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# Trypanocidal and antifungal activities of *p*-hydroxyacetophenone derivatives from *Calea uniflora* (Heliantheae, Asteraceae)

Andréa Mendes do Nascimento, Marcos José Salvador, Regina Celia Candido, Sérgio de Albuquerque and Dionéia C. R. de Oliveira

## Abstract

The dichloromethane extract of underground parts of *Calea uniflora* (Heliantheae, Asteraceae) exhibited trypanocidal and antifungal activities. Four *p*-hydroxyacetophenone derivatives were isolated as the main compounds: 2-senecioyl-4-(hydroxyethyl)-phenol (**1**), 2-senecioyl-4-(angeloyloxyethyl)-phenol (**2**), and two new derivatives, 2-senecioyl-4-(methoxyethyl)-phenol (**3**) and 2-senecioyl-4-(pentadecanoyloxyethyl)-phenol (**4**). **1** and **4** were active towards *Trypanosoma cruzi* trypomastigotes, reducing their number by 70 and 71% at 500 $\mu$g mL$^{-1}$, whereas **2** and **3** were inactive. All the compounds tested showed antifungal activity with minimal inhibitory concentration values between 500 and 1000 $\mu$g mL$^{-1}$ against pathogenic *Candida* spp. and dermatophytes. The isolation, structure elucidation, NMR spectral assignments and bioactivity results of these compounds are reported.

Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n, 14040-903, Ribeirão Preto, SP, Brazil

Andréa Mendes do Nascimento, Dionéia C. R. de Oliveira

Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida dos Bandeirantes 3900, 14040-901, Ribeirão Preto, SP, Brazil

Marcos José Salvador

Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n, 14040-903, Ribeirão Preto, SP, Brazil

Regina Celia Candido, Sérgio de Albuquerque

**Correspondence:** Dionéia C. R. de Oliveira, Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n, 14040-903, Ribeirão Preto, SP, Brazil. E-mail: drolivei@usp.br

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## Introduction

The chemical and biological investigation of plants for use in the treatment of numerous human diseases has led to the search for their active principles (Ho et al 2001). Human infections constitute a serious problem and microorganisms such as parasites, fungi, bacteria and viruses are the most frequent pathogens described as etiological agents of infectious diseases. Thus, interest in research into antimicrobial substances has intensified (Portillo et al 2001; Salvador et al 2002).

Several antimycotic drugs are available at present because of the development of opportunistic and superficial mycoses, such as candidiasis, cryptococcosis and aspergillosis, especially in immunocompromised patients. The antifungal agents currently used in therapeutics are toxic, with low potency and poor solubility, and they are not always effective because of the emergence of resistant strains (Candido et al 1998; Penna et al 2001). The discovery of new safer and more effective antifungal agents is therefore necessary.

Chagas' disease occurs in Latin America, affecting 16–18 million people, with more than 100 million exposed to the risk of infection (WHO 2000). Its etiological agent is *Trypanosoma cruzi*, a flagellate protozoan transmitted by triatomine insects and blood transfusion. The prevention of infection by blood transfusion has been attempted through the clinical and serologic screening of blood donors (Araya et al 2003). Gentian violet is used such as a chemoprophylatic agent in blood banks, however it confers a blue colour to the recipients and its use has been restricted in endemic areas (Ribeiro et al 1997; Araya et al 2003). Because of the high costs involved in the development and registration of new drugs for the treatment of tropical diseases, there is an urgent need to support novel research on natural products with trypanocidal activity (WHO 2000).

*Calea uniflora* Less is a plant belonging to the tribe Heliantheae or sunflower, family Asteraceae (Karis & Ryding 1994). The genus contains about 110 species, which occur at mid-elevations in both tropical and subtropical regions of the New World (Pruski & Urbatsch 1988). The literature reports the antifungal activity of essential oil from

*C. clematidea* and its major isolated compound clemateol (Flach et al 2002). Other biological activities, such as anti-inflammatory, cytotoxic, larvicidal, antiplasmodial and antihypertensive actions, have also been reported for the genus *Calea* (Vichnewski et al 1982; Cerain et al 1996; Bork et al 1997; Köhler et al 2002; Guerrero et al 2002). In the preliminary evaluation of *C. uniflora*, the crude dichloromethane extract (dry, underground parts) showed trypanocidal and antifungal activities. In this work we report the isolation and structure elucidation of two known (**1** and **2**) and two novel (**3** and **4**) *p*-hydroxy-acetophenone derivatives, and present the results of the evaluation of the in-vitro trypanocidal and antifungal activities of these compounds and the crude extract of *C. uniflora*.

