



Original Article

Chromenes from leaves of *Calea pinnatifida* and evaluation of their leishmanicidal activity



Tamires C. Lima^a, Alan Diego C. Santos^b, Danielle T.M. Costa^c, Rafaela J. Souza^a, Andersson Barison^b, Mário Steindel^c, Maique W. Biavatti^{a,*}

^a Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

^b Departamento de Química, Universidade Federal do Paraná, Curitiba, PR, Brazil

^c Departamento de Microbiologia e Parasitologia, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

ARTICLE INFO

Article history:

Received 23 October 2014

Accepted 26 January 2015

Available online 12 February 2015

Keywords:

Asteraceae

Calea pinnatifida

Chromenes

Leishmanicidal activity

ABSTRACT

Calea pinnatifida (R. Br.) Less., Asteraceae, is popularly known as “quebra-tudo”, “cipó-cruz” or “aruca”. This species is used in the folk medicine for the treatment of stomach pain, giardiasis and amoebiasis. The aim of this study was to isolate and identify chromenes from leaves of *C. pinnatifida* and evaluate their leishmanicidal activity. A fraction from leaves of *C. pinnatifida* was analyzed for their chemical constituents, resulting in the isolation and characterization of four known chromenes: 6-acetyl-7-hydroxy-2,2-dimethylchromene (**1**), 6-acetyl-7-methoxy-2,2-dimethylchromene (**2**), 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**). Structure identification of isolated compounds involved analysis of spectral data of 1D and 2D-NMR. The isolated compounds are here reported for the first time in *C. pinnatifida*, and the chromenes **1** and **3** show a moderate leishmanicidal activity.

© 2014 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

Calea L. is a large genus of the Asteraceae family (tribe Heliantheae, subtribe Melampodiinae), containing approximately 125 species distributed essentially in tropical and subtropical zones of the Americas (Roque and Carvalho, 2011), with the greatest number of species being recorded in Brazil (Mondin and Bringel Jr., 2010). This genus has been reported in the literature to possess various biological properties, such as anti-inflammatory (Gomes and Gil, 2011), antiplasmodial (Kohler et al., 2002), antileishmanial (Wu et al., 2011), acaricidal (Ribeiro et al., 2011), antifungal (Flach et al., 2002), antidiabetic (Ramos et al., 1992), antimicrobial (Do Nascimento et al., 2004), antihypertensive (Guerrero et al., 2002), and cytotoxic activities (Nakagawa et al., 2005).

Calea pinnatifida (R. Br.) Less. is popularly known as “aruca”, “cipó-cruz” or “quebra-tudo” (Mors et al., 2000). This species is used in the folk medicine for treating digestive disorders, giardiasis and amoebiasis (Malhado Filho, 1947; Prusk and Urbatsch, 1988; Mors et al., 2000). Previous phytochemical investigations of

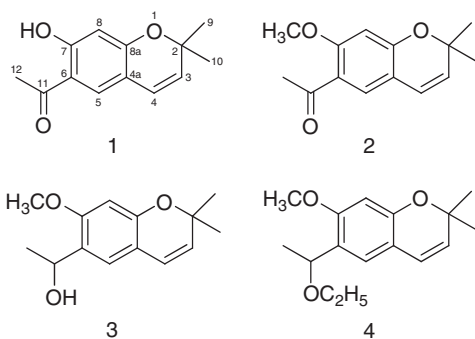
the petroleum ether and ethyl acetate extracts from aerial parts of this plant and its essential oil revealed the presence of fatty esters, phenolic acids, sterols, monoterpenes, one polyacetylene, and one sesquiterpene lactone (Ferreira et al., 1980a,b; Kato et al., 1994).

Chromenes (benzopyrans) represent a class of secondary metabolites that have generated great attention because of their interesting biological and pharmacological properties (Ribeiro et al., 2011; Thomas and Zachariah, 2013). Several studies have demonstrated the insecticidal, antibacterial, fungicidal and cytotoxic activities of these substances (Bandara et al., 1992; Burkhardt et al., 1994; Iqbal et al., 2004; Chen et al., 2005). Furthermore, some compounds of this class of natural products have been described to have notable antiprotozoal effect (Alizadeh et al., 2008; Batista Jr. et al., 2008; Harel et al., 2011).

