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Chemical composition and antioxidant activity of essential oil from Algerian *Anacyclus clavatus*

Original Article

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Abstract:

This study included the volatile composition and antioxidant potential of an Algerian Auresian *Anacyclus clavatus*. *Anacyclus clavatus* essential oil (ACEO) and crude methanol extract (ACME) were obtained by steam distillation and maceration, respectively. Phenols and flavonoids were found in moderate amounts in ACME. ACEO exhibited moderate antioxidant capacity using β-carotene bleaching assays (40.09±3.7% and 78.1±5.28%, respectively). The main volatile constituents of ACEO were octadecanol (33.08%), caryophyllene oxide (5.10%), *cis*-thujone (4.81%), tetracosane (4.36%), nonanal (4.05%), and phytone (3.66%), respectively, with nonanal and phytone as key compounds in ACEO for the first time. These results will certainly enrich our knowledge of the currently limited information on the chemical composition of ACEO and suggest that this species is a viable source of bioactive compounds with potential uses in medicines and nutraceuticals.

Key words:
Anacyclus clavatus, essential oil, GC-MS, phenolic content, flavonoid content, antioxidant activity

Apstrakt:

Hemijski sastav i antioksidativna aktivnost etarskog ulja *Anacyclus clavatus* iz Alžira

Ovo istraživanje obuhvata ispitivanje isparljivih jedinjenja i antioksidativnog potencijala *Anacyclus clavatus* poreklom iz oblasti Aures u Alžiru. Etarsko ulje *Anacyclus clavatus* (ACEO) i sirovi metanolni ekstrakt (ACME) dobijeni su postupkom destilacije vodenom parom, odnosno maceracijom. Fenoli i flavonoidi su utvrđeni u umerenim količinama u ACME. ACEO je pokazalo umerenu antioksidativnu sposobnost primenom testa izbjeljivanja β-karotena (40,09±3,7% i 78,1±5,28%). Glavne isparljive komponente ACEO bile su oktadekanol (33,08%), kariofilen oksid (5,10%), *cis*-tujon (4,81%), tetrakozan (4,36%), nonanal (4,05%) i fiton (3,66%), pri čemu su nonanal i fiton po prvi put identifikovani kao ključna jedinjenja u ACEO. Ovi rezultati svakako doprinose proširenju postojećih, ograničenih podataka o hemijskom sastavu ACEO i ukazuju na to da ova vrsta može predstavljati značajan izvor bioaktivnih jedinjenja sa potencijalnom primenom u medicini i nutraceutici.

Ključne reči:
Anacyclus clavatus, etarsko ulje, GC–MS, sadržaj fenola, sadržaj flavonoida, antioksidativna aktivnost

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Introduction

People have traditionally valued aromatic and therapeutic herbs because they recognize their importance in preserving health and averting illness (Mahato et al., 2025). Despite the development of synthetic medicines, these natural resources remain essential in both traditional and modern medicine, providing a wide range of medicinal compounds (Buragohain et al., 2025). Aromatic and therapeutic species contain a wide range of secondary metabolites, including polyphenols and flavonoids, which are widely used in the culinary, cosmetics, and pharmaceutical sectors (Dimitrijević et al., 2025; Šovljanski et al., 2025).

The Asteraceae is one of the largest families, comprising 250,000 species and approximately 1,600 genera (Paksoy et al., 2016). It is known

for its wide range of uses, not only in medicine but also in the cultivation of ornamental plants and the production of natural rubber, colorants, insecticides, and spices (Mechergui et al., 2017). Asteraceae plants have been distinguished by their capitula, which include closely arranged flowers on a receptacle encircled by bracts. Asteraceae plants are distributed worldwide, including in subtropical, arid, and semi-arid climates, and most species have been used traditionally in conventional medicine due to their phytochemicals, including polyphenols, flavonoids, and volatile compounds (Chroho et al., 2022). The Asteraceae family includes a wide range of herbaceous plants, shrubs, and trees, many of which are economically and medicinally important (Heywood et al., 2007).

Anacyclus, a species of Asteraceae, is found in the Mediterranean basin, including Algerian regions, and is known for its adaptability to challenging, dry environments. The genus *Anacyclus* (Quezel & Santa, 1963a) is distributed across the Mediterranean basin, including Algerian zones, and is known for its adaptability to challenging, dry environments (Vitales et al., 2018). *Anacyclus* species, encompassing both annual and perennial varieties, have historically been used to treat various ailments, including sore throat, toothache, and rhinitis (Baslam et al., 2023). They have various biological capacities, including antimicrobial, anticonvulsant, anxiolytic, anabolic, aphrodisiac, immunostimulating, and antioxidant properties (Sissi et al., 2024).

Anacyclus clavatus (Desf.) Pers., also known as "white *Anacyclus*" (Quezel & Santa, 1963b), is an annual herb found in Mediterranean countries (Mifsud, 2002). Its flowering season starts in March and extends to May (Chroho et al., 2022). The plant grows in fields, on roads, and in wastelands. Its disc achenes are winged and have large capitula. The plant is used for skin, nervous system, digestive, reproductive, and renal issues. The aerial parts are used for fever, digestive problems, and stomach pain (Mechergui et al., 2017). Roots are used for rheumatism, respiratory problems, diabetes, toothaches, and paralysis (Manouze et al., 2017). Powdered roots are also used for kidney diseases, skin problems, reproductive issues, and allergies (Ouasti et al., 2024).

In Algeria, limited research has examined the volatile content of *A. clavatus*, with the main volatile chemicals being germacrene D, δ-elemene, α- and β-thujone, and artemisia ketone (Aliboudhar et al., 2013; Aliboudhar & Tigrine-Kordjani, 2014). Thus, the current study aimed to enrich knowledge on the Algerian *A. clavatus* from the Aures area regarding its essential oil composition by using GC-MS, and to evaluate its antioxidant capacity.

Materials and Methods

Chemicals

Methanol (95%) was purchased from Sigma-Aldrich (Deisenhofen, Germany); DPPH (Sigma, St. Louis, USA); H2O2 was obtained from Sigma-Aldrich, USA; and the analytical standards of ascorbic acid, gallic acid, Folin-Ciocalteu, Na2CO3, and α-tocopherol were obtained from Sigma-Aldrich, USA (purity ≥ 98%).

Plant material

The leaves, stems, and flowers of *A. clavatus* were collected in April 2023 from the Algerian region of Aures (Timgad, 35 km east of Batna, 35° 29′ 05″ north, 6° 28′ 07″ east, altitude: 1072 m). A voucher specimen (AC/118/VAR/04-23) was verified and placed in the VARENBIOMOL Research Unit's Herbarium at the University of Constantine 1.

