PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF *KOELREUTERIA ELEGANS* (SEEM.) LEAVES

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ABSTRACT

A new galloylquinic acid butyl ester**;** 1, 3, 4, 5-tetra-*O*-galloylquinic acid butyl ester (**1**) and other ten known phenolic compounds : 1,3,4,5-tetra-*O*-galloylquinic acid (**2**) , methyl gallate (**3**), gallic acid (**4**) , 3"-galloyl quercetrin (**5**), isorhamnetin-3-*O*-β-D-$^{4}C_{1}$-arabinopyranoside **(6)**, isorhamnetin-3- *O*-α-L-$^{1}C_{4}$-rhamnopyranoside (**7**), quercetrin (**8**), guaijaverin (**9**), quercetin (**10**), azaleatin (**11**) as well as, three triterpenoidal compounds oleanolic acid (**12**), oleanolic acid-3-*O*-β-D-$^{4}C_{1}$-glucopyranoside (**13**), 3-*O*-[*O*-α-L-rhamnopyranosyl-(1-2)-*O*-β-D-glucopyranosyl-(1-2)- β-D-$^{4}C_{1}$-glucopyranosyl] oleanolic acid **(14)** were isolated from the 70 % methanol extract of leaves of *Koelreuteria elegans* (Seem.). The structures of the isolated compounds were elucidated on the basis of chemical and spectroscopic analysis (NMR, -ESIMS and UV). Total phenolic and flavonoid contents were found to be 8.19 g of GAE (gallic acid equivalents) and 6.22 g of RE (rutin equivalents) per 100 g dry extract respectively. The extract significantly improved the main liver function enzymes (ALT, AST) and total bilirubin in addition to the significant hepatoprotective action against the depletion of hepatic GSH and serum vitamin C levels , as well as the changes of hepatic antioxidant enzymes levels (SOD, CAT) induced by CCl_4 toxicity. It non-significantly reduced the level of ALP and GGT. Moreover, compound **1** exhibited higher activity than aqueous methanol extract against both Gram positive and Gram negative organisms and *Geotricum candidum* (fungi).

Key world: *Koelreuteria elegans;* Flavonols; Triterpenoidal saponin; Hepatoprotective effect and Antimicrobial activity**.**

1. I NTRODUCTION

Family Sapindaceae (Soapberry family) comprises about 1900 species mainly found in the tropical regions, with only a few genera being restricted to temperate areas (Buerki, Forest et al. 2009). Members of this family serve as important sources of nuts, oils and drugs. Several species contain saponins in their fruits, seeds and other tissues; hence they serve as soap substitute. Many other members are grown for their edible fruits (Adeyemi, Ogundipe et al. 2012). *Koelreuteria*; a genus in the tribe *Koelreuterieae* (Sapindaceae), is considered a famous ornamental tree species used in landscape architecture (Xin L, Weibing J et al. 2009). Some compounds isolated from *Koelreuteria* species have been reported to have bacteriostatic (Loncin, Kozulis et al. 1970), insecticidal (Contribs. 1947) and antitumor activities (Abou-Shoer, Ma et al. 1993) as well as, anti-inflammatory activity in case of gout (Hatano, Yasuhara et al. 1991).

Koelreuteria elegans (Seem.) is native to Taiwan and Fiji and cultivated in Egypt as ornamental tree; its leaves are used as a black hair dye, its roots, bark, twigs, and leaves have been used in traditional folk medicine for the treatment of diarrhea, malaria, and urethritis (Meyer 1976),

A huge number of different chemical constituents were reported in *Koelreuteria* species *e.g.* flavonoids (Mahmoud, Moharram et al. 2001, Qu, Zhang et al. 2011), cyclolignans (Song, Zhang et al. 1994, Lee, Chiang et al. 2009) and saponins (Sutiashvili 2000, Lei, Li et al. 2007). The present work revealed the isolation and structural elucidation of a new galloylquinic acid butyl ester (**1**) and other ten phenolic compounds (**2-11**) as well as, three triterpenoids (**12-14**) from *Koelreuteria elegans* (Seem.) leaves. The hepatoprotective effect of aqueous methanol extract of *K. elegans* (Seem.) leaves against CCl_4 induced hepatotoxicity in mice was studied, the (LD_{50}) has been determined together with the evaluation of the antimicrobial activity of aqueous methanol extract of *K. elegans* leaves and some isolated pure compounds against fungi, Gram positive and Gram negative bacteria.

2. EXPERIMENTAL

2.1. General procedures

NMR analysis was carried out using Bruker spectrometer operating at (400 and 500 MHz for ^{1}H & 100 MHz for ^{13}C). All samples have been prepared in DMSO-d_6 or pyridine-d_5 with TMS as internal reference, with the chemical shifts expressed in ppm, and coupling constants *(J)* in Hertz. (The analysis was done in the Central Service Lab-NRC, Dokki, Giza, Egypt and in Chemistry Department, College of Science, King Saud University, Riyadh, KSA). Electrospray (ESI)-MS was carried out using Thermo Finnigan LCQ Advantage MAX (ion trap) instrument (Finnigan, Bremen, Germany). UV Spectrophotometer Shimadzu UV 240 (P/N 240-58000) was used for recording different UV spectra. Spectrophotometer (4802 UV/VIS double beam) was used in determination of total phenolic and flavonoid contents. Glass chromatography columns of different sizes were packed with polyamide S6 (Fluka, Switzerland), microcrystalline cellulose (E. Merck, Darmstadt, Germany), and sephadex LH-20 (Pharmacia, Uppsala,

Sweden). For Thin Layer Chromatography, Silica gel F_{254} plates (Fluka, Switzerland) and cellulose glass plates (E. Merck, Darmstadt, Germany) were used. Paper chromatography was performed on Whatmann No. 1 sheets for PC (Whatmann Ltd., Maidstone, Kent, England). HPLC-grade methanol (Merck) was used for sample preparation. All other solvents used for extraction and separation processes were of analytical grade (El-Nasr Chemicals Co., Abou Zaabal, Egypt).

