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Full Length Research Paper

Some alkaloids and flavonoids from *Cissampelos capensis*

Jelili Olalekan Babajide[1]*, Wilfred Thozamile Mabusela[1,2] and Ivan Robert Green[3]

[1]Department of Chemistry, University of the Western Cape, Private Bag X17, Bellville 7535, Cape Town, South Africa.
[2]South African Herbal Science and Medicine Institute, University of the Western Cape, Private Bag X17, Bellville 7535, Cape Town, South Africa.
[3]Department of Chemistry, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch 7600, South Africa.

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Following the screening of several plant species from an inventory of common medicinal plants from South Africa for medicinal properties, *Cissampelos capensis* was selected for further investigation due to its interesting and useful ethnomedicinal properties. This study attempts to relate specific constituents present in this plant with its widespread ethnomedicinal uses. Six compounds were isolated and their structures were unambiguously established by spectroscopic methods. The compounds are: 5,6-dehydro-4,5-dihydroxy-1,3,6-trimethoxy-17-methylmorphinan-7-one (1); 1,2-methylenedioxy-3-hydroxy -9,10-dimethoxyaporphine (2); 5,6-didehydro-4-hydroxy-3,6-dimethoxy-17-methylmorphinan-7-one (3); 3,7,8,3'-tetramethoxy- 6 - C-methyl- 5,4'-dihydroxyflavone (6 -C-methylquercetin 3, 3',7, 8 -tetramethyl ether) (4); 5, 7, 8 -trihydroxy-2′, 5′-dimethoxy-3′,4′-methylenedioxyisoflavanone (5); 3 -methoxy-6 -C-methyl-3',4',5,7,8 -pentahydroxyflavone (6 -C-methylquercetin -3-methyl ether) (6). Five of the isolated compounds, (viz., 1,2,4,5 and 6) have, to our knowledge, not been reported previously. The crude fractions and isolates were tested for cytotoxicity using the brine shrimp lethality test and for antimicrobial properties using nine microbes, including three Gram –ve, three Gram +ve bacteria and three fungi. The Gram-negative bacteria were *Pseudomonas aeruginosa* (NCTC 10332), *Proteus vulgaris* (NCTC 4175) and *Escherichia coli Sero type 1* (NCTC 09001), while the Gram-positive bacteria were *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (NCTC 13134) and *Bacillus licheniformis* (NCTC 01097). The Fungal species used were *Candida albicans* (ATCC 90028), *Candida eropiralis* (ATCC 750) and *Aspergillus niger* (ATCC 10578). The n-Hex fractions were not active while the highest activities were found in the methanolic extracts. The total tertiary alkaloid fraction (TTA) showed the highest activity against the *Bacillus substillis.* Compounds 1, 2 and 5 appear to be the most promising with regards to the prospects of drug development.

Key words: Phytochemistry, *Cissampelos capensis*, antimicrobial, cytotoxicity, isoflavanone, aporphine, morphinandienone.

INTRODUCTION

Medicinal plants, in a variety of forms have been recorded to cure, manage and control conditions in people for at least four thousand years, and it may be assumed that this practice extends even longer (Christophersen et al., 1991). The World Health Organisation (WHO) has recognized this and has accepted

it as an essential building block for primary health care (Akerele, 1988; WHO, 2005). The vibrant healing power of *C. capensis* has been recognized in recent times (Van and Gericke, 2000; Iwu, 1993; WHO, 2002), and especially by traditional healers in South Africa, who are known by the Zulu people as inyangas, herbalists, isangomas or diviners while they are also known as amaxhwele, amagqira (Xhosa), ngaka (Sotho),nanga, mungome or maine (Vhavenda) (Van, et al., 1997; Mander, 1998; De Wet and Van, 2008; Van, 2008; Van et al., 2008).

The plant is one out of 520 species in 75 genera (Van et al., 1997), which belongs to the family of menispermaceae. It is widely distributed in the sandy slopes and scrublands of the Northern, Western and Eastern Cape Provinces of South Africa and northwards into Namibia. It is called by different local name such as Dawidjiewortel (Afrikaans) and Mayisake (Xhosa). Due to its richness in bisbenzylisoquinoline alkaloids, this family is used worldwide in traditional medicine to treat a variety of ailments especially cancer (Barbosa et al., 2000; DeWet et al., 2004, 2005; DeWet, 2006; Watt and Breyer-Brandwijk, 1962; Smith, 1966; Rood, 1994; Cillie, 1992; Dykman, 1891; Iwu, 1993). It is a woody dioecious perennial climbing vine with a twining stem, shrublet without tendrils and supports itself by twining around the stems of other plants, fence or walls. The leaves are rounded, bright green, almost without hairs. The flowers, which usually sprout between February and May, are axillary with velvety-hairy and greenish in colour. The fruit is inedible, dark, grape-sized berries. The root may be up to 2.5cm in diameter with a grey-brown bark (Botha, 1980). The plant is usually confused with *Zehneria scabra* (Cucurbitaceae), which in some parts is known by the same local name (Smith, 1966).

The latter resembles a cucumber and can be distinguished by the spirally coiled tendrils. Nearly all parts of the plant are used; from the whole vine, seed, bark, leaf to the root. It is traditionally used in South Africa to treat a variety of ailments such as dysentery, menstrual problems, prevention of miscarriage, cholera, colic, snakebite, measles, fever, diabetes, tuberculosis, stomach and skin cancers, etc (Watt and Breyer-Brandwijk, 1962; Rood, 1994; Van Wyk and Gericke, 2000; VanWyk et al., 1997; De Wet and Van Wyk, 2008; Barbosa- Filho et al., 2000; De Wet et al., 2004, 2005). In 1962, it was reported that *C. pareira* demonstrated anti-inflammatory, smooth muscle relaxant, antispasmodic, diuretic effect and uterine relaxant actions in various laboratory animals and this was further documented by Pillay et al. (2008) and Taylor (2005). Ssegawa and Kasenene, (2007) described its antiulcerous actions and mild hypoglycemic activity. Further studies have also shown that the roots described its antiulcerous actions and shown that the roots of cissampelos species have anticonvulsant activity, antioxidant properties, antimicrobial activity, antihypertensive, antimalarial effects, antispasmodic and antitumour properties (Dic. Nat. prod., 1996,2006; Bruneton, 1995; Wu, 2007; Amresh et al., 2007; Amresh et al., 2004; Gessler et al., 1995; Ramirez et al., 2003; Sanchez et al., 2001; Taylor, 2005; Graham et al., 2000; Hamill et al., 2003). The antinociceptive and antiarthritic activity of Cissampelos pareira roots were also highlighted by Amresh et al., (2007) while the antifertility activity and anti-inflammatory activity were described by Ganguly et al. (2007) and Amresh et al. (2007) respectively. The Khoi-San and the Cape Dutch recognised the importance of this plant in Cape herbal medicine as one of the major distinct plants used for the treatment of most diseases (Van Wyk, 2008), while during the evaluation of some South African medicinal plants for antimalarial properties, it was discovered that C. capensis possesses antiplasmodial activity (Pillay et al., 2008). The medicinal values of Cissampelos species in the Sango bay area in Southern Uganda was also recorded by Ssegawa and Kasenene (2007).

Phytochemical screening showed the presence of alkaloids, saponins, flavonoids, phenolics and tannins while cardiac glycosides and anthraquinones were not detected in the different extracts (Babajide et al, 2010). A large number of biologically active alkaloids have been isolated from several Cissampelos species among these were cissampareine and magnoflorine, (Jittra et al., 2005; Freitas et al., 1995). Other alkaloids such as cissaglaberrimine and oxobuxifoline were isolated from Cyathea glaberrima (Barbosa-filho et al., 1997) while Milonine, an 8, 14-dihydromorphinandienone alkaloid was also isolated from the dried leaves of Cissampelos sympodialis (Freitas et al., 1995). Similarly, Sloan et al. (2007) showed the isolation of two known aporphine alkaloids, (S)-dicentrine and (S)-neolitsine from methanolic extract of C. capensis, which were found to be potent anthelmintics. To the best of our knowledge no flavonoidal compounds isolated from C. capensis has been published.