Extraction and preparation of samples

The powdered, air-dried aerial part of *A. clavatus* (100 g) was extracted with 80% aqueous methanol for 72 hours at room temperature. Following filtration through cotton, the crude methanolic extract ACME was concentrated to dryness under reduced pressure (20.2 g, yield = 20.2%, *w/w*). The steam distillation method (Yazıcı & Sevgili, 2024) was used to extract the volatile components from the fresh plant material (240 g), which was broken into small pieces and processed for three hours. The obtained ACEO was weighed (32.0 mg; yield = 0.01%, *w/w*) and stored at 4 °C until analysis. The ACEO and ACME yields were calculated in proportion to the plant weight using the following formula: Yield (%) = (W1 × 100) / W2; where W1: weight of the obtained sample; W2: initial weight of the plant materials.

GC-MS and GC-FD analyses of ACEO

The ACEO was analyzed using a SHIMADZU GCMS-QP2010 chromatograph with an RXI-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The flow rate of the carrier gas, helium, was 1.44 mL/min. After 10 minutes at 45 °C, the oven was heated to 180 °C at a rate of 3 °C per minute and held there for 5 minutes. Following that, it was heated to 280 °C at a rate of 5 °C per minute and held for 5 minutes. Finally, it was heated to 330 °C at a rate of 10 °C/min for 2 minutes. The injector and detector (FID) temperatures were set at 330 °C. In split/splitless mode, 1 µL of diluted sample (in dichloromethane) was injected at a 30:1 split. For GC-MS detection, an electron ionization apparatus with an ionization energy of 70 eV was used. The

capillary column components were identified by comparing their mass spectra fragmentation patterns and calculated retention indices (RI) with those available in commercial databases (Adams, 2017; Babushok et al., 2011) and literature.

DPPH radical scavenging assay

A methanolic solution of DPPH was prepared at a concentration of 0.04 mg/mL. The samples ranged in different concentrations. 50 µL of each sample was combined with 3 mL of the prepared DPPH solution under the same conditions. A spectrophotometer was used to measure absorbance at 517 nm after a 30-minute incubation at room temperature. Under the same circumstances, the ascorbic acid was used as a positive standard. All measurements were carried out in duplicate (n = 2), and the following formula was used to determine the anti-radical capacity: IP (%) = $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$, where IP (%) is the inhibition percentage, Abs_{sample} is the absorbance of the sample, and $Abs_{control}$ is the absorbance of the negative control. The obtained values were expressed as IC_{50} (inhibitory concentration required to inhibit 50% of DPPH) from linear regression in Microsoft Excel (Nadji et al., 2024).

β-Carotene bleaching assay

0.5 mg of β-carotene was dissolved in 2 mL of chloroform with 25 µL of linoleic acid and 200 mg of Tween 40 to prepare a β-carotene/linoleic acid solution. After the chloroform evaporated, 100 mL of oxygenated water was added, and the mixture was vigorously stirred until an emulsion formed. Next, 0.5 mL of each aliquot was mixed with 3.5 mL of the previously prepared emulsion. The same conditions were used to test the α-tocopherol standard as a positive control. The decay of β-carotene can be rationalized in the presence of an excess of linoleic acid and O_2 by assuming that a small amount of peroxyl radical generated readily reacts with both β-carotene and antioxidant (Takada et al., 2006). The bleaching of β-carotene was monitored at 490 nm at regular intervals for 120 minutes at 50 °C (Mahdavi et al., 2017). All determinations have been carried out in duplicate. The antioxidant activity was determined using the following formula: Inhibition = $[Abs_{sample\ t=2h} / Abs_{sample\ t=0h}] \times 100$, where: $Abs_{samplet=2h}$ is the absorbance of the emulsion containing the sample after 2 hours. $Abs_{sample\ t = 0h}$ is the initial absorbance of the emulsion containing the sample.

Determination of total phenolic content

The polyphenol content in ACME was evaluated using the colorimetric technique published by Singleton et al. (1999), with minor modifications. 250 µL of sample (1 mg/mL) was mixed with 500 µL of Folin-Ciocalteu (1 N) reagent. After 4 minutes of incubation at 25 °C, 20 µL of 20% sodium carbonate (Na_2CO_3) solution was added to the mixture, bringing the total volume to 770 µL. The resulting mixture was incubated in a dark environment for 120 minutes before being measured at 760 nm. Gallic acid at varied amounts was used as the standard. All measurements have been carried out in duplicate. The total phenol content was estimated in µg GAE/mg by extrapolating from a calibration curve of varied gallic acid concentrations.

Determination of total flavonoid content

Flavonoids were quantified using a procedure that involves the formation of a very stable combination between aluminum chloride and the oxygen atoms present on flavonoids' carbon structures (Kim et al., 2003). ACME was prepared at a concentration of 1 mg/mL in methanol. 1 mL of the sample was then mixed with 1 mL of aluminum trichloride solution ($AlCl_3$, 2%), to obtain a total volume of 2 mL. Following one hour of incubation, absorbance was measured at 420 nm. This experiment was performed in duplicate. The absorbance of a quercetin standard solution was measured under the same circumstances. The results were expressed as the calibration curve for quercetin in µg QE/mg.

Statistical analysis

For the antioxidant evaluation, results are represented as mean values ± standard deviation (SD). A one-way analysis of variance (ANOVA, $p<0.05$) was performed to compare group means. Statistical analyses were performed using Microsoft Excel 2007 (Microsoft, Redmond, WA, USA).

Results and Discussion

GC-MS analysis

ACEO was isolated by steam distillation and then analyzed by GC-FID and GC-MS. Thirty-seven volatile chemicals were found in the acquired data (**Tab. 1**, **Fig. 1**), accounting for 95.78% of the total detected oil composition. Among these, 35.59% were fatty alcohols, 19.86% were sesquiterpenoids, 14.03% were monoterpenoids, and 9.43% were hydrocarbons. The main abundant compounds were octadecanol (33.08%), caryophyllene oxide (5.10%), *cis*-thujone (4.81%), tetracosane (4.36%), nonanal (4.05%), and phytone (3.66%), respectively. Nonanal and phytone, the two main ingredients, have not been described before in the ACEO, according to the literature. Some *Anacyclus* species, including *A. maroccanus*, *A. radiatus*, *A. cyrtolepidioides*, and *A. pyrethrum*, have been

