ORIGINAL ARTICLE

Biological potential of the seed extract and fractions of *Artocarpus heterophyllus*

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ABSTRACT

Artocarpus heterophyllus is a tree of Indian origin that was adapted in Brazil and spread throughout the country. Popularly known as jackfruit, it is an invasive species with sweetened fruits that can reach up to 35 kg and is consumed by various animals. Its pulp is rich in carbohydrates, vitamins, lipids, minerals, and secondary compounds. In addition to pulp consumption, the seeds are cooked or roasted and represent a great source of proteins, vitamins, and carbohydrates. The objective of this study was to evaluate the biological potential of the ethanolic crude extract and the aqueous, chloroform, ethyl acetate, and hexane fractions of *A. heterophyllus* seeds. Antioxidant and antibacterial activities, secondary compounds and total phenolic were also evaluated. All fractions evaluated showed antibacterial activity. The hexane fraction showed better results in two antioxidant activities tested. The ethanolic extract and hexane fraction showed high content of phenolic compounds. *A. heterophyllus* seeds have high antioxidant and antibacterial potential, which may be associated with the presence of secondary metabolites, such as phenols, flavones, flavonols, and xanthones.

Keywords: *Artocarpus heterophyllus*, Plant extract, Antibacterial activity, Antioxidant capacity

INTRODUCTION

Artocarpus heterophyllus Lam. is an angiosperm of the family Moraceae, popularly known as jackfruit. It was brought to Brazil by the Portuguese crown in the 17th century, has adapted to the climate and temperature, and is currently distributed throughout the country, mainly in the Brazilian Northeast (Pereira and Kaplan 2013).

A. heterophyllus is considered an exotic species and causes changes in the soil in which it grows; these changes can influence the growth of other plant species, indicating that it has allelopathic action (Perdomomo and Magalhães 2007). It is a large tree, whose fruit can weigh up to 35 kg, and this fruit is recognized as the largest fruit in the world (Chowdhury et al. 1997).

Its fruit is sold to nature and is also associated with other ingredients used in the preparation of cakes, liqueurs, cookies, and sweets. Its pulp and seeds are rich in primary compounds such as proteins, carbohydrates, fibers, vitamins, and lipids. In addition to the primary compounds, its leaf, bark, pulp, and seed contain secondary metabolites, responsible for various biological activities, such as healing (Jagtap and Bapat 2010), antibacterial (Yuan et al. 2017), antifungal (Trindade et al. 2006), and antioxidant action (Burri et al. 2017).

These secondary metabolites are substances produced to protect the plant against internal and external agents. Some factors influence the production of these compounds, including the environment in which the plant is located and the time of year (Saxena et al. 2015; Caser et al. 2019). Secondary metabolites have significant medicinal importance due to the growing need for the prospection of new compounds with preventive

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action or for the treatment of diseases, whether antioxidant, antimicrobial, or anticancer, which arouses the interest of the food and pharmaceutical industries (Azmir et al. 2013; Boy et al. 2018; Chavez-Santiago et al. 2022).

In view of the above, this study aimed to evaluate the antioxidant and antibacterial activities; identify the presence of groups of secondary metabolites; determine the total phenolic content in ethanolic crude extract and its aqueous, chloroform, ethylacetate and hexane fractions of *A. heterophyllus* seed.

MATERIAL AND METHODS

Collection of plant material

Samples of *A. heterophyllus* (totaling 14 ripe jackfruits) were obtained from a local fair in the municipality of Garanhuns. They were then transported to the Research Support Laboratory Center of the Academic Unit of Garanhuns, CENLAG, located at the Federal University of Agreste of Pernambuco (UFAPE), under SISGEN authorization A84B4E7.

Processing of plant material

The pulp from *A. heterophyllus* was manually separated from the seeds. The seeds were washed and submitted to the oven drying method with forced air circulation at 60 ºC for nine days and then ground in a knife mill.

