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Chemical characterization, antimicrobial activity and cardiac effects in rat heart of the ethyl acetate fraction of Syzygium cumini (L.) Skeels

Caracterização química, atividade antimicrobiana e efeitos cardíacos em coração de rato da fração acetato de etila de *Syzygium cumini* (L.) Skeels

Caracterización química, actividad antimicrobiana y efectos cardiacos en corazón de rato de la fracción de acetato de etilo de *Syzygium cumini* (L.) Skeels

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Abstract
Plants are important sources of secondary metabolites responsible for various biological activities. In this research, was evaluated the effect action ethyl acetate fraction of *Syzygium cumini* leaves (Sc-AcOEt) in the antimicrobial activity, contractile and electrical effects in isolated rat hearts and the chemical characterization. Antimicrobial activity was assessed using the disk-diffusion method and the determination of the minimum inhibitory concentration. The contractile and electrical effects were tested using the Langendorf method. The chemical characterization was evaluated through quantification and HPLC analyzes. *P. aeruginosa* was not viable in any concentration of the fraction (MIC 0,125 mg/mL), while *S. aureus* showed a MIC of 0.5 mg/mL. *K. pneumoniae* and *E. coli* had the highest MIC (1 mg/mL). A reduction in LVDP was observed after heart perfusion with 0.1 mg/mL of Sc-AcOEt. No change was observed in the duration of systole and diastole at all concentrations and in the electrocardiographic parameters. Total phenols, total flavonoids and total flavonols contents of Sc-AcOEt, calculated from the respective calibration curves, were 21.556µg/mg AGE, 617.222µg/mg QE and 315,222µg/mg RE, respectively. In addition, the HPLC analysis of Sc-AcOEt showed the presence of gallic acid, quercetin-3-glucoside, naringin and myricetin as main components. Thus, the Sc-AcOEt has important antimicrobial and cardiac activities possibly due to its chemical constituents.
Keywords: Jambolão; Phenolic compounds; Antimicrobial; Cardiac contraction.

Resumo
As plantas são importantes fontes de metabólitos secundários responsáveis por diversas atividades biológicas. Nesta pesquisa, foi avaliado o efeito da fração acetato de etila das folhas de *Syzygium cumini* (Sc-AcOEt) na atividade

antimicrobiana, efeitos contráteis e elétricos em corações isolados de ratos e a caracterização química. A atividade antimicrobiana foi avaliada pelo método de disco-difusão e pela determinação da concentração inibitória mínima. Os efeitos contráteis e elétricos foram testados pelo método de *Langendorf*. A caracterização química foi avaliada por meio de quantificação e análises de HPLC. *P. aeruginosa* não foi viável em nenhuma concentração da fração (CIM 0,125 mg/mL), enquanto *S. aureus* apresentou CIM de 0,5 mg/mL. *K. pneumoniae* e *E. coli* tiveram a CIM mais alta (1 mg/mL). Observou-se um efeito inotrópico negativo após perfusão cardíaca com 0,1 mg/mL de Sc-AcOEt. Não houve alteração na duração da sístole e diástole e nos parâmetros eletrocardiográficos em todas as concentrações. Os teores de fenóis totais, flavonoides totais e flavonóis totais de Sc-AcOEt, calculados a partir das respectivas curvas de calibração, foram 21,556µg/mg AGE, 617,222µg/mg QE e 315,222µg/mg RE, respectivamente. Além disso, a análise por HPLC de Sc-AcOEt mostrou a presença de ácido gálico, quercetina-3-glicosídeo, naringina e miricetina como componentes principais. Assim, a Sc-AcOEt possui importantes atividades antimicrobiana e cardíaca possivelmente devido aos seus constituintes químicos.
Palavras-chave: Jambolão; Compostos fenólicos; Antimicrobiano; Contração cardíaca.

Resumen
Las plantas son fuentes importantes de metabolitos secundarios responsables de diversas actividades biológicas. En este investigación se evaluó el efecto de la fracción de acetato de etilo de hojas de *Syzygium cumini* (Sc-AcOEt) sobre la actividad antimicrobiana, efectos contráctiles y eléctricos en corazones aislados de rata y caracterización química. La actividad antimicrobiana se evaluó por el método de difusión en disco y por la determinación de la concentración inhibitoria mínima. Los efectos contráctiles y eléctricos se probaron mediante el método de *Langendorf*. La caracterización química se evaluó mediante cuantificación y análisis HPLC. *P. aeruginosa* no fue viable a ninguna concentración de la fracción (MIC 0,125 mg/mL), mientras que *S. aureus* tuvo una MIC de 0,5 mg/mL. *K. pneumoniae* y *E. coli* tuvieron la MIC más alta (1 mg/mL). Se observó un efecto inotrópico negativo después de la perfusión cardíaca con 0,1 mg/mL Sc-AcOEt. No hubo cambios en la duración de la sístole y la diástole y en los parámetros electrocardiográficos en todas las concentraciones. Los contenidos de fenoles totales, flavonoides totales y flavonoles totales de Sc-AcOEt, calculados a partir de las respectivas curvas de calibración, fueron de 21,556 µg/mg AGE, 617,222 µg/mg QE y 315,222 µg/mg RE, respectivamente. Además, el análisis HPLC de Sc-AcOEt mostró la presencia de ácido gálico, quercetina-3-glucósido, naringina y miricetina como componentes principales. Por lo tanto, el Sc-AcOEt tiene importantes actividades antimicrobianas y cardíacas posiblemente debido a sus constituyentes químicos.
Palabras clave: Jambolan; Compuestos fenólicos; Antimicrobiano; Contracción cardíaca.