Table 1. Chemical composition of ACEO using GC-MS analysis

Peak N°	RT	KICalc	KI theo	Component	Area %
1	22.992	1017	1042	Artemisia ketone	1.98
2	**25.476**	**1062**	**1062**	***cis*-Thujone**	**4.81**
3	**25.559**	**1064**	**1069**	**Nonanal**	**4.05**
4	26.137	1074	1112	*trans*-Thujone	0.54
5	29.575	1141	1177	4-Terpineol	0.97
6	30.554	1160	1194	Myrtenal	0.9
7	30.656	1162	1195	Myrtenol	0.66
8	34.01	1232	1233	Chrysanthenyl acetate	2.55
9	39.025	1345	1365	Neryl acetate	1.64
10	39.525	1357	1374	α-Copaene	0.89
11	39.952	1374	1388	β-Elemene	0.58
12	40.114	1404	1408	Caryophyllene Z	1.04
13	43.152	1444	1452	β-E-Farnesene	2.44
14	44.268	1472	1485	β-Copaene	2.7
15	46.068	1519	1523	δ-Cadinene	0.61
16	48.382	1580	1578	Spathulenol	3.39
17	48.499	1583	1582	Neryl isovalerate, butanoic acid	1.96
18	**48.606**	**1585**	**1587**	**Caryophyllene oxide**	**5.1**
19	49.036	1597	1595	Salvial-4(14)-en-1-one	1.28
20	49.654	1614	1616	1,3,12-Hexadecatriene	1.02
21	50.891	1648	1641	α-epi-Muurolol	1.47
22	51.294	1660	1649	β-Eudesmol	0.51
23	51.449	1664	1654	α-Cadinol	1.13
24	53.584	1724	1701	Pentadecanal	0.89
25	**58.918**	**1864**	**1841**	**Phytone**	**3.66**
26	60.667	1906	1884	Hexadecanol	0.87
27	64.266	2001	1977	Hexadecanoic acid	3.32
28	66.048	2062	1999	Octadecanal	0.75
29	**67.989**	**2134**	**2081**	**Octadecanol**	**33.08**
30	68.349	2149	2102	Heneicosane	0.53
31	68.763	2166	2106	Phytol	1.43
32	72.85	2360	2365	Heneicosanol	1.64
33	**73.076**	**2371**	**2400**	**Tetracosane**	**4.36**
34	73.695	2404	2412	Methyl-20-heneicosonoate	0.47
35	76.894	2596	2600	Hexacosane	2.27
36	80.22	2822	2800	Octacosane	1.35
37	83.882	3048	3000	Triacontane	0.92
Oil yield					0.01
Total identified					95.78
Fatty alcohols					35.59
Sesquiterpenoids					19.86
Monoterpenoids					14.03
Hydrocarbons					9.43
Others					16.87

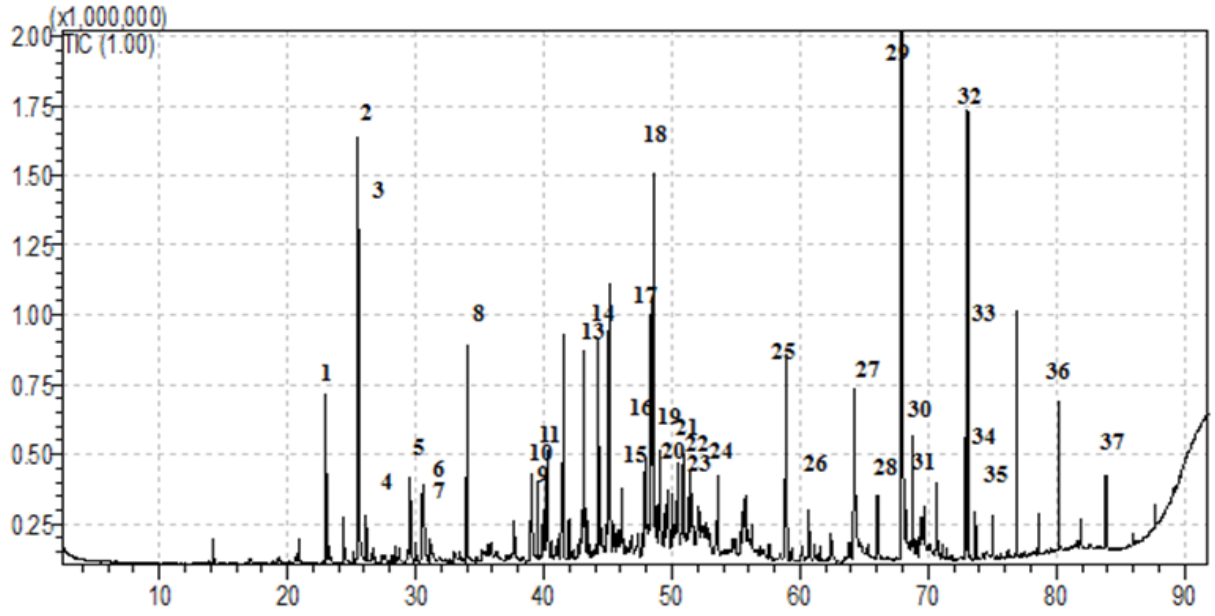

Fig 1. GC-FID Chromatogram of ACEO

shown to contain tetracosane and caryophyllene oxide in their essential oils (El Baz et al., 2024; Sissi et al., 2024; Zardi-Bergaoui et al., 2008).

Tab. 2 shows the presence of the main components of our sample in the ACEO collected from different regions of the world. We can note variability in the volatile chemical composition of our ACEO species compared to that studied in other regions. Only a few published studies have reported the chemical composition of ACEO in the Algerian and Tunisian regions (Aliboudhar et al., 2013; Aliboudhar & Tigrine-Kordjani, 2014; Aliboudhar et al., 2015; Hammami et al., 2013). By comparing the data (**Tab. 2**), a significant difference

