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Full Length Research Paper

Antioxidant and antibacterial activity of leaves and stem extracts of *Bridelia cathartica* Bertol

Paulo Cumbane[1,2] and François Munyemana[1*]

[1]Department of Chemistry, Faculty of Science, Eduardo Mondlane University, Av. Julius Nyerere Nr 3453, P. O. Box 257, Maputo, Mozambique.
[2]Higher Institute of Science and Technology of Mozambique (ISCTEM), School of Health, Pharmacy Course, Rua 1394-Zona da FACIM, 322-Maputo, Mozambique.

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The *Bridelia cathartica* Bertol (Euphorbiaceae) species is used in Mozambican folk medicine for the treatment of various ailments including infectious and inflammatory diseases, but scientific investigations aiming to determine its therapeutic potential are scarce. In the present study, the total phenolics, antioxidant and antibacterial activities of the crude hydroethanolic extracts and ethyl acetate fractions of the leaves and stem of *B. cathartica* were determined using standard procedures. The phenolics found did not show significant variations in the different analyzed samples ($p < 0.05$) and showed maximum values of 435.090 ± 15.507 and 436.970 ± 15.310 milligrams of gallic acid equivalents (mgGAE) per gram of the dry extract for the samples of stem and leaves respectively. The highest antioxidant activity was exhibited by the leaves sample in the DPPH method (EC50 = 3.63 ± 0.12 µg/mL) and phosphomolybdenum complex method (57.88 ± 0.57%). Most of the extracts and fractions from leaves and stem exhibited remarkable antibacterial activity with predominance of minimum inhibitory concentration (MIC) values between 250 - 500 µg/mL. The results found in this study reveal high levels of phenolic compounds as well as a potent antioxidant effect and considerable antibacterial activity, which may justify the use of this plant in traditional medicine for the treatment of various infectious and inflammatory diseases.

Key words: Total phenolics, antibacterial, DPPH, *Bridelia cathartica*, extracts antioxidant.

INTRODUCTION

The history of the development of human societies has always been intimately linked to people's use of the natural resources at their disposal. It was from these resources that different people, through the observation of animals and experimentation, through the method of trial and error, were selecting useful plants for their diverse needs (Cunha, 2005). Therefore, the search for relief and cure of diseases by ingestion of herbs and leaves may have been one of the first purpose for using natural products (Viegas et al., 2006). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Vashvaei et al., 2015). The extracts and essential oils of many herbs have been shown to exert biological activity *in vitro* and *in vivo*, which justifies research on traditional medicine focused on the characterization of their antimicrobial activity. The therapeutic benefit of plants is often attributed to their antimicrobial and antioxidant properties (Burt, 2004; Jafari et al., 2014; Keykavousi et al., 2016). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Bajpai et al., 2005; Vashvaei et al., 2015). Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human

*Corresponding author. E-mail: munyfranc@yahoo.co.uk or francoism@uem.mz. Tel.:+258823175440.

diseases (Anderson et al. 2001; Djeridane et al., 2006). Natural phenolic compounds are widespread in the plant kingdom, and are the plant secondary substances that are synthesized in response to stressful condition to protect plant against oxidative and bacterial aggression (Nitiema et al., 2012). Polyphenols exhibit a wide range of physiological properties, such as anti-inflammatory, antimicrobial and antioxidant effects (Balasundram et al., 2006; Abah, 2010; Nitiema et al., 2012).

Plant preparations are used in traditional African medicine to treat an endless list of diseases, which has attracted the attention of researchers in the ongoing search for detailed information on the activity and toxicity of different plants used in these preparations. Various extracts prepared from selected plants based on information provided by practitioners of traditional medicine are or have been tested against various models of bioactivities. Studies on a variety of isolated secondary metabolites showed that most of them possess therapeutic properties. Some medicinal species used in Mozambican traditional medicine demonstrated biological activities including antioxidant, antimicrobial, antitumor and anti-inflammatory. However, most of them do not have deepened studies on such properties.