The present study involves a more intense investigation of the constituents present in C. capensis as well as their biological activities.

MATERIALS AND METHODS

General experimental proceduresAll laboratory grade solvents were distilled prior to use. Spectroscopic grade solvents were used as such. Adsorption column chromatography was performed using Merck Silica gel 60 H (0.040 -0.063 mm particle size, Merck).

Unless otherwise specified, a column with internal diameter of 25

*Corresponding author. E-mail: jobras2003@gmail.com. Tel: +27714662735.

mm was used and volumes of 10 ml were collected in Pyrex test tubes. Size-exclusion column chromatography was performed using Sephadex LH-20 (Sigma) pre-swollen in the specified solvent before loading of sample.

Preparative Thin Layer Chromatography (TLC) was performed using Merck Silica gel 60 PF_{254} on glass plates (20 cm x 20 cm) and with a thickness of 0.5 mm. Analytical TLC was conducted on normal-phase Merck Silica gel 60 PF_{254} on precoated aluminium plates. Separated compounds on TLC and Preparative Thin Layer Chromatography (PTLC) plates were visualized under Ultraviolet (UV) light at (254 and 366 nm), and spraying of the plates where required were carried out using 2% vanillin in H_2SO_4 followed by heating at 110°C for 2 to 4 mins. Quercetin, rutin and catechin were used as reference standards for flavonoids, gallic acid for tannins and oleanolic acid for essential oils. In the case of the Alkaloids, the dragendorff spraying reagent was used for visualization while quinine and berberine were used as standards.

Reversed phase TLC (RPTLC) was performed using pre-coated plates (Merck, RP-18. F254 2, 0.25 mm thickness) and spots were detected as described above. R_f values were determined at room temperature using different solvent systems as applicable. Melting points were determined on a Fisher – John's melting point apparatus (Fisher-Scientific). All melting points are uncorrected. Mass spectra were obtained using JEOL JMS-AX505HA double-focusing probe at 70 eV while UV spectra with a Unicam UV 4 -100 UV/Vis recording spectrophotometer. The spectra were recorded over the range of 200 to 550 nm, at a concentration of 0.02 mg/ml in spectroscopic grade methanol. All infra-red (IR) spectra were recorded on a Perkin Elmer universal ATR Spectrum 100 series FT-IR spectrometer while Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AMX-400 (400 MHz for 1H and 100 MHz for 13C) spectrometer, using a triple resonance probe head with self-shielded gradient coils and a Bruker Z-gradient accessory delivery squared gradients. The chemical shifts are expressed in ppm relative to tetramethylsilane (TMS).

Plant

The aerial shoot and root of *C. capensis* were collected in May 2007 from the University of the Western Cape (UWC) Cape Nature Conservation Reserve, Bellville, Cape Town, South Africa, authenticated by a plant systematist and a voucher specimen [number Weitz 1056(UWC)] deposited in the hebarium of the University of the Western Cape. Preparation of the extracts was performed as described by Babajide et al. (2010).

Extraction and isolation

Each of the extracts that is, hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), TTA and residual aqueous fraction (BTA) were separately evaporated under reduced pressure at 40°C while aqueous extracts were concentrated by freeze-drying. The preliminary phytochemical screening (Wagner and Bladt, 2001) was carried out for detection of classes of secondary plant metabolites as shown in Babajide et al. (2010)

Fractionation of the TTA

The TTA (1.6g) was adsorbed on silica gel and the constituents were separated by column gradient chromatography (CGC) using an increasing gradient of Hex and $CHCl_3$ and finally MeOH was employed. The fractions collected were analyzed by TLC using Hex– $CHCl_3$ – MeOH (1: 5 : 1). Tubes showing similar TLC characteristics were bulked together and concentrated. Seven fractions coded A to G were obtained and the bioactivities of each were monitored using the brine shrimp cytotoxicity test. Fraction E (0.30g) with LC_{50} value of 2.4050 was selected for further fractionation. Fraction E (0.25 g) was rechromatographed using CGC to afford five fractions: TI to TV and fractions TIII (0.122g) with an LC_{50} value of 3.0226 and TIV (0.047g) with an LC_{50} value of 0.8655 were further investigated. Repeated preparative TLC of TIII gave compound (1) a brownish yellow powder (0.058 g) and that of TIV afforded compound (2) as a brown amorphous solid (0.021g).

Extraction and fractionation of the aerial shoot

The aerial shoot (1 kg) was sequentially extracted to afford the hexane (17.35g, 1.74%), dichloromethane (26.33g, 2.63%), ethyl acetate (32.84g, 3.28%), methanol (56.24g, 5.62%) and water extracts (25.71g, 2.57%). The methanol extract (25.6g) was chromatographed using same method as described for TTA. Ten fractions coded A to J were obtained and fraction F (1.96g) with an LC_{50} value of 1.2332 was selected for further fractionation which afforded five fractions viz., FI to FV of which FII (0.520g) with LC_{50} of 66.73, FIII (0.211g) with LC_{50} of 3.442, FIV (0.115g) with LC_{50} of 17.56 and FV (0.106g) with LC_{50} of 45.46 were further investigated. Fraction FII (0.5 g) was chromatographed using CGC and was further purified using sephadex LH-20 and repeated preparative TLC. This gave a golden brown powder (0.04g) of compound (3). Fraction FIII (0.20 g) was also rechromatographed and further subjected to repeated preparative TLC to afford a brownish yellow powder which was recrystallized from EtOH to give a further quantity of compound (1) (a brownish yellow powder, 0.028 g) while fraction FIV (0.10 g) after preparative TLC afforded (4) as a yellow amorphous powder (0.026 g) and fraction FV (0.10 g) gave compound (5) as a yellow powder (0.024g).

Extraction and fractionation of the root

The same extraction procedure was repeated for the dried root (1 kg). This afforded a hexane (7.38g, 0.74%), dichloromethane (12.65g, 1.27%), ethyl acetate (27.19g, 2.72%), methanol (53.05g, 5.31%) and water extract (26.44g, 2.64%). The methanol extract (30.0g) was chromatographed as described for the aerial shoot. Out of the five fractions collected, fractions M (5.68g) with LC_{50} value of 5.2375 and N (3.67g) with LC_{50} of 19.296 were selected for further fractionation. The fractionation of M (5.0 g) by repeated CGC and monitoring of their LC_{50} values resulted more in compounds of (1) (0.078g), (2) (0.065g) and (5) (0.088g). Similarly, fraction N (3.2 g) was rechromatographed and accorded more of compound (4) (0.068g) and a new compound viz., (6) (0.135g) a creamy yellow solid was obtained from subfractions NIII (0.73 g) with LC_{50} of 76.112.

Dihydromorphinandienone alkaloid (1)

A reddish yellow powder with m.p. 85 to 87°C; UV λ_{max} nm (log ε): 222, 260, 304; IR ν_{max} cm^{-1} pronounced peaks: 3485, 2940, 1685, 1615, 1500; EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 375.0216 (calculated for $C_{20}H_{25}O_6N$: 375.1682). The ^1H NMR and ^{13}C NMR see Table 1

9, 10 – Dimethoxyaporphine alkaloid (2)

A reddish-brown fluffy powder with m.p. 221 – 223°C; UV λ_{max} nm(log ε): 225, 243, 277, 300 (shoulder). IR ν_{max} cm^{-1} pronounced peaks: 3420, 3050, 2925, 1636, 1458, 1387, 1060. EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 341.0112. (calculated for $C_{19}H_{19}O_5N$: 341.1263). For the ^1H NMR and ^{13}C NMR (Table 2).