Table 2. Major compounds of ACEO from different regions

Major compounds	Percentage (%)				
	Algeria				Tunisia
	Batna	Boumerdes	Boumerdes	Boumerdes	Sousse
		(Aliboudhar et al., 2013)	(Aliboudhar & Tigrine-Kordjani, 2014)	(Aliboudhar et al., 2015)	(Hammami et al., 2013)
Octadecanol	33.08	0.06	-	0.1±0.0 to 0.2±0.1	0.8
Caryophyllene oxide	5.1	1.28	1.3±0.3 to 2.5±0.6	0.2±0.2 to 0.3±0.1	-
***cis*-Thujone**	4.81	-	-	0.9±0.4 to 1.3±0.5	9.8
Tetracosane	4.36	tr	-	-	0.5
Nonanal	4.05	-	-	-	-
Phytone	3.66	-	-	-	-
Germacrene D	-	16.84	-	12.3±1.6 to 16.1±1.4	2
β-Thujone	-	11.16%	-	-	-
Artemisia ketone	1.98	0.53	6.5±0.5 to 10.0±0.8	0.4±0.3	0.4
α-Thujone	-	0.3	10.6±1.0 to 11.9±1.1	-	-
δ-Elemene	-	0.17	0.4±0.0 to 4.0±0.7	9.1±1.1 to 10.4±1.3	-
***trans*-Chrysanthenyl acetate**	-	-	0.5±0.1 to 3.6±0.8	-	12.3
Chrysanthenone	-	0.1	-	0.1±0.1	8.2

in chemical composition was observed, where the main compounds in our sample, octadecanol, caryophyllene oxide, *cis*-thujone, and tetracosane, were absent or present at low concentrations in the previously mentioned samples. As for the two compounds, nonanal and phytone, they were completely absent in the previously published samples. In contrast, germacrene D, β-thujone, α-thujone, δ-elemene, *trans*-chrysanthenyl acetate, and chrysanthenone, which were predominant in all published samples (Aliboudhar et al., 2013; Aliboudhar & Tigrine-Kordjani; Aliboudhar et al., 2015; Hammami et al., 2013), were absent in our sample, except for artemisia ketone compounds. This chemical composition variation could be linked to several factors, including geographical and environmental factors (Lahmar et al., 2025; Sarmah & Bora, 2025; Burczyk et al., 2024), time of collection (Hazrati et al., 2024; Boaventura et al., 2025), extraction technique (Acosta-Vega et al., 2025; Suttiarporn et al., 2025; Ferraz & Silva, 2024), and genetic background (Jakovljević, 2025; Ganić et al., 2025; Solgi et al., 2025; Mustafa et al., 2024). Moreover, the high concentration of octadecanol in ACEO, a fatty alcohol known as a vaginal drug-delivery vehicle (Yuan et al., 2010), and caryophyllene oxide, a potent antioxidant, anti-inflammatory (Kumar et al., 2025), and antiparasitic (López-López et al., 2025) sesquiterpenoid, suggests this particular chemotype may exhibit an enhanced transethosomal system for enhanced transdermal delivery and therapeutic biological effects.

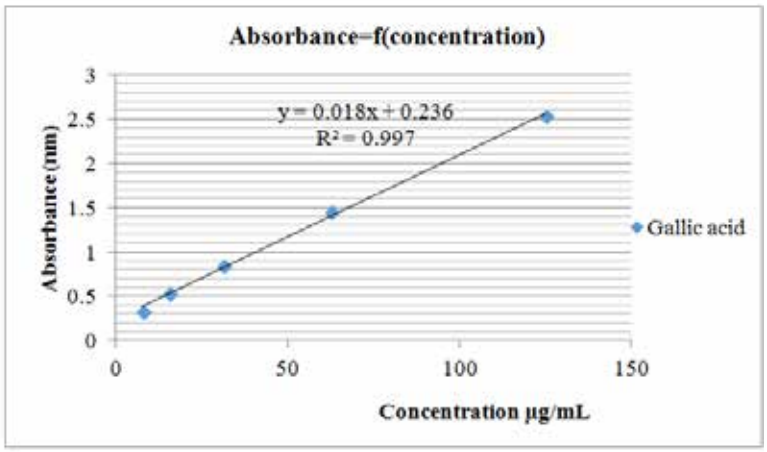

Fig. 2. Gallic acid calibration curve for TPC

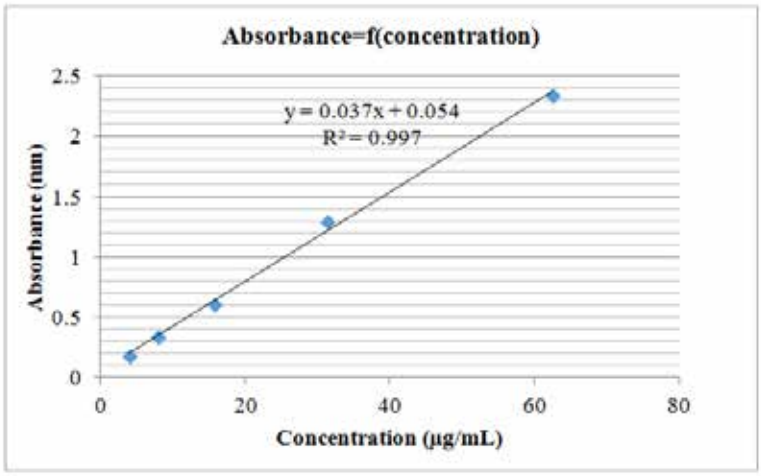

Fig. 3. Quercetin calibration graph for TFC

Antioxidant capacity

The percentage yields (**Tab. 3**) of ACEO and ACME were calculated based on the initial mass of the plant material as follows: Yield (%) = $m_1 \times 100 / m_2$, where m_1 is the final obtained mass of the oil/extract, and m_2 is the initial mass of the species. The equation $y = 0.018x + 0.236$ was used to determine phenol concentrations at 765 nm (**Fig. 2**), with a correlation coefficient (R^2) of 0.997. Gallic acid was used as the standard for the linear curve. The obtained results (**Tab. 3**) showed that the ACME had a moderate amount of phenols with a TPC of 19.28±4.93 µg GAE/mg. The flavonoid amount was measured using the calibration curve equation $y = 0.0337x + 0.054$ at 420 nm (**Fig. 3**), with a correlation

Table 3. Antioxidant activity, total phenol and flavonoid contents of ACEO and ACME (Mean values ± SD, n = 2)

Samples	Yield (%)	Total phenolic content µg GAE/mg	Total flavonoid content µg QE/mg	DPPH radical scavenging IC50 (mg/mL)	β-carotene (%)
ACME	20.2	19.28±4.93	6.53±0.70	6.96±0.06	40.09±3.79
ACEO	0.01				78.1±5.28
Ascorbic acid				0.22±0.02	
α-Tocopherol					61.42±3.64

