ARTÍCULO ORIGINAL

Antimicrobial activity of essential oil from *Baccharis trimera* (Less.) DC. (carqueja-amarga)

Aceite esencial de *Baccharis trimera* (Less.) DC. (carqueja-amarga): actividad antimicrobiana

Érika Yoko Suzuki, César Augusto Caneschi, Romário Costa Fochat, Marcos Antônio Fernandes Brandão, Nádia Rezende Barbosa Raposo

Faculdade de Farmácia, Universidade Federal de Juiz de Fora, Juiz de Fora-MG, Brazil.

Financial support

The authors acknowledge the financial support from CAPES.

ABSTRACT

Introduction: the recent enhancement of interest in 'green' consumerism has given rise to a renewed scientific awareness towards essential oils. Essential oil from *Baccharis trimera* (Less.) DC. (*B. trimera*) (Asteraceae) is cited as one of the ten most consumed oils by the cosmetic and other industries in Brazil.
Objective: to investigate the antimicrobial activity of the essential oil from the leaves of *B. trimera* against *Staphylococcus epidermidis* ATCC 12228, *Proteus vulgaris* ATCC 13315, *Micrococcus luteus* ATCC 7468 and *Corynebacterium xerosis* IAL105, which are the main bacteria responsible for bad perspiration odor.
Methods: the gas chromatography (GC) analysis was performed and the antimicrobial activity was evaluated by means of the turbidimetric method, using a microdilution assay.
Results: ywenty constituents were identified, being that β-pinene (23.4%) was the major compound found. The minimum inhibitory concentration (MIC) values of the essential oil ranged from 500 µg/mL to 1,000 µg/mL. A detrimental effect of the essential oil was observed on the morphology of cell membranes of the bacteria studied by scanning electron microscopy (SEM).

Conclusions: yhe results demonstrate the essential oil of *B. trimera* has potential in the application of antimicrobial agents in personal care products.

Key words: *Baccharis*; bacteria; medicinal plants; personal hygiene products; products with antimicrobial action; scanning electron microscopy.

RESUMEN

Introducción: el reciente aumento del interés por el consumo "verde" ha dado lugar a una renovada conciencia científica hacia a los aceites esenciales. El aceite esencial de *Baccharis trimera* (Less.) DC. (*B. trimera*) (Asteraceae) es considerado uno de los diez aceites más consumidos por la industria cosmética del Brasil.
Objetivos: valorar la actividad antimicrobiana del aceite esencial de hojas de *B. trimera* frente al *Staphylococcus epidermidis* ATCC 12228, *Proteus vulgaris* ATCC 13315, *Micrococcus luteus* ATCC 7468 y *Corynebacterium xerosis* IAL105, que son las principales bacterias responsables del mal olor que es consecuencia de la transpiración.
Métodos: se realizó la cromatografía de gases (GG) y la actividad antimicrobiana fué valorada por el método turbidimétrico, usando el ensayo de microdilución.
Resultados: se identificarón veinte constituyentes, siendo el β-pineno (23,4%) el principal compuesto encontrado. Los valores de la concentración mínima inhibitoria (CMI) del aceite esencial variaron de 500 μg/mL a 1,000 μg/mL. Se observó un efecto perjudicial del aceite esencial en la morfología de las membranas celulares de las bacterias estudiadas por microscopía electrónica de barrido (SEM).
Conclusión: los resultados demuestran que el aceite esencial de *B. trimera* tiene potencial en la aplicación de los agentes antimicrobianos en productos de higiene personal.

Palabras Clave: *Baccharis*; bacterias; plantas medicinales; productos de higiene personal; productos con acción antimicrobiana; microscopía electrónica de barrido.

INTRODUCTION

Many people use numerous personal care products between them deodorant, which are carefully prepared by using intricate recipes and a variety of substances with diverse functions. Such substances include active ingredients, solvents, preservatives and additives, some of which are suspected to affect the consumer's health, such as phthalates, parabens or antimicrobials (triclosan/triclocarban).[1]

Deodorants deserve special attention, in its formulation including antimicrobial substances and fragrances whose aim to control the bacterial growth and avoid bad body odor caused by them. According to Euromonitor International, the global deodorant market stood at almost $20.4 bn in 2011, up 6.7% from $19.1 bn in 2010. The deodorant sector is on the rise both in active growth as saturated markets due to innovative of the targeted products, such as Latin America, Pacific Asia, the Middle East, and Africa were major growth areas.[2]

