Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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ORIGINAL ARTICLE

Evaluation of Biological Activity, Toxicity, and Phytochemical Content of *Bowdichia virgilioides* (*Fabaceae*) Aqueous Extract

Isabela Bacelar Assis, Eliana Maria Mauricio da Rocha, Daniel Silqueira Martins Guimarães, Glaécia Aparecida do Nascimento Pereira[1], Fabiana Paula Pereira, Jaqueline Maria Siqueira Ferreira, Emiliano O. Barreto[2]

Central-West Campus, Federal University of São João del-Rei, Divinopolis, MG, Brazil, [1]Centro de Pesquisas René Rachou,Oswaldo Cruz Foundation (FIOCRUZ), Belo Horizonte, Minas Gerais, Brazil, [2]Laboratory of Cell Biology, Federal University of Alagoas, Maceió, Brazil

Submitted: 26-06-2017 Revised: 09-08-2017 Published: 10-09-2018

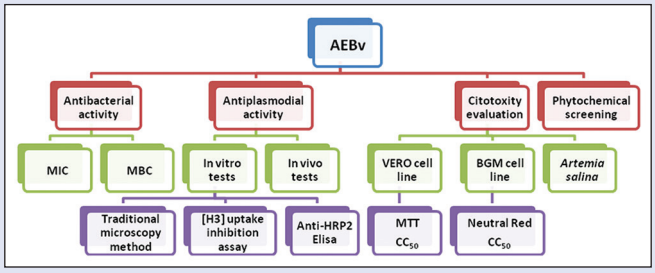
ABSTRACT

Background: Antibiotic resistance is a worldwide problem that poses a serious threat to human health, limiting the therapeutic options for bacterial infections. The spread of *falciparum*-resistant malaria is also concerning, making the patient treatment an extremely difficult task. Those facts have heightened the interest to find alternate options to treat infections caused by drug-resistant microorganisms. **Objective:** Considering the importance of the development of new substances with antibacterial and antimalarial properties, the present study aimed to investigate the activity of the aqueous extract of stem bark of *Bowdichia virgilioides* (AEBv). This plant is commonly used in Brazilian folk medicine to treat a wide range of illnesses, including signs and symptoms associated with malaria. **Materials and Methods:** The AEBv was assayed for toxicity against two cell lines and *Artemia salina* larvae. *In vitro* activity of the extract was screened against a panel of Gram-positive and Gram-negative bacteria, a chloroquine-resistant (W2) and a chloroquine-sensitive (3D7) *Plasmodium falciparum* strains. The extract was also tested as antimalarial *in vivo* against *Plasmodium berghei*. **Results:** The AEBv presented no significant toxicity and was found to exert *in vitro* growth inhibitory effect against the tested bacterial species. The lowest minimal inhibitory concentration was reported for *Staphylococcus aureus* (0.125 mg/ml) followed by *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* (0.50 mg/ml). *B. virgilioides* extract showed weak *in vitro* antimalarial activity against *P. falciparum*. A preliminary phytochemical analysis revealed the presence of flavonoids, phenolic groups, terpenoids, saponins, and tannins and the absence of alkaloids. **Conclusion:** The AEBv showed promising activity against Gram-positive microorganisms.

Key words: Antimalarial, antimicrobial, biological activity, *Bowdichia virgilioides*

SUMMARY

- Bowdichia virgilioides Kunth, Fabaceae, is a plant species commonly used as herbal medicine for the treatment of different health conditions.
- Antimicrobial and antimalarial properties of the aqueous extract of stem bark of B. virgilioides (AEBv) were evaluated.
- Potential anti Gram-positive microorganisms were reported.



Abbreviation used: AEBv: Aqueous extract of stem bark of Bowdichia virgilioides; FBS: fetal bovine serum; CC50: 50% cytotoxic concentration; LC_{50}: Median lethal concentration; ATCC: American Type Culture Collection; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentrations; CQR: Chloroquine resistant; CQS: Chloroquine-sensitive; HRP2 Histidine rich protein 2; ELISA: Enzyme linked immunosorbent assay; PBS T: Phosphate buffer saline with 0.05% Tween 20; ANOVA: Analysis of variance; TLC: Thin layer chromatography; Rf: Retention factor; SI: Selectivity índex; MRSA: Methicillin resistant *Staphylococcus aureus*.