## Materials and Methods

### Plant material

*C. uniflora* Less was collected in March 1997 in the Washington Luis highway, 1 km from Posto Castelo. The plant was identified by Dr Jose L. Panero of the Department of Botany, University of Texas, and a voucher specimen (SPFR 04003) was deposited at the herbarium of the Department of Biology, FFCLRP/USP, Ribeirão Preto, Brazil.

### Extraction and isolation

Dried and powdered underground parts (200 g) were exhaustively extracted with dichloromethane at room temperature, affording 4.2 g of crude extract. The crude extract was chromatographed over a silica gel column eluted with hexane, hexane/ethyl acetate (gradient), ethyl acetate/methanol (gradient) and finally methanol, yielding 14 fractions. Some 11 mg of **3** were purified from fractions 12–13. Fractions 3, 4 and 5 were purified over silica gel followed by preparative TLC affording 72 mg of **1**, 4 mg of **2** and 19 mg of **4**. The structures of all compounds were determined by spectrometric methods (IR, EI-MS, $^{1}$H and $^{13}$C NMR) and carbon attributions were made by HMBC and HMQC correlation spectra.

### Trypanocidal activity in vitro

The bioassays were carried out using the blood of Swiss albino mice in the parasitemy peak (seventh day; $2 \times 10^6$ trypomastigote forms mL$^{-1}$) after infection with the Y strain of *T. cruzi* (Bastos et al 1999). The blood was obtained by cardiac puncture using heparin as anticoagulant in a 7:3 blood/anticoagulant ratio. Crude extract was mixed in microtitre plates (96 wells) with DMSO and infected blood to give a final concentration of 4 mg mL$^{-1}$. Stock solutions of the compounds to be tested were prepared by dissolution of 1 mg of each in 25 $\mu$L of DMSO and 225 $\mu$L of saline (NaCl 0.9%). To each sample compound aliquots of the stock solutions were mixed with the diluted blood in such quantities as to give final concentrations of 100, 250 and 500 $\mu$g mL$^{-1}$. Controls were the blood of infected mice without any addition, infected blood containing DMSO in equivalent amounts as in the samples and infected blood containing gentian violet (positive control) at a concentration of 250 $\mu$g mL$^{-1}$. The plates were kept at 4 °C for 24 h and the number of parasites determined according to Brener (1962). The bioassays were performed in triplicate. Results are shown as lysis percentages of *T. cruzi* trypomastigote forms, which represent the trypanocidal activity. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of São Paulo University.

### Antifungal activity in vitro

Antifungal activity was measured using a modified agar-well diffusion method according to Matar et al (2003), Okeke et al (2001), Pujol et al (1996), Espinel-Ingroff et al (1995) and Grove and Randall (1955). The test fungi strains were inoculated into RPMI-1640 Medium (Sigma) with MOPS (USB) buffer solutions in agar glucosed plates, containing an inoculum size of $10^6$ cfu mL$^{-1}$ (0.5 McFarland scale). Subsequently, 20 $\mu$L aliquots of each test-drug solution were applied to 5.0 mm diameter wells (duplicate wells for each test-drug solution). The crude extract and the isolated compounds **1**–**4** were dissolved in propyleneglycol/RPMI-1640 (1:9) to yield concentrations of 1000 $\mu$g mL$^{-1}$ for extracts and pure compounds in the screening. After incubation at 37 °C for 48 h for yeast strains and at 30 °C for 6 days for the dermatophytes, the diameter of the zone of inhibition was measured in millimetres. In the tests, ketoconazole (500 $\mu$g mL$^{-1}$) was used as experimental positive control for microorganism strains and propyleneglycol/RPMI-1640 (1:9) as negative control. The minimal inhibitory concentration (MIC) was determined for each isolated compound in $\mu$g mL$^{-1}$, evaluated in the range of 50 and 1000 $\mu$g mL$^{-1}$ (Tereschuk et al 1997; Okeke et al 2001; Salvador et al 2002). All experiments involved the use of at least two plates for each test and the results are presented as mean values. A total of four American-type culture collection (ATCC) standard strains (*Candida albicans* ATCC 64548, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 22019 and *C. glabrata* ATCC 90030) and four field strains (*Trichophyton rubrum* Tr-5 and Tr-19, and *T. mentagrophytes* Tr-9 and Tr-17) were used. The microorganisms were obtained from stock cultures of the mycology laboratory of the Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

