Antifungal Properties of Some Mexican Medicinal Plants

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Abstract: The antifungal properties of some extracts from *Artemisia ludoviciana* Nutt., *Heliopsis longipes* 'A. Gray' Blake., *Satureja macrostema* Benth. and *Tagetes lucida* Cav. were analyzed, using the agar disc diffusion method. After 72 h incubation, the plant extracts inhibited the growth of fungi, but the ethyl acetate and methanol-chloroform extracts from *A. ludoviciana, H. longipes* and *T. lucida* inhibited all the fungi assayed: *Candida albicans*, *Colletotrichum lindemuthianum, Mucor circinelloides, Saccharomyces cerevisiae,* and *Sporothrix schenckii*. The spore germination inhibition assay suggested that methanol-chloroform extract from *A. ludoviciana* was the most active against the fungi tested. The methanol-chloroform and ethyl acetate extracts from *A. ludoviciana* and *T. lucida* had pronounced antifungal activity against fungi tested. Compounds found in the leaves methanol-chloroform extracts from *A. ludoviciana* were analyzed by using GC/MS; the major compounds were: 1-8-cineole, camphor, borneol, *cis*-verbenol, myrtenol, eugenol, cariophyllene, α-farnesene, spathulenol, derivatives of caryophyllene and derivatives of spathulenol.

Keywords: *A. ludoviciana, H. longipes, S. macrostema, T. lucida*, antifungal properties.

INTRODUCTION

The treatment of human mycosis has been a great challenge for clinicians and dermatologists. On one hand, opportunistic fungal infections are increasing at an alarming rate, while on the other, allergic reactions of the skin are also increasing day to day. The latter is due to a higher rate of power sensitization of the present generation of antimycotic agents. Human and plant pathogenic fungi in general, are usually treated by the use of synthetic compounds, mainly the drugs belonging to the imidazole family [1]. We believe, it is the time to search for new antifungal agents of herbal origin, which are relatively economically affordable, safer and easily available to common men. Moreover, sometimes imidazole derivatives are not effective, depending on which alternative drugs are required [2]. A perusal of literature indicates that many investigators have reported fungistatic and bacteriostatic properties of extracts of higher plants [3]. Plants produce a lot of secondary metabolites with pharmacological activity, although the exact role of some secondary metabolites in the life processes of the plant is unknown. They are a source of pharmacologically active principles against pathogenic microorganisms, e.g. most members of the Asteraceae family are known to contain sesquiterpene lactones, which usually have antifungal and cytotoxic effect [4, 5].

Mexico has a rich tradition in medicinal plant utilization, among its varied folk healing practices [6, 7]. A total of 3,000 species have been compiled in an atlas of medicinal plants, employed by diverse ethnic groups. Incredibly, of these only approximately 1% of them have been studied in depth regarding their potential medicinal properties [8]. *S. macrostema* Benth. (nurite), a member of Labiateae family and *T. lucida* Cav. (Santamaría), *A. ludoviciana* Nutt. (estafiate) and *H. longipes* 'A. Gray' Blake (chilcuague), members of Asteraceae family, have been described as microbiocides [9-13]. All of them are Mexican medicinal plants that grow throughout central Mexico and were selected on the basis of medicinal folklore reports to investigate their fungitoxic properties.

MATERIALS AND METHODS

Biological Material

A. ludoviciana Nutt., *H. longipes* 'A. Gray' Blake, *S. macrostema* Benth. and *T. lucida* Cav. plants, were collected from INIFAP-Michoacán at the Uruapan Campus and were identified by Facultad de Biologia Herbarium, Universidad Michoacana de San Nicolás de Hidalgo. The material plant was collected during early flowering stage (the months of March-July) and was dried.

A filamentous isolate phytopathogen *Colletotrichum lindemuthianum* and yeast *Saccharomyces cerevisiae,* were cultured on potato dextrose agar (Difco, USA) medium. Two dimorphic clinical isolates, *Candida albicans* and *Sporothrix schenckii* were cultured on Sabouraud medium and dimorphic saprophytic fungus *Mucor circinelloides* cultured on yeast peptone dextrose medium. The spore inocula were prepared in distillated sterile water and yeast inocula were prepared in Sabouraud broth and the inocula were adjusted to 1 X 10^6 cells·ml^{-1}. Fungi were obtained from the fungi collection of the Instituto de Investigaciones Químico Biológicas.

