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Antimicrobial activity of essential oils obtained from *Stachytarpheta cayennensis*, (Rich.) Vahl. (Verbenaceae) collected in the South-west region of Paraná - Brazil

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Essential oils extracted from plants are currently being used in chemical syntheses or as new materials, with various medical, pharmaceutical, technological and business applications. In this context, this study was carried out to evaluate the antimicrobial activity of the essential oil obtained from the verbena - *Stachytarpheta cayennensis,* (Rich.) Vahl. - (Verbenaceae) - collected in the Southwest region of Paraná - Brazil, on the pathogenic bacteria *Escherichia coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923 and *Pseudomonas aeruginosa* ATCC-9027. The oil was extracted through hydro distillation in a Clevenger apparatus. The Agar diffusion method was used to evaluate the antimicrobial activity. Based on the obtained results, the bacterium *E. coli* ATCC-25922 was observed to be susceptible to the action of the oil, presenting a minimum inhibitory concentration (MIC) of 1.56%. The *S. aureus* ATCC-25923 strain, on the other hand, was also susceptible to the action of oil, but more resistant, since its MIC was a concentration of 12.5%. The bacterium *P. aeruginosa* ATCC-9027 proved to be resistant to the components of the evaluated oil at concentrations from 25 to 0.78%, but it was susceptible to concentrations ranging from 100 to 50%, with the MIC for this strain being 50% of oil.

Key words: Medicinal plants, antimicrobials, metabolites, drugs.

INTRODUCTION

The genus *Stachytarpheta* (Verbenaceae), popularly known as, "gervão-roxo", has 133 species. It is distributed throughout Brazil. The species of this genus are generally shrubs, branching subshrubs or, in rare cases, herbs that range from 0.5 to 1.5 m in height, although certain species may reach up to 4 m (Salimena

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and Giulietti, 1998). Its flowers are arranged in a spiral along the axis of the inflorescence in a very compact way, reaching up to 60 cm in length. Its corolas are quite striking and easily located at a distance in the field. Usually they are blue, but they can have several colors depending on the species, such as red, violet, orange, white or black (Atkins, 2005). The "gervão-roxo"- *Stachytarpheta cayennensis* (L. C. Rich) Vahl, (Figure 1) is an erect, perennial, branching, somewhat angular, fibrous subshrub that is very resistant to traction. It usually has opposite, ovate leaves with a distinct petiole and serrated and indented edges, an acute or subacute apex, a slightly wrinkled appearance, green color, terminal inflorescence with linear stalks, sessile flower with a gamosepalous calyx, pilose on the dorsum, a corolla with five petals welded at the base, of a lilac coloring, with an androecium with two fertile stamens and two staminodes.

This plant is native to Tropical America, occurring in Australia, Hawaii, India (Dias et al., 1995) and in Brazil, where it has a wide distribution from the Amazon to Rio Grande do Sul, Brazil (Castro and Chemale, 1995). It is specially common in the Brazilian Amazon in regions that are considered to be drier (Dias FIlho et al., 1995) and with a poor cover of vegetation (Dias, 1994; Kissmann and Groth, 1995). It is been considered as a weed in pastures and areas of cultivation (Carib é and Campos, 1991; Kissmann and Groth, 1995). The pharmacological study of the "gervão-roxo" – *S. cayennensis* (L. C. Rich) Vahl, was carried out by Costa (1960), who determined its active compounds (stachytarphine, citral, geraniol, verbascoside, dextrins and ascorbic acid). One of the virtues of the plant, both from its leaves and roots, is its effect in treating chest and stomach pains, in addition to being a stimulant, febrifuge (Pio Corrêa, 1926), sudorific and diuretic (Almeida, 1993). Later, Caribe and Campos (1991) reported that the decoction of the leaves also had insecticidal properties.

The genus *Stachytarpheta* is used in folk medicine for the treatment of stomach ulcers, pain, fevers, diarrhea. It is characterized by the presence of flavonoids, triterpenes, phenylpropanoids, iridoids and steroids as chemical constituents.

