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## Chemical and Biological Investigation of Essential Oil of *Oroxylum indicum* L. Leaves Cultivated in Egypt.

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### ABSTRACT

Hydrodistilled essential oil of fresh leaves of *Oroxylum indicum* L. family Bignoniaceae cultivated in Egypt was analyzed by gas chromatography–mass spectrometry (GC/MS). The yield of essential oil was (0.2 %). Thirty nine components were identified representing (89.85%). Ar-tumerone (19.37%), a sesquiterpene ketone, was the major constituent in the oil followed by methyl hexadecanoate (6.2%), laurenan-2-one (5.59%) and isopropyl butanoate (5.57%). The oxygenated constituents represents (63.81 %) while non- oxygenated constituents represents (26.04 %). The oil showed significant cytotoxic activity on MCF7 (breast carcinoma cell line), HEPG2 (liver carcinoma cell line) and HELA (cervix carcinoma cell line), in addition to good *in vivo* and *in vitro* antioxidant activity. The oil also exhibited both hepatoprotective and hepatocurative action, but showed weak antimicrobial activity against tested bacteria with no effect against fungi.

**Keywords :** *Oroxylum indicum* L., essential oil, antioxidant, cytotoxic, hepatoprotective and hepatocurative activity.

### INTRODUCTION

*Oroxylum indicum* L. Vent. belongs to the family Bignoniaceae which includes about 116–120 genera and 650–750 species. Genus *Oroxylum* contains only one species which is *indicum*. *Oroxylum indicum* L. Vent tree is distributed in India, Ceylon, Malaysia, Cochin, China, Philippines, Thailand, and Indonesia. The plant is an important herbal medicine in many Asian countries and is used in folk medicines as a cure of various diseases. Root bark is used in fever, bronchitis, intestinal worms, leucoderma, asthma, inflammation, anal troubles, etc. The fruits and seeds are used as expectorant, purgative, and bitter tonic. In Hindu medicine, the root bark, stem, and leaf are prescribed for snake bite, diarrhea, and dysentery Yadav, A. K. *etal* (2012)<sup>1</sup>. Plant is reported to possess anti-inflammatory, analgesic, diuretic, gastroprotective, anti-arthritis, antimutagenic, antifungal, and antibacterial activities Zaveri and Jain (2010)<sup>2</sup>. It supplements traditional ayurvedic medicines to alleviate thirst, rheumatism, dysentery, anorexia, bronchitis, eruptive fevers, and dropsy. Phytochemically, the plant contains flavonoids like baicalein, chrysin, oroxylin A, apigenin, tetuin, and scutellarin. Plant was also reported to contain pterocarpanoids, phenylethanoids, cyclohexylethanoids, sterols and volatile oil. Ali *et al* (1999)<sup>3</sup>

Nothing was traced about the essential oil as a constituent of the plant under investigation so, the following research was carried out to study the chemical composition of the essential oil present and its bioactivity

### MATERIALS AND METHODS

#### Plant material

The leaves of *Oroxylum indicum* L. were obtained from trees cultivated in Zoo Garden, Giza. The plant was kindly authenticated by the agriculture engineer, Terasa Labib, Orman Garden. Voucher specimen were kept at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University voucher no. (1.12.2014.1)

#### Materials for testing the antimicrobial activity

**Microorganism:** the following microorganisms were used from Department of Microbiology, Faculty of Science, Cairo university:

Gram-positive bacteria *viz Staphylococcus aureus* and *Bacillus subtilis*.

Gram-negative bacteria *viz Esherichia coli* and *Pseudomonas aeruginosa*

Fungi *viz Candida albicans* and *Aspragillus niger*.

Culture media: Mueller-Hinton agar

#### Standard antimicrobial agents

Standard discs of Tetracycline (antibacterial agent) (Sigma Chemical Co., St. Louis, Mo, and U.S.A)

Amphotericin B (antifungal agent) (Sigma Chemical Co., St. Louis, Mo, and U.S.A)

DMSO (Sigma Chemical Co., St. Louis, Mo, and U.S.A)

#### Materials for cytotoxic activity

Three human cancer cell lines: MCF7 (breast carcinoma cell line), HEPG2 (liver carcinoma cell line) and HELA

(cervix carcinoma cell line ) from National Cancer Institute, Cairo.

