Scientific note

Antifungal activity in vitro of *Sapranthus microcarpus* (Annonaceae) against phytopathogens

Actividad antifúngica in vitro de *Sapranthus microcarpus* (Annonaceae) contra hongos fitopatógenos

Iván De-la-Cruz-Chacón[1,2], Nora Yazmin López-Fernández[1], Christian Anabí Riley-Saldaña[1], Marisol Castro-Moreno[1], Alma Rosa González-Esquinca[1]

Abstract:

Background and Aims: The phytochemistry study of Annonaceae has intensified in the last decades due to the discovery of secondary metabolites with antimicrobial activities. The inhibitory activities of extracts and compounds from these species for phytopathogens are less known. The antifungal activity of *Sapranthus microcarpus* was determined *in vitro* against six fungal pathogens of important crops.

Methods: Hexane, methanol, and alkaloid extracts of the leaves and root and stem bark of *S. microcarpus* were evaluated to determine whether they could inhibit the mycelial growth of the phytopathogens. The combined activity of the most active extracts for each phytopathogen was also determined. The alkaloid liriodenine was isolated and identified as an antifungal principle.

Key results: All extracts inhibited the growth of the six phytopathogens to some extent. The root bark alkaloid extract showed the highest activity. *Fusarium oxysporum* f. sp. *lycopersici* and *Curvularia lunata* were the most sensitive phytopathogens. Liriodenine was active against all the plant pathogens (minimum inhibitory concentrations of 125-500 nmol ml^{-1}).

Conclusions: *Sapranthus microcarpus* is a native natural resource with great phytochemical potential.

Key words: antifungal alkaloid, botanical extracts, secondary metabolites.

Resumen:

Antecedentes y Objetivos: El estudio de la fitoquímica de Annonaceae se ha intensificado en las últimas décadas debido al descubrimiento de metabolitos secundarios con actividades antimicrobianas. Las actividades inhibidoras de extractos y compuestos de estas especies para fitopatógenos son menos conocidas. Se determinó la actividad antifúngica *in vitro* de *Sapranthus microcarpus* contra seis hongos fitopatógenos de cultivos importantes.

Métodos: Se evaluaron extractos metanólicos, hexánicos y alcaloidales de las hojas, y corteza de tallos y raíces de *S. microcarpus* por el método de inhibición micelial. También se determinó la actividad combinada de los extractos más potentes contra cada fitopatógeno. Se aisló e identificó al alcaloide liriodenina como uno de los metabolitos antifúngicos.

Resultados clave: Todos los extractos, en alguna medida, inhibieron el crecimiento de los seis fitopatógenos. Los extractos alcaloidales mostraron la mayor actividad. *Fusarium oxysporum* f. sp. *lycopersici* y *Curvularia lunata* fueron los fitopatógenos más sensibles. Liriodenina fue activo contra todos los fitopatógenos (a concentraciones inhibitorias mínimas de 125-500 nmol ml^{-1}).

Conclusiones: *Sapranthus microcarpus* es un recurso nativo con alto potencial fitoquímico.

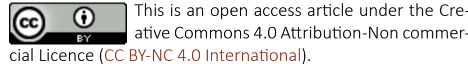
Palabras clave: alcaloides antifúngicos, extractos botánicos, metabolitos secundarios.

1 Universidad de Ciencias y Artes de Chiapas, Instituto de Ciencias Biológicas, Laboratorio de Fisiología y Química Vegetal, 29039 Tuxtla Gutiérrez, Chiapas, México.

2 Author for correspondence: ivan.cruz@unicach.mx

Received: July 6, 2018.
Reviewed: August 16, 2018.
Accepted by Marie-Stéphanie Samain: September 3, 2018.
Published Online first: December, 2018.
Published: Acta Botanica Mexicana 126 (2019).

Cite as:
De-la-Cruz-Chacón, I., N. Y. López-Fernández, C. A. Riley-Saldaña, M. Castro-Moreno and A. R. González-Esquinca. (2018)2019. Antifungal activity *in vitro* of *Sapranthus microcarpus* (Annonaceae) against phytopathogens. Acta Botanica Mexicana 126: e1420. DOI: 10.21829/abm126.2019.1420



This is an open access article under the Creative Commons 4.0 Attribution-Non commercial Licence (CC BY-NC 4.0 International).

e-ISSN: 2448-7589

Introduction

An alternative for controlling phytopathogens is the use of plant secondary metabolites, which leads to a search for antifungals with faster rates of biodegradation, either because they are rapidly hydrolysed or because they exhibit a higher absorption rate (Eksteen et al., 2001; Leiss et al., 2011).

Species of the Annonaceae family biosynthesise various biologically active secondary metabolites, including benzylisoquinoline alkaloids and annonaceous acetogenins. Together, these compounds comprise approximately one thousand molecules, characterised by potent cytotoxic, insecticidal, neuropharmacological, antibacterial, and antifungal activities (González-Esquinca et al., 2014; Lúcio et al., 2015). The inhibitory activities of extracts and compounds from these species for phytopathogens are less known. A review on this topic has reported the growth inhibition of 15 phytopathogenic fungi (Dang et al., 2011; De-la-Cruz-Chacón et al., 2011).

