ANTIMICROBIAL ACTIVITY OF EXTRACTS, ESSENTIAL OIL AND METABOLITES OBTAINED FROM *TAGETES MENDOCINA*

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ABSTRACT

Extracts from the Argentinean herb *Tagetes mendocina* Phil. (Asteraceae) were tested for antimicrobial activity against fungi, bacteria and protozoa. The different extracts (PE, DCM, MeOH and EtOAc) and the essential oil displayed activity mainly against dermatophytes, a group of fungi that produces skin infections. The bioassay-guided fractionation of PE, DCM and EtOAc extracts led to the isolation of eight compounds including two thiophenes, two acetophenone derivatives, dihydrorosefuran and three flavonoids. The thiophenes 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH) (**1**) and its acetate (BBTOAc) (**2**) proved to be highly active against the dermatophytes *Microsporum gypseum*, *Trichophyton rubrum* and *T. mentagrophytes* with MIC values of 3.12, 3.12 and 6.25 µg/mL for compound **1** and 25, 3.12 and 6.25 µg/mL for compound **2**, respectively. In turn, the essential oil displayed a weak to moderate antifungal effect against *M. gypseum* with MIC = 250 µg/mL. The essential oil was examined by GC and GC-MS and 21 constituents (97.3%) were identified. (*E*) β-ocimene, (*Z*)-tagetone, (*E*)-tagetone, (*Z*)-ocimenone, α-pinene and (*E*)-ocimenone were the major components. In addition, thiophenes **1** and **2** and 2-hydroxyacetophenone **3** showed antiprotozoal activity against *Leishmania amazonensis*, *L. brasiliensis* and *L. infantum* promastigotes with 100% lysis at 100 µg/mL.

Keywords: *Tagetes mendocina*, antimicrobial activity, essential oil analysis, dermatophytes, *Leishmania* spp., thiophenes

INTRODUCTION

The genus *Tagetes* (Asteraceae) comprises 12 species in Argentina, ranging from the northwestern part of the country to the southwest in the province of San Juan^{1,2}. Some *Tagetes* species are used as medicinal plants, including *Tagetes mendocina* Phil. (Asteraceae), “Quinchihue” which is recommended in infusion as a diuretic in Argentina³. Previous work on Argentinean *Tagetes* spp. reported the chemotaxonomy, distribution, and free radical scavenging activities^{4,5,6}. Some studies on the antimicrobial effect of several Argentinean *Tagetes* spp. has been published previously^{7,8,9}.

In a screening undertaken by our group, we found that the petroleum ether (PE) and dichloromethane (DCM) extracts of a *T. mendocina* sample collected in the San Juan province was active against dermatophytes, suggesting a great potential of the plant constituents, as antimicrobial agents. In this work the effects of extracts, metabolites and essential oil of *T. mendocina* against selected yeasts, hialohyphomycetes and dermatophytes as well as Gram (+) and Gram (-) bacteria are reported. In addition, the effect of extracts and compounds against the protozoa *Leishmania* spp. is presented for first time.

EXPERIMENTAL

Plant material

The plant *Tagetes mendocina* Phil. (Asteraceae), vernacular name “Quinchihue” was collected in January 2005 in the Bauchaceta district, San Juan province (Argentina), with assistance of the local traditional healer Rodolfo Cabello. The plant material was identified by L. Ariza Espinar at Instituto Multidisciplinario de Biología Vegetal (IMBIV) CONICET, Universidad Nacional de Córdoba, Argentina and a voucher herbarium specimen (BT5) has been kept at the same Instituto.

Isolation and chemical characterization of essential oil

The aerial parts of *T. mendocina* (500 g) was hydrodistilled for 4 hours in a Clevenger-type apparatus resulting in essential oil yield of 0.66% (3.3 mL). δ^{25} : 0.942 µg/mL, n_D^{25} : 1.499 and $[\alpha]_D^{25}$: -2.70 (in CHCl₃, c= 0.074). The essential oil was dried over anhydrous sodium sulfate and stored at -18°C in the dark for antimicrobial assays and chemical analyses.

