



## Chemical Constituents from the Aerial Parts of *Jasminum auriculatum* Vahl and Seeds of *Holarrhena pubescens* Wall. ex G. Don

Shahnaz Sultana<sup>1,2</sup>, Mohammed Ali<sup>1\*</sup> and Showkat Rassol Mir<sup>1</sup>

<sup>1</sup>Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delh, India

<sup>2</sup>College of Pharmacy, Jazan University, Jazan, Saudi Arabia

\*Corresponding Author: Mohammed Ali, Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India.

Received: December 29, 2017; Published: February 05, 2018

### Abstract

*Jasminum auriculatum* Vahl (Oleaceae) is found in south eastern Asia as a small, evergreen and climbing shrub and used to treat bile problems, burning sensation, constipation, flatulence, kidney stones, leprosy, skin diseases, strangury, stomatitis, ulcers and wounds. *Holarrhena pubescens* Wall. ex G. Don (Apocynaceae) is distributed in the Indian subcontinent. Its seeds are effective to treat asthma, colic pain, chest infections, common cold and fever. Our study was planned to isolate chemical constituents from the aerial parts of *J. auriculatum* and seeds of *H. pubescens* and to characterize their structures. The air-dried plant materials were exhaustively extracted separately with methanol in a Soxhlet apparatus. The concentrated methanol extracts were adsorbed on silica gel (60 - 120 mesh) for the preparation of slurries. The dried slurries were chromatographed individually over silica gel columns. The columns were eluted with petroleum ether, chloroform and methanol, successively, in order of increasing polarity to isolate the chemical constituents.

Phytochemical investigation of a methanol extract of the aerial parts of *J. auriculatum* afforded five new phytoconstituents identified as (*Z*)-*n*-dotriacont-6-enyl piperate [(*Z*)-*n*-dotriacontenyl piperate, **1**], (*Z*)-4-pentadecanoxyferulic acid (**2**), (*Z*)-*n*-tri-acont-8-enoic acid [(*Z*)-8-dehydromelissic acid, **3**], (*Z*)-dodec-6-enoyl - O-β-D-glucopyranosyl - (6'→1'')-O-β-D-glucopyranosyl- (6''→1''')-O-β-D-glucopyranosyl- (6'''→1'''')-O-β-D-glucopyranoside [(*Z*)-6-lauroleiy l β-D-tetraglucoside, **5**] and (*Z*)-dodec-6-eno-yl - O-α-D-glucopyranosyl -(6'→1'')-O-α-D-glucopyranosyl-(6''→1''')-O-α-D-glucopyranosyl- (6'''→1'''')-O-α-D-glucopyranoside [(*Z*)-6-lauroleiy l α-D-tetraglucoside, **6**] along with β-D-xylose (**4**). The seeds of *H. pubescens* gave a new aromatic ester (*Z*)-5'-(2-hydroxyphenyl)-pent-4-enyl ricinoleate (**7**). Their structures were established on the basis of spectral data analysis and chemical reactions.

**Keywords:** *Jasminum auriculatum* Aerial Parts; *Holarrhena pubescens* Seeds; Chemical Constituents; Isolation; Characterization

### Introduction

*Jasminum auriculatum* Vahl, syn. *J. ovalifolium* Wight, *Mogorium trifoliatum* Lam. (Oleaceae), known as juhi, banamallika and Indian jasmine, is found in India, Nepal, Bhutan, Sri Lanka, Andaman Islands and Thailand. It is a small, evergreen and climbing shrub used as an analgesic, anthelmintic, antidepressant, anti-inflammatory, anti-oxidant, antiseptic, aphrodisiac, astringent, deobstruent, depurative, diuretic, emollient, expectorant, sedative, stomachic and stimulant and to treat bile problems, burning sensation, constipation, flatulence, kidney stones, leprosy, skin diseases, strangury, skin diseases, stomatitis, ulcers and wounds [1-3]. The leaves are beneficial to cure mouth ulcers. The flowers are effective to relieve tuberculosis. An essential oil of the flowers is utilized in perfumery and to make incense sticks. The roots are useful to cure burning micturition, eye diseases, headache, leprosy, gum and mouth diseases, pimples, ringworm, scabies, renal calculi and wounds [1,2].

The plant contained benzyl acetate, jasmone, resin, glycoside, phenols, salicylic acid, terpenoids, jasmoline, tannins, saponins and flavonoids. The leaves yielded lupeol, aliphatic hydrocarbons (C<sub>20</sub> - C<sub>34</sub>), aliphatic alcohols (C<sub>21</sub> - C<sub>32</sub>), hentriacontane, *n*-trica-tanol, fatty acids, jasminol, D-mannitol, inositol, sorbitol, xylitol, malvalic acid and jasmine. The flower buds possessed indole, benzyl acetate and methyl anthranilate. Jasmine flower is a rich source of essential oils, indole and jasminol [1,4,5]. The concretes of the flower volatile oils were composed mainly of 17-pentatriacontene, 22-tricosenoic acid, 1-hexacosene, propyl oleate, 1,54-dibromo-tetrapentacontane, octadecane, 4-methyl-2-propyl 11-pentanol, 3,5-dimethyl 1-hexene, 3,4-dimethyl 1-hexene, 1-butoxy 2-pen-tene and 3,5,5-trimethyl 1-hexene [6].

*Holarrhena pubescens* Wall. ex G. Don, syn. *H. antidysenterica* (Roth) Wall. ex A. DC., *Echites antidysentericus* Roth (Apocynaceae), known as kurchi, kutaja and bitter oleander, is a small deciduous

tree found throughout the dry forests of Indian subcontinent [7]. Its stem bark is used as an anthelmintic, anti-dysenteric, anti-periodic, antitoxin, astringent, bitter, digestive, expectorant, febrifuge, tonic and to treat anaemia, arthritis, asthma, boils, bowel syndrome, cold, cough, diarrhea, amoebic dysentery, liver disorders, malaria, piles and skin diseases. The seeds are taken as anti-dysenteric, antidiarrhoeal, antibilious, anthelmintic, carminative, febrifuge, stomachic and to treat asthma, colic pain, chest affections, cold, fever and for promoting conception and toning up vaginal tissues after delivery [8,9]. The stem bark contained steroidal alkaloids mainly conessine, kurchinine, kurchinine, kurchinidine, holarrifine, holadiene, regholarrhenines, pubescine, norholadiene, pubescimine, kurchilidine, kurchamide, kurcholessine, kurchessine, conessimine, regholarrenines A - F, isoconessimine and lignoceric, linolenic, linoleic, oleic, palmitic and stearic acids [10-16]. Keeping in view the high reputation and application of *J. auriculatum* and *Holarrhena pubescens* in the many indigenous medicinal systems, it has been aimed to carry out isolation and characterization of chemical constituents from these plant materials procured from Delhi.

