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Myracrondruon urundeuva Allemão: Chemical composition, antioxidant activity, antimicrobial activity and inotropic effect

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Caatinga biome features species with high therapeutic potential such as Aroeira do Sertão (*Myracrodruon urundeuva* Allemão) which has long history in folk medicine. This study aimed to chemically characterize the secondary metabolites of hydroethanolic extract and hexane, chloroform, acetate, hydroethanolic fractions from *Myracrodruon urundeuva* (Allemão) possibly responsible antioxidant and antimicrobial activity, besides to evaluate the inotropic effect of crude extract in the left atrium of *Cavia porcellus*. Phytochemical composition was evaluated by spectrophotometry and high-performance liquid chromatography (HPLC). Antioxidant activity was assessed by 2,2-diphenyl-1-picryl hidrazyl (DPPH•) scavenging and reactive substances to thiobarbituric acid methods. Inotropic effect was evaluated using the isolated organ model. Data were expressed as mean ± SEM and differences determined by ANOVA followed by Tukey test. Phytochemical screening and the HPLC confirmed the presence of flavonoids, terpenes, tannins. Acetate and Hydroethanolic extract showed inhibition of DPPH values of 81.56 and 93.28% respectively. On the other hand, TBARS reduced in extract were 64.13% in a concentration of 150 µg/mL. Hydroethanolic extract and acetate fraction with concentrations of 50 and 100 mg/mL showed inhibition zones between 11.00 and 20.50 mm suggesting antimicrobial activity against *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae* bacteria and *Candida albicans*. The isolated atrium model demonstrated that the extract acts non-competitively antagonizing muscle calcium currents, decreasing the force of contraction by about 60%. Flavonoids, terpenes and tannins are responsible for *M. urundeuva* (Allemão) biological effects.

Key words: *Myracrondruon urundeuva*, chemical composition, antioxidant activity, antimicrobial activity, inotropic effect.

INTRODUCTION

The ethnobotanical study allows rescuing the popular knowledge, supporting research in related fields, while contributing to prioritize species in need of conservation since these have a therapeutic and economic potential that are aimed especially by the pharmaceutical industry which prospects new products using these species.

In this context, the studies about microbial action of natural compounds are of utmost importance not only for the rise of bacterial strains resistant to several types of antibiotics is increasing, but also the occurrence of side effects such as: Diarrhea, vomiting and dental stains common by the use of reference drugs (Menezes et al., 2004).

A number of human diseases are caused by oxidative stress due to imbalance between the formation and neutralization of pro-oxidants. Oxidation reactions may produce free radicals, which play a vital role in damaging various cellular macromolecules. This damage results into various diseases like arthritis, diabetes, asthma, liver damage, atherosclerosis, inflammation and carcinogenesis (Mushtaq et al., 2017). The neutralization of free radicals produced in the organism is related to diseases like cancer, diabetes, Alzheimer's and Parkinson's, as well as heart problems (Paixão et al., 2013) according to the World Health Organization, "cardiovascular diseases will be in 2015, main causes of death in developed and developing countries" (Oliveira et al., 2009).

Plants from Caatinga have great pharmacological potential, among them *Myracrondruon urundeuva* (Allemão) stands out. Its height varies according to the occurrence region, reaching about 30 m high in deciduous forests, although in Caatinga it does not exceed 10 m). The crown is wide, with composed leaves, 5 to 7 pairs of obtuse dovate leaflets, pubescent on both sides when young and with up to 5 cm long. Its fruits are the drupe globular or ovoid, with persistent calyx, considering fruit seed. The seed is single (0.2 to 0.4 cm in diameter), globose, devoid of endosperm with epicarp dark brown, brown flesh, fleshy, with characteristic odor and membranous integument (Lorenzi and Matos, 2002; Nunes et al., 2008)

The bark and leaves can be used in the form of decoctions, macerations and bottles; and the powder of the dried leaves can be used as anti-inflammatory and wound healing (Cordeiro and Félix, 2014). A chemical study has demonstrated that the ethyl acetate extract has predominance of substances with a chalcone nature and the other has predominance of catechic tannins (Viana et al., 2003). Chalcones and dihydrochalcones belong to the class of flavonoids which, apart from their antioxidant activity, are known for their ability to strengthen capillary walls, thus assisting circulation and helping to prevent and treat bruising, varicose veins, bleeding gums and nosebleeds. *In vitro*, catechins are involved in antimicrobial, antioxidant activity and anti-inflammatory effects in this species (Viana et al., 2003; Sá et al., 2009; Machado et al., 2012). Still when being reported on the use of aroeira in accent baths against vaginal itching, it is used to boil its shells by women in post-partum baths, such as cicatrizant and anti-inflammatory (Silvino, 2014).

This study aimed to chemically characterize the secondary metabolites of hydroethanolic extract and fractions from *M. urundeuva* responsible for its possible antioxidant activity, antimicrobial activity and to evaluate the inotropic effect of the crude extract in the left atrium of guinea pig (*Cavia porcellus*).

MATERIALS AND METHODS

Collection and identification of plant material

The bark and stem bark of the plant were collected outside the flowering period on November 23, 2012, Olho D'agua do Casado village, Piranhas municipality, Alagoas State, bordering Sergipe, Brazil located at 9º32'110'' latitude and 37º17'38'' longitude. One specimen was recorded in the herbarium of the Federal University of Sergipe (UFS) on the ID number ASE 29,606. The samples were placed in an incubator at 37°C with air circulation for 48 h until complete dehydration.

Collection and maintenance of animals

The experiments designed to evaluate changes in cardiac contractility and effects on calcium channels induced by the hydroalcoholic extract of *M.urundeuva* were made using guinea pigs (*Cavia porcellus*) of both sexes weighing between 400 and 700 g. This research was approved by the Committee on Ethics in Animal Research (CEPA) of UFS on protocol 43/13. The animals were kept in the UFS Central Vivarium, with a temperature between 20 and 25ºC, with a natural light-dark cycle (12 h/12 h).