Preparation of Plant Extracts

One hundred grams of dried plant materials were finely ground and they were extracted separately as follow: a) macerated, incubated at 4 °C for 5 days with methanol-chloroform

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(2:1 $v \cdot v^{-1}$), b) extracted with ethyl acetate using soxhlet extractor for 2 h (79 °C), and c) infusion with distilled water. The extracts were separated by filtration and the solvent was evaporated to dryness under vacuum to 45 °C. The residues were dissolved in 1 ml of absolute ethanol and were stored at 4 °C for further analysis.

Antifungal Test

Spore germination test: Sterile filter paper discs (Whatman No. 1) of 10 mm in diameter were impregnated with about 10 µl (1 $mg \cdot ml^{-1}$) of extracts, which have been dissolved in absolute ethanol and placed in triplicates on the potato dextrose or Sabouraud agar plates, previously seeded with 100 µl of spores of fungal suspension or yeast cells ($1x10^6$ $cells \cdot ml^{-1}$). The plates were then incubated at 25 °C for 72 h.

Growth inhibition test: Agar plates inoculated with spores or yeast cells were incubated for 4 to 8 h at 25 ºC, when the hypha rose two or three times the size of spore or yeast produced one or two buds, than the filter papers with plant extracts were put on culture and were incubated at same conditions. For three days after every 12 h the diameter of inhibition zone on the cultures was measured, but the diameter of paper disk (10 mm) was not included. Absolute ethanol (100 µl) and water were used as reference standards. The results of both the tests were presented as mean ± SD of the zone of inhibition of three independent experiments.

GC/MS Technique

GC/MS analysis of plant extracts were analyzed by using a Hewlett Packard 6890 gas chromatograph, HP 5973 mass detector, and capillary column Equity 5 (30m x 20 mm). Oven temperature was 50 ºC for 5 min and programmed to 250 ºC for 2 min. Injector temperature initially was 40 ºC, increased a rate of 2 ºC to 250 ºC. Mass spectra were taken at 70 eV with a rank $m \cdot z^{-1}$ of 20 to 450. The total yield of essential oil ($w \cdot v^{-1}$) was 0.94%. Components of the peaks were identified by comparison of their mass spectra and relative retention time with those reported in NIST spectral database.

Statistical Analysis

In order to show the biocide activity of extracts, at least ten isolates of each species were tested, because of the different responses and sensitivity to extracts observed in the fungi test. Finally all the data were presented as mean ± standard error for fungi species and were analyzed with the PROBIT program and for factorial treatment with $P < 0.01$ was used for the extracts of all plants.

RESULTS

The results of antifungal screening of extracts of four plant species are presented in Tables **1** and **2**. The growth inhibitory effect of plant extract was in the following order; ethyl acetate and methanol-chloroform extracts from *A. ludoviciana, H. longipes, T. lucida* and *S. macrostema* showed high growth inhibitory effects against all fungi tested. The aqueous extracts of all the plants showed growth inhibitory effects against *C. albicans*, and a weak or no effect against the rest of the fungi tested. The most sensitive fungi to plant extracts were *C. albicans* and *S. schenckii,* vertebrate pathogens and the phytopathogen *C. lindemuthianum,* the causal agent of bean anthracnose (Table **1**).

The fungi spore germination inhibitory effect of plant extract was similar (Table **2**). The extracts from *A. ludoviciana* inhibited the germination of yeast-like fungi, while spore germination of filamentous fungi were only severely affected by leaves and root methanol-chloroform extracts. The leaves ethyl acetate extract from *T. lucida* was effective on spore germination of four fungi (filamentous and yeast-like fungi), while the leaves methanol-chloroform extract did not inhibit spore germination of filamentous fungi. The methanol-chloroform extracts from leaves of *A. ludoviciana* and root of *H. longipes*, both inhibited the spore germination of fungi test. The flowers ethyl acetate extract inhibited the germination of all the pathogenic fungi, while germination inhibition of the leaves methanol-chloroform extract was weak (Table **2**). All extracts from *S. macrostema* did not inhibit the growth of *C. lindemuthianum,* and *M. circinelloides*. Based on these results, half of the inhibitory concentration (IC_{50}) values of plant extracts were calculated as having significant effects against *C. albicans, C. lindemuthianum*, *M. circinelloides*, *S. cerevisiae* and *S. schenckii,* and are presented in Table **3**. The IC_{50} of extracts from *A. ludoviciana* ranged from 4 to 250 $mg \cdot ml^{-1}$. The root ethyl acetate and the flowers aqueous extracts were the most active, against *C. albicans* with IC_{50} value of 4 $mg \cdot ml^{-1}$, the leaves ethyl acetate and the flowers methanol-chloroform extracts with IC_{50} values of 25 and 33 $mg \cdot ml^{-1}$, respectively.