Among the Annonaceae, *Sapranthus* Seem. is one of the least-studied genera, consisting of eight species that are mainly distributed in Mexico and Central America (De-la-Cruz-Chacón et al., 2016; Schatz et al., 2018). In particular, there are no reports on the presence of secondary metabolites in *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. or their biological activities. The species, known as chufle, and Chac Nich Max in the Mayan language, is native to Mexico, Guatemala, El Salvador and Honduras (Schatz et al., 2018).

This is the first study to examine the biological and phytochemical potential of *S. microcarpus*. The study was designed to evaluate the *in vitro* antifungal activities of organic (hexane and methanol) and alkaloid extracts of the leaves and root and stem bark of *S. microcarpus* against six plant pathogenic fungi (*Aspergillus glaucus* (L.) Link, *Colletotrichum acutatum* var. *fioriniae* Marcelino & Gouli, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Curvularia lunata* (Wakker) Boedijn, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen and *Rhizopus stolonifer* (Ehrenb.) Vuill.). The combined activity of the most active extracts for each phytopathogen was also determined.

Materials and Methods

Leaves and the stem and root bark of *Sapranthus microcarpus* were collected in January 2015 (fruiting stage) in the town of Cintalapa, Chiapas, Mexico. A voucher specimen was deposited under reference #104204 at the Eizi Matuda Herbarium (HEM) of the Universidad de Ciencias y Artes de Chiapas, Mexico.

Fresh plant samples (250 g) were consecutively extracted for maceration, three times for 48 h with hexane and methanol at room temperature in the dark. The solvents were removed through distillation under reduced pressure. To obtain alkaloids, the plant material was extracted via an acid-base technique (De-la-Cruz-Chacón and González-Esquinca, 2012).

The test fungi were *Aspergillus glaucus* NACF0010, *Colletotrichum gloeosporioides* NCBI HM562712, *Colletotrichum acutatum* var. *fioriniae* ATCC 56897, *Fusarium oxysporum* f. sp. *lycopersici* ATCC 9848, *Curvularia lunata* NACF0030, and *Rhizopus stolonifer* NACF0011. The NACF and NCBI strains were obtained from the Grupo de Estudios Moleculares aplicados a la Biología (GeMBio), laboratory of the Centro de Investigación Científica de Yucatán (CICY), Mexico.

Antifungal activities of the plant extracts (250 and 500 µg ml^{-1}) were determined based on the inhibition of mycelial growth according to Eksteen et al. (2001). The hexane extracts were dissolved in dimethyl sulfoxide, and the methanol and alkaloid extracts were dissolved in ethanol; the solvents did not exceed 2.5% of the culture medium (potato dextrose agar). Mycelial growth was recorded every 24 h until that in the control (solvent without an extract) completely covered the surface of the culture medium. For each phytopathogen, the extracts that showed the highest antifungal activity were selected, combined, and then evaluated to determine whether their potencies were additive. The experiments were repeated twice with three replicates (n=6). The activity was expressed using the following formula: % Inhibition = ((dc-de)/dc) × 100, where dc=the fungal growth diameter on the control medium, and de=the fungal growth diameter on a medium with the extract.

The root bark alkaloid extract was analysed by bioautography (Rios et al., 1998), which included G60-F_{254} silica gel (200 µm) chromatography (Sigma-Aldrich®, Fluka®, St. Louis, USA), with a mobile phase of $CHCl_3$: methanol (9:1), to obtain the chemical profile of the extract. The activities of the separated constituents were evaluated against *As-*

pergillus glaucus and *Curvularia lunata*. The active compound was identified based on the area of fungal growth inhibition, followed by its isolation. To this end, preparative thin-layer chromatography (Sigma-Aldrich®, Fluka®, St. Louis, USA) of the root alkaloid extract was conducted on silica gel (1500 µm) under the same conditions. The area of the inhibitory compound was extracted with $CHCl_3$ under constant stirring for 1 h, followed by filtration (Fig. 1).

The compound, isolated as yellow needles, was analysed, identified, and compared with a previously obtained liriodenine reference, based on the melting point (275-276 °C), an ultraviolet absorption spectrum obtained by high-performance liquid chromatography with a diode-array detector (Perkin Elmer Flexar, Norwalk, USA) (De-la-Cruz-Chacón and González-Esquinca, 2012), and mass spectrometry (MS) data. The mass spectral data were obtained by gas chromatography/MS according to Riley-Saldaña et al. (2017), performed on a PerkinElmer Clarus 680 gas (Perkin Elmer, Waltham, USA) chromatograph coupled to a Clarus SQ8T mass spectrometer (Perkin Elmer, Waltham, USA), with a 1:30 split ratio. A Perkin Elmer Elite-1 capillary column (32 m, 0.32 mm, 0.25 mm film thickness) was used as the stationary phase (Perkin Elmer, Waltham, USA). Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The column temperature was programmed as follows: held at 150 °C for one min, raised at a rate of 10 °C/min to 280 °C, and then held at 280 °C for 16 min. The injector temperature was 300 °C. MS was carried out at 70 eV with 2.89 scans per second, fragment detection from 50 to 500 Da, the source temperature of 250 °C, and the quadrupole temperature of 100 °C. The retention time of liriodenine was 17.2±0.1 min, and EM (*m/z*) (relative abundance) values were 275(100), 247(27), 188(45) and 123(24) (Fig. 2).