Plants of genus *Bridelia* are used throughout Africa and Asia in traditional medicine to treat various ailments such as antiamebic, antianemic, antibacterial, antidiabetic, antidiarrhoeal, antihelmintic, anti-inflammatory, antimalarial, antiviral, hypoglycemic, for abdominal pain, cardiovascular, gynecological and sexual diseases among others (Ngueyem et al., 2009). Ethnobotanical, phytochemical, biological and toxicological studies have been reported to support the indigenous knowledge (Ngueyem et al., 2009). Okeleye et al. (2011) reported the presence of flavonoids, steroids, tannins, alkaloids and saponins in the stem bark of *Bridelia micrantha*, and linked those phytochemical compounds to its antimicrobial activity. Mburu et al. (2016) confirmed the presence of the same phytochemical compounds through determination of functional groups using FT-IR spectroscopy and through quantitative analysis using UV-Vis spectroscopy determined the total phenolics and flavonoids, saponin and alkaloid contents. Total phenolics and flavonoids were found highest in barks while saponin content was found highest in leaves and alkaloid content on the other hand was found the highest in roots. He also linked those phytochemicals to the antimalarial activity exhibited by the plant. Ngueyem et al. (2009) revealed that bark and leaves of Bridelia species are particularly rich in phenolic compounds. Adefuye et al. (2011), Adefuye and Ndip (2013) in their studies, showed that extracts of the stem bark of *B. micrantha* revealed antimicrobial activity against *Staphylococcus aureus*, *Shigella sonnei*, *Salmonella typhimurium* and *Helicobacter pylori*, and showed through phytochemical analysis and antibacterial evaluation that ethyl acetate extract of the stem bark possess potent bioactive phytocompounds that may be developed into new antimicrobials.

B. cathartica Bertol (Euphorbiaceae), a species traditionally known as "Munuangati or Thlanthangati" is used in Mozambican folk medicine for the treatment of various diseases, but scientific investigations aiming to determine its medicinal potentialities are still rare.

Previous studies on bioactivity of *B. cathartica* reported its antimalarial activity (Jurg et al., 1991; Ramalhete et al., 2008), and the identification and quantification of iron levels in different parts, justifying its use in traditional medicine for the treatment of anemia (Omolo et al., 1997). The recent studies of Madureira et al. (2012) on *B. cathartica*, led to the detection of some secondary metabolites of major pharmacological interest and highlighted the antibacterial activity of the root extracts against gram positive and gram negative strains. The present study was focused on the determination of total phenolics, total flavonoids, hydrolysable and condensed tannins as well as the screening of the antibacterial and antioxidant activities of the crude hydroethanolic extracts and the respective ethyl acetate fractions of the stem and leaves of *B. cathartica* Bertol.

MATERIALS AND METHODS

Plant material

The fresh plant material (*B. cathartica*) used in the present study was collected in the Marracuene District, in the dunes of Macaneta beach, Maputo Province and the species was authenticated at the Herbarium unit, Department of Biological Sciences - Faculty of Science of Eduardo Mondlane University, by comparing with an existing specimen with voucher No. 1772. The leaves and roots were dried, pulverized and stored at room temperature until the preparation of the extracts.

Preparation of plant extracts

The extraction was carried out using the method described by Djeridane et al. (2006) with some modifications.

50 grams of powdered stem and leaves of *B. cathartica* were extracted with 500 mL of 70% ethanol at 50°C for 5 days with renewal of the solvent every day (24 h). The resulting extracts were combined and the resulting extract filtered by suction with milipore membrane and divided into two portions (A and B). The 'A' portion was subjected to concentration under reduced pressure at 50°C to dryness in a rotary evaporator.

The 'B' portion of the extract was concentrated to ¼ volumes and the resulting extract was partitioned with n-Hexane in a 500 mL separatory funnel (5x200 mL) for removal of lipids. The aqueous fraction was acidified with 2% (m / v) metaphosphoric acid solution in the presence

of 20% ammonium sulfate to pH 5, then transferred to a 500 mL separatory funnel and partitioned with ethyl acetate (10x200 mL). The resulting organic phase (Ethyl acetate) was treated with an aliquot of anhydrous sodium sulfate (to remove traces of water), filtered with Whatman™ filter paper and concentrated to dryness under reduced pressure. The dried extracts were stored in a desiccator.

