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Compounds isolation and antioxidant activity of *Faidherbia albida* fruit extract

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Abstract
Five flavonoidal compounds were successively isolated and identified from the ethanolic extract of the fruits of *Faidherbia albida*. Two of them were previously isolated from the leaves of *Faidherbia albida*, such as Rhamnocitrin (F-1) and Quercetin (F-4), the other three are firstly reported for isolation from the fruit, and named Dihydrokaempferol (F-2), Luteolin (F-3) and Rutin (F-5). The structures were identified and confirmed through different spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR 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 the total ethanolic extract, chloroform and ethyl acetate fractions of the fruits.

Keywords: *Acacia*, *Faidherbia albida*, flavonoids, dihyroflavonols, antioxidant activity

Introduction
Acacia (Mill.) is the largest genus in the Leguminosae with approximately 1300 species [1, 2]. It is represented in Egypt by ten species; which are widely distributed in various phytogeographical regions of Egypt, where they are immensely useful as sources of food, fodder, fire-wood and as a source of natural products such as gum exudates [2].
Acacia sp. have many purposes as being valuable wood for industries, decorations, sources for gum, tannin, perfumes, ink, protein, paint and to prepare disinfectant for micro-organisms and hand washes [3]. The trees of *Acacia albida* Delile are indigenous to Africa [4] and are considered a prominent feature in the flora of Nile valley and the Eastern Desert [2].
Reviewing the available literature, *Faidherbia albida* (Del.) A. Chev. few studies were carried on the chemistry of this species and that provoked this chemical study of the plant fruits which is demonstrated by isolation and identification of the main bioactive constituents, as well as evaluation of the antioxidant activity of the different fruit extracts.

Material and Methods
Equipment's and chemicals
^{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) and Sephadex LH-20 (25-100 mm mesh size, E-Merck) 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 and methanol. 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: dichloromethane-methanol (95:5 v/v (sys 1)), (90:10 v/v (sys 2)) and (80:20 v/v (sys3)). DPPH (Diphenyl-picryl-hydrazine) was purchased from Sigma-Aldrich Chemicals Co. Germany and Quercetin, Rhamnocitrin luteolin and Rutin as authentic was purchased from El-Nasr Pharmaceutical and Chemical Co., Egypt) (Adwic).

The plant material
Fresh fruits 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 fruit (1.100 Kg) were extracted till exhaustion with ethanol 70% by maceration at room temperature. The ethanolic extracts were combined together and concentrated under reduced pressure to give 153.2 g residue (13.92%) which was suspended in distilled water (100 ml), transferred to a separating funnel and extracted with successive portions of n-hexane (4×1 L), chloroform (2×1 L) and ethyl acetate (4×1 L). Then concentrated under reduced pressure to give the corresponding fractions; n-hexane 16.1 g (10.51% w/w), chloroform 3.6 g (2.35% w/w), ethyl acetate 13 g (8.48% w/w) and the remained aqueous extract was 115 g (75.07% w/w).
(3.6 g) of the chloroform fraction was slurried with 8 g of silica gel, dried, powdered and transferred to the top of column packed with silica gel (120g, 110× 3 cm). Elution was performed initially with CH_2Cl_2 followed by gradient systems of CH_2Cl_2-MeOH. Similar fractions were combined together and concentrated under reduced pressure where three groups

(FC-I to FC-III) were obtained. The residue of Group FC-I (1.2 g) was dissolved in the least amount of CH_2Cl_2, slurried with 3 g of silica gel, dried and then inserted on the top of a silica gel column (40 g, 100 x 2 cm). The column was initially eluted with CH_2Cl_2 followed by CH_2Cl_2-MeOH gradient systems. The sub-fractions eluted with CH_2Cl_2-MeOH (90:10) were collected together and subjected to further separation and purification by sephadex LH-20 column chromatography using 100% MeOH as eluent to obtain pure compounds F-1 (12 mg) and F-2 (30 mg).

