IN VITRO EVALUATION OF THE ANTIOXIDANT EFFECTS OF SOME BOTANICAL ETHANOLIC EXTRACTS AND ISOLATION OF THE ANTIOXIDANT CONSTITUENT(S) FROM *RUTA CHALEPENSIS* LEAVES

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ABSTRACT

The antioxidant effects of aqueous ethanolic extracts (80 %)from the leaves of four plants i.e. *Ruta chalepensis*, *Gomphocarpus sinaicus*, *Segertia thea* and *Acacia holoseriaca* were evaluated in vitro by determining their effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) decoloration, inhibition of DNA-sugar damage and inhibition of lipid peroxidation. Among the four extracts tested, *Ruta chalepensis* and *Gomphocarpus sinaicus* extracts showed the strongest antioxidant activity. The ethanolic extract of *Ruta chalepensis* leaves was subjected to isolation and structural characterization of the active constituent(s) of this plant. Two active flavonoid compounds were isolated by using a combination of chromatographed methods (TLC and column chromatography). Their chemical structures were characterized by spectroscopic methods (UV, ^{1}H and ^{13}C-NMR) as Quercetin 3-O-[rhamnosyl (1-6) glucoside] (**I**) and Isorhamnetin 3-O-glucoside-7-O-rhamnoside (**II**).

Key words: **Natural antioxidants, *Ruta chalepensis*, lipid peroxidation, flavonoids, DPPH.**

INTRODUCTION

Living organisms are exposed to arrange of oxidizing species that have the potential to damage cellular tissues. Oxygen derived free radicals i.e reactive oxygen species (ROS) produced during normal metabolism or induced by exogenous damage is the most dangerous species **(Cross *et al.* 1987).**

These ROS oxidize physiological substrates of crucial biological significance (membrane lipids, nucleic acids and proteins) and cause cellular damage. Such damage is implicated in a wide range of diseases including coronary arteriosclerosis, diabetes mellitus, cancer, hepatotoxicity and neurodegenerative disease **(Jackson *et al.* 1993, Simonian and Coyle1996, Haraguchi *et al.* 2002 and Aniya *et al.*2005)**.

The use of antioxidants, both natural and synthetic, in the prevention and cure of various diseases is gaining a wide importance in the medicinal field. Currently there is considerable interest in the natural antioxidants activity than the synthetic ones because they are seen as being safer and causing fewer adverse reactions **(Han *et al.* 2004).**

In the course of our search investigation for finding new sources of natural antioxidants from plants **(Moussa *et al.* 2005)** we report in this paper the antioxidant effects of ethanolic extracts (80%) from leaves of four folk medicinal plants, *i.e. Ruta chalepensis*, *Gomphocarpus sinaicus*, *Acacia holoseriaca* and *Sagertia thea* were evaluated in vitro by determining their effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) decolouration, inhibition of DNA-sugar damage and inhibition of lipid peroxidation in mitochondria and

microsomes along with the isolation and structural elucidation of the active constituent(s) responsible for the antioxidant activity of *Ruta chalepensis* leaves.

MATERIALS AND METHODS

Plant materials

Leaf samples of 4 plants were collected, three of them i.e *Ruta chalepensis, Sagertia thea* and *Acacia holoseriaca* from the experimental farm of the Faculty of Agriculture, Cairo University, Giza whereas the fourth, i.e. *Gomphocarpus sinaicus* from Sinai region. The four plants were identified by the Botany Department, Faculty of Science Cairo University. A voucher specimen deposited in the Biochemistry Department, Faculty of Agriculture, Cairo University, Giza. A portion (50g) of the leaf samples from each of the plant species collected was air dried in the shade, ground into a fine powder and then were extracted with 80% ethanol. The aqueous ethanolic extracts of the leaf samples were evaporated to dryness under reduced pressure and tested for their antioxidant activity.

Tests for antioxidant activity

Lipid peroxidation, DPPH decoloureation and DNA-sugar damage assays were used for measuring the antioxidant activity of the aqueous ethanolic extracts of the four plants collected for this study.

1-Lipid peroxidation assay

The antioxidant activity of the four ethanolic plant extracts on inhibition lipid peroxidation in mitochondria and microsomes as follows:

Animals

Young mail Spargue-Dawley rats weighing 100-120g were housed at 25±1.0ºc with 60% relative humidity, illuminated for 12h a day starting at 7.0 a.m. and were given free access to food and water.