Determination of the total phenolic contents

The total phenolic content was determined using the classic Folin–Ciocalteu colorimetric method as described by Amorim et al. (2012) with some modifications. Methanolic solution of the extract in the concentration of 1mg/mL was used in the analysis. 500 µL of 10% Folin-Ciocalteu reagent was added to 500 µL of methanolic solution of extract. After 5 min, 1 mL of 7.5% sodium carbonate and 8 mL of distilled water were added and the mixture was allowed to stand at room temperature for 120 min. The absorbance of the mixture was then measured at 760 nm. Standard calibration curve (R^2 =0.998) for gallic acid in the range of 0 – 5 mg/L was prepared as described by Waterhouse (2012), and the results were expressed as milligram of gallic acid equivalents per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate and values were means ± SD.

Determination of the total flavonoid contents

The total flavonoid content of the extracts was determined using the aluminium chloride colorimetric method as described by Amorim et al. (2012) with some modifications. 500 µL of methanolic solution of extract (1 mg/mL in methanol) was transferred to a test tube. 500 µL of the acetic acid solution in methanol (60% v/v), 2 mL of the pyridine solution in methanol (20% v/v), 1 ml of the aluminium chloride solution in ethanol (5% m/v) and 6 ml of methanol 80% were added. The mixture was left to stand at room temperature for 30 min. The absorbance was measured at 420 nm with a spectrophotometer UV-Vis Shimazu 1601. Standard calibration curve (R^2 = 0.993) for rutin in the range of 0 – 12 µg/mL was prepared as described by Amorim et al. (2012) and the results were expressed as milligrams of rutin equivalent per gram of dry weight of extract (mg of RE/g of extract). All samples were analyzed in triplicate and values were means ± SD.

Determination of condensed tannins: Cyanidin equivalent

Proanthocyanidin content was determined with a Butanol/HCl test as described by Zemmouri et al. (2014). 500 µL of methanolic solution of extract (1 mg/mL in methanol) was added to 5 mL of an acidic ferrous sulphate solution (77 mg of $FeSO_4.7H_2O$ in 500 mL of HCl/Butanol (2/3)). The tube was covered and placed in a water bath at 95°C for 15 min. The absorbance was read at 530 nm with a spectrophotometer UV-Vis Shimazu 1601 and results were expressed as milligrams of cyanidin equivalent per gram of dry extract (mg CyaE/g). All measures have been done in triplicate and values were means ± SD. The condensed tannins content was calculated using the formula given below in the equation (1):

$$\text{mg CyaE/g dry extract} = [A \cdot V \cdot M \cdot V2] / [l \cdot \varepsilon \cdot v \cdot m] \quad (1)$$

where A is the absorbance of the sample at 530 nm; V is the total volume of the reaction (7 mL); M is the molar mass (g / mol) of cyanidin-3-glucoside; V2 is the total volume of the extract solution (25 mL); l is the width of the cuvette (cm); ε is the molar extinction coefficient (34.700 L/mol. cm); v is the volume of the extract solution used in the assay (2 mL) and m is the mass of dry weight of extract (0,025 g).

Determination of hydrolysable tannins contents

Hydrolysable tannins content was determined with potassium iodate test method described by Zemmouri et al. (2014). 5 ml of aqueous solution of KIO_3 (2.5% m/v) was heated for 7 min at 30°C, then 1 mL of the extract solution (1 mg/mL in methanol), was added. The mixture was then incubated at 30°C in a water bath for 2 min. The absorbance was read at 550 nm on a Shimazu 1601 UV / Vis spectrophotometer. Standard calibration curve (R^2 =0.992) for tannic acid in the range of 0 – 5 µg/mL was prepared as described by Amorim et al. (2012) and the results were expressed as milligrams of tannic acid equivalents per gram of dry extract (mg TAE/g). All samples were analyzed in triplicate.

Evaluation of total antioxidant capacity

Phosphomolybdenum method

The total antioxidant capacity of samples was determined using the phosphomolybdenum method as described by Prieto et al. (1999) with some modifications. An aliquot of 500 µL of sample solution (extract solution 500 µg/mL in methanol or quercetin solution 500 µg/mL in methanol) was combined in a test tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the

aqueous solution of each was measured at 695 nm against a blank. The total antioxidant activity results were the ratio between absorbance of the extracts under study and the absorbance of the positive control (quercetin). The results are expressed as percentage (Souza, 2008). All samples were analyzed in triplicate.