(13 g) of the ethyl acetate fraction was slurried with 40 g of silica gel, dried, powdered and transferred to the top of column packed with silica gel (400g, 150×4 cm). Elution was performed initially with CH_2Cl_2 followed by gradient systems of CH_2Cl_2-MeOH where five groups (FE-I to FE-V) were obtained. The residue of Group FE-II (2.4 g) was dissolved in the least amount of CH_2Cl_2, slurried with 7.5 g of silica gel, dried and then inserted on the top of a silica gel column (100 g, 100 x 3 cm) which was initially eluted with CH_2Cl_2followed by CH_2Cl_2-MeOH gradient systems.The sub-fractions eluted with CH_2Cl_2-MeOH (90:10) were collected together and subjected to further separation and purification by sephadex LH-20 column chromatography using 100% MeOH as eluent to obtain pure compounds F-3 (27 mg) and F-4 (15 mg). The residue of Group FE-III (300 mg) was re-chromatographed on sephadex LH-20 column using 100% MeOH as eluent to obtain pure compound F-5 (19 mg).

DPPH· Radical Scavenging Activity (DPPH· assay)

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

Antioxidant activity was determined by the DPPH method [5]. The method is based on the reduction of alcoholic DPPH· 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:

The actual decrease in absorption induced by the test extract or compound was calculated by subtracting that of the control. The concentration of DPPH· 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 fruits (0.0625, 0.125, 0.25, 0.5, 1 mg/ml) with 2 ml of methanolic solution of DPPH· (100µM). Similarly 2 ml methanolic solutions of quercetin is added to 2 ml DPPH· and used as a positive control. A mixture of 2ml of methanol and 2 ml of methanolic solution of DPPH· (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:

Five flavonoidal compounds were successively isolated and identified from the ethanolic extract of the fruits of *Faidherbia albida*. Two of them were previously isolated from the leaves of *Faidherbia albida*, such as (F-1) and (F-4), the other three are firstly reported for isolation from the fruit (F-2), (F-3) and (F-5).

Compound F-1: obtained as yellow amorphous powder. R_f 0.44 with sys 1. By co-chromatography with an authentic sample which showed the same R_f value and colour reaction, compound F-1 was identified as Kaempferol 7-methy ether (Rhamnocitrin).

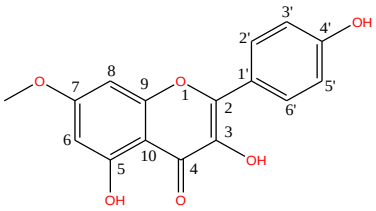
Compound F-2: obtained as yellow amorphous powder. R_f 0.40 with sys 1. UV (MeOH): λ_{max} 292, 329sh; +NaOMe: 327; +$AlCl_3$: 314; +$AlCl_3$/HCl: 312; +NaOAc: 324; +NaOAc/H_3BO_3: 293 nm; The ^{1}H-NMR and DEPT-Q ^{13}C-NMR spectral data of compound F-2 (CD_3OD, 400 MHz) were listed in Tables 1,2 and illustrated in Figs. 1,2.

From the previously mentioned physical, chemical, chromatographic studies as well as by comparison of its spectral data (UV, ^{1}H-NMR and DEPT-Q ^{13}C-NMR) with those reported in the literature [6], compound F-2 was identified as Dihydrokaempferol (Aromadendrin).

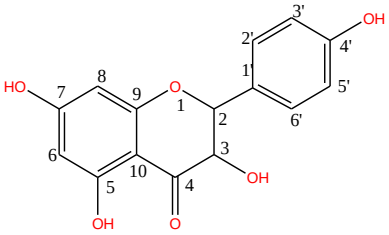
Compound F-3: obtained as yellow amorphous powder. R_f 0.35 with sys 1. UV (MeOH): λ_{max} 255, 343; +NaOMe: 267, 401; +$AlCl_3$: 273, 415; +$AlCl_3$/HCl: 275, 378; +NaOAc: 269, 350; +NaOAc/H_3BO_3: 259, 365 nm; The ^{1}H-NMR and DEPT-Q ^{13}C-NMR spectral data of compound F-3 (CD_3OD, 400 MHz) were listed in Tables 3,4 and illustrated in Figs. 3,4. From the previously mentioned physical, chemical, chromatographic studies as well as by comparison of the spectral data (UV, ^{1}H-NMR and DEPT-Q ^{13}C-NMR) with those reported in the literature [7] in addition to co-chromatography with an authentic sample of luteolin, compound F-3 was identified as Luteolin

Compound F-4: obtained as yellow amorphous powder. R_f 0.32 with sys 1. By co-chromatography with an authentic sample which showed the same R_f value and colour reaction, compound F-4 was identified as Quercetin.