GC analyses of essential oil were performed on a Shimadzu GC-R1A (FID) gas-chromatograph, fitted with a fused silica capillary column (30 m x

0.25 i.d. mm; coating thickness 0.25 µm film) coated with a 5% phenyl 95% dimethylpolysiloxane (non polar DB-5) phase. The GC operating conditions were as follows: Oven temperature was programmed from 40°C to 230°C at 2°C/min; injector and detector temperatures were set at 240°C. The carrier gas was nitrogen at a constant flow of 0.9 mL/min. Identification of the components was performed by comparison of their retention index (RI) with reference to a homologous series of *n*-alkanes (C₉-C₂₅)¹⁰ and with those of pure authentic samples. GC-MS analyses were performed with a Perkin Elmer Q-700 spectrometer equipped with an apolar fused silica capillary column coated with a phase 5% phenyl 95% dimethylpolysiloxane (DB-5) (30 m x 0.25 i.d. mm; coating thickness 0.25 µm film). The analytical conditions were: oven temperature from 40°C to 230°C at 2°C/min, the carrier gas was helium at a constant flow of 0.9 mL/min, the ion source was maintained at 70 eV. The oil components were identified by using two computer MS libraries (Wiley and Nist) using retention indices as a pre-selection routine, visual inspection of the mass spectra from literature for confirmation¹¹ and co-injection with authentic compounds.

Preparation of extracts from aerial parts: A second representative sample of the air-dried plant material (2.0 kg) was successively extracted at room temperature (3 x 96 h) with petroleum ether (PE), dichloromethane (DCM) and methanol (MeOH). After extraction, each extract was evaporated to dryness to give semisolid residues to afford a PE, DCM and MeOH extracts. The w/w yields in terms of dry starting material for the PE, DCM and MeOH extract were 1.28% (22.56 g), 0.59% (11.86 g) and 6.0% (120.17 g) respectively.

Hydrolate EtOAc extract: The remaining hydrolate, after essential oil hydrodistilled, was extracted with ethyl acetate (EtOAc; 3 x 500 mL). EtOAc phase was dried over anhydrous Na₂SO₄, filtered and taken to dryness under reduced pressure to afford 3.5 g of hydrolate EtOAc extract (HEtOAc) with a w/w yield of 0.7%.

Isolation of antimicrobial compounds

The PE and DCM extracts and the HEtOAc, presenting antifungal activity with MICs ≤ 250 µg/mL against at least one microbial isolate, were further investigated for antifungal compounds.

The PE extract (15 g) was applied onto a column (column length 70 cm, diameter 5 cm) containing 500 g silica gel (0.063-0.2 mesh, Merck 60) and eluted with a gradient of PE, PE:EtOAc to EtOAc affording 130 fractions of 150 mL each. After TLC comparison (silica gel, PE:EtOAc 95:5:0.5), fractions

with similar TLC patterns were combined. The following groups of fractions were obtained: H1 (475 mg), H2 (18 mg), H3 (6 mg), H4 (2409 mg), H5 (261 mg), H6 (151 mg), H7 (81 mg), H8 (108 mg), H9 (151 mg), H10 (80 mg), H11 (65 mg), H12 (60 mg), H13 (187 mg), H14 (91 mg), H15 (847 mg), H16 (635 mg), H17 (217 mg), H18 (108 mg) and H19 (346 mg). The antifungal activity was found mainly in fractions H13, H16 and H17. After column chromatography of H13 (187 mg), 5 mg of thiophene **2** was obtained. The pooled fractions 16-17 afforded 50 mg of β -sitosterol, after permeation on Sephadex LH-20 (column length 26 cm, diameter 2.5 cm, equilibrated with PE:CHCl₃:MeOH 2:1:1).

The DCM extract (7.5 g) was permeated on a Sephadex LH-20 column (column length 33 cm, diameter 5.5 cm, equilibrated with MeOH). Twenty five fractions of 35 mL each were obtained. After TLC comparison (silica gel, PE:EtOAc 95.5:0.5), fractions with similar TLC patterns were combined. The following groups of fractions were obtained: 1 (3 mg, fractions 1-9), 2 (1 g, fractions 10-16), 3 (0.7 g, fractions 17-21), 4 (3 mg, fractions 22-25).

Some 300 mg of the fraction pool 3 was chromatographed on a silica gel column (0.063-0.2 mesh, Merck 60; column length 50 cm, diameter 3.5 cm) and eluted with a PE-PE:EtOAc gradient to obtain 432 fractions of 5 mL each. After TLC comparison, fractions with similar TC patterns were pooled yielding 3 mg thiophene **1**, 5 mg thiophene **2** and 15 mg of 4-hydroxyacetophenone **4**. The EtOAc extract (756 mg) was applied onto a silica gel (60 g) column (column length 55 cm, diameter 2 cm) and eluted with DCM:EtOAc to EtOAc gradient, followed by MeOH. Two hundred fractions of 15 mL each were obtained. Fractions with similar TLC patterns were combined to get 45 mg of **3** and 51 mg of compound **5**. From the MeOH extract, the flavonoids patuletin **6**, quercetagenin 7-*O*- β -D-glucoside (**7**) and axillarin 7-*O*- β -D-glucoside (**8**) were obtained.

