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Yield and phytochemical screening of the peel aqueous extract of *Annona squamosa* Linn

Rendimento e triagem fitoquímica do extrato aquoso da casca de *Annona squamosa* Linn

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ABSTRACT

Annona squamosa is a fruit plant of gastronomic interest, with great potential for bioprospecting. Parts of the plant are used by folk medicine for the treatment of various diseases, and studies have already proven many of its activities, such as antioxidant, antimicrobial, antitumor and anti-inflammatory. This study aimed to evaluate the phytochemical content of the aqueous extract of the fruit peel. Samples were acquired from local stores during the first harvest period of the year, for three consecutive years. The extract was performed with dehydrated and pulverized samples and the phytochemical screening followed the specifications for each chemical class. The average yield of the aqueous extract was 36.6%, ranging from 35.3% to 38.1%. There was no significant difference in the yield of the aqueous extract over the three years analyzed. The screening indicated the presence of carbohydrate and several phenolic compounds. The aqueous extract of *Annona squamosa* showed consistency in terms of yield and its phytochemical profile presents a vast number of compounds of biotechnological interest.

Keywords: Carbohydrates. Phenolic compounds. Pinecone. Secondary metabolites.

RESUMO

Annona squamosa é uma planta frutífera de interesse gastronômico, com grande possibilidade de bioprospecção. Partes da planta são utilizadas pela medicina popular para o tratamento de diversas enfermidades e estudos já comprovam muitas de suas atividades, como antioxidante, antimicrobiana, antitumoral e anti-inflamatória. Este estudo objetivou avaliar o conteúdo fitoquímico do extrato aquoso da casca do fruto. Amostras foram adquiridas de comércio local durante o primeiro período de safra do ano, por três anos consecutivos. O extrato foi obtido a partir de amostras desidratadas e pulverizadas e a triagem fitoquímica seguiu as especificações para cada classe química. O rendimento médio do extrato aquoso foi de 36,6%, variando entre 35,3% e 38,1%. Não houve diferença significativa no rendimento do extrato aquoso ao longo dos três anos analisados. A triagem indicou a presença de carboidratos e diversos derivados fenólicos. Esse extrato aquoso de *Annona squamosa* mostrou constância quanto ao rendimento e seu perfil fitoquímico apresenta vasta quantidade de substâncias de interesse biotecnológico.

Palavras-chave: Carboidratos. Compostos fenólicos. Metabólitos secundários. Pinha.

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INTRODUCTION

In Brazil, the popular names of *Annona squamosa* are *ata*, *fruta-do-conde* and *pinha* (Cordeiro, Pinto & Ramos, 2000). The pinecone is a tropical fruit plant well distributed in the world because it has good adaptation and pleases the taste of different cultures (Quílez, Fernández-Arche, García-Gímenez & Puerta, 2018). This plant has a fleshy and extremely sweet fruit, which helps in the preparation of ice cream and candies, and can be used in the preparation of juices or consumed in natura (Kill & Costa, 2003).

In addition to the gastronomic possibilities, the plant has several pharmacological effects and high biotechnological potential (Ma, Y. Chen, J. Chen, Li & Chen, 2017). Ethnopharmacological studies describe the use of roots, seeds, fruits and leaves in the treatment of various diseases (Quílez et al., 2018), and there are already investigations that prove the antioxidant and antimicrobial potential of leaves (Kalidindi et al., 2015), the antioxidant (Vikas, Akhil, Remani & Sujathan, 2017) and the antitumor action of the seeds (Chen et al., 2012), in addition to the immunomodulatory and antioxidant action of the pulp (Tu et al., 2016). This is due to the rich chemical constitution of the plant (Ma et al., 2017).

Species of the genus *Annona* have several secondary metabolites, being phenolic compounds the vast majority among them (Quílez et al., 2018). The main classes of metabolites, alkaloids, tannins, terpenes and flavonoids have already been described (Ma et al., 2017). All of them are well described in the literature as agents of biotechnological interest (Sreenivasulu & Fernie, 2022).

