Journal of Biosciences and Medicines, 2016, 4, 87-99
Published Online April 2016 in SciRes. http://www.scirp.org/journal/jbm
http://dx.doi.org/10.4236/jbm.2016.44012

Antimicrobial and Antioxidant Activity of *Anacardium occidentale* L. Flowers in Comparison to Bark and Leaves Extracts

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Received 6 January 2016; accepted 25 April 2016; published 28 April 2016

Abstract

***Anacardium occidentale* L., Anacardiaceae, is frequently used to treat infections. We evaluated the antimicrobial and antioxidant activity of extracts obtained from aerial parts of the plant in comparison with the extract prepared with the flowers. Ethanol extracts of the leaves (EEL), stem bark (EEB), and flowers (EEF) were prepared separately. The antimicrobial activity was evaluated by agar diffusion and broth dilution methods. The minimum bactericidal and fungicidal concentrations were determined against: *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, MRSA, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Helicobacter pylori*, *Salmonella choleraesuis*, *Candida albicans*, and *Candida tropicalis*. The antioxidant activity of the extracts was evaluated based on their scavenger capacity of 2,2-diphenyl-1-picrylhydrazyle. The phytochemical profile was analyzed by colorimetric methods and by HPLC with UV detection. All extracts exhibited antioxidant and antimicrobial activity. Although, the EEF was the most effective since it inhibited the growth of all 14 microorganisms tested. Anacardic and galic acids were identified in all extracts as well phenolic compounds, triterpenes, flavones and xanthones. In conclusion, the extracts obtained from the aerial parts of *A. occidentale*, mainly the extract of flowers that was the most effective, are rich in bioactive metabolites that exert a potent antioxidant and antimicrobial effect. Taken together, the results indicate an important biotechnological potential of *A. occidentale* as a**

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How to cite this paper: da Silva, R.A., *et al.* (2016) Antimicrobial and Antioxidant Activity of *Anacardium occidentale* L. Flowers in Comparison to Bark and Leaves Extracts. *Journal of Biosciences and Medicines*, **4**, 87-99.
http://dx.doi.org/10.4236/jbm.2016.44012

source of compounds with broad-spectrum antimicrobial and of antioxidant activity to be used in the food and cosmetics industry.

Keywords

***Anacardium occidentale*, Antimicrobial Agents, Flowers, Cashew, Caju, Antioxidant**

1. Introduction

Anacardium occidentale L. (Anacardiaceae), popularly known as cashew tree, is native from Brazil. The plant is mainly found along the coast of the northeastern states [1] [2] and is of great economic and medicinal value [3]. The main ethnopharmacological applications of the species include the treatment of infectious and inflammatory diseases and pain conditions, such as venereal diseases, skin diseases, diarrhea, stomatitis, aphthae, bronchitis, intestinal cramps, muscle weakness, diabetes, tooth pain, weakness, inflammation, psoriasis, and cough [4]-[9].

Studies have described the antimicrobial activity of different parts of *A. occidentale*, such as the **fruits** [3] [10]-[15], **stem bark** [16]-[25], **leaves** [16]-[29] and **gum** [30] [31], but there are no reports of similar activity in the flowers.

The most frequent antimicrobial activity was described to the leaves and barks against bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Escherichia coli*, *Enterococcus faecalis* among others) and fungi (*Candida albicans*) [32].

Similarly, antioxidant, considering the activity in comparison to quercetin, has been described else whereby fruits, pseudofruis and roots [28] [33]-[38], but not for the flowers.

With respect to the chemical components found in the aerial parts of the cashew tree, phenolic compounds such as anacardic acids, cardanols, cardols and 2-methycardols have been isolated from the cashes nut shell liquid (CNSL) [12] [39]. On the basis of these considerations, we evaluated comparatively the antimicrobial and antioxidant activity of extracts obtained from the flowers, leaves and stem bark of *A. occidentale* in order to determine which aerial part exhibits higher activity, since most studies have evaluated these components separately.

2. Materials and Methods

2.1. Plant Material

The flowers, leaves and stem bark of *A. occidentale* were collected with a cutting instrument at the Campus of the Federal University of Maranhão (Universidade Federal do Maranhão – UFMA), São Luís, Maranhão, Brazil as described previously [40] [41]. The species was identified at the Ático Seabra Herbarium of UFMA and a voucher specimen was deposited (No. 1050/SLS017213).