1. Introduction

Plants are an inexhaustible source of phytoconstituents. These have important biological activities and are used by the population to treat various diseases (Batiha et al., 2020). Among these biological activities, the anti-inflammatory, antimicrobial, virucidal, fungicidal, spasmolytic, sedative, analgesic, and local anesthetic are stand outs (Beshbishy et al., 2019). In addition, to identify phytochemicals has been the target of science for the discovery of new drugs to treat diseases.

In the search for therapeutic substances of plant origin, we can highlight those that have antimicrobial activity. In recent decades, the resistance of microorganisms to conventional antibiotics has been increasing and this has been one of the greatest global threats to health, food safety and development (Han & Parker, 2017; Santos et al., 2020). Antibiotic resistance is natural and occurs through genetic mutations in microorganisms. However, the indiscriminate use of these substances has accelerated this process and an increasing number of infections, such as pneumonia, are becoming increasingly difficult to treat, as the antibiotics used have become less effective (Santos et al., 2020; B. Wu et al., 2020). Antibiotic resistance leads to longer hospital stays, increased medical costs, and increased mortality. Given this scenario, the search for new substances with antimicrobial potential becomes urgent. It is known that some groups of secondary metabolites, such as tannins, have the ability to inhibit bacterial proliferation through cell wall degradation, being considered as promising antimicrobial agents (Salim, 2017; Santos et al., 2020).

As observed for antimicrobial compounds, another area that has gained prominence is that related to cardiovascular diseases, as these are currently the main causes of death in the world. An estimated 17.9 million people died from cardiovascular disease in 2016, representing 31% of all deaths globally. Of these deaths, an estimated 7.4 million are due to cardiovascular disease and 6.7 million are due to stroke (Who, 2020). Although there are several drugs for these diseases, the

search for substances that act on the cardiovascular system becomes fundamental in the search for new therapeutic alternatives as well as to potentiate existing substances in combined therapies (B. Wu et al., 2020).

Syzygium cumini (L.) skeels, popularly known as jambolão, is a plant belonging to the Myrtaceae family, which is originally from the Middle East and widely distributed in Brazil, is used by the population to treat rheumatism, diabetes and diseases of the digestive system (Qamar, et al., 2022; Singh et al., 2016). Several studies have tried to prove the pharmacological properties of this plant, as well as chemically characterize its components, and the literature reports the occurrence of compounds such as tannins, flavonoids, alkaloids, and anthocyanidins (Chhikara et al., 2018) and polyphenolic compounds (Qamar, et al., 2022) that are effective against cancer, cardiovascular disease and diabetes (Li et al., 2021; Monteiro et al., 2018; Nahid et al., 2017).

In the present research, the antimicrobial activity, and contractible and electrical effects in rat heart of ethyl acetate fraction of *Syzygium cumini (l.)* skeels were evaluation. In addition, chemical characterization was performed to identify the main compounds involved in biological activities.

2. Methodology

Plant extract

Syzygium cumini (L.) Skeels leaves were collected in September 2018, at the Federal University of Sergipe, located at latitude -10.925675 and longitude -37.099631 WGS84, municipality of São Cristóvão (Sergipe, Brazil). The specimen was compared to one collected at the same location and identified by the Herbarium of the Federal University of Sergipe (voucher ASE6765). The samples were washed with sterile distilled water and kept in an oven with air circulation at 37ºC until complete dehydration. Afterwards, they were crushed separately and the resulting powder was mixed with 80% Ethanol. After the extraction period, the hydroethanolic extract obtained was filtered and concentrated in a rotary evaporator under reduced pressure at ± 50°C. To obtain the ethyl acetate fraction (Sc-AcOEt), part of the hydroethanolic extract concentrated (27.9 g) was dissolved in 40% methanol (in water, v/v) and subjected to liquid-liquid extraction with ethyl acetate.

Antibacterial Activity

Microorganisms

Antimicrobial activity was evaluated against *Staphylococcus aureus* ATCC25923 (Gram+), *Streptococcus agalacteae* ATCC13813 (Gram+), *Enterococcus durans/hirae* SS1225/IAL03/10 (Gram+), *Klebisiella pneumoniae* derived ATCC700603 (Gram-), classic enteropathogenic *Escherichia coli* 0111 (Gram-), *Pseudomonas aeruginosa derived* ATCC27853 (Gram-) and *Enterobacter aerogenes* ATCC13048 (Gram-). The bacteria were maintained in Müller-Hinton Agar medium, in an inclined test tube, under refrigeration at 4°C.

Disc difusion test

The evaluation of antimicrobial activity was performed using the agar diffusion test proposed by (Bauer et al., 1966), which is a qualitative assay. Cultures were seeded with sterile swab in Petri dishes containing Muller-Hilton Agar medium at pH 7.2 - 7.4. Subsequently, sterile paper discs were deposited on the surface of the culture medium inoculated with microorganisms and soaked with 20µL of the sample at different concentrations. As a negative control, all reagents in use for sample dilution were used, while the antibiotic gentamicin (GENT) was used for the positive control. After incubation, measurements of inhibition halos were performed and the interpretation of results was given according to NCCLS standards (2018).

