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Antibacterial activity of *Momordica charantia* (Curcubitaceae) extracts and fractions

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ABSTRACT: *Momordica charantia* L. belongs to the family Curcubitaceae and it is very common in many Brazilian regions. The plant is a liana with flowers and yellow fruits that present red seeds when are ripe. Popularly known as "melão-de-são-caetano", "melão amargo" or "cabaço-amargo", it possesses many uses: antidiabetic, antihelmintic, antmicrobial, anticancerigenous and antioxidant. The phytochemical prospection of the fresh and dried leaves extracts showed the presence of different classes of secondary metabolites, as flavonoids, alkaloids and tannins, that have demonstrated antimicrobial action. Fresh and dried leaves presented significantly antimicrobial activity against all bacterial strains tested, specially *Escherichia coli*. Ethyl acetate fractions were effective against *Escherichia coli* and *Bacillus cereus*. The modulatory activity was significative too.

KEYWORDS: *Momordica charantia*, Bioprospection, antibacterial activity

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ABBREVIATIONS

IBAMA: Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis
EtOH: Ethanol
NCCLS: National Committee for Clinical Laboratory Standards
ATCC: American Type Culture Collection
CFU: Colony Forming Unit
BHI: Brain Heart Infusion
DMSO: dimethylsulfoxide
MIC: Minimum inhibitory concentration
FLE: fresch leaves extract;
FLE HEX: fraction hexane of the fresch leaves extract;
FLE CLO: fraction chloroform of the fresch leaves extract;
FLE ACET: fraction ethyl acetate of the fresch leaves extract;
FLE MET: fraction methanol of the fresch leaves extract;
DLE: dried leaves extract;
DLE HEX: fraction hexane of the dried leaves extract;
DLE CLO: fraction chloroform of the dried leaves extract;
DLE ACET: fraction ethyl acetate of the dried leaves extract;
DLE MET: fraction methanol of the dried leaves extract.
(AMIC) Amikacin;
(KAN) Kanamycin;
(GEN) Gentamicin;
(NEO) Neomicin;
(C+) Positive control;

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INTRODUCTION

The Curcubitaceae family is composed by 90 genera and about 700 species, mainly in tropical regions (Asia, Amazonia, Oriental Africa and Caribe), and subtropical. The species can be found in temperate regions too. Many species are cultivated because their comestible properties, as pumpkin (*Cucurbita sp*), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.) and West Indian gherkin (*Cucumis anguria* L.) [1,2]. The "melão-de-são-caetano" (*Momordica charantia* L.), also belongs to this family, is very common in many Brazilian regions. This specie is a liana with flowers and yellow fruits that present red seeds when are ripe [3]. Popularly known as "melão-de-são-caetano", "melão amargo" or "cabaço-amargo", it possesses many uses, as antidiabetic, carminative, antihelmintic, antimalaral and antimicrobial, antiviral, anticancerigenous, contraceptive, immunostimulant and laxative, antioxidant and insecticidal, besides its indication in skin treatments (eczema, acne, mycoses, scabies, hemorrhoid and furuncules [4-6].

According to Omoregbe et al. (1996) [7] aqueous, ethanolic and methanolic extracts of *M. charantia* leaves presented antimicrobial activity against *Escherichia coli*, *Salmonella paratyphi*, *Shigella dysenterae*, *Streptomyces griseus* and *Mycobacterium tuberculosis*. On the other hand Prabakar and Jebanesan (2004) have shown that the leaves methanolic extracts were effective against *Culex quinquefasciatus* larvae [8].

Recently, the antiviral and antihelmintic activities of glycosidic triterpenoids mormodicine I and II were demonstrated, with special regard to nematicidal properties of these substances [9].

As reported by Ritter et al (2002) [10], "melão-de-são-caetano" can´t be used internally, because the known toxicity of its seeds that led to an inhibition of pregnancy in mice [11-13], besides the fact that its popular use is not the same that ones recommended by literature.

The purpose of this work was to perform the chemical prospection of the fresh and dry leaves extracts and to evaluate the antibacterial activity of *M. charantia* extracts and fractions.

MATERIALS AND METHODS

Ethanolic extract obtention

Leaves of *M. charantia* were collected on July 2009 at Instituto Chico Mendes/IBAMA, Crato, Ceará, Brazil. A voucher specimen is deposited at the herbarium Prisco Bezerra (Federal University of Ceará; accession number # 44172).