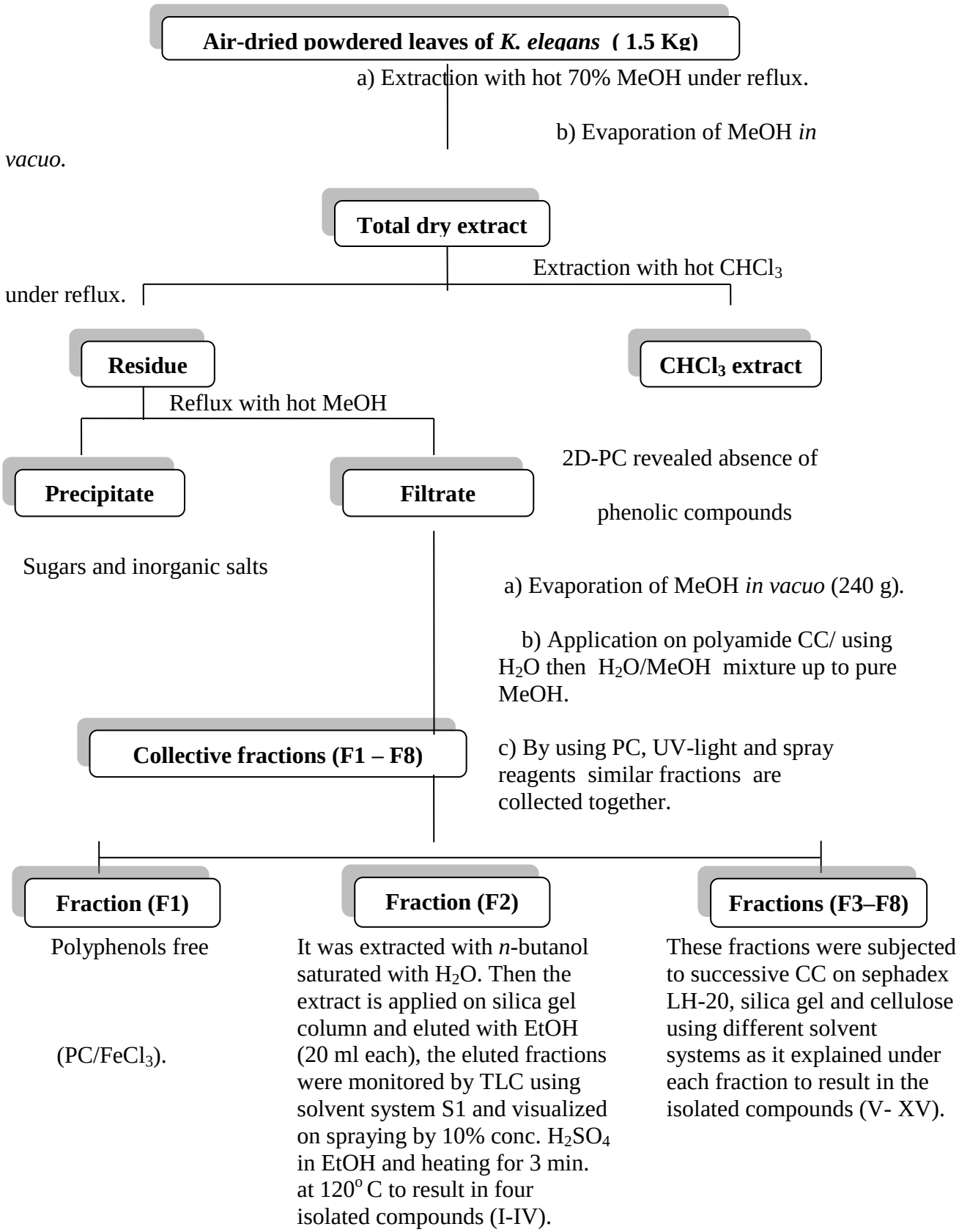
2.2. Plant material

Fresh leaves of *Koelreuteria elegans* (Seem) were collected from plants cultivated in the Zoo, Giza, and in the Agricultural museum, Dokki, Giza, during September (2009). The identity of the plant was established by Prof. Dr. Wafaa M. Amer, Botany Department, Faculty of Science, Cairo University. Voucher specimens (Reg. No.: K-5) are kept in herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University. The plant material was air- dried, powdered and kept in tightly closed container.

2.3. Extraction and Isolation

Air dried defatted powder of the aerial parts (1.5 kg) was exhaustively extracted with 70% aqueous methanol under reflux (5 x 6L; at 60°C for 4hr). The residue (300 g) left after evaporation of the solvent was extracted with $CHCl_3$ under reflux (3 x 1.5L; 60°C) afforded 40 g of dry $CHCl_3$ extract and 230 g of dry methanol extract. Its concentrated water solution was precipitated with excess EtOH (1:10), filtered and dried (180 g). Methanol soluble portion was subjected to 2D-PC using S_1: *n*-BuOH-HOAc-H_2O (BAW 4:1:5, top layer) in the first run and S_2: 15% aqueous HOAc in the second run followed by visualization in visible and UV light and spraying with different spray reagent *vis*, $FeCl_3$ and Naturstoff methanol soluble (rich in flavonoids and phenolic acids) extract was subjected to chromatographic fractionation on polyamide column (300 g). Elution was started by 100 % distilled H_2O followed by decreasing the polarity by MeOH until 100% MeOH. Similar fractions were collected together on the bases of Co-PC using solvents systems S_1 and S_2 and visualization as above or silica gel F_{254} plates using S_1; sprayed with EtOH/H_2SO_4 reagent. The main collective fractions obtained from polyamide column were subjected to repeated columns chromatography on cellulose and/or sephadex LH-20 using suitable solvents for elution as illustrated in **Figure (1).**

Figure (1): Extraction, isolation and purification of phytoconstituents of *K. elegans* (Seem.) leaves:



2.4. Determination of median lethal dose (LD_{50}) was done according to (Kerber 1941). The LD_{50} of the extract was found to be 3 g / Kg b.wt.

2.5. Methods for evaluation of hepatoprotective effect:

Male Albino mice (18-20 g) were randomly selected and purchased from animal house of National Research Centre and kept in the animal house of Al-Azhar University, they were housed in an environmentally control room and maintained at uniform laboratory conditions of light and temperature as well as, provided with food and water *ad libitum* for one week.

51 animals were divided into 3 groups as following:

- ***Normal control group*** (15 mice), receiving nothing, except *ad libitum*, water and intraperitoneal (i.p.) injection of 200 µl / Kg b.wt paraffin oil every 3 days for 2 weeks.
- ***The second group*** (18 mice) was injected i.p. with CCl_4 in paraffin oil (50%) in a dose of 200 µl / Kg b.wt every 3 days.
- ***The third group*** (18 mice) was given daily oral 70% aqueous methanol extract of *K. elegans* (Seem.) leaves in a dose of 300 mg / Kg b.wt simultaneously with i.p. injection of CCl_4 in paraffin oil (50%) in a dose of 200 µl / Kg b.wt every 3 days.

Blood samples were collected after 14 days. They were divided into 2 aliquots, the first aliquot received on EDTA in dry clean tubes for SOD, CAT and reduced glutathione (GSH) in RBC_S. While, the second one was received in dry clean tubes and allowed to clot at 37^{o}C for one hour. Then centrifuged at 3000 rpm for 15 minutes. Serum was separated and divided into aliquots and vitamin C determination was done at once. Other serum aliquots were stored at -20^{o}C for one week during which the other biochemical parameters were done.

2.5.1. Quantitative determination of aspartate transaminase (AST) and alanine transaminase (ALT) in mice serum were measured according to the method of (Reitman and Frankel 1957), using commercial kits obtained from Marcy L'Etoile, France.

2.5.2. Alkaline phosphatase (ALP) in mice serum was measured according to the method of (Kachmar and Moss 1976) using commercial kit obtained from Stanbio Company, USA.

2.5.3. Quantitative determination of gamma glutamyl transferase (GGT) in mice serum was measured according to the method of (Szasz 1969), using commercial kit obtained from Stanbio Company, USA.

2.5.4. Quantitative determination of total bilirubin (TB) in mice serum was measured according to the method of (Doumas, Perry et al. 1973), using commercial kit obtained from QCA (Quimica Clinica Aplicada), USA.

2.5.5. Quantitative determination of superoxide dismutase (SOD) in mice RBC_S was measured according to the method of (Woolliams, Woolliams et al. 1983), using commercial kit obtained from Randox Company, UK.

2.5.6. Quantitative determination of reduced glutathione (GSH) in mice RBC_S was measured according to the method of (Woolliams, Woolliams et al. 1983), using automated chemicals.

