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Original Research Article

Chemical constituents from the leaves of *Fraxinus excelsior* L., *Senna sulfurea* (Collad.) H. S. Irwin et Barneby and *Prosopis cineraria* (L.) Druce

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ABSTRACT

Fraxinus excelsior L. (Oleaceae) is used to treat diarrhoea, dysentery, jaundice, joint pain, malaria, sores, swelling and wounds. The leaves of *Senna sulfurea* (Collad.) H. S. Irwin et Barneby (Leguminosae) are effective to cure blennorrhagia, diabetes, dysentery, gonorrhoea and skin diseases. *Prosopis cineraria* (L.) Druce, (Fabaceae) is taken to alleviate anxiety, asthma, bronchitis, dysentery, dyspepsia, fever, leprosy, leucoderma, muscle tremors, piles, rheumatism and tremors. Our study was planned to isolate chemical constituents of the methanolic extracts obtained from the leaves of *F. excelsior*, *S. sulfurea* and *P. cineraria* and to characterize their structures. The air-dried plant materials were exhaustively extracted with methanol separately in a Soxhlet. The concentrated methanolic extracts were adsorbed on silica gel (60-120 mesh) for the preparation of slurries. The dried slurries were chromatographed over silica gel columns individually packed in petroleum ether. The columns were eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate a variety of phytoconstituents. Phytochemical investigation of the leaves of *F. excelsior* afforded (*Z,Z,Z*)-*n*-tetratriacont-3,5,15-triene (**1**), *n*-hexatriacontane (**2**), (*Z,Z,Z*)-*n*-octatriacont-11,13,20-triene (**3**), phytanic acid (3,7,11,15-tetramethylhexadecanoic acid, **4**), 26-hydroxystigmastanol-18-oic acid (**5**) and α -L-xylose (**6**). The leaves of *S. sulfurea* furnished isoliquiritigenin (**7**) and 4-methoxy- α -L-xylopyranosyl-(3 \rightarrow 1')-*O*- α -L-4'-methoxyxylopyranoside (di-4-methoxy- α -L-xyloside, **8**). The leaves of *P. cineraria* on subjection to silica gel column chromatography led to isolate glyceryl-1-oleoyl-2-myristoyl-3-*O*-hydroxydihydrocinnamate (**9**), 2,3,4-trihydroxybenzyl *n*-hexadecanyl ether (**10**) and salicyloyl *O*- α -D-glucopyranosyl-2'-oleate (**11**). Their structures were established on the basis of spectral data analysis and chemical reactions.

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1. Introduction

Fraxinus excelsior L., syn. *F. acutifolia* Dippel, *F. aurea* Willd., *F. heterophylla* Vahl, *F. stricta* Beissner (Oleaceae), known as European ash, common ash, weeping ash, bird's tongue and kum, is distributed throughout Europe and cultivated as an ornamental plant in New Zealand, United States and Canada. It is a large tree, up to 40 m tall, with domed crown, ascending branches, bark smooth, pale grey, thick, fissured; leaves compound, 9-13 leaflets, odd pinnate, serrated and stalkless;

flowers open before the leaves unfold; seeds flattened, 2-5 cm long, in bunches, ripen individually in oval-shaped samaras. (Kerr, 1998; Anonymous, 2012). The ash bark is used to treat arthritis, diarrhoea, dysentery, gout, rheumatism, sores, swelling and wounds. It is substituted for quinine to cure malaria. The leaves are taken as a diuretic, laxative and recommended against fever, rheumatism, gout, oedema, stones, constipation, liver problems, jaundice, nephrolithiasis, kidney pain, oedema, cervical pain, worm infestations, intoxications, urinary stones and worm infestation; and externally

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for leg ulcers and wounds (Nadkarni, 1982; Gaedcke, 1993; Bruneton, 1999; Duke et al., 2002; Gruenwald et al., 2007; Kostova and Iossifova, 2007; Tahirović and Bašić, 2016). The plant sap is useful as an antidote for snake bites. The previous phytochemical studies on the leaves evidenced the presence of coumarins, coumarins, iridoids, seco-iridoids, rutin, kaempferol and quercetin-3-O-glucoside, their respective 3-O-rhamnoglucosides, β -sitosterol, betulin, betulinic acid and ursolic acid (Carnat et al., 1990; Damtoft et al., 1992; Gaedcke, 1993; Iossifova et al., 1997; Egan et al., 2004; Gruenwald et al., 2007; Kostova and Iossifova, 2007). Ferulic, caffeic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic and vanillic acids, hentriacontane, nonacosane, tetratriacontane, tannins and mannitol were found in different parts of *F. excelsior* (Carnat et al., 1990; Gaedcke, 1993; Oddo et al., 2002). The seeds afforded seco-iridoids excelsides A and B, muzhenide, G13, G15, ligstroside, oleoside 11-methyl ester, oleoside dimethyl ester, 1''-O- β -D-glucosyl formoside and salidroside (Bai et al., 2010). Phenolic compounds were detected in the heartwood (Sanz et al., 2012).

Senna sulfurea (Collad.) H. S. Irwin et Barneby, syn., *Cassia glauca* Lam., *C. arborescens* Vahl, *C. enneaphylla* Wight et Arn. and *Senna arborescens* (Vahl) Roxb. (Leguminosae), known as glaucous cassia, glossy sulphur-flowered senna, kalamona, scrambled eggs and smooth senna, is distributed in India, Sri Lanka, south eastern Asia, Australia, Polynesia, Gabon and China. It is commonly planted as an ornamental plant due to its beautiful flowers. It is up to 3 m high, evergreen, perennial shrub or small tree, with long linear, acute, curved leaves; inflorescence at axillary raceme, yellow flowers; flat strap shaped pods, black shiny seeds. (Quattrocchi, 2012). The leaves mixed with milk and sugar are taken to cure blennorrhagia. The bark and leaves are useful to relieve diabetes, dysentery and gonorrhoea (Kirtikar and Basu, 1990; Quattrocchi, 2012). This plant is also a good pollution tolerant and reduces chemical pollutants from the atmosphere (Warrier et al., 1996). The flowers and pods are purgative; the pods are used as an antiseptic to subside skin diseases (Quattrocchi, 2012). The stem contained chrysothanol, physcion, stearic acid, β -sitosterol and its 3-O-D-glucoside (Hemlata and Kalidhar, 1994). The leaves yielded di-(2-ethyl hexyl) phthalate, apigenin, luteolin, quercetin, its 3-glycosides, kaempferol 3-O-rutinoside, D-(+)-pinitol and rutin (El-Sayed et al., 2013; Kittur et al., 2015). The pods afforded 5,7-dihydroxy-4'-methoxyflavono-3-O- β -D-galactoside, chrysophenol, physcion, kaempferide and quercetin. (Rai et al., 1997). The seeds yielded methyl esters of fatty acids. (Kumar et al., 2013).