Although there are indications of its potential for bioprospecting, little is known about the possibilities of the *Annona squamosa* fruit peel. Thus, this study aimed to evaluate the yield of the aqueous extract of the fruit peel, as well as the investigation of possible biotechnological agents.

MATERIAL AND METHODS

Fruits and peel preparation

Pinecones produced in Petrolina, PE, were purchased from a local merchant at the Pernambuco Food Supply Center (CEASA-PE). This study is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), under registration A58E095. The fruits were quickly washed and had their pulp removed. The peels were cut and conditioned in an oven with an exhaust fan, at 30ºC, to complete theirs drying. Then, they were ground in a commercial blender.

Aqueous extract and yield

The aqueous extract was obtained by adding 100 g of peel powder to 1 L of distilled water, ensuring a ratio of 1:10 (solute/solvent). The material was stirred for 8 hours and then filtered and dried by lyophilization. To investigate reproducibility, extractions were carried out from three samples acquired at different times (February 2018, February 2019 and February 2020), with each time having three extractions to verify the average yield. The yield was the result of the calculation: weight of aqueous extract after lyophilization/weight of powder used for extraction x 100.

Investigation of the presence of phenolic compounds

A 5 mL portion of the aqueous extract was treated with 10 drops of 5% ferric chloride in distilled water (w/v) and the presence of phenolic compounds was confirmed with the formation of a dark blue or black color (Kloss, Albino, Souza & Lima, 2016).

Investigation of the presence of alkaloids

A 5 mL portion of the aqueous extract received 5 drops of Wagner's reagent (1.27 g of iodine + 2 g of potassium iodide in 100 mL of distilled water). Confirmation of alkaloids was given by the formation of a reddish-brown precipitate (Kloss et al., 2016).

Investigation of the presence of carbohydrates

A 5 mL portion of the aqueous extract received 1 mL of α-naphthol. Then, it was slowly added 500 µL of sulfuric acid through the tube wall. The presence of carbohydrates was confirmed by the formation of a violet ring between the two liquid phases (Kloss et al., 2016).

Investigation of the presence of deoxysugars

A 5 mL portion of the aqueous extract received 2 mL of glacial acetic acid (PA). Then, it was added 5 drops of ferric chloride. Subsequently, it was added through the tube wall 1 mL of sulfuric acid and the formation of a brown ring between the liquid phases indicated the presence of deoxysugars (Kloss et al., 2016).

Investigation of the presence of flavonoids

A 5 ml portion of the aqueous extract received 2 ml of 20% sodium hydroxide. The presence of flavonoids was confirmed with the color change to an intense yellow, which becomes transparent when 2 mL of hydrochloric acid is added (Kloss et al., 2016).

Investigation of the presence of oxalates

A 5 mL portion of the aqueous extract received 1 mL of glacial acetic acid. The presence of oxalate was due to the formation of a dark green to black color (Kloss et al., 2016).

Investigation of the presence of quinones

A 5 mL portion of the aqueous extract received 1 mL of hydrochloric acid. The formation of a yellow precipitate indicated the presence of quinones (Kloss et al., 2016).

Investigation of the presence of saponins

It was added 2 ml of aqueous extract to a long test tube containing 6 ml of distilled water. The tube was shaken vigorously for 1 min and the persistence of a layer of foam indicated the presence of saponins (Kloss et al., 2016).

Investigation of the presence of steroids and triterpenes

A 5 mL portion of the aqueous extract received 10 drops of chloroform. Subsequently, it received 10 drops of acetic anhydride and 10 drops of sulfuric acid later. The immediate formation of a red or pink color in the organic phase indicated the presence of steroid and triterpene groups (Kloss et al., 2016).

Investigation of the presence of tannins

A 5 ml portion of the aqueous extract received 1 ml of 10% ferric chloride in an alcoholic solution. The formation of a blue or dark green precipitate confirmed the presence of tannins (Kloss et al., 2016).