Table 1. ^{1}H NMR (400 MHz) data of (**1**) and ^{13}C NMR (100 MHz) data of (**1**) in C_5D_5N.

C	^{13}C	^{1}H
1	146.4	--
2	111.2	6.91 s (1H)
3	148. 4	--
4	145.6	--
5	125.3	--
6	152.2	--
7	194.7	--
8	40.3	ax 3.63 dd (J = 14.2, 17.4 Hz) eq. 2.64 dd (J = 17.4, 4.6 Hz)
9	56.9	2.83 *br* d (J = 5.6 Hz)
10	28.5	ax 3.12 d (J = 17.0 Hz) eq 2.74 dd (J = 17.0, 5.6 Hz)
11	131.2	--
12	128.7	--
13	38.5	--
14	42.7	2.40 m
15	32.3	2.21 – 2.11 m (2H)
16	46.5	2.40 m (1H)/2.30 m (1H)
MeO-1	57.2	3.83 s (3H)
MeO-3	56.5	3.76 s (3H)
MeO-6	55.3	3.69 s (3H)
Me-N	43.1	2.26 s (3H)
HO-4	--	10.75 br s (1H)
HO-5	--	11.50 br s (1H)

8,14-Dihydromorphinandienone alkaloid (3)

A golden brown powder with m.p 77 to 80°C. UV λ_{max} nm (log ***ε***): 226, 261,302; IR (v cm^{-1}) pronounced peaks: 3517; 2952, 1685, 1616, and 1510. EIMS: the molecular ion base peak $(M)^+$ *m/z* 329.2133 (calculated for $C_{19}H_{23}O_4N$: 329.1627). The ^{1}H NMR and ^{13}C NMR (Table 3)

6 -C-methylquercetin -3, 3',7, 8 -tetramethyl ether (4)

A yellow amorphous powder with m.p. 234 to 236°C. UV λ_{max} nm (log ***ε***): 255 (4.64), 357 (4.62); +NaOAc: 271, 365; +NaOH: 276, 343, 410. +$AlCl_3$: 279, 440. IR (v cm^{-1}) pronounced peaks: 3401, 2932, 1653, 1612, 1556 EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 388.0103. (calculated for $C_{20}H_{20}O_8$: 388.1158). ^{1}H NMR: δ 2.14 (3H, s, Me-6), 3.74 (3H, s, OMe-3), 3.96 (3H, s, OMe-3'), 4.01 (3H, s, OMe-7); 4.56 (3H, s, OMe-8), 6.91 (1H, d, J= 8.5 Hz, H-5'), 7.60 (1H, *dd,* J = 2.3, 8.4 Hz, H-6'), 7.70 (1H, d, J= 2.3 Hz, H-2'), 10.90 (1H, s, 4'-OH), 12.92 (1H, s, 5-OH). ^{13}C NMR (Table 4)

Methylenedioxyisoflavanone (5)

A yellow waxy powder with m.p. 185 to 187°C; UV $\boldsymbol{\lambda}_{max}$ nm (log ***ε***): 214(4.10), 218 (4.12), 288 (4.00), 333 (sh) (3.25). IR $\boldsymbol{\nu}_{max}$ cm^{-1} pronounced peaks at : 3421, 2914, 1634, 1472, 1386, 1358, 1257, 1224, 1161, 1100, 1068, 925; EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 378.0625 (calculated for $C_{18}H_{18}O_9$, 378.0623). ^{1}H NMR, ^{13}C NMR and HMBC are as shown in Tables 5 and 6 respectively.

6-C-Methylquercetin 3-methyl ether (6)

A creamy yellow powder with m.p. 217 to 219°C. UV λ_{max} nm (log ***ε***): 260 (4.62), 351 (4.60); +NaOAc: 260, 355; +NaOH: 270, 405; +A1$C1_3$: 277, 435. IR (v cm^{-1}) pronounced peaks: 3500 – 3400, 1652, 1613, 1568, 1556 EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 346.0752 (calculated for $C_{17}H_{14}O_8$: 346.0688). ^{1}H NMR: δ 2.11 (3H, s, Me-6), 3.87 (3H, s, OMe-3), 7.03 (1H, d, J=8.6Hz, H-5'), 7.62 (1H, dd, J= 2.3, 8.6 Hz, H-6'), 7.75 (1H, d, J= 2.3Hz, H-2'), 10.23 (1H, s, 4'-OH), 11.15 (1H, s, 3'-OH).12.05 (1H, s, 8-OH), 12.90 (1H, s, 7-OH), 13.05 (1H, s, 5-OH). ^{13}C NMR: see Table 7.

Brine shrimp lethality biossay

The brine shrimp lethality biossay was carried out as previously described (Babajide et al., 2008, 2010) where about 2 g of the Brine shrimp eggs (*Artemia salina* Leach) were hatched in 2 L of sea water using a large plastic case as an artificially partitioned dam. The number of dead nauplii were counted and recorded (lethality data) after 24 h. The numbers of dead nauplii were used for calculating the LC_{50} at 95% confidence limit by the Finney Probit analysis program. LC_{50} values greater than 800 ppm or in that range were considered inactive.

Antimicrobial activity

The organisms used in the screening tests were as follows: The gram-negative bacteria were *P. aeruginosa* (NCTC 10332), *P. vulgaris* (NCTC 4175) and *E. coli* sero type 1 (NCTC 09001), while the gram-positive bacteria were *B. subtilis* (NCTC 8236), *S. aureus* (NCTC 13134) and *B. licheniformis* (NCTC 01097). These species are considered as the most important pathogens (NCCLS, 1990). Fungal species used were *C. albicans* (ATCC 90028), *C. eropiralis* (ATCC 750) and *A. Niger* (ATCC 10578). Cultures were grown in sabouraud dextrose (SD) broth at 37°C and maintained on SD agar at 4°C. A colony of each bacterial strain was suspended in 1 ml of

Mueller–Hinton broth and incubated for 18 h at 37°C. A subculture was again made after 6 h. The subculture was diluted 1/50 in the same broth before use. A disc diffusion assay was used to determine the inhibition of bacterial growth by the plant extracts, fractions, sub fractions and isolates (Rasoanaivo et al.,1993). Plant extracts, fractions and isolates were dissolved in appropriate solvents at a concentration of 100 mg/ml for extracts and 10 mg/ml for isolates. 20 µl were dispensed on a 9 mm sterile paper disc (Munktell/Lasec, Numb. FLAS3526009). Amoxicillin was used as a positive control (40 µg/ml) for bacteria while Fluconazole (120 µg/ml) was used for fungi. The diluted cultures were spread on sterile Muller–Hinton agar plates. The plates were then incubated at 37°C for 18 to 24 h for bacterial pathogens and 3 days for fungal pathogens. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated. Antimicrobial inhibition activities measured were compared to that of Amoxicillin and Fluconazole standards for antifungal (Vlietinck, 1997).

DISCUSSION AND RESULTS

Aerial shoots and roots of *C. capensis* were used in this study. Extractions and isolations were specifically targeted for alkaloids and flavonoids indicated by the preliminary phytochemical screening results (Babajide et al., 2010) where high concentrations of flavonoid and alkaloids in the methanolic extracts of both the root and the aerial shoot were noted. A total of 6 compounds, viz-1 to 6 were isolated from the fractionation of the TTA, methanolic extract of the aerial shoot and root. The TLC investigations of these 6 compounds revealed 1, 2 and 3 to be alkaloids as demonstrated by their positive test to dragendorff reagent using berberine as marker, while 4, 5 and 6 were flavonoids due to their positive test to vanillin in sulphuric acid using quercetin as a marker.

