# Chemical constituents from the aerial parts of *Salsola inermis*

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**Background and objective**

The hydroalcoholic extract from the aerial parts of *Salsola inermis* exhibited antioxidant, anti-inflammatory, and antinociceptive effects. The present study deals with the isolation and identification of the chemical constituents of this hydroalcoholic extract.

**Materials and methods**

The aerial parts of *S. inermis* (Forsskal) were collected from wild plants growing near the El-Alamein area in October 2005. Air-dried and powdered aerial parts of *S. inermis* were extracted with 70% alcohol in $H_2O$. The extract was partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH. The structures of the isolated compounds were determined by chemical and spectroscopic analyses.

**Results and conclusion**

Phytochemical investigation of the alcoholic extract from the aerial parts of *S. inermis* revealed 12 compounds, identified as long chain hydroxyl fatty acid 9,12,13-trihydroxydecosan–10,15,19-trienoic acid; *trans-N*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside; umbelliferone; scopoletin; 3-methyl kaempferol; olean-12-en-3,28-diol; olean-12-en-28-oic acid; stigmasterol-3-β-*O*-D-glucopyranoside; 3-*O*-[β-D-glucopyranosyl]oleanolic acid; kaempferol 3-O-β-glucopyranoside; and isorhamnetin 3-*O*-β-glucopyranoside, in addition to β-sitosterol, stigmasterol, and stigmastanol. Some of these compounds have hydroxyl groups, which help in scavenging free radicals and inhibit COX and various mediators involved in the pathogenesis of pain relief.

**Keywords:**

aerial parts, coumarins, flavonoids, NMR, *Salsola inermis*, terpenes

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## Introduction

The genus *Salsola*, family *Chenopodiaceae* (Goosefoot family), includes over 100 species found in the dry regions of Asia, Europe, and Africa [1]. The *Salsola* species represents 16 species in Egypt, most of which grow in the Egyptian deserts [2]. Previous phytochemical investigation of the genus resulted in the isolation of alkaloids, saponins, sterols and their glucosides, comarinolignan, isoflavonoids, and flavonoids [3–10]. Some *Salsola* plants are widely used as folk medicine for the treatment of hepatitis [11] or infections caused by tapeworm and parasites [12]; they also have pronounced vasoconstrictive, hypertensive, and cardiac stimulant action [13] and can act as an allergenic substance [14,15]. Reactive oxygen species (ROS) are always present in cells as metabolic products of normal cellular respiration. However, oxidative stress, an imbalance caused by excessive ROS originating from endogenous and exogenous sources, might cause inflammation and therefore play a pivotal role in many diseases [16]. Cytopreventive antioxidants prevent the formation of free radicals and scavenge them or promote their decomposition [17]. In chemical terms, polyhydroxy flavonoids efficiently modulate the redox status and thus may play a critical role in regulating the inducible gene expression of inflammatory mediators in the lipopolysaccharide-stimulated mouse leukemic monocyte macrophage cell line (RAW 2647macrophages) [18]. As a continuation of our previous studies that showed that the ethanol extract of *Salsola inermis* has antioxidant and anti-inflammatory properties [19], the present study deals with the isolation and identification of chemical constituents of the hydroalcoholic extract from the aerial parts of *S. inermis*.

## Materials and methods

Electron impact mass spectra (EIMS) were obtained using Varian MAT 711 (Germany), Finnigan SSQ 7000 (San Jose, California, USA), and OMM 7070 E spectrometers (Maryland, USA). $^1$H-NMR and $^{13}$C-NMR spectra were recorded at 500 MHz on a JEOL 500 A spectrometer (JEOL Inc., USA). The $^1$H-NMR and $^{13}$C-NMR chemical shifts are expressed in ppm relative to tetramethylsilane. Infrared (IR) spectra were measured on a Perkin Elmer FT-IR1700 spectrometer (Perkin Elmer, USA) at the National Research Centre, Cairo, Egypt. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-Vis spectrophotometer (Shimadzu, USA). Thin layer chromatography (TLC) plates (aluminum sheets) precoated with silica gel G 60 (F 254; Merck) were used for chromatography. Special reagents used were iodine–potassium iodide for detection of coumarins and chlorosulfonic acid spray reagent for the detection of sterols and triterpens. The two-dimensional paper chromatographic technique using the solvent system

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BuOH : HOAc : $H_2O$ (4 : 1 : 5) and HOAc (15%) was also used [20].