coefficient (R^2) of 0.997. Quercetin was used as the standard for the linear curve. The results showed that the ACME had moderate flavonoid content (6.53±0.70 µg QE/mg). Regarding the antioxidant evaluation of ACME using the DPPH assay, ascorbic acid was used as a reference, and the results were expressed as IC_{50} values (**Tab. 3**). The linear curve equation y =7.208x–0.136 (R^2 = 0.993) was used to determine the IC_{50} (**Fig. 4**), and the linear curve equation y=240.8x+0.145 (R^2=0.999) was used to determine the IC_{50} of the ascorbic acid (**Fig. 5** and **Fig. 6**). The values indicate a weak antioxidant capacity for ACME compared to ascorbic acid (IC_{50} = 6.96±0.06 and 0.22±0.02 mg/ml, respectively), whereas, the β-carotene assay (**Fig. 7**), revealed a moderate antioxidant capacity (40.09±3.7%) and a high antioxidant capacity of ACEO (78.1±5.28%) compared to the α-tocopherol standard with 61.42±3.64% (ACEO > α-tocopherol > ACME). This could be explained by the difference in antioxidant mechanisms (Famutimi et al., 2025). Several published works reported the antioxidant capacities of *A. clavatus* extracts and essential oils (Chroho et al., 2022; Bouriche et al., 2016; Aliboudhar et al., 2013). Furthermore, *A. clavatus* extracts and essential oils have been shown, according to many studies, to possess various biological activities, including anticancer, anti-inflammatory, antibacterial, antifungal, and antioxidant capacity (Adiba et al., 2019; Hammami et al., 2013; Hasan et al., 2025). A number of published works demonstrated the direct relationship between the phenols and biological potentials, such as antioxidant capacity (Vlocskó et al., 2025; Hazarika et al., 2025), including the primary constituents of ACEO, caryophyllene oxide (Shabana et al., 2023), phytone (Gao et al., 2025), and compounds that follow (**Tab. 1**), such as spathulenol (do Nascimento et al., 2018), hexadecanoic acid (Ganesan et al., 2024), and chrysanthenyl acetate (Di Napoli et al., 2020).

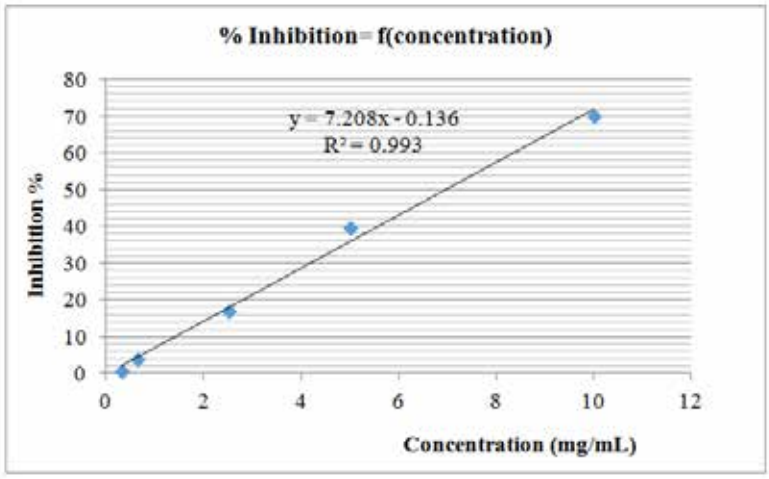

Fig. 4. Linear curve of ACME

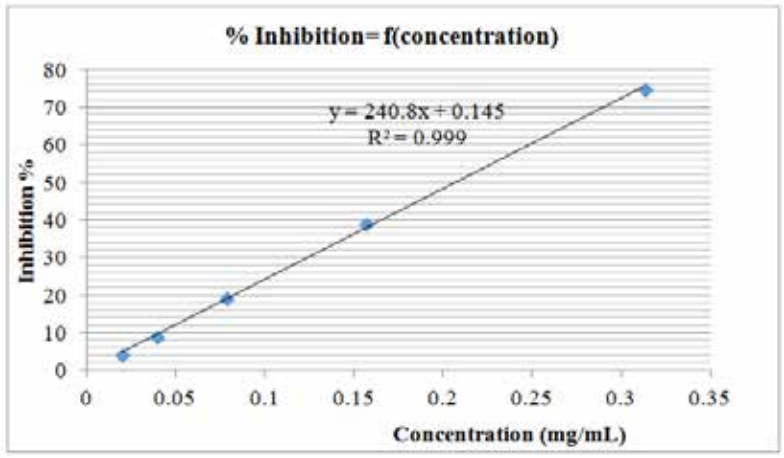

Fig. 5. Linear curve of ascorbic acid standard

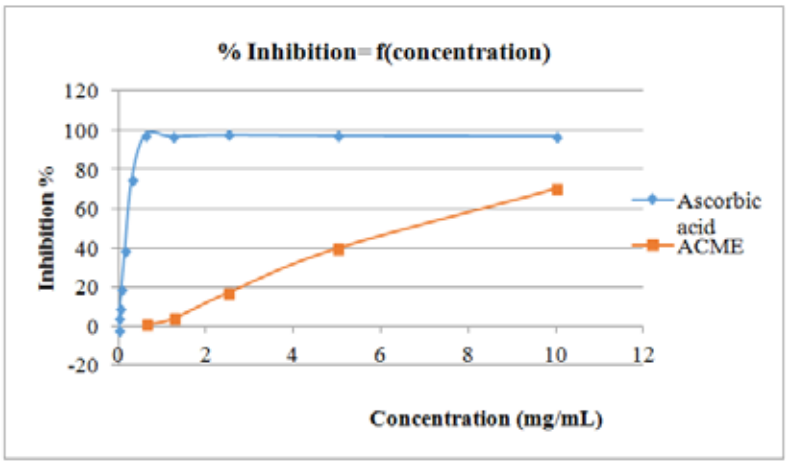

Fig. 6. Antioxidant activity by DPPH assay of ACME and ascorbic acid

Conclusion

This study's aim was to enrich knowledge of the volatile composition of *Anacyclus clavatus*, a member of the Asteraceae family collected in

Algeria's Aures zone, and to assess its crude methanol extract for total phenol and flavonoid contents. Using GC-MS and GC-FID, the primary constituents of ACEO were identified as octadecanol, caryophyllene oxide, thujone, tetracosane, nonanal, and phytone. The notably high antioxidant activity of ACEO and moderate amounts of flavonoids and phenols found in ACME imply that this species could be a useful natural resource for medicinal and nutritional uses. Future phytochemical and *in vivo* biological studies should be focused on the isolation of major compounds, particularly octadecanol and caryophyllene oxide, to confirm their individual contributions to the observed strong antioxidant activity and to test for other potential bioactivities, such as anti-inflammatory, antiparasitic, and vaginal drug-delivery vehicle activities.

