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Phytochemical Investigation and Antiviral Activity of *Duranta repens*

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Abstract: 24-ethylcholest-5-en-3-β-ol (β-sitoserol) 1, naringenin 2, 3,4-dihydroxy-bphenethyl-O-α-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranosid (acteoside) 3, lamiide 4, α-glucopyranosyl (1→2) β-fructopyranoside (sucrose) 5, α-galactopyranosyl(1→6) α-glucopyranosyl-(1→2) fructopyranoside (raffinose) 6 were isolated from *Duranta repens* Linn. var. *variegate* (Verbenaceae) and identified by spectral analyses. Antiviral activity against Hepatitis A virus was studied. The total ethanol extract showed 76% inhibition, while the ethylacetate/methanol fraction of celite column showed 88% inhibition of the virus by the plaque reduction assay. Compounds 2, 5, and 6 were reported for the first time from this species. Also, the petroleum ether extract was studied. Both unsaponifiable fraction and fatty acid methyl esters were subjected to GLC for identification of their constituents. The unsaponifiable fraction was found to be a mixture of hydrocarbons ranging from C15 - C27. The fatty acids methyl esters composed of 15 fatty acids in which palmitic acid represent the main component (46%).

Keywords: acteoside, antiviral activity, b-sitoserol, *Duranta repens*, lamiide, naringenin, raffinose, sucrose, Verbenaceae.

INTRODUCTION

Duranta repens Linn. var. *variegate* (syn: *Duranta plumieri* Jacq.) (Verbenaceae) is native to scrub and open woodlands in the West Indies and northern part of Pakistan and central and South America. The genus *Duranta* comprises about 35 species which are evergreen shrubs distributed in tropical and sub-tropical regions[3,8,9]. It was introduced to Egypt as an ornamental plant in the 1920s[4]. The fruits showed *in vivo* antimalerian activity against *Plasmodium berghei*[5]. Thrombin inhibitory coumarins were isolated from fruits[2]. From the genus *Duranta* several iridoid glycosides as durantosides I, II, III, IV, and lamiide were isolated[21,14]. Flavonoids and C-alkylated flavonoids[16,2] and some alkaloids[18] were isolated. *Duranta repens* (*variegata*) is one of the most common varieties of *D. repens* and little phytochemical work has been carried out on the plant grown in Egypt. In a previous communication[17] this plant and its isolated fractions showed strong antioxidant activity which is considered as a prerequisite for other biological activities. On searching for new antiviral agents from medicinal plants the crude methanol extract of *D. repens* showed 76% inhibition for the viral titre of Hepatitis A virus. This evidence encouraged the authors to carry out biologically guided fractionation for the plant which resulted in the isolation of the bioactive components; acteoside and lamiide, and other less active components like β-sitoserol 1, naringenin 2, α-glucopyranosyl(1→2) ß-fructopyranoside(sucrose) 5, α-galactopyranosyl(1→6) α-glucopyranosyl-(1→2) fructopyranoside (raffinose) 6. Compounds 2, 5, and 6 were reported for the first time from this species.

MATERIALS AND METHODS

Experimental:

General: TLC was carried out on precoated silica gel F_{254} plates (Merck) (Darmstadt, Germany) developed with $CHCl_3$- MeOH- H_2O (90:10:1, 70:30:3, 65:35:5, solvents a1, a2, a3), Hexane:ethyl acetate (9:1) (solvent b, for β-sitoserol), Whatman 3MM Pc. eluted by n-Bu: AcOH: H_2O (4:1:5) upper phase (solvent c) for flavonoid and n-butanol-benzene-pyridine-H_2O (5:1:3:3), (solvent d for sugars). Spots were detected using vanillin-H_2SO_4 (vanillin 1% in methanol and 5% H_2SO_4) followed by heating the plates to 110 ˚C for 15-20 min, Neu's spray reagent (1% diphenylboric acidethanolamine complex) or 5% $AlCl_3$ in MeOH were used to visualize flavonoids; and aniline phathalate [aniline (9.2 ml) and phthalic acid (16 g in) n-butanol (490 ml), Et_2O (490 ml) and H_2O (20 ml)] for sugars. Invertase [EC3.2.1.26] and a-galactosidase [EC.3.2.1.22] were obtained from sigma chemicals Co, St. Louis, MO, USA.

