



## Chemical Constituents From the Aerial Roots of *Ficus benghalensis* L., Leaves of *Nyctanthes arbor-tristis* L. and Roots of *Verbesina encelioides* (Cav.) Benth. et Hook. f.

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### Abstract

*Ficus benghalensis* L. (Moraceae) is a native to tropical Asia. Its aerial roots are styptic and taken to alleviate biliousness, dysentery, liver inflammation, jaundice, spermatorrhoea, syphilis and obstinate vomiting. *Nyctanthes arbor-tristis* L. (Oleaceae) is distributed in the eastern Asia including India, Nepal, Pakistan, Thailand and Indonesia. Its leaves are used to treat acidity, asthma, bronchitis, cough, dyspepsia, fevers, hypertension, malaria, menstrual cramps, piles, rheumatism, sciatica, snake bites, strangury and to expel intestinal worms. *Verbesina encelioides* (Cav.) Benth. et Hook. f. (Asteraceae) is a native to southeastern North America and one of the most common weeds in northern India after the rainy season. Its roots are used to cure bladder inflammation and also as a blood purifier. The air-dried plant parts were exhaustively extracted with methanol individually in a Soxhlet apparatus. The concentrated methanol extracts were adsorbed on silica gel for column and chromatographed over silica gel column separately. The columns were eluted with petroleum ether, chloroform and methanol successively to isolate the phytoconstituents. Phytochemical investigation of the aerial roots of *F. benghalensis* afforded *n*-tritriacontan-10-one (**1**), 30-lauryloxy-urs-12-en-3 $\beta$ -olyl butyrate (30-lauryloxy- $\alpha$ -amyirin 3-butyrate, **2**) and urs-12-en-23,6 $\alpha$ -olide 3 $\beta$ -olyl palmitate (3-palmityl  $\alpha$ -amyirin-23,6 $\alpha$ -olide, **3**). The leaves of *N. arbor-tristis* furnished two vanillyl glycosidic disters characterized as oleiyl-O- $\alpha$ -D-xylopyranosyl-(2a $\rightarrow$ 1b)-O- $\alpha$ -D-xylopyranosyl-2b-vanillyl-4b-caproate (oleiyl-O- $\alpha$ -D-dixylosyl vanillyl caproate, **4**) and oleiyl-O- $\alpha$ -D-arabinopyranosyl-(2a $\rightarrow$ 1b)-O- $\alpha$ -D-arabinopyranosyl--(2b $\rightarrow$ 1c)-O- $\alpha$ -D-arabinopyranosyl--(2c $\rightarrow$ 1d)-O- $\alpha$ -D-arabinopyranosyl-2d-vanillyl-4d-caproate (oleiyl-O- $\alpha$ -D-tetra-arabinosyl vanillyl caproate, **5**). The roots of *V. encelioides* produced tetracosan-1-olyl 1-tetradecanoate (lignoceryl myristate, **6**),  $\beta$ -amyirin palmitate (**7**), urs-12-en-3 $\beta$ -olyl oleate ( $\beta$ -amyirin oleate, **8**) and  $\beta$ -amyirin stearate (**9**). The structures of these phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

### 1 Introduction

*Ficus benghalensis* L., syn. *F. banyana* Oken, *F. indica* L., *F. procera* Salisb., *Urostigma benghalense* (L.) Gasp. (Moraceae), known as bargad, bar and Indian banyan, is a native to tropical Asia from India through Myanmar, Thailand, southern China and Malaysia. It is a large, fast growing,

evergreen to deciduous tree up to 30 m tall, with spreading branches and many aerial roots; leaves stalked, ovate-cordate, 3-nerved, entire; petiole with a broad smooth greasy gland at the apex, compressed, downy; female flowers sessile, small; gall flowers numerous, pedicellate; fruits in axillary pairs, of a cherry size, pinkish-red, round and downy<sup>1</sup>. The leaves are

used as an aphrodisiac, astringent, diaphoretic and to treat abdominal pain, abscesses, biliousness, cuts, dysentery, diarrhoea, fever, inflammation, ulcer, vaginal complaints and vomiting. The plant latex is aphrodisiac, maturant, tonic and vulnerary; useful to relieve bruises, dysentery in children, fever, gonorrhoea, inflammations, lumbago, mumps, nose-diseases, piles, rheumatism, ringworm, cracked soles, spermatorrhoea, toothache and wounds. The fruits are regarded as an aphrodisiac, refrigerant and tonic. The aerial roots are styptic and taken to alleviate biliousness, dysentery, inflammation of liver, jaundice, spermatorrhoea, syphilis and obstinate vomiting. The bark is antidiabetic, astringent, diuretic and tonic; effective to cure leucorrhoea. The fruit is ingested as a refrigerant and tonic. The stem bark with the fruits of *Embellia ribes* is given to prevent pregnancy. An infusion of the twigs is drunk as a remedy for haemoptysis<sup>2-4</sup>. A stem decoction is drunk to cure dysentery<sup>5</sup>.

The leaves of *F. benghalensis* contained quercetin-3-galactoside, rutin, catechin, genistein, friedelin, taraxoseterol, lupeol,  $\beta$ -amyrin, psoralen, bergapten, proteins and  $\beta$ -sitosterol<sup>3,4,6</sup>. The bark yielded 5,7-dimethyl ether of anthocyanidins, perlagonidin, long chain aliphatic ketones,  $\beta$ -sitosterol- $\alpha$ -D-glucose, phytosterols, meso-inositol, linolyl and oleyl glucosides, keto-*n*-cosanyl stearate, hydroxypentacosanyl palmitate and phenyl tetradecanyl oleate<sup>7-9</sup>. Taraxasterol tiglate was isolated from the heart wood<sup>3,4</sup>. The seeds afforded lectins<sup>10</sup>. The seed oil was consisted of palmitic, oleic, linoleic, linolenic, vernolic, stearic, malvalic, sterculic, lauric and myristic acids<sup>11</sup>. The aerial roots possessed flavonoids, bengalensinone, benganoic acid, lupanyl acetate, 3-acetoxy-9(11),12-ursandiene, stigmasterol, 4-hydroxyacetophenone, 4-hydroxybenzoic acid, 4-hydroxymellein and *p*-coumERIC acid<sup>12,13</sup>. The leaf essential oil was composed mainly of  $\alpha$ -cadinol, germacrene-D-4-ol,  $\gamma$ -cadinene and  $\alpha$ -muurolene<sup>14</sup>.

*Nyctanthes arbor-tristis* L., syn. *N. dentata* Blume, *N. tristis* Salisb., *Bruschia macrocarpa* Bertol., *Scabrita triflora* L. (Oleaceae), known as parijat, har singar, night-flowering jasmine and queen of the night, is distributed in the eastern Asia including India, Nepal, Pakistan, Thailand and Indonesia. It is a shrub or a small tree up to 10 m tall, with flaky grey bark; leaves are opposite, simple, with an entire margin; flowers fragrant, corolla white with an orange-red centre, in clusters of two to seven; fruits brown, cordate capsule with two sections each containing a single seed<sup>15</sup>. The leaves are bitter tonic, cholagogue, diaphoretic, diuretic, febrifuge, anti-inflammatory, antispasmodic, hypotensive, laxative and respiratory stimulant; used to treat congestion caused by asthma, dry cough, bronchitis, acidity, dyspepsia, fevers, hypertension, malaria, menstrual cramps, piles, rheumatism, sciatica, snake bites and strangury. A leaf extract is given to children to expel roundworms and threadworms<sup>15,16</sup>. The flowers are emmenagogue and

consumed to provoke menstruation. The flower essential oil is effective to relieve dandruff, irritation, swellings associated with arthritis, stress, muscle tension, rheumatism, sore muscles, headaches, injuries, lice infection, menstrual pains, pimples, rashes, sciatica, vertigo and wounds<sup>2,15</sup>.

The leaves of *N. arbor-tristis* contained  $\beta$ -sitosterol, hentriacontane, astragalol, nicotiflorin, nyctanthine, nyctanthic acid,  $\beta$ -amyrin, friedelin, lupeol, oleanolic, tannic, ascorbic and fatty acids, methyl salicylate, resin, volatile oil, carotene, D-mannitol, glucose, fructose, iridoids, benzoic acid and arborides A – D<sup>15, 17-19</sup>. The leaf epicuticular wax was consisted of higher aliphatic hydrocarbons<sup>20</sup>. The flowers yielded nyctanthin, D-mannitol, tannins, glucose, carotenoids,  $\alpha$ -crocetin glycosides, rengyolone, 6-O-trans-cinnamoyl-7 O-acetyl-6- $\beta$ -hydroxyloganin and iridoid glucosides<sup>21</sup>. The flower oil was composed of  $\alpha$ -pinene, p-cymene, 1-hexanol, methyl heptanone, phenyl acetaldehyde, 1-decenol, phytol, 2-methyl octadecane, nonadecane, methyl myristate, cis-9-tricosene, geranyl geraniol and anisaldehyde<sup>15,19, 22-24</sup>. The seeds possessed lipids, nyctanthic acid, 3,4-secotriterpene acid, a polysaccharide,  $\beta$ -sitosterol, arbortristosides A, B, D and E and 6- $\beta$ -hydroxyloganin<sup>25</sup>. The stems furnished naringenin-4'-O- $\beta$ -glucopyranosyl- $\alpha$ -xylopyranoside and  $\beta$ -sitosterol<sup>26</sup>.