The micro-environment was composed of metal cages (for up to 6 animals), lined with cardboard. The animals were fed commercial ration (Nutricobaia Agribrands do Brasil LTDA, Brazil) provided at will. The water was also supplied at will, in plastic containers placed adjacent to the feed.

Preparation of the alcoholic extract and fractions

The dried plant material was pulverized in a blade mill to obtain the powder of bark and stem bark, which was extracted by maceration in 90% ethanol for five days. Afterwards the solvent was concentrated in a rotary evaporator under reduced pressure to give the crude hydroethanolic extract (EHE). Part of EHE (400 g) was suspended in a methanol/water solution (2:3) and subjected to liquid-liquid extraction using organic solvents hexane to give the

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fractions (FHX), chloroform (FCL), ethyl acetate (FAE) and hydromethanol (FHM).

Phytochemical screening

For determining the chemical compounds were applied methods and classical chemical reactions that result in the development of color and/or characteristic precipitate, according to the methodology described by Matos (2009). These reactions were held in numbered test tubes containing ethanol solutions 3 to 4 ml, and the fractions of the extract from the stem bark of *M. urundeuva* Allemão.

Detection of metabolites by high pressure liquid chromatography (HPLC)

The HPLC system used consisted of a series of liquid chromatograph Shimadzu Prominence LC comprises two pumps 6AD, photodiode array detector (DAD) and a SPD M20A analytical C18 column (25.0 × 0.46 cm, 5 µm particles). The solvents used were HPLC grade H_2O and CH_3OH.

From ethanolic extracts and fractions were made solutions with final concentration of 1 µg/ml subjected to elution through gradient 5 to 100% CH_3OH exploration, exploratory gradient: 20 to 100% CH_3OH up to 40 min followed by isocratic gradient of 40 to 60 min 100% CH_3OH to investigate the chromatographic profile. The substances were detected using a photodiode array detector (DAD - UV / Vis) using four wavelengths: 254, 280, 360 and 370 nm (Queiroz et al., 2002). For the acquisition and processing of chromatographic data was used LC Solution software. To elucidate the chemical samples were used retention times present in the chromatograms besides their UV spectral data were compared with the literature.

1,1-Diphenyl-2-picryl hidrazyl (DPPH) method

Quantitative evaluation of the antioxidant activity of the crude extract and fractions was performed according to the methodology described by Chaves et al. (2010). The radical consumption was monitored by the decrease of their absorbance Ultraviolet-visible spectroscopy (UV-VIS) spectrophotometer (UV BEL PHOTONICS 1105) at 515 nm, using glass cuvettes with 1 cm optical path. Methanol was used as blank. Absorbance measurements were made in triplicate in times of 1, 5, 10, 20, 30, 40, 50 and 60 min. To evaluate the antioxidant activity were used efficient concentration 50 (EC_{50}), the index of antioxidant activity (IAA) (Scherer and Godoy, 2009) and the percentage inhibition (PI).

The EC50 was calculated by linear regression using a straight line equation y = ax + b where y represents the remaining DPPH (50%) and x is the concentration. Antioxidant Activity Index was calculated dividing the concentration of the DPPH solution by EC50 for each extract or fraction. Percentage inhibition (PI) was calculated according to Equation 1 which DPPHi is absorbance of negative control and DPPHf is the absorbance of the extracts and fractions after 60 min after sample:

$$PI = 100 * \left(\frac{DPPHi - DPPHf}{DPPHi}\right) \quad (1)$$

Thiobarbituric acid reactive species (TBARS) method

The ability of the extracts or fractions in inhibiting lipid peroxidation was determined by monitoring the production of reactive thiobarbituric acid reactive species (TBARS) as malondialdehyde (MDA) in a lipid solution (Budni et al., 2007).

The spectrophotometric absorbance reading was performed at 532 nm. For all the tests, Trolox was used in the same concentrations as positive control. The result was expressed as inhibition percentage calculated according to Equation 2. The MDA formed index was calculated from the average absorbance obtained for each sample divided by the molar extinction coefficient (154000 L/mol.cm):

$$PI = 100 * \left(\frac{MDAi - MDAf}{MDAi}\right) \quad (2)$$

Antimicrobial analysis

Methods to verify the antimicrobial activity were based on protocols developed and determined by the Clinical and Laboratory Standards Institute (CLSI, 2005), using the agar diffusion technique. For this purpose, American Type Culture Collection (ATCC) standard strains provided by the Central Public Health Laboratory of Sergipe (LACEN/SE) was used: Gram+ bacteria *Enterococcus faecalis* (ATCC 51299), *Staphylococcus aureus* (ATCC 29213), and Gram- *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 51446), *Klebissiela pneumoniae* (ATCC 13883), *Proteus mirabilis* (ATCC 7002) and *Candida albicans* fungi (ATCC 14053).

Samples of microorganisms were placed on Brain Heart Infusion (BHI) agar followed by incubation at 35 ± 2°C for 24 h, after that period, it was kept under refrigeration until the time of the tests. The microbial cell suspension was prepared at a concentration of 1.0 MacFarland standard scale in sterile saline, CFU/mL (CLSI, 2005).

Inotropic effect of left atrium experimental model

For the evaluation of inotropic activity was used the method of Cerqueira et al. (2011) with modifications. A total of 18 animals was used (guinea pigs - *Cavia porcellus*), divided into two groups each one with 6 animals, Group 1 was intended to evaluate the inotropic effect of EHE on the contractility of the atrial myocardium and the determination of the EC_{50}. Group 2 was used to evaluate the mechanism of action of the crude extract on concentration-effect curve of the calcium chloride, the EC_{50} of the inotropic effect were expressed in duplicate with three replications.

The animals were anesthetized and were not killed by decapitation. The experiments were performed with the left atrium which was mounted on an isolated organ tank. There atrium was bathed with a modified Tyrode solution containing 120 mmol/L NaCl; 2.7 mmol/L KCl; 0.9 mmol/L $MgCl_2$; 11.9 mmol/L $NaHCO_3$; 1.37 mmol/L $CaCl_2$; 5.5 mmol/L $C_6H_{12}O_6$; 0.4 mmol/L NaH_2PO_4 and pH 7.2. The mixture was maintained at 30 ± 0.1°C and oxygenated with a carbogennic solution (95% O_2 and 5% CO_2).