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Column chromatography (CC) was performed using Silica gel (Merck) and Sephadex LH-20 (Pharmacia (Merck) (Darmstadt, Germany).

NMR was recorded on a Bruker DRX-400 spectrometer operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. Chemical shifts are presented in ppm downfield of TMS.

EI/MS and FAB/MS were obtained on JEOL JMS-AX500 mass spectrometer.

GLC analyses were carried out according to the following conditions:

For the unsaponifiable matter, Column: HP-1(methyl siloxane) 30 m length (0.53 x 2.65 µm).Temp. program:- Ini. temp. 60˚C, Ini. time 2 min, program rate 10˚C/min,

F. temp. 280˚C, final time 30 min, Injection temp: 260˚C, Detector (FID), T = 300˚C, flow rate of carrier gas N_2:30 ml/min, H_2: 35ml/min, Air: 300 ml/min.

Methyl esters of fatty acids were prepared by BF_3/MeOH complex 12% in methanol (Merck) and analyzed on HP-6890 GC. Column: HP-5(phenyl methyl siloxane) 30 m length (0.32 x 0.25 µm). Temp. program:- Ini.temp.70˚C, Ini. time 2 min, program rate 8˚C/min, Final temp. 270˚C, Final time 10 min, Injection temp. 270˚C, Detector (FID), T = 300˚C, flow rate of carrier gas N_2: 30ml/min, H_2: 35 ml/min, Air: 300 ml/min.

Plant material: Arial parts of *Duranta rep*ens var. *variegate*, were collected from AL-Zohria garden, Giza, Egypt in September 2005, and kindly identified by professor Dr. K. H. Batanouny, professor of Botany, Faculty of Science, Cairo University, Cairo, Egypt, (to whom the authors are deeply indebted). A voucher specimen was kept in the Phytochemistry Department, National Research Center, Cairo, Egypt (DR, 2005). The plant was shade dried and minced.

Extraction and Isolation: About 1 Kg of minced air dried aerial parts of *Duranta repens* were exhaustively extracted with petrolium ether in a Soxhlet apparatus then extracted with 80% methanol in a percolator. The collected methanol extract was concentrated under vacuum at 40˚C, and left overnight in the refrigerator then filtered. About 50 g of the alcohol extract was mixed with 250 g of celite (545) and dried under *vacuo* at 40˚C. The mixture was then applied onto a column (60 x 5 i.d). Elution was affected using different solvents, hexane, hexane/chloroform (1:1), chloroform, chloroform/ethylacetate (1:1), ethylacetate, ethylacetate/methanol (1:1), methanol.

The petrolium ether extract was dried under *vacuo* (20 g) and dissolved in boiling acetone (100 ml),

cooled and the amorphous precipitate formed was separated out (acetone precipitate, 1.8 g). The acetone soluble fraction (5 g) saponified (N/2 alc. KOH), and the saponifiable matter (1.1 g) was separated. The liberated fatty acid mixture, after acidification of saponifiable fraction was extracted, methylated [methanol, BF_3 (boron triflouride) 12%], samples of the isolated unsaponifiable fraction and methyl esters of fatty acids were subjected to GLC analysis.

The $CHCl_3$ fraction of celite column was subjected for column chromatographic fractionation using silica gel as an adsorbent and hexane with increasing proportions of EtOAc as mobile phase. Fraction eluted by hexane/EtoAc 98:2 v/v was found to contain one major spot with R_f value of 0.51 (solvent b). The fraction was subjected for further purification by crystallization with hot methanol to give white amorphous ppt. having m.p. of 266-269 ˚C (compound 1).

Fraction eluted by ethyl acetate from celite column was found to contain one main spot and some minor ones. It subjected to PTLC on silica gel plates eluted by $CHCL_3$: MeOH (85:15). It further purified by passing through Sephadex column eluted by MeOH to give pure compound appeared as dark purple spot (R_f 0.86) on PC eluted by solvent (c). It gave yellowish green color after exposure to NH_3 vapour (compound 2).

