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In Vitro Antimicrobial Activity of Natural Essence and Distilled Extract of Bergamot against Drug Resistance Clinical Isolates

Quirino A*, Morelli P, Marano V, Cortese G, Barreca GS, Liberto MC and Focà A
Institute of Microbiology, Department of Health Sciences, "Magna Graecia" University, Viale Europa, Catanzaro, Italy

Abstract

Bergamot (*Citrus bergamia*) belongs to the *Citreae* tribe in the *Aurantioideae* subfamily of the *Rutaceae* plant family cultivated almost exclusively along the coast of the Calabria region, Southern Italy. Bergamot essential oil is widely employed in cosmetics such as in aromatherapy and its antimicrobial effects against bacteria, yeasts and virus are also demonstrated. This study evaluates the "*in vitro*" antimicrobial activity of natural essence and distilled extract of bergamot on drug resistance pathogens isolated from clinical specimens of patients admitted to University Hospital of Catanzaro, Italy. Bactericidal and fungicidal activity was analyzed using a micro well dilution assay and the efficacy of tested substances was verified by acridine orange staining procedures and confocal microscopy. Data revealed that the distilled extract of bergamot possessed greatest antimicrobial efficacy when compared with essential oil particularly against drug resistant Gram negative bacteria such as *Acinetobacter baumannii, Stenotrophomonas maltophilia, Escherichia coli and Klebsiella pneumoniae* responsible for nosocomial infections representing a worldwide public health problem. This study suggests that alternative antimicrobial substances as well as distilled extract of bergamot could be of great utility to prevent microbial contamination on hospital devices and healthcare personnel hand skin.

Keywords: *Citrus bergamia*; Bergamot natural essence; Antimicrobial activity; A romatherapy

Introduction

Citrus bergamia belongs to the *Citreae* tribe in the *Aurantioideae* subfamily of the *Rutaceae* plant family and is defined as a hybrid between a sour orange (*C. aurantium* L.) and lemon (*C. lime* L. Burm. f.) or a mutation of the latter. Trees are cultivated almost exclusively along the coast of the Calabria region, Southern Italy [1,2].

Bergamot fruit is principally used for its essential oils that are extracted from the peel of the fruit by rasping and cold-pressing procedure. Bergamot essential oil is one of the main basic constituents for the manufacture of perfumes and it is widely used in cosmetics such as in aromatherapy, mainly for reducing stress and anxiety [3]. Anti-inflammatory, anti-proliferative activities [4,5] and analgesic effects [6,7] were also demonstrated.

Popular tradition has always ascribed antimicrobial properties to bergamot oil, and these features were firstly studied in the 19 th century by Francesco Calabrò, a physician coming from Calabria region [8].

At present several studied report antimicrobial effects against bacteria, yeasts, filamentous fungi, of different type of derivative from bergamot essence as well as varied bergamot oil extraction [9,10]. Focà et al. [8] has also evaluated the antiviral activity of the essential oil against Herpes Simplex type 1, clearly showing its inhibitory capacity on viral replication during single-cycle growth curves. The aim of this study was to evaluate the "*in vitro*" antimicrobial activity of natural essence and distilled extract of bergamot on drug resistance pathogens isolated from clinical specimens. The activities were tested using a micro well dilution assay. To verify the killing effects of the above substances on microorganisms we used acridine orange staining procedures and confocal studies.

Materials and Methods

Antimicrobial activities of natural essence and distilled extract of bergamot, a kindly gift by Prof. G. Sindona from the Department of Chemistry and Chemical Technology, University of Calabria, Cosenza (Italy), were tested on a range of clinical pathogens isolated from patients admitted to University Hospital of Catanzaro, Italy, by using different methods such as a micro well dilution assay and co focal microscopy. Pathogens included Gram-positive *(S. epidermidis, S. pyogenes, S. aureus, C. striatum, E. faecalis),* Gram-negative bacteria *(S. maltophilia, A. baumannii, K. pneumoniae, P.aeruginosa, E. coli, E. aerogenes)* and yeasts *(C. albicans, C. glabrata, C. lypolytica, C. tropicalis, C. lusitaniae).* Strains were conventionally identified by biochemical testing (Vitek-2, bioMérieux, France). Antibiotic sensitivity was evaluated by Vitek System (bioMérieux, France).