2.5.7. Quantitative determination of Catalase (CAT) in mice RBC_S was measured according to the method of (Cohen, Dembiec et al. 1970), using automated chemicals.

2.5.8. Quantitative determination of ascorbic acid (Vit. C) in mice serum was measured according to the methods of (Denson and Bowers 1961), **&** (Loh and Wilson 1971), using automated chemicals. (All results are compiled in (**Tables 5, 6**)

2.6. Method for evaluation of antimicrobial activity:

Antimicrobial activities were investigated using agar well diffusion method (Bauer AW, Kirby WMM et al. 1966) **(Table 7).**

Determination of minimum inhibitory concentration (MIC):

The agar plate method was used to determine the MIC of tested samples; two-fold serial dilutions of each sample were added to nutrient broth for bacteria and to sabourad dextrose broth for fungi, DMSO was used as control. The MIC was considered to be the lowest concentration that completely inhibits against inoculums comparing with the control, disregarding a single colony or a faint haze caused by the inoculums.

3. Results

3.1. Identification of the isolated compounds

1, 3, 4,5-tetra-O-galloyl quinic acid butyl ester (**1**) Dark buff amorphous powder (100 mg). Chromatographic properties*:* R_f values; 0.49 (S_1) and 0.85(S_2), dark shine violet color under UV light and deep blue color with $FeCl_3$ spray reagent. Complete acid hydrolysis gave gallic acid and quinic acid in organic phase using CoPC (R_f values; 0.79 for gallic acid and 0.81 for quinic acid in solvent system S_2). UV spectrum showed a characteristic absorption band of gallic acid esters at λ_{max} = 230 and 276 nm. Negative ESI-MS showed molecular ion peak at *m/z* 855 $[M-H]^-$ corresponding to a molecular weight of 856 which is compatible with $C_{39}H_{36}O_{22}$. ^{1}H NMR spectral data (500 MHz, DMSO-d_6) showed quinic acid moiety: δ 5.73 (2H, *m*, H-3/5), 5.58 (1H, br*d*, H-4), 2.12, 2.65 (4H, two *m*, H-2/6), butyl group: 0.88 (3H, *t*), 1.3 (2H, *m*), 1.38 (2H, *m*), 3.39 (2H, *t*), galloyl moieties: H-2/6 at δ 6.81, 6.84, 6.94, 6.95 ppm each represent 2H for each galloyl moiety.^{13}C NMR spectral data (100 MHz, DMSO-d_6) showed δ 14.41 (C-11), 19.19 (C-10), 35.64 (C-2), 39.52 (C-9), 39.72 (C-6), 60.95 (C-8), 68.17 (C-3), 69.40 (C-5), 76.93 (C-1), 77.33 (C-4), 171.20 (C-7) [COOR of quinic acid], 109.29, 109.43, 109.62 (represent 2 carbons) [galloyl 2,6], 119.39, 119.46, 119.48, 119.56 [galloyl 1], 139.02 (represent 2 carbons), 139.42 (represent 2 carbons) [galloyl 4], 146.08 (represent 2 carbons), 146.16 (represent 2 carbons) [galloyl 3,5], 165.68 (represent 2 carbons), 165.99 (represent 2 carbons) [galloyl COO] ppm.

1, 3, 4, 5-tetra-O-galloylquinic acid (**2**) Off-white amorphous powder (35 mg).Chromatographic properties: R_f values; 0.41(S_1) and 0.89 (S_2), shine violet color

under UV light and deep blue color with $FeCl_3$ spray reagent. UV spectrum showed a characteristic absorption band of gallic acid esters at λ_{max} = 273 nm. Acid hydrolysis gave gallic acid and quinic acid in organic phase using CoPC. Negative ESI-MS of compound **2** showed molecular ion peak at *m/z* 799 $[M-H]^-$ corresponding to a molecular weight of 800 ($C_{35}H_{28}O_{22}$ **) .** ^{1}H NMR spectral data (500 MHz, DMSO-d_6) Quinic acid moiety δ 5.7 (2H, *m*, H-3/5), 5.4 (1H, *t*, H-4), 2.21, 2.6 (4H, two *m*, H-2/6), the four galloyl moieties H-2/6 at δ 6.77, 6.79, 6.89, 6.92 ppm each represent 2H for each galloyl moiety.^{13}C NMR spectral data (100 MHz, DMSO-d_6) showed δ 33.29 (C-2), 39.78 (C-6), 68.29 (C-3), 69.43 (C-5), 77.34 (C-4), 80.24 (C-1), 174.06 (C-7) [COO of quinic acid]. 109.30, 109.43 (represent 2 carbons), 109.58 [galloyl 2, 6], 119.24, 119.45, 119.54, 119.75 [galloyl 1], 138.78, 138.99, 139.25, 139.46 [galloyl 4], 145.89, 146.11 (represent 2 carbons), 146.20 [galloyl 3, 5], 165.26, 165.54, 165.64, 166.00 [galloyl COO] ppm. Acid hydrolysis gave gallic acid and quinic acid in organic phase using CoPC.

Gallic acid (**3**) is off-white amorphous powder (30 mg) with m.p 252^{o}C.*Chromatographic properties* R_f values; 0.56 (S_1) and 0.79 (S_2), shine violet fluorescence under short UV light which turned to deep blue color with $FeCl_3$ spray reagent. UV spectral data showed a characteristic band at λ_{max} = 272 nm. Negative ESI-MS showed molecular ion peak at *m/z* 169 $[M-H]^-$ corresponding to a molecular weight of 170 which is compatible with $C_7H_6O_5$. ^{1}H NMR spectral data (500 MHz, DMSO-d_6) δ ppm; 6.88 (2H, s, H-2 and H-6). ^{13}C NMR spectral data (100 MHz, DMSO-d_6) δ ppm;109.02, 109.11 (C-2, C-6), 119.88 (C-4), 138.95 (C-1), 146.13 (C-3, C-5), 166.89 (COO).

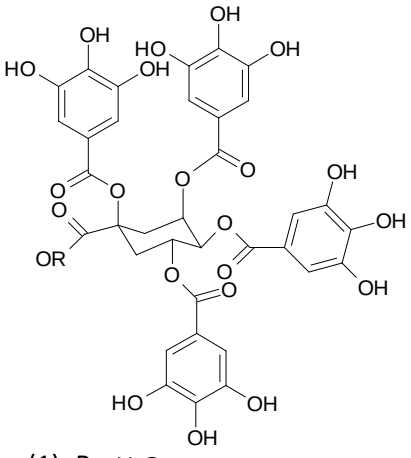
Methyl gallate (**4**) is off-white amorphous powder (65 mg) with m.p 204^{o}C. Chromatographic properties: R_f values; 0.63(S_1) and 0.76 (S_2), shine violet fluorescence under short UV light which turned to deep blue color with $FeCl_3$ spray reagent. Negative ESI-MS showed molecular ion peak at *m/z* 183 $[M-H]^-$ corresponding to a molecular weight of 184 which is compatible with $C_8H_8O_5$. ^{1}H NMR spectral (500 MHz, DMSO-d_6) δ ppm; 6.91 (2H, brs, H-2 and H-6), 3.7 (3H, *s*, OCH_3). ^{13}C NMR spectral (100 MHz, DMSO-d_6) δ ppm; 52.17 (OCH_3), 109.04 (C-2, C-6), 119.89 (C-4), 138.96 (C-1), 146.08 (C-3, C-5), 166.94 (COO).

