Chemical composition and antinociceptive, anti-inflammatory and antiviral activities of *Gallesia gorazema* (Phytolaccaceae), a potential candidate for novel anti-herpetic phytomedicines

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ABSTRACT

Ethnopharmacological relevance: In traditional medicine, teas made from leaves and bark of *Gallesia gorazema* are used as antispasmodic, anthelmintic, antihemorrhagic and febrifuge agents. Crude leaves of this plant are also employed as a remedy in the treatment of abscesses, orchitis, gonorrhea and for rheumatic pain relief. this study investigates the presumed antinociceptive and anti-inflammatory activities of leaves and roots *Gallesia gorazema* (Phytolaccaceae) extracts. The most active extract and its isolated compound, a new natural product, are also evaluated against viruses HSV-1 and HSV-2.
Materials and methods: In vivo experiments with mice were used to assess the analgesic and anti-inflammatory activities of *Gallesia gorazema* extracts. Antiviral activity of extracts and the new natural product was investigated by *in vitro* experiments.
Results: Results show that dichloromethanic root (**DRE**) and ethanolic leaf (**ELE**) extracts displayed significant antinociceptive and anti-inflammatory activities in *in vivo* experiments with mice. Both extracts were also assayed against the herpes simplex viruses HSV-1 and HSV-2, but only **DRE** was highly active, showing a selective antiviral effect against HSV-1. Phytochemical fractionation of **DRE** led to the isolation of 28-hydroxyoctacosyl ferulate, a novel natural product, which displayed strong antiviral activity against HSV-1 ($EC_{50}=21.6$ µg/mL) with a selective index above 9, justifying, at least in part, the high selective antiviral activity observed for **DRE**.
Conclusion: These results suggest that the plant *Gallesia gorazema* is a potential candidate for the development of novel anti-herpetic phytomedicines.

1. Introduction

HSV-1 and HSV-2 are two types of *Herpes simplex*, a double-stranded DNA virus that has very high rates of infection in humans (Fleming et al., 1997; Strand et al., 2012). While the primary site of infection by HSV-1 is usually the orolabial mucosa, HSV-2 mainly infects the genital tract. In both cases, the clinical manifestation of the disease exhibits different severity in normal and immuno-competent hosts, but it can cause serious systemic illnesses in immuno-compromised patients and neonates (Khan et al., 2005).

Acyclovir and penciclovir, and their respective derivatives valacyclovir and famciclovir, are the main drugs/prodrugs used for the chemotherapy of HSV infections. However, drug resistance in treated patients and the high cost of these drugs are among the main problems associated with long-term use of acyclovir and related nucleoside analogues as chemotherapeutical agents. There is, therefore, a great and urgent need for the development of new antiviral drugs with novel targets and different mechanisms of action (Khan et al., 2005; Brandão et al., 2010).

Medicinal plants have long been used to treat many infectious diseases and human disorders. Recently, there has been a growing

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emphasis, among the scientific community, on determining the scientific evidence and rationale for the use of preparations made from medicinal plants, with various research efforts focused on identifying plants and their active components against a wide array of pathogens, including viruses (Khan et al., 2005), bacteria (Rios et al., 1988) and protozoa (Rocha et al., 2005). Natural products have indeed been used as valuable scaffolds in the preparation of novel bioactive compounds with optimized biological activities, playing an important role in the discovery of leads for the development of drugs for the treatment of human diseases (Newman, 2008; Batista et al., 2009; Newman and Cragg, 2012).

Gallesia gorazema (sin. *Gallesia integrifolia* Spreng, Phytolaccaceae) is a shrub with glossy, elliptical leaves. It is widely distributed in the Atlantic Rain Forest of Brazil, occurring mainly in the states of Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo and Paraná. It is commonly known as "pau-d'alho" or "garlic plant" due to the strong odor of garlic peculiar to all parts of the plant (Akisue et al., 1986). Its presence is often seen as an indication that the land is very fertile (Lorenzi, 2000). In traditional medicine, teas made from its leaves and bark are used as antispasmodic, anthelmintic, antihemorrhagic and febrifuge agents (Barbosa et al., 1997), while its crude leaves are employed as a remedy in the treatment of abscesses, orchitis and gonorrhea, as well as a folk medicine for rheumatic pain relief (Akisue et al., 1986; Balbach, 1992). The essential oil from the fresh leaves of *Gallesia gorazema* contains vitamin E and more than 80 other components including sulfur-containing compounds such as dimethylsulfone, methylmethanethiosulfonate and 1-methylsulfonyl-2,3-dithiabutane, among others, which explain the strong garlic odor that is characteristic of the entire plant (Barbosa et al.,1997). GC/MS analysis of the essential oil from the bark also revealed the presence of numerous sulfurated compounds (Barbosa et al., 1999).