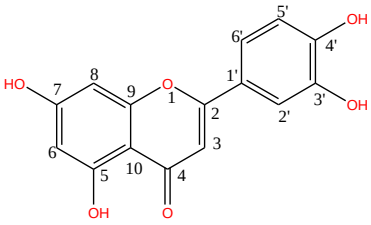
Compound F-5: obtained as yellow amorphous powder. R_f 0.17 with sys 3. UV (MeOH): λ_{max} 258, 353; +NaOMe: 273, 406; +$AlCl_3$: 269, 427; +$AlCl_3$/HCl: 279, 370; +NaOAc: 269, 380; +NaOAc/H_3BO_3: 265, 372 nm; The ^{1}H-NMR spectral data of compound F-5 (DMSO-d_6,400 MHz) were listed in Table 5 and illustrated in Fig. 5. From the previously mentioned physical, chemical, chromatographic studies as well as by comparison of the spectral data (UV, ^{1}H-NMR) with those reported in the literature [8] in adition to co-chromatography with an authentic sample of quercetin-3-O-rutinoside (Rutin), compound F-5 was identified as Quercetin-3-O-rutinoside (Rutin).



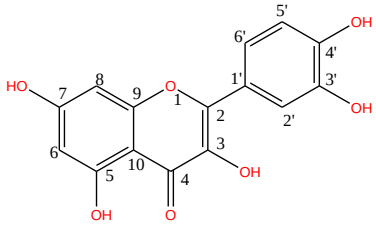
Rhamnocitrin (F-1)



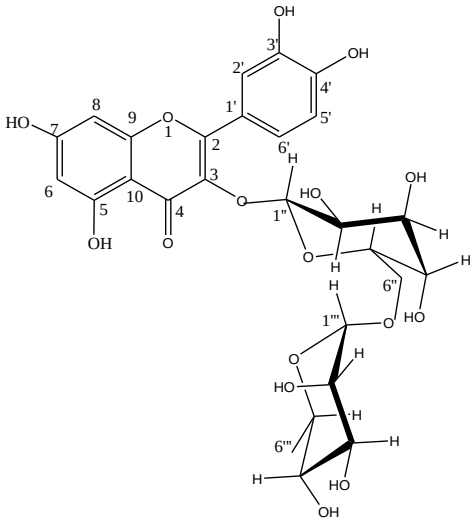
Aromadendrin (F-2)



Luteolin (F-3)



Quercetin (F-4)



Quercetin-3-O-rutinoside (F-5)

Table 1: ^{1}H-NMR spectral data of compound F-2 (CD_3OD, 400 MHz)

Chemical shift (δ) ppm	No. of protons	Coupling constant (Hz)	Assignment
4.56	1H (d)	11.6	H-3
4.99	1H (d)	11.6	H-2
5.90	1H (br.s)	-	H-6
5.94	1H (br.s)	-	H-8
6.85	2H (d)	8	H-3',5'
7.37	2H (d)	8	H-2',6'

Table 2: DEPT-Q ^{13}C-NMR spectral data of compound F-2 (CD_3OD, 100 MHz)

Carbon No.	Type of carbon	δ_C (ppm)	Reported data [6] (DMSO-d_6)
2	CH	83.9	83.0
3	CH	72.2	71.6
4	C	197.0	198
5	C	164.0	163.5
6	CH	96.2	96.2
7	C	167.2	166.9
8	CH	95.0	95.2
9	C	163.1	162.7
10	C	100.5	100.6
1'	C	127.8	127.7
2',6'	CH	129.0	129.6
3',5'	CH	114.6	115.1
4'	C	157.8	157.9