### Plant material

The aerial parts of *S. inermis* (Forsskal) were collected from wild plants growing near the El-Alamein area in October 2005. The plant specimen was authenticated by Dr N. El-Hadidi, Faculty of Science, Cairo University, and was compared with reference herbarium specimens.

### General procedure for extraction and isolation

Air-dried and powdered aerial parts of *S. inermis* were extracted with 70% alcohol in $H_2O$ after evaporation of the solvent under reduced pressure. It was essential that the extract (200 g) be partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH.

The $CHCl_3$ fraction (8 g) was applied onto a silica gel column and eluted with a gradient of *n*-hexane, $CHCl_3$, and MeOH (100–0, 90–10, 80–20, 70–30, 60–40, 50–50, 40–60, 20–80, 0–100) to give five fractions $A_1$–$A_5$. Further purification of $A_1$ (0.8 g) by preparative TLC with *n*-hexane/$CHCl_3$ as an eluent afforded compounds I (0.05 g) and II (0.02 g). Moreover, column chromatography of $A_2$ with $CHCl_3$ afforded compounds III (0.28 g) and IV (0.10 g). Column chromatography of $A_4$ with gradient elution using EtOAc/MeOH yielded compound V (0.18 g). The EtOAc fraction (7 g) was chromatographed over silica gel with successive petroleum ether/EtOAc (80–20, 20–80, 0–100) and EtOAc/MeOH (90–10, 0–100) elution to give eight fractions, $B_1$–$B_8$. Column chromatography of $B_6$ (1.24 g) with $CHCl_3$/MeOH elution (9–1 and 9–2) afforded compounds VI (5 mg) and VII (3 mg). Further, column chromatography of $B_8$ with $CHCl_3$/MeOH elution (9–1 and 8–2) afforded compound VIII (6 mg). BuOH (12 g) applied onto a flash column chromatography column with $H_2O$/MeOH gradient elution afforded three fractions, $C_1$–$C_3$. Purification of $C_2$ and $C_3$ carried out on a Sephadex LH-20 column with $CHCl_3$/MeOH elution (1–9 and 0–10) afforded compounds IX, X, XI, and XII. The physical and spectral data of the isolated compounds are as follows.

**Table 1 $^1$H-NMR (300 MHz) and $^{13}$C-NMR (300 MHz) for compound II ($CHCl_3$-$d_6$)**

| Position | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1 | | 175 |
| 2 | 6.47 | 121.7 |
| 3 | 7.43 | 140.98 |
| 1′ | | 129.18 |
| 2′ | 7.17 br d (1.6) | 114.0 |
| 3′ | | 148 |
| 4′ | | 148.6 |
| 5′ | 6.74 d (8.5) | 115.64 |
| 6′ | 6.95 dd (8.2, 1.7) | 121.4 |
| 1″ | 3.34 t (7.3) | 40.55 |
| 2″ | 2.76 t (7.3) | 40.39 |
| 1‴ | | 132.0 |
| 2‴, 6‴ | 7.17 d (8.5) | 130 |
| 3‴, 5‴ | 7.1 2 d (8.5) | 115.0 |
| 4‴ | | 156 |
| Glc-1 | 4.2 d (7.4) | 101.3 |
| 2 | 3.33 (overlap) | 74.01 |
| 3 | 3.39 (overlap) | 77.44 |
| 4 | 3.25 (overlap) | 70.62 |
| 5 | 3.25 (overlap) | 67.93 |
| 6 | 3.99 dd (12.0, 1.9) | 63.61 |
| | 3.73 dd (12.0, 5.5) | |
| $OCH_3$ | 3.81s | 56.42 |