Gas chromatography analysis

The chemical composition of tested substances (essential oil and distillate) was analyzed with Hewlett Packard (Agilent) gas chromatograph (model HP 5890A) - mass spectrometer (model HP 5972A) equipped with a HP-35MS column. Helium was used as carrier gas and ionization was obtained by electron impact. Temperature of the column was maintained at 60°C for 5 minutes, and then raised to 280°C in 10°C/minutes increments. Nineteen compounds, which were available reference standards, were identified and quantified. Other compounds were identified by comparison of mass spectra of each peak with those of authentic samples in the NIST (National Institute of Standard and Technology) library.

Micro well dilution assay

Aliquots of 1 ml of each substance were placed in sterile glass

***Corresponding author:** Quirino A, , University "Magna Graecia" of Catanzaro, Viale Europa, 88100 Catanzaro, Italy, Tel: +3909613697748; Fax: +3909613697760; E-mail: quirino@unicz.it

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test tubes and were emulsified with Tween 20, an inert, non ionic tensioactive agent with no inherent bactericidal and fungicidal activity (900 µl of substance plus 100 µl of Tween 20). Mixture was then vortexes and the first dilution of the emulsion was performed in a liquid medium (Nutrient Broth, BioMérieux), adding 100 ml of emulsion in 2 ml of liquid medium (dilution 1:20). 100 µl of the first dilution were dispensed into the first well of a sterile micro titration plate and twofold serial dilutions were then performed.

Microorganisms identification was performed by biochemical testing (Vitek-2, bioMèrieux, France). The microorganism under investigation were prepared in test tubes containing sterile physiological solution, diluting colonies grown on solid medium to obtain a bacterial suspension of 0.5 McFarland (about 10^8 colony-forming units [CFU]/ml). Each microbial suspension was then diluted 1:100 in liquid medium (100 µl bacterial suspension in 9.9 ml Nutrient Broth, BioMérieux), and then 50 µl were added in all plate micro wells.

Each plate was incubated for 24 hours at 37°C. After incubation, to determine antimicrobial effect of tested substances, aliquots of 1 µl from each dilution, were inoculated in plates (Blood agar and Sabouraud agar) and incubated for 24 hours at 37°C, before colony counting.

In all experiments we included the substance alone to exclude possible contamination (negative control) and bacterial suspension, without substance, as positive control of microbial growth.

Confocal microscopy

To assess the bactericidal and fungicidal activity of bergamot natural essence and bergamot distilled extract, 10 µl, containing bacterial or fungal inoculums plus substance to be tested at different dilutions was mixed with 10 µl of acridine orange, a nucleic acid selective metachromatic stain. During acridine orange staining procedures, the fluorescent dye discriminates live from dead cells giving out a green and a red fluorescence respectively. Images were acquired under a 63x objective using a confocal microscope (Leica TC-SP2 Confocal System, Leica Microsystems Srl, and Milan, Italy).

Results

Gas chromatography analysis

The chromatograms of essential oil and distillate are reported in Figures 1 and 2, respectively. Figure 1 and Table 1 show that the major component of the essential oil was lynalyl acetate (TR=12:03 min, 42.22%). Probably this result can be attributed to the period of harvest of the fruits. Among cyclic monoterpenes mainly representative were limonene (tR=7.48 min, 24.32%), linalool (tR=9.47 min, 10.86%) and β-pinene (tR=5.99 min, 6.56%). Figure 2 and Table 1 show that in the distillate the main component was limonene (tR=7:48 min, 30.20%), followed by linalool (tR=9.47 min, 21.82%), linalyl acetate (tR=12:03 min, 16.21%) and ɣ-terpinene (tR=8:37 min, 11.95%).

