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Antibacterial activity of *Cordia dentata* Poir, *Heliotropium indicum* Linn and *Momordica charantia* Linn from the Northern Colombian Coast

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Summary

Cordia dendata Poir, Heliotropium indicum Linn and Momordica charantia Linn are used for treatment of the most common human diseases and health disorders in folk medicine of the population from the northern Colombian coast. In this study, chemical composition and antibacterial activity of the ethanol extract and fractions from C. dentata, H. indicum and M. charantia were investigated. The chemical constituents of qualitative detection were examined by Thin Layer Chromatography (TLC). The antibacterial activity was determined by agar gel diffusion and broth microdilution method. The main identified compounds were flavonoids, cardiac glycosides, alkaloids, saponins, terpenoids, tannins and coumarins. The minimal inhibitory concentration (MIC) was 31.25 to 1000 μ g/mL for Gram-positive and Gram-negative bacteria, respectively. These results indicated that ethanol extract and fractions from C. dentata, H. indicum and M. charantia significantly inhibited the growth of standard strains. Potent antibacterial activities of C. dentata, H. indicum and M. charantia may be considered in future study, particularly against antibiotic-resistant cases.

Keywords: Cordia dendata Poir, Heliotropium indicum Linn, Momordica charantia Linn and Antibacterial activity.

RESUMEN

Actividad antibacterial de *Cordia dentata* Poir, *Heliotropium indicum* Linn y *Momordica charantia* Linn de la costa norte de Colombia

Cordia dendata Poir, Heliotropium indicum Linn y Momordica charantia Linn son usadas para el tratamiento de las enfermedades más comunes y trastornos de salud en la medicina popular de la población de la costa norte de Colombia. En este estudio se investigó la composición química y la actividad antibacterial de los extractos etanólicos y fracciones de C. dentata, H. indicum y M. charantia. Los constituyentes químicos se examinaron por detección cualitativa cromatografía de capa fina (TLC, por sus siglas en inglés). La actividad antibacterial se determinó por difusión en agar y microdilución en caldo. Los mayores compuestos identificados fueron flavonoides, glucósidos cardiotónicos, alcaloides, saponinas, terpenoides, taninos y cumarinas. La concentración inhibitoria mínima (MIC, por sus siglas en inglés) fue de 31,25 a 1000 μg/mL para bacterias Gram-positivas y Gram-negativas, respectivamente. Estos resultados indican que el extracto etanólico y las fracciones de C. dentata, H. indicum y M. charantia inhibieron de manera significa el crecimiento de las cepas estándar. La potente actividad antibacterial de C. dentata, H. indicum y M. charantia puede considerarse en posteriores estudios, en particular frente a casos de antibióticos resistentes.

Palabras clave: Cordia dendata Poir, Heliotropium indicum Linn, Momordica charantia Linn y actividad antibacterial.

Introduction

According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care [1]. Colombia accounts for approximately 10% of the world's biodiversity and is home to about 50,000 species of plants [2]. The population of the northern coast, specifically in department of Bolívar use traditional medicine for treatment of skin affections, inflammation of the respiratory tract, and gastro-intestinal disorders (Figure 1) [3]. However, there is little scientific information about the properties of medicinal plants in this region of Colombia.



Figure 1. Photographs of plants: A. *Cordia dentata* Poir, B. *Heliotropium indicum* Linn, C. *Momordica charanatia* Linn.

Cordia dentata Poir, is one of the species of plants to the family Boraginaceae little studied in the phytochemical and ethnopharmacological [4]. The genus Cordia has about 250 species [5, 6], C. dentata Poir is native to South and Central America [7], in Colombia it's distributed in the regions of Atlántico, Cundinamarca, Guajira, Magdalena, Meta and Bolívar, its local name is "uvito" [8]. The plant fruits are widely used as a traditional remedy for diseases such as intestinal parasites [4]. Several recent studies have revealed that the leaves and fruits of C. dentata contain a wide range of active compounds that are responsible for leishmaniacidal properties [9] and antinutritional factors in the diet of ruminants [10]. The accumulation of certain polyphenols (such as flavonoids, tannins, phenol acids and others) and their derivatives are mainly responsible for these potential health benefits [9, 11, 12]. Heliotropium indicum Linn (Boraginaceae) is an herbaceous plant popularly known as Indian heliotrope, has a pantropical distribution and is usually regarded as a weed. Widely distributed in South America, Asia [13] and Africa [14]. In Colombia it's distributed in the regions of Atlántico, Amazonas, Bolívar, Cauca, Cundinamarca, Meta and Norte de Santander, its local name is "Rabo de alacrán" and "verbena" [3, 8]. This plant is widely used as traditional remedy for several diseases such as intestinal parasites [3], amenorrhea, high blood pressure [15], abdominal pain, convulsion, cataract, conjunctivitis [16] and treatment cancer in Peru [17]. The chemical components of H. indicum included flavonoids, terpenoids, glycosides, steroids, saponins, tannins, pyrrolizidine alkaloid and phenolic compounds related to anti-glaucomas [14], anthelmintic [18], anti-inflammatory, rheumatism, anti-tumor [19], antileishmanial, hemolytic and toxicological activities [9].

