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CHEMICAL AND BIOLOGICAL STUDIES OF *RUSSELIA EQUISETIFORMIS* (SCH.&CHAM.) AERIAL PARTS

Eman M. Ahmed, Samer Y. Desoukey, Mostafa A. Fouad*, Mohamed S. Kamel

Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, 61519 Egypt

ABSTRACT

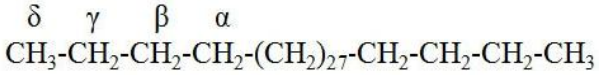
From the aerial parts of *Russelia equisetiformis*, eight compounds were isolated (1–8) and identified by different spectral techniques as well as comparison with reported data or authentic samples. The isolated compounds included an alkane (n-pentatriacontane) (1), a mixture of β-sitosterol and stigmasterol (2), a triterpene (lupeol) (3), a mixture of β-sitosterol- and stigmasterol-1-O-β-D-glucopyranosides (4), a phenylpropanoidglucoside (4-Allyl-2,6-dimethoxyphenol-1-O-β-D-glucopyranoside(5), a flavonoid (vitexin) (6) and the two closely related phenylethanoids (acteoside) (7) and isoacteoside (8). The anti-inflammatory, antipyretic and antihyperglycemic activities were carried out on different plant fractions.

Key words: sterols, triterpene, anti-inflammatory, antipyretic, antihyperglycemic activity.

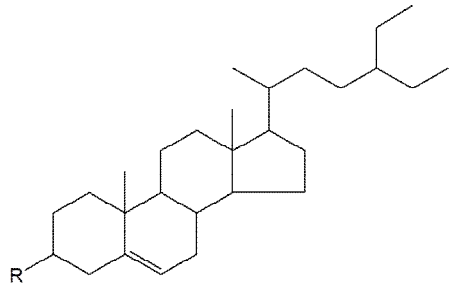
***Corresponding author**: Mostafa A. Fouad, Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, 61519 Egypt. Tel: 00201154527131, fax: 0020862369075, Email: m_fouad2000@yahoo.com

1. Introduction

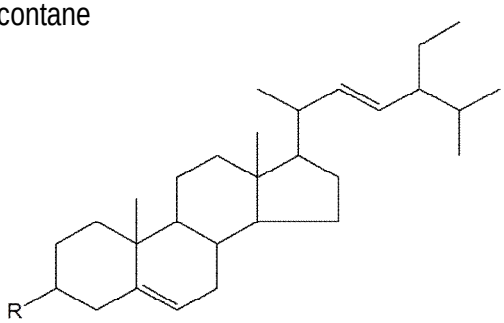
Russelia equisetiformis Schlechtendalet Chamisso. (*Russelia juncea* Zucc.) belonging to family scrophulariaceae, recently introduced into the new family Plantaginaceae is native to Tropical South America especially in Mexico[1-3]. It is an evergreen, perennial, weeping shrub with attractive looking, green arching stems and tubular red blossoms, commonly named as fire cracker plant, coral plant, coral blow and fountain plant[4]. *R. equisetiformis* is traditionally used in Nigeria to cure malaria, cancer, inflammatory disorders, diabetes, leukemia, and in hair preparations to promote hair growth[5-6]. In Colombia, the fresh entire plant decoction is taken orally to cure kidney stones[7] , while the whole plant and its aerial infusion is utilized as a complementary therapy for DM2 patients in Mexico[8-9]. An anti-oxidant, anti-inflammatory[6], antinociceptive and analgesic properties[10-11] were observed for different extracts of *R. equisetiformis*, together with antibacterial[12], antimicrobial, cytotoxic[13], CNS depressant[14], hepatic functions activity[5], hair growth promoter[15] and membrane stabilizing activities[16]. The present study reported the separation and structure elucidation of eight compounds (Fig 1) from different fractions of the aerial parts of *R. equisetiformis*, in addition to evaluate the LD_{50}, anti-inflammatory, antipyretic and antihyperglycemic activities of it.