Correspondence:

Dr. Eliana Maria Mauricio da Rocha,
Laboratory of Parasitology, Federal University of São João del-Rei, Divinopolis, MG, Brazi, CEP 35501-296, Divinópolis, Minas Gerais, Brazil.
E-mail: eliana.rocha@pq.cnpq.br

DOI: 10.4103/pm.pm_273_17

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INTRODUCTION

The resistance of different pathogens to available drugs is becoming increasingly dangerous. The ESKAPE pathogens, which is an acronym to describe a multi-resistant group of bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), are responsible for majority of nosocomial infections. Resistant microorganisms can provoke serious and potentially fatal infections in humans and cause financial difficulties for the public health systems.[1] Similarly, antimalarial drug resistance to commonly available medications, such as chloroquine and sulfadoxine-pyrimethamine, remains a major problem in malaria-affected areas. Patients with a delayed *Plasmodium falciparum* parasite clearance response to artemisinin, a potent antimalarial drug, have been reported in endemic areas around the world, which leads to increased morbidity and mortality from this disease.[2] These public health problems demand efforts to develop new therapeutic alternatives to overcome resistance and to prevent the spread of resistant microorganisms to uninfected individuals.[3,4]

Cite this article as: Assis IB, Mauricio da Rocha EM, Martins Guimarães DS, do Nascimento Pereira GA, Pereira FP, Siqueira Ferreira JM, *et al*. Evaluation of biological activity, toxicity, and phytochemical content of *Bowdichia virgilioides* (Fabaceae) aqueous extract. Phcog Mag 2018;14:S403-8.

One promising strategy for novel drug discovery is to search for plants used empirically in the prevention and treatment of infectious diseases. Ethnomedicinal information can be collected by documented or undocumented expertise from traditional healers and literature search. Indigenous knowledge, coupled with scientific research, can contribute to the discovery of new alternative treatments.[5] The interest in drugs derived from natural products has increased significantly because several marketed chemotherapeutic agents have chemical structures related to a molecule extracted from the plants used in popular medicine.[6] It should be taken into account that infectious disease transmission is recorded in the poorest countries, in tropical and subtropical regions. Drugs derived from medicinal plants are usually available and affordable to those who need it, increasing the chances of distribution, and adherence to therapy.[7]

Bowdichia virgilioides Kunth, *Fabaceae*, popularly known as "Sucupira Preta" in Brazil, is a plant species commonly used in traditional medicine to treat inflammation, wound healing, general pain, sore throat, rheumatism, arthritis, skin diseases, and fever.[8-10] This plant species is also used in folk phytotherapy against malaria in Brazil and Bolivia.[11,12] Experimental studies using this plant demonstrated its effectiveness as antidiabetic, antinociceptive, antimalarial, and anti-inflammatory and also as treatment to anxiety disorders.[8,13-15]

Different strategies are used to identify new alternative drugs for malaria, including the test of medicinal plants used by traditional health practitioners and the search among compounds that are potentially active against other diseases.[16] There are antibiotics used against plasmodia, particularly in combination with standard antimalarials to treat drug-resistant parasites.[17,18] Therefore, antimicrobial agents may be also active for malaria treatment and prophylaxis.[19,20]

Since antibactericidal and antimalarial multidrug resistance is a challenge to public health, the identification of new effective drugs remains as an important tool to the infectious diseases control effort.

MATERIALS AND METHODS

Plant material

The stem bark of *B. virgilioides* Kunth (Family *Fabaceae*) was collected at the Arboretum of the Federal University of Alagoas (Brazil), and the plant was identified by the taxonomic expert Rosângela P. Lyra Lemos. An authenticated voucher specimen was deposited (number MAC29914) at the environment institute (IMA) Herbarium, in Alagoas, Brazil.