The antifungal activity of liriodenine (100 and 200 nmol ml^{-1}) was evaluated based on mycelial growth inhibition under the same conditions as those used for the extracts. The fungicide Captan® was used as a positive control at 200 and 1000 nmol ml^{-1}.

The mycelial growth inhibition data were analysed for the extracts, mixtures, and liriodenine using the Kruskal-Wallis non-parametric analysis, followed by the Mann-Whitney multiple comparison test at $p<0.5$. To detect a general pattern of inhibition as a result of the evaluated factors, a principal component analysis (PCA) was performed, and the groups were confirmed by permutational multivariate analysis of variance (PERMANOVA). The Past statistical software (Hammer et al., 2001) was used (Natural History Museum, University of Oslo, Oslo, Norway).

Minimum inhibitory concentrations (MICs) of liriodenine were determined by a macrobroth (Sabouraud dextrose) dilution technique (Moussa et al., 2013). Liriodenine was dissolved in ethanol at a final concentration of 1% (v/v)

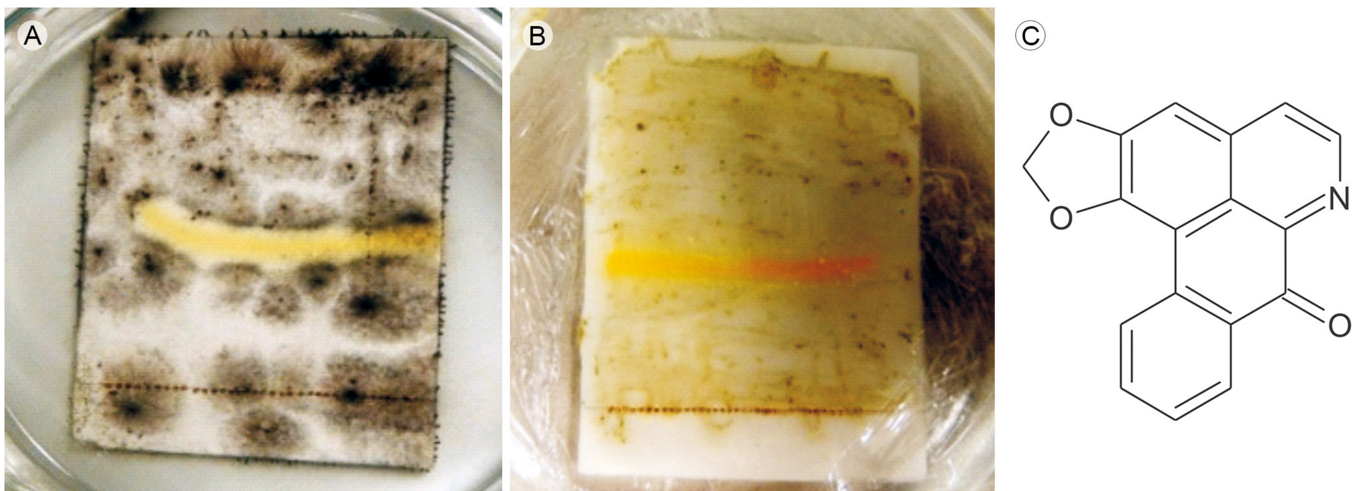


Figure 1: Liriodenine, antifungal alkaloid from *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. Bioautography of liriodenine against A. *Curvularia lunata* (Wakker) Boedijn and B. *Aspergillus glaucus* (L.) Link; yellow areas indicate inhibition of fungal growth by liriodenine; C. chemical structure of liriodenine.