Since no previous work was found in the literature describing any experimental investigation on the presumed biological activities of this plant, this study was proposed to investigate the antinociceptive and anti-inflammatory activities of *Gallesia gorazema* extracts, seeking to explain the use of this plant in the treatment of the abovementioned painful disorders, and as a rheumatic pain relief agent in traditional medicine. Additionally, the most active extract and its isolated constituent, a novel natural product, were assayed against HSV-1 and HSV-2 viruses, also with the aim of investigating this plant as an alternative candidate for the development of novel anti-herpetic phytomedicines.

2. Material and methods

2.1. General procedures

Thin layer chromatography (TLC) was performed on 0.25 mm thick silica gel Merck 60 F254, and column chromatography on silica gel 60 (70–230 mesh) from Acros Organics. Spots were visualized under UV light or with a solution (1 L) containing H_2SO_4 (28 mL) and ceric sulfate (42 g) followed by charring. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz and Carbon-13 nuclear magnetic resonance (^{13}C NMR) at 75 MHz using deuterochloroform as solvent and tetramethylsilane (TMS) as internal reference, on a Bruker DPX300 apparatus. Chemical shift values are expressed in ppm and coupling constants (J) in Hz. Infrared spectrum was recorded on a Bomen MB 100 spectrophotometer with KBr pressed plates containing the sample. A low resolution mass spectrum was produced on a Shimadzu GC–MS-QP2010S apparatus. The solvents and reagents were purified by standard procedures as necessary.

2.2. Plant material

Leaves and roots of *Gallesia gorazema* were collected in Itapetinga, Bahia, Brazil, in January 2009, and identified by the botanist Prof. M. Sc. Antônio F.P.C. Guimarães. A voucher specimen was deposited at the Herbarium of the State University of Feira de Santana, Bahia, Brazil, under the code HUEFS186988.

2.3. Preparation of plant extracts

The leaves and roots of *Gallesia gorazema* were dried in an oven at 60 °C and powdered separately to a fine grade, affording the corresponding dried sprayed leaves (570 g) and roots (180 g). The plant material was subjected to thorough soaking with ethanol 96% (leaves) and dichloromethane (roots), yielding the ethanol leaf (**ELE**, 82 g) and dichloromethane root (**DRE**, 19 g) extracts, both with a pasty consistency. Part of **ELE** (60 g) was suspended in water (150 mL), extracted with chloroform (3 × 150 mL) and evaporated to dryness on a rotary evaporator, yielding the chloroform leaf extract (**CLE**, 16 g).

2.4. Analysis of samples by GC–MS and MS

Samples were analyzed according to the following method. **DRE** was dissolved in acetone and a 1-μL aliquot of the solution was injected onto the GC–MS system. GC–MS analysis was performed using a Shimadzu GC–MS-QP2010S apparatus with a Shimadzu AOC-20i autosampler. A RTX-1 30 m × 0.25 mm, 0.10 μm, dimethylpolysiloxane analytical column (Agilent, Palo Alto, CA, USA) was used for the separation. Helium was used as the carrier gas, at a flow rate of 0.8 mL/min. The injector (Splitless mode, 20:1 Split ratio) was maintained at 300 °C. The initial column temperature was set at 80 °C and maintained for 2 min. It was then ramped by 30 °C/min up to 290 °C, then 25 °C/min up to 300 °C and, finally, 20 °C/min up to 310 °C, where it was maintained for 15 min. Detector MS in a scan ($m/z = 30$ to 450 Da) and positive electron impact ionization (EI) modes was used, and data were collected using single ion monitoring (SIM). Compound 1 (solid) was injected by direct insertion onto the MS apparatus (350 °C), with detector in scan ($m/z = 30$ to 750 Da) and positive electron impact ionization (EI) modes.

