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Marwa M Mohammed
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Ahmed A Ali
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Ezz-eldin K Desoky
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Lourin G Gobraeil
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Correspondence
Marwa M Mohammed
Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Compounds isolation and *in vitro* antioxidant activity evaluation of *Faidherbia albida* (Del.) A. Chev. Leaves ethanolic extract

Marwa M Mohammed, Ahmed A Ali, Ezz-eldin K Desoky and Lourin G Gobraeil

Abstract
Eight phenolic compounds were successively isolated and identified from the ethanolic extract of the leaves of *Faidherbia albida*. Five of them were previously isolated from the genus *Acacia,* such as β-amyrin (L-1), β-sitosterol (L-2), β-sitosterol-3-*O*-β-*D*-glucopyranoside.(L-3), Quercetin (L-5) and Gallic acid (L-6), the other three are firstly reported for isolation from the genus *Acacia,* and named Rhamnocitrin (L-4), Afzelin (L-7) and (6*S*, 9*S*)- Roseoside (L-8). The structures were identified and confirmed through different spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, HMBC and UV spectroscopy, in addition to comparison with authentic samples. Antioxidant activity was determined by the DPPH method revealed that all the tested extracts and fractions exhibited strong antioxidant activity especially ethyl acetate and aqueous fractions of the leaves.

Keywords: *Acacia, Faidherbia albida,* flavonoids, C_{13}-norisoprenoid glycoside, antioxidant activity

Introduction
Acacia (Mill.) is the largest genus in the Leguminosae - Mimosoideae with approximately 1300 species distributed mainly in tropical and subtropical regions [1, 2]. The trees of *Acacia albida* Delile are indigenous to Africa, where they are widely distributed in Senegal and Gambia [3]. In Egypt, they are considered a prominent feature in the flora of Nile valley and the Eastern Desert [2].
There has been considerable discussion to *Acacia albida* taxonomic status, as evidence indicates that this taxon is not closely related to the other African species of *Acacia* as it has distinct botanical characters, also quite different chemical constituent and the pollens also differs [3]. So, it has been excluded from the genus *Acacia* and transferred to a new monotypic genus, *Faidherbia*, within tribe Acacieae [3]. Molecular studies confirm that this taxon should be removed from *Acacia*. While accepting the distinct nature of the species, some authorities have reservation about keeping *Faidherbia* separate from the genus *Acacia* and still prefer to keep the name *Acacia albida* [4, 6]. Reviewing the available literature, *Faidherbia albida* (Del.) A. Chev. has many biological activities and few studies were carried on the chemistry of this species and that provoked this chemical study of the plant leaves which is demonstrated by isolation and identification of the main bioactive constituents, as well as evaluation of the activity of the different leaves extracts.

Material and Methods
Equipments and chemicals
Melting Point measured by Electrothermal 9100 Digital Melting Point Instrument (England Ltd., England), ^{1}H-NMR, ^{13}C-NMR measured using JEOL Oxford YH-400 (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR), UV spectra were recorded in methanol on Ultrospec 1000, UV-VIS spectrometer, Pharmacia Biotech, Cambridge, England, Silica gel (70-230 mesh, E-Merck, Germany), Sephadex LH-20 (25-100 mm mesh size, E-Merck) and Prepacked flash chromatography column (12g, silica Redi-sperf) for column chromatography, TLC Silica gel $G_{60}F_{254}$ precoated plates (E-Merck, Germany), The solvents used in this work include, *n*-hexane, dichloromethane, ethyl acetate, ethanol, methanol and butanol. Also, CD_3OD and DMSO-d_6 have been used in the NMR spectral analysis using TMS as internal standard, the solvent systems used for TLC analysis include: *n*-Hexane-Ethylacetate (95:5 v/v (sys 1)), (90:10 v/v (sys2)), dichloromethane-methanol (95:5 v/v (sys 3)), (90:10 v/v (sys 4)) and (80:20 v/v (sys5)). DPPH (Diphenyl-picryl-hydrazine) was purchased from Sigma-Aldrich Chemicals Co. Germany and Quercetin as a reference antioxidant was purchased from El-Nasr

Pharmaceutical and Chemical Co., Egypt) (Adwic).