Herein, we report the isolation and the structure determination of four known chromenes, named as 6-acetyl-7-hydroxy-2,2-dimethylchromene (**1**), 6-acetyl-7-methoxy-2,2-dimethylchromene (**2**), 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**). In addition, the isolated compounds were selected for leishmanicidal assays based on previously reported activity of related structurally compounds in other trypanosomatid protozoa (Harel et al., 2011).

* Corresponding author.

E-mail: maique.biavatti@ufsc.br (M.W. Biavatti).



Materials and methods

General experimental procedures

Melting point was determined using an MQAPF-301 melting point apparatus. Optical rotation was measured in the solvent CHCl_3 on a Jasco P-2000. The ^1H and ^{13}C NMR spectra was obtained using a high resolution Bruker AVANCE-400 and Ascend 600 spectrometers, frequency of 400 and 600 MHz for ^1H , and 100 and 150 MHz for ^{13}C , respectively. NMR spectroscopic data were acquired in CDCl_3 , TMS was used as internal standard, chemical shifts (δ) were given in ppm, and coupling constants (J) in Hz. 2D NMR experiments (HSQC, HMBC) were also performed using Bruker AVANCE-400 and Ascend 600 spectrometers.

Plant material

The leaves from *Calea pinnatifida* (R. Br.) Less., Asteraceae, were collected in September 2012, at the “Costa da Lagoa”, Florianópolis, Santa Catarina, Brazil. Plant identification was performed by Dr. John F. Pruski, New York Botanical Garden, and a voucher specimen (MO-2383318 number) is deposited in Missouri Botanical Garden Herbarium (MO), St. Louis, Missouri, USA.

Extraction and isolation

Fresh leaves from *C. pinnatifida* (800 g) were extracted by maceration for 15 days at room temperature (ca. 25 °C) with ethanol 92%. After evaporation of the solvent under reduced pressure, 12 g of the ethanol extract of *C. pinnatifida* were obtained. The ethanol extract was re-dissolved in H_2O and fractionated with solvents of increasing polarity. The partitioning of this extract was performed with *n*-hexane, dichloromethane and ethyl acetate, respectively, yielding *n*-hexane (4.5 g), dichloromethane (0.5 g) and ethyl acetate (1.5 g) fractions, as well as a residual aqueous fraction (5.5 g). Initially, an aliquot of the hexane fraction (2.0 g) of the extract was subjected to column chromatography with silica gel 60. Elution was carried out using a solvent gradient of *n*-hexane:acetone in increasing polarities (100:0, 98:2, 95:5, 90:10, 70:30, 50:50, 0:100, respectively), obtaining some sub-fractions rich in chromenes (subfraction 1: 35 mg, sub-fraction 2: 20 mg, subfraction 3: 15 mg). Subsequently, these sub-fractions were purified by preparative TLC (*n*-hexane:acetone 85: 15). Sub-fraction 1 afforded 8.0 mg of **1** and 4.0 mg of **4**, sub-fraction 2 yielded 18.0 mg of **2**, and sub-fraction 3 afforded 5.0 mg of **3**.

6-Acetyl-7-hydroxy-2,2-dimethylchromene (**1**)

Yellow needles; mp: 78–80 °C; ^1H NMR (CDCl_3 , 400 MHz): δ 7.31 (br s, 1H, H-5), 6.33 (br s, 1H, H-8), 6.28 (d, 1H, J 9.9 Hz, H-4), 5.58 (d, 1H, J 9.9 Hz, H-3), 2.54 (s, 3H, H-12), 1.44 (s, 3H, H-9), 1.44 (s, 3H, H-10); ^{13}C NMR (CDCl_3 , 100 MHz): δ 77.9 (C-2), 128.9 (C-3), 121.0

(C-4), 113.5 (C-4a), 128.5 (C-5), 113.8 (C-6), 165.2 (C-7), 104.5 (C-8), 160.4 (C-8a), 28.6 (C-9), 28.6 (C-10), 202.3 (C-11), 26.1 (C-12).