About 5 g of the ethylacetate/methanol (1:1) fraction was subjected for column chromatography (CC) on silica gel column [200 g of silica gel 70-230 mesh (Merck), packed onto glass column (75 x 3.5 cm)]. Elution of the column was affected by hexane, hexane/chloroform, chloroform, chloroform/ethyl acetate, ethyl acetate, ethyl acetate/methanol, methanol; 50 ml fractions each were collected.

The first 21 fractions come with ethyl acetate/methanol were collected and subjected to further CC on Sephadex LH_{20} (solvent MeOH) to give compound 3

Fractions 25-45 eluted with ethyl acetate/methanol were collected and subjected to CC on silica gel using solvent systems hexane/chloroform/methanol increasing polarity, the fractions eluted with chloroform/methanol (90:10) were collected and purified on Sephadex LH-20 using methanol afforded compound 4.

Fractions 65-72 eluted by $CHCl_3$:MeOH (80:20) subjected to PTLC on silica gel plates eluted by solvent (a2) gave compounds 5 and 6 which were subjected to further purification on Sephadex LH-20 using methanol/water as an eluting solvent.

Antiviral Bioassay: The antiviral bioassay technique was done in Virology Lab. NRC, Water Pollution Department, in which the authors are deeply indebt.

Cells: Human hepatoma (HepG2) cells were propagated in Dulbeccos' Minimum essential medium, DMEM supplemented with 10% Foetal bovine serum, 1% antibiotic-antimycotic mixture.The pH was adjusted at 7.2-7.4 by 7.5% sodium bicarbonate solution.

Viruses: Hepatitis A virus cell culture adapted strain HAV-MBB kindly provided by Prof. Dr. Verena Gauss-Muller, Luebeck University of Medicine, Institute of Molecular Virology, Germany.

Antiviral activity: Plaque infectivity reduction assay for rapid screening was used[20].

Preparation of Plant Extracts for Bioassay: Extracts were dissolved as 100 mg each in 1 ml of 10% DMSO in water. The final concentration was 100 µg/µl (Stock solution). The dissolved stock solutions were sterilized by addition of 50 µg/ml antibiotic-antimycotic mixture (10,000U penicillin G sodium, 10,000 µg streptomycin sulfate and 250 µg amphotericin B).

Plaque Reduction Assay: A 6-well plate was cultivated with HepG2 cell culture (10^5cell/ml) and incubated for 2 days at 37 ˚C. HAV was diluted to give 10^4 PFU/ml final concentrations for each virus and mixed with the plant extracts at the previous concentration and incubated overnight at 4 ˚C. Growth medium was removed from the multiwell plate and virus-compound mixture was inoculated (100 µl /well). After 1 h contact time, the inoculum was aspirated and 3 ml of MEM with 1% agarose was overlaid the cell sheets. The plates were left to solidify and incubated at 37 ˚C until the development of virus plaques. Cell sheets were fixed in 10% formalin solution for 2 h, and stained with crystal violet stain. Control virus and cells were treated identically without plant extracts. Virus plaques were counted and the percentage of reduction was calculated[20].

RESULTS AND DISCUSSIONS

Unsaponifiable Fraction: GLC analysis of the unsaponifiable fraction proved to be a mixture of hydrocarbons, sterols and triterpenes. Identification of the compounds was carried out by comparison of their retention time with the available reference compounds Table 1.

Fatty acid fraction: GLC analysis of the fatty acid methyl esters resulted in the identification of 15 fatty acids in which palmitic acid (46.149%), docosaeinoic acid (11.758%) and myristic acid (10.04%) represent the main components as illustrated in Table 2.

Compound 1: The *EI* mass spectrum of this compound showed molecular ion at m/z 414 and other fragment peaks at m/z 396, 273, 255, and 213. ^{1}H-NMR and ^{13}C-NMR spectrum Table (3) were found to be identical with that published for β-sitoserol[6].

Table 1: GLC analysis of unsaponifiable fraction of *Duranta repens*.

