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ORIGINAL RESEARCH

Antimicrobial Potential of *Momordica charantia* L. against Multiresistant Standard Species and Clinical Isolates

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ABSTRACT

Aim: The aim of the present study was to evaluate the antibacterial and antifungal potential *in vitro* of *Momordica charantia* L. against the microorganisms of clinical interest (standard strains and multiresistant isolates) in order to aggregate scientific information in relation to its use as a therapeutic product.

Materials and methods: *M. charantia* L. plant material was acquired in municipality of Malta, Paraiba, Brazil. The extract was obtained through maceration, filtration and then concentrated under reduced pressure in a rotary evaporator, resulting in a dough, and was then dried in an oven for 72 hours at 40°C. Antimicrobial action of ethanolic extract of seed *M. charantia* L. was evaluated based on the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) against standard strains of bacteria, isolates multiresistant bacteria and *Candida* species, by microdilution in broth method.

Results: All organisms were sensitive to the extract, being considered strong antimicrobial activity (MIC and MBC/MFC < 0.125 mg/ml).

Conclusion: The *M. charantia* L. showed strong antimicrobial potential, with bactericidal and fungicidal profile, there is the prospect to constitute a new therapeutic strategy for the control of infections, particularly in multiresistant strains.

Clinical significance: The use of medicinal plants in treatment of infectious processes have an important function nowadays, due to the limitations of the use of synthetic antibiotics available, related specifically to the microbial resistance emergence.

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INTRODUCTION

The search for new antimicrobial agents from medicinal plants has increased in recent years, mainly because of the emergence of microbial resistance.[1] Hospital infections, as well as community-acquired infections, especially caused by bacteria, such as *Pseudomonas euruginosa, Proteus mirabilis, Providencia rettgeri, Staphylococcus aureus* and *Escherichia coli* are worrisome conditions, due to the high morbidity and mortality,[2-4] plus the associated antibiotic resistance.[3,5-7]

Other infections, such as those caused by *Candida* species are also a concern in healthcare. The most suitable drugs for their treatment are the azoles (fluconazole, itraconazole and miconazole) and polyene (amphotericin B and nystatin). Fluconazole, for example, is much prescribed for the treatment of candidiasis, however, some limitations have been identified in its use, such as low solubility in water and the emergence of resistant strains. These observations have conducted studies in an attempt to overcome its drawbacks.[8] Nystatin is one of the most important active agents against fungal infections of the oral cavity,[9] on the other hand, cases of resistance of *Candida albicans* strains against this drug have already been observed.[10] Amphotericin B is the most used drug for the treatment of invasive fungal infections, especially in immunocompromised patients, despite being one of

the most toxic drugs of antimicrobial therapy.[11] Also noteworthy is the emergence of the non- *C. albicans* yeast, since they can be highly resistant to antifungal agents, such as *C. tropicalis, C. parapsilosis, C. glabrata, C. krusei* and *C. guillermonde.*[12,13]

Thus, there is a great demand for new antimicrobials of different structural classes, acting selectively on new targets, with fewer side effects. In this perspective, some studies have investigated the antimicrobial activity of medicinal plants aiming at developing new drugs.[14,15]

Among the medicinal plants available, there is *Momordica charantia* L., which is considered 'revolutionary' because of its versatility as food and therapeutic product.[16]

Especially from the identification, in its seeds, of a ribosome inactivating protein with antiherpes and antipoliovirus activity,[17] *M. charantia* L. became the object of study for many researchers.[18] Phytochemical studies revealed the presence, in its structure, of glycosides, saponins, alkaloids, triterpenes, proteins and steroids, which are considered biologically active compounds.[19] The fruits have a high lycopene content,[16] vitamins A, B1, B2 and C, as well as some minerals (calcium, magnesium, phosphorus and iron).[20]

Momordica charantia L. is a wild plant species, popularly known in Brazil as the melão-de-são-caetano.[21] It is widely used in traditional medicine in several countries, including Brazil. Studies have investigated its use as antidiabetic product,[22,23] antiviral against different subtypes of influenza A,[24] anti-HIV activity,[25] antiproliferative in human tumor cells[26], antioxidant and anti-inflammatory,[27] antimicrobial[28,29] and wound healing of gastric ulcers.[30]

The studies seeking to verify the antimicrobial potential of medicinal plants represent a challenge in the discovery and identification of new drugs. Whereas there are still few studies on the antimicrobial potential of *M. charantia* L. fruits, this work aims at evaluating the antibacterial and antifungal potential of this plant, *in vitro*, against the microorganisms of clinical interest (standard strains and multiresistant isolates) in order to aggregate scientific information in relation to its use as a therapeutic product.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Momordica charantia L. plant material (fruit) was acquired through direct sampling in the high Mesoregion of Paraiba Hinterland, in the municipality of Malta (6° 54' 14" S, 37° 31' 19" W), Brazil. The testimony specimen is deposited in the Herbarium collection of Manuel Arruda Camara (ACAM) at the State University of Paraiba (UEPB), Campus I, Campina Grande, Paraiba, Brazil (No. 003/ACAM).