## Materials and Methods

### General procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined 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  $^1\text{H}$  NMR (300 MHz) and  $^{13}\text{C}$  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 plates were run on silica gel G 60 F<sub>254</sub> precoated TLC sheets (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

### Plant material

The aerial parts of *J. auriculatum* and the seeds of *H. pubescens* were collected locally from Delhi and authenticated by Professor MP Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of the plant materials were preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

### Extraction and Isolation

The aerial parts of *J. auriculatum* and the seeds of *H. pubescens* (1 kg each) were coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 147.3 g and 116.4 g, respectively. The dried residue (100 g each) was dis-

solved in minimum amount of methanol and adsorbed on silica gel column grade (60 - 120 mesh) separately to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns individually loaded in petroleum ether (b. p. 60 - 80°C). Each column was 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). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same  $R_f$  values were combined and crystallized with solvents. The isolated compounds were recrystallized to get the following pure compounds:

### Isolation of phytoconstituents from the rhizomes of *Jasminum auriculatum*

#### (Z)-n-Dotriacontenyl piperate (1)

Elution of the column with chloroform yielded pale yellow crystals of **1**, yield 214 mg, m. p. 228 - 229°C, UV  $\lambda_{\text{max}}$  (MeOH): 335 nm, IR  $\nu_{\text{max}}$  (KBr): 2925, 2839, 1727, 1637, 1549, 1442, 1318, 1267, 1135, 1085, 930, 725  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.48 (1H, d, J = 9.6 Hz, H-5''), 7.24 (1H, dd, J = 9.6, 2.4 Hz, H-6''), 7.08 (1H, d, J = 2.4 Hz, H-2''), 6.86 (1H, d, J = 14.8 Hz, H-5'), 6.57 (1H, dd, J = 14.8, 13.9 Hz, H-4'), 6.23 (1H, d, J = 14.8 Hz, H-2'), 5.79 (1H, dd, J = 14.8, 13.9 Hz, H-3'), 5.75 (1H, d,  $w_{1/2}$  = 8.9 Hz, H-6), 5.67 (1H, m,  $w_{1/2}$  = 8.7 Hz, H-7), 4.98 (2H, s, O-CH<sub>2</sub>-O), 4.92 (2H, t, J = 9.2 Hz, H<sub>2</sub>-1), 2.26 (2H, m, H<sub>2</sub>-5), 1.99 (2H, m, H<sub>2</sub>-8), 1.55 (2H, m, CH<sub>2</sub>), 1.36 (4H, brs, 2 x CH<sub>2</sub>), 1.28 (12H, brs, 6 x CH<sub>2</sub>), 1.25 (34H, brs, 17 x CH<sub>2</sub>), 0.85 (3H, t, J = 6.4 Hz, Me-32);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  68.91 (C-1), 34.70 (C-2), 34.58 (C-3), 34.28 (C-4), 33.84 (C-5), 123.93 (C-6), 119.14 (C-7), 56.46 (C-8), 34.83 (C-9), 33.78 (C-10), 31.94 (C-11), 31.48 (C-12), 30.23 (C-13), 29.71 - 29.17 (C-14 to C-29), 28.95 (C-30), 22.67 (C-31), 14.11 (C-32), 168.84 (C-1'), 113.46 (C-2'), 117.54 (C-3'), 114.08 (C-4'), 115.96 (C-5'), 143.25 (C-1''), 124.43 (C-2''), 149.25 (C-3''), 147.12 (C-4''), 139.25 (C-5''), 124.09 (C-6''), 103.29 (OCH<sub>2</sub>O); ESI MS  $m/z$  (rel. int.): 664 [M]<sup>+</sup> (C<sub>44</sub>H<sub>72</sub>O<sub>4</sub>) (25.1), 543 (2.4), 463 (3.9), 447 (2.8), 377 (17.2), 217 (4.1).

#### (Z)-4-Pentadecanoxyferulic acid (2)

Further elution of the column with chloroform yielded yellow crystals of **2**, yield 187 mg, m. p. 252 - 253°C, UV  $\lambda_{\text{max}}$  (MeOH): 223, 282, 325 nm (log  $\epsilon$  2.3, 1.9, 2.2); IR  $\nu_{\text{max}}$  (KBr): 3327, 2928, 2841, 1692, 1643, 1523, 1436, 1265, 1163, 1055, 936, 726  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.71 (1H, d, J = 8.9 Hz, H-5), 7.37 (1H, dd, J = 2.1, 8.9 Hz, H-6), 7.04 (1H, d, J = 2.1 Hz, H-2), 6.86 (1H, d, J = 8.3 Hz, H-7), 6.26 (1H, d, J = 8.3 Hz, H-8), 3.95 (3H, s, OMe), 3.91 (2H, t, J = 12.3 Hz, H<sub>2</sub>-1'), 2.63 (2H, m, CH<sub>2</sub>), 2.33 (2H, m, CH<sub>2</sub>), 2.05 (2H, m, CH<sub>2</sub>), 1.63 (2H, m, CH<sub>2</sub>), 1.35 (2H, m, CH<sub>2</sub>), 1.29 (16H, m, 8 x CH<sub>2</sub>), 0.88 (3H, t, J = 6.7 Hz, Me-15');  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  153.41 (C-1), 143.86 (C-2), 161.15 (C-3), 160.58 (C-4), 113.19 (C-5), 107.19 (C-6), 127.03 (C-7), 115.66 (C-8), 180.27 (C-9), 56.25 (OMe), 73.07 (C-1'), 33.72 (C-2'), 31.94 (C-3'), 30.29 (C-4'), 29.65 (C-5'), 29.65 (C-6'), 29.46 (C-7'), 29.33 (C-8'), 29.22 (C-9'), 26.07 (C-10'), 25.61 (C-11'), 24.73 (C-12'), 23.95 (C-13'), 22.65 (C-14'), 14.15 (C-15'); ESI MS  $m/z$  (rel. int.): 404 [M]<sup>+</sup> (C<sub>25</sub>H<sub>40</sub>O<sub>4</sub>) (1.7).