Obtaining the extract

The extract was obtained by Soxhlet extraction using 5 l of 96° GL ethyl alcohol, and the jackfruit seed powder 600 g was bathed for 7 h, with a total of six extraction cycles at reflux temperature (80 °C). The solvent was removed under reduced pressure at a maximum temperature of 40 °C, and the residues were combined. This combination is referred to as crude ethanolic extract. Finally, 104.85 g (17.8% yield) of crude extract were obtained, from which 20 g was removed, frozen and lyophilized, and the remainder was passed through the partition process with different solvents.

Partition with solvents

To obtain the hexane fraction, the crude extract (84.85 g) was dissolved in a mixture of methanol and water at a ratio of 2:3 (v/v) and the solution was placed in a separatory funnel. Therefore, a volume of 5 l of hexane was added to extract low-polarity compounds. The hexane addition procedure was repeated twice. Thereafter the hexane-soluble material was combined, and the solvent was removed under reduced pressure at a maximum temperature of 40 °C.

After the above procedure, the extraction processes were repeated with the addition of other solvents to obtain low-polarity compounds, such as ethyl acetate and chloroform, with the highly polar-compounds concentrated in the aqueous residue. The crude extract and fractions were subjected to lyophilization, and the obtained materials were frozen at -20 °C until subsequent analysis.

Antibacterial activity

The antibacterial activity of the ethanolic crude extract and the aqueous, chloroform, ethyl acetate, and hexane fractions was determined using the methodology previously described by Wu et al. (2013), with some modifications. In the present study, concentrations from 1.5 to 25 mg/ml of the crude extract and fractions were analyzed against four bacterial strains: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Streptococcus agalactiae* ATCC 13813, and *Staphylococcus aureus* ATCC 25923. All strains were cultivated in tryptic soy broth (TSB) for 24 h in a bacteriological oven at 37 °C. The trials consisted of 100 µl of sample diluted in 1% DMSO (w/v), 90 µl of Mueller-Hinton broth, and 10 µl of bacterial suspension, previously standardized at 0.1 optical density under a wavelength of 600 nm in absorbance mode in a spectrophotometer (Biochrom Libra S22 UV/Vis, Cambridge, UK), corresponding to 10^8 CFU/ml, or 0.5, on the McFarland scale. Assays were performed in sterile, polystyrene 96-well round-bottom microplates. For calculation purposes, the positive control for bacterial growth inhibition was performed with the addition of chloramphenicol 30 µg/ml, and the negative control was performed with the addition of sterile distilled water in place of the sample. Both controls followed the same conditions as the other tests. Assays were performed in triplicates and incubated for 24 h at 37 °C. Time passed, and the absorbance of the samples was measured at 600 nm using an Asys UVM 340 microplate reader (Biochrom). Bacterial growth inhibition activity was expressed as a percentage of growth inhibition and was calculated using Equation 1:

$$GrowthInhibition(\%) = \left(\frac{A_{control} - A_{sample}}{A_{negativecontrol}} \right) \text{x}100$$

where $A_{negative\ control}$ is the absorbance of the assay containing sterile water in place of the sample and A_{sample} is the absorbance of the tested assays.

Antioxidant activity

ABTS, DPPH, and hydroxyl radical scavenging assay

The ABTS radical scavenging assay was performed by oxidation of 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) at

7 mM with potassium persulfate (2.45 mM) pre-incubated in shelter from light for 12 h before use. The ABTS solution was adjusted to an absorbance of 0.700±0.02 at 734 nm in a spectrophotometer, by dilution in 5 mM phosphate buffer, according to the methodology previously described by Re et al. (1999) and modified by Hernández-Ledesma et al. (2005). A 50 µl aliquot of the sample was mixed with 150 µl of the diluted ABTS solution, and the reaction mixture was incubated for 10 min in the dark at room temperature. The absorbance of the reaction was measured at 734 nm.

The DPPH radical scavenging activity was determined according to the methodology previously described by Li et al. (2008), with some modifications. The reaction mixture consisted of 100 µl of sample and 100 µl of DPPH solution (0.1 mM in 95% methanol) in a 96-well microplate. The reaction mixture was incubated for 30 min at room temperature and protected from light. Absorbance was measured at 517 nm.