The plant material

Fresh leaves of *Faidherbia albida* (Del.) A. Chev. (*Acacia albida* (Del.)) was collected during the fruiting stage in the period of September to December 2013 from the fields of Kom-ombo garden of medicinal plant, Aswan, Egypt. The plant was kindly identified and authenticated by Prof. Dr. kotb Amer, Botany Department, Faculty of Science, Assiut University.

Extraction and isolation

The air-dried powdered leaves (1.625 kg) were extracted with 70 % ethanol by maceration at room temperature till exhaustion (6×3 L). The ethanolic extracts were combined together and concentrated under reduced pressure to give 403 g residue (24.8 % w/w) which was suspended in distilled water (500 ml), transferred to a separating funnel and extracted successively with *n*-hexane (7×1 L), chloroform (4×1 L), and ethyl acetate (5×1 L). The extracts were concentrated separately under reduced pressure to give the corresponding fractions, *n*-hexane 88 g (21.8 % w/w), chloroform 23 g (5.7 % w/w), ethyl acetate 30 g (7.4 % w/w). The remained aqueous extract was 247 g (61.2 % w/w).

About 50 g of the *n*-hexane fraction were slurried with 150 g silica gel, dried, powdered and transferred to a Buchner (10× 14 cm), packed with a relatively narrow layer of silica gel for column followed by a wide layer of silica gel $G_{60}F_{254}$ for TLC and a negative pressure was applied by a water jet pump. It was eluted with *n*-Hexane and ethyl acetate in gradient elution where five fractions (H-I to H-V) were obtained. Group H-II eluted with *n*-Hex: EtOAc (95:5) was re-chromatographed over silica gel column and eluted with *n*-Hexane and ethyl acetate in gradient elution where three sub-fractions were obtained; H-II-1 (0.5 g eluted with *n*-Hex: EtOAc 97:3), H-II-2 (0.7 g, eluted with *n*-Hex: EtOAc 95:5) and H-II-3 (1.8 g, eluted with *n*-Hex: EtOAc 90:10). Each of H-II-1 and H-II-2 was purified by repeated crystallization from MeOH to obtain the pure compounds L-1 (170 mg) and L-2 (123 mg).

The chloroform fraction (23 g) was chromatographed on a silica gel column (700 g, 120 x 5 cm) eluted with gradient systems of CH_2Cl_2-MeOH where five groups (C-I to C-V) were obtained. Group C-II eluted with CH_2Cl_2-MeOH (95:5) was re-chromatographed over silica gel column then the sub-fraction eluted with CH_2Cl_2-MeOH (95:5) was subjected to crystallization from MeOH to obtain the pure compound L-3 (180 mg). Group C-III eluted with CH_2Cl_2-MeOH (90:10) was re-chromatographed over silica gel column then sub-fractions eluted with CH_2Cl_2-MeOH (90:10) was subjected to further separation and purification by sephadex LH-20 column chromatography using 100% MeOH to obtain the pure compounds L-4 (16 mg) and L-5 (13 mg).

