INTERACTIONS BETWEEN CARDAMOM ESSENTIAL OIL AND CONVENTIONAL ANTIBIOTICS AGAINST *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES

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Abstract

The aim of this study was to investigate the effects of cardamom essential oil and antibiotics combinations against methicillin-resistant clinical isolates and ATCC 25923 reference strain of *Staphylococcus aureus*. The interaction of the essential oil with the antibiotics was assessed by the checkerboard method. The chemical composition of essential oil was analysed by gas chromatography with mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID) and α-terpinyl acetate (39.59%) and 1,8-cineole (31.27%) were identified as main compounds. Combinations of cardamom essential oil with amoxicillin or ciprofloxacin exerted mainly additive and indifferent effects on methicillin-resistant clinical isolates while combinations of 1,8-cineol with antibiotics generated antagonistic and indifferent effects. Additive effects on *Staphylococcus aureus* ATCC 25923 were generated by the following combinations: cardamom essential oil – ciprofloxacin, 1,8-cineol – amoxicillin and 1,8-cineol – ciprofloxacin. The antagonistic interactions noted here, particularly for 1,8-cineol, suggest that its concomitant administration with amoxicillin or ciprofloxacin, depending on the combination ratios, can diminish the antibacterial activity of these antibiotics in infections with *Staphyloccocus aureus* isolates.

Rezumat

Acest studiu a avut drept obiectiv investigarea efectelor combinațiilor între uleiul volatil de cardamom și antibiotice asupra unor izolate clinice meticilin-rezistente și tulpinii standard ATCC 25923 a patogenului *Staphylococcus aureus*. Interacțiunea uleiului volatil cu antibiotice a fost evaluată prin metoda *checkerboard*. Compoziția chimică a uleiului volatil a fost analizată prin GC-MS și GC-FID, iar acetatul de α-terpinil (39,59%) și 1,8-cineolul (31,27%) au fost identificați drept constituenți majoritari. Combinațiile uleiului volatil de cardamom cu amoxicilina sau ciprofloxacina au exercitat, in principal, efecte aditive și indiferente asupra izolatelor clinice meticilin-rezistente de stafilococ auriu în timp ce combinațiile 1,8-cineolului cu antibiotice au generat efecte antagonice și indiferente. Următoarele combinații: ulei volatil de cardamom-ciprofloxacina; 1,8-cineol-amoxicilina și

1,8-cineol-ciprofloxacina au generat efecte aditive asupra tulpinii standard *Staphylococcus aureus* ATCC 25923. Interacțiunile de tip antagonist semnalate aici, mai ales pentru 1,8-cineol, sugerează că administrarea sa concomitentă cu amoxicilina sau ciprofloxacina, dependent de raportul de combinare, poate diminua activitatea acestor antibiotice în infecțiile cu izolate de *Staphylococcus aureus*.

Keywords: Cardamom essential oil, antibiotics, *Staphylococcus aureus*, interactions

Introduction

Elletaria cardamomum (L.) Maton (Zingiberaceae), known as cardamom, is a perennial aromatic plant, native to the evergreen forests of southern India [9, 19]. Its dried fruits have great economic importance, being one of the most highly priced spices [10]. Cardamom fruits have stomachic, spasmolytic, anti-foaming, expectorant and anti-inflammatory properties [16, 20]. Besides medicinal purposes, cardamom fruits are used as a condiment and flavouring agent in food industry [19, 20]. Cardamom essential oil is used in medicine as antimicrobial [18], anti-inflammatory and analgesic agent [2], but also in cosmetic industry and perfumery [5]. Recently there has been a considerable interest in plants extracts and essential oils as antimicrobial agents. *Staphylococcus aureus* is one of the pathogens whose resistant strains have created a real public health issue. Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are some of the main agents involved in the production of nosocomial infections [11]. One of the approaches currently investigated to overcome resistant bacteria is the use of combinations between antibiotics and bioactive vegetal extracts/compounds. Different interactions (additive, synergistic or antagonistic) between essential oils and antibiotics have already been reported in literature. Essential oils of *Thymus maroccanus* and *Thymus broussonetii* in combination with conventional antibiotics (ciprofloxacin, gentamicin, pristinamycin, cefixime) showed synergistic effects against *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [7]. The combinations of norfloxacin and *Pelargonium graveolens* essential oil acted synergistically against three bacterial strains: *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 29213 and *Bacillus cereus* ATCC 11778 [15]. Synergistic effects between vancomycin and *Zataria multiflora* essential oil against clinical isolates of *Staphylococcus aureus* were also reported [13]. Essential oil of *Origanum vulgare* in combination with amoxicillin, polymycin and lincomycin displayed addition effects against extended-spectrum β-lactamase-producing *Escherichia coli* isolated from chicken; when combined with fluoroquinolones, doxycycline, maquindox,

