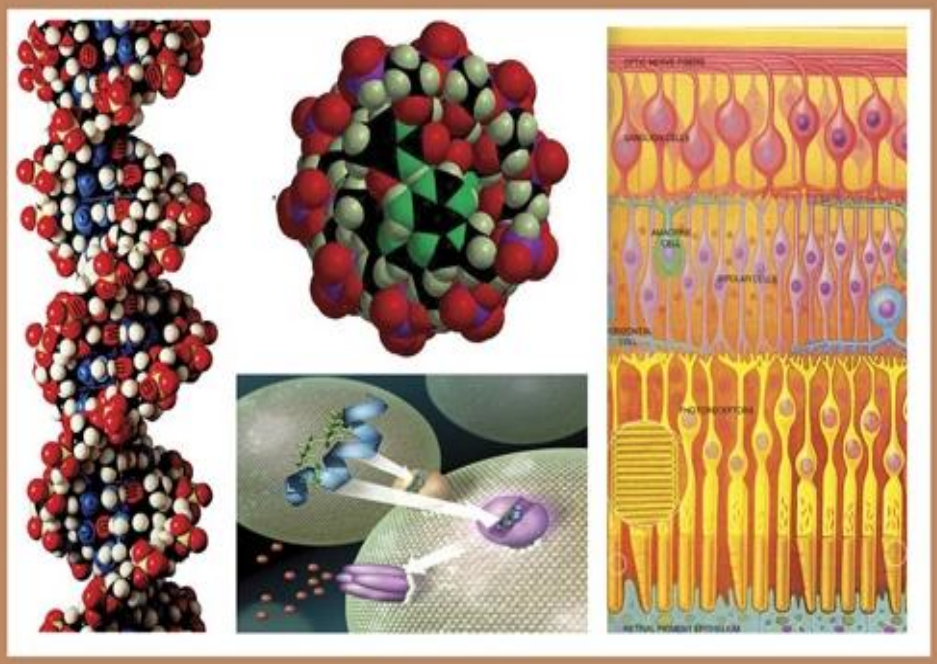
EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
C
PHYSIOLOGY & MOLECULARBIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(1) pp423-433 (2022)*
DOI: 10.21608/EAJBSC.2022.240730

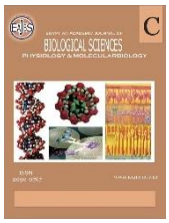
Egypt. Acad. J. Biolog. Sci., 14(1):423-433 (2022)

Egyptian Academic Journal of Biological Sciences

C. Physiology & Molecular Biology

ISSN 2090-0767

www.eajbsc.journals.ekb.eg



Evaluation of the Antioxidant Activity of Essential Oils of *Calamintha officinalis* and *Abies numidica*

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ARTICLE INFO

Article History

Received:10/4/2022

Accepted:28/5/2022

Available:1/6/2022

Keywords:

Antioxidant activity, essential oils, DPPH, ABTS, FRAP, Calamintha officinalis, Abies numidica, ANOVA.

ABSTRACT

Background: oxidative stress forms a worldwide problem of public health it is considered the origin of most human diseases, and free radicals are responsible for this oxidative stress.**Objective:** our study evaluates the antioxidant activity of the essential oils extracted from *Clamintha officinalis* and *Abies numidica*. The three tests, ABTS radical scavenging activity, antiradical activity DPPH and ferric reducing ability allowed us to study the antioxidant activity of the extracted essential oil.**Results:** doing the ABTS scavenging activity test, essential oils have shown a different antioxidant activity that increases in the following ways: A Numidia<Geraniol<Garavone<C officinalis<Thymol**.** The result obtained by the anti-free radical activity by the anti-free radical activity test DPPH increase as follows: *A. numidica*. **Conclusion:** The results show that the antioxidant activity of essential oils extracted from *Calamintha officinalis* is more efficient compared to *Abies numidica*. Geraniol, carvone and thymol are used as reference compounds.

INTRODUCTION

In recent years, medicinal plants have shown considerable effectiveness in the treatment of many human diseases. (Gilani et al., 2004). Essential oils extracted from plants have many pharmacological properties such as antioxidant properties. (Lemaoui, 2011). Oxidative stress is a global public health problem and is the source of most human diseases. Free radicals are responsible for oxidant stress resulting from disruption of the oxidant/ antioxidant ratio, resulting in biochemical damage to body cells and molecular destabilization. (Lenzi, 2011). Interest in natural antioxidants, in relation to their therapeutic properties, has increased considerably. Scientific research in several specialties has been developed for the extraction, identification and quantification of these compounds from medicinal plants. (Sanchez Moreno., 2002). Because antioxidant activity plays a key role in preventing chronic diseases such as heart disease, cancer, diabetes, hypertension, and Alzheimer's disease by combating oxidative stress. (Riboli and Norat, 2003). The objective of our study is to evaluate the antioxidant activity of *Calamintha officinalis* and *Abies numidica* essential oils using in vitro chemical systems.