Preparation of the extract

The stem barks of *B. virgilioides* were dried at room temperature in a light-protected area. Then, the dried product was triturated and 50 g of the plant material was infused into 300 mL of boiling distilled water for 20 min.[8,15] After filtration, the extract was concentrated, to remove the water using a rotary evaporator, and subsequently lyophilized. The yield of the infusion was 17.2%.

Cytotoxicity assay

The aqueous extract of stem bark of *B. virgilioides* (AEBv) was screened for its toxicity at different concentrations against two monkey kidney cell lines (renal cells from the African green monkey): Vero and BGM. Vero cells were cultured in Dulbecco's Modified Eagle Medium (Cultilab, Brazil) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich, Brazil) and 50 μg/mL gentamicin (Schering-Plough, EUA). BGM cell line was cultured in RPMI 1640 medium (Gibco, Brazil) supplemented with 5% FCS and 40 mg/L gentamicin. Both cell lines were kept at 37°C in a humid atmosphere containing 5% CO_2. After reaching 80%–90% confluence, the cell layers were collected by adding trypsin/ethylenediaminetetraacetic acid 0.25% (Gibco, Brazil). The cells were seeded and plated into 96-well plates at a density of 5×10^3 cells/well in 180 μL of culture medium containing 10% FBS and incubated at 37°C and 5% CO_2. After 16 h to permit their adhesion, the cells were exposed to the AEBv (20 μL) at various concentrations (3.9–1000 μg/mL) and incubated for 24 h, in triplicate.

The viability of the cells was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT)[21] for Vero cells and by the neutral red uptake assay for BGM cell line.[22] The optical density was measured at a wavelength of 540 nm in a microtiter plate reader (SpectraMa × 340 PC 384, Molecular Devices). Relative cell viability was expressed as a percentage relative to the absorbance of the untreated control wells (100% of absorbance).

The 50% cytotoxic concentration (CC_{50}) was defined as the extract concentration (mg/mL) required for the reduction of cell viability by 50%. The CC_{50} was obtained from nonlinear regression analysis of concentration–effect curve by plotting the logarithm of extract concentrations on the X axis and the corresponding % cell growth inhibition on the Y axis using Prism software (GraphPad 5.0). A sigmoid dose–response with variable slope regression curve was generated for determination of CC_{50} value.

Artemia salina lethality bioassay

The cytotoxicity of extracts was evaluated by *Artemia salina* lethality test, according to the procedure described previously.[23] *A. salina* (brine shrimp) eggs were incubated in artificial seawater (3.8%, w/v AquaSalt-Aqua One) under light at room temperature. After 24–36 h, the hatched nauplii were collected and used in bioassays conducted in 96-well microplates. In each assay, ten active shrimp nauplii were exposed to the AEBv in different concentrations (0.125; 0.250; 0.5; 1; 1.25; 1.5; 1.75; 2 mg/mL) prepared by diluting the extract in artificial seawater solution to obtain a final volume 250 μL/well.[23] After 24 h under artificial lighting, the number of surviving nauplii was counted and the percent (%) of lethality was calculated for each well. *Artemia* were considered dead if they did not exhibit any sign of internal or external movement during at least 30 s of observation. Each concentration, including positive control (thymol 0.01% Sigma-Aldrich) and negative control (artificial seawater), had three replicates.

The median lethal concentration (LC_{50}) was determined by linear regression method (probit regression) plotting the logarithm of the tested concentrations of the AEBv against the corresponding probit of *Artemi*a lethality percentage in the ordinate axis (Probit Software Ltd.). The intercept on the abscissa corresponding to probit 5 yields the LD_{50}.

Bacterial samples

The microorganisms employed in the study originated from the American Type Culture Collection (ATCC) and were kindly provided by the Reference Microorganisms Laboratory of the Oswaldo Cruz Foundation (FIOCRUZ, Brazil). Antibacterial tests were conducted with nine strains, which included five Gram-positive (*S. aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus saprophyticus* ATCC 15305, *Streptococcus mutans* ATCC 25175, and *Streptococcus agalactiae* ATCC 13813) and four Gram-negative bacteria species (*K. pneumoniae* ATCC 4352, *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 25619, and *Salmonella typhi* ATCC 19430). All bacteria were stored at −80°C in glycerinated (25%) nutrient broth (Lab Impex®, HiMedia®, India) until used.