Studies conducted by Robinson et al. (1990) and Vargas (1995) indicated that the triterpenoids obtained from the *S. cayennensis* have anti-inflammatory, antimicrobial, antiviral and analgesic effects. An *in vivo* experiment in rats has confirmed the analgesic and anti-inflammatory activity.

The isolation in the extract of *S. cayennensis* of a glycosylated phenylpropanoid with anti-histaminic activity and of an iridoid, ipolamiide, with anti-histaminic and anti-bradykinin effect, contributed to confirming the afore-mentioned effects. The aqueous extract of *S. cayennensis* has an antiulcerogenic effect and inhibits the secretion of gastric acid. This last effect is a consequence of the inhibition of the activity of the kinase protein dependent of AMPc (PKA). The anti-ulcer effect seems to involve the activation of defense mechanisms of the gastric mucosa, which is independent of the effect that inhibits the secretion of gastric acid (Vella, 1999; Penido et al., 2006).

The aqueous extract of *S. cayennensis* presents activity protective activity against strains of *Staphylococcus aureus*, *Micrococcus luteus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimurium* (Duarte et al., 2002). In general, it presents weak to moderate antibacterial activity *in vitro* for Gram-positive and Gram-negative bacteria (Rosas, 2004). A study with the aqueous extract of the leaves of *S. cayennensis* (gervão-roxo) to check the intestinal transport of water in rats and the effect on gastrointestinal propulsion in mice showed a reduction in intestinal transit in relation to the control group.

This same study revealed that the extract of S. cayennensis leaves have a potential anti-diarrheal effect in infections by enteropathogens (Rosas, 2004). Because of the scant information available in the technical and scientific literature on the chemical and biological properties of the species, *S. cayennensis*, (Rich.) Vahl. -(Verbenaceae), possibly as a result of the limited and scarce ethno-botanical knowledge about the localities where this flora is usually present, the objective of this work was to evaluate the antimicrobial activity of the essential oil obtained from the gervão-roxo - *S. cayennensis,* (Rich.) Vahl. (Verbenaceae), collected in the southwest region on Paraná- Brazil.

MATERIALS AND METHODS

Study location

The experiments were carried out in the Chemistry and Microbiology Laboratories of the institutions União de Ensino do Sudoeste do Paraná - UNISEP - Francisco Beltrão - Paraná - Brazil and Universidade Comunitária da Região de Chapeco - UNOCHAPECÓ - Chapecó - Santa Catarina - Brazil, in the period between July, 2013 and May, 2014. All procedures were performed in an aseptic way in a laminar flow cabinet

Obtaining the essential oil

The oils used in this study were obtained through the hydro distillation process using a Clevenger type apparatus. The botanical species used in this study was *S. cayennensis,* (Rich.) Vahl. -(Verbenaceae) collected in the Southwest region of the State of Paraná in the period between July, 2013 and May, 2014. Identified and stored under the voucher number 12.643. All the activities involving the collection of the botanical material were approved by IBAMA under the permit number 13234-2 (from 1 August, 2012). The essential oil was extracted from the leaves and stems of the plant *in natura*, with the temperature set at 98°C for the 3 h of the process. After the extraction, this oil was stored in an amber glass vial at ambient temperature, sheltered from light so as to prevent the deterioration of any component.

Figure 1. Botanical characteristics of "gervão-roxo" - *Stachytarpheta cayennensis* (L. C. Rich) Vahl.

Analysis of oil yields

The yield of the essential oils was given in volume/mass percentage (%), i.e. volume (ml) of essential oil per mass (g) of plant material (Farmacopéia Brasileira, 1988; Fabrowski, 2002).