The ethyl acetate fraction (30 g) was dissolved in the least amount of CH_2Cl_2, slurried with 90 g of silica gel, dried, powdered and transferred to a Buchner (10× 14 cm), packed with a relatively narrow layer of silica gel for column followed by a wide layer of silica gel $G_{60}F_{254}$ for TLC, negative pressure was applied by a water jet pump. The Buchner was eluted with CH_2Cl_2-MeOH gradient systems where four groups (E-I to E-IV) were obtained. Group E-II eluted with CH_2Cl_2-MeOH (90:10) (15 g) was re-chromatographed on silica gel column using gradient elution system of CH_2Cl_2-MeOH. The sub-fractions eluted with CH_2Cl_2-methanol (90:10) were collected together to afford a residue of 4 g where only (1g) of the residue was chromatographed on flash column where it was mixed with a small amount of silica used and applied to a sample specified cartridge of the flash apparatus which is connected through a tube to a disposable prepacked 12 gm flash column which was developed and eluted using gradient elution systems of *n*-Hex-EtOAc under suitable pressure (maximum pressure 400 psi) and flow rate (30 mL/min) to produce five sub-fractions E-II-1 to E-II-5. Sub-fractions E-II-1, E-II-3 and E-II-4 were subjected to further separation and purification by sephadex LH-20 column chromatography using 100% MeOH as eluent to obtain three pure compounds L-6 (75mg), L-7 (37mg) and L-8 (44mg).

DPPH$^{\cdot}$ Radical Scavenging Activity (DPPH$^{\cdot}$ assay)

Antioxidant activity was determined by the DPPH method [7]. The method is based on the reduction of alcoholic DPPH$^{\cdot}$ solutions at 517 nm in the presence of a hydrogen donating antioxidant (AH) due to the formation of the non-radical from DPPH-H by the reaction:

$$DPPH^{\cdot} + AH \longrightarrow DPPH\text{-}H + A^{\cdot}$$

The actual decrease in absorption induced by the test extract or compound was calculated by subtracting that of the control. The concentration of DPPH$^{\cdot}$ was kept at 100 µM in MeOH. The radical scavenging activity was measured by spectrophotometric method. Mix 2 ml of methanolic solutions of total extract and the fractions: *n*-hexane, chloroform, ethyl acetate and aqueous fractions of leaves (0.0625, 0.125, 0.25, 0.5, 1 mg/ml) with 2 ml of methanolic solution of DPPH$^{\cdot}$ (100µM). Similarly 2 ml methanolic solutions of quercetin is added to 2 ml DPPH$^{\cdot}$ and used as a positive control. A mixture of 2ml of methanol and 2 ml of methanolic solution of DPPH$^{\cdot}$ (100 µM) served as control. After mixing, all the solutions were incubated in dark for 20 minutes and absorbance was measured at 517 nm. The experiments were performed in triplicate and percent scavenging activity was calculated as follows:

$$\text{Scavenging \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Results

Eight compounds (L-1 to L-8) were successively isolated from the ethanolic extract of the leaves of *Faidherbia albida*. Three of them (L-5, L-6 and L-7) were previously detected by HPLC-DAD but there are no report for their isolation from the plant [8].

Compound L-1: obtained as white fine needles (acetone), m.p. 198-200°C. From this data in addition to mixed melting point as well as co-chromatography with authentic samples, it showed the same R_f value and colour reaction as β-amyrin.

Compound L-2: obtained as white needles (acetone), m.p. 134-136°C. From this data in addition to mixed melting point as well as co-chromatography with authentic samples, it showed the same R_f value and colour reaction as *β*-sitosterol.

Compound L-3: obtained as white granular powder (methanol), m.p. 263-265°C. From this data in addition to mixed melting point as well as co-chromatography with authentic samples, it showed the same R_f value and colour reaction as *β*-sitosterol-3-*O*-*β*-*D*-glucopyranoside.

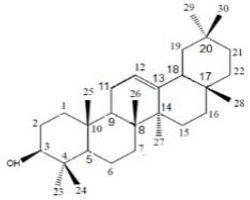
Compound L-4: obtained as yellow amorphous powder. R_f 0.45with sys 3. UV (MeOH): λmax 269, 361; +NaOMe: 277, 408; +$AlCl_3$: 271, 421; +$AlCl_3$/HCl: 270, 419; +NaOAc: 271, 366; +NaOAc/H_3BO_3: 270, 364 nm; ^{1}H-NMR (CD_3OD, 400 MHz): two signals at δ_H 6.21 and 6.43 (each 1H, br.s) assigned for H-6 and H-8, two signals at δ_H 6.93 and 8.11 (each 2H, d, J = 8.8 Hz) assigned for H-3', 5' and H-2', 6' and a signal at δ_H 3.71 (3H, s) assigned for methoxyl group and it was determined by the UV spectral study to be at position-7. From the previous data as well as by comparison with literature [9], compound L-4 was identified as Kaempferol 7-methy ether (Rhamnocitrin).