The atrium were stretched to a tension of 10 mN (1.0 gf) and subjected to field stimulation of 1 Hz (Digitimer D3040, 3072). The force was recorded in a isometrical transducer: FTA10 HP / Sunborn and HUGO-SACHS F30) and the signals from the transducer were sent to an HP 8805B amplifier being then recorded in thermal application polygraph (HP 7754A, 7754B) and stored in computer (DATAQ DI400, DI 205, WINDAQ PRO) for processing "off-line".

Inotropic effect of left atrium EC_{50} determination

It was added to the bath increasing concentrations of the EHE to determine their effect on the strength of atrial contraction. To determine the variables mentioned, 50 successive contractions was selected in control situation and in test situation (EHE different concentrations) and after the 'washout'. These atrial contractions

Table 1. Metabolites and chemical reactions used in detection the extracts and fractions of *Myracrondruon urundeuva* Allemão.

Compound	Chemical reaction	Result				
		EHE	FHX	FCL	FAE	FHM
Hydrolysable tannins	Iron chloride III Alcoholic Solution	+	-	+	+	+
Flavones, flavonols and xanthones	pH change (HCl / NaOH)	-	-	-	+	-
Flavonols	pH change (HCl / NaOH)	+	-	-	+	+
Leucoanthocyanidins	pH change (HCl / NaOH) and heating	-	-	-	+	-
Flavonones	pH change (HCl / NaOH) and heating	+	-	-	+	-
Saponins	Extraction with chloroform and shaking with water	+	-	-	+	+
Triterpenoids	Liebermann-Buchard	-	-	+	-	-
Steroids	Liebermann-Buchard	-	+	-	-	-

Table 2. Antioxidant activity parameters and phenolic compounds and flavonoids contents.

Sample	IP (%)	AAI	EC_{50}(µg/mL)	µgEAG/mg of extract	µgEQ/mg of extract
Gallic Acid	93.68	32.0	1.25 ± 0.55^{c}	-	-
Hydroethanolic extract	93.28	2.47	12.11 ± 1.50^{bc}	3.13 ± 0.85^{b}	23.71 ± 0.57^{b}
Hexanic fraction	69.79	0.25	162.29 ± 5.47^{a}	0.50 ± 0.17^{a}	13.09 ± 0.20^{a}
Chloroformic fractions	77.93	1.98	20.23 ± 0.93^{b}	1.50 ± 0.10^{a}	12.92 ± 3.30^{a}
Ethylacetate fraction	81.56	5.85	6.84 ± 0.19^{c}	3.61 ± 0.10^{b}	44.62 ± 0.50^{d}
Hydromethanolic fraction	76.35	5.59	7.15 ± 0.32^{c}	3.27 ± 0.16^{b}	36.65 ± 0.90^{c}

PI was calculated concentrations of 6, 25, 200, 25, 10 and10 µg/mL respectively. Total phenolic compounds was calculated from the equation y = 0.0661 x + 0.0183 R^2 = 0.9974 and flavonoids compounds y = 0.0177 +0.066 x R^2 = 0.9957. Column were compared by one way ANOVA and Tukey post-hoc p<0.05.

were processed by CONEXON software. EC_{50} (concentration able to produce 50% of maximum effect) of the EHE was also determined in order to estimate its power. The EC50 was calculated by nonlinear regression (Cerqueira et al., 2011).

Action of extracts on concentration-effect of $CaCl_2$

To evaluate the possible calcium entry antagonism into the cells of the atrial myocardium of EHE concentration-response curves were raised by adding $CaCl_2$ to the bath cumulatively, so that the calcium concentration stay in the range of 100 to 8000 g/ml. $CaCl_2$ curves were obtained in the absence and presence of EHE. For each situation, the curve was raised inotropic response against the logarithm of the concentration of extracellular $CaCl_2$ (Cerqueira et al., 2011).

Statistical analysis

Data were expressed as a repetition in triplicate and calculated as mean ± standard deviation. To determine significant differences between means one-way ANOVA was used followed by Turkey post-test with a significance level p<0.05. Calculations were performed using Microsoft Excel 2007 and Graph PadPrism 5.0 software.

RESULTS AND DISCUSSION

Phytochemical screening

Samples from EHE, FAE and FHM had evidences of flavones, flavonols, xanthones and flavonones (Table 1), compounds known to have, among other effects, antioxidant and antimicrobial activity (Gyawali and Ibrahim, 2014). The presence of tannins has also been described in this study (Table 1) which were responsible for its anti-ulcer, anti-inflammatory, antifungal, antioxidant and repellent actions justifying the popular use of the species (Carlini et al., 2010; Vianna et al., 2003; Sa et al., 2009).

DPPH assay

AAI values <0.5 corresponds to a low antioxidant activity, AAI between 0.5 and 1.0 corresponds to a moderate antioxidant activity, IAA between 1.0 and 2.0 is strong antioxidant activity and IAA> 2.0 very strong antioxidant (Scherer and Godoy, 2009). Thus, the hydroethanolic extract and the ethyl acetate and hydrometanolic fractions can be considered strong antioxidants, the chloroform fraction very strong and the hexane fraction showed a weak antioxidant (Table 2). About antioxidant activity, the FAE and FHM and EHE had an efficient concentration 50 similar to gallic acid, showing that these samples are as potent as the positive control, but the most efficient sample was EHE 93.28% gallic acid efficiency was 93.68%.

The content of phenolic compounds exert some influence in effect, since the samples had higher

Table 3. Lipoperoxidation inhibition percentage of the extracts and fractions in different concentrations.

