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MICROBIOLOGY

Phytochemical screening and antimicrobial activity testing of crude hydroalcoholic extract from leaves of *Sphagneticola trilobata* (Asteraceae)

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ABSTRACT: *This study aimed to perform phytochemical analysis and to test the antimicrobial activity of the crude hydroalcoholic extract obtained from the leaves of Sphagneticola trilobata. Classes of secondary metabolites present in the extract were identified through phytochemical screening using analytical thin-layer chromatography. Antimicrobial activity was evaluated by testing cultures of Staphylococcus aureus, S. epidermidis, Staphylococcus spp., Escherichia coli, Serratia marcescens, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella Typhimurium, and Klebsiella pneumoniae isolated from human skin and those of Staphylococcus spp. isolated from dog skin using the broth microdilution method. In the phytochemical screening, classes of anthracenic derivatives and mono-, sesqui-, and diterpenes were identified. Colorimetric analysis showed total phenol and total flavonoid contents of 21.7 ± 0.009 mg of gallic acid equivalents per gram of sample and 0.23 ± 0.005 mg of catechin equivalents per gram of sample, respectively. Microbiological analysis revealed that the hydroalcoholic extract of S. trilobata exhibited antimicrobial activity against cultures of Staphylococcus spp., E. coli, S. marcescens, and E. faecalis isolated from human skin and those of Staphylococcus spp. isolated from dog skin. Thus, crude hydroalcoholic extract of leaves of S. trilobata contained flavonoids and terpenoids as secondary metabolites, which contributed to its antimicrobial activity against skin bacteria isolated from different sources.*
Key words: *Antimicrobial, Phytochemical Screening, Phenolic compounds.*

Triagem fitoquímica e atividade antimicrobiana do extrato hidroalcoólico bruto das folhas de *Sphagneticola trilobata* (Asteraceae)

RESUMO: *Este estudo teve como objetivo realizar a triagem fitoquímica preliminar e testar a atividade antimicrobiana do extrato hidroalcoólico bruto das folhas de Sphagneticola trilobata. A identificação das classes de metabólitos secundários presentes no extrato foi realizada através da cromatografia em camada delgada analítica (CCDA). Para determinar a quantidade de fenóis e flavonoides totais foram utilizados os métodos espectrofotométricos de Folin-Ciocalteu e complexação com $AlCl_3$, respectivamente. Para avaliar a atividade antimicrobiana foram testadas culturas de Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus spp., Escherichia coli, Serratia marcescens, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella Typhimurium, Klebsiella pneumoniae isoladas de pele humana e culturas de Staphylococcus spp. isoladas de pele de cães pelo método de microdiluição em caldo. Na triagem fitoquímica foi verificada reação positiva para a presença de derivados antracênicos, mono, sesqui e diterpenos. As análises colorimétricas mostraram conteúdos de fenóis totais e flavonoides totais de 21,7 ± 0,009 miligramas de equivalentes de ácido gálico por grama de amostra e 0,23 ± 0,005 miligramas de equivalentes de catequina por grama de amostra, respectivamente. Na análise microbiológica, o extrato hidroalcoólico das folhas de Sphagneticola trilobata apresentou atividade antimicrobiana frente às culturas de Staphylococcus spp., Escherichia coli, Serratia marcescens e Enterococcus faecalis. Todas as culturas de Staphylococcus spp. isoladas de pele de cães foram sensíveis ao extrato. Conclui-se que o extrato hidroalcoólico bruto das folhas de Sphagneticola trilobata possui entre seus metabólitos secundários os flavonoides e terpenoides que contribuíram com a atividade antimicrobiana frente às bactérias isoladas de pele de diferentes origens.*
Palavras-chaves: *antibacteriano, estudo fitoquímico, compostos fenólicos.*

INTRODUCTION

Two decades ago, CECHINEL-FILHO & YUNES (1998) reported advances in phytotherapy and growth in the market for phytotherapeutic agents, highlighting the importance of conducting chemical and pharmacological studies to determine the efficacy of medicinal plants. Several medicinal plants are used without particularly confirming their pharmacological properties, which raises the possibility of these plants being harmful to health due to their adverse effects and toxicity (VEIGA JÚNIOR et al., 2005).

