgesic activity), whereas in tail immersion method (central analgesic activity), at same dose levels, the isolates exhibited significant ( $P < 0.01$ ) analgesic activity at 180 min and moderate ( $P < 0.05$ ) analgesic activity at 120 min as compared to control. Conclusion: We concluded that, stigmosterol-3-glycerol-2'-linoleate, campesterol and daucosterol compounds possess potential analgesic effects which are most likely mediated by their anti-inflammatory activity rather than through opioid receptor system.

**Keywords:** *Aerva lanata*, Antinociceptive, Writhing, Formalin, Stigmosterol, Campesterol, Daucosterol.**INTRODUCTION**

*Aerva lanata* belongs to the Family Amaranthaceae, known as Kurandaka (Sanskrit). It is a bushy, prostrate tomentose herb. The plant is used by Ayurvedic practitioners for a number of ailments. It is used for the treatment of gonorrhoea, kidney disorders, cutaneous infections, sugar in urine and eye complaints. The roots are used in the treatment of headache. The plant is regarded as a demulcent on the Malabar Coast<sup>1,2</sup>. A leaf decoction is prepared as gargle for treating sour-throat and used in various complex treatment against guinea-worms<sup>3</sup>. It is valued for cough in Ceylon; also as a vermifuge for children. The Meena tribals of the Sawaimadhopur district of Rajasthan give orally the juice of the roots to patients of liver congestion, jaundice, biliousness and dyspepsia. They also give decoction of the whole plant to cure pneumonia, typhoid and other prolonged fevers<sup>4</sup>. Root and flower decoction is given to treat headache. Root decoction is used as an antidote for snakebite. Root powder is used as tooth paste to treat toothache. The flowers are used in dysentery, diarrhoea and bronchitis<sup>5</sup>. Benzene and alcoholic extracts of *A. lanata* was investigated in the rat to evaluate the anti-inflammatory activity<sup>6</sup>. The alcoholic extract of *A. lanata* was tested for Anti-diabetic activity<sup>7</sup>. Studies have shown hydroalcoholic extract of *A. lanata* possesses hepatoprotective activity against paracetamol-induced hepatotoxicity in rats<sup>8</sup>. This paper describes the isolation, characterization of phytoconstituents and evaluation of antinociceptive activity from isolates of *Aerva lanata* aerial parts.

**MATERIALS AND METHODS****Experimental**

The melting points were determined on a perfit apparatus. The IR spectra were recorded in KBr pellet in Win IR FTS 135 instrument (Biorad, USA), <sup>1</sup>H NMR (300MHz) and <sup>13</sup>C NMR (300MHz) spectra were recorded by Bruker spectropin NMR instrument in CDCl<sub>3</sub> using as TMS as internal standard. ESIMS were scanned at 70 eV on Jeol D-300 instrument (Jeol, USA). Column chromatography was performed on silica gel (Merck, 60-120 mesh) and thin layer chromatography on silica gel G-coated TLC plates (Merck).

**Plant material**

The whole plant of *Aerva lanata* Linn was collected from local areas of Hyderabad dried in the shade at room temperature. The plant was authenticated by Dr. B. Bhadraiah, HOD, Department Of Botany, Osmania University, Hyderabad. The Voucher specimen (ALL/MRCP/2011/11) was deposited in herbarium at the Malla Reddy College of Pharmacy, Dhulapally, Hyderabad, A.P., India.

**Extraction and isolation of compounds**

The dried and coarsely powdered aerial parts of plant (2.0 kg) were extracted with methanol in soxhlet apparatus. The extracts were combined and the solvent evaporated under reduced pressure to obtain a dark brown viscous mass (109 g). The dried methanolic extract was dissolved in minimum amount of methanol and adsorbed on silica gel (95 g) to form slurry. The slurry was air-dried and chromatographed over silica gel column prepared in petroleum ether (60-80°). The column was eluted with petroleum ether, chloroform and ethyl acetate in order of increasing polarity to isolate the following compounds.