Experimental data ***compounds*** **5-11** are compiled in Tables 1-3. Negative ESI-MS of compounds **5, 7, 8, 10, 11** were m/z 599 $[M-H]^-$, m/z 461 $[M-H]^-$, m/z 447.1 $[M-H]^-$, *m/z* 301 $[M-H]^-$**,** m*/z* 315 $[M-H]^-$respectively while positive ESI-MS of compound **9** was m/z 457 $[M+Na]^+$, m/z 435 $[M+H]^+$, m/z 303 $[M+H\text{-arabinosyl}]^+$.

3β-hydroxy olean-12-en-28-oic acid (Oleanolic acid) **(13)** White needle crystals (25 mg) with m.p 304-306°C, gives positive Liebermann-Burchard's test but didn't respond to Molisch's test Chromatographic properties : R_f values; 0.5(S_3: Benzene- Ethyl acetate- Acetic acid 12 : 4 : 0.5 v/v) and 0.46(S_4: Chloroform- Tolune- Methanol 7 : 3 : 1 v/v) pink spot on TLC after spraying with 10% conc. H_2SO_4 in EtOH and heating for 3 min. at 120^o C.^{1}H NMR spectral data (400 MHz, DMSO-d_6) δ ppm 4.13 (1H, *t*, H-12), 3.35 (1H, *t*, H-3), 1.35, 1.28, 1.23, 0.89, 0.87, 0.86, 0.85 (each 3H, *s*). ^{13}C NMR spectral data (100 MHz, DMSO-d_6) **(Table 4).**

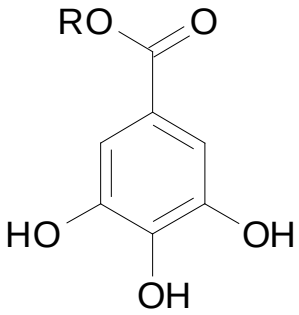
Oleanolic acid-3-O-β-D-$^4C_1$-glucopyranoside **(14)** White amorphous powder (50 mg) with m.p 227-230°C, gives positive Liebermann-Burchard's test and Molisch's test which indicate the triterpenoid glycosidic nature of the molecule. Chromatographic properties: R_f value; 0.53 in solvent system S_5 (Chloroform- Methanol- Water 9: 4: 0.5 v/v). It was observed as pink spot on TLC after spraying with 10% conc. H_2SO_4 in EtOH and heating for 3 min. at 120°C. Complete acid hydrolysis gave oleanolic acid (organic phase) and glucose (aqueous phase). Alkaline hydrolysis: the aqueous layer was free from any sugar.^{1}H NMR spectral data (400 MHz, DMSO-d_6) δ ppm 4.87 (1H, *d*, J=7.8 Hz, H-1′), 4.53 (1H, *t*, H-12), 4.12 (1H, *t*, H-3), 1.33, 1.23, 1.11, 1.05, 1.01, 0.98, 0.85 (each 3H, *s*).^{13}C NMR spectral data (100 MHz, DMSO-d_6) **(Table 4).**

3- O - [O - α - L- rhamnopyranosyl-(1-2)-O-β-D-glucopyranosyl-(1-2)-β-D-$^4C_1$ gluco-pyranosyl]- oleanolic acid. **(14)** ; White amorphous powder (40 mg) which gives positive Liebermann-Burchard's test and Molisch's test Chromatographic properties: R_f value; 0.48 (S_5), pink spot on TLC after spraying with 10% conc. H_2SO_4 in EtOH and heating for 3 min. at 120° C. Complete acid hydrolysis; oleanolic acid (organic phase), glucose and rhamnose (aqueous phase). Alkaline hydrolysis: the aqueous layer was free from any sugar moieties.^{1}H NMR spectral data (400 MHz, DMSO-d_6) δ ppm 5.24 (1H, d, J=7.5 Hz, H-1′), 5.01(1H, d, J=7.2 Hz, H-1″), 4.71 (1H, brs, H-1‴), 4.50 (1H, t, H-12), 4.35 (1H, t, H-3), 1.87, 1.49, 1.40, 1.24, 1.07, 0.97, 0.75 (each 3H, s), .087 (3H, d, J =5.8 Hz, CH_3-6‴).^{13}C NMR – DEPT spectrum (100 MHz, Pyridin-d_5) **(Table 4)**



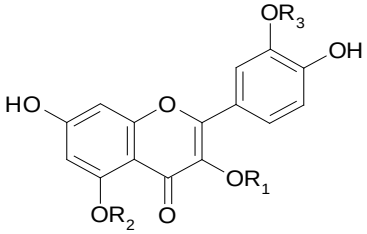
(1) R= H_3C

(2) R=H

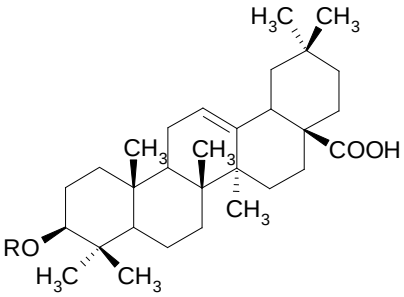


(3) R=H

(4) R=CH_3



	R_1	R_2	R_3
(5)	Rh-3$^{//}$-Galloyl	H	H
(6)	Arabinose	H	CH_3
(7)	Rhamnose	H	CH_3
(8)	Rhamnose	H	H
(9)	Arabinose	H	H
(10)	H	H	H
(11)	H	CH_3	H



(12) R=H

(13) R= β-D- glucopyranose

(14) R= rhamnopyranosyl-(1-2)-*O*-β-D-glucopyranosyl-(1-2)-β-D-glucopyranose

Table (1) Chromatographic properties and UV spectral data of 5-11

Cpd No.	R_f-values		Fluorescence	Spray reagent		UV λ_{max}, nm					
	S_1	S_2	UV	Amm	$FeCl_3$	NP	MeOH	+NaOMe	+NaOAc	+NaOAc/H_3BO_3	+$AlCl_3$	+$AlCl_3$/HCl
5	0.65	0.7	d. pr	d. y	dp. gr	dp. or	-	-	-	-	-	-
6	0.62	0.48	d. pr	d. y	dp. gr	dp. y	255, 268, 303 (sh), 357	272, 327 (sh), 415	275, 325sh, 380	274, 316 (sh), 387	267 , 307 (sh), 381	267, 298 (sh), 403
7	0.63	0.5	d. pr	d. y	dp. gr	dp. y	-	-	-	-	-	-
8	0.72	0.52	d. pr	d. y	d. gr	or	258, 270, 290 (sh), 353	270, 325, 398	270,324 (sh), 399	267, 391	269, 313(sh), 362, 425	269, 312, 355(sh), 398.
9	0.7	0.56	d. pr	d. y	d. gr	or	-	-	-	-	-	-
10	0.67	0.1	d. pr	d. y	d. gr	or	255, 297.5 (sh), 301(sh), 370	274, 297, 324 (sh), 398	57, 274, 321 (sh), 388	262, 326 (sh), 391	269, 295(sh), 314(sh), 445	255 (sh), 275, 299 (sh), 428
11	0.79	0.02	bl	y. gr.	gr.	y. gr.	-	-	-	-	-	-

d. pr = dark purple, d. y = dark yellow, dp. gr = deep green, dp. or = deep orange.