2.5. Isolation of substance 1 from **DRE**

The dichloromethane root extract of *Gallesia gorazema* (**DRE**, 1.3 g) was chromatographed on silica gel column eluted with increasingly polar mixtures of dichloromethane and ethyl acetate, resulting in ninety fractions of 30 mL each. Fraction 44, eluted with dichloromethane—ethyl acetate (8:2), afforded substance 1 (28 mg) which was pure under TLC and spectroscopic analysis.

28-Hydroxyoctacosyl ferulate (1). Amorphous powder. IR (KBr) ν_{max}(cm^{-1}): 3423, 2917, 2849, 1708, 1634, 1601, 1592, 1519, 1472, 1032. ^{1}H NMR (CDCl$_3$, δ, J, 300 MHz): δ 7.63 (1*H*, d, 15.9 Hz, H-7′); 7.03(1*H*, dd, 8.1, 1.9 Hz, H-6′); 7.01 (1*H*, m, H-2′); 6.89 (1*H*, d, 8.1 Hz, H-5′); 6.32 (1*H*, d, 15.9 Hz, H-8′); 4.18 (2*H*, t, 6.6 Hz, H-1); 3.90 (3*H*, s, O–CH$_3$); 3.63 (2*H*, t, 6.6 Hz, H-28); 1.68 (2*H*, m, H-2); 1.55 (2*H*, m, H-27); 1.25 (48*H*, bs, H-3 to H-26). ^{13}C NMR (CDCl$_3$, δ, 75 MHz): δ 126.9 (C-1′), 109.3 (C-2′), 146.7 (C-3′), 147.9 (C-4′), 115.5 (C-5′), 123.0 (C-6′), 144.6 (C-7′), 114.7 (C-8′), 167.3 (C-9′), 64.5 (C-1), 63.0 (C-28), 55.9 (O–CH$_3$). LRMS, m/z(%): 603(18) [M+1]$^+$, 575(47), 546(6), 516 (1), 362(1), 207(1), 194(53), 177(100), 150(42), 137(50), 83(20), 55 (60).

2.6. Evaluation of antinociceptive and anti-inflammatory effects

2.6.1. Animals

Swiss mice (25–35 g), housed at 22 ± 2 °C under a 12-h light/12 h dark cycle with access to food and water *ad libitum*, were acclimatized to the laboratory for at least 1 h prior to testing. One group of animals was used in each experiment. The experiments reported here followed the current ethical guidelines on the care of laboratory animals and the investigation of experimental pain in conscious animals (Zimmermann, 1983). The experiments were approved by the local Ethics Committee of UNIVALI (113/2005-03). The number of animals (6–8 for treatment group) and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments.

2.6.2. Acetic acid-induced writhing

Abdominal constriction was induced by intraperitoneal injection of acetic acid (0.6%), according to the procedure described previously (Collier et al., 1968) with minor modifications. Male Swiss mice (25–30 g) were pre-treated with extracts (3–20 mg/kg), intraperitoneally (i.p.), 30 and 60 min respectively, before acetic acid injection (six to eight animals in each group). The control animals received a similar volume of 0.9% NaCl (10 mL/kg, i.p.). All the experiments were carried out at 23 ± 2 °C. After the challenge, pairs of mice were placed in separate glass funnels and the number of contractions of the abdominal muscles, together with stretching, were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal contractions between the control animals and the mice pretreated with the test materials.

2.6.3. Formalin-induced nociception

The observation chamber is a cylinder of 20 cm in diameter, equipped with a mirror at a 45° angle to allow clear observation of the animals' legs. Mice were treated with a solution of the extracts (10 mg/kg) or saline (control animals) intraperitoneally 30 min before receiving the formalin injection. Each animal was placed in the chamber 5 min before the treatment to allow them to acclimatize to the new environment. A volume of 20 mL of 2.5% solution of formalin in 0.9% saline was injected intraplantarly into the animal's right hindpaw. The animal was then returned to the chamber and the time spent licking the paw that had received the injection was considered as indicative of pain. Two distinct phases were identified: an acute phase, 0–5 min, and a chronic phase, 15–30 min after formalin injection.