Preparation of mitochondria and microsomes in rat livers:

Rats were killed by decapitation after fasting for 24 h; and their liver tissue was quickly removed. Microsomal and mitochondria fractions were isolated from liver tissue by the method of **Kimura *et al.* (1984),** and the protein was determined by the method of **Lowry *et al*. (1951).**

Measurement of lipid peroxidation:

A mixture of mitochondrial suspension (0.5 ml), containing 5 mg protein, 50 mM 2-[4-(2-hydroxyethyl) 1-piperazinyl] ethanesulfonic acid (HEPEs) buffer (pH 7.4, 0.1 ml), 20mM KCl (0.1 ml), 10 µM $FeSO_4$ (0.1 ml), 0.2 mM ascorbate (0.1 ml) and the indicated amounts of various plant extracts were incubated at 37°C for 30min in a final volume 1.0 ml **(Haraguchi *et al* . 2002).** A mixture of microsomal suspension (10 µl) containing 100 µg protein, $FeSO_4$ (1.0 ml) combined with ascorbic acid (10 µM) and the indicated amounts of various plant extracts were incubated at 37 °C for 30 min in a final volume 1.5 ml (Aboul-Enein *et al*. 2003).

Determination of lipid peroxidation products:

Lipid peroxidation was measured using the reaction with thiobarbituric acid, TBA **(Houghton *et al*. 1995 and Burits and Bucar; 2002).** All reagents used for this assay were prepared freshly and all reactions were carried out in triplicate. Silymarin was used as a positive control. Inhibition (I) of lipid peroxidation in percent was calculated by the following equation.

$$\mathbf{I\ (\%) = 100\ x\ (A^o - A^1 / A^o)}$$

Where**:** A^o was the absorbance of the control reaction and A^1 was the absorbance in the presence of the tested extract at 532nm.

2-DPPH decolouration assay

The free radical scavenging effect of the ethanolic plant extracts and the isolated compounds was assessed by the decolouration of a methanolic solution of 1,1diphenyl-2-picryl hydrazyl (DPPH) according to **Brand-Williams *et al*. 1995.** A freshly prepared DPPH solution (20 mg/L) was used for the assay. Samples were dissolved in methanol and the methanolic solution of DPPH served as a control. The degree of decolouration indicates the free radical scavenging efficiency of the susbstances. Silymarin was used as a reference of free radical scavenger. The percentage of DPPH decolouration was calculated as follows:

$$\mathbf{Decolouration\ (\%) = 100\ x\ (A^o - A^1 / A^o)}$$

The percentage DPPH decoloration of the extracts and the isolated compounds were triplicatly assessed at 100, 50 and 10 µg/ml.

3-DNA-sugar damage assay

The DNA- sugar damage was assayed by the method of Sultana *et al.* 1995. Inhibition %(I) of DNA-sugar damage was calculated by the following equation:

$$\mathbf{I\ (\%) = 100\ x\ (A^1 - A^2 / A^2)}$$

Where: $A^1$was the absorbance of the control reaction.

A^2 was the absorbance in the presence of the tested extract at 532nm.

Extraction and isolation of the bioactive constituent(s) from *Ruta chalepensis* leaves:

1-Extraction:

Ground air dried leaves (350g) were extracted three times with 80% ethanol (each 700ml)at room temperature (25±2 ºC). After filtration, the combined extracts were evaporated under reduced pressure to afford 55.2g of dry extract. A portion of the aqueous ethanolic extract (40g) was suspended in water (150 ml), and extracted with $CHCl_3$ (3×50ml) to give $CHCl_3$ soluble components (Fr. A, 6.5g). The aqueous layer was freeze dried (33.5g) and were then extracted with $CHCl_3$-MeOH-H_2O (70:30:5; 150 ml). After centrifugation both the supernatant and the precipitate were dried under reduced pressure to afford 4.6g (Fr.B) and 28.8g (Fr.C) respectively.

The three fractions, i.e. A, B and C were tested for their free radical scavenging activity.