Table 3: ^{1}H-NMR spectral data of compound **F-3** (CD_3OD, 400 MHz):

Chemical shift (δ) ppm	Integration and (Multiplicity)	Coupling constant (Hz)	Assignment
7.41	1H (s)	-	H-2'
7.39	1H (s)	-	H-6'
6.92	1H (d)	8.8	H-5'
6.55	1H (s)	-	H-3
6.46	1H (br.s)	-	H-8
6.22	1H (br.s)	-	H-6

Table 4: DEPT-Q^{13}C-NMR spectral data of compound F-3 (CD_3OD, 100 MHz)

Carbon No.	Type of carbon	δ_C (ppm)	Reported data [7]
2	C	164.6	164.3
3	CH	102.5	103.2
4	C	182.6	181.7
5	C	162.00	161.5
6	CH	98.7	99.1
7	C	164.94	164.3
8	CH	93.6	94.2
9	C	157.9	157.6
10	C	103.9	104.1
1'	C	122.3	122.0
2'	CH	112.7	112.8
3'	C	145.7	146.1
4'	C	149.5	150.0
5'	CH	115.4	115.3
6'	CH	118.9	119.2

Table 5: ^{1}H-NMR spectral data of compound **F-5** (DMSO-d_6,400 MHz)

Chemical shift (δ) ppm	Integration and (Multiplicity)	Coupling constant (Hz)	Assignment
12.59	1H (br. s)	-	5-OH
7.56	1H (d)	2	H-2'
7.54	1H (dd)	2, 8	H-6'
6.83	1H (d)	8	H-5'
6.35	1H (br.s)	-	H-8
6.16	1H (br.s)	-	H-6
5.33	1H (d)	7.2	H-1"
4.39	1H(s)	-	H-1"'
3.06-3.72	9H (m)	-	other sugar protons
0.994	3H (d)	6.4	CH_3

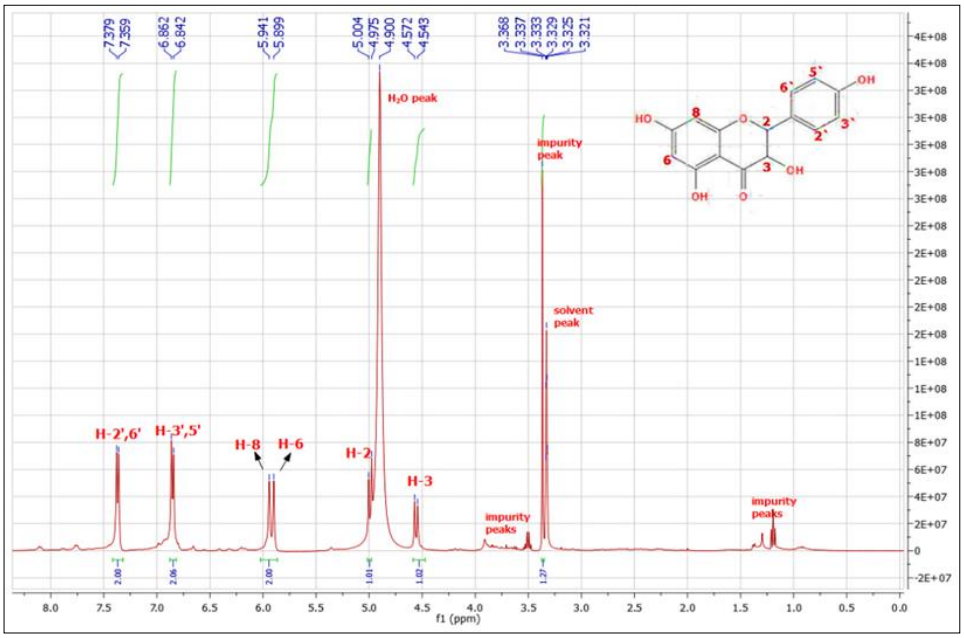


Fig. 1: ^{1}H-NMR spectrum of compound **F-2** (CD_3OD, 400 MHz)