Essential oils are frequently incorporated into these kinds of products because of the complexity of their active compounds, strong fragrant properties and 'natural' marketing image.[3,4] Essential oils are present in almost 3,000 different species of plants, 10% of this total being of commercial interest in agriculture, food industry and the pharmaceutical area.[5]

Although some essential oils have long been researched for their medicinal properties, the recent enhancement of interest in 'green' consumerism has given rise to a renewed scientific awareness towards them.[6] The growing 'green' consciousness is pushing to the consumers to choose premium natural personal care products, instead of products containing synthetic ingredients. Natural cosmetics exhibited an increased revenue of 20.9%, partly as a result of the increased awareness towards the chemicals contained in some commercial products.[7] Moreover, essential oils have shown activity as penetration enhancers for antiseptics and could be applied as antimicrobial actives against antiseptic-resistant species.[8]

Among the different species of the plants studied, *Baccharis trimera* (Less.) DC. (Asteraceae) is native to South America and it is popularly known as "carqueja-amarga". The leaves of *B. trimera* are widely used in the popular medicine in the form of infusion in order to obtain an anti-inflammatory, antacid, antiulcer and digestive extract.[9-12] The hydroalcoholic extract of the leaves and flowers of the plant also shows potential as an antioxidant and an antiulcer,[13] and it is used in the treatment of rheumatism, hepatobiliary disorders,[14] diabetes, as well as an antimicrobial,[15] antifungal[16] and antigenotoxic agent.[14] The essential oil extracted from the shrub presents activity against *Schistosoma mansoni*[11] and *Candida albicans.*[17] It is currently employed in the food and in the pharmaceutical industries.[11] Additionally, it has been reported antimicrobial activity of the essential oil obtained from aerial parts of many species of *Baccharis* gender indicating action against Gram-negative and Gram-positive bacteria.[18-22] Moreover, essential oil from *B. trimera* is cited as one of the ten most consumed oils by the cosmetic and other industries in Brazil.[15]

Based on this context, the aim of this work was to evaluate the antimicrobial activity of the essential oil of *B. trimera* against four main bacteria responsible for bad perspiration odor, as a possible alternative for application as an antimicrobial agent in personal care products.

METHODS

Essential oil

The essential oil from *B. trimera* leaves (lot BATRI0111) was obtained from Lazlo Aromatologia Ltda, which was extracted by vapor entrainment.

Gas chromatography

In order to qualitatively and quantitatively characterize the main chemical constituents from essential oil, an aliquot was subjected to analysis by high-resolution gas chromatography (HP 5890) equipped with flame ionization detector. A BP-1 (SGE) 25 m x 0.25 mm column was used, with a temperature gradient of 60°C/1 minute, 3°C/minute to 220°C; injector (split of 1/50) at 220°C and detector at 220°C. The carrier gas used was hydrogen (2 mL/minute) and the injection volume was of 1 µL. Samples were diluted to 0.5% in chloroform. Identification of essential oil components was based on the retention times of sample components and a mixture of n-alkanes from C_{10}-C_{18} and the calculated Kovats Index compared with the available literature.[23] In the biological assays, essential oil concentration is referenced as an equivalent of its major component.

Antimicrobial activity

Microorganisms

Micrococcus luteus (ATCC 7468), *Proteus vulgaris* (ATCC 13315) and *Staphylococcus epidermidis* (ATCC 12228) were obtained from the American Type Culture Collection. *Corynebacterium xerosis* (IAL105) was obtained from Adolfo Lutz Institute Culture Collection.

Antimicrobial screening and minimum inhibitory concentration (MIC)

A total of four microorganisms, including three Gram-positive (*Corynebacterium xerosis* IAL105, *Micrococcus luteus* ATCC 7468 and *Staphylococcus epidermidis* ATCC 12228) and a Gram-negative (*Proteus vulgaris* ATCC 13315) bacteria were tested. The inhibition of microorganism growth was determined by means of turbidimetric method by using a microdilution assay in a sterile 96-well microplate (Sarstedt, Germany). [24] Each well contained 100 µL of the essential oil (62.5 to 1,000 µg/mL of β-pinene) and 100 µL of Brain Heart Infusion (BHI) for *C. xerosis* or Mueller Hinton Broth (MHB) for the others with the bacteria representing, approximately, 4×10^3 colony-forming units (CFU)/mL. The microplates were incubated at 35ºC for 24 hours. Next, 30 µL of aqueous solution of 0.01 mg/mL resazurin were added in each well and the microplate was reincubated for 4 hours. The MIC values were determined by change in color, with the highest dilution remaining blue indicating the MIC. In addition, chloramphenicol (0.025 - 250 µg/mL) and neomycin (0.0125 - 125 µg/mL) were used as drugs references. Tests were carried out in triplicate.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration value was determined in the wells with absence of growth obtained in the MIC assay and 20 µL of culture of each well were transferred to tubes with Tryptone Soy Broth (TSB). The tubes were incubated at 35ºC for 24 hours. The MBC value was regarded as the lowest concentration of the essential oil where no visible growth was observed.

