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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:
Citrullus colocynthis (L.) Schrad , volatile oil , antioxidant , free radical , ascorbic acid , DPPH

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ABSTRACT

The current study was included the separation and identification of many volatile oil compounds from the seeds and peels of the fruit of 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, P-cymene, 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 concetrations 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) $Mg.ml^{-1}$ respectively compared to the separated volatile oils from seeds.

فصل وتشخيص عدد من الزيوت الطيارة لبذور وقشور ثرة نبات الحنظل العراقي ودراسة تاثيرها ا لمضاد 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 لـــــ 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 valuable plant commonly known as Colocynth is belongs to Cucurbitaceae, reported among all arid regions of world, it is native to Mediterranean area and Asia,geographically it is distributed in deserts of North Africa, South Europe and whole of Asia (Kapoor *et al*., 2020). It has traditionally been used in the treatment many of diseases such as cough, constipation, leprosy, diabetes, asthma, and toothache, *C. colocynthis* fruits have been used in the treatment of pulmonary and urinary infections and its seeds are commonly used as antidiabetic and antihypertension agents (Bourhia *et al*., 2021).

Plants have main nutritional importance by their content of proteins, carbohydrates, fats, oils, minerals, vitamins and water responsible for growth and development in human and animals, phytochemicals are classified to primary and secondary components, that depended on their role in plant metabolism, Primary metabolism is important for growth and development of plants include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlrophyll, while secondary metabolism in a plant plays a main role in the survival of the plant in its environment, attractions of pollinators, natural defense system against predators and diseases (Velavan *et al*., 2007)., Moreover, secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids have found commercial application as drug, dye, flavour, fragrance, insecticide,plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect human against diseases like cancer, cardiovascular, arthritis, diabetic, aging (Velavan, 2011 ;Velavan, 2015). Essential oils, that are complex mixtures of volatile oil compounds particularly abundant in aromatic plants, are composed of terpenes biogenerated by the mevalonate pathway. These volatile molecules include monoterpenes and sesquiterpenes (Dhifi *et al*., 2016).

Oxidations is chemical reactions that transfers hydrogen or electrons from a substance to an oxidizing agent, and oxidation reactions can generate free radicals. Oxidative stress plays a significant role in diverse diseases like cardiovascular conditions, cancer, inflammatory diseases and early ageing, antioxidant components of plants are also effective in preventing many diseases, and antioxidants protect biological systems against free radical damage. (Akar *et al*., 2017).

Kumar *et al*. (2008) was confirmed that free radical scavenging effect of fruit of *Citrullus colocynthis* increases with increasing concentration and maximum antioxidant activity which was showed at 2500 $Mg.ml^{-1}$, thus the high antioxidant and free radical scavenging ability of the fruit extract was observed at the highest concentration (2500 $Mg.ml^{-1)}$, this is may be due to phenolic compounds. And this plant was classified according to (ITIS, 2010), Table (1)

MATERIAL AND METHODS

Collection of the fruits:

Citrullus colocynthis (L.) Schrad seeds were collected from the Mosul Dam region and then classified in the Directorate of the Midicinal plants Development project in the Mosul Dam of the Iraqi Minstry of Agriculture and Agriculture Reform. After the seeds were cleaned from the dust, they were grinded and put in a paper batch and kept in conditions away from moisture until use.

Volatile oils extracted by converted Clevenger Apparatus.

The volatile oil compounds was extracted from the both of the seeds and peels of the fruit of this plant separately, by using Clevenger device to extract the light oil and connected with a volumetric flask with a capacity of 500 ml and used 15gm of the seeds and peels of *Citrullus colocynthis* (L.) Schrad as powder separately and then mixed with 200ml of distilled water after that the distillation process was carried out with the boiling point 100°C and the process of distillation lasted between (1– 2hrs.). The distilled water was put in separating funnel (100ml) and 50ml of ether was added to it for two stage, shaking the mixture well and then left to settle, two layers were formed, when we obtained the upper layer (ether) was also concentrated by using rotary vacuum evaporator. The crude oil was placed in the bottle and kept in the refrigerator until it used and identified (British, 1958; Ismael and Khorsheed, 2021).

Identification of volatile oils by using GLC- analysis.