**(Z)-8-Dehydromelissic acid (3)**

Elution of the column with chloroform - methanol (99:1) furnished cream - color flakes of **3**, yield 327 mg, m. p. 119 - 121°C; IR  $\nu_{\max}$  (KBr): 3288, 2923, 2841, 1697, 1643, 1425, 1327, 1286, 935, 725  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  5.31 (1H, m,  $w_{1/2}$  = 8.1 Hz, H-8), 5.11 (1H, m,  $w_{1/2}$  = 9.5 Hz, H-9), 2.31 (2H, t, J = 6.9 Hz,  $\text{H}_2$ -2), 2.21 (2H, m,  $\text{H}_2$ -7), 2.07 (2H, m,  $\text{H}_2$ -10), 1.68 (2H, m,  $\text{CH}_2$ ), 1.55 (2H, m,  $\text{CH}_2$ ), 1.28 (8H, brs,  $3 \times \text{CH}_2$ ), 1.23 (36H, brs,  $18 \times \text{CH}_2$ ), 0.86 (3H, t, J = 6.7 Hz, Me-30);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  180.58 (C-1), 135.97 (C-8), 124.75 (C-9), 55.95 ( $\text{CH}_2$ ), 35.91 ( $\text{CH}_2$ ), 35.59 ( $\text{CH}_2$ ), 34.31 ( $\text{CH}_2$ ), 34.01 ( $\text{CH}_2$ ), 33.71 ( $\text{CH}_2$ ), 31.93 ( $\text{CH}_2$ ), 31.59 ( $\text{CH}_2$ ), 30.72 ( $\text{CH}_2$ ), 30.29 ( $\text{CH}_2$ ), 29.65 ( $12 \times \text{CH}_2$ ), 29.31 ( $\text{CH}_2$ ), 29.16 ( $\text{CH}_2$ ), 24.73 ( $\text{CH}_2$ ), 22.69 ( $\text{CH}_2$ ), 14.16 (Me-30); ESI MS  $m/z$  (rel. int.): 450  $[\text{M}]^+$  ( $\text{C}_{30}\text{H}_{58}\text{O}_2$ ) (2.7), 321 (10.5), 295 (38.3), 155 (11.2), 129 (3.1).

 **$\beta$ -D-xylose (4)**

Elution of the column with chloroform - methanol (19 : 1) afforded colourless needles of **4**, recrystallized from methanol, yield 590 mg,  $R_f$ : 0.15 (toluene - ethyl acetate - formic acid, 5:4:1.8), UV  $\lambda_{\max}$  (methanol): 210 nm, m. p. 143 - 145°C;  $[\alpha]_D^{22} + 22.5^\circ$  (10, water); IR  $\nu_{\max}$  (KBr): 3495, 3305, 2936, 2841, 1633, 1458, 1378, 1185, 1026, 895  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  5.30 (1H, d, J = 7.2 Hz, H-1), 4.21 (1H, m, H-2), 3.87 (1H, m, H-3), 3.71 (1H, m, H-4), 3.31 (1H, d, J = 10.3 Hz,  $\text{H}_2$ -5a), 3.22 (1H, d, J = 10.5 Hz,  $\text{H}_2$ -5b);  $^{13}\text{C NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  92.51 (C-1), 78.48 (C-2), 71.73 (C-3), 69.57 (C-4), 63.77 (C-5); ESI MS  $m/z$  (rel. int.): 150  $[\text{M}]^+$  ( $\text{C}_5\text{H}_{10}\text{O}_5$ ) (6.1).

**(Z)-6-Lauroleyl  $\beta$ -D-tetra-glucoside (5)**

Elution of the column with chloroform - methanol (9:1) gave colorless mass of **5**, yield 398 mg, UV  $\lambda_{\max}$  (MeOH): 212 nm, m. p. 170 - 173°C; IR  $\nu_{\max}$  (KBr): 3521, 3446, 3353, 2928, 2847, 1723, 1646, 1442, 1387, 1263, 1149, 1051, 778  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  5.32 (1H, m,  $w_{1/2}$  = 8.9 Hz, H-6), 5.16 (1H, m,  $w_{1/2}$  = 8.6 Hz, H-7), 2.83 (2H, t, J = 7.3 Hz,  $\text{H}_2$ -2), 2.34 (2H, m,  $\text{H}_2$ -5), 2.06 (2H, m,  $\text{H}_2$ -8), 1.55 (2H, m,  $\text{CH}_2$ ), 1.25 (8H, brs,  $4 \times \text{CH}_2$ ), 0.83 (3H, t, J = 6.6 Hz, Me-12), 5.08 (1H, d, J = 7.3 Hz, H-1'), 4.58 (1H, m, H-5'), 3.94 (1H, m, H-2'), 3.72 (1H, m, H-3'), 3.58 (1H, m, H-4'), 3.36 (2H, d, J = 8.5 Hz,  $\text{H}_2$ -6'), 4.97 (1H, d, J = 7.5 Hz, H-1''), 4.46 (1H, m, H-5''), 3.87 (1H, m, H-2''), 3.70 (1H, m, H-3''), 3.54 (1H, m, H-4''), 3.31 (2H, d, J = 8.8 Hz,  $\text{H}_2$ -6''), 4.89 (1H, d, J = 7.2 Hz, H-1'''), 4.37 (1H, m, H-5'''), 3.83 (1H, m, H-2'''), 3.66 (1H, m, H-3'''), 3.51 (1H, m, H-4'''), 3.25 (2H, d, J = 8.3 Hz,  $\text{H}_2$ -6'''), 4.85 (1H, d, J = 7.1 Hz, H-1'''), 4.29 (1H, m, H-5'''), 3.79 (1H, m, H-2'''), 3.63 (1H, m, H-3'''), 3.46 (1H, m, H-4'''), 3.09 (2H, d, J = 8.8 Hz,  $\text{H}_2$ -6''');  $^{13}\text{C NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  169.11 (C-1), 48.29 (C-2), 29.62 (C-3), 28.51 (C-4), 34.25 (C-5), 138.81 (C-6), 128.37 (C-7), 33.51 (C-8), 28.45 (C-9), 25.33 (C-10), 22.67 (C-11), 14.18 (C-12), 103.96 (C-1'), 73.06 (C-2'), 71.29 (C-3'), 66.37 (C-4'), 76.73 (C-5'), 63.63 (C-6'), 101.96 (C-1''), 72.91 (C-2''), 70.61 (C-3''), 65.59 (C-4''), 75.69 (C-5''), 63.07 (C-6''), 96.85 (C-1'''), 72.82 (C-2'''), 70.21 (C-3'''), 65.50 (C-4'''), 75.75 (C-5'''), 62.83 (C-6'''), 92.21 (C-1'''), 71.63 (C-2'''), 69.64 (C-3'''), 65.47 (C-4'''), 74.75 (C-5'''), 61.12 (C-6'''); ESI MS  $m/z$  (rel. Int.): 846  $[\text{M}]^+$  ( $\text{C}_{36}\text{H}_{62}\text{O}_{22}$ ) (13.1), 341 (58.9), 181 (32.4), 179 (23.2).