Concentration (µg/mL)	EHE (%)	FHX (%)	FCL (%)	FAE (%)(%)	FHM (%)	Trolox (%)
50	60.85	0.00	16.74	32.98	33.09	94.26
100	62.20	0.00	23.41	26.11	29.41	94.77
150	69.85	4.64	25.16	26.11	61.76	95.58
200	63.64	15.19	29.39	26.39	52.94	100.00

secondary metabolites contents and higher free radical inhibitions The scavenging of DPPH radicals can be correlated with the number of available hydroxyl groups. But we realize that the EHE, FAE and FHM shows differ significantly in capturing radicals, but showed no differences in total flavonoid levels ($p<0.05$). The chemical composition and chemical structure of the active component of the extract are important factors that influence the effectiveness of natural antioxidant. It is believed that the ortho-dihydroxylation contributes to the antioxidant activity of the compound (Melo et al., 2006), as in the molecule of gallic acid and its derivatives which showed the highest antioxidant potentials higher than molecules that do not exhibit ortho-dihydroxylation as ascorbic acid, kaempferol and trolox (Villaño et al., 2007).

It can be observed in Tables 1 and 2 that the samples with large variety of phenolic compounds were those which showed more antioxidant effect suggesting a possible synergism between phenolic and antioxidant effects. This behavior has been shown in phenolic compounds such as BHT (Butyl hydroxyl toluene) and BHA (Butylated hydroxyl anisole) that are synergistic with each other and BHA showing synergy with propyl gallate (Ramalho and Jorge, 2006), but more evidence is needed before exploring this hypothesis.

Induced lipid peroxidation by TBARS (thiobarbituric acid reactive substances)

Malondialdehyde (MDA) is the most frequently used biomarker of oxidative stress in many health problems such as cancer, psychiatry, chronic obstructive pulmonary disease, asthma, or cardiovascular diseases. The assay is based on a condensation reaction of two molecules of TBA with one molecule of MDA, in which the reaction rate depends on temperature, pH and concentration of TBA (Khoubnasabjafari et al., 2015).

Although there is a wide variety of phenolic compounds in extracts and fractions it was not observed by any direct proportional relationship between the amount of these chemicals and lipid peroxidation activity. Other mechanisms may be responsible for this phenomenon such as the three-dimensional structure of the molecule and the electronic stabilization derived from benzene rings (Sroka and Cisowski, 2003; Budni et al., 2007).

A single plant extract may have a different behavior depending on the evaluation method of capturing free radicals, since it may have selectivity to a specific type of radical, proving that the electron scavenging activity also depends on the radical nature and its mechanism specific action (Edenharder and Grünhage, 2003).

Only hydroethanolic extract (all concentrations) and hydromethanol fraction (150 to 200 µg/mL) were able to reduce more than 50% of the lipid peroxidation induced by ferrous sulfate (Table 3), showing that they are the most active against the peroxide hydrogen and hydroxyl radicals produced by ferrous sulfate during the Fenton reaction (Lima et al., 2013).

Antimicrobial activity (model agar diffusion)

Flavonoids are phenolic compounds known to be synthesized by plants in response to microbial infection (Scalbert, 1991; Cowan, 1999). The antimicrobial mechanism of action is based on three assumptions: (1st) They inhibit bacterial enzymes directly or complexing with the substrate of them; (2nd) The hydroxyl (eOH) groups in phenolic compounds can interact with the cell membrane of bacteria to disrupt membrane structures and cause the leakage of cellular components; (3rd) They complex with metallic ions decreasing the availability of essential ions to microbial metabolism. Other mechanism that could be pointed out is the delocalization of electrons which then act as proton exchangers and reduce the gradient across the cytoplasmic membrane of bacterial cells. This will cause the collapse of the proton motive force and depletion of the ATP pool and ultimately leading to cell death (Scalbert, 1991; Gyawali and Ibrahim, 2014).

Overall, the extract and its fractions showed no difference between the mean inhibition zones between Gram+ and Gram-, suggesting that the peptidoglycan barrier is of little relevance to confer resistance to extract and *M. urundeva* fractions. On the other hand, it cannot be unconsidered of other protection mechanisms are involved such as efflux pump, enzymatic modifications and modifications of target sites that can be resistant to microorganism metabolites (Garvey et al., 2011). The specific mechanisms involved in the antimicrobial action of terpenes remain poorly characterized (Helander et al., 1998; Silveira et al., 2006).

For *Candida albicans,* as is shown in Table 4 that hydroethanolic extract and its ethyl acetate fraction showed the best results against the growth of this fungi

Table 4. Inhibition zone measurements (mm) of hydroethanolic extract, ethyl acetate fraction and hydromethanol fraction of the stem bark of *M. urundeuva* (Allemão) by agar diffusion method against Gram (+) and Gram (-) microorganisms and fungus.

Microorganism	EHE 50 mg/mL	EHE 100 mg/mL	FAE 50 mg/mL	FAE 100 mg/mL	FHM 50 mg/mL	FHM 100 mg/mL	Tetraciclin® 25 mg/mL
E. faecalis (+)	15.5±0.71^{b}	16.0±1.41^{b}	16.0±1.41^{b}	18.0±1.41^{b}	-	12.5±2.12^{c}	23.0±0.00^{a}
S. aureus (+)	14.0±0.00^{b}	15.50±0.71^{b}	16.5±2.20^{b}	19.0±0.00^{b}	13.5±3.54^{b}	12.5±2.12^{b}	28.0±0.00^{a}
E. coli (-)	-	-	-	-	-	-	24.0±1.41
P. aeruginosa (-)	16.0±1.41^{b}	14.0±1.41^{b}	16.0±0.00^{b}	18.5±0.71^{b}	-	-	24.5±0.71^{a}
P. mirabilis(-)	-	-	-	-	-	-	16.5±0.71
K. pneumonia (-)	15.0±0.00^{b}	16.0±1.41^{b}	16.0±1.41^{b}	20.5±0.71^{c}	11.0±0.00^{d}	15.5±2.12^{b}	32.0±0.00^{a}
C. albicans (Fungus)	17.0±1.41^{b}	16.5±0.71^{b}	17.0±0.00^{b}	19.5±1.41^{a}	14.0±0.00^{b}	14.0±0.71^{b}	23.5±0.71^{a}

Fields with traces indicate that the halo in this extract or fraction replaces values less than 8.0 mm. Positive Control: Tetracycline (25 mg / mL). These samples are not antimicrobial effect for that microorganism. Table lines were compared by one way ANOVA and Tukey post-hoc $p<0.05$.