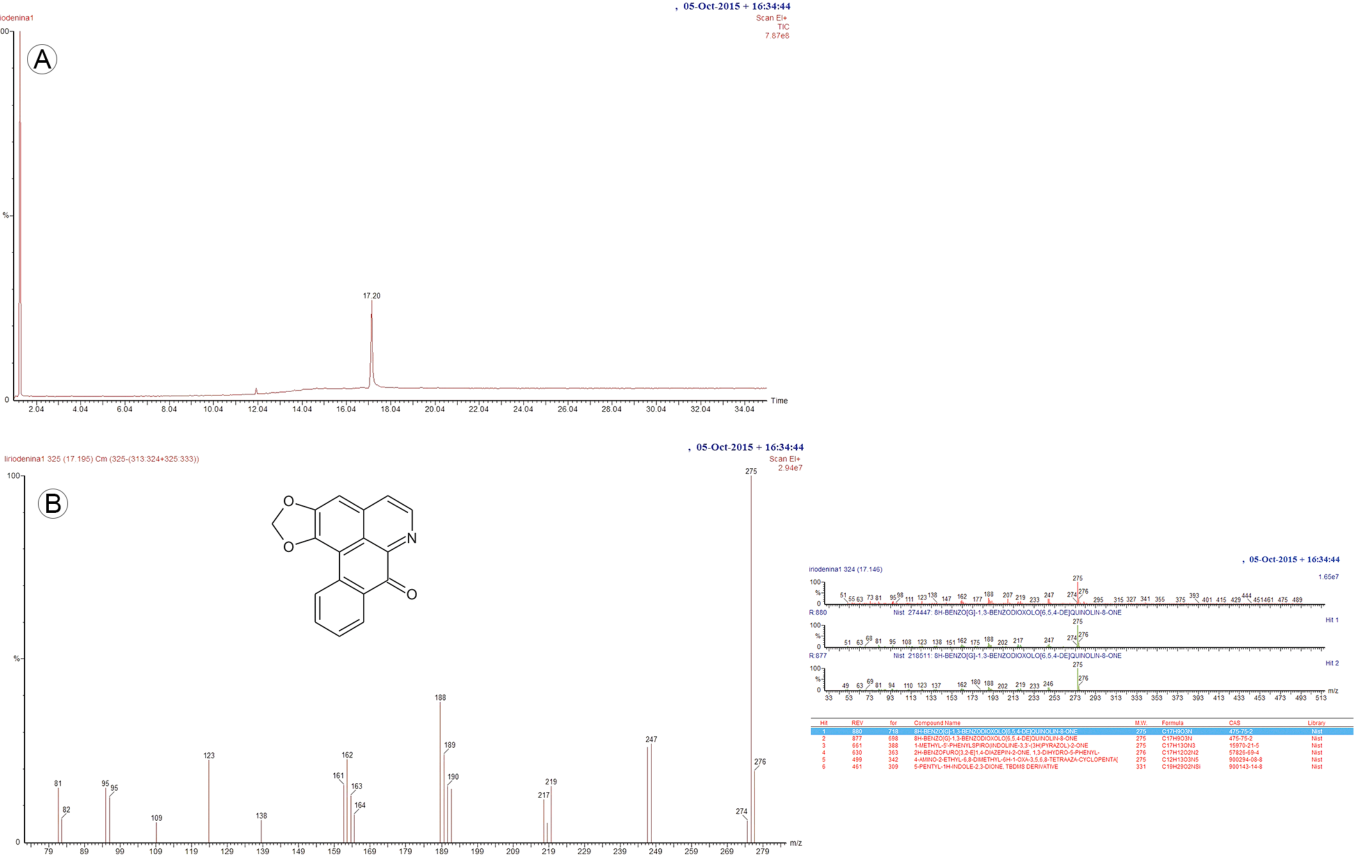


Figure 2: Analytical GC-MS chromatogram of liriodenine alkaloid isolated *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. A. the retention time of compound is 17.196 min using the GC-MS method we set; B. chemical structure and EM-MS fragmentation pattern of liriodenine (8H-Benzo(g)-1,3-benzodioxolo(6,5,4-de)quinolin-8-one).

and then evaluated in serial dilutions (500 to 1.0 nmol ml^{-1}). All experiments were performed in triplicate.

Results

The growth of all six phytopathogens tested was inhibited by at least one type of *Sapranthus microcarpus* extract, although not with the same potency (Table 1). The PCA (Fig. 3) and PERMANOVA confirmed that the alkaloid extracts were the most potent, whereas the hexane extracts showed less activity (F=25.85, p=0.0001); the root and leaf tissues showed higher activity than the stems (F=5.84, p=0.003), and the highest concentration was the most effective (F=48.5, p=0.001).

For the extracts that showed the highest antifungal activity, the growth inhibition rate was calculated (Table 2). The growth rate of all fungi was attenuated by the treatments (60-95% of control); the alkaloid root extract was the most potent and fast acting, except for *Colletotrichum gloeosporioides*.

The combined extracts displayed similar or lower activities (25-59% inhibition) compared with those of the individual extracts (Table 3).

Liriodenine was more potent than the most active extracts were for all of the phytopathogens, except *Rhizopus stolonifer* (Table 4), although it was superior to Captan® against all tested species. The most sensitive fungus was *Fusarium oxysporum* f. sp. *lycopersici,* whose mycelial growth on agar was completely inhibited at 200 nmol ml^{-1} (MIC: 125 nmol ml^{-1}).

Discussion

The results indicated that the tissues of *Sapranthus microcarpus* biosynthesise and accumulate substances with antifungal activities against phytopathogens. This property has rarely been reported for other Annonaceae, which have been mostly studied against fungi of medical importance (Rios et al., 2003; Lago et al., 2007; Akendengué et al., 2009). The fractionation of plant secondary metabolites

Table 1: Antifungal activity of extracts from *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. The values are means ± DE (n=6). The separation of groups was performed with the Mann-Whitney test (p<0.05). Capital letters represent comparisons in columns, and lowercase letters represent comparisons in rows.