*Verbesina encelioides* (Cav.) Benth. et Hook. f., syn. *V. australis* Baker, *V. scabra* Benth., *Ximenesia encelioides* Cav. (Asteraceae), known as golden crownbeard, gold weed, wild sunflower, cowpen daisy, butter daisy, American dogweed and South African daisy, is a native to southeastern North America and parts of Central and South America. It is naturalized in the Middle East, Spain, Argentina, India, Australia, the Pacific islands and other warm regions of the world<sup>27</sup>. It is one of the most common weeds in northern India, germinating after the rainy season and invading crop fields<sup>28</sup>. It is an annual herb, up to 150 cm high; with short-hairy stem, alternate, lanceolate to triangular-ovate leaves, bases broadly cuneate to truncate, dull green, 3-veined, with dentate margin, and strigose-canescens hairs; inflorescence has 1-many heads, ray flowers orange-yellow, disk flowers yellow to light brown; achenes grayish brown, obovate, flattened, with wide wing<sup>29,30</sup>. The plant is used to treat cancer, fevers, gastrointestinal disturbance, itch, gum sores, piles, skin problems, snake and spider bites and warts. The roots are used for retention of water, bladder inflammation and also as a blood purifier. Leaf juice is taken as a laxative, a leaf paste is applied to cure rheumatism. It is a toxic plant for livestock<sup>31,32</sup>.

*V. encelioides* plant contained ceryl alcohol, galegine,  $\beta$ -sitosterol, stigmasterol, their 3- $\beta$ -D-glucosides,  $\beta$ -sitosterol galactoside, hentriacontol,  $\alpha$ - and  $\beta$ -amyrins, benzyl-2, 6-dimethoxy benzoate, bornyl ferulate, *p*-coumaric, linoleic and linolenic acids, phytol, taraxasterol acetate, pseudotaraxasterol, its acetate, pseudotaraxastenone, 16 $\beta$ -hydroxy-

pseudotaraxasterol-3 $\beta$ -palmitate and quercetin-3-O- $\beta$ -D-galactopyranoside<sup>33-36</sup>. The flowers yielded quercetin-3-galactoside, quercetin-3-galactoside-7-glucoside and quercetin-3-xyloside-7-glucoside<sup>37,38</sup>. The flower essential oil was composed of pseudolimonene,  $\gamma$ -cadinene, 9-epicaryophyllene,  $\gamma$ -eudesmol,  $\delta$ -3-carene, and viridiflorol; the leaf essential oil consisted of  $\gamma$ -caryophyllene,  $\gamma$ -muurolene, germacrene-D,  $\gamma$ -cadinene,  $\delta$ -elemene and borneol as the major constituents<sup>39</sup>. Keeping in view the high reputation and wide application of herbal drugs *F. benghalensis*, *N. arbortristis* and *V. encelioides* in many indigenous medicinal systems, it has been aimed to describe establishment of structures of the phytoconstituents isolated from these plants.

## 2 Materials and Methods

### 2.1. General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were measured on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The <sup>1</sup>H NMR (300 MHz) and <sup>13</sup>C NMR (75 MHz) spectra were scanned on a Bruker DRX instruments using TMS as an internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with direct inlet prob system. The *m/z* values of the more intense peaks are mentioned and the figures in bracket attached to each *m/z* values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F254 precoated TLC plates (Merck, Mumbai, India). Spots were visualized by exposing to iodine vapors and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

### 2.2. Plant materials

The aerial roots of *Ficus benghalensis* were procured from a tree located in Ghaziabad (U.P.), India. The leaves of *Nyctanthes arbor-tristis* were purchased from a local market of Khari Baoli, Delhi. The roots of *Verbesina encelioides* were collected from the Inderprasth Park, Delhi. The plant materials were authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of these plant parts are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

### 2.3. Extraction and isolation

The plant parts (1 kg each) were coarsely powdered and extracted exhaustively with methanol individually in a Soxhlet apparatus. The extracts were concentrated under reduced

pressure to get dark brown masses, 121.4 g, 142.9 g and 112.5 g, respectively. The dried residue (100 g each) was dissolved in a minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) individually to obtain a slurry. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether (b. p. 60 - 80°C) separately. The columns were eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform and chloroform - methanol (99:1, 49:1, 19:5, 9:1, 17:3, 4:1 7:3, 1:1, v/v) mixtures. Various fractions were collected singly and matched by TLC to check homogeneity. Similar fractions having the same R<sub>f</sub> values were combined and crystallized with solvents. The isolated compounds were recrystallized to get the following pure compounds:

### 2.4 Isolation of phytoconstituents from the aerial roots of *Ficus benghalensis*

#### 2.4.1 *n*-Tritriacontan-10-one (1)

Elution of the column with petroleum ether furnished colorless powder of **1**, 208 mg, recrystallized from chloroform-methanol (1:1), m. p. 109 - 110 °C, UV  $\lambda_{max}$  (MeOH): 213 nm; IR  $\nu_{max}$  (KBr): 2921, 2854, 1710, 1635, 1462, 1377, 1260, 1196, 1096, 804, 726 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.53 (2H, m, H<sub>2</sub>-9), 2.15 (2 H, m, H<sub>2</sub>-11), 1.72 (2 H, m, CH<sub>2</sub>), 1.63 (2 H, m, CH<sub>2</sub>), 1.33 (2 H, m, CH<sub>2</sub>), 1.27 (6 H, br s, 3 x CH<sub>2</sub>), 1.25 (44 H, br s, 22 x CH<sub>2</sub>), 0.87 (3 H, t, J = 6.1 Hz, Me-1), 0.83 (3 H, t, J = 6.3 Hz, Me-33); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  193.65 (C-10), 31.19 (CH<sub>2</sub>-11), 30.31 (CH<sub>2</sub>-8), 29.94 (25 x CH<sub>2</sub>), 28.62 (CH<sub>2</sub>), 26.32 (CH<sub>2</sub>), 22.67 (CH<sub>2</sub>), 19.16 (Me-1), 13.55 (Me-33); ESI MS *m/z* (rel. int.): 478 [M]<sup>+</sup> (C<sub>33</sub>H<sub>66</sub>O) (26.3), 351 (2.3), 323 (29.6), 155 (5.7), 127 (4.6).

#### 2.4.2 30-Lauryloxy- $\alpha$ -amyirin 3-butyrate (2)

Elution of the column with chloroform gave colourless crystals of **2**, recrystallized from acetone, 167 mg, m. p. 129 - 130 °C; UV  $\lambda_{max}$  (MeOH): 215 nm (log  $\epsilon$  4.9); IR  $\nu_{max}$  (KBr): 2921, 2853, 1732, 1641, 1463, 1375, 1247, 1182, 1028, 757 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.34 (1H, d, J = 5.1 Hz, H-12), 4.15 (2H, d, J = 7.2 Hz, H<sub>2</sub>-30), 4.06 (1H, dd, J = 4.7, 7.5 Hz, H-3 $\beta$ ), 2.33 (2H, t, J = 5.7 Hz, H<sub>2</sub>-2'), 2.29 (2H, t, J = 4.6 Hz, H<sub>2</sub>-2''), 2.23 - 1.20 (43H, m, 5 CH, 19 x CH<sub>2</sub>), 1.07 (3H, brs, Me-23), 0.98 (3H, brs, Me-25), 0.95 (3H, brs, Me-27), 0.93 (3H, d, J = 6.2 Hz, Me-29), 0.90 (3H, brs, Me-24), 0.88 (3H, brs, Me-28), 0.86 (3H, brs, Me-26), 0.83 (3H, t, J = 6.1 Hz, Me-4'), 0.79 (3H, d, J = 6.2 Hz, Me-12''); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  38.27 (C-1), 27.29 (C-2), 81.04 (C-3), 38.81 (C-4), 55.24 (C-5), 18.31 (C-6), 33.26 (C-7), 39.12 (C-8), 47.36 (C-9), 38.16 (C-10), 23.24 (C-11), 124.40 (C-12), 139.86 (C-13), 52.76 (C-14), 27.91 (C-15), 23.49 (C-16), 36.78 (C-17), 42.13 (C-18), 42.85 (C-19), 30.71 (C-20), 29.57 (C-21), 36.94 (C-22), 28.36 (C-23), 15.98 (C-24), 15.51 (C-25), 17.21 (C-26), 17.16 (C-27), 27.32 (C-28), 24.27 (C-29), 64.31 (C-30), 174.03 (C-1'), 55.92 (C-2'), 31.44 (C-3'), 14.26 (C-4'), 171.12 (C-1''), 40.09 (C-2''), 32.15 (C-3''), 30.18 (C-4''), 29.69

(C-5"), 29.46 (C-6"), 29.18 (C-7", C-8"), 28.59 (C-9"), 25.11 (C-10"), 22.68 (C-11"), 14.21 (C-12"); ESI MS *m/z* (rel.int.): 694 [M]<sup>+</sup> (C<sub>46</sub>H<sub>76</sub>O<sub>4</sub>) (25.6), 623 (5.3), 511 (9.2), 416 (12.8), 278 (11.3), 207 (21.7), 199 (23.4), 191 (8.1), 183 (34.8).