2-Analytical Thin Layer Chromatography (TLC)

TLC analysis was carried out on precoated silica gel plates (kiesel gel G-60, F-254.0.25mm Merck) using the following systems:

1) n-Butanol- Acetic acid-Water (4:1:5) upper layer.
2) Chloroform- Methanol-Water (70:30:5)
3) Chloroform-Methanol (80:20)
4) Ethylacetate- Acetic acid- Formic acid-Water (100:11:11:27)
5) Dichloromethane-Methanol- Water (50:25:5)
6) Chloroform-Acetone (50:6)

Zones were detected under UV light (254 and 365nm) and by spraying with concentrated H_2SO_4 followed by heating at 105ºC for 5 min or with NH_3. Sugars were detected by spraying with naphthoresorcinol phosphoric acid followed by heating at 105ºC for 10 min.

3-Isolation of the bioactive component(s)

The bioactive fraction (Fr. B, 4.5g) was subjected to the isolation of the bioactive component (s) as follows:-

Fraction B (4.5g) was chromatographed over silica gel column (100g, 230-400 mesh, Merck) and eluted with the solvent mixtures of $CHCl_3$-MeOH-H_2O (80:20:0 and 70:30:5, 200 ml for each eluent). Twenty fractions of each eluent were collected. The eluates were combined on the basis of similarity of TLC profiles to afford 7 fractions and were then tested for free radical scavenging activity. The bioactive fractions No. 6 and 7 were further purified several times over Sephadex LH-20and silica gel columns as shown in (Fig.1) yielded two active compounds **I** and **II**. The purity of these two compounds were established by the resolution of each one as a single spot in four different TLC systems.

4-Structure identification of the isolated compounds.

The isolated compounds were characterized by chemical investigation (detection tests and acid hydrolysis) and spectroscopic methods.

Chemical Investigation

The phytoconstituents classes of the isolated compounds were detected according to the methods described by **Farnsworth (1966).**

Acid hydrolysis of the isolated compounds

Compound **I** or **II** (2mg) was heated with aqueous 10% HCl (2ml) in a 100ºC water-bath for 4 hours. The aglycone was extracted with diethyl ether and analyzed by TLC with $CHCl_3$-Acetone (50:6). The aqueous layer was neutralized with N,N-dioctylamine (10% in $CHCl_3$). After evaporation to dryness, the sugars were identified by TLC with CH_2Cl_2-MeOH-H_2O (50:25:5) by comparison with authentic samples.

Spectroscopic methods

Nuclear Magnetic Resonance (NMR) Spectroscopy

^1H and ^{13}C-NMR spectra were recorded in CD_3OD on a varion Mercury VXR300 (300 MHz for ^1H and 75 MHz for ^{13}C). Chemical shifts (ppm) were related to that of the solvent.

Ultraviolet Spectroscopy (UV)

The UV-spectra were registered with a spectrophotometer CeCil 3000 series according to **Mabry *et al.* (1970).**

RESULTS AND DISCUSSION

Effect of the plant extracts on mitochondrial and microsomal lipid peroxidation:

The peroxidation of polyunsaturated fatty acid can proceed through processes that are enzymatically or through non- enzymatic auto oxidative pathway **(Slater, 1984).** As shown in (Table 1) 80% ethanol extract of *Ruta chalepensis, Sagertia thea Gomphocarpus sinaicus,* and *Acacia holoseriaca* respectively, were effective in preventing Fe^{2+}/ ascorbate induced non-enzymatic lipid peroxidation in mitochondria. It was observed that the highest inhibition values resulted in the high concentration i.e. 1 mg/ml of each extract. In microsomes the obtained data (Table 2) showed that ethanolic extract of *Ruta chalepensis,* and *Gomphocarpus sinaicus* gave the highest inhibition values and the highest inhibition percentage resulted in the high concentration (i.e. 1 mg/ml).