2.6.4. Glutamate-induced nociception

The animals were intraperitoneally treated with extracts at 10 mg/kg or saline (10 mL/kg, i.p.) 30 min before the glutamate injection. A volume of 20 mL of glutamate solution (30 μmol/paw) was injected intraplantarly under the surface of the right hindpaw, as described by Beirith et al. (1998, 2002). After injection, the animals were observed from 0 to 15 min. The time spent licking or biting the injected paw was timed with a chronometer and considered as indicative of pain.

2.6.5. Statistical analysis

The results are presented as the means of six experimental values, except for the mean ID_{50} values (*i.e.*, the dose of extracts or fractions reducing the algesic responses by 50% compared with the control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance between groups was analyzed by one-way ANOVA followed by Dunnett's post-test, unless otherwise stated. *P* values of less than 0.05 were considered as indicative of significance.

ID_{50} values were determined by graphical interpolation from individual experiments.

2.7. Evaluantion of antiviral activity against HSV-1 and HSV-2

2.7.1. Cells and virus

Vero cells (African green monkey kidney) were grown in Eagle's minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 50 μg/mL gentamicin, 2.5 μg/mL fungizone and 10% heat-inactivated fetal bovine serum (FBS) and maintained at 37 °C in 5% CO_2 atmosphere. *Herpes simplex virus* type 1 and type 2 strains were isolated from typical oral and genital lesions, respectively, at the Virology Department of the Federal University of Rio de Janeiro (UFRJ), Brazil. The isolates were typed by polymerase chain reaction (PCR) using specific primers to identify HSV-1 and HSV-2 (Markoulatos et al., 2001) and propagated in a Vero cell. The titers were assessed by the cytopathic end-point assay and were expressed as 50% tissue culture infective dose ($TCID_{50}$) per mL (Reed and Muench, 1938). The virus suspensions were stored at -70 °C until use.

2.7.2. Cytotoxicity assay

ELE, **DRE** and compound **1** were dissolved in dimethyl sulfoxide (DMSO), using slight warming to dissolve compound **1**. Stock solutions were prepared in water at 400 μg/mL and sterilized by filtration using a 0.22 μm Millipore membrane filter. The cytotoxicity assay was performed by incubating triplicate Vero cell monolayers cultivated in 96 well microplates with two-fold serial dilutions of extracts/compound **1** for 48 h at 37 °C in 5% CO_2 atmosphere. The morphological alterations of the treated cells were observed in an inverted optical microscope (Leitz) and the maximum non-toxic concentrations (MNTC) were determined (Walker et al., 1971). Cellular viability was further evaluated by the neutral red dye-uptake method (Borefreund and Puerner, 1985). The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration that caused a 50% reduction in the number of viable cells.

2.7.3. Antiviral activity assay

Vero cell monolayers were treated with **ELE**, **DRE** and compound **1** at the MNTC and 100 $TCID_{50}$/mL of HSV-1 or HSV-2 suspensions were added to treated and untreated cell cultures and incubated at 37 °C for 48 h in a 5% CO_2 atmosphere. After incubation, the supernatants were collected and virus titers in treated and untreated cells were determined. The antiviral activity was expressed as the percentage inhibition (PI) using antilogarithmic $TCID_{50}$ values as follows: $PI = [1 - (\text{antilogarithmic test value/antilogarithmic control value})] \times 100$. For compound **1** only, the dose–response curve was established starting from the MNTC, and the 50% effective concentration (EC_{50}) was defined as the concentration required to achieve 50% protection against virus-induced cytopathic effects. The selectivity index (SI) was determined as the ratio of CC_{50} to EC_{50}.