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Sphagneticola trilobata is a plant that has been very commonly used in folk medicine in Brazil; however, few studies have evaluated its activity. It is a herbaceous plant from the Asteraceae family and is popularly known as *malmequer*, *vedélia*, and *picão-da-praia* in Brazil. It is native to Brazil, easily grows, and quickly spreads on several different types of soil. It grows well both under the sun and in the shade and can easily be found in dark places, on seashores, and in vacant lots, forming a carpet of foliage (CORREIA, 1984).

Although, phytochemical analysis studies of *S. trilobata* have been conducted (CECHINEL FILHO, 2000; CARVALHO et al., 2001; FIDELIS et al., 2005; SILVA et al., 2012; SHANKAR & TOMAS, 2014), it is important to identify the chemical composition of extracts from this plant collected from different regions of Brazil because the composition may vary with changes in soil and climate. Thus, the antimicrobial activity of the extract from this plant against bacteria isolated from human and animal skin is directly related to the variation in its chemical components under different environmental conditions.

Therefore, this study aimed to perform a preliminary phytochemical screening and to evaluate the antimicrobial activity of hydroalcoholic extract from the leaves of *S. trilobata* against skin isolates of Gram-positive and Gram-negative bacteria from different sources.

MATERIALS AND METHODS

Seven samples of the plant species were collected at blooming from the campus of the Federal Rural University of Pernambuco (UFRPE) located at geographic coordinates 8° 04' 03" S and 34° 55' 00" W, in the morning in May 2015. The material was sent to the Geraldo Mariz Herbarium of the Federal University of Pernambuco, where the botanical dehydrated material was deposited, identified, and catalogued under the registration number 78.782.

To produce the hydroalcoholic extract, the plant's leaves were dehydrated in an oven at 40°C and then ground and macerated in 70% ethanol for 72 h. The extract/plant proportion was 2 mL of the solvent—70% ethanol (Merck ethanol PA)—for 1 g of plant material, according to the method described by MATOS (1997). The extracting solution was filtered and then concentrated in a rotary evaporator under reduced pressure at 45°C, which produced a crude hydroalcoholic extract with a concentration of 140 mg/mL. Procedures were performed at the UFRPE Laboratory of Pharmacology.

Phytochemical assays were performed at the Center for Studies and Research on Medicinal Plants of the University of the São Francisco River Valley (NEPLAME-UNIVASF). Preliminary phytochemical screening was performed using analytical thin-layer chromatography. The extract was applied with a glass capillary tube onto plates with an aluminum support using silica gel 60 F_{254} as an adsorbent and then eluted using different solvent systems, as described by WAGNER & BLADT (1996).

For determining the total phenol content, an aliquot (40 µL) of the extract was added to 3.16 mL of distilled water and 200 µL of Folin– Ciocalteu reagent, mixed immediately in succession. The mixture was made to stand for 6 min, and 600 µL of a stock Na_2CO_3 solution was then added. The final solutions were made to stand at 20°C for 2 h. At the end of the process, the absorbance of each solution was determined using a spectrophotometer at 756 nm against blank (all components, except the sample being analyzed), and results were plotted on a graph correlating the absorbance of the samples with their concentration. The total phenolic content of extracts was expressed as milligrams of gallic acid equivalents per gram of sample (mg EqGA/g) using the gallic acid calibration curve, which was obtained in concentrations ranging from 50 to 1,000 mg/L (SLINKARD & SINGLETON, 1977). All assays were performed in triplicate.

Total flavonoid content was determined using the metallic complexation method previously described by ZHISHEN et al. (1999). First, 300 µL of the crude hydroalcoholic extract and the same volume of (+)-catechin standard solution were added to 1.5 mL of distilled water, following which 90 µL of $NaNO_2$ solution was added. After letting the mixture react for 6 min, 180 µL of 10% $AlCl_3 \cdot H_2O$ solution was added to it. After reacting for 5 min, 600 µL of 1 M NaOH solution was added to the previous mixture. Finally, the volume was topped with 330 µL of distilled water, and the system was completely homogenized. Immediately after obtaining the final mixture, absorbance was measured against the blank at 510 nm on a spectrophotometer and compared with standard solutions containing (+)-catechin at known concentrations. Results were expressed as milligrams of catechin equivalents per gram of sample (mg EqC/g) by comparison with the standard catechin curve, which was obtained in concentrations ranging from 50 to 1,000 mg/L. All assays were performed in triplicate (ZHISHEN et al., 1999).