florfenicol, the essential oil showed synergy against the same bacterial strain [17]. The aim of this study was to determine the effects of the combinations of cardamom essential oil and conventional antibiotics (amoxicillin, ciprofloxacin) against some MRSA clinical isolates. In addition, the effects of these combinations on *Staphylococcus aureus* reference strain were also determined. The antimicrobial activity of 1,8-cineole and its combinations with antibiotics was evaluated against the same bacterial strains.

Materials and Methods

Chemicals

1,8-cineole (purity 99%) was purchased from Sigma-Aldrich (Milwaukee, USA). An alkane standard solution C8-C20 and dimethylsulphoxide (DMSO) were obtained from Sigma-Aldrich (Steinhem, Germany).

Plant material and essential oil isolation

Dried cardamom fruits were purchased from a local supermarket. The botanical identity was confirmed in the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania. A voucher specimen (EC no. 12012) was deposited in the same Department. The essential oil was isolated by the hydrodistillation of powdered dried fruits (100 g) in a Clevenger type apparatus (5 h). The essential oil was dried over anhydrous sodium sulphate and stored in dark glass tubes at 4°C until analysis.

GC-MS and GC-FID analysis

Gas chromatography with mass spectrometry (GC-MS) analysis of the oil was carried out on an Agilent type 7890A gas cromatograph, equipped with an Agilent 5975C mass spectrometer and a DB-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 μm film thickness). The injection volume was 0.3 μL of pure essential oil. The analyses were performed using the following temperature program: the oven temperature was raised at a rate of 3°C min^{-1} from 40°C to 250°C (isothermal for 4 min), then raised to 280°C at 10°C min^{-1} and the final temperature was held for 2 min [4]. The compounds were identified by comparing their recorded mass spectra with those stored in the Wiley mass spectral library and their retention indices relative to n-alkanes with those mentioned in literature [1, 3, 8, 14]. The gas chromatography with flame ionization detection (GC-FID) analysis was performed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector and a DB-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 μm film thickness). The GC-FID conditions were the same as the ones described above.

Antimicrobial assays

Microbial test strains

Cardamom essential oil (CEO) was tested against *Staphylococcus aureus* ATCC 25923 reference strain (MediMark Europe-Grenoble, France) and two methicillin resistant clinical isolates, namely: *S. aureus* 37 and *S. aureus* 4185. The clinical isolates were obtained from the Microbiology Laboratory of the Lung Hospital, Iasi, Romania.

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC values were assessed by the broth dilution method on plates with 24 wells [21]. CEO and 1,8-cineole serial dilutions, ranging from 25 mg/mL to 0.0015 mg/mL and 5 mg/mL to 0.015 mg/mL respectively, were prepared into Mueller Hinton broth (Biolab Zrt.-Budapest, Hungary) containing the inoculum adjusted to 0.5 McFarland standard turbidity. Amoxicillin and ciprofloxacin (Bioanalyse Tibbi Malzemeler-Ankara, Turkey) were used as positive controls in a dilution range of 16 µg/mL to 0.007 µg/mL and 8 µg/mL to 0.003µg/mL, respectively. The incubation was performed at 35°C, for 24 hours. The MIC is defined as the lowest concentration of essential oil/1,8-cineole that completely inhibited visible growth of the tested microorganisms. The MBC represents the lowest concentration of the essential oil/1,8-cineole at which a percentage of 99.9% of bacteria have been killed.

The assessment of CEO/1,8-cineole and antibiotics combinations effects

The checkerboard method on 96-well plates was performed to evaluate the effects of CEO/1,8-cineole and antibiotics combinations [12]. 50 µL of each dilution were dispensed in each well, the final volume being 100 µL. The inoculum, adjusted to 0.5 McFarland standard of bacterial concentration, was added in each well of the plate. The plates were incubated at 35°C, for 18-24 hours. The interaction of the essential oil with the antibiotics was interpreted according to the fractional inhibitory concentration indexes (FICIs) values. The combination of two substances acts synergistically if FICI≤0.5, additively if $0.5 < FICI < 1$, indifferently if $1 < FICI < 2$ and antagonistically if $FICI \geq 2$ [6].