3. Results and discussion

The ethanol leaf (**ELE**), chloroform leaf (**CLE**) and dichloromethane root (**DRE**) extracts of *Gallesia gorazema* revealed significant antinociceptive effects in mice, displaying 57.8%, 37.4% and 76.0% inhibition respectively, against acetic acid-induced abdominal constrictions (Fig. 1). These results indicate that low polarity compounds are responsible for the antinociceptive effects observed for **CLE** (37.4%) and **DRE** (76%), and that this activity is also attributed to more polar constituents present in **ELE** (57.8%). Although the writhing test is a non-specific antinociceptive model
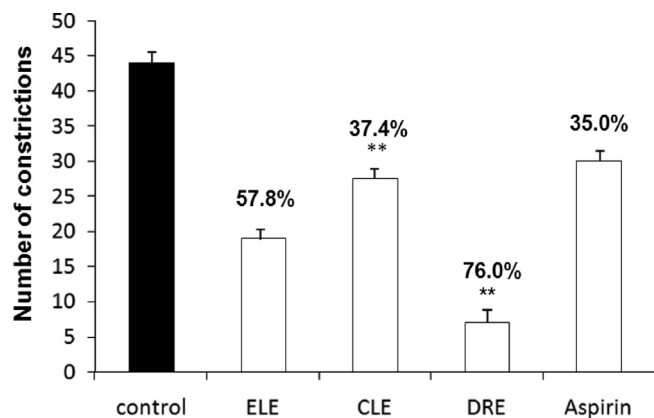
Fig. 1. Effects of the ethanol leaf (**ELE**), chloroform leaf (**CLE**) and dichloromethane root (**DRE**) extracts of *Gallesia gorazema* and acetyl salicylic acid (Aspirin®) at the dose of 10 mg/kg, administrated intraperitonealy, against acetic acid-induced abdominal constrictions in mice. Each column represents the mean of six experimental values. $**P < 0.01$.

due to the fact that anticholinergic and antihistaminic agents, among others, can also be active, this assay is widely used for antinociceptive screening and involves local peritoneal receptors (cholinergic and histamine receptor) as well as the mediators of acetylcholine and histamine (Choi et al., 2003). In addition, all extracts **ELE**, **CLE** and **DRE** also displayed more potent antinociceptive activity than acetyl salicylic acid, a non-steroidal anti-inflammatory and analgesic drug used here for comparison, which caused inhibition of 35% in the same model and dose (Fig. 1).Thus, the significant reduction in the number of acetic acid-induced writings by **ELE**, **CLE** and **DRE** indicates the antinociceptive potential of this plant and confirms its traditional use for relief of rheumatic pain.

Since **DRE** showed the most potent antinociceptive activity (76.0% inhibition of constrictions) at 10 mg/kg (Fig. 1), this extract was selected for evaluation at different doses against 0.6% acetic acid-induced constrictions. Administered intraperitoneally, **DRE** exhibited grade dose-dependent inhibition of abdominal constrictions with an ID_{50} value of 5.68 (4.80–6.73) mg/kg, which corresponds to an activity about five times more potent than those observed for acetyl salicylic acid and acetaminophen, both of which presenting an ID_{50} value of approximately 25 mg/kg by same route. However, when administered orally, **DRE** did not provoke any significant antinociceptive activity up to the dose of 100 mg/kg (data not shown), discouraging us from taking further studies focused on oral administration.

Extracts **ELE**, **CLE** and **DRE** were also analyzed by the formalin-induced pain test, which is a model characterized by the first phase (neurogenic) evoked by the direct formalin stimulation of the nerve endings followed by substance *P* release, and by the second phase caused by subsequent inflammation reaction in peripheral tissue (Hunskaar et al., 1985). As depicted in Fig. 2, only **ELE** and **CLE** inhibited the first phase of pain, causing reductions of 39.9% and 30.3%, respectively. **DRE** was inactive during this phase. Concerning the second phase of pain, **ELE** (29.5%) and **CLE** (30.3%) showed modest anti-inflammatory activity, whereas **DRE** (54.7%) was shown to be the most potent extract, displaying superior inhibition to that observed for acetyl salicylic acid (39.3%), the reference drug. These results suggest that **DRE** has significant anti-inflammatory activity mainly due to the inhibition of peripheral nociceptors. Moreover, this anti-inflammatory response justifies the popular use of this plant as a remedy in the treatment of inflammatory disorders such as orchitis, gonorrhea, rheumatism and abscesses.