### Statistical analysis

Results of trypanocidal activity are presented as the mean (coefficient of variation, CV (%) = (s/m) × 100; where s = standard deviation, m = mean). Statistical significance was determined by Kruskal–Wallis' test, with the level of

**Table 1** $^{1}$H NMR data of compounds **1, 2, 3** and **4** ($CDCl_3$, $\delta$ values).

| H | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 3 | 7.78 d (2.2) | 7.76 d (2.3) | 7.62 d (2.0) | 7.66 d (2.1) |
| 5 | 7.43 dd (2.2, 8.6) | 7.49 dd (2.3, 8.6) | 7.32 dd (2.0, 8.6) | 7.39 dd (2.1, 8.6) |
| 6 | 6.95 d (8.6) | 6.98 d (8.6) | 6.90 d (8.6) | 6.89 d (8.6) |
| 7 | 4.87 q (6.5) | 5.92 q (6.6) | 4.19 q (6.3) | 5.78 q (6.6) |
| 8 | 1.49 d (6.5) | 1.58 d (6.6) | 1.36 d (6.3) | 1.46 d (6.6) |
| 10 | 6.81 m | 6.78 s | 6.74 m | 6.71 m |
| 12 | 2.06 d (1.1) | 2.06 d (1.0) | 1.99 d (1.2) | 1.99 d (1.2) |
| 13 | 2.21 d (1.1) | 2.21 d (0.8) | 2.15 d (1.2) | 2.14 d (1.2) |
| $OCH_3$ | – | – | 3.15 s | – |
| OCOR | – | 6.07 qq (1.5, 7.2) | – | 2.22 dt |
| | – | 1.97 qd (1.5, 7.3) | – | 1.51 m, 1.18 ls |
| | – | 1.91 m | – | 0.81 t (7.2) |
| OH-1 | 12.78 s | 12.79 s | 12.72 s | 12.74 s |

significance set at $P < 0.05$ (Kruskal & Wallis 1952). In antifungal activity all experiments involved the use of at least two plates for each test (n = 4 wells for each test drug). The results are presented as the mean value of inhibition (CV%). Comparison of the MIC values of all test drugs were performed using Kruskal–Wallis' test, $P < 0.05$ denoted significance. Individual differences between the test drugs were statistically examined using a post-hoc test (Tukey's test) with the level of significance set at $P < 0.05$ and chemometric analysis (cluster analysis single linkage method and principal components analysis). In these analyses the diameter of inhibition values of test drugs and positive control were used as otus (variables) and microorganism strains as characters (Alves 1986; Hair Jr et al 1995; Bolton 1997; Alcaráz et al 2000).

## Results

From the dichloromethane extract of underground parts of *C. uniflora*, four *p*-hydroxyacetophenone derivatives were isolated. The NMR data for the compounds are presented in Tables 1 and 2, and their structures are shown in Figure 1. Compounds **1** and **2** were respectively identified as 2-senecioyl-4-(hydroxyethyl)-phenol and 2-senecioyl-4-(angeloyloxyethyl)-phenol, previously isolated from *C. cuneifolia* and *C. villosa* by Lourenço et al (1981) and Bohlmann et al (1982). IR, EI-MS and $^{1}$H NMR data for **1** and **2** are in agreement with published data (Bohlmann et al 1978 (**1**), 1981 (**2**)). The $^{13}$C NMR data of both compounds, not reported previously, are of value for reference purposes. Two other molecules were detected for the first time and were denoted 2-senecioyl-4-(methoxyethyl)-phenol (**3**) and 2-senecioyl-4-(pentadecanoyloxyethyl)-phenol (**4**).