Table (2): ^{1}H-NMR Spectral data of compounds (400 MHz, DMSO-d_6) **5-11).**

Protons	5	6	7	8	9	10	11
6	6.12, *d*, *J*=2 Hz	6.21	6.15	6.21 (1H, *d*, *J*=2.1Hz	6.20, *d*, *J*=3.5 Hz,	6.18, *d*, *J*=2.1 Hz	6.39
8	6.36, *d*, *J*=2 Hz	6.41	6.36	6.39, *d*, *J*=2.1Hz	6.40, *d*, *J*=3.5 Hz	6.38, *d*, *J*=2.1 Hz	6.37
5'	6.85*d*, *J*= 8.1 Hz	6.84, *d*, *J*= 8.8 Hz	6.79,*d*, *J*= 8.8 Hz,	6.87,*d*, *J*= 8.4 Hz,	6.85, *d*, *J*= 8.5 Hz	6.87,*d*, *J*=8.4 Hz	6.49, *d*, *J*=8.5 Hz
2'	7.27, *d*, *J*= 2.2 Hz	7.51 , brs	7.49,brs	7.30, *d*, *J*=1.8 Hz,	7.67, *d*, *J*=2.1 Hz	7.67, *d*, *J*=2.1Hz	7.32, brs
6'	7.25, *dd*, J=8.1, 2.2 Hz	7.66, *d*, *J*=8.1 Hz	7.67, *d*, J=8.1 Hz,	7.26, *dd*, *J*=8.4, 1.8 Hz	7.51, *dd*, *J*=8.5, 2.1 Hz	7.48, *dd*, *J*=4.8, 2.1 Hz	6.87, br*d*, *J*=8.8 Hz,
1"	5.46, *d*, *J*=1.6 Hz	5.28, *d*, *J*=6.4 Hz	5.34 brs	5.26	5.28, *d*, *J*=6 Hz	-	-
Rh- Me **6"** (CH_3) -	0.89 ,*d*, *J* = 6 Hz,	-	0.9, *d*, *J*= 5.5 Hz,	0.82 (3H, *d*, *J* =5.7 Hz,	-	-	-
2'''/6'''	7.00, *s* (gallic)	-	-	-	-	-	-
O-CH_3	-	3.52	3.52, *s*	-	-	-	3.76
2"	4.25, *dd*, J= 3.4, 1.7 Hz,	-	-	3.98, brs	-	-	-
3"	5.11 ,*dd*, J= 9.8, 3.4 Hz,	-	-	-	-	-	-
4",5"	3.0-3.7, *m*, H-4" and H-5"),	-	-	-	-	-	-
2",3",4",5"	-	3.6-3.4 m	3.1-3.69 m	3.00-3.8 m	3.1-3.7	-	-

Table (3): ^{13}C- NMR Spectral data of compounds (5-11).

Carbons	5	6	7	8	9	10	11
2	156.30	156.82	156.22	158.0	156.83	156.70	156.50
3	134.82	134.28	134.18	134.88	134.27	135.60	138.8
4	176.20	178.06	178.00	178.37	178.04	175.72	174.63
5	161.55	161.76	161.44	161.90	161.76	160.58	164.15
6	99.35	99.23	99.44	99.24	99.27	98.18	96.98
7	164.30	164.74	164.14	164.76	164.92	164.03	163.04
8	94.25	94.06	94.12	94.20	94.08	93.33	95.6
9	156.44	156.82	156.32	156.99	156.77	156.05	155.84
10	104.22	104.45	104.10	104.62	104.4	102.83	107.69
1'	120.92	121.1	121.1	120.068	121.43	121.83	121.60
2'	116.14	115.5	115.6	116.04	115.9	114.97	115.8
3'	145.50	149.15	149.10	145.79	149.16	145.03	146.8
4'	147.42	145.53	145.33	147.49	146.72	147.67	146.64
5'	115.99	116.35	116.52	116.24	116.3	115.57	116.2
6'	121.88	121.44	121.42	121.69	122.57	119.88	121.3
OCH_3	-	56.2	55.4	-	-	-	56.38
Sugar moiety	**Rhamnose**	**Arabinose**	**Rhamnose**	**Rhamnose**			
1"	101.82	101.99	100.92	102.54	101.9	-	-
2"	68.82	72.19	69.8	70.89	66.6	-	-
3"	74.48	71.28	70.07	70.98	71.27	-	-
4"	70.00	65.5	69.7	71.14	72.20	-	-
5"	70.61	66.62	68.82	70.61	64.8	-	-
6"	18. 4	-	18.54	18. 1	-	-	-
galloyl moiety							
1"'	119.19						
2"'/6"'	109.12	-	-	-	-	-	-
3"'/5"'	145.49	-	-	-	-	-	-
4"'	139.24	-	-	-	-	-	-
7"'	65.40	-	-	-	-	-	-

Table (4): ^{13}C NMR of compound (13) and (14) in comparison with oleanolic acid published data [48]:

Compound / Position	Oleanolic acid 12	13	14	Compound Position	Oleanolic acid 12	13	14
	δ C	δ C	δ C		δ C	δ C	δ C
1	39	38.1	38.1	28	180.2	181	181
2	28.8	26.2	27.3	29	33.3	32.8	32.8
3	**78.1**	**87.59**	**89.3**	30	23.8	23.1	23.1
4	39.4	39.6	38.8	**Glucose (A)**			
5	55.8	55.1	55.1	**1′**		**102.09**	**104**
6	18.8	18.15	17.8	**2′**		77.36	**78.5**
7	33.3	33.43	32.8	3′		76.7	76.2
8	39.8	39.33	39.2	4′		73.8	73.3
9	48.2	46.7	46.7	5′		76.92	78.5
10	37.4	37	37	6′		61.6	63.0
11	23.7	23.96	23.1	**Glucose (B)**			
12	122.6	122.7	122.7	**1″**			**103.7**
13	144.8	143.4	143.4	**2″**			**78.4**
14	42.2	40.69	41.5	3″			76.2
15	28.4	27.3	27.3	4″			72.3
16	23.8	24.5	24.5	5″			78.5
17	46.7	46.7	46.5	6″			61.3
18	42.0	46.2	40.9	**Rhamnose**			
19	46.5	30.16	46.2	**1‴**			**102.2**
20	31.0	34.4	30.5	2‴			72.3
21	34.3	32.8	34.8	3‴			72.0
22	33.2	29.2	29.2	4‴			74.2
23	28.8	15.5	15.5	5‴			69.4
24	16.6	15.1	15.1	6‴			17.7
25	15.6	16.1	16.1				
26	17.5	16.9	16.9				
27	26.2	25.7	25.7				

3.2.Biological activity

In this study the level of **ALT, AST, GGT** and **TB** was significantly increased in acute liver injury control mice compared with normal mice ($p < 0.001$, < 0.001, < 0.05 and < 0.001 respectively) while, there was a slight increase in level of **ALP** enzyme. However, pre-treatment with aqueous methanol extract of *K. elegans* (Seem.) leaves in a dose of 300 mg / Kg b wt. reduced significantly the alterations in the activity levels of **ALT, AST** and **TB** ($p < 0.001$ in all) compared with liver injury control mice but, there was a slight reduction in levels of both **GGT** and **ALP** enzymes compared with liver injury control group. [**Table** (**5, 6**)].