Elution of column with petroleum ether: chloroform (9.5:0.5) gave yellowish viscous mass of compound 1, R<sub>f</sub>: 0.91 (n:hexane :ethyl acetate; 8.5:1.5); m.p. 52-55°C, yield 1.54 g, whereas the elution of column with petroleum ether -chloroform (5:5) gave orange yellowish needles of compound 2, R<sub>f</sub>: 0.44 (n:hexane :ethyl acetate: glacial acetic acid; 7.5:2.0:0.5); m.p. 157-159 °C, yield 1.82 g and finally the elution of column with chloroform: ethyl acetate (4:6) afford the pale yellowish white solid of compound 3 R<sub>f</sub>: 0.63 (n:hexane :ethyl acetate: glacial acetic acid; 6.5:3.0:0.5); m.p. 157-159 °C, yield 1.69 g respectively.

**Animals**

Institutional Animal ethical committee (IAEC) approval was obtained from Malla Reddy Group of Institutions before conducting the experiments. Albino mice (25-30 g) of either sex were housed under standard laboratory conditions. The animals had free access to food and water. The Animal Ethical Committee of the Institute approved all the protocols of the study (Registration No. 1201/ac/09/CPSCSE).

**Antinociceptive activity****Acetic acid-induced writhing**

Mice were divided into eight groups with five in each group. Control group I was interaperitoneally treated with 0.6 % w/v of sodium CMC suspension orally, standard group II received diclofenac sodium at a dose of 50mg/kg and test groups III-VIII received stigmosterol-3-glycerol-2'-linoleate, campesterol and daucosterol compounds at dose of 100 and 200 mg/kg body weight (b. w) respectively (Table no.1). After 30 min, all the groups were administered with 0.6 % acetic acid at a dose of 10mL/kg intraperitoneally. The number of writhing for each mouse was counted for 20 min starting 10 min after injection of acetic acid and the percentage inhibition of writhing was calculated<sup>9</sup>.

**Table 1: Effect of isolated compounds from *Aerva lanata* aerial parts on acetic-acid induced writhing method.**

Treatment	Dose (mg/kg)	Number of writhing	% inhibition
Control	-----	82.20±2.01	----
Diclofenac sodium	50	25.50±1.74**	68.98
Stigmasterol-3-glyceryl-2'-linoleate	100	33.20±1.08**	59.61
	200	27.45±0.81**	66.14
Campesterol	100	34.80±1.08**	53.28
	200	29.60±1.56**	63.99
Dacusterol	100	35.20±0.66**	57.17
	200	30.38±0.76**	62.08

All the values were expressed as mean ±SEM. \*\*P<0.01 when compared to control group.

#### Tail immersion method

The mice were divided into eight groups with five in each group. Control group I was intraperitoneally treated with 0.6 % w/v of sodium CMC suspension orally, standard group II received aspirin at a dose of 50 mg/kg and test groups III -VIII received stigmasterol-3-glyceryl-2'-linoleate, campesterol and daucosterol compounds at dose of 100 and 200 mg/kg b.w respectively. 1-2 cm of the mice tail

was immersed in warm water and kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latent period of 20 sec was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60, 120 and 180 min (Table no.2) after the administration of test drugs<sup>10</sup>.

**Table 2: Effect of isolated compounds from *Aerva lanata* aerial parts on tail immersion method.**

Treatment	Dose (mg/kg)	Tail flick time in min				
		After 0 min	After 30 min	After 60 min	After 120min	After 180 min
Control	-----	4.3 ± 0.6	5.5 ± 0.14	6.0 ± 0.21	7.0 ± 0.3	6.9±0.23
Diclofenac sodium	50	6.1±0.22	8.2 ± 0.11	9.0 ± 0.8*	23.2 ± 2.0**	34.3±7.0**
Stigmasterol-3-glyceryl-2'-linoleate	100	5.2 ± 0.72	5.9 ± 0.38	6.4 ± 0.43	17.7 ± 0.28*	28.9±0.75**
	200	5.8 ± 0.66	6.7 ± 0.39	7.8 ± 0.61	19.5 ± 0.34*	32.5±0.80**
Campesterol	100	4.6 ± 0.48	4.9 ± 0.75	5.9 ± 0.32	16.4 ± 0.73*	26.5±0.58**
	200	5.1 ± 0.31	5.8 ± 0.85	6.2 ± 0.53	17.0 ± 0.42*	30.3±0.08**
Dacusterol	100	5.0 ± 0.55	5.4 ± 0.29	6.0 ± 0.95	16.8 ± 0.67*	27.8±0.19**
	200	5.5 ± 0.18	6.2 ± 0.67	6.9 ± 0.49	18.5 ± 0.54*	31.9±0.06**

All the values were expressed as mean ±SEM. \*P<0.05, \*\*P<0.01 when compared to control group.