Compound **3** was obtained as a yellow gum. The EI spectrum indicated a molecular ion peak at *m/z* 234 compatible with the molecular formula $C_{14}H_{18}O_3$. The IR spectrum revealed important absorption bands at $\nu_{max}$ 3450 (OH), 1640 (C=O) and 1580–1480 $cm^{-1}$ (C=C). The $^{1}$H NMR spectrum (Table 1) showed the presence of a 1,2,4-trisubstituted benzene ring ($\delta$ 7.62 (H-3, d, $J = 2.0$ Hz),

**Table 2** $^{13}$C NMR data of compounds **1, 2, 3** and **4** ($CDCl_3$, $\delta$ values).

| C | 1 (75 MHz) | 2 (100 MHz) | 3 (100 MHz) | 4 (100 MHz) |
|---|---|---|---|---|
| 1 | 162.57 s | 163.29 s | 161.74 s | 162.98 s |
| 2 | 120.20 s | 120.47 s | 119.33 s | 120.07 s |
| 3 | 126.55 d | 128.33 d | 126.27 d | 128.00 d |
| 4 | 135.91 s | 131.32 s | 132.54 s | 131.88 s |
| 5 | 133.13 d | 134.02 d | 132.87 d | 133.71 d |
| 6 | 118.50 d | 119.19 d | 117.58 d | 118.77 d |
| 7 | 69.77 d | 71.94 d | 78.01 d | 71.53 d |
| 8 | 25.18 q | 22.72 q | 22.77 q | 21.97 q |
| 9 | 196.09 s | 196.46 s | 195.13 s | 196.01 s |
| 10 | 119.89 d | 120.31 d | 118.96 d | 119.86 d |
| 11 | 158.35 s | 158.71 s | 157.19 s | 158.49 s |
| 12 | 28.24 q | 28.66 q | 27.23 q | 28.31 q |
| 13 | 21.44 q | 21.85 q | 20.45 q | 21.49 q |
| $OCH_3$ | – | – | 55.33 q | – |
| OCOR | – | 167.67 s | – | 173.17 s |
| | – | 21.01 q | – | 34.63 t |
| | – | 16.20 q | – | 29.11–31.93 t |
| | – | 138.30 d | – | 24.98 t, 22.70 t |
| | – | 132.50 s | – | 14.14 q |

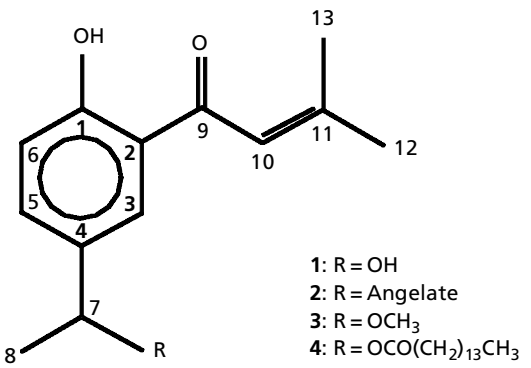


**Figure 1** Structures of compounds **1, 2, 3** and **4**.

7.32 (H-5, dd, $J = 8.6$, 2.0 Hz) and 6.90 (H-6, d, $J = 8.6$ Hz)); an olefinic hydrogen at $\delta$ 6.74 (H-10, m); a methoxy signal at $\delta$ 3.15 (s); two doublets at $\delta$ 2.15 (H-13, $J = 1.2$ Hz) and 1.99 (H-12, $J = 1.2$ Hz), indicative of methyl groups, and another methyl group at $\delta$ 1.36 (H-8, d, $J = 6.3$ Hz) coupled to a quartet signal at $\delta$ 4.19 (H-7, $J = 6.3$ Hz). The $^{13}$C NMR spectrum (Table 2) showed four quartets that resonated at $\delta$ 55.33, 27.23, 22.77 and 20.45, and were assigned to the carbons of the one methoxyl-substituent at C-7 and three methyl groups (C-12, C-8 and C-13, respectively). Six aromatic carbon atoms were also observed at $\delta$ 161.74, 132.87, 132.54, 126.27, 119.33 and 117.58. The signal at $\delta$ 195.13 was attributed to the carbonyl carbon. The structure of **3** was deduced from detailed analysis of the $^{1}$H NMR and $^{13}$C NMR data aided by 2D NMR experiments (HMQC and HMBC). The HMBC spectrum showed the correlation of C-1 ($\delta$ 161.74) with H-3 ($\delta$ 7.62) and H-5 ($\delta$ 7.32); C-2 ($\delta$ 119.33) with H-6 ($\delta$ 6.90); C-4 ($\delta$ 132.54) with H-6 ($\delta$ 6.90) and H-8 ($\delta$ 1.36); and C-11 ($\delta$ 157.19) with H-12 ($\delta$ 1.99) and H-13 ($\delta$ 2.15).