However, the IC_{50} values of the leaves methanol-chloroform extract against filamentous and yeast like fungi ranged from 91 to 100 $mg \cdot ml^{-1}$.

The IC_{50} values of the aqueous and ethyl acetate extracts from *T. lucida* against *C. albicans, M. circinelloides* and *S. schenckii,* ranged from 11 to 88.7 $mg \cdot ml^{-1}$. The best IC_{50} values of ethyl acetate and aqueous extracts from *H. longipes* against *C. lindemuthianum*, *M. circinelloides*, *S. cerevisiae* and *S. schenckii,* ranged from 4 to 9 $mg \cdot ml^{-1}$. The IC_{50} values of extracts from *S. macrostema* against *S. cerevisiae* ranged from 10 to 76 $mg \cdot ml^{-1}$, and against *C. albicans* was ranged from 10 to 51 $mg \cdot ml^{-1}$.

Based on these results and because the leaves methanol-chloroform extract from *A. ludoviciana* inhibited all the fungi tested, this extract was selected as the best antifungal extract and because of this we made the identification of the major compounds by GC/MS.

The chemical analysis showed that the methanol-chloroform extract of *A. ludoviciana* Nutt. had the major compounds: borneol (6.19 %), spathulenol (3.09 %), derivatives of caryophyllene (5.09 %), and derivatives of spathulenol (1.16 %) (Table **4**). These compounds have been identified in other species of *Artemisia* with other minor compounds, for example: 1,8-cineole, camphor, *cis*-verbenol, myrtenol, eugenol, cariophyllene, α-farnesene, derivatives of caryophyllene, and derivatives of spathulenol.

DISCUSSION

The antifungal properties of ethyl acetate, methanol-chloroform and water extracts from *A. ludoviciana* Nutt., *H. longipes* 'A. Gray' Blake., *S. macrostema* Benth. and *T. lucida* Cav., showed that fifty percent of the extracts inhibited the fungi growth.

Table 1. Antifungal Activities of Crude Plant Medicinal Extract Determinate by Mycelial or Yeast-Like Growth Inhibition Test. *C. albicans* (*Ca*), *C. lindemuthianum* (*Cl*), *M. circinelloides* (*Mc*), *S. cerevisiae* (*Sc*) and *S. schenckii* (*Ss*)

Species	Organ	Extract	Inhibition				
			C a	*C l*	*M c*	*S c*	*Ss*
A. ludoviciana	Flowers	Aqueous	+ + + +	+	+	+	+
	Leaves	Aqueous	+ + + +	+	+	+	+
	Root	Aqueous	+ + + +	+	+	+	+
	Flowers	Ethyl acetate	+ + +	+ + +	+ + + +	+ + + +	+ + + +
	Leaves	Ethyl acetate	+ + + +	+ + +	+ + +	+ + + +	+ + + +
	Root	Ethyl acetate	+ + + +	+ + + +	+ + +	+ + + +	+ + + +
	Flowers	Methanol/chloroform	+ + + +	+ + +	+ + +	+ + + +	+ + + +
	Leaves	Methanol/chloroform	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
	Root	Methanol/chloroform	+ + +	+ + +	+ + + +	+ + + +	+ + + +
H. longipes	Flowers	Aqueous	+	+	+	+	+
	Leaves	Aqueous	+ + +	+	+	+	+
	Root	Aqueous	+ + + +	+	+	+	+
	Flowers	Ethyl acetate	+ + +	+ + + +	+ + + +	+	+ + + +
	Leaves	Ethyl acetate	+ + +	+ + +	+ + + +	+	+ + + +
	Root	Ethyl acetate	+ + + +	+ + +	+ + +	+ + + +	+ + + +
	Flowers	Methanol/chloroform	+	+	+	+	+
	Leaves	Methanol/chloroform	+ + + +	+ + +	+ + +	+ + + +	+ + +
	Root	Methanol/chloroform	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
S. macrostema	Flowers	Aqueous	+	+	+	+	+
	Leaves	Aqueous	+ + + +	+	+	+	+
	Root	Aqueous	+ + + +	+	+	+	+
	Flowers	Ethyl acetate	+ +	+	+	+ + + +	+
	Leaves	Ethyl acetate	+ + + +	+ + +	+	+ + + +	+ + +
	Root	Ethyl acetate	+ + + +	+ + +	+	+ + + +	+ + + +
	Flowers	Methanol/chloroform	+	+	+	+	+
	Leaves	Methanol/chloroform	+	+	+	+ + + +	+ + +
	Root	Methanol/chloroform	+ + +	+ + +	+	+ + + +	+ + + +
T. lucida	Flowers	Aqueous	+ + + +	+ + +	+ +	+	+
	Leaves	Aqueous	+ + + +	+ + + +	+ +	+	+
	Root	Aqueous	+ + + +	+ + + +	+	+	+
	Flowers	Ethyl acetate	+ + +	+ + +	+ + + +	+ + + +	+ + +
	Leaves	Ethyl acetate	+ + + +	+ + +	+ + + +	+ + + +	+ + + +
	Root	Ethyl acetate	+ + + +	+ + +	+	+ + + +	+ + + +
	Flowers	Methanol/chloroform	+ + + +	+ + +	+ + + +	+ + + +	+ + + +
	Leaves	Methanol/chloroform	+ + + +	+ + +	+ + + +	+ + + +	+ + +
	Root	Methanol/chloroform	+ + + +	+ + + +	+ + + +	+ + + +	+ + +

