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Antifungal and Antibacterial Activities of Mexican Tarragon (*Tagetes lucida*)

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Mexican tarragon (*Tagetes lucida* Cv. Asteraceae: Campanulatae) is an important, nutritious plant and an effective herbal medicine. Seven coumarins, 7,8-dihydroxycoumarin (**4**), umbelliferone (7-hydroxycoumarin) (**5**), scoparone (6,7-dimethoxycoumarin) (**7**), esculetin (6,7-dihydroxycoumarin) (**11**), 6-hydroxy-7-methoxycoumarin (**12**), herniarin (7-methoxycoumarin) (**13**), and scopoletin (6-methoxy-7-hydroxycoumarin) (**14**), and three flavonoids, patuletin (**18**), quercetin (**19**), and quercetagetin (**20**), were isolated from CH_2Cl_2 and MeOH extracts from aerial parts of *T. lucida*. In addition, 6,7-diacetoxy coumarin (**15**), 6-methoxy-7-acetylcoumarin (**16**), and 6-acetoxy-7-methoxycoumarin (**17**) derivatives were synthesized. 8-Methoxypsoralen (**1**), 8-acetyl-7-hydroxycoumarin (**2**), 7,8-dihydroxy-6-methoxycoumarin (**3**), 6,7-dimethoxy-4-methylcoumarin (**6**), 5,7-dihydroxy-4-methylcoumarin (**8**), 4-hydroxycoumarin (**9**), 4-hydroxy-6,7-dimethylcoumarin (**10**), naringenin (**21**), glycoside-7-rhamnonaringin (**22**), and rutin (**23**) were commercially obtained (Sigma-Aldrich). All of these compounds and extracts (M_1 and M_2) were assayed against bacteria and fungi. The antibacterial activity was determined on *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Salmonella* sp., *Shigella boydii*, *Shigella* sp., *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Sarcina lutea*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Yersinia enterolitica*, *Vibrio cholerae* (three El Tor strains, CDC-V12, clinic case, and INDRE-206, were obtained from contaminated water), and *V. cholerae* (NO-O1). The evaluated fungi were *Aspergillus niger*, *Penicillium notatum*, *Fusarium moniliforme*, *Fusarium sporotrichum*, *Rhizoctonia solani*, and *Trichophyton mentagrophytes.* The most active compounds against Gram-positive and -negative bacteria were the dihydroxylated coumarins **3** and **4**. In addition, **2**−**4**, **6**, **7**, and **11** showed an interesting activity against *V. cholerae*, a key bacterium in the contaminated water; **2**−**4** were the most active. Coumarins were the most effective compounds against Gram-negative bacteria. The extract MeOH/CH_2Cl_2 (1:4) M_2 at 0.4 μg/disk inhibited the growth of *E. coli* and *P. mirabilis* (40%), *K. pneumoniae* (31.1%), *Salmonella* sp. (35.5%), and *Shigella* sp. (0%) at 72 h of culture. The dimethoxy compounds **6** and **7** showed a strong activity against fungal strains, especially *T. mentagrophytes* and *R. solani* (100% of inhibition at 125.0 and 250.0 μg/mL, respectively).

KEYWORDS: ***Tagetes lucida*; Mexican tarragon; antibacterial; antifungal; simple coumarins; spice**

INTRODUCTION

Plants produce a great diversity of substances that could be active in many fields of medicine. However, the chemical nature of plant compounds contained naturally and in the extracts is unknown. It is important to study plant extracts, such as *Tagetes lucida*, because their biocidal, nutritional, nutraceutical, and other properties have not been extensively investigated.

A nutraceutical is defined as any substance that may be considered a food or part of a food and also provides medical or health benefits including the treatment and prevention of disease. Nutraceuticals may range from single nutrients, dietary supplements, and secondary metabolites (*1*). There are a large number of secondary metabolites acting as plant chemical defenses against pathogen organisms. In the present circumstances, plant research with antimicrobial effects is extended

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(*2*−*4*). Many medicines have come from botanical sources (*5*). Approximately 25% of the active substances of prescriptions in the United States come from plant material, including therapeutic agents with anticholinergic, antihypertensive, and antileukemic properties (*6*, *7*). It is estimated that an amount of 20000 species is useful for these purposes (*8*).

T. lucida is an herbaceous, perenne, and endemic plant in Mexico and Central America, known as Santa Maria, pericon, hierbanis, Mexican tarragon, sweet dragon, yauhtli, periquillo, anicillo, curucumin, and Mexican marigold. It is used widely in traditional medicines of Central America to cure gastrointestinal disorders (*9*). Mexican tarragon is bushy with many smooth, upright, and unbranched stems. The leaves are linear or oblong, about 4.6 cm long and shiny green in color, not blue-green as in real French tarragon (*Artemisia dracunculus*). It is cultured for its pungent aromatic foliage, which is used as a flavoring. In the late summer, Mexican tarragon bears clusters of small yellow flower heads on the ends of the stems. The flower heads are about 1.3 cm across and have 3−5 golden-yellow ray florets. In Mexico, it is also traditionally used as a tea to reduce stomachache, to calm mental agitation, to alleviate the symptoms of a hangover, and to diminish the harsh manifestations of smoking Turkish tobaccos (*10*−*15*).