Minimum Inhibitory Concentration (MIC) test

The MIC was determined by the dilution technique in microplates (96 wells) according to the methodology described according for the M7-A6 standard of the Manual 38 of the Clinical and Laboratory Standards Institute (CLSI, 2015) for aerobic bacteria, with some modifications. The microplate wells (96 wells) were filled with 80 µL of Mueller Hinton Broth. Then, 100 µL of the solutions of the most prominent fraction in the disc diffusion test were added and a serial dilution from 1 to 0.125 mg/mL (2:2) was performed. To these wells, 20 µL of the microorganism suspensions were added. Culture medium control, bacterial growth control, sample control and negative control (solvents) were performed. GENT was used as a positive control at an adequate concentration for each microorganism. The sample was tested in triplicate on each microplate. The MIC was considered as the lowest concentration of the sample or controls capable of inhibiting the growth of 90% of the strains (Hörner et al., 2008). The microplates were incubated in an oven at 37ºC for 24 h. Resazurin developer (100 µg/mL) was added to each well of the microplates with the bacteria (30 µL). In the course of 2 h, the presence of blue color represents absence of growth and pink color, presence of bacterial growth (Palomino et al., 2002). The test was performed in triplicate.

Contractile and electrical effects on the rat heart

Five Wistar rats (250 - 300 g) were obtained from the Sectorial Animal Facility of the Federal University of Sergipe and allocated in the Animal Facility of the Laboratory of Biophysics of the Heart. The animals, kept in a 12-hour light-dark cycle at room temperature (20 - 25ºC), had free access to water and food. The use of animals in this experiment was approved by the Animal Research Ethics Committee of the FUS (Protocol number: 7227120520). Its execution was carried out under the regulations of the National Council for the Control of Animal Experimentation (CONCEA) and the recommendations of the Department of Physiology/Center of Biological Sciences and Health/FUS.

After 30 minutes of administration of heparin (1000 I.U/kg, i.p), the rats were euthanized (guillotine decapitation). The thorax was opened and the heart was mounted in a Langendorff constant flow aortic perfusion system (10 mL/min, peristaltic pump, Milan), perfused with Krebs-Henseleit (K-H) solution, aerated by a carbogenic mixture (95% O_2 and 5% CO_2 oxygen), heated to 37 ± 0.1 °C (HAAKE C/F3 pump) and filtered in millipore (0.45 mm). To record the electrocardiogram (ECG), three electrodes (Ag/AgCl/NaCl 1 M) were placed on the heart to sense electrical signals. The signals were amplified and digitalized (PowerLab 4/35 ADInstrument, USA). Left ventricular developed pressure (LVDP) was measured using a water-filled balloon (15 cm/Hg) introduced into the cavity of the left ventricle. The signals were capted by pressure transducer (MLT0699/A), amplified (Bridge Amp FE221 ADInstrument, USA) and sent to an AD converter (PowerLab 4/35 26 ADInstrument, USA). The system was calibrated using a column of mercury. Contractile parameters were evaluated in 30 consecutive beats using LabChart 8.0 ProSoftware (ADInstruments, USA) in control situation, after *Sc-AcOEt* perfusion (0.001 to 0.1 mg/mL) and during the washout.

Quantification of total phenols, flavonids and flavonols

Total phenols (TP)

The determination of the total phenol content present in the Sc-AcOEt was carried out through spectrophotometry in the visible region using the Folin-Ciocalteau reagent, according to Sousa et al., 2007 with minor modifications.

For this purpose, 1 mg/mL solutions of the tested sample were prepared. A 0.4 mL aliquot of these solutions was transferred to a Falcon tube where 2.5 mL of 10% Folin-Ciocalteau reagent were added. Then, the solution was homogenized by stirring. Afterwards, the Falcon tube was placed in a water bath at 50°C for 7-10 minutes. Subsequently, 2 mL of 15% Na_2CO_3 were added to the mixture and it was stirred for 30 seconds before the absorbance was read at 750 nm. As a blank, water and the other reagents were used, except for Sc-AcOEt. Analyzes were carried out in triplicate and in three replications,

whose total phenols content was determined by interpolation of the average absorbance against a calibration curve constructed with gallic acid standards (100 to 500 mg/mL) and expressed as mg of gallic acid equivalents per mg of sample (GAE mg/mg sample). The resulting equation for this calibration curve was Y = 0.0158X +0.0912; r^2 = 0.9966, where Y is absorbance and X is gallic acid concentration. The test was performed in triplicate.

Total flavonoids (TF)

To determine the total flavonoids (TF) present in the Sc-AcOEt, the method that leads to the formation of the flavonoid-aluminum complex was used (Mbaebie et al., 2012). For that, 0.5 mL of 2% $AlCl_3$ solution (w/v in ethanol) was mixed with 0.5 mL of the sample at a concentration of 1 mg/mL, separately. Absorbance was measured at 420 nm in a UV-VIS spectrophotometer. The analyzes were carried out in triplicate and in three replications. The TF content was determined by interpolation of the average absorbance of the sample against a calibration curve constructed with the quercetin standard (200 to 1000 μg/mL). The TF content was expressed as mg of quercetin equivalent per mg of sample (QE μg/mg sample). The equation obtained was Y=0.0008X+0.03; r^2 = 0.9998, where Y is the absorbance and X is the concentration of quercetin. The test was performed in triplicate.

