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Separation and Identification of many Volatile oils from the Seeds and Peels of the Fruit of Iraqi *Citrullus colocynthis* (L.) Schrad Plant and Study its Antioxidant Effect

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KEY WORDS:
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ABSTRACT

A current study was included the separation and diagnosis of a number of volatile oils components from the seeds and the peels of a fruit from the Iraqi *Citrullus colocynthis* (L.) Schrad Plant , by using Clevenger apparatus for light compounds and the results were confirmed by using GLC technique .The volatile compounds were identified which included the following : (α-pinene, Limonene, Camphene, Carvacrol, Camphor, Pcymene, Terpinene, Linalool, Menthol, Sabinen and phellanodrene). Terpinene was the highest compound concentration in the seeds and peels (12.14,16.58)% respectively , whereas the lowest concentration in the seeds and peels was Menthol (0.08,0.14) % respectively. The study was also included studying the antioxidant effect of volatile oil separated from the seeds and peels at different concentrations and compared them with the ascorbic acid as a control sample, it was observed that the separated volatile oils from the peels were significantly superior of the free radical inhibition of DPPH (76.1,76.8) % at a concentration (300, 400) $\mu g.ml^{-1}$ respectively compared to the separated volatile oils from seeds.

فصل وتشخيص عدد من الزيوت الطيارة لبذور وقشور ثمرة نبات الحنظل العراقي ودراسة تاثيرها المضاد للأكسدة

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الخلاصة

تضمنت الدراسة الحالية فصل وتشخيص العديد من مركبات الزيوت الطيارة من بذور وقشور ثمرة نبات الحنظل العراقي ، باستخدام جهاز كليفنجر للزيت الخفيف و اثبتت النتائج تشخيص العديد من مركبات الزيوت الطيارة باستخدام تقنية كروماتوغرافيا الغاز السائل والتي تضمنت مايلي : (ألفا- بنين ، اللايمونين ، الكامفين ، الكارفاكرول ،الكامفور ، بارا – سايمين ، التربنين ،اللينالول ، المنثول ، السابنين ، الفيلاندرين) ، وكان التربنين المركب الاعلى تركيز في البذور والقشور (12.14 و16.58)% على التوالي ، بينما اقل تركيز في البذور والقشور كان المنثول (0.08 و 0.14) % على التوالي. وتضمنت

الدراسة ايضاً دراسة التأثير المضاد للأكسدة للزيوت الطيارة المفصولة من البذور والقشور بتراكيز مختلفة ، ومقارنتها مع حامض الاسكوربك بوصفه عينة قياسية ، وقد لوحظ ان الزيوت الطيارة المفصولة من القشور تفوقت معنوياً في تثبيط الجذور الحرة لـــــــ DPPH (76.1 و % 76.8) بتركيز (300 و 400) مايكروغرام / مل على التوالي مقارنة بالزيوت الطيارة المفصولة من البذور.

الكلمات المفتاحية: نبات الحنظل ، الزيوت الطيارة ، مضادات الاكسدة ، الجذر الحر ، حامض الاسكوربك ، DPPH

INTRODUCTION

Citrullus colocynthis (L.) Schrad, a precious plant generally known as Colocynth , which is belong to Cucurbitaceae, reported among all dry regions of world , it is native to Mediterranean area and Asia, as well as it is distributed in deserts of North Africa, South Europe and all Asia (Kapoor *et al*., 2020). It has usually been in the treatment many of diseases like constipation, cough, diabetes, leprosy, toothache, and asthma, *Citrullus. colocynthis* fruits have been utilized in the treatment of pulmonary and urinary infections and its seeds are generally used as antidiabetic and antihypertension agents (Bourhia *et al*.,2021).