Prosopis cineraria (L.) Druce, syn. *Adenanthera aculeata* Roxb., *Mimosa cineraria* L., *Prosopis spicata* Burm. f. (Fabaceae), called as shami, khejri, kandi, jand, Indian mesquite and sponge tree, is distributed in Afghanistan, India, Iran, Oman, Pakistan, Sri Lanka,

Saudi Arab and Yemen. It is a small, 3-5 m tall tree, with bipinnate leaves, 7-14 leaflets on each of one to three pinnae; thorny branches along the internodes; small, creamy-yellow flowers; seeds in pods. The bark is considered as an abortifacient, anthelmintic, laxative, refrigerant, tonic and vermifuge, used to treat anxiety, asthma, bronchitis, dysentery, dyspepsia, fever, leprosy, leucoderma, muscle tremors, piles, rheumatism and tremors (Kirtikar and Basu, 1984). A leaf paste is applied to cure boils, blisters, sores and mouth ulcers. Smoke from the leaves is suggested for eye troubles (Nadkarni, 2000). The flowers are taken as an antidiabetic and mixed with sugar are given to prevent miscarriage (Anonymous, 2003). The plant material is used to treat snake bite and scorpion sting. The wood ash is rubbed over the skin to remove hair. (Janbaz et al., 2012). A pod extract is used to relieve earache, toothache and fractured bone pain (Ghazanfar and Alsabahi, 1993). The whole plant contained methyl heptacosanoate, heneicosanoic acid, 4-hydroxybenzoic acid, methyl 4-hydroxycinnamate, methyl 2-methoxy-5-hydroxycinnamate, methyl 2,5-dihydroxy-cinnamate and 1-O-coumaroylglycerol (Khan et al., 2006). The leaves possessed phytosterols, spicigerine, hentriacontane, methyl docosanoate, diisopropyl-10,11-dihydroxyicosane-1,20-dioate, tricosan-1-ol and 7,24-tirucalladien-3-one. (Malik and Kalidhar, 2007; Garg and Mittal, 2013). The roots afforded methyl tritriacontanoate, β -sitosterol, diethyl 2,3,6,7-tetraethoxydodecan-1,12-dioate and β -glucose (Malik and Kalidhar, 2007). The seeds furnished fatty acids, prosogerins C, D and E, gallic acid, patuletin, patulitrin, luteolin, rutin and galactomannan (Sharma and Soni, 1994; Gangal et al., 2009; Garg and Mittal, 2013). The immature pods yielded 3-benzyl-2-hydroxy-urs-12-en-28-oic acid, maslinic acid 3-glucoside, linoleic acid, prosophylline, 5,5'-oxybis-1,3-benzene diol, 3,4,5-trihydroxycinnamic acid 2-hydroxyethyl ester and 5,3',4'-trihydroxyflavanone 7-glucoside (Liu et al., 2012). The flowers contained patulitrin, β -sitosterol, spicigerine and prosogerins A and B (Garg and Mittal, 2013). Keeping in view the high reputation and application of *Fraxinus excelsior*, *Senna sulfurea* and *Prosopis cineraria* in the indigenous medicinal systems, it has been aimed to carry out isolation and characterization of chemical constituents from the leaves of these plants.

2. Experimental

2.1. General procedures

Melting points were recorded using one end open capillary tubes on a thermoelectrically heated melting point M-560 apparatus (Perfit, India) without correction. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded by using KBr pellets with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^1H (400 MHz)

and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using CDCl_3 and DMSO-d_6 as solvents and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) instrument with a +ve and -ve ESI techniques. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size and petroleum ether, chloroform, methanol and other solvents used were purchased from Merck Specialties (E. Merck, Pvt. Ltd. New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

2.2. Plant materials

The leaves of *Fraxinus excelsior*, *Senna sulfurea* and *Prosopis cineraria* were collected from the Jahan Panah garden of South Delhi and authenticated by Prof. M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. Their voucher specimens numbers (PRL/JH/2015/04), (PRL/JH/2016/21) and (PRL/JH/2015/12), respectively, are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

2.3. Extraction and isolation

One kilogramme (1.0 kg) each of the leaves of *F. excelsior*, *S. sulfurea* and *P. cineraria* were coarsely powdered and extracted separately and exhaustively with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 123.4 g, 132.6 g and 116.2 g, respectively. Small portion of each extract was analyzed chemically to determine the presence of different chemical constituents. The dried extract (100 g each) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of slurries. Each slurry was dried in air and chromatographed individually over silica gel columns (1.6 m x 16 mm x 2 mm) packed in petroleum ether. Each column was eluted successively in increasing order of polarity in various combinations with petroleum ether, petroleum ether-chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform, chloroform-methanol (19.9: 0.1; 99: 1; 97: 3; 19: 1; 93: 7; 9: 1; 17: 3; 4:1; 3: 1; 3: 2; 2: 3, v/v) and methanol. The fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get the following pure compounds:

2.3.1. Isolation of phytoconstituents from the leaves of *Fraxinus excelsior*

2.3.1.1. *n*-Tetratriacont-3,5,15-triene (**1**)