Mexican tarragon has been used since the Aztec times, for both religious and medical purposes. Today, it is a popular herb in the Southern states of the United States, since it can be a substitute for the well-known European tarragon and can be grown easily in a hot climate. Mexican tarragon is stronger and more robust than its analogous part (*14*, *16*).

Our studies concerning the chemical structure of biologically active secondary metabolites obtained from aerial part extracts of some other herbaceous species have shown the presence of a series of compounds including flavonoids, stilbenes, phenylpropanoids, and terpenes (*4*, *17*−*27*). Recent papers reported that flavonoids and coumarins isolated from the *Tagetes* genus have different biological activities such as antiinflammatory, antioxidant, antiviral, platelet antiaggregant, and nematicidal activities (*28*−*42*). Coumarins are made from L-phenylalanine or *p*-coumaric acid residues by the action of phenylalanine ammonia lyase involving (trans−cis) rearrangements to produce different isomers. These isomers are widely distributed in the *Tagetes* genus (*38*−*43*). The formation of these compounds involves a direct stereochemically controlled coupling glycosylation, followed by a light-mediated *E*−*Z* isomerization (*43*). This may require the presence of two stereoselective enzymes with different activities. The subsequent processes are dependent on the regiochemistry and stereochemistry of the initial linkage (*43*, *44*).

Analytical methods for detecting and studying the simple coumarins have been reviewed, and these compounds are common in many plant families (*43*, *45*). In contrast, furanocoumarins are distributed in the genera Apiaceae, Moraceae, Pittosporaceae, Rosaceae, Rutaceae, and Thymelaceae (*43*). The most well-known and representative coumarins are coumarin, umbelliferone, scopoletin, and scoparone. They are important parent compounds, which exhibit potent biological activities, and are chemical mediators in insect−plant relationships (*45*). Most known coumarins possess potent biological properties (*43*, *44*, *46*). However, the studies are limited to structure determination, biotransformation, phytoalexin and anticancer activities, insect−plant relationships, and biosynthetic pathways (*43*−*45*, *47*). In some cases, coumarins are responsible for toxicity in mammals (*43*, *48*), insects, fungi, and bacteria.

Some species of the *Tagetes* genus have been studied in America and Asia (*28*−*36*, *38*−*42*, *49*−*51*); however, there are not studies of the entire phytochemical composition and antimicrobial properties as well as the possible uses as a nutraceutical. This article contributes to the phytochemical knowledge of this plant and continues the study of native species of Mexico. The aims of this investigation were to evaluate the antibacterial and antifungal activities of the MeOH/CH_2Cl_2 and CH_2Cl_2 extracts, coumarins, and flavonoids present in *T. lucida*.

EXPERIMENTAL PROCEDURES

Plant Material. Samples of *T. lucida* were collected in native forests in ecological areas of Michoacán State, Mexico, particularly near the Morelia-Cointzio-Uruapan highway, at 1980 m altitude, on the slopes of the hills at Sierra Central, during October−November 2003. Voucher specimens were deposited in the herbarium collection of IZTA (FES-Iztacala Universidad Nacional Autónoma de México, UNAM, México D. F., México).

Isolation and General Procedures. Dried aerial parts (flowers, leaves, and stems; 10 kg) were finely chopped and milled. They were extracted three times with methanol at 40 °C for 48 h each. The crude extract was then evaporated to dryness under vacuum conditions. The total extract was partitioned between water/MeOH (1:1) and CH_2Cl_2, the CH_2Cl_2/MeOH (4:1) fraction (M_2) was separated from the aqueous MeOH (extract M_1) phase, and both were concentrated. The phase M_2 was fractionated by fast column chromatography (CC) (silica gel 60, 0.063−0.200 mm particle size, 1 g of extract:20 g of silica gel). The column was eluted with increasing polarity solvent mixtures to obtain a total of 10 fractions: F-1−10. Compound purification was carried out from the 10 fractions, using conventional chromatographic techniques [i.e., CC, thin-layer chromatography, high-performance liquid chromatography (HPLC), gas−liquid chromatography, and gas chromatography−mass spectrometry (GC-MS)]. The compound purity was controlled by HPLC with a diode arrangement LC 10 A-VP detector.