DPPH radical scavenging assay

The antioxidant activity of the extract was measured with the DPPH method. A solution of DPPH was freshly prepared by dissolving 6 mg DPPH in 50 mL methanol (about 0.3 mM) (Do et al., 2014). 5 mL of a freshly prepared DPPH solution were treated with varying volumes (40 - 600 µL) of samples (extracts solutions or quercetin) at a concentration of 1 mg / mL in 10 mL volumetric flasks. The volumes were adjusted to 10 mL with methanol and the final concentrations ranged from 2 - 30 µg / mL, incubated for 20 min at room temperature. The absorbance was measured at 517 nm and the data used to determine the percent inhibition using equation 2. The control solution of DPPH was prepared from 5 mL of the initial solution (300 µM) and 5 mL of methanol and its absorbance was also measured at 517 nm using methanol as a blank. The inhibition percentages obtained from the different dilutions of the extracts calculated by equation 2 were used to determine the IC_{50} values in the analyzed samples. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50% (Do et al., 2014).The low value of IC_{50} is indicative of high antioxidant capacity of the sample (Zemmouri et al., 2014). All tests were performed at least in triplicate, and graphs were plotted using the average of three determinations.

% Inhibition= [(Acontrol – Asample) / Acontrol] x 100 (2)

where $A_{control}$ is the absorbance of DPPH solution without extract and A_{sample} is the absorbance of sample with DPPH solution.

Determination of the antibacterial activity by the disc diffusion method

The antimicrobial activity of the extracts was evaluated by the disc diffusion method, following the methodology described by Klančnik et al. (2010) and Farjana et al. (2014) with some modifications. In Brief, five bacterial strains (gram negative and gram positive) were used, namely, *Escherichia coli* ATCC 25922 (gram negative), *Pseudomonas aeruginosa* ATCC 27853 (gram negative), *S. aureus* ATCC 29213 (gram positive), *Enterococcus faecalis* ATCC 29212 (gram positive) and *Streptococcus pneumoniae* ATCC 49619 (gram positive). Microbial strains were obtained from the Microbiology Laboratory of Maputo Central Hospital (courtesy of Dr. Calvina Langa), and were spiked in the nutrient broth contained in test tubes and incubated at 37ºC for 24 h, standardized for turbidity of 0.5 on the Mcfarland scale in 0.9% saline solution by visual comparison with standard tube containing a standard suspension of barium sulphate equivalent to a suspension of *E. coli* containing 1.5×10^8 cells / mL. For the test, 500 µL of the suspension of each microorganism was uniformly spread on a Petri dish containing Muller Hinton agar using a sterile swab. Four 6 mm diameter sterile paper disks were placed and impregnated with 10 µL of each sample at concentrations ranging from 50 µg to 2 mg. Dimethylsulfoxide (DMSO) was used as a negative control. 5 µg ciprofloxacin or 10 µg gentamycin were used as positive controls. Plates were incubated in a bacteriological oven at 37°C for 24 h. All assays were done in 4 replicates and the minimum inhibitory concentration determined to be the minimum concentration capable of causing a halo (mm) greater than or equal to 8 mm. The criterion adopted for the classification of activity is the proposed by Holetz et al. (2002).

Statistical analysis

All the experiments were replicated three times to evaluate the statistical quality of the results. The data were expressed as means ± SD values. The treatment of the results for experimental designs was carried out using software *GraphPad Prism 7.0*. The polyphenolic content and the condensed tannins content were compared using ANOVA test, and Student test and the difference of mean test used when required. All the statistical analyses were carried out at P values < 0.05 significance level.

RESULTS AND DISCUSSION

Polyphenolic contents

The results of the total phenolics, total flavonoids and tannins content of the leaves and stem extracts of *B.cathartica* are shown in Table 1.

The total phenolics were measured by the Folin-Ciocalteu method and their contents obtained through the calibration curve Y = 0.00483X + 0.0693 (R^2 = 0.998), where Y is the absorbance and X is the concentration of gallic acid solution in mg/L and expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE / g DE). No significant differences were found between the phenolic contents of the stem and leaves samples of *B. cathartica* ($p < 0.05$). The partitioning of the crude extracts with ethyl acetate does not exert a significant influence on the phenolic variation in the extracts of the leaves and the stem.

Table 1. Total phenolics, flavonoids, hidrolysable tannins and condensed tannins content in the hydroethanolic extract and ethyl acetate fraction of *Bridelia cathartica* Bertol.