Compound L-5: obtained as yellow amorphous powder. R_f 0.31 with sys 3. UV (MeOH): λmax 256, 371; +NaOMe: 275, 416; +$AlCl_3$: 271, 449; +$AlCl_3$/HCl: 266, 421; +NaOAc: 268, 378; +NaOAc/H_3BO_3: 259, 385 nm; ^{1}H-NMR (CD_3OD, 400 MHz): two signals at δ_H 6.20 and 6.41 (each 1H, d, J =2 Hz) which are assigned for H-6 and H-8 and signals for a typical ABX pattern at δ_H 6.91 (1H, d, J =8.8 Hz), 7.65 (1H, dd, J =8.8, 2 Hz) and 7.76 (1H, d, J =2 Hz) which are assigned for H-5', H-6' and H-2'. From the previous data as well as by comparison with literature [10], in addition to co-chromatography with an authentic samples which showed the same R_f value and colour reaction, compound L-5 was identified as Quercetin.

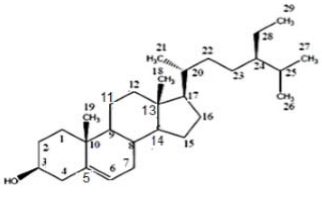
Compound L-6: obtained as colorless needle crystals (methanol), m.p. 160-162°C. R_f 0.35 using sys 4. ^{1}H-NMR (DMSO-d_6, 400 MHz) revealed the presence of one aromatic signal at δ_H 6.91 (2H, *s*), ^{13}C-NMR (CD_3OD, 100 MHz), which displayed 5 signals equivalent to 7 carbon atoms including downfield shifted signal for carbonyl group at δ_C 169.2. In addition to 4 signals corresponding to 6 aromatic carbons at δ_C 120.9 (C-1), 108.9 (C-2, C-6), 145 (C-3, C-5) and 138.1 (C-4). From the previous data as well as by comparison with literature [11], Compound L-6 was identified as Gallic acid.

Compound L-7: obtained as yellow amorphous powder. R_f 0.30 using sys 5**.** UV (MeOH): λmax 264, 354; +NaOMe: 274, 400; +$AlCl_3$: 273, 407; +$AlCl_3$/HCl: 274, 406; +NaOAc: 274, 370; +NaOAc/H_3BO_3: 267, 356 nm; ^{1}H-NMR (CD_3OD, 400 MHz): showed the pattern of kaempferol skeleton which is noted from the presence of two signals at δ_H 6.20 and 6.40 (each 1H, d, J = 1.6 Hz) assigned for H-6 and H-8, two signals at δ_H 6.92 and 7.76 (each 2H, d, J = 8.8 Hz) which are assigned for H-3', 5' and H-2', 6'. Also a signal at δ_H 5.30 (1H, d*,* J = 0.8 Hz) which was characteristic for α- anomeric sugar proton and a signal at δ_H 0.80 (3H, *d, J* = 6 Hz) indicating the presence of α-rhamnopyranosyl moiety, along with other sugar signals at δ_H 3.47 (1H, dd*,* J = 3.2, 9.9 Hz) assigned for H-3" and at δ_H 3.12- 3.17 (2H, *m*)assigned for H-4", H-5". The glycosidic linkage at position-3 was determined by the UV spectral study. From the previous data as well as by comparison with literature [12, 14], compound L-7 was identified as Kaempferol 3-*O*-α-L-rhamnoside (Afzelin).

