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Original Article

Antifungal activity of *Crotalaria longirostrata* Hook. & Arn. extracts against phytopathogen fungi from maize

Actividad antifúngica de extractos de *Crotalaria longirostrata* Hook. & Arn. contra hongos fitopatógenos de maíz

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ABSTRACT

In the present study, the hexane, acetone and methanol extracts of stem, branches and roots from *Crotalaria longirostrata* were evaluated for their antifungal activity against *Fusarium* sp.*, Fusarium verticillioides* and *Aspergillus flavus.* The variables analyzed were inhibition halos, mycelial growth inhibition, sporulation and minimum inhibitory concentration. Phytochemical analysis revealed the presence of saponins, coumarins, anthrones, anthraquinones, flavonoids and alkaloids on different organic extracts. The hexane extract showed zones of inhibition between 6.3 and 10.5 mm. The mycelia growth and sporulation of *A. flavus* were reduced to 90% with methanol extract. The minimum inhibitory concentration values obtained with hexane extracts were 6.75 mg mL^{-1} and with methanol extracts were 50 and 25 mg mL^{-1} for *Fusarium* strains. This is the first study reporting of phytochemical composition and biological activity of *C. longirostrata* that could be used as a natural alternative to control *in vitro* of certain important pathogenic fungi.

Keywords: Fungicidal properties, extracts, *Fusarium verticillioides*, *Fusarium* sp., *Aspergillus flavus.*

RESUMEN

En el presente estudio se evaluó la actividad antifúngica de los extractos hexánicos, cetónicos y metanólicos de tallo, ramas y raíces de *Crotalaria longirostrata* frente a los hongos *Fusarium* sp.*, Fusarium verticillioides* y *Aspergillus flavus*. Las variables analizadas fueron halos de inhibición, la inhibición del crecimiento micelial, la inhibición de la esporulación y la concentración mínima inhibitoria. El análisis fitoquímico reveló la presencia de saponinas, cumarinas, antronas, antraquinonas, flavonoides y alcaloides en los diferentes extractos orgánicos. Los extractos hexánicos mostraron zonas de inhibición entre 6,3 y 10,5 mm. El crecimiento micelial y la esporulación de *A. flavus* se redujo 90% con los extractos metanólicos. El valor de la concentración mínima inhibitoria obtenida con los extractos hexánicos fue 6,75 mg mL^{-1} y con los extractos metanólicos fueron 50 y 25 mg ml^{-1} para las cepas de *Fusarium*. Este es el primer reporte del estudio de la composición fitoquímica y la actividad biológica de *C. longirostrata* que podría ser utilizada como una alternativa natural para el control *in vitro* de ciertos hongos patógenos importantes.

Palabras clave: Propiedades fungicidas, extractos, *Fusarium verticillioides*, *Fusarium* sp., *Aspergillus flavus.*

INTRODUCTION

Phytopathogenic fungi cause severe losses in plants and crop production and significant reduction in seed germination and seedling emergence. The genus *Fusarium* is one of the most important fungi that include many pathogenic species, causing a wide range of plant diseases (Roncero *et al. 2*003). Microorganisms belonging to *Fusarium* genus live inside plant tissue as endophytes (Schulz & Boyle 2005) or in the rhizosphere (Cotxarrera *et al. 2*002) and they have the advantage of being able to adapt to the same environmental conditions. Maize is a crop attacked by various fungi in the early stages of plant growth due the high levels of moisture that occur in the field, this causes reduced performance. Annual maize production (*Zea mays* L.) in Mexico decreases 30 %, and *Fusarium* species are primarily responsible González *et al*. 2007, Briones-Reyes *et al*. 2015. *Fusarium verticillioides* is the predominant pathogen associated with the crop worldwide (Nelson 1992). A viable option to reduce crop losses due to diseases is the use of botanical species extracts, traditionally used for their wide variety of organic compounds that provide the ability to act as repellents or fungicides, besides being biodegradable and not harmful to humans. For instance, Rahman *et al*. (2011) reported that organic extract of *Piper chaba* exhibited a great antifungal potential for growth inhibition of *Fusarium oxysporum, Phytophthora capsici, Colletotrichum capsici, Fusarium solani,* and *Rhizoctonia solani* in a range of 55.1-70.3% and the values of their minimum inhibitory concentration were from 125 to 500 µg mL^{-1}. Meanwhile Adejumo & Langenkämper (2012) found that partially and completely purified methanolic extracts of seeds of *Piper guineense, Garcinia kola,* and *Aframomum melegueta* produced a reduction of mycelial growth of *Fusarium verticillioides*. The partially purified extracts produced a growth reduction of 82, 80 and 73% respectively, while the completely purified extracts reduced mycelial growth at 76, 54 and 43% respectively. Therefore the use of plant extracts could be an available, effective, and sustainable alternative to control diseases caused by fungi in crops.