Administration of CCl_4 to the mice resulted in significant depletion of the levels of hepatic **GSH** and serum **Vit. C**, as were the activities of hepatic antioxidant enzymes; **CAT** and **SOD** ($P < 0.001$ in all) as compared to the normal control group. Co-

adminstration of aqueous methanol extract of *K. elegans (*Seem.) leaves in a dose of 300 mg / Kg b.wt. was significantly ($P < 0.001$ in all) effective in protecting hepatocytes and serum against such depletion. [**Table** (**5, 6**)].

Antimicrobial activity of aqueous methanol extract of *K. elegans* leaves and other isolated pure compounds (**1, 3** and **9**) are shown in **Table (7), Figure (2)** .The results showed that the aqueous methanol extract and the isolated pure compounds **1** and **3** were effective against both Gram positive and Gram negative organisms and *Geotricum candidum* (fungi). The highest activity (diameter of zone of inhibition = 27.2 mm) was demonstrated by compound **1** against *Bacillus subtilis* while, the lowest activity (diameter of zone of inhibition = 9.3 mm) was demonstrated by the aqueous methanol extract against *E. coli.* Tested compound **9** has no antimicrobial activity. All tested samples showed no activity against *Candida albicans* and *Pseudomonas aeruginosa.* As some of the antimicrobial results are promising, so the minimum inhibitory concentration (MIC) is important to be determined. [MIC results are shown in **Table (7)].**

Table (5): Effects of aqueous methanol extract of *K. elegans* leaves on certain serum biochemical parameters and Vit. C in CCl_4 induced hepatotoxicity in mice:

Parameters / Groups	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	GGT (U/L)	Total bilirubin (mg/dl)	Vitamin C (µg/ml)
Normal control	**168±1.18**	**71.8 ± 2.03**	**43.9 ± 1.83**	**9.9 ± 1.17**	**0.33 ± 0.01**	**2.49 ± 0.09**
CCl_4 control	**274 ± 5.9**	**113.3 ± 2.69**	**47.7 ± 2.38**	**14.2 ± 1.54**	**0.88 ± 0.07**	**1.79 ± 0.07**
***K. elegans* + CCl_4**	**188.2±4.5*****	**76.4 ± 3.4*****	**46.1 ± 2.75^{ns}**	**13.5 ± 1.84***	**0.58 ± 0.05*****	**2.45 ± 0.1*****

Results are expressed in the form of mean ± S.E.

When experimental groups were compared with CCl_4 control:

* $p < 0.05$(significant), ** $p < 0.01$(highly significant), *** $p < 0.001$(very highly significant), ns = non-significant.

Table (6): Effects of aqueous methanol extract of *K. elegans* leaves on hepatic antioxidant enzymes (SOD and CAT) and GSH in CCl_4 induced hepatotoxicity in mice:

Parameter / Group	SOD (U/ml)	CAT (U/ml)	GSH (mg/dl)
Normal control	**295.8 ± 12.65**	**17850 ± 824**	**45.9 ± 1.72**
CCl_4 control	**163.5 ± 11.07**	**13535 ± 535**	**26.8 ± 1.84**
***K. elegans* + CCl_4**	**299.5 ± 16*****	**16929 ± 715*****	**41.24 ± 1.97*****

Results are expressed in the form of mean ± SE.
When experimental groups were compared with CCl_4 control:
* $p < 0.05$(significant), ** $p < 0.01$(highly significant), *** $p < 0.001$(very highly significant

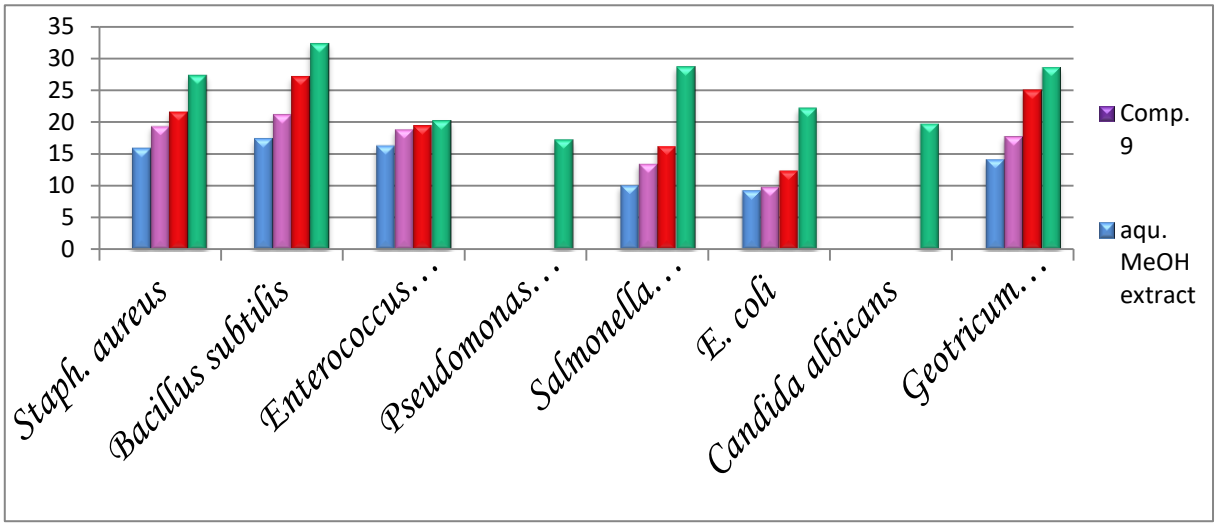
Table (7): Antimicrobial activities of tested samples against tested microorganisms:

Microorganisms	Inhibition zone (mm)				
	Aqueous methanol extract	**Compound 1**	**Compound 3**	**Compound 9**	**Control**
Fungi					**Amphotericin B**
Candida albicans (RCMB 05031)	NA	NA	NA	NA	19.8 ± 0.2
Geotricum candidum (RCMB 05097)	14.1 ± 0.2	25.1 ± 0.16	17.8 ± 0.22	NA	28.7 ± 0.22
Gram-positive bacteria					**Ampicillin**
Staphylococcus aureus (RCMB 010028)	15.9 ± 0.24	21.7 ± 0.18	19.4 ± 0.34	NA	27.4 ± 0.18
Bacillus subtilis (RCMB 010067)	17.4 ± 0.19	27.2 ± 0.22	21.3 ± 0.24	NA	32.4 ± 0.1
Enterococcus faecalis (RCMB 010068)	16.3 ± 0.17	19.5 ± 0.21	18.9 ± 0.31	NA	20.3 ± 0.13
Gram-negative bacteria					**Gentamicin**
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	NA	17.3 ± 0.15
Salmonella typhimurium (RCMB 010072)	10.1 ± 0.31	16.3 ± 0.1	13.5 ± 0.19	NA	28.8 ± 0.24
Escherichia coli (RCMB 010052)	9.3 ± 0.11	12.4 ± 0.25	9.9 ± 0.23	NA	22.3 ± 0.18

Results are expressed in the form of mean ± S.D.

