



DEVELOPMENT OF A NOVEL OIL FORMULATED FROM THIRTEEN CHEMICAL COMPOUNDS: ECONOMIC SIGNIFICANCE OF ACTIVE COMPOUND QUANTITIES BASED ON SYNERGY STUDIES

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In this work, oil formulated from thirteen standard compounds is developed. These compounds include seven monoterpene hydrocarbons (limonene, α -pinene, β -pinene, p-cymene, myrcene, camphene, ocimene), two monoterpenic alcohols (menthol, linalool), two monoterpenic phenols (carvacrol, thymol), one monoterpene ketone (camphor), and one phenylpropene (eugenyl acetate). The evaluation of the antioxidant activity of these pure compounds, using the free radical trapping method 2,2-diphenyl-1-picrylhydrazyl (DPPH), shows that phenolic compounds are the most active; however, their activities remain inferior to those of reference antioxidants (BHT, BHA, and guaiacol). The activity of the formulated oil demonstrates a significant positive synergistic effect, closely matching the activity of guaiacol. This synergistic effect results in a marked decrease in the EC₅₀ of the formulated oil to a value of 27 μ mol/ml, with the interactions resulting from the difference in antioxidant activity between experimental and theoretical values (percentage difference in DPPH) equal to 94.62%. This corresponds to a decrease in the overall active dose of 99.95%. Although the partial synergistic contributions of phenolic compounds are the most significant—carvacrol (79.69%), thymol (64.87%), and eugenyl acetate (19.81%)—those of other phenolic compounds are generally less than or equal to 1%. Nonetheless, these compounds induce a material gain in the quantity of compounds with the highest reducing potential, yielding decreases (that is, gains in active materials) of 20.31%, 35.13%, and 80.19%, respectively.

Keywords: Formulated Oil; DPPH; Antioxidant activity; Reducing potential; synergistic contributions

INTRODUCTION

Essential oils are natural mixtures of highly complex volatile compounds. They consist of terpenes and aromatic compounds derived from phenylpropene. Several studies have highlighted their biological activities, particularly their antifungal¹, antibacterial²⁻³, antioxidant, and insecticidal⁴⁻⁶ properties. This has given them an important place in

aromatherapy⁷, pharmacy⁸, perfumery, cosmetics^{9,10}, and food preservation¹¹. The biological activity of these oils is related to their chemical compositions, the functional groups potentially endowed with these activities (such as alcohols, phenols, terpenes, and ketones), and their synergistic effects¹².

The compounds with the greatest antioxidant activity are phenols. Thymol, carvacrol, and eugenol are the most studied¹³⁻²⁰.

Carvacrol and thymol are the main components found in the essential oils of *Origanum vulgare*²¹, *Lippia gracilis*²², and *Thymus vulgaris*²³, which have already been reported for their medicinal benefits against several diseases, including cancer²⁴. Known to be non-toxic, they are used as preservatives and flavorings in food products²⁵. Monoterpene alcohols follow phenols in terms of antioxidant activity, with the best-known being geraniol, linalool, thujanol, myrcene, terpineol, menthol, and piperitol^{26, 27}.

Monoterpene aldehydes also possess antioxidant properties, albeit weaker; the most commonly used are nerol, geranial, citronellal,

and cuminal²⁸. The food, cosmetics, and pharmaceutical industries are very interested in the properties of these compounds, especially since they serve as natural flavoring agents. Many researchers around the world are studying their potential as preservatives²⁹.

Our work aims to study the antioxidant activity of certain components of essential oils and a formulated oil, named 'HF', derived from the equimolar mixture of these components. This could allow us to estimate the synergistic effect of the components of essential oils. **Table 1** presents the raw and chemical formulas of the selected chemical species.

Table 1: Elements of the formulated oil, their families and molecule number.