Antimicrobial activity was evaluated using the broth microdilution method at the Federal University of the São Francisco River Valley (UNIVASF)

Laboratory of Microbiology and Animal Immunology. First, 0.25 mg of the crude hydroalcoholic extract from the leaves of *S. trilobata* was weighed and diluted in alcohol and water (3:7), which resulted in a stock solution with a concentration of 25,000 µg/mL. Fifteen isolates of Gram-positive and Gram-negative bacteria from different sources, including isolates from human skin, such as *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Staphylococcus* spp. (MRSA 8536 and MRSA 9606), *E. coli* (ATCC 35218), *S. marcescens* (ATCC 13880), *E. faecalis* (ATCC 19433), *P. aeruginosa* (ATCC 27853), *S.* Typhimurium (ATCC 10708 and ATCC 14023), and *K. pneumoniae* (ATCC 13883), and those from dog skin, including four strains of *Staphylococcus* spp. (244, 246, 250, and 256) during clinical consultations at the UNIVASF University Veterinary Hospital. Bacterial isolates used in this experiment were chosen for their importance in several dermatological diseases occurring in both human and veterinary clinical practice. The strains were available at the bacteria collection of the UNIVASF Laboratory of Microbiology and Animal Immunology. All isolates were reseeded in trypticase soy agar medium for 24 h at 37°C.

For preparing bacterial inoculum, four colonies were inoculated into tubes containing 5 mL of saline until the McFarland turbidity scale value of 0.5 was obtained. Then, 0.1 mL of the suspension was inoculated into tubes containing 9.9 mL of Mueller–Hinton broth. In a 96-well microplate, 200 µL of Mueller–Hinton broth was added to each well, and a serial dilution was performed in the same wells starting with 200 µL of the extract followed by a 1:2 dilution, discarding the final 200 µL. The serial dilutions were tested at the following final concentrations: 12,500, 6,250, 3,125, 1,562.5, 781.3, 390.6, 195.3, and 97.6 µg/mL. Wells were then inoculated with 20 µL of the Mueller–Hinton broth containing the tested bacterium, separately in each well. The last wells received the diluent control (the diluent plus Mueller–Hinton broth and the tested bacterium), positive control (the Mueller–Hinton broth and the tested bacterium), and negative control (the Mueller–Hinton broth alone). Plates were incubated in a bacteriological oven at 37°C, and the growth conditions of microorganisms were checked after 24 h (CLSI, 2014).

The antimicrobial activity was evaluated considering the most dilute concentration of the crude hydroalcoholic extract that inhibited bacterial growth in the tested tube (minimum inhibitory concentration, MIC), assessed by a negative result in the colorimetric reaction produced by adding 10 µL of 2,3,5-triphenyl tetrazolium chloride (TTC) at 2% to each well. A change in the color of TTC from colorless to red indicates bacterial metabolic activity. All assays were performed in triplicate (CLSI, 2014).

RESULTS

Phytochemical screening of the crude hydroalcoholic extract from leaves of *S. trilobata* revealed the presence of phenolic compounds, anthracene derivatives, and mono-, sesqui-, and diterpenes (Table 1). The total phenol and total flavonoid content was 21.7 ± 0.009 mg EqGA/g and 0.23 ± 0.005 mg EqC/g, respectively.

The following bacterial cultures isolated from human skin exhibited susceptibility to the crude hydroalcoholic extract from the leaves of *S. trilobata*: *Staphylococcus* spp. (MRSA 8536 and MRSA 9606), *E. coli* (ATCC 35218), *S. marcescens* (ATCC 13880), and *E. faecalis* (ATCC 19433) (Table 2).