Antibacterial assay

The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of the AEBv were determined. The

extract was serially diluted into five concentrations (0.125–2 mg/mL) and tested against each bacterium strain, except for *S. aureus*, which was assayed against six concentrations (0.0625–2 mg/mL).

The MIC of the AEBv was determined for each microorganism using the microdilution method, according to the document M07-A9 of the Clinical and Laboratory Standards Institute guidelines.[24] Different concentrations of the extract were distributed in 96-well plate in contact with bacterial suspension. The microorganisms' inocula were adjusted to match 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml). Each microplate included the following as quality controls: bacterial suspension without antimicrobial or extract treatment, medium only, and bacteria strains incubated with penicillin (10,000 U/mL) or streptomycin (10 mg/mL) as positive controls. The microplates were incubated for 24 h at 37°C. MIC is defined as the lowest concentration of an antimicrobial agent that prevented visible bacterial growth. All the experiments were performed in triplicate and in three independent assays.

MBC was determined by inoculating Muller-Hinton (HiMedia®, India) agar plates with 25 µL of samples taken from the wells that showed no apparent bacterial growth on MIC. The plates were further incubated at 37°C for 24 h. The MBC is considered the lower concentration on the plate presenting no bacterial growth indicating bactericidal activity. The MBC_{50} and MBC_{90} were the concentrations of the AEBv that resulted in 50% and 90% killing of the microorganism relative to the concentration of the bacteria that were present in test wells at 0 h. All the experiments were performed in triplicate and in three independent assays.

In vitro antiplasmodial activity

The human malarial parasites *P. falciparum* species were kept in continuous culture in human erythrocytes in medium supplemented with human plasma according to the method described by Trager and Jensen.[25] Plates were maintained in desiccators at 37°C in an atmosphere of 3%–5% of CO_2. Chloroquine-resistant (CQR) *P. falciparum* W2 and Chloroquine sensitive (CQS) 3D7 strains were used for the *in vitro* antiplasmodial studies. The tests were conducted on three different techniques at the AEBv concentrations ranging from 0.09 to 50 µg/mL.

In the traditional microscopic method, extract testing was performed in 96-well microtiter plates with cultures mostly at ring stages at 1% parasitemia.[26] The parasites were distributed in the wells and grown *in vitro* in the presence of the AEBv for 48 h. After this period, thin blood smears of each well were stained with Giemsa and parasitemia was quantified under microscopy. Negative controls were prepared with a suspension of intraerythrocytic parasites kept in the culture medium. Chloroquine was used as positive control. The anti-*P. falciparum* AEBv activity was measured by comparing parasite growth in treated wells with that in the extract-free control cultures.

The hypoxanthine uptake inhibition assay was conducted using ring-stage parasites cultured in 96-well microplate at 1% parasitemia and 1% hematocrit.[27] AEBv was added to the wells in increasing concentrations (0.09–50 µg/mL). The negative control without extract and the positive control treated with chloroquine were run in parallel. After an incubation period of 24 h, 20 µL of medium containing (^{3}H) hypoxanthine was added to each well, followed by incubation for 18 h at 37°C. The plates were frozen and thawed to lyse the red blood cells, and the content of each well was aspirated using a cell harvester (Harvester 96 Mach III, TomTec Imaging Systems GmbH, Unterschleissheim, Germany) on filter papers (Perkin Elmer). After drying the filters on microwave oven, they were drenched in 4 ml of scintillation fluid. Radioactive emission was counted using a 1450 MicroBeta Reader (Perkin Elmer). The results were recorded as counts per minute (cpm) per well at each extract concentration. The inhibition of parasite growth was evaluated by comparing the [^{3}H] hypoxanthine incorporation in drug-free control cultures.