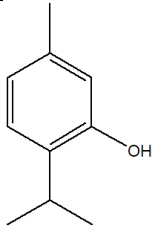
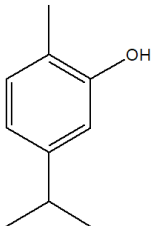
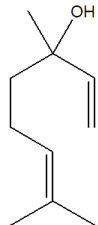
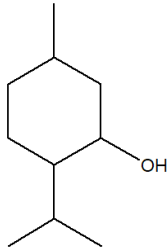
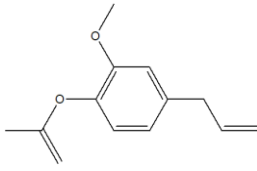

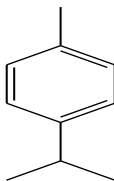
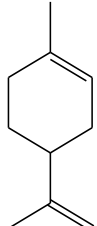
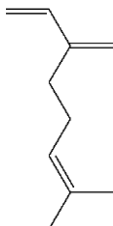
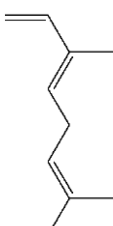
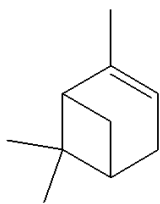
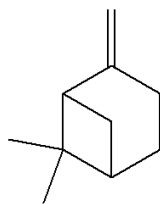
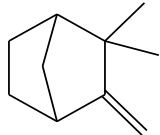
Element	structure	molecule number	element	structure	molecule number
Thymol		1	Caracole		2
Linalool		3	Menthol		4
Eugenyl-acetat		5	Camphor		6
P-cymene		7	Limonene		8

Table 1: Continued.

Myrcene		9	Ocimene		10
α -pinene		11	β -pinene		12
Camphene		13			

Experimental Section

Chemicals and equipment

The solvents and chemicals used were of the brand names Fluka or Prolabo. The chemicals were of reagent grade with a purity exceeding 95%. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

UV-visible analyses were conducted using a "Shimadzu UV/ VIS Spectrophotometer UV-1601" "Obtained from the supplier of equipment Prochima Sigma, Algeria, with ethanol serving as the solvent for the DPPH test.

Free radical trapping with DPPH

In this method, a color change occurs in the DPPH alcoholic solution upon its reduction by the antioxidant, shifting from deep purple (dark violet) to faint yellow. The deep purple color is attributed to the DPPH radical form, which absorbs at $\lambda_{\max} = 517$ nm, while the faint yellow color corresponds to the reduced form of DPPH. The decline in the intensity of the absorbance band at $\lambda_{\max} = 517$ nm of the DPPH radical form is indicative of antioxidant activity^{15,18,19}.

A solution of the DPPH radical was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol. For each compound studied and for each control antioxidant used (3,5-di-tert-4-butylhydroxytoluene (BHT), a commercial mixture of 2-tert-butyl-4-hydroxyanisole (2-BHA) and 3-tert-butyl-4-hydroxyanisole (3-BHA), guaiacol, and eugenol), 5×10^{-3} M solutions in ethanol were prepared. Different concentrations of these solutions were introduced into dry and sterilized test tubes, which were then filled to 1 mL with ethanol. Subsequently, 1 mL of the DPPH solution was added to the resulting mixtures. The entire system was stirred until vortex formation, and then each tube was kept in the dark at 25°C for 30 minutes. After this period, the UV absorbance at $\lambda_{\max} = 517$ nm was measured. Three trials were performed for each solution. The antioxidant activity (AA) was calculated using Equation 1 :

$$AA (\%) = (Abs_{blank} - Abs_{test}) / Abs_{blank} \times 100 \quad (1)$$

Where, Abs_{blank} and Abs_{test} represent the absorbances of the blank solution (DPPH in ethanol) and the test solution, respectively.

Formulation of composed oil

Many works have been conducted to evaluate the antioxidant activity¹⁹⁻²⁵. In this paper the formulated oil represents an equimolar mixture of thirteen chemical compounds, at a concentration of 10^{-2} mol/l in 250 ml of ethanol. These compounds include: limonene, α -pinene, β -pinene, p-cymene, myrcene, camphene, ocimene, menthol, linalool, carvacrol, thymol, camphor, and eugenyl acetate.