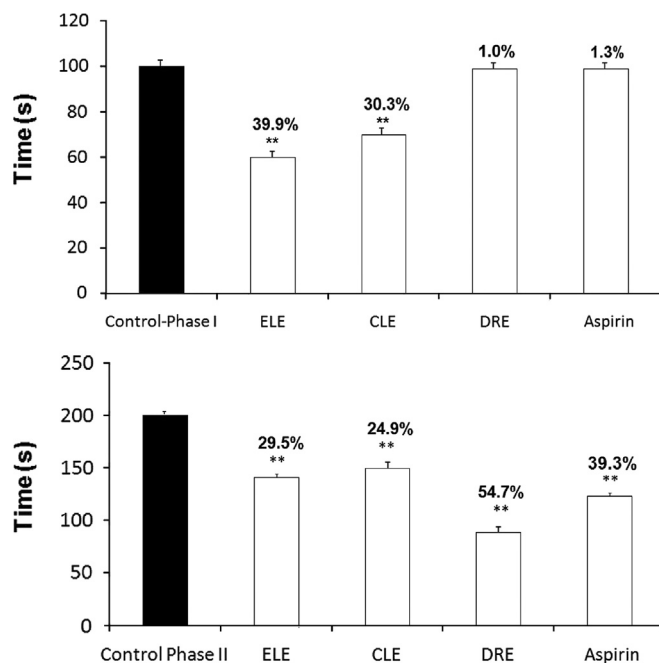Finally, when analyzed by the glutamate-induced pain assay, **ELE** and **CLE** showed a pain inhibition of 56.5% and 47.9% at the

Fig. 2. Effects of the ethanol leaf (**ELE**), chloroform leaf (**CLE**) and dichloromethane root (**DRE**) extracts of *Gallesia gorazema* and acetyl salicylic acid (Aspirin®) at the dose of 10 mg/kg, administered intraperitoneally, against formalin-induced pain in mice (phases I and II). Each column represents the mean of six experimental values. $*P < 0.05$; $**P < 0.01$.

doses of 10 mg/kg, while **DRE** caused an inhibition of 31.5% against glutamate-induced edema, suggesting that such extracts might be inhibiting the liberation of neuropeptides from sensory fibers, namely NKs and kinins involved in the peripheral role of controlling the nociceptive process (Beirith et al., 1998, 2002).

A preliminary ¹H NMR analysis of **DRE** indicated a very intense broad singlet at δ 1.25, attributed to $(CH_2)_n$ groups, along with triplets at around δ 3.6 typical of primary alcohols, revealing a characteristic profile of long-chain alcohols, which suggests these are chemical constituents of this extract. Considering that behenyl alcohol [CH_3–$(CH_2)_{20}$–CH_2OH], also known as docosanol, is a long-chain alcohol and one of the main therapeutic drugs of choice for treating HSV infections (Richardson et al., in press), it was assumed that **DRE** might also display antiviral activity against HSV-1 and HSV-2.

Based on these results, the next step of our study was to assess the antiviral activity of the most promising extracts against *Herpes* viruses. **ELE** and **DRE**, which showed more potent analgesic and anti-inflammatory properties, were selected for evaluation against HSV-1 and HSV-2. The results presented in Table 1 show that **DRE** (100 μg/mL) was active only against HSV-1, with a percentage of inhibition of 93% and a maximum non-toxic concentration (MNTC) of 100 μg/mL, presenting a 50% cytotoxic concentration (CC_{50}) above 200 μg/mL. On the other hand, **ELE** did not present any antiviral activity against either HSV-1 or HSV-2 and was discarded from further studies.

At this point, **DRE** was selected as the most promising extract of *Gallesia gorazema*. Its analysis by GC/MS revealed the presence of minor constituents such as tetradecanal, palmitic acid, octadecanal, γ-sitosterol and β-amyrin, whereas the major peak at 16.1 min was attributed to α-amyrin. On the other hand, three other major peaks with corresponding retention times at 8.9, 15.9 and 17.2 min could not be identified by similarity of their MS spectral profile to any known compound from the NIST library.

This fact made us to submit **DRE** to chromatographic fractionation over silica gel in order to isolate and identify possible novel

Table 1
Cytotoxicity and antiviral activities for extracts **ELE** and **DRE** and for compound **1**.

Sample	Viral inhibition		CC_{50}[c] (μg/mL)	EC_{50}[d] (μg/mL) [SI][e]	
	MNTC[a] (μg/mL)	PVI[b] (HSV-1/HSV-2)		HSV-1	HSV-2
ELE	200	0/0	> 200	–	–
DRE	100	93/0	> 200	–	–
Compound 1	200	99.8/97.8	> 200	21.6 [SI > 9.2]	86.5 [SI > 2.3]
Acyclovir	200	99/99	> 200	0.8 [SI > 250]	1.38 [SI > 145]

[a] Maximum non-toxic concentration.
[b] Percentage of viral inhibition.
[c] Concentration for 50% cytotoxic activity.
[d] Concentration for 50% effective antiviral activity.
[e] Selectivity index (ratio CC_{50}/EC_{50}); HSV-1, *Herpes simplex virus* type 1; HSV-2, *Herpes simplex virus* type 2; acyclovir, standard compound.