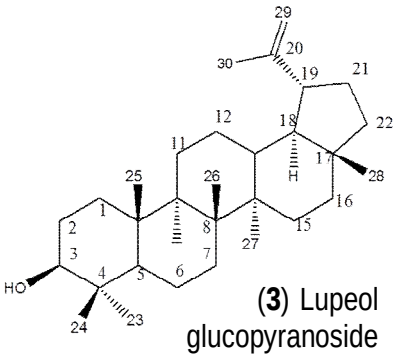
(**1**) n-Pentatriacontane



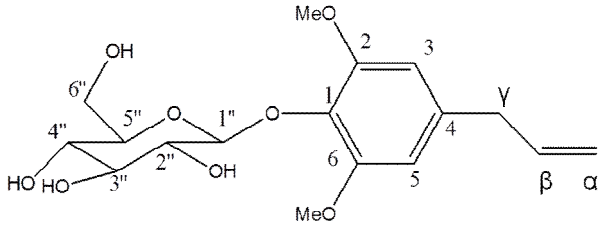
(**2**) R = OH (major) β-Sitosterol
(**4**) R= Glc (major) β-Sitosterol-1-O-β-D-glucopyranoside



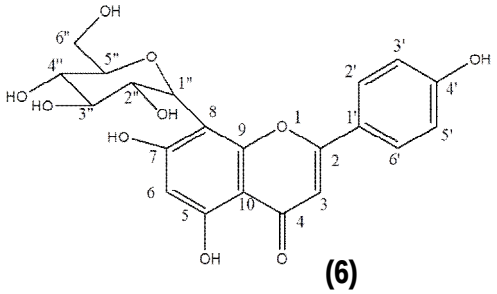
(**2**) R = OH (minor) Stigmasterol
(**4**) R= Glc (minor) Stigmasterol-1-O-β-D-glucopyranoside



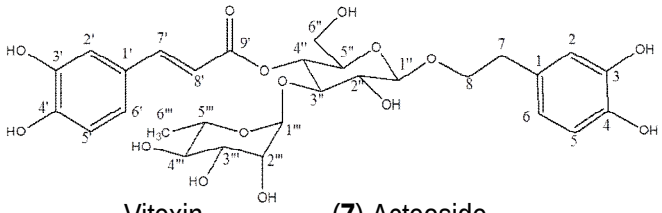
(**3**) Lupeol



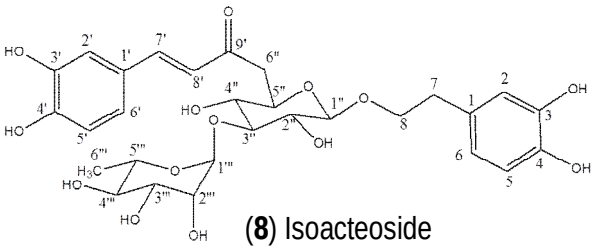
(**5**) 4-Allyl-2,6-dimethoxyphenol-1-O-β-D-glucopyranoside



(6)



Vitexin



(**7**) Acteoside

(**8**) Isoacteoside

Fig. 1. Structures of the isolated compounds

2. Materials and Methods

2.1 General

The EI-MS was measured on JEOL, JMS 600 Hz (Japan). 1D NMR was measured on both (JEOL JNM-LA 400 MHz and BRÜKER 600 MHz NMR spectrometers, Japan and Germany, respectively). Column chromatography (CC) was performed using silica gel (60-120 μm mesh size, Nile chemicals, Egypt) and sephadex LH-20 (25-100 mm mesh size, GE Health care, Sweden). TLC was carried on pre-coated silica gel plates ($G_{60}F_{254}$, E-Merck and FLUKA, Germany). The plates were examined under UV light via a portable UV lamp at λ_{max} 365 and 254 nm (UVP, USA) and visualized by spraying with10% v/v H_2SO_4 in ethyl acetate, then allowed to dry at room temperature followed by heating at 110–140°C using a circulating hot-air oven (CARBOLITE, Germany) for 1–2 min. The following solvent systems were used for TLC: (I): n-Hexane-EtOAc (8.5:1.5 v/v), (II): n-Hexane-EtOAc (9:1 v/v), (III): $CHCl_3$-MeOH (9.2:0.8), (IV):$CHCl_3$-MeOH (9:1), (V):$CHCl_3$-MeOH-H_2O (8.5:1.5:0.1), (VI):EtOAc-MeOH (7:3). Authentic reference materials (*β*-Sitosterol and stigmasterol) were obtained from Pharmacognosy department, Faculty of Pharmacy, Minia University, Minia, Egypt. For biological studies, a vernier caliber, digital thermometer and a glucometer (One Touch Horizon, LIFESCAN, Johnson and Johnson, Ltd) were used.

