ORIGINAL PAPER

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *CYMBOPOGON CITRATUS* AND *SYZYGIUM AROMATICUM* ESSENTIAL OILS ALONE AND IN COMBINATION

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Abstract. *Essential oils are now getting more importance in food industry, being used to prevent food spoilage and to promote food preservation. The present study was carried out so as to evaluate the antioxidant and antimicrobial effect of the essential oils of two aromatic plants, namely Cymbopogon citratus (Lemongrass) and Syzygium aromaticum (Clove), and to investigate a possible synergistic effect of the combination of these oils. The evaluation was performed for individual oils and the combination of the two oils in 1:1, 2:1 and 1:2 ratios, against two bacterial pathogens, namely Escherichia coli and Staphylococcus aureus. The study showed promising results for the use of Cymbopogon citratus and Syzygium aromaticum oils, as antioxidant (individually and in combination) and antimicrobial (individual) agents. Cymbopogon citratus and Syzygium aromaticum oils are good candidates to the biopreservation of foods.*

Keywords: *Cymbopogon citratus essential oil, Syzygium aromaticum essential oil, antioxidant activity, antibacterial activity*

1. INTRODUCTION

The International Organization for Standardization (ISO) defines essential oil as a product obtained from a natural raw material of plant origin, obtained by steam distillation, by mechanical processes from the epicarp of citrus fruits, or by dry distillation, after the separation of the aqueous phase [1]. Essential oils (EOs) consist of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300, which can be physically separated from other plant components or membranous tissues [2]. EOs are considered a green alternative in the nutritional, pharmaceutical, and agricultural fields due to their antimicrobial (antibacterial, antifungal, and antiviral), insecticidal, and antioxidant properties [2, 3]. Nowadays, EOs have acquired great popularity over the years as consumers have developed a growing awareness toward the use of natural ingredients, especially in foods and cosmetics [3]. EOs are natural mixtures consisting of about 20-60 components at extremely different concentrations, some components (*e.g.* terpenes, terpenoids) being present at fairly high concentrations (20-70), while other components are present in trace amounts [4]. The components at high concentrations like terpenes and terpenoids possess a major role in

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the antimicrobial and biological effect of EOs, promoting food preservation, and alternatives to treating infectious diseases [4]. *Cymbopogon citratus* (lemongrass) is a perennial plant that grows spontaneously around the world, mainly in the tropical and savannah regions. *Cymbopogon citratus* essential oil is used in the food industry, fragrances, cosmetics and pharmaceuticals, and has a strong antibacterial activity against numerous bacteria such as *Acinetobacter baumanii, Aeromonas veronii, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Salmonella enterica serotip typhimurium, Serratia marcesens, Proteus vulgaris, Enterobacter aerogenes, Corynebacterium equii, Staphylococcus aureus, and Candida albicans* [5-7]. *Syzygium aromaticum* (clove) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. This plant is one of the richest sources of phenolic compounds like eugenol, eugenol acetate and gallic acid, and possesses a great potential for pharmaceutical, cosmetic, food and agricultural applications [8]. *Syzygium aromaticum* essential oil inhibits many microbes including *Lactobacillus* sp., *Bacillus thermoacidurans*, *Salmonella* sp., *Corynebacterium michiganense*, *Pseudomonas striafaciens*, *Clostridium botulinum*, *Alternaria* sp., *Aspergillus* sp., *Canninghamella* sp., *Fusarium* sp., *Mucor* sp., and *Penicillium* sp. [9-11]. Most of the antimicrobial activity of EOs is found in the oxygenated compounds (*e.g.* alcohols, phenolic terpenes), while some hydrocarbons also have antimicrobial effects [12-21]. The interactions between these components may lead to antagonistic or synergistic effects [12-17]. As a rule, combinations, either single EOs or combinations of purified primary components, influence many biochemical processes in the bacteria, producing a plethora of interactive antibacterial effects [12]. In recent years, there has been an increased interest in the use of natural antimicrobials from EOs, and the use of EOs combinations is an important strategy in controlling food-borne bacteria and other pathogenic microorganisms [12]. In EOs combinations, the interaction between antimicrobials can result in three different outcomes *i.e.*, synergistic (the antibacterial activity greater than the sum of the antibacterial activity of individual oil), additive (the antibacterial activity is equal to the sum of the individual compounds of individual oil), or antagonistic (a decreased antimicrobial activity of the combination as compared to their individual antimicrobial activity). The aim of this study is to evaluate the antioxidant and antimicrobial activities of *Cymbopogon citratus* and *Syzygium aromaticum* EOs, and to investigate a possible synergistic effect of the mixtures of these oils.