Dimethylsulphoxide (DMSO) (Sigma Chemical Co., St. Louis, Mo, and U.S.A)

RPMI-1640 medium (Sigma Chemical Co., St. Louis, Mo, and U.S.A)

Sodium bicarbonate (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Trypan blue (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Fetal Bovine Serum (FBS) (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Penicillin/Streptomycin (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Trypsin (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Acetic acid (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Sulphorhodamine-B (SRB) (Sigma Chemical Co., St. Louis, Mo, U.S.A)

Trichloroacetic acid (TCA) (Sigma Chemical Co., St. Louis, Mo, U.S.A)

*Materials for in vitro antioxidant activity*

Vitamin C (Sigma Chemical Co., St. Louis, Mo, U.S.A)

1,1-Diphenyl-2-picryl-hydrazil (DPPH) (Sigma Chemical Co., St. Louis, Mo, U.S.A)

*Materials for in vivo antioxidant ,hepatoprotective and hepatocurative activity*

Table 1: Results of identified components by GC/MS of the essential oil of *Oroxylum indicum* L. leaves

Peak No.	KI	M <sup>+</sup>	Base peak M/z	Identification Common name(Chemical name)	Formula	%
1	844	130	43	Isopropyl butanoate (butanoic acid, 1-methyl ester)	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub>	5.57
2	1065	120	105	Acetophenone (phenylethanone)	C <sub>8</sub> H <sub>8</sub> O	1.68
3	1241	148	133	Cuminaldehyde (benzaldehyde,4-(1-methylethyl)	C <sub>10</sub> H <sub>12</sub> O	4.84
4	1364	190	69	Damascenone<(Z)-β-> (2-buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-,(2z)	C <sub>13</sub> H <sub>18</sub> O	0.56
5	1408	204	41	Caryophyllene<(Z)-> (bicycle(7.2.0)undec-4-ene,4,11,11-trimethyl-8-methylene-,(1R-(1R*,4Z,9S*))	C <sub>15</sub> H <sub>24</sub>	0.60
6	1436	204	121	Elemene<δ-> (cyclohexane,1-ethenyl-1-methyl-2-(1-methylethylene)-4-(1-methylethlidene)-,(1R-trans)	C <sub>15</sub> H <sub>24</sub>	1.16
7	1441	204	41	Aromadendrene (1H-cyclopropylazulene,decahydro-1,1,7-trimethyl-4-methylene-,(1aR-(1aα,4aα,7a,7aβ,7bα))	C <sub>15</sub> H <sub>24</sub>	0.52
8	1487	192	177	Ionone<(E)- β-> (3-buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-,(E)	C <sub>13</sub> H <sub>20</sub> O	0.61
9	1496	198	43	Tridecanone<2-> (2- tridecanone)	C <sub>13</sub> H <sub>26</sub> O	0.67
10	1505	204	41	Farnesene<(E,E)- α -> (1,3,6,10-dodecatetraene,3,7,11-trimethyl-,(E,E)	C <sub>15</sub> H <sub>24</sub>	0.78
11	1505	204	41	Bisabolene< β> (cyclohexene,4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-,(Z)	C <sub>15</sub> H <sub>24</sub>	0.79
12	1515	204	119	Curcumene< β -> (1,4-cyclohexadiene,1-(1-1,5-dimethyl-4-hexenyl)-4-methyl)	C <sub>15</sub> H <sub>24</sub>	1.12
13	1583	220	41	Caryophyllene oxide (5-oxatricyclo(8.2.0.0.4,6) dodecane,4,12,12-trimethyl-9-methylene-,(1R-(1R*,4R*,6R*,10S*))	C <sub>15</sub> H <sub>24</sub> O	1.84
14	1585	230	55	Octanedioicacid,diethyl ester (Octanedioicacid,diethyl ester)	C <sub>12</sub> H <sub>22</sub> O <sub>4</sub>	1.37
15	1594	222	161	Carotol (3a(1H)-azulenol,2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-,(3R-(3α,3αα,8αα))	C <sub>15</sub> H <sub>26</sub> O	1.59
16	1595	218	119	Tumerone<ar-dihydro-> (4-heptanone,2-methyl-6-(4-methylphenyl)	C <sub>15</sub> H <sub>22</sub> O	1.30
17	1667	220	41	Caryophyllene<14-hydroxy-(z)->	C <sub>15</sub> H <sub>24</sub> O	1.17