#### Statistical Analysis

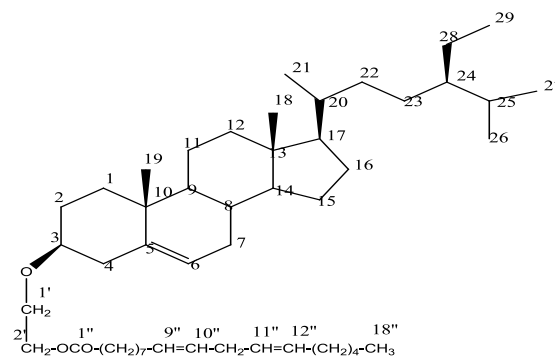
All observations were presented as mean± SEM. The data was analyzed by one way ANOVA and followed by Dunnett's multiple comparison test. A difference of P<0.01 was considered as statistically significant.

#### RESULT AND DISCUSSION

##### Structure Elucidation of isolated compounds

##### Compound 1

Elution of column with petroleum ether: chloroform (9.5:0.5) gave yellowish viscous mass of compound 1,  $R_f=0.91$  (n:hexane :ethyl acetate; 8.5:1.5); m.p.52-55°C, yield 1.54 g; IR (KBr):3430, 2921, 2815, 1736,1655, 1460, 1377 $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): $\delta$  5.49 (m, 1H, H-10"), 5.48 (m, 1H, vinylic protons, H-6), 5.43 (m, 1H, H-12"), 5.42 (m, 1H, H-6), 5.40 (m, 1H, H-9'), 5.39 (m, 1H, H-22), 5.37 (m, 1H, H-13"), 3.70 (m, 2H, H-1'), 2.82 (m, 1H, CH-O- group, protons, H-3), 2.40 (s, 2H, H-2"), 2.38 to 1.2 (m, 18H, 19 X  $\text{CH}_2$  & 1H of  $\text{CH}_3$  group), 1.06 (s, 3H,  $\text{CH}_3$  group, H-18"), 1.14 and 1.03 (m, 6H, 2X $\text{CH}_3$  group H-19, 18), 0.96 to 0.71 (m, 9H, 3X  $\text{CH}_3$  group, H-26, 27, 29);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ): $\delta$  37.8 (C-1 & 10), 31.9 (C-2), 70.1 (C-3), 41.3 (C-4), 141.9 (C-5), 124.3 (C-6), 32.1 (C-7), 30.1 (C-8), 37.9 (C-9), 21.1 (C-11), 42.3 (C-12), 46.1 (C-13), 58.6 (C-14), 24.1 (C-15), 27.2 (C-16), 62.0 (C-17), 11.3 (C-18), 22.9 (C-19), 40.9 (C-20), 20.5 (C-21), 135.3 (C-22), 130.1 (C-23), 59.0 (C-24), 30.9 (C-25), 20.1 (C-26), 20.6 (C-27), 30.3 (C-28), 19.1 (C-29), 196.8 (C-1'), 37.3 (C-2'), 30.1 (C-2" to 7"), 39.2 (C-8"), 135.1 (C-10"), 121.1 (C-10"), 30.1 (C-11"), 128.1 (C-12"), 135.2 (C-13"), 25.3 (C-14" to 17"), 14.2 (C-18"). EIMS (m/z) (rel.int): 705 [M+H], ( $\text{C}_{48}\text{H}_{80}\text{O}_3$ ), (Figure 1). From the M. P, I. R,  $^1\text{H NMR}$ ,  $^{13}\text{C NMR}$  and FABMASS spectra compound 1 was ascertained as " Stigmasterol-3-glyceryl-2'-linoleate".