The separated volatile oil compounds were identified in the laboratories of the ministry of Science and Technology / Dept. of Environment and water by GLC model Shimadzu, Japanese, 2010 using ionized flame detector and with the injection area and the detector temperatures (295 and 330 ° C) while the column temperature starts from (100- 250 ° C) at rate of 8° C / min. using passive nitrogen gas as a carrier gas at a rate of 100 kp.

Antioxidant assays

To assay the activity of the volatile oil compounds separated from the both of the seeds and peels of the fruit of this plant as an antioxidants to know the effects of their activity against free radical, the method of (DPPH) was utilized, that is a common abbreviation for the organic chemical compound (2,2-Diphenyl-1-picrylhydrazil), that is a free fixed radical, and the chemical formula ($C_{18}H_{12}N_5O_6$) (Ahmed *et al*., 2019). Where the weight of 15.8 mg was taken and dissolved in 200 ml of methanol to obtain 200 mM. Different concentrations of the separated volatile oil compounds at ranged from (100,200,300, 400) $Mg.ml^{-1}$, also ascorbic acid was utilized as a control sample,1 ml of (DPPH) solution was added to each concentration besides the control sample, after which the samples were incubated at room temperature for 30 min. in the dark, each sample was measured at a wavelength of 517 nm. by a (Jenway -UV- 6705) Spectrophotometer of British origin, unicellular, and applied the following equation to find out the ratio percentage for inhibiting free radicals (Bourhia *et al*., 2019)

% = (AbB-AbS)/ AbB*100 were: % is an inhibition percentage of (DPPH), AbB is the absorbance of the control at 30 min AbS is the absorbance of the sample at 30 min, the analysis of all the sample were done in triplicate. A high percentage (%) value showed the higher antioxidant activity of the plant extracts.

Statistical Analysis

Date of the experiment were analyzed using Completely Randomized Design (C.R.D) and using the electronic computer according to the SAS(2002) system programs in factorial experiments to find the analysis of variance and obtain the significant differences by Duncan method .

RESULTS AND DISCUSSION

Chromatographic analysis of the charts were obtained in which the CAS No. of each compound and the retention time of each compound for the study samples compared to the standard sample retention, and the identification by using GLC technique 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 ($C_{10}H_{16}$) is a group of monoterpenes containing a double bond - are very suitable renewable building blocks for a variety of sustainable polymers and materials (Winnacker , 2018).

It has various applications and uses, such as fungicidal agents, flavors, fragrances, and antiviral and antimicrobial agents , in addition, it is a components of renal and hepatic drugs , also, pinene are used as antibacterials due to their toxic effects on membranes ,moreover, it has been found to have inhibitory effects on breast cancer and leukemia , the application of pinene goes beyond natural medicine; for instance, they have been proven to be very flexible in the synthesis of polymers (Salehi *et al* ., 2019).

The monoterpene terpinene is used as a fragrance compound and is present in different essential oils. It is one of the components responsible for the antioxidant activity of tea tree oil (Rudbäck *et al*.,2012). Limonene or 4-isopropenyl-1-methylcyclohexene ($C_{10}H_{16}$) is a monocyclic monoterpene hydrocarbon . It constitutes one of the most abundant monocyclic monoterpenes in the plant kingdom (Ibáñez *et al* ., 2020) , it is a commonly used flavor additive in food, beverages and fragrances for its pleasant 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 (2,3), Figs (1,2) and Fig (3).

Table (2): The concentration (%) of volatile oil compounds by using GLC technique of normal aqueous extract from the seeds of the fruit of *Citrullus colocynthis* (L.) Schrad

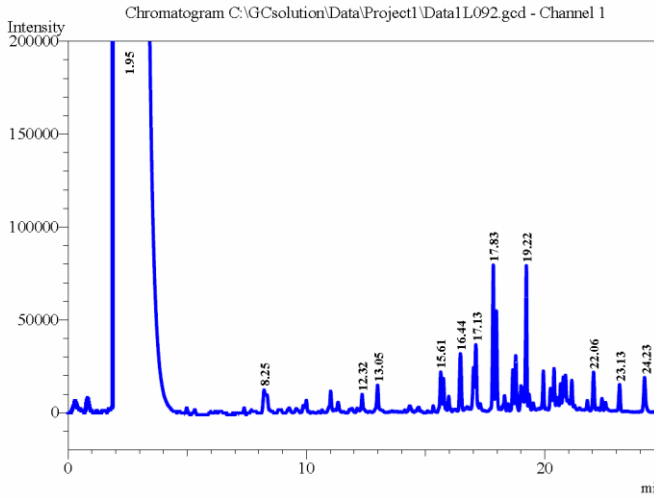
No.	the standard volatile oil	the retention time (min)	area	CAS No.	The concentration (%)
1	α-pinene	8.25	1025	7785-70-8	3.25
2	Limonene	12.32	1895	5989-27-5	5.11
3	Camphene	13.05	2658	79-92-5	2.59
4	Carvacrol	15.61	1014	499-75-2	4.11
5	Camphor	16.44	7524	76-22-2	9.25
6	P-Cymene	17.13	5324	99-87-6	6.25
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 (3) The concentration (%) of volatile oil compounds by using GLC technique of normal aqueous extract from the peels of 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