Total flavonols (TFL)

The total flavonol (TFL) content was determined according to the method of Kumaran & Karunakaran, (2007). A volume of 2 mL of Sc-AcOEt at a concentration of 1 mg/mL was mixed with 2 ml of 2% $AlCl_3$ (w/v in ethanol) and 3 ml of 50 g/l sodium acetate. The mixture was incubated at 20°C for 2.5 h. Soon after, the absorbances were read at 440 nm in a UV-VIS spectrophotometer. The analyzes were carried out in triplicate and in three replications. The total flavonols (TFL) content was expressed as μg rutin equivalent per mg sample (RE μg/mg sample) using the calibration curve Y=0.0012X+0.1072; r^2 = 0.9967 against a rutin standard (100 to 500 μg/mL), where Y is absorbance and X is concentration. The test was performed in triplicate.

High performance liquid chromatography

The chromatographic analysis was performed using a Shimadzu HPLC System constituted of two Shimadzu pump (LC-20AD and LC-20AT), degasser (DGU-20A3), autosampler (SIL-20AT) and diode array detection (DAD) module (SPD-M20A). The software was Lab Solutions (versão 1.24 SP2) from Shimadzu Technologies. The compounds were analyzed in the range of 190-800 nm. A Phenomenex Kinetex C18 column (250 cm x 4.60 mm, 5 μm) and mobile phases consisting of 1% acetic acid in water (pump A) and 1% acetic acid in acetonitrile (pump B) were used. For the gradient elution, the following program was used: 0.0 - 1.0 min, 5% B; 1.0 - 5.0 min, 10% B; 5.0 - 7.0 min, 10% B; 7.0 - 12.0 min, 20% B; 12.0 -15.0 min, 20%; 15.0 - 20.0 min, 30%; 20.0 - 22.0 min, 30% B; 22.0 - 27.0 min, 40% B; 27.0 -30.0 min, 40% B; 30.0 -35.0 min, 5% B (mixture with A). The elution flow was 0.6 mL/min and injection volume was 10 μL.

Statistical treatment of data

For the systematization and observation of the results, data were tabulated and the relevant graphs were made with them (Excel, GraphPad Prism). All data were expressed as mean ± Mean Standard Error. For the statistical analyses, the one-way ANOVA test was used followed by the Tukey test. Probability values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

Antibacterial Activity

Disc difusion test

The disc diffusion test revealed that Sc-AcOEt inhibited the bacterial growth. Silva et al. (2011) reported that the sensitivity of a microorganism to an antibiotic can be classified, according to the diameter of the growth inhibition halo, as: low (≤8 mm), moderate (8.1–10 mm), high (10.1–15 mm) and very high (≥15.1 mm). Thus, based on this classification, it is possible to observe in Table 1 that Sc-AcOEt presented moderate to very high sensitivities.

Table 1 - Growth inhibition halos from different bacteria of medicinal importance against the Ethyl acetate fraction (Sc-AcOEt) of the *S. cumini* (L.) Skeels leaf by the disk diffusion test.

Bacterium	**Inhibition halos (mm)**	
	GENT	Sc-AcOEt
S. aureus (+)	23	16
S. agalacteae (+)	24	7
E. durans (+)	21	9
K. pneumoniae (-)	23	20
P. aeruginosa (-)	22	15
E. coli (-)	24	16
E. aerogenes (-)	21	9

Source: Authors.

In this context, among the gram + bacteria, *S. aureus* stands out for presenting very high sensitivity to Sc-AcOEt with the highest inhibition halo (16 mm), while *S. agalacteae* presented low sensitivity towards the extract with the smallest inhibition halo (7 mm). As for Gram-, *K. pneumonia, P. aeruginosa* and *E. coli* bacteria showed very high sensitivity to Sc-AcOEt, with inhibition halos ranging from 15 to 20 mm, while *E. aerogenes* showed moderate sensitivity with an inhibition halo of 9 mm.

Minimum Inhibitory Concentration (MIC) test

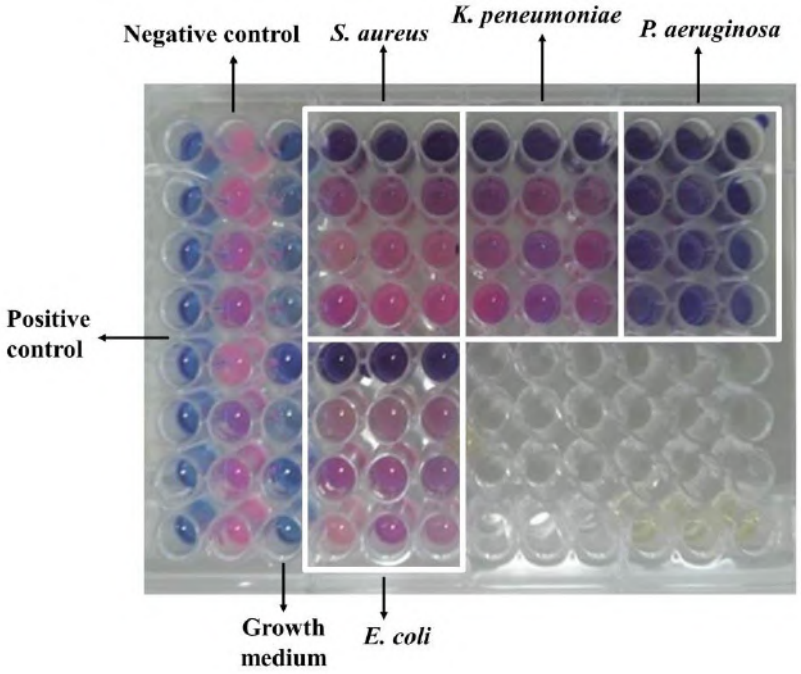
Sc-AcOEt MICs were determined using resazurin as an indicator for cell viability in 96 well microplates. This phenoxazine dye has a blue color and can be reduced to pink colored resorufin in the presence of viable cells as examples shown in Figure 1. Table 2 shows that *P. aeruginosa* was not viable in any concentration of the extract (MIC 0,125 mg/mL), while *S. aureus* showed a MIC of 0.5 mg/mL. *K. pneumoniae* and *E. coli* had the highest MIC (1 mg/mL).