The Fabaceae family is a primary source of potentially active secondary metabolites such as alkaloids, triterpenoids, flavonoids, isoflavonoids, coumarin, phenylpropanoids, anthraquinones, cyanogenic glycosides, and protease inhibitors; all these compounds have antifungal activity (Boulogne *et al. 2*012). *Crotalaria longirostrata,* belongs to this family, is an edible legume whose aroma and flavour have been part of southern Mexican and Central American cuisine, and has been used to treat gonorrhea, insomnia, and rheumatism. Its hypnotic, narcotic, purgative, and emetic properties are attributed to its leaves (Cáceres 1996).

The aim of this study was to investigate the antifungal activity *in vitro* of extracts from *Crotalaria longirostrata* on growth of *Fusarium* sp.*, Fusarium verticillioides* and *Aspergillus flavus*.

MATERIALS AND METHODS

Plant material

Stems, branches and roots of *C. longirostrata* were collected in the municipality of Suchiapa, Chiapas, México, geographic location: latitude 16° 37' 59.4'' north and longitude 93° 06' 50.8''. The plant material was dried in the shade at room temperature. Then samples were milled and stored in closed containers in the dark at room temperature until used.

Preparation of extracts

Each plant powder was successively extracted with different organic solvents in order of increasing polarity according to protocol reported by Arokiyaraj *et al*. (2009). Ten grams of each powder were briefly soaked in 100 ml hexane and shaken for 24 h. They were filtered through Whatman No. 1 filter paper and air dried. After, a second extraction was performed with acetone and then a third extraction with methanol. Finally, solvents were removed from the extracts using a rotary under reduced pressure (Büchi-Switzerland) and the residue was suspended in 10 mL of each solvent. The extracts were stored at 4 °C until used for further study.

Phytochemical analysis

Chemical analysis to detect the presence of major classes of secondary metabolites was determined using silica gel thin-layer chromatography (TLC) plates (silica gel 60 F_{254} Merck) and the samples were eluted with chloroform: methanol: ammonium hydroxide (8.5:1.4:0.1). The chromatography plates were revealed as was reported by Wagner and Bladt (1996).

Quantitative analysis of the groups of secondary metabolites present in the extracts was done using a visible light spectrophotometer (DR5000-03 HACH-USA). The chemical standards used were from Sigma-Aldrich, USA. Total phenol content was estimated as gallic acid equivalents (Singleton *et al*. 1999); saponin content was estimated as diosgenin equivalents (Sim *et al.* 2012); flavones and flavonols were estimated as quercetin equivalents (Chang *et al.* 2002), and total flavonoid content was estimated as rutin equivalents (Robertson & Hall 1989).

Fungal strains

Fusarium sp. strain F0708 and *Aspergillus flavus* strain CA06/6.5.2 were donated by 'Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias' *(*INIFAP)-Ocozocoautla. *Fusarium verticillioides* was obtained as a monoconidial culture from SERR symptomatic root tissue;

this was molecularly identified (Genbank accession no. GU982311.1) and tested for pathogenicity, showing high aggression to maize in seedling assays (Figueroa-López *et al.* 2013).