Davicino and Col demonstrated that limonene's antioxidant activity could help protect healthy lymphocytes from diseases associated with oxidative stress. Studies have also shown that monoterpenes, such as limonene, exhibit antioxidant properties in the DPPH model. Moreover Piccialli et al. demonstrated the neuroprotective effects of limonene, a key compound found in the Citrus genus, against the neurotoxicity caused by A β 1-42 oligomers, which are believed to play a central role in triggering Alzheimer's disease²⁶.

Ciftci et al. Treated female Sprague Dawley rats exposed to the environmental contaminant 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) with myrcene [with a high dose of up to 200 mg/kg bw per day (1,468 μ mol/kg) for 30 or 60 days]. These rats had a decrease in hepatic lipid peroxidation via activation of antioxidant and radical scavenger properties (97). Myrcene, again at a high dose of 200^{27,28}.

The Tulsi plant contains numerous active compounds and the major compounds are linalol, eugenol, methylchavicol, methylcinnamat, linolen, ocimene, pinene, cineol, anethol, estragol, thymol, citral, and camphor.³⁵ Different parts of the O. sanctum plant, mostly leaves,

Mahdian and col E. platyloba essential oil with (Z)- β -Ocimene (26.7%), δ -3-carene (16.2%) and limonene (6.6%) as the main components showed the IC₂₀ of 1.1 mg/ml in DPPH system. The oil with thymol (27.2%), trans-ocimene (20.9%) and carvacrol (7.2%) had the IC₅₀ of 50 μ g/ml²⁹.

RESULTS AND DISCUSSION

The principle of the method used to assess antioxidant activity, as described by Blois³⁰, is based on the rate of trapping of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanolic or methanolic solution. DPPH has an

unpaired electron on a nitrogen atom in the hydrazyl bridge. Because of this delocalization, the radical molecules do not form dimers, and DPPH remains in its relatively stable monomer form at room temperature. This delocalization also gives rise to its dark violet color, characterized by an absorbance in ethanol solution at 517 nm. The addition of an antioxidant, which can contribute a hydrogen atom or a free electron, leads to the formation of the reduced form DPPH-H, resulting in a color transition from violet to yellow. The intensity of this color change is directly proportional to the antioxidant capacity of the added product. This change can be monitored by spectrophotometry by measuring the decrease in absorbance at 517 nm.

To classify the pure compounds studied based on their antioxidant power, we determined the effective concentration that produces 50% of the trapping activity of the DPPH radical (EC_{50}) for each compound individually. These results are grouped in **Table 2**.

Table 2: Values of the effective concentration, which gives 50% of the free radical DPPH trapping activity (EC_{50}^i), for each compound studied individually.

N°	compound (i)	EC_{50}^i μ mol/ml
01	Camphor	1437,96
02	Caracole	2,54
03	Thymol	3,12
04	Limonene	316,1
05	Menthol	520,06
06	β -pinene	271,89
07	α -pinene	1174,48
08	Myrcene	-
09	Ocimene	126,48
10	Linalool	691,41
11	p-cymene	469,75
12	Camphene	1453,01
13	eugenyl acetate	10,33

Similarly, to compare the reducing potential of our thirteen compounds to that of synthetic and/or natural antioxidants widely used in various industrial fields, we performed a comparative study between their activity and that of four reference antioxidants: BHA (butylhydroxyanisole), BHT (butylhydroxytoluene), guaiacol, and eugenol. **Table 3** lists the EC_{50} values of the reference compounds.

The antioxidant capacity of a compound is higher when its EC_{50} is lower. The analysis in **Table 3** shows that the reference antioxidants have the lowest EC_{50} values. This indicates that their antioxidant power is greater than that of the compounds present in the formulated oil.

Table 3: Values of the effective concentration, which gives 50% of the free radical trapping activity DPPH ($EC_{50}^{j_{ref}}$), for each j_{ref} compound studied individually.

N°	reference compound	EC_{50}^{ref} $\mu\text{mol/ml}$
01	BHA	$5.24.10^{-4}$
02	BHT	$3.05.10^{-4}$
03	Guaiacol	0.655
04	Eugenol	97.10^{-4}

Among these compounds, the most active are carvacrol and thymol, and this activity is attributed to their phenolic functional groups. These results are consistent with those obtained by Sharapov et al.²⁷, where they found that carvacrol, both experimentally and theoretically, demonstrates superior antioxidant activity compared to thymol. They showed that the trapping activity of the DPPH free radical by these molecules is due to the dissociation of the phenolic bond rather than the C-H bond in the benzene ring.

