Chemical Composition of Essential Oils Total Phenols and Antioxidant Activity of Achillea fragrantissima and A. santolina Grown in Syria

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ABSTRACT

The aim of this study is to investigate the active components in the essential oils and determine the total phenol content and antioxidant activity of flowers of *Achillea fragrantissima* and *A. santolina* collected from Al-Kalamoon (Damascus countryside, Syria). Flower oils were extracted and analyzed using gas chromatography-mass spectrometry (GC-MS). Three extracts were prepared using distilled water, methanol, and chloroform. Total phenol content and antioxidant activity were determined for the essential oils as well as for the aqueous, methanolic, and chloroformic extracts. The results revealed the presence of 20 components in the essential oil of *A. fragrantissima*. The major compounds identified were beta-thujone (39.63%), santolina alcohol (15.54%), artemisia ketone (15%), and alpha-thujone (10.58%). Sixteen components were identified in the essential oil of *A. santolina*, with the primary compounds being camphor (49.13%), eucalyptol (17.13%), and terpine-4-ol (8.29%). The essential oil and aqueous, methanolic, and chloroformic extracts of *A. santolina* contained 414.2, 1388.4, 2084.2, and 965.7 mg of TAE/g of dry extract, respectively. In interaction with 2,2-Diphenyl-1-picrylhydrazyl (DPPH), the IC50 values were 105, 120, and 110 µg/L for the aqueous, methanolic, and chloroformic extracts of *A. fragrantissima*, respectively, and 720 and 320 µg/L for the aqueous and methanolic extracts of *A. santolina*, respectively. The essential oils of *A. fragrantissima* and *A. santolina*, as well as the chloroformic extract of *A. santolina*, did not show antioxidant activity. The study demonstrated that the aqueous and methanolic extracts of *A. fragrantissima* exhibit good free radical scavenging activity.

Keywords: *Achillea fragrantissima, A. santolina*, essential oil, total phenols, antioxidant acivity.

1. INTRODUCTION

The genus *Achillea*, belonging to the Asteraceae family, comprises approximately 115 species predominantly found in the temperate regions of the Northern Hemisphere, particularly in northern Africa, southeastern Europe, and southwestern Asia [1]. *Achillea fragrantissima* (Forssk.) Sch. Bip. is a small shrub that grows to a height of 30-60 cm. The leaves are oval with shallow, toothed edges, and the flower heads are disc-shaped terminals composed of yellow tubular flowers [2]. It is commonly used as an antispasmodic to treat rheumatic pain, acute cough, and to lower blood sugar levels [3]. Terpine-4-ol, linalool, carvone, β-phellandrene, artemisia ketone, and α-thujone have been identified as important bioactive compounds in the essential oil of *A. fragrantissima* [4] [5] [1]. Studies have shown that the essential oil can inhibit the growth of many Gram-positive and Gram-negative bacteria [5]. In addition, the aqueous extract has demonstrated cytotoxic activity against cell lines [6] and antitumor activity [7].

A. santolina L. is a herbaceous plant with yellow tubular flowers arranged in disc-shaped inflorescences. The leaves are deeply divided transversely several times,

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giving them a worm-like shape [8]. It is commonly used to treat toothaches, as a tonic and carminative, for the treatment of colic, kidney stones, diabetes, and dysentery [2]. The major compounds of the essential oil of *A. santolina* are camphor, α-pinene, linalool, and fragranyl acetate [9] [10] [1] [11]. Other identified compounds include santolin, stigmasterol, clionasterol, salvigenin, eupatorin, poriferasterol, leukodin, artemistin, and 6,7,3',4'-tetramethoxy-5-hydroxy flavone [12] [8]. Studies have demonstrated its hypoglycemic effect [13] and its ability to inhibit the growth of *Candida albicans* [2]. Medicinal plants are known for their therapeutic and antioxidant effects [14] [15] [16].