Evaluation of in vitro antifungal activity

Activity of organic *C. longirostrata* extracts were tested against *Fusarium* sp.*, F. verticillioides* and *A. flavus* by the agar-well diffusion method reported by Ndukwe *et al*. (2006). Briefly, Petri plates containing Czapek agar were inoculated with 100 µl of a solution of $1x10^7$ spores ml^{-1}. The wells (5 mm diameter) were done in the plates using a sterile Pasteur pipettes. After, 60 µl of the extracts (50 mg ml^{-1}), were added into each well and incubated for 48 h at 28 °C. The solvents (hexane, acetone and methanol) were also evaluated. Diameters of inhibitory zones were measured in millimeters. The experiment was done three times with six replicates and means values were presented.

Inhibition of mycelial growth

The extracts were mixed 1:10 with Czapeck agar at 45-50 °C. The mixture was poured into Petri dishes. These Petri plates were inoculated at the centre with the pathogens Control plates contained only the solvent of extraction (hexane, acetone, and methanol). The experiment was repeated three times with six replicates. Treatments and controls were incubated for seven days at 28 °C. Radial growth diameter of the fungi was measured at the end of the incubation period as was reported by Kumar *et al*. (2011) and Taiga *et al*. (2008), and then the inhibition (%) of each extract was determined using the formula:

$$\text{Mycelia growth inhibition (\%)} = \left[\frac{dc-dt}{dc}\right] x\ 100\ (\%)$$

Where dc=average diameter of the fungal colony in the control, and dt=average diameter of the fungal colony in the treatment.

Sporulation inhibition

The number of spores of a culture was determined after seven days. The final spores ml^{-1} concentration was determined using a Neubauer chamber and the percentage of inhibition of sporulation was evaluated for each extract using the formula:

$$\text{Sporulation inhibition (SI)} = \left[\frac{sc-st}{sc}\right] x\ 100\ (\%)$$

Where sc=number of spores in the control, and st=number of spores in the treatment.

Minimum Inhibitory Concentration (MIC)

Sensitivity of *Fusarium* sp.*, F. verticillioides,* and *A. flavus* to different organic extracts of *C. longirostrata* was determined using a common broth microdilution method of 96 wells in microtiter plates (Rashmi & Rajkumar 2011). The experiment was repeated three times with six replicates.

Experimental design and statistical analysis

Factorial design of type 3^3 was used for the experiment. The factors were type of solvent (three types; hexane, acetone, and methanol), part of plant (three parts; stem, branches, and root) and fungi strains (*Fusarium* sp.*, F. verticillioides,* and *A. flavus*). Six replicates were used for each combination, and the total number of runs was 162. For statistical analyses, one-way ANOVA at 5% level of significance using the STATGRAPHICS PLUS program was used (1999). The results expressed in percentages were subjected to an angular transformation (arcsine square root) to normalize the variance prior to analysis (Hwang *et al. 2*001).

RESULTS

Phytochemical study

Qualitative analysis of organic extracts of *C. longirostrata* showed the presence of flavonoids, coumarins, saponins, alkaloids, anthrones, and anthraquinones (Table 1). Root extracts (Rz2 and Rz3) had higher abundance of phytochemical components such as flavonoids, coumarins, and anthrones; while a moderate content of flavonoids and coumarins were found in root hexane extract (Rz1). Presence of flavonoids and saponins were observed in all extracts, mainly in acetone and methanol.

The content of total flavonoids in branches was higher in methanol extracts (Rs3) than in acetone and hexane extracts (Rs2 and Rs1); while in roots and stems, their values were higher in acetone extracts than in methanol and hexane extracts. The root extract in methanol (Rz3) had the highest content of total phenols, followed by root extract in acetone (Rz2); however this was not statistically significantly different for branches extract in methanol (Rs3). Meanwhile, hexane extracts (Rs1, T1, and Rz1) had lowest values due to the non-polar nature of the solvent. The content of saponins was determined only in the methanol extracts, because hexane and acetone reaction false positive and highest values were found in root extract (Table 2).

Table 1. Qualitative identification of phytochemical components of organic extracts from *Crotalaria longirostrata*. / Identificación cuantitativa de los componentes fitoquímicos de los extractos orgánicos de *Crotalaria longirostrata*.