Preparation of Plant Extract

After collection and cleaning, the fruits were packed in paper bags, left in the oven on continuous air flow at 40°C for a period of 7 days. After drying, the material was ground and immersed in 96% ethanol alcohol at a ratio of 1:3 for 72 hours at room temperature, in order to obtain the extract through maceration. After this period, the extract was filtered and stored in a glass bottle, protected from light, in the refrigerator. This process of submersion, maceration and filtration was performed three times on intervals of 72 hours. The total filtrate was then concentrated under reduced pressure between (40° and 43°) in a rotary evaporator until maximum solvent's evaporation, resulting in a dough, and was then dried in an oven for 72 hours at 40°C.

Strains of Microorganisms and Susceptibility Testing

The microorganisms used in this study were *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *C. albicans* ATCC 18804, *Candida glabrata* ATCC 2001, *Candida guillermond* ATCC 6260, *Candida krusei* ATCC 34135, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 13803 and over multiresistant bacterias (National Program of Quality Control): *P. mirabilis*, *P. rettgeri* and *E. coli*, isolated from hospitalized patients and donated for this study by the Laboratory for antimicrobial activity, from the Pharmacy Department of State University of Paraiba.

The antimicrobial activity of the ethanol extract of *M. charantia* L. was identified by determining the minimum inhibitory (MIC), bactericidal (MBC) and fungicide (MFC) concentrations, in accordance with the clinical and Laboratory Standards Institute.[31,32] The test was performed in plates of 96 well containing 100 µl/culture medium well Brain Heart Infusion (BHI Liquid, Himedia®) for bacteria and sabouraud dextrose (SD Liquid medium, Himedia®) for yeasts. The dry extract was diluted in sterile distilled water at the 1:1 gm/ml ratio, being transferred to the first well-poço and serially diluted in order to achieve concentrations of 0.5 mg/ml and 0.003 mg/ml performed. The positive controls were chlorhexidine 0.12% (bacteria), nystatin (yeast) and ciprofloxacin 10% (multiresistant bacteria). The bacterial (1.0×10^6 UFC/ml) and fungi (5.0×10^3 UFC/ml) inocula were added to the wells (10 µl) and the plates wee incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of extract that inhibited visible microbial growth, being confirmed with the addition of 10 µl of resazurin 0.01% (Sigma-Aldrich, St Louis, MO, USA). The MIC is defined as the lowest concentration of

the sample, capable of preventing the red color appearance on the medium when cells have respiratory activity.

To determine MBC/MFC, a rate of 50 µl of the corresponding and of the following two well of highest and lowest concentration relative to the MIC—as well as of the controls were subcultured on BHI Agar (bacteria) and agar sabouraud dextrose (yeasts) and incubated at 37°C for 24 hours. The MBC/MFC was defined as the lowest concentration which inhibited visible growth on solid medium used. Assays were performed in triplicate in three independent experiments.

The sensitivity profile of multiresistant bacteria were screened using a series of polidiscos Cefar® for Gram negative microorganisms.

RESULTS

The results presented in Tables 1 and 2 can be considered promising results, since the extract showed antimicrobial activity against all the species used, including the multiresistant bacteria. Table 3 shows the behavior of different strains against antimicrobials.

DISCUSSION

Momordica charantia L. is widely used in traditional medicine, however, studies investigating their biological properties and mechanisms of action are still insufficient. Different therapeutic indications in traditional medicine are found in literature, especially for its pharmacological activities in the control of diabetes, viral infections and anticancer.[18] The present study investigated the antimicrobial potential of *M. charantia* L. against different species of clinical interest, common pathogens in opportunistic infections in the oral cavity and hospital infections; finding bactericidal and fungicidal activity with MIC, MBC and MFC values less than 0.125 mg/ml, representing strong antimicrobial activity.[33]

Table 1: Minimum inhibitory and bactericidal concentrations of *M. charantia* L. ethanol extract assessed against bacteria species (ATCC) and multidrug-resistant clinical isolates

	M. charantia L.	
Microorganisms	*CIM (mg/ml)*	*CBM (mg/ml)*
Proteus mirabilis (PNCQ)	0.003	0.007
Providência rettgeri (PNCQ)	0.007	0.007
Staphylococcus aureus ATCC 25923	0.031	0.062
Eucherichia coli (PNCQ)	0.031	0.062
Eucherichia coli ATCC 25922	0.062	0.125
Pseudomonas aeruginosa ATCC 27853	0.062	0.062

Table 2: Minimum inhibitory and fungicide concentrations of *M. charantia* L. ethanol extract assessed against C. species (ATCC)

	M. charantia L.	
Microorganisms	*CIM (mg/ml)*	*CFM (mg/ml)*
Candida parapsilosis ATCC 22019	0.015	0.031
Candida guillermond ATCC 6260	0.031	0.031
Candida glabrata ATCC 2001	0.031	0.062
Candida tropicalis ATCC 13803	0.062	0.062
Candida albicans ATCC 18804	0.062	0.125
Candida krusei ATCC 34135	0.125	0.125

Table 3: Profile of susceptibility of *E. coli*, *P. mirabilis* and *P. rettgeri* strains against antimicrobial agents