Compound 1 was obtained as a reddish-yellow powder with melting point of 85 to 87°C. The UV spectrum had a λ_{max} values at 222 nm known to be associated with α,β-unsaturated carbonyl chromophore (Stuart, K.L., 1971) and that at 260 nm is typical for an aromatic ring system. The shoulder observed at 304 nm is a general characteristic of 8,14-dihydromorphinandienone alkaloids (Freitas et al., 1995). The IR spectrum showed pronounced bands at 3485 cm^{-1} (hydroxyl group), 2940 cm^{-1} (C-H of an olefinic group), 1685 cm^{-1} (α, β-unsaturated carbonyl), 1615 cm^{-1} (C=C olefinic) and 1500 cm^{-1} (C=C aromatic). The El mass spectrum indicated the molecular formula to be $C_{20}H_{25}NO_6$ from the peak observed at M^+ (375). The following fragments were observed: 360 $(M- CH_3)^+$, 332 $(360 – CO)^+$, 238 $(M – C_8H_90_2)^+$ and 42 $(H_2C=C=O)^+$, being characteristic of the morphinandienone alkaloids (Wheeler et al.,1968). The complete analysis of ^1H NMR, ^{13}C NMR, HMBC, COSY and NOESY were used in assigning the structure of 1. The ^1H NMR (400 MHz, pyridine- d_5) showed only one aromatic signal at δ6.91 which implies that the aromatic ring is penta-substituted. The four singlets integrated for three protons each were observed at δ 2.26 for (N-Me), with the other three at δ3.69 for MeO-6, 3.76 for MeO-3 and 3.83 for MeO-1. The two single proton signals at 10.75 and 11.50 were attributable to HO-4 and HO-5 respectively.

The ^{13}CNMR (100MHz, pyridine-d_5) similarly showed a signal at δ194.7 (s, C-7) for the α,β-unsaturated carbonyl, δ43.1 (s, N-Me), δ55.3 (s, C-6-OMe), δ56.5 (s, C-3-OMe) and δ57.2 (s, C-1-OMe). The complete assignments of all the protons and carbons are given in Table 1. This data was found to be in agreement with the literature (Blasko and Cordell, 1988; Vecchietti et al., 1981) thus confirming compound 1 to be 5,6-dehydro-4,5-dihydroxy-1,3,6-trimethoxy- 17-methylmorphinan-7-one (Figure 1). Compound 1 to the best of our knowledge, has not been reported previously.

Compound 2 was obtained as a reddish-brown fluffy powder with m.p of 221 to 223°C. Its UV spectrum showed absorptions at 225, 243, 277 and 300 (shoulder) nm which are characteristic of 1, 2, 3-trisubstituted aporphinoid systems (Guinaudeau et al., 1979; Guinaudeau and Bruneton, 1993). The IR spectrum showed bands at 3420, 3050, 2925, 1636, 1458, 1387 and 1060 cm^{-1} confirming the aporphinic skeleton of 2. The EIMS showed an $[M]^+$ at 341.0112 which suggested the molecular formula of $C_{19}H_{19}O_5N$. ^{1}HNMR, ^{13}CNMR, HMBC, COSY and NOESY spectra were used in assigning the structure. The ^1H NMR (400 MHz, C_5D_5N) spectrum showed an AB set of doublets at δ5.94 and 6.13 (J = 1.2 Hz), characteristic of a methylenedioxy group at positions C-1 and C-2 in aporphines (Guinaudeau and Bruneton, 1993). The presence of an ABCD tetra substituted ring system with signals at δ 7.31 (br s) and 8.32 (br s) is a strong indication that ring D (the dimethoxysubstituted ring) is disubstituted. The ^{13}C NMR (100 MHz, C_5D_5N) spectrum showed a signal at δ101.3 (s, C-1, 2 methylenedioxy). In the ^1H - ^1H NOESY spectrum one of the methylenedioxy protons (δ 6.13) showed a correlation with H-11 (δ8.32) while H-8 (δ7.31) correlated with the H-7 methylene protons at (δ2.95-3.10). Complete assignments of all protons and carbon atoms are listed in Table 2. The spectroscopic analysis above established the structure of 2 as 1, 2-methylenedioxy-3- hydroxy -9, 10-dimethoxyaporphine (Figure 2) and to the best of our knowledge it is being reported for the first time.

Compound 3 was isolated as a golden brown powder with m.p of 77 to 80°C and runs as a single spot in Hex: $CHCl_3$: MeOH (3:10:2). This spot showed a characteristic orange colour of an alkaloid that is closely similar to the berberine standard TLC plate marker used when sprayed with dragendorff reagent. The IR spectrum showed a pronounced band at 3517 cm^{-1} due to the presence of a hydroxyl group while that at 2952 cm^{-1} was assigned to C-H of an olefinic system. The band at 1685 cm^{-1} is a characteristic of an α,β-unsaturated carbonyl system as observed for **1**, the band at1616 cm^{-1} is due to the olefinic C=C while that at 1510 cm^{-1} is due to the aromatic

Table 2. ^{1}H NMR (400 MHz) data of (**2**) and ^{13}C NMR (100 MHz) data of (**2**) in C_5D_5N.

C	δ ^{13}C	^{1}H
1	144.8	-
2	134.3	-
3	140.2	-
3a	118.0	-
4	25.3	2.92-3.12 2H m
5	44.1	2.92-3.12 1Hm, 3.45 1H m
6a	54.7	4.06 1H dd, (J = 5.2, 12.4 Hz)
7	37.1	2.95-3.10 2H m
7a	135.6	-
8	128.9	7.31 1H (br s)
9	160.2	-
10	161.9	-
11	127.1	8.32 1H (br s)
11a	133..7	-
11b	107.1	-
11c	130.2	-
O-CH_2-O	101.3	5.94, 6.13 2H A-B *qJ* = 1.2 Hz
MeO-9	56.5	3.76 s (3H)
MeO-10	55.3	3.69 s (3H)
HO-3	-	10.75 *br s* (1H)

C=C bond. Its UV spectrum showed absorptions at 226 nm which is known to be characteristic of an α,β-unsaturated carbonyl chromophore and at 261 nm due to an aromatic ring while the band at 302 nm is a characteristic of the 8,14-dihydromorphinandienone alkaloids as was noticed for **1** (Stuart, K.L., 1971). The El mass spectral peak of 329.2133 suggested a molecular formula of $C_{19}H_{23}O_4N$ for **3,** for which the following fragments were observed: - (1) 314 $[M- CH_3]^+$, (2) 286 $[314 – CO]^+$, (3) 192 $[M – C_8H_9O_2]^+$ and (4) 42 $[H_2C=C=O]^+$ confirming the characteristics of a morphinandienone alkaloid as observed for **1** (Wheeler et al., 1967). The ^{1}H NMR spectrum showed an AB pair of doublets at δ 6.90 and 6.78 (J = 8.4 Hz) for H-2 and H-1 respectively, while the three 3-proton singlets at δ 2.28 (N-Me), δ 3.62 (MeO-6) and δ 3.74 (MeO-3) (with similar numbering system as shown in 1) were assigned as indicated. The singlet at δ 10.72 was attributable to HO-4. The ^{13}C NMR spectrum showed 19 carbon signals which supported the calculated molecular formula of $C_{19}H_{23}O_4N$. The carbons were assigned as shown in Table 3 where δ 43.3 is for N-Me, δ 55.2 for C6-OMe, δ 56.6 for C3-OMe and δ 194.5 for the C7-C=O. Complete assignments of all the protons and carbons are given in Table 3.

The structure of compound 3 was therefore established to be 5, 6-didehydro-4-hydroxy-3, 6-dimethoxy-17-methylmorphinan-7-one as shown in Figure 3. Literature reveals that 3 has been previously isolated from *Croton* (Vecchietti et al., 1981) and some *Cissampelos* species such as *C. sympodialis* (Freitas et al., 1995) and *C. pareira* (Amresh et al., 2007). This is however the first time that the alkaloid has been reported as found in *C. capensis*.