This research investigates the chemical composition of essential oils by GC-MS. The phenol content and antioxidant activity of the aqueous, methanolic, and chloroformic extracts, as well as the essential oils of *A. fragrantissima* and *A. santolina*, were also measured.

2.METHODOLOGY

2.1 Materials

All chemicals and reagents used in the study were of analytical grade. Solvents were purchased from Panreac, Spain; Folin-Ciocalteu reagent from Merck KGaA, Germany; anhydrous sodium carbonate from AvonChem, United Kingdom; tannic acid from Rhône-Poulenc LTD Prolabo; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Tokyo Chemical Industry; and butylated hydroxytoluene (BHT) from Titan Biotech.

2.2 Instruments

Gas chromatography instrument: GC-plus MS2010 (Shimadzu), sensitive electronic balance of Shimadzu AX200 (Japan), UV-VIS Spectrophotometer ((T80+), PG Instruments, United Kingdom), rotatory evaporator (RV 10 digital IKA, Germany).

2.3 Sourcing and preparation of extracts

Flowering aerial parts were collected from AL-Kalamoon (Damascus countryside, Syria) between May and June (2018-2020). The identification of the plant was verified by the Department of Plant Biology, Faculty of Science, Damascus University. The plant material was dried at room temperature for 10 days in the shade, then powdered and stored in closed containers until extraction. Essential oil was extracted from 500 g of the plant material using the steam distillation method [17]. Powdered flowers (30 g) were placed in a Soxhlet apparatus, and extraction was performed by adding 250 mL of solvent (methanol or chloroform) at 60°C for 4 hours. The extract was then filtered through filter paper [18]. Flowers (30 g) were also extracted with 200 mL of freshly distilled water using a condenser distillation method [19] for 90 minutes. The extracts were concentrated using a rotary vacuum evaporator, and the extraction yield was calculated using the following equation [10]: Extraction yield % = (weight of dry extract/ weight of dry powdered plant material) × 100.

2.4 GC-Ms analysis

The analysis of the volatile oil components was conducted according to the method described in [17], with some modifications. A gas chromatography instrument with a mass spectrometer detector (GC-MS), GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) was used. The column was an OPTIMA 5 (100% dimethylpolysiloxane USP G1, G2, G38) with a film thickness of 0.25 µm, a column length of 25 m, and a diameter of 0.25 mm. The carrier gas was helium at 54 kilopascals (KPa) pressure. The split ratio was set at 1:30. The injector was automatic, with the injector temperature set at 250°C. The injection mode was split, and the injected sample volume was 1 µL.

Thermal program: The oven temperature was programmed from 80°C to 240°C at a rate of 3°C/min, with a total program time of 62 minutes. The mass spectrometer was operated with an ion source temperature of 200°C and an ionization voltage of 70 eV. Identification

of volatile oil components was performed using the Wiley electronic library.

2. 3 Determination of the total phenolic content in plant extracts

The method published by Abdeltaif et al. was applied to determine the total phenolic content in the samplesaliquot of extract was mixed with 1.58 mL of distilled water, 300 µL of 20% sodium carbonate, and 100 µL of Folin-Ciocalteu reagent. A blanked simultaneously, containing 2 mL of ethanol. The samples were mixed and then left in a dark place at room temperature for 45 minutes. The absorbance was measured at 765 nm. Total phenols were quantified using a calibration curve of tannic acid in ethanol, within the concentration range of 0-500 mg/L (standard curve equation: y=0.0011x + 0.0698 R2=0.9961). Means were calculated from three parallel analyses.