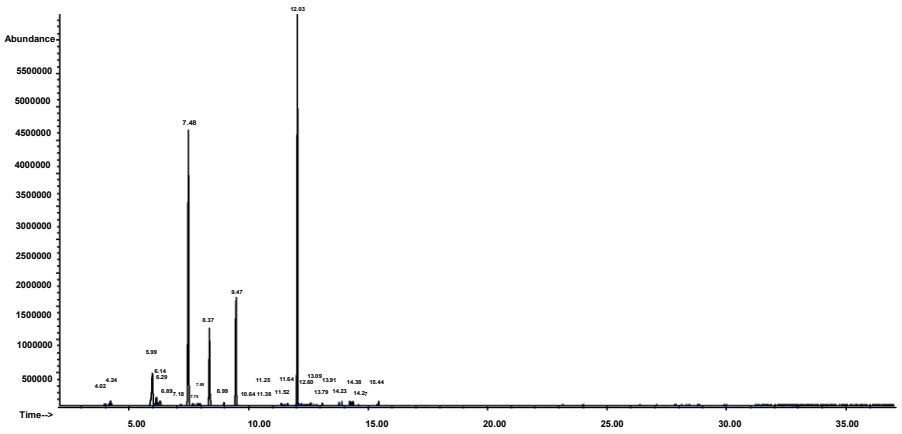

Figure 1: Chromatogram of essential oil of bergamot. Chemical composition of essential oil of bergamot analyzed with Hewlett Packard (Agilent) gas chromatograph (model HP 5890A) - mass spectrometer (model HP 5972A) equipped with a HP-35MS column.

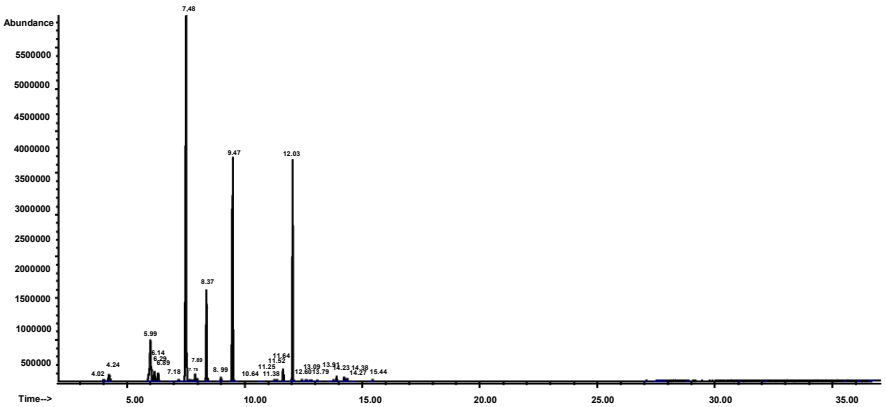

Figure 2: Chromatogram of distilled extract of bergamot. Chemical composition of distilled extract of bergamot analyzed with Hewlett Packard (Agilent) gas chromatograph (model HP 5890A) - mass spectrometer (model HP 5972A) equipped with a HP-35MS column.

Microwell dilution assay

The activity of different dilutions of natural essence and distilled extract of bergamot has been tested on Gram-positive and Gram-negative bacteria. In particular *S. epidermidis* (three strains), *S. aureus* (two strains) *S. pyogenes* (one strain), *C. striatum* (one strain) , *E. faecalis* (one strain), *S. maltophilia* (one strain), *A. baumannii* (one strain), *K. pneumoniae* (three different strains), *P. aeruginosa* (one strain), *E. coli* (one strain), *E. aerogenes* (one strain). All bacteria, their isolation site as well as antibiotic resistance profile are listed in Tables 2 -4.

Moreover substance activities have been tested on different Candida species such as *C. albicans* (three strains isolated from b lood, bronchoalveolar lavage and sputum respectively), *C. lypolytica* (two strains both isolated from sputum), *C.tropicalis* isolated from blood, *C. glabrata,* isolated from vaginal swab and resistant to Fluconazole and Voriconazole, and *C. lusitaniae* isolated from bronchoalveolar lavage, also resistant to Fluconazole.