#### 2.4.3 3-Palmityl $\alpha$ -amyirin-23,6 $\alpha$ -olide (3)

Further elution of the column with chloroform gave colourless crystals of **3**, recrystallized from acetone, 216 mg, m. p. 191 - 193 °C; UV  $\lambda_{\max}$  (MeOH): 221 nm (log  $\epsilon$  5.1); IR  $\nu_{\max}$  (KBr): 2925, 2855, 1736, 1640, 1459, 1372, 1245, 1162, 1029, 758 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.12 (1H, t, J = 3.6 Hz, H-12), 4.51 (1H, ddd, J = 4.6, 4.8, 5.7 Hz, H-6 $\beta$ ), 4.13 (1H, dd, J = 5.6, 8.3 Hz, H-3 $\beta$ ), 2.30 (2H, t, J = 7.6 Hz, H<sub>2</sub>-2'), 2.26 (2H, d, J = 7.6 Hz, H<sub>2</sub>-11), 2.23 - 1.31 (19H, m, 5 x CH, 7 x CH<sub>2</sub>), 1.29 (8H, brs, 4 x CH<sub>2</sub>), 1.27 (8H, brs, CH<sub>2</sub>), 1.25 (4H, brs, 2 x CH<sub>2</sub>), 1.23 (4H, brs, 2 x CH<sub>2</sub>), 1.13 (3H, brs, Me-25), 1.06 (3H, brs, Me-24), 1.01 (3H, brs, Me-26), 0.97 (3H, brs, Me-28), 0.91 (3H, brs, Me-27), 0.87 (3H, d, J = 6.1 Hz, Me-29), 0.84 (3H, J = 6.0 Hz, Me-30), 0.79 (3H, t, J = 6.6 Hz, Me-16"); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  38.87 (C-1), 25.90 (C-2), 80.07 (C-3), 37.80 (C-4), 54.60 (C-5), 80.05 (C-6), 33.09 (C-7), 40.84 (C-8), 46.95 (C-9), 36.12 (C-10), 22.95 (C-11), 123.66 (C-12), 138.88 (C-13), 41.38 (C-14), 28.39 (C-15), 27.50 (C-16), 38.94 (C-17), 59.55 (C-18), 39.08 (C-19), 37.02 (C-20), 30.57 (C-21), 36.92 (C-22), 169.96 (C-23), 15.12 (C-24), 16.24 (C-25), 16.94 (C-26), 22.70 (C-27), 20.86 (C-28), 20.69 (C-29), 20.43 (C-30), 170.11 (C-1'), 59.34 (C-2'), 32.44 (C-3'), 30.57 (C-4'), 29.56 (C-5'), 29.41 (C-6'), 29.27 (C-7'), 30.18 (C-8'), 29.69 (C-9'), 29.46 (C-10'), 29.18 (C-11'), 28.56 (C-12'), 25.43 (C-13'), 24.28 (C-14'), 22.64 (C-15'), 13.64 (C-16'); ESI MS *m/z* (rel.int.): 692 [M]<sup>+</sup> (C<sub>46</sub>H<sub>76</sub>O<sub>4</sub>) (5.4), 473 (20.1), 453 (11.4), 436 (23.7), 256 (12.8), 239 (7.5), 234 (14.2), 218 (6.2).

### 2.5 Isolation of phytoconstituents from the leaves of *Nyctanthes arbor tristis*

#### 2.5.1 Oleiyl-O- $\alpha$ -D-dixylosyl vanillyl caproate (4)

Elution of the column with chloroform-methanol (19 : 1) afforded pale yellow crystals of **4**, purified from chloroform-methanol (1:1), 258 mg, m. p. 116 - 117 °C, UV  $\lambda_{\max}$  (MeOH): 210 nm (log  $\epsilon$  3.6); IR  $\lambda_{\max}$  (KBr): 3510, 3405, 3365, 2922, 2853, 1730, 1629, 1522, 1458, 1375, 1272, 1170, 1097, 985, 769 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.36 (1H, m, H-9), 5.32 (1H, m, H-10), 2.34 (2H, t, J = 7.6 Hz, H<sub>2</sub>-2), 2.05 (2H, m, H<sub>2</sub>-8), 2.01 (2H, m, H<sub>2</sub>-11), 1.82 (2H, m, CH<sub>2</sub>), 1.55 (2H, m, H<sub>2</sub>-3), 1.29 (8H, m, 4 x CH<sub>2</sub>), 1.25 (4H, brs, 2 x CH<sub>2</sub>), 1.21 (8H, m, 4 x CH<sub>2</sub>), 0.86 (3H, t, J = 6.8 Hz, Me-18), 5.02 (1H, d, J = 6.4 Hz, H-1a), 4.34 (1H, dd, J = 6.4, 7.2 Hz, H-2a), 4.17 (1H, m, H-3a), 4.15 (1H, m, H-4a), 3.63 (2H, d, J = 9.3 Hz, H<sub>2</sub>-5a), 4.95 (1H, d, J = 6.7 Hz, H-1b), 4.63 (1H, dd, J = 6.7, 7.6 Hz, H-2b), 4.13 (1H, m, H-3b), 4.20 (1H, m, H-4 b), 3.80 (2H, d, J = 9.1 Hz, H<sub>2</sub>-5b), 7.50 (1H, d, J = 1.2 Hz, H-2'), 6.73 (1H, d, J = 8.5 Hz, H-5'), 6.83 (1H, dd, J = 1.2, 8.5 Hz, H-6'), 3.67 (3H, br s, OMe), 2.29 (2H, t, J = 7.2

Hz, H<sub>2</sub>-2"), 1.53 (2H, m, H<sub>2</sub>-3"), 1.27 (2H, m, H<sub>2</sub>-4"), 1.18 (2H, m, H<sub>2</sub>-5"), 0.84 (3H, t, J = 6.3 Hz, Me-6"); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  173.89 (C-1), 52.01 (C-2), 37.19 (C-3), 33.83 (C-4), 31.93 (C-5), 29.70 (C-6), 29.46 (C-7), 48.03 (C-8), 129.51 (C-9), 124.83 (C-10), 47.16 (C-11), 29.37 (C-12), 29.27 (C-13), 29.21 (C-14), 29.16 (C-15), 27.21 (C-16), 22.70 (C-17), 14.98 (C-18), 101.07 (C-1a), 74.51 (C-2a), 71.90 (C-3a), 64.71 (C-4a), 61.83 (C-5a), 97.21 (C-1b), 79.55 (C-2b), 71.90 (C-3b), 81.03 (C-4b), 60.29 (C-5b), 145.69 (C-1'), 138.08 (C-2'), 153.72 (C-3'), 152.18 (C-4'), 137.13 (C-5'), 131.39 (C-6'), 172.15 (C-7'), 56.01 (OMe), 169.22 (C-1"), 55.08 (C-2"), 37.16 (C-3"), 29.37 (C-4"), 29.27 (C-5"), 14.13 (C-6"); ESI MS *m/z* (rel. int.): 794 [M]<sup>+</sup> (C<sub>42</sub>H<sub>66</sub>O<sub>14</sub>) (1.6), 627 (11.6), 397 (12.1), 281 (8.2), 230 (9.7), 167 (9.3).