Susceptibility test by disk diffusion

The method used was based on the disk diffusion technique. The principle of this method is based on inoculation on the surface of a standardized solution of a specific microorganism's agar. On top, paper disks are placed that were previously soaked with solutions of the samples for which the antimicrobial activity is to be investigated. The substances soaked in the paper disks diffuse in the culture medium and if the sample in question has an inhibitory effect on the tested micro-organism, a halo without growth is formed around the impregnated disc. After the period of incubation, following the specific conditions for the micro-organism, the zones of inhibition around each disk are measured (Bauer et al., 1966; Isenberg, 1992; Vandepitte et al., 1994; Koneman et al., 1997).

Analysis of oil dilution

Tubes scaling from 1 to 10 were prepared according to the different concentrations of *S. cayennensis* oil. For this, dimethyl sulfoxide (DMSO) was used as solvent. The following concentrations were prepared: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19% of oil. These dilutions were stored in an amber bottle for later assessment.

Preparation of the paper discs

Discs of filter paper (6 mm) were saturated with the dilutions in the concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19% of oil. These discs were then kept for a time in a sterile environment so that the excess solvent was removed by evaporation.

Table 1. Yields of the essential oil of *Stachytarpheta cayennensis,* (Rich.) Vahl. - (Verbenaceae) - using the hydro distillation process in a Clevenger apparatus.

Sample	Yield (ml/ 100 g)	Time (h)
Plants	0.45	3

Preparation of the inoculum

The following microorganisms were used: *S. aureus* - ATCC-25923 (susceptible to oxacillin and penicillin); *E. coli* - ATCC-25922 (beta-lactamase negative) and *P. aeruginosa* ATCC-9027. The microbial cultures were standardized at 108 cells/ml which is equivalent to the 0.5 tube on the MacFarland scale.

Disk diffusion test

The bacterial suspensions were inoculated on plates containing Mueller-Hinton agar with the aid of a swab. After this procedure, the disks prepared beforehand were transferred to media containing the inocula. The plates were incubated at 35 ± 1°C for 24 h. After this period, the dishes were evaluated for the presence of inhibition halos (measured in mm). The diameters of inhibition halos were interpreted according to standard M2-A8 recommended by the Clinical Laboratory Standards International (CLSI) (2003). The minimum inhibitory concentration (MIC) was considered to be the lowest concentration of oil capable of inhibiting bacterial development.

Statistical analysis

The analyses of variance were carried out according to the standards of analysis of variance (ANOVA). The significant differences between the means were determined by Tukey's Test.

RESULTS AND DISCUSSION

Establishment of essential oil yields

The data relating to the yields of the essential oil of *S. cayennensis,* (Rich.) Vahl. - (Verbenaceae), are shown in Table 1. Analyzing the data in Table 1 regarding the hydro distillation process, one can see that 0.45 ml of essential oil was produced for each 100 g of *S. cayennensis* extracted from the leaves and stems of the plant *in natura.* This methodology made it possible to observe that a large part of the essential oil is extracted at the beginning of the distillation, maintaini ng satisfactory yield levels in the first hour of extraction, but decreasing considerably in the course of the hydro distillation process, which in this case lasted for 3 h. When these data are compared with those obtained by Mejdoub and Katsiotis (1998), who indicated three hours as the ideal period for the distillation of essential oils from *Eucalyptus citriodora*, a similarity in the time periods can be observed between these two works. Fabrowski (2002) also used the same amount of time to perform the extraction of chamomile oil, and his data also coincided with this study in relation to the extraction time and greater yields of oil during the initial hours of the process.

The data obtained in this study are similar to those obtained in studies with the same objectives by Benigni et al. (1971), who studied the levels of essential oils present in peduncles and flower heads of chamomile. The values obtained in these studies are accepted by the Farmacopéia Brasileira (1998) and the mean values of essential oils contained in the samples of Brazilian chamomile, which presented 0.407% of essential oil. This value is considered to exceed the minimum requirement of 0.40% for essential oils (Borsato et al., 2007). The values obtained in this study are comparable to the levels of essential oil found and produced in various traditional producer countries: Germany 0.50%; France: 0.50%; Hungary: 0.40%; Austria: 0.40%; Greece: 0.40% and Spain: 0.30% (Pharmazentischen, 1969; Benigni et al., 1971).