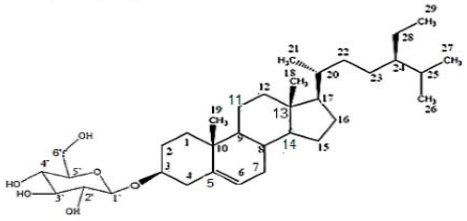
Compound L-8 (Figs: 1-3): obtained as brown amorphous powder (methanol). R_f 0.20 using sys 5. The ^{1}H- and ^{13}C-NMR (DMSO-d_6, 400 MHz): δ_H 5.96 (1H, d, J = 15.2 Hz, H-7, δ_c 132.1), 5.66 (1H, dd, J= 15.2, 6.4 Hz, H-8, δ_c 131.9), 4.45 (1H, *qui*, J=6.4 Hz, H-9, δ_c 73.7), 1.19 (3H, *d*, J = 6.4 Hz, H-10, δ_c 22.5), 0.92 (3H, *s*, H-11 δ_c 23.5) and 0.94 (3H, *s*, δ_c H-12, δ_c 24.5), 1.83 (3H, *s*, H-13, δ_c 19.1), 5.77 (1H, *s,* H-4, δ_c 126.0), 2.06 and 2.56 (each 1H, *d*, J = 16.8 Hz, H-2, δ_c 49.8), 4.10 (1H*, d,* J = 7.6 Hz, H-1', δ_c 100.4), 2.94-3.65 (6H, *m*, δ_c 61.5- 77.6). The ^{13}C-NMR spectrum also showed four quaternary signals at δ_c 198.0 (C-3), 41.4 (C-1), 78.4 (C-6) and 164.4 (C-5). The structure was further confirmed by the HMBC correlations between H-13 (δ_H 1.83) and C-3, C-4, C-5 and C-6 which confirmed the presence of α,β-unsaturated carbonyl groups and the correlations between H-11 (δ_H 0.92), H-12 (δ_H 0.94) and C-1, C-2, C-3 and C-6 which confirmed the presence of six-membered ring moiety. In addition to the correlations between H-8 (δ_H 5.66) and C-6, C-9 and C-10 which confirmed that the side chain is attached to C-6 of the six membered ring. Also, the correlations between anomeric proton H-1' (δ_H 4.10) of the sugar moiety to δ_c 73.7 (C-9) and between the oxymethine protons at δ_H 4.45 (H-9) and C-1' (δ_c 100.4) confirmed that the linkage position of the sugar moiety was to be at C-9. The chemical shift value of C-9 was important for deducing the configuration of C-9 $\Delta^{7,8}$- type of 9- hydroxyl megastigmane -9-*O*-β-D-glucopyranoside. For the 9*S*, the carbon resonatse near δ_c (74-76) while in 9R, the chemical shift appears near δ_c (77-79) [15]. Thus, the absolute configuration of C-9 of compound L-8 was confirmed to be (*S*) based on its chemical shift at δ_c 73.7. From the previous data as well as by comparison with reported literature [16], compound L-8 was identified as (6*S*, 9*S*)- Roseoside which is known as Vomifoliol 9-*O*-β-D-glucopyranoside, a C_{13}-norisoprenoid glycoside, and this is the first report for its isolation from genus *Acacia*, but it was previously isolated from family Fabaceae [17].



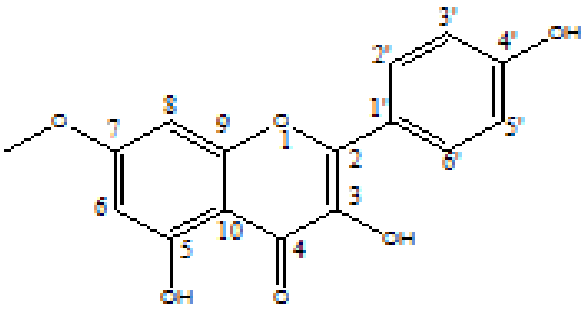
β- Amyrin (L-1)