Samples	Total phenolics (mg GAE/g DE)	Flavonoids (mg RE/g DE)	Hydrolysable tannins (mg TAE/g DE)	Condensed tannins (mg C-3-GE/g DE)
CHES	429.114 ± 5.975[a]	0.495 ± 0.077[a]	1.431 ± 0.085[a]	35.814 ± 0.351[a]
CHEL	427.527 ±10.406[a]	5.187 ± 0.096[b]	1.507 ± 0.085[a]	48.020 ± 0.062[b]
EAFS	435.090 ± 15.507[a]	4.433 ± 0.057[c]	1.943 ± 0.069[b]	34.903 ± 0.432[a]
EAFL	436.970 ± 15.310[a]	25.531± 0.106[d]	2.688 ± 0.100[c]	65.296± 0.577[c]

Legend: mg GAE/g DE: milligrams of gallic acid equivalents per gram of dry extract; mg RE/g DE: milligrams of rutin equivalents per gram of dry extract; mg TAE/g DE: milligrams of tannic acid equivalents per gram of dry extract; mg C-3-GE/g DE: milligrams of cyanidin 3-glucoside equivalents per gram of dry extract.
CHEL: crude hydroethanolic extract of Leaves; CHES: crude hydroethanolic extract of stem; EAFL: Ethyl acetate fraction of leaves; EAFS: ethyl acetate fraction of stem.
Means followed by the same letter in each column are not significantly different ($p < 0.05$).

The total flavonoids were measured by the aluminum chloride complexation method and their contents quantified through the calibration curve Y = 0.02188X + 0.03712 (R^2 = 0.993), where Y is the absorbance and X is the concentration of the rutin solution in mg / L and were expressed in milligrams of rutin equivalents per gram of dry extract (mg RE/g DE).
Significant differences in flavonoids content were found in the different analyzed samples ($p < 0.05$). The highest value was found for the ethyl acetate fraction of the leaves (25,531 ± 0,106 mg RE/g DE) while the lowest value was obtained for the crude stem extract (0.495 ± 0.077 mg RE/g DE). The partitioning with ethyl acetate showed a significant increase ($p < 0.05$) in the flavonoid contents in the samples analyzed. For the stem sample, an increase occurred from 0.495 ± 0.077 mg RE/g DE (crude hydroethanolic extract) to 4.433 ± 0.057 mg RE/g DE (ethyl acetate fraction). For the leaves sample, the flavonoid contents increased from 5.187 ± 0.096 mg RE/g DE (crude hydroethanolic extract) to 25.531 ± 0.106 mg RE/g DE (ethyl acetate fraction).

Hydrolysable and condensed tannins were also estimated and their results expressed in milligrams of tannic acid equivalents per gram of dry extract (mg TAE/g DE) and milligrams of cyanidin 3-glucoside equivalents per gram of dry extract (mg C-3-GE/g DE) respectively. The hydrolysable tannins contents were calculated from the calibration curve Y = 0.07069 X + 0.006466; (R^2 = 0.992) where Y corresponds to absorbance and X is the concentration of the tannic acid solution in mg / L. No significant differences ($p < 0.05$) were found for the hydrolysable tannins between the crude stem extract and the crude leaves extract. However, differences were found between these crude extracts with their respective ethyl acetate fractions. There was an increase from 1.431 ± 0.085 mg TAE/g DE (crude extract of stem) to 1.943 ± 0.069 mg TAE/g DE (ethyl acetate fraction of stem) and from 1.507 ± 0.085 mg TAE/g DE (crude extract of leaves) to 2.688 ± 0.100 mg TAE / g DE (ethyl acetate fraction of leaves). The condensed tannins showed significant differences in the two samples ($p < 0.05$). The highest value was exhibited by the leaves sample (crude extract of the leaves: 48.020 ± 0.062 mg C-3-GE/g DE and ethyl acetate fraction of leaves: 65.296 ± 0.577 mg C -3-GE/g DE).