Table (1) Effect of the plant extracts on lipid peroxidahion in rat liver mitochondria:

Sample	Conc.(mg/ml)	X ± S.E	I %
Control	----	90.5 ± 0.4	----
Silymarin	1.0	4.5 ± 0.1	96.4
	0.5	15 ± 0.2	88.0
	0.25	18 ± 0.2	85.4
R. chalepensis	1.0	14.8 ± 0.2	83.7
	0.5	20.5 ± 0.4	77.3
	0.25	44.3 ± 0.4	51.1
G. sinaicus	1.0	17.5 ± 0.6	80.6
	0.5	22.8 ± 0.9	74.8
	0.25	49.5 ± 1.4	45.3
S. thea	1.0	37.8 ± 0.8	58.2
	0.5	43.0 ± 1.3	52.4
	0.25	85.5 ± 1.9	5.5
A. holoseriaca	1.0	46.3 ± 1.6	48.9
	0.5	48.3 ± 0.8	46.6
	0.25	70.5 ± 1.9	22.1

Table (2) Effect of the plant extracts on lipid peroxidahion in rat liver microsomes:

Sample	Conc.(mg/ml)	X ± S.E	I %
Control	----	124 ± 1.7	----
Silymarin	1.0	3.8± 0.02	97.0
	0.5	8.3 ± 0.08	93.3
	0.25	15 ± 0.3	88.0
R. chalepensis	1.0	29.8 ± 0.4	76.0
	0.5	47.8 ± 0.7	61.5
	0.25	50.3 ± 2.3	59.5
G. sinaicus	1.0	18.5 ±1.9	85.1
	0.5	35.8 ± 0.8	71.1
	0.25	62.8 ±1.7	49.4
S. thea	1.0	50.3 ± 2.3	59.5
	0.5	58.5 ± 0.9	52.8
	0.25	90 ± 0.4	27.4
A. holoseriaca	1.0	64.8 ± 0.9	47.7
	0.5	68.8 ±1.0	44.5
	0.25	99.5 ± 2.0	19.7

Free radical scavenging activity of plant extracts:

In order to evaluate the free radical- scavenging activity of these plant extracts we used a method based on the reduction of DPPH, a stable free radical. Table (3) summarizes the percentage of DPPH coloration with 100, 50, and 10 µg/ml of material extracted from the four plants under study. All plant extracts showed free radical scavenging activity. *Gomphocarpus sinaicus* and *Ruta chalepensis* were found to be significantly more efficient free radical scavengers than *Sagertia thea* and *Acacia holoseriaca* at the three concentrations used.

Table (3) Free radical scavenging activity of the plant extracts and isolated compounds

Sample	DPPH decoloration %		
	10µg/ml	**50µg/ml**	**100µg/ml**
Silymarin	78.9	86.3	98.0
R. chalepensis	24.8	50.1	73.2
G. sinaicus	35.9	40.3	75.7
S. thea	5.9	20.1	40.3
A. holoseriaca	2.6	11.0	35.0
Compound I	40.4	87.5	100
Compound II	20.6	28.1	33.3

Effect of the plant extracts on free radical-mediated DNA-sugar damage:

The results of the effect of the four plant extracts on free radical mediated DNA-sugar damage are presented in Table (4). The inhibitory effect of the plant extracts was dose-dependent at concentrations 50, and 100 µg/ml. All plant extracts exerted damage.

Table (4) Effect of the plant extracts on free radical-mediated DNA-sugar damage

Plant extract	I % of DNA-sugar damage	
	50 (µg/ml)	**100 (**µg/ml)
R. chalepensis	39.5	59.0
G. sinaicus	53.9	66.0
S. thea	25.8	37.2
A. holoseriaca	31.8	40.7

The premise that oxidative stress plays a role in the pathology of human diseases has provoked that evaluation of natural and synthetic antioxidant compounds for the treatment of diseases. Lipid peroxidation, as well as simple DPPH and DNA-sugar damage assays are established methods used for measuring antioxidant activity, to predict antioxidant behavior in biological systems and to quantify the total antioxidant capacity of body fluids.

Incubation of rat liver mitochondria suspensions in the presence of low concentrations of ascorbic acid resulted in the formation of lipid peroxides and the oxidation of the ascorbic acid **(Ottolenghi, 1959).** The present study showed the protective effect of ethanol extract of these plants on lipid peroxidation induced by Fe^{+2} / ascorbate in rat liver mitochondria and microsomes at the three concentrations. *Ruta chalepensis* and *Gomphocarpus sinaicus* are apparently good free radical, and probably have the ability to inhibit autoxidation of lipid, e.g. in the treatment of liver disease in which lipid peroxidation is an important component.

Effect of these plant extracts on DNA-sugar damage are observed. *Ruta chalepensis* and *Gomphocarpus sinaicus* exerted the most effect. These results suggest that the plant extracts could probably contain compounds that reduce free radical mediated DNA-sugar damage. Also observed the scavenging activity of these plant extracts against DPPH radical. All plant extracts scavenge DPPH radical. *Ruta chalepensis* and *Gomphocarpus sinaicus* had the potent activity.