Plant has nutritional importance by their content of protein, carbohydrates , fats, oils, mineral, vitamin and water dependable for growth and development in human and animal, phytochemicals are classified to primary and secondary components, that depended on their action in metabolism of plants , the primary metabolism is significant to growth and evolution of plant contain protein , the general sugars, amino acids, , nucleic acids (purines and pyrimidines), chlrophyll, while the metabolites of secondary in plants play a great function in the permanence of the plants in their environments , attract pollinators , natural defenses systems from diseases as well as predators (Velavan *et al*., 2007) Moreover, secondary metabolites like flavonoids, alkaloids, tannin , saponin, steroids, anthocyanin , terpenoids have found commercial applications as drug, dye, flavour, fragrance, insecticide, plants produce those chemicals to safeguard themselves , but recent research shows which numerous phytochemicals can maintain human from diseases such as cancer , diabetic , arthritis , aging , cardiovascular disease (Velavan, 2011 ;Velavan, 2015). Volatile oils, that are complex mixtures of volatile oil component particularly abundant in aromatic plants, are consist of terpenes biogenerated by pathway of the mevalonate. These volatile molecules comprise monoterpenes and sesquiterpenes (Dhifi *et al*.,2016). The oxidation is include the chemical reactions that carry an electron or hydrogen from the material to the oxidizing agents , and the reactions of oxidation may generate the free radicals. There are various diseases in which oxidative stress plays an important role , such as cancer , inflammatory diseases, cardiovascular diseases, and premature aging. The antioxidant ingredient from the plant is efficient in prevention numerous diseases, and antioxidants save biological systems from the free radicals damages (Akar *et al*.,2017).

Kumar *et al*., (2008) was confirmed which free radicals scavenging effect of fruit of *Citrullus colocynthis* increases when increasing concentrations and the biggest antioxidant efficiency, which was be shown at 2500 $\mu g.ml^{-1}$, so the high antioxidant and the free radicals scavenging capability from the fruit extracts were shown at the highest concentrations (2500 $\mu g.ml^{-1}$) , this is may be due to phenolic compounds.

MATERIAL AND METHODS

1- Collection of the fruits:

The seeds of *Citrulluss colocynthis* (L.) Schrad were collected of the Dam region of Mosul and they were classified in the management of the Midicinal plants Development project in the Mosul Dam in Iraqi Minstry of Agriculture as Agricultural Reform . the seeds were grinded after they were cleaned from the soils , and were set their in a paper bags , and then was kept within the conditions away of moisture until used **.**

2- The extraction of Volatile oils with converted Clevenger Apparatus.

Essential oils compounds were extracted of the both from the seeds and peels of the fruit of this plant separately, by using Clevenger apparatus for separated the light oils and connected together with the volumetric flask whose the 500 ml and utilized15 gm from the seeds and peels of *Citrullus colocynthis* (L.) Schrad like powder separately and then mixed for 200ml of distilled water after that the process of distillation was done at the boiling degree100°C and the process of distillation lasted among (1– 2hrs.). 100 ml of the distilled water were put in the separating funnel and 50 ml of ether was added to it for two stage , and the mixture were shaked well and left to settle , the two layers were created, when we obtained the upper layer (ether) was also concentrated with using rotary vacuum evaporator. After that the crude oils were put in the bottle and were protected in the refrigerator until they utilized and diagnosis (British,1958 ; Ismael and Khorsheed,2021).

3- Identification of volatile oil compounds by GLC- analysis.

After the essential oil compounds were separated by using Clevenger Apparatus , they were classified in ministry of the Science and Technology / Dept. of Environment and water by Gas Liquid Chromatography model Shimadzu , from Japanese , 2010 by using ionized flame detector with the temperatures of injection zone and the detector (295 - 330° C) whilst the temperatures of column started from (100- 250°C) at the rate of 8°C / min. and using inefficient nitrogen gas as a carrier gas at the rate of 100 kp.