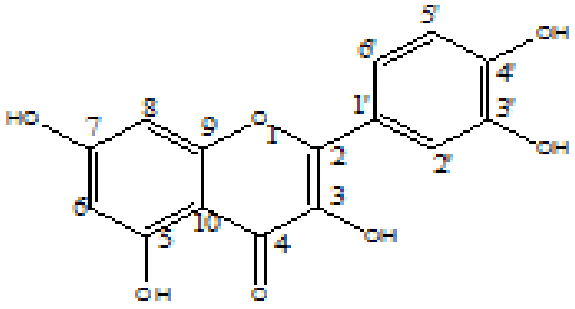
β- Sitosterol (L-2)



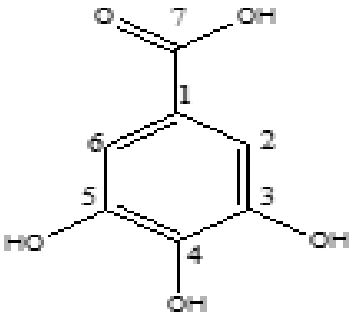
β- Sitosterol-3-O- β-D-Glucopyranoside (L-3)



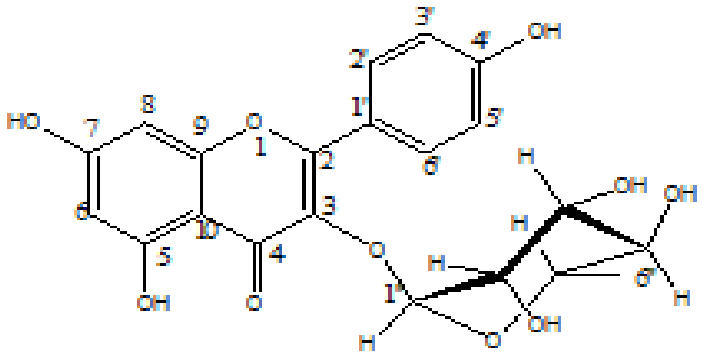
Rhamnocitrin (L-4)



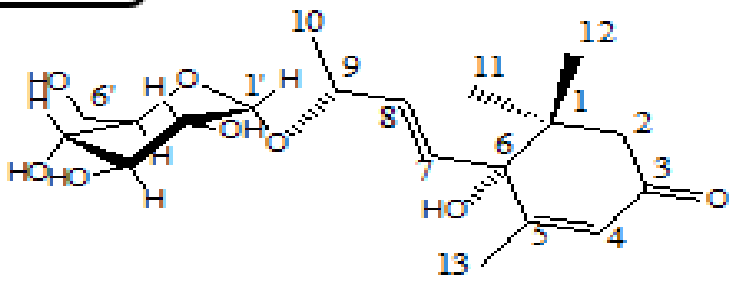
Quercetin (L-5)



Gallic acid (L-6)



Afzelin (L-7)



(6*S*, 9*S*)- Roseoside (L-8)

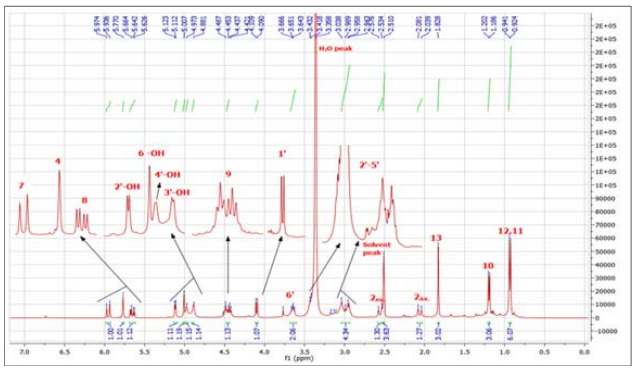


Fig 1: ^{1}H-NMR spectrum of compound **L-8** (DMSO-*d*$_6$, 400 MHz)