These findings align with the literature, which describes that phenolic compounds

possess excellent antioxidant properties due to their ability to trap free radicals and reactive oxygen species. Carvacrol can induce significant hepatoprotective and antioxidant effects by improving the activity of enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and increasing levels of non-enzymatic antioxidants (vitamin C, vitamin E, and reduced glutathione)³¹.

Additionally, examining the EC_{50} values reveals that menthol is less active than p-cymene. The latter consistently exhibits aromaticity in its chemical structure, contributing to greater stability of the free radicals formed. Therefore, for compounds with the same carbon skeleton, aromaticity enhances antioxidant activity more effectively than a hydroxyl group. Nevertheless, limonene, with an EC_{50} of $316.1 \mu\text{mol/ml}$, shows better activity than both p-cymene and menthol, despite the absence of aromaticity and hydroxyl functionality. This is likely due to the number of radical sites that can form and be stabilized by the two unconjugated double bonds in limonene's structure.

Returning to the structure of eugenyl acetate, it shares a similar chemical structure with eugenol and guaiacol. With a methoxy group in the meta position of the phenolic function and a prop-2-enyl group in the para position, the stabilization of the phenoxyl radical by resonance is reduced in the case of eugenol compared to that of guaiacol. This is attributed to the donor mesomeric effect of these groups (**Fig. 1**); therefore, guaiacol is expected to exhibit greater antioxidant activity, which is contrary to the findings. The EC_{50} value shifts from $0.0097 \mu\text{mol/ml}$ for eugenol to $0.655 \mu\text{mol/ml}$ for guaiacol, indicating an activity nearly 70 times lower. This discrepancy can only be explained by the presence of an isolated double bond in the prop-2-enyl group. As a result, the intramolecular synergistic effect between the isolated double bond and the hydroxyl group outweighs the donor mesomeric effect.

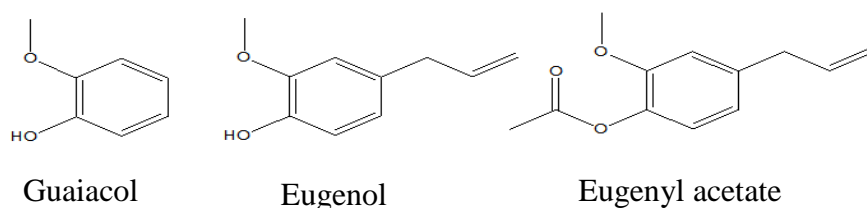


Fig. 1: Chemical structure of guaiacol, eugenol and eugenyl acetate.

The substitution of the hydroxyl function on the benzene ring with an ester function supports this conclusion, as the EC_{50} increases to 10.33 $\mu\text{mol/ml}$ for eugenyl acetate, a value closer to that of carvacrol and thymol (**Fig. 1**).

In contrast, meta-substituents, isopropyl in thymol and methyl in carvacrol, do not exert a donor mesomeric effect, making these compounds more active than guaiacol and eugenyl acetate. Myrcene and ocimene are positional isomers; these two compounds exhibit the same activity at low concentrations (15% activity for 36 $\mu\text{mol/ml}$). However, at higher concentrations, myrcene becomes insoluble, making analysis by UV-Vis spectrophotometry impossible. The antioxidant activity of ocimene can be attributed to the presence of multiple mobile hydrogens, which can give rise to several resonance-stabilized radical sites. The significance of this resonance is especially apparent in the case of linalool, where the absence of resonance leads to decreased activity, with an EC_{50} of 691.41 $\mu\text{mol/ml}$.

While the hydrogen from the hydroxyl function is generally more mobile, the generated alkoxy radical is not stabilized by resonance. Thus, the transition from the aliphatic form of ocimene to the cyclic form does not enhance its activity, since higher EC_{50} values are obtained with α - and β -pinene, at 1174.48 $\mu\text{mol/ml}$ and 271.89 $\mu\text{mol/ml}$, respectively.