(presenting no significant difference in comparison with the positive control in 100 mg/mL concentration). Botelho et al. (2007) found that combined extracts of *M. urundeuva* and Lippia sidoides gel decrease of periodontal microorganism growth. Botelho et al. (2007) and Fontenele et al. (2007) which are largely attributed to the presence of thymol and carvacrol. In accordance with our data, a double-blind clinical trial has demonstrated that a mouth-rinse of *Lippia sidoides* EO decreases salivary *Streptococcus mutans* levels as well the clinical scores of gingival health in humans as compared to 0.12% chlorhexidine.

Within the samples FAE has the larger variety of phenolic compounds, presenting 6 subclasses, suggesting that there is some synergistic effect of these metabolites on antimicrobial activity, these data agrees with Sato et al. (1996) which says the more hydroxylation, the greater the antimicrobial activity. However Nitiema et al. (2012) point out that crud extracts has lower antimicrobial activity than isolated molecules such as quercetin and coumarin against enteric bacteria. Cowan (1999) supposed that phenolic compounds without free hydroxyl groups have more antibacterial activity than those which are provided that increases their chemical affinity to microbial lipid membrane. It is safe to say that there is no clear predictability for the degree of hydroxylation and toxicity to microorganisms.

The increasing concentration of phenolic compounds enhanced antibacterial activities. These results are in agreement with earlier investigations which showed that antimicrobial agents with high activity against an organism has a low minimum inhibitory concentration while a low active antimicrobial agent gives a high minimum inhibitory concentration (Banso and Adeyemo, 2007; Nitiema et al., 2012).

High performance liquid chromatography (HPLC) and identification by UV

Fractions that showed better antioxidant effects in both lipid peroxidation and DPPH were analyzed by HPLC in order to obtain a better definition of its chemical components, which are possibly responsible for the redox-active power.

The UV spectra of the substances found in the hydroethanolic extract (Figure 1) exhibited flavones characteristics, presented since I band (300 to 400 nm) with shoulder presence and flavonoids, with I band (352 to 285 nm). Since there is no significant structural difference in the chemical structures of these two classes, like its spectrophotometric signals is difficult to identify with this technique (Ugaz, 1994; Grotewold, 2006).

The spectrum peak found at 38.94 min in the chromatogram of the hydroethanolic extract (Figure 2) has a similar to standard found by terpenoid compounds found in essential oils. For terpenes, the UV spectrum showed strong peak absorption between 202 and 210 nm and longer wavelengths to be indicative of saturated compounds or of the presence of isolated unsaturation between 215 and 250 nm, the presence of unsaturated compounds, and between 250 and 270 nm, the presence of aromatic compounds (Ugaz, 1994; Simões et al., 2001).

Although it is common to obtain these compounds from extractions vapor dragging, maceration with nonpolar organic solvents maceration in 90% ethanol has an extractive power high enough to remove polar and nonpolar compounds from plants, which makes it a good method for investigating a large number of compounds to plants. Like Pietta et al. (1990) who purified major diterpene extracts in acetone *Ginkgo biloba* L. (bilobalídeo a ginkgolídeo, ginkgolídeo B and C) from a C18 column by isocratic elution using 2-propanol: water (10:90) as the eluent and a DAD detector identification, despite the limitations of using this detection technique for terpenes (Ugaz, 1994).

Signals obtained from the hydromethanol fraction (Figure 3) and hydroethanolic extract (Figure 4) showed profiles similar to those of tannins as gallic acid, tannic