2.2. Preparation of the Extracts

The flowers, leaves and stem bark were separately dried at room temperature (24˚C ± 1˚C) for 24 hours, followed by drying in an oven at 37˚C for 7 days. The dried material was triturated in an electrical grinder (TENAL TE 340) and extracted by maceration in absolute ethanol P.A. The process was repeated weekly for 3 weeks according to Melo *et al.* [22], with some modifications. The extracts were concentrated under reduced pressure, lyophilized, and stored in sterile flasks at 4˚C, for the subsequent assays. The mean yield was 15% for the flower extract (EEF), 13% for the (EEL), and 18% for the (EEB). For the *in vitro* microbiological assays, 20 g of each lyophilized extract was resuspended in 100 mL sterile distilled water and sterilized by filtration through a 20-µm membrane (Sartorius). For chemical analysis, 10 g of the lyophilized extracts were resuspended in absolute ethanol P.A. (Merck).

2.3. Chromatographic Analysis (HPLC/UV) of the *Anacardium occidentale* Extracts

The extracts were analyzed by high-performance liquid chromatography with UV detection (HPLC/UV) with an analytical scale. The chromatograms of EEF, EEL and EEB were obtained with the Varian system (Pro Star 310,

Varian Star Workstation, 6.0) using the following program parameters: elution time of 40 min, detection at 278 nm, and a flow rate of 1 mL/min. A Polaris C-18 reverse-column, protected by a 2.5-cm pre-column, and a 20-µL loop were used. The mobile phase consisted of ultrapure water (Milli-Q) containing 2% acetic acid (A) or acetonitrile (Merck) (B) as follow: 0 min - 30 min, 70% A and 30% B until 50% A and 50% B. Time of 30 min - 40 min, 50% A and 50% B until 30% A and 70% B. The results are expressed as retention time (Rt) in minutes and concentrations are expressed as percentage (%) based on integration of the peak areas.

2.4. Evaluation of Antimicrobial Activity

2.4.1. Microorganisms

Antimicrobial activity was determined against reference strains of the following microorganisms:

Gram-positive bacteria: *Streptococcus mutans* (ATCC 25175), *Lactobacillus acidophilus* (ATCC 4356), *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus*-MRSA (ATCC 3438402), *Enterococcus faecalis* (ATCC 29212), and *Streptococcus pyogenes* (ATCC 19615).

Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC7002) *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 51446), *Klebsiella pneumoniae* (ATCC 13883), *Klebsiella pneumoniae* (ATCC 700503), and *Helicobacter pylorim* (ATCC 43504). *Salmonella choleraesuis* was isolated from a case of human infection.

Fungi: *Candida albicans* (ATCC 18804), *Candida albicans* No.37, *Candida albicans* No.12, *Candida albicans* No. 1, *Candida tropicalis* No.84, *Candida tropicalis* No.82, *Candida tropicalis* No. 59 and *Candida tropicalis* No.39, isolated from cases of human infection.

The ATCC strains used were obtained from the Oswaldo Cruz Foundation (Fundação Oswaldo Cruz), Rio de Janeiro.

The bacteria were cultured in brain-heart infusion (BHI) broth (Difco) at 37˚C for 24 hours. The fungal strains were cultivated on Sabouraud-dextrose agar (Difco) and incubated at 37˚C for 24 hours. The microbial suspensions used for susceptibility testing were prepared in sterile saline (0.9% NaCl). Turbidity was adjusted to a 0.5 McFarland standard (10^8 CFU/mL) [42].

2.4.2. Agar Diffusion

Antimicrobial activity was evaluated by the agar diffusion method using the cavity technique for screening [43]. Plates containing BHI agar were used for *Streptococcus mutans* and *Lactobacillus acidophilus*, blood agar for *Helicobacter pylori*, *Streptococcus pyogenes* and *Enterococcus faecalis*, Mueller-Hinton agar for the other bacteria, and Sabouraud-dextrose agar for *Candida albicans* and *Candida tropicalis*. Perforations (wells) measuring 5 mm in diameter were made in the agar media. The wells were then filled with the standard microbial suspensions using a sterile swab and with 25 µL of the extracts (EEF, EEB, and EEL) and control solutions. Absolute ethanol was used as solvent control, 0.12% chlorhexidine gluconate was used as positive control for bacteria, fluconazole (128 µg/mL) as positive control for fungi, and distilled water as negative control in all assays.