Scanning electron microscopy analysis

The scanning electron microscopy (SEM) was used to investigate morphological changes in the strains of interest submitted to the treatment with the essential oil, chloramphenicol and neomycin. The SEM was carried out by a method adapted from Gao et al.[25] The bacteria cells were incubated for 24 hours in MHB (*S. epidermidis*, *P. vulgaris*, *M. luteus*) or BHI (*C. xerosis*) at 35ºC. The suspension was treated with essential oil (1,000 µg/mL of β-pinene) or the drugs reference (chloramphenicol and neomycin at MBC value), and then the samples were reincubated at 35ºC for 24 hours. After incubation, cells were harvested by centrifugation for 10 minutes at 5,000 x *g* and transferred onto slides. The cells were fixed with 2.5% glutaraldehyde for 12 hours. After that, the slides were washed with 0.1 M phosphate buffer solution (pH 7.4), dehydrated with increasing concentrations of ethanol (50 to 100%) with an interval of 20 minutes between each exchange, and dried at room temperature. The slides were assembled in aluminum stubs with double-faced carbon and then covered with gold in a sputter (2 mm) for 2 minutes in Balzers Union FL - 9496 (Balzers, Germany). Subsequently, they were analyzed in the scanning electron microscope JSM 5310 (Jeol, Japan) in high vacuum in secondary electron mode.

RESULTS

Chemical composition of the essential oil

Twenty constituents were identified by GC in the essential oil, accounting for 89.7% of all components in the essential oil. Other components not listed are present in amounts of less than 0.1%. β-pinene (23.4%) and carquejyl acetate (19.0%) were the constituents in highest concentrations (Fig 1 and table 1).

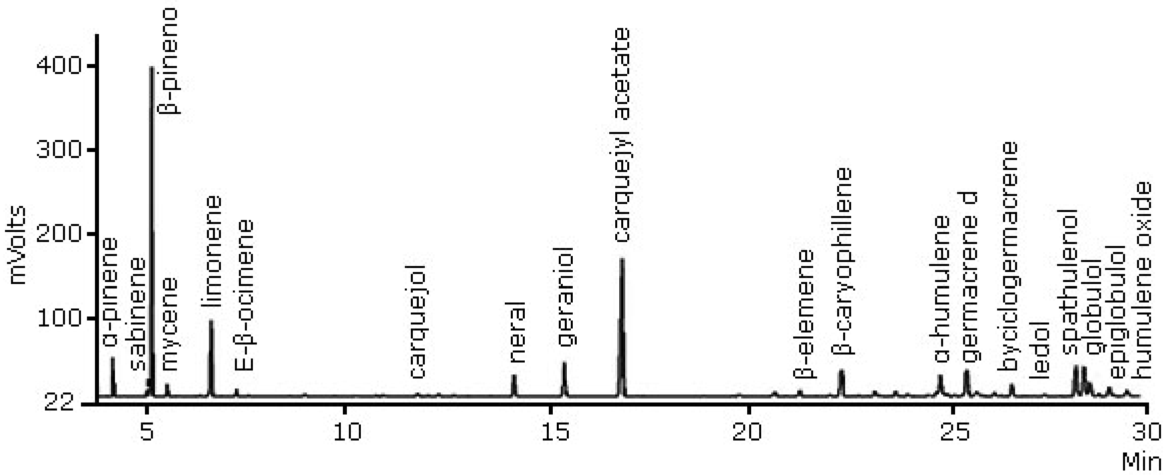

Fig. 1. Chromatographic profile of the essential oil of B. trimera. Peaks lower than 0,1 % were not documented.