**(Z)-6-Lauroleyl  $\alpha$ -D-tetra-glucoside (6)**

Further elution of the column with ethyl acetate - methanol (9:1) yielded colorless mass of **6**, recrystallized from methanol, yield 249 mg, UV  $\lambda_{\max}$  (MeOH): 211 nm; m. p. 176 - 177°C; IR  $\nu_{\max}$  (KBr): 3522, 3413, 3346, 2927, 2851, 1725, 1647, 1413, 1349, 1081, 719  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  5.37 (1H, m,  $w_{1/2}$  = 8.5 Hz, H-6), 5.24 (1H, m,  $w_{1/2}$  = 8.9 Hz, H-7), 2.81 (2H, t, J = 7.2 Hz,  $\text{H}_2$ -2), 2.11 (2H, m,  $\text{H}_2$ -5), 2.08 (2H, m,  $\text{H}_2$ -8), 1.49 (2H, m,  $\text{CH}_2$ ), 1.23 (8H, brs,  $4 \times \text{CH}_2$ ), 0.84

(3H, t, J = 6.5 Hz, Me-12), 5.19 (1H, d, J = 2.9 Hz, H-1'), 4.29 (1H, m, H-5'), 3.88 (1H, m, H-2'), 3.65 (1H, m, H-3'), 3.49 (1H, m, H-4'), 3.29 (2H, d, J = 8.9 Hz,  $\text{H}_2$ -6'), 4.95 (1H, d, J = 3.1 Hz, H-1''), 4.21 (1H, m, H-5''), 3.81 (1H, m, H-2''), 3.60 (1H, m, H-3''), 3.46 (1H, m, H-4''), 3.25 (2H, d, J = 8.5 Hz,  $\text{H}_2$ -6''), 4.83 (1H, d, J = 2.8 Hz, H-1'''), 4.18 (1H, m, H-5'''), 3.76 (1H, m, H-2'''), 3.57 (1H, m, H-3'''), 3.42 (1H, m, H-4'''), 3.20 (2H, d, J = 8.6 Hz,  $\text{H}_2$ -6'''), 4.63 (1H, d, J = 3.8 Hz, H-1'''), 4.11 (1H, m, H-5'''), 3.70 (1H, m, H-2'''), 3.53 (1H, m, H-3'''), 3.39 (1H, m, H-4'''), 3.07 (2H, d, J = 8.8 Hz, H-6''');  $^{13}\text{C NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  169.27 (C-1), 47.98 (C-2), 33.51 (C-3), 30.37 (C-4), 29.09 (C-5), 137.95 (C-6), 119.89 (C-7), 28.93 (C-8), 28.75 (C-9), 24.28 (C-10), 22.74 (C-11), 14.12 (C-12), 103.93 (C-1'), 72.59 (C-2'), 71.63 (C-3'), 70.25 (C-4'), 82.45 (C-5'), 63.58 (C-6'), 101.91 (C-1''), 72.73 (C-2''), 71.56 (C-3''), 69.64 (C-4''), 77.08 (C-5''), 63.45 (C-6''), 96.89 (C-1'''), 73.06 (C-2'''), 71.27 (C-3'''), 69.72 (C-4'''), 78.69 (C-5'''), 62.10 (C-6'''), 92.41 (C-1'''), 74.77 (C-2'''), 70.52 (C-3'''), 65.51 (C-4'''), 75.21 (C-5'''), 61.15 (C-6'''); ESI MS  $m/z$  (rel. Int.): 846  $[\text{M}]^+$  ( $\text{C}_{36}\text{H}_{62}\text{O}_{22}$ ) (7.8), 341 (48.4), 181 (66.7), 179 (7.6).

**Isolation of phytoconstituents from the seeds of *Holarrhena pubescens*****5'-(2-Hydroxyphenyl)-pent-4-enyl ricinoleate (7)**

Elution of the column with chloroform yielded pale yellow crystals of **7**, yield 208 mg, m. p. 186 - 187°C, UV  $\lambda_{\max}$  (MeOH): 209, 271 nm (log  $\epsilon$  2.3, 3.6); IR  $\nu_{\max}$  (KBr): 3410, 2922, 2845, 1726, 1650, 1525, 1456, 1375, 1241, 1125, 1012, 826, 725  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (MeOD):  $\delta$  8.04 (1H, d, J = 10.8 Hz, H-3), 7.95 (1H, m, H-6), 7.33 (1H, m, H-4), 6.91 (1H, m, H-5), 6.23 (1H, d, J = 10.2 Hz, H-5'), 6.11 (1H, m, H-6'), 5.73 (1H, m, H-9''), 5.31 (1H, m, H-10''), 4.03 (2H, t, J = 5.7 Hz,  $\text{H}_2$ -1'), 3.66 (1H, m,  $w_{1/2}$  = 14.8 Hz, H-12'' $\alpha$ ), 2.27 (2H, t, J = 7.5 Hz,  $\text{CH}_2$ -1''), 2.20 (2H, m,  $\text{CH}_2$ -3'), 2.18 (2H, m,  $\text{CH}_2$ -8''), 2.07 (2H, m,  $\text{CH}_2$ -11''), 1.88 (2H, brs,  $\text{CH}_2$ -2'), 1.64 (2H, m,  $\text{CH}_2$ ), 1.55 (4H, m,  $2 \times \text{CH}_2$ ), 1.32 (6H, brs,  $3 \times \text{CH}_2$ ), 1.28 (10H, brs,  $5 \times \text{CH}_2$ ), 0.86 (3H, t, J = 6.5 Hz, Me-18'');  $^{13}\text{C NMR}$  (MeOD):  $\delta$  143.81 (C-1), 160.14 (C-2), 141.25 (C-3), 138.08 (C-4), 130.15 (C-5), 139.62 (C-6), 64.57 (C-1'), 33.23 (C-2'), 35.09 (C-3'), 122.57 (C-4'), 24.19 (C-5'), 172.67 (C-1''), 35.07 (C-2''), 120.23 (C-9''), 117.67 (C-10''), 33.21 (C-11''), 74.01 (C-12''), 30.91 ( $10 \times \text{CH}_2$ ), 26.24 ( $\text{CH}_2$ ), 23.88 ( $\text{CH}_2$ ), 14.60 (Me-18''); ESI MS  $m/z$  (rel. int.): 458  $[\text{M}]^+$  ( $\text{C}_{29}\text{H}_{46}\text{O}_4$ ) (2.8), 281 (8.1), 177 (27.6).