Organic extracts	Secondary metabolites					
	Flavonoids	Coumarins	Saponins	Alkaloids	Anthrones	Anthraquinones
Rs3	+++	+	++	++	-	++
T3	++	+	++	+	+	++
Rz3	++	+++	++	++	+++	-
Rs2	+	++	++	-	+	++
T2	+	+	++	+	-	++
Rz2	+++	+++	++	+	+++	-
Rs1	+	-	+	+	-	+
T1	+	-	+	-	-	+
Rz1	++	++	+	-	-	-

The presence and qualitative abundance of phytochemical compounds is shown: Very abundant (+++), moderate (++), low (+), not available (-), as was reported by Kamatenesi-Mugisha *et al*. 2013. T: stem, Rs: branches, Rz: root, 1: hexane, 2: acetone, 3: methanol. / Presencia y abundancia cualitativa de compuestos fitoquímicos: muy abundante (+++), moderada (++), leve (+), nula (-), de acuerdo con Kamatenesi-Mugisha *et al.* 2013. T:tallo, Rs: ramas, Rz: raíz, 1: hexano, 2: acetona, 3: metanol.

Table 2. Quantification of secondary metabolites in organic extracts from *Crotalaria longirostrata*. / Cuantificación de metabolitos secundarios en extractos orgánicos de *Crotalaria longirostrata*.

Organic extracts	Secondary metabolites			
	Flavones and flavonols	Total flavonoids	Total phenols	Saponins
Rs3	309.56 ± 21.52 a	1012.34 ± 05.74 a	1332.79 ± 46.06 b	3228.97 ± 184.45 b
T3	102.49 ± 06.89 c	225.66 ± 11.48 f	985.46 ± 56.26 c	1602.06 ± 52.39 c
Rz3	109.00 ± 06.77 c	369.22 ± 05.74 d	1690.33 ± 127.78 a	3925.52 ± 129.24 a
Rs2	222.50 ± 17.72 b	492.77 ± 24.41 c	545.51 ± 35.75 d	ND
T2	183.21 ± 02.46 b	307.11 ± 05.98 e	487.62 ± 07.80 d	ND
Rz2	345.57 ± 33.00 a	896.64 ± 13.16 b	1337.21 ± 51.08 b	ND
Rs1	ND	133.16 ± 05.17 g	296.66 ± 18.39 e	ND
T1	ND	34.39 ± 00.99 h	289.51 ± 19.94 e	ND
Rz1	ND	38.41 ± 01.72 h	287.46 ± 18.39 e	ND

T: stem, Rs: branches, Rz: root, 1: hexane, 2: acetone, 3: methanol. ND: not determined. Values expressed in micrograms (equivalent of quercetin, rutin, gallic acid, diosgenin as appropriate) per milliliter (µg mL-1). Means of six replicates followed by at least one same letter are not significantly different at $P< 0.05$, Tukey test. / T: tallo, Rs: ramas, Rz: raíz, 1: hexano, 2: acetona, 3: metanol. ND: no determinado. Valores expresados en microgramos (equivalentes de quercetina, rutina, ácido gálico, diosgenina según corresponda) por mililitro (µg mL-1). Valores promedio de seis repeticiones seguidas de al menos una misma letra no son significativamente diferentes a $P < 0.05$ (prueba de Tukey).

Table 3. *In vitro* antifungal activity of organic extracts from *Crotalaria longirostrata* against *Fusarium* sp.*, F. verticillioides* and *Aspergillus flavus* using the agar-well diffusion method. / Actividad antifúngica *in vitro* de extractos orgánicos de *Crotalaria longirostrata* contra *Fusarium* sp.*, F. verticillioides* y *Aspergillus flavus* usando el método de difusión en agar-pozo.