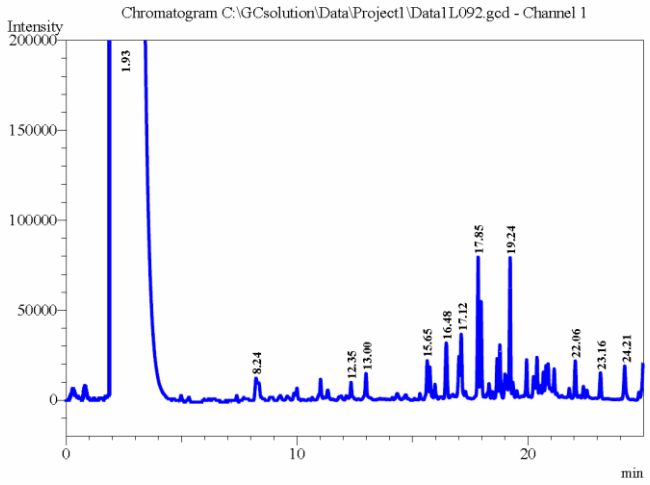
Peak Table - Channel 1

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
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

The results of Table (4) and Fig indicated that using the 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)

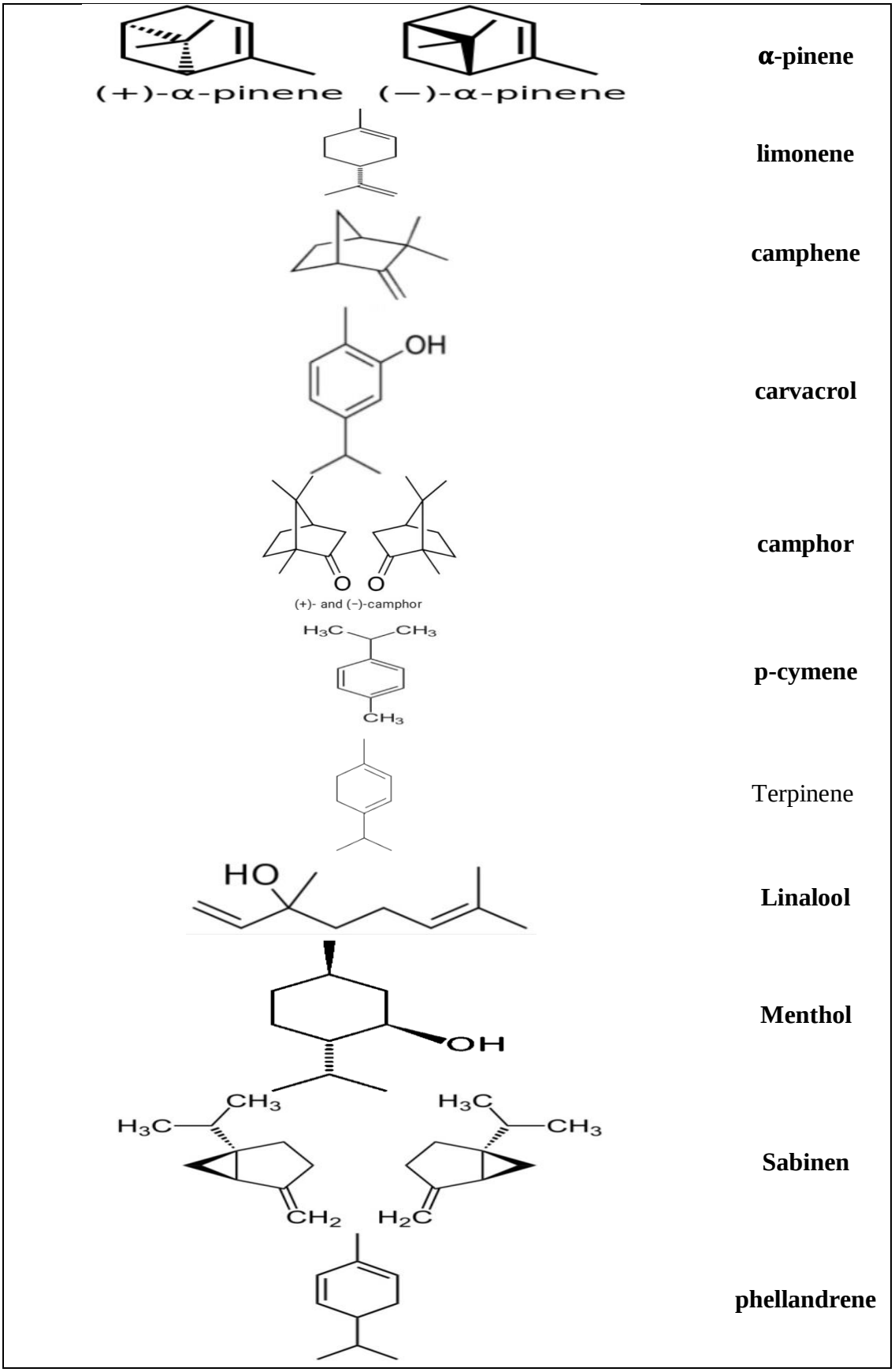


Fig (3): the chemical structures of some volatile oil compounds that identified by using GLC technique for the seeds and peels of the fruit of *Citrullus colocynthis* (L.) **Schrad**

Table (4): The antioxidant activity by DPPH for the separated volatile oil compounds from the seeds and peels of fruit of *Citrullus colocynthis* (L.) Schrad

***Citrullus colocynthis* (L.) Schrad**			
volatile oil compounds			