6-Acetyl-7-methoxy-2,2-dimethylchromene (**2**)

Yellow oil; ^1H NMR (CDCl_3 , 400 MHz): δ 7.54 (s, 1H, H-5), 6.38 (s, 1H, H-8), 6.30 (d, 1H, J 9.8 Hz, H-4), 5.53 (d, 1H, J 9.8 Hz, H-3), 3.88 (s, 3H, H-13), 2.56 (s, 3H, H-12), 1.44 (s, 3H, H-9), 1.44 (s, 3H, H-10); ^{13}C NMR (CDCl_3 , 100 MHz): δ 77.7 (C-2), 128.4 (C-3), 121.4 (C-4), 114.0 (C-4a), 129.1 (C-5), 120.8 (C-6), 161.2 (C-7), 99.7 (C-8), 158.5 (C-8a), 28.4 (C-9), 28.4 (C-10), 197.6 (C-11), 31.9 (C-12), 55.6 (C-13).

6-(1-Hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**)

Green gum; $[\alpha]_D^{20} = +45.49$ ($c = 0.2133$ g ml^{-1} ; CHCl_3 ; ca. 20 °C); ^1H NMR (CDCl_3 , 400 MHz): δ 6.94 (d, 1H, J 0.3 Hz, H-5), 6.37 (s, 1H, H-8), 6.27 (dd, 1H, J 9.7 0.3 Hz, H-4), 5.47 (d, 1H, J 9.7 Hz, H-3), 5.02 (q, 1H, J 6.5 Hz, H-11), 3.82 (s, 3H, H-13), 1.48 (d, 3H, J 6.5 Hz, H-12), 1.42 (s, 3H, H-9), 1.42 (s, 3H, H-10); ^{13}C NMR (CDCl_3 , 100 MHz): δ 76.5 (C-2), 127.9 (C-3), 121.9 (C-4), 113.7 (C-4a), 123.9 (C-5), 125.6 (C-6), 157.5 (C-7), 99.9 (C-8), 153.2 (C-8a), 28.2 (C-9), 28.2 (C-10), 65.8 (C-11), 22.9 (C-12), 55.6 (C-13).

6-(1-Ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**)

Green oil; ^1H NMR (CDCl_3 , 600 MHz): δ 7.00 (s, 1H, H-5), 6.34 (s, 1H, H-8), 6.29 (d, 1H, J 9.7 Hz, H-4), 5.45 (d, 1H, J 9.7 Hz, H-3), 4.74 (q, 1H, J 6.4 Hz, H-11), 3.77 (s, 3H, H-13), 3.39 (dq, 1H, J 9.4 7.0 Hz, H-1a'), 3.37 (dq, 1H, J 9.4 7.0, 1b'), 1.43 (s, 3H, H-9), 1.43 (s, 3H, H-10), 1.35 (d, 3H, J 6.4 Hz, H-12), 1.18 (dd, 3H, J 7.0 7.0 Hz, H-2'); ^{13}C NMR (CDCl_3 , 150 MHz): δ 76.3 (C-2), 127.5 (C-3), 122.2 (C-4), 113.9 (C-4a), 123.9 (C-5), 124.6 (C-6), 157.6 (C-7), 99.3 (C-8), 152.8 (C-8a), 28.2 (C-9), 28.2 (C-10), 70.9 (C-11), 22.8 (C-12), 55.5 (C-13), 63.8 (C-1'), 15.5 (C-2').

Leishmanicidal screening

Human macrophage cell line THP-1 (ATCC TIB202) was grown in RPMI-1640 without phenol red (Sigma-Aldrich, CO. St. Louis, MO, USA) supplemented with 10% FBS (Life Technologies, USA), 12.5 mM HEPES, penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and Glutamax (2 mM), at 37 °C in a 5% CO_2 incubator. *L. amazonensis* MHOM/BR/77/LTB0016 promastigotes, expressing β -galactosidase, were grown at 26 °C in Schneider's insect medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 5% heat inactivated fetal bovine serum FBS and 2% of human urine.