Compound **4** was also obtained as a yellow gum. The EI of **4** corresponded to the molecular formula $C_{28}H_{44}O_4$, with a molecular ion peak at $m/z$ 444. A fragment ion at $m/z$ 220 showed a prominent eliminated product [M—$CH_3(CH_2)_{13}CO_2H$(242)], confirming the presence of 13 methylene groups in R. The IR spectrum exhibited absorption bands due to hydroxyl group, ester, enone and aromatic ring functionalities at $\nu_{max}$ 3500, 1730, 1640 and 1580–1450 cm$^{-1}$, respectively. The $^{1}$H and $^{13}$C NMR data (Tables 1 and 2) of **4** were closely related to those of **3**, having the following main differences: the spectra showed the presence of an additional ester group in **4**; the signals of H-7 ($\delta$ 5.78) and H-8 ($\delta$ 1.46) were deshielded and the C-7 signal ($\delta$ 71.5) shielded in comparison with the signals for **3**. The $^{1}$H and $^{13}$C NMR attributions are consistent with the observations made in the HMQC and HMBC experiments. The correlations observed by HMBC were basically the same for **3** and **4**. The carbonyl carbon of the ester group ($\delta$ 173.17) showed correlation with the methylene group at $\delta$ 2.22.

The results of the trypanocidal studies of these four *p*-hydroxyacetophenone derivatives and crude extract against *T. cruzi* trypomastigotes are shown in Table 3. The assays obtained indicate a marked trypanocidal activity of the dichloromethane extract of underground parts. This crude extract was able to reduce the number of trypomastigotes by 99%. However, only the isolated compounds **1** and **4** exhibited trypanocidal activity, lysing 70 and 71% of the parasites at 500 $\mu$g mL$^{-1}$. This suggests that these compounds contribute to the high activity of crude extract. Gentian violet at 250 $\mu$g mL$^{-1}$ was used as a positive control with 100% lysis of *T. cruzi* tripomastigotes, while the negative controls, mice infected with blood without any added compound and mice infected with blood containing the same DMSO concentration ($\leq$ 2.5%) used in the stock solutions did not show any reduction in parasite numbers.

Additionally, the crude extract and isolated compounds were assayed for antifungal activity (Tables 4 and 5). The results show that all the compounds were bioactives, mainly against the dermatophytes (all the strains of *Trichophyton rubrum* and *T. mentagrophytes*). Only **2** and **3** demonstrated any effect against two yeasts, *Candida albicans* and *C. glabrata*, while **4** was active against *C. albicans*. The ketoconazole (500 $\mu$g mL$^{-1}$), here used as a positive control against all fungi strains assayed, produced a diameter of inhibition zone in the range 18–40 mm (Table 1). The solution containing propyleneglycol/RPMI-1640 (1:9) was used as negative control, in which any inhibitory effect could be observed.

**Table 3** In-vitro trypanocidal activity of crude extract and isolated compounds from *Calea uniflora*.

| Tested material | Trypanocidal activity, % lysis (CV)[a,b,c] | | | |
|---|---|---|---|---|
| | Dose ($\mu$g mL$^{-1}$) | | | |
| | 100 | 250 | 500 | 4000 |
| Extract | – | – | – | 98.8 (1.2) |
| **1** | 18.1 (19.2) | 37.7 (26.6) | 70.3 (12.1) | – |
| **2** | 27.5 (30.8) | 26.8 (20.6) | 36.2 (8.6) | – |
| **3** | 27.5 (10.0) | 28.9 (4.9) | 42.0 (6.5) | – |
| **4** | 8.8 (4.7) | 24.7 (25.7) | 70.9 (13.9) | – |

[a]Gentian violet, the positive control, was used at a 250 $\mu$g mL$^{-1}$ concentration. [b]The controls, mice with infected blood without any added compound and mice with infected blood containing the same DMSO concentration used in the stock solutions, did not show any reduction of the parasite numbers. [c] The activity is expressed as lysis percentages of *T. cruzi* trypomastigote forms (CV%). –, not evaluated in this concentration.