2.4 Evaluation the DPPH scavenging activity

The free radical scavenging activity was measured using a modified version of the method described by . Eight concentrations for each extract and essential oil were prepared (25, 50, 75, 100, 125, 250, 500, and 750 µg/mL in methanol). A series of BHT concentrations (1.5, 3.1, 6.25, 12.5, 25, 50, 75, and 100 µg/L in methanol) were also prepared. The DPPH solution in methanol was prepared at a concentration of 0.04 g/L. The reaction was initiated by adding 0.5 mL of the sample (BHT or extract) to 3.5 mL of DPPH solution. A control was prepared by adding 0.5 mL of methanol to 3.5 mL of DPPH solution. The reaction mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-visible spectrophotometer (Model: T80+, PG Instrument Ltd, United Kingdom). A decrease in absorbance indicates greater radical scavenging activity. Each measurement was repeated three times, and the average was calculated. A calibration curve was generated, and the percentage of scavenging activity was calculated using the following equation:

DPPH scavenging activity
(%) = (Acontrol – Asample) / Acontrol × 100.

Where:
Acontrol: Absorbance of control
(Ethanol + DPPH)
Asample: The absorbance of the sample
(Extract + DPPH).

Butylated hydroxytoluene (BHT) was used as the positive control, and IC_{50} was calculated. Means were calculated from three parallel analyses.

3.RESULTS AND DISCUSSION

3.1 GC-Ms analysis

The essential oil yield from *A. fragrantissima* is 2.4%, wheres that from *A. santolina* is 0.533%.

20 Components were identified in the essential oil of *A. fragrantissima* (Figure1.a). The major compounds found are β-thujone (39.63%), followed by santolina alcohol (15.54%), artemisia ketone (15%), and α-thujone (10.58%) (Table1) (Figure2).

This composition closely resembles the oil studied in wild-grown Egypt [1]. In contrast, essential oil from plants cultivated in Jordan has a different composition, mainly containing terpene-4-ol, linalool, and carvone [4], Which shows the difference in the chemical composition of essential oil depending on the region in which the plant grows and whether it is cultivated or wild-grown.