Tables 2 and 3 summarized antimicrobial activity of essential oil and distilled extract respectively. Essential oil was mostly active on a multi drug resistant *Acinetobacter baumannii* (up to 1:320 dilutions moreover a slight effect on *Candida* spp growth was detected. Data revealed that the distilled extract of bergamot possessed greatest antimicrobial efficacy when compared with essential oil. In particular

Compounds	t_R (min.)	Distillate w/w %	Essential oil w/w %	Bp °C
Cyclic monoterpenes				
α-pinene	4.24	1,03	1.06	155
β-pinene	5.99	6.56	5.37	167-167
α-phellandrene	6.89	0.04	0.02	171-172
α-terpinene	7.18	0.16	0.11	173-175
Limonene	7.48	30.20	24.32	176
p-cimene	7.89	0.18	0.11	177
ɣ-terpinene	8.37	11.95	11.44	182
Terpinolene	8.99	0.27	0.26	184-185
Acyclic monoterpenes				
Myrcene	6.29	0.82	0.64	165
Ocimene	7.75	0.08	0.12	65-66
Oxygenated acyclic monoterpenes				
Linalool	9.47	21.82	10.86	196-198
Lynalyl acetate	12.03	16.21	42.22	220
Neral	12.60	0.21	0.32	103
Geranial	13.09	0.11	0.14	229
Neryl acetate	13.91	0.28	0.43	134 (25 mmHg)
Oxygenated cyclic monoterpenes				
Terpineol	11.64	0.87	0.19	213-218
Ester				
Octyl acetate	11.38	0,10	0.13	203-213
Aldehydes				
Decanal	11.52	Trace	0.09	93-95
Sesquiterpenes				
Cariofillene	14.38	0.14	0.35	128-129

Table 1: Composition of essential oils and distilled extract of Bergamot.

Strains code	Isolation site	Antibiotic resistance profiles
Gram-positive		
Staphylococcus epidermidis 4/15	Catheter tip	Clindamycin, Erythromycin, Levofloxacin, Oxacillin, Rifampicin, Tetracycline
Staphylococcus epidermidis 11/15	Catheter tip	Clindamycin, Erythromycin, Levofloxacin, Oxacillin, Rifampicin, Gentamicin
Staphylococcus epidermidis 15/15	Surgical sponge	Clindamycin, Erythromycin, Levofloxacin, Oxacillin, Rifampicin, Tetracycline, Gentamicin, Fusidic Acid
Staphylococcus aureus 9/15	Skin swab	Benzylpenicillin
Staphylococcus aureus 18/15	MRSA nasal swab	Benzylpenicillin, Oxacillin, Tetracycline,
Streptococcus pyogenes 3/15	Throat swab	No resistance
Corynebacterium striatum 5/15	Peritoneal fluid	Clindamycin, Erythromycin, Levofloxacin, Oxacillin, Rifampicin, Tetracycline, Trimethoprim/Sulfamethoxazole

Enterococcus faecalis 10/15	Blood	Cefuroxime, Cefuroxime-Axetil, Gentamicin, Levofloxacin, Erythromycin, Clindamycin, Trimethoprim/Sulfamethoxazole, Quinupristin/Dalfopristin
Gram-negative		
Acinetobacter baumannii 147/15	Urine	Amoxicillin/Clavulanic Acid, Ampicillin, Cefotaxime, Ciprofloxacin, Ertapenem, Imipenem, Meropenem, Trimethoprim/Sulfamethoxazole
Stenotrophomonas maltophilia 1/15	Bronchoalveolar lavage	Trimethoprim/Sulfamethoxazole
Enterobacter aerogenes 2/15	Bronchoalveolar lavage	Amoxicillin/Clavulanic Acid, Cefoxitin, Fosfomycin
Escherichia coli 8/15	Blood	Ampicillin, Amoxicillin/Clavulanic Acid, Piperacillin/Tazobactam, Cefuroxime, Cefuroxime-Axetil, Cefoxitin, Cefepime, Cefotaxime, Ceftazidime, Gentamicin, Ciprofloxacin, Levofloxacin, Trimethoprim/Sulfamethoxazole
Klebsiella pneumoniae 6/15	Sputum	Ampicillin
Klebsiella pneumoniae 16/15	Rectal swab	Amikacin, Amoxicillin/Clavulanic Acid, Ampicillin, Cefoxitin, Cefepime, Cefotaxime, Ceftazidime, Ciprofloxacin, Ertapenem, Fosfomycin, Imipenem, Meropenem, Piperacillin/TazobactamTrimethoprim/Sulfamethoxazole, Tigecycline
Klebsiella pneumoniae 17/15	Bronchoalveolar lavage	Amikacin, Amoxicillin/Clavulanic Acid, Ampicillin, Cefoxitin, Cefepime, Cefotaxime, Ceftazidime, Ciprofloxacin, Ertapenem, Fosfomycin, Imipenem, Meropenem, Piperacillin/TazobactamTrimethoprim/Sulfamethoxazole,
Pseudomonas aeruginosa 7/15	Throat swab	Ampicillin, Amoxicillin/Clavulanic Acid, Cefotaxime, Ertapenem, Trimethoprim/ Sulfamethoxazole, Tigecycline