For the leishmanicidal screening against intracellular *L. amazonensis* amastigotes, THP-1 cells (3.0×10^4 per well) were cultivated in 96 well plates in RPMI-1640 medium supplemented as described above and treated with 100 ng/ml of phorbol 12-myristate 13-acetate (PMA) for 72 h at 37 °C in a 5% CO_2 , to allow THP-1 cells differentiation into non-dividing macrophages (Schwende et al., 1996).

Four days culture promastigotes (4.0×10^6 parasites/ml) were washed with phosphate buffered saline, pH 7.4 (PBS) and incubated in RPMI-1640 supplemented with 10% human AB+ serum heat-inactivated for 1 h at 34 °C to parasite opsonization. THP-1 cells were incubated with a parasite/cell ratio of 10:1 for 3 h at 34 °C and 5% CO_2 . After this period non-adherent parasite were removed by one wash with PBS and infected cells were incubated with 180 μl of full supplemented RPMI-1640 medium for another 24 h to allow the transformation of promastigotes into intracellular amastigotes.

Compounds **1–4** were solubilized in dimethyl sulfoxide (DMSO) and serially diluted (50–0.8 $\mu\text{g}/\text{ml}^{-1}$). Infected cell layer were

treated by addition of 20 μl of each sample, in triplicate, followed by incubation for 48 h at 34 °C at 5% CO_2 . After treatment, cells were carefully washed with PBS and incubated for 16 h 37 °C with 250 μl of chlorophenolred- β -D-galactopyranoside (Sigma–Aldrich Co., St. Louis, MO, USA) (CPRG) at 100 μM and Nonidet P-40 (Amresco Inc, Solon, Ohio, USA) (NP-40) 0.1%. Optical density was read at 570/630 nm in an Infinite M200 TECAN, Austria. Amphotericin B (Bristol-Myers, Squibb) was used as positive control and DMSO 1% as negative control.

Results and discussion

The hexane fraction of the leaves from *C. pinnatifida* was phytochemically studied by column chromatography on silica gel and the chromene-rich fractions obtained were further purified by preparative TLC to isolate compounds **1–4**. Their chemical structures were established by physical data and 1D and 2D-NMR spectroscopy, and their spectral data were in agreement with published data (Steelink and Marshall, 1979; Fang et al., 1988; Zhai et al., 2010; Harel et al., 2011).

Compounds **1–4** showed closely related NMR data. The 1D and 2D-NMR spectral data of these compounds are consistent with those of a chromene skeleton. Thus, compounds **1**, **2**, **3** and **4** were identified as 6-acetyl-7-hydroxy-2,2-dimethylchromene (eupatoriocromene) (Harel et al., 2011), 6-acetyl-7-methoxy-2,2-dimethylchromene (methyleupatoriocromene) (Zhai et al., 2010), 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (encecalinol) (Fang et al., 1988) and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene (ethyl encecalol) (Steelink and Marshall, 1979), respectively. There are no spectroscopic or optical evidences to define the absolute stereochemistry of C-11 from compounds **3** and **4**. Optical rotation of encecalinol (**3**) was determined as +45.49 (see details above).

Compound **4** ($\text{C}_{16}\text{H}_{22}\text{O}_3$) was isolated as a green oil. Its ^1H NMR spectrum showed the presence of two singlets at δ 7.00 (s, 1H, H-5) and δ 6.34 (s, 1H, H-8), indicating the presence of a 1,2,4,5-tetrasubstituted benzene ring. These signals associated with a pair of AM doublets at δ 6.29 (d, 1H, J 9.7 Hz, H-4) and δ 5.45 (d, 1H, J 9.7 Hz, H-3), typical of a *cis*-olefin, suggested a benzopyran moiety that together with the presence of a singlet at δ 1.43 (s, 6H, H-9 and H-10), indicated a chromene skeleton. Furthermore, it was also possible to observe a singlet at δ 3.77 (s, 3H, H-13), typical of a methoxy group; a doublet at δ 1.35 (d, 3H, J 6.4 Hz, H-12) and a quartet at δ 4.74 (q, 1H, J 6.4 Hz, H-11), corresponding to a carbinol hydrogen. This compound also exhibited two doublet of quartets at δ 3.39 (dq, 1H, J 9.4 7.0 Hz, H-1a') and 3.37 (dq, 1H, J 9.4 7.0, 1b') coupled to a doublet of doublets at δ 1.18 (dd, 3H, J 7.0 7.0 Hz, H-2'), corresponding to an ethyl group attached to an oxygenated carbon δ C-11. This is the first report of the isolation of the ethyl encecalol in the genus *Calea*.