4- Antioxidant assays

To assay the efficiency of the volatile oils compounds that separated from the seeds and the peels of the fruit of these plants like an antioxidant to see the effect of their efficiency against free radicals , (DPPH) style was utilized , whose is a general abbreviation to an organic chemical compound 2,2-Diphenyl-1-picrylhydrazil , these are free fixed radicals , and the chemical formula (C18H12N5O6) (Ahmed et al., 2019). Where 15.8 mg was taken as weight and then it was dissolved in methanol (200 ml) to get (200 mM) . The different concentrations of the isolated volatile oils compounds at ranged from (100,200,300, 400) Mg.ml $^{-1}$, also the ascorbic acid was utilized as the control samples , as well as (1 ml) of DPPH solutions were added to each concentration and the control samples , next that these samples were incubated in room temperature until (30 min) in the dark , then at a wavelength of (517 nm) , the each sample was measured by - Jenway -UV- 6705 - Spectrophotometer of the British origin , an unicellular , and used the following equation for get the ratio percentage to an inhibiting the free radical (Bourhia et al., 2019)

$$\% = (AbB - AbS) / AbB \times 100$$

: % an inhibition percentage for DPPH

: AbB the absorbance of for the control in (30 min)

: AbS is the absorbance for the sample in (30 min)

The analysis for all the samples were carried out for triplicate. A top percentage (%) value saw a higher antioxidant efficiency in these plant extracts**.**

5- The Statistical Analysis :

The date of the experiment was analyzed utilizing (C.R.D) Completely Randomized Design and utilizing an electronic computer as to the SAS (2002) system program in the factorial experiment to find the analysis of variance and get the significant differences by Duncan method .

RESULTS AND DISCUSSION:

1- The identification of volatile oil compounds of the seeds and peels from fruit of *C. colocynthis* (L.) Schrad by using GLC-analysis:

From the chromatographic analysis of the charts were got in which the CAS No. of the each compound and the time of retention for each compound for current study samples compared to the time of retetion of standard sample retention , and by using GLC technique the identification showed that the study samples agreed in their contents of volatile oil compounds which identified in the seeds and peels and included : (α-pinene, Limonene, Camphene, Carvacrol, Camphor, P-cymene, Terpinene, Linalool, Menthol, Sabinen and phellandrene), the CAS No. (7785- 70- 8, 5989 -27- 5, 79 -92- 5, 499- 75- 2, 76- 22- 2, 99- 87- 6, 562- 74 -3, 78- 70 -6, 2216- 51- 5, 3387- 41- 5, 99- 83- 2) respectively.

α- Pinene (C10H16) is one of a group of monoterpenes whose containing a double bond and they are so appropriate renewable building blocks for a diversity of sustainable materials and polymers (Winnacker , 2018). It has different applications and uses, like flavors , fragrances , fungicidal agent as well as antimicrobial and antiviral agent , in addition, it is one of an ingredient of hepatic and renal drugs , also due to their toxic effects on membranes , pinene are utilized as antibacterial , furthermore , it was found to have inhibitory effects on the leukemia and breast cancers , the implementation of pinene goes beyond natural drugs ; such as they comfirmed to be so adaptable in synthesis of polymers (Salehi *et al* ., 2019). that monoterpene terpnene was found in diverse the volatile oil compounds and it was utilized as a perfume compounds and it is one of the constitutes in charge of for the antioxidants vitality of tea tree oils (Rudbäck *et al* .,2012). The Limonene C10H16 (4-isopropenyl-1-methylcyclohexene) , it is monocyclic monoterpenes hydrocarbons . It is constitute one of a most abundant monocyclic monoterpene in the kingdom of the plants (Ibáñez et al ., 2020) , it is ordinarily utilized flavor additive in the beverages , foods and fragrances so as its cute lemon-like odor (Ravichandran.*et al* ., 2018) .

The highest concentration in the seeds and peels was in Terpinene (12.14,16.58) % respectively, whereas the lowest concentration in the seeds and peels was in Menthol (0.08,0.14) % respectively, that Terpinene was appeared at the highest concentration in the seeds and peels because of Terpinene is not contain the polar hydroxyl group and the opposite of Menthol which appeared at lowest concentration that contain hydroxyl group ,Table (1,2), and Figs (1,2) and Fig (3).