The hydroxyl radical scavenging assay was performed according to the methodology previously described by Zhang et al. (2012), with some modifications. Thereafter, 335 µl of the sample, 100 µl of $FeSO_4$ (8 mM), 335 µl of salicylic acid (3 mM), and 80 µl of H_2O_2 (20 mM) were mixed. The reaction mixture was incubated for 30 min at 37 °C, and the reaction was interrupted in an ice bath. Subsequently, 150 µl of distilled water was added, and the reaction mixture was centrifuged at 1200 ×*g* for 10 min, after which the absorbance was measured at 510 nm. All oxidant radical-scavenging assays were calculated according to Equation 2:

$$RadicalEliminationAssay(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \text{x}100$$

where $A_{control}$ is the absorbance of the negative control containing phosphate buffer instead of the sample, and A_{sample} is the absorbance of the sample. The results were presented as percentages.

Superoxide radical scavenging activity

The superoxide radical scavenging assay was performed according to the methodology previously described by Pownall et al. (2010). The fraction (80 µl) was mixed with 80 µl 50 mM Tris-HCl buffer (pH 8.3, containing 1 mM EDTA) in a 96-well microplate. Next, 40 µl of pyrogallol solution (1.5 mM) was added to each well of the plate. Subsequently, the absorbance was measured at 420 nm immediately and after 4 min of reaction. The percentage of superoxide radical scavenging activity (AERS) was calculated using Equation 3:

$$AERS(\%) = \left[\frac{\Delta A_{control}/min - \Delta A_{sample}/min}{\Delta A_{control}/min}\right] x100$$

Where, $\Delta A_{control/min}$ is the absorbance per minute of the control solution containing the buffer, and $\Delta A_{sample/min}$ is the absorbance per minute of the sample.

Phytochemical screening

The phytochemical screening was performed according to the methodology previously described by Matos (1997). Initially, seven test tubes were used for the crude ethanolic extract, and seven tubes were used for each fraction. The crude extract and fractions were diluted with ethanol to reach a final concentration of 1 mg/ml. In tube one, 3 ml of the sample and three drops of ferric chloride ($FeCl_3$) were added, and a color change from blue to red indicated the presence of phenols. In tubes two, three, and four, pH corrections were performed by adding HCl and NaOH, where the final pH corresponded to 3.0 for tube two, 8.5 for tube three, and 11.0 for tube four. A non-change in colour of tubes two and three, together with the yellow color of tube four, indicated the presence of flavones, flavonols, and xanthones.

Catechins and flavanones were performed in tubes five and six. Tube five was acidified to pH 3 and tube six was alkalized to pH 11. A non-change in the color of the alkalized solution and a change in the acidified solution to yellowish-brown was indicative of the presence of catechins. A color change of the alkalized solution to red or orange and a non-change in the color of the acidified solution was indicative of the presence of flavanones.

In tube seven, a few milligrams of magnesium and half a milliliter of concentrated hydrochloric acid were added, and effervescence was observed. At the end of effervescence, the appearance or intensification of the red color was indicative of the presence of flavonols, flavanones, flavanonols, and xanthones. To identify the presence of saponins, 5 ml of the ethanolic extract is added to a beaker and dried in a water bath. The dry residue is dissolved in water, transferred to a test tube and shaken vigorously for two minutes. The formation of a persistent and abundant foam is indicative of the presence of saponins.

Tests for alkaloids, anthraquinones, anthrones and coumarins are performed using thin layer chromatography (TLC). For the alkaloid indicative test, the extracts are solubilized in methanol, applied to the TLC plate, and eluted with a 9:1 (v/v) chloroform/methanol mixture. After elution, Dragendorff's reagent is sprayed, and the appearance of an orange color indicates the presence of alkaloids. For the other substances, proceed in the same way, except that instead of

spraying the Dragendorff reagent, 10% potassium hydroxide is sprayed, it is allowed to dry, and the presence of the indicative colors is observed in ultraviolet light in the length wavelength of 365 nm, blue being indicative of coumarin; red, from anthraquinone; and yellow, from anthrone.

Statistical analysis

Assays were performed in triplicate, and data were expressed as the mean and standard deviation. Significant differences between fractions were obtained through one-way analysis of variance, followed by the evaluation of differences between means using Tukey's test of multiple comparisons in SISVAR software version 5.6 (Ferreira 2011).