Antimicrobial activity

This study was developed and used the essential oil produced by *S. cayennensis*, (Rich.) Vahl. - (Verbenaceae) in 10 different concentrations. The results obtained in this study are summarized in Table 2 and in Figures 2 and 3. Analyzing the results obtained in Table 2, one can observe that the best results were obtained for the bacterium *S. aureus* with the concentration of 100% of oil. That is with the pure oil, without any dilution. The inhibition halos for this concentration were 38.32 ± 6.57 mm, which can be considered as statistically superior to the other halos obtained with other concentrations. The remaining results observed for the effect of the essential oil of *S. cayennensis* on *S. aureus* revealed values that varied between 21.05 ± 3.56; 12.23 ± 2.10 and 10.56 ± 1.89 mm for the concentrations of 50, 25 and 12.5%, respectively. An analysis of this data reveals that the value obtained at a concentration of 50% was higher than the other results at concentrations of 25 and 12.5%, and these last two results did not differ among themselves according to the Tukey Test at the level of 5%.

The value of the (MIC) was found to be 12.5% for the bacterium *S. aureus*. At lower concentrations, the bacterium proved resistant. This result is in line with those obtained by Silveira et al. (2007) who evaluated the antimicrobial activity of hydroalcoholic extracts of verbena on *S. aureus* and observed a minimum inhibitory concentration

Table 2. Distribution in millimeters (mm) of the antibacterial inhibition effect halo of the essential oil produced by *Stachytarpheta cayennensis,* (Rich.) Vahl. - (Verbenaceae) - as assessed on pathogenic bacteria, according to the scale of dilution.

Concentration (%)	*E. coli*	*S. aureus*	*P. aeruginosa*
100	18.53 ± 2.12^{Ab}	38.32 ± 6.57^{Aa}	12.29 ± 2.16^{Ac}
50	14.67 ± 2.09^{Ab}	21.05 ± 3.56^{Ba}	7.12 ± 0.89^{Bc}
25	11.21 ± 2.18^{Ba}	12.23 ± 2.10^{Ca}	R
12.5	9.98 ± 1.98^{Ba}	10.56 ± 1.89^{Ca}	R
6.25	9.34 ± 1.02^{B}	R	R
3.12	7.22 ± 0.78^{B}	R	R
1.56	7.11 ± 0.67^{B}	R	R
0.78	R	R	R

*Capital letters on the vertical and small case letters on the horizontal do not differ statistically between themselves by the Tukey Test at the level of 5%. R = The obtained values were less than 6.0 mm - considering the bacterium resistant (R) to the components of the oil.