MATERIALS AND METHODS

Extraction of Essential Oils: The method used is to introduce 100g of the Plant material into a glass balloon with enough distilled water to cover the plant material, then the mixture is brought to boiling. The oil vapors come out passthrough the glass cooling coil where condensation will take place. Water and essential oil are separated by density difference. Finally, the oil obtained is stored in an opaque bottle well-sealed at4°C. The extraction operation lasts a few hours.

Calculate Yields %: The yield is the maximum amount of essential oil that a given mass of plant during a given period. Performance is calculated by the formula: (%) = $\boldsymbol{m}(\boldsymbol{he})\ \boldsymbol{m}(\boldsymbol{s})/\times\ \mathbf{100}$

Determination of Antioxidant Activity: Three tests were used to assess the antioxida nt activity of oils essential to the two plants studied (Calamintha Officinalis and Abies nu midica). DPPH · Free Radical Scavenger activity (2,2-diphenyl-1-picryl hydrazyl) the scavenger activity of cationic radical ABT · +(22 acid, azino-bis(3ethylebenzothiazoline) -6 sulfonic). Reductive power to potassium ferricyanide. And three standard rooms (Thymol, Carvone, Geraniol).

ABTS Radical Scavenger Activity: The total antioxidant activity of a molecule is der ived from its ability to inhibit the ABT.+ radical obtained from ABTS. Getting Radicalcation results from ABTS contact with:

- metmyoglobin peroxidase. (Miller and Ric e-Evans, 1997).
- Horseradish peroxidase (HRP). (Arnao *et a l.*, 2001).
- H2O2 or manganese dioxide. (Benavente-Garcia et al., 2000); Miller *et al.*, 1997).
- Potassium persulfate (Re *et al.*, 1999).

ABT.+ radical, in contact with a H · donor leads to ABTS+and discoloration at 734 nm of the solution (Lien *et al.*, 1999).

Radical Scavenger Activity DPPH: In the DPPH test, the synthetic-free radical 2,2-diphenyl-1-picryl-hydrazyl is the most commonly used method for evaluating antioxidant activity. DPPH is characterized by purple coloration in the absence of the ant ioxidant. In the presence of an antioxidant, the characteristic purple color of DPPH is transformed into a yellow color, this color change represents the ability of the plant extract to trap this radical. (Akrout *et al.*, 200 9).Color intensity is inversely proportional to the capacity of antioxidants present in the medium to give protons are measured at 517 nm. (Sanchez-Moreno, 2002).

Resolving Reducing Power: This method is used to evaluate the ability of the s ample to give an electron to convert Fe3+ to Fe2+, this form is quantified by measuring the color of the complex absorbed at 700 nm. A high absorbance indicates that the sample has high reducing power (Barros *et al.*, 2007).

Fe^{3+}_L + antioxidant ⟶ Fe^{2+}_L + oxidized antioxidant.

Expression of Results: Calibration curves are used to determine the effective concentration at 50% (IC50), which is a concentration that yields an absorbance of 0.5.

Statistical Study: The calculation of mean and standard deviations is based on the results of three tests with Microsoft Excel 2013.

ANOVA variance analysis using the Least Significant Difference (LSD)test is per formed using IBM SPSS 25.0 software.

Microsoft Excel 2013 software is also used to make graphics**.**

RESULTS AND DISCUSSION

Yields on Essential Oils: We used the hydrodistillation method to extract essential oils from our plants (*Calamintha Officinalis* and *Abies numidica*). Results are expressed as a mass percentage of dry matter (Fig. 1). The aerial portion of *Calamintha officinalis* showed a high return (0.28%), while the *Abies numidica* yielded a high return (0.21%). Several studies have been carried out on other plants of the Pinaceaeand Lamiaceae families.