Some cultures exhibited resistance to the extract, including *P. aeruginosa* (ATCC 27853), *S.* Typhimurium (ATCC 10708 and ATCC 14023), and *K. pneumoniae* (ATCC 13883).

All bacterial cultures isolated from dog skin obtained from the UNIVASF University Veterinary Hospital, i.e., *Staphylococcus* spp. (244, 246, 250, and 256), were susceptible to the crude hydroalcoholic extract from the leaves of *S. trilobata* (Table 2).

DISCUSSION

S. trilobata contains several classes of secondary metabolites exhibiting pharmacological

Table 1 - Determination of compounds present in the crude hydroalcoholic extract from the leaves of *Sphagneticola trilobata* by phytochemical screening.

Class	Result
Alkaloids	–
Anthocyanins	–
Anthraquinones	–
Flavonoids	++
Coumarins	–
Anthracene derivatives	+
Lignans	–
Saponins	–
Condensed tannins	–
Hydrolyzable tannins	–
Triterpenes and steroids	–
Mono-, sesqui-, and diterpenes	+

(–) negative; (+) weakly positive; (++) moderately positive.

properties, including tannins, saponins, flavonoids, phenolic compounds, and terpenoids (BALEKAR et al., 2014). SOUZA-MOREIRA et al. (2010) have highlighted the importance of determining the chemical constituents of a plant and identifying the active compounds among them and their possible adverse effects.

BALEKAR et al. (2012) analyzed the ethanolic extract from the leaves of *S. trilobata* collected from the campus of the Prince of Songkla University in Thailand in February and reported that the total phenol content was 74.38 ± 1.03 mg/g, which was measured using the Folin–Ciocalteu method, and the total flavonoid content was 16.67 ± 0.74 mg/g in quercetin equivalents. Higher contents obtained in their study than those obtained in the present study are attributable to the solvent used in extraction and differences in climate and soil because the extracts were produced from plants collected from different locations.

Other studies have analyzed the chemical composition of the aerial parts of *S. trilobata* and identified the main chemical constituents of this species, such as kaurenoic acid, luteolin, flavonoids, tannins, and essential oils (CECHINEL FILHO, 2000; FIDELIS et al., 2005; SILVA et al., 2012). In addition to these, other compounds have been isolated from the flowers, such as stigmasterol and its glucosides, β-sitosterol, oleanolic acid ester derivatives (CARVALHO et al., 2001), phenolic compounds, flavonoids, and terpenoids (SHANKAR & TOMAS, 2014).

The presence of these compounds in the chemical composition of *S. trilobata* makes it a widely used species in folk medicine. Phenols and flavonoids are potent antioxidants (PRIOR & CAO, 2000; VIEIRA et al., 2015), and flavonoids also exhibit antimicrobial activity (RAJARATHINAM & DRONAMRAJU, 2018). Terpenes exhibit anti-nociceptive, anti-inflammatory, and antimicrobial properties (SARTORI, 2005), and anthracene derivatives, the presence of which has not been reported in this species to date, exhibit astringent activity (SOUSA et al., 2003).

In addition to phytochemical studies, advances have been made in research on pharmacological actions of plants exhibiting antimicrobial activity with an aim of obtaining novel compounds with biological activity. Although, the bactericidal properties of several species have been empirically acknowledged for centuries, these properties have started to be scientifically confirmed only in the last few decades (HAIDA et al., 2007).

One of the bacterial genera tested in the present study was *Staphylococcus* spp. The crude hydroalcoholic extract from the leaves of *S. trilobata* inhibited the growth of cultures isolated from human and dog skin. Similar results were observed in a study by SARTORI (2005) in which fractions of extracts from flowers of the same species inhibited the growth of *S. aureus*; that study evidenced that the hexane, dichloromethane, and butanol fractions of the extract exhibited antimicrobial activity with an MIC between 250 and 1,000 µg/mL. BALEKAR et al. (2012) observed that the ethyl acetate fraction of the ethanolic extract from leaves of *S. trilobata* was active against *S. aureus* and *S. epidermidis*, with an MIC of 62.5 and 31.25 µg/mL, respectively. SHANKAR & TOMAS (2014) tested the antimicrobial activity of various extracts from the flowers of *S. trilobata* against other bacterial species, such as *Vibrio parahaemolyticus*,

Table 2 - Minimum inhibitory concentration of crude hydroalcoholic extract from the leaves of *Sphagneticola trilobata* against bacterial cultures isolated from human and dog skin.