The ^{13}C NMR assignments were aided by heteronuclear shift correlation experiments such as HSQC and HMBC. The HSQC and HMBC spectra demonstrated the presence of 16 carbon signals, including two sp^2 aromatic carbons (δ_{C} 99.3, 123.9), two aromatic quaternary carbons (δ_{C} 113.9, 124.6), two oxygenated aromatic quaternary carbons (δ_{C} 152.8, 157.6), two olefinic carbons (δ_{C} 122.2, 127.5), three sp^3 oxygenated carbons (δ_{C} 55.5, 63.8, 70.9), one sp^3 oxygenated quaternary carbons (δ_{C} 76.3) and four methyl groups (δ_{C} 15.5, 22.8, 28.2, 28.2).

Eupatoriocromene has been previously isolated from *Calea* species, such as *C. serrata* (Steinbeck et al., 1997), *C. hispida* (Bohlmann et al., 1982b), *C. oxylepis* (Bohlmann et al., 1982a), *C. rotundifolia* (Bohlmann et al., 1981a) and *C. peckii* (Castro et al., 1989). Moreover, this compound has been tested on *Trypanosoma* (Harel et al., 2013) and insect larvae (Klocke et al., 1985).

Table 1

Leishmanicidal activity of isolated compounds from *Calea pinnatifida*.

Material tested 50 $\mu\text{g ml}^{-1}$	% Growth inhibition <i>L. amazonensis</i> amastigotes
Compound 1	39.3 (± 7.8)
Compound 2	No activity
Compound 3	32.3 (± 5.7)
Compound 4	No activity
Negative control (DMSO 1%)	0
Positive control (amphotericin B 2 μM)	86.6 (± 5.0)

Methyleupatoriocromene was earlier isolated from *C. oxylepis* (Bohlmann et al., 1982a), *C. teucrifolia* (Bohlmann et al., 1981b), *C. rotundifolia* (Bohlmann et al., 1981a) and *C. morii* (Bohlmann et al., 1981b) and tested against some protozoan parasites (Harel et al., 2013) and insect species (Klocke et al., 1985).

Encecalinol was previously obtained from *C. morii* (Bohlmann et al., 1981b) and was evaluated against protozoan parasites (Harel et al., 2013), pathogenic bacteria (Rios et al., 2003), insect larvae (Klocke et al., 1985) and dermatophytes fungi (Aguilar-Guadarrama et al., 2009).

Ethyl encecalol has been described as an artifact produced during ethanol extraction of species *Encelia farinosa* Gray (Steelink and Marshall, 1979). In general, ethoxylated derivatives are unlikely from the biogenetic point of view. However, this particular compound was also isolated from the *n*-hexane extract of *Ageratum conyzoides*, whose separation and purification steps did not involve any ethanol in the isolation procedure (González et al., 1991). With the aim to confirm if the compound **4** is or not an artifact produced during the extraction with ethanol, a small portion of the plant material was extracted using MeOH and subjected to the same partition procedure. A comparison performed by TLC demonstrated that the ethyl encecalol was not identified in the hexane fraction obtained from MeOH extract, therefore suggesting that this compound may be really an artifact of extraction with ethanol.