2. MATERIALS AND METHODS

2.1. MATERIALS

EOs. The EOs used in this study were commercial samples of *Cymbopogon citratus* and *Syzygium aromaticum*. The *Cymbopogon citratus* EO was obtained from Herbavit (Romania), while the *Syzygium aromaticum* EO was obtained from Fares (Romania). Both EOs were manufactured by hydrodistillation.

Reagents. 2.2 diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu, gallic acid, sodium nitrite, and sodium molybdate were supplied by Sigma-Aldrich (Germany). The aqueous solutions were prepared with ultra-pure water. All reagents were of analytical grade and highest purity available.

Bacteria and culture media. The EOs and their combination were tested on the *Escherichia coli* and *Staphylococcus aureus. Escherichia coli* and *Staphylococcus aureus* were cultured on Muller Hinton Agar and Chapman medium, respectively.

2.2. METHODS

2.2.1. Determination of total phenolic compounds (TPC)

The content of TPC in *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their combinations were determined using a modified El-Maati's method [22]. Briefly, an amount of 400 µL of every diluted essential oil (10 mg in 10 mL solvent), 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 mL of Na_2CO_3 were added to a tube and then homogenized. The reaction was kept for 30 minutes in the dark, after which the absorbance was read at 760 nm by means of a Camspec spectrophotometer. For the control sample, 400 µL of distilled water was used. The TPC content expressed as the gallic acid equivalent (GAE) was calculated based on the calibration curve using the linear equation (1).

$$y = 0.015x + 0.0533, R^2 = 0.9966 \quad (1)$$

where *y* is the absorbance; *x* is the concentration (mg GAE·g^{-1} extract); R^2 - correlation coefficient. The concentration of phenolic compounds was measured five times. The results were reported as mean standard deviation and expressed as mg of gallic acid equivalents (GAEs) per 100 g sample (essential oils alone or in combination).

2.2.2. Determination of phenolic acids

The content of phenolic acids was measured by a spectrophotometric method with Arnov's reagent (10.0 g sodium molybdate, 10.0 g sodium nitrite in 100.0 mL water). The sample (1.0 mL) was pipetted into 10.0 mL volumetric flask containing 5.0 mL water; next, 1.0 ml HCl (18 g/L), 1.0 mL Arnov's reagent and 1.0 mL NaOH (40g/L) were added. The volume was brought to 10.0 mL with distilled water. The total phenolic acid content was calculated according to the equation (2).

$$(\%) = A \cdot \frac{0.877}{m} \quad (2)$$

where A is the absorbance of the mixture analysed at 490 nm, and m is the mass of the mixture, in grams. The results are averages of five measurements, expressed as the caffeic acid equivalent (mg CAE / 100g) [23].