Conc(𝓜g. 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 different letters mean that there are significant differences at the 0.0001 probability level according to the Duncan Multiple Range Test.

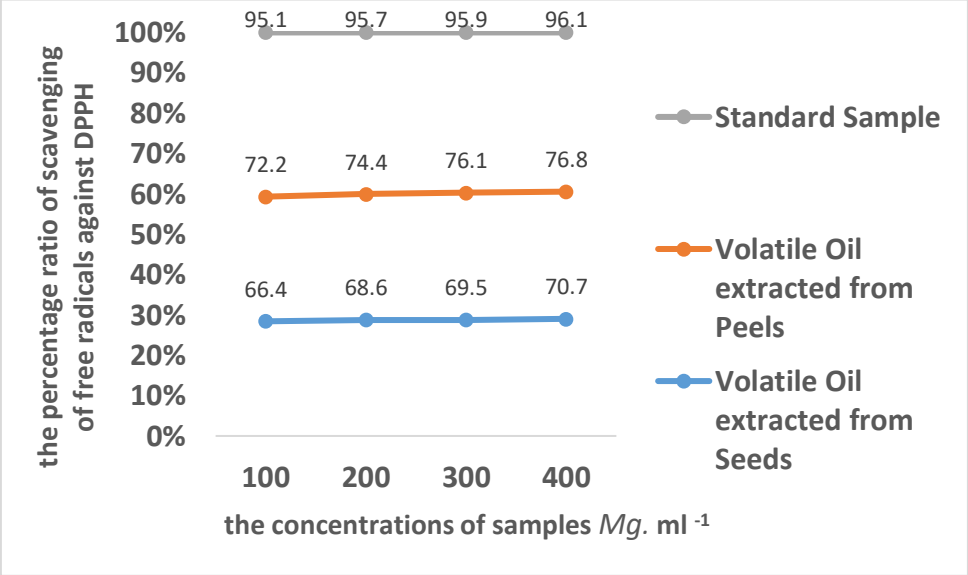


Figure (4): The effectiveness of the separated volatile oil compounds from the seeds and peels of the fruit of *Citrullus colocynthis* (L.) Schrad as an antioxidant compared with ascorbic acid as a standard sample according to the DPPH method.

CONCLUSION

From the results that included (Tables and Figures), it was 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.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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