The leishmanicidal screening data of the chromenes **1–4** against the amastigote forms of *Leishmania amazonensis* are shown in Table 1. Compounds **2** and **4** did not displayed leishmanicidal activity, while **1** and **3** exhibited moderate effect against *Leishmania* intracellular amastigotes. At a concentration of 50 $\mu\text{g ml}^{-1}$ compounds **1** and **3** inhibited the parasite intracellular growth by 39.3% and 32.3%, respectively. Amphotericin B was used as positive control, producing inhibition of 86.6% at concentration 2 μM , and the negative control (DMSO 1%) did not show any inhibition. Comparing the structure of the four chromenes was found that the polar chromenes (**1** and **3**) were more bioactive than the non-polar chromenes (**2** and **4**), indicating that the polarity may play an important role in the leishmanicidal effect of these compounds.

Natural and synthetic chromenes have been reported to exhibit interesting antiprotozoal effect. A molecular docking analysis was carried out to evaluate potential *Leishmania* protein targets of antiprotozoal plant-derived chromenes and other phenolic compounds (Ogungbea et al., 2014). In this study, a chromene exhibited selective docking to *Leishmania major* N-myristoyltransferase, showing that chromenes may be promising as antiparasitic based on *in-silico* analysis. In other study, it was evaluated the leishmanicidal activity of twelve synthetic chromenes against *in vitro* *L. major* promastigotes, and some compounds exhibited important leishmanicidal effect (% growth inhibition >70%), showing the potential biological of this chemical class (Alizadeh et al., 2008).

Conclusions

The phytochemical investigation of the fresh leaves of *C. pinnatifida* afforded four known chromenes: 6-acetyl-7-hydroxy-2,2-dimethylchromene (**1**), 6-acetyl-7-methoxy-2,

2-dimethylchromene (**2**), 6-(1-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (**3**) and 6-(1-ethoxyethyl)-7-methoxy-2,2-dimethylchromene (**4**), which are being reported in this species for the first time. Regarding the leishmanicidal activity, compounds **2** and **3** demonstrated moderate leishmanicidal effect.

Authors' contribution

TCL (PhD student) contributed in all steps of this study. DTMC and MS contributed to the biological studies. RJS contributed to isolation and purification of the compounds. AB and ADCS contributed to spectroscopic analysis. MS contributed to critical reading of the manuscript. MWB supervised the laboratory work and contributed to design of the study and critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are grateful to physician César Simmionato for the help in the collection of the species, the Dr. John Pruski for identification of the plant, the CAPES and CNPq for financial support and the Federal University of Santa Catarina.