The isolated and purified compounds were characterized and identified through their spectroscopic data and compared with authentic samples. The melting points were determined on a Kofler block. Infrared spectra were determined in an IR-408 Shimadzu spectrophotometer. Ultraviolet spectra were determined with an UV-160 Shimadzu spectrometer. NMR spectra (^1H and ^{13}C) were recorded with a JEOL ECLIPSE-300 spectrometer (at 300 and 75 MHz, respectively), and mass spectra were carried out in a 5972 series Hewlett-Packard mass spectrometer. The GC-MS technique (MS detection at 70 eV) was performed under the following conditions: column HP-5, 30 m × 0.25 mm × 0.25 μm; temperature, 100 °C, isothermal for 5 min, with 10° increments per min up to 275 °C, which was held constant for 20 min; split injection, 100:1; injector temperature, 275 °C; detector temperature, 300 °C; and helium, carrier.

7,8-Dihydroxycoumarin (**4**), umbelliferone (7-hydroxycoumarin) (**5**), scoparone (6,7-dimethoxycoumarin) (**7**), esculetin (6,7-dihydroxycoumarin) (**11**), 6-hydroxy-7-methoxycoumarin (**12**), herniarin (7-methoxycoumarin) (**13**), scopoletin (6-methoxy-7-hydroxycoumarin) (**14**), patuletin (**18**), quercetin (**19**), and quercetagetin (**20**) were isolated from M_2 and M_1 extracts. 6,7-Diacetylcoumarin (**15**), 6-methoxy-7-acetylcoumarin (**16**), and 6-acetyl-7-methoxycoumarin (**17**) were semisynthetically obtained. 8-Methoxypsoralen (**1**), 8-acetyl-7-hydroxycoumarin (**2**), 7,8-dihydroxy-6-methoxycoumarin (**3**), 6,7-dimethoxy-4-methylcoumarin (**6**), 5,7-dihydroxy-4-methylcoumarin (**8**), 4-hydroxycoumarin (**9**), 4-hydroxy-6,7-dimethylcoumarin (**10**), naringenin (**21**), glycoside-7-rhamnonaringin (**22**), and rutin (**23**) were purchased from Sigma-Aldrich (Toluca, Mexico). The chemical structures of all natural compounds were determined by analyzing their spectrometric and spectroscopic data and compared with authentic samples.

Ketoconazole was used as a positive control in the antifungal bioassay at a concentration of 0.9 μg/mL. Kanamycin and chloramphenicol (Sigma-Aldrich) were used as positive controls in the antibacterial assay at concentrations of 30 μg/mL in the quantitative assay in culture medium, and in the Kirby−Bauer qualitative bioassay, 30 μg/disk was used.

Evaluation of Biological Activity (Microorganisms and Growth Medium). The antibacterial and antifungal activities of the M_1 and M_2 extracts, coumarins, and flavonoids were determined. Because of a very little amount of **15**−**17**, these compounds were not examined. Paper disks (6 mm, Whatman #1) were impregnated with 10 μL of solution containing 100 μg of each compound to perform the test against the Gram-negative bacteria, *Escherichia coli* (ATCC25922, and wild-type 3), *Enterobacter aerogenes* (wild-type 1), *Enterobacter agglomerans* (ATCC27155), *Proteus mirabilis* (wild-type 3), *Klebsiella pneumoniae* (wild-type 3), *Salmonella typhi* (ATCC19430), *Salmonella* spp. (wild-type 3), *Shigella* spp. (wild-type 3), *Shigella boydii* (ATCC8700), *Yersinia enterocholitica* (wild-type 2), *Vibrio cholerae* [all strains correspond to biotype *El-tor*, three collections: CDC-V12, INDRE-206 (isolated from contamined water), and CC (a wild-type 1, isolated from a clinic case)], and *V. cholerae* NO-O1 (wild-type 1), and the Gram-positive bacteria, *Bacillus subtilis* (ATCC6633), *Sarcina lutea* (wild-type 1), *Staphylococcus aureus* (ATCC12398), and *Staphylococcus epidermidis* (wild-type 1).

The fungi strains were *Aspergillus niger* (ATCC64958), *Fusarium moniliforme* (ATCC96574), *Fusarium sporotrichum* (wild-type 2), *Rhizoctonia solani* (wild-type 2a), *Trichophyton mentagrophytes* (ATCC9972), *A. niger* (wild-type 2), and *Penicillium notatum*.

Wild-Type 1. The strain was cultured and donated by Laboratorio de Microbiología of FES-Cuautitlán (UNAM).

Wild-Type 2. The strain was cultured and donated by Laboratorio de Análisis Clínicos of FES-Iztacala (UNAM).

Wild-Type 2a. The strain was isolated from infected bean cultures by Prof. Dr. Rodolfo de la Torre, Laboratorio de Microbiología, FES-Iztacala (UNAM).

Wild-Type 3. Strains were cultured and donated by Prof. Q. F. B. Sandra Maria Suárez M., Laboratorio de Microbiología y Patología, Hospital Infantil (Morelia, Michoacán, Mexico).