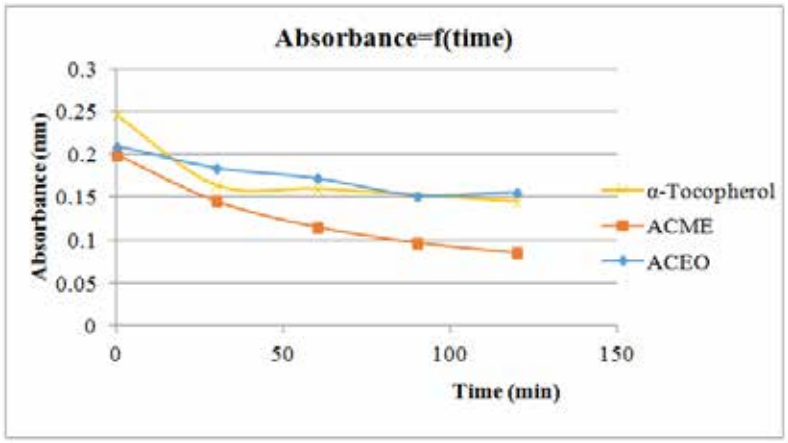

Fig. 7. Antioxidant activity by β-carotene test of ACME and ACEO

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References

Acosta-Vega, L., **Cifuentes, A.**, **Ibáñez, E.**, & **Galeano Garcia, P.** (2025). Exploring natural deep eutectic solvents (nades) for enhanced essential oil extraction: current insights and applications. *Molecules, 30*(2), 284. https://doi.org/10.3390/molecules30020284

Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/ mass spectrometry* (ed. 4.1). Biology Department Baylor University.

Aliboudhar, H., & **Tigrine-Kordjani, N.** (2014). Effect of extraction technique on the content and antioxidant activity of crude extract of *Anacyclus clavatus* flowers and their essential oil composition. *Natural Product Research, 28*(23), 2140–2149. https://doi.org/10.1080/14786419.2014.927872

Aliboudhar, H., **Tigrine-Kordjani, N.**, & **Youcef Meklati, B.** (2015). Competition of microwave-assisted hydro-distillation in highlighting volatile phytochemicals of *Anacyclus clavatus* species. *Journal of Essential Oil Research, 27*(4), 355–362. https://doi.org/10.1080/10412905.2015.1029083

Aliboudhar, H., **Tigrine-Kordjani, N.**, **Hanifi, N.**, & **Meklati, B. Y.** (2013). Volatiles profiling and antioxidant activity evaluation of different parts of a medicinal plant: *Anacyclus clavatus*. *Journal of Herbs, Spices and Medicinal Plants, 19*(1), 33–47. https://doi.org/10.1080/10496475.2012.735215

Babushok, V. I., **Linstrom, P. J.**, & **Zenkevich, I. G.** (2011). Retention indices for frequently reported compounds of plant essential oils. *Journal of Physical and Chemical Reference Data, 40*(4), 043101–043147. https://doi.org/10.1063/1.3653552

Baslam, A., **Aitbaba, A.**, **Aboufatima, R.**, **Agouram, F.**, **Boussaa, S.**, **Chait, A.**, & **Baslam, M.** (2023). Phytochemistry, antioxidant potential, and antibacterial activities of *Anacyclus pyrethrum*: promising bioactive compounds. *Horticulturae, 9*(11), 1196. https://doi.org/10.3390/horticulturae9111196

Boaventura, T. P., **dos Santos, F. A. C.**, **de Oliveira, P. E. C. M.**, **Braga, N. G.**, **Assis, Y. P. A. S.**, **de Oliveira, J. E.**, **Luz, R. L.**, & **Gisele Cristina Favero, G. C.** (2025). Use of the essential oil of *Thymus vulgaris* (Thyme) and its nanoemulsion as sedatives during tambaqui (*Colossoma macropomum*) transport: water quality, survival and physiology. *Aquaculture International, 33*(2), 150. http://dx.doi.org/10.2139/ssrn.4846016

Buragohain, D., **Kundu, P.**, **Nath, R.**, **Kityania, S.**, **Giri, A.**, & **Das Talukdar, A.** (2024). Importance of traditional resources in pharmaceutical industries. In **Das Talukdar, A.**, **Patra, J. K.**, **Das, G.**, & **Nath, D.** (Eds.), *Traditional resources and tools for modern drug discovery. Interdisciplinary Biotechnological Advances*. https://doi.org/10.1007/978-981-97-4600-2_15

Burczyk, J., **Wierzchowska-Renke, K.**, **Głowniak, K.**, **Głowniak, P.**, & **Marek, D.** (2024). Geographie and environmental influences on the variation of essential oil and coumarins in *Crithmum maritimum*

L. *Breeding Research on Aromatic and Medicinal Plants, 17*(2), 305–311. https://doi.org/10.1300/J044v09n04_07

Chroho, M., **Aazza, M.**, **Bouymajane, A.**, **Majdoub, Y. O. E.**, **Cacciola, F.**, **Mondello, L.**, **Zair, T.**, & **Bouissa, L.** (2022). HPLC-PDA/ESI-MS analysis of phenolic compounds and bioactivities of the ethanolic extract from flowers of Moroccan *Anacyclus clavatus*. *Plants, 11*(24), 3423. https://doi.org/10.3390/plants11243423

Di Napoli, M., **Maresca, V.**, **Varcamonti, M.**, **Bruno, M.**, **Badalamenti, N.**, **Basile, A.**, & **Zanfardino, A.** (2020). (+)-(E)-Chrysanthenyl acetate: A molecule with interesting biological properties contained in the *Anthemis secundiramea* (Asteraceae) flowers. *Applied Sciences, 10*(19), 6808. https://doi.org/10.3390/app10196808

Dimitrijević, M., **Stojanović-Radić, Z.**, **Radulović, N.**, **Nešić, M.**, & **Pejčić Pejić, M. P.** (2025). Chemical composition and antifungal effect of the essential oil of *Helichrysum italicum* (Roth) G. Don against clinical isolates of *Candida* spp. *Biologica Nyssana, 16*(1), 177–187. http://dx.doi.org/10.46793/BiolNyss.16.1.6D

do Nascimento, K. F., **Moreira, F. M. F.**, **Santos, J. A.**, **Kassuya, C. A. L.**, **Croda, J. H. R.**, **Cardoso, C. A. L.**, **Carmo Vieira, M.**, **Tasca Góis Ruiz, A. L.**, **Foglio, M. A.**, **de Carvalho, J. E.**, & **Formagio, A. S. N.** (2018). Antioxidant, anti-inflammatory, antiproliferative and antimycobacterial activities of the essential oil of *Psidium guineense* Sw. and spathulenol. *Journal of Ethnopharmacology, 210*, 351–358. https://doi.org/10.1016/j.jep.2017.08.030