Investigation of the presence of terpenes

A 5 mL portion of the aqueous extract received 1 mL of chloroform and 500 µL of sulfuric acid. The formation of a brown precipitate indicated the presence of terpenoids (Kloss et al., 2016).

Statistical analysis

Yield data were presented as mean and standard deviation, and were submitted to one-way analysis of variance followed by Tukey's test to analyze statistical difference between the yields of the extracts. The test was conducted using the GraphPad Prism 8.0 software (GraphPad, USA) and significance was considered if $p < 0.05$.

RESULTS AND DISCUSSION

The extraction process resulted in similar responses across all realizations. The aqueous extract of *Annona squamosa* is a golden-brown liquid (Figure 1), which becomes a yellowish-brown powder after lyophilization.

The extraction yield showed slight variance between samples, but there was no statistical difference in any of the samples evaluated (Table 1), with an average yield of 36.6%. This indicates the possibility of maintaining the production of extract, if the extraction is maintained periodically.

It is worth mentioning that the samples come from the same season, the first crop of the year, which does not rule out the possibility of yield variation at other times of the year, especially in fruits harvested in the off-season.

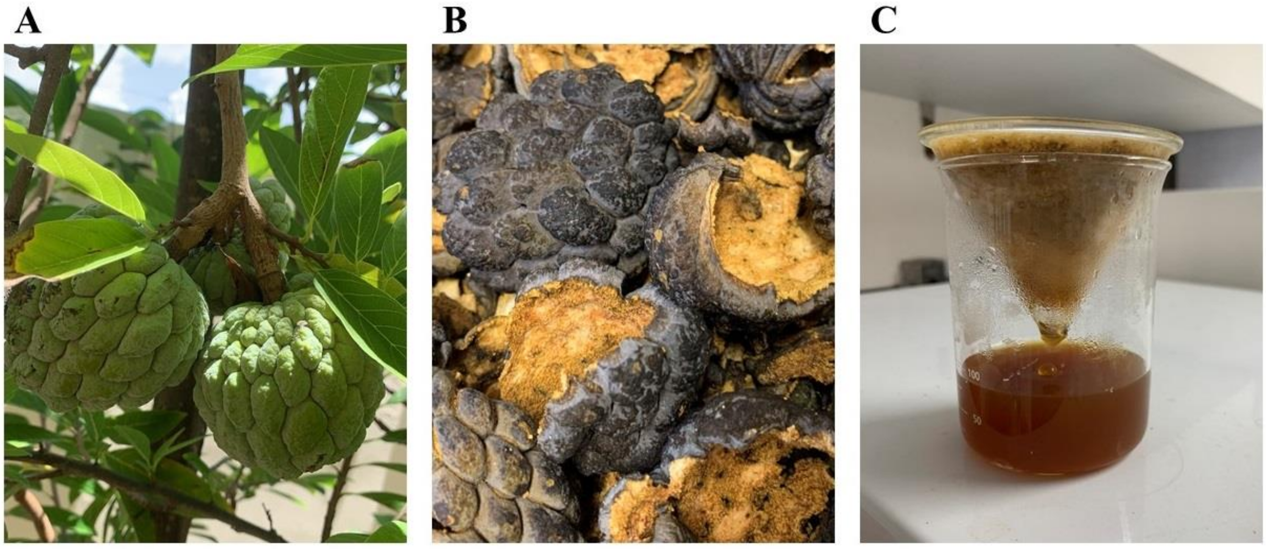

Figure 1. Fruit of *Annona squamosa* and aqueous extract of its bark.
Source: The authors.
Notes: a) fruits of *Annona squamosa*; b) dehydrated fruit peels; c) aqueous extract being filtered.