The plates were incubated at 37˚C, for 1 hour, for diffusion of the extracts and control solutions and then inverted for incubation. The BHI and blood agar plates were incubated under microaerophilic conditions in a 5% CO_2 atmosphere at 37˚C, for 48 hours. The blood agar plates with the *Helicobacter pylori* were incubated under microaerophilic conditions in a 5% CO_2 atmosphere, at 37˚C, for 72 hours. The Sabouraud and Mueller-Hinton agar plates were incubated under aerobic conditions for 24 hours. All assays were carried out in duplicate for each microorganism. After incubation, the inhibition halos were measured in millimeter.

2.4.3. Determination of Minimum Bactericidal and Fungicidal Concentrations

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by the broth dilution method [43], using serial dilutions of EEF, EEL and EEB in BHI broth at concentrations ranging from 0.31 to 2 mg/mL. Each tube was inoculated with 1 µL of the microbial suspension (10^8 CFU/mL) and incubated at 37˚C, for 24 hours. Next, aliquots (1 µL) of the tubes were streaked onto plates containing BHI agar for *S. mutans* and *Lactobacilus acidophilus*, blood agar for *Helicobacter pylori*, *Streptococcus pyogenes* and *Enterococcus faecalis*, Mueller-Hinton agar for the other bacteria, and Sabouraud agar for *Candida albicans* and *Candida tropicalis*. The MBC and MFC were determined considering the lowest concentrations of the each extract that completely inhibited microbial growth. It was not possible to determine the minimum in-

hibitory concentration because of the color and turbidity of the extracts.

2.5. Determination of Antioxidant Activity

Aliquots (5 µL) of each extract (200 µg) and of quercetin used as the standard (1 mg/mL in methanol) were applied to silica gel 60 F_{254} chromatography plates (0.2 mm, Merck). The mobile phase consisted of a mixture of n-butanol, acetic acid and water (67:23:10). The chromatograms were developed using a methanol solution of 0.2% 2,2-diphenyl-1-picrylhydrazyle (DPPH, Sigma). A purple color was seen within approximately 30 min, and white or yellow spots were indicative of DPPH free radical-scavenging activity [44]. The retention factor (Rf) of the spots was calculated and the chromatograms were photographed and stored in the CAMAG system.

Antioxidant activity was quantified in 96-well plates in an ELISA reader (KC4-Bio-Tek Instruments, Inc., USA). For this purpose, 70 µL of a methanol solution of DPPH (0.3 mM) and 180 µL of the extract (concentrations of 4, 2, 1 and 0.5 µg/mL) were added to each well. Quercetin (4 µg/mL) was used as positive control. Analyses were carried out in triplicate. The microplate was kept at room temperature and the intensity of absorbance (515 nm) was measured after 15 and 30 min. Antioxidant activity was calculated as percentage (%AA) based on the absorbance values as follows: $AA = (Abs^{sample} - Abs^{blank}) \times 100/Abs^{control}$ [45].

The effective concentration (EC_{50}) of the extracts was calculated from the dose-response curve of DPPH radical-scavenging activity in relation to quercetin (Merck) after measurement in a spectrophotometer (Shimadzu, Japan) at 512 nm (n = 3). The EC_{50} was defined as the concentration of the extract necessary to scavenge 50% of the initial DPPH concentration, and was also called inhibitory concentration (IC_{50}) [45].

2.6. Statistical Analysis

The data were processed and analyzed using the Stata 10.0 program (Stata Corporation, College Station, TX, USA).The variables are expressed as mean ± standard deviation. The Mann-Whitney test was used for comparison between two variables and the Kruskal-Wallis test for multiple comparisons. The level of significance was set at 5% ($p < 0.05$).