Peak NO.	RT	Percentage	Constituents
1	8.703	3.817	C_{15} n- pentadecane
2	9.270	2.598	C_{16} n- hexadecane
3	11.027	2.990	C_{17} n- heptadecane
4	11.614	6.469	C_{18} n- octadecane
5	13.117	7.640	C_{19} n- nonadecane
6	14.097	2.536	Non identified
7	14.699	5.637	C_{20} n- cosane
8	16.255	3.198	C_{21} n- uncosane
9	18.232	10.995	C_{22} n- dodacosane
10	19.365	3.133	Non identified
11	20.079	4.934	C_{23} n- tricosane
12	21.000	5.346	C_{24} n- tetracosane
13	21.894	10.737	C_{25} n- pentacosane
14	22.792	25.926	C_{26} n- hexacosane
15	23.863	2.633	C_{27} n- heptacosane

Table 2: GLC analysis of the fatty acid methyl esters of *Duranta repens*.

Peak No.	RRT*	Percentage	Constituents
1	0.607	3.836	C_{10} decanoic acid
2	0.620	1.2056	$C_{10\ (1)}$ decanoic acid
3	0.689	0.946	C_{12} lauric acid
4	0.808	10.004	$C_{14\ (.)}$ myristic acid
5	0.809	9.223	$C_{14\ (1)}$ Tetradecaenoic acid
6	1.00	46.149	$C_{16\ (.)}$ palmitic acid
7	1.181	1.049	$C_{18\ (.)}$ stearic acid
8	1.213	3.462	$C_{18\ (1)}$ oleic acid
9	1.271	0.359	$C_{18\ (2)}$ linoliec acid
10	1.341	1.612	$C_{18\ (3)}$ linolenic acid
11	1.361	1.160	Non identified acid
12	1.386	2.247	Non identified acid
13	1.517	4.813	$C_{20\ (.)}$ arachidonic acid
14	1.550	11.758	$C_{22\ (1)}$ docosaeinoic acid
15	1.81	2.183	Non identified acid

RRT*= Relative to retention time of palmitic acid 18.100 min

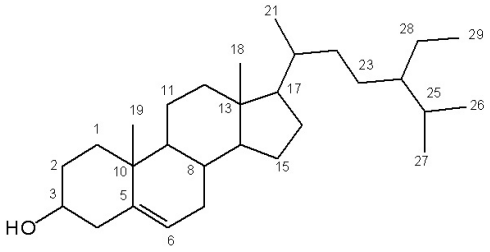


Fig. 1: β-sitoserol (compound 1)

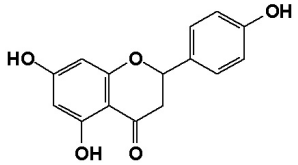


Fig. 2: Naringenin. (compound 2)

Table 3: ^{1}H- and ^{13}C-NMR assignment of compound 1 (β-sitoserol).

C. NO.	^{1}H-NMR	^{13}C-NMR	C. NO.	^{1}H-NMR	^{13}C-NMR
1	-	37.1	16	-	28.2
2	-	31.5	17	-	56
3	3.52 m	71.6	18	Me-0.68 s	11.9
4	-	42.2	19	Me-1.008 s	19..3
5	-	140.6	20	-	36.1
6	5.35 m	121.6	21	0.919 d	18.8
7	-	31.5	22	5.043	33.9
8	-	31.5	23	5.119	26.6
9	-	50	24	-	45.8
10	-	36.4	25	-	29.1
11	-	20.9	26	0.817 d	19.8
12	-	39.79	27	0.829 d	18.9
13	-	42.3	28	-	23
14	-	56.6	29	0.851 t	12
15	-	24.3			

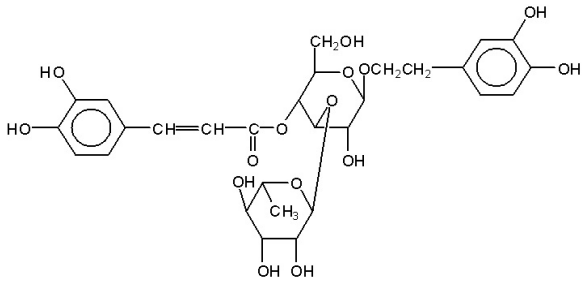


Fig. 3: Acteoside (compound 3)

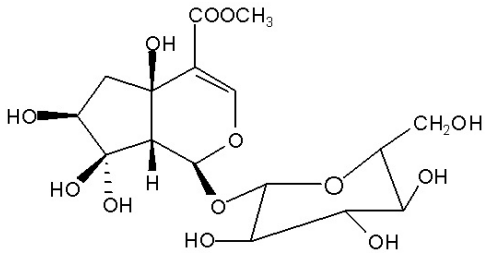


Fig. 4: Lamiide (compound 4)

Compound 2: The UV absorption spectrum showed $\lambda_{max\ (MeOH)}$ 291, 330$_{(sh).}$ Band II at 291 nm suggesting the compound to be flavanone or dihydroflavonol.