Elution of the column with petroleum ether produced pale yellow semisolid mass of **1**, yield 158 mg, UV λ_{max} (MeOH): 211 nm; IR ν_{max} (KBr): 2956, 2848, 1637, 1483, 1376, 1247, 1170, 723 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.26 (1H, m, $w_{1/2}$ =8.6 Hz, H-4), 5.17 (1H, m, $w_{1/2}$ =9.1 Hz, H-5), 5.14 (1H, m, $w_{1/2}$ =8.2 Hz, H-6), 5.11 (1H, m, $w_{1/2}$ =8.8 Hz, H-15), 5.08 (1H, m, $w_{1/2}$ =7.6 Hz, H-3), 5.05 (1H, m, $w_{1/2}$ =8.1 Hz, H-16), 2.13 (2H, m, H₂-7), 2.09 (2H, m, H₂-17), 2.04 (2H, m, H₂-2), 2.01 (2H, m, H₂-14), 1.98 (2H, m, H₂-2), 1.75 (2H, m, CH₂), 1.63 (4H, brs, 2 x CH₂), 1.29 (36H, br s, 18 x CH₂), 0.91 (3H, t, J =6.2 Hz, Me-1), 0.87 (3H, t, J =6.5 Hz, Me-34); ^{13}C NMR (CDCl_3): δ 134.96 (C-4), 134.84 (C-5), 131.29 (C-6), 124.47 (C-15), 124.30 (C-16), 124.26 (C-3), 39.75 (C-7), 39.72 (C-14), 37.19 (CH₂), 33.75 (CH₂), 31.94 (2 x CH₂), 29.87 (CH₂), 29.78 (CH₂), 29.73 (4 x CH₂), 29.67 (2 x CH₂), 29.55 (3 x CH₂), 29.37 (2 x CH₂), 29.32 (CH₂), 29.27 (CH₂), 28.30 (CH₂), 26.77 (CH₂), 26.68 (CH₂), 25.71 (CH₂), 22.72 (CH₂), 16.07 (Me-1), 14.12 (Me-34); ESI MS m/z (rel. int.): 472 [M]⁺ (C₃₄H₆₄) (32.3), 443 (48.2), 391 (4.8), 279 (6.2), 253 (5.1).

2.3.1.2. *n*-Hexatriacontane (**2**)

Further elution of the column with petroleum ether gave colorless powder of **2**, 146 mg, m.p. 75 -76 °C, UV λ_{max} (MeOH): 209 nm; IR ν_{max} (KBr): 2954, 2845, 1467, 1367, 1255, 1176, 726 cm^{-1} ; ^1H NMR (CDCl_3): δ 2.14 (2H, m, CH₂), 1.62 (2H, m, CH₂), 1.31 (2H, m, CH₂), 1.26 (32H, br s, 16 x CH₂), 0.88 (3H, t, J =6.4 Hz, Me-1), 0.83 (3H, t, J =6.5 Hz, Me-36); ^{13}C NMR (CDCl_3): δ 31.89 (CH₂), 29.78 (26 x CH₂), 25.34 (CH₂), 22.69 (CH₂), 14.18 (Me-1), 14.13 (Me-36); ESI-MS m/z (rel. int.): 506 [M]⁺ (C₃₆H₇₄) (16.2).

2.3.1.3. *n*-Octatriacont-11,13,20-triene (**3**)

Further elution of the column with petroleum ether afforded yellow semisolid mass of **3**, 121 mg, UV λ_{max} (MeOH): 218 nm; IR ν_{max} (KBr): 2925, 2853, 1638, 1463, 1378, 994, 721 cm^{-1} ; ^1H NMR (CDCl_3): δ 5.86 (1H, m, $w_{1/2}$ =8.7 Hz, H-12), 5.74 (1H, m, $w_{1/2}$ =9.2 Hz, H-13), 5.16 (1H, m, $w_{1/2}$ =8.1 Hz, H-14), 5.09 (1H, m, $w_{1/2}$ =6.6 Hz, H-11), 5.04 (1H, m, $w_{1/2}$ =9.1 Hz, H-20), 4.99 (1H, m, $w_{1/2}$ =8.7 Hz, H-21), 2.05 (2H, m, H₂-2), 2.01 (2H, m, H₂-10), 1.99 (2H, m, H₂-15), 1.97 (2H, m, H₂-19), 1.95 (2H, m, H₂-22), 1.68 (2H, m, CH₂), 1.61 (2H, brs, CH₂), 1.38 (2H, m, CH₂), 1.35 (2H, m, CH₂), 1.33 (2H, m, CH₂), 1.31 (2H, m, CH₂), 1.28 (4H, m, 2 x CH₂), 1.25 (34H, br s, 17 x CH₂), 0.86 (3H, t, J =6.4 Hz, Me-1), 0.82 (3H, t, J =6.5 Hz, Me-38); ^{13}C NMR (CDCl_3): δ 139.29 (C-12), 135.33 (C-13), 131.26 (C-14), 124.4 (C-11), 124.29 (C-20), 114.08 (C-21), 39.76 (C-2), 33.83 (C-10), 32.66 (C-15), 31.97 (2 x CH₂), 29.74 (2 x CH₂), 29.67 (10 x CH₂), 29.63 (3 x CH₂), 29.53 (CH₂), 29.28 (CH₂), 29.18 (CH₂), 28.97 (CH₂), 28.29 (CH₂), 26.78 (CH₂), 26.66 (CH₂), 25.70 (CH₂), 22.72 (CH₂), 16.04 (C-1), 14.15 (C-38); ESI MS m/z (rel. int.): 528 [M]⁺ (C₃₈H₇₂) (36.2), 387 (34.2), 335 (5.8), 265 (21.6), 239 (6.9).