El Baz, A., **Mrabti, H. N.**, **Ashmawy, N. S.**, **Khan, S. A.**, **Abdallah, E. M.**, **Al-Mijalli, S. H.**, **Alenazy, R.**, **Alshabrmi, F. M.**, **Bouyahya, A.**, **El Hachlafi, N.**, **Ardianto, C.**, **Ifadotunnikmah, F.**, & **Hmimid, F.** (2024). Phytochemical characterization, antimicrobial properties and in silico modeling perspectives of *Anacyclus pyrethrum* essential oil. *Heliyon, 10*(16), e35079. https://doi.org/10.1016/j.heliyon.2024.e35079

Famutimi, O. G., **Masha, S.**, **Maluleke, R.**, **Ncapayi, V.**, **Lebepe, T. C.**, **Mgedle, N.**, **Kungwa, C. M.**, **Fanoro, O. T.**, **Adewale, I. O.**, & **Oluwafemi, O. S.** (2025). Assessment of antioxidant potential of carbon-based nanomaterials from different sources. *Antioxidants, 14*(10), 1227. https://doi.org/10.3390/antiox14101227

Ferraz, L. P., & **Silva, E. K.** (2024). Pulsed electric field-assisted extraction techniques for obtaining vegetable oils and essential oils: Recent progress and opportunities for the food industry. *Separation and Purification Technology, 354*(2), 128833. https://doi.org/10.1016/j.seppur.2024.128833

Ganesan, T., **Subban, M.**, **Christopher Leslee, D. B.**, **Kuppannan, S. B.**, & **Seedevi, P.** (2024). Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. *Biomass Conversion and Biorefinery, 14*(13), 14547–14558. https://doi.org/10.1007/s13399-022-03576-w

Ganić, T., **Pećinar, I.**, **Nikolić, B.**, **Kekić, D.**, **Tomić, N.**, **Cvetković, S.**, **Vuletić, S.**, & **Mitić-Ćulafić, D.** (2025). Evaluation of Cinnamon essential oil and its emulsion on biofilm-associated components of *Acinetobacter baumannii* clinical strains. *Antibiotics, 14*(1), 106. https://doi.org/10.3390/antibiotics14010106

Gao, J. Y., **Zou, Y. Y.**, **Liang, Y.**, **Zhang, Y. Y.**, & **Zhang, Y.** (2025). The essential oil composition of *Lycopodium japonicum* and its antioxidant activities. *Chemistry of Natural Compounds*, 1–3. https://doi.org/10.1007/s10600-025-04813-0

Hammami, R., **Fernandez, B.**, **Lacroix, C.**, & **Fliss, I.** (2013). Anti-infective properties of bacteriocins: an update. *Cellular and Molecular Life Sciences, 70*(16), 2947–2967. https://doi.org/10.1007/s00018-012-1202-3

Hazarika, T. K., **Debbarma, P.**, **Malsawmkima, G.**, **Barman, K.**, & **Nath, P. C.** (2025). Biochemical profiling and antioxidant potential of fruit tissues: a comparative study of Citrus cultivars indigenous to Northeast India. *Journal of Food Processing and Preservation, 2025*(1), 8833248. https://doi.org/10.1155/jfpp/8833248

Hazrati, S., **Mousavi, Z.**, & **Nicola, S.** (2024). Harvest time optimization for medicinal and aromatic plant secondary metabolites. *Plant Physiology and Biochemistry, 212*, 108735. https://doi.org/10.1016/j.plaphy.2024.108735

Heywood, V. H., **Brummitt, R. K.**, **Culham, A.**, & **Seberg, O.** (2007). *Flowering plant families of the world*. Ontario: Firefly Books.

Jakovljević, D. (2025). Genetic diversity, cultivation, and utilization of aromatic plants. In *Industrial Crops Improvement: Biotechnological Approaches for Sustainable Agricultural Development, 19*, 171–182. https://doi.org/10.1007/978-3-031-75937-6_10

Kumar, G. S., **Sholapuri, P.**, **K, D.**, **Shaily Enugonda, M.**, & **BP, G.** (2025). In vivo and in silico anti-inflammatory activity of *Artemisia vulgaris* and β-caryophyllene oxide in carrageenan-induced paw edema in Wistar rats. *Drug and Chemical Toxicology, 48*(2), 426–439. https://doi.or

g/10.1080/01480545.2024.2415349

Lahmar, I., **Yotova, L.**, & **Belghith, K.** (2025). Environmental impact on phonological stages in *Lavandula officinalis*: Chemical profiling of essential oil and extract, antioxidant activity, and acetylcholinesterase inhibition potential. *Euro-Mediterranean Journal for Environmental Integration, 7*, 1–12. https://doi.org/10.1007/s41207-024-00714-w

López-López, L. P., **Hernández-Cuevas, N. A.**, **Acosta-Viana, K. Y.**, **Arana-Argáez, V. E.**, **Torres-Romero, J. C.**, & **Polanco-Hernández, G. M.** (2025). Activity of β-Caryophyllene oxide and benznidazole mixture against *Trypanosoma cruzi* and in silico prediction of anti-trypanocidal interaction. *Scientia Pharmaceutica, 93*(3), 40. https://doi.org/10.3390/scipharm93030040

Mahato, D., **Mahto, H.**, & **Kumari, S.** (2025). Medicinal and aromatic plant cultivation and sustainable development. In *Industrial Crops Improvement. Sustainable Landscape Planning and Natural Resources Management*. https://doi.org/10.1007/978-3-031-75937-6_8

Mahdavi, B., **Yaacob, W. A.**, & **Din, L. B.** (2017). Chemical composition, antioxidant, and antibacterial activity of essential oils from *Etlingera sayapensis* AD Poulsen & Ibrahim. *Asian Pacific Journal of Tropical Medicine, 10*(8), 819–826. https://doi.org/10.1016/j.apjtm.2017.08.006

Manouze, H., **Bouchatta, O.**, **Gadhi, A. C.**, **Bennis, M.**, **Sokar, Z.**, & **Ba-M'hamed, S.** (2017). Anti-inflammatory, antinociceptive, and antioxidant activities of methanol and aqueous extracts of *Anacyclus pyrethrum* roots. *Frontiers in Pharmacology, 8*, 598. https://doi.org/10.3389/fphar.2017.00598

Mechergui, K., **Khaldi, S.**, & **Jaouadi, W.** (2017). Assessment of phenology and morphological diversity of 3 species of Asteraceae: *Anacyclus clavatus, Chamaemelum fuscatum* and *Tanacetum parthenium*. *Asian Journal of Biology, 3*(2), 1–12. https://doi.org/10.9734/AJOB/2017/34533

Mifsud, S. (2002). *Anacyclus clavatus* (White Anacyclus). MaltaWildPlants.com—the online Flora of the Maltese Islands.

