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Research Article

Chemical composition and antibacterial activity of the essential oil from *Stachytarpheta cayennensis* leaves grown in Brazil Southeast

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Abstract

The antibacterial activity of the essential oil from the leaves of *Stachyterpheta cayenensis* (Verbenaceae) (SC-EO) grown in Brazil against a panel of cariogenic bacteria was investigated using the broth microdilution method. SC-EO displayed strong activity against *Streptococcus mutans*, *S. mitis*, *S. salivarus*, and *S. sanguinis*, with minimal inhibitory concentration (MIC) values of 300, 300, 400, and 400 μg/mL, respectively. Oct-1-en-3-ol (37.8%), 2,6-di-*tert*-butyl-4-methylphenol (20.9 to 20.6%), (*E*)-phytol (8.6 to 8.3%), eugenol (5.1 to 4.8%), and borneol (4.9 to 4.6 %) were identified as the main chemical compounds of SC-EO. To the best of our knowledge, this is the first report on the antibacterial activity of the essential oil of *S. cayenensis*.

1. Introduction

Dental caries is a major public health problem worldwide. It is characterized by the demineralization of the tooth's hard tissues, cavitation, and tooth loss [1]. This pathology is associated with acidogenic and aciduric bacteria that adhere to the tooth surface and form a structurally and functionally organized biofilm (dental plaque) [2]. Biofilm removal by brushing and flossing is the most efficient procedure to prevent dental caries [3]. However, most people fail to control biofilm at a sufficient level through mechanical removal only. Therefore, the use of chemicals incorporated into mouth rinses as a complementary measure is also necessary and has been demonstrated to be of great value to diminish the tooth surface biofilm [3]. Currently, chlorhexidine (CHX) is considered to be the gold anti-cariogenic standard [4], however, the regular use of oral care products containing this chemical is often associated with several side effects [5]. In this scenario, the search for new potential chemotherapeutic agents that can be incorporated into dental products has escalated in recent years.

Essential oils (EOs) are mixtures of volatile compounds produced by plants as part of their secondary metabolism [6]. These compounds are mainly monoterpenes, sesquiterpenes, and phenylpropanoids, which can occur as functionalized derivatives such as alcohols, ketones, aldehydes,

and esters [7]. EOs and their compounds are known to display antibacterial activities against a wide range of bacteria, including cariogenic bacteria [8, 9]. However, the antibacterial activity of EOs is related to their chemical composition, which depends on intrinsic (e.g., genetics and morphology) and extrinsic (e.g., humidity, soil composition, stage of the vegetative cycle, geographic variations, and seasonal variations) factors [10]. Thus, it is crucial to determine the EO composition each time it is obtained from a plant collected in different places or conditions [11-13].

Stachytarpheta cayennensis (Rich.) Vahl (Verbenaceae) is an herbaceous plant commonly found in tropical and subtropical America. In Brazil, *S. cayennensis* is popularly known as "gervão roxo", "vassourinha de botão" or "gervão do campo" [14]. The infusion and decoction of its leaves are used in folk medicine to treat malaria, inflammation, pain, fever, hypertension, stress, diabetes, constipation, and hepatic and renal disorders [14]. Recently, the popular use of *S. cayennensis* for the treatment of COVID-19 in Jamaica has been also reported [15]. In the literature, extracts of *S. cayennensis* have been reported to display anti-inflammatory [16, 17], antinociceptive [17], antiulcerogenic [16], hypoglycemic [18], immunomodulatory [19], sedative and anxiolytic [20], anticancer [21], antimalarial [22], antileishmanial [23], and antimicrobial [16, 24, 25]. However, data on the antimicrobial activities of the essential oil of *S. cayennensis* are still scarce [25, 26].

As part of our ongoing research on the antibacterial activities of essential oils (EOs) and other natural products as potential leads for incorporation into dental products [27-30], here we report the antibacterial activity of the essential oil from *Stachytarpheta cayennensis* leaves grown in Brazil Southeast against a panel of cariogenic bacteria. The chemical composition of the essential oil is also reported.

2. Materials and methods

2.1 Plant material, EO extraction, and Gas chromatography-mass spectrometry (GC-MS) analysis

Stachytarpheta cayennensis (Rich.) Vahl (Verbenaceae) was collected at "Sítio 13 de Maio" near the city of Franca (20°26'S 47°27'W 977 m, State of São Paulo, Brazil) in April 2021. A voucher specimen (SPFR 10005) was deposited at the Herbarium of Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil (Herbarium SPFR).