Wild-Type 3 Strains Cultivation. The bacteria *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Salmonella* sp., and *Shigella* sp. were maintained under freezing and, before the bioassays were done, were cultured in sterile Erlenmeyer flasks with 10 mL of YEB liquid medium (flasks were maintained in incubation for 72 h at 37 °C). The bioassay was made by paper disk method (AOAC, 1070); disks of 6 mm in diameter were soaked with M_1 and M_2, respectively. These disks were impregnated with 10 μL of a 40 μg/μL solution of M_2 and with 10 μL of a 400 μg/μL solution of M_1 and placed in Petri dishes containing YEB medium previously inoculated with bacteria (100 μL). Cultures were incubated at 37 °C for 24 h. The diameter of the growth inhibition zone was determined (mm, included the paper disk). The mean value of at least three different experiments was used for statistical analysis, and each experiment was made in triplicate.

Bioassays with ATCC, Wild-Types 1 and 2 Strains. Bacteria that were grown in brain heart infusion broth (Bioxon, Mexico City, Mexico, 112-1) for stock cultures and Mueller−Hinton broth (Bioxon, 260-1) were used as test media due to their low interaction with the assayed compounds. The antibacterial activity of the extract and pure compounds was assessed with the disc−diffusion method using Mueller−Hinton agar (*52*), and the determination of inhibition zones at different dilutions of compounds and extracts was evaluated. In this case, filter paper disks (6 mm diameter, Whatman #1) with pure compounds were impregnated with 10 μL of a 10 μg/μL solution of each sample, and the filter paper disks of the crude total extract were impregnated with 10 μL of a 40 μg/μL solution of M_2 and 10 μL of a 400 μg/μL solution of M_1, respectively, and placed in Petri dishes containing the test organisms. Cultures were incubated at 37 °C, and after 72 h, the diameter of inhibition zone was determined (mm). The mean value of at least three different experiments was used for statistical analysis, and each experiment was made in triplicate. The treatments were evaluated with a completely randomized design. The treatments were subjected to a one-way analysis of variance (ANOVA), and means were compared with the Student−Newman−Keuls (SNK) test ($P = 0.05$) under MicroCal Origin 6.2 Microsoft statistical program. Kanamycin and chloramphenicol were used as positive controls.

The antifungal property of the extract and compounds was tested by the agar−well diffusion method using Sabouraud dextrose agar. Standard reference antibiotics were used in order to control the sensitivity of the tested microorganisms, which were inoculated in Czapek−Dox broth medium. Plates containing only the culture medium, with the addition or not of the solvents (methanol or water 10 μL/disk), were used as viability controls for each fungus studied. The fungi inocula (10 μL of 3×10^6 spores/mL) were placed in a hole (0.4 mm^2) made in the center of each Petri dish after solidification of the agar. Extract doses were until 4 mg/disk (0.4 mg of M_2 and 4.0 mg of M_1, respectively); positive control, 10 μg/disk ketoconazole. The cultures were incubated at 28 °C for 14 days and checked every 24 h. Inhibition of radial mycelial growth diameters was measured daily and recorded as mean percentages (%) of growth (*53*).

Antifungal Assays (FC_{50}) and Minimum Fungicide Concentration (MFC). These tests were carried out to analyze the fungicidal activity exhibited by each compound and extract. For quantitative assays of the extracts, three doses were added to Czapek−Dox agar (4 mL) at 45 °C, mixed rapidly, and poured into three separated 6 cm Petri dishes. After the agar had cooled at room temperature, a small amount of mycelia (1 mm × 1 mm) was inoculated, and the same amount to each plate was added. DMFA was only employed as a negative control. After incubation at 23 °C for 72 h, the area of the mycelial colony was measured and the inhibition of fungal growth and hence the IC_{50} were determined. Fourteen days after the beginning of the assay, a circle of agar around the central hole was obtained. Ketoconazole (0.9 μg/mL) was used as a positive control. At the end of this period, the MFC values were recorded (*53*).

Statistical Analysis. Data shown in tables are the average of three replicates and independent experiments and are presented as averages ± standard errors of the mean. Data were evaluated by variance analysis (ANOVA). Significant differences between means were identified by GLM procedures. Results are given in the text as probability values, with $p < 0.05$ adopted as the criterion of significance; differences between treatments means were established with a SNK test. The I_{50} (CF_{50}) values for each activity were calculated by PROBIT analyses based on percentage of inhibition obtained at each concentration of the samples. I_{50} is the concentration producing 50% inhibition. The complete statistical analysis was performed by means of the OriginLab, Origin 7.0, statistical and graphs PC program.

RESULTS AND DISCUSSION

In our screening program looking for biological activities of plants from Mexico and North America regions, it was found that *T. lucida* showed antifungal and antibacterial activities in a preliminary trial. On the basis of this information and in the high resistance to insect and pathogen attack of this plant, we carried out a biodirected phytochemical study on the aerial parts of *T. lucida*.