2.3.1.4. Phytanic acid (**4**)

Elution of the column with petroleum ether-chloroform (1:1) produced colorless semisolid mass of **4**, 153 mg, UV λ_{\max} (MeOH): 213 nm; $[\alpha]_{\text{D}}^{25} -1.97^{\circ}$ (conc. 10, CHCl_3); IR γ_{\max} (KBr): 3357, 2932, 2870, 1695, 1457, 1386, 1281, 1032, 997, 763 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 2.14 (2H, dd, $J=4.0, 10.4$ Hz, H_2-2), 1.92 (1 H, m, H-3), 1.71 (1 H, m, H-7), 1.68 (2 H, m, H_2-4), 1.65 (2 H, m, H_2-5), 1.61 (2H, m, H_2-6), 1.59 (2 H, m, H_2-8), 1.57 (2 H, m, H_2-9), 1.50 (2 H, m, H_2-10), 1.45 (1 H, m, H-11), 1.36 (1 H, m, H-15), 1.25 (4 H, m, $\text{H}_2-12, \text{H}_2-13$), 1.08 (2 H, m, H_2-14), 0.95 (3 H, d, $J=6.8$ Hz, Me-17), 0.92 (3 H, d, $J=6.6$ Hz, Me-18), 0.84 (3 H, d, $J=6.5$ Hz, Me-19), 0.73 (3 H, d, $J=7.2$ Hz, Me-16), 0.71 (3 H, d, $J=6.9$ Hz, Me-20); $^{13}\text{C NMR}$ (CDCl_3): δ 179.83 (C-1), 55.28 (C-2), 32.73 (C-3), 39.89 (C-4), 25.17 (C-5), 36.72 (C-6), 32.75 (C-7), 37.49 (C-8), 24.58 (C-9), 37.29 (C-10), 32.87 (C-11), 37.46 (C-12), 24.98 (C-13), 39.46 (C-14), 28.07 (C-15), 22.82 (C-16), 19.87 (C-17), 19.75 (C-18), 16.42 (C-19), 22.72 (C-20); ESI MS m/z (rel. int.): 312 $[\text{M}]^+$ ($\text{C}_{20}\text{H}_{40}\text{O}_2$) (3.5).

2.3.1.5. 26-Hydroxystigmasterol-18-oic acid (**5**)

Elution of the column with chloroform yielded yellow crystals of **5**, recrystallized from chloroform-methanol (1:1), 157.2 mg, m. p. 249 - 251 $^{\circ}\text{C}$; UV λ_{\max} (MeOH): 221 nm ($\log \epsilon$ 3.2); IR γ_{\max} (KBr): 3420, 3365, 3280, 2931, 2852, 1693, 1636, 1455, 1381, 1242, 1183, 1035, 764 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.25 (1H, m, H-6), 5.05 (1H, m, H-22), 5.01 (1H, m, H-23), 3.81 (1H, brm, $w_{1/2}=18.5$ Hz, H-3 α), 3.22 (2H, d, $J=7.2$ Hz, H_2-26), 1.08 (3H, brs, Me-19), 0.92 (3H, d, $J=6.1$ Hz, Me-21), 0.87 (3H, d, $J=6.0$ Hz, Me-27), 0.77 (3H, t, $J=6.5$ Hz, Me-29), 2.17 - 1.12 (25 H, m, 9 x CH_2 , 7 x CH); $^{13}\text{C NMR}$ (CDCl_3): δ 38.25 (C-1), 29.70 (C-2), 73.25 (C-3), 39.28 (C-4), 140.07 (C-5), 120.18 (C-6), 31.88 (C-7), 32.25 (C-8), 49.27 (C-9), 37.82 (C-10), 23.11 (C-11), 37.91 (C-12), 43.26 (C-13), 53.77 (C-14), 24.28 (C-15), 29.26 (C-16), 52.93 (C-17), 181.22 (C-18), 19.13 (C-19), 33.96 (C-20), 18.49 (C-21), 138.21 (C-22), 129.32 (C-23), 47.82 (C-24), 29.21 (C-25), 63.43 (C-26), 19.36 (C-27), 23.06 (C-28), 11.84 (C-29); ESI MS m/z (rel. int.): 458 $[\text{M}]^+$ ($\text{C}_{29}\text{H}_{46}\text{O}_4$) (3.8), 440 (18.2), 413 (12.7), 303 (4.7), 155 (6.1).

2.3.1.6. α -L-xylose (**6**)

Elution of the column with chloroform-methanol (4:1) afforded colourless needles of **6**, recrystallized from methanol, yield 208 mg, R_f : 0.15 (toluene -ethyl acetate-formic acid, 5:4:1.8), UV λ_{\max} (methanol): 210 nm, m. p. 150 - 151 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{24} -18.7^{\circ}$ (conc 4, H_2O); IR γ_{\max} (KBr): 3390, 3296, 2935, 2841, 1617, 1460, 1384, 1317, 1082, 1020, 878 cm^{-1} ; $^1\text{H NMR}$ ($\text{DMSO}-d_6$): δ 4.38 (1H, d, $J=5.4$ Hz, H-1), 4.27 (1H, dd, $J=5.4, 5.7$ Hz, H-2), 4.11 (1H, m, H-3), 3.61 (1H, m, H-4), 3.57 (1H, d, $J=7.2$ Hz, H_2-5a), 3.44 (1H, d, $J=5.6$ Hz, H_2-5b); $^{13}\text{C NMR}$ ($\text{DMSO}-d_6$): δ 100.21 (C-1), 71.28 (C-2), 69.73 (C-3), 69.65 (C-4), 63.76

(C-5); ESI MS m/z (rel. int.): 150 $[\text{M}]^+$ ($\text{C}_5\text{H}_{10}\text{O}_5$) (6.8).