These findings suggest that *Ruta chalepensis* and *Gomphocarpus sinaicus* may be useful for therapeutic use and as source of natural antioxidants.

The *Ruta chalepensis* leaves were subjected to isolation and structural identification of the active constituent (s) because the quantity of leaves collected was sufficient to isolate the active component (s), as well as its high activity.

Bioactivity guided fractionation of the 80% ethanol extract of *Ruta chalepensis* leaves carried out by chromatographic method as described in materials and methods (Fig.1) led to the isolation of two compounds **I** and **II**

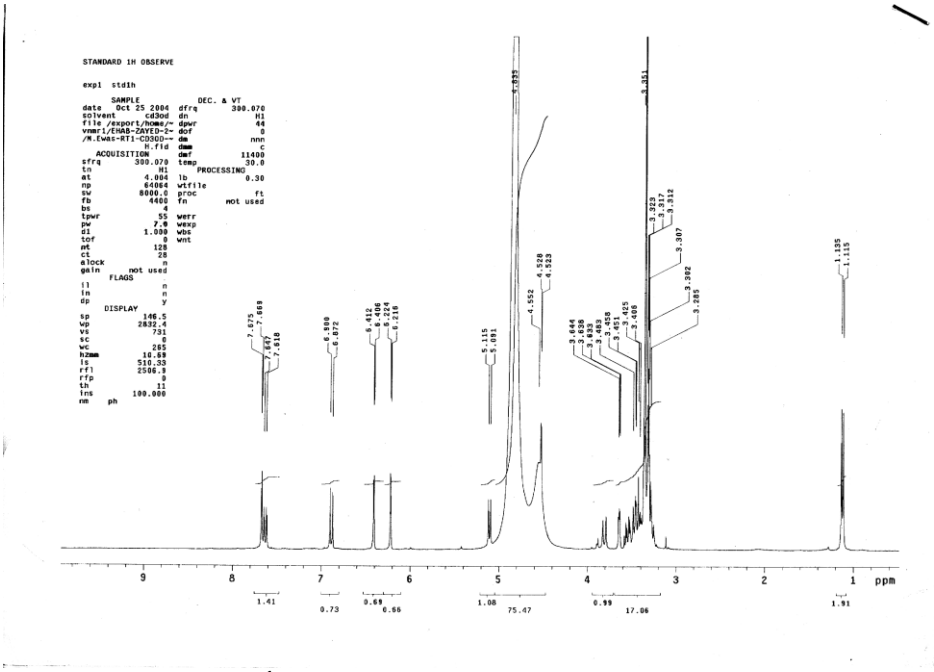


Fig. (2) ^{1}H-NMR spectrum of compound I in CD_3OD

Compound I

It was obtained as a yellow fine crystal, which appeared purpule on TLC under UV light, 365nm and turned yellow in NH_3 suggesting it is a flavonoid compound. This compound also showed two distincit bands at λ 259 and 359 nm in the UV spectrum suggesting it is a flavonol compound **(Mabry *et al.* 1970).** On acidic hydrolysis, it gave D-glucose and L-rhamnose on TLC by direct comparison with authentic samples. The presence of the two sugars β-D-glucose and α-L-rhamnose were confirmed by the appearance of two anomeric proton signals in the ^{1}H-NMR spectrum (Fig. 2) at δ5.1 (1H,d,J =7.2Hz) for glucose and at δ 4.52 (1H,d,J=1.5Hz) for rhamnose , as well as the carbon signals in the ^{13}C-NMR spectrum (Fig.3 and Table 5) at δ104.66, δ 68.57 ppm (C-1 and C- 6, glu), δ 102.4 and δ 17.89 ppm (C-1 and C-6 , rha). The ^{13}C- NMR spectrum also showed 27 carbon signals out of which 12 carbons accounted for the two sugars (glu, rha).