A study of the species Abies koreana of Sout

h Korea showed a yield of (0.9%). (Jeong *et al.*,2007). However, another trial in Turkey in Abies Cilicia showed a rate of return of (3.47%). (Dayisoylu *et al.*, 2009). The results obtained for Calamintha Officina lis differ widely from our results, a study on Satureja Calamintha from the Sefrou region of morocco by Ech-Chahad *et al.*, (2013) raised a return of (0.082%). On the other hand, ELAjjouri *et al.* (2015), evaluated the yield of Thymusalgeriensis collected in the region of Rachida, Morocco, they noted a rate of return close to our results (0.3%). However, other results were postponed, such as *Salvia officinalis* de France (2.05%) (Bentaarit *et al.*, 2009), Origanumthorouflorum (4.4%) (Rocha *et al.*, 2007), *Thymus ciliatus* de Maroc (1.2%) (EL Ajjouri *et al.*, 22219) 015), According to These articles, we have noticed that the Lamiaceae family yield higher than the Pinaceae family. Variations encountered, both qualitatively and quantitatively, may be due to plant origin, period of the plant cycle, plant age, and also extraction technique. (Kokkini *et al.*, 1994). On the other hand, plant species can influence the quantity and quality of essential oils extracted. (Russo *et al.*, 1998).

Activity Scavenging of the ABTS • + radical: The ABTS method has a turquoise blue color when trapped in antioxidant substances. The reduced form gives the solution a yellow color, the intensity of the color depends on the nature, concentration, and power of the anti-radical substance (Miguel, 2010). The results of the ability to scavenger ABTS • + radical of essential oils of *A. numidica* and *C. Officinalis*, as well as the standards us ed, are shown in Figure (1).

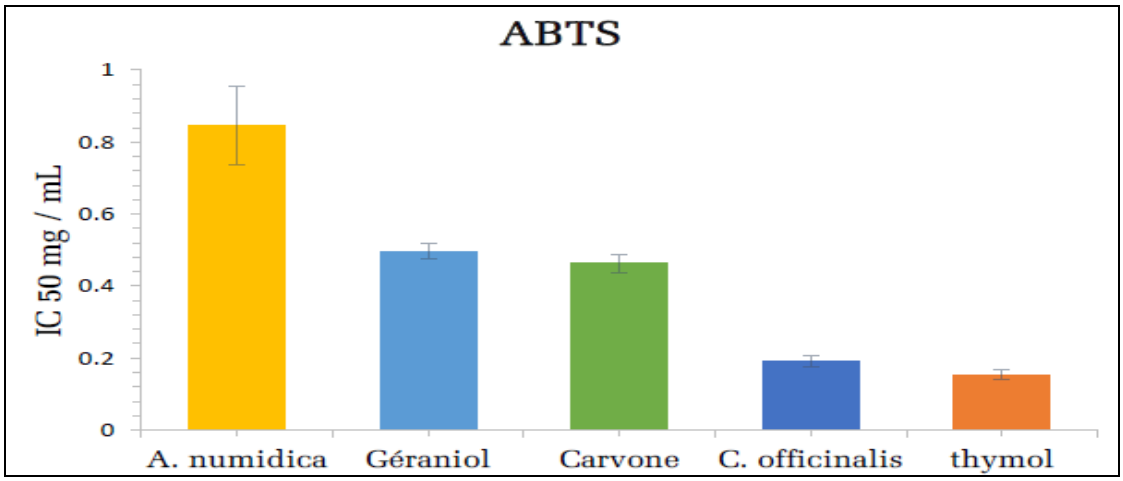

Fig.1: Activity scavenging of the radical ABTS.

The IC50 is defined as the concentration that causes a 50% reduction in the concentration of free radical ABTS • +. (Petlevski *et al.*, 2003). Samples with low IC50 have better antioxidant activity (Pokorny et al., 2001). According to the results, essential oils have different antioxidant activities which increase as follows:
A. numidica < Geraniol < Carvone < C. officinalis < Thymol.
Their respective IC50 values are 0.84±0.10 0 .43±0.003 0.46±0.02; 0.19±0.01and 0.15±0.0 1 mg/mL. These results show that essential oils of C. Officinalis have high antioxidant activity compared to *A. numidica*, Thymol, Carvone and Geraniol are used as standard.

The statistical study (Table 1) shows that there is a significant difference ($p<0.05$) between the latter.