From the M_1 and M_2 extracts, seven coumarins and three flavonoids were isolated and identified from the aerial part of the Mexican tarragon species *T. lucida*: **4**, **5**, **7**, **11**−**14**, and **18**−**20** (**Figures 1** and **2**). The structures of these compounds were established by cochromatography with standards and spectroscopic evidences. Compounds **1**−**3**, **6**, **8**−**10**, and **21**−**23** were purchased from Sigma-Aldrich.

The antibacterial activity was carried out against Gram-positive and Gram-negative bacteria. Compounds **3** and **4** were active against almost all Gram-positive and -negative bacteria assayed. Compound **3** was quantitatively the most active compound. Compounds **2**, **6**, **7**, and **11** were active only against the four collections of *V. cholerae*—El-tor (**Table 3**).

Tables 1 and **2** shows the zone (mm) and percentage of inhibition of the M_1 and M_2 extracts against enterobacteria and fungi, respectively. M_2 showed an inhibition effect against *P. mirabilis*, *E. coli*, *Salmonella* sp., and *K. pneumoniae*; this effect was noteworthily higher in *E. coli* and *P. mirabilis* than the other bacteria assayed; the inhibition diameter was 18 mm (40% at 72 h) in *Salmonella* sp., and (16 mm) in *K. pneumoniae*, *Shigella* sp., *B. subtilis*, and *S. lutea*, there was not a response

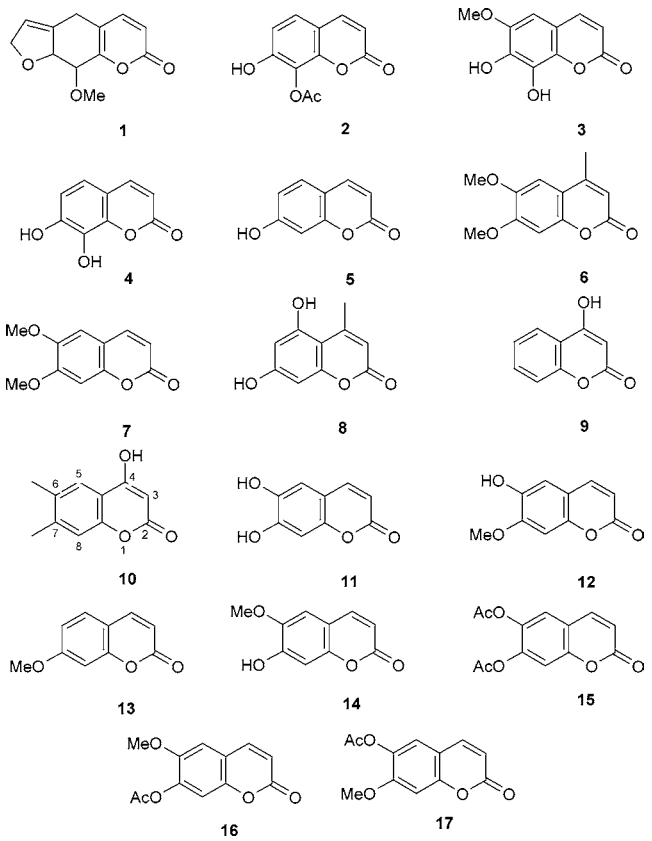

Figure 1. Chemical structures of coumarins used in this study.

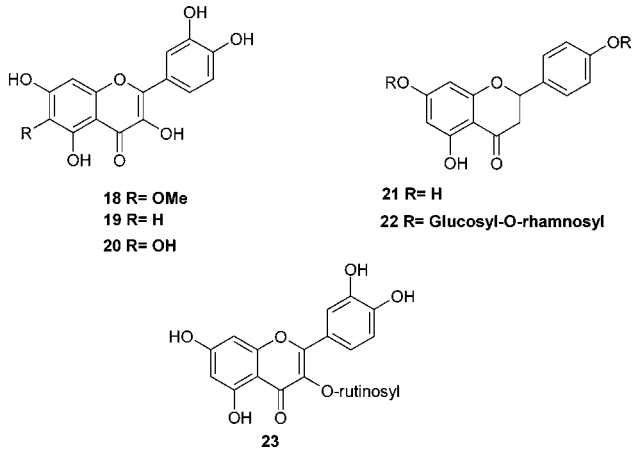

Figure 2. Chemical structures of flavonoids used in this work: **18**–**23**.

(**Table 1**). These results shows that the M_2 extract and one of its components (**13**) had a significant inhibitory activity against Gram-negative bacteria and did not have the same activity against Gram-positive bacteria (there was no zone of inhibition at the concentration of 400 μg/disk) (data not shown).