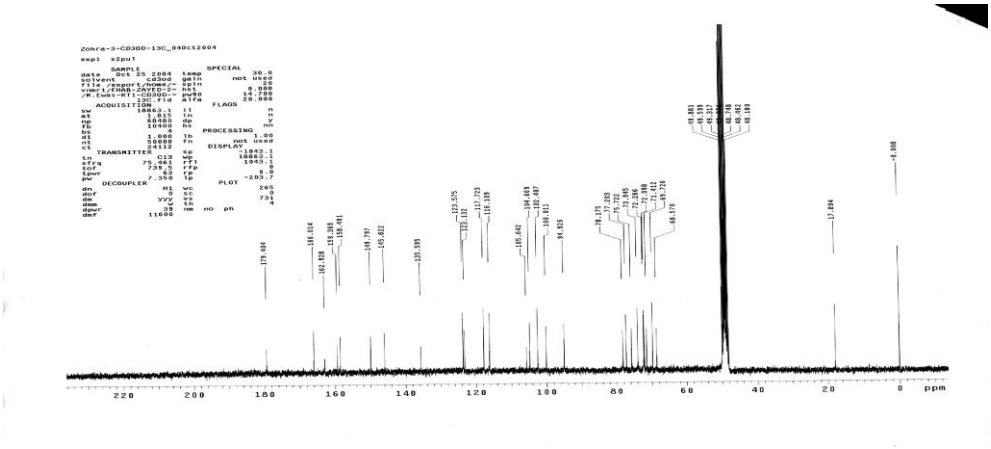


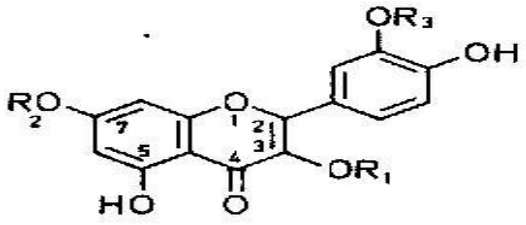
Fig. (3) ^{13}C-NMR spectrum of compound I in CD_3OD

The down field shift of C-6 of glucose (about 5.0 ppm) in comparing with previously reported data (Markham *et al.* 1978) indicated that rhamnose and glucose linked to each other through a (1-6) linkage. The glycosylation site of the disaccharide at C-3 hydroxyl was confirmed through the down field resonance of C-2 at δ 158.49 ppm and the up field signals of C-3 at δ135.5 ppm **(Markham *et al*.1978).**

Table 5: ^{13}CNMR Chemical shifts (ppm) of compounds I and II in CD_3OD

C-atom		I	II
	2	158.49	158.05
	3	135.59	135.25
	4	179.40	178.48
	5	162.92	162.60
	6	100.01	102.06
	7	166.01	166.03
	8	94.92	96.36
	9	159.36	158.96
	10	105.64	105.13
	1’	123.57	122.91
	2’	116.10	114.47
	3’	145.82	148.44
	4’	149.79	151.81
	5’	117.72	116.21
	6’	123.13	123.95
	OCH_3	--------	56.72
Glu	1	104.66	103.48
	2	75.72	75.91
	3	78.17	78.31
	4	69.72	69.79
	5	77.20	77.35
	6	68.57	64.32
Rha	1	102.40	102.54
	2	73.94	73.89
	3	72.26	72.27
	4	72.09	72.08
	5	71.41	71.59
	6	17.89	17.91

Thus the structure of compound **I** (Fig.4) was characterized as Quercetin-3- [rhamnosyl (1-6) glucoside] This result was confirmed by comparing the present spectroscopic data with those previously reported **(Markham *et al.* 1978).**



Compound	R1	R2	R3
I	Glu (6-1) Rha	H	H
II	Glu	Rha	CH_3

Fig. (4) Structural formula of the isolated compounds I and II

Compound II

It was obtained as yellow fine powder, which showed two distinct bands at λ 253 and 355nm in the UV spectrum. This compound exhibited the same color behavior on TLC as compound **I** but it is less polar, suggesting it is also a flavonol compound. On acid hydrolysis, it afforded D-glucose and L-rhamnase as detected by TLC with authentic samples. The presence of the two sugars β-D-glucose and α-L-rhamnose were confirmed by the carbon chemical shifts of sugars (Fig. 5 and Table 5) in the ^{13}C-NMR spectrum and by the ^{1}H-NMR spectrum (Fig.6) due to the appearance of two anomeric protons at δ5.1 (d, J= 7.5Hz) for glucose and δ 4.5 (d,J= 1.5Hz) for rhamnose.