3. Results

3.1. Chromatographic Profile (HPLC/UV) of the Leaf, Flower and Bark Extracts of *Anacardium occidentale*

Figure 1 shows the chromatographic profile of EEF with 37 peaks and their respective retention times (T_r). The chromatographic profile of EEL presented 33 peaks and that of EEB 35 peaks. In all chromatograms, smaller peaks were detected below 4%.

3.2. Antimicrobial Activity

The antimicrobial activity of the *A. occidentale* EEF, EEL and EEB was tested against 14 bacterial species and/or strains using chlorhexidine as standard

As shown in **Table 1** EEL exhibited bactericidal activity against 10 microorganisms and EEB against 9, whereas EEF was effective in inhibiting the growth of all 14 bacteria strains tested (*Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus*—MRSA, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli* (ATCC 25922 and ATCC 51446), *Klebsiella pneumoniae* (ATCC 13883 and ATCC 700503), *Helicobacter pylori* and *Salmonella choleraesus* previously isolated from a human infection).

The antimicrobial efficacy of EEF was also demonstrated by the Minimum Bactericidal Concentration (MBC), since the values found to the flowers were significantly higher than the values found to the leaves and bark extracts as shown in **Table 2**, since it was not possible to determine the MBC the other extracts EEL and EEB to *Klebsiella pneumonia; Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus pyogenes.*

EEF exhibited antimicrobial activity against the different *Candida albicans* and *Candida tropicalis* strains as demonstrated by the inhibition halos (**Table 3**) or the determination of MFC. In contrast EEL and EEB are unable to form inhibition halo to all *Candida tropicalis* samples and to the majority of tested samples of *Candida albicans*, being effective only to the strain 37 (**Table 4**).