Table 2 - Minimum inhibitory concentration (MIC) observed for the ethyl acetate fraction (Sc-AcOEt) obtained from *S. cumini* leaf. (-) indicates no growth and (+) indicates growth.

Bacterium	Sc-AcOEt **(mg/mL)**				GENT **(mg/mL)**				Negative control **(mg/mL)**			
	1	**0.5**	**0.25**	**0.125**	**1**	**0.5**	**0.25**	**0.125**	**1**	**0.5**	**0.25**	**0.125**
S. aureus	-	-	+	+	-	-	-	-	+	+	+	+
K. pneumoniae	-	+	+	+	-	-	-	-	+	+	+	+
E. coli	-	+	+	+	-	-	-	-	+	+	+	+
P. aeruginosa	-	-	-	-	-	-	-	-	+	+	+	+

Source: Authors.

Figure 1 - Bacteria cell viability shown by resazurin in culture medium supplemented with Sc-AcOEt.

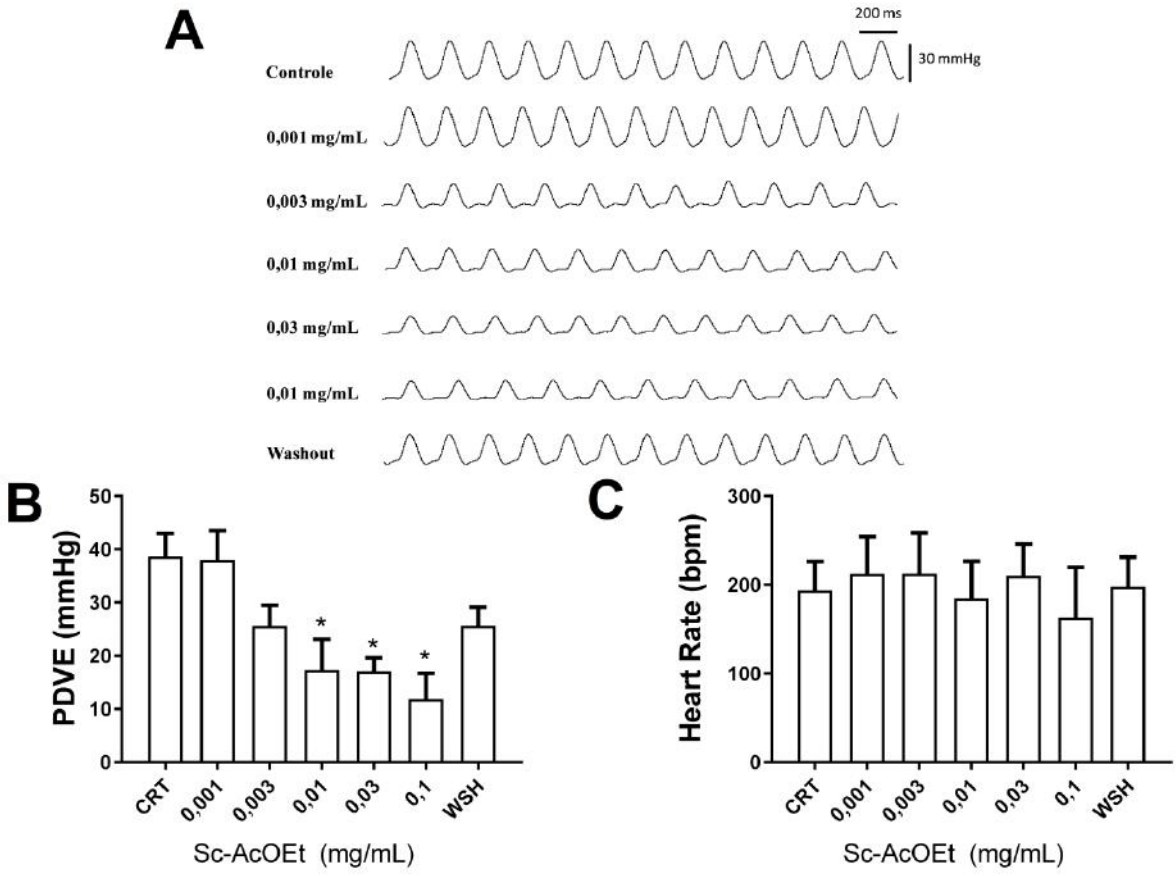


Source: Authors.