n = 9, standard errors < 16 % all cases; 1.99 < + + + + ≥ 1.0 cm; 0.99 < + + + ≥ 0.5 cm; 0.49 < + + ≥ 0.2 cm; 0.2 < + ≥ 0.1.

Table 2. Antifungal Activities of Crude Plant Medicinal Extract Determinate by Germination Inhibition Test. *C. albicans* (*Ca*), *C. lindemuthianum* (*Cl*), *M. circinelloides* (*Mc*), *S. cerevisiae* (*Sc*) and *S. schenckii* (*Ss*)

Species	Organ	Extract	Inhibition of Germination (%)				
			C a	*C l*	*M c*	*S c*	*Ss*
A. ludoviciana	Flowers	Aqueous	53	-	-	-	-
	Leaves	Aqueous	50	-	-	-	-
	Root	Aqueous	55	-	-	-	-
	Flowers	Ethyl acetate	-	-	99	57	56.25
	Leave	Ethyl acetate	50	-	-	53.5	53.75
	Root	Ethyl acetate	51	50	-	54	61.25
	Flowers	Methanol/chloroform	50	-	-	75	60
	Leaves	Methanol/chloroform	52.5	50	61.2	52.5	55
	Root	Methanol/chloroform	-	-	62.5	77.5	50
H. longipes	Root	Aqueous	50	-	-	-	-
	Flowers	Ethyl acetate	-	65	72	-	56
	Leaves	Ethyl acetate	-	-	73.7	-	54
	Root	Ethyl acetate	53.5	-	-	60	52
	Leaves	Methanol/chloroform	52	-	-	58.5	-
	Root	Methanol/chloroform	54	60	62.5	53.7	50
S. macrostema	Leaves	Aqueous	64	-	-	-	-
	Root	Aqueous	51	-	-	-	-
	Flowers	Ethyl acetate	-	-	-	100	-
	Leaves	Ethyl acetate	50	-	-	63.5	-
	Root	Ethyl acetate	53	-	-	50	53
	Leaves	Methanol/chloroform	-	-	-	60	-
	Root	Methanol/chloroform	-	-	-	57.5	50
T. lucida	Flowers	Aqueous	54	-	-	-	-
	Leaves	Aqueous	60	58.5	-	-	-
	Root	Aqueous	65	62	-	-	-
	Flowers	Ethyl acetate	-	.	76	90	-
	Leaves	Ethyl acetate	50	-	78.7	81	58.75
	Root	Ethyl acetate	50.5	-	-	50	53.75
	Flowers	Methanol/chloroform	62	-	78	78.7	55
	Leaves	Methanol/chloroform	53	-	78.7	50	-
	Root	Methanol/chloroform	52	50.5	77.5	56	-

Also, the ethyl acetate and methanol-chloroform extracts from *A. ludoviciana, H. longipes* and *T. lucida* inhibited all the fungi assayed: *C. albicans*, *C. lindemunthianum, M. circinelloides, S. cerevisiae,* and *S. schenckii*. However, the results of the spore germination inhibition assay suggested that the methanol-chloroform extract from *A. ludoviciana,* was the most active against the fungi tested. An observation similar to antifungal effect of the extracts obtained from *Artemisia giraldi* on *Aspergillus flavus* and *Trichoderma viride* [14]. It was reported that *A. mexicana* had a strong antifungal activity against *C. albicans*, too [15]. The essential oils of *A. absinthium, A. santonicum,* and *A. spicigera*