Table 2: Isolation site and antibiotic resistance profiles of bacterial clinical isolates.

Strains code	**Dilutions**							
	1:20	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**1:2560**
Gram-positive								
Staphylococcus epidermidis 4/15	-	-	-	-	+	+	+	+
Staphylococcus epidermidis 11/15	+	+	+	+	+	+	+	+
Staphylococcus epidermidis 15/15	+	+	+	+	+	+	+	+
Staphylococcus aureus 9/15	+	+	+	+	+	+	+	+
Staphylococcus aureus 18/15	+	+	+	+	+	+	+	
Streptococcus pyogenes 3/15	-	+	+	+	+	+	+	+
Corynebacterium striatum 5/15	+	+	+	+	+	+	+	+
Enterococcus faecalis 10/15	+	+	+	+	+	+	+	+
Gram-negative								
Acinetobacter baumannii 147/15	-	-	-	-	-	+	+	+
Stenotrophomonas maltophilia 1/15	-	+	+	+	+	+	+	+
Enterobacter aerogenes 2/15	+	+	+	+	+	+	+	+
Escherichia coli 8/15	+	+	+	+	+	+	+	+
Klebsiella pneumoniae 6/15	+	+	+	+	+	+	+	+
Klebsiella pneumoniae 16/15	+	+	+	+	+	+	+	+
Klebsiella pneumoniae 17/15	+	+	+	+	+	+	+	
Pseudomonas aeruginosa 7/15	+	+	+	+	+	+	+	+
Yeasts								
Candida albicans 26/15	-	-	-	-	-	+	+	+
Candida albicans 20/15	-	+	+	+	+	+	+	
Candida albicans 22/15	-	-	+	+	+	+		

Candida glabrata 12/15	-	-	+	+	+	+	+	+
Candida lypolytica 13/15	-	+	+	+	+	+	+	+
Candida lipolytica 23/15	-	+	+	+	+	+		
Candida tropicalis 14/15	-	+	+	+	+	+	+	+
Candida lusitaniae 19/15	+	+	+	+	+	+		

Table 3: Antimicrobial activity of essential oil of bergamot.

Strains code	**Dilutions**							
	1:20	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**1:2560**
Gram-positive								
Staphylococcus epidermidis 4/15	-	-	-	+	+	+	+	+
Staphylococcus epidermidis 11/15	+	+	+	+	+	+	+	+
Staphylococcus epidermidis 15/15	+	+	+	+	+	+	+	+
Staphylococcus aureus 9/15	-	+	+	+	+	+	+	+
Staphylococcus aureus 18/15	+	+	+	+	+	+	+	
Streptococcus pyogenes 3/15	+	+	+	+	+	+	+	+
Corynebacterium striatum 5/15	-	-	-	+	+	+	+	+
Enterococcus faecalis 10/15	-	+	+	+	+	+	+	+
Gram-negative								
Acinetobacter baumannii 147/15	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia 1/15	-	-	+	+	+	+	+	+
Enterobacter aerogenes 2/15	+	+	+	+	+	+	+	+
Escherichia coli 8/15	-	-	-	+	+	+	+	+
Klebsiella pneumoniae 6/15	-	+	+	+	+	+	+	+
Klebsiella pneumoniae 16/15	-	+	+	+	+	+	+	+
Klebsiella pneumoniae 17/15	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa 7/15	+	+	+	+	+	+	+	+
Yeasts								
Candida albicans 26/15	-	-	-	-	-	-	-	-
Candida albicans 20/15	-	-	+	+	+	+	+	
Candida albicans 22/15	-	-	-	-	+	+		
Candida glabrata 12/15	-	-	+	+	+	+	+	+
Candida lypolytica 13/15	-	-	+	+	+	+	+	+
Candida lipolytica 23/15	-	-	-	+	+	+		
Candida tropicalis 14/15	-	-	-	+	+	+	+	+
Candida lusitaniae 19/15	-	-	+	+	+	+	+	