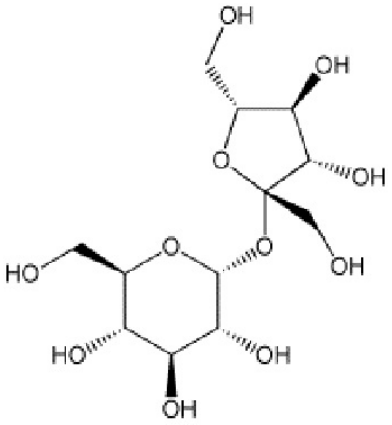


Fig. 5: Sucrose (α- glucopyranosyl (1-2) ß fructopyranoside) (compound 5)

There is 36 nm bathochromic shift of the major absorption band II with increase in intensity on addition of NaOH shift reagent predicting the presence of 5,7-dihydroxy-flavanone. Also on addition of NaOAc shift reagent, the compound showed 34 nm bathochromic shift which confirms the presence of 5, 7-dihydroxy system. The presence of 5- hydroxyl group was confirmed by a bathochromic shift of 24 nm of band II on addition of $AlCl_3$ shift reagent. EI/MS showed M^+ at 272 constitutes to molecular formula

Table 4: ^{13}C and ^{1}H NMR of compound 3 (acteoside).

	C no.	^{13}C *d* (ppm) mult.	DEPT	^{1}H (ppm), mult., *J* (Hz)
Aglycone	1	130.147 s	C	-
	2	114.966 d	CH	6.69 (br.5, 1 H)
	3	143.306 s	C	-
	4	144.761 s	C	-
	5	115.727 d	CH	6.67 (d, 2.0, 1H)
	6	119.909 d	CH	6.56 (dd, 8.0, 2.0, 1H)
	α	70.881 t	CH_2	3.72 (dd, 14.8, 7.9, 1H)
	β	35.194 t	CH_2	2.78, (m, 2H)
Caffeic acid	´1	126.310 s	C	-
	´2	113.897 d	CH	7.05 (d, 2.0,1H)
	´3	148.428 s	C	-
	´4	145.469 s	C	-
	´5	115.168 d	CH	6.77 (d, 8.1, 1H)
	´6	121.841 d	CH	6.95 (dd, 8.2, 1.9, 1H)
	´α	146.653 s	C	-
	´ß	113.357 d	CH	6.26 (d, 15.9, 1H)
	χ	166.949 d	CH	7.59 (d, 12.6, 1H)
glucose	″1	102.846 d	CH	4.37 (d, 7.9,1H)
	″2	74.846 d	CH	3.35 (dd, 9.8, 1H)
	″3	80.287 d	CH	3.81 (t, 9.2, 1H)
	″4	69.255 d	CH	4.91 (t, 9.3, 1H)
	″5	74.676 d	CH	nd
	″6	61.015 t	CH2	3.6 (dd, 14.2, 4.3) 3.4 (dd, 8.9, nd)
rhamnose	′″ 1	101.652 d	CH	5.18 (br.s, 1H)
	′″ 2	70.989 d	CH	3.91 (dd, 4.8, 1.8, 1H)
	′″ 3	70.712 d	CH	3.57 (dd, 9.5, 3.3, 1H)
	′″ 4	72.447 d	CH	3.28 (t, 9.6)
	′″ 5	69.050 d	CH	nd
	′″ 6	17.074 q	CH3	1.08 (d, 6.2)

(C_{15} H_{12} O_5). From all these data and CO-chromatography with authentic samples the compound was found to be naringenin (4, 5, 7-trihydroxyflavanone)[13,12].

Compound 3: The ^{1}H and ^{13}C NMR spectra of compound 3 (Table 4) showed characteristic signals for a disaccharide moiety (2 anomeric protons at δ 4.37 (d)) and δ 5.18 (br s), and 2 anomeric carbons at δ

Table 5: ^{13}C and ^{1}H NMR of compound 4 (lamiide).