General experimental procedures

Melting points were determined on a Kofler hot stage apparatus (Electrothermal 9100). IR spectra were recorded on a Nicolet Nexus FT-IR instrument. Mass spectra were measured on a MAT 95XP, Thermo Finnigan operating at 70 eV. ¹H-NMR spectra were recorded at 400 MHz and ¹³C-NMR data were obtained at 100 MHz on a Bruker spectrometer (δ scale). TLC spots were visualized by spraying the chromatograms with *p*-anisaldehyde:acetic acid:H₂SO₄:EtOH (1.5:20:10:170) and heating at 110°C for 1 min. Column chromatography was performed over Merck Kieselgel 60, particle size 0.063-0.200 mm.

Antimicrobial activity evaluation

Microorganisms For the antimicrobial evaluation, the strains detailed below were used. Bacteria: *Staphylococcus aureus* methicillin-sensitive ATCC 29213, *Staphylococcus aureus* methicillin-resistant ATCC 43300, *Escherichia coli* ATCC 25922, *Escherichia coli* LM 1 (LM: Laboratorio de Microbiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina), *Escherichia coli* LM 2, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* PI (PI: Pasteur Institute), *Salmonella enteritidis* MI (MI: Malbrán Institute), and *Salmonella* sp. LM. Fungi: *Candida albicans* ATCC 10231, *Candida tropicalis* C 131 (C: Centro de Referencia en Micología, FCByF, UNR), *Saccharomyces cerevisiae* ATCC 9763, *Cryptococcus neoformans* ATCC 32264, *Aspergillus flavus* ATCC 9170, *Aspergillus fumigatus* ATCC 26934, *Aspergillus niger* ATCC 9029, *Trichophyton rubrum* C 110, *Trichophyton mentagrophytes* ATCC 9972 and *Microsporium gypseum* C 115. Parasites: The following protozoa from the Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, Paraguay, were used: *Leishmania amazonensis* (IFLA/BR/67/PH8), *L. brasiliensis* (MHOM/BR/75/M2903) promastigotes and *L. infantum* (2259).

Antibacterial activity

Mueller Hinton broth was inoculated with cultures of less than 30 h old and incubated at 37°C (3 h) to prepare the inoculum, the culture was adjusted in a spectrophotometer with sterile physiological solution to give a final organism density of 0.5 Mc Farland scale (1-5 x 10⁵ CFU/mL)^{12,13}. The antibacterial activity was evaluated with the agar dilution method using Mueller Hinton agar medium for Gram (+) and Gram (-) bacteria. Stock solutions of the compounds/extracts in DMSO were diluted to give serial two-fold dilutions that were added to each medium resulting in concentrations ranging from 1000 to 10 μ g/mL. The final concentration of DMSO in the assay did not exceed 1%. The antimicrobial agent Cefotaxime (Argentia Pharmaceutica) was included in the assays as positive control. The plates were incubated for 24 h at 37°C in static conditions. Minimum Inhibitory Concentration (MIC) was defined as the lowest compounds/extracts concentration showing no visible bacterial growth after incubation time. Tests were done in triplicate. Extracts and compounds with MICs $\leq$ 250 μ g/mL were considered of interest¹⁴.

Antifungal activity

The MIC of each extract/compound was determined by using broth microdilution techniques in 96 wells microtiter plates as described by the Clinical and Laboratory Standards Institute (CLSI) formerly National Committee for Clinical and Laboratory Standards (NCCLS) for yeasts (M27-A2) as well as for filamentous fungi (M 38 A)¹⁵. MIC values were determined in RPMI-1640 medium (Sigma, St. Louis, MO, USA) buffered to a pH 7.0 with morpholinepropanesulfonic acid (MOPS) at 0.165 M.

