Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.02221

Phenolic content, antioxidant and antifungal activity of jackfruit extracts (*Artocarpus heterophyllus* Lam.).

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Abstract

Jackfruit (*Artocarpus heterophyllus* Lam.) is a fruit of tropical and subtropical zones of the world, is an important source of phytochemicals (phenolic acids, flavonoids and tannins). Several studies have associated phytochemicals with antioxidant and antimicrobial properties. The objective of this work was to analyze the polyphenolic content, the antioxidant and antifungal properties of jackfruit extracts on phytopathogenic fungi. Two phenolic extracts of jackfruit of different maturity level (J1-J2) were used. The total polyphenol content (TPC) was determined by Folin-Ciocalteu method and total flavonoids (TFC) by the aluminum chloride method, the free radical trapping potential was measured using FRAP and ORAC methods. The results showed a TPC of 844 and 1,178 mgEAG/100 g and TFC of 37 and 68 mgQE/100 g, of dry jackfruit, the antioxidant potential analysis by FRAP was 7,575 and 8,691, by ORAC was 13.369 and 14.728 μmol Trolox/100 g, of dry jackfruit for J1 and J2 respectively. Additionally, it was observed that the phenolic extracts of jackfruit reduced the mycelial growth of: *Penicillium digitatum* (20-14%), *Geotrichum candidum* (56-55%), *Aspergillus niger* (72-67%) and *Botrytis cinerea* (100%-100%) for J1 and J2 respectively. We conclude that regardless of the degree of maturity, jackfruit has antioxidant and antifungal properties on phytopathogens important in agriculture.

Keywords: *Artocarpus heterophyllus*; antifungal; antioxidant; polyphenols.

Practical Application: Jackfruit extracts have properties as a protective agent against phytopathogens in agriculture.

1 Introduction

Artocarpus heterophyllus Lam. is a plant from the Moraceae family commonly known as jackfruit or "tree bread" in Latin America, a common name of Artocarpus genus like: *Artocarpus brasiliensis* Gomez., *Artocarpus heterophylla* Lam., *Artocarpus maxima* Blanco, *Artocarpus philippinensis* Lam., among others. Native to Southeast Asia and is widely cultivated in Malaysia and the Western Ghats of India. (Prakash *et al*., 2009; Vazhacharickal *et al*., 2016; Nayak *et al*., 2017). Jackfruit is not a very widespread crop in America. However, it is considered of importance in Brazil, Puerto Rico and in some Caribbean islands such as Jamaica and Bahamas, also is cultivated in southern Florida, California, and Hawaii in the United States (Crane *et al*., 2016). Jackfruit cultivation was introduced in Mexico in 1985 and is currently distributed in the states of Nayarit, Jalisco, Veracruz, Tabasco, and Chiapas, among others (Luna *et al*., 2016). The fruits are compounds or aggregates with a weight of 4.5 to 27.3 kg each; some varieties produce small fruits that weigh from 1.4 to 4.5 kg. The period between flowering and fruit ripening varies between 150 and 180 days (Jagtap & Bapat, 2010). The edible part surrounds each seed and is composed of a sweet, aromatic, crisp and smooth pulp; its exotic characteristic resides in its flavors, which is a mixture of tropical fruits such as pineapple (*Ananas comosus*), banana (*Musa paradisiaca*), mango (*Mangifera indica*), orange (*Citrus sinensis*), melon (*Cucumis melo*) and papaya (*Carica papaya*) (Servicio de Información Agroalimentaria y Pesquera, 2017). *A. heterophyllus* Lam. has been shown to have a wide variety of secondary metabolites, including various types of flavonoids, carotenoids, prenylflavones, and sterols; of which stand out artocarpine, artocarpetine, norartocarpetine, morine, artonin, isocarpine, artocapesine, tannins and sapogenins (Hari *et al*., 2014; Vazhacharickal *et al*., 2016). In addition to the above, this species contains lectins (jacaline and jackin), which have been shown to inhibit the growth of *Fusarium moniliforme* and *Sacharomyces cerevisiae*, due to their affinity for chitin, which is a key component of the cell wall of these microorganisms, altering the synthesis and / or arrangement of chitin in the cell wall (Prakash *et al*., 2009). Isoprenyl flavones, artocarpine and artocarpesin, obtained from methanolic extracts, have also been shown to inhibit the formation of cariogenic bacteria *Streptococcus mutans* and plaque formation in the prevention of dental caries (Theivasanthi *et al*., 2011). There is a current