Table (1) The concentrations (%) for volatile oils component with using (GLC) technique of ordinary aqueous extracts of the seeds from the fruit of *Citrullus colocynthis* (L.) Schrad

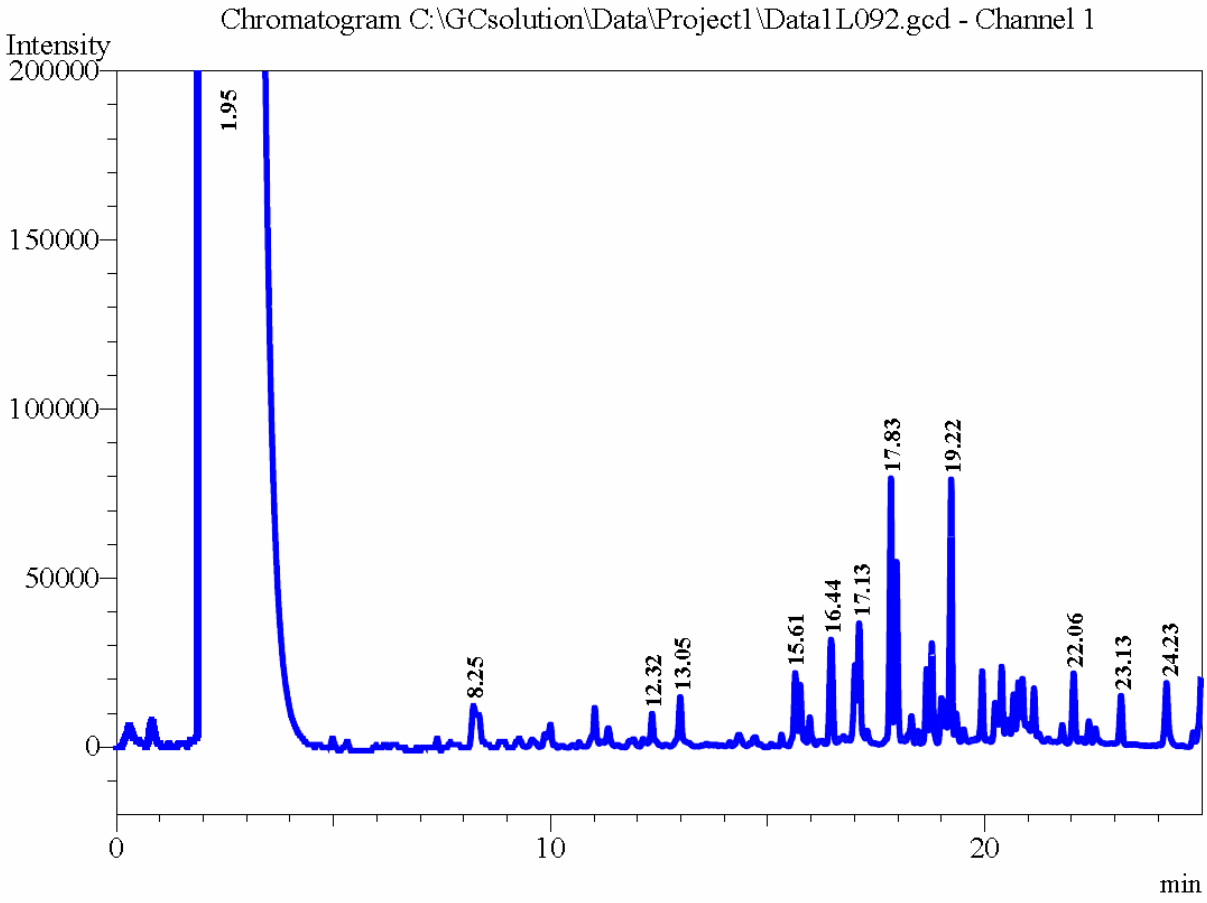
No.	the standard volatile oil	the retention time (min)	area	CAS No.	The concentration (%)
1	α-pinene	8.25	1025	7785-70-8	3.25
2	Limonene	12.32	1895	5989-27-5	5.11
3	Camphene	13.05	2658	79-92-5	2.59
4	Carvacrol	15.61	1014	499-75-2	4.11
5	Camphor	16.44	7524	76-22-2	9.25
6	P-Cymene	17.13	5324	99-87-6	6.25
7	Terpinene	17.83	7456	562-74-3	12.14
8	Linalool	19.22	6521	78-70-6	0.88
9	Menthol	22.06	1895	2216-51-5	0.08
10	Sabinen	23.13	2124	3387-41-5	6.25
11	Phellandrene	24.23	1896	99-83-2	1.00
					50.91