RESULTS AND DISCUSSION

Antibacterial activity

The ethanolic crude extract and fractions of the *A. heterophyllus* seeds exhibited antibacterial activity against all microorganisms tested. The percentage of inhibition of bacterial growth is presented in Table 1. According to the data, the ethyl-acetate fraction showed better antibacterial activity up to a concentration of 1.5 mg/ml, with a percentage of inhibition of bacterial growth above 90% for all strains tested.

Regarding the minimum inhibitory concentration (MIC), that is, the lowest concentration evaluated capable of inhibiting 100% of the visible growth of the microorganism, it was possible to determine the MIC for *E. faecalis* and *S. agalactiae*. For *E. faecalis*, the MIC values were 25 and 0.776 mg/ml for the aqueous and ethyl-acetate fractions, respectively. For *S. agalactiae*, the MIC was 25 mg/ml for the ethyl-acetate fraction.

Table 1. Antibacterial activity (%) at different concentrations of extract and fractions of *Artocarpus heterophyllus* seed.

Concentration (mg/ml)	*Enterococcus faecalis*					*Escherichia coli*				
	ECE	AF	CF	EAF	HF	ECE	AF	CF	EAF	HF
25.0	66.6±23.1^{Ca}	100.0±0.3^{Aa}	93.3±3.6^{Ba}	100.0±3.1^{Aa}	81.9±3.0^{Ba}	36.5±12.7^{Ca}	99.7±0.2^{Aa}	67.3±7.9^{Ca}	99.4±1.3^{Aa}	86.0±6.8^{Ba}
12.5	55.2±28.3^{Cb}	93.3±2.2^{Aa}	84.9±5.1^{Bab}	98.6±0.5^{Aa}	79.4±7.5^{Ba}	28.4±0 7.0^{Dab}	93.7±7.3^{Aa}	55.7±18.3^{Cab}	98.2±7.2^{Aa}	76.0±2.7^{Ba}
6.3	53.7±14.5^{Cb}	76.1±3.3^{Bb}	70.0±3.8^{Bb}	98.3±0.5^{Aa}	53.6±6.7^{Cb}	15.4±2.5^{Cbc}	92.5±6.7^{Aa}	47.0±29.5^{Bbc}	98.2±0.1^{Aa}	48.0±5.8^{Bb}
3.1	32.2±4.0^{Cc}	77.1±1.3^{Bb}	70.0±15.6^{Bb}	98.0±0.4^{Aa}	49.5±4.2^{Cb}	14.4±9.1^{Dc}	21.1±19.7^{Cb}	42.6±28.7^{Bbc}	97.7±0.2^{Aa}	46.5±7.5^{Bb}
1.5	24.4±2.4^{Ad}	76.6±2.2^{Bb}	63.04±19.1^{Bc}	95.7±1.0^{Aa}	56.3±12.7^{Cb}	10.4±4.0^{Cc}	15.4±3.7^{Cb}	37.4±12.4^{Bc}	94.7±0.9^{Aa}	38.7±2.4^{Bc}
Concentration (mg/ml)	***Staphylococcus aureus***					***Streptococcus agalactiae***				
	ECE	**AF**	**CF**	**EAF**	**HF**	**ECE**	**AF**	**CF**	**EAF**	**HF**
25.0	73.9±9.2^{Ba}	99.9±1.0^{Aa}	87.5±16.5^{Bab}	99.2±2.4^{Aa}	99.0±0.6^{Aa}	76.2±5.6^{Ca}	99.6±0.1^{Aa}	91.3±1.8^{Aa}	100.0±0.0^{Aa}	91.3±2.7^{Ba}
12.5	49.0±2.0^{Cb}	90.4±1.1^{Aa}	85.5±15.6^{Bab}	98.5±0.8^{Aa}	89.6±7.2A^{Bb}	53.9±3.5^{Cb}	78.1±0.2^{Bb}	80.9±6.1^{Bab}	99.5±0.5^{Aa}	76.9±3.7^{Bb}
6.3	28.4±4.4^{Dc}	73.5±7.1^{Bb}	77.5±2.6^{Bb}	98.5±0.4^{Aa}	63.5±2.7^{Cc}	32.0±8.9^{Ad}	56.4±4.8^{Cc}	79.3±12.1^{Bb}	99.2±0.0^{Aa}	50.7±4.4^{Cc}
3.1	23.1±4.9^{Ccd}	39.6±2.6^{Cc}	60.3±7.6^{Bc}	98.0±0.7^{Aa}	50.4±1.8^{Bc}	30.0±10.4^{Ec}	51.6±8.2^{Cc}	73.2±7.0^{Bb}	98.7±0.5^{Aa}	47.7±6.7^{Cc}
1.5	15.9±3.6^{Cd}	19.5±0.7^{Cd}	52.5±10.2^{Bc}	97.0±1.3^{Aa}	47.5±10.9^{Bc}	25.0±1.2^{Ec}	32.0±1.0^{Dd}	71.6±4.2^{Bb}	96.3±0.6^{Aa}	47.5±1.2^{Cc}