Bacterial cultures	Minimum inhibitory concentration
Human skin	
Staphylococcus aureus (ATCC 25923)	3,125 µg/mL
Staphylococcus epidermidis (ATCC 12228)	3,125 µg/mL
Staphylococcus spp. (MRSA 8536)	6,250 µg/mL
Staphylococcus spp. (MRSA 9606)	6,250 µg/mL
Escherichia coli (ATCC 35218)	12,500 µg/mL
Serratia marcescens (ATCC 13880)	12,500 µg/mL
Enterococcus faecalis (ATCC 19433)	6,250 µg/mL
----------Dog skin----------	
Staphylococcus spp. (244)	3,125 µg/mL
Staphylococcus spp. (246)	1,562.5 µg/mL
Staphylococcus spp. (250)	3,125 µg/mL
Staphylococcus spp. (256)	6,250 µg/mL

Streptococcus haemolyticus, *Proteus rettgeri*, and *P. vulgaris*, and concluded that the ethanolic extract from the flowers exhibited antimicrobial activity.

Other studies that reported the bactericidal activity of medicinal plants and importance of natural products can easily be reported in the literature, such as the study conducted by DUARTE (2006) who observed that extracts from *Mikania glomerata* and *M. laevigata* inhibited *S. aureus* and *S. faecium*, with MIC values between 0.04 and 0.1 mg/mL, which is similar to the MIC of chloramphenicol (0.12 mg/mL). PARENTE et al. (2009) observed that the ethanolic extract from the flowers of *Calendula officinalis* L. exhibits antimicrobial activity against *S. aureus.* Likewise, HAIDA et al. (2007) observed that the crude extract from the aerial parts of *Rosmarinus officinalis* exhibited inhibitory activity against *S. aureus*.

E. coli is an important bacterium that is commonly used to study the antimicrobial activity of phytotherapeutic products and was found to be inhibited by the crude hydroalcoholic extract from the leaves of *S. trilobata*. Other plants have been studied and found to exhibit this activity, such as various extracts from the aerial parts of *Bidens pilosa* and *Origanum majorana* (HAIDA et al., 2007). However, SILVA & ALMEIDA (2014) did not find the same result when they tested the crude ethanolic extract from the bark of *Carapa guianensis* Aubl.

E. faecalis was another bacterium tested in the present study with the crude hydroalcoholic extract from the leaves of *S. trilobata*, which exhibited an MIC of 6,250 µg/mL for this bacterial species. The same assessment was conducted by SOARES et al. (2008) using the crude hydroalcoholic extract from the dried bark of *Stryphnodendron adstringens*, with MIC values >400 µg/mL. Another studied bacterium in the present study was *S. marcescens*, which was inhibited with an MIC of 12,500 µg/mL. In another study conducted by NURTJAHJA et al. (2013), *S. marcescens* was shown to be susceptible to the methanolic extract from the leaves of *Callicarpa candicans*.

In the present study, in addition to the cultures that were inhibited, there were also some that exhibited resistance against the tested extract, namely *S.* Typhimurium, *K. pneumoniae*, and *P. aeruginosa*. According to BATISTA et al. (2011) and NURTJAHJA et al. (2013), plant products exhibit a stronger antimicrobial activity against Gram-positive bacteria, and the antimicrobial activity of the extracts may be attributed to the presence of secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolic compounds.

CONCLUSION

Results obtained in the present study indicated that the crude hydroalcoholic extract from the leaves of *S. trilobata*, which contains terpenes and flavonoids among its major secondary metabolites, exhibit antimicrobial activity against bacterial cultures isolated from human and dog skin.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

AGBL, LRME and JEN conceived and designed experiments. AGBL, ETNF, APO, REFA, MMC and JRGSA performed the experiments. AGBL carried out the lab analyses. AGBL and LRME performed statistical analyses of experimental data. AGBL and LRME prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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