Table (2) The concentrations by % of volatile oils components with using (GLC) technique from ordinary aqueous extracts of the peels from the fruit of *Citrullus colocynthis* (L.) Schrad

No.	the standard volatile oil	the retention time (min)	area	CAS No.	The concentration (%)
1	α-pinene	8.24	1259	7785-70-8	5.58
2	Limonene	12.35	2569	5989-27-5	7.12
3	Camphene	13.00	3241	79-92-5	3.65
4	Carvacrol	15.65	1458	499-75-2	5.44
5	Camphor	16.48	9854	76-22-2	12.25
6	P-Cymene	17.12	6255	99-87-6	8.46
7	Terpinene	17.85	9652	562-74-3	16.58
8	Linalool	19.24	8547	78-70-6	1.36
9	Menthol	22.06	2145	2216-51-5	0.14
10	Sabinen	23.16	2568	3387-41-5	9.58
11	Phellandrene	24.21	2214	99-83-2	1.25
					71.41

Sample Information

Sample Name = volatile oils seeds
Injection Volume = 1 uL
Tem Injector = 295 C
Tem Detector (FID) = 330 C
Column Oven (ZB - 1) = 100 (hold 2 min) - 250 c (8 c / min)
pressure= 100kpa



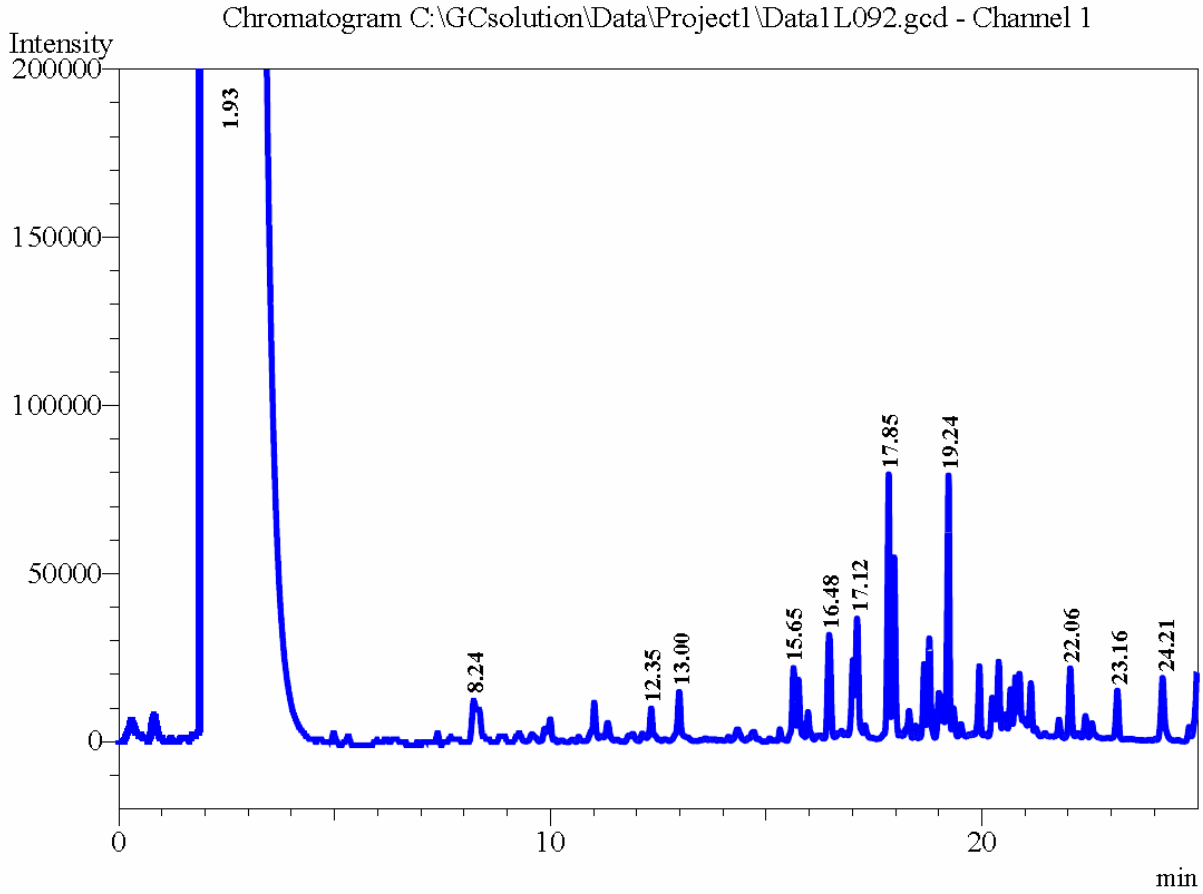
Peak Table - Channel 1

Peak#	Ret.Time	Area	Con %	CAS NO.	Name
1	1.95	45895899			
2	8.25	1025	3.25	7785-70-8	α-pinene
3	12.32	1895	5.11	5989-27-5	Limonene
4	13.05	2658	2.59	79-92-5	Camphene
5	15.61	1014	4.11	499-75-2	Carvacrol