2.2 Plant Material

Aerial parts of *R. equisetiformis* were collected in February 2008 from El-Zohria garden, Cairo, Egypt. The plant was identified by Dr. Mamdoh Shokry, director of El-Zohria garden. A voucher specimen was deposited in the herbarium of the Pharmacognosy department, Faculty of Pharmacy, Minia University under the number (REMIN08).

2.3 Extraction and Isolation

Dried powdered aerial parts of *R. equisetiformis* (1.5 Kg) were extracted with 70% aqueous MeOH and concentrated to dryness in a rotary evaporator. The dried methanolic extract (**A**) was suspended in water and successively partitioned with three organic solvents to obtain n-hexane (11 gm) (**B**), chloroform (9 g) (**C**) and ethyl acetate (22.9 g) (**D**) fractions, together with the remaining concentrated aqueous fraction (**E**). A part of the n-hexane fraction (9 g) was subjected to silica gel CC (330 g). Elution was started with n-hexane followed by increasing polarity in gradient manner between n-hexane and EtOAc. Fractions of 200 ml each were collected and monitored using TLC and 10% v/v H_2SO_4 in MeOH as spraying reagent; similar fractions were pooled together where three groups (I-III) were obtained. Group I, fraction eluted with n-hexane:EtOAc (95:5), was further subjected to silica gel CC using mixture of n-hexane:EtOAc in gradient manner to give two sub fractions (I-1, I-2), compound (**1**) (25 mg) was precipitated from the latter one (I-2). Group II, fraction eluted with n-hexane:EtOAc (90:10), was further subjected to silica gel CC using mixture of n-hexane:EtOAc in gradient manner to give compound (**2**) (20 mg) after crystallization with methanol and and compound (**3**) (40 mg).

On the other hand, a part of the chloroformic fraction (8 g) was chromatographed on silica gel CC (270 g). Elution was started with n-hexane followed by mixture of n-hexane:EtOAc in gradient manner, fractions of 200 ml were collected, and three groups of fractions (I-III) were obtained. Group II, fraction eluted with n-hexane:EtOAc (20:80), was further chromatographed over silica gel CC using $CHCl_3$:MeOH in gradient manner to give compound (**4**) (30 mg). Group III, fraction eluted with n-hexane:EtOAc (5:95) precipitated compound (**5**) (60 mg). Finally, a part of the EtOAc fraction (12 g) was subjected to silica gel CC (360 g), running with $CHCl_3$:MeOH in gradient manner, where four groups of fractions (I-IV) were obtained. Group II, fraction eluted with $CHCl_3$:MeOH (85:15), was further subjected to silica gel CC using the same solvent mixture to yield two sub fractions (1,2); compound (**6**) (12 mg) was precipitated from sub-fr. 2 (80:20). Group III, fraction eluted with $CHCl_3$:MeOH (80:20), was subjected to Sephadex gel LH-20 column using

$CHCl_3$:MeOH (1:1), followed by silica gel CC using EtOAc: MeOH in gradient manner to yield three sub fractions (1-3); subfr. 3 (84:16) gave compound (**7**) (14 mg). Group IV, fraction eluted with $CHCl_3$:MeOH (70:30), was subjected to Sephadex LH-20 column using $CHCl_3$:MeOH (1:1), followed by silica gel CC using EtOAc: MeOH to give compound (**8**) (40 mg).

2.4 Chemicals for Biological Assays

Indomethacin was obtained as Liometacin from El-Nile Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. Acetyl salicylic acid was obtained as (Aspirin 500, Bayer, Egypt). Metformin was obtained as (Cidophage 500, Chemical Industries Development Company "CID", Egypt). Alloxan monohydrate was obtained from Sigma Company, U.S.A, while other chemicals were obtained from El-Nasr Company for Pharmaceutical Chemicals "ADWIC", Egypt.

2.5 Animals

Adult Albino rats (each 150–200 g) of either sex were bred and housed under standardized environmental conditions in the pre-clinical animal house, Pharmacology Department, Faculty of Medicine, Assiut University, Assiut. The animals were fed with standard diet and free access to water and were kept for one week to acclimatize to the environmental conditions. The animals were handled only at the time of experiments and during cage cleaning. All conditions were made to minimize animal suffering.