Table 3. Inhibitory Growth Concentration Half (IC_{50}) of Crude Plant Medicinal Extract Against *C. albicans* (*Ca*), *C. lindemuthianum* (*Cl*), *M. circinelloides* (*Mc*), *S. cerevisiae* (*Sc*) and *S. schenckii* (*Ss*)

Species	Organ	Extract	IC_{50} on Test Fungi (mg/ml)				
			C a	*C l*	*M c*	*S c*	*Ss*
A. ludoviciana	Flowers	Aqueous	4	-	-	-	-
	Leaves	Aqueous	108	-	-	-	-
	Root	Aqueous	38	-	-	-	-
	Flowers	Ethyl acetate	-	-	-	160	140
	Leaves	Ethyl acetate	25	-	-	250	250
	Root	Ethyl acetate	4	107	-	107	27
	Flowers	Methanol/chloroform	33	-	-	20	27
	Leaves	Methanol/chloroform	95	100	80	95	91
	Root	Methanol/chloroform	-	-	-	65	100
H. longipes	Root	Aqueous	168	-	-	-	-
	Flower	Ethyl acetate	-	9	-	-	-
	Root	Ethyl acetate	5	-	-	4	4
	Leaves	Methanol/chloroform	500	-	-	430	-
	Root	Methanol/chloroform	100	83	80	93	100
S. macrostema	Leaves	Aqueous	34	-	-	-	-
	Root	Aqueous	51	-	-	-	-
	Leaves	Ethyl acetate	25	-	-	18	-
	Root	Ethyl acetate	10	-	-	10	10
	Leaves	Methanol/chloroform	-	-	-	76	-
	Root	Methanol/chloroform	-	-	-	43	64
T. lucida	Flower	Aqueous	60	-	-	-	-
	Leaves	Aqueous	11	12	-	-	-
	Root	Aqueous	60	68	-	-	-
	Flowers	Ethyl acetate	-	-	21	31	-
	Leaves	Ethyl acetate	88.7	-	51	50	70
	Root	Ethyl acetate	16	-	46	11	14
	Flowers	Methanol/chloroform	37	-	-	250	330
	Leaves	Methanol/chloroform	49	-	310	500	-

showed toxic effect against phytopathogen fungi. In these species the 1,8-cineol and the borneol were found to be the major compounds.

A lot of *Artemisia* species have many references, however *A. ludoviciana* does not. In *Artemisia* genus, more than 96 compounds were determined, the most significant being terpenes: artemisia ketone, 1,8-cineole, camphor, santolin alcohol, borneol, camphene, caryophyllene, sabinene, *p*-cymene, and *γ*-terpinene [16]. These compounds have been reported to show antifungal activity against *C. albicans* and *S. cerevisiae* [17]. Likewise, it was reported that borneol and its derivatives, camphor and 1,8-cineole (eucalyptol), have demonstrated low antifungal activity against *Malassezia furfur*, *Trichophyton rubrum* and *T. beigelii* [18-21]. Other antifungal compounds such as vulgarone B and verbenone, have been obtained from *A. douglasiana* [22].

However, it changes have been observed in the amount and quality of some antifungal metabolites in some plant species e.g. camphor, *cis*-verbenone and verbenol have not been detected in *A. afra*, but their essential oil inhibited yeast growth [23, 24].

Table 4. Qualitative Composition from Leaves *A. ludoviciana* Extract Obtained with Methanol-Chloroform

Compounds Identified	Relative Abundance (%)
Camphor	0.35
1,8-Cineole	0.14
Borneol	6.19
Cis-Verbenol	0.69
Myrtenol	0.52
Eugenol	0.73
Cariophyllene	0.84
α-Farnesene	0.63
Spathulenol	3.09
Derivatives of caryophyllene	5.09
Derivatives of spathulenol.	1.16

Other authors reported fungitoxic effect against *C. albicans* and soil born plant pathogens by eucalyptol and other major compounds detected in *Artemisia* species [12]. A lot of species of *Artemisia* are used medicinally, e.g. *A. aryii* is most commonly used in the treatment of pain in the Chinese medicine, its main chemical constituents are borneol, camphor and cineole [25].