## Discussion

The study of the extracts of plants is a promising alternative for finding active products with pharmacological activity. In the past few decades there has been a movement named 'back to Nature', in which phytotherapy has been revived (Coura & Castro 2002). This trend has been accentuated by the success obtained by taxol in cancer chemotherapy, and among parasitic diseases the examples are quinine and artemisin.

In this study, we first examined the trypanocidal and antifungal effect of the dichloromethane extract of underground parts from *C. uniflora*. The crude extract exhibited higher trypanocidal activity, lysing 99% of the parasites (Table 3). However, the crude extract only showed an inhibition zone for two dermatophytes (*Trichophyton rubrum* Tr-5 and *T. mentagrophytes* Tm-17, Table 4).

Next, we examined the trypanocidal and antifungal effect of the isolated compounds (*p*-hydroxyacetophenone derivatives) from crude extract. The results for activity against *T. cruzi* trypomastigotes demonstrated that two *p*-hydroxyacetophenone derivatives isolated from dichloromethane crude extract of underground parts of *C. uniflora* possess in-vitro trypanocidal properties (Table 3). These results corroborate the trypanocidal effects observed for other

**Table 4** In-vitro antifungal activity of crude extract and isolated compounds from *Calea uniflora*.[a]

| Microorganisms | Extract | 1 | 2 | 3 | 4 | P[d] |
|---|---|---|---|---|---|---|
| *Candida albicans* ATCC 64548[b] | – | – | 7 (0.0) | 8 (1.0) | 7 (0.0) | 40 (2.0) |
| *Candida krusei* ATCC 6258[b] | – | – | – | – | – | 18 (4.6) |
| *Candida parapsilosis* ATCC 22019[b] | – | – | – | – | – | 35 (0.0) |
| *Candida glabrata* ATCC 90030[b] | – | – | 7 (7.1) | 7 (0.0) | – | 22 (2.3) |
| *Trichophyton rubrum* Tr-5[c] | 7 (11.7) | 6 (0.0) | 6 (1.4) | 6 (0.0) | 7 (0.0) | 30 (0.0) |
| *Trichophyton rubrum* Tr-19[c] | – | 7 (11.7) | 6 (0.0) | 7 (7.1) | 7 (1.4) | 30 (2.7) |
| *Trichophyton mentagrophytes* Tm-9[c] | – | 6 (8.3) | 8 (6.3) | 7 (7.1) | 8 (10.2) | 20 (0.0) |
| *Trichophyton mentagrophytes* Tm-17[c] | 6 (8.3) | 9 (9.0) | 7 (0.0) | 6 (0.0) | 6 (0.0) | 35 (1.4) |

[a]Values are the mean of diameter of inhibition zone (mm) of four replicates in the screening phase using the agar-well diffusion method (CV%); –, not active at 1000 $\mu$g mL$^{-1}$. [b]Standard strain. [c]Field strain. [d]Positive control: ketoconazole (500 $\mu$g mL$^{-1}$).

**Table 5** MIC for compounds **1**–**4** isolated from *Calea uniflora*.

| Microorganisms | MIC[c] ($\mu$g mL$^{-1}$ (CV)) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| *Candida albicans* ATCC 64548[a] | – | 500 (8.2) | 500 (0.0) | 500 (4.1) |
| *Candida krusei* ATCC 6258[a] | – | – | – | – |
| *Candida parapsilosis* ATCC 22019[a] | – | – | – | – |
| *Candida glabrata* ATCC 90030[a] | – | 500 (3.0) | 500 (1.6) | – |
| *Trichophyton rubrum* Tr-5[b] | 1000 (0.0) | 1000 (2.2) | 1000 (6.2) | 1000 (0.0) |
| *Trichophyton rubrum* Tr-19[b] | 1000 (4.1) | 1000 (0.0) | 1000 (4.1) | 1000 (1.2) |
| *Trichophyton mentagrophytes* Tm-9[b] | 1000 (2.9) | 1000 (0.0) | 1000 (0.0) | 1000 (4.1) |
| *Trichophyton mentagrophytes* Tm-17[b] | 1000 (10.2) | 1000 (1.2) | 1000 (4.2) | 1000 (0.0) |

[a]Standard strain. [b]Field strain. [c]Each value is a mean of at least four replicate experiments (CV%). –, not active at 1000 $\mu$g mL$^{-1}$.

derivatives of *p*-hydroxyacetophenone obtained from *Senecio graveolens* and reported by González et al (1990).