2.6 Toxicity Study

The acute toxicity study of the methanolic extract of *Russelia equisetiformis* Sch. & Cham. was performed by measuring the lethal dose for 50% of the laboratory animals (LD_{50} method)[17]. The rats were kept fasting overnight with free access to water. They were divided into seven different groups (six rats per group) and administered different doses of the extract (0.5, 1, 2, 4, 8 and 16 g/Kg, p.o.) respectively suspended in the vehicle (0.5% carboxymethyl cellulose aqueous solution "CMC") beside control group which received an equivalent dose of the vehicle, orally. All of the groups' animals were then allowed free access to food and water and observed a period of 48 hrs for signs of acute toxicity. The number of death within this period was recorded.

2.7 Anti-inflammatory Activity (yeast-induced Paw Oedema Method)

Different fractions of *R. equisetiformis* were evaluated for their anti-inflammatory activity[18]. Rats were randomly divided into seven groups (six rats per group). Group1 (negative control) was administered the vehicle (0.5 % CMC solution) orally, Group 2 was administered indomethacin (8 mg/kg) as the reference drug in vehicle orally, while groups 3-7 were administered 300 mg/kg of fractions **A**-**E**, respectively, suspended in the vehicle orally. After 30 minutes, inflammation was induced by subcutaneous injection of an equal volume of 20% w/v yeast aqueous suspension in the right hind paw of each rat under the sub-plantar region. The increase in linear paw circumference measured by a vernier caliber (at 0, 1, 2, 3, 4 and 5hrsafter yeast injection) was taken as a measure of oedema.

$$\textbf{(\%Inhibition)} = [(V_o - V_t)/ V_o] \cdot 100,$$

Where, V_o– the average paw thickness of the control group,

V_t– the average paw thickness of the treated group.

2.8 Antipyretic Activity

For screening of the antipyretic activity, pyrexia was induced by subcutaneous injection of 20% w/v yeast aqueous suspension in the back, below the nape of the neck. After 18 hours, the same grouping of animals in the anti-inflammatory study and their respective treatment were followed except group 2 (positive control) which received acetyl salicylic acid 100 mg/kg as the reference drug in vehicle orally. The rectal temperature before and after treatment, which was recorded with the help of a digital thermometer at every hour up to four hours, was compared with control[19].

2.9 Antihyperglycemic Activity (Alloxan-induced hyperglycemia method)

For evaluation of antihyperglycemic activity of the different fractions of *R. equisetiformis*, the rats were subjected to a 12-hour fast. Diabetes was induced by intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg. Diabetic rats were divided into seven groups (six rats each). Group 1 (negative control) was given the vehicle, group 2 (positive control) was given metformin 500 mg/kg as the reference drug in vehicle, while the other five groups received the tested fractions at a dose level of 300 mg/kg of fractions **A**-**E**, respectively, suspended in the vehicle. All treatments were given orally. Blood glucose level was measured by a glucometer at every hour up to five hours and compared with the control[20].

2.10 Statistical analysis

The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) test using the statistical package of the social science (SPSS) program. The results are presented as the mean ± S.E. (standard error). Group means were compared by a one-way analysis of variance and Duncan's multiple range tests. Statistical differences were considered significant at $P<0.05$ and very significant at $P<0.01$.

Compound (**1**) (n-pentatriacontane) was obtained as a white waxy powder, m.p. 74-76 °C, R_f =0.37 (system II). ^{1}H-NMR spectral data ($CDCl_3$, 600 MHz) δ_H: 0.86 (6H, t, H_3-1, H_3-35), 1.23 (64H, brs, 32×H_2), 1.59 (2H, brs, H_2). ^{13}C-NMR spectral data ($CDCl_3$, 150 MHz) δ_C: 14.2 (C-δ), 22.7 (C-γ), 29.4 (C-α), 29.7 (27×C), 32.0 (C-β). EI-MS showed a molecular ion peak at *m/z* 494 $[M]^+$, corresponding to the molecular formula ($C_{35}H_{72}$).

Compound (**2**) (a mixture of β-Sitosterolandstigmasterol) was obtained as colourless needles, m.p. 159-160 °C, R_f =0.44 (system I).The mixture was identified by comparing its NMR data with those previously reported[21].