Received 18 Jan., 2021
Accepted 10 May, 2021
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need in agriculture to offer new alternatives in the control of diseases caused by phytopathogens, mainly those of fungal and bacterial origin. A great variety of genera of fungi of agronomic and food importance cause losses in cultivation, post-harvest, storage, and distribution; damage can reach the fruit, stem, leaves, roots, or tubers of the plant (Andrade-Bustamante *et al*., 2017). In recent years, the use of pesticides has increased, worldwide, especially in countries with intensive production schemes; the abuse and ignorance of the side effects of these products have caused problems of environmental imbalance, human health and pest resistance (Vázquez, 2018). This has boosted the search for new sources of biocidal agents, friendly to the environment, among which essential oils and plant extracts with antioxidant and antimicrobial properties (Juárez-Becerra *et al*., 2010). The objective of this work was to evaluate the polyphenolic, antioxidant, and antimicrobial chemical composition of jackfruit extracts on phytopathogenic fungi.

2 Materials and methods

2.1 Materials

The jackfruit (*A. heterophyllus* Lam.) was obtained from the local market of the city of Reynosa, Tamaulipas, Mexico, during the winter period 2018-2019 (fruits specimens were chosen randomly). The samples were identified as J1 and J2, according to characteristics and sensorial and visuals (surface structure, solidity, external and internal visual color), of each fruit (Kader, 2002), it stands out that J2 was the fruit with the highest degree of ripeness (see Figure 1). The samples were cleaned, and the seeds were separated from the pulp, the latter being used for extraction. The pulp was weighted and subsequently oven-dehydrated (DHG-9145A Drying Oven) at 65 °C for 72 h. The dried jackfruit pulp was pulverized and sieved on No. 40 mesh (<420 µm). The samples were stored in a dry environment until further analysis.

2.2 Extracts

A 1:10 (m:v) solution of dried jackfruit was prepared with a 70% (v/v) ethanol-water solution and stored in the dark for 15 days according to Barrientos *et al*., 2013. Subsequently, the mixture was vacuum filtered using filter paper (Munktell) grade 929ª. The mixture was concentrated with a rotary evaporator (40 ºC; 100 RPM), until obtaining one tenth of the initial volume. The phenolics extracts of jackfruit (JFE) were sterilized using a 0.22 µm pore filter (Syringe). Finally, they were stored at -18 °C until its analysis.

Figure 1. Jackfruit (a) J1, (b) J2 and cutted bulbs (c) J1 and (d) J2.

2.3 Determination of total phenolic content (TPC)

Total phenolic analyzes were performed on a Cytation 5 multimode microplate reader from BioTek Instruments, Inc. (Winooski, VT, USA), using 96-well polystyrene microplates. 125 µL of Folin-Ciocalteu reagent, 25 µL of diluted JFE (1: 100) and 100 µL of 7.5% Na_2CO_3 were added to each well (Bridi *et al*., 2019; Folin & Ciocalteu, 1927). The samples were incubated for 60 min at 37 °C in the microplate reader and its absorbance was subsequently measured at 765 nm. Quantification was carried out by linear regression from a standard curve of the gallic acid (G7384-100G Sigma-Aldrich). The results were expressed as milligrams (mg) gallic acid equivalents of (GAE) per 100 g of dried jackfruit (mg GAE / 100 g of dried jackfruit).

2.4 Total flavonoid content (TFC)

In a 96-well microplate were added: 105 µL of methanol, 20 µL of diluted JFE (1:10) and 125 µL of 2% $AlCl_3$. The mixture stood for 60 min at room temperature and subsequently absorbance at 420 nm was measured using a Cytation 5 multimode microplate reader (Bridi *et al*., 2019; Heimler *et al*., 2005). Quantification was carried out by linear regression from a standard curve using quercetin (Q4951-100G Sigma-Aldrich). Total flavonoid content was reported as mg of quercetin equivalents (QE) per 100 g of dried jackfruit (mg QE / 100 g of dried jackfruit).

2.5 Ferric reducing antioxidant potential (FRAP)

The ferric reducing power of JFE was determined according to Bridi *et al*., 2019. The FRAP solution was prepared daily: 10 parts of acetate buffer (0.3 M; pH 3.6), one part of TPTZ (2,4,6-Tripyridyl-S-triazine) 10 mM (Sigma), and one part of ferric chloride 20 mM. Aliquots of 270 µL of FRAP solution were blended with 30 µL of diluted JFE (1: 250). The samples were incubated for 30 min at 37 °C, and its absorbance was measured at 594 nm using a Cytation 5 multimode microplate reader. As positive controls, a solution of pure ethanol and Trolox (0- 30 µM) (Benzie & Strain, 1996; Bridi *et al*., 2019). The results were reported as µmol Trolox equivalent per 100 g of dried jackfruit (µmol TE / 100 g of dried jackfruit).