In histidine-rich protein-2 (HRP2) enzyme-linked immunosorbent assay (ELISA),[28] *P. falciparum* cultures (0.05% parasitemia and 1.5% hematocrit) were distributed in 96-well microplates and incubated in the presence of different concentrations of AEBv and the control-positive drug (chloroquine). The control-negative wells contained only parasites in culture medium. After 24 h, the contents of six control drug-free medium wells were harvested and frozen for later use to exclude the background value. After 72 h incubation of the parasites in the presence of the AEBv, the plates were frozen and thawed twice to lyse the erythrocytes. Hemolysed cultures (100 µL) were placed in an ELISA plate previously coated with primary antibody anti-HRP2 (MPFM-55A ICLLAB®, USA) and incubated 1 h at room temperature. Following washing with phosphate buffer saline with 0.05% Tween-20 (PBS-T), the plates were incubated with the secondary antibody (MPFG-55P ICLLAB®, USA) and incubated for 1 h at room temperature. Wells were washed again with PBS-T, and the final reaction was initiated by incubating with 3,3',5,5'-tetramethylbenzidine chromogen in the dark for 10 min. The reaction was stopped by adding 50 µl/well of 1M sulfuric acid to each well. The absorbance was read at 450 nm (Spectra Max 340PC384, Molecular Devices). All antiplasmodial assays were performed in triplicates.

For data analyses of the *in vitro* antiplasmodial activity of the extract, analysis of variance (ANOVA) was performed using GraphPad 5.0 Prism program (GraphPad Software, Inc., San Diego, CA, USA). Data were considered statistically significant at $P < 0.05$.

In vivo antiplasmodial activity

The experiments involving the use of laboratory animals in this study were approved by the Ethics Committee for Animal Use of the Oswaldo Cruz Foundation (FIOCRUZ). Swiss mice (20 g ± 2 g body weight) were inoculated by the intraperitoneal route with 1×10^6 infected erythrocytes with *Plasmodium berghei* NK65 strain. After 24 h, the animals were randomly divided into groups of five mice per cage and treated orally with the AEBv (100 and 200 mg/kg), the control drug (chloroquine), or with the vehicle used for dissolving the extract, for 3 consecutive days. Blood smears were prepared from the tail of each mouse on days 5 and 7, fixed with methanol, stained with Giemsa, and then examined by light microscopy to estimate parasitemia. Results were expressed as means ± standard error of the mean. Comparisons of blood parasitemia and mouse survival time in treated and untreated mice were analyzed statistically using one-way ANOVA. $P < 0.05$ was considered statistically significant.

Phytochemical investigation

The AEBv was analyzed for the presence of alkaloids, flavonoids, phenolic compounds, anthraquinones, and terpenes. Qualitative phytochemical screening was conducted by thin-layer chromatography (TLC) using as stationary phase activated silica gel, according to the standard methods.[29,30] The AEBv was dissolved in methanol, applied on a precoated plate (silica gel GF254 Merck®), and developed in different solvent systems as mobile phase. After the separation of phytochemicals, specific spray reagents were used. Dragendorff solution was used to reveal alkaloids and NP/PEG (1% diphenylboriloxyethylamine in methanol p/v, 5% polyethylene glycol 4000 in ethanol p/v) to flavonoids and phenolic compounds. Anthraquinones were visualized by spraying with 10% KOH in ethanol, and terpenes were revealed using vanillin-sulfuric acid reagent.[29] Visualization was performed under UV light (UVP Model UVGL-25 Compact Split Tube UV Lamp, 254/365 nm wavelength). The presence of the phytochemical groups was investigated by comparison with the standards. The retention factor (Rf) of each standard was compared with spots exhibited by the AEBv sample.

To test the presence of saponins, the AEBv was vigorously stirred with water in a tube for approximately 20 min. Persistent foam, compared to a

standard (Saponin Weiss Rein; Merck, Darmstadt, Germany), indicated the presence of saponins.[31]

The AEBv was also treated with 5% ferric chloride and the development of a dark bluish gray color indicated the presence of tannins. Precipitation reaction with aqueous solution of gelatin (1%) and sodium chloride (10%) was also performed to verify the presence of tannin.[32]

RESULTS

Cytotoxic activity of the aqueous extract of stem bark of *Bowdichia virgilioides* on Vero and BGM cell lines

The AEBv exhibited no significant toxicity based on the high CC_{50} values observed: 1.8 mg/mL for Vero cells and >1.0 mg/mL for BGM cells.[33,34]