Notably, the antioxidant activity of β -pinene is four times higher than that of α -pinene. This may be attributed to the presence of an exo-cyclic free double bond in β -pinene's bicyclic structure, which could better stabilize the resulting radicals than the endo-cyclic free double bond found in α -pinene. Interestingly, camphene, which also features an exo-cyclic double bond in a bicyclic structure, displays lower activity than α -pinene ($EC_{50} = 1453.01$ $\mu\text{mol/ml}$), likely due to the two methyl groups adjacent to the double bond. Furthermore, camphor, which contains a cyclic ketone function, also exhibits low activity, with an EC_{50} of 1437.96 $\mu\text{mol/ml}$, comparable to that of camphene.

Limonene (316.1 $\mu\text{mol/ml}$), although devoid of aromaticity, demonstrates higher activity than p-cymene. This is similar to ocimene, as it can present multiple radical sites.

Activity of the formulated oil

It is accepted that there is a synergistic interaction between the constituents of a mixture endowed with a common biological property if, and only if, the measurement of this property for the mixture is greater than the sum of the measurements made individually on the components of the mixture. This is known as the synergistic effect. In the case of additive interaction regarding the common effect, the measurement made on the mixture is equal to the sum of the measurements obtained separately for the compounds that constitute it. Finally, antagonistic interactions imply that the value of the common effect for the mixture is less than the sum of the values obtained for the same effect in the individual compounds.

In our work, we are interested in antioxidant activity, which is inversely proportional to the value of EC_{50} . We can therefore say that there will be a synergistic effect if the EC_{50} of the formulated oil—i.e., the overall molar amount of all the components of the mixture is less than the sum of the EC_{50} values of the components taken in their pure states (equation 2):

$$EC_{50}^{HF} < \sum_{i=1}^n EC_{50}^i \quad (2)$$

With:

EC_{50}^{HF} : EC_{50} of formulated oil; EC_{50}^i : EC_{50} of component i of the HF individually; n : the number of HF constituents. In our work, it is equal to 13 compounds.

By representing the sum $\sum_{i=1}^n EC_{50}^i$ by the abbreviation S_1^n , we can rewrite the equation 2 as below (equation 3):

$$EC_{50}^{HF} < S_1^n \quad (3)$$

Table 4 provides the concentrations in $\mu\text{mol/ml}$ of the various components of the formulated oil for an activity level of 50%.

Based on the values in **Table 4**, the EC_{50}^{HF} equal to 27 $\mu\text{mol/ml}$, this value is much lower than the sum S_1^n which is equal to 647,7 $\mu\text{mol/ml}$, and is close to that of the phenolic compound eugenyl acetate (10.33 $\mu\text{mol/ml}$).

According to the previously described postulate, and based on the results obtained, the formulated oil exhibits a significant synergistic effect, most likely due to interactions between reducers and oxidants that promote the antioxidant role.

Table 4: Concentrations of the various components of the formulated oil for an activity of 50%.

compound	n_i^{HF} $\mu\text{mol/ml}$
camphor	2,128
caracole	2,024
Thymol	2,024
limonène	2,144
menthol	2,04
β -pinène	2,026
α -pinène	2,04
Myrcene	2
ocimene	2,22
linalol	2,08
p-cymene	2,012
camphene	2
Eugenylacétate	2,046

Calculation of percentage difference (DPPH)

Hidalgo and his co-authors³¹ describe the interactions arising from the difference in antioxidant activity between experimental and theoretical (calculated) values to enhance the understanding of the synergistic phenomenon.

In the case of the free radical trapping method DPPH, this difference, expressed as a percentage, is given by the equation below (equation 4).

% difference (DPPH)

$$= 100 - \left(\frac{EC_{50}^{HF} \times n \times 100}{S_1^n} \right) \quad (4)$$

n: represent the number of constituents of the mixture under study. EC_{50}^{HF} and S_1^n have already been described.

Positive difference percentages (DPPH) are considered to indicate synergistic values, while negative values represent antagonistic effects. Values close to 0% imply additive effects³¹. For n equal to thirteen compounds, we find:

$$\% \text{ difference (DPPH)} = 94.62 \%$$

As the difference in antioxidant activity between experimental and theoretical values is significantly positive, we can conclude that the interactions among the various constituents of the formulated oil exhibit a notable and promising synergistic effect.