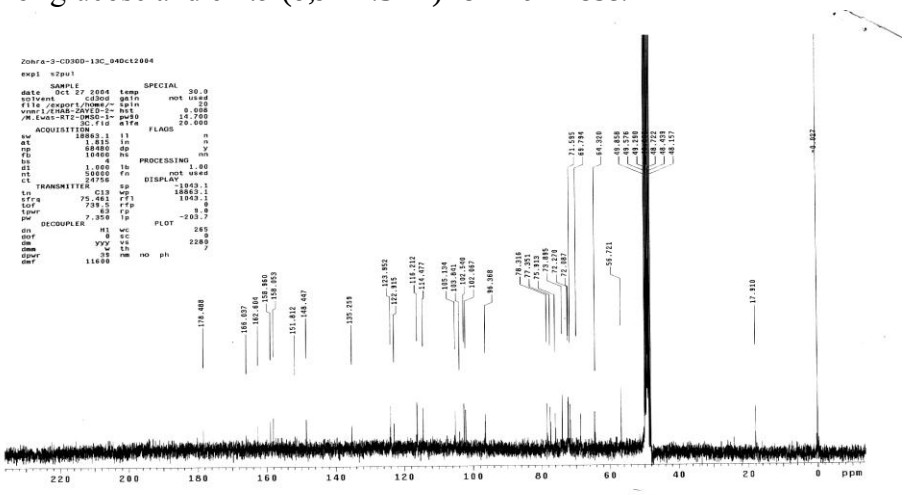


Fig. (5) ^{13}C-NMR spectrum of compound II in CD_3OD

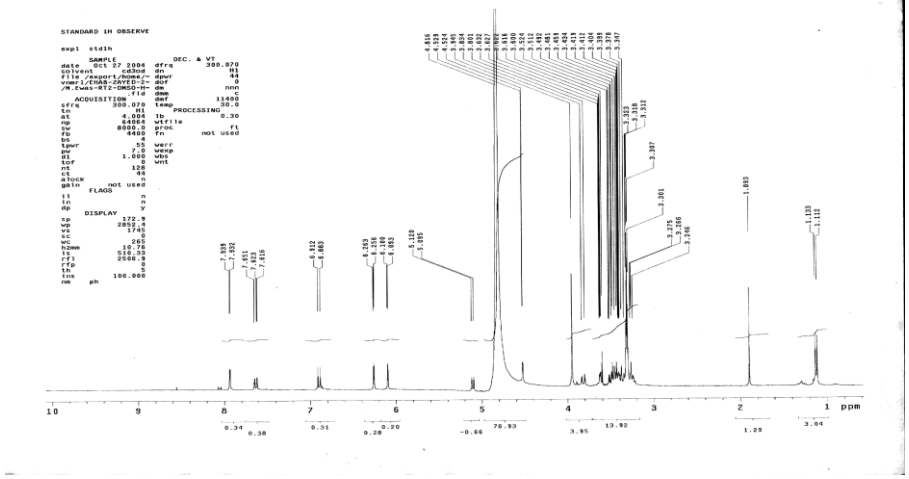


Fig. (6) ^{1}H-NMR spectrum of compound II in CD_3OD

The aglycone was clearly deduced as Isorhamnetin from the following proton signals, in the ^{1}H-NMR spectrum (Fig.6) three proton signals at δ 3.94 (OCH_3) two meta-coupled proton H-6 and H-8 of ring A (δ 6.1,d,J=2.1Hz, δ 6.2 d, J=2.1Hz) respectively and three aromatic proton(ABX system) at δ 6.89(1H, d, J=8.7Hz, H-5`) δ 7.63(1H,dd,J =2.1, 8.4 Hz,H-6`) and δ 7.93 (1H,d,J=2.1 Hz,H-2`) attributed to the protons of the B ring substituted at C-3' and C-4'.