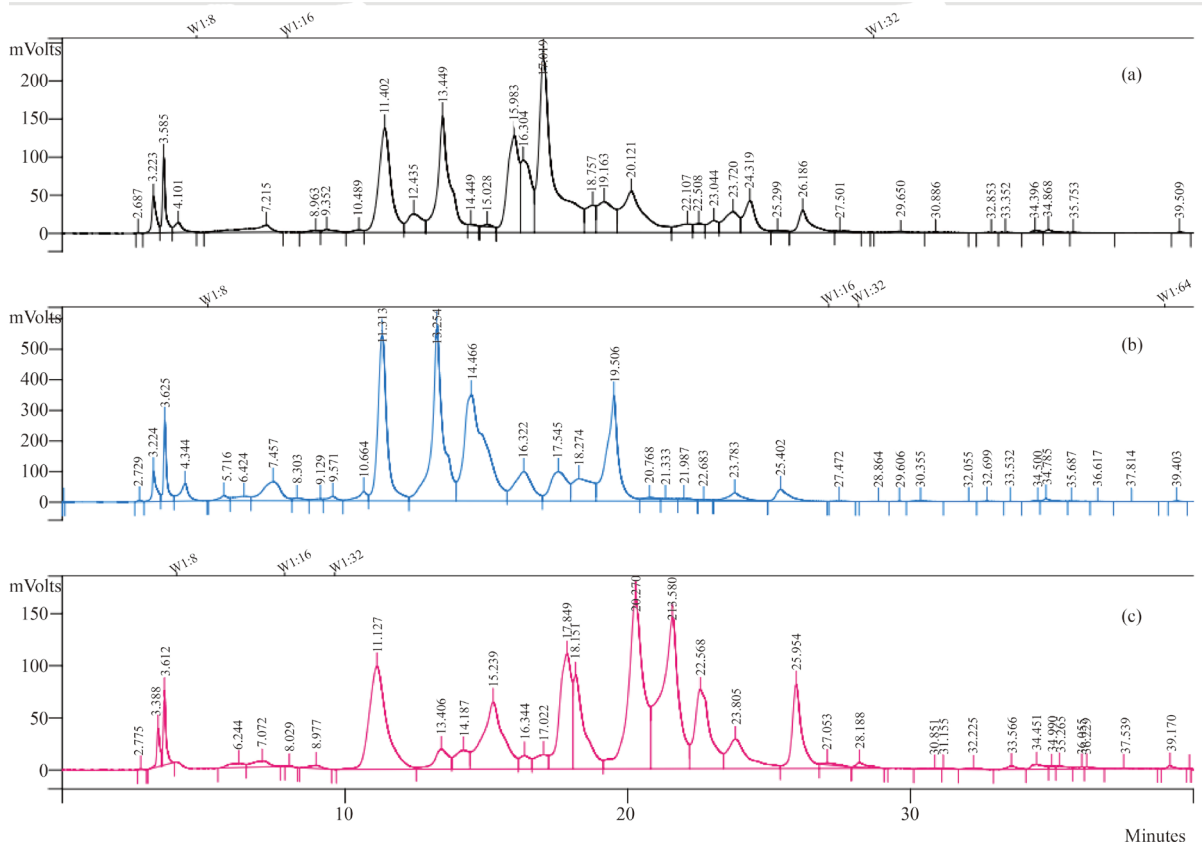

Figure 1. Chromatographic profiles (HPLC/UV, 278 nm) of ethanol extracts prepared from the stem bark (a), flowers (b) and leaves (c) of *Anacardium occidentale*.

Table 1. Antimicrobial activity of ethanol extracts prepared from the stem bark, leaves and flowers of *Anacardium occidentale* against Gram-positive and Gram-negative bacteria.

Microorganism	Crude extract/inhibition halo (mm)			Chlorhexidine[a]
	Bark[a]	Leaves[a]	Flowers[a]	
Enterococcus faecalis (ATCC 29212)	13 ± 3	10 ± 1	15 ± 1	20 ± 1
Escherichia coli (ATCC 25922)	10 ± 1	11 ± 1	21 ± 1	20 ± 1
Escherichia coli (ATCC 51446)	NI[b]	10 ± 1	18± 1	20 ± 1
Helicobacter pylori(ATCC 43504)	11 ± 1	12 ± 1	19 ± 1	24 ± 1
Klebsiella pneumoniae (ATCC 700503)	8 ± 1	8 ± 1	13 ± 3	11 ± 1
Klebsiella pneumoniae (ATCC 13883)	NI	NI	12 ± 1	11 ± 1
Lactobacillus acidophilus (ATCC 00076)	NI	NI	15 ± 1	20 ± 1
Proteus mirabilis (ATCC 7002)	14± 1	12 ± 1	17 ± 1	9 ± 1
Pseudomonas aeruginosa (ATCC 27853)	NI	NI	19 ± 1	12 ± 1
MRSA (ATCC 3438402)	16 ± 1	15 ± 1	18 ±1	20 ± 1
Salmonella choleraesuis	15 ± 1	14 ± 2	33 ± 1	20 ± 1
Staphylococcus aureus (ATCC 25923)	17 ± 1	16 ± 1	22 ± 1	21 ± 1
Streptococcus mutans (ATCC 25175)	NI	NI	18 ± 1	25 ± 1
Streptococcus pyogenes(ATCC 19615)	12 ± 1	10 ± 1	16 ± 1	19 ± 1

[a]The results are reported as the mean ± standard deviation of strains tested in duplicate. [b]NI: no inhibition halo. MRSA: methicillin-resistant *Staphylococcus aureus*.

Table 2. Minimum bactericidal concentration of ethanol extracts prepared from the flowers, leaves and stem bark of *Anacardium occidentale* against Gram-positive and Gram-negative bacteria.

Microorganism	Crude extract (mg/mL)		
	Flowers	Leaves	Bark
Escherichia coli (ATCC 25922)	1.25 ± 1	20 ± 1	20 ± 1
Escherichia coli (ATCC 51446)	1.25 ± 1	10 ± 1	NR
Enterococcus faecalis (ATCC 29212)	10 ± 1	20 ± 1	20 ± 1
Helicobacter pylori (ATCC 43504)	5 ± 1	20 ± 1	10 ± 1
Klebsiella pneumoniae (ATCC 13883)	20 ± 1	NP*	NP
Klebsiella pneumoniae (ATCC 700503)	20 ± 1	NP	NP
Lactobacillus acidophilus (ATCC 4356)	5 ± 1	NP	NP
*Pseudomonas aeruginosa (*ATCC 27853)	1.25 ± 1	NP	NP
*Proteus mirabilis (*ATCC 7002)	20 ± 1	20 ± 1	20 ± 1
*Staphylococcus aureus (*ATCC 25923)	10 ± 1	10 ± 1	20 ± 1
MRSA (ATCC 3438402)	2.5 ± 1	10 ± 1	5 ± 1
Streptococcus mutans (ATCC 25175)	2.5 ± 1	NP	NP
Streptococcus pyogenes(ATCC 19615)	5 ± 1	NP	5 ± 1
Salmonella choleraesuis	0.625 ± 0	5 ± 1	5 ± 1

*NP: Not performed since no inhibition halo was formed; MRSA: methicillin-resistant *Staphylococcus aureus*.