Calculation of the rate of reduction of the overall active dose

It clears that, positive synergistic interactions lead to a decrease in the overall amount of matter (in $\mu\text{mol/ml}$) necessary to achieve 50% activity, which represents the overall active dose.

To calculate the rate of decrease ($Td_s^{EC_{50}}$) of this dose (reflecting the contribution of the combination of our thirteen compounds to synergistic power), equation (5) is proposed:

$$Td_s^{EC_{50}} = \left(\frac{S_1^n - EC_{50}^{HF}}{S_1^n} \right) \times 10 \quad (5)$$

With:

$Td_s^{EC_{50}}$: Rate of decrease in the overall amount of n constituents in the formulated oil, for 50% activity

Applying the previous relationship, we find $Td_s^{EC_{50}} = 99.95\%$

This value confirms the existence of an important synergistic effect, due to interactions that govern the dose-activity relationship by significantly lowering the activity dose for 50%.

Calculation of partial synergetic contribution

The decrease in the overall amount of matter that induces the antioxidant activity of the formulated oil is directly related to the more or less significant decrease in the quantity of each component of the mixture corresponding to the studied activity percentage, such as 50%.

These partial decreases symbolized, for each component i, by the abbreviation $Td_i^{EC_{50}}$ are given by the equation(6) below:

$$Td_i^{EC_{50}} = \left(\frac{EC_{50}^i - n_i^{HF}}{EC_{50}^i} \right) \times 100 \quad (6)$$

$Td_i^{EC_{50}}$: Rate of reduction of the partial quantity of component i in the compound oil,

for 50% activity compared to the quantity responsible for the same percentage of activity if component *i* is taken individually

EC_{50}^i : Effective concentration of the constituent *i* individually for an activity of 50%.

n_i^{HF} : Amount of constituent *i* material (in $\mu\text{mol/ml}$) in HF to have an activity of 50%

The results obtained for the thirteen compounds are summarized in **Table 5**.

To estimate the contribution of each compound to the synergistic effect of the mixture, the partial contribution of each compound is calculated individually using the equation 6 given as follows:

$$C_{s-i}^{EC_{50}} = 100 - Td_i^{EC_{50}} \quad (7)$$

With:

$C_{s-i}^{EC_{50}}$: Contribution of constituent *i* to the synergistic power of the mixture for 50% activity.

$Td_i^{EC_{50}}$: As described earlier.

Table 5: Rate of reduction of the partial quantity of component for the thirteen compounds.

N°	composé	$Td_i^{EC_{50}}$
1	camphor	99,85
2	caracole	20,20
3	Thymol	35,03
4	limonène	99,32
5	menthol	99,61
6	β -pinène	99,25
7	α -pinène	99,83
8	Myrcene	-
9	ocimene	98,24
10	linalol	99,70
11	p-cymene	99,57
12	camphene	99,86
13	Eugenylacétate	80,19

The results for the thirteen compounds are summarized in **Table 6** below. **Table 6** clearly shows that the phenolic compounds—namely,

carvacrol (79.69%), thymol (64.87%), and eugenyl acetate (19.81%)—continue to dominate the antioxidant activity of the mixture, despite a significant reduction in their effective concentrations required for 50% activity, with respective reduction rates of 20.31%, 35.13%, and 80.19%.

Additionally, we note that the partial contribution of the non-phenolic compounds studied does not exceed 1%, except in the case of ocimene (1.76%). This small contribution was sufficient to create a significant synergistic effect. This demonstrates that, although non-phenolic compounds have a reducing potential lower than that of phenolic compounds, they still play a tangible role at various stages of the redox reaction between the oxidant and the antioxidant.

Table 6: Contribution of the constituent *i* in the mixture.