Fresh leaves (1,200 g) of *S. cayennensis* were divided into three samples (3 x 400 g) and accommodated into three 1-L round bottom flasks containing 500 mL distilled water each. The flasks were coupled to a Clevenger-type apparatus and submitted to hydrodistillation for four hours. After the manual collection of the essential oil of *S. cayennensis* (SC-EO), traces of water were removed by freezing the collected sample below 0°C, followed by the transfer of the unfrozen SC-EO to a new vial. The SC-EO yield (w/w) was calculated from the weight of the fresh leaves.

SC-EO was diluted in ethyl ether (Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 1.0 mg/mL and analyzed by gas chromatography-mass spectrometry (GC-MS) on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an OAC-20i autosampler under the previously reported conditions [30]. The column temperature was programmed to increase from 60°C to 240°C at 3°C/min and held at 240°C for 5 min [30]. Helium (99.999%) was used as carrier gas at a flow rate of 1.0 mL/min. The injector and ion-source temperatures were set at 240°C and 280°C, respectively. The injection volume was 0.1 μL and a split ratio of 1:10 was used. The mass spectrometer operated in the electron ionization (EI) mode at 70 eV. Mass spectra were recorded with a scan interval of 0.5 s over the mass range of 40-600 Da. Quantification of each SC-EO constituent was estimated from GC-MS chromatograms by internal normalization (%) and expressed as the average of three replicates. The chemical compounds of SC-EO were identified on the basis of their retention indices (RI) on an Rtx-5MS (30 m x 0.25 mm x 0.25 μm) capillary column (Restek Co., Bellefonte, PA, USA) relative to a homologous series of *n*-alkanes (C_8-C_{20}) under the same operating conditions as well as computer matching with the Wiley 7, NIST 08 and FFNSC 1.2 spectral libraries. Moreover, the constituents were determined by comparison of their mass spectra with those reported in the literature.

2.2. Bacterial strains and antimicrobial assays

The minimum inhibitory concentration (MIC) values of SC-EO were determined by using the broth

microdilution method in 96-well microplates. The following standard strains from the ATCC were employed: *Streptococcus mutans* (ATCC 25175), *Streptococcus mitis* (ATCC 49456), *Streptococcus salivarius* (ATCC 25975), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus sobrinus* (ATCC 33478), *Enterococcus faecalis* (ATCC 4082), and *Lactobacillus casei* (ATCC 11578). Individual 24-hour colonies from blood agar (Difco Labs, Detroit, MI, USA) were suspended in 10.0 mL tryptic soy broth (Difco Labs, Detroit, MI, USA). Suspensions of each microorganism were standardized as previously described [31]. Samples of SC-EO were dissolved in DMSO (Merck, Darmstadt, Germany) and diluted in tryptic soy broth (Difco Labs, Detroit, MI, USA) to achieve concentrations in the range of 400 to 20 μg/mL. The final DMSO concentration was 5% (v/v), and this solution was used as the negative control. One inoculated well was included to control the adequacy of the broth for organism growth. One non-inoculated well free of the antimicrobial agent was also included to ensure medium sterility. Chlorhexidine dihydrochloride (CHD) (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in tryptic soy broth (Difco Labs, Detroit, MI, USA) and used as a positive control at concentrations ranging from 59 to 0.115 μg/mL. The microplates (96 wells) were sealed with plastic film and incubated at 37°C for 24 h as described above. After that, 30 μL of 0.02% resazurin (Sigma-Aldrich, St. Louis, MO, USA) aqueous solution was poured into each microplate reservoir, to indicate microorganism viability. The MIC values were determined as the lowest concentration of SC-EO capable of inhibiting microorganism growth [32].

Table 1. *In vitro* antibacterial activity (MIC, in μg/mL) of the essential oil of *S. cayenensis* (SC-EO).

Microorganism	SC-EO	CHD
Streptococcus mutans	300	0.92
Streptococcus mitis	300	0.36
Streptococcus sanguinis	>400	0.36
Streptococcus salivarus	400	0.92
Streptococcus sobrinus	400	0.92
Lactobacillus casei	>400	0.18
Enterococcus faecalis	>400	0.36

CHD: chlorhexidine dihydrochloride. The assays were performed in three replicates assays for each microorganism.