ECE, ethanol crude extract; AF, aqueous fraction; CF, chloroform fraction; EAF, ethyl-acetate fraction; HF, hexane fraction. Same capital letters on the same line do not differ significantly from each other. The same lowercase letters in the same column do not differ significantly from each other ($p < 0.05$).

The ethanolic crude extract showed a lower proportion of inhibition against the strain *E. coli* when compared with the others tested. The highest percentage of inhibition of the ethanol extract was observed against *S. agalactiae*, with activity up to a concentration of 1.5 mg/ml.

For *E. faecalis*, the aqueous, chloroform, ethyl acetate, and hexane fractions showed the best results up to concentrations of 1.5 mg/ml, when compared with the ethanol extract. The results found for the ethyl acetate fraction were similar in all inhibition assays, showing significant results at the lowest concentration tested. This fraction was the most active when compared with the crude extract and the other fractions in all assays.

Jagtap and Bapat (2013), observed inhibition effects, mainly on gram-positive bacteria, similar to the results found here. When screening for the antimicrobial activity of *A. heterophyllus* seed oil, Bhat et al. (2017), found inhibition results for *S. aureus* and *E. coli* strains, as well as those found by Nagala et al. (2013).

Tests performed by Cavalcante et al. (2013), showed negative results for hydroalcoholic extracts of the bark and stem regarding the inhibition of *E. coli* and *Streptococcus pneumoniae* strains. Reports on the antibacterial activity of jackfruit seed extracts are scarce.

Antioxidant activity

In general, the crude extract and the fractions tested showed good results as biological compounds with antioxidant action (Table 2). With regards to DPPH· radical scavenging activity, it was observed that the ethanolic crude extract and, chloroform and hexane fractions did not show antioxidant activity at the concentrations tested. The aqueous fraction and ethyl acetate presented results of 37.1±7.2% and 51.3±1.3%, at concentration of 1.5 mg/ml.

In the ABTS radical scavenging assay, the concentration of the different extracts evaluated did not influence the antioxidant activity; the results were statistically similar in the crude extract and fractions, except for the hexane fraction, which did not present significant results in the test performed. For scavenging of hydroxyl radicals and superoxide, there was an increase in activity while the concentration increased, showing better results at concentrations of 25 and 12.5 mg/ml. According to Nagala et al. (2013), this result may be due to the higher concentration of secondary metabolites found in jackfruit seeds at these concentrations.

For the capture of hydroxyl radicals, the hexane fraction presented a result of 100% at all concentrations tested, followed by the ethyl-acetate fraction 100% activity up to a concentration of 3.1 mg/ml. The ethanolic crude extract and other tested fractions showed similar results at all concentrations.