The fresh leaves extract was obtained by extraction with cold EtOH (^{TR}Dinamica), for 72 h at room temperature and after this the distillated solvent was rotaevaporated (yield 3.92%).

In order to obtain the dried leaves extract, leaves were dried for 48h at 40±2°C, picked and immersed in EtOH for 72h. After this, the solvent distillation was performed (yield 11.75%).

The fresh leaves extract was submitted to filtration using a Büchner funnel and four solvents: hexane, chloroform, ethyl acetate and methanol (^{TR}Dinamica) leading the obtention of four fractions. The dried leaves extract was submitted to the same procedure, using the same solvents and obtaining four fractions.

Chemical prospection

The fresh and dried leaves extract, and the respective fractions were submitted to phytochemical tests in order to detect the presence of heterosides, saponnins, tannins, flavonoids, steroids, triterpens, cumarines, quinones, organic acids and alkaloids were performed following the method described by Matos (1997) [14]. These tests are based on visual observation of color modification or precipitate formation after addition of specific reagents

Minimal Inhibitory Concentration (MIC) Determination

The antibacterial activity was investigated by employing a microdilution method, recommended by NCCLS M7-A6. Previously to the tests, bacterial strains were activated in Brain Hear Infusion Broth (BHI, Difco) for 24h at 35 ± 2°C. Two gram-positive standard strains were used: *Staphylococcus aureus* (ATCC 12692) and *Bacillus cereus* (ATCC 33018); three strains obtained from clinical material: *Staphylococcus aureus* (358), *Escherichia coli* (10536) and *Escherichia coli* (27). The inoculum was an overnight culture of each bacterial species in BHI broth diluted in the same media to a final concentration of approximately 1 x 108 UFC/mL (0.5 nephelometric turbidity units - McFarland scale). After this, the suspension was diluted to 1 x 106 UFC/mL in 10% BHI. 100 µL of each dilution were distributed in 96-well plates plus extracts and fractions in different concentrations, achieving 5 x 105 UFC/mL as final concentration of the inoculums [15-17].

Extracts and fractions were dissolved in distilled water and dimethyl sulfoxide (DMSO, Merck) to concentration of 1024 µg/mL. Further serial dilutions were performed to reach a final concentration in the range of 512 to 8 µg/mL. All experiments were performed in triplicate and the microdilution trays were incubated at 35 ± 2ºC for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 µL of resazurin staining (0.01%) aqueous solution in each well at the end of the incubation period. The minimal inhibitory concentration (MIC) was defined as the lowest extract or fraction concentration able to inhibit the bacteria growth, as indicated by resazurin staining (bacteria died cells are not able to change the staining color by visual observation – blue to red) [18]. The negative control was BHI.

Modulatory Activity Determination

In order to evaluate the extracts and fractions as modulators of antibiotic resistance, the MICs of aminoglycosides (neomycin, kanamycin, amikacin and gentamicin (Sigma Chemical Co.) against the analyzed strains were determined in the presence or absence of extracts and fractions using the microdilution test. Subinhibitory concentrations (MIC 1/8) in 10% BHI were used [19, 20].

RESULTS AND DISCUSSION

The chemical prospection of *M. charantia* fresh leaves extracts and fractions have indicated the presence of various secondary metabolites classes (Table 1), that are known to present different therapeutic applications, for example, tannins (antimicrobial, antiviral, moluscicidal and antitumoral), flavonoids (anticarcinogenic, antiviral, antihemorrhagic and antioxidant) [21-25]. The dried leaves extract and its fractions revealed the presence of many metabolites (Table 1), some of them were found in fresh leaves extract too.

Regarding the antibacterial activity (Table 2), fresh leaves extract inhibited the growth of all tested strains. The lowest MIC (32 µg/mL) was against *E. Coli* (27). The chloroform fraction also presented the lowest MIC against the same microorganism (64 µg/mL). The ethyl acetate fraction presented a similar result, showing activity against *E. coli* (27) and *B. cereus* (ATCC 33018) at 64µg/mL. The methanol fraction was effective against all tested strains and the lowest MIC (128 µg/mL) was against *S. aureus* (358). The hexane fraction was ineffective against all tested strains.

The dried leaves extract was effective against all tested strains and the lowest MIC (128 µg/mL) was against *E. coli* (27). The chloroform fraction presented the best results against *E. coli* (27) and *S. aureus* (ATCC 12692). The ethyl acetate fraction was effective against all tested strains and, similarly to ethyl acetate fraction, the lowest MICs were against was effective against all tested strains and the lowest MIC (32µg/mL) was against *E. coli* (27) and *B. cereus* (ATCC 33018). The methanol fraction had a MIC of 512 µg/mL, indicating activity against (ATCC 12692), *E. coli* (10536) and *E. coli* (27). The hexane fraction, as the fresh leaves extract, had no effect against the strains.