#### 2.5.2 Oleiyl-O- $\alpha$ -D-tetra-arabinosyl vanillyl caproate (5)

Elution of the column with chloroform - methanol (9 : 1) gave a pale yellow crystals of **5**, recrystallized from chloroform-methanol (1:1), 307 mg, m. p. 125 - 127 °C, UV  $\lambda_{\max}$  (MeOH): 213 nm (log  $\epsilon$  2.8); IR  $\lambda_{\max}$  (KBr): 3523, 3420, 3364, 2926, 2855, 1732, 1717, 1630, 1517, 1442, 1374, 1277, 1173, 1036, 862, 767 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.35 (1H, m, H-9), 5.31 (1H, m, H-10), 2.30 (2H, t, J = 7.6 Hz, H<sub>2</sub>-2), 2.19 (2H, m, H<sub>2</sub>-8), 2.06 (2H, m, H<sub>2</sub>-11), 1.53 (2H, m, H<sub>2</sub>-3), 1.51 (2H, m, H<sub>2</sub>-7), 1.49 (2H, m, H<sub>2</sub>-12), 1.27 (4H, m, 2 x CH<sub>2</sub>), 1.25 (8H, brs, 4 x CH<sub>2</sub>), 0.86 (3H, t, J = 6.3 Hz, Me-18), 5.03 (1H, d, J = 3.6 Hz, H-1a), 4.20 (1H, dd, J = 3.6, 5.3 Hz, H-2a), 3.97 (1H, m, H-3a), 3.89 (1H, m, H-4a), 3.74 (2H, d, J = 8.2 Hz, H<sub>2</sub>-5a), 4.91 (1H, d, J = 4.4 Hz, H-1b), 4.17 (1H, dd, J = 4.4, 5.8 Hz, H-2b), 3.95 (1H, m, H-3b), 3.87 (1H, m, H-4 b), 3.72 (2H, d, J = 8.0 Hz, H<sub>2</sub>-5b), 4.89 (1H, d, J = 6.4 Hz, H-1c), 4.15 (1H, dd, J = 6.4, 6.8 Hz, H-2c), 3.94 (1H, m, H-3c), 3.86 (1H, m, H-4 c), 3.70 (2H, d, J = 7.8 Hz, H<sub>2</sub>-5c), 4.81 (1H, d, J = 4.8 Hz, H-1d), 4.22 (1H, dd, J = 4.8, 6.7 Hz, H-2d), 3.92 (1H, m, H-3d), 4.03 (1H, m, H-4d), 3.76 (2H, d, J = 10.4 Hz, H<sub>2</sub>-5d), 7.33 (1H, d, J = 1.2 Hz, H-2'), 6.78 (1H, d, J = 7.5 Hz, H-5'), 7.50 (1H, dd, J = 1.2, 7.5 Hz, H-6'), 3.78 (3H, br s, OMe), 2.21 (2H, t, J = 7.2 Hz, H<sub>2</sub>-2"), 1.49 (2H, m, H<sub>2</sub>-3"), 1.23 (4H, m, H<sub>2</sub>4", H<sub>2</sub>-5"), 0.82 (3H, t, J = 6.1 Hz, Me-6"); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  171.56 (C-1), 56.35 (C-2), 39.20 (C-3), 38.76 (C-4), 33.91 (C-5), 29.66 (C-6), 29.52 (C-7), 48.01 (C-8), 129.98 (C-9), 127.64 (C-10), 48.01 (C-11), 37.83 (C-12), 31.92 (C-13), 29.11 (C-14), 27.20 (C-15), 26.26 (C-16), 22.69 (C-17), 14.96 (C-18), 108.79 (C-1a), 79.07 (C-2a), 74.75 (C-3a), 64.75 (C-4a), 60.19 (C-5a), 102.69 (C-1b), 79.03 (C-2b), 72.47 (C-3b), 69.58 (C-4b), 61.12 (C-5b), 101.15 (C-1c), 75.63 (C-2c), 72.40 (C-3c), 64.75 (C-4c), 61.09 (C-5c), 97.15 (C-1d), 86.89 (C-2d), 64.73 (C-3d), 84.50 (C-4d), 64.69 (C-5d), 147.19 (C-1'), 130.25 (C-2'), 153.75 (C-3'), 152.51 (C-4'), 127.64 (C-5'), 114.52 (C-6'), 170.44 (C-7'), 51.99 (OMe), 170.42 (C-1"), 55.92 (C-2"), 37.15 (C-3"), 34.39 (C-4"), 29.69 (C-5"), 14.87 (C-6"); ESI MS *m/z* (rel. int.): 1058 [M]<sup>+</sup> (C<sub>52</sub>H<sub>82</sub>O<sub>22</sub>) (1.5), 529 (8.3), 397 (21.8), 281 (2.8), 230 (7.3), 167 (11.6), 99 (1.9).

## 2.6 Isolation of phytoconstituents from the roots of *Verbesina encelioides*

### 2.6.1 Lignoceryl myristate (6)

Elution of the column with petroleum ether – chloroform (1 : 1) furnished a colourless mass of **6**, yield 161 mg, recrystallized from chloroform-methanol (1:1), m. p. 63 - 65 °C; IR  $\nu_{\max}$  (KBr) : 2919, 2851, 1739, 1641, 1464, 1374, 1167, 724  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  4.06 (2H, t, J = 7.1 Hz, H<sub>2</sub>-1'), 2.27 (2H, t, J = 7.2 Hz, H<sub>2</sub>-2), 2.03 (2H, m, CH<sub>2</sub>-3), 1.53 (2H, m, CH<sub>2</sub>-2'), 1.29 (6H, brs, 3 x CH<sub>2</sub>), 1.25 (56H, brs, 28 x CH<sub>2</sub>), 0.88 (3H, t, J = 5.7 Hz, Me-14), 0.84 (3H, t, J = 6.8 Hz, Me-24');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  172.98 (C-1), 68.41 (C-1'), 32.81 (CH<sub>2</sub>), 29.60 (30 x CH<sub>2</sub>), 27.69 (CH<sub>2</sub>), 25.24 (CH<sub>2</sub>), 22.62 (CH<sub>2</sub>), 14.19 (Me - 14), 14.16 (Me - 24'); ESI MS  $m/z$  (rel.int.): 564 [M]<sup>+</sup> (C<sub>38</sub>H<sub>76</sub>O<sub>2</sub>) (13.1), 353 (63.9), 337 (8.5), 308 (8.2), 227 (7.6), 211 (28.7).

### 2.6.2 $\beta$ -Amyrin palmitate (7)

Elution of the column with petroleum ether – chloroform (1 : 3) gave colourless crystalline powder of **7**, recrystallized from chloroform – methanol (1 : 1), yield 189 mg, m. p. 76 - 77 °C;  $[\alpha]_{\text{D}}^{25} +54$  (c = 1.3, benzene); UV  $\lambda_{\max}$  (MeOH): 209 nm (log  $\epsilon$  3.4); IR  $\nu_{\max}$  (KBr): 2919, 2851, 1729, 1635, 1635, 1468, 1380, 1361, 1265, 1198, 1174, 1096, 989, 720  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.18 (1H, t, J = 3.6 Hz, H-12), 4.50 (1H, dd, J = 5.5, 8.8 Hz, H-3 $\alpha$ ), 2.30 (2H, t, J = 7.2 Hz, H-2'), 1.86 to 1.29 (23H, m, 3 x CH, 10 x CH<sub>2</sub>), 1.27 (2H, m, H<sub>2</sub>-3'), 1.25 (6H, brs, 3 x CH<sub>2</sub>), 1.23 (4H, brs, 2 x CH<sub>2</sub>), 1.13 (4H, brs, 2 x CH<sub>2</sub>), 1.10 (10H, brs, 5 x CH<sub>2</sub>), 1.06 (3H, brs, Me-23), 0.97 (3H, brs, Me-29), 0.95 (3H, brs, Me-30), 0.91 (3H, brs, Me-25), 0.89 (3H, brs, Me-27), 0.87 (3H, brs, Me-24), 0.85 (3H, brs, Me-28), 0.83 (3H, t, J = 6.1 Hz, Me-16'), 0.80 (3H, brs, Me-26);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  38.30 (C-1), 23.58 (C-2), 80.63 (C-3), 37.82 (C-4), 55.30 (C-5), 18.21 (C-6), 34.96 (C-7), 41.76 (C-8), 48.70 (C-9), 36.90 (C-10), 23.61 (C-11), 121.71 (C-12), 145.29 (C-13), 39.86 (C-14), 26.20 (C-15), 26.98 (C-16), 32.02 (C-17), 47.60 (C-18), 47.26 (C-19), 31.17 (C-20), 32.64 (C-21), 34.79 (C-22), 28.49 (C-23), 16.13 (C-24), 17.15 (C-25), 23.68 (C-26), 23.77 (C-27), 28.13 (C-28), 33.43 (C-29), 26.05 (C-30), 173.81 (C-1'), 46.84 (C-2'), 37.21 (C-3'), 32.57 (C-4'), 29.78 (C-5', C-6'), 29.67 (C-7'), 29.56 (C-8'), 29.47 (C-9'), 29.36 (C-10'), 29.27 (C-11'), 29.25 (C-12'), 29.16 (C-13'), 29.12 (C-14'), 22.63 (C-15'), 14.65 (C-16'); ESI MS  $m/z$  (rel.int.): 664 [M]<sup>+</sup> (C<sub>46</sub>H<sub>80</sub>O<sub>2</sub>) (1.8), 425 (8.3), 393 (6.8), 256 (11.7), 218 (12.3), 206 (5.8), 175 (39.7), 159 (15.6).

### 2.6.3 $\beta$ -Amyrin oleate (8)

Further elution of the column with petroleum ether- chloroform (1 : 3) produced colourless crystals of **8**, recrystallized from chloroform - methanol (1 : 1), yield 138 mg, m. p. 178 - 180 °C;

UV  $\lambda_{\max}$  (MeOH): 213 nm (log  $\epsilon$  3.1); IR  $\nu_{\max}$  (KBr): 2943, 2850, 1735, 1645, 1457, 1374, 1246, 1025, 979, 873, 725  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.27 (2 H, m, H-9', H-10'), 5.18 (1H, t, J = 3.8 Hz, H-12), 4.48 (1H, dd, J = 3.6, 8.4 Hz, H-3 $\alpha$ ), 2.27 (2H, t, J = 7.1 Hz, H<sub>2</sub>-2'), 2.05 to 1.32 (35 H, m, 3 x CH, 16 x CH<sub>2</sub>), 1.28 (8H, m, 4 x CH<sub>2</sub>), 1.25 (4H, brs, 2 x CH<sub>2</sub>), 1.20 (4H, brs, 2 x CH<sub>2</sub>), 1.15 (4H, brs, 2 x CH<sub>2</sub>), 1.12 (3H, brs, Me-23), 1.06 (3H, brs, Me-25), 0.97 (3H, brs, Me-29), 0.95 (3H, brs, Me-30), 0.93 (3H, brs, Me-27), 0.90 (3H, brs, Me-26), 0.87 (3H, brs, Me-28), 0.85 (3H, t, J = 6.1 Hz, Me-18'), 0.82 (3H, brs, Me-24);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  38.49 (C-1), 24.89 (C-2), 81.04 (C-3), 38.30 (C-4), 55.43 (C-5), 18.24 (C-6), 34.78 (C-7), 41.75 (C-8), 48.68 (C-9), 36.89 (C-10), 22.63 (C-11), 121.69 (C-12), 145.19 (C-13), 39.85 (C-14), 26.96 (C-15), 27.08 (C-16), 32.06 (C-17), 47.26 (C-18), 47.61 (C-19), 31.16 (C-20), 32.62 (C-21), 34.44 (C-22), 28.47 (C-23), 15.64 (C-24), 16.85 (C-25), 23.59 (C-26), 23.77 (C-27), 28.01 (C-28), 32.56 (C-29), 26.02 (C-30), 171.15 (C-1'), 46.82 (C-2'), 37.75 (C-3'), 32.56 (C-4'), 29.23 (C-5', C-6'), 29.66 (C-7'), 36.74 (C-8'), 118.94 (C-9'), 109.07 (C-10'), 36.72 (C-11'), 26.73 (C-12'), 29.96 (C-13'), 27.61 (C-14'), 26.06 (C-15'), 25.23 (C-16'), 22.68 (C-17'), 14.77 (C-18');