NO.	^{13}C (δ, ppm)	^{1}H (δ, ppm), multi, *J* (Hz)
1	94.50	5.81, (br. S, 1H)
2	-	-
3	152.45	7.42, (s, 1H)
4	115.42	-
5	69.25	-
6	46.75	2.35, dd, 15.1, 5.2// 15.1, 3.3(m,2H)
7	77.86	3.51, dd, 5.2, 3.3
8	79.11	-
9	58.10	2.78, br s
10	21.29	1.08, s
11	168.03	3.72, s
-OCH_3	51.67	4.58, d , 7.9
1	99.63	3.18, dd, 9.2, 7.9
2	74.40	3.37, m
3	77.45	3.27, m
4	71.67	3.31, m
5	78.38	3089, dd, 12.0, 2.1/: 3.66, dd, 12.0
6	62.78	5.7

Table 6: NMR of compound 4 (lamiid)

^{13}C (δ, ppm)	DEPT	^{1}H (δ, ppm), multi, *J* (Hz)
168.03	-	-
152.45	CH	7.42, s
115.42	-	-
99.63	CH	4.59, d, 7.9
94.50	CH	5.81, d, 0.75
79.11	-	-
78.38	CH	3.31, m
77.86	CH	3.41, dd, 5.2, 3.3
77.45	CH	3.37, dd
74.40	CH	3.18, dd, 9.2, 7.9
71.67	CH	3.27, dd
96.25	-	-
62.78	CH_2	3.89, dd, 11.0, 2.1
58.10	CH	3.66, dd, 12.0, 5.7
51.67	CH_3	3.72, s
46.75	CH_2	2.35, dd, 15.1, 5.2 2.24, dd, 15.1, 3.3
21.29	CH_3	1.08, s

Table 7: ^{1}H- and ^{13}C-NMR assignment of compound (5) Sucrose (α- glucopyranosyl (1-2) fructopyranoside).

C No.	^{1}H (δ ppm), multi,	^{13}C-NMR
1	5.39 d	93.63 d
2	3.563 d	74.38 d
3	3.762 d	71.35 d
4	3.86 M	75.75 d
5	3.476 d	73.27 d
6	3.817 M	62.77 t
1	3.89 M	83.77 d
2	4.055 d	74.65 d
3	4.219 d	79.36 d
4	--	105.32 s
5	3.679 d	63.37 t
6	3.826 T	64.06 t

102.85 and δ 101.65); showed that rhmnose is α- and glucose is b-linked a methyl signal at δ 17.07 pointed towards rhamnose as one of the sugar. In addition, the presence of two aromatic residues was obvious, and

Table 8: ^{1}H- and ^{13}C-NMR assignment of compound (6) Raffinose (α-galactopyranosyl (1→6)α- glucopyranosyl- (1→2) fructopyranoside)

Carbon NO.	^{13}C (δ ppm,	^{1}H (δ, ppm), multi, *J* (Hz)
1	62.665	3.66 d
2	105.8	-
3	77.96	4.48 d
4	74.803	4.51, d, 5.6
5	83.25	3.87
6	64.056	3.84 t
7	93.883	5.129, d, 3.6
8	71.7	3.50 ,d, 3.2
9	73.727	3.70
10	69.353	3.86
11	72.94	3.47
12	65.761	3.81 m
13	99.183	5.129, d, 3.6
14	69.00	3.52
15	71.123	3.79
16	71.679	3.67
17	64.55	3,46
18	65.761	3.81

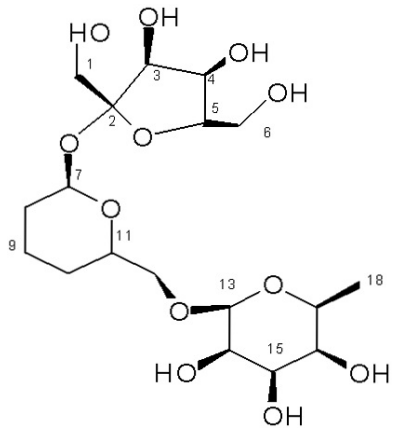
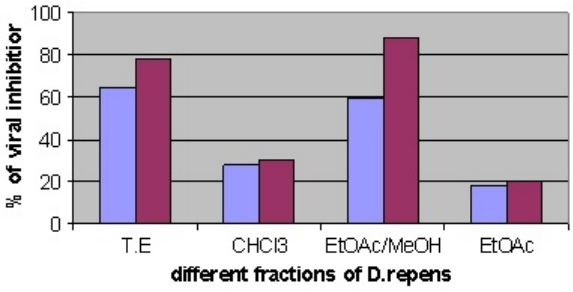