The extracts M_1 and M_2 and compound (**13**) were also assayed against *R. solani*, *F. sporotrichum*, *F. moniliforme*, *A. niger*, and *P. notatum* (**Table 2**). The activity level shown by compound **13** and M_2 extract against these fungi was relatively high as compared with the positive control (ketoconazole) (data not shown); M_2 was the most active, with a inhibition at 400.0 μg/disk of 89.25% against *F. moniliforme*, 77.5% against *F. sporotrichum*, 55.8% against *A. niger*, and 55.5% against *R. solani*. *P. notatum* did not show sensitivity (**Table 2**). In addition, compound **13** and M_2 extract showed a total inhibition (100%) against these fungi at concentrations above of 400 μg/disk (data not shown). A similar effect was shown by M_1 extract, which completely inhibited the mycelial growth of these fungi above 4000.0 μg/disk. The growth of *P. notatum* only was partially inhibited (39%) by M_1 assayed at 4500.0 μg/disk (data not shown). The growth of *A. niger*, *F. moniliforme*, *F. sporotrichum*, and *R. solani* was completely inhibited by M_1 in a range of 5000.0−10000.0 μg/disk (data not shown).

In view of the strong activity of M_2 extract against bacteria, this extract and compounds **1**−**14** and **18**−**23** were assayed against different fungal and bacterial strains and their minimum inhibitory concentration (MIC), minimum bactericide concentration (MBC), FC_{50}, and MFC values were obtained. The derivatives **15**−**17** were not assayed because of low yield. The M_1 extract was used in all measurements.

The antibacterial inhibitory activities of **1**−**4**, **6**, **7**, and **11** are shown in **Table 3**. Thus, M_1 and M_2 extracts, **13**, and the other compounds did not show a significant activity at the concentration used (100.0 μg/disk). Compounds **3** and **4** are dihydroxylated coumarins at positions C-7 and C-8, and these were the most active with antibacterial inhibitory activity. Nonetheless, compounds **2** and **11**, which are coumarins mono- and dihydroxylated at positions C-7 and C-6 and C-7, respectively, only showed a partial activity against *V. cholerae*. On the other hand, compounds **1**, **6**, and **7** showed only a restricted activity on some *V. cholerae* strains assayed. These facts probably prove that the vicinal diol moieties at C-7 and C-8 positions could be important for antibacterial activity. Noteworthy, compound **3** is quantitatively more active than **4**. The MICs of compounds **3** and **4** against *V. cholerae* (Tor: strain CDC-V12) were 25 and 200 μg/mL, respectively, and against *E. coli*, they were 250 and 450 μg/mL, respectively (**Table 4**). In this case, it is probable that the methoxyl group at the C-6 position, together with the two vicinal hydroxyl groups, electronically activate the aromatic ring and increase the lipophilicity of the compound; consequently, these facts could induce a large growth inhibition on the bacteria strains assayed by these compounds.

Besides, the fungicidal qualitative evaluation showed that the coumarins **6** and **7**, methoxylated at C-6 and C-7 positions, were the most active followed by **13** in a significant minor potency (**Table 5**). By this reason, the FC_{50} and MFC values were determined for **6** and **7** only; this result shows that **6** and **7** are the most potent fungicides of the simple coumarin assayed in this study. Their FC_{50} and MFC values against *R. solani*, *F. sporotrichum*, *F. moniliforme*, *A. niger*, and *T. mentagrophytes* are presented in **Table 6**. The values for these compounds are close to those of the positive control; this renders them quite important due to the possible use of these substances as nutraceuticals.

The M_2 extract showed a composition mainly of coumarin and flavonoids; **13** (>35.0%) was the most abundant compound in the *T. lucida* extracts, followed by **7** (>20.0%), **18** and **20** (both with ≥10.0%), **19** (≈5.0%), and other secondary metabolites in a very minor amount (≈1.5%). These compounds have also been previously reported for other species of the *Tagetes* genus (*28*−*36*, *38*−*42*, *49*−*51*).

Interestingly, the M_2 extract has the highest percentage of the coumarins **13** and **7** together with some flavonoids, and from these compounds, only **7** has an important biocidal effect, and **13** only showed a partial inhibition on bacteria and fungi, but M_2 nevertheless showed the highest antibacterial and antifungal activities. Then, these results permit us to assume a possible synergistic effect of M_2 extract (**Tables 1** and **2**).