The carbon chemical shifts of the sugars of compound 2 (Table 5) indicated that there is no linkage between the two sugars and these sugars must occupy two different positions. The^{13}C-NMR chemical signals of the C-2 and C-3 positions (δ 158.05 and 135.25 ppm) indicated that one of the two sugars moieties must occupy the hydroxyl group at C-3 **(Markham *etal*.1978).** The lack of a bathochromic shift on the addition of NaOAc in the UV spectrum indicated the absence of a free hydroxyl group at the C-7 position **(Mabry *et al* 1970).** The significant enhancement of the ^{13}C NMR chemical shifts of the C-6 and C-8 positions (Table 5) as compared with the same positions of compound **I** indicating that the C-7 position of compound **II** must be occupied with one of the sugar moieties. The positions of the two sugars, i.e. glucose and rhamnose at C-3 and C-7 respectively were established from comparing the ^{13}CNMR signals of the two compounds **I** and **II** as well as from the spectroscopic data previously reported **(Markham *et al.* 1978).**

Thus the structure of compound **II** (Fig. 4) was characterized as Isorhamnetin 3- β-D-O-glucoside -7- α-L-O-rhamnoside.

The two isolated flavonoids (**I**) and (**II**) exerted a free radical scavenging effect in the DPPH decoloration assay (Table 3). It was noticed that the decoloration percentage was increased proportionally to flavonoid concentration. The flavonoid **I** ; Quercetin-3- [rhamnosyl (1-6) glucoside] was stronger than flavonoid **II**; Isorhamnetin 3-O- gluoside 7-O- rhamnoside, as its decoloralion percentage at high concentrations 100 and 50 µg/ml was more three times (Table 3). The degree of hydroxylation of the two flavonoids (Fig. 4) is responsible for the difference in their free radical scavenging activity **(Daniel 1989).** The antioxidant activity of flavonoid compounds is essentially due to the ease with which a hydrogen atom from an aromatic hydroxyl (OH) group can be donated to a free radical and the ability of an aromatic compound

to support an unpaired electron as a result of delocalization around the μ electron system **(Pratt and Hudson, 1990).** Structural activity relationship of flavonoids **(Pathak *etal.* 1991)** revealed that a C-2, C-3 double bond, a ketone function at C-4, two hydroxyl groups meta to each other on ring A and two hydroxyl groups ortho to each other on ring B enhanced the activity.

Ethanol extract of *Ruta chalepensis* has a protective effect against Fe^{+2}/ ascorbate model system and has also scavenging activity against DPPH radical and free radical mediated DNA-sugar damage. This effect is in part due to the two isolated flavonoids, i.e Quercetin 3- [rhamnosyl (1-6) glucoside] (**I**) and Isorhamnetin 3-O-glucoside 7-O-rhamnoside (**II**) which isolated from the ethanol extract of the leaves of this plant.

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التقييم المعملى لتأثير بعض مستخلصات الايثانول النباتية كمضادات للاكسدة وفصل المركبات الفعالة كمضادات للاكسدة من أوراق نبات الروتا كاليبنينسس

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تم إجراء التقييم المعملى لتأثير مستخلص الإيثانول المائى ٨٠% لاوراق نباتات الروتا كاليبنينسس وجومفوكاربس سينيكس وسيجرتيا ثيا وأكاسيا هولوسيركيا كمضادات للاكسدة عن طريق تقدير تاثيراتها على إزالة اللون الناتج عن الشق الحر ثنائى فينيل بكريل هيدرازيل و تثبيط اتلاف سكريات الحمض النووى الداى أوكسي ريبوز وكذلك تثبيط اكسدة الدهون. أوضحت الدراسة أن مستخلص أوراق نباتات الروتا كاليبنينسس وجومفوكاربس سينيكس لهما أقوى تاثير كمضادات للاكسدة. ولقد تم إخضاع مستخلص الإيثانول المائى لاوراق نبات الروتا كاليبنينسس لفصل وتعريف التركيب الكيميائي للمركبات المسئولة عن فعالية هذا النبات وتم فصل مركبان من الفلافونويدات ذو فعالية كمضادات للاكسدة بواسطة طرق الفصل الكروماتوجرافى (الطبقة الرقيقة والأعمدة) وأمكن التعرف علـى التركيب الكيميائي للمركبان الفعالان عن طريق إستخدام طرق التحليل الطيفى (الأشعة الفوق بنفسجية والـرنين المغناطيسي للكربون ١٣ والبروتـون ١) حيث وجد أنهما على النحو التالى: (I) كرسيتين ٣- [رامنوزيل (١-٦) جلوكوسيد] (**II**) أيزورامنيتين ٣- جلوكوسيد-٧- رامنوسيد.