The inocula were adjusted to 1-5 x 10³ CFU/mL following the guidelines of CLSI (formerly NCCLS). Microtiter trays were incubated at 35°C for yeasts and hialophomycetes and at 28-30°C for dermatophyte strains in a moist, dark chamber. For the assay, stock solutions were two-fold diluted with RPMI from 1000 to 0.98 μ g/mL (final volume = 100 μ L) and a final DMSO concentration $\leq$ 1%. A volume of 100 μ L of inoculum suspension was added to each well with the exception of the sterility control where sterile water was added to the well instead. The time of incubation was the same of that of the control fungus growth (a well containing culture medium and fungal inoculum, but devoid of extract or compound). MIC was defined as the minimum inhibitory concentration of the extract/compound which resulted in total inhibition of the fungal visible growth. Ketoconazole, terbinafine and amphotericin B were used as positive controls. MICs $\leq$ 250 μ g/mL were considered of interest¹⁴.

Leishmanicidal activity

Promastigote inhibition studies were performed on *Leishmania amazonensis* (IFLA/BR/67/PH8), *L. brasiliensis* (MHOM/BR/75/M2903) and *L. infantum* (2259) grown at 22°C in Schneider's *Drosophila* medium containing 20% foetal bovine serum. Promastigote cultures in the logarithmic phase were transferred at a concentration of 10⁶ cells/mL. Compounds (1 mg) were dissolved in 40 μ L DMSO and added to 1 mL of the medium from which aliquots were drawn. 200 μ L from this stock solution were dissolved in 800 μ L of medium. A 100 μ L sample of this second solution was mixed with 100 μ L of parasite culture reaching a compound concentration of 100 μ g/mL and 0.4% of DMSO. The solution was placed in microtiter plates. All assays were carried out in triplicate. The activity of the compounds was evaluated after 72 h by observation of a drop of each culture with a microscope and compared with control cells. Assays to assess the drug concentration required to inhibit parasite growth were performed in triplicate¹⁶. Amphotericin B and pentamidine were used as reference compounds.

RESULTS AND DISCUSSION

From the PE, DCM and EtOAc extracts, 5 compounds (Figure 1) were isolated and identified as 5-(4-hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH, compound **1**), 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc, compound **2**), 2-hydroxyacetophenone (**3**), *p*-hydroxyacetophenone (**4**) and dihydrorosefuran (**5**). The spectroscopic data of the isolated compounds is in agreement with literature¹⁷.

5-(4-Hydroxy-1-butynyl)-2,2'-bithienyl (BBTOH) (**1**): colorless oil. HRMS *m/z* (rel. int.%): 234.340 (calc. for C₁₂H₁₀OS₂: 234.017).

5-(4-Acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) (**2**): colorless oil. MS *m/z* (rel. int.%): 276 (13) [M]⁺ (C₁₄H₁₂O₂S₂), 217 (15), 216 (100), 203 (14), 43 (21).

2 β -(3-methylbut-2-enyl)-4-methyl-2,5-dihydrofuran (Dihydorosefuran) (**5**): colorless oil. MS *m/z* (rel.int.%): 152 [M]⁺ (C₁₀H₁₆O) (8), 137 [M-Me]⁺ (15), 112 [M-Me]⁺ (24), 83 [M-C₃H₉]⁺ (20), 69 [C₅H₉]⁺ (100). ¹H-NMR: 5.66 t (8) (H-3), 5.13 t (8) (H-4), 4.03 t (8) (H-5), 4.21 dd (H-6), 2.24 m (H-7), 1.75 s (H-8), 1.66 s (H-9), 1.69 s (H-10). ¹³C-NMR: 140.427 s (C-1), 135.300 s (C-2), 124.523 d (C-3), 119.737 d (C-4), 76.382 d (C-5), 59.137 t (C-6), 34.202 t (C-7), 25.926 q (C-8), 18.027 q (C-9), 12.218 q (C-10). [α _D]₂₀: -2.2, (c=0.09; CHCl₃).

Figure 1. Compounds isolated from *Tagetes mendocina*

1: 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOH); **2:** 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc); **3:** 2-hydroxyacetophenone; **4:** 4-hydroxyacetophenone; **5:** dihydrorosefuran; **6:** patuletin; **7:** quercetagenin 7-*O*- β -D-glucoside; **8:** axillarin 7-*O*- β -D-glucoside

The thiophenes **1** and **2** were reported as constituents of *Tagetes* spp., while dihydrorosefuran was isolated from *Artemisia pallens*^{17,18}.

The composition and quantification of thiophenes of *T. minuta* collected in Mendoza province (Argentina) was reported, the main thiophene identified was BBTOH¹⁹. In our *T. mendocina* sample, the main thiophenes were BBTOH and BBTOAc, confirming the trend observed in other species belonging to the genus.