Table 1: Results of identified components by GC/MS of the essential oil of *Oroxylum indicum* L. leaves

Peak No.	KI	M <sup>+</sup>	Base peak M/z	Identification Common name(Chemical name)	Formula	%
18	1669	216	83	(bicyclo(7.2.0)undec-4-ene-4-methanol,11,11-dimethyl-8-methylene-,(1R,4Z,9S)) Tumerone<ar>	C <sub>15</sub> H <sub>20</sub> O	*19.37
19	1672	214	55	(2-heptene-4-one,2-methyl-6-(4-methylphenyl)) Tetradecanol<n->	C <sub>14</sub> H <sub>30</sub> O	1.08
20	1675	122	120	(1-tetradecanol) Salicylic acid,hexyl ester	C <sub>13</sub> H <sub>18</sub> O <sub>3</sub>	1.59
21	1723	242	74	(benzoic acid,2-hydroxy-,hexyl ester) Methyl tetradecanoate	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	0.66
22	1800	254	57	(tetradecanoicacid,methyl ester) Octadecane<n->	C <sub>18</sub> H <sub>38</sub>	3.49
23	1833	240	41	(octadecane) Cyclopentadecanolide	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	3.42
24	1900	268	57	(oxacyclohexadecan-2-one) Nonadecane<n->	C <sub>19</sub> H <sub>40</sub>	1.50
25	1921	270	74	(nonadecane) Methyl hexadecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	6.20
26	1934	254	41	(hexanoicacide,methyl ester) Cyclohexadecanolide	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	0.60
27	2000	282	57	(oxacycloheptadecan-2-one) Eicosane<n->	C <sub>20</sub> H <sub>42</sub>	1.94
28	2085	294	67	(eicosane) Methyl linoleate	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	2.44
29	2116	288	55	(9,12-octadecadienoic acid,(Z,Z)-,methyl ester) Laurenan-2-one	C <sub>20</sub> H <sub>32</sub> O	5.59
30	2125	298	74	(not assigned) Methyl octadecanoate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	0.66
31	2200	310	57	(octadecanoic acid ,methyl ester) Docosane<n->	C <sub>22</sub> H <sub>46</sub>	1.91
32	2300	324	57	(docosane) Tricosane<n->	C <sub>23</sub> H <sub>48</sub>	1.58
33	2338	308	43	(tricosane) Manool oxide<3-α14,15-dihydro->	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	1
34	2800	394	57	(1H-naphtho(2,1-b)pyran-8-ol,3-ethyl)dodecahydro-3,4a,7,7,10a-pentamethyl-,(3S-(3α,4αβ,6αα,8α,10αβ10βα)) Octacosane	C <sub>28</sub> H <sub>58</sub>	0.54
35	2900	408	57	(octacosane) Noncosane	C <sub>29</sub> H <sub>60</sub>	0.80
36	3000	422	57	(noncosane) Triacontane	C <sub>30</sub> H <sub>62</sub>	1.43
37	3200	450	57	(triacontane) Dotriacontane	C <sub>32</sub> H <sub>66</sub>	4.61
38	3300	464	57	(dotriacontane) Tritriacontane	C <sub>33</sub> H <sub>68</sub>	1.97
39	3400	478	57	(tritriacontane) Tetratriacontane	C <sub>34</sub> H <sub>70</sub>	1.30
Total identified compounds						89.85 %
* Major component of the oil.						

*Experimental animals*

Adult male albino rats of Sprague Dawely Strain of 130-150 g body weight. The animals were kept under the same hygienic conditions and on a standard laboratory diet

consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%),cellulose (0.2%),casein-95% pure (10.5%) and starch(54.3%).

*Drugs,chemicals and kits*

Alloxan; Sigma Co.

Vitamin E (dl  $\alpha$ -tocopheryl acetate); Pharco Pharmaceutical Co., it is available in the form of gelatinous capsules ;each contains 400 mg vitamin E.

Silymarin (Sedico Pharmaceutical Co., 6 October, Egypt).  
Carbon tetrachloride (analar)

Biodiagnostic kit : used for the assessment of blood glucose level.