2.3.2. Isolation of phytoconstituents from the leaves of *Senna sulfurea*

2.3.2.1. Isoliquiritigenin (**7**)

Elution of the column with chloroform-methanol (99:1) mixture afforded colourless amorphous powder of **7**; recrystallized from chloroform-methanol (1:1); 148 mg, m. p. 206 - 208 $^{\circ}\text{C}$; UV λ_{\max} (MeOH): 218, 241, 347 nm ($\log \epsilon$ 2.8, 5.3, 4.9); IR γ_{\max} (KBr): 3428, 3381, 2926, 2856, 1680, 1623, 1587, 1512, 1483, 1413, 1349, 1243, 1213, 1040, 853 cm^{-1} ; $^1\text{H NMR}$ ($\text{DMSO}-d_6$): δ 8.31 (1H, d, $J=8.7$ Hz, H-6'), 8.26 (1H, d, $J=8.7$ Hz, H-8), 7.72 (2H, d, $J=7.5$ Hz, H-3, H-5), 7.66 (1H, d, $J=8.7$ Hz, H-7), 6.78 (1H, dd, $J=2.1, 8.7$ Hz, H-5'), 6.34 (1H, d, $J=2.1$ Hz, H-3'), 6.29 (2H, d, $J=7.5$ Hz, H-2, H-6); $^{13}\text{C NMR}$ (CDCl_3): δ 144.13 (C-1), 130.24 (C-2), 132.90 (C-3), 152.16 (C-4), 138.27 (C-5), 123.99 (C-6), 122.54 (C-7), 129.75 (C-8), 194.06 (C-9), 146.61 (C-1'), 157.28 (C-2'), 117.41 (C-3'), 158.88 (C-4'), 116.69 (C-5'), 118.76 (C-6'); ESI MS m/z (rel. int.): 256 $[\text{M}]^+$ ($\text{C}_{15}\text{H}_{12}\text{O}_4$) (2.2).

2.3.2.2. Di-4-methoxy- α -L-xyloside (**8**)

Elution of the column with chloroform-methanol (9:1) afforded colourless crystals of **8**, yield 126 mg, m. p. 202-203 $^{\circ}\text{C}$; IR γ_{\max} (KBr): 3403, 3318, 3265, 2952, 2849, 1453, 1341, 1250, 1128, 1103, 1071, 962 cm^{-1} ; $^1\text{H NMR}$ ($\text{DMSO}-d_6$): δ 4.65 (1H, d, $J=2.8$ Hz, H-1 α), 4.46 (1H, dd, $J=2.8, 4.4$ Hz, H-2), 4.28 (1H, m, H-3), 3.72 (2H, m, H_2-5), 3.57 (1H, m, H-4), 4.55 (1H, d, $J=3.0$ Hz, H-1'), 4.38 (1H, dd, $J=3.0, 6.5$ Hz, H-2'), 3.75 (1H, m, H-3'), 3.30 (2H, m, H-5'), 3.42 (1H, m, H-4'), 3.37 (3H, br s, OMe), 3.34 (3H, br s, OMe); $^{13}\text{C NMR}$ ($\text{DMSO}-d_6$): δ 99.02 (C-1), 79.01 (C-2), 78.35 (C-3), 72.20 (C-4), 70.99 (C-5), 93.53 (C-1'), 78.68 (C-2'), 72.55 (C-3'), 71.73 (C-4'), 70.12 (C-5'), 55.61 (OMe), 55.32 (OMe); ESI MS m/z (rel. int.): 310 $[\text{M}]^+$ ($\text{C}_{12}\text{H}_{22}\text{O}_9$) (2.9), 163 (11.3), 147 (4.1).

2.3.3. Isolation of a glyceride from the seeds of *Prosopis cineraria*

2.3.3.1. Glycerol-1-oleiyl-2-myristoyl-3-O-hydroxy dihydrocinnamate (**9**)

Elution of the column with petroleum ether furnished a yellow semisolid mass of **9**, purified by preparative TLC using petroleum ether - chloroform (1:1), UV λ_{\max} (MeOH): 212, 273 nm ($\log \epsilon$ 2.4, 3.7); IR γ_{\max} (KBr): 3350, 2925, 2865, 1727, 1605, 1509, 1459, 1377, 1272, 1246, 1181, 1029, 826, 726 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 7.72 (1H, m, H-6'''), 7.52 (1H, m, H-7'''), 7.13 (1H, m, H-8'''), 6.82 (1H, d, $J=7.6$ Hz, H-9'''), 5.37 (1H, m, H-9'), 5.28 (1H, m, H-10'), 4.30 (1H, m, H-2), 4.19 (2H, d, $J=6.0$ Hz, H_2-1), 4.13 (2H, m, H_2-3), 2.28 (2H, t, $J=7.2$ Hz, H_2-2'), 2.19 (2H, t, $J=7.5$ Hz, H_2-2''), 2.05 (2H, t, $J=7.2$ Hz, H_2-2'''), 2.01



3.2. Spectroscopic characteristics of compound **3**

Compound **3**, $[M]^+$ at m/z 528 ($C_{38}H_{72}$), showed IR absorption bands for unsaturation (1638 cm^{-1}) and long aliphatic chain (721 cm^{-1}). The production of the ion peaks at m/z 387 [C_{10} - C_{11} fission, $CH_3(CH_2)_{16}CH=CH-(CH_2)_5(CH=CH)_2^+$], 335 [C_{14} - C_{15} fission, $CH_3(CH_2)_{16}CH=CH-(CH_2)_5^+$], 265 [C_{19} - C_{20} fission, $CH_3(CH_2)_{16}CH=CH^+$] and 239 [C_{21} - C_{22} fission, $CH_3-(CH_2)_{16}^+$] indicated the presence of the vinylic linkages at C-11, C-13 and C-20 positions in the alkatriene chain. The 1H NMR spectrum of **3** displayed six multiplets integrating each for one proton in the deshielded region between δ 5.86-4.99 with half-widths from 9.2 to 6.6 Hz assigned to cis-oriented vinylic H-12, H-13, H-14, H-11, H-20 and H-21 protons. Two triplets integrating each for three protons at δ 0.86 ($J=6.4$ Hz) and 0.82 ($J=6.5$ Hz) were due to C-1 and C-38 primary methyl protons, respectively. The remaining methylene protons resonated in the range of δ 2.05 - 1.25. The ^{13}C NMR spectrum of **3** showed signals for vinylic carbons from δ 139.29 to 114.08, methylene carbons between δ 39.76 - 22.72 and methyl carbons at δ 16.04 (C-1) and 14.15 (C-38). The absence of any signal from δ 4.99 to 2.05 in the 1H NMR spectrum and between δ 114.08-39.76 in the ^{13}C NMR spectrum ruled out the existence of any carbinol proton in the molecule. The 1H - 1H COSY spectrum of **3** showed correlations of H-12 with H-11, H₂-10, H-13, H-14 and H₂-15; and H-20 with H₂-19, H-21 and H₂-22. On the basis of the foregoing account, the structure of **3** was formulated as (*Z,Z,Z*)-*n*-octatriacont-11,13,20-triene, a new alkatriene (Fig. 1). Compound **4** was a known diterpenic constituent characterized as phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) (Ellinghaus et al., 1999; van den Brink and Wanders, 2006).