6	16.44	7524	9.25	76-22-2	Camphor
7	17.13	5324	6.25	99-87-6	*P*-Cymene
8	17.83	7456	12.14	562-74-3	Terpinene
9	19.22	6521	0.88	78-70-6	Linalool
10	22.06	1895	0.08	2216–51–5	menthol
11	23.13	2124	6.25	3387-41-5	Sabinen
12	24.23	1896	1.00	99-83-2	Phellandrene
Total		45935231	% 50.91		

Fig(1): The separated volatile oil compounds and identified from the seeds by GLC technique

Sample Information

Sample Name = volatile oils peels
Injection Volume = 1 uL
Tem Injector = 295 C
Tem Detector (FID) = 330 C
Column Oven (ZB - 1) = 100 (hold 2 min) - 250 c (8 c / min)
pressure= 100kpa



Peak Table - Channel 1

Peak#	Ret.Time	Area	Con %	CAS NO.	Name
1	1.93	58932056			
2	8.24	1259	5.58	7785-70-8	α-pinene
3	12.35	2569	7.12	5989-27-5	Limonene
4	13.00	3241	3.65	79-92-5	Camphene
5	15.65	1458	5.44	499-75-2	Carvacrol
6	16.48	9854	12.25	76-22-2	Camphor
7	17.12	6255	8.46	99-87-6	*P*-Cymene
8	17.85	9652	16.58	562-74-3	Terpinene
9	19.24	8547	1.36	78-70-6	Linalool
10	22.06	2145	0.14	2216–51–5	menthol)l
11	23.16	2568	9.58	3387-41-5	Sabinen
12	24.21	2214	1.25	99-83-2	Phellandrene
Total		58981818	% 71.41		

Fig(2): The separated volatile oil compounds and identified from the peels by GLC technique