Tissue	Extract: ug ml^{-1}	% Inhibition Mycelial Growth					
		Aspergillus glaucus (L.) Link NACF0010	*Colletotrichum gloeosporoides* (Penz.) Penz. & Sacc NCBI HM562712	*Colletotrichum acutatum* var. *fioriniae* Marcelino & Gouli ATCC 58697	*Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen ATCC 9848	*Curvularia lunata* (Wakker) Boedijn NACF0030	*Rhizopus stolonifer* (Ehrenb.) Vuill. NACF0011
Roots	Hexanic 250	10±5 Ac	5±3 Ad	0±0 Cd	6±3 Ae	20±5 Ae	0 Bd
	500	5±3 Ccd	28±7 Bc	8±2 Bc	28±7 Bd	37±5 Ad	41±6 Ab
	Metanolic 250	1±2 Ad	36±10 Bb	6±1 Cc	40±10 Bc	37±6 Ad	0 Ac
	500	38±6 Ab	65±2 Aa	6±3 Bc	65±2 Ab	62±5 Ab	60±1 Aa
	Alkaloidal 250	47±3 Aab	44±3 Ab	60±1 Ab	55±3 Ac	47±1 Ac	36±2 Ac
	500	48±2 Aa	60±2 Aa	73±2 Aa	90±3 Aa	76±5 Aa	56±2 Aa
Stem	Hexanic 250	8±2 Ac	26±5 Ab	7±1 Bc	15±5 Ad	12±3 Bc	0 Bc
	500	30±10 Ab	24±4 Bb	15±4 Ab	28±4 Bc	16±3 Cc	25±5 Bb
	Metanolic 250	3±2 Ad	18±3 Cc	10±6 Ac	20±5 Cd	0±0 Cd	0 Ac
	500	13±2 Bc	26±2 Bb	17±3 Ab	58±2 Bb	23±6 Cb	24±4 Cb
	Alkaloidal 250	21±2 Bb	26±1 Bb	3±1 Cd	32±2 Bc	37±5 Ba	0±0 Ac
	500	43±1 Ba	47±4 Ba	32±5 Ca	70±4 Ba	38±5 Ba	55±1 Aa
Leaves	Hexanic 250	10±6 Ac	25±5 Ac	15±2 Ac	25±5 Ac	23±2 Ad	25±3 Ab
	500	14±2 Bc	45±2 Ab	24±6 Ab	35±2 Ab	27±1 Bd	41±14 Aa
	Metanolic 250	0 Ad	50±9 Aa	0 Cd	30±5 Abc	7±2 Be	0 Ad
	500	18±1 Bb	59±8 Aa	13±3 Bc	62±8 Aa	42±3 Bb	29±8 Bb
	Alkaloidal 250	20±1 Bb	26±2 Bc	20±5 Bb	30±2 Bbc	34±1 Bc	1±1 Bc
	500	46±2 Aa	41±2 Cb	47±1 Ba	68±2 Ca	64±5 Aa	42±3 Ba

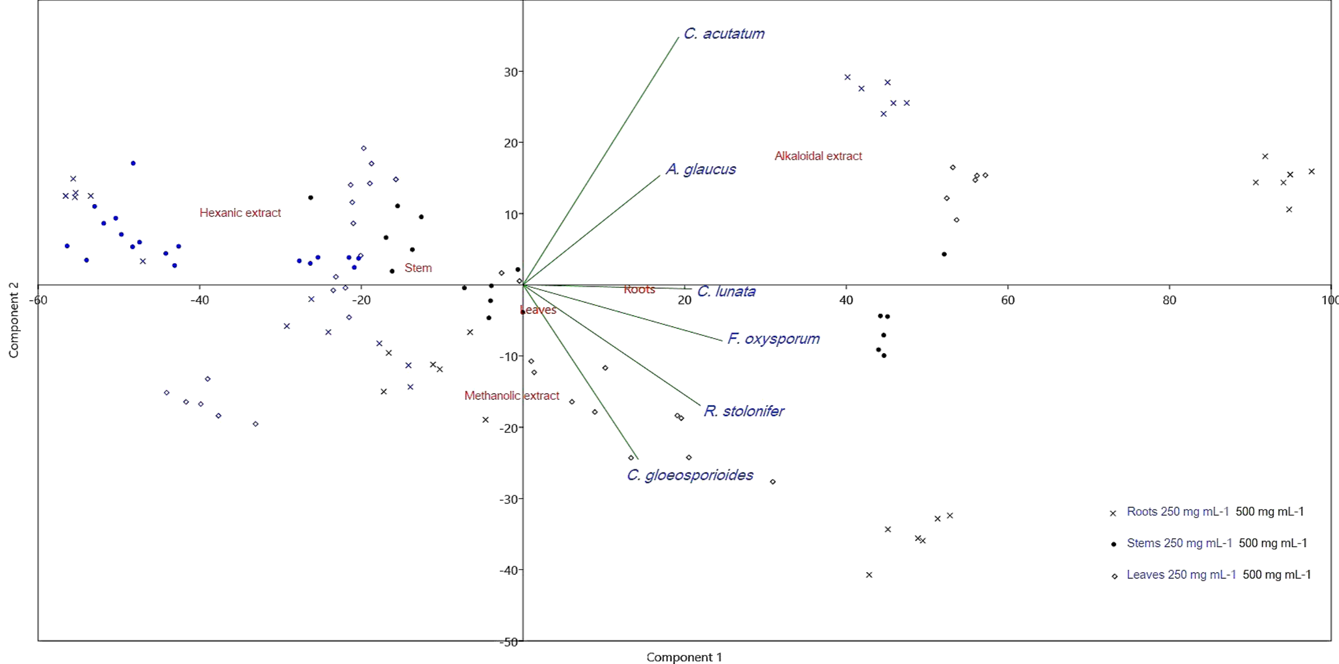


Figure 3: Principal component analysis of antifungal activity of *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. PCA bi-plot based on fungal inhibition percent of three types of extracts in two concentrations from three plant tissues. PC1 and PC2 accounted for over 80% of the total variance. The arrows indicate the direction of maximum correlation by fungus species.

Table 2: Effect of extracts from *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. on the growth rate of fungi. For extracts that showed the highest antifungal activity, the growth rate was calculated from the slope of the linear regression (y=a + bx, where "a" is the intercept and "b" is slope). E=Evaluated extracts (E). A=Alkaloidal, M=Methanolic; H=Hexanic; L=Leaf; S=Stem; R=Root.