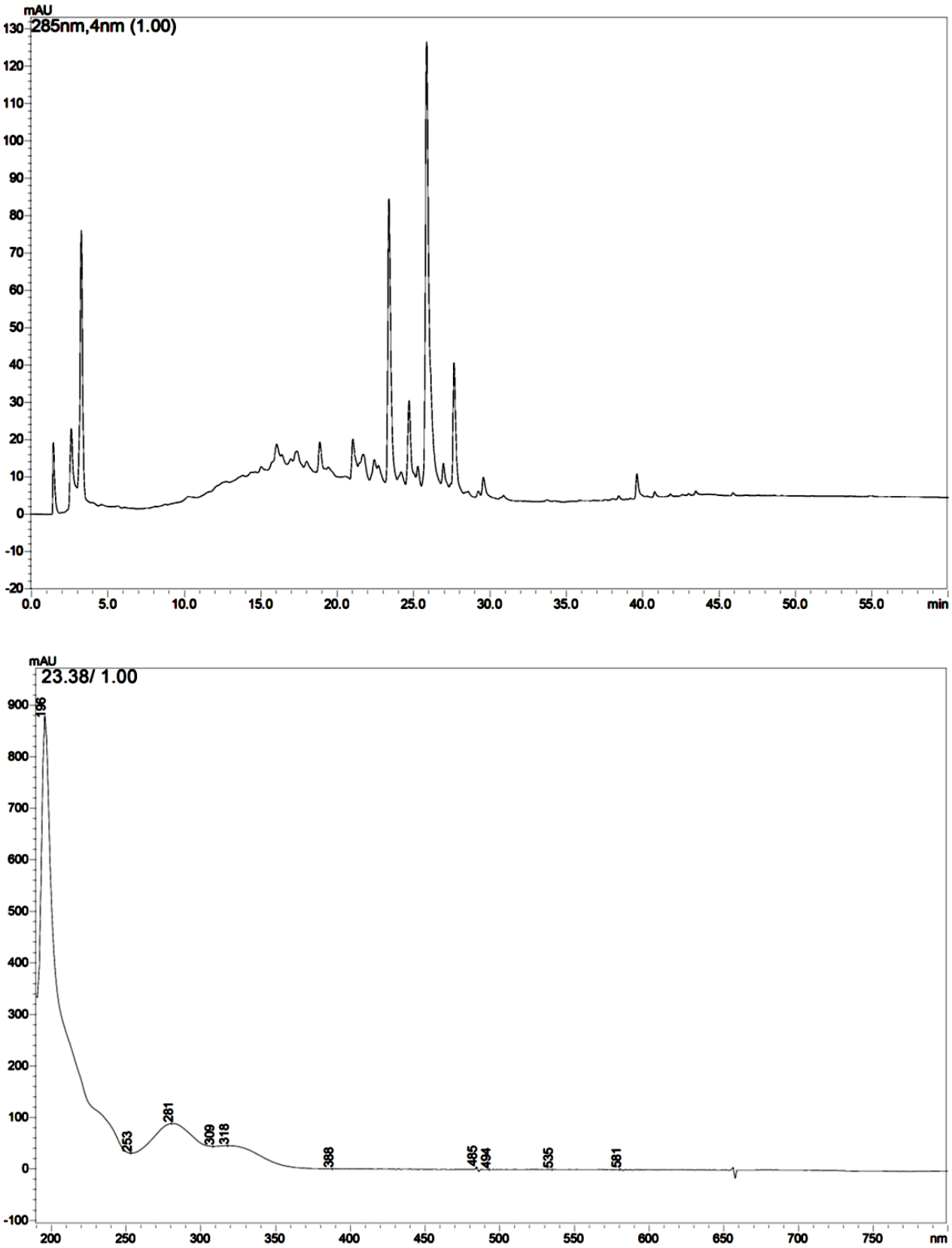

Figure 1. Chromatogram of the hydroethanolic extract at a wavelength of 285 nm and UV spectrum at 23.38 min retention time. Analysis conditions were exploratory gradient 20-100% CH_3OH from 0 to 40 min and isocratic condition using 100% CH_3OH from 40 to 60 min.

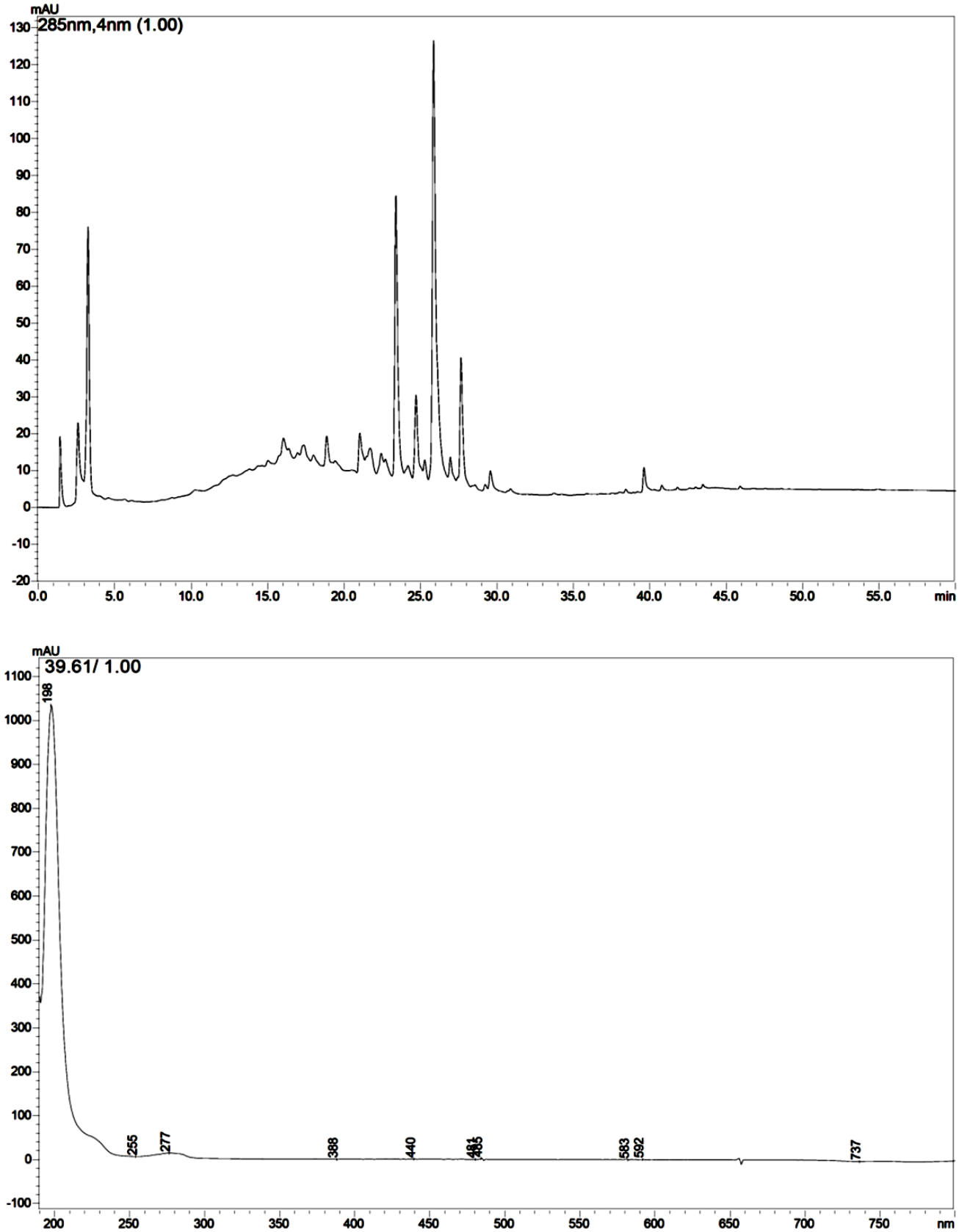

Figure 2. Hydroethanolic extract the chromatogram and UV spectrum at 38.94 min retention time. Analysis conditions were exploratory gradient 20 to 100% CH_3OH from 0 to 40 min and isocratic condition using 100% CH_3OH from 40 to 60 min.