**Results and Discussion**

Compound **1**, designated as (*Z*)-*n*-dotriacontenyl piperate, showed characteristic IR absorption bands for ester group (1727  $\text{cm}^{-1}$ ), unsaturation (1637  $\text{cm}^{-1}$ ), aromatic ring (1549, 1085  $\text{cm}^{-1}$ ) and a long aliphatic chain (725  $\text{cm}^{-1}$ ). On the basis of mass and  $^{13}\text{C NMR}$  spectra, the molecular ion peak of **1** was established at  $m/z$  664 consisting to a molecular formula of piperic acid ester,  $\text{C}_{44}\text{H}_{72}\text{O}_4$ . The ion peaks arising at  $m/z$  543 [ $\text{C}_{1''}$  -  $\text{C}_{5'}$  fission] $^+$ , 463 [ $\text{C}_{1'}$  - O fission] $^+$ , 217 [O -  $\text{C}_1$  fission,  $\text{C}_6\text{H}_3(\text{OCH}_2\text{O})\text{-CH=CH-CH=CH-COO}^+$ , 447 [M - 217, ( $\text{CH}_2$ ) $_5\text{-CH=CH-(CH}_2$ ) $_{24}\text{-Me}^+$ ] and 377 [CH=CH-( $\text{CH}_2$ ) $_{24}\text{-Me}^+$ ] indicated that piperic acid was esterified with dotriacont-6-enol. The  $^1\text{H NMR}$  spectrum of **1** exhibited two one-proton doublets at  $\delta$  7.48 (J = 9.6 Hz) and 7.08 (J = 2.4 Hz) and a one-proton double doublet at  $\delta$  7.24 (J = 9.6, 2.4 Hz) assigned to aromatic *ortho*-coupled H-5'', *meta*-coupled H-2'' and *ortho*-, *meta*-coupled H-2'' protons, respectively, four one-proton signals as doublets at  $\delta$  6.23 (J = 14.8 Hz) and 6.86 (J = 14.8 Hz) and as double doublets at  $\delta$  5.79 (J = 14.8, 13.9 Hz) and 6.57 (J = 14.8, 13.9 Hz) ascribed to *trans*-oriented vinylic H-2', H-5', H-3' and H-4' protons, respectively, two one-proton multiplets at  $\delta$  5.75 ( $w_{1/2}$  = 8.9 Hz) and 5.67

( $w_{1/2} = 8.7$  Hz) attributed correspondingly to *cis*-oriented H-6 and H-7 protons, a two-proton singlet at  $\delta$  4.98 accounted to dioxomethylene protons, a two-proton triplet at  $\delta$  4.92 ( $J = 9.2$  Hz) due to oxymethylene H<sub>2</sub>-1 protons, a three-proton triplet at  $\delta$  0.85 ( $J = 6.4$  Hz) associated with terminal C-32 primary methyl protons and the remaining methylene protons from  $\delta$  2.26 to 1.25. The <sup>13</sup>C NMR spectrum of **1** showed signals for the ester carbon at  $\delta$  168.84 (C-1'), aromatic and vinylic carbons between  $\delta$  149.25 - 113.46, oxymethylene carbon at  $\delta$  68.91 (C-1), other methylene carbons from  $\delta$  34.70 to 22.67, dioxomethylene carbon at  $\delta$  103.29 and a methyl carbon at  $\delta$  14.11 (C-32). On the basis of the foregoing discussion the structure of **1** has been established as (*Z*)-*n*-dotriacont-6-enyl piperate, a new aromatic ester (Figure 1).

Compound **2**, [M]<sup>+</sup> at  $m/z$  404 (C<sub>25</sub>H<sub>40</sub>O<sub>4</sub>), produced effervescences with sodium bicarbonate solution, showed UV absorption maxima at 282 and 325 nm for aromatic compounds and IR absorption bands for carboxylic group (3327, 1692 cm<sup>-1</sup>), unsaturation (1643 cm<sup>-1</sup>), aromatic ring (1523, 1055 cm<sup>-1</sup>) and long aliphatic chain (726 cm<sup>-1</sup>). The <sup>1</sup>H NMR spectrum of **2** displayed a one-proton doublet at  $\delta$  7.37 ( $J = 2.1, 8.9$  Hz) and four one-proton doublets at  $\delta$  7.71 ( $J = 8.9$  Hz), 7.04 ( $J = 2.1$  Hz), 6.86 ( $J = 8.3$  Hz) and 6.26 ( $J = 8.3$  Hz) assigned to aromatic H-6, H-5 and H-2 and *cis*-oriented vinylic H-7 and H-8 protons, respectively. A three-proton signal at  $\delta$  3.95, a two-proton triplet at  $\delta$  3.91 ( $J = 12.3$  Hz) and a three-proton triplet at  $\delta$  0.88 ( $J = 6.7$  Hz) were ascribed correspondingly to methoxy, oxymethylene H<sub>2</sub>-1' and terminal C-15' primary methyl protons. The remaining methylene protons resonated from  $\delta$  2.63 to 1.29. The <sup>13</sup>C NMR spectrum of **2** exhibited signals for aromatic and vinylic carbons between  $\delta$  161.15 - 107.19, carboxylic carbon at  $\delta$  180.27 (C-9), oxymethylene carbons at  $\delta$  73.07 (C-1'), methoxy carbon at  $\delta$  56.25 (OMe), methyl carbon at  $\delta$  14.15 (C-15') and methylene carbons from  $\delta$  33.72 to 22.65. These evidences led to establish structure of **2** as (*Z*)-4-pentadecanoxyferulic acid, a new aromatic acid (Figure 1).