ESI MS  $m/z$  (rel.int.): 690 [M]<sup>+</sup> (C<sub>48</sub>H<sub>82</sub>O<sub>2</sub>) (1.4), 265 (1.3), 218 (12.4), 207 (9.8), 203 (11.2), 189 (5.2), 174 (21.7).

### 2.6.4 $\beta$ -Amyrin stearate (9)

Elution of the column with petroleum ether - chloroform (1 : 3) afforded colourless crystalline powder of **9**, recrystallized from chloroform - methanol (1 : 1), yield 218 mg, m. p. 56 - 58 °C; UV  $\lambda_{\max}$  (MeOH): 212 nm (log  $\epsilon$  5.1); IR  $\nu_{\max}$  (KBr): 2914, 2848, 1728, 1635, 1469, 1270, 1208, 1173, 1112, 1036, 889, 719  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  5.18 (1H, t, J = 3.6 Hz, H-12), 4.50 (1H, dd, J = 5.5, 8.8 Hz, H-3 $\alpha$ ), 2.30 (2H, t, J = 7.2 Hz, H-2'), 1.86 to 1.29 (23H, m, 3 x CH, 10 x CH<sub>2</sub>), 1.27 (2H, m, H<sub>2</sub>-3'), 1.25 (6H, brs, 3 x CH<sub>2</sub>), 1.23 (4H, brs, 2 x CH<sub>2</sub>), 1.13 (4H, brs, 2 x CH<sub>2</sub>), 1.10 (10H, brs, 5 x CH<sub>2</sub>), 1.01 (3H, brs, Me-23), 0.97 (3H, brs, Me-29), 0.95 (3H, brs, Me-30), 0.91 (3H, brs, Me-25), 0.89 (3H, brs, Me-27), 0.87 (3H, brs, Me-24), 0.85 (3H, brs, Me-28), 0.83 (3H, t, J = 6.1 Hz, Me-16'), 0.80 (3H, brs, Me-26);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  38.32 (C-1), 23.54 (C-2), 80.65 (C-3), 37.83 (C-4), 55.32 (C-5), 18.34 (C-6), 34.94 (C-7), 41.78 (C-8), 47.62 (C-9), 36.92 (C-10), 23.64 (C-11), 121.72 (C-12), 145.28 (C-13), 39.88 (C-14), 26.21 (C-15), 27.01 (C-16), 32.05 (C-17), 47.30 (C-18), 47.31 (C-19), 31.15 (C-20), 32.66 (C-21), 34.81 (C-22), 28.47 (C-23), 15.63 (C-24), 16.86 (C-25), 23.69 (C-26), 23.79 (C-27), 28.15 (C-28), 33.41 (C-29), 26.03 (C-30), 173.76 (C-1'), 46.86 (C-2'), 37.23 (C-3'), 32.59 (C-4'), 29.76 (C-5', C-6', C-7'), 29.68 (C-8', C-9', C-10'), 29.57 (C-11'), 29.47 (C-12'), 29.34 (C-13'), 29.26 (C-14'), 25.24 (C-15', C-16'), 22.78 (C-17'), 14.21 (C-18'); ESI MS  $m/z$  (rel.int.): 692 [M]<sup>+</sup> (C<sub>48</sub>H<sub>84</sub>O<sub>2</sub>) (1.3), 425 (6.2), 284 (12.1), 218 (5.2), 207 (3.5).

### 3 Results and Discussion

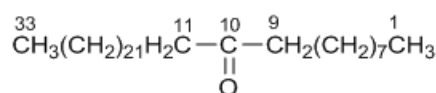
Compound **1** showed IR absorption bands for carbonyl group ( $1710\text{ cm}^{-1}$ ) and long aliphatic chain ( $726\text{ cm}^{-1}$ ). Its mass spectrum displayed a molecular ion peak at  $m/z$  478 corresponding to a molecular formula of an aliphatic ketone,  $C_{33}H_{66}O$ . The ion peaks generating at  $m/z$  351 [ $C_9 - C_{10}$  fission,  $CH_3(CH_2)_{22}CO$ ]<sup>+</sup>, 127 [ $M - 351$ ]<sup>+</sup>, 323 [ $C_{10} - C_{11}$  fission,  $CH_3(CH_2)_{22}$ ]<sup>+</sup> and 153 [ $M - 323$ ]<sup>+</sup> suggested the presence of the carbonyl function at  $C_{10}$  carbon. The <sup>1</sup>H NMR spectrum of **1** exhibited five two-proton multiplets from  $\delta$  2.53 to 1.33 and two broad singlets at  $\delta$  1.27 (6H) and 1.25 (44 H) assigned to methylene protons. Two three-proton triplets at  $\delta$  0.87 ( $J = 6.1$  Hz) and 0.83 ( $J = 6.3$  Hz) were accounted to terminal C-1 and C-33 primary methyl protons, respectively. The <sup>13</sup>C NMR spectrum of **1** displayed signals for the carbonyl carbon at  $\delta$  193.65 (C-10), methylene carbons between  $\delta$  31.19 - 22.67 and methyl carbons at  $\delta$  19.16 (C-1) and 13.55 (C-33). The absence of any signal beyond  $\delta$  2.53 in the <sup>1</sup>H NMR spectrum and between  $\delta$  193.65 - 31.19 in the <sup>13</sup>C NMR spectrum ruled out the unsaturated nature of the molecule. On the basis of foregoing spectral data analysis, the structure of **1** has been elucidated as *n*-triacontan-10-one, a new aliphatic ketone (Fig. 1).

Compound **2**, named 30-lauryloxy- $\alpha$ -amyrin 3-butyrate, responded to Liebermann-Burchardt test positively for triterpenoids and exhibited characteristic IR absorption bands for ester group ( $1732\text{ cm}^{-1}$ ), unsaturation ( $1641\text{ cm}^{-1}$ ) and aliphatic chain ( $757\text{ cm}^{-1}$ ). On the basis of mass and <sup>13</sup>C NMR spectra the molecular ion peak of **2** was established at  $m/z$  456 consistent with a molecular formula of pentacyclic triterpenic ester,  $C_{46}H_{78}O_4$ . The mass spectrum showed important ion fragments at  $m/z$  623 [ $M - 71$ ,  $OC(CH_2)_2CH_3$ ]<sup>+</sup>, 511 [ $M - 183$ ,  $OC(CH_2)_{10}CH_3$ ]<sup>+</sup>, and 199 [ $OOC(CH_2)_{10}CH_3$ ]<sup>+</sup> indicating that butyrate and laurate groups were linked to the triterpenic unit. The ion peaks arising at  $m/z$  278 and 416 generated due to retro-Diels Alder fragmentation, 207 [ $278 - OC(CH_2)_2CH_3$ ]<sup>+</sup> and 191 [ $278 - OOC(CH_2)_2CH_3$ ]<sup>+</sup>, suggested  $\Delta^{12}$  olefinic linkage in ring C<sup>40</sup>, butyrate function in the ring A/B placed at C-3 on the basis of biogenetic consideration and laurate group in the ring D/E. The <sup>1</sup>H NMR spectrum of **2** displayed a one-proton downfield doublet at  $\delta$  5.34 ( $J = 5.1$  Hz) assigned to vinylic H-12 proton, a one-proton double doublet at  $\delta$  4.06 ( $J = 4.7, 7.5$  Hz) ascribed to oxymethine H-3 $\alpha$  proton, a two - proton doublet at  $\delta$  4.15 ( $J = 7.2$  Hz) attributed to oxymethylene H<sub>2</sub>-30, methylene and methine protons in the range from 2.33 to 1.20, six three-proton singlets at  $\delta$  1.07, 0.98, 0.95, 0.90, 0.88 and 0.86 associated with tertiary C-23, C-25, C-27, C-24, C-28 and C-26 methyl protons, respectively, a three-proton doublet at  $\delta$  0.93 ( $J = 6.2$  Hz) accounted to secondary C-29 methyl protons of ursene-type triterpene, and two three-protons triplets at  $\delta$  0.83 ( $J = 6.1$  Hz) and 0.79 ( $J =$