3.3. Spectroscopic characteristics of compound **5**

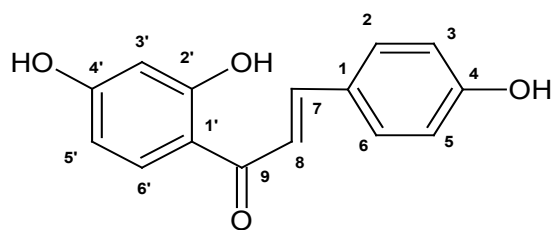
Compound **5** produced effervescence with sodium bicarbonate solution due to the presence of a carboxylic group and had IR distinctive absorption bands for hydroxyl functions ($3420, 3365\text{ cm}^{-1}$), carboxylic group ($3280, 1693\text{ cm}^{-1}$) and unsaturation (1636 cm^{-1}). Its molecular ion peak was established at m/z 458 on the basis of mass and ^{13}C NMR spectra corresponding to the molecular formula of a sterol $C_{29}H_{46}O_4$. The ion peaks arising at m/z 440 [$M-H_2O, C_{29}H_{44}O_3^+$] and 413 [$M-COOH, C_{28}H_{45}O_2^+$] supported the presence of one each of carbinol and carboxylic functions in the molecule. The expulsion of a side chain with mass unit of 155 produced an ion peak at m/z 303 [$M-C_{10}H_{19}O, \text{side chain}^+$] indicating the existence of the saturated C_{10} side chain with a hydroxyl group. The 1H NMR spectrum of **5** exhibited three multiplets integrating each for one proton at δ 5.25, 5.05 and 5.01 assigned correspondingly to vinylic H-6, H-22 and H-23 protons of a stigmasterol-type molecule, a one-proton broad multiplet at δ 3.81 with half-width of 18.5 Hz ascribed to α -oriented

carbinol H-3 proton, a two-proton doublet at δ 3.22 ($J=7.2$ Hz) accounted to hydroxymethylene H₂-26 protons, a three-proton singlet at δ 1.08 due to tertiary C-19 methyl protons, two doublets integrating each for three protons at δ 0.92 ($J=6.1$ Hz) and 0.87 ($J=6.0$ Hz) attributed to secondary C-21 and C-27 methyl protons and a three-proton triplet at δ 0.77 ($J=6.5$ Hz) associated with primary C-29 methyl protons. The remaining methylene and methine protons resonated from δ 2.17 to 1.12. The ^{13}C NMR spectrum of **5** displayed 29 carbon signals including carboxylic carbon at δ 181.22 (C-18), vinylic carbons at δ 140.07 (C-5), 120.18 (C-6), 138.21 (C-22) and 129.32 (C-23), carbinol carbon at δ 73.25 (C-3), hydroxymethylene carbon at δ 63.43 (C-26) and methyl carbons at δ 19.13 (C-19), 18.49 (C-21), 19.36 (C-27) and 11.84 (C-29). The 1H and ^{13}C NMR spectral data of the steroidal nucleus were compared with the reported spectral values of similar compounds (Akhtar et al., 2010; Mustafa and Ali, 2011; Jung et al., 2012). The DEPT spectrum of **5** displayed the presence of four methyl, ten methylene, eleven methine and four quaternary carbons. The 1H - 1H COSY spectrum of **5** exhibited correlations of H-3 with H₂-1, H₂-2 and H₂-4; H-6 with H₂-4, H₂-7 and H-8; H-22 with H-17, H-20, H₃-21, H-23 and H-24; and H-25 with H-24, H₂-26 and H₃-27. Its HMBC spectrum showed that H-3, H₂-4, H-6 and H₂-7 interacted with C-5; H-14, H₂-12 and H-17 interacted with C-18; and H-23, H-24, H₂-26 and H₃-27 interacted with C-25. On the basis these evidences the structure of **5** has been elucidated as 26-hydroxystigmasterol-18-oic acid, a new steroidal acid (Fig. 1). Compound **6** (Fig. 1) was a known monosaccharide identified as α -L-xylose (Usvalampi et al., 2012; Sultana et al., 2018).

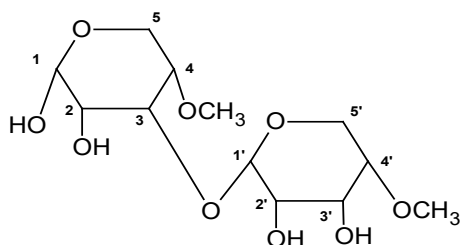
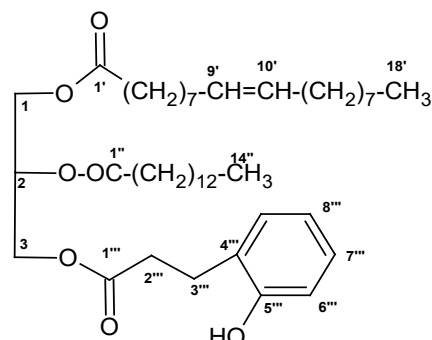
Compound **7** was a chalcone derivative characterized as isoliquiritigenin (Ma et al., 2005; Sato et al., 2007).