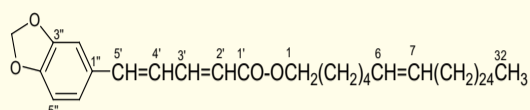
Compound **3**, named (*Z*)-8-dehydromelissic acid, gave effervescences with sodium bicarbonate indicating carboxylic nature of the compound. Its IR spectrum showed characteristic absorption bands for carboxylic group (3288, 1697 cm<sup>-1</sup>), unsaturation (1643 cm<sup>-1</sup>) and long aliphatic chain (725 cm<sup>-1</sup>). On the basis of mass and <sup>13</sup>C NMR spectra its molecular ion peak was established at  $m/z$  450 corresponding to a molecular formula of a long chain unsaturated fatty acid, C<sub>30</sub>H<sub>58</sub>O<sub>2</sub>. The ion peaks arising at  $m/z$  295 [CH<sub>3</sub>(CH<sub>2</sub>)<sub>20</sub>]<sup>+</sup>, 155 [M - 295, CH=CH(CH<sub>2</sub>)<sub>15</sub>COOH]<sup>+</sup>, 321 [CH<sub>3</sub>(CH<sub>2</sub>)<sub>20</sub>CH=CH]<sup>+</sup> and 129 [M - 321, (CH<sub>2</sub>)<sub>6</sub>COOH]<sup>+</sup> indicated the existence of the vinylic linkage at C-8 carbon. The <sup>1</sup>H NMR spectrum of **3** displayed two one-proton multiplets at  $\delta$  5.31 ( $w_{1/2} = 8.1$  Hz) and 5.11 ( $w_{1/2} = 9.5$  Hz) assigned to *cis*-oriented vinylic H-8 and H-9 protons, respectively. A two-proton triplet at  $\delta$  2.31 ( $J = 6.9$  Hz) was ascribed to methylene H<sub>2</sub>-2 protons adjacent to the carboxylic group. The other methylene protons appeared between  $\delta$  2.21 - 1.23. A three-proton triplet at  $\delta$  0.86 ( $J = 6.7$  Hz) was accounted to the C-30 primary methyl protons. The <sup>13</sup>C NMR spectrum of **3** exhibited important signals for carboxylic carbon at  $\delta$  180.58 (C-1), vinylic carbons at  $\delta$  135.97 (C-8) and 124.75 (C-9), methyl carbon at  $\delta$  14.16 (C-28) and methylene carbons between  $\delta$  55.95 - 22.69. On the basis of these observations the structure of **3** has been elucidated as (*Z*)-*n*-triacont-8-enoic acid, a new fatty acid (Figure 1).

Compound **4** was a known monosaccharide identified a  $\beta$ -D-xylose (Figure 1).

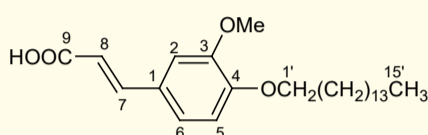
Compound **5**, named (*Z*)-6-lauroleil  $\beta$ -D-tetra-glucoside, gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3521, 3446, 3353 cm<sup>-1</sup>), ester function (1723 cm<sup>-1</sup>) and unsaturation (1646 cm<sup>-1</sup>). On the basis of mass and <sup>13</sup>C NMR spectral data, the molecular ion peak of **5** was established at  $m/z$  846 consistent with a molecular formula of an acyl tetraglycoside, C<sub>36</sub>H<sub>62</sub>O<sub>22</sub>. An ion peak generating at  $m/z$  181 [O-C<sub>1</sub> fission, C<sub>12</sub>H<sub>21</sub>O]<sup>+</sup> suggested that 6-lauroleic acid was esterified with a tetraglycosidic unit. The ion fragments arising at  $m/z$  179 [C<sub>6</sub>H<sub>11</sub>O<sub>6</sub>]<sup>+</sup> and 341 [C<sub>6</sub>H<sub>11</sub>O<sub>6</sub> - C<sub>6</sub>H<sub>10</sub>O<sub>4</sub>]<sup>+</sup> indicated the attachment of hexose units in the tetraglycosidic chain. The <sup>1</sup>H NMR spectrum of **5** exhibited four one - proton doublets at  $\delta$  5.08 ( $J = 7.3$  Hz), 4.97 ( $J = 7.5$  Hz), 4.89 ( $J = 7.2$  Hz) and 4.85 ( $J = 7.1$  Hz) assigned correspondingly to  $\beta$ -oriented sugar anomeric H-1', H-1'', H-1''' and H-1'''' protons. The other sugar protons resonated from  $\delta$  4.58 to 3.09. Two one-proton multiplets at  $\delta$  5.32 ( $w_{1/2} = 8.9$  Hz) and 5.16 ( $w_{1/2} = 8.6$  Hz) were ascribed to *cis*-oriented vinylic H-6 and H-7 protons, respectively. A two-proton triplet at  $\delta$  2.83 ( $J = 7.3$  Hz) was attributed to methylene H<sub>2</sub>-2 protons adjacent to the ester group. A three-proton triplet at  $\delta$  0.83 ( $J = 6.6$  Hz) was accounted to terminal C-12 primary methyl protons. The remaining methylene protons appeared between  $\delta$  2.83 - 1.25. The <sup>13</sup>C NMR spectrum of **5** displayed signals for ester carbon at  $\delta$  169.11 (C-1), vinylic carbons at  $\delta$  138.81 (C-6) and 128.37 (C-7), anomeric carbons at  $\delta$  103.96 (C-1'), 101.96 (C-1''), 96.85 (C-1''') and 92.21 (C-1'''), remaining sugar carbons between  $\delta$  76.73 - 61.12, methylene carbons from  $\delta$  48.29 to 22.67 and terminal methyl carbon at  $\delta$  14.18 (C-12). The presence of downfield signals of oxymethylene protons as two-proton doublets at  $\delta$  3.36 ( $J = 8.5$  Hz, H<sub>2</sub>-6'), 3.31 ( $J = 8.8$  Hz, H<sub>2</sub>-6'') and 3.25 ( $J = 8.3$  Hz, H<sub>2</sub>-6''') in the <sup>1</sup>H NMR spectrum and carbon signals at  $\delta$  63.63 (C-6'), 63.07 (C-6'') and 62.83 (C-6''') in the <sup>13</sup>C NMR spectrum suggested the (6 $\rightarrow$ 1) linkages among sugar units. Acid hydrolysis of **5** yielded D-glucose, R<sub>f</sub> 0.26 (*n*-butanol- acetic acid - water, 4: 1: 5). On the basis of above discussion, the compound **5** was structurally elucidated as (*Z*)-dodec-6-enoyl - O- $\beta$ -D-glucopyranosyl - (6' $\rightarrow$ 1'')-O- $\beta$ -D-glucopyranosyl-(6'' $\rightarrow$ 1''')-O- $\beta$ -D-glucopyranosyl-(6''' $\rightarrow$ 1'''')-O- $\beta$ -D-glucopyranoside, a new acyl tetraglycoside (Figure 1).