3.1 Contractile and electrical effects in rat heart

Figure 2A shows recordings of left ventricular developed pressure (LVDP) in control situation, at different concentrations of Sc-AcOEt and washout. Figure 2A shows the negative inotropic effect of Sc-AcOEt at concentrations above 0.01 mg/mL. A reduction in LVDP was observed after heart perfusion with 0.1 mg/mL of Sc-AcOEt. The decrease of cardiac contraction may have occur may be due to several mechanisms, such as blockage of Na^+ or Ca^{2+} channels that are essential for cardiomyocyte contraction (Bacha et al., 2019). Monteiro et al. (2018) reported that standardized hydroalcoholic extract prepared from the leaves of S. cumini (HESc) has antidiarrhoeal and antispasmodic activity was mediated blockage of calcium influx through voltage-gated Ca^{2+} channels (CaV). It is known that calcium channel blockers are substances with antihypertensive and antiarrhythmic action (R. M. Ribeiro et al., 2014). Thus, the study of the cardioprotective effect of this extract in a model of cardiovascular disease will be very promising. It can also be observed that there was no significant change in heart rate (Figure 2C).

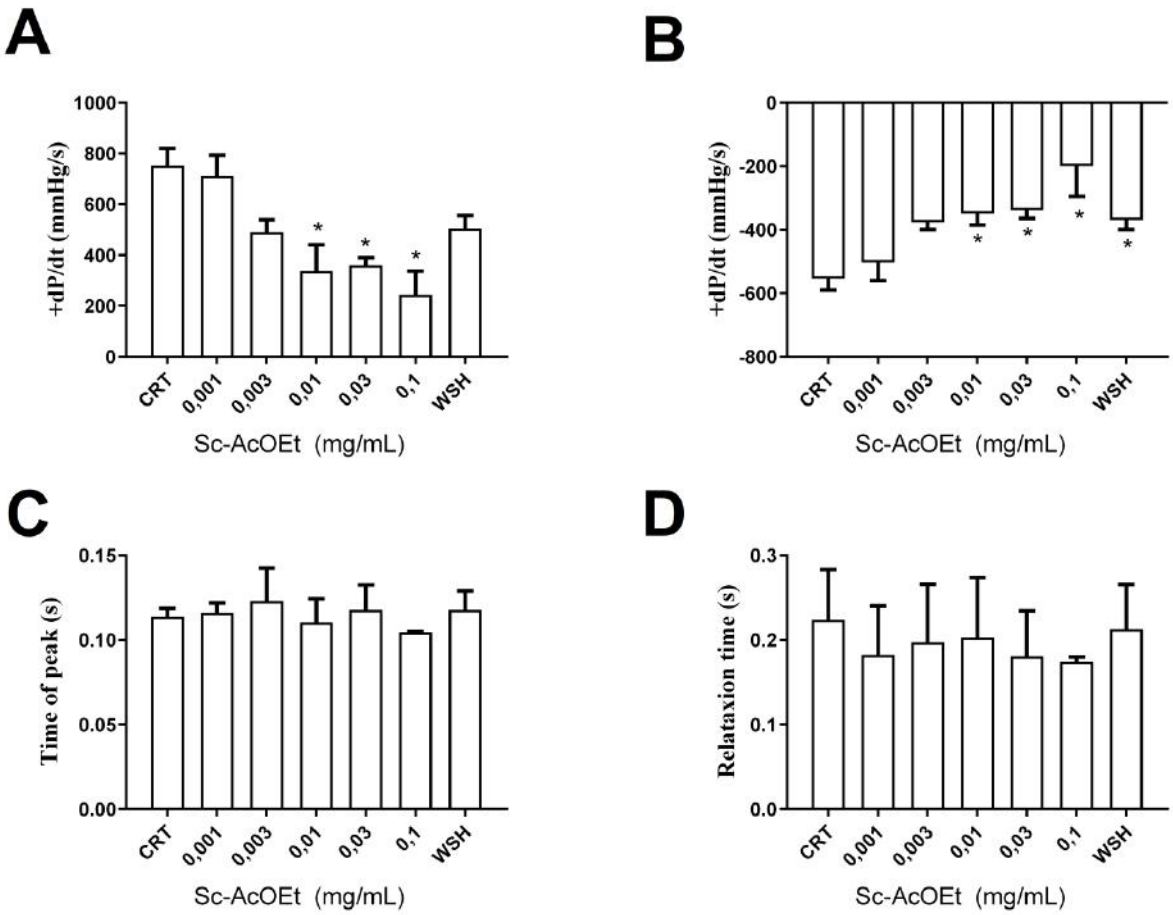
Figure 2 - Contractile effects of Sc-AcOEt in rat isolated heart. (A) Representative traces of left ventricular developed pressure (LVDP) in control, with Sc-AcOEt (0.001 to 0.01 mg/mL) and washout, (B) LVDP and (C) cardiac frequency. *p < 0.05 vs. control; One-way ANOVA followed by Tukey post-test; n=5



Source: Authors.

In addition to reducing LVDP, the extract promoted a reduction both maximum derivative of left ventricular pressure (+dP/dt) and minimum derivative of left ventricular pressure (-dP/dt) at concentration above 0.01 mg/mL (Figure 3A and B). No change was observed in the duration of systole and diastole at all concentrations of the Sc-AcOEt (Figure 3C and 3D).