*Compound I*

Gummy white solid, EIMS, *m/z* 386: $[M]^+$ calculated for $C_{22}H_{42}O_5$; IR (KBr) $\nu_{max}$ $cm^{-1}$ 3437, 2925, 2854, 1740, 929; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta_H$ 0.9 (3H, t, $J = 7.3$, H-22), 1.35 (11 H, bs, H-4, H-5, H-6, H-7, H-8a), 1.45 (1H, m, H-8b), 1.61 (2H, m, H-3), 2.05 (2H, t, $J = 6$, 8 Hz, H-21), 2.17 (1H, m, H-14a), 2.33 (1H, m, H-14b), 3.46 (1H, m, H-13), 3.98 (1H, t, $J = 5.3$ Hz, H-12), 4.05 (1H, m, H-9), 5.42, O, (1H, $J = 11.2$, 5.2 Hz, H-16), 5.47, O, (1H, $J = 11.2$, 5.2 Hz, H-15), 5.68 (1H, dd, $J = 15.7$, 5.2 Hz, H-11), 5.73 (1H, dd, $J = 15.7$, 5.2 Hz, H-10).

*Compound II*

Amorphous powder, IR (KBr) $\nu_{max}$ $cm^{-1}$ 3416, 2925, 1725, 1646, 1515, 1269, 1074. EIMS, *m/z*: 476 $[M]^+$ calcd for $C_{24}H_{30}NO_9$. UV $\lambda_{max}$ (MeOH) nm ($\log_e$): 225 (3.12), 278 (2.99), 311 (3.1). $^1$H-NMR and $^{13}$C-NMR spectral data are presented in Table 1.

*Compound III*

White crystals, m.p. 225–228°C, $R_f$ 0.42 (TLC, $S_1$); UV $\lambda_{max}$ nm (MeOH) 217, 245, $260_{sh}$, $279_{sh}$, and 322 nm; EIMS *m/z* 162 $[M]^+$, $C_9H_6O_3$. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta_H$ 6.15 (1H, d, $J = 9.6$ Hz, H-3), 6.58 (1H, d, $J = 2.6$ Hz, H-8), 6.85 (1H, dd, $J = 8.6$ Hz, H-6), 7.35 (1H, d, $J = 8.6$ Hz, H-5), 7.81 (1H, d, $J = 9.3$ Hz, H-4).

*Compound IV*

Colorless needle crystals ($CHCl_3$), m.p. 221–223°C, $R_f$ 0.5 (TLC, $S_1$); UV $\lambda_{max}$ nm (MeOH) 229, $250_{sh}$, $260_{sh}$, $295_{sh}$ and 342 nm. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta_H$ 6.26 and 7.58 (2H, d, $J = 9.6$ Hz, H-3 and H-4), 6.85 and 6.92 (2H, s, H-8 and H-5) and 3.92 (3H, s, Me-6).

*Compound V*

Colorless needles, m.p. 131–132°C, showed $[M^+]$ peak at *m/z* 412 (25.0%), 414 (17%), and 416 (1.40%) and characteristic fragmentation peaks at *m/z* 275, 255, 231, 213.

*Compound VI*

White needles (0.22 g), m.p. 254°C. $M^+$ peak at *m/z* 441 (9.30%), corresponding to $C_{30}H_{50}O$, and an intensive peak at *m/z* 411 (18.92%), corresponding to $M^+ - CH_2OH$. IR showed characteristic absorption bands at 3395 (OH), 2925 cyclic ($CH_2$), 1730, and 1446 (C = C). $D^{12}$ double bond proved to be readily recognizable by mass spectra and $^1$H-NMR shows seven tertiary methyl proton singlets at 0.81, 0.82, 0.84, 0.86, 1.18, 1.22, and 1.84, an olefin proton at $\delta$ 5.4 (br.s.), and a hydroxyl methylene proton at 5.14.