In terms of the ability to scavenge superoxide radicals, the crude ethanolic extract showed 100% activity when tested at a concentration of 25 mg/ml, decreasing as the concentration decreased. In general, the crude extract showed scavenging of oxidant radicals than the other fractions tested,

Table 2. Antioxidant activities in different concentrations of ethanolic crude extract and fractions of *Artocarpus heterophyllus* seed

Antioxidant activities										
	DPPH (%)					ABTS (%)				
Concentration (mg/ml)	ECE	AF	CF	EAF	HF	ECE	AF	CF	EAF	HF
25	n.d.	52.1±0.2^{Aa}	n.d.	60.4±2.5^{Aa}	n.d.	98.0±0.2^{Aa}	96.5±0.1^{Ba}	96.5±0.2^{Ba}	97.7±0.3^{Ba}	n.d.
12.5	n.d.	42.6±1.2^{Ab}	n.d.	57.4±1.3^{Aa}	n.d.	97.7±0.1^{Aa}	96.8±0.1^{Aa}	96.8±0.1^{Aa}	95.7±0.2^{Ab}	n.d.
6.3	n.d.	33.3±0,7^{Ac}	n.d.	42.6±0,8^{Ab}	n.d.	91.8±0.2^{Bb}	97.2±0.0^{Aa}	97.2±0.1^{Aa}	97.0±0.2^{Aa}	n.d.
3.1	n.d.	21.5±0.4^{Ad}	n.d.	37.9±0.3^{Ac}	n.d.	97.2±0.3^{Aa}	97.5±0.1^{Aa}	97.5±0.2^{Aa}	97.8±0.3^{Aa}	n.d.
1.5	n.d.	n.d.	n.d.	21.4±1.7^{Ad}	n.d.	98.3±0.2^{Aa}	97.7±0.2^{Aa}	97.7±0.2^{Aa}	97.8±0.4^{Aa}	n.d.
CV1 (%) = 30.54				CV2 (%) = 38.74		CV1 (%) = 0.86			CV2 (%) = 1.50	
Antioxidant activities										
	Hydroxyl (%)					Superoxide (%)				
Concentration (mg/ml)	ECE	AF	CF	EAF	HF	ECE	AF	CF	EAF	HF
25	100.0±0.0^{Aa}	78.9±3.7^{Ba}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	95.9±0.9^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
12.5	100.0±0.0^{Aa}	50.7±1.1^{Bb}	100.0±0.0^{Aa}	100.±0.0^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	59.9±5.3^{Cb}	76.8±1.5^{Bb}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
6.3	100.0±0.0^{Aa}	n.d.	80.6±2.5^{Bb}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	47.8±3.3^{Db}	52.9±3.2^{Cb}	61.7±3.2^{Cc}	100.0±0.0^{Aa}	76.5±3.6^{Bb}
3.1	23.4±2.5^{Cb}	n.d.	50.0±3.7^{Bc}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	28.9±2.2^{Cc}	31.6±2.7^{Cc}	46.6±1.1^{Bd}	72.5±3.1^{Ab}	32.1±3.6^{Cd}
1.5	5.0±0.2^{Bc}	n.d.	n.d.	100.0±0.0^{Bb}	100.0±0.0^{Aa}	15.1±1.4^{Cd}	19.92.3^{Cd}	21.6±1.7^{Ce}	39.0±1.3^{Bc}	42.5±4.1^{Ac}
CV1 (%) = 5.37				CV2 (%) = 4.59		CV1 (%) = 7.07			CV2 (%) = 8.71	

ECE, ethanol crude extract; AF, aqueous fraction; CF, chloroform fraction; EAF, ethyl-acetate fraction; HF, hexane fraction. Same capital letters on the same line do not differ significantly from each other. n.d.: not determined. The same lowercase letters in the same column do not differ significantly from each other ($p < 0.05$).

especially at the lowest concentration.

The results found in the present study corroborate those of Burci et al. (2015). When working with ethanolic, hexane, chloroform, and ethyl-acetate extracts of *A. heterophyllus* seeds, the authors found greater antioxidant potential in the elimination of DPPH· and hydroxyl scavenging radicals, when compared with the other plant parts.

The results of ethanolic extract of *A. heterophyllus* stem bark, by Ajiboye et al. (2016), were concentration-dependent; the higher the concentration, the better the antioxidant activity (DPPH and Hydroxyl). This was similar to the results found in the present study.

While examining the seed oil of five jackfruit varieties, Nagala et al. (2013), evaluated the potential for scavenging the DPPH and hydroxyl radical, with extraction by the Soxhlet method, as used in the present study. The results of the activities evaluated by Nagala et al. (2013), showed a dependence between the concentration of the extract and the scavenging of oxidant radicals. The authors demonstrated that the rate of antioxidant activity of samples tested at concentrations of 25, 50, and 100 mg/ml were 19.1, 24.3, and 87.4%, respectively.