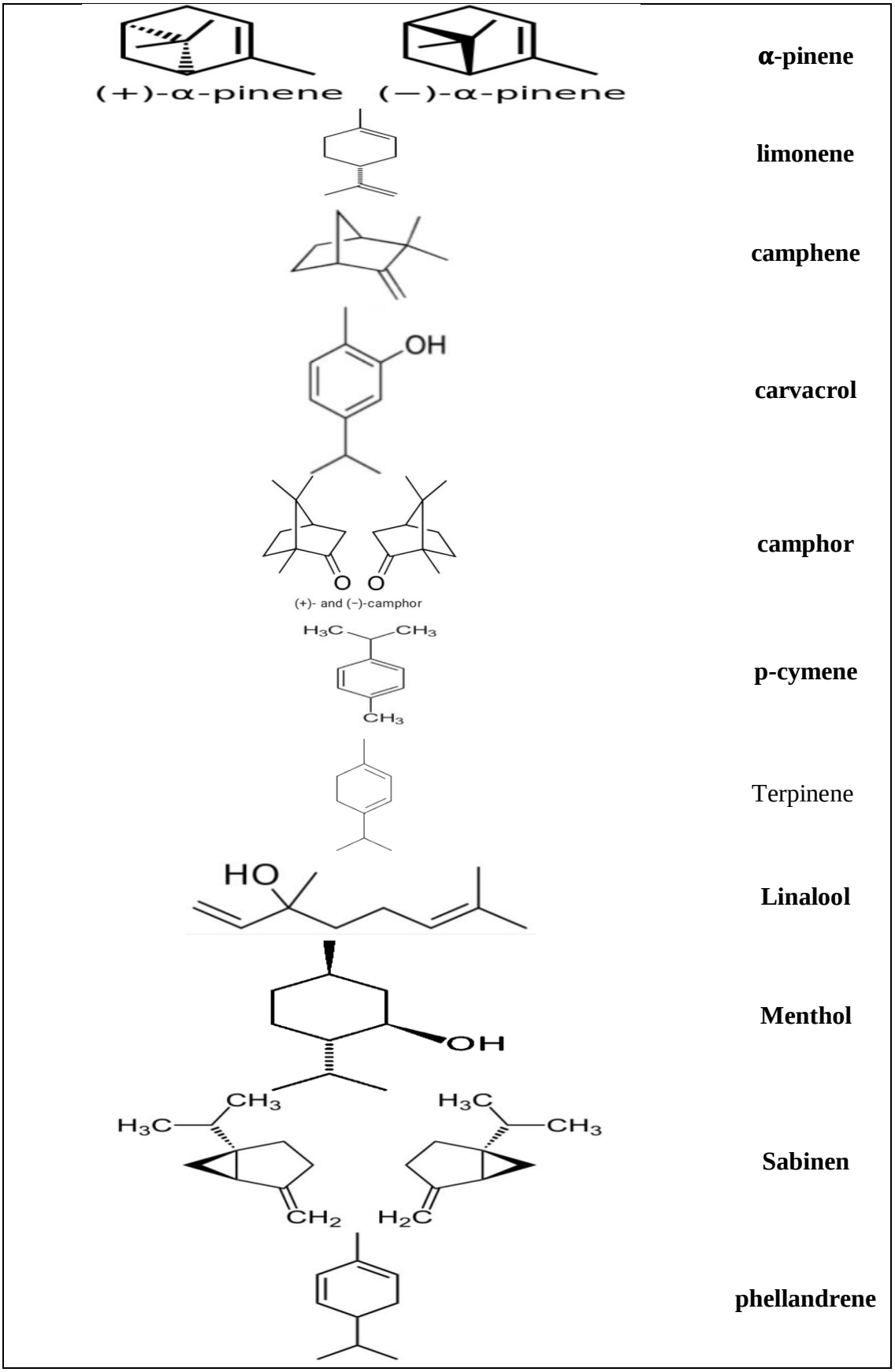


Fig (3): the chemical structures of some volatile oil compounds that identified by using GLC technique for the seeds and peels of the fruit of *Citrullus colocynthis* (L.) Schrad