Mustafa, K. H., **Khorshidi, J.**, **Vafaee, Y.**, **Rastegar, A.**, **Morshedloo, M. R.**, & **Hossaini, S.** (2024). Phytochemical profile and antifungal activity of essential oils obtained from different *Mentha longifolia* L. accessions growing wild in Iran and Iraq. *BMC Plant Biology, 24*(1), 461. https://doi.org/10.1186/s12870-024-05135-z

Nadji, W., **Yousfi, B.**, **Barhouchi, B.**, **Salem, R.**, **Yassad, S.**, & **Djekoun, A.** (2024). Formulation and development of an antiacne patch containing black seed and clove extracts by using chitosan-gelatin as polymers matrix. *Journal of Molecular and Pharmaceutical Sciences, 3*(1), 26–37.

Ouasti, I., **Ouasti, M.**, **Panda, S.**, **Mahanty, D. S.**, **Bussmann, R. W.**, & **Elachouri, M.** (2024). *Anacyclus clavatus* (Desf.), *Anacyclus monanthos* (L.) Thell., *Anacyclus monanthos* (L.) Thell. subsp. *cyrtolepidioides* (Pomel) Humphries, *Anacyclus pyrethrum* (L.) Lag., *Anacyclus radiatus* Loisel., *Anacyclus* × *valentinus* L. – Asteraceae. In *Ethnobotany of Northern Africa and Levant* (pp. 1–7). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-43105-0_31

Paksoy, M. Y., **Selvi, S.**, **Sevindik, E.**, & **Uysal, H.** (2016). Soil content in the *Anacyclus* L. (Asteraceae) genus growing in Turkey. *Turkish Journal of Life Sciences, 1*(1), 8–12.

Quezel, P., & **Santa, S.** (1963a). *Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II.* Paris: Editions du Centre National de la Recherche Scientifique, 15, quai Anatole-France.

Quezel, P., & **Santa, S.** (1963b). *Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II.* Paris: Editions du Centre National de la Recherche Scientifique, 15, quai Anatole-France.

Sarmah, A., & **Bora, D. J.** (2025). Carbon farming and the green economy: a science-based framework for sustainable agricultural transformation. In *Advances in Modern Agriculture: Research and Innovation, 25*(2), 209–225. Bhumi Publishing, India.

Shabana, S. M., **Gad, N. S.**, **Othman, A. I.**, **Mohamed, A. F.**, & **El-Missiry, M. A.** (2023). β-caryophyllene oxide induces apoptosis and inhibits proliferation of A549 lung cancer cells. *Medical Oncology, 40*(7), 189. https://doi.org/10.1007/s12032-023-02022-9

Singleton, V. L., **Orthofer, R.**, & **Lamuela-Raventós, R. M.** (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sissi, S., **Ouhaddou, S.**, **Dilagui, I.**, **Soraa, N.**, **Bellioua, S.**, **Larhsini, M.**, & **Markouk, M.** (2024). Chemical composition, antioxidant and antimicrobial activities of *Anacyclus maroccanus* Ball. and *Anacyclus radiatus* Loisel. essential oils against multidrug resistant microbial

pathogens. *Natural Product Communications, 19*(10), 1934578X241289844. https://doi.org/10.1177/1934578X241289844

Solgi, M., **Bagnazari, M.**, **Mohammadi, M.**, & **Azizi, A.** (2025). *Thymbra spicata* extract and arbuscular mycorrhizae improved the morphophysiological traits, biochemical properties, and essential oil content and composition of Rosemary (*Rosmarinus officinalis* L.) under salinity stress. *BMC Plant Biology, 25*(1), 220. https://doi.org/10.1186/s12870-025-06221-6

Šovljanski, O., Kljakić, A. C., & **Tomić, A.** (2025). Antibacterial and antifungal potential of plant secondary metabolites. In *Plant Specialized Metabolites: Phytochemistry, Ecology and Biotechnology*. https://doi.org/10.1007/978-3-031-51158-5_6

Suttiarporn, P., **Taithaisong, T.**, **Namkhot, S.**, & **Luangkamin, S.** (2025). Enhanced eugenol composition in clove essential oil by deep eutectic solvent-based ultrasonic extraction and microwave-assisted hydrodistillation. *Molecules, 30*(3), 504. https://doi.org/10.3390/molecules30030504

Takada, H., **Kokubo, K.**, **Matsubayashi, K.**, & **Oshima, T.** (2006). Antioxidant activity of supramolecular water-soluble fullerenes evaluated by β-carotene bleaching assay. *Bioscience, Biotechnology, and Biochemistry, 70*(12), 3088–3093. https://doi.org/10.1271/bbb.60491

Vitales, D., **Feliner, G. N.**, **Valles, J.**, **Garnatje, T.**, **Firat, M.**, & **Alvarez, I.** (2018). A new circumscription of the Mediterranean genus *Anacyclus* (Anthemideae, Asteraceae) based on plastid and nuclear DNA markers. *Phytotaxa, 349*(1), 1–17. https://doi.org/10.11646/phytotaxa.349.1.1

Vlocskó, R. B., **Mastyugin, M.**, **Török, B.**, & **Török, M.** (2025). Correlation of physicochemical properties with antioxidant activity in phenol and thiophenol analogues. *Scientific Reports, 15*(1), 73. https://doi.org/10.1038/s41598-024-83982-4

Yazıcı, Ö., & **Sevgili, L. M.** (2024). Obtaining cumin (*Cuminum cyminum*) essential oils by steam distillation. Part I: Investigation of operation parameters on essential oil yield and distribution of oil composition. *Journal of Chemical Technology and Biotechnology, 99*(2), 481–498. https://doi.org/10.1002/jctb.7550

Yuan, D., **Ju, C.**, **Ding, S.**, **Jing, X.**, & **Zhang, C.** (2010). Synthesis of 1-octadecanol-modified water-swelling polyurethane hydrogels as vaginal drug-delivery vehicle. *Journal of Biomaterials Science, Polymer Edition, 21*(4), 493–505. https://doi.org/10.1163/156856209X427032

Zardi-Bergaoui, A., **Harzallah-Skhiri, F.**, **Hammami, S.**, **Chreaif, I.**, **Cheriaa-Issa, J.**, **Jannet, H. B.**, & **Mighri, Z.** (2008). Composition and antibacterial activity of the essential oil from flowerheads of *Anacyclus cyrtolepidioïdes* Pomel from Tunisia. *Journal of Essential Oil Bearing Plants, 11*(6), 577–585. https://doi.org/10.1080/09720560X.2008.10643669