*Compound VII*

Isolated as white crystals (0.01 g), m.p. 259–60°C, IR spectrum showed strong bands near 3415 $cm^{-1}$ (OH), 1735 $cm^{-1}$ (CO), two bands 1390–1375 and 1369–1354 $cm^{-1}$ in the 'A-region', and three bands at 1328–1318, 1303–1296, and 1267–1248 $cm^{-1}$ in the 'β-region'; its

mass gave an $M^+ + 1$ peak at *m/z* 457 (2.02%), corresponding to $C_{30}H_{48}O_3$, fragmentation characteristic with respect to oleanane triterpenoids having $D^{12:13}$ unsaturation. The ion at *m/z* 189 stands for rings A and B in the dehydrated form [21].

*Compound VIII*

Yellow powder, m.p. 275–278°C, $R_f$ 0.47 (TLC, $S_2$); UV $\lambda_{max}$ nm (MeOH) 268 and 363, (MeOH/NaOMe) 279 and 423, (MeOH/$AlCl_3$) 269 and 423; $^1$H-NMR (500 MHz, DMSO) $\delta_H$ 6.16 and 6.42 (2H, d, $J$ = 2.2 Hz, H-6 and H-8), 6.90 and 8.0 (each 2H, d, $J$ = 8.7 Hz, H-3′, -5′ and H-2′,-6′).

*Compound IX*

White powder, m.p. 265–268°C, $R_f$ 0.47 (TLC, $S_2$); EIMS, $M^+$ peak at *m/z* 576, 9.45%, corresponding to the molecular formula $C_{35}H_{60}O_6$, *m/z* 163 (13.54) of one hexose sugar; IR spectrum (KBr) $V_{max}$ $cm^{-1}$, 3421 (OH), 1730–1446 (C = C), 1129, 1076, 1055, and 1015 (ether linkage of glycoside); $^1$H-NMR (500 MHz, DMSO-$d_6$) $\delta_H$ 0.64 and 1.02 (each 3H, s, H-18 and H-19), 0.78–0.85 (9H, m, H-26, 27 and H-29), 0.89 (3H, d, $J$ = 6.6 Hz, H-21), 0.94 (3H, m, H-29), 4.39 (1H, m, H-3), 5.38 (1H, broad s, H-6), and 4.30 (1H, d, $J$ = 7.7 Hz, H-1′).

*Compound X*

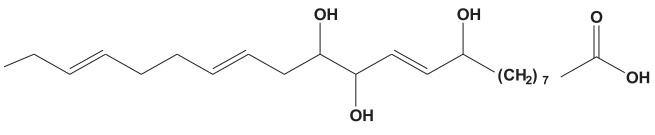
White powder, m.p. 260–263°C, $R_f$ 0.57 (TLC, $S_2$); EIMS, $M^+$ at *m/z* 618 compatible with $C_{36}H_{58}O_8$, *m/z* 456 ascribe to the mass of triterpene (aglycone), corresponding to $C_{30}H_{47}O_3$, *m/z* 438 (aglycone-$H_2O$), 426 (aglycone-2Me), 410 (aglycone-COOH + Me), 248 and 189, 133, the ion at *m/z* 161 stands for a hexose sugar.