3. Results and discussion

The antibacterial activity of EOs and their constituents have been extensively reported and reviewed in the literature [8, 9, 33]. Many EOs have displayed promising activities against cariogenic bacteria, such as those obtained from *Cinnamomum verum* [34], *Plectranthus neochilus* [35], and *Salvadora persica* [36]. Recently, studies on the combination between EOs and herbal toothpastes have also been carried out [37]. On the other hand, a few studies have reported the antibacterial activity of the essential oil of *S. cayennensis*. Onofre and coworkers obtained the EO of *S. cayennensis* grown in Brazil Southwest and assessed its antibacterial activity against the pathogenic bacteria *Escherichia coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923 and *Pseudomonas aeruginosa* ATCC-9027 using the agar diffusion method [25]. Duarte and coworkers investigated the antibacterial activity of the EO of *S. cayennensis* grown in Brazil Southeast against different *Eschericia coli* serotypes [26]. However, to date, the antibacterial activity of the EO of *S. cayennensis* against cariogenic bacteria has not been reported.

The antibacterial activity of SC-EO against a representative panel of cariogenic bacteria was investigated in terms of their MIC values (Table 1). SC-EO was not active against *S. sanguinis*, *L. casei*, and *E. faecalis* in the range of concentrations tested in this study (MIC > 400 μg/mL). On the other hand, SC-EO displayed activity against *S. mutans* (MIC = 300 μg/mL), *S. mitis* (MIC = 300 μg/mL), *S. sobrinus* (MIC = 400 μg/mL), and *S. salivarus* (MIC = 400 μg/mL). According to the literature [8], these MIC values denote a strong antibacterial activity against cariogenic bacteria. This is also an interesting result because very few natural compounds are known to inhibit *S. mutans*, which is one of the primary causative agents of dental caries [38].

SC-EO was obtained in 0.10% (w/w) yield. The chemical composition of SC-EO was determined by GC-MS. A representative GC-MS chromatogram of SC-EO is shown in Figure 1. A total of thirteen compounds were identified, as listed in Table 2. The major compounds were identified as being oct-1-en-3-ol (37.8 to 37.5 %), 2,6-di-*tert*-butyl-4-methylphenol (20.9 to 20.6 %), (*E*)-phytol (8.6 to 8.3 %), eugenol (5.1

Table 2. Chemical composition of the essential oil from *Stachytarpheta cayennensis* leaves (SC-EO).

Compound	RT (min)	RI_{exp}	RI_{lit}	%RA
α-Thujene	5.78	930	931	0.9
α-Pinene	5.98	937	939	3.6
1-Octen-3-ol	7.11	977	978	37.5
Octan-1-ol	7.60	995	993	2.0
Limonene	8.73	1031	1031	2.0
Eucalyptol	8.83	1034	1033	3.6
Borneol	13.76	1163	1165	4.6
Cinnamaldehyde	14.00	1263	1266	1.2
p-Vinyl-phenol	18.80	1305	1309	3.9
Eugenol	22.40	1355	1356	4.8
(*E*)-Caryophyllene	24.69	1417	1418	2.2
2,6-*di*-*tert*-Butyl-4-methylphenol	28.50	1526	1527	20.6
(*E*)-Phytol	44.48	1947	1949	8.3
Monoterpene hydrocarbons				6.5
Oxygenated monoterpenes				8.2
Sesquiterpene hydrocarbons				2.2
Oxygenated diterpenes				8.3
Phenylpropanoids				6.0
Others				64.0
Not identified				4.8

RT: retention time in an Rtx-5MS column; **RI_{exp}:** retention indices relative to *n*-alkanes C_8-C_{20} on Rtx-5MS capillary column; **RI_{lit}**: retention indices from the literature (https://webbook.nist.gov/); **RA:** relative area (peak area relative to the total peak area in the GC-MS chromatogram), an average of three replicates.