Compound (**3**) (lupeol) was obtained as white crystals, m.p. 215-216 °C, R_f =0.51 (system I). ^{1}H-NMR spectral data (C_5D_5N, 400 MHz) δ_H: 0.83 (3H, s, H-28), 0.89 (3H, s, H-27), 0.98 (3H, s, H-23), 1.03(3H, s, H-25), 1.05 (3H, s, H-26), 1.23 (3H, s, H-24), 1.74 (3H, s, H-30), 2.48 (H, m, H-19), 3.46 (H, m, H-3), 4.70 (H, s, H-29a), 4.90 (H, s, H-29b). ^{13}C-NMR spectral data (C_5D_5N, 100 MHz) δ_C: 14.5 (C-27), 16.0(C-24), 16.1(C-26), 16.2(C-25), 18.0 (C-28), 18.6 (C-6), 19.5 (C-30),20.9(C-11), 25.4 (C-12), 27.6 (C-15), 28.1(C-2), 28.5 (C-23), 30.0 (C-21), 34.5 (C-7), 35.6 (C-16), 37.3 (C-10), 38.2 (C-13), 39.1 (C-1), 39.3 (C-4), 40.0 (C-22), 40.9 (C-8), 43.0 (C-14), 43.0 (C-17), 48.1 (C-19), 48.4 (C-18), 50.6 (C-9), 55.7 (C-5), 77.9 (C-3), 109.7 (C-29), 150.8 (C-20).

Compound (**4**) (a mixture of β-sitosterol- and stigmasterol-1-O-β-D-glucopyranoside) was obtained as a white amorphous powder, m.p. 265 °C, R_f0.23 (system III). The mixture was identified by comparing its NMR data with those previously reported[22].

Compound (**5**) (4-allyl-2,6-dimethoxyphenol-1-O-β-D-glucopyranoside) was obtained as colourless needles (methanol), m.p. 159-161 °C, R_f 0.54 (system IV). ^{1}H-NMR spectral data (CD_3OD, 600 MHz) δ_H: 3.33 (2H, d, *J*=6.6, H-α), 3.66 (H, dd, *J*=12, 5.4, H-6'α), 3.77 (H, dd, *J*=12, 2.4, H-6'β), 3.81 (2×H_3, s, OCH_3), 4.80 (H, d, *J*=7.8, H-1**'**), 5.10 (2H, m, H-γ), 5.90 (H, m, H-β), 6.52 (2×H, s, H-3, H-5). ^{13}C-NMR spectral data (CD_3OD, 150 MHz) δ_C: 41.4 (C-α), 56.9 (2×OCH_3), 62.6 (C-6'), 71.3 (C-4'), 75.7 (C-2'), 77.8 (C-5'), 78.3 (C-3'), 105.5 (C-1'), 107.4 (C-3, C-5), 116.2 (C-γ), 134.6 (C-4), 138.4 (C-1), 138.7 (C-β), 154.2 (C-2, C-6).

Compound (**6**) (vitexin) was obtained as a yellow powder from methanol, m.p. 262-264 °C, R_f 0.38 (system V). ^{1}H-NMR spectral data (DMSO, 600 MHz) δ_H: 4.67 (H, d, *J*=10.2, H-1"), 6.25 (H, s, H-6), 6.76 (H, s, H-3), 6.88 (2×H, d, *J*=8.4, H-3', H-5'), 8.01 (2×H, d, *J*=8.4, H-2', H-6'), 13.15 (H, brs, OH). ^{13}C-NMR spectral data (DMSO, 150 MHz) δ_C: 61.4 (C-6"), 70.6 (C-4"), 70.9 (C-2"), 73.5 (C-1"), 78.7 (C-3"), 81.9 (C-5"), 98.3 (C-6), 102.5 (C-3), 104.1 (C-10), 104.7 (C-8), 115.9 (C-3', C-5'), 121.7 (C-1'), 129.1 (C-2', C-6'), 156.1 (C-9), 160.5 (C-5), 161.2 (C-4'), 162.8 (C-7), 164.1 (C-2), 182.2 (C-4).