Biodiagnostic transaminase Kits: used for assessment of serum AST (Aspartate Amino Transferase), ALT (Alanine Amino Transferase) and ALP (Alkaline Phosphatase) enzymes

Biodiagnostic glutathione Kit: used for assessment of antioxidant activity.

#### Preparation of essential oil

Fresh leaves of *Oroxylum indicum* L.(500 g) were covered with sufficient water in a round flask and subjected to hydrodistillation in Clavenger's apparatus for two hours. The collected essential oil was subsequently dried over anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and stored under refrigeration at  $< 4^\circ\text{C}$  until analyzed by GC/MS. Egyptian Pharmacopea (2012)<sup>4</sup>.

#### GC/MS analysis

GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness).Electron ionization mass detector (EI/MS) was used for GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at  $280^\circ\text{C}$ . The oven temperature was programmed at an initial temperature  $40^\circ\text{C}$  (hold 3 min) to  $280^\circ\text{C}$  as a final temperature at an increasing rate of  $5^\circ\text{C}/\text{min}$  (hold 5 min).

#### Identification of components

A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the Wiley7 , Nist05 library data of the GC/MS system, as well as, by comparing their mass fragmentation with published data [Adams, (2009)]<sup>5</sup>. The quantification of all the identified components was investigated using a percent relative peak area. Results are compiled in table (1& 2).

#### Antimicrobial activity

Antimicrobial activity of the tested samples were determined using a modified Kirby-Bauer disc diffusion method Bauer, A.W. *et al* (1966)<sup>6</sup> and National Committee for Clinical Laboratory Standards. (2002)<sup>7</sup>. This was achieved using discs made from Whatman filter paper (5mm diameter).The oil was diluted 1/5 v/v in DMSO (10  $\mu\text{l}$  of pure oil/disc) and added to the discs.50  $\mu\text{l}$  of DMSO was used as a negative control and discs of both tetracycline and amphotericin B (5  $\mu\text{l}$  /disc) were used as positive controls. Results are compiled in table (3).

#### In vitro cytotoxicity

The potential cytotoxicity of the essential oil of leaves against MCF7 (breast carcinoma cell line ),HEPG2 (liver carcinoma cell line )and HELA (cervix carcinoma cell line ) was performed adopting sulforhodamine B stain (SRB) assay Skehan. P. *etal* (1990)<sup>8</sup>. The dose of the oil which

reduces survival to 50 % (IC<sub>50</sub>) was calculated from graphed dose response data Fig.(1) using table (4) .

#### In vitro antioxidant activity

The free radical scavenging activity of the essential oil of leaves of *Oroxylum indicum* L. was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Shimada *et al.* (1992)<sup>9</sup>, Oktay, M., *et al.*(2003)<sup>10</sup>. The oil was dissolved in 50 % methanol at concentrations of 25,50,100,200,300 and 400  $\mu\text{g}/\text{ml}$ , then mixed with 1 ml of DPPH in methanol (0.02 g/1000 ml methanol) .The solutions were incubated for 30 min in the dark at room temperature; the reduction of DPPH free radicals was measured by reading the absorbance at 517 nm against the blank. Ascorbic acid (vitamin C) was used as a standard for antioxidant activity, different concentrations of vitamin C was prepared from stock solution (0.1 g/100 ml methanol).Results are illustrated at table (5) and Fig (2).

#### In vivo antioxidant activity

The antioxidant activity was estimated by determination of blood glutathione level in the blood of alloxan-induced diabetic rats adopting the method of Beutler *et al* (1963)<sup>11</sup> and using vitamin E as standard (positive control).

Twenty four adult male albino rats of Sprague Dawely Strain (130-140 g) were used. One group (6 animals) received 1ml saline and kept as a negative control, while diabetes was induced in eighteen rats according to the method described by Eliasson and Samet (1969)<sup>12</sup> using a single dose of 150 mg alloxan/Kg body weight by a single intraperitoneal injection . Hyperglycemia was assessed after 72 hours by measuring blood glucose level (Trinder 1969)<sup>13</sup>. Eighteen diabetic rats were divided into three groups (6 animals each)

First group: normal rats received 1ml saline and kept as a negative control

Second group: diabetic untreated rats receive 1 ml saline

Third group: diabetic rats received the reference drug, 7.5 mg/kg b.wt Vitamin E (positive control).