Results and Discussion

Chemical composition of essential oil

The hydrodistillation of cardamom fruits gave a yellowish and pleasant-scented essential oil with a yield of 9.25% (v/w). Twenty-three compounds were identified representing 94.34% of the total oil (Table I). α-

terpinyl acetate (39.59%) and 1,8-cineole (31.27%) were the major constituents in CEO. Oxygenated monoterpenes were dominant (84.54%) followed by monoterpenes (8.27%). The higher level of α-terpinyl acetate compared to 1,8-cineole gives a superior odour quality of CEO. In addition to α-terpinyl acetate, other terpenoids such as α-terpineol, linalool, linalyl acetate and geraniol impart a sweet flavour which counterbalances the camphorated-sharp touch of 1,8-cineole.

Table I.

Chemical composition of the essential oil isolated from cardamom fruits

Compound	RI[a]	%	Identification
Monoterpene hydrocarbons			
α-thujene	921	0.17	GC-MS
α-pinene	929	1.35	GC-MS
camphene	943	0.03	GC-MS
sabinene	970	3.53	GC-MS
myrcene	987	1.86	GC-MS
Δ-3-carene	1004	0.02	GC-MS
α-terpinene	1015	0.38	GC-MS
trans-β-ocimene	1045	0.14	GC-MS
γ-terpinene	1067	0.62	GC-MS
α-terpinolene	1081	0.17	GC-MS
Monoterpene alcohols			
linalool	1104	4.73	GC-MS
terpinen-4-ol	1179	1.75	GC-MS
α-terpineol	1198	3.43	GC-MS
carveol	1215	0.08	GC-MS
geraniol	1250	0.25	GC-MS
trans-anethole	1279	0.32	GC-MS
Monoterpene esters			
linalyl acetate	1256	3.21	GC-MS
bornyl acetate	1277	0.91	GC-MS
α-terpinyl acetate	1360	39.59	GC-MS
Monoterpene ketones			
carvone	1238	0.18	GC-MS
Monoterpene ethers			
1,8-cineole	1038	31.27	GC-MS
Sesquiterpene hydrocarbons			
β-selinene	1490	0.32	GC-MS
Sesquiterpene alcohols			
nerolidol	1539	0.03	GC-MS
Total		**94.34**	

[a]Retention indices on a DB-5MS column.

Antimicrobial activity

Cardamom essential oil inhibited the growth of all *Staphylococcus aureus* strains at a concentration of 6.25 mg/mL (Table II). 1,8-cineol was

more active than the cardamom essential oil (MIC=1.25-2.5 mg/mL). The methicillin-resistant clinical isolates of *Staphylococcus aureus* were most susceptible to 1,8-cineol (MIC=1.25 mg/mL). The essential oil and 1,8-cineol showed an antistaphylococcal activity much lower than that of amoxicillin or ciprofloxacin.

Table II.

MIC and MBC values of cardamom essential oil (CEO), 1,8-cineole and conventional antibiotics (mg/mL)

Microorganisms		**CEO**	**1,8-cineole**	**Standard drugs**	
		MIC/MBC	**MIC/MBC**	**MIC amoxicillin**	**MIC ciprofloxacin**
G+	*Staphylococcus aureus* ATCC 25923	6.25/12.5	2.5/2.5	2×10^{-3}	0.5×10^{-3}
G+	*S. aureus* 37	6.25/12.5	1.25/5	2×10^{-3}	4×10^{-3}
G+	*S. aureus* 4185	6.25/12.5	1.25/5	4×10^{-3}	4×10^{-3}

The combinations of cardamom essential oil and amoxicillin determined additive effects (FICI = 0.56-1) on MRSA 37 and MRSA 4185 isolates. On the reference strain, the combinations exhibited antagonistic (FICI = 2) and indifferent effects (FICI = 1.06) (Table III). An interaction of additive type was generated by the combination of cardamom essential oil with ciprofloxacin on MRSA 4185 (FICI=1) and *Staphylococcus aureus* reference strain (FICI=0.62). When tested on MRSA 37 strain, the cardamom essential oil-ciprofloxacin combination exhibited antagonistic or indifferent interactions depending on the combination ratios (Table III). One dose pair combination of 1,8-cineole and amoxicillin elicited additive effects on *Staphylococcus aureus* reference strain and MRSA 4185 (FICI= 0.56 and 0.74, respectively) (Table IV). Therefore, an additive interaction was demonstrated at one dose pair combination of 1,8-cineole and ciprofloxacin on *Staphylococcus aureus* reference strain (FICI = 0.62). Antagonist and indifferent interactions were found for the combinations 1,8-cineole-amoxicillin and 1,8-cineole-ciprofloxacin against MRSA 37 isolate. A similar interaction profile was noticed for the combination 1,8-cineol-ciprofloxacin against MRSA 4185 (Table IV).