References

- Aguilar-Guadarrama, B., Navarro, V., Leon-Rivera, I., Rios, M.Y., 2009. Active compounds against tinea pedis dermatophytes from *Ageratina pichinchensis* var. *bustamenta*. *Nat. Prod. Res.* 23, 1559–1565.
- Alizadeh, B.H., Fouroumadi, A., Ardestani, S.K., Poorrajab, F., Shafiee, A., 2008. Leishmanicidal evaluation of novel synthetic chromenes. *Arch. Pharm. Chem. Life Sci.* 341, 787–793.
- Bandara, B.M.R., Hewage, C.M., Karunaratne, V., Wannigama, G.P., Adikaram, N.K.B., 1992. An antifungal chromene from *Eupatorium riparium*. *Phytochemistry* 31, 1983–1985.
- Batista Jr., J.M., Lopes, A.A., Ambrosio, D.L., Regasini, L.O., Kato, M.J., Bolzani, V.D.S., Cicarelli, R.M.B., Furlan, M., 2008. Natural chromenes and chromene derivatives as potential anti-trypanosomal agents. *Biol. Pharm. Bull.* 31, 538–540.
- Bohlmann, F., Bapuji, M., King, R.M., Robinson, H., 1982a. Naturally occurring terpene derivatives. Part 421. New heliangolides *Calea oxylepis*. *Phytochemistry* 21, 1164–1166.
- Bohlmann, F., Gupta, R.K., Jakupovic, J., King, R.M., Robinson, H., 1982b. Furanoheliangolides and farnesol derivatives from *Calea hispida*. *Phytochemistry* 21, 2899–2903.
- Bohlmann, F., Gupta, R.K., Jakupovic, J., King Robert, M., Robinson, H., 1981a. Naturally occurring terpene derivatives. Part 338. Eudesmanolides and heliangolides from *Calea rotundifolia*. *Phytochemistry* 20, 1635–1637.
- Bohlmann, F., Zdero, C., King, R.M., Robinson, H., 1981b. Naturally occurring terpene derivatives. Part 343. Heliangolides, and nerolidol and *p*-hydroxyacetophenone derivatives from *Calea* species. *Phytochemistry* 20, 1643–1647.
- Burkhardt, G., Becker, H., Grubert, M., Thomas, J., Eicher, T., 1994. Bioactive chromenes from *Rhynchosia penicillata*. *Phytochemistry* 37, 1593–1597.
- Castro, V., Tamayo-Castillo, G., Jakupovic, J., 1989. Sesquiterpene lactones and other constituents from *Calea prunifolia* and *C. peckii*. *Phytochemistry* 28, 2415–2418.
- Chen, J.J., Duh, C.Y., Chen, I.S., 2005. Cytotoxic chromenes from *Myriactis humilis*. *Planta Med.* 71, 370–372.
- Do Nascimento, A.M., Salvador, M.J., Candido, R.C., Ito, I.Y., de Oliveira, D.C., 2004. Antimicrobial activity of extracts and some compounds from *Calea platylepis*. *Fitoterapia* 75, 514–519.
- Fang, N., Yu, S., Mabry, T.J., 1988. Chromenes from *Ageratina arsenii* and revised structures of two epimeric chromene dimers. *Phytochemistry* 27, 1902–1905.
- Flach, A., Gregel, B., Simionatto, E., da Silva, U.F., Zanatta, N., Morel, A.F., Linares, C.E., Alves, S.H., 2002. Chemical analysis and antifungal activity of the essential oil of *Calea clematidea*. *Planta Med.* 68, 836–838.
- Ferreira, Z.S., Roque, N.F., Gottlieb, O.R., Oliveira, F., Gottlieb, H.E., 1980a. Structural clarification of germacranolides from *Calea* species. *Phytochemistry* 19, 1481–1484.
- Ferreira, Z.S., Roque, N.F., Gottlieb, O.R., Oliveira, F., 1980b. Chemical study on *Calea pinnatifida*. *Cien. Cult.* 32, 83–85.
- Gomes, M., Gil, J.F., 2011. Topical anti-inflammatory activity of *Calea prunifolia* HBK (Asteraceae) in the TPA model of mouse ear inflammation. *J. Braz. Chem. Soc.* 22, 2391–2395.
- González, A.G., Aguiar, Z.E., Grillo, T.A., Luis, J.G., Rivera, A., Calle, J., 1991. Chromenes from *Ageratum conyzoides*. *Phytochemistry* 30, 1137–1139.
- Guerrero, M.F., Puebla, P., Carrón, R., Martín, M.L., Arteaga, L., Román, L.S., 2002. Assessment of the antihypertensive and vasodilator effects of ethanolic extracts of some Colombian medicinal plants. *J. Ethnopharmacol.* 80, 37–42.
- Harel, D., Khalid, S.A., Kaiser, M., Brun, R., Wünsch, B., Schmidt, T.J., 2011. Enecalol angelate, an unstable chromene from *Ageratum conyzoides* L.: total synthesis and investigation of its antiprotozoal activity. *J. Ethnopharmacol.* 137, 620–625.