Momordica charanatia Linn (Cucurbitaceae) is an herbaceous plant popularly known as bitter gourd or karela in India. This plant is distributed in Asia, South America, and East Africa [20]. In Colombia it's distributed in all national territory, its local name is "balsamina" [3]. The fruit is used as traditional remedy for the treatment of diabetes, cardiovascular diseases [21] and its aerial parts in infusion for intestinal parasites

diseases), it often used as a vegetable in diet [3]. Several studies have demonstrated antibacterial, antiviral, anticancer, and antidiabetic activities [21, 22-24].

The aim of the present study was to evaluate the antibacterial activities of the ethanol extract and various fractions with chloroform, ethyl acetate, hexane and methanol obtained from the leaves of *Cordia dendata* Poir, *Heliotropium indicum* Linn and *Momordica charantia* Linn by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Methods

Plants materials

Plants were collected in the department of Bolívar, on the northern coast of Colombia. The study region is located between 75°15′ and 75°45′ West longitude and between 10°10′ and 10°40′ North latitude; sector the Boquilla in the city of Cartagena and San Basilio de Palenque, in February and March 2014. Botanical identification was performed by botanists at the Institute of Botany from the University of Antioquia-Colombia and voucher specimens were retained in the herbarium (*C. dentata P.* number: 19558, *H. indicum L.* number: 95580, *M. charantia L.* number: 85570).

Preparation of extracts

The extraction process was performed using the cold percolation method. The *C. dentata*, *H. indicum* and *M. charantia* leaves were dried at room temperature, and then were ground with a blender. A sample (300 g) of each powder was macerated in 98% ethanol (3L) during 4 days in room temperature. The crude extracts were obtained after of the filtration after filtration with whatman N° 1 filter paper, and were evaporated under at low pressure at below 55 °C in a rotor evaporator *Heidolph* model Hei-Vap precision (250 mbar) [11, 25].

Preparation of fraction

The EtOH extract of C. dentata, H. indicum and M. charantia was concentrated and then partitioned with Hexane, CH_2Cl_2 , EtOAc and Methanol respectively. The Hexane soluble extract was subjected to silica gel chromatography column and eluted with mixture of Hexane- CH_2Cl_2 (1:1), CH_2Cl_2 (1), CH_2Cl_2 - EtOAc (1:1), EtOAc (1), EtOAc - MeOH (1:1) allows obtaining five fractions.

Phytochemical screening

The qualitative detection of secondary metabolites was examined by thin Layer Chromatography (TLC) silica gel 60 GF254 plate. TLC plates were detected under UV $\lambda = 254$ and $\lambda = 366$ nm [9, 11, 26].

Test microorganisms

The microorganisms used in the study were obtained from the American Type Culture Collectionn (ATCC): *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