The activity showed by these coumarins could be due to the lipophilic and antioxidant capacities of the components of M_2,

Table 1. Effects of M_1 and M_2 Extracts from *T. lucida* on Growth Inhibition of Bacteria (Diameter in mm)[a]

extract	*E. coli*	%	*P. mirabilis*	%	*K. pneumoniae*	%	*Salmonella* sp.	%	*Shigella* sp.	%	*B. subtilis*	%	*S. lutea*	%
M_1	4.1 ± 0.6 a	9.1	4.2 ± 0.6 a	9.3	3.9 ± 0.47 a	8.6	4.0 ± 0.45[a]	9.0	0[b]		0[b]		0[b]	
M_2	18.1 ± 3.1 b	40.0	18.1 ± 3.3 b	40.0	14.2 ± 2.8 b	31.3	16.3 ± 2.4 b	35.5	0[b]		0[b]		0[b]	
13	16.5 ± 2.1 d	36.5	17.1 ± 2.6 b	37.8	12.3 ± 1.9 b	27.2	14.4 ± 2.2 b	31.8	0[b]		0[b]		0[b]	
kanamycin	25.5 ± 1.4 c	56.4	22.5 ± 0.9 c	49.7	27.9 ± 1.6 c	61.7	30.1 ± 1.1 c	66.5	23.3 ± 0.8	51.5	22.4 ± 0.9	49.6	21.7± 1.4	47.9

[a] Inhibitory effects at an equivalent concentration of 4000 μg/disk with M_1, 400 μg/disk with M_2, and 100 μg/disk with **13** are represented as millimeters of growth; the mean value of the diameter of the inhibition zone is mm ± standard error of $N = 21$, and its significant difference from the control is $p < 0.01$. [b] Activity not present.[c] Mean of three replicates. Means followed by the same letter within a column after ± standard error values are not significantly different in a SNK test (treatments are compared by concentration to control); 95% confidence limits.

Table 2. Effect of Extracts M_1 and M_2 of *T. lucida* as Antifungal Growth Inhibition Activity on Fungi Preparation Inocula (Diameter in mm)[c]

	fungi								
sample	*R. solani*	%	*F. sporotrichum*	%	*F. moniliforme*	%	*A. niger*	%	*P. notatum*
M_2	16.4 ± 1.7b	36.2	35.1 ± 4.1b	77.5	40.4 ± 4.5b	89.2	25.3 ± 2.3b	55.8	NA[b]
M_1	8.1 ± 0.5a	17.9	17.9 ± 2.2a	39.5	23.05 ± 2.4a	50.9	15.5 ± 1.9a	34.2	NA[b]
13	18.1 ± 0.9b	40.0	25.1 ± 1.9c	55.5	36.1 ± 3.1b	79.8	33.9 ± 2.7c	75.0	NA

[a] Inhibitory effects at an equivalent concentration of 4000 μg/disk with M_1, 400 μg/disk with M_2, and 100 μg/disk with **13** are represented as millimeters of growth; the mean value of the diameter of the inhibition zone is mm ± standard error of $N = 21$, and its significant difference from the control is $p < 0.01$. [b] Activity not present.[c] Mean of three replicates. Means followed by the same letter within a column after ± standard error values are not significantly different in a SNK test (treatments are compared by concentration to control); 95% confidence limits.

Table 3. Antibacterial Inhibitory Activities[a] of the Compounds Evaluated in This Study[b]

bacteria	**1**	**2**	**3**	**4**	**6**	**7**	**11**	C+
Tor	*c*	10.0 ± 0.1 a	17.3 ± 0.5 a	17.2 ± 0.3 a		10.1 ± 0.1 a	15.3 ± 0.4 b*	31.1 ± 0.4 a
N	22.5 ± 0.5	16.0 ± 0.3 b	17.5 ± 0.5 a	20.3 ± 0.3 b	10.2 ± 0.2	15.3 ± 0.4 b	14.0 ± 0.1 a	40.1 ± 0.5 b
A		9.0 ± 0.1 a	15.2 ± 0.4 b	15.0 ± 0.1 a		8.0 ± 0.3 c	14.0 ± 0.3 a*	30.0 ± 0.3 a
CC		11.0 ± 0.2 a	17.1 ± 0.3 a	14.1 ± 0.1 c		11.2 ± 0.2 a	12.0 ± 0.2 c*	27.0 ± 0.5 a
E.c.				10.0 ± 0.1 d				20.0 ± 0.3 c
E.ae.			8.2 ± 0.3 c	10.1 ± 0.2 d				23.1 ± 0.5 c
E.agg.				10.2 ± 0.3 d				25.2 ± 0.4 a
S.t.			14.1 ± 0.3 b	13.3 ± 0.3 c			11.0 ± 0.3 c	24.0 ± 0.5 a
S.b.							15.2 ± 0.3 b	25.0 ± 0.5 a
Y.e.			18.0 ± 0.3 d	18.4 ± 0.4 b				20.0 ± 0.5 c
B.s.				11.1 ± 0.3 d				27.4 ± 0.5 a
S.l.			11.4 ± 0.3 b	15.3 ± 0.3 a				37.5 ± 0.5 b
S.e.			17.1 ± 0.3 a	14.4 ± 0.2 c				18.5 ± 0.4 c
S.a.			16.0 ± 0.3 a	15.0 ± 0.3 a				20.2 ± 0.5 c