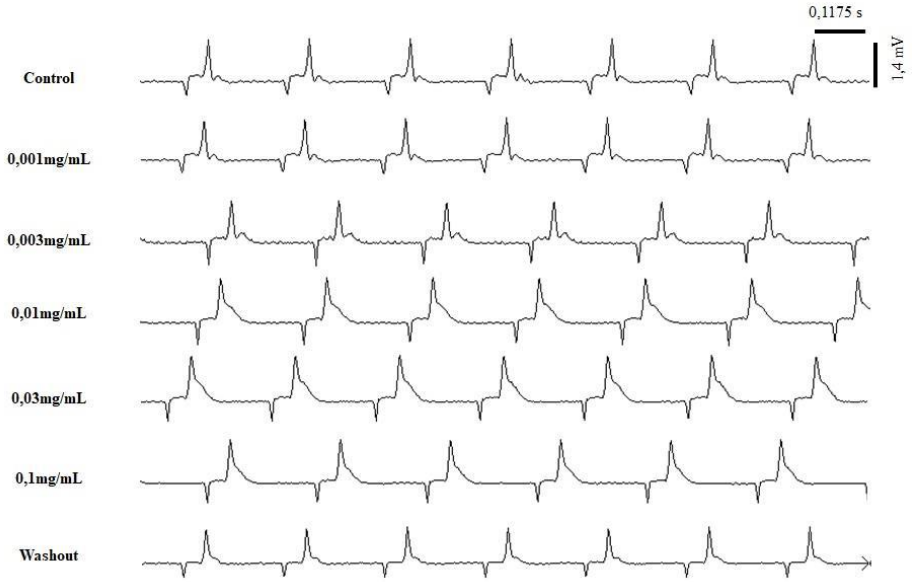
Figure 3 - Effects of Sc-AcOEt on contractile parameters in rat isolated heart. (A) maximum (+dP/dt) and (B) minimum (-dP/dt) derivative of left ventricular pressure, (C) time do peak (C) and (D) relaxation time. *p < 0.05 vs. control; One-way ANOVA followed by Tukey post-test; n=5



Source: Authors.

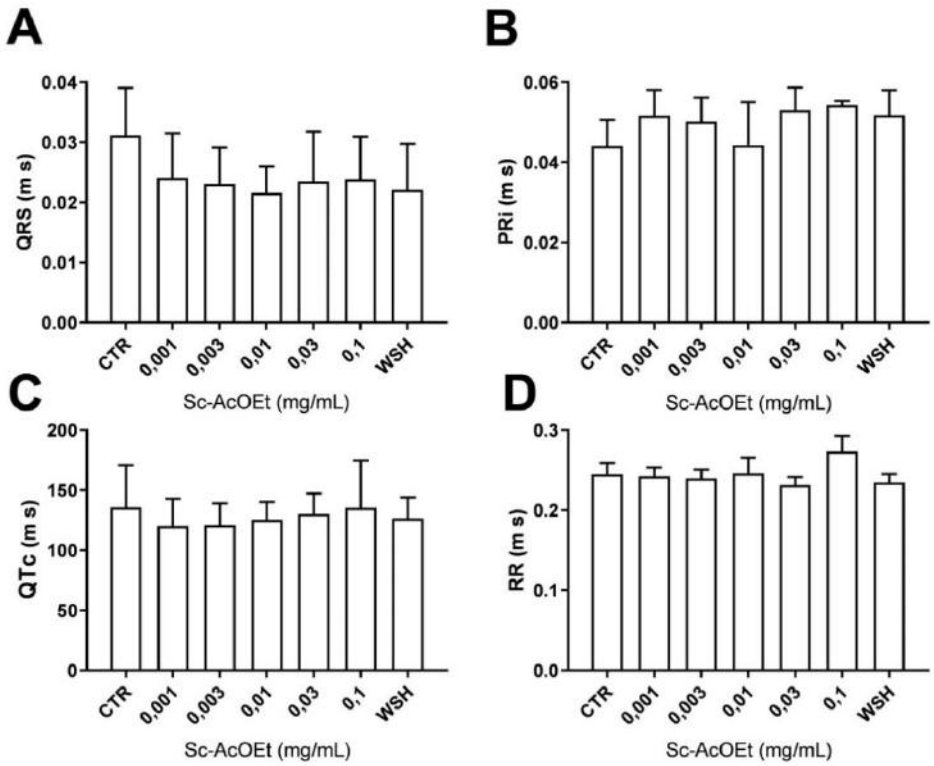
The electrocardiogram is a simple and low-cost test that provides data on electrical aspects of the myocardium, allowing the diagnosis of cardiac problems such as myocardial infarction, ischemia and cardiac hypertrophy, and risk evaluation for future cardiac events or mortality from heart disease (Rose et al., 1982; Pastore et al., 2016). In the present study, there were no significant changes in the electrocardiographic parameters of rat heart in the presence of Sc-AcOEt such as the electrocardiogram (Figure 4) and its parameters QRS, PRi, QTc and RR (Figure 5). Since electrocardiographic changes can indicate serious alterations in cardiac physiology due to the exposure to potential drugs for heart illness treatment, Sc-AcOEt can be considered as a potential natural product capable of induce contractile force without affecting the electrical aspects of the myocardium.