Fig. 6: Raffinose (α-galactopyranosyl(1→6)α-glucopyranosyl- (1→2) fructopyranoside) (compound 6)



T.E=total extract, CHCl3= chloroform fraction, EtOAc/MeOH=ethyleacetae/methanol fraction, EtOAc= ethylacetate fraction

Fig. 7: Antiviral activity of different fractions of D. repens.

one of these could easily identified as caffeic acid moiety e.g. by the typical double bond signals at δ 146.65 (C-7) and δ 113.36 (C-8) in ^{13}C NMR, and at δ 6.26 (d) and 7.59 (d) in ^{1}H NMR, the coupling constant (15.9 Hz) indicating the trans stereochemistry. The remaining signals were readily distributed to a phenylether moiety (two CH_2 units at δ 35.19 and δ 70.88 in ^{13}C-NMR). ^{13}C NMR library search gave a complete agreement with published assignment for acteoside (or verbascoside)[7,19,11].

Compound 4: The ^{1}H- and ^{13}C-NMR and HSQC spectra of compound 4 Table (5 and 6) showed same typical signals for iridoids, such as C-1 at δ 94.50 and C-3 at δ 152.45 in ^{13}C NMR, with the corresponding protons at δ 5.81 (d, j = 0.8 HZ) and δ 7.42 (s), respectively. The presence of a methoxycarbonyl and a glucosyl substituent, present in many iridoids, could easily be detected. In addition to the carbohydrate moiety, three oxygenated carbons were present, a quaternary one at δ 69.25 and two methane carbons at δ 77.86 and δ 79.11 in ^{13}C NMR. Careful analysis of two-dimentional COSY, HSQC, HMBC and MS[10] spectra allowed identifying the compound as lamiide[11].

Compound 5: The ^{1}H- and ^{13}C-NMR spectra of compound (5) Table (7) showed characteristic signals for a disaccharide (2 anomeric protons at δ 5.39(d) j = 3.8 and δ 4.219 (d) and 2 anomeric carbons at δ 93.625 and δ 105.32). The coupling constant j = 3.8-Hz) indicating the α-linked sugar moiety. The ^{1}H and ^{13}C library search gave a complete agreement with published assignment for α-glucopyranosyl(1→2)-β-fructopyranoside(sucrose)[1]. Enzymatic hydrolysis by invertase (sucharase enzyme) gave rise to α-glucose and β-fructose.

Compound 6: The acid hydrolysis, followed by paper chromatography with authentic sugars gave rise to glucose, galactose and fructose, confirms the structure of the compound to be raffinose. On selective enzymatic hydrolysis by invertase (α β-fructofuranosidase) hydrolyzes the sucrose part of the molecule to give melibiose and D-fructose. On hydrolysis by α-galactosidase, which hydrolyzes the melibiose residue to yield D-galactose and sucrose. The ^{1}H- and ^{13}C-NMR library search gave a complete agreement with published data for α-galactopyranosyl(1→6)α-glucopyranosyl-(1→2) fructopyranoside (Raffinose)[15].

Antiviral activity: The total ethanol extract of *D. repens* var. vareigata showed 76% reduction in the viral titer of virus Hepatitis A at concentration of 40 μg ml^{-1}, while at 20 μg ml^{-1} the inhibition reached 64% by plaque reduction assay Fig 7. On fractionation of total alcohol extract by celite column and each fraction subjected for antiviral activity study, the ethyl acetate/methanol fraction showed the highest inhibition percentage. It showed 88% inhibition for 40 μg ml^{-1} while 20 μg ml^{-1} concentrations the inhibition percentage reached 59%. This activity may be attributed to the acteoside or lamiide content in this fraction, which showed high antioxidant activity before[17].

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