2.2.3. Determination of antioxidant activity ($DPPH^{\bullet}$ radical scavenging activity)

The antioxidant properties of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their combinations were also investigated by determining the free radical-scavenging activity of the $DPPH^{\bullet}$ radical based on the method proposed by El-Maati et al. [13]. Briefly, 100 µL of each oil (10 mg extract/10 mL solvent) was added to 3 mL of 0.1 mM $DPPH^{\bullet}$ dissolved in ethanol according to the solvent used for extraction. After the incubation time of 30, 60 and 120 minutes at room temperature, the absorbance was measured against the control at 517 nm. The percentage of the antioxidant potential of $DPPH^{\bullet}$ radicals was calculated as follows, according to equation (3).

$$Antioxidant\ activity\ (Inhibition)\% = \frac{(Acontrol - Asample)}{Acontrol} * 100 \quad (3)$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance in the presence of the sample. BHT (butylated hydroxytoluene) was used as a reference substance.

2.2.4. Disk diffusion assay

The antibacterial activity of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their combinations was evaluated as in the traditional antibiotic susceptibility testing using the disc diffusion method. An amount of 20 mL Mueller-Hinton Agar Medium was transferred to a Petri dish. After cooling and solidification, inoculation was performed by spreading Drigolski disc of 1 µL of cell suspension in 0.9% NaCl. Then the sterile paper discs (6 mm in diameter) were impregnated with 5 µL essential oil and placed on the medium surface. Erythromycin and Norfloxacin were used as controls. Plates were then incubated at 37 °C for 24 h using a Stericell thermostat (Germany). Following incubation, the growth inhibition zones were observed. Each experiment was done in triplicate.

2.2.5. Statistical analysis

All assays were carried out five times and results were reported as mean ± standard error. The statistical significance between the phenolic content, antioxidant activity and antibacterial values of the essential oils and their mixtures was evaluated with ANOVA. Values of p lower than 0.05 were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. RESULTS

To evaluate the antioxidant and antimicrobial activities of *Cymbopogon citratus* and *Syzygium aromaticum EOs*, and to investigate a possible synergistic effect of the mixtures consisting of these oils, the following samples were used in the experiment (Table 1).

Table 1. The samples used in experiment.

Sample	***Cymbopogon citratus* EO**	***Syzygium aromaticum EO***	***Combinations of Cymbopogon citratus* and *Syzygium aromaticum* EOs**
P1	X	-	-
P2	-	X	-
P3	-	-	1:1
P4	-	-	2:1
P5	-	-	1:2

Table 2 shows the total phenolic content (TPC) and phenolic acids amount of the analysed EOs and their combinations.

Table 2. Total phenolic content (TPC) and phenolic acids of EOs and their mixtures.

Sample	TPC [mg GAE[a] / 100 g]	Phenolic acids [mg CAE[b] / 100 g]
P1	146.27±0.51	2.84±0.74
P2	138.71±0.93	2.59±0.98
P3	143.93±1.05	3.44±1.42
P4	133.34±0,84	3.20±1.11
P5	132.44±0.56	3.05±0.65

[a]Gallic acid equivalent; [b]Caffeic acid equivalent

Figs. 1 and 2 show the DPPH$^{\bullet}$ radical scavenging activity of *Cymbopogon citratus* and *Syzygium aromaticum* EOs alone and in combination.

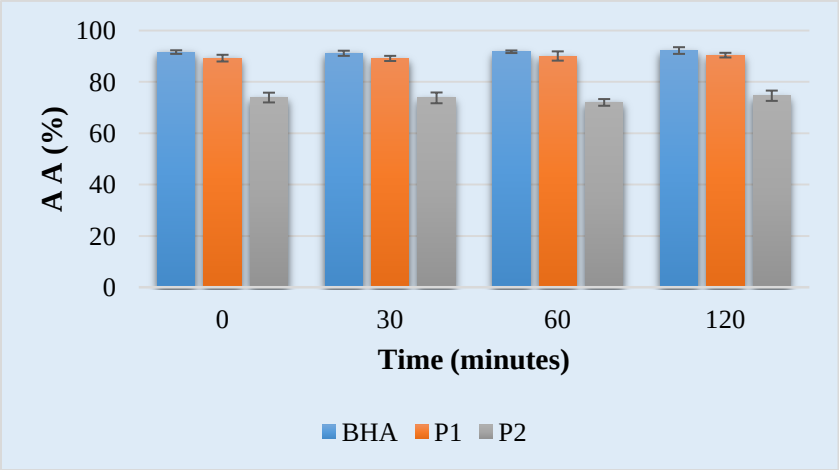

Figure 1. DPPH$^{\bullet}$ radical scavenging activity of Cymbopogon citratus and *Syzygium aromaticum* EOs.