NA = No Activity RCMB = Regional Centre for Mycology and Biotechnology

Figure (2): Antimicrobial activities of tested samples against tested microorganisms



4. DISCUSSION

On the basis of the data discussed above, compound **1** showed chromatographic properties of gallotannin. UV spectrum showed a characteristic absorption band of gallic acid esters at λ_{max} = 230 and 276 nm and negative ESI-MS analysis showed a molecular ion peak at *m/z* 855 $[M-H]^-$ corresponding to a molecular weight of 856 which is compatible with $C_{39}H_{36}O_{22}$ **,** so compound **1** was tentatively identified as gallic acid ester of quinic acid. The presence of four galloyl moieties in this compound was easily deduced from its ^{1}H-NMR and ^{13}C-NMR data since ^{1}H-NMR spectrum (500 MHz, DMSO-d_6) showed four singlet resonances each integrated for two protons at δ 6.81, 6.84, 6.94, 6.95 ppm which were characteristic for the orthoprotons (H2/6) of the four galloyl moieties, and signals at δ 5.73 (2H, *m*, H-3/5), 5.58 (1H, br*d*, H-4), 2.12, 2.65 (4H, two *m*, H-2/6) which were attributed to the methine and methylene protons of the quinic acid moiety. The location of the four galloyl groups at C-1, C-3, C-4 and C-5 of quinic acid were determined from the down field multiplet resonance at 5.73 ppm of H-3 and H-5 and the down field shift of H-4 at 5.58 ppm relative to ^{1}H-NMR resonances of quinic acid which suggesting that esterification positions in compound **1** at C-3, C-4 and C-5. Also, the spectrum showed the presence of butyl group resonances which esterified on carboxylic group of quinic acid proved by two multiplets of two methene groups at δ 1.3, 1.38, downfield shifted triplet signal of third methene group at 3.4 ppm due to oxygenation and the presence of another triplet signal at 0.88 ppm of CH_3. The presence of four gallic acid moieties and their substitution positions on quinic acid were evidenced also by ^{13}C-NMR which showed six aliphatic signals due to two methylenes (δ 35.64, 39.72) and three methine carbons bearing oxygen function at (δ 68.17, 69.40, 73.33 ppm) and a quaternary carbon atom at (δ 76.93) together with carboxyl carbonyl carbon at δ 171.20 ppm, also, this spectrum indicated the presence of oxygenated butyl group by the presence of one oxygenated methene carbon at δ 60.95,

two methenes at δ 39.52 and 19.19 and one methyl radical at δ 14.41 ppm. The upfield shift of C-1 of quinic acid at δ 76.93 ppm and the ester carbonyl carbon at δ171.20 ppm, which is upfield shifted (3.8 ppm) as compared to carboxyl carbonyl carbon of compound **2**, is due to esterification.

From the above data, this phenolic compound was identified as 1, 3, 4, 5-tetra-*O*-galloylquinic acid butyl ester as a new phenolic compound in nature which isolated from *K. elegans* (Seem.) leaves. [Butyl ester derivative of 3-*O*-galloyl quinic acid reported only in Chinese olive fruit (He, W. et al. 2009).

^{1}H NMR and ^{13}C NMR spectra of compound **2** were similar to previous data of compound **1** except absence of resonances of butyl group that esterified on carboxylic group of quinic acid and Negative ESI-MS of compound ***2*** showed molecular ion peak at *m/z* 799 $[M-H]^-$ corresponding to a molecular weight of 800 which is compatible with $C_{35}H_{28}O_{22}$ (John P M, Kim L W et al. 2005). Accordingly, from the above data and comparison with published data (John P M, Kim L W et al. 2005) compound **2** was identified as 1, 3, 4, 5-tetra-*O*-galloylquinic acid.

Compounds **3** and **4** were identified as methyl gallate and gallic acid respectively from the above data and comparison with authentic samples (using CoPC and m.m.p) and published data (Ekaprasada MT, Nurdin H et al. 2009).

Compounds **5-9** showed chromatographic properties, Molisch's test and UV spectral data of quercetin glycosides, (Tables 1) (Mabry TJ, Markham KR et al. 1970) and were identified as quercetin-3-*O*-(3$^{//}$-gallate-α-L-$^{1}C_4$-rhamnopyranoside), [(3$^{//}$-galloyl quercetrin)] **(5)** (Wen-Han L, Zhi-Wei D et al. 2002)**,** isorhamnetin-3-*O*-β-D-$^{4}C_1$-arabinopyranoside (6) (Wen-Han L, Zhi-Wei D et al. 2002), isorhamnetin-3-O-α-L-rhamnopyranoside **(7),** quercetin -3-*O*-α-L-rhamnopyranoside (Quercetrin) **(8)** (Harborne JB 1982), quercetin -3-*O*-β-D-arabinopyranoside (Guaijaverin) **(9)** (Lee TH, Chiang YH et al. 2009), while compounds **10, 11** were identified as quercetin and azaleatin respectively (Mabry TJ, Markham KR et al. 1970) and CoPC with authentic samples and proved by their comparison with the reported data of structural related compounds (Harborne JB 1982). Also, on the basis of above spectral and chemical evidences and comparison with published data compound **12-14** were identified as 3β-hydroxy olean-12-en-28-oic acid (Oleanolic acid**) (12)** (Bhatt. 2011), oleanolic acid-3-*O*-β-D-glucopyranoside. **(13)** (Gohari AR, Saeidnia S et al. 2009), 3-*O*-[*O*-α-L-rhamnopyranosyl-(1-2)-*O*-β-D-glucopyranosyl-(1-2)-β-D-glucopyranosyl] Oleanolic acid**. (14)** (Gohari AR, Saeidnia S et al. 2009).

Chronic liver diseases commonly result in liver fibrosis and oxidative stress is the common mechanism contributing in the initiation and progression of hepatic damage in a variety of liver disorders. Carbon tetrachloride (CCl_4) is widely used for experimental induction of liver fibrosis. CCl_4 is a potent hepatotoxin producing centrilobular necrosis which causes liver injury (Khalaf AA, Mekawy MEM et al. 2009). Since the changes associated with CCl_4-induced liver damage are similar to those of acute viral hepatitis (Suja, Latha et al. 2004), CCl_4-mediated hepatotoxicity was chosen as the experimental model. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which

have been disturbed by a hepatotoxin, is the index of its protective effects (Yadav and Dixit 2003).

Liver damage induced by CCl_4 involves biotransformation of free radical derivatives, increased lipid peroxidation and excessive cell death in liver tissue (Gilani and Janbaz 1995). Serum AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar 2002), so increased levels of ALT, AST, ALP and TB are conventional indicators of liver injury. The prevention of this phenomenon can be considered as hepatoprotective activity (Gilani and Janbaz 1995).

Aqueous methanol extract of *K. elegans* (Seem.) leaves protects against diseases that are caused by reactive oxygen species (ROS) because it has radical scavenging ability based on its antioxidant activity against CCl_4 in mice which developed significant hepatic damage as manifested by a significant increase in activities of AST, ALT, ALP, GGT and TB concentration that are indicators of hepatocyte damage and loss of functional integrity (Li, Guo et al. 2011).

Bilirubin is the main bile pigment that is formed from breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Malfunctioning of the liver was evidenced by the significant increase in the level of total bilirubin in the serum of CCl_4 treated group when compared with normal control group. The ability of simultaneous administration of aqueous methanol extract of *K. elegans* (Seem.) leaves with CCl_4 to significantly reduce the level of serum total bilirubin when compared with that of CCl_4 control group of animals confirming the potential of this extract in clearing bilirubin from the serum when its level elevated which may be attributed to high content of polyphenolic compounds (Iniaghe, Malomo et al. 2008).