Toxicity against the *Artemia salina* leach

The LC_{50} recorded in TAS bioassay was 1.69 ± 0.1 mg/mL. Extracts that have LC_{50} ≥1.0 mg/mL are accepted as nontoxic.[35]

Antibacterial assay

The AEBv showed different degrees of inhibition against the nine bacterial strains tested [Table 1]. The lowest MIC (0.125 mg/mL) was obtained with the extract on *S. aureus* strain, followed by MIC values of 0.50 mg/ml obtained against *S. epidermidis* and *S. saprophyticus*. The bactericidal (MBC) AEBv effect was observed at the highest concentration against *S. epidermidis* and *S. mutans* [Table 1]. The data of bacterial sensitivity to the positive control, along with their IC_{50} values (concentration required to inhibit 50% of bacterial growth), are shown in Table 1.

The selectivity indices (SIs), defined as the ratio of the CC_{50} to the IC_{50} (SI = CC_{50} for Vero cells/IC_{50}), were determined. The AEBv displayed *in vitro* antibacterial SI from 5 to 60, with SI values for *S. aureus* (SI = 60) higher than those of *E. coli* and *S. mutans* strains (SI < 10).

Antimalarial activity

The results from the *in vitro* tests showed poor antiplasmodial activity of the AEBv against both *P. falciparum*-resistant (W2) and chloroquine-sensitive (3D7) strains. The extract at all tested doses did not significantly inhibit parasitemia relative to negative control ($P > 0.05$). IC_{50} value was ≥50 µg/mL. Based on the literature, extracts demonstrating IC_{50} values of >50 µg/ml were considered weak.[36,37]

No antiplasmodial activity was demonstrated *in vivo* against *P. berghei*. The extract tested at 100 and 200 mg/kg/day did not significantly decrease parasitemia or increase survival time of the infected mice compared to untreated control group.

Phytochemical screening

Phytochemical screening of AEBv revealed the presence of flavonoids, phenols, terpenes, tannins, and saponins. No alkaloids and anthraquinones were detected.

DISCUSSION

B. virgilioides is used in Brazilian folk medicine to treat diseases such as inflammation, wound healing, general pain, back pain, and sore throat, among others.[8-10,15] Scientific evidence of *B. virgilioides* anti-inflammatory effectiveness correlates with its traditional use by the population.[38,39]

The plant extract showed no cytotoxic effect when tested *in vitro* toward two cell lines and also when evaluated using the *A. salina* lethality bioassay. These results are consistent with toxicity information from previous studies that reported no acute *in vivo* toxicity of AEBv.[15]

The *in vitro* evaluation of the antimicrobial activities of AEBv demonstrated that Gram-positive bacteria were more susceptible when compared to Gram-negative bacteria. The outer membrane, a characteristic found only in the cell wall of Gram-negative bacteria, functions as a selective barrier that influences its sensitivity to many types of compounds.[40]

S. aureus was the bacterial isolate most inhibited by the AEBv, presenting satisfactory SI value. Previous investigation showed wound healing activity in mice infected with *S. aureus* and treated with the AEBv.[41] The same authors showed antimicrobial potential of the AEBv against methicillin-resistant *S. aureus* (MRSA). In the United States, MRSA mortality rates are higher than the rates of HIV/AIDS and tuberculosis deaths combined.[42,43] MRSA-colonized American hospital-associated settings are variable, but reports indicate that up to 85% of patients are infected with *S. aureus* antibiotic-resistant strains.[44]

In Brazil, a study carried out in hospitals monitored by the SENTRY Antimicrobial Surveillance Program, from 2005 to 2008, showed that *S. aureus* was the major cause of bacteremia, skin, and soft-skin infections.[45] It is also the second most common causative pathogen of nosocomial pneumonia. The worrisome data presented in the same study indicate that 31.0% of the isolates were MRSA.