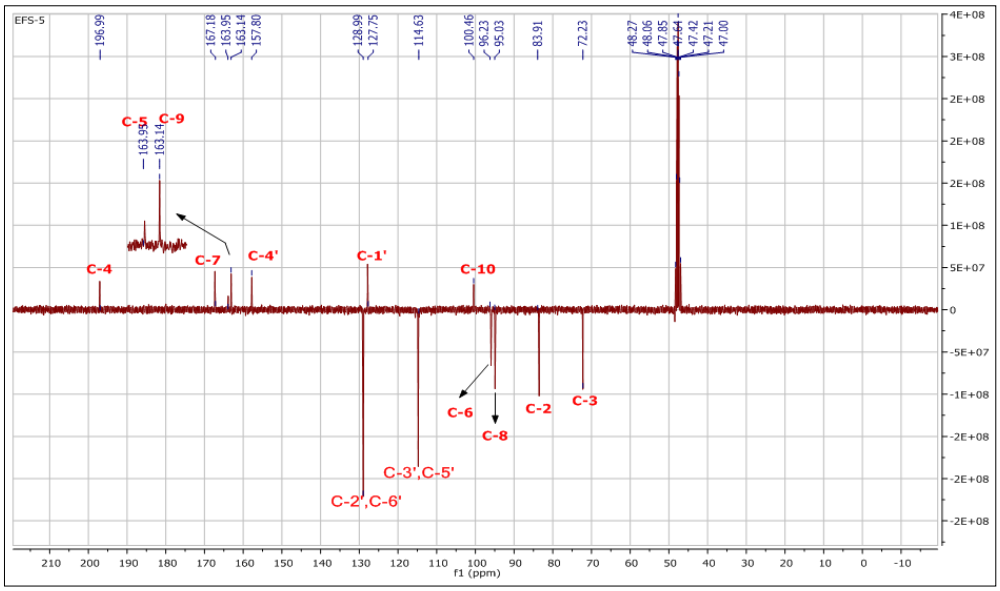


Fig. 2: DEPT-Q ^{13}C-NMR spectrum of compound **F-2 (**CD_3OD, 100 MHz)