2.6 Oxygen Radical Absorbing Capacity (ORAC)

The antioxidant capacity of JFE was determined using the ORAC-fluorescein assay (ORAC-FL) using a fluorescent microplate reader (Cytation 5), according to Bridi *et al*. (2019). Fluorescein consumption was assessed by decreasing the fluorescence intensity of the sample (excitation 493 nm; emission 515 nm). AAPH (2,2'-azo-bis (2-amidino-propane) dihydrochloride) was used as peroxyl ion generator and Trolox µM as standard (0–100 µM). Results are expressed as µmol equivalent Trolox per 100 g dried jackfruit (µmol TE / 100 g dried jackfruit).

2.7 Antifungal activity

The strains of *Penicillium digitatum*, *Aspergillus niger*, *Geotrichum candidum* and *Botrytis cinerea*, from the collection of the Laboratorio de Patología Frutal of the Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, were used. The phytopathogens were activated in potato dextrose agar medium (PDA) at 20 °C ± 2 °C, with 12 h light / 12 h darkness periods for 7 days. Bioassays were performed as follows: one part of the micellar growth of the strains was used and they were resuspended in 10 mL of a solution with 0.05% Tween 80, mixing until a homogeneous solution. The solution was filtered with a sterile two-layer gauze to remove hyphae or mycelium of phytopathogens, then a count of the conidia was carried out in the Neubauer chamber until the following concentrations were obtained: *P. digitatum* (2×10^5), *A. niger* (2.7×10^7) *G. candidum* (3.2×10^5) and *B. cinerea* (3.4×10^6) conidia / mL respectively. A random design was used evaluating 4 treatments for each fungus: I) PDA medium in which J1 extract was added until reaching a concentration of 40% v/v; II) PDA medium with the addition of J2 extract until reaching a concentration of 40% v/v; III) a negative control that consisted of a PDA medium supplemented with a commercial solution based on the fungicide Iprodione (Agrospec) at 100 µL / L and IV) a positive control without inhibitors. Each treatment had 6 repetitions. Subsequently, 10 µL of conidial suspension of each strain were incorporated into each Petri dish and incubated at 20 °C ± 2 °C, with 12 h light / 12 h dark periods for 7 days. Measurements of the diameter of mycelial growth were carried out for each of the phytopathogens on days 1, 3, 5 and 7 (Cabrera & Montenegro, 2013).

2.8 Statistical data analysis

All experiments were carried out in triplicate twice (n=6). All data are the mean ± standard deviation (S.D.). All data were analyzed using the Student's t-Test and the analysis of variance (One-way ANOVA) and comparisons using the Tukey Test, employing the Origin Pro 8 statistical program (SRO v8. 0724 B724, Massachusetts, USA).

3 Results and discussion

3.1 Determination of total phenolic content (TPC)

The TPC concentration in JFE are shown in Table 1. The J2 extract had a concentration of 1,178 mg EAG per 100 g of dried jackfruit, this value was 28% higher than the J1 extract ($p = 0.0475$). There are variations of the TPC reported in studies of *A. heterophyllus*: Jagtap & Bapat (2010) mentioned for jackfruit pulp from West Ghats, India, a concentration of 46 mg EAG / 100 g dried jackfruit in ethanolic extracts. Shafiq *et al*. (2017) evaluated TPC in jackfruit pulp from Lahore, Pakistan and found 239.87 mg EAG / 100 g of dried jackfruit, in methanolic extracts, which represents 20% of amount quantified in this study. Jalal *et al*. (2015), reported results of the analysis of *Artocarpus altilis* from Kuantan, Malaysia, in which methanolic

Table 1. Total phenol content (TPC), total flavonoid (TFC) and antioxidant activity by ORAC and FRAP of jackfruit.

