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Research Article

Evaluation of the Pharmacological Potential of the Combination of Two Essential Oils from Eucalyptus and Lemongrass

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Abstract

This study aims to assess the pharmacological potential of essential oils extracted from two aromatic plants, *Cymbopogon citratus* and *Eucalyptus globulus*, cultivated in Lebanon, taken individually and in combination in order to determine possible synergistic interactions. The extraction of these essential oils was carried out by hydrodistillation. Then, an organoleptic characterization and an evaluation of their antioxidant, repellent/insecticidal and antibacterial activities have been evaluated. The obtained results showed that the two oils had an antioxidant activity dependent on the dilution used and reaching 85% for eucalyptus and 60% for lemongrass. While the combination of these oils showed an antioxidant activity of 65%. In addition, they showed an insecticidal effect when used individually, and an increase in this effect when were used in combination. Also, these two essential oils demonstrated an antibacterial effect which increases when they are in combination according to the types of bacteria.

Keywords: Eucalyptus globulus; *Cymbopogon citratus*; Essential oils; Hydrodistillation; Antioxidant; Antibacterial; Insecticidal effect

Introduction

Essential oils (EO) are aromatic oily liquids extracted from different parts of plants by several methods like hydrodistillation, steam distillation, expression, and so on. Additionally, the amount of essential oil from different plants and from different plant parts is different. Due to the increasing attention in natural additives, essential oils have been used more widely [1]. They have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. They are commonly used as flavouring agents in food products, drinks, perfumeries, pharmaceuticals and cosmetics [2]. The individual effects of EOs of spices and herbs on antimicrobial activity as well as their antioxidant activity have been extensively reported [1], but their combination effects seem to be dubious. For that, the present study was carried out to evaluate, for the first time, the antioxidant, antibacterial and insecticidal effects of the essential oils obtained from the leaves of eucalyptus and lemongrass grown in Lebanon, taken individually and combined together.

Materiel and Methods

Essential oil extraction

The leaves of *Eucalyptus globulus* and *Cymbopogon citratus* were collected very early in the morning in order to preserve the integrity of their volatile molecules. Eucalyptus leaves were collected in Hadat, south east of Beirut, in May 2021. Lemongrass leaves were collected in Chehim-Chouf, south east of Beirut in June 2021. They were washed and cut into small pieces. The extraction of essential oils from leaves (500g) was done by hydrodistillation using

a Clevenger type apparatus under optimal operating conditions for 3 hours. At the end of the process, the essential oils were collected, dried over anhydrous sodium sulphate, and stored in sealed vials in the dark, at 4 °C, until used.

Yield of essential oils

The yield of the obtained essential oil was calculated according to the following equation:

$$Yeild(\%) = \frac{Amount\ of\ extracted\ oil(g)}{Amonut\ of\ dry\ vegetalmatter\ mass(g)} \times 100$$

Organoleptic characterization

The appearance, color and smell of the extracted essential oils were studied.

DPPH free radical scavenging assay

The method of Rammal et al. [3] has been used for the scavenging ability of DPPH antioxidant test of the two essential oils, alone and in combination. Briefly, 1mL of different dilutions (2%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1% and 0.005%) of each essential oil alone from each plant and of the combined essential oils was added to 1mL of DPPH (0.15mM in ethanol) and at the same time, a control consisting of 1mL DPPH with 1mL ethanol was prepared. The reaction mixtures were mixed very well by hand and then allowed to stand in dark at room temperature for 30 minutes. Then, the absorbance was measured at 517nm by a Gene Quant 1300UV-Vis spectrophotometer. The gallic acid was used as a positive control and the ethanol was used as blank.

The DPPH scavenging ability of plant essential oils was calculated using the following equation:

% Scavenging activity = [(Abs control−Abs sample)]/ (Abs control)] ×100

The Abs control is the absorbance of DPPH+ethanol; Abs sample is the absorbance of DPPH radical + sample.

Determination of the Antioxidant Combination Index (CI)

To study the possible synergistic antioxidant activity between the two essential oils, an isobologram analysis (interaction study; dose-effect relationship) based on the principle of the median effect (IC_{50}) was performed [4,5]. The classical isobologram-combination index (CI) equation was used to analyze the data.

$$CI = [(D)1/(Dx)1] + [(D)2/(Dx)2]$$

where (D)1 and (D)2 are the doses (IC_{50} values) of the essential oils in combination. (Dx)1 and (Dx)2 are the doses (IC_{50} values) of the essential oils individually. According to the values of CI, the type of antioxidant interactions was interpreted as follows:

IC < 1 : synergistic ; IC = 1 : additive ; IC > 1 : antagonist [4,5].

Determination of the repulsive/insecticidal effect of essential oils

A volume of 5mL of the different dilutions (2%, 1.6% and 0.8%) of the EO of each plant was placed in glass jars. For the combination, 2.5mL of the different concentrations (2%, 1.6% and 0.8%) of the EOs of each plant are mixed and placed in glass jars. At the same time, the negative control consists of 5 mL of ethanol (0.5%). Then the fruit flies (drosophila) are collected inside the jars. The time required for total drosophila mortality was observed and recorded. If mortality was observed after a very short term (<5 minutes), we have increased the dilution of the essential oils.