Aqueous extracts from other plants also showed similar yields to the custard apple. Most crude aqueous extracts yield around 40%. Specifically, the aqueous extracts of *Annona muricata* and *Annona cherimola* leaves presented 28% and 9%, respectively (Aguilar-Villalva et al., 2021). Other aqueous plant extracts, such as flowers of *Calendula officinalis*, *Helianthus annus* and *Rosa x grandiflora* showed yields of 38%, 58% and 42%, respectively (Franzen et al., 2018), and crude aqueous extract of cashew leaves yielded 18.7% (Costa et al., 2021).

Table 1
Yield of the aqueous extract of *Annona squamosa* fruit peel.

Sample	2018	2019	2020
Yield (%)	36.4 ± 3.5	38.1 ± 2.9	35.3 ± 3.8

Source: The authors.
Note: Mean ± standard deviation. $p = 0.84$, ANOVA followed by Tukey Test.

In relation to the peel of other fruits, the pinecone presented a higher yield than the passion fruit peel, described as less than 30% (Cazarin, Silva, Colomeu, Zollner, Maróstica, 2014), but the aqueous extract of jabuticaba has a high yield according to the literature, with little more than 70% (Oliveira et al., 2018).

The yield of a crude aqueous extract is due to the large number of water-soluble components present in plant tissues (Nobre, Mendes, Porto, Azevedo & Brandão, 2014). In addition, the great variability of its yield is due to the variation in the content of compounds such as carbohydrates and proteins, although the content of phenols is also very varied depending on the genus of the plant and the part used (Amarante, Souza, Benincá & Steffens, 2017).

In addition, the dehydration and grinding process helps release hydrophobic compounds and increases the contact surface of the solvent with the chemical compounds of the plant material. Thus, there is a greater possibility of extraction, with an average saturation point around 40% yield (Roesler et al., 2007).

In the evaluation of chemical compounds, the aqueous extract of the pinecone peel showed carbohydrates and several classes of secondary metabolites (Table 2). The same classes of compounds were present in all samples evaluated, indicating their richness of constituents and the stability of their presence.

Table 2
Phytochemical screening Aqueous extract of *Annona squamosa* fruit peel.

Samples	2018	2019	2020
Phenolic compounds	+	+	+
Alkaloids	+	+	+
Carbohydrates	+	+	+
Deoxysugars	+	+	+
Steroids and triterpenes	+	+	+
Flavonoids	+	+	+
Oxalates	Absent	Absent	Absent
Quinones	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Terpenes	+	+	+

Source: The authors.
Notes: +: present.

There are already studies that describe the variety of constituents, mainly secondary metabolism, in other parts of *Annona squamosa* (Ravaomanarivo et al., 2014; Kalidindi et al., 2015). Specifically in the peel, the aqueous extract showed a greater variety of components when compared to studies of other parts of the plant, such as the leaves, which do not have alkaloids, tannins and steroids (Kalidindi et al., 2015). Besides, the pulp, which does not have triterpenes and steroids (Nandhakumar & Indumathi, 2013), and seeds, which lack alkaloids, deoxysugars and saponins (Ravaomanarivo et al., 2014).

Due to the presence of several different classes of chemical constituents, the aqueous extract of the pinecone fruit peel shows promises for activities such as antioxidant and anti-inflammatory, due to the presence of flavonoids (Beigh et al., 2022). The presence of tannins indicates the possibility of healing potential (Su et al., 2017). Also, there are great chances of analgesic potential, due to the presence of alkaloids (Li et al., 2021) and terpenes (Carvalho et al., 2019). Most of these activities are already attributed to the fruit and other parts of the plant in its popular use (Quílez et al., 2018). Therefore, these findings indicate the possibility of using the fruit peel, often neglected, as a source of compounds of plant origin with great value for bioprospecting.

CONCLUSION

The fruit peel of the conifer cone is an underused plant product. Its aqueous extract has good yield and is made up of a rich variety of compounds with biotechnological potential. The findings of this study raise the need for studies that identify the chemical components and isolate them, as well as an investigation of the action of these components against the various activities already carried out in their popular use, in order to validate their use.

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