*Compound XI*

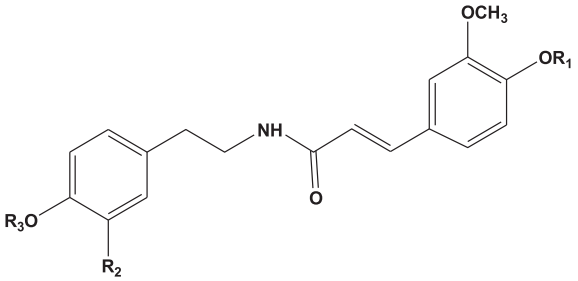
Yellow powder, UV $\lambda_{max}$ nm: (MeOH) 256, 267.1, $292_{sh}$, 357; MeOH + NaOMe, 272, 291, $325_{sh}$, 415; MeOH + NaOAc, 273, 315, 390; MeOH + $AlCl_3$, 274, 292, $340_{sh}$, 425 MeOH + $AlCl_3$ + HCl; 272, $303_{sh}$, $360_{sh}$, 403. $^1$H-NMR (500 MHz, DMSO) $\delta_H$ 7.82 (2H, d, $J$ = 8.2 Hz, H-2′, 6′), 6.84 (2H, d, $J$ = 8.2 Hz, H-3′,5′), 6.28 (1H, d, $J$ = 1.9 Hz, H-6), 5.30 (1H, d, $J$ = 7.6 Hz, H-1″ of glucose), 3.27–3.57 (m, rest of glucose protons). Acid hydrolysis gave kaempferol and glucose.

*Compound XII*

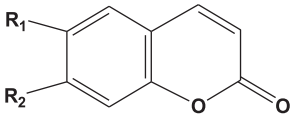
Yellow powder, m.p. 224–226°C; brown fluorescence in UV, $R_f$ 0.34, UV $\lambda_{max}$ nm: (MeOH) 254, $265_{sh}$, 353; (NaOMe) 270, $331_{sh}$, 415; (NaOAc) 271, $311_{sh}$, 394; ($AlCl_3$) 264, 296, $366_{sh}$, 400; ($AlCl_3$ + HCl) 262, 300, 366, 400. Acid hydrolysis gave isorhamnetin and glucose.



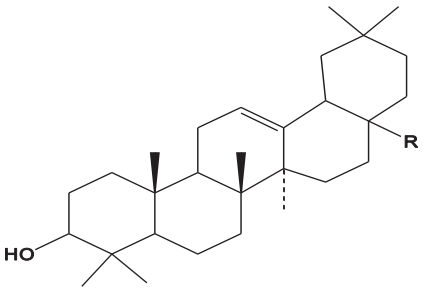

I



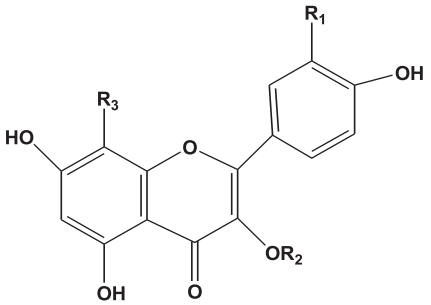

II: trans feruloyl tyramine glucoside
$R_1$, $R_2$ = H , $R_3$= Glc



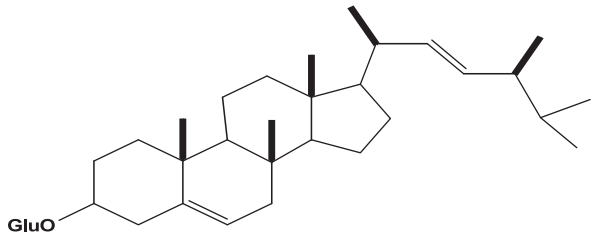

III: $R_1$= H, $R_2$= OH
IV: $R_1$= $OCH_3$, $R_2$ = OH




VI: R= $CH_2OH$
VII: R=COOH
X: R=COOglc




VIII: $R_1$, $R_3$ = H, $R_2$ = $CH_3$
XI: $R_1$, $R_3$ = H, $R_2$ = glc
XII: $R_1$=$OCH_3$, R3 = H , $R_2$ = glc




IX: Stigmasterol 3- β -O-D- glucopyranoside

## Results and discussions

The aqueous ethanolic extract was successively partitioned in $H_2O/CHCl_3$, $H_2O$/EtOAc, and $H_2O$/*n*-BuOH. The three fractions were then subjected to a sequence of column chromatography procedures to yield compounds I–V, VI–VIII, and IX–XII, respectively.