Antioxidant activity

Several studies have described the reducing power of phenolic compounds present in several plant species, correlating them with the capacity of sequestration of free radicals produced in various pathological processes in the biological system (Procházková et al., 2011; Carocho and Ferreira, 2013). In this way the quantification of phenolic compounds in plants is useful to assign them these functional properties. The high levels of phenolics found in the *B. cathartica* species may suggest a potent antioxidant power, since this activity is usually linked to the phenolic content present in plant samples with this power. Several studies evaluated the relationship between the antioxidant activity of the plant products and their phenolic content and in some studies, this correlation was found (Jafari et al., 2014; Zemmouri et al., 2014), but should be interpreted with caution, since the Folin-Ciocalteu reagent can also react positively with different antioxidant compounds present in plant samples.

Increasing epidemiological evidence on the role of antioxidant foods in the prevention of certain diseases has led to the development of a large number of methods for determining antioxidant capacity (Rufino et al., 2007).

In the present study, different extracts of *B. cathartica* were used as source of bioactive substances and their potential antioxidant capacities were measured and quantified using the DPPH free radical sequestration

Table 2. Antioxidant activity of *Bridelia cathartica* extracts.

Samples	DPPH (EC_{50}, µg/mL)	Phosphomolybdenum method (% AAT)
CHES	10.45 ± 0.41[a]	42.36 ± 0.07[a]
CHEL	3.63 ± 0.12[b]	57.88 ± 0.57[b]
EAFS	14.60 ± 0.06[c]	45.98 ± 0.19[c]
EAFL	4.93 ± 0.44[d]	50.51 ± 0.08[d]
Quercetin	1.50 ± 0.03	100.00 ± 0.00

Legend: DPPH: free radical 2,2-diphenyl-1-picryl-hydrazyl; EC_{50} - efficient concentration in µg / mL; % AAT - total antioxidant activity in percentages of quercetin. CHEL: crude hydroethanolic extract of Leaves; CHES: crude hydroethanolic extract of stem; EAFL: Ethyl acetate fraction of leaves; EAFS: ethyl acetate fraction of stem. Means followed by the same letter in each column are not significantly different ($p < 0.05$).

method and reduction of the phosphomolybdenum complex and its results are shown in Table 2. The phosphomolybdenum method or test of total antioxidant capacity is a method used in the quantitative determination of the antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Molybdenum (VI) to Molybdenum (V) by antioxidants present in the sample and subsequent formation of the green color of phosphomolybdenic complex (V) at acidic pH (Alam, 2013) which is determined spectrophotometrically at 695 nm. The absorbances of the extracts were compared with the standard (quercetin) and expressed as a percentage (%). The quercetin was assigned a percentage of 100% (Souza, 2008). The DPPH assay, widely used as a fast, reliable and reproducible parameter to look for in vitro antioxidant activity of pure compounds as well as plant extracts (Aliyu et al., 2012) is based on the ability of this radical to react with substances which are donors of hydrogen (H) including phenolic compounds. This method is one of the most used and consists of evaluating the sequestering activity of the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Alam, 2013; Sousa et al., 2007).

The delocalization of the electron in the DPPH molecule gives rise to the dark violet color, which absorbs in the band of about 517 nm (Alam, 2013) and that by action of an antioxidant (RH) or a radical species (R•), changes to Yellow coloration, with consequent decay of the absorption, thus being able to be monitored spectrophotometrically (Alam, 2013; Sousa et al., 2007). In this way the percentages of inhibition of different dilutions of the samples have been determined and later were used to determine the effective concentration (EC_{50}). The results showed that there were significant differences ($p < 0.05$) in the capacity to reduce the DPPH free radical between the crude extract of the stem (EC_{50} = 10.45 ± 0.41 µg/mL) and the crude extract of the leavest (EC_{50} = 3.63 ± 0.12 µg/mL). The partition with ethyl acetate causes a significant decrease of this capacity from 10.45 ± 0.41 µg/mL (crude extract of the stem) to 14.60 ± 0.06 µg/mL (Ethyl acetate fraction of the stem) and 3.63 ± 0.12 µg/mL (crude extract of the leaves) to 4.93 ± 0.44 µg/mL (ethyl acetate fraction of the leaves).

In the phosphomolybdenum complex assay, the potent reducing capacity of the crude extract of the leaves (57.88 ± 0.57%) is also confirmed in comparison with the crude extract of the stem (42.36 ± 0.07%). The partition with ethyl acetate causes a significant decrease for the leaves sample, from 57.88 ± 0.57% (crude extract) to 50.51 ± 0.08% (ethyl acetate fraction). On the contrary for the stem sample, there was an increase of this capacity from 42.36 ± 0.07% (crude extract) to 45.98 ± 0.19% (Ethyl acetate fraction).