The chemical prospection carried out with fresh leaves extract and dried leaves extract showed that many secondary metabolites of various classes occur in both extracts, as tannins, flavonoids and alkaloids. These metabolites are reported to have many biological actions, including antimicrobial [21].

When the modulatory activity was evaluated, the extracts and fractions presented synergistic effect, with few exceptions, against the aminoglycosides tested. In some cases, no effect was observed (Table 3). In the most cases, a synergistic effect was observed, as shown by fresh leaves plus gentamicin or kanamycin against *S. aureus* (358). The same effect was observed for methanol fraction plus all aminoglycosides tested against *E. coli* (27) (Table 4).

In general, the toxic effect to the bacterial membrane and function, because the lipophilic membrane structure, has been used to explain the antimicrobial effect of essential oils and extracts [26, 27].

The results obtained here reveal that *M. charantia* extracts have presented significative antibacterial activity in vitro and this effect could be associated to the chemical constituents of the extracts and their ability to penetrate into lipidical layers.

Significative results were obtained for both extracts, but the fractions had the lowest MICs against *S. aureus* (358), *E. coli* (27) and *B. cereus* (ATCC 33018). This suggests a possible extract antagonistic effect and this activity can be related to the constituents of the extracts and fractions. This result shows the relevance of the study of efflux pump inhibition effect of native species extracts as potential antibiotic adjuvant.

Table 1: Identification of the main chemical classes of the extracts and fractions

Metabolites	FLE	FLE HEX	FLE CLO	FLE ACET	FLE MET	DLE	DLE HEX	DLE CLO	DLE ACET	DLE MET
Catequic tannins	+	+	+	+	+	+	-	+	+	+
Flavones	+	+	+	+	+	+	-	+	+	+
Flavonols	+	+	+	+	+	+	-	+	+	+
Xantones	+	+	+	+	+	+	-	+	+	+
Flavanonols	+	-	+	-	+	+	-	+	+	-
Flavanones	+	-	+	-	+	-	-	-	+	-
Alkaloids	+	-	-	-	+	+	-	-	-	+
Steroids	+	-	+	-	-	+	-	-	+	-
Triterpenes	-	-	-	+	-	-	-	+	-	+

(+) positive; (-) negative; FLE: fresh leaves extract; FLE HEX: fraction hexane of the fresh leaves extract; FLE CLO: fraction chloroform of the fresh leaves extract; FLE ACET: fraction ethyl acetate of the fresh leaves extract; FLE MET: fraction methanol of the frech leaves extract; DLE: dried leaves extract; DLE HEX: fraction hexane of the dried leaves extract; DLE CLO: fraction chloroform of the dried leaves extract; DLE ACET: fraction ethyl acetate of the dried leaves extract; DLE MET: fraction methanol of the dried leaves extract.

Table 2: Values of the minimal inhibitory concentration (MIC) of fresh and dried leaves extract, and the respective fractions

	Sample / Obtained concentrations (µg/mL)									
Strains	FLE	FLE HEX	FLE CLO	FLE ACET	FLE MET	DLE	DLE HEX	DLE CLO	DLE ACET	DLE MET
S. aureus	64	-	512	128	512	256	-	64	64	512
*S. aureus**	256	-	-	128	128	256	-	-	128	-
E. coli	512	-	512	512	512	512	-	512	512	512
*E. coli**	32	-	64	64	256	128	-	64	32	512
B. *cereus*	512	-	512	64	512	512	-	128	32	-

* multiresistant strains; FLE: fresh leaves extract; FLE HEX: fraction hexane of the fresh leaves extract; FLE CLO: fraction chloroform of the fresh leaves extract; FLE ACET: fraction ethyl acetate of the fresh leaves extract; FLE MET: fraction methanol of the fresh leaves extract; DLE: dried leaves extract; DLE HEX: fraction hexane of the dried leaves extract; DLE CLO: fraction chloroform of the dried leaves extract; DLE ACET: fraction ethyl acetate of the dried leaves extract; DLE MET: fraction methanol of the dried leaves extract.