Table 4: Antimicrobial activity of distilled extract of bergamot.

the effects were evident on *S.pyogenes* strain (up to 1:80 dilutions) as well as on *S. epidermidis* clinical isolate (up to 1:80 dilutions) Concerning antifungal activity, bergamot distilled extract was effective against all yeast strains studied, in particular when it was tested on blood isolated *Candida albicans* strain (up to 1.2560 dilution). Finally the activity on a multi drug resistant *Acinetobacter baumannii* was extremely successful (up to 1:2560 dilutions). Although data differed among microorganisms, a reduction of total number of colony forming units was always observed (data not shown).

Confocal microscopy

Concerning the bactericidal/fungicidal activity, visualized by confocal microscopy during acridine orange staining procedures, images of *E. coli* strain, treated with bergamot distilled extract (Figure 3) and *C. albicans* strain, treated with bergamot distilled extract (Figure 4) are reported. Pictures show the dose dependent bactericidal/fungicidal effects on both microorganisms.

Discussion

Our data show a more powerful activity of bergamot distilled extract when compared with bergamot essential oil, moreover bergamot distilled extract were found to be more active against Gram-negative bacteria and yeast tested.

Chemical composition of substances tested can affect the mode of action and antimicrobial activity, so the most abundantcompounds, found in both mixtures, are limonene, a cyclic monoterpenes, linalool and lynalyl acetate, oxygenated acyclic monoterpenes, which together account for about 77% and 70% of their composition respectively. The major variation between the two mixtures includes difference in concentration/combination of the three components mentioned before and this condition could affect the different antimicrobial activities of the substances. On the other hand the antimicrobial activity of the monoterpenes has been previously investigated and it is related to the relative lipophilicity and water solubility, then significantly influenced by their physicochemical characteristics, is and by the composition of bacterial membranes [11]. Moreover, interaction between the different constituents can occur, causing antagonistic, additive and synergistic antimicrobial effects [12-15]. Indeed, essential oils show an antimicrobial activity greater than their main components suggesting a possible interaction between all of its constituents [16,17].

The hydrophilic permeability barrier, due to lipopolysaccharide molecole, in the outer membrane of Gram-negative bacteria, confers protection against the effects of highly hydrophobic drugs [18]. Even so, we found *in vitro* activity of bergamot distilled extract on drug resistant clinical isolates such as *Acinetobacter baumanni, Stenotrophomonas maltophilia, Escherichia coli and Klebsiella pneumoniae.* Interestingly, such bacteria cause nosocomial infection and represent a worldwide public health problem.

Notably the effectiveness on *Acinetobacter baumanni (A. baumannii)*, an emerging pathogen responsible for community-and hospital-acquired infections that are difficult to control and treat.

It is thought that infections are acquired after exposure to *A. baumannii*, which is able to survive on contaminated hospital equipments or by contact with healthcare personnel exposed to this microorganism through contact with a colonized patient. Indeed this species can be isolated from human skin or faeces as well as from hospital bed rail also many days after discharge of an infected patient from the hospital. Therefore, alternative antimicrobial substances, such as distilled extract of bergamot, could be of great importance to prevent *A. baumannii* contaminations on hospital devices and healthcare personnel hand skin.