From the MeOH extract, the three flavonoids **6-8**, previously isolated from this species, were obtained⁶. The light green essential oil of *T. mendocina* was obtained in a yield of 0.6 mL/100 g of fresh aerial parts. Results of the GC and GC-MS analysis of the essential oil are shown in Table 1, where the chemical components are listed in the order of elution of the peaks. Some 21 constituents were identified representing 97.3% of the total. The major constituents (concentrations higher than 3.5%) were (*E*) β -ocimene (28.6%), (*Z*)-tagetone (28.0%), (*E*)-tagetone (11.7%), (*Z*)-ocimenone (7.4%), α -pinene (5.5%) and (*E*)-ocimenone (4.7%).

Table 1. Chemical composition of *Tagetes mendocina* essential oil

Components	Retention Index ^a	Composition (%) ^b	Methods of identification
α pinene	939	5.5	Ms ₁ , Co
sabinene	975	3.7	Ms ₂
β pinene	979	1.0	Ms ₁ , Co
δ 2 carene	1002	tr	Ms ₂
α terpinene	1017	1.1	Ms ₁ , Co
β phellandrene	1030	tr	Ms ₂
(<i>Z</i>) β ocimene	1037	tr	Ms ₂
(<i>E</i>) β ocimene	1050	28.6	Ms ₁ , Co
dihydrotagetone	1053	2.7	Ms ₁ , Co
γ terpinene	1060	tr	Ms ₂
terpinolene	1089	0.5	Ms ₂
(<i>E</i>)- tagetone	1144	11.7	Ms ₁ , Co
myrcenone	1150	0.2	Ms ₁ , Co
(<i>Z</i>)- tagetone	1152	28.0	Ms ₁ , Co
citronellal	1153	tr	Ms ₂
isoborneol	1162	1.2	Ms ₁ , Co
borneol	1169	0.5	Ms ₁ , Co
dihydrocitronellol	1196	1.8	Ms ₂
(<i>Z</i>) ocimenone	1229	7.3	Ms ₁ , Co
(<i>E</i>) ocimenone	1238	4.7	Ms ₁ , Co
geranial	1267	0.1	Ms ₁ , Co

Ms₁: identical with those of pure reference compounds. Ms₂: identical with published data. Co: co injection authentic compounds; a: retention indices relative to homologous alkanes; b: Percentages were calculated from the peak area without correction; tr: trace < 0.1%.

Antimicrobial activity

The antimicrobial activity of the extracts, essential oil and compounds isolated from *T. mendocina* was assessed *in vitro* against several yeasts, filamentous fungi, dermatophytes, Gram positive, Gram negative bacteria and protozoa. Results are showed in Table 2.

Regarding antifungal activity, the essential oil of *T. mendocina* was active against all dermatophytes assayed, being *Microsporum gypseum* the most susceptible species (MIC = 250 µg/mL). On the other hand PE, DCM and HEtOAc extracts were active against the dermatophytes *M. gypseum*, *T. mentagrophytes* and *T. rubrum*. The best effect was elicited by the HEtOAc extract towards *T. mentagrophytes* with a MIC value of 125 µg/mL and DCM extracts with MICs 250 µg/mL (Table 2). None of the extracts from *T. mendocina* was active towards the yeasts *C. albicans*, *C. tropicalis*, *C. neoformans* and *S. cerevisiae* or the filamentous fungi *A. flavus*, *A. fumigatus* and *A. niger* with MIC values >1000 µg/mL (data not shown).

From PE and DCM *T. mendocina* extracts, two antifungal constituents were isolated. Compound **1** showed strong activity against the dermatophytes with MICs values of 3.12, 3.12 and 6.25 µg/mL while for compound **2**, MIC values were 25, 3.12 and 6.25 µg/mL towards *M. gypseum*, *T. rubrum* and *T. mentagrophytes*, respectively. The activity is considered very interesting in the case of MICs < 100 µg/mL for extracts and 10 µg/mL for isolated compounds²⁰.

Thiophene **1** was previously reported as a constituent of *Tagetes*, *Porophyllum* and *Echinops* species, while the corresponding acetate **2** was isolated from species of the genera *Berkheya*, *Dyssodia*, *Echinops* and *Tagetes*²¹. The activity of thiophenes against the dermatophytes *T. mentagrophytes* and *T. rubrum* is of interest considering that these two fungi are responsible for approximately 80-93% of chronic and recurrent non-scalp skin infections in humans²². *T. mentagrophytes* and *T. rubrum* are the ethiological agents of tinea unguium, producer of invasive nail infections, tinea manuum (infections of palmar and interdigital areas of the hand) and tinea pedis (athlete's foot), the last one being the most prevalent fungal infection all over the world²³. *T. rubrum* is also the most frequent ethiological agent of tinea cruris, which affect the perianal and perineal areas of adults producing highly contagious bilateral erythematous lesions²⁴.