Antibiotic	*E. coli*	*P. mirabilis*	*P. rettgeri*
Sulfamethoxazole (SUT)	S	S	S
Ciprofloxacin (CIP)	S	S	S
Cefalotin (CFL)	R	S	I
Gentamicin (GEN)	S	S	S
Ampicillin (AMP)	R	R	R
Amikacin (AMI)	S	S	S
Cefepime (CPM)	S	S	S
Tetracycline (TET)	S	R	R
Ceftriaxone (CRO)	S	S	S
Amoxicillin/clavulanic acid (AMC)	R	R	R
Cefoxitin (CFO)	S	S	S
Ceftazidime (CAZ)	S	S	S
Chloramphenicol (CLO)	S	S	S
Aztreonam (ATM)	S	S	S
Pipenocitalina (PIT)	S	S	S

R: Resistant; S: Sensible; I: Intermediate

Results found for *P. euruginosa*, *P. mirabilis*, *P. rettgeri* and *S. aureus* bacteria should be highlighted, given the clinical importance of each one of them. *Pseudomonas euruginosa* are Gram-negative cocci, responsible for serious infections in humans and can lead to death of the patient.[34] It presents hidrolizis enzymes capable of promoting antibiotics, such as imipenem, aztreonam, amikacin and cefepime, which confers it a resistance to such antibiotics.[5] Its spread in hospitals is a worrying condition, representing the main risk factor for isolated infections toward the use of antibiotics in large quantities;[5] and is also considered an important source of bacteremia and a predominant cause of morbidity and mortality in patients, for example, with cystic fibrosis.[2] The *P. mirabilis* and *P. rettgeri* are Gram-negative bacteria, commonly associated with urinary-tract infections in hospitalized patients from the intensive care unit and is associated with the use of urinary catheters,[35,36] being one of the main etiological agents responsible for much of nosocomial infections affecting the urinary tract, a special concern when we consider the reports of antibiotic

resistance.[6] The extract of *M. charantia* L. also showed a strong antimicrobial activity potential against *S. aureus* and *E. coli.* These microorganisms are one of the most common causes of nosocomial infections and community infections with high morbidity and mortality.[3,4] Systemic infections caused by methicillin-resistant *S. aureus* (MRSA) infections are increasingly common in the hospital environment[7] and infections caused by extended-spectrum *E. coli* Beta-lactamase productors (ESBL) appear to increase in outpatients in different countries.[3]

It is also worth mentioning the results against *Candida* species. Despite being part of the normal flora of the oral cavity, they are considered important pathogens, especially *C. albicans, C. tropicalis, C. parapsilosis, C. krusei, C. glabata, C. guillermond.* Oral candidiasis is one of the most common opportunistic infections in patients with compromised immune system, cancer patients, diabetics and patients with total prosthesis.[37-41] Most of the clinically used antifungal agents have several drawbacks in terms of toxicity, efficacy and cost, and its frequent use has favored the emergence of resistant species,[1] which render the promising results found in this study.

The antimicrobial activity of *M. charantia* L. leaf extract was observed against *E. coli, Salmonella paratyphi, Shigella dysenterae*.[42] The extract produced from the fruits also showed activity against *Helicobacter pylori*.[43] Recently[28] was found an antifungal effect of the leaf extract of *M. charantia* L. against *C. albicans, C. tropicalis* and *C. krusei,* which minimal inhibitory concentration was 1024 µg/ml. On the other hand[29] did not find activity on the ethanol extract of *M. charantia* L. *against C. albicans* and *E. coli*, showing strong activity against *Bacillus subtilis* (CIM = 0.625 mg/ml). This difference in results can be attributed to several factors, including the methods used,[44,45] as well as environmental factors of cultivation and collection of the plant, which influence the production of secondary metabolites.[46] The both microdilution has been considered an excellent method for analyzing the activity of antimicrobials with greater sensitivity, when compared to other methods.[44,45]

The study of medicinal plants as a source of new types of drugs has grown in recent years due to the search for new drugs with greater therapeutic activity, lower toxicity, better biocompatibility and more accessibility to the population, which, due to cultural aspects, has a good acceptance, reflecting good prospects in the market of therapeutic products made from active natural ingredients.

CONCLUSION

The ethanol extract of the *M. charantia* L. fruits has antimicrobial activity against species of standard and multiresistant bacteria and against *Candida* species responsible for significant local and systemic infections in humans, which represent important prospects for obtaining new strategies for infectious treatment. We ratify the importance of studies with the referred plant, aiming at the definition of the plant's phytochemical characteristics, mechanism of action, toxicity, among others, and thus determining its therapeutic potential.

CLINICAL SIGNIFICANCES

Despite the widespread use of *M. charantia* L. by the population, few studies have sought to demonstrate its properties scientifically. Bacterial and fungal infections pose a challenge to researchers and clinicians, especially in immunocompromised patients. This situation is further complicated by the development of resistance to conventional medications on the part of a large number of microorganisms. Therefore, studies are needed for the discovery of safe, stable drugs from sources found in nature that are effective against resistant bacteria and fungi.

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