Table 1. Chemical composition of the essential oil from *B. trimera* leaves

No.	Compound	%	Kovat's index calculated
1	α-pinene	2.2	928
2	Sabinene	0.5	967
3	β-pinene	23.4	973
4	Myrcene	0.8	986
5	Limonene	5.9	1021
6	E-β-ocimene	0.6	1039
7	Carquejol	0.4	1150
8	Neral	2.3	1231
9	Geranial	4.1	1258
10	carquejyl acetate	19.0	1295
11	β-elemene	0.8	1388
12	β-caryophyllene	6.4	1412
13	α-humulene	3.1	1477
14	germacrene D	5.0	1494
15	bicyclogermacrene	1.6	1523
16	ledol	4.6	1563
17	spathulenol	4.4	1569
18	globulol	2.3	1575
19	epiglobulol	1.3	1586
20	humuleno oxide	1.0	1596
Total		89.7	

Antimicrobial activity

Minimum Inhibitory Concentration (w/v) and Minimal Bactericidal Concentration (w/v)

According to the results given in table 2, the oil exhibited antimicrobial activity against all bacteria, except *S. epidermidis* ATCC 12228. The MIC values of the essential oil ranged from 500 µg/mL to 1,000 µg/mL. The findings of our study showed bactericidal activity only against *P. vulgaris* (MBC=1000 µg/mL) and bacteriostatic activity against *P. vulgaris*, *M. luteus* and *C. xerosis*.

Table 2. *In vitro* susceptibility of bacteria to the essential oil of *B. trimera* and the reference drugs

Microorganisms	Essential oil of *B. trimera*		Chloramphenicol		Neomycin	
	MIC[a]	MBC[a]	MIC[b]	MBC[b]	MIC[b]	MBC[b]
S. epidermidis ATCC 12228*	-	-	2.5	25	1.25	1.25
P. vulgaris ATCC 13315**	1,000	1,000	2.5	25	12.5	12.5
M. luteus ATCC 7468*	500	-	2.5	25	12.5	125
C. xerosis IAL105*	500	-	25	250	1.25	1.25

Notes: (-) not detected at all tested concentrations (62.5 to 1,000 µg/mL of β-pinene); MIC: Minimum inhibitory concentration; MBC: minimum bactericidal concentration; * Gram-positive; ** Gram-negative; [a]: Results expressed as µg/mL of β-pinene; b: Results expressed as µg/mL.

Scanning electron microscopy

The SEM analysis allowed for morphologically identifying structures and to perceive morphological changes of the studied bacteria. There was a change in the structure of the treated bacteria when compared to the negative control. The untreated cells of all microorganisms tested (negative control) showed cell membrane with a regular and smooth surface, whereas the treated ones revealed a severe detrimental effect on the morphology of the cell membrane.

The cells exposed to the essential oil and the reference drugs (neomycin and chloramphenicol) were capable to alter the bacterial cell membrane. The cells exposed to the action of antimicrobial agents have shown deformities in the structure (orifices and appearance of fissures, disintegration of the cell membrane, aggregated cells and cell extravasation) with probable cell destruction (Fig 2).