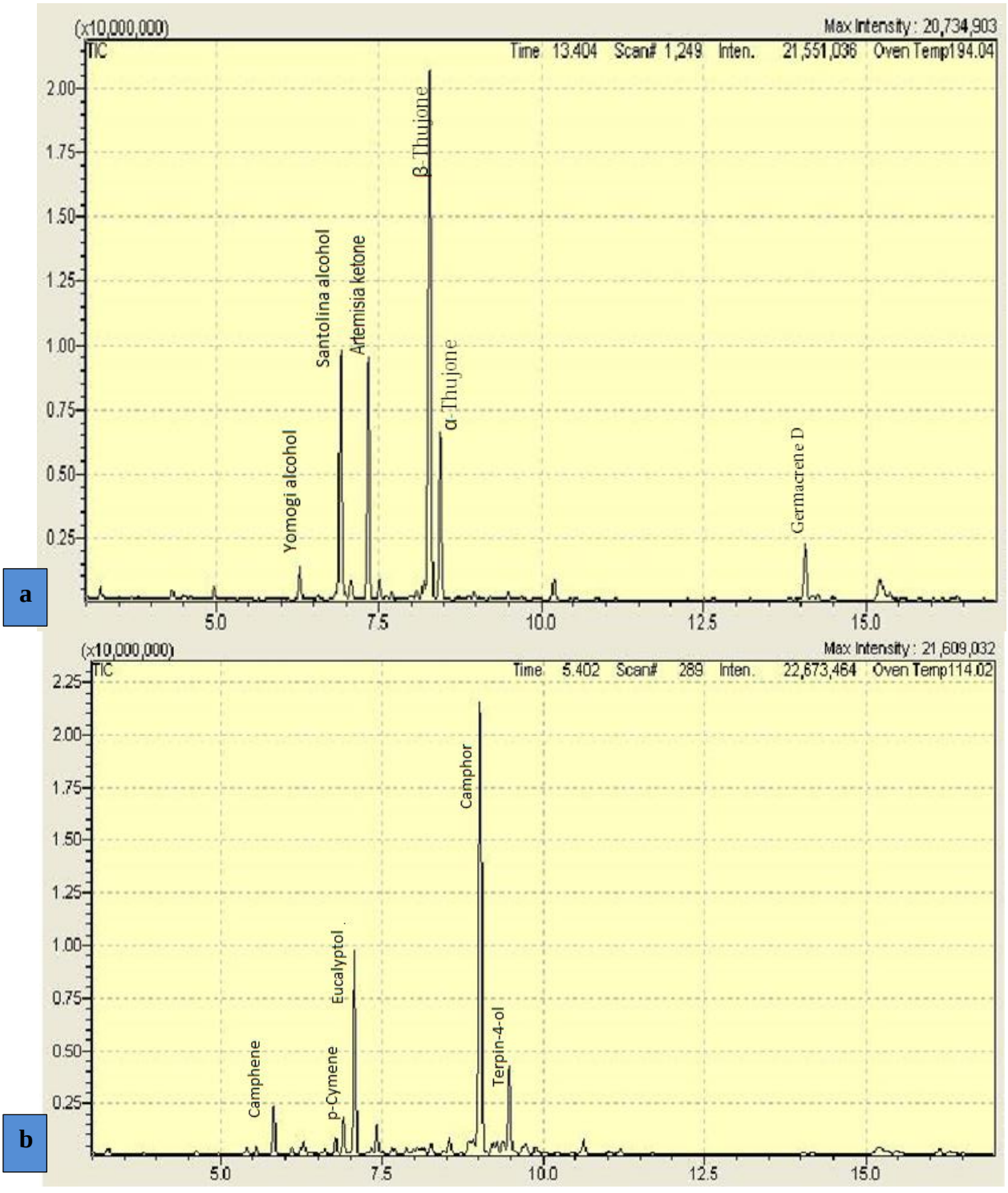

Figure1. a. Chromatogram of the essential oils a. the essential oils *A. fragrantissima*, b. the essential oils *A. santolina.*

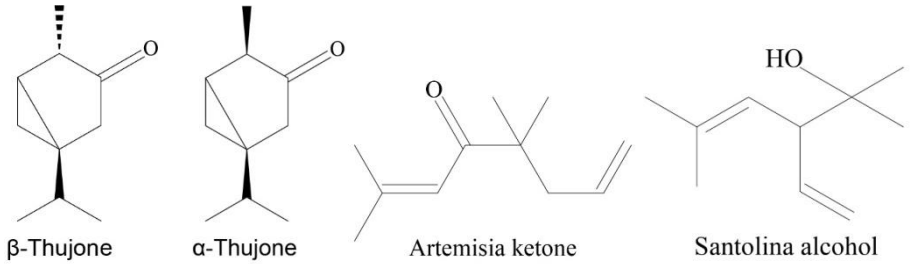

Figure2. Chemical structure of β-thujone, α-thujone, santolina alcohol, and artemisia ketone.

16 Components were identified in the essential oil of *A. santolina* (Figure1.b). The primary compounds are camphor (49.13%), eucalyptol (17.13%), and terpine-4-ol (8.29%) (Table1).

In comparison with other studies on the composition of essential oils, the essential oil studied in Iran contained camphor and eucalyptol, while terpene-4-ol and camphene were absent, and other compounds were present. For example, linalool [9]. Another study conducted in Iran also revealed a different composition, with the essential oil mainly containing fragranyl acetate and fragranol, along with small amounts of camphor, eucalyptol, and terpene-4-ol [10]. This composition is similar to that of the essential oil of the wild plant studied in Egypt [1]. The difference in chemical composition can be explained by the variation of genotypes.