Figure 4 - Representative electrocardiograms of isolated rat hearts at different concentrations of Sc-AcOEt.



Source: Authors.

Figure 5 - Effects of Sc-AcOEt on electrocardiographic parameters of isolated rat heart. (A) QRS complex, (B) PRi, (C) QT corrected and (D) RR range.



Source: Authors.

Phytochemical analysis

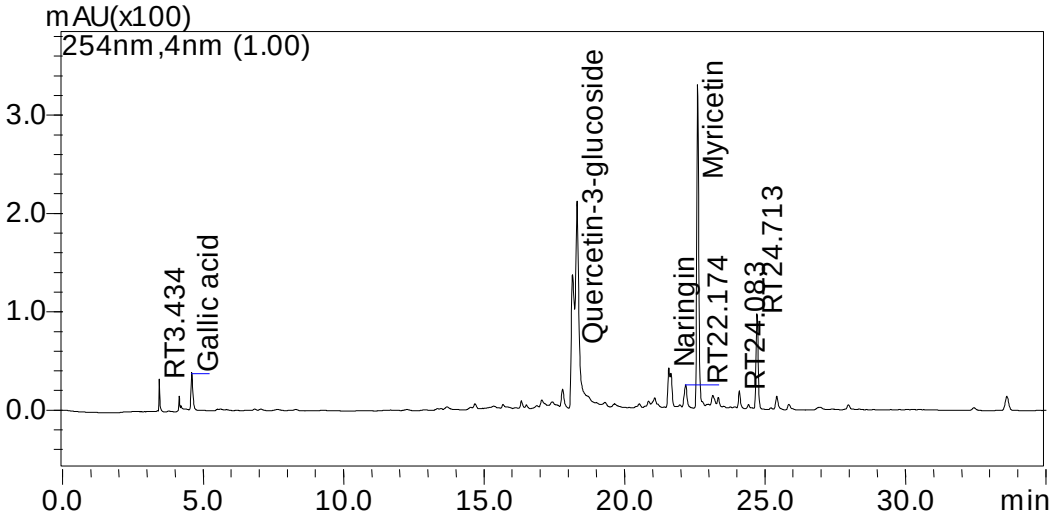
Total phenol, total flavonoids and total flavonols contents of Sc-AcOEt, calculated from the respective calibration curves, were 21.556 ± 1.095 AGE µg/mg, 617.222 ± 39.869 QE µg/mg and 315,222 ± 25,720 RE µg/mg, respectively. In

addition, the HPLC analysis of Sc-AcOEt showed the presence of gallic acid, quercetin-3-glucoside, naringin and myricetin as main components (Figure 6).

Phenolic compounds are known for important biological activities such as immune system regulation, anti-inflammatory effect, chemoprevention, neuroprotection, cardioprotection, antibacterial and also in the treatment of diseases such as diabetes, Parkinson's disease and cancer (L. F. Ribeiro et al., 2021; Salih et al., 2017). Phenols are important components of plants and contributes directly to the antioxidant activity due to their free radical scavenging properties, which are attributed to the hydroxyl groups present in their structure, which is mostly comprised of aromatic rings (Kumar et al., 2018; Subramanian et al., 2015; Zaiter et al., 2016). Specifically, flavonoids and flavonols have important antioxidant activity and can reduce oxidative stress related to cardiovascular diseases (Iwashina, 2013; Nijveldt et al., 2001; Stangl et al., 2007). In addition, flavonoids have also been recognized for their antimicrobial activity and many researchers have isolated and identified the structures of flavonoids with antimicrobial properties. Quercetin and apigenin, for example, are among the most studied flavonoids because they exhibit relevant antibacterial activity (D. Wu et al., 2008).

Gallic acid was previously show as potential agent to treat heart dysfunctions and fibrosis after heart failure by reducing hypertrophy and final diastolic and systolic diameters and suppressing the synthesis of natriuretic peptides, skeletal α-actin, and β-myosin heavy chain (Jin et al., 2018). Cardiac hypertrophy, which is associated with hypertensive heart disease, is also attenuated by quercetin, naringin and myricetin (Chen et al., 2021; Liao et al., 2019; Park et al., 2018). Quercetin can also improve the diastolic function of left ventricle in patients with high blood pressure (Kondratiuk & Synytsia, 2018). In addition, myricetin was show to reduce cardiac inflammation (Zhang et al., 2018). It can also improve the maximum up/down rate of left ventricular pressure and reduce infarct size as well as the level of cardiomyocyte apoptosis (Qiu et al., 2017). Thus, considering that these compounds are present in Sc-AcOEt, this may explain the results observed in the present study.

Figure 6 - HPLC chromatogram of Sc-AcOEt.



Source: Authors.

4. Conclusion

The Sc-AcOEt has important biological activities. As for the antimicrobial activity, it was able to inhibit the growth of *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* bacteria, demonstrating its antimicrobial potential. When tested to verify the effects on left ventricular pressure, it was observed that it reduced left ventricular strength without changing heart rate, showing that the plant has antiarrhythmic and antihypertensive potential by reducing contractile strength. Regarding the

chemical composition, HPLC analysis of Sc-AcOEt showed the presence of gallic acid, quercetin-3-glucoside, naringin and myricetin, important constituents for these biological activities. Thus, this work evidences a potential antimicrobial and heart negative inotropic plant fraction. Futures research are needed to isolate the bioactive compound.

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