These biological activities are evidenced by the presence of secondary metabolites; however, to date, there have been no specific reports on the antioxidant and antibacterial activities of extracts and fractions of jackfruit seeds or reports on their chemical composition.

Phytochemical screening

Phytochemical screening verified the presence of phenols, flavones, xanthones, flavanones, and flavonols in the ethyl-acetate fraction, and their absence in the extract and other fractions. Catechins, saponins, alkaloids, and anthraquinones were absent in the extract and fractions (Table 3).

The absence of some classes of secondary metabolites may occur as a result of the extraction or dilution method of the samples. Degradation of chemical constituents occurs when using the Soxhlet extraction method (Duan et al. 2006). Other extraction methods can be used to solve the low extraction content, such as the percolation and maceration processes, demonstrating the presence of several secondary compounds, such as those found by Fernandes et al. (2017), when studying *A. heterophyllus*. However, in our work, the yield of the extract obtained by maceration was extremely low, which led us to perform the extraction using the soxhlet technique.

Qualitative studies carried out by Cavalcante et al. (2013), on hydroalcoholic extracts from the bark and stem of *A. heterophyllus* showed the presence of alkaloids, flavonoids, saponins, tannins, and terpenes. Some studies have used the pulp, leaf, and bark (Cavalcante et al. 2013; Nagala et al. 2013), but studies on the chemical composition of the seed are scarce. In the present work, we performed a qualitative colorimetric test, which often presents false-negative results due to the low concentration

Table 3. Class of secondary metabolites identified in the ethanolic crude extract and aqueous, chloroform, ethyl-acetate, and hexane fractions of *Artocarpus heterophyllus* seed.

	Extract / Fractions				
Class of secondary metabolites	**ECE**	**AF**	**CF**	**EAF**	**HF**
Phenols	-	-	-	+	-
Flavones/Flavonols/Xanthones	-	-	-	+	-
Flavanones	-	-	-	+	-
Flavonols/Flavanones/Xanthones	-	-	-	+	-
Xanthones	-	-	-	+	-
Catechins	-	-	-	-	-
Saponins	-	-	-	-	-
Alkaloids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Anthrones	-	-	-	-	-
Coumarins	-	-	-	-	-

ECE, ethanol crude extract; AF, aqueous fraction; CF, chloroform fraction; EAF, ethylacetate fraction; HF, hexane fraction. (-) indicates the absence of a chemical constituent and (+) indicates the presence of a chemical constituent.

of the tested substances in the sample or even due to the color of the solution being tested, which would prevent the visualization of the color change. On the other hand, the positive result indicates that those substances would be present in relatively high amounts. In this perspective, we can consider the ethyl-acetate fraction as being the richest in potentially bioactive compounds.

CONCLUSION

Phenols, flavones, flavonols, xanthones, and flavanones were found in the ethylacetate fraction of *A. heterophyllus* seed. Antioxidant and antibacterial activities are concentration-dependent; the higher the concentration, the higher the activity. The extract and fractions showed antioxidant activity against the radicals tested, with emphasis on the hexane fraction that showed the best activity in most assays, except for the capture of the DPPH and ABTS radical. The inhibition of bacterial growth was effective both in the extract and fractions; the ethyl-acetate fraction stood out for presenting activity at the lowest concentrations tested. Hence, jackfruit has the potential to be used in food and pharmaceutical preparations; to reinforce the results found in this study, further search for methodologies and research to this end are required.

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AUTHOR'S CONTRIBUTION

M.E.B.S.: literature review, phytochemical screening, phenolic content, and discussion of results; E.F.T.S: literature review, statistical analysis, and discussion of results; M.T.A.E.: discussion of results; G.C.L.: language translation, and discussion of results; P.G.V.A.: evaluation of the antimicrobial and antioxidant test; J.E.G.G.: statistical analysis, and discussion of results; R.E.A.F.: evaluation of the antimicrobial and antioxidant test; K.A.M.: evaluation of the antimicrobial and antioxidant test, and discussion of the results.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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