Fungus	Extract at 500 µg ml^{-1}	Slope	Intercept	Correlation
Aspergillus glaucus (L.) Link NACF0010	Control	0.367	6.713	r=0.9902; r^2=0.9804; P=8.92E^{-30}
	EAR	0.186	4.226	r=0.9569; r^2=0.9157; P=2.70E^{-19}
	EAS	0.203	5.600	r=0.9699; r^2=0.9408; P=7.88E^{-22}
	EAL	0.181	3.257	r=0.9766; r^2=0.9537; P=4.21E^{-12}
Colletotrichum acutatum var. *fioriniae* Marcelino & Gouli ATCC 58697	Control	0.131	4.254	r=0.9916; r^2; 0.9833; P=4.45E^{-63}
	EAR	0.032	4.526	r=0.9661; r^2; 0.9333; P=2.63E^{-42}
	EAS	0.106	5.265	r=0.9889; r^2; 0.9779; P=6.72E^{-59}
	EAL	0.079	6.001	r=0.9881; r^2; 0.9764; P=7.20E^{-58}
Colletotrichum gloeosporoides (Penz.) Penz. & Sacc NCBI HM562712	Control	0.415	3.530	r=0.9829; r^2; 0.9661; P=7.76E^{-26}
	EAR	0.131	3.287	r=0.9566; r^2; 0.9149; P=3.12E^{-19}
	EMR	0.085	4.435	r=0.9648; r^2; 0.9309; P=1.01E^{-20}
	EAS	0.204	2.956	r=0.9740; r^2; 0.9487; P=7.35E^{-23}
	EML	0.169	3.348	r=0.9766; r^2; 0.9538; P=1.31E^{-23}
Curvularia lunata (Wakker) Boedijn NACF0030	Control	0.269	4.582	r=0.9929; r^2; 0.9859; P=2.76E^{-43}
	EAR	0.039	5.029	r=0.9003; r^2; 0.8105; P=7.25E^{-18}
	EAS	0.085	4.435	r=0.9648; r^2; 0.9309; P=1.03E^{-20}
	EAL	0.086	5.164	r=0.9718; r^2; 0.9441; P=7.01E^{-30}
Fusarium oxysporum f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen ATCC 9848	Control	0.240	9.296	r=0.9575; r^2; 0.9167; P=3.42E^{-29}
	EAR	0.025	4.047	r=0.8444; r^2; 0.7129; P=1.97E^{-15}
	EAS	0.171	2.375	r=0.9789; r^2; 0.9583; P=7.34E^{-37}
	EAL	0.077	4.359	r=0.9691; r^2; 0.9392; P=1.10E^{-32}
Rhizopus stolonifer (Ehrenb.) Vuill. NACF0011	Control	0.915	11.516	r=0.9089; r^2; 0.8261; P=442E^{-07}
	EAR	0.395	5.387	r=0.9655; r^2; 0.9322; P=3.59E^{-10}
	EMR	0.354	5.774	r=0.9676; r^2; 0.9363; P=2.25E^{-10}
	EAS	0.358	5.903	r=0.9805; r^2; 0.9614; P=5.15E^{-12}
	EHL	0.512	9.452	r=0.8686; r^2; 0.7545; P=6.15E^{-06}
	EAL	0.493	7.000	r=0.9583; r^2; 0.9184; P=1.45E^{-09}

into two groups, nonpolar (hexane extracts) and polar (alkaloid and methanolic extracts), yielded information on the presence of active substances of a different nature in each tissue. Species of the Annonaceae family produce nonpolar and polar metabolites (annonaceous acetogenins, essential oils, benzylisoquinoline alkaloids, etc.) with antimicrobial activity (Rios et al., 2003; Lago et al., 2007; Dang et al., 2011; González-Esquinca et al., 2014).

One assessed hypothesis was that it might be possible to increase the antifungal activity by combining extracts. The combined extracts showed good activity, although it was never greater than that of the individual extracts, reflecting the average activity of the combined extracts. Nevertheless, combined extracts are an alternative that offers the advantage of phytochemical diversity and that, according to Reuveni and Sheglov (2002), is suitable

Table 3: Antifungal activity of the combined extracts of *Sapranthus microcarpus* (Donn. Sm.) R.E. Fr. Combined extracts. E=Extract; A=Alkaloidal, M=Methanolic; L=Leaf; S=Stem; R=Root. The values are means ± DE (n=6). The separation of groups was performed with the Mann-Whitney test (p<0.05). Capital letters represent comparisons in columns, and lowercase letters represent comparisons in rows.

Phytopathogens	Combined activity		Combined extracts
	250 µg ml^{-1}	500 µg ml^{-1}	
Aspergillus glaucus (L.) Link NACF0010	14±1 Cb	30±2 Ca	EAL, EAS, EAR
Colletotrichum gloeosporoides (Penz.) Penz. & Sacc NCBI HM562712	0±3 Db	25±2 Da	EAL, EAS, EAR
Colletotrichum acutatum var. *fioriniae* Marcelino & Gouli ATCC 58697	19±1 Bb	35±5 Ba	EAL, EAS, EAR, EMR, EML
Fusarium oxysporum f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen ATCC 9848	50±5 Aa	63±2 Ab	EAL, EAS, EAR, EMR, EML
Curvularia lunata (Wakker) Boedijn NACF0030	45±5 Aa	59±1 Ab	EAL, EAS, EAR, EMR, EML
Rhizopus stolonifer (Ehrenb.) Vuill. NACF0011	0±0 Eb	2±1 Ea	EAL, EAS, EAR, EMR, EML

Table 4: Antifungal activity of liriodenine. The values are means ± DE (n=6). The separation of groups was performed with the Mann-Whitney test (p<0.05). Capital letters represent comparisons in columns, and lowercase letters represent comparisons in rows.