Antibacterial activity assay

Antibacterial activity was determined by modified agar gel diffusion method [27]. Autoclaved Mueller-Hinton agar (MHA) was poured into sterile Petri dishes and solidified into plate. The inoculated plates were incubated at 37 °C for 24 hours. $10\,\mu\text{L}$ of ethanol extract or fractions were aseptically placed on top of the agar layer, and the plates were incubated at 37 °C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. Standard antibiotic kanamycin, tetracycline and ampicillin ($20\,\mu\text{g/mL}$), $10\,\mu\text{L}$ DMSO solution used as a negative control. All the antimicrobial tests were performed in triplicate and the results were reported as mean \pm standard deviation of three replicates.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The MIC and MBC of extracts and fractions were evaluated by the microdilution method with slight modifications [28-30], and the Clinical and Laboratory Standards Institute [31]. In brief, a series of ethanol extract and fraction concentrations were prepared with the broth and $100\,\mu\text{L}$ of these solutions were added to sterile 96-well plates. Then $100\,\mu\text{L}$ aliquots of culture containing approximately $106\,\text{CFU/mL}$ of the tested microorganisms were added to set the final concentration of ethanol extract or each fraction at $1000, 500, 250, 125, 62.5\,\text{y}\,31.25\,\mu\text{g/mL}$. The well only containing broth and inoculate was used as a negative control. The inoculated 96-well plates were incubated at 37 °C for 24 hours, cell growth was measured in a Multiskan Ascent spectrophotometer (Thermo Labsystems Oy, UK) at OD 620 nm. In this assay, each experiment was performed in triplicate. The MBC value was defined as the lowest concentration of ethanol extract or its fraction able to kill at least 99.9% of the bacterial inoculum within 24 hours [32]. In brief, the contents of well with value MIC and those with value above to MIC for each microorganism were plated sterile Petri dishes containing

MHA [33]. After incubation at 37 °C for 24 hours, the bacterial colonies on the Petri dishes were counted. In this assay, each experiment was performed in triplicate.

Statistical analysis

Each experiment was repeated three times. The results were expressed as the mean \pm SD. The data were subjected to analysis of variance (ANOVA) and the differences between means were determined by Tukey test ($P \le 0.05$), using Graph Pad Prism 5.0.

RESULTS AND DISCUSSION

Phytochemical screening of C. dendata Poir, H. indicum Linn and M. charantia Linn

Phytochemical constituents are shown in Table 1. Terpenoids were observed in all the extract and fractions of three medicinal plants, while alkaloids were present in ethanol extract. Further analysis showed that dichloromethane fractions contained rich saponins and cardiac glycosides. Ethyl acetate fraction enriched flavonoid, tannins and coumarins.

Antibacterial activity

The antibacterial activity is shown in Table 2, 3 and 4. Our results showed *C. dendata*, *H. indicum* and *M. charantia* ethanol extract and fractions exhibited varying degrees of antibacterial activity against the tested microorganisms. *C. dendata* inhibited 4 out of 5 of the tested microorganisms. Hexane fraction showed high inhibitory activity against *P. aeruginosa* (21.3 \pm 1.2 mm), ethyl acetate fraction activity against *S. epidermidis* (19.8 \pm 1.1 mm) and *K. pneumoniae* (12.8 \pm 0.5 mm), dichloromethane fraction against *S. aureus* (15.2 \pm 1.3 mm). Ethanol extract and fractions were not effective against *E. coli* (Table 2). *H. indicum* hexane fraction showed antimicrobial effects only against *S. aureus* (9.2 \pm 1 mm) and *P. aeruginosa* (10.8 \pm 0.8 mm). Ethanol extract and fractions, Dichloromethane fraction, Ethyl acetate fraction, Methanol fraction were not effective against *S. epidermidis*, *K. pnuemoniae* and *E. coli* (Table 3). *M. charantia* dichloromethane fraction showed notable antibacterial activity against *S. aureus* (9.8 \pm 1.2 mm), *S. epidermidis* (9.5 \pm 0.6 mm), *P. aeruginosa* (8.9 \pm 1.0 mm), ethanol extract and fractions exhibited against *K. pneumoniae* (7.7 \pm 1.2 mm), ethanol extract and fractions were not effective against *E. coli* (Table 4).

Table 1. Phytoconstituents of the extract and fractions of C. dendata Poir, H. indicum Linn and M. charantia Linn.