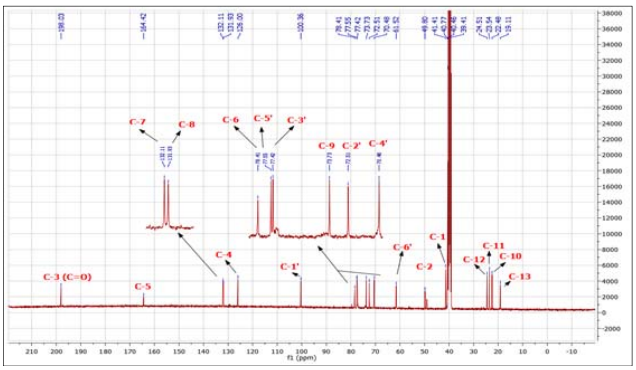


Fig 2: ^{13}C-NMR spectrum of compound L-8 (DMSO-*d*$_6$, 100 MHz)

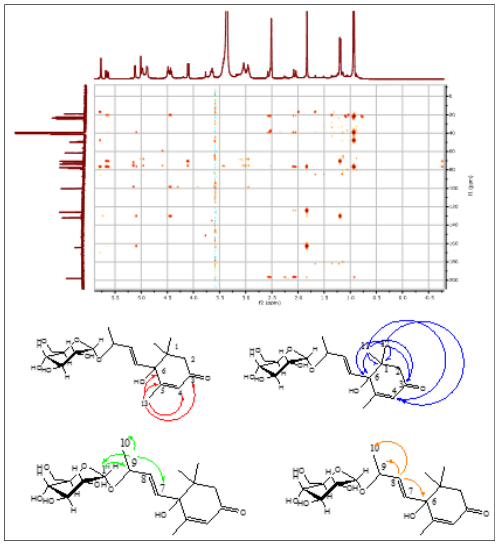


Fig 3: HMBC spectrum of compound L-8 (DMSO-d_6, 400/100 MHz)

Antioxidant activity

The results of the antioxidant activity of different extracts and fractions were shown in Table 1 and Figure 4.

Table 1: Antioxidant activity of the different extracts and fractions of *Faidherbia albida* leaves

Extracts / Concentrations	Antioxidant %				
	1 mg	0.5 mg	0.25 mg	0.125 mg	0.0625mg
DPPH (Blank)	-	-	-	-	-
Quercetin (Reference)	100	100	99.8	99.7	98
Total EtOH extract	87.6	81.74	56.8	55.3	42.8
n-Hexane fraction	75.4	74.93	49.8	41.69	29.83
Chloroform fraction	78.74	67.1	64.3	62.8	45.4
Ethyl acetate fraction	94.9	92.23	91.8	88.9	69.1
Aqueous fraction	94.96	92.77	91.44	44.9	27.52

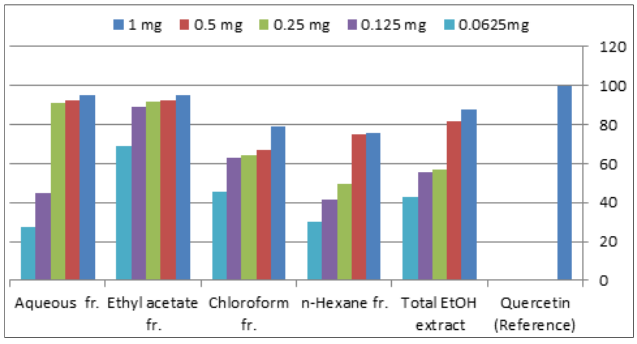


Fig. 4: Chart for the antioxidant activity of the different extracts and fractions of *Faidherbia albida* leaves

Discussion

The listed results in table 1, revealed that all the tested extracts and fractions exhibited strong antioxidant activity especially ethyl acetate and aqueous fractions of the leaves.

Conclusion

Eight compounds were isolated and identified from the leaves extracts of *Faidherbia albida* by different spectroscopic methods, including sterols, triterpenes, phenolic acids, C_{13}-norisoprenoid glycoside, flavonoidal aglycons and glycoside. The antioxidant activity is probably due to the presence of phenolic compounds as flavonoidal compounds.

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