	Liriodenine			Captan®	
Phytopathogens	% Inhibition Mycelial Growth (nmol ml^{-1}/µg ml^{-1})		CMI in broth	% Inhibtion Mycelial Growth (nmol ml^{-1}/µg ml^{-1})	
	100/27.5	200/55.1	nmol ml^{-1}	200/60.1	1000/300.6
Aspergillus glaucus (L.) Link NACF0010	38±2 Cb	50±2 Ca	250	15±1 Bd	29±1 Cc
Colletotrichum acutatum var. *fioriniae* Marcelino & Gouli ATCC 58697	43±2 Bb	52±2 Ca	250	43±4 Ab	50±7 Ba
Colletotrichum gloeosporoides (Penz.) Penz. & Sacc NCBI HM562712	35±2 Cb	30±3 Db	500	5±3 Cc	59±2 Aa
Fusarium oxysporum f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen ATCC 9848	70±2 Ab	100±2 Aa	125	43±4 Ad	50±7 Bc
Curvularia lunata (Wakker) Boedijn NACF0030	46±2 Bc	85±2 Ba	250	12±7 Bd	65±4 Ab
Rhizopus stolonifer (Ehrenb.) Vuill. NACF0011	0±0 Dc	10±0 Eb	500	3±0 Cc	47±7 Ba

for fungicides, since different modes of action can minimise the risk of the development of resistance by target microorganisms, improving disease control.

The presence of liriodenine as an active substance in alkaloid and even methanol extracts is a recurring phenomenon in the Annonaceae (Feitosa et al., 2009; Alias et al., 2010) since it is one of the most widely distributed benzylisoquinoline alkaloids in plants (González-Esquinca et al., 2014; Lúcio et al., 2015). This is the second report on the genus *Sapranthus*, as Etse and Waterman (1986) reported the presence of liriodenine in the stem bark of *Sapranthus palanga* R.E. Fr.

Resistance of phytopathogens to fungicides is a frequently occurring biological phenomenon. One strategy to find new effective fungicides is to start with *in vitro* tests of secondary metabolites. Spore germination and mycelial growth are central to the life cycle of a fungus. Therefore, a plant metabolite that inhibits either of these two processes can reduce the ability of phytopathogens to cause the disease in host plants (Morrisey and Osbourn, 1999; Balkis et al., 2002). Our results suggest that *S. microcarpus* tissues and its bioactive alkaloids can be promising alternative fungicides for the control of various plant diseases.

Author contributions

IDC and ARGE designed the study. NYLF, CARS and IDC conducted experiments. IDC, CARS and MCM analyzed the data. IDC, ARGE and MCM wrote the article. IDC and ARGE

contributed reagents and equipment. All authors read and approved the manuscript.

Funding

This work was funded by the Secretaría de Educación Pública de México (SEP) (PRODEP/103.5/13/6583) and the Consejo Nacional de Ciencia y Tecnología (CONACyT), México (INFR-2014-01:00226293).

Acknowledgements

We are grateful to the Instituto de Ciencias Biológicas of the Universidad de Ciencias y Artes de Chiapas for the support provided in the research. We remain thankful to the anonymous reviewers of the paper for constructive criticisms and suggestions.

Literature cited

Akendengué, B., P. Champy, J. Nzamba, F. Roblot, P. M. Loiseau and C. Bories. 2009. Antifungal and Anthelmintic Activities of *Cleistopholis patens* (Annonaceae). Planta Medica 75(10): 1143-114. DOI: https://dx.doi.org/10.1055/s-0029-1185510

Alias, A., H. Hazni, F. M. Jaafar, K. Awang and N. H. Ismail. 2010. Alkaloids from *Fissistigma latifolium* (Dunal) Merr. Molecules 15(7): 4583-4588. DOI: https://doi.org/10.3390/molecules15074583

Balkis, M. M., S. D. Leidich, P. K. Mukherjee and M. A. Ghannoum. 2002. Mechanisms of Fungal Resistance Drugs 62(7): 1025-1040. DOI: https://doi.org/10.2165/00003495-200262070-00004

Dang, Q. L., W. K. Kim, C. M. Nguyen, Y. H. Choi, G. J. Choi, K. S, Jang, M. S. Park, C. H. Lim, N. H. Luu and J. C. Kim. 2011. Nematicidal and antifungal activities of annonaceous acetogenins from *Annona squamosa* against various plant pathogens. Journal of Agricultural and Food Chemistry 59(20): 11160-11167. DOI: https://doi.org/10.1021/jf203017f

De-la-Cruz-Chacón, I. and A. R. González-Esquinca. 2012. Liriodenine alkaloid in *Annona diversifolia* during early development. Natural Product Research 26(1): 42-49. DOI: https://doi.org/10.1080/14786419.2010.533373

De-la-Cruz-Chacón, I., A. R. González-Esquinca, P. Guevara Fefer and L. F. Jiménez García 2011. Liriodenine, early antimicrobial defence in *Annona diversifolia*. Zeitschrift für Naturforschung C 66(7-8): 377-384. DOI: https://doi.org/10.1515/znc-2011-7-809

De-la-Cruz-Chacón, I., M. Castro-Moreno M., L. M. Luna-Cazáres and A. R. González-Esquinca. 2016. La familia Annonaceae Juss. en México. Lacandonia 10(2): 71-82.