Compound **6**, designated as (*Z*)-6-lauroleil  $\alpha$ -D-tetra-glucoside, [M]<sup>+</sup> at  $m/z$  846 (C<sub>36</sub>H<sub>62</sub>O<sub>22</sub>), was an  $\alpha$ - analogue of **5**. Its <sup>1</sup>H NMR spectrum exhibited four one-proton doublets at  $\delta$  5.19 ( $J = 2.9$  Hz), 4.95 ( $J = 3.1$  Hz), 4.83 ( $J = 2.8$  Hz) and 4.63 ( $J = 3.8$  Hz) assigned to  $\alpha$ -oriented sugar anomeric H-1', H-1'', H-1''' and H-1'''' protons, respectively. The other sugar protons appeared from  $\delta$  4.29 to 3.07. Two one-proton multiplets at  $\delta$  5.37 ( $w_{1/2} = 8.5$  Hz) and 5.24 ( $w_{1/2} = 8.9$  Hz) were ascribed to *cis*-oriented vinylic H-6 and H-7 protons, respectively. A three-proton triplet at  $\delta$  0.84 ( $J = 6.5$  Hz) was due to terminal C-12 primary methyl protons. The remaining methylene protons appeared between  $\delta$  2.81 - 1.23. The <sup>13</sup>C NMR spectrum of **6** displayed signals for ester carbon at  $\delta$  169.27 (C-1), vinylic carbons at  $\delta$  137.95 (C-6) and 119.89 (C-7), anomeric carbons between  $\delta$  103.93 - 92.41, other sugar carbons from  $\delta$  82.45 to 61.15, methylene carbons in the range of  $\delta$  47.98 to 22.74 and terminal methyl carbon at  $\delta$  14.12 (C-12). The presence of downfield signals of oxymethylene protons as two-proton doublets at  $\delta$  3.36 ( $J = 8.5$  Hz, H<sub>2</sub>-6'), 3.31 ( $J = 8.8$  Hz, H<sub>2</sub>-6'') and

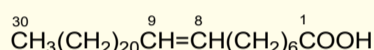
3.25 ( $J = 8.3$  Hz,  $H_2-6'''$ ) in the  $^1H$  NMR spectrum and carbon signals at  $\delta$  63.63 (C-6'), 63.07 (C-6'') and 62.83 (C-6''') in the  $^{13}C$  NMR spectrum suggested the (6 $\rightarrow$ 1) linkages among sugar units. These spectral data led to establish the structure of **6** as (*Z*)-dodec-6-enoyl-O- $\alpha$ -D-glucopyranosyl-(6' $\rightarrow$ 1'')-O- $\alpha$ -D-glucopyranosyl-(6'' $\rightarrow$ 1''')-O- $\alpha$ -D-glucopyranosyl-(6''' $\rightarrow$ 1'''')-O- $\alpha$ -D-glucopyranoside, a new acyl tetraglucoside (Figure 1).



*n*-Dotriacontenyl piperate (**1**)



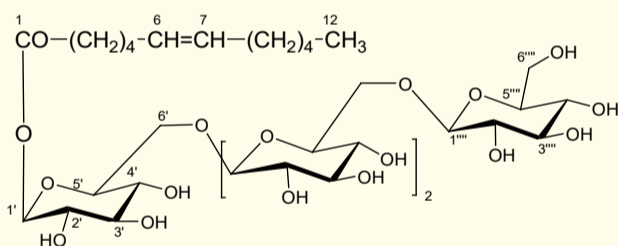
4-Pentadecanoxyferulic acid (**2**)



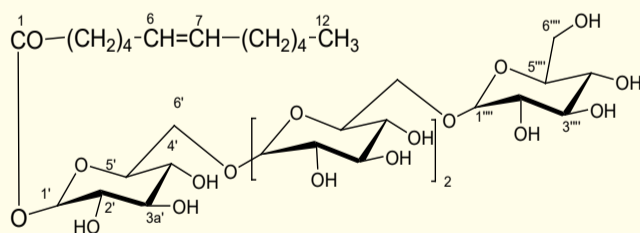
(*Z*)-8-Dehydromelissic acid (**3**)



$\beta$ -D-Xylose (**4**)



(*Z*)-6-Lauroleyl  $\beta$ -D-tetraglucoside (**5**)

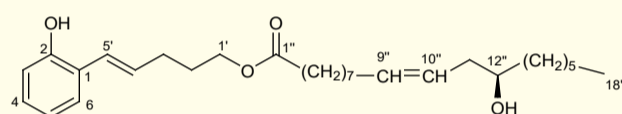


(*Z*)-6-Lauroleyl  $\alpha$ -D-tetraglucoside (**6**)