Sample	TPC* (mg EAG)	TFC* (mg QE)	ORAC* (µmol Trolox)	FRAP* (µmol Trolox)
J1	844 ± 32[a]	37 ± 1[a]	13,369 ± 660[a]	7,575 ± 301[a]
J2	1,178 ± 4[b]	68 ± 2[b]	14,728 ± 5751[a]	8,691 ± 181[b]

The values represent mean ± SD (n = 6), different letters indicate significant differences between groups ($p < 0.05$); *: per 100 g of jackfruit dry weight.

extractions were performed and found 78,100 mg EAG / 100 g of dried jackfruit. In 2011 Almeida *et al.* (2011) report a 29.0 mg EAG per 100 g of fresh weight *Artocarpus integrifolia*. These differences are closely related to the complex nature of polyphenols, their extraction method, as well as intrinsic factors (genus, species, or cultivar) and extrinsic (agronomic, environmental or storage) (Kalt *et al*., 2001; Tomás-Barberán & Espín, 2001). The phenolics compounds in fruits are diverse and abundant group of metabolites such as phenolic acids, flavonoids, or tannins. They have responsible of color and flavor characteristics and are related to antioxidant and antiradical activities. However its content decreases during maturity stages (Redondo *et al*., 2021). The decrease in phenolic content in fruits is attributed to a series of chemical and enzymatic alterations of some polyphenols during ripening, mainly hydrolysis of glycosides by glycosidase, oxidation of polyphenols by phenoloxidases and polymerization of free phenols (Zheng *et al*., 2012). The results obtained in this study have a similar tendency to that reported in studies related to alcoholic extractions. Due to the polarity of primary alcohols (mainly methanol and ethanol), they are excellent as solvents for phytochemicals, especially those with high polarity (Zhang *et al*., 2007). Similar studies have been reported in tropical fruits, the total phenolic content in pineapple (*Ananas comosus* L.) fluctuate between 38.1 and 67.2 mg of GAE / 100 g fresh weight, in plum (*Spondias purpurea* L.) 55.0 mg of GAE / 100 g fresh weight, soursop (*Annona muricata* L.) 54.8 to 120 mg GAE / 100 g, papaya (*Carica papaya* L.) 53.2 mg of GAE / 100 g fresh weight (Almeida *et al*., 2011; Hassimotto *et al*., 2005) and sapota fruit (*Achras sapota* Linn.) 134.6 mg GAE / 100 g (Kulkarni *et al*., 2007).

3.2 Determination of total flavonoids content (TFC)

Flavonoids are a family of polyphenols, content in fruits and vegetables. They are the main components of fruits with yellow, red, and blue colors (Erlund, 2004; Hýsková & Ryšlavá, 2019). The concentration of total flavonoids determined in JFE is shown in Table 1; where it was observed that J2 had the highest content with 68 mg QE / 100 g of dried jackfruit, this value was 45% higher with respect to J1 ($p = 0.0472$). The difference between J1 and J2 is their degree of maturation. Jagtap & Bapat (2010) reported high solubility of flavonoids in methanolic extracts of mature jackfruit. Some studies relate the degree of maturation with the increase in the concentration of polyphenolic compounds such as flavonoids in fruits such as: watermelon cultivars (Tlili *et al*., 2011), custard apple (Harris & Brannan, 2009) and jackfruit (Jagtap & Bapat, 2010). The polyphenolic compounds reported in jackfruit include phenolic acids such as gallic acid, ferulic acid and tannic acid and flavonoids mainly catechin, rutin and myricetin. (Singh *et al*., 2015; Sharma *et al*., 2015; Anaya-Esparza *et al*., 2018).

3.3 Antioxidant potential using FRAP assay

The antioxidant potential determined by the FRAP method showed a difference of 12% between J1 (7,575 µmol Trolox / 100 g dry weight) and J2 (8,691 µmol Trolox / 100 g dry weight), finding significant statistical differences between both extracts ($p = 0.0079$). Loizzo *et al*. (2010) reported the analysis of extracts of *A. heterophyllus* Lam. in relation to the reduction of the antioxidant ability when reacting with the ferric tripyridyltriazine complex (Fe^{3+} -TPTZ) and producing ferrous tripyridyltriazine (Fe^{2+} -TPTZ). The reducing ability of JFE is strongly related to the presence and concentration of polyphenolic compounds (Jagtap & Bapat, 2010). This chemical behavior is similar to the observed in this study. On the other hand, the redox properties that polyphenols can have (reducing agents, donors of hydrogens or oxygen reactants through processes such as excitation reactions, energy transfer, complex formation and collisional quenching) must be considered (Soong & Barlow, 2004). The redox potential of polyphenols plays a crucial role in determining antioxidant properties (Rice-Evans *et al*., 1997).