3.4. Spectroscopic characteristics of compound **8**

Compound **8**, named di-4-methoxy- α -L-xyloside, $[M]^+$ at m/z 310 ($C_{12}H_{22}O_9$), gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups ($3403, 3318, 3265\text{ cm}^{-1}$). The ion fragments generated at m/z 163 [$O-C_1$ fission, $C_6H_{11}O_5^+$] and 147 [$M-163, C_6H_{11}O_4^+$] indicated that two methoxypentose units were linked in the molecule. The 1H NMR spectrum of **8** exhibited two doublets integrating each for one proton at δ 4.65 ($J=2.8$ Hz) and 4.55 ($J=3.0$ Hz) assigned to anomeric H-1 and H-1' protons, respectively, supported the existence of α -glycosidic units of the disaccharide. The other sugar protons resonated between δ 4.46 - 3.34. Two singlets integrating each for three protons at δ 3.37 and 3.34 were associated with the methoxy protons. The ^{13}C NMR spectrum of **8** displayed signals for anomeric carbons at δ 99.02 (C-1) and 93.53 (C-1'), other sugar carbons from δ 79.01 to 70.12 and methoxy carbons δ 55.61 and 55.32. The presence of the sugar H-3 signal in the deshielded region as a one-proton multiplet at



Isoliquiritigenin (7)


 Di-4 methoxy- α -L-xyloside (8)


Glycerol-1-oleyl-2-myristoyl-3-O-hydroxydihydrocinnamate (9)

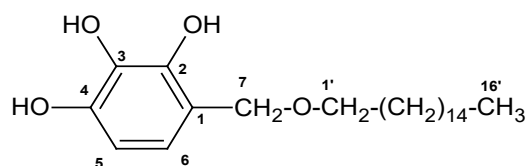
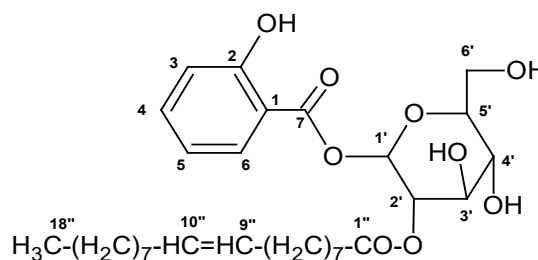

 2,3,4-Trihydroxybenzyl *n*-hexadecanyl ether (10)

 Salicyloyl O- α -D-glucosyl-2'-oleate (11)

Fig. 2. Chemical constituents **7** and **8** isolated from the leaves of *Senna sulfurea*.

δ 4.28 in the ^1H NMR spectrum and C-3 carbon signal at δ 78.35 in the ^{13}C NMR spectrum suggested (3 \rightarrow 1') linkage of the sugar units. The ^1H - ^1H COSY spectrum of **8** exhibited correlations of H-3 with H-1, H-2, H-4, H₂-5, OMe and H-1'; and H-3' with H-1', H-2', H-4', OMe and H₂-5'. Its HMBC spectrum showed that H-1, H-2, H-4, H₂-5 and H-1' interacted with C-3; and H-1', H-2', H-4', OMe and H₂-5' interacted with C-3'. On the basis of these evidences the structure of **8** has been formulated as 4-methoxy- α -L-xylopyranosyl-(3 \rightarrow 1')-O- α -L-4'-methoxyxylopyranoside, a new dixyloside (Fig. 2).

3.5. Spectroscopic characteristics of compound **9**

Compound **9** had UV absorption maximum at 273 nm for aromatic compounds, showed IR absorption bands for a hydroxyl group (3350 cm^{-1}), ester function (1727 cm^{-1}), aromaticity (1605, 1509, 1029 cm^{-1}) and long aliphatic chain (726 cm^{-1}) and gave positive tests for phenols. Its mass spectrum showed a molecular ion peak at m/z 714 consistent with the molecular formula of a lipid glyceride, $\text{C}_{44}\text{H}_{74}\text{O}_7$. The ion peaks arising at m/z 265 [$\text{C}_{17}\text{-O}$ fission, $\text{CH}_3(\text{CH}_2)_7\text{-CH=CH-(CH}_2)_7\text{CO}^+$], 211 [$\text{C}_{14}\text{-O}$ fission, $\text{CH}_3(\text{CH}_2)_{12}\text{-CO}^+$] and 165 [$\text{C}_6\text{H}_4(\text{OH})\text{-CH}_2\text{-CH}_2\text{-COO}^+$] indicated that oleic acid, myristic acid and o-hydroxydihydrocinnamic acid were linked with glycerol unit. The ^1H NMR spectra of **9** exhibited three multiplets integrating each for one proton at δ 7.72, 7.52 and 7.13 and a one - proton doublet at δ 6.82 ($J=7.6$ Hz) assigned to aromatic H-6''' to H-9''' protons, two multiplets integrating each for one proton at δ 5.37 and 5.28 accounted correspondingly to vinylic protons H-9' and H-10' protons, a one - proton multiplet at δ 4.30 due to oxymethine H-2, oxymethylene protons as a two-proton doublet at δ 4.19 ($J=6.0$ Hz, H₂-1) and a

Fig. 3. Structures of the chemical constituents **9-11** isolated from the leaves of *Prosopis cineraria*.

two-proton multiplet at δ 4.13 (H₂-3), other methylene protons from δ 2.28 to 1.25 and two triplets integrating each for three protons at δ 0.86 ($J=6.6$ Hz) and 0.83 ($J=6.3$ Hz, Me-14'') ascribed to primary C-18' and C-14'' methyl protons, respectively. The HMBC spectrum of **9** displayed interactions of H₂-1 and H₂-3 with C-2; H₂-8', H-9' and H₂-11' with C-10'; H-2''' and H-3''' with C-1'''; and H-6''' and H-7''' with C-5'''. On the basis of these evidences, the structure of **9** has been characterized as glyceryl-1-oleoyl-2-myristoyl-3-o-hydroxydihydrocinnamate, a new lipid component (Fig. 3).