**Figure 1:** Chemical compounds **1** – **6** isolated from the aerial parts of *Jasminum auriculatum*.

Compound **7** responded to phenolic tests positively, had UV absorption maximum at 271 nm for an aromatic compound and IR absorption bands for hydroxyl groups ( $3410\text{ cm}^{-1}$ ), ester function ( $1726\text{ cm}^{-1}$ ), aromaticity ( $1650, 1525, 1012\text{ cm}^{-1}$ ) and long aliphatic chain ( $725\text{ cm}^{-1}$ ). Its mass spectrum showed a molecular ion peak at  $m/z$  458 consistent with the molecular formula of an aromatic

ester,  $C_{29}H_{46}O_4$ . The ion peaks arising at  $m/z$  281 [ $C_{17}H_{31}O_2$ ] $^+$  and 177 [ $M - 281, C_{18}H_{33}O_2$ ] $^+$  indicated that ricinoleic acid was esterified with a phenolic ring substituted pentenyl alcohol. The  $^1H$  NMR spectrum of **7** exhibited a one-proton doublet at  $\delta$  8.04 ( $J = 10.8$  Hz) and three one-proton multiplets between  $\delta$  7.95 - 6.91 assigned to aromatic H-3 to H-6 protons, a one-proton doublet at  $\delta$  6.23 ( $J = 10.2$  Hz) and a one-proton multiplet at  $\delta$  6.11 ascribed to *cis*-oriented vinylic H-5' and H-6' protons, respectively, two one-proton multiplets at  $\delta$  5.73 (1H, m) and 5.31 (1H, m, H-10'') attributed correspondingly to vinylic H-9'' and H-10'', a two-proton triplet at  $\delta$  4.03 ( $J = 5.7$  Hz) accounted to oxymethylene  $H_2-1'$  protons, a one-proton multiplet at  $\delta$  3.66 with half-width of 14.8 Hz due to carbinol H-12'' $\alpha$  proton, methylene protons from  $\delta$  2.27 to 1.28 and a three-proton triplet at  $\delta$  0.86 ( $J = 6.5$  Hz) associated with terminal primary C-18'' methyl protons. The  $^{13}C$  NMR spectrum of **7** displayed the presence of signals for benzene and vinylic carbons between  $\delta$  160.14 - 117.67, ester carbon at  $\delta$  172.67 (C-1''), carbinol carbon at  $\delta$  74.01 (C-12''), oxymethylene carbon at  $\delta$  64.57 (C-1') and methyl carbon at  $\delta$  14.60 (C-18''). On the basis of these evidences, the structure of **7** has been characterized as 5'-(2-hydroxyphenyl)-pent-4-enyl ricinoleate, a new aromatic ester (Figure 2).



5'-(2-Hydroxyphenyl)-pent-4-enylricinoleate (**7**)

**Figure 2:** Compound **7** isolated from the seeds of *Holarrhena pubescens*.

## Conclusion

Phytochemical investigation of a methanolic extract of the aerial parts of *Jasminum auriculatum* gave two aromatic compounds *n*-dotriacontenyl piperate and 4-pentadecanoxyferulic acid, a fatty acid viz., (*Z*)-8-dehydromelissic acid,  $\beta$ -D-xylose and two (*Z*)-6-lauroleyl  $\beta/\alpha$ -D-tetraglucosides. The seeds of *Holarrhena pubescens* afforded 5'-(2-hydroxyphenyl)-pent-4-enyl ricinoleate. This work has enhanced understanding about the phytoconstituents of the plants. These secondary metabolites can be used as analytical markers for quality control of these plant materials. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view to supplementing conventional drug development especially in developing countries.

## Bibliography

- Anonymous. "The wealth of India, Raw material". New Delhi: NISCAIR, Council of Scientific and Industrial Research 5 (2003): 279-291.
- Vaidyaratnam PS. "Indian medicinal plants-A compendium of 500 species". Orient Longman Private Ltd, Hyderabad 3 (2003) 164-165.
- Bedi S., et al. "A hand book of aromatic and essential oil plants". Agrobios Pvt Ltd, Mumbai (2008): 280-284.
- Srivastava DN. "Isolation and identification of constituents of *Jasminum auriculatum* (Vahl) leaves". *Journal of Applied Chemistry and Biotechnology* 27.1 (1977): 55-57.

5. Gowdhami T and Rajalakshmi AK. "Phytochemical analysis of *Jasminum auriculatum* Vahl. using FT-IR and GC-MS". *International Journal of Multidisciplinary Research and Development* 2.8 (2015): 268-273.
6. Ranchana P, et al. "Investigation of volatile compounds from the concrete of *Jasminum auriculatum* flowers". *International Journal of Current Microbiology and Applied Science* 6.11 (2017): 1525-1531.
7. Chouhan AS, et al. "*Holarrhena pubescens* Wall ex.Don: A review on ethnobotanical, phytochemical and pharmacological profile". *Indian Journal of Drugs* 5.2 (2017): 71-77.
8. Quattrocchi U. "CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology". Boca Raton, Florida (2012): 1994 -1995.
9. Jamadagni PS, et al. "Review of *Holarrhena antidysenterica* (L.) Wall. ex A. DC.: pharmacognostic, pharmacological, and toxicological perspective". *Pharmacognosy Reviews* 11.22 (2017): 141-144.
10. Siddiqui S and Shamsuddin BA. "Isolation and structure of holarrifine, a new alkaloid from the bark of *Holarrhena antidysenterica* Linn". *Pakistan Journal of Scientific and Industrial Research* 32 (1989): 1-3.
11. Siddiqui BS, et al. "Steroidal alkaloids and an androstane derivative from the bark of *Holarrhena pubescens*". *Phytochemistry* 33.4 (1993): 925-928.
12. Siddiqui BS, et al. "Further constituents from the bark of *Holarrhena pubescens*". *Phytochemistry* 58.8 (2001): 1199-1204.
13. Kumar N, et al. "Steroidal alkaloids from *Holarrhena antidysenterica* (L.) Wall". *Chemical and Pharmaceutical Bulletin* 55.6 (2007): 912 -914.
14. Raman MS, et al. "In Vitro antimicrobial activity of holarrifine 24-ol isolated from the stem bark of *Holarrhena antidysenterica*". *International Journal of Agricultural Biology* 6 (2004): 698-700.
15. Kumar A and Ali M. "A new steroidal alkaloid from the seeds of *Holarrhena antidysenterica*". *Fitoterapia* 71.2 (2000): 101-104.
16. Bhattchartya S, et al. "Triterpenoids and steroids from *Holarrhena pubescens* seeds". *Pharmacognosy Magazine* 5.20 (2009): 407-411.

**Volume 2 Issue 3 March 2018**

**© All rights are reserved by Mohammed Ali, et al.**