Compound 4 was a yellow amorphous powder with a m.p of 234 – 236^{o}C. It showed EI mass spectral peak $(M)^{+\cdot}$ at *m/z* 388.0103 suggesting a molecular formula of $C_{20}H_{20}0_8$. The IR spectrum showed pronounced bands at 3401 cm-^{1} (hydroxyl group), 2932 cm-^{1} (C-H olefinic), 1653 cm-^{1} (carbonyl group), 1612 cm-^{1} (C=C olefinic) and 1556 cm-^{1} (C=C aromatic). The UV spectrum showed two peak maxima at 357 and 255 nm respectively. The band at 271 nm showed a large bathochromic shift in the presence of sodium acetate, and an additional band appeared at 343 nm in the methanol NaOH spectrum which suggested a flavonol with a 7-OMe group. The ^{1}H NMR spectrum showed an aryl methyl singlet at δ 2.14 and four distinct methoxyl singlet peaks at δ 3.74 (OMe-3), 3.96 (OMe-3'), 4.01 (OMe-7) and 4.56 (OMe-8). The spectrum further showed the presence of only three 1-proton aromatic signals at δ 6.91 (d, J = 2.3 Hz), 7.60 (dd, J = 8.5 and 2.3 Hz) and 7.70 (d, J = 8.5 Hz) suggesting a 3',4'-disubstituted aromatic ring C, and a fully substituted aromatic ring A. The characteristically chelated 5-OH group was observed at δ 12.92 while that of 4'-OH was observed δ 10.90. By careful comparison of the ^{13}C NMR data of 4 with existing literature (Roitman and James, 1985; Breitmeier and Voelter, 1990; Babajide et al., 2008), the 4'-substituent was assigned to a hydroxyl

Table 3. ^{1}H NMR (400 MHz) data of (**3**) and ^{13}C NMR (100 MHz) data of (**3**) in C_5D_5N.

C	^{1}H	NOESY	HMBC
1	119.2	6.78 br d (J = 8.4 Hz)	C10, C12, C3
2	112.0	6.90 d (J = 8.4 Hz)	C1, C11, C4
3	147.2	-	-
3	145.6	-	-
4	125.3	-	-
5	8.30 s	-	C14, C12, C7 C6
6	152.2	-	-
7	194.5	-	-
8	40.5	ax 3.64 dd (J = 14.0, 17.6 Hz) eq. 2.60 dd (J = 17.6, 4.4 Hz)	C14, C7 C13
9	57.4	2.80 *br* d (J = 5.8 Hz)	C10, C14 C13, C14, C16, C11
10	28.4	ax 3.12 d (J = 17.2 Hz) eq 2.75 dd (J = 17.2, 5.8 Hz)	C9, C11 C14, C1, C12
11	132.2	-	-
12	128.2	-	-
13	38.5	-	-
14	42.0	2.40 m	-
15	33.2	2.22 - 2.12 m (2H)	C16 C12
16	47.6	2.42 m (1H)/2.30 m (1H)	C15 C13, C9
MeO-3	56.6	3.74 s (3H)	C3
MeO-6	55.2	3.62 s (3H)	C6
Me-N	43.3	2.28 s (3H)	C16, C9
HO-4	10.72 br s (1H)	-	-

hydroxyl group while that at the 3' position was assigned as a methoxyl group. Corroboration of this deduction was evident from chemical shifts of the substituted carbons, viz., C-3' at δ 147.2 and C-4' at 148.5. The other methoxyl groups were attached at C-3 (δ 137.5), C-7 (δ 145.4) and to C-8 (δ at 149.5 ppm which was in agreement with the HMBC correlations OMe-3/C-3 and OMe-7/C-7 and OMe-8/C-8 (Table 4).

The only non-oxygenated methyl group present was assigned as being attached to C-6, (which appeared at 106.6 ppm in the ^{13}C spectrum). Other peaks as demonstrated by the HMBC cross peaks were OH-5/C-5, C-6, C-4a; Me-6/C-5, C-6, C-7. Other carbon peaks were similarly assigned as shown in Table 4. Based on the above information the structure of 4 was suggested to be 3,7,8,3'-tetramethoxy-6-*C*-methyl-5,4'-dihydroxyflavone (6-*C*-methylquercetin 3, 3',7, 8 -tetramethyl ether) as shown in Figure 4 and to the best of our knowledge it is being reported for the first time.

Compound 5 was obtained as a yellow waxy powder with m.p of 185 to 187°C. A molecular formula of $C_{18}H_{18}O_9$ was suggested based on the molecular ion peak obtained at *m*/*z* 378.0625 and the fragmentation pattern in the EIMS. An exhaustive comparative study of the data obtained, revealed the general features of an isoflavanone nucleus (Mabry et al., 1970; Markham and Chari, 1982; Rahman and Gray, 2005; Rahman et al., 2007). The UV absorption spectrum with maxima at 333 and 288 nm suggested an isoflavonoid. The IR spectrum had a strong absorbance at 3421 cm^{-1} due to an O-H group present, 2914 cm^{-1} for the C=C-H group, and 1634 cm^{-1} for the C=O system. The ^{1}H NMR spectrum showed an ABC pattern of 1-proton signals viz., *δ* 4.69 (*t*, *J* = 11.0 Hz), *δ* 4.50 (*dd*, *J* = 11, 5.6 Hz) and *δ* 4.43 (*dd*, *J* = 11.0, 5.6 Hz) assigned to the H-2 and H-3 protons respectively, which was similar to that observed in Markham and Chari's work as reported in 1982 for an isoflavanone system. The ^{1}H NMR spectral data (Table 5) also exhibited three H-bonded groups viz., C-5 hydroxyl at *δ* 12.89, C-7 hydoxyl at 12.98 and C-8 hydroxyl at *δ* 13.02. Two 1-proton singlets at δ 6.52 and *δ* 6.80 were assigned to H-6 of ring A and H-6′ of ring C of the isoflavanone. The presence of 2 methoxyl groups as well as methylenedioxy group were apparent from their signals at *δ* 3.96 (2′-OMe), 3.87 (5′-OMe) and *δ* 5.90 (–OCH_2O–) respectively. The ^{13}C NMR spectrum demonstrated that compound 5 contained a total of 18 carbons including a carbonyl group as indicated by the IR absorption peak at 1634 cm^{-1}. The assignment of all carbons and the placement of the 2-methoxyl and methylenedioxy groups within the molecule were achieved by 2D NMR spectroscopic analysis. The HMBC data (Table 6) showed correlation of the C-5 hydroxyl group with a methine at *δ* 97.7 and a quaternary carbon at *δ* 103.3 which were assigned to C-6 and C-4a respectively.

The signals at *δ* 164.7, 168.6 and 169.3 were assigned to C-8a, C-7, and C-8 respectively as shown in Table 5.

Table 4. NMR spectroscopic data for compound (4).