[a] An amount of 100 μg/disk of compounds was used. Key to bacteria: Tor, *V. cholerae* CDC-V12; N, *V. cholerae* NO-O1; A, *V. cholerae*, INDRE-206; CC, *V. cholerae* clinic case; E.c., *E. coli*; E.ae., *E. aerogenes.* E.agg., *E. agglomerans*; S.t., *S. typhi*; S.b., *S. boydii*; Y.e., *Y. enterocholitica*; B.s., *B. subtilis*; S.l., *S. lutea*; S.e., *S. epidermidis*; and S.a., *S. aureus.* [b] The mean of three replicates is represented as millimeters of growth. Means followed by the same letter within a column after ± standard error values are not significantly different in a SNK test (treatments are compared by concentration to control); 95% confidence limits. Negative control, 5 μL/disk *N*,*N*-Dimethylformamide (DMFA); positive control (C+), 30 μg/disk chloramphenicol. *Diminution of population. [c] Activity not present (in this table are shown only the more significant inhibitory effects, above 8.0 mm).

Table 4. Results of MIC and MBC of Compounds **3** and **4** (μg/mL)[a]

	3		**4**	
bacteria	MIC	MBC	MIC	MBC
E. coli	250	300	450	↑450
V. cholerae CDCV12	25	50	200	250
S. aureus	400	↑800	450	↑450

[a] Negative control, 70 μL/mL DMFA; positive control, 30 μg/mL chloramphenicol.

Table 5. Fungal Qualitative Evaluation of Coumarins[a]

fungi/compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**13**	C^+	C^-
F. sporotrichum	–	–	–	–	–	+++	+++	–	–	–	+	+++	–
R. solani	–	–	–	–	–	+++	+++	–	–	–	+	+++	–
F. monoliforme	–	–	–	–	–	+++	+++	–	–	–	+	+++	–
A. niger	–	–	–	–	–	+++	+++	–	–	–	+	+++	–
T. mentagrophytes	–	–	–	–	–	+++	+++	–	–	–	+	+++	–

[a] This evaluation was carried out using 1000 μg/disk; the positive control (C^+) was 10 μg/disk ketoconazole, and the negative control (C^-) was DMFA, with a maximum dilution of 20 μL/disk. Key: +++, evident inhibition of radial growth (30% inhibition of radial growth with respect to control); +, partial inhibition of radial growth (15% inhibition of radial growth with respect to control); and –, complete growth of mycelium on the agar.

according to those reported by Schultz et al. (*54*, *55*). The compounds assayed showed an inhibitory effect against some human and phytophatogenic fungi. The presence of compounds **1**−**23** has already been described in many botanical families, and it is well-established that these compounds have different biological properties. Nonetheless, to the best of our knowledge, until now, the fungitoxic and bactericidal activities of extracts from this plant have not been described completely in the literature. Among the above-mentioned compounds, only compounds **3**, **4**, **6**, **7**, and **13** showed a significant fungal inhibitory effect in our guided bioassay procedure. The results show that the activities of **6** and **7** could be explained in view of their liposolubilities since they have an activity higher than **13** that

Table 6. Antifungal Bioassay of the Compounds **6** and **7** and Evaluation of FC_{50} and MFC[a]

compounds	μg/mL					
	6		**7**		ketoconazole	
fungi	FC_{50}	MFC	FC_{50}	MFC	FC_{50}	MFC
R. solani	62.5	125	125	250	12.5	25
F. sporotrichum	125	250	125	250	7.5	15
F. monoliforme	125	250	125	250	12.5	25
A. niger	125	250	125	250	12.5	25
T. mentagrophytes	62.5	125	62.5	125	5.0	10

[a] MFC is defined as the lowest concentration providing complete inhibition of mycelial growth. The average of three replicates was measured for 14 days after incubation.

has only one methoxyl group at the C-7 position and that the other compounds such as **3**, **4**, **8**, **9**, **11**, or **12** present hydroxyl groups that decrease their liposolubilities. Thus, extract M_2 emerged as the substance responsible for the antibacterial and antifungal activities of Mexican tarragon, *T. lucida*.

In summary, when the antifungal activity was assayed with methoxylated compounds **6** and **7**, the FC_{50} values were five and 10 times higher than those observed with the positive control ketoconazole. This result shows clearly a possible synergistic effect of the coumarin and/or flavonoids composition of the extract M_2, which had not been observed until now. The synergistic effect is one of the most important characteristics exhibited by natural extracts, and this fact increases the efficacy in contrast to that which could be obtained with the equivalent amount of the active constituents alone. The synergistic effect of these compounds is under study.

These results reveal that *T. lucida* extracts act on bacteria and phytophatogenic fungi. This could indicate that this plant can play an important role in food preservation and food preparation and as an excellent food spice.

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