Eksteen, D., J. C. Pretorius, T. D. Nieuwoudt and P. C. Zietsman. 2001. Mycelial growth inhibition of plant pathogenic fungi by extracts of South African plant species. Annals of Applied Biology 139(2): 243-249. DOI: https://doi.org/10.1111/j.1744-7348.2001.tb00400.x

Etse, J. T. and P. G. Waterman. 1986. Alkaloids and an acetylenic lactone from the stem bark of *Sapranthus palanga*. Phytochemistry 25(8): 1903-1905. DOI: https://doi.org/10.1016/S0031-9422(00)81171-1

Feitosa, E. M. A., Â. M. C. Arriaga, G. M. P. Santiago, T. L. G. de Lemos, M. C. F. de Oliveira, J. N. Vasconcelos, J. Q. Lima, G. T. Malcher, R. F. do Nascimento and R. Braz-Filho. 2009. Chemical composition and larvicidal activity of *Rollinia leptopetala* (Annonaceae). Journal of the Brazilian Chemical Society 20(2): 375-378. DOI: https://dx.doi.org/10.1590/S0103-50532009000200024

González-Esquinca, A. R., I. De-la-Cruz-Chacón, M. Castro-Moreno, J. A. Orozco-Castillo and C. A. Riley-Saldaña. 2014. Alkaloids and acetogenins in Annonaceae development: biological considerations. Revista Brasileira de Fruticultura 36(spe1): 1-16. DOI: https://dx.doi.org/10.1590/S0100-29452014000500001

Hammer, Ø., D. A. T. Harper and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm (consulted September, 2017).

Lago, J. H., M. H. Chaves, M. C. Ayres, D. G. Agripino and M. C. Young. 2007. Evaluation of antifungal and DNA-damaging activities of alkaloids from branches of *Porcelia macrocarpa.* Planta Medica 73(3): 292-295. DOI: https://dx.doi.org/10.1055/s-2007-967108

Leiss, K. A., Y. H. Choi, R., R. Verpoorte and P. G. L. Klinkhamer. 2011. An overview of NMR-based metabolomics to identify secondary plant compounds involved in host plant resistance. Phytochemistry Reviews 10(2): 205-216. DOI: https://doi.org/10.1007/s11101-010-9175-z

Lúcio, A. S. S. C., J. R. G. da Silva Almeida, E. V. L. da-Cunha, J. F. Tavares and J. M. Barbosa Filho. 2015. Alkaloids of the Annonaceae: occurrence and a compilation of their biological ac-

tivities. In: Knölker, H. J. (ed.). The Alkaloids: Chemistry and Biology, vol. 74, cap. 5. Academic Press. Elsevier. Cambridge, MA, USA. Pp. 233-409. DOI: https://doi.org/10.1016/bs.alkal.2014.09.002

Morrisey, J. P. and A. E. Osbourn. 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiology and Molecular Biology Reviews 63(3): 708-724.

Moussa, S. H., A. A. Tayel, A. A. Al-Hassan and A. Farouk. 2013. Tetrazolium/Formazan test as an efficient method to determine fungal chitosan antimicrobial activity. Journal of Mycology ID753692. DOI: https://dx.doi.org/10.1155/2013/753692

Reuveni, M. and D. Sheglov. 2002. Effects of azoxystrobin, difenoconazole, polyoxin B (polar) and trifloxystrobin on germination and growth of *Alternaria alternata* and decay in red delicious apple fruit. Crop Protection 21(10): 951-955. DOI: https://doi.org/10.1016/S0261-2194(02)00073-X

Riley-Saldaña, C. A., M. R. Cruz-Ortega, M. M. Vázquez, I. De-la-Cruz-Chacón, M. Castro-Moreno and A. R. González-Esquinca. 2017. Acetogenins and alkaloids during the initial development of *Annona muricata* L. (Annonaceae). Zeitschrift für Naturforschung C 72(11-12): 497-506. DOI: https://doi.org/10.1515/znc-2017-0060

Rios, M. Y., F. Castrejón, N. Robledo, I. Leon, G. Rojas and V. Navarro. 2003. Chemical composition and antimicrobial activity of the essential oils from *Annona cherimola* (Annonaceae). Revista de la Sociedad Química de México 47(2): 139-142.

Rios, J. L., M. C. Recio and A. Villar. 1998. Screening methods for natural products with antimicrobial activity: a review of literature. Journal of Ethnopharmacology 23(2-3): 127-149. DOI: https://doi.org/10.1016/0378-8741(88)90001-3

Schatz, G. E., P. J. M. Maas, H. Maas-van de Kamer, L. Y. T. Westra and J. J. Wieringa. 2018. Revision of the Neotropical genus *Sapranthus* (Annonaceae). Blumea 63(1): 54-66. DOI: https://doi.org/10.3767/blumea.2018.63.01.06