Compound (**7**) (acteoside) was abtained as a yellowish amorphous powder, m.p. 135-136 °C, R_f 0.56 (system VI). ^{1}H-NMR spectral data (CD_3OD, 600 MHz) δ_H: 1.08 (3H, d, *J*=6.2, H-6‴), 2.79 (2H, m, H-7), 3.28 (H, m, H-4‴), 3.38 (H, t, H-2"), 3.52-3.63 (5H, m, overlapped, H-5", H-6", H-3‴, H-5‴), 3.71 (H, m, H-8α), 3.80 (H, t, *J*=9.2, H-3"), 3.91 (H, m, H-2‴), 4.05 (H, m, H-8β), 4.37 (H, d, *J*=7.9, H-1"), 4.92 (H, t, *J*=9.2, H-4"), 5.17 (H, brs, H-1‴), 6.27 (H, d, *J* = 15.9, H-8'), 6.55 (H, dd, *J*=2, 8, H-6), 6.68 (H, d, *J*=8, H-5), 6.70 (H, d, *J*=2, H-2), 6.76 (H, d, *J*=8, H-5'), 6.95 (H, dd, *J*=2, 8, H-6'), 7.05 (H, d, *J*=2, H-2'), 7.58 (H, d, *J*=15.9, H-7'). ^{13}C-NMR spectral data (CD_3OD, 150 MHz) δ_C:18.5 (C-6‴), 36.6(C-7), 62.4 (C-6"), 70.4 (C-5‴), 70.6 (C-4"), 72.1 (C-3‴), 72.3 (C-8), 72.4 (C-2‴),73.8 (C-4‴), 76.1 (C-5"), 76.2 (C-2"), 81.7 (C-3"), 103.1 (C-1‴), 104.2 (C-1"),114.7 (C-8'), 115.2 (C-2'), 116.3 (C-5), 116.5 (C-5'),117.11 (C-2), 121.2 (C-6), 123.2 (C-6'), 127.6 (C-1'), 131.4 (C-1), 144.7 (C-4), 146.2 (C-3), 146.9 (C-3'), 148.0 (C-7'), 149.8 (C-4'), 168.3 (C-9').

Compound (**8**) (isoacteoside) was obtained as a yellowish amorphous powder, m.p. 134-135 °C, R_f0.38 (system VI).^{1}H-NMR spectral data (CD_3OD, 600 MHz) δ_H: 1.24 (3H, d, *J*=6.2, H-6‴), 2.78 (2H, m, H-7), 3.28 (H, m, H-4‴), 3.38 (H, t, H-2"), 3.40 (H, t, *J*=9.2, H-4"), 3.52-3.63 (5H, m, overlapped, H-5", H-6", H-3‴, H-5‴), 3.71 (H, m, H-8α), 3.80 (H, t, *J*=9.2, H-3"), 3.92 (H, m, H-2‴), 4.05 (H, m, H-8β), 4.33 (H, d, *J*=7.9, H-1"), 5.18 (H, brs, H-1‴), 6.29 (H, d, *J* = 15.9, H-8'), 6.55 (H, dd, *J*=2, 8, H-6), 6.68 (H, d, *J*=8, H-5), 6.70 (H, d, *J*=2, H-2), 6.75 (H, d, *J*=8, H-5'), 6.88 (H, dd, *J*=2, 8, H-6'), 7.03 (H, d, *J*=2, H-2'), 7.56 (H, d, *J*=15.9, H-7'). ^{13}C-NMR spectral data (CD_3OD, 150 MHz) δ_C: 17.9 (C-6‴), 36.7(C-7), 64.4 (C-6"), 70.0(C-5‴), 70.3 (C-4"), 72.3(C-3‴), 72.4 (C-8), 72.5(C-2‴),74.0 (C-4‴), 75.4 (C-5"), 75.7(C-2"), 84.0(C-3"), 102.7(C-1‴), 104.4(C-1"), 114.8 (C-8'), 115.1(C-2'), 116.3 (C-5), 116.5 (C-5'),117.11 (C-2), 121.2 (C-6), 123.2 (C-6'), 127.6 (C-1'), 131.3(C-1), 144.7 (C-4), 146.2 (C-3), 146.8(C-3'), 147.3(C-7'), 149.7(C-4'), 169.1(C-9').

3. RESULTS

From the aerial parts of *R. equisetiformis*, eight compounds were isolated using different chromatographic techniques and identified by different physical, chemical, and spectroscopic methods in addition to comparison with reported data.

Compound (**1**) was obtained as a white waxy powder from the n-hexane fraction and was identified with the aid of 1D (^{1}H and^{13}C) NMR data and mass spectroscopy as n-pentatriacontane[23]. Compounds (**2-4**) were obtained from the n-hexane and chloroform fractions and gave positive results with Salkowski's and Liebermann-Burchard's tests indicating their triterpenoidal and/or steroidal nature, andfrom their NMR

data together with co-chromatography, the compounds were identified as a mixture of β-sitosterol and stigmasterol(**2**)[21], lupeol (**3**)[24], a mixture of β-sitosterol- and stigmasterol-1-O-β-D-glucopyranosides (**4**)[22]. Compound (**5**) was obtained as colourless needles from the chloroform fraction and was identified as 4-allyl-2,6-dimethoxyphenol-1-O-β-D-glucopyranoside[25]. From the ethyl acetate fraction, compounds (**6**-**8**) were isolated and identified as vitexin (**6**)[26], acteoside (**7**) and isoacteoside (**8**)[27-28].