Table 1. Composition of the essential oil of A. fragrantissima, and A. santolina

A. fragrantissima			*A. santolina*		
Component	**RT**	**Percentage**	**Component**	**RT**	**Percentage**
Butanol	3.230	0.67	α-Pinene	5.543	0.54
Methanone	4.325	0.51	Champhene	5.823	3.78
Citronellal	4.505	0.25	Yomogi alcohol	6.288	0.98
Camphene	4.965	0.65	-Terpineneα	6.783	1.32
Yomogi alcohol	6.290	1.76	p-Cymene	6.903	3.03
Iso-amyliso-butyrate	6.562	0.25	Eucalyptol (1.8-Cineole)	7.074	17.13
Santolina alcohol	6.914	15.54	-Terpeneγ	7.424	2.21
Propanoic acid	7.074	1.03	Trans-Sabinene hydrate	8.158	2.50
Artemisia ketone	7.339	15.00	-Thujoneβ	8.265	0.85
Butanoic acid	7.510	1.44	p-Menth-2-en-ol	8.850	1.47
Epoxylinalol	7.617	0.13	Trans-Pinocarveol	8.904	2.54
Artemisia alcohol	7.700	0.40	Camphor	9.026	49.13
Iso-cetronellol	7.800	0.13	-Fenchyl alcoholβ	9.281	1.10
β-Thujone	8.288	39.63	Borneol	9.380	1.26
α-Thujone	8.452	10.58	Terpin-4-ol	9.475	8.29
Artemisyl acetate	8.968	0.34	Myrtanol	9.719	3.31
Norborineol	10.199	1.24			
Germacrene D	14.063	3.94			
Spathulenol	15.367	0.48			
Rosifoliol	16.394	0.19			

3.3 *Yield of extraction*

The yield of extraction ranged from 13.4% in the methanolic extract of *A. fragrantissima* to 4.87% in the aqueous extract of *A. santolina* (Table 2).

3.3 Determination of the total phenolic content

Phenolic compounds ranged from 345.5 µg Tannic acid equivalents/g dry plant in the essential oil of A. fragrantissima to 2084.2 µg Tannic acid equivalents/g dry plant in the methanolic extract of *A. santolina* (Table 2).

In A. fragrantissima, the aqueous extract contains the highest amount of phenols, followed by the chloroform extract, then the methanolic extract, and finally the essential oil. This indicates the presence of both polar and non-polar phenols. Regarding A. santolina, the methanolic extract contains the highest concentration of phenols. The aqueous extract is followed by the chloroform extract, and finally the essential oil, indicating the polar nature of phenols. The following chart displays the total phenol content of both plants.

The inhibitory Concentration (IC_{50}) ranged from 720 µg/L in the aqueous extract of *A. santolina* to 105 µg/L in the aqueous extract of *A. fragrantissima*, whereas BHT had IC_{50} value 16 µg/L (Table 2) [24].

Antioxidant activity was assessed by calculating the IC_{50} of the tested extracts. The results indicate that the methanolic extract of *A. fragrantissima* has the smallest value, followed by the two chloroform extracts, followed by the methanolic extract of the same plant, followed by the methanolic extract, and finally the aqueous extract of *A. santolina*. Note that it was not possible to calculate the IC_{50} values within the studied concentrations for both the essential oils and the chloroform extract of *A. santolina*.

Aqueous extract of *A. fragrantissima* contains the largest content of phenols, and have the highest antioxidant activity. As for the *A. santolina*, methanolic extract contains the largest amount of phenols and its antioxidant activity is greater than that of the other extracts.

The antioxidant activity of different extracts varies according to the composition of the extracts, which may give an idea about the nature of the compounds that have an antioxidant effect. Aqueous, methanolic, and chloroform extracts of *A. fragrantissima* have similar antioxidant activities. This results indicates that the plant contains polar and non-polar antioxidant compounds. As for *A. santolina*, the chloroformic extract did not show antioxidant activity at the concentrations studied, while the aqueous and methanolic extracts have antioxidant activities that can be attributed to the polar compounds present in the plant.