Table 3. Antifungal activity of ethanol extracts prepared from the flowers, leaves and stem bark of *Anacardium occidentale* against *Candida* sp.

Species/strain		Extract/inhibition halo (mm)*			Fluconazole[a]
		Flowers[a]	Leaves[a]	Bark[a]	
Candida albicans	ATCC 18804	16± 1	NI[b]	NI	15± 1
	37	19± 1	12 ± 1	11 ± 1	13 ± 1
	12	17 ± 1	NI	NI	13 ± 1
	1	13 ± 1	NI	NI	12 ± 1
Candida tropicalis	39	15 ± 1	NI	NI	15 ±1
	59	14 ± 1	NI	NI	15 ± 1
	84	14 ± 1	NI	NI	13 ± 2
	82	14 ± 1	NI	NI	14 ± 0

[a]The results are reported as the mean ± standard deviation of strains tested in duplicate. [b]NI: no inhibition halo. *$p < 0.05$.

Table 4. Minimum fungicidal concentration of ethanol extracts prepared from the flowers, leaves and stem bark of *Anacardium occidentale* against *Candida* sp.

Species /strain		Crude extract(mg/mL)		
		Flowers	Leaves	Bark
Candida albicans	ATCC 18804	20 ± 1	NP*	NP
	37	20 ± 1	>20	>20
	12	20 ± 1	NP	NP
	1	20 ± 1	NP	NP
Candida tropicalis	39	20 ± 1	NP	NP
	59	20 ± 1	NP	NP
	84	20 ± 1	NP	NP
	82	20 ± 1	NP	NP

*NP: Not performed since no inhibition halo was formed.

3.3. Antioxidant Activity

Qualitative analysis of the antioxidant activity of EEB, EEL and EEF suggested the presence of compounds with antioxidant activity as indicated by the free radical-scavenging capacity similar a quercetin. The retention factor (Rf) was 0.64 for EEF and EEB and 0.74 for EEL, values similar to that of quercetin (0.85) which was used as the standard. The same was observed for free radical-scavenging capacity, with EC_{50} values of 0.99 for EEF, 1.47 for EEL, 1.12 for EEB, and 1.71 for quercetin (**Table 5**). **Figure 2** shows the percentage of the DPPH free radical-scavenging activity of the *A. occidentale* extracts and positive control (quercetin) after a reaction time of 30 min. According the results the three extracts showed similar anti-oxidant activity as shown in **Figure 2** and detailed in **Table 5**.

4. Discussion

4.1. Chemical Composition of the *Anacardium occidentale* Extracts

Comparison of the chemical composition of EEF, EEL and EEB showed that all extracts are rich in bioactive secondary metabolites, with more strongly positive results observed for the flowers.

Similarly previous results it was possible to identify the presence of saponins, alkaloids, phenolic compounds and tannins in the extracts of *A. occidentale* [25] [46]-[49]. Differences in the qualitative or semi-quantitative chemical composition of the same plant species may occur between studies because of the influence of factors

Table 5. Antioxidant activity of ethanol extracts prepared from the stem bark, leaves and flowers of *Anacardium occidentale* compared to quercetin.

Sample	Concentration of the extract (µg/mL)				EC_{50}
	0.5	1	2	4	
Flowers	24 ± 2[a]	48± 1	85 ± 3	94 ± 0.2	0.99
Leaves	17 ± 1	30 ± 1	57 ± 1	90 ± 0.4	1.47
Bark	22 ± 2	42± 1	76 ± 2	94 ± 0.5	1.12
Quercetin	-	-	-	94 ± 0.5	1.71

[a]The results are reported as the percentage of inhibition of 2,2-diphenyl-1-picrylhydrazyle (DPPH) after a reaction time of 30 min and represent mean ± SD from quadruplicates.