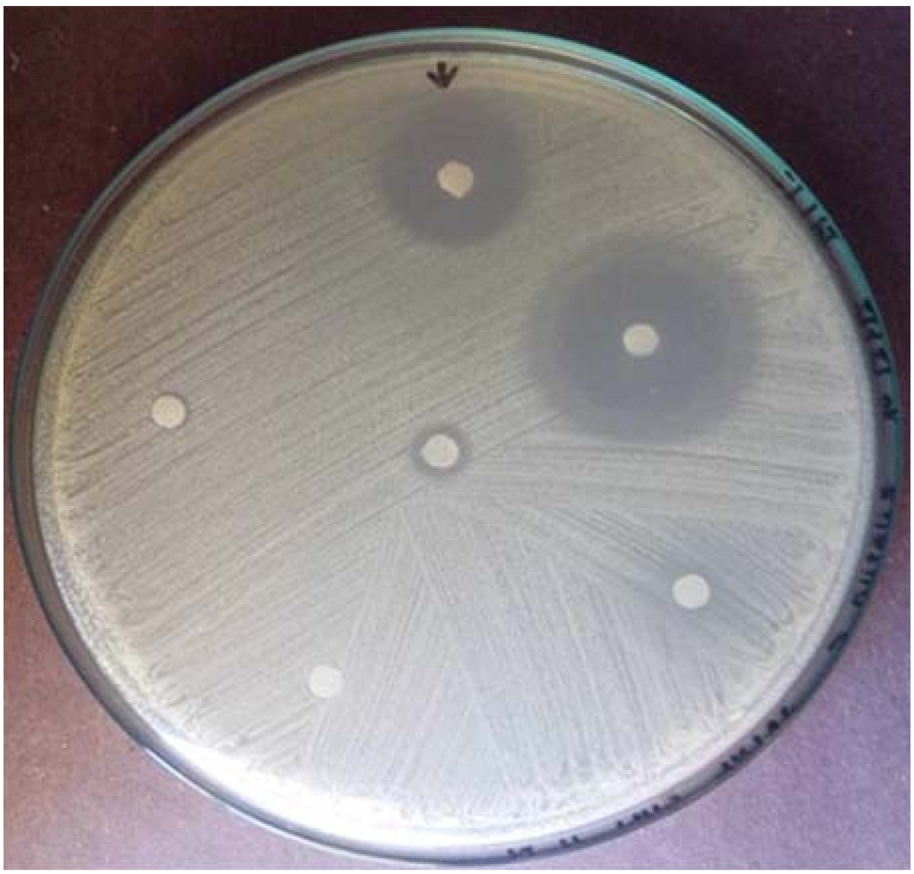

Figure 2. Antimicrobial activity test of the essential oil of *Stachytarpheta cayennensis,* (Rich.) Vahl. - (Verbenaceae) for the Gram-positive bacterium *Staphylococcus aureus.*

for this bacterium of 12.5 mg/ml for the evaluated extract. The trials performed with the bacterium *E. coli* showed that this bacterium was more susceptible to various concentrations of the essential oil of *S. cayennensis*, since even a concentration of 1.56% revealed inhibition halos for this bacterium. Lower concentrations did not reveal any antimicrobial activity for this bacterial strain. The values obtained ranged from 18.53 ± 2.12 to 7.11 ± 0.67 cm for the concentrations of 100 to 1.56%, respectively. It should be stressed that the mean of