13C signal (δ)	DEPT	1H – 13C COSY	HMBC	^{13}C Assignment
155.5	C	-	H-2', H-6'	2
137.5	C	-	3-Ome	3
178.8	-	-	-	4
156.3	C	-	-	5
106.6	C	-	-	6
145.4	C	-	7-Ome	7
149.5	C	-	C-7, C-9, C-10	8
151.4	C	-	8-Ome	8a
103.9	C	-	5-OH	4a
121.6	C	-	H- 5'	1'
115.3	CH	7.70d (J = 2.3 Hz)	H-6'	2
147.2	C	-	3'-OMe, H-5'	3'
148.5	-	-	H-5', H-6'	4'
115.8	CH	6.91d (J = 8.5 Hz)	-	5'
120.6	CH	7.60dd (J = 2.3 and 8.4 Hz)	H-2'	6'
59.5	CH_3	3.74 s	C-5, C-6	3-OMe
60.2	CH_3	4.01 s	C-7	7-Ome
63.5	CH_3	4.56 s	C-8	8-OMe
56.5	CH_3	3.96 s	C-3'	3'-Ome
8.1	CH_3	2.14 s	-	6-Me

Correlation of the methoxyl protons with the carbons at *δ* 142.5 and 145.6 in the HMBC data proved the presence of C-2′ and C-5′ methoxy groups at these positions. The methylenedioxy group exhibited J_3 correlation with carbons at *δ* 137.8 and 149.8 and the occurrence of a J_3 correlation of H-6′ to *δ* 149.8 suggested location of the methylenedioxy between C-3′ and C-4′. The assignment of C-6′ at *δ* 125.1 was also confirmed by the coupling observed in the HMQC which suggested the methine carbon at *δ* 48.5 as C-3 as observed in the J_3 for H-6′. Furthermore, the peak at *δ* 71.1 was assigned to C-2 as it showed a J_2 correlation to H-3 in the HMBC spectrum, and direct coupling with H-2 in the HMQC spectrum. The COSY spectrum showed correlation between H-3 and the two non-equivalent protons at C-2. The large diaxial coupling (*J* = 11.0 Hz) observed between H-3 and H-2 placed the aryl substituent at C-3 in the pseudo-equatorial position. Based on the above information the structure of compound 5 was therefore suggested to be 5,7,8-trihydroxy-2′,5′-dimethoxy-3′,4′methylenedioxy-isoflavanone (Figure 5) and to the best of our knowledge it is being reported for the first time.

Compound 6 was isolated as a creamy yellow powder was assigned as $C_{17}H_{14}0_8$ based on the molecular ion peak at *m/z* 346.0752. The UV spectrum with maxima at 351 and 260 nm suggested a flavonol with a 7-OH group. A deep broad band observed in the IR spectrum at 3500 to 3400 cm^{-1} was indicative of the presence of a hydrogen-bonded hydroxyl groups. The ^{1}H NMR spectrum exhibited two 3-proton singlets assignable to one aryl methyl group at δ 2.11 (Me-6), very similar to that of 4 and one methoxyl group at δ 3.87 due to 3-OMe. The aromatic region showed three 1-proton signals typical of a 3', 4'-disubstituted ring C similar to that observed in 4 viz., δ 7.03 (d, J = 2.3 Hz), δ 7.62 (dd, J = 8.6 and 2.3 Hz) and δ 7.75 (d, J = 2.3 Hz). A broad cluster of 1-proton singlets at δ 10.23 (4'-OH), 11.15 (3'-OH), 12.05 (8-OH), 12.90 (7-OH) and 13.05 (5-OH) were due to five OH groups. The ^{13}C spectrum (Table 7) demonstrated a pattern similar to that of compound 4, in which the C ring is di-substituted, carrying two hydroxyl groups at C-3' (163.6) and C-4'(164.5). The HMBC data for ring A was expected to be slightly different from that of 4 with the C-7(159.2) position showing the presence of an OH group. The HMBC cross peaks observed for OH-5/C-5, C-6, C-4a and Me-6/C-5, C-6, C-7, OH-8/C-7, C-8, C-8a indicated that the OH groups were located at C-7 and C-8 due to their respective coupling effects. The location of the single available methoxyl group and methyl group at C-3 and C-6 respectively, were further verified by their HMBC correlations with C-3 and C-6 respectively. Consequently the structure of 6 was with m.p of 217 to 219°C and the molecular formula of 6 assigned as 3-methoxy-6-*C*-methyl-3',4',5,7,8 -pentahydroxyflavone (6 -*C*- methylquercetin -3-methyl ether) (Figure 6). Compound 6 has, to the best of our knowledge, not been reported previously. It should be noted that there were several other extracts, fractions and subfractions that were not further analyzed due to insufficient quantities and time.

Table 5. ^{1}H NMR (400 MHz) data of (**5**) and ^{13}C NMR (100 MHz) data of (**5**) in C_5D_5N.

Position	^{1}H	^{13}C
2	4.69, *t*, *J* = 11.0 Hz	71.1
-	4.50, *dd*, *J* = 10.5, 5.6 Hz	-
3	4.43, *dd*, *J* = 11.0, 5.6 Hz	48.5
4	-	197.6
5	-	166.0
6	6.52, s	97.7
7	-	168.6
8	-	169.3
8a	-	164.7
4a	-	103.3
1′	-	121.4
2′	-	142.5
3′	-	137.8
4′	-	149.8
5′	-	145.6
6′	6.80, s	125.1
5-OH	12.89, *br. S*	-
7-OH	12.98, *br. S*	-
8-OH	13.02, *br. S*	-
2′-OMe	3.96, *s*	59.6
5′-OMe	3.87, s	60.2
$–OCH_2O–$	5.90, *s*	102.0

Table 6. HMBC data (400 MHz, C_5D_5N) of (**5**).

Proton	(**5**) (^{1}H - ^{13}C)	
	J_2	J_3
H-2	C-3	C-8a, C-4, C-1′
H-3	C-2, C-4, C-1′	C-2′, C-6′
H-6	C-5, C-7	C-4a, C-8
H-6′	C-5′	C-3, C-2′, C-4′
HO-5	C-5	C-6, C-4a
HO-7	C-7	C-6, C-8
HO-8	C-8	C-7, C-8a
MeO-2′	-	C-2′
MeO-5′	-	C-5′
$–OCH_2O–$	-	C-3′, C-4′

Brine shrimp lethality bioassay

The brine shrimp lethality bioassay as described earlier was performed on all the extracts and fractions as shown in Babajide et al. (2010) and also on the isolates (1 to 6) estimating their LC_{50} with 95% confidence. As shown in Table 8, where three test bands were used viz., Inactive (LC_{50} > 700), active (LC_{50} < 700) and very active (LC_{50} < 10). The results demonstrated that all the hexane extracts showed no activity. Clearly evident was the finding that the highest bioactivity appeared in the methanolic extracts, both in the root and the aerial shoot of this plant. The TTA showed a very high active value of 0.3155 (Table 8) thus indicating better activity than the BTA with a value of 64.87. This could be correlated with the isolation of the highly active compound 1 from the TTA. All the extracts and isolates (1, 3 and 5) that were very active should be considered as good candidates for antimicrobial and antiviral agents, since the lethality of a test substance to brine shrimp nauplii has been linked to the possible ability of such substances to kill cancer cells (antitumor activity) and possession of anti-inflammatory

Table 7. The NMR Spectroscopic Data for Compound (**6**).

^{13}C Signal (δ)	HMBC	^{1}H (δ, J Hz)	^{13}C assignment
155.0	H-2', 6'	-	2
137.3	3-OMe	-	3
177.9	-	-	4
157.1	5-OH, Me-6	-	5
107.3	5-OH, Me-6	-	6
159.2	Me-6, 8-OH	-	7
162.8	-OH, Me-6	-	8
154.4	8-OH	-	8a
104.8	5-OH	-	4a
120.4	H- 5'	-	1'
115.4	H-6'	7.75 (1H, d, 2.3)	2'
163.6	H-2', H-5'	-	3'
164.5	H-2', H-5', H-6'	-	4'
115.4	-	7.03 (1H, d, 8.6)	5'
120.8	H-2'	7.62 (1H, dd, 2.3 and 8.6)	6'
7.2	-	2.11 (3H, s)	6-Me
59.2	-	3.87 (3H, s)	3-OMe

Table 8. Brine shrimp lethality test (average number dead counted after 24 hours).