Organic extract	Inhibition halos (mm)		
	Fusarium sp.	*F. verticillioides*	*Aspergillus flavus*
Rs3	6.67 ± 0.82 d	7.5 ± 0.55 bc	7.16 ± 0.98 ab
T3	6.33 ± 0.52 d	6.83 ± 0.75 c	0 ± 0.00 c
Rz3	6.67 ± 0.82 d	6.83 ± 0.75 c	0 ± 0.00 c
Rs2	7.67 ± 0.52 c	10.5 ± 1.38 a	0 ± 0.00 c
T2	7.83 ± 0.75 c	9.67 ± 0.82 a	0 ± 0.00 c
Rz2	6.33 ± 0.52 d	9.83 ± 1.47 a	0 ± 0.00 c
Rs1	8.5 ± 0.55 bc	7.67 ± 0.82 bc	7.33 ± 0.82 a
T1	8.67 ± 0.52 b	8.17 ± 0.68 b	7.67 ± 1.03 a
Rz1	10.5 ± 1.37 a	10.17 ± 0.75 a	6.33 ± 0.52 b
methanol	0 ± 0.00 e	0 ± 0.00 d	0 ± 0.00 c
acetone	0 ± 0.00 e	0 ± 0.00 d	0 ± 0.00 c
hexane	0 ± 0.00 e	0 ± 0.00 d	0 ± 0.00 c

T: stem, Rs: branches, Rz: root, 1: hexane, 2: acetone, 3: methanol. Means of six replicates followed by at least one same letter are not significantly different at $P < 0.05$, Tukey test. / T: tallo, Rs: ramas, Rz: raíz, 1: hexano, 2: acetona, 3: metanol. Valores promedio de seis repeticiones seguidas de al menos una misma letra no son significativamente diferentes a $P < 0,05$ (prueba de Tukey).

In vitro antifungal evaluation

The value of inhibition halos was the highest with treatment Rz1 compared with other treatments when we applied different organic extracts against *Fusarium* sp.*;* while such treatment had no significant statistical difference with Rz2, Rs2, and T2 against *F. verticillioides,* we have found significant statistical difference when we used Rs3, T3, Rz3, Rs1, and T1. The value inhibition halos were highest for Rs3, T1, and Rs1 compared with other treatments when we used different organic extracts against *A. flavus*. The hexane extracts (Rs1, T1, and Rz1) were effective against the three fungi compared with other treatments (Table 3).

Mycelial growth and sporulation inhibition

The methanolic extracts (Rs3, T3, and Rz3) showed the highest inhibitory effects on the mycelia growth (IMG) with 90, 70.53, and 62.41% against *A. flavus*, *F. verticillioides,* and *Fusarium* sp., respectively. Statistical analysis indicated that there wasn't a significant statistical difference between the methanol extracts. The sporulation inhibition (SI) in *A. flavus* was higher in the presence of the methanolic extracts (Rs3, T3, and Rz3) with 90% while in *Fusarium* sp. in the presence of root, branches, and stem extracts in acetone (Rz2, Rs2, and T2) we obtained values above 61% and no have significant statistical difference between these treatments, finally *F. verticillioides* had the highest values for treatments in methanol Rs3, T3, and Rz3 with a range of 66.3-71.3% (Table 4).

Minimum inhibitory concentration

The extracts of roots, branches, and stems from *C. longirostrata* obtained with hexane and acetone have the same effect on the fungi with the minimum inhibitory concentration lower (6.75 mg mL^{-1}), and we also found that methanol extracts completely inhibited the growth of *A. flavus* when applied at lower concentrations (6.75 and 12.5 mg mL^{-1}), whereas to inhibit the *Fusarium* species growth higher concentrations were necessary (25 and 50 mg mL^{-1}) (Table 5).

Table 4. Effect of organic extracts from *Crotalaria longirostrata* in inhibiting mycelial growth (IMG) and sporulation (SI) of *Fusarium* sp.*, F. verticillioides* and *Aspergillus flavus*. / Efecto de los extractos orgánicos de *Crotalaria longirostrata* en la inhibición del crecimiento micelial (ICM) y la esporulación (IS) de *Fusarium* sp., *F. verticillioides* y *Aspergillus flavus.*