Evaluation of the antibacterial activity

Bacterial strains: The strains used in this study were Gram positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram-negative strains (*Escherichia coli* ATCC 35218).

MIC and MBC assays: Essential oils of plants were tested for their corresponding Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) by broth microdilution assay, as recommended by the Clinical Laboratory and Standard Institute (CLSI) [6]. In a 96-well plate (200µl/well) (Greiner Bio-One, Essen, Germany), serial two-fold dilutions in MHB of the different essential oils were done. The wells were inoculated with 5×10^5 bacteria/ml. After incubating the plates at 37 °C for 24 hours, the MIC (which is defined as the lowest concentration that yielded no growth) was determined. In addition, the wells with no visible growth, were plated on BHA in order to determine the MBC (which is defined as the lowest concentration which killed ≥99.9% of the initial inoculum). The Petri plates were incubated overnight at 37 °C, and the MBC was determined.

Results and Discussion

Yield of essential oils

After three hours of extraction, the essential oils were collected, and their yields are estimated at 1.17% for eucalyptus and 0.86% for lemongrass. The yield depends mainly on the season. It was documented that the eucalyptus used in september gave a higher yield than that used in may [7].

Organoleptic characterization

The characterization of the essential oils of eucalyptus and lemongrass focused on three parts presented in Table 1. The organoleptic characteristics of our essential oils are comparable to those of [7] Mpiana Kibwela (2020).

Table 1: Organoleptic characteristics of eucalyptus and lemongrass essential oils.

	EO Eucalyptus	EO Lemongrass	Combined EOs
Aspect	Liquide	Liquide	Liquide
Color	Pale Yellow	Light Yellow	Light Yellow
Odor	Strong and Fresh	Lemony, characteristic	Very Strong

Antiradical DPPH assay

The results shown in the figures below illustrate the percentages of anti-free radical inhibition of the essential oils of eucalyptus and lemongrass alone and in combination. The obtained results indicated that the % antioxidant activity increased in a concentration dependent manner for the essential oils to reach a certain value. Also, from the foregoing findings, it was observed that the tested essential oils showed promising antioxidant activity (Figure1). These active essential oils were then subjected to antioxidant combination study with a view to elucidate their possible synergistic potential, if any. Eucalyptus and lemongrass essential oils have reached inhibition activities of 90% ± 0.06 and 63% ± 0.5 respectively at 2% v/v. Also, our results demonstrated values of IC_{50} of 0.1% and 1.5% for Eucalyptus and lemongrass essential oils respectively (Figure 2).

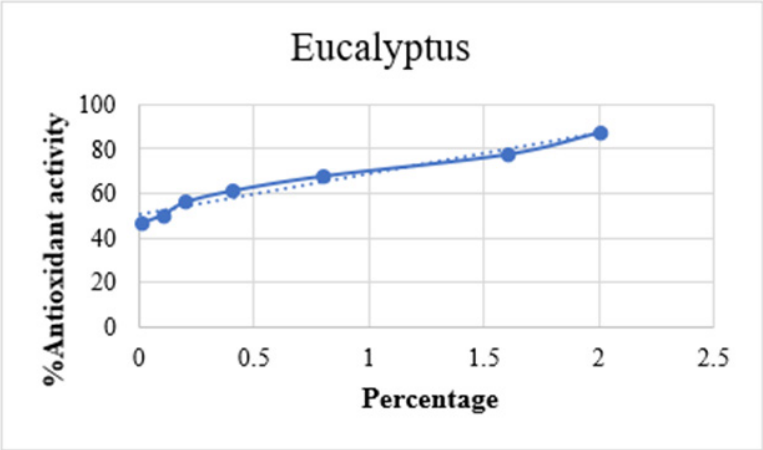

Figure 1: Antioxidant activity of the different concentrations (percentage) of the essential oil of eucalyptus.

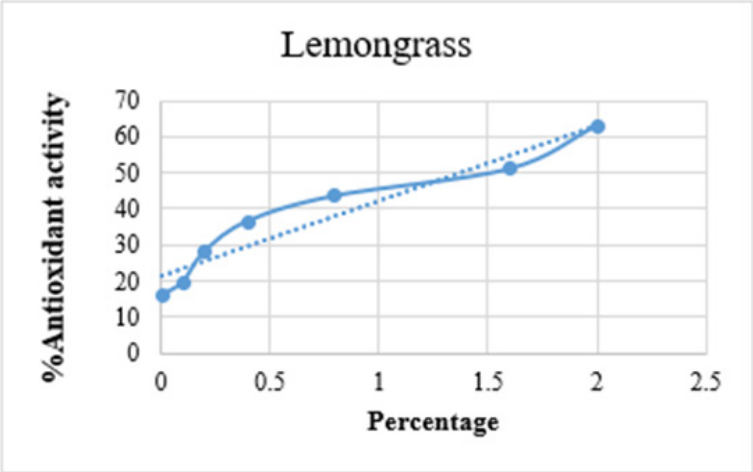

Figure 2: Antioxidant activity of the different concentrations (percentage) of the essential oil of lemongrass.