In the *Tagetes* genus, it has been looking for new insecticides with specific characteristics, two of them are that, its possible use does not harm the environment and they have a similar or a better effect than the conventional insecticides, [4, 26]. In this search, *T. patula* turned out to be a good candidate, because it presents larvicide activity against *Aedes aegypti* and the majority of its components are terpenes, specially limonene, caryophyllene and ocimene, which are directly responsible for this activity [27].

On the other hand, it was reported that peperitone and piperitenone obtained from *T. patula* had antifungal activity against *Botrytis cinerea* and *Penicillium digitatum*. However, in this antifugal effect it was reported a synergy of these and others components of essential oil [28, 29]. Likewise, in the antifungal effect of extracts from *T. lucida* and *T. filifolia* its antifungal metabolites and metals such as Cu, Zn, Ca and Mg have been involved [30].

On the other hand it is known that *T. lucida* is an important source of antioxidant compounds [31].

In the *Tagetes* genus, there are few reports on its antifungal activity, some of them involved to piperitone, piperitenone, terpinolene, dihydro tagetone, *cis*-tagetone, limonene and *allo*-ocimene [29, 31].

Other reports are related with their bactericide activity or including enterobactericide activity [29, 32, 33]. However, we observed that extracts from *T. lucida* inhibited the growth and germination of all the fungi assayed. *T. lucida* is one of the most used species by the Latin-American population for the stomachache sickness [34]. Sometimes it is possible to confuse *T. filifolia, T. micrantha* or even *Artemisia dracunculus*, that is why it is necessary to confirm the specie on which someone is working [35].

In *H. longipes*, some alkamides have been identified in the root of this plant, the affinin and bornyl decatrienate are responsible for the antifungal activity of this species [36, 37]. These metabolites are concentrated in the root because different root, extracts of *H. longipes* showed this activity, whereas other organs did not show any fungitoxic activity except the leaves methanol-chloroform extract which was observed to inhibit the growth of yeast and flowers ethyl acetate extract also inhibited the growth of dimorphic fungi (Table **1**).

Several species of *Satureja* genus have been reported that possess antifungal components. The essential oil from *S. timbra* showed a good antifungal activity against soil borne microorganisms and foliage pathogen [38]. Likewise the methanol extracts of herbal parts and callus from *S. hortensis* have shown fungicidal activity and this property has been attributed to thymol, carvacrol and eugenol [39, 40]. However in this work, the *S. macrostema* extracts showed a low fungitoxic effect (Tables **1** and **2**).

Essential oil from *S. parnassica,* subsp. *parnassica*, *S. timbra* and *S. spinosa*, had strong antibacterial effect. The major compounds identified were 1,8-cineole, camphor, borneol, spathulenol and caryophyllene oxide [41]. These results are similar to ours, where the leaves methanol-chloroform extract of *A. ludoviciana* showed the best fungicide activity, because it possess the same compounds (Tables **2** and **4**). In *S. macrostema* results, no fungitoxic effect was observed and this was probably because of the concentration of compounds, genetic variability or the extraction type.

In other *Satureja* species, like *S. montana* L. and *S. cuneifolia,* the essential oils have shown antifungal activity against *A. fumigates, C. albicans,* and *S. cerevisiae*. These oils contain carvacrol, *p*-cymene, γ-terpinene, borneol, thymol, β-cubebene, limonene, α-pinene, spathulenol, β-caryophyllene, camphor, and borneol [13]. These results are similar to ours, because the *S. macrostema* extracts inhibited the growth and germination of C. *albicans* and *S. cerevisiae* (Tables **1** and **2**).

It has been demonstrated that the activity and chemical composition of a plant vary considerably depending on the geographic location, growing conditions, plant organs and developmental stages of plant, solvent used for extraction, photosensitivity of some compounds in the extract, and the methods used to isolate the essential oils [12, 27].

There are many works about Asteraceae plants and its fungitoxic effect against pathogen fungi, the most important belongs to the *Artemisia, Tagetes* and *Heliopsis* genus, and in this work we have shown the fungitoxic properties of *A. ludoviciana*, *H. longipes*, *S. macrostema* and *T. lucida*, during our search for new more affordable antifungal compounds.

In general, all the extracts showed an antifungal effect against tested fungi in this work, but only two plants were found to be the source of metabolites with antifungal properties: *A. ludoviciana* and *T. lucida.* This is the first work that has reported a screening for antifungal effects of different extracts for these species, where the methanol-chloroform

and ethyl acetate extracts from *A. ludoviciana* and *T. lucida* showed pronounced antifungal activity against tested fungi.

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