Organic extracts	*Fusarium* sp.		*F. verticillioides*		*Aspergillus flavus*	
	IMG (%)	SI (%)	IMG (%)	SI (%)	IMG (%)	SI (%)
Rs3	60.58± 0.77 a	41.62± 1.70 d	69.54± 1.55 a	68.31 ± 1.22 ab	90 ± 0.00 a	90 ± 0.00 a
T3	62.41± 0.46 a	54.44± 0.89 c	67.30 ± 1.85 a	66.30± 2.36 abc	90 ± 0.00 a	90 ± 0.00 a
Rz3	60.85 ± 1.81 a	59.74 ± 2.99bc	70.53± 0.61 a	71.32± 0.00 a	90± 0.00 a	90± 0.00 a
Rs2	39.81± 1.02 b	63.05± 2.09ab	38.23± 2.08 b	63.16± 3.24bc	49.47 ± 0.20 bc	34.27 ± 0.01c
T2	35.03± 1.07 b	61.01± 1.99ab	33.83± 2.14 c	63.07± 0.00bc	52.84 ± 3.22 b	38.16 ± 0.06 b
Rz2	39.14 ± 1.39 b	66.04 ± 3.54 a	39.81 ± 0.39 b	61.31 ± 1.75 c	47.96 ± 6.88bc	34.17± 0.03c
Rs1	2.91 ± 0.96 e	0.0 ± 0.00 g	12.09 ± 2.68 e	42.06 ± 0.74 d	ND	ND
T1	0.0 ± 0.00e	0.0± 0.00g	0.0± 0.00 f	0.0± 0.00 g	ND	ND
Rz1	0.0 ± 0.00 e	33.76± 0.91 e	0.0± 0.00 f	36.04± 0.77 e	ND	ND
methanol	34.74 ± 1.69c	31.62 ± 1.69e	32.90 ± 1.98d	35.25 ± 1.56 e	30.71± 0.35d	31.04 ± 0.12 d
acetone	22.09 ± 1.43 d	21.62 ± 0.97 f	28.09 ± 1.99 e	27.21 ± 2.56 f	22.07 ± 0.26 e	29.60 ± 0.08 d

T:stem, Rs: branches, Rz: root, 1: hexane, 2: acetone, 3: methanol. ND: not determined. Means of six replicates followed by at least one same letter are not significantly different at $P< 0.05$, Tukey test. P values after arcsine transformation. / T:tallo, Rs: ramas, Rz: raíz, 1: hexano, 2: acetona, 3: metanol. ND: no determinado. Valores promedio seguidos de al menos una misma letra no son significativamente diferentes a $P < 0,05$ (prueba de Tukey). Valores de P después de la transformación arcoseno.

Table 5. Minimum Inhibitory Concentration (MIC) of organic extracts from *Crotalaria longirostrata* against *Fusarium* sp.*, F. verticillioides* and *Aspergillus flavus* / Concentración Mínima Inhibitoría (CMI) de los extractos orgánicos de *Crotalaria longirostrata* contra *Fusarium* sp.*, F. verticillioides* y *Aspergillus flavus.*

Organic extracts	MIC in mg mL $^{-1}$		
	Fusarium sp.	*F. verticillioides*	*Aspergillus flavus*
Rs3	25.00	25.00	6.75
T3	50.00	50.00	12.50
Rz3	25.00	25.00	12.50
Rs2	6.75	6.75	ND
T2	6.75	6.75	ND
Rz2	6.75	6.75	6.75
Rs1	6.75	6.75	6.75
T1	6.75	6.75	6.75
Rz1	6.75	6.75	6.75

T:stem, Rs: branches, Rz: root, 1: hexane, 2: acetone, 3: methanol. ND: not determined. Means of six replicates followed by at least one same letter are not significantly different at $P< 0.05$, Tukey test. / T:tallo, Rs: ramas, Rz: raíz, 1: hexano, 2: acetona, 3: metanol. ND: no determinado. Valores promedio de seis repeticiones seguidas de al menos una misma letra no son significativamente diferentes a $P < 0.05$ (prueba de Tukey).