9,12,13-Trihydroxydocosan–10,15,19-trienoic acid (I) was isolated as a white solid with the molecular formula $C_{22}H_{42}O_5$, calculated from the [$M^+$] peak at *m*/z 386. Its IR spectrum showed OH and CO absorptions at 3437 and 1740 $cm^{-1}$, respectively. $^{13}$C-NMR was characteristic of an unsaturated long chain fatty acid with a methyl group at $\delta_C$ 14.3, several methylene carbons from 23.2 to 39.91, two $sp^2$carbons at 139.3 and 157.4, and a substituted carboxyl carbon at $\delta_C$ 166.98, in addition to three low-field oxygenated carbons at $\delta_C$ 72.99 and 77.0, bearing methane protons at $\delta_H$ 4.2, 3.89, and 3.58, respectively, which confirmed the presence of three hydroxyl groups; an olefinic proton signal appeared at $\delta$ 5.27. Analysis of the spectra provided evidence for the fragment and established the structure of compound I [22].

*Trans-N*-feruloyl tyramine-4‴-*O*-β-D-glucopyranoside (II) showed EIMS, $M^+$ at *m*/*z* 476 calculated for the molecular formula $C_{24}H_{30}NO_9$. Its IR spectrum exhibited characteristic absorption bands for a hydroxyl group (3416 $cm^{-1}$), conjugated carbonyl group (1646 $cm^{-1}$), and conjugated double bond (1515 $cm^{-1}$). Acid hydrolysis of II afforded D-glucose as determined by comparing the $R_f$ of the hydrolysis product with that of an authentic sample using the paper chromatographic technique. The $^1$H-NMR spectrum (Table 1) indicated the presence of one 1,4-disubstituted aromatic ring at $\delta_H$ 7.19 (2H, d, $J$ = 8.4 Hz, H-2‴, 6‴) and $\delta_H$ 7.19 (2H, d, $J$ = 8.5 Hz, H-3‴, 5‴); one 1,3,4-trisubstituted aromatic ring at $\delta_H$ 6.95 (1H, dd, $J$ = 8.2, 1.7 Hz, H-6′) and $\delta_H$ 6.75 (1H, d, $J$ = 8.2 Hz, H-5′); one *trans* olefin at $\delta_H$ 6.68 (1H, d, $J$ = 15.2 Hz, H-3) and $\delta_H$ 4.22 (1H, d, $J$ = 12.1 Hz, H-2); and one methoxy proton at $\delta_H$ 3.99 (3H). From the coupling constant of the anomeric proton at $\delta_H$ 4.24 (1H, d, $J$ = 7.4 Hz, Glc-1), C-1 of the D-glucopyranose was determined to be in the β-configuration. Analysis of the $^{13}$C-NMR (Table 1; $\delta_{C\text{-}1''}$ 175, $\delta_{C\text{-}1}$ 40.5) and the molecular formula of II revealed that C-1″ and C-1were linked by a nitrogen atom. The current analysis and comparison with the data in the literature suggested the structure of compound II [23].

The two coumarins III and IV were isolated from the $CHCl_3$ extract. Umbelliferone (III) showed shine blue fluorescence under UV light (366) and when sprayed with $I_2$/KI reagent turned into a colorless spot. From the results of $^1$H-NMR analysis and by cochromatography with the reference substance, compound III was identified.

Scopoletin (IV) showed strong blue fluorescence under UV light (366) and when sprayed with $I_2$/KI reagent turned into brown spot. The UV spectrum of IV in MeOH showed absorption bands at 229, $250_{sh}$, $260_{sh}$, $295_{sh}$, and 342 nm, which suggested a 6,7-dioxgenated coumarin skeleton. From the results of $^1$H-NMR analysis and by cochromatography with the reference substance, compound IV was identified [24].

Three known sterols (V) isolated from the $CHCl_3$ extract gave positive results for the Liebermann test for sterols and showed an [$M^+$] peak at *m*/*z* 412 (25.0%), 414 (17%), and 416 (1.40%) corresponding to $C_{29}H_{48}O$, $C_{29}H_{50}O$, and $C_{29}H_{52}O$, respectively. Because of its occurrence with the identified sterols [25], the sterol with $M^+$ at *m*/*z* 414 (17.0%) was identified as β-sitosterol, the sterol with $M^+$ at *m*/*z* 412 (25.0%) was identified as stigmasterol, and the sterol with $M^+$ at *m*/*z* 416 was identified as sitostanol.