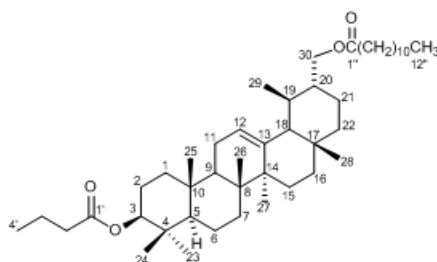
6.2 Hz) allocated correspondingly to primary C-4' and C-12'' methyl protons. The presence of two doublets at  $\delta$  0.93 (3H, Me-29) and 4.15 (2H, CH<sub>2</sub>O-30) supported the existence of one oxymethylene function in ring E. The <sup>13</sup>C NMR spectrum of **2** exhibited signals for forty six carbons including ester carbons at  $\delta$  174.03 (C-1') and 171.12 (C-1''), vinylic carbons at  $\delta$  124.40 (C-12) and 139.86 (C-13), oxymethine carbon at  $\delta$  81.04 (C-3), oxymethylene carbon at  $\delta$  64.31 (C-30) and methyl carbons resonated from  $\delta$  28.36 to 14.21. The absence of the C-30 carbon signal near  $\delta$  21.0 indicated the location of the lauryl group at C-30. The assignments of the <sup>1</sup>H NMR and carbon chemical shifts of **2** were compared with  $\delta$  values of the corresponding positions of urs-12-enes<sup>40,41</sup>. On the basis of above discussion and literature values the structure of **2** was elucidated as 30-lauryloxy-urs-12-en-3 $\beta$ -olyl butyrate, a new  $\alpha$ -amyryn diester (Fig. 1).

Compound **3**, designated as 3-palmityl  $\alpha$ -amyrin-23,6 $\alpha$ -olide, 692 [ $M$ ]<sup>+</sup> at  $m/z$  692 ( $C_{46}H_{76}O_4$ ), showed distinctive IR absorption bands for lactone ring ( $1736\text{ cm}^{-1}$ ), unsaturation ( $1640\text{ cm}^{-1}$ ) and aliphatic chain ( $758\text{ cm}^{-1}$ ). Its mass spectrum displayed important ion fragments at  $m/z$  239 [ $CH_3-(CH_2)_{14}-CO$ ]<sup>+</sup>, 453 [ $M - 239$ ]<sup>+</sup>, 256 [ $CH_3-(CH_2)_{14}-COOH$ ]<sup>+</sup> and 436 [ $M - 256$ ]<sup>+</sup> indicating that palmitic acid was esterified with the triterpenic unit. The ion peaks generating at  $m/z$  473 and 218 due to retro-Diels Alder fragmentation and 234 [ $473 - 239$ ]<sup>+</sup> suggested the existence of  $\Delta^{12}$  olefinic linkage in ring C<sup>40</sup> and palmitate unit in the ring A/B placed at C-3 on the basis of biogenetic analogy and saturated nature of the rings D and E. The <sup>1</sup>H NMR spectrum of **3** displayed a one-proton downfield triplet at  $\delta$  5.12 ( $J = 3.6$  Hz) assigned to vinylic H-12 proton, a one-proton triple doublet at  $\delta$  4.51 ( $J = 4.6, 4.8, 5.7$  Hz) and a one - proton double doublet at  $\delta$  4.13 ( $J = 5.3, 8.3$  Hz) attributed to  $\alpha$ -oriented oxymethine H-6 and H-3 protons, respectively, a two-proton triplet at  $\delta$  2.30 ( $J = 7.6$  Hz) ascribed to methylene H<sub>2</sub>-2' protons adjacent to the ester function, other methylene protons from  $\delta$  2.26 to 1.23, five three-proton singlets at  $\delta$  1.13, 1.06, 1.01, 0.97 and 0.91 associated correspondingly with the tertiary C-25, C-24, C-26, C-28 and C-27 methyl protons, two three - protons doublets at  $\delta$  0.87 ( $J = 6.1$  Hz) and 0.84 ( $J = 6.0$  Hz) allocated to secondary C-29 and C-30 methyl protons, respectively, and a three - protons triplet at  $\delta$  0.79 ( $J = 6.6$  Hz) due to primary C-16' protons. The <sup>13</sup>C NMR spectrum of **3** exhibited signals for forty six carbons including ester carbon at  $\delta$  170.11 (C-1'), lactone carbon at  $\delta$  169.96 (C-23), vinylic carbons at  $\delta$  123.66 (C-12) and 138.88 (C-13), oxymethine carbons at  $\delta$  80.07 (C-3) and 80.05 (C-6) and methyl carbons from  $\delta$  22.70 to 13.64. The assignments of the <sup>1</sup>H NMR and carbon chemical shifts of **3** were compared with  $\delta$  values of the corresponding positions of urs-12-ene-type triterpenoids<sup>40,41</sup>. On the basis of these evidences the structure

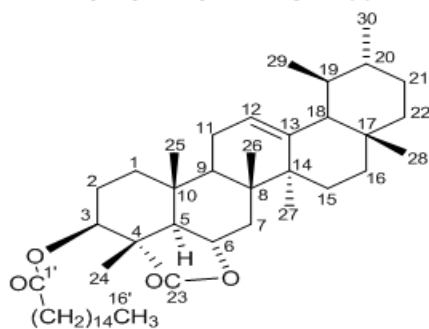
of **3** was elucidated as urs-12-en-23,6 $\alpha$ -olide 3 $\beta$ -olyl palmitate, a new  $\alpha$ -amyrin lactonic ester (Fig. 1).



***n*-Tritriacontan-10-one (1)**



**30-Lauryloxy- $\alpha$ -amyrin-3-butyrate (2)**



**3-Palmityl- $\alpha$ -amyrin-23,6 $\alpha$ -olide (3)**

**Fig 1: Structural formulae of the compounds 1 - 3 isolated from the aerial roots of *Ficus benghalensis***

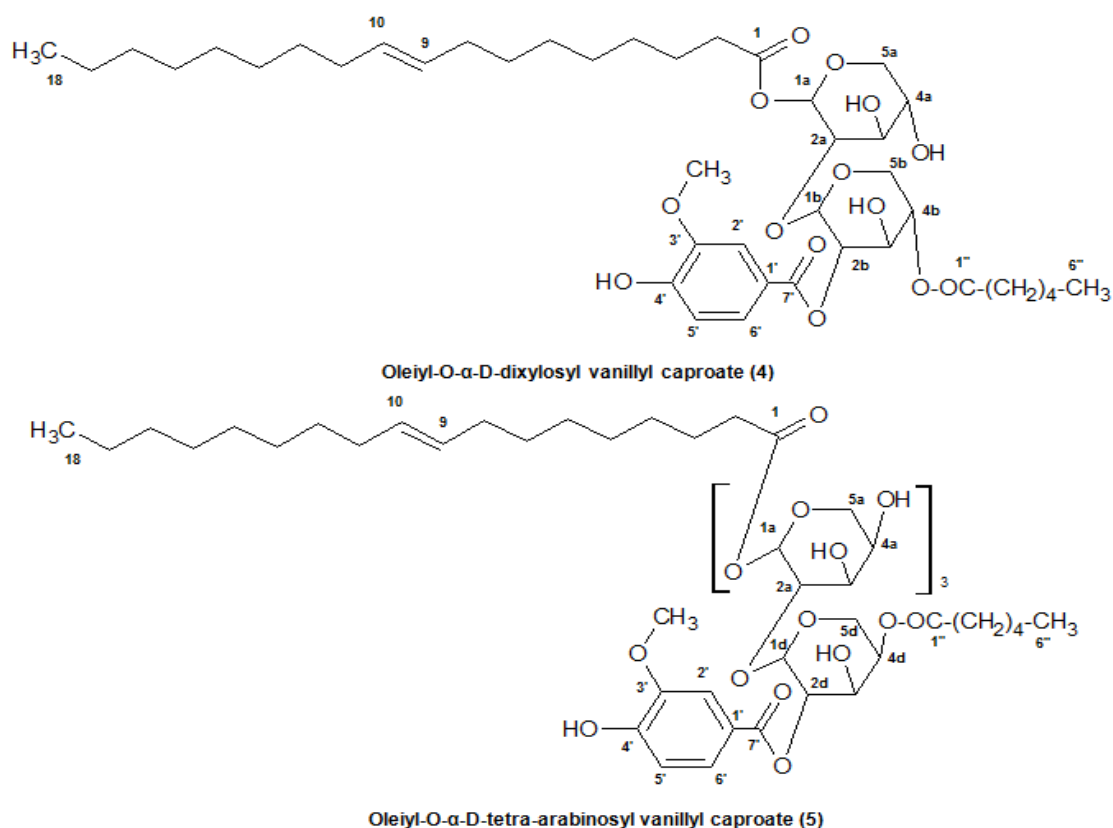
Compound **4**, named oleiyl-O-  $\alpha$ -D-dixylosyl vanillyl caproate, gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3510, 3405, 3365  $\text{cm}^{-1}$ ), ester function (1730  $\text{cm}^{-1}$ ) and unsaturation (1629  $\text{cm}^{-1}$ ). On the basis of mass and  $^{13}\text{C}$  NMR spectral data, the molecular ion peak of **4** was established at  $m/z$  794 consistent with a molecular formula of an acyl diglycosidic ester,  $\text{C}_{42}\text{H}_{66}\text{O}_{14}$ . An ion peak generating at  $m/z$  281 [O - C<sub>1</sub> fission,  $\text{C}_{18}\text{H}_{33}\text{O}_2$ ]<sup>+</sup> suggested that oleic acid was esterified with a diglycosidic ester unit. The ion fragments arising at  $m/z$  397 [C<sub>2a</sub> - O fission,  $\text{C}_5\text{H}_7\text{O}_4-(\text{C}_6\text{H}_3-(\text{OH})(\text{OMe})-\text{CO}-(\text{CH}_3-(\text{CH}_2)_4-\text{CO}))$ ]<sup>+</sup>, 167 [C<sub>2b</sub> - O fission,  $\text{C}_6\text{H}_3-(\text{OH})(\text{OMe})-\text{CO}$ ]<sup>+</sup> and 230 [397 - 167]<sup>+</sup> indicated the attachment of vanillyl and hexanoyl units with the second sugar moiety. The  $^1\text{H}$  NMR spectrum of **4** exhibited two one - proton multiplets at  $\delta$  5.36 and 5.32 assigned to vinylic H-9 and H-10 protons, respectively, methylene protons between  $\delta$  2.34 - 1.18 and two three - proton triplets at  $\delta$  0.86 (J = 6.8 Hz) and 0.84 (J = 6.3 Hz) ascribed to terminal C-18 and C-6'' primary methyl protons. Two one - proton doublets at  $\delta$  5.02 (J = 6.4 Hz) and 4.95 (J = 6.7 Hz) were ascribed correspondingly to  $\alpha$ -oriented anomeric H-1a and H-1b protons. The other sugar protons resonated as one - proton double doublets at  $\delta$  4.34 (J = 6.4, 7.2 Hz, H-2a) and 4.63 (J = 6.7, 7.6 Hz, H-2b), as one - proton multiplets