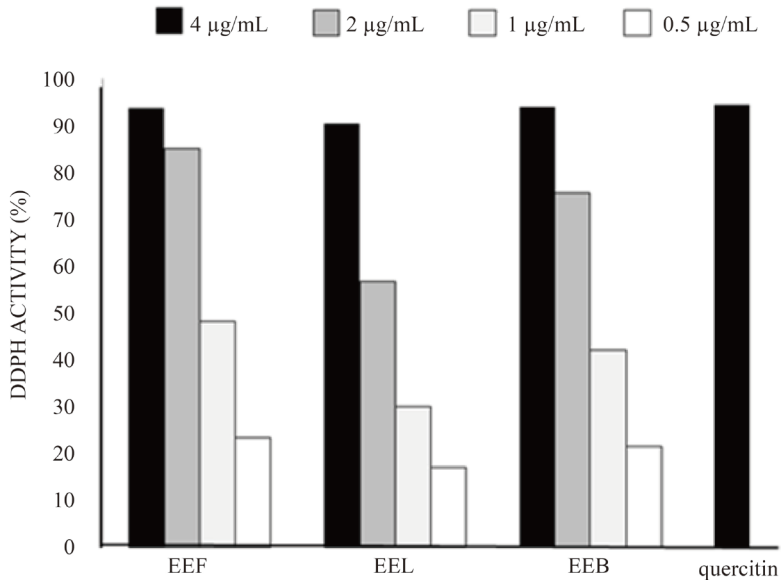

Figure 2. Antioxidant activity of ethanol extracts prepared from the flowers (EEF), leaves (EEL) and stem bark (EEB) of *Anacardium occidentale* compared to quercetin (Merck; 4 µg/mL) used as a positive control. Antioxidant activity was evaluated based on DPPH free radical-scavenging activity after 30 min. The results are reported as percent inhibition obtained in quadruplicate for each extract at concentrations of 0.5, 1, 2 and 4 µg/mL.

such as plant development, seasonality, circadian rhythm, temperature, rainfall, radiation, nutrients, and altitude, among other environmental factors [34], for this reason We therefore compared extracts collected from different aerial parts during the same period and using the same methods. Although qualitative analysis of the chemical composition showed no significant differences between the extracts, the chromatographic profiles of EEF, EEL and EEB of *A. occidentale*, obtained under the same conditions of analysis (**Figure 1**), indicate the presence of different bioactive secondary metabolites in the three extracts, a finding that may explain the higher antimicrobial activity of the extract.

4.2. Antimicrobial Activity

Antimicrobial activity against bacteria and fungi, particularly *Candida*, has been reported for extracts obtained from the fruits, pseudofruits, leaves and bark of *A.occidentale* [5] [15] [16] [20] [21] [25] [26] [29] [50] [51]. This activity was attributed mainly to the presence of flavonoids, tannins, organic acids, alkaloids, saponins, terpenes, and especially to the presence of phenolic compounds, including anacardic acids, cardol and cardanol [10] [52]-[55]. These compounds have shown antimicrobial activity when tested separately, although their mechanism of action is not fully understood [56] [57].

The antibacterial effect against different bacterial species was observed in the present study for the three extracts of *A. occidentale* (EEF, EEL and EEB). Comparison showed that EEF was more effective than the other extracts (EEL and EEB), inhibiting the growth of all bacteria (**Table 1**) and fungi (**Table 3**) tested as indicated by greater inhibition halos, which were often compatible to those formed by the controls (chlorhexidine and fluconazole). Although EEL and EEB also exhibited antimicrobial activity, they were not effective against some bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*) and against most of the fungi tested (*Candida albicans*-ATCC 18804; 12; 1; *Candida tropicalis*-39; 59; 84; 82). These findings indicate the presence of compounds with broad-spectrum antimicrobial activity in EEF. The higher antimicrobial efficacy of EEF was demonstrated by the MBC which ranged from 0.626 mg/mL for *Salmonella choleraesuis* to 20 mg/mL for *Klebsiella pneumonia* and *Proteus mirabilis*. *S. choleraesuis* was the bacterium most susceptible to EEF considering the inhibition halo (33 mm) and MBC (0.625 mg/mL).