Test materials	Vials at 1000 µg/ml	Vials at 100 µg/ml	Vials at 10 µg/ml	LC_{50}	General remarks
CCRH	1	0	0	3526.22	Inactive
CCRM	10	10	9	3.75	Very active
-	-	-	-	-	-
CCAH	4	3	1	*	Inactive
CCAM	10	10	8	6.1523	Very active
-	-	-	-	-	-
TTA	10	10	9	0.3155	Very active
BTA	7	5	3	64.87	Active
-	-	-	-	-	-
1	10	10	10	0.8229	Very active
2	9	6	3	320.66	Active
3	10	9	7	6.201	Very active
4	8	6	3	199.55	Active
5	10	9	6	3.234	Very active
6	4	1	0	1159.62	Inactive

*****Data did not converge and therefore could not be regressed by the finnery probit analysis programme.
CCRH = *C. capensis* root nHex extract; CCRM = *C. capensis* root MeOH extract; CCAH = *C. capensis* aerial shoot nHex extract; CCAM = *C. capensis* aerial shoot MeOH extract; TTA = *C. capensis* aerial shoot nHexane extract; BTA = *C. capensis* aerial shoot neutralized aqueous residual fraction (Babajide et al., 2010)

properties (Mc Laughlin et al., 1991; Farthing, 2000). However, this does not mean that the inactive compounds (6 and hexane extracts) would not be useful for some other purposes such as a good antioxidant agents.

Antimicrobial activity evaluations

The antimicrobial evaluation was carried out using the diffusion method in which three organisms were used in each case. For gram-negative bacteria: *P. aeruginosa*, *P. vulgaris* and *E. coli* Sero type 1 were used while for gram-positive, *B. subtilis*, *S. aureus* and *B. licheniformis* were used. The fungal species used were *C. albicans*, *C. eropiralis* and *A. niger*. The assay was set up as described earlier. The inhibition zones for both the bacteria and the fungi were measured in triplicate for all the extracts and the 6 identified isolates. The average was

Table 9. Antimicrobial profile of *Cissampelos capensis.*

Parameter	Psa	Prv	Esc	Bas	Sta	Bal	Caa	Cae	Asn
CCRH	0	0	0	0	0	0	0	0	0
CCRM	32	28	28	43	35	27	30	36	23
CCAH	0	0	0	0	0	0	0	0	0
CCAM	25	21	20	35	40	44	23	22	19
TTA	36	38	37	45	40	36	38	33	35
BTA	15	17	17	23	28	24	30	32	29
1	41	34	29	32	30	33	0	0	0
2	16	19	19	22	19	14	19	15	17
3	18	0	26	19	0	18	10	11	11
4	10	13	11	12	10	11	0	0	14
5	15	13	19	22	28	30	35	29	27
6	10	11	15	0	0	21	20	26	29
Amx	61	44	49	42	53	47	0	0	0
Flu	0	0	0	0	0	0	46	53	39

*Gram –ve bacteria: - *Pseudomonas aeruginosa* (Psa) , *Proteus vulgaris* (Prv) and *Escherichia coli* (Esc) Gram +ve bacteria: - *Bacillus subtilis* (Bas), *Staphylococcus aureus* (Sta) and *Bacillus licheniformis* (Bal) Fungi: - *Candida albicans* (Caa), *Candida eropiralis* (Cae) and *Aspergillus niger* (Asn) Amx:- Ammoxicilin and Flu:- Fluconazole.

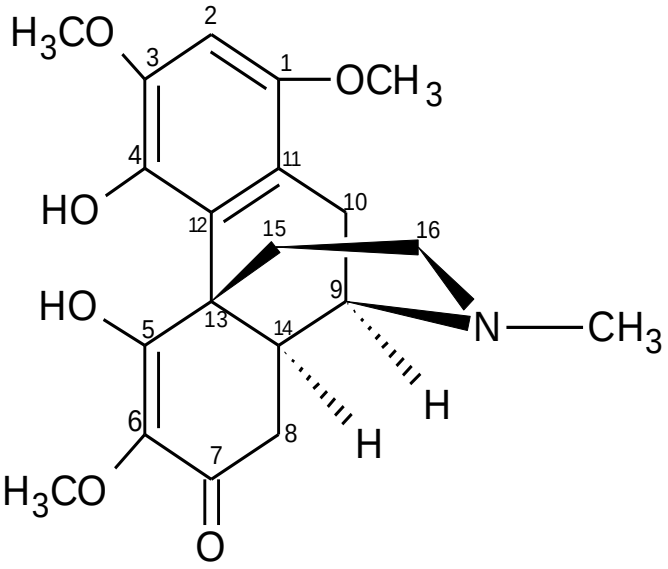

Figure 1. (1) 5, 6-dehydro-4,5-dihydroxy-1,3,6-trimethoxy- 17-methylmorphinan-7-one.

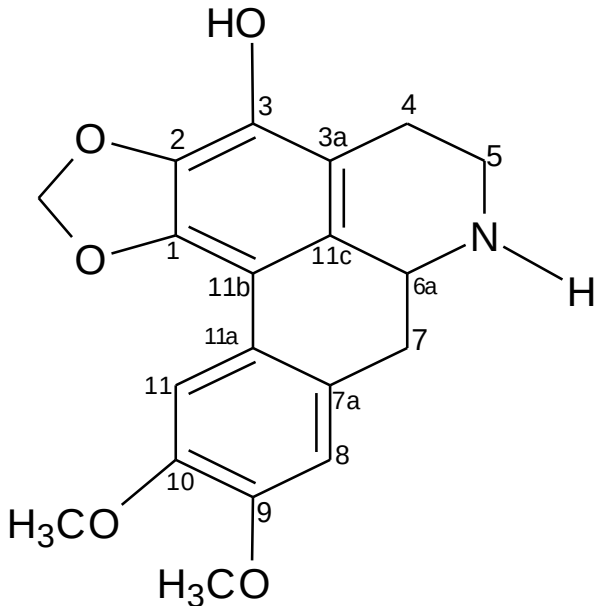

Figure 2. (2) 1,2-methylenedioxy-3- hydroxy -9,10-dimethoxyaporphine .

determined and compared to the positive controls used (Amoxicillin; 40µg/ml for bacteria and Fluconazole; 120µg/ml for fungi). Similar patterns were observed in the microbial analysis except in very few cases as shown in Table 9. The selection of the three (3) sets of test organisms was designed to allow for the study of a reasonably wide spectrum of antimicrobial activities of the extracts and isolates.

The results showed different levels of activity for both the extracts and the isolates. Any value > 20 mm is assumed to be moderately active. There was more antibacterial than antifungal activity. The highest activity was recorded for the gram +ve bacteria while that of gram –ve bacteria was more than the fungi. The maximum zone (45 mm) of antibacterial activity was observed in the TTA fraction against the gram +ve organism *B. subtilis.* This may be due to the strong antimicrobial and antiviral activities of the alkaloids in the plant that is well known for the treatment of malaria, fungal infections,

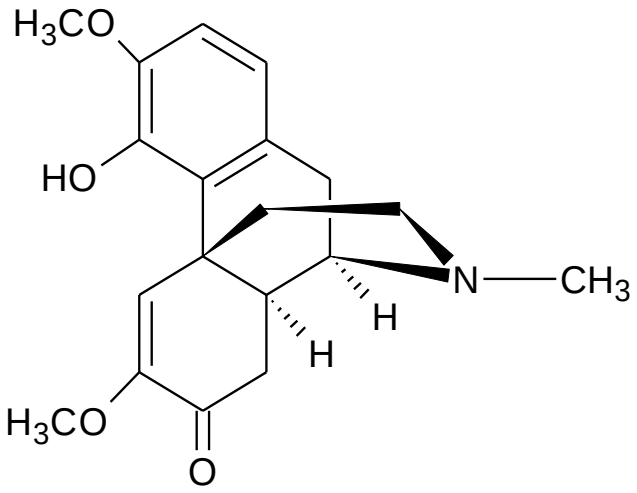

Figure 3. (3) 5, 6-didehydro-4-hydroxy-3, 6-dimethoxy-17-methylmorphinan-7-one.