Concerning antimicrobial activity against Gram-positive bacteria, it is well known that the presence of polysaccharides, at the

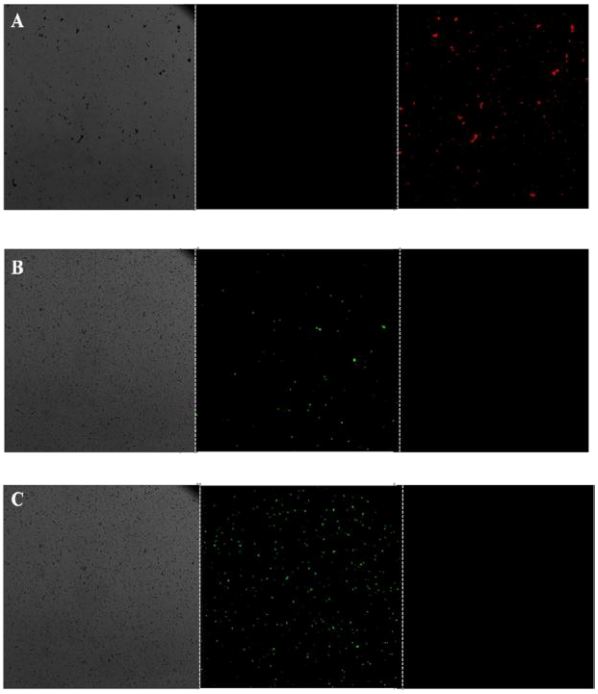

Figure 3: Confocal microscopy during acridine orange staining procedure of *E. coli* clinical isolate treated with bergamot distilled extract. Bactericidal activity of distilled extract of bergamot vs. *E. coli* (1:40 dilution) (Panel A); No Bactericidal activity (1:640 dilution) (Panel B); Positive control of *E. coli* growth (Panel C).

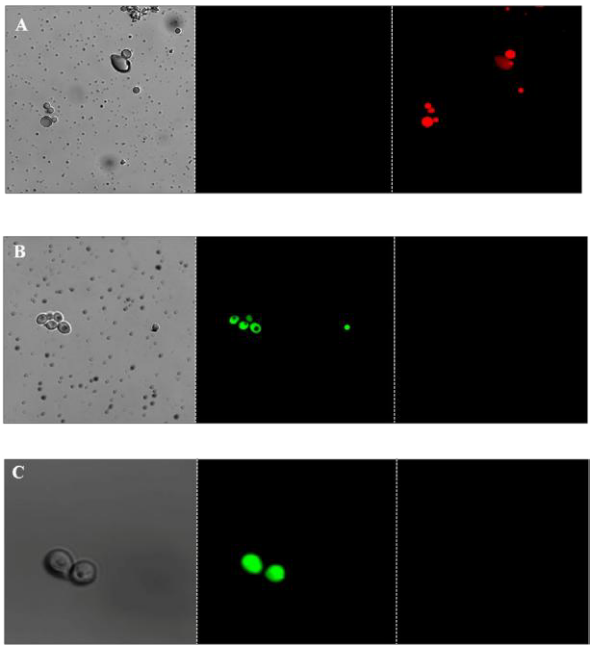

Figure 4: Confocal microscopy during acridine orange staining procedure of *C. albicans* clinical isolate treated bergamot distilled extract. Fungicidal activity of distilled extract of bergamot vs. *C. albicans* (1:40 dilution) (Panel A); No fungicidal activity (1:640 dilution) (Panel B); Positive control of *C. albicans* growth (Panel C).

microorganisms capsule surface can confer a certain resistance to the microbicidal action of the substances. This could be the case of both Gram-positive and Gram-negative bacteria, like *Pseudomonas aeruginosa*. Indeed a *S. epidermidis* strain, isolated on a catheter tip and a *Corynebacterium striatum* strain, isolated in peritoneal fluid were the only Gram-positive microorganism inhibited.

Finally, regarding *Candida* spp strains, which possess the abundance of sterols on their exteriorenvelope, leading their chemical and physical affinity for different bergamot constituents, we confirm previously observations and suggest bergamot potential role in topical treatment of Candida infections [8].

Moreover, considering bactericidal/fungicidal activity, established through observation of dead cells by acridine orange staining, our data give a further scientific support to frequent opinion in the usefulness of treating skin infections with bergamot oils and its derivatives [19,20]. Therefore, this underlines the need for additional studies to better understand bergamot inhibition mechanisms.