to 4.8 %), and borneol (4.9 to 4.6%). This chemical composition significantly differs from the chemical composition reported by Lima and coworkers for the EOs isolated from *S. cayennensis* leaves grown in Brazil North [39]. The authors identified methyl 7-methylcyclopentan[c]pyran-4-carboxylate (48.4%) and citronellol (89.5%) as the major compounds in the EOs isolated by hydrodistillation followed by extraction with ethyl acetate and hexane, respectively [39]. Moreover, none of the compounds identified in SC-EO were previously reported by the authors. α-Pinene and oct-1-en-3-ol were detected as minor compounds in the EO of *Stachytarpheta gesnerioides* grown in Brazil Southeast, but its major compound – the sesquiterpene guaiol (56.5%) was not detected in SC-EO [40]. Differences in the EO composition can be due to geographic factors, which affect growth conditions, climate, altitude, and soil type, as well as to agricultural methods and practices, developmental stage, and harvesting time [41]. It is well-established that the antibacterial activity of EOs cannot be assignable to a single mechanism of action only [6]. In Gram-positive bacteria, lipophilic EO components can easily diffuse across the cell wall and act upon they are within the cytoplasm [8]. Indeed, the anti-cariogenic potential of some SC-EO compounds has been demonstrated. The anti-biofilm activity and the inhibitory effect of β-caryophyllene on biofilm have been recently demonstrated [42]. Eugenol was reported to inhibit the acid production by *S. mutans*, reduce the synthesis of water-insoluble glucans by glucosyltransferases, and suppress the adherence of *S. mutans* to saliva-coated hydroxyapatite beads, besides reducing the incidence and severity of carious lesions in rats [43]. Limonene significantly reduces caries lesions caused by *S. sobrinus* as compared to the control group [44]. However, the antibacterial activity of SC-EO cannot be assigned to the activity of its compounds only, since possible additive or synergistic interactions between the SC-EO compounds can also occur.

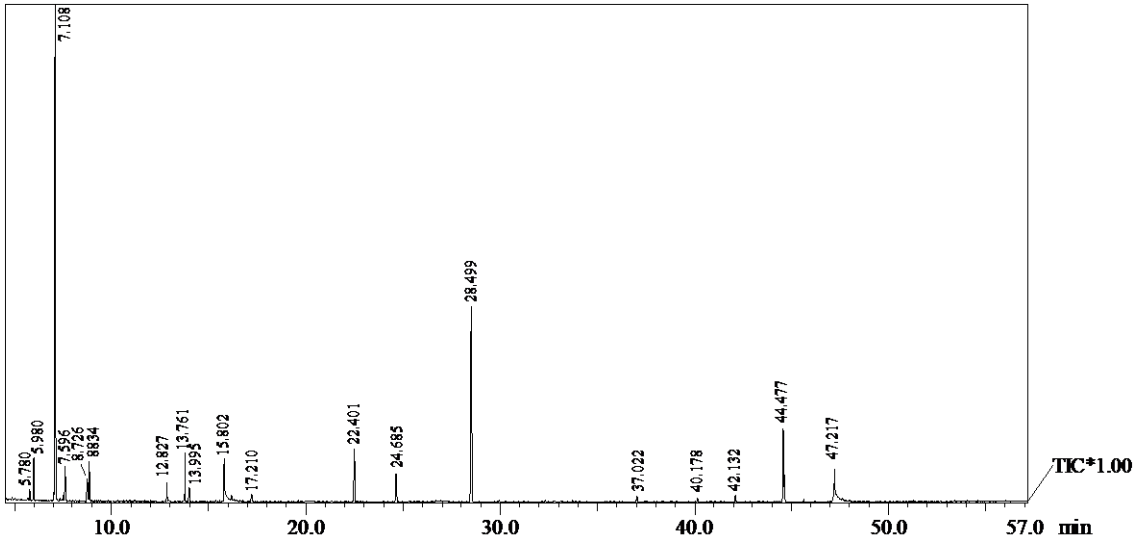

Figure 1. Representative GC-MS chromatogram of SC-EO.

These interactions are beneficial for antibacterial activity due to the difficulties bacteria have in developing resistance to them [45].

4. Conclusions

SC-EO displayed strong activity against *S. mutans*, *S. mitis*, *S. salivarus*, and *S. sobrinus*, with MIC values of 300, 300, 400, and 400 μg/mL, respectively. The chemical composition of SC-EO significantly differs from previous reports in the literature, with the predominance of 1-octen-3-ol and 2,6-di-*tert*-butyl-4-methylphenol, which were not detected in the EO isolated from other *S. cayennensis* specimens. This is the first report on the antibacterial activity of the essential oil from the leaves of *S. cayennensis* up to date and highlights an interesting anti-cariogenic potential that could be further exploited for the development of new oral care products.

Authors' contributions

Conceptualization, A.E.M.C and C.H.G.M.; Methodology, T.A.S.O, J.B.A.S., and J.G.B.; Investigation, T.A.S.O, J.B.A.S., J.G.B., S.M.S., and M.G; Writing – Original Draft Preparation, T.A.S.O., J.B.A.S., and J.G.B., Writing – Review & Editing, A.E.M.C. and C.H.G.M.; Supervision, A.E.M.C and C.H.G.M.; Funding Acquisition, A.E.M.C. and C.H.G.M.

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Conflicts of interest

The authors declare no conflict of interest.

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