Three compounds VI, VII, and VIII were isolated from the EtOAc extract.

Olean-12-en-3,28 diol (VI) gave a positive Liebermann test for triterpenes. The compound with $M^+$ at *m*/*z* 441 (8.02%) was identified as $C_{30}H_{50}O_2$, with a peak at 411 (45.0%). Spectral analysis suggested the structure of the compound [21].

Olean-12-en-28-oic acid (VII): the IR spectrum showed strong bands near 3415 (OH) and 1735 $cm^{-1}$ (CO): two bands, 1390–1375 and 1369–1354 $cm^{-1}$, in the so called 'A-region' and three bands at 1328–1318, 1303–1296, and 1267–1248 $cm^{-1}$ in the 'β-region'; its mass gave an $M^+ + 1$ peak at *m*/*z* 457 (2.02%), corresponding to $C_{30}H_{48}O_3$, fragmentation characteristic with respect to oleanane triterpenoids having $D^{12:13}$ unsaturation. The ion at *m*/*z* 189 represents rings A and B in the dehydrated form. Previous spectral data and chemical analysis elucidate the structure of this compound [21].

3-Methyl kaempferol (VIII) was identified from the analysis of its UV spectra in MeOH before and after the addition of different shift reagents and from the analysis of its $^1$H-NMR spectral data [20]; this was further confirmed by cochromatography with a reference substance.

Stigmasterol-3-β-*O*-D-glucopyranoside (IX) showed an EIMS $M^+$ peak at *m*/*z* 576 (9.45%), corresponding to the molecular formula $C_{35}H_{60}O_6$, *m*/*z* 163 (13.54) of one hexose sugar. IR spectroscopy revealed bands $V_{max}$ $cm^{-1}$, 3421 OH, 1730–1446 (C = C), 1129, 1076, 1055, and 1015 (ether linkage of glycoside). $^1$H-NMR revealed one anomeric proton at 4.43 (d, $J$ = 6.78), indicating the sugar to be in the β-configuration. Thus, from the large $J_{H1,H2}$

coupling constant, the structure of this compound was elucidated as stigmasterol-3-β-*O*-D-glucopyranoside [25].

3-*O*-[β-D-glucopyranosyl]oleanolic acid (X) showed an $M^+$ peak at *m/z* 618, corresponding to the molecular formula $C_{36}H_{58}O_8$, and a fragment ion at *m/z* 456, corresponding to $H_{30}H_{48}O_3$. This is ascribed to the mass of triterpene acid having a $\Delta^{12}$ aglycone. IR revealed bands $V_{max}$ $cm^{-1}$, 3421 OH, 1730–1446 (C = C), 1129, 1076, 1055, and 1015 (ether linkage of glycoside).

Kaempferol 3-*O*-β-glucopyranoside (XI) and isorhamnetin 3-*O*-β-glucopyranoside (XII) gave typical brown fluorescence under UV for the C-3-substituted flavonoid glycosides. Acid hydrolysis yielded glucose and kaempferol or isorhamnetin, respectively. The structures of compounds XI and XII were confirmed by $^1$H-NMR and cochromatography with authentic reference samples [26]. Isolation of these compounds from *S. inermis* has not been reported previously.

## Conclusion

Twelve compounds were isolated and identified for the first time from the 70% ethanolic extract of *S. inermis*. Some of these compounds contain different hydroxyl groups and the others were terpenoids, which help scavenge free radicals and inhibit COX and various mediators involved in the pathogenesis of pain relief. The chloroform fraction showed more potent inhibitory activity than the ethanol extract, whereas the 70% ethanolic extract was more potent than the chloroform fraction in antinociceptive activity.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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