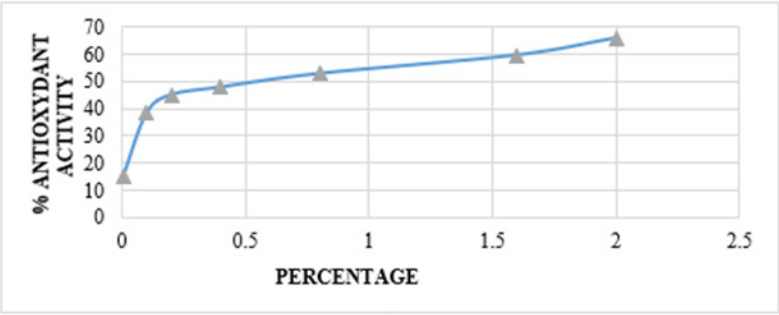

Figure 3: Antioxidant activity of the different concentrations (percentage) of the combined essential oils.

The combination of these two essential oils did not increase the percentage of antioxidant activity as shown in Figure 3. The IC_{50} was of 0.5%, a value more important than those of both essential oils taken individually.

To study the type of interaction between eucalyptus and citronella essential oils in antioxidant activity, we determined the combination index:

$$CI = [(D)1/(Dx)1] + [(D)2/(Dx)2] = 5.3$$

As this value is greater than 1 therefore their interaction is antagonistic and not synergistic.

Repellent /Insecticidal Activity

By definition, repellents are substances that act locally or at a distance, avoiding an insect from flying to, landing on or biting human or animal skin [8,9]. Usually, insect repellents work by providing a vapor barrier preventing the insects from coming into contact with the surface [10]. In our study, two essential oils (eucalyptus and citronella) are used individually and in combination to evaluate its repellent capacity. The obtained results indicated that once the insects are placed in the jar, they quickly moved away from the added oils and avoided coming close to it. They die a few minutes later depending on the concentration (Table 2). The results show that the time required to kill drosophila increases with increasing dilutions of essential oils in all three cases. As shown in the table above, lemongrass oil kills fruit flies faster than eucalyptus oil at all dilutions used. These results agree with those obtained by others indicating that Cymbopogon plants have been traditionally used to repel mosquitoes in jungle regions such as the Bolivian Amazon. This genus produces the most used natural repellents in the world. Also, our obtained results are in agreement with those obtained by others concerning the repellent properties of essential oils from genus Eucalyptus [1].

Table 2: Necessary time to kill drosophila according to essential oils type.

	2%	1.60%	0.80%	0.40%	0.20%	0.10%	0.05%
Eucalyptus	2min	4min	9min	11min	17min	26min	40min
Lemongrass	1min	2min	7min	9min	13min	20min	31min
Combination	1min	1min	4min	6min	9min	13min	21min

On the other hand, the combination of these two essential oils takes a shorter time than that of each oil taken individually, to kill fruit flies. So, the combination of essential oils increases their effectiveness by killing fruit flies faster, compared to the time taken by each oil alone. The results show that the essential oils of eucalyptus and lemongrass have almost similar repulsive effects, where these oils work by repelling the Drosophila within a few minutes, and this leads to their death in a closed jar where the Drosophila cannot escape from it. But the combination of these essential oils increases their effectiveness by killing fruit flies more quickly, compared with the time taken by each oil alone. So, we can then say that the combination of these two oils has a synergistic effect as the combination is more effective.

Antibacterial activity

Table 3: MIC & MBC of essential oils of eucalyptus, citronella and their combination against *E. coli* and *S. aureus*.

	MIC		MBC	
Plants	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Eucalyptus	0.50%	1.50%	0.50%	1%
Citronnelle	1.50%	2%	2%	2%
Combinaison	0.50%	1%	0.50%	1%

The obtained results (Table 3) show that the essential oils of eucalyptus and lemongrass, and their combination, have an antibacterial effect against both *E. coli* and *S. aureus* (inhibitory and bactericidal effects). Eucalyptus oil has an antibacterial effect for a lower concentration than that of citronella. In combination, the inhibitory and bactericidal effects of essential oils are obtained for similar or lower concentrations than those of eucalyptus against the same bacteria with MIC and MBC of 0.5% against *E. coli* and MIC and MBC of 1% against *S. aureus*.

Conclusion

The objective of the present study was to evaluate, for the first time, the possible synergistic interactions on repellent, antibacterial and antioxidant efficacy of essential oils of eucalyptus and lemongrass grown in Lebanon. The results show that these oils have important antioxidant and antibacterial activities allowing them to be used for therapeutic applications, knowing that antioxidants contribute very effectively to the prevention of some diseases related to oxidative stress. In addition, the combination of these essential oils demonstrated a synergistic insecticidal effect. Also, the obtained results showed that the essential oils of eucalyptus and lemongrass have an antibacterial effect. Therefore, their combination can sometimes have a synergistic effect.

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