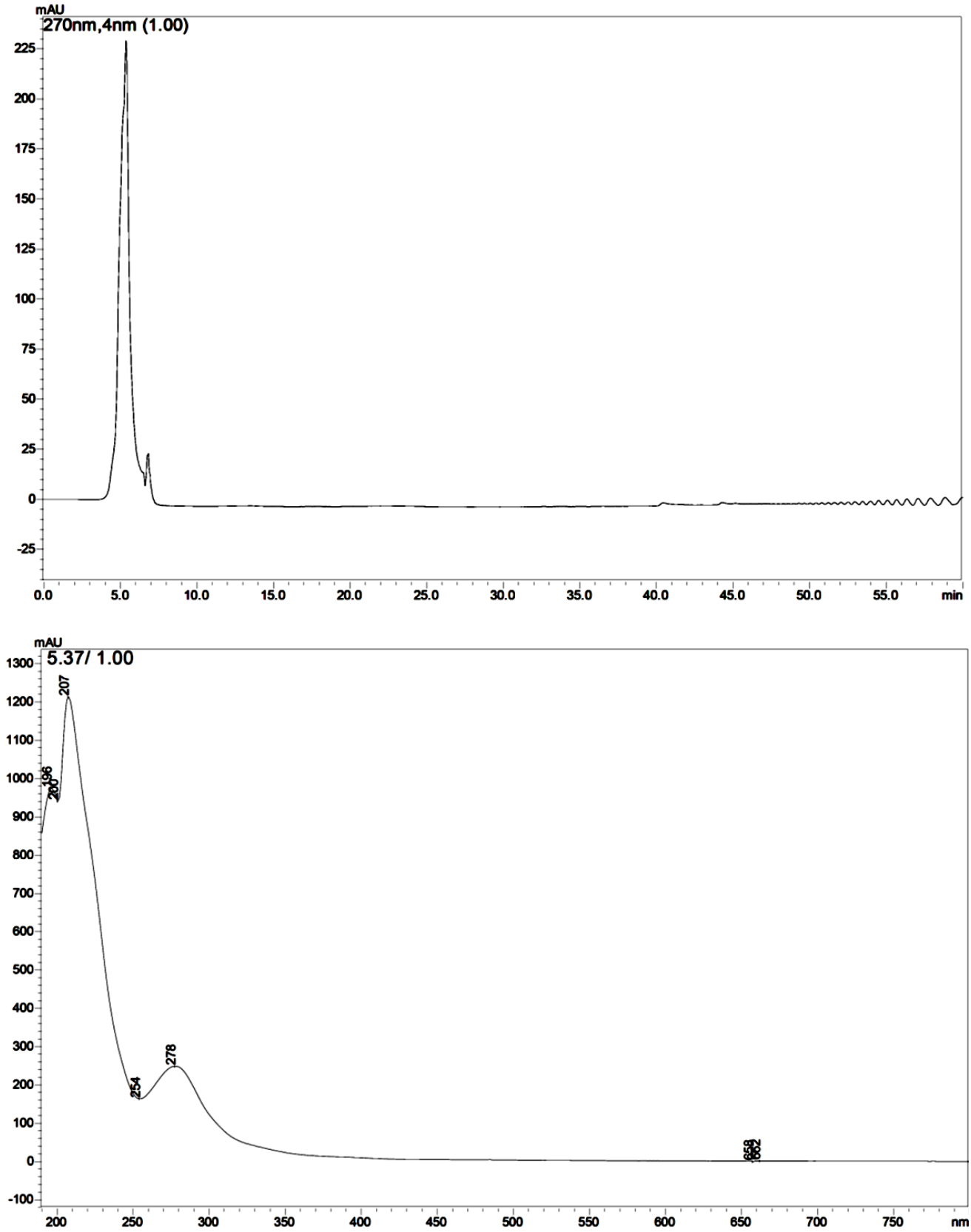

Figure 3. Chromatogram of hydromethanolic fraction at a wavelength of 270 nm and UV spectrum at 5.37 min retention time. Analysis conditions were exploratory gradient 20 to 100% CH_3OH from 0 to 40 min and isocratic condition using 100% CH_3OH from 40 to 60 min.

