ORIGINAL ARTICLE

Antibacterial and antioxidant potential of ethanol extract from *Momordica charantia* leaves

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ABSTRACT

Momordica charantia popularly known as São Caetano melon, belonging to the Curcubitaceae family is considered an effective plant against various diseases. Many studies have reported its antimicrobial and antioxidant potential, especially in its fruits. Therefore, this study evaluated the antioxidant and antibacterial activity in ethanol extract of *M. charantia* leaves. It was selected the DPPH method to evaluate the antioxidant activity. Antibacterial activities were assessed against three strains - *Klebsiella pneumoniae* ATCC 29665, *Enterococcus faecalis* ATCC 6057 and *Pseudomonas aeruginosa* UFPEDA 416 using the well diffusion method. The percentage of free radical capture increased as the concentration of the extract also increased. At the concentration of 100 mg/ml the extract showed low antibacterial activity against the strains, and at the concentration of 50 mg/ml there was no inhibition of bacterial growth. Therefore, it is necessary a phytochemical study for the detection of the compounds responsible for these activities.

Keywords: Medicinal plants, São Caetano melon, Alternative medicine, DPPH.

INTRODUCTION

Popularly known plants with healing properties are used in several preparations to heal or to prevent diseases in humans and animals. The species with these qualities are called medicinal plants. There is a growing number of investigations related to plant extracts, due to the increasing popularity of natural products (Martins and Casali 2019).

Among the plants used for their healing properties, the species *Momordica charantia* L. It belongs to the Cucurbitaceae family and is commonly known in India as bitter gourd or karela (Ceballos et al. 2017), it is popularly known in Brazil as São Caetano melon. Some studies have demonstrated its larvicidal (Mituiassu et al. 2022), antidiabetic (Jiang et al. 2020) and anticancer action in Wistar rats (Ranasinghe et al. 2021), in addition to antioxidant and anti-apoptotic properties (Kim et al. 2018).

Bacterial resistance to antibiotics has become a persistent problem in infectious diseases, forcing science to constantly investigate new drugs with this property. In this perspective, medicinal plants can be used to combat microbial growth, as stated by Gonçalves et al. (2011). The bacterial resistance is promoted by the virulence present in it, which gives it the ability to adapt to hostile environments (Rocha et al. 2018). This is the result of an evolutionary process of the species triggered by the exposure of strains to antibiotics, even those correctly prescribed (Sampaio et al. 2018).

Another important pharmacological property of interest by researchers is the antioxidant ability present in different parts of the plants. Antioxidant substances are capable of inhibiting the oxidation of free radicals in substrates (Morais et al. 2013). Free radicals are atoms or ions that have unpaired electrons and can favor health problems such as degenerative diseases and premature aging

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(Miranda et al. 2014). Oxidative stress is based on the alteration of homeostasis, during this period occurs the production of these radicals (Bortolatto et al. 2020).

In view of the great potential of the flora in the search for secondary compounds that demonstrate therapeutic action such as antibacterial and antioxidant activity, this study aimed to evaluate such activities in ethanolic extract of leaves of *M. charantia*.

MATERIAL AND METHODS

Botanical collection and identification

The plant samples were collected in the municipality of Garanhuns, in the southernmost region of Pernambuco, Brazil. The botanical identification was made through a plant voucher and deposit at the Herbário Dárdano de Andrade Lima at the Agronomic Institute of Pernambuco (IPA) identified by O. Cano as *M. charantia*, obtaining the tipping #92887. The recently collected leaves were cleaned and immersed in ethanol p.a., in a 10% proportion (w/v). After a period of five days, the ethanolic extract passed through a rotary evaporator at a temperature of 50 ºC until the solvent was completely eliminated.

Antimicrobial activity

The methodology used for the antimicrobial activity was described by Moody et al. (2004) with modifications. The microorganisms *Klebsiella pneumoniae* ATCC 29665, *Pseudomonas aeruginosa* UFPEDA 416 and *Enterococcus faecalis* ATCC 6057 were provided by the Federal University of Pernambuco Agreste and standardized at 0.5 using the McFarland scale.

The extract concentrations used were 100 mg/ml and 50 mg/ml. After weighing, the ethanolic extract was dissolved in distilled water and dimethyl sulfoxide (DMSO) 1:1 (500 µL of DMSO and 500 µL of distilled water). After bacteria were inoculated in a Petri dish, wells were drilled measuring 5 mm, where 20 µL of the ethanolic extract were deposited. The cephalexin antibiotic was used as a positive control at a concentration of 100 mg/ml. The plates were incubated at 37.0 ºC for 24 h. DMSO was used as a negative control. The diameter of the formed halo was measured with a caliper.

Antioxidant activity

The antioxidant activity of the radical 2, 2-diphenyl1-picrilhidrazil (DPPH) was based on the methodology employed by Brand-Williams et al. (1995) and Rufino et al. (2007) 0.1 mg/ml of ascorbic acid was used as a positive standard. In this step, DPPH was used in a concentration of 0.5 to 6.0 µg/ml. Ethanol and DPPH were added to the sample in a final concentration of 5.0 to 200 µg/ml. The experiment was carried out in a 96-well plate. The reading was conducted using UV-visible at 517 nm. The hijacking activity (HA) was defined using the formula showed bellow and the results of the antioxidant activity were expressed as EC_{50} which refers to the concentration required to contain 50% of the DPPH radical.

$$HA\ (\%) = 100\ \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}$$

For the statistical analyzes, the Excel program was used to calculate the averages and standard deviations.

RESULTS AND DISCUSSION

According to the results presented in table 1, the extract was not successful in inhibiting bacterial growth at a concentration of 50 mg/ml. At the concentration of 100 mg/ml, the averages obtained from the halos formed were still lower compared to the halos formed from the positive control. In the antibacterial test presented in table 1, the extract of *M. charantia* against *K. penumoniae*, can be classified as lack of activity, whereas with the other two strains a low activity was observed, according to the classification pattern adopted by Matsuura (2004).