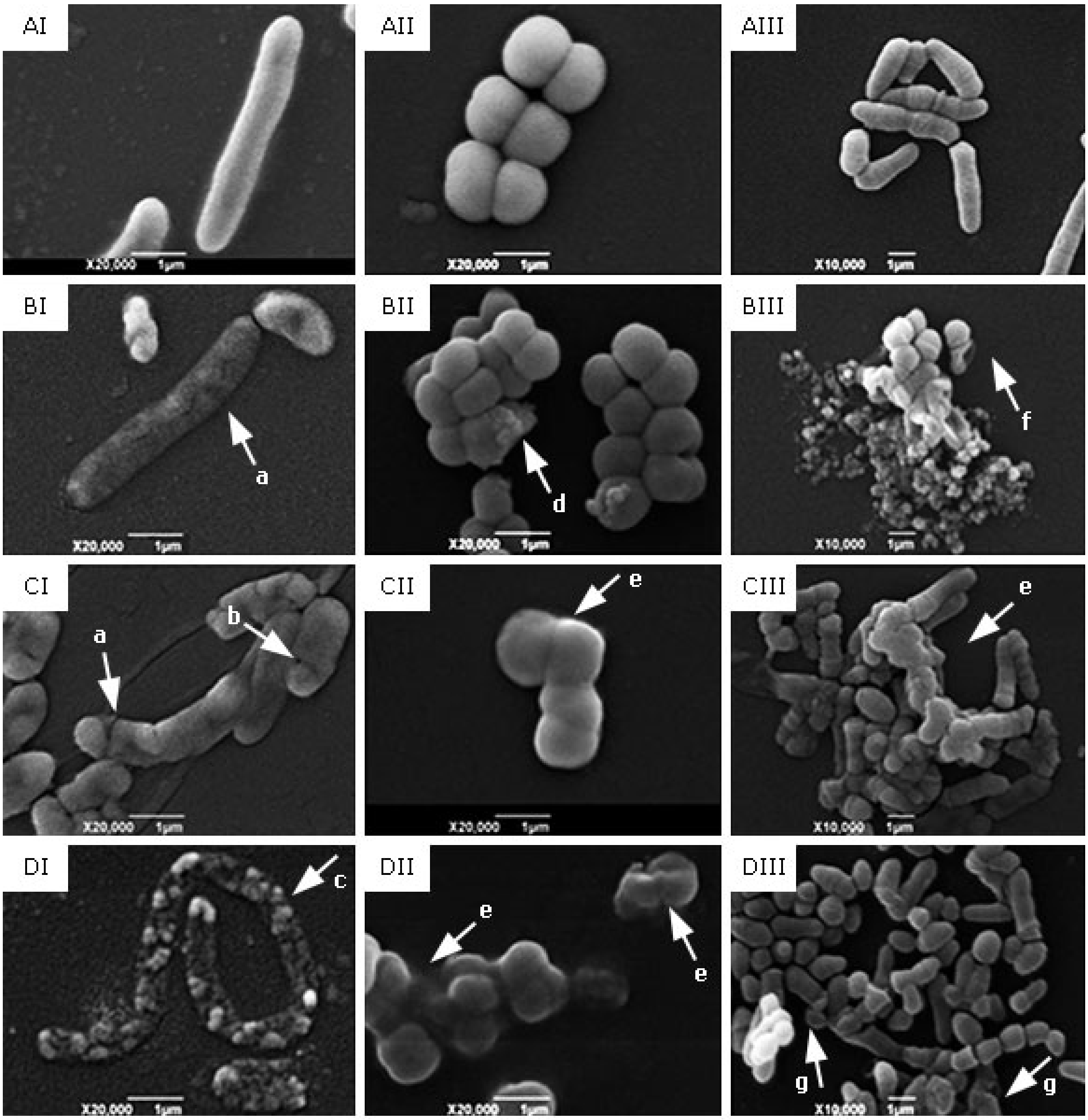

Fig. 2.1. SEM images of *P. vulgaris* ATCC 13315. II: SEM images of *M. luteus* ATCC 7468. III: SEM images of *C. xerosis* IAL105. A: untreated bacterial cells, B: treatment with chloramphenicol, C: treatment with neomycin, D: treatment with essential oil of *B. trimera*. "a": indicates cleft formation. "b": shows pore formation. "c": shows destroyed cell. "d": shows wrinkled abnormalities. "e": indicates aggregated and deformed cells. "f": indicates disruption and lysis of the membrane integrity. "g": indicates deformed cell. Magnifications: X20,000 (I and II) and X10,000 (III).

DISCUSSION

According to Bakkali et al.[26] essential oils are characterized by two or three major components at high concentrations (20 - 70%) compared to other components present in trace amounts. The major compound found in this study was β-pinene (23.4%). It is a bicyclic monoterpene which has been identified as one of the most important bioactive constituents of many essential oils. The antimicrobial effect of β-pinene has been attributed to alterations produced at the membrane level.[27] This work has been developed according to the percentage of β-pinene. However, there is some evidence that minor components of essential oils have contributed to the antimicrobial activity, possibly by producing a synergistic or antagonistic effect between other components.[28,29]

In addition, carquejyl acetate, which accounted for 19.0% of the essential oil, can also make a contribution to the antimicrobial activity. It has been reported that the great commercial value of the essential oil of "carqueja" produced in Brazil has been associated with high carquejyl acetate content.[30] Carquejyl acetate and carquejol, which are present in the essential oil, were proposed as a chemotaxonomic marker of this species. Nevertheless, some populations of *B. trimera* without these compounds have been recently reported, suggesting the existence of a novel chemotype or chemical variations which arise as a result of different environmental conditions.[31]

Amaral et al.[32] found β-pinene, palustrol and carquejyl acetate as the major compounds in the essential oil of *B. trimera*. Such findings are in agreement with our results which demonstrated β-pinene and carquejyl acetate in higher percentages.