from  $\delta$  4.20 to 4.13 and as two - proton doublets at  $\delta$  3.63 (J = 9.3 Hz, H<sub>2</sub>-5a) and 3.80 (J = 9.1 Hz, H<sub>2</sub>-5b). Two one - proton doublets at  $\delta$  7.50 (J = 1.2 Hz) and 6.73 (J = 8.5 Hz), a one - proton double doublet at  $\delta$  6.83 (J = 1.2, 8.5 Hz) and a three - proton singlet at  $\delta$  3.67 were accounted to aromatic *meta*-coupled H-2', *ortho*-coupled H-5', *meta*, *ortho*-coupled H-6' and methoxy protons, respectively. The  $^{13}\text{C}$  NMR spectrum of **4** displayed signals for ester carbons at  $\delta$  173.89 (C-1), 172.15 (C-7') and 169.22 (C-1''), aromatic and vinylic carbons between  $\delta$  153.72 - 124.83, anomeric carbons at  $\delta$  101.07 (C-1a) and 97.21 (C-1b), remaining sugar carbons from  $\delta$  81.03 to 60.29, methylene carbons in the range of  $\delta$  52.01 - 22.70, methoxy carbon at  $\delta$  56.01 and terminal methyl carbons at  $\delta$  14.98 (C-18) and 14.13 (C-6''). The presence of H-2a in a deshielded region as a one-proton double doublet at  $\delta$  4.34 in the  $^1\text{H}$  NMR spectrum and C-2a carbon signal at  $\delta$  74.51 suggested attachment of the second sugar unit at C-2a. The existence of H-2b and H-4b at  $\delta$  4.63 and 4.20 in further downfield field indicated the attachment of vanylic group at C-2b and hexanoyl function at C-4b. Acid hydrolysis of **4** yielded oleic acid, R<sub>f</sub> 0.34 (glacial acetic acid, 85%), vanillic acid, m. p. 210 - 212 °C, R<sub>f</sub> 0.56 (benzene - acetic acid - water, 37:45:18, v/v); D-xylose, R<sub>f</sub> 0.81 (*n*-butonal - pyridine - water, 6 : 4 : 3, v/v), m. p. 153 - 156 °C,  $[\alpha]_D^{20} + 91^\circ$  (water, 10%) and caproic acid (hexanoic acid), R<sub>f</sub> 0.82 (methanol-acetic acid-tetralin, 10: 2: 1, v/v). On the basis of above discussion, the compound **4** was structurally elucidated as oleiyl-O- $\alpha$ -D-xylopyranosyl-(2a $\rightarrow$ 1b)-O- $\alpha$ -D-xylopyranosyl-2b-vanillyl-4b-caproate, a new acyl dixyloxy diester (Fig. 2).

Compound **5**, designated as oleiyl-O-  $\alpha$ -D-tetra-arabinosyl vanillyl caproate, [M]<sup>+</sup> at  $m/z$  1058 ( $\text{C}_{52}\text{H}_{82}\text{O}_{22}$ ), was a tetra-arabinosyl  $\alpha$ - homologue of **5**. It showed IR absorption bands for hydroxyl groups (3523, 3420, 3364  $\text{cm}^{-1}$ ), ester functions (1732, 1717  $\text{cm}^{-1}$ ), unsaturation (1630  $\text{cm}^{-1}$ ), aromaticity (1517, 1036  $\text{cm}^{-1}$ ) and long aliphatic chain (767  $\text{cm}^{-1}$ ). The ion peaks produced at  $m/z$  281 [O - C<sub>1</sub> fission,  $\text{C}_{18}\text{H}_{33}\text{O}_2$ ]<sup>+</sup> and 529 [C<sub>2b</sub> - O fission,  $\text{C}_{18}(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{CO}-\text{C}_5\text{H}_8\text{O}_4-\text{C}_5\text{H}_8\text{O}_4$ ]<sup>+</sup> indicated that oleiyl was linked to the sugar chain. The ion fragments arising at  $m/z$  397 [C<sub>2a</sub> - O fission,  $\text{C}_5\text{H}_7\text{O}_4-(\text{C}_6\text{H}_3-(\text{OH})(\text{OMe})-\text{CO}-(\text{CH}_3-(\text{CH}_2)_4-\text{CO}))$ ]<sup>+</sup>, 167 [C<sub>2b</sub> - O fission,  $\text{C}_6\text{H}_3-(\text{OH})(\text{OMe})-\text{CO}$ ]<sup>+</sup> and 230 [397 - 167]<sup>+</sup> indicated the attachment of vanillyl and hexanoyl units with the second sugar moiety. The ion peaks generated at  $m/z$  397 [C<sub>2c</sub> - O fission,  $\text{C}_5\text{H}_7\text{O}_4-(\text{C}_6\text{H}_3-(\text{OH})(\text{OMe})-\text{CO}-(\text{CH}_3-(\text{CH}_2)_4-\text{CO}))$ ]<sup>+</sup>, 167 [C<sub>2d</sub> - O fission,  $\text{C}_6\text{H}_3-(\text{OH})(\text{OMe})-\text{COO}$ ]<sup>+</sup> and 230 [397 - 167]<sup>+</sup> supported the existence of vanillyl and caproyl groups attached to the last sugar unit. Its  $^1\text{H}$  NMR spectrum displayed two one-proton multiplets at  $\delta$  5.35 and 5.31 assigned to vinylic H-9 and H-10 protons, respectively, four one proton doublets at  $\delta$  5.03 (J = 3.6 Hz), 4.91 (J = 4.4 Hz), 4.89 (J = 6.4 Hz) and 4.81 (J = 4.8 Hz) ascribed correspondingly to  $\alpha$ -oriented anomeric H-1a, H-1b, H-1c and H-1d protons, other sugar protons

between  $\delta$  4.22 - 3.70, two one - proton doublets at  $\delta$  7.33 ( $J = 1.2$  Hz), 6.78 ( $J = 7.5$  Hz) and a one - proton double doublet at  $\delta$  7.50 ( $J = 1.2, 7.5$  Hz) due to aromatic H-2', H-5' and H-6' protons, respectively, a three - proton singlet at  $\delta$  3.78 accounted to methoxy protons, two three - proton triplets at  $\delta$  0.86 ( $J = 6.3$  Hz) and 0.82 ( $J = 6.1$  Hz) associated with the primary C-18 and C-6'' primary methyl protons and the methylene protons in the range of  $\delta$  2.30 - 1.23. The  $^{13}\text{C}$  NMR spectrum of **5** exhibited signals for ester carbons at  $\delta$  171.56 (C-1), 170.44 (C-7') and 170.42 (C-1''), aromatic and vinylic carbons from  $\delta$  153.75 to 114.52, anomeric carbons in the range of  $\delta$  108.79 - 97.15, other sugar carbons between  $\delta$  86.89 - 61.09, methylene carbons from  $\delta$  56.35 - 22.65, methoxy carbon at  $\delta$  51.99 and terminal methyl carbons at  $\delta$  14.96 (C-18) and 14.87 (C-6''). The presence of proton H-2a, H-2b and H-2c signals in the deshielded region as a one-

proton double doublets from  $\delta$  4.20 to 4.15 and their respective carbon signals at  $\delta$  79.07 (C-2a), 79.03 (C-2b) and 75.63 (C-2c) suggested attachment of the sugar units through (2a $\rightarrow$ 1b), (2b $\rightarrow$ 1c) and (2c $\rightarrow$ 1d), respectively. The existence of H-2d as a one - proton double doublet at  $\delta$  4.22 ( $J = 4.8, 6.7$  Hz) and H-4d as a multiplet at  $\delta$  4.03 (1H) in further downfield field region indicated the attachment of vanillyl group at C-2b and caproyl function at C-4b. Acid hydrolysis of **5** yielded oleic, vanillic and caproic acids (co-TLC comparable) and D-arabinose,  $R_f$  0.77 (*n*-butanol - pyridine - water, 6 : 4 : 3), m. p. 162 - 164  $^{\circ}\text{C}$ ,  $[\alpha]_D^{20} - 103^{\circ}$  (water, 4%). On the basis of above discussion, the compound **5** was structurally elucidated as oleilyl-O- $\alpha$ -D-arabinopyranosyl-(2a $\rightarrow$ 1b)-O- $\alpha$ -D-arabinopyranosyl--(2b $\rightarrow$ 1c)-O- $\alpha$ -D-arabinopyranosyl--(2c $\rightarrow$ 1d)-O- $\alpha$ -D-arabinopyranosyl-2d-vanillyl-4d-caproate, a new acyl tetra-arabinosyl diester (Fig. 2).