**Figure 1: Stigmasterol-3-2'-linoleate**

##### Compound 2

Elution of column with petroleum ether -chloroform (5:5) gave orange yellowish needles of compound 2,  $R_f=0.44$  (n:hexane :ethyl acetate: glacial acetic acid; 7.5:2.0:0.5); m.p.157-159 °C, yield 1.82 g; IR(KBr): 3405, 2942, 2853, 1712, 1461, 376, 1048 $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 5.42 (s, 1H, olefinic proton), 3.41 (s, 1H, OH group), 3.28 (m, 1H, vinylic proton H-3), 2.42 to 1.02 (m, 20H 10 X  $\text{CH}_2$  protons), 1.65 (s,  $\text{CH}_2$  protons), 0.91 to 0.75 (m, 6 X  $\text{CH}_3$  group H-18, 19, 21, 26, 27 & 28), 0.88 (m, 9H, 3 X  $\text{CH}_3$  group H-30, 32 & 33);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ): $\delta$  33.6 (C-1 & C-12), 34.1 (C-2), 77.0 (C-3), 62.1 (C-4), 130.1 (C-5), 117.8 (C-6), 32.0 (C-7), 31.6 (C-8), 68.4 (C-9, C-14 & C-17), 37.2 (C-10 & C-20), 24.3 (C-11), 32.4 (C-13 & C-23), 25.1 (C-15), 25.3 (C-16), 13.9 (C-18), 14.5 (C-19), 29.5 (C-21), 30.1 (C-22), 39.1 (C-24), 32.6 (C-25), 28.1 (C-26 & 27), 15.3 (C-28); EIMS (m/z) (rel.int): 400 [M<sup>+</sup>] ( $\text{C}_{28}\text{H}_{40}\text{O}$ ), (Figure 2). From the M. P, I. R,  $^1\text{H NMR}$ ,  $^{13}\text{C NMR}$  and FABMASS spectra compound 2 was ascertained as " Campesterol".

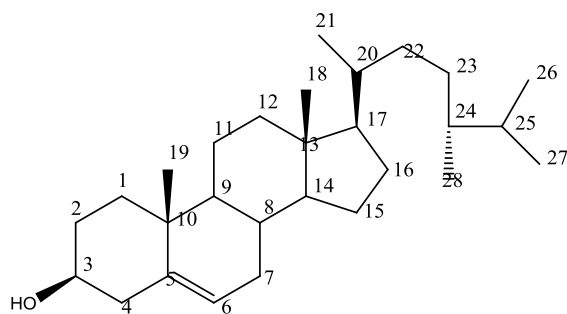


Figure 2: Campesterol

### Compound 3

The elution of column with chloroform: ethyl acetate (4:6) afford the pale yellowish white solid of compound 3  $R_f=0.63$  (n:hexane :ethyl acetate: glacial acetic acid; 6.5:3.0:0.5); m.p.157-159 °C, yield 1.69 g; IR(KBr): 2922, 2825, 1735,1462, 1376, 1171 $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR ( $\text{CDCl}_3$ ): 5.41 (s, 1H, CH group C-6), 5.23 (d, 1H, O-CH group C-1'), 3.73 to 3.41 (m, 6H, CH groups of sugar moiety), 3.65 to 3.54 (m, 4H, OH groups oH-2', 3', 4', 6'), 2.84 (m, 1H, CH group H-3), 2.38 to 1.04 (m, 2H,  $\text{CH}_2$  &  $\text{CH}_3$  group), 0.91 (m, free  $\text{CH}_3$  group, sugar moiety, H-26, 27, 29);  $^{13}\text{C}$ NMR( $\text{CDCl}_3$ ): 37.5 (C-1), 29.8 (C-2), 82.9 (C-3), 39.9 (C-4), 140.5 (C-5), 121.9 (C-6), 32.1 (C-8), 31.8 (C-9), 50.5 (C-10), 21.1 (C-11), 40.5 (C-12), 42.7 (C-13), 59.6 (C-14), 30.3 (C-15), 24.6 (C-16), 57.3 (C-17), 12.5 (C-18), 19.9 (C-19), 37.1 (C-20), 20.4 (C-21), 35.6 (C-22), 32.7 (C-23), 41.2 (C-24), 26.5 (C-25), 11.9 (C-26), 20.3 (C-27), 27.1 (C-28), 11.8 (C-29), 109.9 (C-1'), 75.5 (C-2'), 72.3 (C-3'), 71.5 (C-4'), 81.5 (C-5'), 6.5 (C-6'); EIMS (m/z) (rel.int): 577 [M+H] ( $\text{C}_{35}\text{H}_{60}\text{O}_6$ ), (Figure 3). From the M. P, I. R,  $^1\text{H}$ NMR,  $^{13}\text{C}$ NMR and FABMASS spectra compound 3 was ascertained as "Daucosterol".