- Harel, D., Schepmann, D., Prinz, H., Brun, R., Schmidt, T.J., Wuensch, B., 2013. Natural product derived antiprotozoal agents: synthesis, biological evaluation, and structure–activity relationships of novel chromene and chromane derivatives. *J. Med. Chem.* 56, 7442–7448.
- Iqbal, M.C.M., Jayasinghe, U.L.B., Herath, H.M.T.B., Wijesekera, K.B., Fujimoto, Y., 2004. Fungistatic chromene from *Ageratum conyzoides*. *Phytoparasitica* 32, 119–126.
- Kato, E.T.M., Akisue, M.K., Matos, F.J.A., Craveiro, A.A., Alencar, J.M., 1994. Constituents of *Calea pinnatifida*. *Fitoterapia* 65, 377.
- Klocke James, A., Balandrin Manuel, F., Adams Robert, P., Kingsford, E., 1985. Insecticidal chromenes from the volatile oil of *Hemizonia fitchii*. *J. Chem. Ecol.* 11, 701–712.
- Kohler, I., Jenett-Siems, K., Siems, K., Hernández, M.A., Ibarra, R.A., Berendsohn, W.G., Bienzle, U., Eich, E., 2002. *In vitro* antiparasitological investigation of medicinal plants from El Salvador. *Z. Naturforsch. C.* 57, 277–281.
- Malhado Filho, 1947. *Novo antidiarreico vegetal; Calea pinnatifida* Less. *Arq. Cir. Clin. Exp.* 31, 43.
- Mondin, C.A., Bringel Jr., J.B.A., 2010. In: Forzza, R.C., et al. (Eds.), *Calea*. Available from: <http://floradobrasil.jbrj.gov.br/2010/FB103751> (accessed June 2014).
- Mors, W.B., Rizzini, C.T., Pereira, N.A., 2000. Medicinal Plants of Brazil. Reference Publications, Algonac.
- Nakagawa, Y., Linuma, M., Matsuura, N., Yi, K., Naoi, M., Nakayama, T., Nozawa, Y., Akao, Y., 2005. A potent apoptosis-inducing activity of a sesquiterpene lactone, arucanolide, in HL60 cells: a crucial role of apoptosis-inducing factor. *J. Pharm. Sci.* 97, 242–252.
- Ogungbea, I.V., Erwin, W.R., Setzer, W.N., 2014. Antileishmanial phytochemical phenolics: molecular docking to potential protein targets. *J. Mol. Graph. Model.* 48, 105–117.
- Prusk, J.F., Urbatsch, L.E., 1988. Five new species of *Calea* (Compositae: Heliantheae) from planaltine Brazil. *Brittonia* 40, 341–356.
- Ramos, R.R., Alarcon-Aguilar, F., Lara-Lemus, A., Flores-Saenz, J.L., 1992. Hypoglycemic effect of plants used in Mexico as antidiabetics. *Arch. Med. Res.* 23, 59–64.
- Ribeiro, V.L.S., Santos, J.C., Martins, J.R., Schripsemad, J., Siqueira, I.R., von Poser, G.L., Apel, M.A., 2011. Acaricidal properties of the essential oil and precocene II obtained from *Calea serrata* (Asteraceae) on the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Vet. Parasitol.* 179, 195–198.
- Rios, M.Y., Aguilar-guadarrama, A.B., Navarro, V., 2003. Two new benzofurans from *Eupatorium aschenborniana* and their antimicrobial activity. *Planta Med.* 69, 967–970.
- Roque, N., Carvalho, V.C., 2011. Estudos taxonômicos do gênero *Calea* (Asteraceae, Neurolaeneae) no estado da Bahia, Brasil. *Rodriguésia* 62, 547–561.
- Schwende, H., Fitzke, E., Ambs, P., Dieter, P., 1996. Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D3. *J. Leukoc. Biol.* 59, 555–561.
- Steelink, C., Marshall, G.P., 1979. Structures, syntheses, and chemotaxonomic significance of some new acetophenone derivatives from *Encelia farinosa* Gray. *J. Org. Chem.* 44, 1429–1433.
- Steinbeck, C., Spitzer, V., Starosta, M., von Poser, G., 1997. Identification of two chromenes from *Calea serrata* by semiautomatic structure elucidation. *J. Nat. Prod.* 60, 627–662.
- Thomas, N., Zachariah, S.M., 2013. Pharmacological activities of chromene derivatives: an overview. *Asian J. Pharm. Clin. Res.* 6, 11–15.
- Wu, H., Fronczek, F.R., Burandt Jr., C.L., Zjawiony, J.K., 2011. Antileishmanial Germacranolides from *Calea zacatechichi*. *Planta Med.* 77, 749–753.
- Zhai, H., Zhao, G., Yang, G., Sun, H., Yi, B., Sun, L., Chen, W., Zheng, S., 2010. A new chromene glycoside from *Tithonia diversifolia*. *Chem. Nat. Compd.* 46, 198–200.