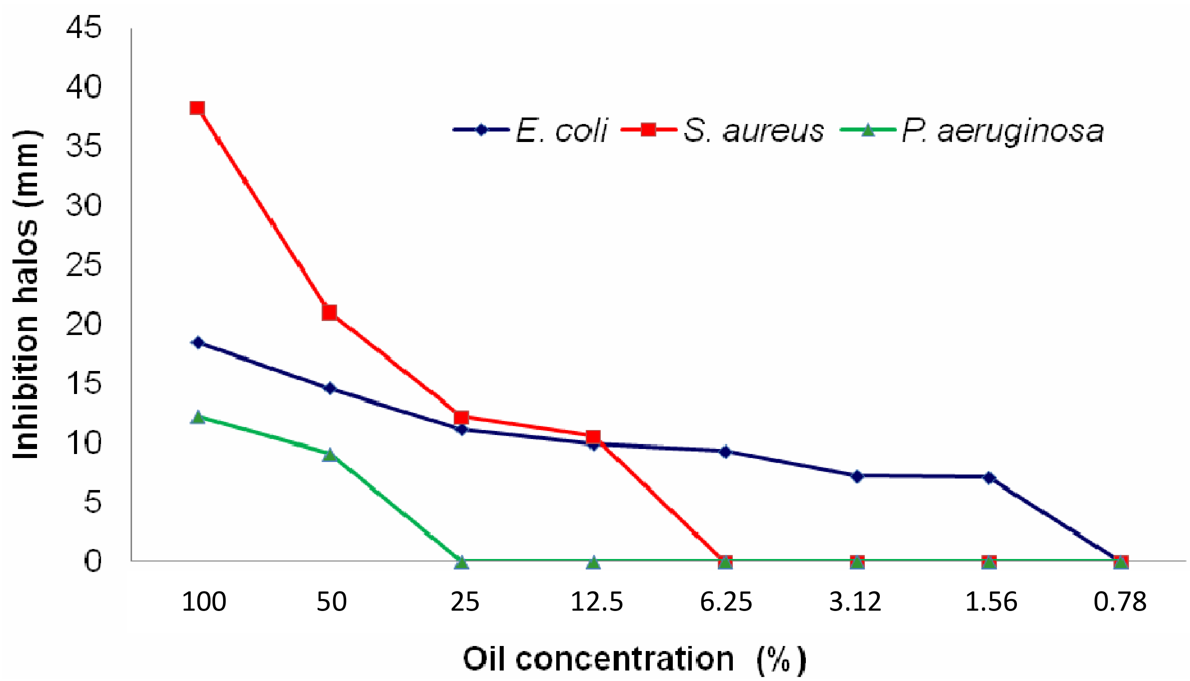

Figure 3. Effect of the various concentrations of the essential oil on the evaluated bacteria - data on the inhibition halos in millimeters.

inhibition halos obtained with the essential oil for the bacterium *E. coli* proved to be higher at concentrations of 100 and 50%, differing from each other statistically by the Tukey Test at the level of 5%, when compared to the other evaluated concentrations.

Taking into account the obtained values, the MIC of the oil on the bacterium *E. coli* could be determined as 1.56% of oil. For lower concentrations no antimicrobial activity was observed on the evaluated strain. With regard to the results obtained for the bacterium *P. aeruginosa,* one can see that this bacterium was susceptible only to the action of the components of essential oils at concentrations of 100 and 50%, developing inhibition halos of 12.29 ± 2.16 and 7.12 ± 0.89 mm, respectively. For other dilutions, this bacterium proved resistant to the components of the essential oil of *S. cayennensis,* which did not present any level of susceptibility.

Considering that the therapeutic activity of *S. cayennensis* is determined by the active compounds contained in the extracts and essential oils from this plant and since, according to the literature, stachytarphine, citral, geraniol verbascoside have an antimicrobial effect (Silveira et al., 2007), it can be suggested that these compounds contributed to the antimicrobial activity of this oil on the bacteria *E. coli, S. aureus* and *P. aeruginosa.* The results obtained in this study are unique for *S. cayennensis*, but they may contribute to the literature, indicating that this species of plant produces metabolites with efficient antimicrobial action and that it could be used in the treatment of infections caused by pathogenic bacteria.

According to Verdi et al. (2005), people have used plants extensively to heal diseases, including infectious diseases. Information and results from the literature show that plants have a great potential for therapeutic treatments, despite the fact that they have not been fully studied. More studies need to be conducted to research the new components contained in their oils. According to these same authors, an antimicrobial activity has been found in some spices derived from plants. Once extracted, and before they can be used as therapeutic treatment, their toxicity must be tested *in vivo*. Tests have demonstrated the toxicity of the extracts of different plants.

Conclusion

After completing all stages of this study, it can be drawn that the bacterium *S. aureus* was susceptible to the action of the components of the oil at a concentration of 100%, showing inhibition halos of 38.32 ± 6.57 mm. For other concentrations of the oil, the mean values of the halos were 21.05 ± 3.56, 12.23 ± 2.10 and 10.56 ± 1.89 mm, corresponding to concentrations of 50, 25 and 12.5%, respectively. The MIC for this bacterium was established at a concentration of 12.5%. For the bacterium *E. coli*, a greater susceptibility was observed, presenting halos between 18.53 ± 2.12 at the concentration of 100% to 7.11 ± 0.67 at the concentration of 1.56% of oil. This last concentration was considered to be the MIC for this strain. The bacterium *P. aeruginosa* proved to be resistant to the components of the evaluated oil at concentrations from 25 to 0.78%, but it was susceptible to concentrations ranging from 100 to 50%, with the MIC for this strain being 50% of oil.

Conflict of interest

The authors declare no conflict of interest.

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