Table 2. Total Phenol Content, and IC_{50} value IC_{50} value *A. santolina*, *A. fragrantissima*, and positive control

	Yield %		**Total Phenols (mg tannic acid equivalents/ g dry extract)**		**IC_{50} (µg/L)**	
	A. santolina	***A. fragrantissima***	***A. santolina***	***A. fragrantissima***	***A. santolina***	***A. fragrantissima***
Methanolic extract	12.46	13.4	2084.2±8.58	657.7±22.5	350±0.1	120±0.1
Chloroform extract	6.76	6.7	965.7±12.6	1402.7±27.81	-	110±0.2
Aqueous extract	4.87	5.1	1388.4±9.55	1490.3±9.43	720±0.2	105±0.3
Essential oil	0.533	2.4	414.2±22.8	345.5±6.12	-	-
BHT						16±0.1

Values are mean ±standard deviation

CONCLUSION

It is concluded that the aqueous, methanolic, and chloroformic extracts of *A. fragrantissima* exhibit antioxidant activity, whereas the essential oil contains compounds, the most prominent of which are β-thujone, santolina alcohol, artemisia ketone, and α-thujone. The aqueous and methanolic extracts of *A. santolina* flowers have demonstrated antioxidant effects, suggesting their potential utility in combating free radicals. In contrast, the chloroformic extract did not exhibit effectiveness at the concentrations tested. The study showed that aqueous and methanolic extracts of *A. fragrantissima* have good free radical scavenging activity. Therefore, extracts are useful for preventing diseases caused by oxidation and free radicals.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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التركيب الكيميائي للزيوت الأساسية الفينولات الكلية والفعالية المضادة للأكسدة لـ *Achillea fragrantissima* و *A. santolina* النامي في سوريا

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ملخص

الهدف من هذه الدراسة هو تحري المكونات الفعالة في الزيوت الأساسية وتحديد المحتوى الفينولي الكلي والفعالية المضاة للأكسدة لأزهار نبات *Achillea fragrantissima* و*A. santolina* التي جمعت من القلمون (ريف دمشق، سوريا). استخلاص الزيت الأساسي لأزهار النباتين وحللت باستخدام جهاز الكروماتوغرافيا الغازية ومطياف الكتلة (GC-MS). حضرت ثلاثة خلاصات باستخدام الماء المقطر والميثانول والكلوروفورم. حدد المحتوى الفينولي الكلي و الفعالية المضادات للأكسدة في الخلاصات. أظهرت النتائج وجود 20 مكوناً في الزيت الأساسي لنبات *A. fragrantissima*. المركبات الرئيسية الموجودة هي بيتا ثوجون (39.63%)، يليها كحول السانتولينا (15.54%)، كيتون الأرتيميسيا (15%)، وألفا ثوجون (10.58%). تم التعرف على 16 مكوناً في الزيت العطري لنبات *A. santolina*. المركبات الرئيسية هي الكافور (49.13%)، والأوكالبتول (17.13%)، والتيربين-4-أول (8.29%). احتوى الزيت الأساسي والخلاصات المائية والميثانولية والكلوروفورمية لـ *A. santolina* على (414.2، 1388.4، 2084.2، و965.7 مغ من TAE/غ من الخلاصة الجافة)، على التوالي. في التفاعل مع 2،2-ثنائي فينيل-1- بيكريل هيدرازيل، كانت قيمة التراكيز المثبطة لنصف الجذور الحرةهي (105، 120، و110 مكغ/لتر) للخلاصات المائية والميثانولية والكلوروفورمية لنبات*A. fragrantissima* على التوالي، و(720 و320 مكغ/لتر). بالنسبة للخلاصات المائية والميثانولية لـ *A. santolina*، على التوالي، لم يظهر الزيت الأساسي لكل من *A. fragrantissima* و*A. santolina* و الخلاصة الكلوروفورمية لـ *A. santolina* فعالية مضادة للأكسدة. أظهرت الدراسة أن الخلاصات المائية والميثانولية لنبات *A. fragrantissima* تمتلك فعالية جيدة في كبح الجذور الحرة.

الكلمات الدالة: *Achillea fragrantissima*، *A. santolina* ، زيت عطري، الفنولات الكلية، الفعالية المضادة للأكسدة.

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