Enhanced lipid peroxidation may be associated with depletion of the antioxidant; GSH that found in the heart tissues of CCl_4 treated mice which are a characteristic observation in CCl_4-intoxicated mice. GSH is important in detoxification of the reactive metabolites of cells; tissue necrosis is initiated when the reserves of GSH are markedly depleted. Thus, the reduced (relative to normal) levels of GSH observed in the heart tissues of CCl_4 intoxicated mice might be a reflection of increased oxidative damage (Mohan, Pallavi et al. 2007, Li, Guo et al. 2011).

Antioxidant enzymes SOD (enzymes that catalyze the conversion of two superoxides into oxygen and hydrogen peroxide which substantially less toxic than superoxide) and CAT (enzymes which degrade hydrogen peroxide to water and oxygen, and hence finish the detoxification reaction started by SOD.) represent one of the protection mechanisms against oxidative tissue-damage (Kehrer and Klotz 2015).

The administration of the aqueous methanol extract of *K. elegans* (Seem.) leaves caused an elevation of the levels of CAT, SOD, GSH in the liver and Vit. C (Vit. C is an antioxidant which can protect cell membranes and lipoproteins particles from oxidative damage by itself and by regenerating the antioxidant form of vitamin E) (Himakar Reddy, Nagi et al. 2010), in serum suggesting that it can restore these antioxidant enzymes and/or re-activate enzyme after the damage caused by CCl_4.

The aqueous methanol extract of *K. elegans* (Seem.) leaves and the three isolated pure compounds **1, 3** and **9** were tested against the pathogenic microorganisms viz., *Staphylococcus aureus,* a wound infecting pathogen which can cause septicemia, endocarditis and toxic shock syndrome; *E. coli*, a most common bacteria of which virulent strains can cause gastroenteritis, urinary tract infections and neonatal meningitis; *Pseudomonas aeruginosa* which infects the pulmonary tract, urinary tract, burns and wounds; *Candida albicans* a causal agent of opportunistic oral and genital infections in humans (Thirumurugan, Shihabudeen et al. 2010). *Geotricum candidum* which normally present in skin and gut flora but can cause diseases as oral, vaginal, skin or systemic infection in immunosuppressed people mainly patients with systemic diseases like diabetes mellitus (Verghese and Ravichandran 2003); *Enterococcus faecalis* which can cause endocarditis and bacteremia, urinary tract infections, meningitis and other infections in humans (Murray 1990); *Salmonella typhimurium* a causal agent of salmonellosis (Swanson, Snider et al. 2007); and *Bacillus subtilis* which used as a model organism in laboratory studies directed at discovering the fundamental properties and characteristics of Gram positive spore forming bacteria (Earl, Losick et al. 2008) by presence or absence of inhibition zones, zone diameters and MIC values. The aqueous methanol extract and each of compound **1** and **3** showed antimicrobial activities against all tested microorganisms except *Candida albicans* and *Pseudomonas aeruginosa* by inhibiting their growth while, compound 9 showed no activity against any microorganism [**Table (7)**.

The least activity was against *E. coli*, a Gram negative bacterium where the inhibition zone diameters ranged between 9.3 and 12.4 mm. This could be attributed to the difference between Gram positive and Gram negative bacteria in the structure of their cell walls. Therefore, it is postulated that the tested samples are unable enough to pass through the lipopolysaccharide layer of Gram negative bacteria which provide a permeability barrier to polar phenolic compounds (Takahashi, Kokubo et al. 2004, Masibo and He 2009).

Minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that results in inhibition of visible growth (i.e. colonies in a plate or turbidity in broth culture) under standard conditions. It is used as a research tool to determine the *in vitro* activity of new antimicrobial agents (Andrews 2001). So, MIC was calculated to indicate the antibacterial potency of the active tested samples [aqueous methanol extract of *K. elegans* leaves and isolated pure compounds **(1** and **3**)]. It was considered that if the tested sample displayed MIC less than 100 µg / ml the antimicrobial activity was good; from 100 to 500 µg / ml the antimicrobial activity was moderate; from 500 to 1000 µg / ml the antimicrobial activity was weak; over 1000 µg / ml the tested sample was considered to be inactive (Saraf, Mishra et al. 2011).

Table (7) showed MIC results of the tested samples. The lowest MIC values were observed for compound **1** while the highest values were observed for the aqueous methanol extract which indicate that compound **1** is the most potent compound of the tested samples and the antimicrobial potency of the aqueous methanol extract may referred to its presence. From above MIC data we can see that, *Bacillus subtilis* was the most sensitive microorganism as it presenting MIC values between 0.06 and 62.5µg/ml.

In conclusion, the tested samples were more active against Gram positive bacteria than Gram negative bacteria and fungi. The tested compound 1 (1, 3, 4, 5-tetra-*O*-galloylquinic acid butyl ester) showed antimicrobial activities very near to the standards used mainly against Gram positive tested microorganisms and *Geotricum candidum* (fungi).

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التحقيق الكيميائي النباتي والبيولوجي لأوراق كوليريتريا اليجانس

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حمض الجالويلكينيك بوتيل إستر جديد ؛ 1 ، 3 ، 4 ، 5- tetra-O-galloylquinic acid butyl ester (1) وعشرة مركبات فينولية أخرى معروفة: 1-،3،4،5- tetra-O-galloylquinic acid (2) ، methyl gallate (3) ، حمض الغاليك (4) ، 3 "- galloyl quercetrin (5)، isorhamnetin-3-O-β-D-4C1-arabinopyranoside (6)، isorhamnetin-3- O-α-L-1C4-rhamnopyranoside (7) ، كيرسيترين (8) ، guaijaverin (9) ، كيرسيتين (10) ، أزالاتين (11) وكذلك ، ثلاثة مركبات ترايتيربينويدال حمض الأولينوليك (12) ، حمض الأولينوليك 3- O-β-D-4C1-glucopyranoside (13)، 3-O- [O-α-L-rhamnopyranosyl- (1-2) -O-β-D-glucopyranosyl- (1-2) - β-D-4C1-glucopyranosyl] حمض الأولينوليك (14) تم فصلهم من 70 ٪ من مستخلص الميثانول من أوراق Koelreuteria elegans (Seem.). تم تعريف المركبات المفصولة على أساس التحليل الكيميائي والطيفي (NMR، ESIMS- و UV) ، وجد أن إجمالي محتويات الفينول والفلافونويد يبلغ 8.19 جم من GAE (مكافئات حمض الجاليك) و 6.22 جم من RE (مكافئات الروتين) لكل 100 جم مستخلص جاف على التوالي. اظهر المستخلص بشكل ملحوظ تحسن في إنزيمات وظائف الكبد الرئيسية (ALT، AST) والبيليروبين الكلي بالإضافة إلى أنها كانت فعالة بشكل كبير في حماية الكبد من استنفاد مستويات GSH الكبدية وفيتامين C في الدم ، وكذلك التغيرات في مستويات إنزيمات الكبد المضادة للأكسدة (SOD، CAT) التي تسببها سمية CCl4. كما اظهر انخفاض بشكل غير ملحوظ مستوى ALP و GGT. علاوة على ذلك ، أظهر المركب 1 نشاطًا أعلى من مستخلص الميثانول المائي ضد كل من الكائنات الحية الموجبة والسالبة الجرام و Geotricum candidum (الفطريات).

الكلمات الرئيسية: كوليريتريا اليجانس ؛ الفلافونول. ترايتيربينويدال سابونين. تأثير وقائي للكبد ونشاط مضاد للميكروبات.

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