Table.1: ANOVA test result between samples and ABTS

(I)the plant	(J) the plant	Mean Difference (I-J)	Std Error	sig	95% Confidence Interval	
					Lower Bound	Upper Bound
A.numidica	C.officinalis	.63500	.06618	.001	.3695	.9005
	Thymol	.67000	.06618	.001	.4045	.9355
	Géraniol	.32500	.06618	.023	.0595	.5905
	Carvone	.36000	.06618	.015	.0945	.6255
C.officinalis	A.numidica	-.63500	.06618	.001	-.9005	-.3695
	Thymol	.03500	.06618	.980	-.2305	.3005
	Géraniol	-.31000	.06618	.027	-.5755	-0445
	Carvone	-.27500	.06618	.044	-.5504	-.0095
Thymol	A.numidica	-.67000	.06618	.001	-.9355	-.4045
	C.officinalis	-.03500	.06618	.980	-.3005	.2305
	Géraniol	-.34500	.06618	.018	-.6105	-.0795
	Carvone	-.31000	.06618	.027	-.5755	-.0445
Géraniol	A.numidica	-.32500	.06618	.023	-.5905	-.0595
	C.officinalis	.3100	.06618	.027	.0445	.5755
	Thymol	.34500	.06618	.018	.0795	.6105
	Carvone	.03500	.06618	.980	-.2305	.3005
Carvone	A.numidica	-.36000	.06618	.015	-.6255	-.0945
	C.officinalis	.27500	.06618	.044	.0095	.5445
	Thymol	.31000	.06618	.027	.0445	.5755
	Géraniol	-.03500	.06618	.980	-.3005	.2305

Anti-Radical Power in DPPH: According to the DPPH method. Free radical has a purp le color, when trapped by antioxidant substances, the reduced form gives the solution a young pale color (Igwe, 2004). The results of the ability to scavenger ABTS ˙ + radical of essential oils of *A. numidica* and *C. officinalis* and the standards used are shown in Figure 1.

The IC50 is defined as the concentration that causes a 50% reduction in the concentration of free radical DPPH ˙ . (Petlevski *et al.*, 2003). The graph (Fig. 1) shows the percentage of inhibition of the DPPH radi cal which increases as follows: *A. numidica* < Geraniol < Carvone < *C. officinalis* <Thymol. Their respective IC50 values are 26.70±9.02; 8.41±0.16; 8.26±0.16 ;1.77±1.66 and 0.69±0.05 mg/mL. The statistical study shows that there is no significant difference ($p<0.05$) between C. officinalis, thymol, Carvone and Geraniol, but there is a highly significant difference between *C. officinalis*, thymol, Carvone, and *A. numidica.*

Table.2: ANOVA test result between samples and DPPH

(I)the plant	(J) the plant	Mean Difference (I-J)	Std Error	Sig	95% Confidence Interval	
					Lower Bound	Upper Bound
A.numidica	C.officinalis	24.96500*	4.10440	.009	8.5002	41.4298
	Thymol	26.00000*	4.10440	.008	9.5352	42.4648
	Géraniol	18.25000*	4.10440	.034	1.7852	34.7148
	Carvone	18.42500*	4.10440	.032	1.9602	34.8898
C.officinalis	A.numidica	--24.96500*	4.10440	.009	-41.4298	-8.5002
	Thymol	1.03500	4.10440	.999	-15.4298	17.4998
	Géraniol	-6.71500	4.10440	.536	-23.1798	9.7498
	Carvone	-6.54000	4.10440	.557	-23.0048	9.9248
Thymol	A.numidica	-26.00000*	4.10440	.008	-42.4048	-9.5352
	C.officinalis	-1.03500	4.10440	.999	-17.4998	15.4298
	Géraniol	-7.75000	4.10440	.424	-24.2148	8.7148
	Carvone	-7.57500	4.10440	.442	-24.0398	8.8898
Géraniol	A.numidica	-18.25000*	4.10440	.034	-34.7148	-1.7852
	C.officinalis	6.71500	4.10440	.536	-9.7498	23.1796
	Thymol	7.7500	4.10440	.424	-8.7148	24.2148
	Carvone	.17500	4.10440	1.000	-16.2898	16.6398
Carvone	A.numidica	-18.42500*	4.10440	.032	-34.8898	-1.9602
	C.officinalis	6.54000	4.10440	..557	-9.9248	23.0048
	Thymol	7.57500	4.10440	.442	-8.8898	24.0398
	Géraniol	-.17500	4.10440	1.000	-16.6398	16.2898

FRAP Iron Reduction Test:

The OX/RED reaction between the extract and the transition metal ions is an ideal way to evaluate the reducing activity of this extract, K3Fe (CN)6 potassium ferricyanide provides ferric ions (Fe3+) that will be reduced to Ferreux (Fe2+) by the antioxidants present in essential oils. (Oyaizu, 1986). The reduction power of our samples is expressed by the effective concentration at 50% (IC50), which is a concentration that allows an absorbance of 0.5. Figure 1 gives an idea of the effectiveness of our samples to reduce the Ferric ions (Fe3+) to Ferreux (Fe2+). Based on the results in Figure 1, Carvone has a high IC50 value (0.49±0.07mg/mL), which means low Reductive power, the second position is Geraniol (0.32±0.03 mg/mL), followed by *A. numidica*; *C. officinalis* and Thymol Theirrespective IC50 values are: 0.16±0.02; 0.12±0.01; 0.09±0.01 mg/mL. The statistical study shows that there is no significant differ ence ($p<0.05$) between *A. numidica*, *C. officinalis* and thymol, but there is a significant difference between them and Geraniol and Carvone.

Table 3: Results of the ANOVA test between samples and Reductive Power

(I)the plant	(J) the plant	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval	
					Lower Bound	Upper Bound
A.numidica	C.officinalis	.04000	.03860	.830	-.1148	.1948
	Thymol	.07000	.03860	.456	-.0848	.2248
	Géraniol	-.16500*	.03860	.039	-.3100	-.0102
	Carvone	-.33000*	.03860	.002	-.4848	-.1752
C.officinalis	A.numidica	-.04000	.03860	.830	-.1948	.1148
	Thymol	.03000	.03860	.927	-.1248	.1848
	Géraniol	-.20500*	.03860	.016	-.3598	-.0502
	Carvone	-.37000*	.03860	.001	-.5248	-.2152
Thymol	A.numidica	-.07000	.03860	.456	-.2248	.0848
	C.officinalis	-.03000	.03860	.827	-.1848	.1248
	Géraniol	-.23500*	.03860	.009	-.3898	-.0802
	Carvone	-.40000*	.03860	.001	-.5548	-.2452
Géraniol	A.numidica	.16500*	.03860	.039	.0102	.3198
	C.officinalis	.20500*	.03860	.016	.0502	.3598
	Thymol	.23500*	.03860	.009	.0802	.3898
	Carvone	-.16500*	.03860	.039	-.3198	-.0102
Carvone	A.numidica	.33000*	.03860	.002	.1752	.4848
	C.officinalis	.37000*	.03860	.001	.2152	.5248
	Thymol	.40000*	.03860	.001	.2442	.5548
	Géraniol	.16500*	.03860	.039	.0102	.3198

A study by Hazzit in 2009 showed t hat thymolrich essential oils containing hydr oxyl group have significant reductive power compared to phenol-poor essential oils. Which justifies our result. The absence of a significant difference between *A. numidica*, *C. officinalis* and Thymol may be justified by the existence of phenolic compounds in the essential oils of these two plants. Consulting scientific data banks, it was found that iron reduction activity may be due to the presence of aggregations

CONCLUSION

This study assessed the antioxidant activity o f the essential oils of these two *Calamintha officinalis* plants belonging to the family of Lamiaceae and Abiesnumidica, the family of Pinaceae, and the three standards: Carvone, Geraniol, and thymol, by the following methods:

ABTS・+, DPPH・ and reductive power.

Obtaining essential oil by hydrodistillation remains a simple and efficient method. *Calamintha officinalis* yields 0.28% and *Abies numidica* 0.21%. First, the antioxidant activity of the essential oils of the two plants and the three standards were studied by the ABTS ・ + reduction method, the IC50 obtained are *A. numidica* (0.84±0.10); Geraniol (0.43±0.003); Carvone (0.46±0.02); C. officinalis (0.19±0.01); Thymol (0.15±0.01). Also, the study of antioxidant activity by the DPPH.Free Radical Trapping method allowed us to identify the IC50 values of our samples, which are classified in the following order:

A. numidica (26.70±9.02); Geraniol (8.41±0. 16); Carvone (8.26±0.16); *C. officinalis* (1.77±1.66) and Thymol (0.69± 0.05). In addition, the FRAP (Iron Reductive Power (Fe3+)) test showed t hat our samples have IC50, which are respectively: Carvone (0.49±0.07); Geraniol (0.32±0.03); *A. numidica* (0.16±0.02); *C. officinalis* (0.12±0.01) and Thymol (0.09±0.01). These results and statistical analyzes show that the essential oil of *Calamintha officinalis* has better antioxidant activity than the essential oil of *Abies numidica.*

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