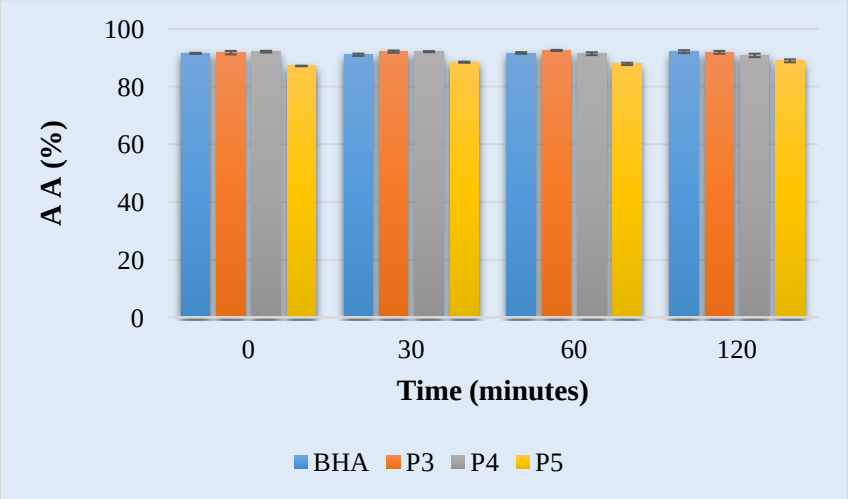

Figure 2. DPPH$^{\bullet}$ radical scavenging activity of mixtures consisting of *Cymbopogon* citratus and *Syzygium aromaticum* EOs.

Table 3 shows the different inhibition zone diameters of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their combinations.

Table 3. Antibacterial activities of Cymbopogon citratus and *Syzygium aromaticum* EOs and their mixtures against Escherichia coli and Staphylococcus aureus. Values are the means of five measurements.

Sample	Inhibition zone diameters (mm)	
	Escherichia coli	***Staphylococcus aureus***
P1	29.0	12.0
P2	12.0	16.5
P3	16.5	12.0
P4	16.0	7.5
P5	15.0	9.0
Control	31.0	26.5

3.2. DISCUSSION

The total phenol content is a very important indicator in the characterization of EOs because most phenolic compounds have both antioxidant and antibacterial properties. The total phenol content was estimated by the Folin-Ciocalteu colorimetric method in comparison with standard gallic acid.

The essential oils studied and their combinations had an important load of phenols (Table 2). Among these, the TPC ranged from 138.71±0.93 (*Syzygium aromaticum* oil) to 146.27±0.51 (*Cymbopogon citratus* oil) gallic acid equivalents (GAE mg / 100 g). Both essential oils under study should be considered as a very good source of phenolic compounds. According to Khadri et al. [5], who reported a total phenolic content of 129 mg GAE / 100 g in the *Cymbopogon citratus* oil, an amount of 146.27±0.51 mg GAE / 100 g could be considered as high.

In the case of *Syzygium aromaticum* oil, El-Maati et al. have reported a total phenolic content ranging from 58.5 to 293 mg GAE / 100g [13]. Many researchers who have been concerned with the antioxidant activity of essential oils have highlighted the correlation between the nature of compounds and their ability to inhibit the oxidation process [14]. For the essential oil of *Cymbopogon citratus* it has been established that the main components responsible for the antioxidant action are: E- Citral/ Geraniol (37.7%), Z- Citral/ Neral (21.2%), Selina- 6- en-4-ol (8.9%), Myrcene (2.5%), and Sesquiterpene (1.7%) [7].