Different biological studies were carried out to evaluate the acute toxicity, anti-inflammatory, antipyretic as well as antihyperglycemic activity of the methanolic extract and all the fractions. For acute toxicity study, the methanolic extract was evaluated and the LD_{50} value was found to be 8 g/kg.

Table 1: Anti-inflammatory activity of the different fractions of *R. equisetiformis* on yeast-induced edema in rats

Group	Dose mg/kg	Thickness of the paw (mm)					
		0h	1h	2h	3h	4h	5h
Control (negative)	-	3.42 ± 0.19	7.28 ± 0.12	7.48 ± 0.16	7.64 ± 0.10	7.62 ± 0.10	7.62 ± 0.10
Indomethacin	8	3.24 ± 0.11	5.78 ± 0.13**	3.9 ± 0.10**	3.76 ± 0.11**	3.32 ± 0.13**	3.4 ± 0.10**
Total extract	300	3.36 ±0.10	7.16 ± 0.10	6.14 ± 0.10**	5.56 ± 0.17**	6.34 ± 0.14**	6.76 ± 0.11**23
n-Hexane fraction	300	3.12 ± 0.10	5.62 ± 0.10**	5.88 ± 0.10**	5.14 ± 0.10**	4.76 ± 0.11**	5.28 ± 0.12**
Chloroform fraction	300	3.26 ± 0.11	5.12 ± 0.10**	4.64 ± 0.10**	4.02 ± 0.02**	4.02 ± 0.02**	3.5 ± 0.03**
Ethyl acetate fraction	300	3.28 ± 0.16	6.12 ± 0.10**	5.62 ± 0.10**	4.76 ± 0.11**	4.86 ± 0.10**	4.88 ± 0.10**
Aqueous fraction	300	3.18 ± 0.10	6.8 ± 0.02*	6.16 ± 0.10**	5.72 ± 0.16**	6.3 ± 0.34**	6.86 ± 0.10**

*P<0.05, **P<0.01

Table 2: Inhibitory effects of the different fractions of *R. equisetiformis* on yeast-induced edema in rats

Group	Dose mg/kg	Percentage inhibition (%)				
		1h	2h	3h	4h	5h
Control (negative)	-	-	-	-	-	-
Indomethacin	8	20.60	47.86	50.79	56.43	55.38
Total extract	300	1.65	17.90	27.23	16.80	11.29
n-Hexane fraction	300	22.80	21.39	32.59	37.53	30.71
Chloroform fraction	300	29.67	37.97	47.38	47.24	54.07
Ethyl acetate fraction	300	15.93	24.87	37.70	36.22	35.96
Aqueous fraction	300	6.59	17.65	25.13	17.32	9.97

For anti-inflammatory activity, different fractions of *R. equisetiformis* were evaluated using yeast-induced paw edema method in rats. The chloroform fraction exhibited a significant anti-inflammatory activity at a dose (300 mg/kg) which significantly reduced the yeast-induced hind paw edema in rats compared with indomethacin at a dose 8 mg/kg. The n-hexane and ethyl acetate fractions exhibited a moderate activity while both the total and aqueous fractions were weakly active (Table 1 & 2).

For antipyretic activity, the ethyl acetate fraction (300 mg/kg) showed a high and significant activity after 1h from pyrexia induction using yeast and up to 4h compared with acetyl salicylic acid (100 mg/kg) as a positive control (Table 3). The other fractions exhibited a moderate and significant activity after 2h and up to the end of the experiment (4h).