Similar to *S. aureus*, *S. epidermidis* is an important commensal inhabitant of the human skin and mucosal surfaces. Despite usually innocuous, *S. epidermidis* is a potential pathogen that can cause opportunistic infections and it is the most frequent cause of nosocomial infections, including

Table 1: Antibacterial activity of the aqueous extract of *Bowdichia virgilioides*

Bacteria	Aqueous extract of *Bowdichia virgilioides*				Positive control[a]	
	MIC (mg/mL)	MBC (mg/mL)	IC_{50} (mg/mL)	SI[b]	MIC (mg/mL)	MBC (mg/mL)
Gram-positive						
Staphylococcus epidermidis	0.50	2	0.07	26	0.125	0.250
Staphylococcus aureus	0.125	>2	0.03	60	0.007	0.125
Staphylococcus saprophyticus	0.50	>2	0.12	15	0.0625	0.125
Streptococcus mutans	1	2	0.21	9	0.007	0.007
Streptococcus agalactiae	>2	>2	-	-	0.125	-
Gram-negative						
Klebsiella pneumoniae	1	>2	0.17	11	0.015	0.015
Escherichia coli	2	>2	0.36	5	0.125	0.125
Pseudomonas aeruginosa	>2	>2	-	-	0.0625	-
Salmonella typhi	>2	>2	-	-	0.125	-

[a]MIC and MBC values to the reference antibiotic (penicillin + streptomycin solution); [b]SI: Selectivity index values of compounds against bacterial pathogens.
MIC: Minimal inhibitory concentration; MBC: Minimum bactericidal concentrations; SIs: Selectivity indices

formation of biofilms on implanted medical devices. Treatment of these conditions has been complicated by the emergence of antibiotic-resistant strains.[46,47]

Plants with antimicrobial properties are rich in tannins, catechins, alkaloids, steroids, flavones (flavonoids, flavonols, quinones), essential oils, lectins, polypeptides, phenolics, polyphenols, and terpenoids.[48,49] Phytochemical analysis of the AEBv in this study identified the presence of some of these compounds (flavonoids, phenolic groups, terpenes, and tannins), suggesting that the antimicrobial activity of the extract is related to the presence of one of these classes or on the synergistic interactions among the components. Previous report also revealed the same classes of phytoconstituents in the AEBv.[50]

B. virgilioides has been used to treat malaria as a folk medicine, and a group of researchers found antimalarial efficacy using stem bark ethanol extract of *B. virgilioides*.[12] However, the aqueous extract of *B. virgilioides* demonstrated to be unpromising as antimalarial. The lack of antiplasmodial activity may be due to the absence of alkaloids in the AEBv. It is known that alkaloids are the main class of natural products from plants responsible for the antimalarial effect. Quinine, the first documented antimalarial, is an alkaloid isolated from natural sources. Thenceforth, over 100 natural alkaloids from higher plants with significant antiplasmodial activity were published.[51,52]

The extraction method is an important step to separate the soluble plant metabolites, therefore determining phytochemical constituents of the plant. The difference concerning the solvent, water or ethanol, may have influenced the chemical composition of the extract qualitatively and/or quantitatively. Five types of alkaloids of the ormosanine and homoormosanine type have already been described in methanolic extract from the stem bark of the Colombian *B. virgilioides*.[53] The results suggest that the form of preparation of the extract altered its biological activity and that the active principles responsible for antimalarial activity are probably better obtained with an organic solvent.

Chemical composition of an herbal preparation is also influenced by a number of environmental factors, including its geographical distribution, atmospheric conditions, soil characteristics, harvesting, and storage conditions. In addition, genetic variations may also impact secondary metabolites production.[54] These aspects might result in different plant-derived natural products synthesis and consequently its bioactivity.

CONCLUSION

The active components of AEBv may provide a new valuable option in the treatment of bacteria of clinical interest. As for the antimalarial potential, although the results obtained with the aqueous extract showed weak anti-*Plasmodium* activity, it is worth investing in further studies on this species, considering its nontoxic characteristic.

The present paper suggests that *B. virgilioides* could be suitable for the treatment of infections caused by *S. aureus* without being toxic. Further chemical and pharmacological investigations are necessary for the isolation and identification of the potential active compound(s).

Acknowledgements

We are grateful to Flávia M. da Rocha Fontes for the English translation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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