Quantitative phytochemical studies showed higher phenolic content in all samples, and there were no significant differences in their occurrence in terms of total phenolic contents but they were different in their distribution in terms of chemical groups. These differences probably dictated the differentiated antioxidant capacity exhibited by each sample analyzed. In this way, the evidenced antioxidant activity of the leaves and stem of *B. cathartica* can support the reports on the uses of this plant as antimicrobial and anti-inflammatory agent. Phytochemical studies of some species of the genus *Bridelia* led to the identification and isolation of a large number of polyphenols, triterpenes, glycosides and lignins (Ngueyem et al., 2009). Many of the species studied belonging to the genus *Bridelia*, including *B. cathartica*, are traditionally used as anti-inflammatories and as antimicrobials. And in most of cases, the results obtained during phytochemical and biological studies served as a tool for the validation of the traditional uses reported for each species. As the excessive production of reactive oxygen species (ROS) leads to damage of lipids, proteins, membranes and nucleic acids and also serves as an important intracellular signaling that enhances the inflammatory responses (Filippin et al., 2008), the antioxidant properties shown for Extracts of *B. cathartica* may have a very important contribution in inflammatory processes generated by ROS, which may partly explain the use of

Table 3. Minimum inhibitory concentration of *Bridelia cathartica* Bertol crude extracts and ethyl acetate fractions against test microorganisms.

	MIC (µg/mL)				
Sample	***E. coli***	***P. aeruginosa***	***S. aureus***	***E. faecalis***	***S. pneumoniae***
CHEL	>300	300	>1000*	>1000*	>1000*
CHES	>300	>250	500	>1000*	>1000*
EAFL	>300	>1000*	250	>1000*	>1000*
EAFS	>250	>250	>250	>1000*	>1000*

*Values were considered as noteworthy antimicrobial activity; MIC: minimum inhibitory concentration; CHEL: crude hydroethanolic extract of Leaves; CHES: crude hydroethanolic extract of stem; EAFL: Ethyl acetate fraction of leaves; EAFS: ethyl acetate fraction of stem.

this species as an anti-inflammatory remedy.

Several phenolic substances are considered as important antioxidants, and this capacity is closely associated with the type of carbon skeleton they have, which is conducive to the stabilization of free radicals. The antioxidant activity of flavonoids, for example, depends on their structure and their antioxidant capacity can be determined by at least five factors namely: reactivity as a hydrogen atom and electron donor, stability of the flavanoyl radical formed, reactivity with other antioxidants, the ability to chelate transition metals and solubility and interaction with biological membranes (Barreiros et al., 2006). The configuration, substitution, and number of hydroxyl groups also substantially influence the various mechanisms of antioxidant activity (Kumar and Pandey, 2013). On the other hand, different metabolites of phenolic nature have their particularities that may dictate a greater or lesser antioxidant capacity, not necessarily depending on their greater or lesser concentration in the samples.

Antibacterial activity

The results of antimicrobial activity performed in the present work are summarized in Table 3 and the criteria chosen for the characterization of this activity as a function of the MIC was the described by Holetz et al. (2002). According to them, it was considered that if the extracts displayed an MIC less than 100 µg/mL, the antimicrobial activity was good; from 100 to 500 µg/mL the antimicrobial activity was moderate; from 500 to 1000 µg/mL the antimicrobial activity was weak; over 1000 µg/mL the extract was considered inactive. It is clear that these criteria are not consensual with those of different authors. In fact, some authors consider the value of 250 µg/mL as strong antimicrobial activity (Madureira et al., 2012).