Eugenol (the active substance) makes up 90-95% of the *Syzygium aromaticum* oil. EOs of *Cymbopogon citratus* and *Syzygium aromaticum* and their combinations have a relatively high content of phenolic acids (Table 2). Phenolic compounds are secondary metabolites that can act as antibacterial agents. The antibacterial activity could be attributed to the hydrophobic character of phenolic content.

Assays based on the use of $DPPH^{\bullet}$ radicals are among the most popular spectrophotometric methods for determination of the antioxidant capacity of essential oils. The $DPPH^{\bullet}$ scavenging methods have been used to evaluate the antioxidant activity of compounds due to their simple, rapid, sensitive, and reproducible procedure. The $DPPH^{\bullet}$

radical scavenging activity of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their mixtures was shown in Figs. 1 and 2, respectively.

Figs. 1 and 2 it show that the samples containing a large amount of phenols manifested a high antioxidant activity similar to that of BHA. For the P1 sample (essential oil of *Cymbopogon citratus*) the antiradical activity was 90.3% after 120 minutes, and for the sample P3 the antiradical activity was 91.85% after 120 min. A high antioxidant activity is influenced by the high content of phenolic acids [24].

The antimicrobial activity was determined by using the agar well diffusion assay. In the present study, the *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their mixtures were tested against two resistant bacteria, i.e. *Escherichia coli* and *Staphylococcus aureus.* The antibacterial activity of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their combinations and their potency was quantitatively assessed by the presence or absence of the inhibition zone and its diameter.

The antibacterial activity of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their combinations can be classified into three levels such as: weak activity (inhibition zone ≤ 12 mm), moderate activity (inhibition zone 12 mm - 20 mm), and strong activity (inhibition zone ≥ 20 mm) [25].

The results indicate the different inhibition zone diameters of *Cymbopogon citratus* and *Syzygium aromaticum* EOs and their mixtures (Table 3). Significant difference in the activities of the investigated samples against the tested bacteria was observed ($p<0.05$). The *Cymbopogon citratus* EO showed a strong activity against *Escherichia coli* (the highest diameter inhibition - 29 mm), and a weak activity against *Staphylococcus aureus* (low zone of inhibition - 12 mm) (Table 3). At the same time, the *Syzygium aromaticum* EO showed a weak activity against *Escherichia coli* (low zone of inhibition - 12 mm), and a moderate activity against *Staphylococcus aureus* (the diameter of inhibition - 16.5 mm) (Table 3).

The *Syzygium aromaticum* EO showed a higher inhibitory activity against *Staphylococcus aureus* than *Cymbopogon citratus* EO. The results are consistent with those presented by Mohd et al., 2010 and Zulfa et al., 2016 [7, 26]. All combinations have no synergistic effect (the antibacterial activity is lower than the sum of the antibacterial activity of the oils taken individually).

4. CONCLUSIONS

The results clearly demonstrate a good antioxidant activity of the *Cymbopogon citratus* and *Syzygium aromaticum* essential oils, similar to that exhibited by the synthetic antioxidant BHT. The results illustrate the effectiveness of *Cymbopogon citratus* and *Syzygium aromaticum* oils alone and in combination as potential natural antioxidants in replacing synthetic antioxidants.

The *Cymbopogon citratus* oil alone exhibited a strong antimicrobial activity against *Escherichia coli,* while *Syzygium aromaticum* oil showed a higher inhibitory activity against *Staphylococcus aureus* than *Cymbopogon citratus* oil. No combinations of these oils have any synergistic effect. The antibacterial activity of the combinations consisting of *Cymbopogon citratus* and *Syzygium aromaticum* oils is lower than the sum of the antibacterial activity of the oils considered individually.

Taking into account the results of this study, it can be concluded that *Cymbopogon citratus* and *Syzygium aromaticum* oils are good candidates for the biopreservation of foods.

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