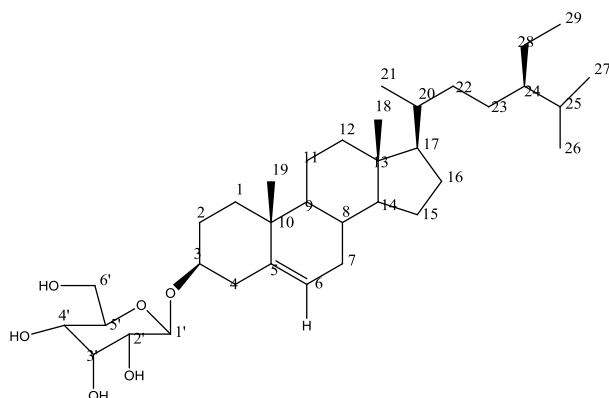


Figure 3: Daucosterol

### Acetic acid-induced writhing

The isolated compounds (Stigmasterol-3-glyceryl-2'-linoleate, campesterol and daucosterol) were screened for peripheral analgesic activity using acetic acid induced writhing method in mice and the results were compared with diclofenac sodium 50 mg/kg b.w. The Stigmasterol-3-glyceryl-2'-linoleate isolate exhibited 62.08 and 66.14 % writhing inhibition at the doses 100 and 200 mg/kg b.w respectively, campesterol isolate exhibited 59.61 and 63.99% writhing inhibition at the doses 100 and 200 mg/kg b.w respectively and daucosterol isolated exhibited 53.28 and 57.17 % writhing inhibition at the doses 100 and 200 mg/kg b.w respectively (Table no.1) as compared to 68.97 % by standard (diclofenac sodium, 50 mg/kg b. w).

The acetic acid-induced writhing test in mice is regarded as a model of inflammation pain, and it is used as screening tool for evaluation of analgesic or anti-inflammatory agents. Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors<sup>11</sup>. It has been suggested that acetic acid acts by releasing endogenous inflammatory mediators or irritation of the visceral

surface, which leads to the liberation of histamine, kinins, prostanoids, serotonin and substance P. It is a sensitive procedure to evaluate peripherally- and centrally- acting analgesics<sup>12-16</sup>. The nociceptive activity of acetic acid may be due to cytokine release, such as TNF- $\alpha$ , interleukin-1 $\beta$  and interleukin-8, by resident peritoneal macrophages and mast cells<sup>17</sup>. The intraperitoneal injection of acetic acid induced an increase in the concentration of glutamate and aspartate in the cerebrospinal fluid<sup>18</sup>. We have reported that the *Aerva lanata* isolated compounds were inhibited, in a dose-dependent manner, the nociception induced by acetic acid, when compared with the well-known NSAID, diclofenac sodium.

### Tail immersion method

The isolates compounds (Stigmasterol-3-glyceryl-2'-linoleate, campesterol and daucosterol) from *Aerva lanata* at dose levels 100 and 200mg/kg b.w exhibited a significant ( $P<0.01$ ) inhibition of tail-flicking response at 180 min and less significant ( $P<0.05$ ) inhibition of tail flicking response at 120 min. The effects were comparable with those of the reference standard (aspirin, 50 mg/kg), that showed significant inhibition of the tail-flicking response (Table no.2).

The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena. But the extend of activity shown by the isolated compounds are less than that of the standard drug nalbuphine but many fold more than that of the control group, which justifies its activity.

Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain<sup>19</sup>. The extract inhibited both mechanisms of pain, suggesting that the isolates from *Aerva lanata* aerial parts may act as a narcotic analgesic.

### CONCLUSION

In conclusion, the study demonstrated the analgesic activity of isolated compounds (stigmasterol-3-glyceryl-2'-linoleate, campesterol and daucosterol) in the test models of nociception induced by chemical stimuli. It is suggested that the observed antinociceptive activity of stigmasterol-3-glyceryl-2'-linoleate and campesterol might be mediated both peripherally and centrally. The antinociceptive activity of daucosterol could be related to its anti-inflammatory activity. These observations merits further studies regarding the precise site and the mechanism of action.

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