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ıtia	JV	+	+	+	+	+	+	١	١	١	,	,	1
M. charantia	DĘ	+	+	+	,	١	١	+	+	+	+	+	+
M.	JН	+	+	+	+	١	+	+	+	+	+	+	+
	Ε¢	+	+	+	+	١	+	+	+	+	+	+	+
	ЭW	+	+	+	+	١	١	+	١	+	+	+	+
un	ĴΥ	,	١	١	+	+	+	+	+	+	+	+	+
H. indicum	DŁ	+	+	+	١	+	+	+	+	+	+	+	+
H.	JН	+	+	+	١	١	+	+	١	+	+	+	+
	Е¢	+	+	+	+	+	+	+	ı	+	+	+	+
	₹W	١	١	١	+	1	١	+	+	,	,	,	-
ta	JУ	,	1	,	+	+	+	+	+	1		,	1
C. dendata	DŁ	ı	١	١	,	+	1	+	+	+	+	+	+
C.	JН	١	١	1	1	1	+	+	+	+	+	+	+
	${ m E}^{ m c}$	+	+	+	,	1	+	+	+	١	,	,	-
	TesT	Mayer's test	Drangandroff's test	Wagner's test	Citric acid boric test	FeCl ₃ test	With aqueous KOH 5% solution	Liebermann Burchard test	Vanillin sulfuric acid test	SbCl ₃ test	Baljet test	Raymond's test	Kedde's test
sį	рһуғосһетісә	Alkaloids			Flavonoids	Tannins	Coumarins	Phytosterols	Saponins		Cardiac	glycosides	

Note. "+" sign denotes presence, and "-' sign denotes absence of the compound the plants. Ethanol extracts (Ee): Hexane fraction (Hf); Dichloromethane fraction (Df); Ethyl acetate fraction (Af); Methanol fraction (Mf). + Present, - Absent.

Table 2. Antibacterial activity of C. dendata by modified agar gel diffusion method.

s														Extra	ıct an	d fra	Extract and fractions													
airain			Eth	Ethanol					Hex	Hexane				Dic	hloro	Dichloromethane	ane			豆	hyl a	Ethyl acetate	4)			~	Methanol	lou		
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S	١	+	+	+	+	+	+	+	+	+	+	+	١	١	١.	١	١,	١	+	+	+	+	+	+	٠,	١	١.	١	+	+
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Table 3. Antibacterial activity of H. indicum by modified agar gel diffusion method.

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		9	١	1	+	١	+
	-	5	١	1	1	١	+
	Methanol	4	١	1	1	١	+
	Met	3	١	1	١	١	١
		2	١	1	١	١	١
		I	١	١	١	١	١
		9	١	+	١	١	+
	9	5	١	1	,	١	+
	Ethyl acetate	3 4 5 6 1	,	1	,	,	+
	thyl a	3	,	1	,	١	+
	Ξ.	2	,	1	1	,	١
		1	١	1	,	,	
		9	١	+	+	+	+
Extract and fractions	ane	3 4 5 6 1	,	1	+	+	+
d frac	Dichloromethane	4	,	1	+	,	+
ct an	hlore	3	,	1	,	,	+
Extra	Dic	1 2	,	,	,	,	,
		1	١	١	١	١	,
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	Hexane	4 5	,	1	,	١	+
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	Strains		Э	Ъ	A	s	K

Strains: Escherichia coli (E); Pseudomonas aeruginosa (P); Staphylococcus aureus (A); Staphylococcus epidermidis (S); Klebsiella pneumoniae (K). Concentrations: 1 = 31.25 µg/mL; 2 = $62.5\,\mu\mathrm{g/mL}; 3 = 125\,\mu\mathrm{g/mL}; 4 = 250\,\mu\mathrm{g/mL}; 5 = 500\,\mu\mathrm{g/mL}; 6 = 1000\,\mu\mathrm{g/mL}$ Inhibition zone = $< 6\,\mathrm{mm}$ no antibacterial activity (-); Inhibition zone = $> 6\,\mathrm{mm}$: high antibacterial activity (+).

Table 4. Antibacterial activity of M. charantia by modified agar gel diffusion method.

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		5	1	,	,	,	,
	anol	4	1	1	,	,	,
	Methanol	3	1	1	,	,	,
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			1	,	,	,	١
		5 6 1	١	١	١	١	١
	te	~	,	١	١.	١	١
	Ethyl acetate	4	1	,	١,	١	١
	thyl	3	١	١.	١.	١	١
	щ	2	١	١	١	١	١
			١	,	١	,	١
		9	1	,	+	,	١
Extract and fractions	Dichloromethane	>		,	,	,	١
d frac	omet	4	١	١	,	١	١
ct an	chlor	3	١	١	١.	١,	١
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		П	١	,	١.	١	١
		9	١	+	+	١,	١
		~	١	١	١	١	١
	Hexane	4		1	١	1	١
	Не	3	١	١	,	,	١
		2	1	1	,	,	١
		1		,	١	,	1
		9	1	,	1	,	١
		>	1	١	١	,	١
	Ethanol	4	1	,	,	,	١
	Eth	3	١	,	١	,	١
		2	1	,	١	١	١
			1	١	١	١.	١
	Strains	3	Э	Ь	A	S	K