Table III.

Combination effects of cardamom essential oil and antibiotics against *Staphylococcus aureus* strains

Strains	CEO[1] + amoxicillin (A)[2]/ ciprofloxacin (C)[2]	MIC_c (mg/mL)	FIC[1]/ FIC[2]	FICI	Outcome
Staphylococcus ATCC 25923	CEO + A	6.25/2 x10^{-3} 6.25/0.125x10^{-3}	1/1 1/0.06	2 1.06	An[a] I[b]
	CEO + C	3.125/0.06x10^{-3} 6.25/0.06x10^{-3}	0.5/0.12 1/0.12	0.62 1.12	**A**[c] I
S.aureus 37	CEO + A	0.7/2 x10^{-3} 0.7/1 x10^{-3} 3.125/1 x10^{-3} 3.125/0.125x10^{-3} 6.25/0.125x10^{-3}	0.11/1 0.11/0.5 0.5/0.5 0.5/0.06 1/0.06	1.11 0.61 1 0.56 1.06	I **A** **A** **A** I
	CEO + C	6.25/4 x10^{-3} 6.25/0.06x10^{-3}	1/1 1/0.01	2 1.01	An I
S.aureus 4185	CEO + A	0.7/4 x10^{-3} 0.7/2 x10^{-3} 6.25/2 x10^{-3} 6.25/0.125 x10^{-3}	0.11/1 0.11/0.5 1/0.5 1/0.03	1.11 0.61 1.5 1.03	I **A** I I
	CEO + C	3.125/4 x10^{-3} 3.125/2 x10^{-3} 6.25/2 x10^{-3} 6.25/0.06 x10^{-3}	0.5/1 0.5/0.5 1/0.5 1/0.01	1.5 1 1.5 1.01	I **A** I I

FIC, fractional inhibitory concentration; FICI, FIC indexes; MIC_c, MIC of essential oil/antibiotic combination; Antagonism[a]; [b]Indifferent; [c]Additive.

Table IV.

Combination effects of cardamom essential oil and 1,8-cineole against *Staphylococcus aureus* strains

Strains	1,8-cineole (Ci)[1] + amoxicillin (A)[2]/ ciprofloxacin (C)[2]	MIC_c (mg/mL)	FIC[1]/ FIC[2]	FICI	Outcome
Staphylococcus aureus ATCC 25923	Ci + A	1.25/0.125x10^{-3}	0.5/0.06	0.56	**A**[c]
	Ci + C	1.25/0.06x10^{-3}	0.5/0.12	0.62	**A**
S.aureus 37	Ci + A	1.25/2 x10^{-3} 1.25/0.125x10^{-3}	1/1 1/0.06	2 1.06	An[a] I[b]
	Ci + C	1.25/4 x10^{-3} 1.25/0.06x10^{-3}	1/1 1/0.01	2 1.01	An I
S.aureus 4185	Ci + A	0.3/4 x10^{-3} 0.3/2 x10^{-3} 1.25/2 x10^{-3} 1.25/0.125 x10^{-3}	0.24/1 0.24/0.5 1/0.5 1/0.03	1.24 0.74 1.5 1.03	I **A** I I
	Ci + C	1.25/4 x10^{-3} 1.25/0.06x10^{-3}	1/1 1/0.01	2 1.01	An I

FIC, fractional inhibitory concentration; FICI, FIC indexes; MIC_c, MIC of essential oil/antibiotic combination; Antagonism[a]; [b]Indifferent; [c]Additive.

Conclusions

Combinations of cardamom essential oil with amoxicillin or ciprofloxacin exerted mainly additive and indifferent effects on methicillin-resistant clinical isolates while combinations of 1,8-cineol with antibiotics generated antagonistic and indifferent effects. Additive effects on *Staphylococcus aureus* ATCC 25923 were generated by the following combinations: cardamom essential oil-ciprofloxacin, 1,8-cineol-amoxicillin and 1,8-cineol-ciprofloxacin. The antagonistic interactions found particularly for 1,8-cineol suggest that its concomitant administration with amoxicillin or ciprofloxacin, depending on the combination ratios, can diminish the antibacterial activity of these antibiotics in infections with *Staphyloccocus aureus* isolates.

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