Antimicrobial resistance has become a leading public health problem in the world. The most common bacteria causing infections, especially nosocomial infections, are *S. aureus*, MRSA, *E. coli* and *Pseudomonas aeruginosa*, all of them resistant to most antibiotics [27] [37] [58]-[63]. The three extracts were effective against *S. aureus* and MRSA, in agreement with other studies testing the stem bark and leaves of *A. occidentale* [18] [19] [61], anacardic acid isolated from the cashew nut [10] [63] and CNSL [11].

MRSA are known for their resistance to a series of antibiotics and are therefore frequently associated with infectious complications in hospitalized patients and health professionals [64]. Resistance is mediated by enzymatic inactivation and changes in penicillin-binding proteins. In addition, alterations in membrane permeability prevent the antibiotic from reaching its receptors [65]. In this respect, the effectiveness of the three extracts against these microorganisms is an important finding and indicates the use of the aerial parts of *A. occidentale* as targets for bioprospection of compounds with antimicrobial activity, especially against MRSA.

Another important finding was the observation that the extracts were effective in inhibiting the growth of extended-spectrum beta-lactamase producers, such as *K.pneumoniae* (ATCC 700503) and *E. coli* (ATCC 51446) [66] [67]. The three extracts tested were effective against *K. pneumoniae* (ATCC 700503), and EEF and EEL were effective against *E. coli* (ATCC 51446). According to Bouttier *et al*. [68], the antimicrobial activity of *A. occidentale* CNSL is related to the inhibition of beta-lactamase by anacardic acids. Beta-lactamases possess considerable hydrolytic activity and can inactivate a variety of beta-lactam antibiotics [69]. In this respect, anacardic acids emerge as an important alternative for the control of microorganisms that use this strategy as an escape and resistance mechanism to most commercially available antibiotics.

Drug resistance is not only limited to bacteria, but is also observed in pathogenic fungi, particularly *Candida albicans* which is frequently associated with infections [70]-[72]. Therefore, studies of new antimicrobial agents that are able to overcome multidrug-resistant mechanisms are urgently needed [73]. Within this context, the present results suggest the aerial parts of *A. occidentale*, especially its flowers, to be promising materials for bioprospection of new broad-spectrum antimicrobial agents that could be used to treat diseases caused by microorganisms which are resistant to commercially available antibiotics.

4.3. Antioxidant Activity

Antioxidants are substances that, when present at low concentrations in relation to the oxidizable substrate, significantly delay or inhibit oxidative processes [45]. In the present study, EEF, EEL and EEB exhibited antioxidant activity compatible to that of quercetin as demonstrated by their ability to scavenge the DPPH free radical. Similar results have been reported for bark [27] [36] and leaf extracts of *A. occidentale* [74] [75]. Antioxidant activity has been described for compounds isolated from the CNSL, including anacardic acids, cardol and cardanol [34] [38] [76]-[78]. Furthermore, antioxidant activity has been detected in sub-products such as peduncle bagasse [79] [80], cashew apple juice [33], sprouts [81], and cashew nut skin [82]. However, this is the first study reporting antioxidant activity in flowers.

Phenolic compounds are secondary metabolites found in all plants. This group includes simple phenols, hydroxybenzoic acid, derivatives of cinnamic acid, flavonoids, tannins, and coumarins, among others [83]. According to Haslam, natural phenolic compounds have received much attention in recent years due to their capacity to inhibit lipid peroxidation and lipoxygenase *in vitro*. It is therefore possible that the antioxidant activity observed here is related to the presence of phenolic compounds in the three extracts tested (**Table 1**).

5. Conclusion

The present results show that extracts obtained from the flowers, leaves and stem bark of *Anacardium occidentale* are rich in bioactive secondary metabolites, exerting a potential antimicrobial effect against Gram-positive and Gram-negative bacteria as well as fungi. The flower extract was the most effective since it inhibited the growth of all microorganisms tested. In addition, the extracts exhibited antioxidant activity compatible to that of quercetin. Taken together, those results suggest an important biotechnological potential of *A. occidentale* as a source of compounds with broad-spectrum antimicrobial activity and of antioxidant compounds to be used in the drug, food and cosmetic industry.

Author's Contribution

All authors equally contribute to this work

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