Fig. 3. Chemical structure of compound **1**.

compounds as chemical constituents of *Gallesia gorazema*. Taking into account the high complexity of this extract on TLC, this approach led us to isolate only substance **1** (28 mg) as a pure compound. Bands at 3423 and 1708 cm^{-1} in its IR spectrum were indicative of a hydroxyl group and an ester carbonyl, respectively (Baldé et al., 1991). The mass spectrum showed a peak $[M+1]^{+}$ at *m*/*z* 603 (18%) which was compatible to the molecular formula $C_{38}H_{66}O_5$. The major peaks at *m*/*z* 194 (53%) and 177 (100%) were attributed to methylcaffeic acid and methylcaffeoyl fragments, respectively (Sakushima et al., 1985), indicating the presence of a 4-hydroxy-3-methoxy cinnamic (ferulic) acid moiety (Dobhal et al., 1999). Indeed, the ^{1}H NMR spectrum of **1** exhibited signals characteristic of a feruloyl moiety: a methoxyl signal at δ 3.92, two *trans* olefinic protons (δ 7.63 and 6.32, $J=15.9$ Hz) and three aromatic protons [ABX, δ 7.03 m, 7.01 d ($J=8.1$) and 6.89 d ($J=8.1$)]. In the aliphatic region, two triplets of equal intensity (2*H*) and coupling constants ($J=6.6$ Hz) were observed at δ 4.18 and δ 3.63 and they were assigned to methylene hydrogens at the ester linkage (OCO–CH$_2$–R) and the hydroxymethyl group (R–CH$_2$–OH), respectively. The signals at δ 1.68 (2*H*, m) and δ 1.55 (2*H*, m) were attributed to hydrogens of methylene groups which are attached to each of the two abovementioned oxygenated methylenes. A broad singlet at δ 1.25 showed the presence of the remaining 48*H* for (CH$_2$)$_{24}$ groups. The ^{13}C NMR spectrum was in agreement with the above assignments and confirmed the structure of compound **1** as 28-hydroxyoctacosyl ferulate (Fig. 3). As far as the authors are aware, this substance is a new natural product.

Although compound **1** was isolated in small amount (28 mg) and, for this reason, could not be assessed for its *in vivo* antinociceptive and anti-inflammatory activities, there is a close similarity of its structure with those of caffeic acid derivatives that were shown to exhibit significant dose-related antinociceptive activities by *in vivo* experiments (Campos-Buzzi et al., 2009). Thus, compound **1** could be considered as one of the likely constituents responsible for the potent antinociceptive activity presented by **DRE** extract.

Compound **1** was also assayed against HSV-1 and HSV-2 viruses, and the results are shown in Table 1. This new natural product was shown to inhibit 99.8% and 97.8% of virus HSV-1 and HSV-2, respectively, at the non-toxic concentration of 200 μg/mL. In addition, it presented a higher selectivity index (SI > 9.2) against HSV-1 than that observed against HSV-2 (SI > 2.3), demonstrating that this compound is, at least partially, the constituent responsible for the selective antiviral activity of **DRE** against HSV-1 virus.

The promising analgesic and anti-inflammatory properties observed for **DRE**, along with the high antiviral activities observed
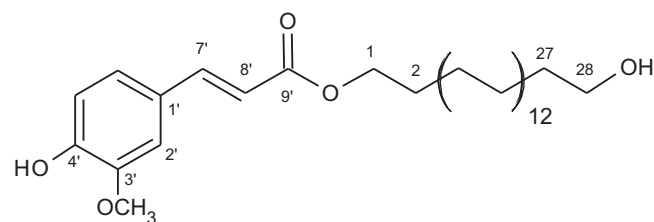
for both **DRE** and compound **1** against HSV-1, indicate that the plant *Gallesia gorazema* is a potential candidate for the development of novel anti-herpetic phytomedicines. Further studies need to be undertaken in order to develop suitable formulations, as well as to investigate other additional pharmaceutical aspects such as those related to toxicity and pharmacokinetics, among others.

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