Table 1 - Antibacterial activity of the ethanolic extract of leaves of *Momordica charantia*. The activity was expressed in mm of the diameter of the inhibition halo.

Species	Sample concentrations (mg/ml)			
	100	50	NC	Cephalexin
Pseudomonas aeruginosa UFPEDA 416	7.3 ± 0.5	-	-	23 ± 0
Klebsiella pneumoniae ATCC 29665	6.3 ± 0.5	-	-	22.6 ± 0.5
Enterococcus faecalis ATCC 6057	7.3 ± 0.5	-	-	24 ± 2.6

Legend: (-) no activity, (NC) negative control (DMSO + distilled water).

Using the well diffusion method, comparing it with disk diffusion, Leelaprakash et al. (2011) found that the methanolic extract of the leaves of São Caetano melon showed antimicrobial activity when compared with the aqueous extract of this plant against four strains, including the species *P. aeruginosa* and *K. penumoniae*. In addition to antibacterial activity, Rakholiya et al. (2014) also analyzed the antifungal capacity in hydroalcoholic extracts (methanol + water) from the leaves, petiole, bark, pulp and fruit of the plant. The authors observed the plant's effectiveness, especially in relation to species of the genus *Pseudomonas,* which were more susceptible when compared to the Gram-positive bacteria tested in this study.

Mada et al. (2013) analyzed the antimicrobial activity in ethanolic and aqueous extract of the leaves of the species under study, obtaining halos of 14 mm for *P. aeruginosa* with ethanol extract. Comparing the results obtained in this present work to the authors mentioned above, it is observed that there is a large difference in the diameter of the halos formed. Hence even though it is the same species, the strains probably have a different resistance profile.

Olufunke (2011) analyzed aqueous, methanolic and ethanolic extracts of three plant species, including leaves of *M. charantia*, performing tests against bacterial species. He showed a result for *K. pneumoniae* of 8 mm in diameter at a concentration of 50 mg/ml, differentiating from the results obtained in the present study, which possibly illustrates changes from the solvent used and the characteristic affinity of each solvent. In an investigation carried out by Saengsai et al. (2015), they isolated a *M. charantia* lactone called plumericin, which was used to perform an antimicrobial test to identify the minimum inhibitory concentration (MIC) against three Gram-negative and five Gram-positive species. Among them, *E. faecalis* and *B subtilis* were more susceptible to the presence of the isolate, presenting better values than the antibiotic cloxacillin.

Regarding studies with antioxidant activity, Yoshime et al. (2019) carried out tests with essential oil of *Punica granatum* and *M. charantia*, obtaining results for the São Caetano melon in the EC_{50}, amount necessary for elimination of 50% of the DPPH radical of 11.8. This value is close to the result shown in table 2. According to Yadav et al. (2016), the antioxidant activity of plants such as São Caetano melon is due to the high content of phenol and flavonoid compounds present in these plants.

According to Chung et al. (2016), the ability to stabilize free radicals such as the peroxyl radical, occurs due to the presence of phenolic compounds in the plant that allow the donation of electrons or hydrogen. Like the result obtained in the antioxidant activity used in this study with the ethanolic extract of the leaves of São Caetano melon, Patel et al. (2011) performed antioxidant tests with the fruit extract, obtaining results for the ethanolic extract in the concentrations of 60 µg/ml a percentage of inhibition of the DPPH radical of 31.675 and in the 200 µg/ml a value of 69.729, results that corroborate the results shown in table 2.

Table 2 - Antioxidant activity of the radical 2,2-diphenyl 11-picrilhidrazil (DPPH), EC_{50} of the ethanolic extract of the leaves of *Momordica charantia* and ascorbic acid pattern.

Concentration (µg/ml)	Hijacking Activity (%)
10.0	1.49 ± 0.01
20.0	8.92 ± 0.02
30.0	19.47 ± 0.02
50.0	32.33 ± 0.01
60.0	36.88 ± 0.01
70.0	42.24 ± 0.00
80.0	47.64 ± 0.01
90.0	56.37 ± 0.03
150.0	67.24 ± 0.00
200.0	73.07 ± 0.01
DPPH· EC_{50} ± S.D. (µg/ml)	
Momordica charantia	6.96±0.02
Ascorbic acid	29.58±0.01

Legend: (EC_{50}) Efficient concentration to eliminate 50% of free radicals.

Rezaeizadeh et al. (2011), analyzed and compared the antioxidant activity of methanolic and chloroform extracts from the fruits of *M. charantia*, obtaining results for DPPH in the concentrations of 35 and 62.5 µg/ml the respective values for the methanol extract 5.57 and 13.10 µg/ml, for chloroform extract 3.70 and 6.62 µg/ml. These values are below the results obtained for the ethanolic extract of the leaves in the concentrations of 30 and 60 µg/ml in this study. This is possibly due to the difference in the environment of leaf collection, as factors such as climate may interfere in the concentration of compounds, as stated by Gobbo-Neto and Lopes (2007), in addition to factors such as the rainfall and altitude.

CONCLUSION

The ethanolic extract of *M. charantia* leaves has low antimicrobial activity, which lead for further investigation with extracts and fractions in different strains and the relationship of these variables with antimicrobial efficiency. Regarding the antioxidant activity, the extract showed a certain similarity

with the other works addressed, which may point to a potential use of secondary compounds of *M. charantia* as a natural product with antioxidant activity. However, more detailed protocols are necessary for a better understanding about the action of the extract on the cells and by what mechanisms this protection acts.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

The authors equally contributed to the manuscript.

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