Fourth group: diabetic rats received 10 mg/kg bwt of volatile oil

Rats were kept one week before the determination of glutathione in blood

Sample: Fresh heparinized blood after seven days for estimation of blood glutathione level Beutler *et al* (1963)<sup>11</sup>. Concentration using biodiagnostic kit and measure the absorbance at 405nm using Shimadzu spectrophotometer. The blood glutathione level was calculated according to the following equation:

$$\text{GSH blood} = A \text{ sample} \times 66.66 \text{ (mg/dl)}$$

The level of blood glutathione in diabetic rats was restored after oral administration of volatile oil.

The data obtained were statistically analyzed using Student's t-test. The results of antioxidant activity were recorded in table (6) and Fig (3) .

#### Hepatoprotective and hepatocurative activity

##### Induction of Liver Damage

Liver damage in rats is induced according to the method of (Klassen and Plaa 1969)<sup>14</sup> by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride ( $\text{CCl}_4$ ) in liquid paraffin.

##### Experimental Design

Table 3: Antimicrobial activity of essential oil of the leaves of *Oroxylum indicum* L. cultivated in Egypt

Sample	% Inhibition Zone					
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Control (DMSO)	-	-	-	-	-	-
Standard Tetracycline	100%	100%	100%	100%	-	-
Amphotericine B	-	-	-	-	100%	100%
Essential oil	30%	37.9%	45%	48.5%	-	-

Table 2: Percentages of the different classes of constituents in the volatile oil of *Oroxylum indicum* L.

Class of volatile constituents	Percentage
<i>Non oxygenated</i>	26.04
Sesquiterpenes	4.97
Aliphatic hydrocarbons	4.54
<i>Oxygenated</i>	63.81
Esters	17.83
Sesquiterpene esters	0.66
Alcohols	1.08
Sesquiterpene alcohols	2.76
Ketones	4.12
Sesquiterpene ketones	24.09
Diterpene ketones	5.59
Monoterpene aldehydes	4.84
Diterpene oxide	1
Sesquiterpene epoxide	1.84

Table 4: IC50 of essential oil on different cell lines (Surviving fraction)

Conc(µg/ml)	HEPG2	MCF7	HELA
0	1	1	1
1	0.795	0.871	0.911
2.5	0.659	0.714	0.919
5	0.601	0.589	0.702
10	0.378	0.401	0.353
IC 50	7.43	7.31	7.86

Table 5: Antioxidant activity and IC50 values of essential oil of leaves of *Oroxylum indicum* L. compared to vitamin C

Conc (µg/ml)	volatile oil	Vitamin C
25	22.17	34.36
50	31.81	49.12
100	58.2	56.42
150	77.8	58.18
200	79.1	62.39
300	83.5	70.8
400	90.4	82.1
IC50	97.79	90.37

Thirty male albino rats, weighting 130-150g were randomly divided into three groups each of 10 rats.

#### Animal Groups

Group 1: Control group received a daily oral dose 1 ml saline for one week before and after liver damage.

Group 2: Liver damaged rats pretreated with daily oral dose of 10 mg/kg b.wt of volatile oil of *Oroxylum indicum* L. for one week. Administration of volatile oil was continued after liver damage for another week.

Group 3: Liver damaged rats pretreated with daily oral dose of 25 mg/kg b.wt Silymarin drug for one week

Administration of the drug was continued after liver damage for another one week, followed by an overnight fast whole blood was obtained from the retroorbital venous plexus through the eye canthus of anaesthetized rat. Blood samples were collected at zero time, 1 week and 72 hours after CCl<sub>4</sub> injection and after one week interval. Serum was isolated by centrifugation. Serum ALT, AST (Thewfweld 1974)<sup>15</sup>, ALP (Kind and King 1954)<sup>16</sup>. The data obtained were analyzed using the student's t- test (Snedecor and Cochran 1971)<sup>17</sup>. Results are recorded in table (7).