On the other hand, Oliveira et al.[11] identified (E) - caryophyllene, germacrene D and bicyclogermacrene as the main compounds. Although those compounds were not the major constituents in the present study, they are present in the chemical composition of the essential oil.

Lago et al.[33] found α-humulene (19.44%) as the major constituent and other compounds in lower percentages, such as myrcene (6.09%) and germacrene D (8.86%). Those constituents were found in the present study, but in different concentrations.

Morais and Castanha[30] suggested the essential oil of *B. trimera* is mainly composed by carquejol, carquejyl acetate, α and β-pinene, trans-β-ocimene, nerolidol and spathulenol. Our study revealed the presence of all those compounds, except nerolidol. In contrast, they observed a higher concentration of sesquiterpenes compared to monoterpenes and the presence of ledol (13.7%) in high concentration. Nevertheless, this compound is frequently found in low concentrations in the essential oil of *B. trimera,* as we found in this study (4.6%).[30,34]

According to Silva et al.[15] the seasonal variations of the essential oil of wild and cultivated *B. trimera* populations indicated differences in the chemical composition of wild and cultivated flowering samples from a period of March-May showed high percentages of globulol and spathulenol. Wild samples collected from June-February exhibited germacrene D and (E)-caryophyllene as the major constituents, while samples cultivated from June-February contained a high content of ledol.

Furthermore, Silva et al.[12] evaluated the effect of different post-harvest processing forms of drugs constituted of parts of "carqueja" on the chemical composition of its essential oil. The concentration of the major constituents of "carqueja" essential oil, spathulenol and ledol, was not affected by the post-harvest treatment. Although they presented different variations, with ledol concentrations decreasing and spathulenol concentrations increasing.

Accordingly, it should be noticed that there are factors which may lead to changes in its chemical composition, such as extraction technique, time of collection, drying of the plant material, soil types, genetic factors and climate.[35]

Although several researchers reported antimicrobial activity of essential oils or extracts of *B. trimera* against some bacteria, such as *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus uberis*,[36-38] the antimicrobial activity with the establishment of the MIC value of the essential oil against the tested bacteria has not been performed up to now.

Pires[34] researched the antimicrobial activity of the essential oil of *B. trimera* against *S. epidermidis* ATCC 12228 and *M. luteus* ATCC 9341. However, this author used the bioautography method as a preliminary biological screening with no quantitative analysis.

Despite the fact that the MIC values of the essential oil were higher than those of chloramphenicol and neomycin, the use of the essential oil of *B. trimera* may be an alternative as a natural antimicrobial agent in personal care products. The MIC values (500 and 1000 μg/mL) demonstrated to be sufficient inhibiting the growth of bacteria. The use of plant extracts or essential oils by consumers and regulatory agencies is expected and reported to be increasing due to "green consumerism".[39] Therefore, as well as some essential oils have pharmacological properties, others can be used as starting compounds which are useful in the chemical, cosmetic and pharmaceutical industries.[11]

The findings of the SEM suggested that the essential oil of *B. trimera* could affect the morphology of cells and destroy cell membranes. Such results demonstrated that the essential oil had a destructive effect on *P. vulgaris* ATCC 13315, *M. luteus* ATCC 7468 and *C. xerosis* IAL105 bacterial cells.

Damages to the cell membrane structure and function of the bacteria are probably due to the presence of monoterpenes, which can cause damage to the cell membrane. According to Florão,[40] these constituents have a lipophilic character, resulting in the expansion and increase of the fluidity of the membrane and enzymatic inhibition.

In conclusion, the essential oil of *Baccharis trimera* has a chemical composition complex with a potential application as antimicrobial agent against to bacteria causal of bad body odor in modern human being. Further studies are necessary.

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41. Érika Yoko Suzuki: Núcleo de Pesquisa e Inovação em Ciências da Saúde (NUPICS), Faculdade de Farmácia, Universidade Federal de Juiz de Fora, Juiz de Fora-MG, Brazil. Correo electrónico: suzukibarbacena@uol.com.br

Recibido: 14 de julio de 2015.
Aprobado: 22 de abril de 2016.

Érika Yoko Suzuki: Faculdade de Farmácia, Universidade Federal de Juiz de Fora, Juiz de Fora-MG, Brazil. Correo electrónico: revistamil@infomed.sld.cu