Strains: Escherichia coli (E); Pseudomonas aeruginosa (P); Staphylococcus aureus (A); Staphylococcus epidermidis (S); Klebsiella pneumoniae (K). Concentrations: 1 = 31.25 µg/mL; 2 = $62.5\,\mu\mathrm{g/mL}; 3 = 125\,\mu\mathrm{g/mL}; 4 = 250\,\mu\mathrm{g/mL}; 5 = 500\,\mu\mathrm{g/mL}; 6 = 1000\,\mu\mathrm{g/mL}$ Inhibition zone = $< 6\,\mathrm{mm}$ no antibacterial activity (-); Inhibition zone = $> 6\,\mathrm{mm}$: high antibacterial activity (+).

MIC and MBC analyses

The MIC and MBC values are shown in Figures 2, 3 and 4. The MIC values of ethanol extract and the fractions ranged from 31.25 to $1000 \,\mu\text{g/mL}$, exhibited significant antimicrobial activity ($P \le 0.05$) when compared to positive control. Overall, ethanol extract and fractions of *C. dendata* and *M. charantia* exhibited a potent antibacterial activity from 50 at 99 % against *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* different at *H. indicum*, ethanol extract and fractions of the plants evaluated were not effective against *E. coli*. Ethyl acetate and hexane fraction of *C. dentata* showed maximum activity with MIC 31.25 μ g/mL for *S. aureus*, *S. epidermidis* ($500 \,\mu$ g/mL), *K. pneumoniae and P. aeruginosa* ($1000 \,\mu$ g/mL). In all cases, most of the MBC values were found to be equal or twice as high as the corresponding MIC values for *S. aureus* (Figure 2). Ethanol extract and fractions of *H. indicum* exhibited activity with MIC $1000 \,\mu$ g/mL against *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* (Figure 3). Dichloromethane and methanol fraction of *M. charantia* exhibited good activity with the MIC $62.5 \,\text{and} \, 125 \,\mu$ g/mL against *S. aureus* and the MBC values were found to be equal or twice as high as the corresponding MIC values (Ethanol extract) (Figure 4).

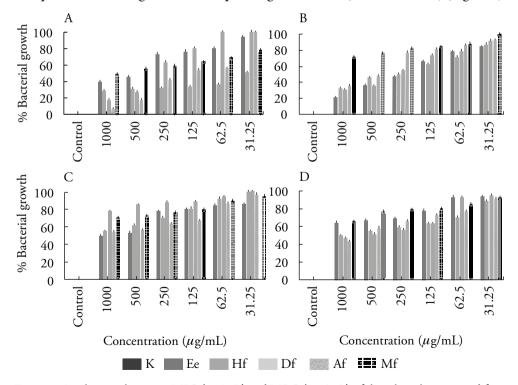


Figure 2. Antibacterial activity, MIC (μ g/mL) and MBC (μ g/mL) of the ethanol extract and fractions of *C. dendata* P. Strains: (A) *S. aureus*; (B) *S. epidermidis*; (C) *P. aeruginosa*; (D) *K. pneumoniae*. Kanamycin (K); Ethanol extract (Ec); Hexane fraction (Hf); Dichloromethane fraction (Df); Ethyl acetate fraction (Af); Methanol fraction (Mf).

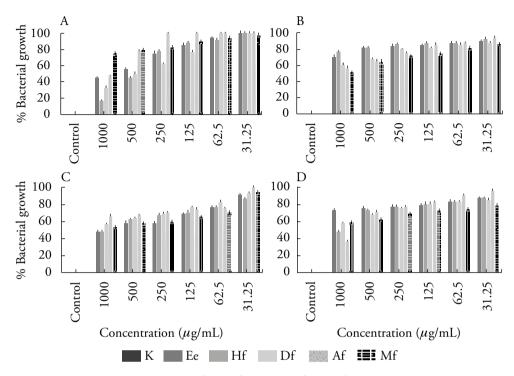


Figure 3. Antibacterial activity, MIC (μ g/mL) and MBC (μ g/mL) of the ethanol extract and fractions of *Heliotropium indicum* L. Strains: (**A**) *S. aureus*; (**B**) *S. epidermidis*; (**C**) *P. aeruginosa*; and *K. pneumoniae* (**D**). Kanamycin (**K**); Ethanol extract (Ec); Hexane fraction (Hf); Dichloromethane fraction (**D**f); Ethyl acetate fraction (Af); Methanol fraction (Mf).