#### RESULTS AND DISCUSSION

Hydrodistillation of fresh leaves of *Oroxylum indicum* L. yielded a viscous yellow essential oil (0.2 % w/w) with an intense aromatic odour. The values of specific gravity and refractive index at 20 °C were determined to be 0.927 and 1.455 respectively. Thirty nine components were identified in the volatile oil representing 89.85% w/w. Results of GC/MS analysis of essential oil were summarized in table (1).

The essential oil of *Oroxylum indicum* L. leaves was found to contain oxygenated constituents (63.81 %) more than non-oxygenated constituents (26.04 %). Oxygenated constituents include esters (17.83%), sesquiterpene esters (0.66 %), alcohols (1.08 %), sesquiterpene alcohol (2.76 %), ketones (4.12 %), sesquiterpene ketones (24.09 %) diterpene ketones (5.59 %), monoterpene aldehyde (4.84%), diterpene oxides (1%), sesquiterpene epoxide (1.84 %) , while non-oxygenated constituents include sesquiterpenes (4.97%) , and aliphatic hydrocarbon represents (4.54 %). The major constituent of volatile oil was Ar-tumerone (19.37%) ( sesquiterpene ketones), followed by methyl hexadecanoate (6.2%) and isopropyl butanoate (5.57%) (ester), while oil contains minor components as laurenan-2-one (5.59%) ( diterpene ketones), damascenone-(Z)-β-(0.56%), ionone-( E)- β-(0.61%) and cyclohexadecanolide (0.6%)( ketones), also caryophyllene-(Z)- (0.6%)(sesquiterpene epoxide).

The antimicrobial activity of essential oil of leaves of *Oroxylum indicum* L. cultivated in Egypt , showed weak

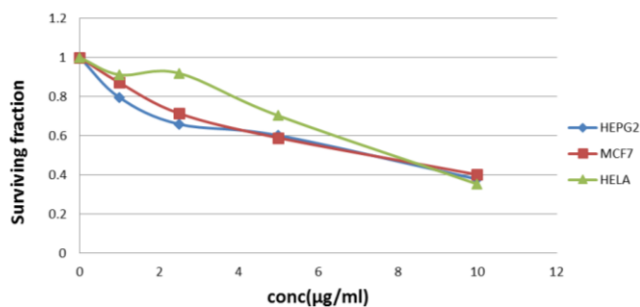


Fig 1: Effect of essential oil of the leaves of *Oroxylum indicum* L. on different cell lines

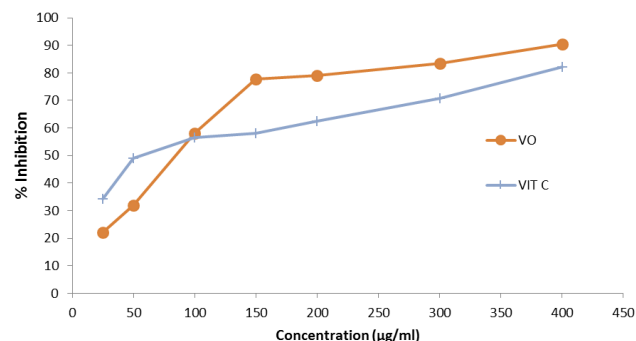


Fig. 2: Assessment of concentration- dependant antioxidant activity of essential oil of leaves of *Oroxylum indicum* L. compared to vitamin C by DPPH assay