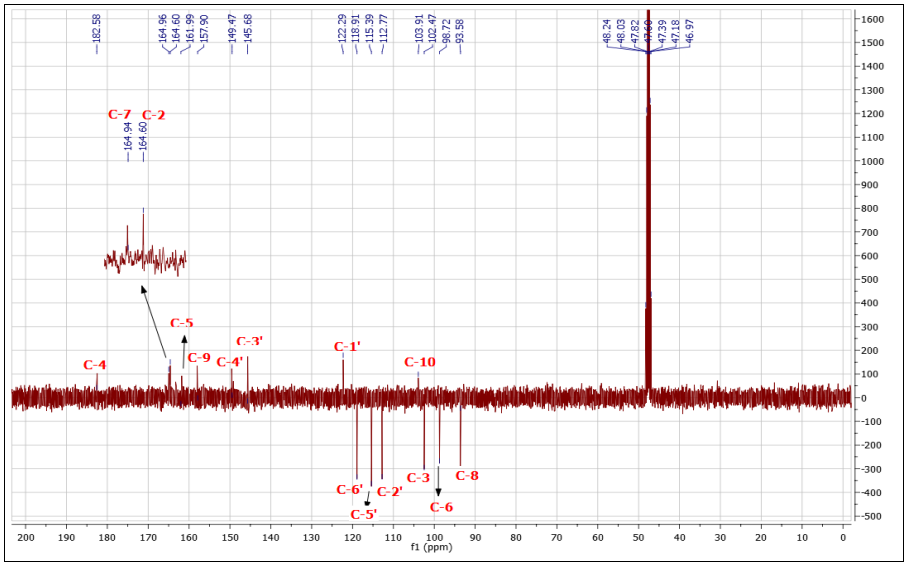


Fig. 4: DEPT-Q ^{13}C-NMR spectrum of compound F-3 (CD_3OD, 100 MH**z)**

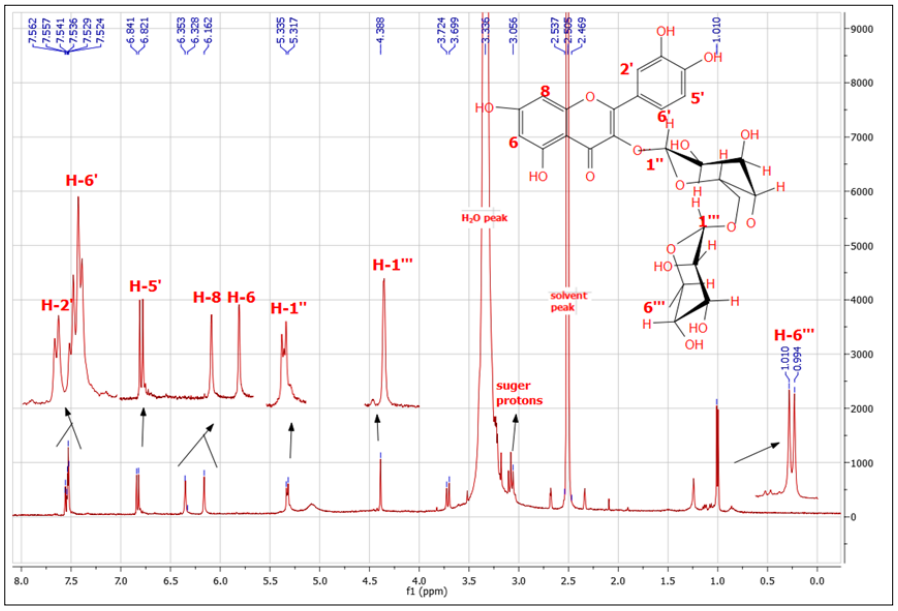


Fig. 5: ^{1}H-NMR spectrum of compound F-5 (DMSO-d_6,400 MHz)

Antioxidant activity

The results of the antioxidant activity of different extracts and fractions were shown in Table 6 and Figure 6.

Table 6: Antioxidant activity of the different extracts and fractions of Faidherbia albida fruits

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	90.1	79.56	77.93	64.06	49.72
n-Hexane fr.	62.56	62.234	58.42	57.65	41.85
Chloroform fr.	89.51	85.64	80.65	61.99	35.29
Ethyl acetate fr.	89.6	86.64	71.66	70.34	66.81
Aqueous fr.	78.33	52.99	30.52	17.16	11.035

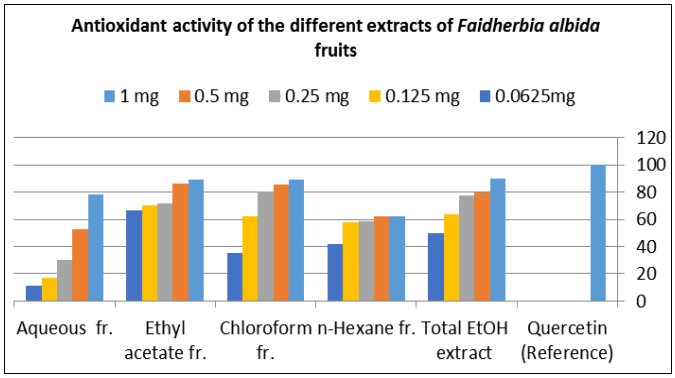


Fig. 6: Chart for the antioxidant activity of the different extracts of *Faidherbia albida* fruits

Discussion

The listed results in table 6, revealed that all the tested extracts and fractions exhibited strong antioxidant activity especially the total ethanolic extract, chloroform and ethyl acetate fractions of the fruits**.**

Conclusion

Five flavonoidal compounds were isolated and identified from the fruits extracts of *Faidherbia albida* by different spectroscopic methods, including dihydroflavonol, flavonoidal aglycons and glycoside.The strong antioxidant activity is probably due to the presence of various flavonoidal compounds.

Acknowledgement

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