Assays made from crude hydroethanolic extracts of stem and leaves of *B. cathartica* and their ethyl acetate fractions in dimethylsulfoxide (DMSO) at concentrations of 50, 100, 250, 500, 700, 1000 and 2000 µg/ml showed considerable activity against gram positive and gram negative human pathogens, namely *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212 and *S. pneumoniae* ATCC 49619. Polyphenolic constituents, flavonoids, esters of simple phenols, among others, may play important antimicrobial activities. This is the case of quercetin, rutin, apigenin, among other chemical compounds, which have strong antimicrobial activity against strains of gram positive and gram negative bacteria (Basile et al., 1999; Orhan et al., 2010). Phytochemical studies of *B. cathartica* performed by Madureira et al. (2012) revealed the presence of these constituents in addition to alkaloids and terpenes. The chemotaxonomic analysis shows that the constituents of phenolic nature are present in several species of the genus *Bridelia* and therefore, different parts (roots, stem and leaves) can perform different types of biological activities. Crude hydroethanolic extracts of leaves and stem and their respective ethyl acetate fractions demonstrated moderate antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* with MIC ≤ 500 µg/mL except the ethyl acetate fraction of the leaves inactive against *P. aeruginosa* and the crude extract of leaves inactive against *S. aureus* (MIC> 1000 µg/mL). The species *E. faecalis* and *S. pneumoniae* were insensitive to all the samples studied, and the ethyl acetate fraction of the leaves showed the highest antibacterial activity against S. aureus.

B. cathartica root preparations are used in traditional medicine for the treatment of various diseases including malaria (Ramalhete et al., 2008) and venereal diseases (Madureira et al., 2012).

Madureira et al. (2012) in his studies on antimicrobial activity of crude methanolic extract and fractions (n-hexane, dichloromethane and ethyl acetate) of roots of *B. cathartica*, described a strong and moderate antibacterial activity against gram positive and gram negative bacteria with MIC in the range 7.5 µg/mL - >250 µg/mL.

The ethyl acetate fraction of leaves, with the lowest MIC (250 µg/mL), exhibited promising activity against Gram-positive bacteria *S. aureus*, but generally gram-negative bacteria (*E. coli* and *P. aeruginosa*) showed to be more sensitive in relation to gram positive, since of the three gram positive bacteria tested only one was sensitive. The results obtained for the gram negative species can be partially explained by the structural and

chemical composition differences of the cell wall in the bacterial cells. Analyzing the quantitative phytochemical results obtained for the flavonoids and tannins (hydrolysable and condensed) of the studied samples, it is evident the occurrence of higher levels in the ethyl acetate fraction of leaves. This may explain the moderate antimicrobial activity associated with the low MIC value obtained for this sample. Several reports suggest that flavonoids generally have multiple mechanisms of antimicrobial action. However, these mechanisms remain largely unknown for most flavonoids, but it is known that they may exert antibacterial activity through cytoplasmic membrane rupture, inhibition of nucleic acid synthesis, inhibition of energy metabolism, inhibition of cell wall synthesis, and the inhibition of cell membrane synthesis (Xiao et al., 2014). It is also believed that the antibacterial activity of tannins may be associated with their strong antioxidant and free radical scavenging ability, ability to complex protein macromolecules (enzymes or microbial proteins) or polysaccharides and metal ions (Cunha et al., 2005 ; Simões et al., 2004). Qualitative phytochemical analysis of the ethyl acetate extract of *B. cathartica* roots revealed the presence of flavonoids, phenolics, terpenes and alkaloids, and the same extract revealed antibacterial activity against *S. aureus*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa* and *M. smegmatis* (Madureira et al., 2012). In our study we didn't detect alkaloids in the ethyl acetate fraction used for antibacterial analysis and phenolic compounds quantification, probably because of the extraction method used which is directed to phenolic compounds.

The results of this study support the use of this species in traditional medicine, for the treatment of various infectious diseases. This correlation can be observed in the last analysis by the capacity demonstrated by extracts of leaves and stem of *B. cathartica* against *E. coli*, *P. aeruginosa* and *S. aureus*.

E. coli, for example is a bacillary enterobacteria and facultative anaerobe that is commonly found in the intestinal tract of humans and other warm-blooded animals. It is often associated with intestinal and urinary infections, which probably explains the use of preparations of this plant in traditional remedies used in the treatment of diarrhea and venereal diseases.

Our findings on phytochemical compounds and antibacterial activity on *B. cathartica* species are similar to those obtained by Okeleye et al. (2011), Adefuye et al. (2011) and Adefuye and Ndip (2013) on *B. micrantha* species.

Conclusion

The results of the present research shows that leaves and stem of *B. cathartica* Bertol have strong antioxidant capacity and remarkable antimicrobial activity which are linked to the high levels of phenolic compounds, flavonoids and tannins exhibited by these samples and allow to validate the ethno-medicinal use of the plant in treating infectious and inflammatory diseases.

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