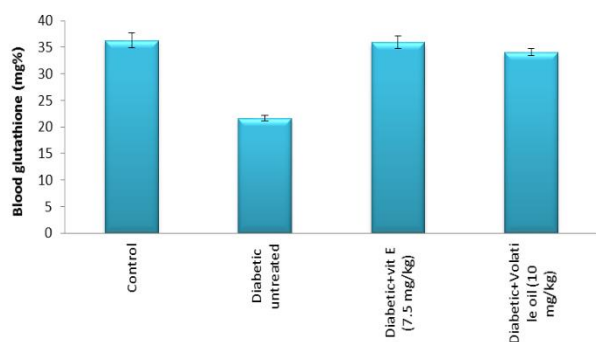


Fig. 3 :Effect of volatile oil of *Oroxylum indicum* L. leaves and vitamin E on blood glutathione level in rats activity against Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) but no activity against tested fungi (*Candida albicans* and *Aspragillus niger*). table (3). This oil under investigation possessed good *in vitro* antioxidant activity as it inhibited the activity of DPPH radicals in a dose dependent manner (IC<sub>50</sub> =97.79 µg/ml) and the activity was similar to that of ascorbic acid (vitamin C), which was used as a standard (IC<sub>50</sub> =90.37 µg/ml),table (5)and Fig (2).The volatile oil also showed good *in vivo* antioxidant activity on comparing its activity to standard Vitamin E , with percent of change 6.1%, table (6)and Fig (3). The oil under investigation showed significant cytotoxic effects on the viability of (HELA) , (MCF7)and (HEPG2) with IC<sub>50</sub> was 7.86 µg/ ml ,7.31 µg/ ml, and 7.43 µg/ ml respectively, table (4) and Fig (1). Concerning the hepatoprotective and hepatocurative activity ,The effect of volatile oil of *Oroxylum indicum* L. in comparison with Silymarin on AST,ALT and ALP serum enzyme levels in liver damaged rats revealed that the volatile oil possessed

Table 6: Antioxidant activity of volatile oil of *Oroxylum indicum* L. leaves and vitamin E drug in male albino rats (n=6)

Group	Blood glutathione (mg%) Mean ± S.E	% change from control
Control (1 ml saline)	36.3±1.4	-
Daibetic	21.6±0.5*	40.5
Diabetic +Vitamin E (7.5mg/kg)	35.9±1.2	1.1
Diab.+ volatile oil (10 mg/kg)	34.1±0.6	6.1

\* Statistically significant from control group at p < 0.01.

both hepatoprotective and hepatocurative activity regarding each enzyme with percentage of change 93.5% and 18.9 % respectively for AST, 74.9 % and 8 % respectively for ALT and 300 %and 28.7 % respectively for ALP, table (7). It is conceivable that the antioxidant and cytotoxic activity of the essential oil of *Oroxylum indicum* L. leaves may be attributed its content of tumerone<ar> (19.37%) Kim, D. *etal* (2013)<sup>18</sup> and Singh,G. *etal* (2010)<sup>19</sup> as a major constituent also may be due to methyl hexadecanoate (6.2%) Wei, L.S. *etal* (2011)<sup>20</sup>, cuminaldehyde(4.84%) Nitoda, T. *etal* (2008)<sup>21</sup> and Sharififar, F. , *etal* (2010)<sup>22</sup>,caryophylleneoxide(1.84 %) Ali, N.A. *etal* (2012)<sup>23</sup>, acetophenone(1.68 %) Opletalova , V. *etal* (2008)<sup>24</sup> and Gonzalez ,A.M. *etal* (2012)<sup>25</sup>,tricosane<n->(1.58 %) Gürsoy,N. *etal* (2012)<sup>26</sup> and Walia,M.,*etal*(2012)<sup>27</sup>,and elemene<δ->(1.16 %)

Table 7: Effect of volatile oil of *Oroxylum indicum* L. in comparison with Silymarin on serum enzyme levels (AST, ALT and ALP) in liver damaged rats

Group	AST (u/L)				ALT (u/L)				ALP (KAU)			
	Zero	7d	72h	7d	Zero	7d	72h	7d	Zero	7d	72h	7d
Control	35.9±1.1	36.3±1.5	139.7±4.9*	145.6±6.2•*	37.8±7.4	37.9±1.6	146.1±7.8*	151.3±6.8*	7.4±0.1	7.5±0.1	52.3±3.4*	59.2±3.8*
Volatile oil	33.7±1.4	33.4±1.1	65.2±2.6	52.9±2.1	35.1±1.2	34.9±1.3	61.4±2.2	56.5±2.3*	7.4±0.7	7.3±0.1	29.6±0.9*	21.1±0.7*
Silymarin 25mg/kg	38.4±1.5	37.6±1.2	51.2±2.8*	36.2±1.4•	41.2±1.7	40.8±1.5	53.6±1.8*	38.2±1.3•	7.8±0.1	7.7±0.1	18.6±0.9*	7.9±0.1•

\* Statistically significant from zero time at p < 0.01; Statistically significant from 72h after CCl<sub>4</sub> at p < 0.01

Chen, C.etal (2013)<sup>28</sup> and Xie, X.F. etal(2014)<sup>29</sup>.The antioxidant and cytotoxic activity of oil can also explain its hepatoprotective and hepatocurative activity .

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