DISCUSSION

In Mexico about 4,000 species of plants have medicinal attributes, however little scientific evidence is known to validate such applications due to the fact that only approximately 5% of the diversity of plants has been studied for their biological, chemical, and biomedical activity (Ocegueda *et al. 2*005). The genus *Crotalaria* is a member of the Fabaceae family and has 300 species worldwide. This genus produces mainly alkaloids and flavonoids, which are specifically found in the leaves and stems of *C. burhia* Halmilt (Kumar *et al. 2*011). The saponins were found in the leaves of *C. pulchra* Andr, *C. lachnosema* Stapf and *C. juncea* L (Rama Devi *et al.* 2010, Dinakaran *et al.* 2011, Ibrahim *et al.* 2012), while Kwaji *et al*. (2013) reported the presence of saponins in the roots of *C. pallida* Aiton. Bhakshu *et al*. (2008) reported that in the leaves of *C. madurensis* var. *kurnoolica* were also found other metabolites, such as anthraquinones and coumarins. In this study a phytochemical screening revealed that *C. longirostrata* contained all these phytochemical component and also anthrones, they were found in all extracts at different amounts, regardless of the section of the plant from which they were obtained. The production or synthesis of secondary metabolites depends greatly on the physiological and developmental stage of the plant (Rao & Ravishankar 2002). It has been reported that diverse factors affect the yield of the extraction process of phytochemical compounds, mainly the solvent polarity and organ nature (Trabelsi *et al. 2*010, Falleh *et al. 2*011).

Many plant phenolic compounds are known to be antimicrobial; their function as precursors to structural polymers such as lignin, and as signal molecules was reported (Akila *et al. 2*011), and many reports indicate these compounds alter membrane functionality (Cox *et al. 2*000). This membrane disruption causes the leakage of specific ions, affecting proton motive force, reduction of the intracellular ATP content, and the overall activity of microbial cells, including the control of turgor pressure, solute transport, and regulation of metabolism (Lanciotti *et al. 2*004). For instance, Yadav *et al*. (2013) reported the antimicrobial activity of isolated anthraquinones from *Cassia nodosa* against various bacterial and fungal strains, while Srinivasan & Sarada (2012) reported the antifungal activity of a phenyl derivative of pyranocoumarin (PDP) isolated from *Psoralea corylifolia* L. against *F. oxysporum, F. moniliforme,* and *F. graminearum.* Govindappa *et al*. (2011) reported the antifungal activity from *Crotalaria pallida* Aiton against different fungi, incluiding *A. nidulans, A. flaviceps, F. solani, F. oxysporum, F. verticilloides,* and *N. oryzae*; the positive inhibition was attributed to the presence of polyphenolic compounds, such as alkaloids, flavonoids, tannins, steroids, terpenoids, phenols, and saponins. The secondary metabolites found in the plant materials used in this study could be responsible for antifungal activity from *C. longirostrata* that was effective at inhibiting the mycelial growth and sporulation of *A. flavus, F. verticillioides, and Fusarium* sp. although it hasn't been defined, the mechanism of action of these compounds could be considered an inactivation of cellular enzymes. Guzmán-de-Peña *et al.* (1998) reported that when the decarboxylase ornithine enzyme is inhibited, mycelium and asexual and sexual sporulation is blocked which may induce stress in the cell membrane and lead to reactions that suppress proteins related to cell growth, the development of hyphae, and spore germination (Sannazzaro *et al.* 2004). Others studies have shown that natural phenolic compounds can serve as potent redox cyclers that inhibit microbial growth through destabilization of cellular redox homeostasis and/or antioxidation systems (Jacob 2006, Hernández-Ortega *et al*. 2011). To our knowledge, these results are the first experimental evidence demonstrating antifungal activity of this species.

Values of MIC of the extracts of *C. longirostrata* against *Fusarium* sp., *F. verticillioides,* and *A. flavus* showed that they vary widely in their degree of susceptibility to the nature of the compounds present in the extracts. The MIC values of hexane and acetone extracts against *A. flavus* were lower than in methanol extracts, suggesting that is more sensitive to low polarity biomolecules.

CONCLUSIONS

The findings of the study revealed that extracts from *C. longirostrata* possess phytochemicals with biological activity, mainly phenolic compounds (flavonoids, coumarins, anthraquinones, and anthrones). Also it was confirmed that this species has antifungal activity *in vitro* against *F. verticillioides, Fusarium* sp. and *A. flavus,* demonstrating its potential for control of various economically important phytopathogenic microorganisms.

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