The phytochemical analysis of the plants evaluated revealed the presence of flavonoids, cardiac glycosides, alkaloids, saponins, terpenoids, tannins and coumarins in the extract and fractions, as shown in Table 1. Each extract or fraction contains at least seven types of secondary metabolites, and some of the extract and fractions exhibited substantial antibacterial activities as shown by low MIC and MBC values against the tested microorganisms (Figures 2, 3 and 4). The secondary metabolites such as flavonoids and phenols are effective antibacterial substances due to their ability to form complexes with extra cellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria [34]. Terpenoids are known to have antibacterial property by affecting the synthesis of cell membranes components, prenylation of proteins and the use of carbon source [35]. The activities of these phytochemical components may be responsible for the antibacterial activities observed in the study. Largely, ethanol extract of M. charantia and ethyl acetate fractions of C. dendata with the MIC $1000 \,\mu\text{g/mL}$ (95 at 100%) showed a broad spectrum antimicrobial activity against the tested bacteria, and polar fraction exhibited better bacterial activity against some tested bacteria like S. aureus (ATCC 25923). However, few studies antibacterial of *C. dendata* and *H. indicum* activity. The leaf extracts (water, ethanol, and methanol) of *M. charantia* showed the broad-spectrum antimicrobial activity [36]. The studies *M. charantia* extracts/essential oils [37, 38], which possessed potential activity against *S. aureus*. Some of fractions, even at higher concentration ($1000 \mu g/mL$), could not inhibit or kill most of the bacteria. We speculated these fractions or extract might have undiscovered inhibitory mechanism, or they only have bacteriostatic and no bactericidal effect. Most antibacterial studies have focused on activity of plant extracts, but few on the time-dependent stability of the activity [39]. This indicated the active principles in the ethanol extract and fractions had a relative short shelf life [40]. The leaves of *C. dentata* and *H. indicum* used in folk medicine of the population from the northern Colombian coast for the treatment of internal parasites, skin affections as eczema, pruritic ailments, abscess or other inflamed wounds, boils, dermatosis presumably caused by fungal or yeast infections, dry skin condition [3].

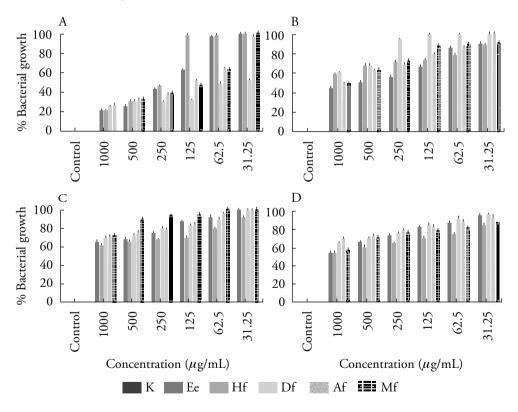


Figure 4. Antibacterial activity, MIC (µg/mL) and MBC (µg/mL) of the ethanol extract and fractions of *Momordica charantia* L. Strains: (A) *S. aureus*; (B) *S. epidermidis*; (C) *P. aeruginosa*; and *K. pneumoniae* (D). Kanamycin (K); Ethanol extract (Ec); Hexane fraction (Hf); Dichloromethane fraction (Df); Ethyl acetate fraction (Af); Methanol fraction (Mf).

Conclusion

The results of this assay showed that the ethanol extract and fractions of *C. dentata*, *H. indicum* and *M. charantia* the leaves exhibited activity bacteriostatic against the tested microorganisms *S. aureus*, *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* and not effective against E. coli. *C. dentata*, *H. indicum* and *M. charantia* medicinal plants from the Northern Colombian Coast have been shown to have promising *in vitro* antibacterial activity.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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