Table 3: Antipyretic activity of the different fractions of *R. equisetiformis* on yeast-induced edema in rats

Group	Dose mg/kg	Average rectal temperature (°C)					
		Before yeast injection	Pre-drug	1h	2h	3h	4h
Control (negative)	-	36.92 ± 0.03	39.15 ± 0.09	39.17 ± 0.12	39.82 ± 0.03	39.83 ± 0.02	39.83 ± 0.02
Acetylsalicylic acid	100	36.92 ± 0.04	38.87 ± 0.04*	37.48 ± 0.09**	36.50 ± 0.02**	35.42 ± 0.03**	35.52 ± 0.03**
Total extract	300	36.90 ± 0.04	38.88 ± 0.05*	38.85 ± 0.05*	36.78 ± 0.03**	36.40 ± 0.19**	36.10 ± 0.03**
n-Hexane fraction	300	36.92 ± 0.04	38.90 ± 0.04*	38.9 ± 0.04	37.93 ± 0.08**	36.17 ± 0.02**	36.10 ± 0.03**
Chloroform fraction	300	36.88 ± 0.03	39.03 ± 0.04	39.03 ± 0.04	37.05 ± 0.03**	35.90 ± 0.04**	35.90 ± 0.04**
Ethyl acetate fraction	300	36.92 ± 0.04	38.60 ± 0.11**	38.60 ± 0.11**	36.90 ± 0.04**	36.00 ± 0.05**	35.63 ± 0.04**
Aqueous fraction	300	36.90 ± 0.04	38.93 ± 0.04	38.93 ± 0.04	37.38 ± 0.28**	35.93 ± 0.03**	35.90 ± 0.04**

*P<0.05, **P<0.01

Table 4: Antihyperglycemic activity of the different fractions of *R. equisetiformis* on alloxan-induced hyperglycemia in rats

Group	Dose mg/kg	Blood glucose level (mg./dl)					
		0h	1h	2h	3h	4h	5h
Control (negative)	120	476.17 ± 4.93	470.83 ± 9.12	471.5 ± 11.56	465 ± 15.17	465 ± 22.84	481 ± 14.66
Metformin	500	447.83 ± 6.43	384 ± 11.67**	312.17 ±13.76**	231.5 ± 4.63**	143.33 ± 2.95**	124.5 ± 2.98**
Total extract	300	461 ± 17.03	395 ± 2.22**	325 ± 7.65**	255.33 ± 8.04**	167.5 ± 2.45**	191.33 ± 5.74**
n-Hexane fraction	300	483.17 ± 6.26	470.17 ± 7.31	476.5 ± 5.81	458.17 ± 14.39	472 ± 11.60	494.5 ± 15.60
Chloroform fraction	300	470.67 ± 17.37	392.33 ± 4.90**	303 ± 18.05**	239.17 ±10.55**	173.5 ± 5.81**	196 ± 9.68**
Ethyl acetate fraction	300	480.17 ± 7.02	381.5 ± 7.75**	279.17 ±4.23**	204 ± 10.53**	150.67 ± 4.99**	183.33 ± 4.37**

Aqueous fraction	300	457.67 ± 15.34	442.83 ± 8.39*	430 ± 14.68	444 ± 15.66	482.33 ± 7.97	484.33 ± 7.89

*P<0.05, **P<0.01

For the antihyperglycemic activity, the total extract, chloroform, and ethyl acetate fractions (300 mg/kg) exhibited a high and significant activity after 1h from hyperglycemia induction using alloxan and up to 5h, compared with metformin (500 mg/kg) as a positive control (Table 4). Both n-hexane and aqueous fractions were inactive.

4. DISCUSSION

Eight known compounds including sterols, triterpenoids, flavonoids and phenethyl caffeoyl glycosides were isolated from the methnolic extract of the aerial parts of *R. equisetiformis*. The isolation of compounds (**1**-**4**) is considered as the first report for the isolation of such compounds from *R. equisetiformis* which could be helpful and can contribute in the chemotaxonomic analysis of this genus which has no previous studies. These compounds can also contribute in explanation of many of the biological activities exhibited by *R. equisetiformis*. The acute toxicity study showed that the methanolic extract with its high LD_{50} value showed a wide safety margin which could explain the wide use of *R. equisetiformis* in folkloric medicine. The different biological assays for the total extract and the different fractions showed that the chloroform fraction, together with the n-hexane and ethyl acetate ones were the most active against inflammation. Furthermore, ethyl acetate fraction showed a significant antipyretic activity could be attributed to its high content of flavonoids[29].

Finally, the antihyperglycemic activity showed that the total extract, chloroform and ethyl acetate fractions were significantly active against hyperglycemia, especially the latter which had the best effect reducing BGL up to 5h, and in comparison with metformin, having a better effect after 2 and 3h. From the overall results of the current study, it can conclude that both the ethyl acetate and chloroform fractions possess promising anti-inflammatory, antipyretic and antihyperglycemic activities.

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