3.6. Spectroscopic characteristics of compound **10**

Compound **10**, $[\text{M}]^+$ at m/z 380 ($\text{C}_{23}\text{H}_{40}\text{O}_4$), responded positive tests for phenols, showed UV absorption maximum at 276 nm for aromatic compounds and had IR absorption bands for hydroxyl groups (3378, 3265 cm^{-1}), aromaticity (1576, 1091 cm^{-1}) and long aliphatic chain (722 cm^{-1}). The ion peaks arising at m/z 155 [$\text{C}_7\text{-O}$ fission, $\text{C}_6\text{H}_2(\text{OH})_3\text{-CH}_2\text{O}^+$] and 225 [M -



155, $\text{CH}_2(\text{CH}_2)_{14}\text{CH}_3^+$ indicated that *n*-hexadecane was linked with trihydroxybenzyl alcohol. The ^1H NMR spectra of **10** exhibited two doublets integrating each for one proton at δ 7.09 ($J=7.8$ Hz, H-5) and 6.82 ($J=7.8$ Hz, H-6) assigned to aromatic *ortho*-coupled H-5 and H-6 protons, respectively, two singlets integrating each for one proton at δ 4.15 and 4.05 accounted to oxymethylene H_2 -7 protons attached to the aromatic ring, a two-proton triplet at δ 3.61 ($J=8.0$ Hz) due to oxymethylene H_2 -1', other methylene protons from δ 2.12 to 1.25 and a three-proton triplet at δ 0.83 ($J=6.5$ Hz) ascribed to primary C-16' methyl protons. The ^1H - ^1H COSY spectrum of **10** showed correlations of H-5, H-6, H_2 -1' with H_2 -7. The HMBC spectrum of **10** displayed interactions of H-5, H-6 and H_2 -7 with C-1; and H_2 -7 and H_2 -1' with C-2'. On the basis of these evidences, the structure of **10** has been characterized as 2,3,4-trihydroxybenzyl *n*-hexadecanyl ether, a new phenolic ether (Fig. 3).

3.7. Spectroscopic characteristics of compound **11**

Compound **11**, named salicyloyl O- α -L-glucosyl-2'-oleate, gave positive tests for phenols and glycosides and had UV absorption maximum at 284 nm for aromaticity. Its IR spectrum showed absorption bands for hydroxyl groups (3403, 3365 cm^{-1}), ester function (1727 cm^{-1}), unsaturation (1620 cm^{-1}) and aromatic ring (1575, 1087 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at m/z 548 consisting of a molecular formula of an aromatic carboxylic glucosidic ester, $\text{C}_{31}\text{H}_{48}\text{O}_8$. An ion peak arising at m/z 121 [$\text{C}_7\text{-O}$ fission, $\text{HO-C}_6\text{H}_4\text{-CO}^+$] indicated that salicyloyl unit was linked with a glycosidic moiety. The ion peak generated at m/z 265 [$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{CO}^+$] indicated that oleic acid was esterified with the glycosidic unit. The ^1H NMR spectrum of **11** showed three multiplets integrating each for one proton at δ 7.69, 7.59 and 7.09 and a one-proton doublet at δ 6.80 ($J=8.4$ Hz) assigned to aromatic H-3, H-4, H-5 and H-6 protons, respectively. Two multiplets integrating each for one proton at δ 5.31 and 5.29 were ascribed correspondingly to vinylic H-9'' and H-10'' protons. A one-proton doublet at δ 5.23 ($J=5.6$ Hz) was accounted to α -oriented anomeric H-1' proton. The other sugar protons appeared as a one-proton doublet at δ 4.17 ($J=5.6, 5.7$ Hz, H-2'), as one-proton multiplets at δ 4.03 (H-5'), 3.81 (H-3') and 3.70 (H-4') and as a two-proton doublet at δ 3.30 ($J=9.2$ Hz, H-6'). The presence of the sugar H-2' proton in the deshielded region at δ 4.17 indicated attachment of the ester linkage at C-2'. The methylene protons of the fatty acid chains resonated in the range of δ 2.29 - 1.27. A three-proton triplet at δ 0.84 ($J=6.7$ Hz, Me-18'') was associated with the terminal C-18'' primary methyl protons. The ^1H - ^1H COSY spectrum of **11** showed correlations of H-3, H-5 and H-6 with H-4; H-2', H-3' and H-5' with H-1'; and H_2 -8'', H-9'' and H_2 -11'' with H-10. The HMBC spectrum of **11** exhibited

that H-3 and H-4 interacted with C-2; H-6 and H-1' interacted with C-7; H-2' and H-2'' interacted with C-1''; H-2-8'', H-9'' and H_2 -11'' interacted with C-10''. Acid hydrolysis of **11** yielded salicylic acid, m. p. 157-158 $^\circ\text{C}$; R_f 0.70 (benzene-acetic acid-diethylether-methanol, 60:9:30:5); α -D-glucose acid, R_f 0.78 (*n*-butanol-acetic acid-water, 4:1:1.6) and oleic acid (R_f 0.34, 85% glacial acetic acid). The absolute configuration of α -D-glucose was determined by measuring its specific rotation, $[\alpha]_D^{25} + 112^\circ$ (water). On the basis of the foregoing account, the structure of **11** has been established as salicyloyl O- α -D-glucopyranosyl-2'-oleate, a new phenolic acid glucosidic ester (Fig. 3).

4. Concluding remarks

Phytochemical investigation of the leaves of *Fraxinus excelsior* afforded three aliphatic constituents viz., (*Z,Z,Z*)-*n*-tetratriacont-3,5,15-triene (**1**), *n*-hexatriacontane (**2**) and (*Z,Z,Z*)-*n*-octatriacont-11,13,20-triene (**3**), phytanic acid (**4**), 26-hydroxystigmasterol-18-oic acid (**5**) and α -L-xylose (**6**). The leaves of *Senna sulfurea* furnished isoliquiritigenin (**7**) and di-4-methoxy- α -L-xyloside (**8**). The leaves of *Prosopis cineraria* yielded glyceryl-1-oleoyl-2-myristoyl-3-O-hydroxydihydrocinnamate (**9**), 2,3,4-trihydroxybenzyl *n*-hexadecanyl ether (**10**) and salicyloyl O- α -D-glucopyranosyl-2'-oleate (**11**). This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the tubers of the plant.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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