N°	composé	$C_{s-i}^{EC_{50}}$ (%)
1	camphor	0,15
2	caracole	79,69
3	Thymol	64,87
4	limonène	0,68
5	menthol	0,39
6	β -pinène	0,75
7	α -pinène	0,17
8	Myrcene	-
9	ocimene	1,76
10	linalol	0,30
11	p-cymene	0,43
12	camphene	0,14
13	Eugenylacétate	19,81

Conclusion and perspectives

Our study investigates the antioxidant properties of essential oil components and a formulated oil named "HF," composed of an equimolar mixture of these components. Key compounds like carvacrol and thymol

demonstrate potent antioxidant effects due to their phenolic nature, while p-cymene shows heightened activity owing to its stability against free radicals. Interestingly, limonene exhibits significant antioxidant activity despite lacking aromaticity, potentially due to its unconjugated double bonds, which stabilize radicals.

Our comparative analysis reveals that eugenyl acetate has lower antioxidant activity than eugenol and guaiacol, likely due to the isolated double bond in its structure enhancing intramolecular interactions.

In summary, our findings emphasize the antioxidant potential of various essential oil compounds, highlighting that structural features significantly influence efficacy. The significant synergistic effect observed in the formulated oil reduces the required active dose for 50% activity (94.62% difference between experimental and theoretical values), underscoring the importance of interactions in the dose-activity relationship.

Analysis of partial synergistic contributions identifies phenolic compounds, particularly carvacrol and thymol, as key contributors, while non-phenolic compounds also play a minor role. This synergistic interaction suggests the potential for reducing the quantity of antioxidants needed, lowering production costs in various industrial sectors, and paving the way for exploring selective combinations that may mitigate the use of synthetic antioxidants.

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نشرة العلوم الصيدلانية جامعة أسيوط



تطوير زيت جديد مركب من ثلاثة عشر مركباً كيميائياً: الأهمية الاقتصادية لكميات المركبات النشطة بناءً على دراسات التآزر

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في هذا العمل، تم تطوير زيت مركب من ثلاثة عشر مركباً قياسيًّا. تتضمن هذه المركبات سبعة هيدروكربونات أحادية التربين (ليمونين، ألفا بينين، بيتا بينين، ب-سيمين، ميرسين، كمفين، أوسيمين)، واثنين من كحولات أحادية التربين (منثول، لينالول)، واثنين من الفينولات أحادية التربين (كارفاكرول، ثيمول)، وكيثون أحادي التربين (كافور)، وفينيل بروبين (أسيئات يوجينيل). يُظهر تقييم النشاط المضاد للأكسدة لهذه المركبات النقية، باستخدام طريقة حبس الجذور الحرة ٢،٢-ثنائي فينيل-١-بيكريل هيدرازيل (DPPH)، أن المركبات الفينولية هي الأكثر نشاطاً؛ ومع ذلك، تظل أنشطتها أدنى من أنشط مضادات الأكسدة المرجعية (BHT، BHA وguaiacol). يُظهر نشاط الزيت المُصاغ تأثيراً تآزرياً إيجابياً كبيراً، يتطابق بشكل وثيق مع نشاط (guaiacol). يؤدي هذا التأثير التآزري إلى انخفاض ملحوظ فيتركيز مثبط EC₅₀ للزيت المُصاغ إلى قيمة ٢٧ ميكرومول/مل، مع تفاعلات ناتجة عن الاختلاف في نشاط مضادات الأكسدة بين القيم التجريبية والنظرية (الفرق النسبي في DPPH) تساوي ٩٤,٦٢%. وهذا يتوافق مع انخفاض في الجرعة النشطة الكلية بنسبة ٩٩,٩٥%. وعلى الرغم من أن المساهمات التآزرية الجزئية للمركبات الفينولية هي الأكثر أهمية - كارفاكرول (٧٩,٦٩%)، والثيمول (٦٤,٨٧%)، وأسيئات الأوجينيل (١٩,٨١%) - فإن مساهمات المركبات الفينولية الأخرى أقل من أو تساوي ١% بشكل عام. ومع ذلك، فإن هذه المركبات تحفز زيادة مادية في كمية المركبات ذات القدرة الأعلى على الاختزال، مما يؤدي إلى انخفاضات (أي مكاسب في المواد الفعالة) بنسبة ٢٠,٣١% و ٣٥,١٣% و ٨٠,١٩% على التوالي.