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References

1. Watanabe E, Kuchta K, Kimura M, Rauwald HW, Kamei T, et al. (2015) Effects of bergamot (Citrus bergamia (Risso) Wright & Arn.) essential oil aromatherapy on mood states, parasympathetic nervous system activity, and salivary cortisol levels in 41 healthy females. Forsch Komplementmed 22: 43-49.
2. Navarra M, Mannucci C, Delbò M, Calapai G (2015) Citrus bergamia essential oil: from basic research to clinical application. Front Pharmacol 6: 36.
3. Saiyudthong S, Marsden CA (2011) Acute effects of bergamot oil on anxiety-related behaviour and corticosterone level in rats. Phytother Res 25: 858-862.
4. Impellizzeri D, Bruschetta G, Di Paola R, Ahmad A, Campolo M, et al. (2015) The anti-inflammatory and antioxidant effects of bergamot juice extract (BJe) in an experimental model of inflammatory bowel disease. Clin Nutr 34: 1146-1154.
5. Ferlazzo N, Cirmi S, Russo M, Trapasso E, Ursino MR, et al. (2016) NF-kB mediates the antiproliferative and proapoptotic effects of bergamot juice in HepG2 cells. Life Sci 146: 81-91.
6. Sakurada T, Mizoguchi H, Kuwahata H, Katsuyama S, Komatsu T, et al. (2011) Intraplantar injection of bergamot essential oil induces peripheral antinociception mediated by opioid mechanism. Pharmacol Biochem Behav 97: 436-443.
7. Kuwahata H, Komatsu T, Katsuyama S, Corasaniti MT, Bagetta G, et al. (2013) Peripherally injected linalool and bergamot essential oil attenuate mechanical allodynia via inhibiting spinal ERK phosphorylation. Pharmacol Biochem Behav 103: 735-741.
8. Focà A, Liberto MC, Matera G, Barreca GS, Pollio A, et al. (1998) Valutazione dell'attività antimicrobica dell'essenza di bergamotto. Bergamotto 98: Stato dell'arte, Reggio Calabria, pp: 185-207.
9. Sanguinetti M, Posteraro B, Romano L, Battaglia F, Lopizzo T, et al. (2007) In vitro activity of Citrus bergamia (bergamot) oil against clinical isolates of dermatophytes. J Antimicrob Chemother 59: 305-308.
10. Romano L, Battaglia F, Masucci L, Sanguinetti M, Posteraro B, et al. (2005) In vitro activity of bergamot natural essence and furocoumarin-free and distilled extracts, and their associations with boric acid, against clinical yeast isolates. J Antimicrob Chemother 55: 110-114.
11. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, et al. (2005) Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 49: 2474-2478.
12. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol 94: 223-253.
13. Delgado B, Fernández PS, Palop A, Periago PM (2004) Effect of thymol and cymene on Bacillus cereus vegetative cells evaluated through the use of frequency distributions. Food Microbiology 21: 327-334.
14. Nychas GJE (1995) Natural antimicrobials from plants. In: Gould GW (ed.), New methods of food preservation. London: Blackie Academic and Professional, pp: 58-89.
15. Raybaudi-Massilia RM, Mosqueda-Melgar J, Soliva-Fortuny R, MartínBelloso O (2009) Control of pathogenic and spoilage microorganisms in fresh cut fruits and fruit juices by traditional and alternative natural antimicrobials. Comprehensive Reviews in Food Science and Food Safety 8: 157-180.
16. Herman A, Tambor K, Herman A (2016) Linalool Affects the Antimicrobial Efficacy of Essential Oils. Curr Microbiol 72: 165-172.

17. Ou MC, Liu YH, Sun YW, Chan CF (2015) The Composition, Antioxidant and Antibacterial Activities of Cold-Pressed and Distilled Essential Oils of Citrus paradisi and Citrus grandis (L.) Osbeck. Evid Based Complement Alternat Med 2015: 804091.

18. Hancock RE (1997) The bacterial outer membrane as a drug barrier. Trends Microbiol 5: 37-42.

19. Focà A, Liberto MC (2015) Aromatherapy: Basic Mechanisms and Evidence Based Clinical Use. Bagetta G, Cosentino M, Sakurada T (eds.). Chapter: 1 and 15.

20. Makar AB, McMartin KE, Palese M, Tephly TR (1975) Formate assay in body fluids: application in methanol poisoning. Biochem Med 13: 117-126.

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