On the other hand, a remarkable increase in the antifungal activity of isolated compounds was observed as compared with the activity of the crude extract (Table 4). The *p*-hydroxyacetophenone derivative **1** was active against four strains of microorganism (all the strains of *Trichophyton*). Compounds **2** and **3** showed an effect against two yeasts and four dermatophytes, whereas **4** exhibited antifungal activity against five tested fungal strains. Orabi et al (1991) demonstrated the in-vitro antifungal activity of acetophenone derivatives against strains of *Candida albicans* and other microorganisms. Structure modifications by methylation or reduction resulted in diminished activity.

The results documented here indicate that compounds isolated from *C. uniflora* display antifungal activity mainly against human pathogen dermatophytes. Considering that dermatophytic fungi that parasitize keratinized tissues, such as hair, nails and the horny layer of the epidermis, are also related to systemic diseases, it would be interesting to determine the possibility of topical and systemic administration of the crude extract and *p*-hydroxyacetophenone derivative compounds isolated from *C. uniflora* with regard to their use as antifungal agents and to evaluate their toxicity.

In the chemometric analysis the principal components analysis validated the cluster analysis (Figures 2 and 3) and the drug tests were grouped by similarity of antifungal activity. Based on cluster analysis (Figure 2), there was a natural variability of grouping: **2** and **3** formed a group with minor Euclidean distance (0.074 ED); **4** and **1** formed a second group (0.220 ED); **1**–**4** and extract formed another group (0.521 ED) and the positive control, ketoconazole (P), was in a group with larger Euclidean distance (2.380

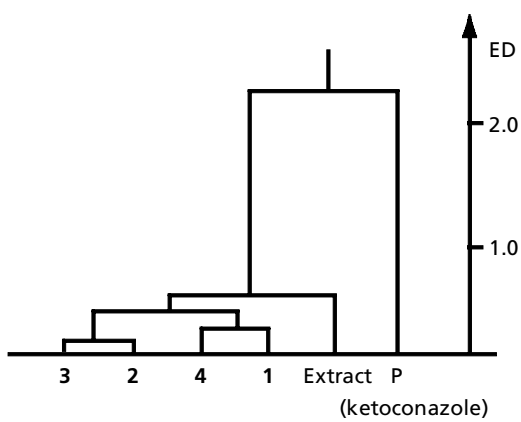


**Figure 2** Cluster analysis for antifungal drug tests.

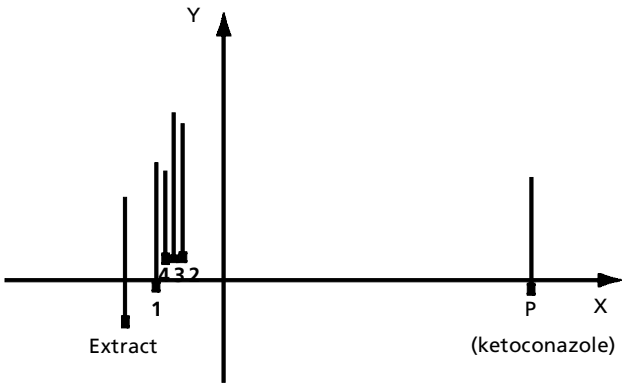


**Figure 3** Principal components analysis for antifungal drug tests.

ED). The principal components analysis (Figure 3) confirmed these groupings. In the groupings the variables were: *C. krusei*-ATCC 6258 (x-axis, first component, $\lambda 1 = 95.47\%$), *T. mentagrophytes*-strain Tm-9 (y-axis, second component, $\lambda 2 = 2.72\%$) and *C. glabrata*-ATCC 90030 (z-axis, third component, $\lambda 3 = 1.44\%$). These results suggest that the antifungal activities of **1** and **4** were similar as well as the bioactivities of **2** and **3**, while the extract and ketoconazole formed distinct groups.

In conclusion, this class of compounds is of particular interest, and detailed studies on both activities should be undertaken to understand their toxicological effects and mechanisms of action. In addition, phytochemical investigations of other species of this genus confer an alternative for the registration of materials that could be useful in the future.

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