**Fig 2: Structural formulae of the compounds 4 and 5 isolated from the leaves of *Nyctanthes arbor-tristis***

Compound **6**, named lignoceryl myristate, showed IR absorption bands for ester group ( $1739\text{ cm}^{-1}$ ) and long aliphatic chain ( $724\text{ cm}^{-1}$ ). Its mass spectrum exhibited a molecular ion peak at  $m/z$  564 consistent with the molecular formula of a fatty acid ester,  $\text{C}_{38}\text{H}_{76}\text{O}_2$ . The generation of the ion peaks at  $m/z$  211 [ $\text{C}_1 - \text{O}$  fission,  $\text{CH}_3(\text{CH}_2)_{12}\text{CO}^+$ ], 353 [ $\text{M} - 211, \text{O}-(\text{CH}_2)_{23}\text{CH}_3^+$ ], 227 [ $\text{C}_{1'} - \text{O}$  fission,  $\text{CH}_3(\text{CH}_2)_{12}\text{COO}^+$ ] and 337 [ $\text{M} - 227, \text{O}-(\text{CH}_2)_{23}\text{CH}_3^+$ ] indicated that a  $\text{C}_{24}$  lignoceryl alcohol was esterified with myristic acid. The  $^1\text{H}$  NMR spectrum of **6** displayed two triplets at  $\delta$  4.06 ( $J = 7.1$  Hz) and 2.27 ( $J = 7.2$  Hz) integrating for two protons each assigned to oxymethylene H<sub>2</sub>-1' and methylene

H<sub>2</sub>-2 protons adjacent to the ester function, respectively. The remaining methylene protons appeared as two - proton multiplets at  $\delta$  2.03 and 1.53 and as broad singlets at  $\delta$  1.29 (6H) and 1.25 (56H). Two three-proton triplets at  $\delta$  0.88 ( $J = 5.7$  Hz) and 0.84 ( $J = 6.8$  Hz) were due to correspondingly C-14 and C-24' primary methyl protons. The  $^{13}\text{C}$  NMR spectrum of **6** showed signals for ester carbon at  $\delta$  172.98 (C-1), oxymethylene carbon at  $\delta$  68.41 (C-1'), other methylene carbons between  $\delta$  32.81 - 22.62 and methyl carbons at  $\delta$  14.19 (C-14) and 14.16 (C-24'). The absence of any  $^1\text{H}$  NMR signal beyond  $\delta$  4.06 and carbon signal between  $\delta$  172.98 - 68.41



supported the saturated nature of the molecule. On the basis of the foregoing account, the structure of **6** has been formulated a tetracosan-1-oyl 1-tetradecanoate (Fig.3).

Compound **7** was a known triterpenic ester identified as  $\beta$ -amyrin palmitate<sup>42,43</sup> (Fig 3).

Compound **8**, named  $\beta$ -amyrin oleate, gave positive tests for triterpenoids and showed characteristic IR absorption bands for ester function ( $1735\text{ cm}^{-1}$ ), unsaturation ( $1645\text{ cm}^{-1}$ ) and long aliphatic chain ( $725\text{ cm}^{-1}$ ). Its molecular ion peak was determined on the basis of mass and  $^{13}\text{C}$  NMR spectra at  $m/z$  690 consistent with a molecular formula of a triterpenic ester  $\text{C}_{48}\text{H}_{82}\text{O}_2$ . The ion peaks arising at  $m/z$  207 and 218 due to Retro-Diels Alder fragmentation of the triterpenic unit after removal of the acyl group suggested that a vinylic linkage was present at  $\text{C}_{12}$  carbon in ring  $\text{C}^{40}$ . The ion peaks produced at  $m/z$  189 [ $207 - \text{H}_2\text{O}$ ]<sup>+</sup>, 174 [ $189 - \text{Me}$ ]<sup>+</sup> and 203 [ $218 - \text{Me}$ ]<sup>+</sup> indicated that a hydroxyl group was present in ring A which was placed at C-3 on the basis of biogenetic consideration. The  $^1\text{H}$  NMR spectrum of **8** exhibited a two-proton multiplet at  $\delta$  5.27 and a one-proton triplet at  $\delta$  5.18 ( $J = 3.8\text{ Hz}$ ) assigned to vinylic H-9' and H-10' and H-12 protons, respectively, a one-proton double doublet at  $\delta$  4.48 ( $J = 3.6, 8.4\text{ Hz}$ ) ascribed to oxymethine H-1 $\alpha$  proton, a two-proton triplet at  $\delta$  2.27 ( $J = 7.1\text{ Hz}$ ) attributed to methylene  $\text{H}_{2-2'}$  protons adjacent to the ester group, other methine and methylene proton signals between  $\delta$  2.05 - 1.15, a three-proton triplet at  $\delta$  0.85 ( $J = 6.1\text{ Hz}$ ) due to primary C-18' methyl protons and eight three-proton singlets from  $\delta$  1.12 to 0.82 accounted to tertiary methyl protons, all attached to saturated carbons in an oleanene-type triterpenic framework. The  $^{13}\text{C}$  NMR spectrum of **8** displayed signals for ester carbon at  $\delta$  171.15 (C-1'), vinylic carbons at  $\delta$  121.69 (C-12), 145.19 (C-13), 118.94 (C-9') and 109.07 (C-10'), oxymethine carbon at  $\delta$  81.04 (C-3), methyl carbons from  $\delta$  28.47 to 14.77 and methine and methylene carbon between  $\delta$  55.43 - 22.68. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data of the isolated compounds were compared with the oleanene-type triterpenoids<sup>44,45</sup>. On the basis of spectral data analysis, the structure of **8** had been formulated as urs-12-en-3 $\beta$ -oyl oleate. This is a new triterpenic ester (Fig. 3).

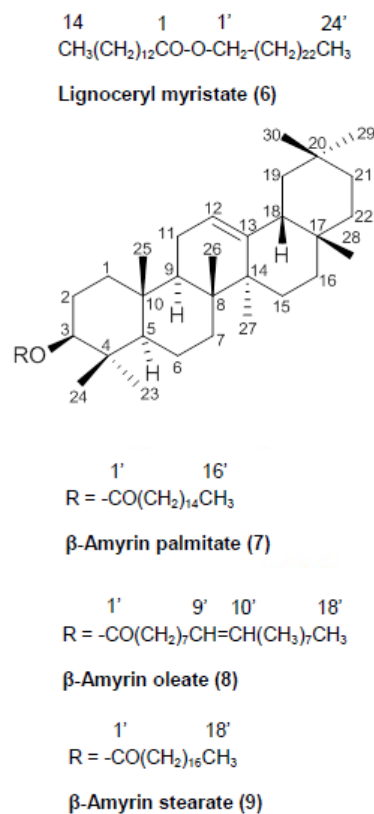
Compound **9** was a known triterpenic ester identified as  $\beta$ -amyrin stearate<sup>46,47</sup>(Fig 3).

#### 4 Conclusion

Phytochemical investigation of the aerial roots of *F. benghalensis* afforded *n*-trtriacontan-10-one (**1**), 30-lauryloxy- $\alpha$ -amyrin 3-butyrate (**2**) and 3-palmityl  $\alpha$ -amyrin-23,6 $\alpha$ -olide (**3**).

The leaves of *N. arbor-tristis* furnished two vanillyl glycosidic disters characterized as oleyl-O-  $\alpha$ -D-dixylosyl vanillyl caproate (**4**) and oleyl-O-  $\alpha$ -D-tetra-arabinosyl vanillyl caproate (**5**). The roots of *V. encelioides* afforded lignoceryl myristate (**6**),  $\beta$ -amyrin palmitate (**7**),  $\beta$ -amyrin oleate (**8**) and  $\beta$ -amyrin stearate

(**9**). This work has enhanced understanding about the phytoconstituents of these plants. These secondary metabolites can be used as analytical markers for quality control of the plants. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.



**Fig 3: Structural formulae of the compounds 6 - 9 isolated from the roots of *Verbesina encelioides***

#### 5 Conflicts of Interests

The authors hereby declare that there are no conflicts of interests.

#### 6 Author's contributions

SS and SRM performed the experimental work. MA and SRM analyzed the spectral data and compiled the manuscript.

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