Table 3: Fresh leaves extract and fractions antibacterial activity by direct contact

		FLE$_{8\mu g/mL}$	FLE Acet$_{16\ \mu g/mL}$	FLE Clo$_{12\ \mu g/mL}$	FLE MET$_{512\ \mu g/mL}$	C+
	AMIC	32	8	-	-	32
	KAN	64	16	-	-	32
S.aureus	GEN	32	32	-	-	8
	NEO	64	32	-	-	32
		FLE$_{32\ \mu g/mL}$	**FLE Acet$_{16\ \mu g/mL}$**	**FLE Clo$_{512\ \mu g/mL}$**	**FLE MET$_{16\ \mu g/mL}$**	**C+**
	AMIC	16	32	-	32	8
	KAN	1	4	-	8	64
S.aureus *	GEN	2	2	-	4	8
	NEO	8	16	-	32	8
		FLE$_{4\ \mu g/mL}$	**FLE Acet$_{8\ \mu g/mL}$**	**FLE Clo$_{8\ \mu g/mL}$**	**FLE MET$_{32\ \mu g/mL}$**	**C+**
	AMIC	32	128	64	1	16
	KAN	64	16	64	0,5	64
E.coli*	GEN	16	4	2	0,5	8
	NEO	64	4	64	2	32
		FLE$_{>512\ \mu g/mL}$	**FLE Acet$_{8\mu g/mL}$**	**FLE Clo$_{>512\ \mu g/mL}$**	**FLE MET$_{>512\ \mu g/mL}$**	**C+**
	AMIC	-	16	-	-	16
	KAN	-	64	-	-	8
B. cereus	GEN	-	16	-	-	8
	NEO	-	64	-	-	16

* multiresistant strains
(AMIC) Amikacin; (KAN) Kanamycin; (GEN) Gentamicin; (NEO) Neomicin; (C+)Positive control.

Table 4: Dried leaves extract and fractions antibacterial activity by direct contact

		$DLE_{32\mu g/mL}$	DLE Hex $_{>512\ \mu g/mL}$	DLE $Clo_{8\ \mu g/mL}$	DLE $Met_{8\ \mu g/mL}$	C+
	AMIC	64	-	32	32	32
	KAN	128	-	32	64	32
S.aureus	GEN	16	-	8	32	8
	NEO	64	-	32	32	32
		$DLE_{32\ \mu g/mL}$	DLE Hex $_{>512\ \mu g/mL}$	DLE $Clo_{>512\ \mu g/mL}$	DLE $Met_{16\ \mu g/mL}$	C+
	AMIC	16	-	-	16	8
	KAN	1	-	-	4	64
S.aureus *	GEN	2	-	-	2	8
	NEO	8	-	-	4	8
		$DLE_{16\mu g/mL}$	DLE Hex $_{>512\mu g/mL}$	DLE $Clo_{8\ \mu g/mL}$	DLE $Met_{4\ \mu g/mL}$	C+
	AMIC	16	-	64	32	16
	KAN	28	-	2	8	64
E.coli*	GEN	16	-	16	32	8
	NEO	32	-	32	4	32
		$DLE_{>512\ \mu g/mL}$	DLE Hex $_{>512\ \mu g/mL}$	DLE $Clo_{16\ \mu g/mL}$	DLE $Met_{4\ \mu g/mL}$	C+
	AMIC	-	-	8	16	16
	KAN	-	-	4	8	8
B. cereus	GEN	-	-	1	2	8
	NEO	-	-	4	8	16

* multiresistant strains
(AMIC) Amikacin; (KAN) Kanamycin; (GEN) Gentamicin; (NEO) Neomicin; (C+)Positive control.

CONCLUSION

The chemical prospection of fresh and dried *M. charantia* leaves extracts have shown the presence of different secondary metabolites, as steroids, flavonoids, alkaloids and tannins, that have comproved antimicrobial action.

Both extracts, fresh and dried leaves, presented significative antibacterial activity against all tested strains, especially against *E. coli* (27). Regarding to fractions MICs, the ethyl acetate fraction was the most effective against gram-negative (EC 27) strains from clinical material and standard gram-positive (BC 33018), besides it presented the most significative MIC. The ethyl acetate fraction presented the same behavior, but in a minor concentration.

The assay to determine the MIC has demonstrated the efficiency of the extracts and of some fractions against the standard strains and from clinical material, showing that there is a relationship when the ethyl acetate fraction from both extracts is compared.

The evaluation of the modulatory activity showed a significative result, and this can be related to a major synergic potential of extracts and fractions.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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