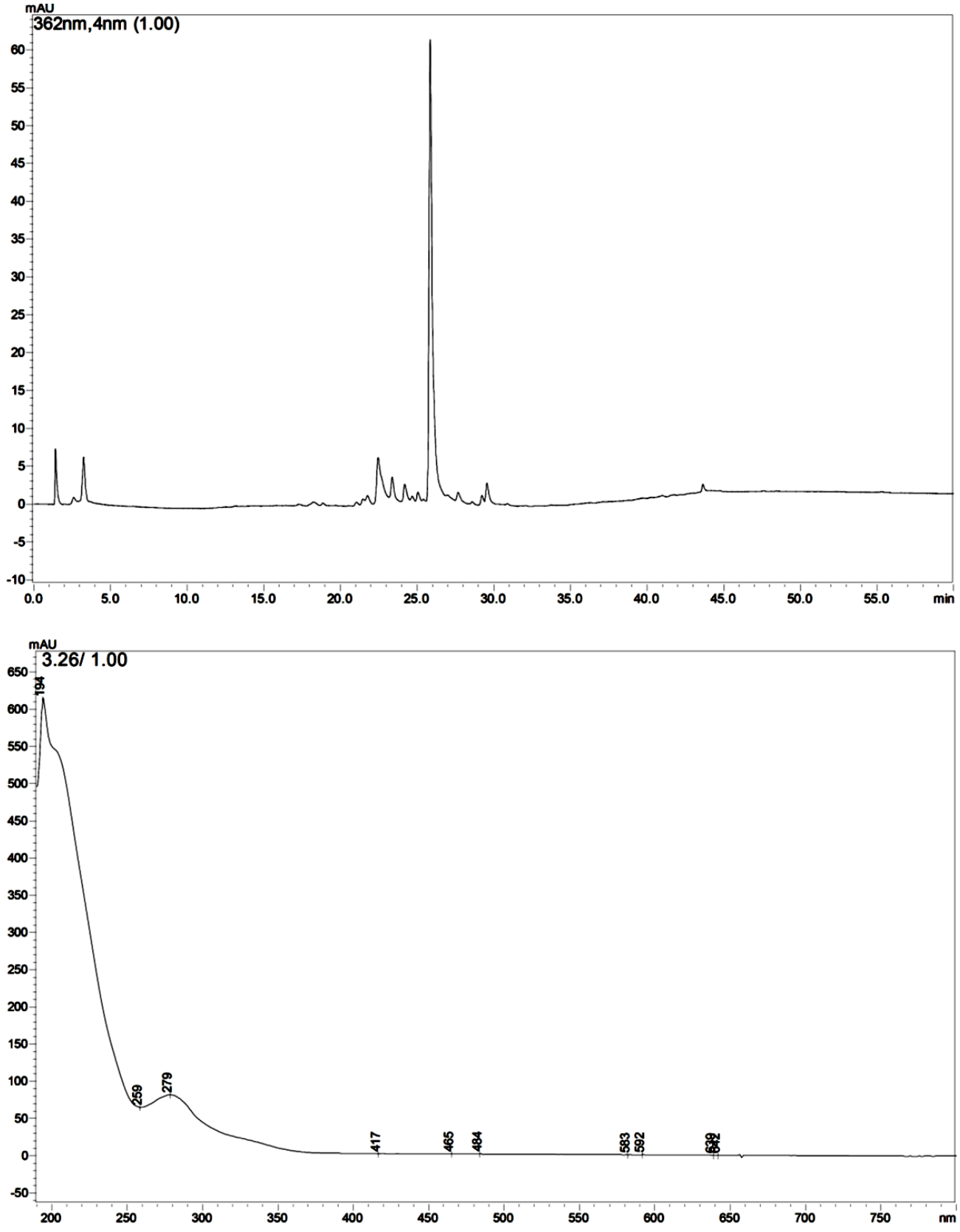

Figure 4. Chromatogram wavelength of 362 nm and UV spectrum of the peak present in the hydroethanolic extract at 3.26 min retention time. Analysis conditions were exploratory gradient 20-100% CH3 OH from 0 to 40 min and isocratic condition using 100% CH_3OH from 40 to 60 min.

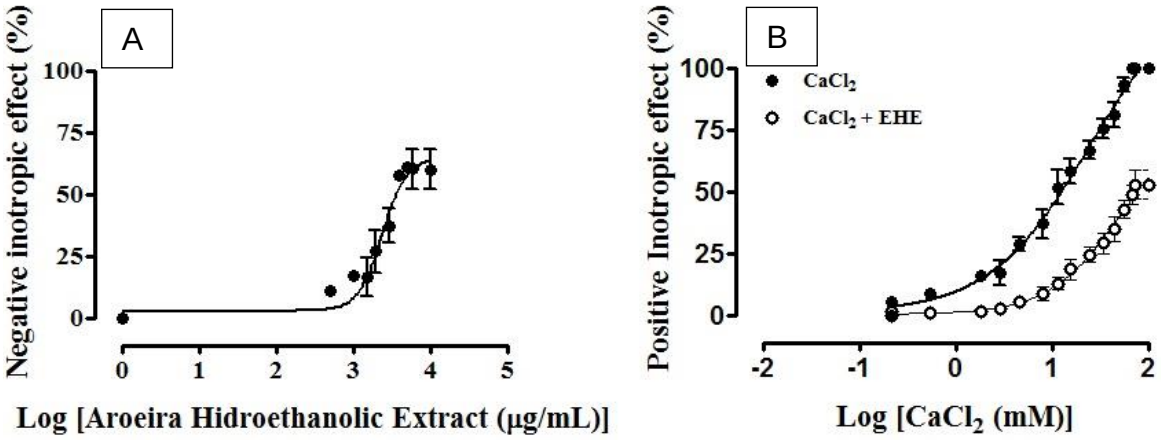

Figure 5. Inotropic effect of Aroeira hidroethanolic extract (A) and positive inotropic effect displacement of Calcium Chloride and Calcium Chloride with Aroeira Hidroethanolic extract/EHE (B).

acid, catechin and pyrogallol Ramos-Tejada et al. (2002). Queiroz et al. (2002) using HPLC, identified fisetin, gallic acid and ellagic acid, in hydromethanolic and hydroacetonic extract *M. urundeuva*. Their results show that aroeira preta contains a high amount of tannins, which was detected in the phytochemical crude extract to polar fractions (Table 1).

Inotropic effect

The development of atherosclerosis depends on the balance between proinflammatory; anti-infl ammatory, and antioxidative defense mechanisms, in this context Hydroethanolic extract has achieved good results in all tests related to antioxidant activity because of this it was chosen to checking it inotropic effect in guinea pig left atrium (Rahman, 2007).

Isometric strength of atrial contraction in the presence of the extract was reduced by 60%, indicating a negative inotropic effect concentration-dependent and reversible after washing, with an EC_{50} of 2080 ± 600 mg / mL (Figure 5A). The negative inotropic medications, that is, beta blockers, diltiazen, verapamil, decrease the heart's effort by reducing the frequency and strength of heartbeat and thereby decreasing the amount of blood pumped by the heart, blood pressure in the vessels and the amount of oxygen that the heart uses (Bombig and Póvoa, 2009). It investigated the possible mechanism of action from a relative efficacy of 50% on the inotropic effects produced by $CaCl_2$. Figure 5B showed that there was a displacement of almost twice the EC50 for the positive inotropic effect, going from 3250 ± 727 µg/ml in the control $CaCl_2$ only, to 6240 ± 854 µg/mL for $CaCl_2$ treatment and the extract, characterized the mechanism as calcium channel antagonism. Because the maximum effect not be maintained with the extract of the increase, it was revealed that this antagonism is not competitive, meaning that the extract acts in a different location agonist ($CaCl_2$) (Vauquelin et al., 2002).

Among the secondary metabolites found in the plant in phytochemical screening (Table 1) and literature are discriminated flavonoids, saponins, terpenoids and tannins. Chen (1994) showed that saponins can reduce the contractility of atrial muscle of guinea pig, and reduce the inotropic effect produced by both isoprenaline mechanism and $CaCl_2$ channel blocking. There are reports that isoflavone gistein directly inhibits the influx of calcium in ventricular myocyte of guinea pig, while reducing the probability of open state of the channel without affecting the average time of opening the channels or variations in conductance of the membrane (Chiang et al., 1996)

Conclusion

The plant in general showed antioxidant, antimicrobial and negative inotropic due to secondary metabolites indicated by chemical studies (phytochemical and UV spectrophotometry) effect.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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