3.4 Antioxidant ORAC assay

The antioxidant potential using the ORAC method proved a 4% superiority of J1 (13,369 µmol Trolox / 100 g dry weight) in comparison to J2 (14, 728.81 µmol Trolox / 100 g dry weight); however, there were no significant statistical differences (Table 1). Pavan *et al*. (2014) reported values of 2,117 µmol Trolox for 100 g of dried jackfruit, approximately 15% of the concentration determined in this study. Phytochemical studies on jackfruit have reported the content of polyphenolic compounds and their antioxidant activity (Baliga *et al*., 2011; Saha *et al*., 2015). The differences in antioxidant capacity are directly related to the phytochemicals contained in the fruit, mainly those having functional groups such as hydroxyl (-OH) and that abound in compounds of the polyphenolic type such as: phenolic acids (gallic acid, ferulic acid and tannic acid), flavonoids (catechin, rutin and myricetin) and/or tannins (condensed/hydrolysable) mainly (González-Aguilar *et al*., 2008; Singh *et al*., 2015; Sharma *et al*., 2015; Anaya-Esparza *et al*., 2018).

3.5 Antifungal activity of the extracts

The inhibitory effect of extracts J1 and J2 did not show significant differences ($p \geq 0.05$), which can be attributed to the fact that the component with antifungal activity is present

Table 2. % Mycelial growth inhibition of *P. digitatum*, *G. candidum*, *A. niger* and *B. cinerea* by jackfruit.

Phytopathogen	Positive control		Negative control		J1		J2	
	mm	%	mm	%	mm	%	mm	%
Penicillium digitatum	75 ± 6.3[a]	N/A	0 ± 0.0[b]	100	60 ± 3.6[c]	20	65 ± 5.8[c]	14
Geotrichum candidum	75 ± 1.0[a]	N/A	56 ±1.7[b]	26	33 ± 1.4[c]	56	34 ± 3.0[c]	55
Aspergillus niger	54 ± 2.6[a]	N/A	6 ± 0.2[b]	88	15 ± 2.6[c]	72	18 ± 2.5[c]	67
Botrytis cinerea	85 ± 0.0	N/A	0 ± 0.0	100	0 ± 0.0	100	0 ± 0.0	100

The values represent mean ± SD ($n = 6$), different letters indicate significant differences between groups ($p < 0.05$); Iprodione (100 µL/L); J1 y J2 (400 mg/mL).

in both extracts. The mycelial growth of the phytopathogens decreased: *P. digitatum* (20% vs. 14%), *G. candidum* (56% vs. 55%), *A. niger* (72% vs. 67%) and *B. cinerea* (100% vs. 100%) for J1 and J2 respectively (Table 2). This suggests that the ripe jackfruit, which is no longer pleasant to the consumer, can be used to obtain extracts with an antifungal effect. Manikandan *et al.* (2017) reported the studies of the development of silver nanoparticles with extracts of *A. heterophyllus* Lam. against phytopathogens and demonstrated to be superior to the control and reference agents, similar behavior to that reported in this work. On the other hand, Tao *et al.* (2010) reported the antifungal activity of polyphenols (gallic acid, catechin and quercetin 3-galactoside) at different concentrations at each stage of development of *B. cinerea* and demonstrated the inhibitory effect at each phase of growth of the pathogen. This indicates that the different compounds have effects on the sensitivity of fungi that can change at different stages of development as well as by various antifungal mechanisms such as the ability to inhibit the germination of fungal spores or effects on deformation and cellular lysis (Pusztahelyi *et al.*, 2015). Phenolic compounds are related to defense response in plants against pathogen attack (Pusztahelyi *et al.*, 2015; Joaquín-Ramos *et al.*, 2020). Some small chemical structure phenolic compounds such as phenolic acids alter the fungal membranes and adhere and polymerize within the wall of the fungal hyphae, thus reducing the plasticity of the fungus for growth (Wang *et al.*, 2008).

4 Conclusions

In this study, the jackfruit (*A. heterophyllus* Lam.) extracts were found to have a high content of polyphenols like flavonoids, and these compounds are related to strong antioxidant capacity. The results showed that, regardless of the degree of maturation, jackfruit has antioxidant phytochemicals with potential antifungal effects on fungi of importance in agriculture. This suggests that ripe jackfruit, which is no longer consumer-friendly, can be harnessed for the formulation of environmentally friendly biofungicides. The jackfruit is a very generous supplier of phenolic compounds and this might be an added value to be considered to develop subproducts with benefits in agriculture.

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