The antifungal effect of *T. patula* methanolic extracts against phytopathogenic fungi was enhanced after UV light exposition²⁵. This is surely due to its thiophene constituents, which become more active after light activation. The mechanism of action of α -terthienyl, a related thiophene, was previously reported. This compound, under UV light, promotes singlet O₂ generation, which is extremely toxic for fungal membranes^{26,27,28}.

Regarding antiprotozoal effect, the DCM extract of *T. mendocina* at 100 µg/mL completely lysed *Leishmania amazonensis* and *L. brasiliensis* promastigotes. The same effect was observed with the compounds **1**, **2** and **3** (Table 2).

Table 2 Antimicrobial activity of extracts, compounds an essential oil from *Tagetes mendocina* (MIC, µg/mL).

Microorganism	PE	DCM	MeOH	HEtOAc	EO	1	2	3	5	Amp B	Pen	Ket	Terb	Cef
Fungus														
<i>Microsporum gypseum</i> C 115 2000	250	250	>1000	250	250	3.12	25	250	250	0.125	-	0.05	0.04	
<i>Trichophyton rubrum</i> C 113 2000	500	250	>1000	250	500	3.12	3.12	250	250	0.075	-	0.025	0.025	
<i>Trichophyton mentagrophytes</i> ATCC 9972	250	250	>1000	125	500	6.25	6.25	250	250	0.075	-	0.025	0.04	
Bacteria														
<i>Staphylococcus aureus</i> methicillin-sensitive ATCC 29213	>1000	>1000	>1000	1000	1000	-	-	-	-	-	-	-	-	0.5
<i>Staphylococcus aureus</i> methicillin-resistant ATCC 43300	>1000	>1000	1000	1000	500	-	-	-	-	-	-	-	-	0.5
<i>Escherichia coli</i> ATCC 25922	>1000	1000	1000	1000	1000	-	-	-	-	-	-	-	-	0.5
LM- <i>Escherichia coli</i>	1000	1000	>1000	500	1000	-	-	-	-	-	-	-	-	5
LM- <i>Escherichia coli</i>	>1000	1000	>1000	1000	750	-	-	-	-	-	-	-	-	0.5
<i>Pseudomonas aeruginosa</i> ATCC 27853	1000	1000	1000	1000	500	-	-	-	-	-	-	-	-	7.5
PI- <i>Yersinia enterocolitica</i>	>1000	1000	1000	500	500	-	-	-	-	-	-	-	-	0.5
MI- <i>Salmonella enteritidis</i>	1000	1000	1000	1000	750	-	-	-	-	-	-	-	-	12.5
LM- <i>Salmonella</i> sp	>1000	1000	>1000	500	750	-	-	-	-	-	-	-	-	0.5
Protozoa (lysis a 100µg/mL)														
<i>Leishmania amazonensis</i> PH8	<90	100	<70	<90	<70	100	100	100	<70	100	100	-	-	
<i>Leishmania brasiliensis</i> 2903	100	100	<70	<90	<70	100	100	100	<70	100	100	-	-	
<i>Leishmania infantum</i> 2259	Nt	Nt	Nt	Nt	Nd	100	100	100	<70	100	100	-	-	

Amp B: Amphotericin B, Pen: Penicillin, Ket: Ketoconazole, Terb: Terbinafine, Cef: Cefotaxime, - : Not tested. Extracts: PE: petroleum ether; DCM: Dichloromethane; MeOH: methanol; HEtOAc: hydrolate ethyl acetate; EO: essential oil. Compounds: **1**: 5-(4-hydroxy-1-butynyl)-2,2-bithienyl (BBTOH); **2**: 5-(4-acetoxy-1-butynyl)-2,2-bithienyl (BBTOAc); **3**: 2-hydroxyacetophenone; **5**: dihydroterosefuran.

Moreover, related to antibacterial activity, the extracts PE, DCM, MeOH, EO and HEtOAc tested, showed less activity against a panel of bacteria assayed with MIC between 500-1000 µg/mL.

The antifungal effect, the identification of the active thiophenes BBTOH and BBTOAc as well as the chemical composition of essential oil of *T. mendocina* collected in San Juan province, Argentine are reported for the first time.

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