2- The volatile oil compounds as an antioxidant

The results of Table (3) and Fig indicated that using a separated volatile oil compounds from the seeds and peels of the fruit of *Citrullus colocynthis* (L.) Schrad as an antioxidant in different concentrations and compared them with the ascorbic acid as a control sample, it led to the free radicals inhibition of DPPH, the separated volatile oils from the peels were significantly superior of the free radical inhibition of DPPH at a concentration (300, 400) $\mu g.ml^{-1}$ (76.1,76.8) % respectively. This indicates that the essential oils separated from the peels have a high ability to inhibition the free radicals because this plant has many aromatic compounds that make it rich in protons granted to free radicals, making it stable compared to oils separated from its seeds , and the concentrations of the volatile oil compounds in peels are higher than in the seeds (park *et al*., 2012).

Table (3): The antioxidant activity by DPPH for the separated volatile oils components of the seed and peel of fruit of *C.colocynthis* (L.) Schrad

***Citrullus colocynthis* (L.) Schrad volatile oil compounds**			
Conc($\mu g.ml^{-1}$)	the separated volatile oil compounds from the seeds	the separated volatile oil compounds from the peels	Standard Sample
100	66.4 g	72.2 d	95.1 a
200	68.6 fg	74.4 c	95.7 a
300	69.5 ef	76.1 bc	95.9 a
400	70.7 de	76.8 b	96.1 a

The diverse letters is mean which there are significant variations at the 0.01 possibility level by using the Duncan Multiple Range Tests .

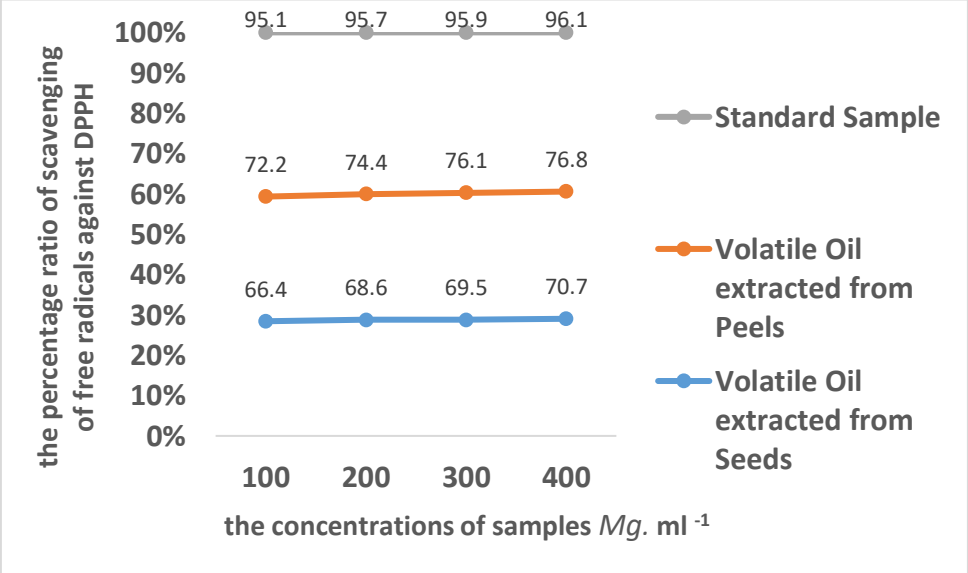


Figure (4): The effectiveness of the separated volatile oil compounds from the seeds and peels of the fruit of *Citrullus colocynthis* (L.) Schrad as an antioxidant compared with ascorbic acid as a standard sample according to the DPPH method.

CONCLUSION

The results that enclosed by (Tables and Figures) , they were confirmed that *Citrullus colocynthis* (L.) Schrad seeds and peels are rich with volatile oil compounds, and that is showed antioxidant activity by measuring their capacity to inhibition the DPPH , and we have the benefit of this study to get the comparison between the xplant (seeds and peels) from the active compounds which were contained .

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