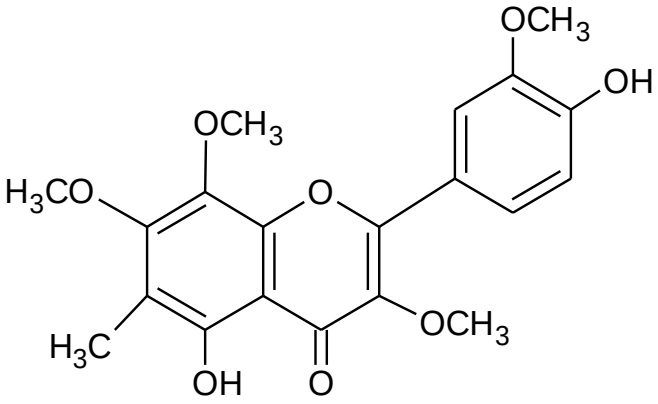

Figure 4. (4) 6 -C-methylquercetin 3, 3', 7, 8 -tetramethyl ether.

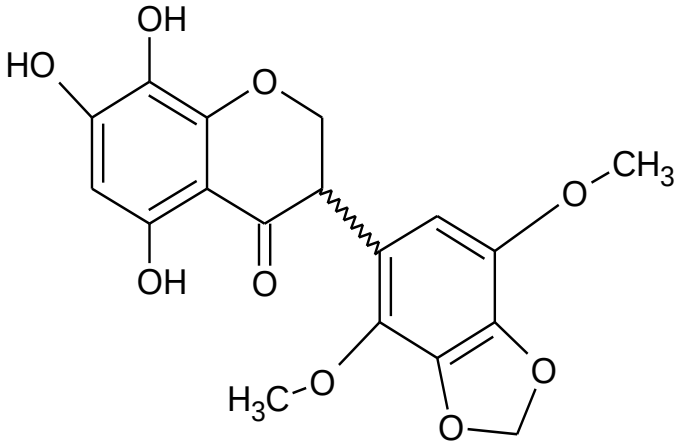

Figure 5. (5) 5, 7, 8- Trihydroxy-2′5′-methoxy-3′, 4′-methylenedioxyisoflavanone.

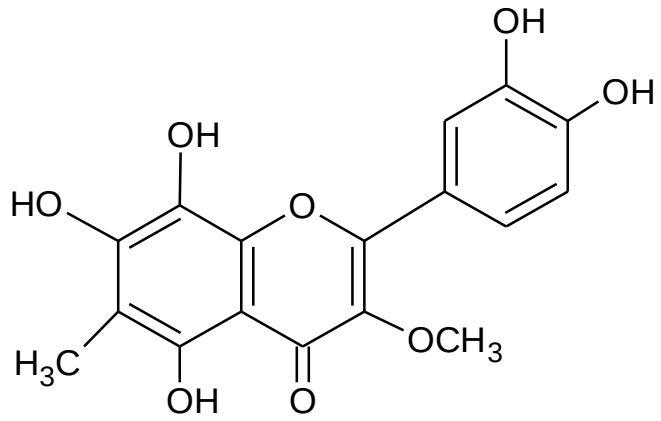

Figure 6. (6) 6-C-Methylquercetin 3-Methyl ether.

inflammations and cancer (Kaur et al., 2009; Mc Gaw et al., 2000). No activity was recorded for the hexane extracts against all nine organisms. High values of activity were recorded for the drug resistant breed of bacteria *P. aeuruginosa*, where an inhibition zone of 36 mm was recorded for TTA, 25 mm for CCAM and 32 mm for CCRM.

Some preferential selective activity was observed in which the compound preferentially act on certain selected organism were inactive against the other set (Babajide et al., 2010; Shai et al., 2008). Similar observations were made in the isolated compounds, where high values were recorded for 1 against gram +ve and –ve bacteria whereas there was no effect on all the three fungi. High fungicidal effect was recorded for 5 while the activities were generally low for compound 4. The observed biological activities of extracts from this plant may thus be due to the presence of both alkaloids and flavonoids, as these compounds are generally known to exhibit a wide range of activities which include anti inflammatory, antithrombotic, anticancer and antiviral, where some of these may be associated with their ability to scavenge free-radicals (Babajide et al., 2008; Shai et al., 2008; Rahman et al., 2007; Liu et al., 1990).

Conclusion

This study successfully relates the specific constituents present in *C. capensis* to its widespread ethnomedicinal uses. The results provide a useful profile of active compounds for the development of useful and promising anticancer, antimicrobial and antioxidant agents in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Akerele O (1988). Medicinal plants and primary health care: an agenda for action. Fitoterapia 59(5):355-363.

Amresh G, Rao CV, Singh PN (2007a). Antioxidant activity of *Cissampelos pareira* on benzo(a)pyrene-induced mucosal injury in mice. Nutri. Res. 27:625-632.

Amresh G, Reddy GD, Rao CV, Singh PN (2007b). Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. J. Ethnopharmacol. 110:526-531.

Amresh G, Reddya GD, Rao CV, Shirwaikar A (2004). Ethnomedical value of *Cissampelos pareira* extract in experimentally induced diarrhea. Acta Pharm. 54(1):27-35.

Amresh G, Singh PN, Rao CV (2007c). Antinociceptive and antiarthritic activity of *Cissampelos pareira* roots. J. Ethnopharmacol. 111:531-536.

Babajide JO, Babajide OO, Daramola AO, Mabusela WT (2008). Flavonols and an oxychromonol from *Piliostigma reticulatum*. Phytochemistry 69:2245-2250.

Babajide JO, Mabusela WT, Green IR, Ameer F, Weitz F, Iwuoha EI (2010). Phytochemical screening and biological activity studies of five South African indigenous medicinal plants. J. Med. Plants Res. 4(18):1924-1932.

Barbosa-Filho JM, Ca-Cunha EVL, Gray AI (2000). Alkaloids of the Menispermaceae. In: Cordell GA (Ed.), The Alkaloids, v. 54. Academic Press, Illinois, USA. pp. 1-190.

Barbosa-Filho JM, Da-Cunha EVL, Cornelio ML, Silva Dias CD, Gray AI (1997). Cissaglaberrimine, an aporphine alkaloid from *Cissampelos glaberrima*. Phytochemistry 44:959-961.

Blasko G, Cordell GA (1988). Morphinandienone Alkaloids. Heterocycles 27(5):1269-1300.

Botha DJ (1980). The identity of *Antizoma harveyana* Miers ex Harv. and *A. capensis* L.f. Diels. J. South Afr. Bot. 46(1):1-5.

Breitmeier E, Voelter W (1990). Carbon-13 NMR Spectroscopy, VCH, New York. P 451.

Bruneton J (1995). Pharmacognosy and Phytochemistry of Medicinal Plants, second ed. Intercept Ltd., Hampshire. pp. 385-386.

Christophersen C, Larsen C, Dimayuga RE (1991). Traditional Medicine-A potential resource exploitation of natural products. The H.C. Orsted institute, Copenhagen. pp. 8-12.

Cillie AM (1992). Kruie op Witblits, Rate, Respte en Feite. Unpublished notes, Worcester Museum.

De Wet H (2006). An ethnobotanical and chemotaxonomic study of South African Menispermaceae. PhD Thesis, University of Johannesburg.

De Wet H, Van Heerden FR, Van Wyk BE (2004). Alkaloids of *Antizoma angustifolia* (Menispermaceae). Biochem. Syst. Ecol. 32:1145-1152.

De Wet H, Van Heerden FR, Van Wyk BE (2005). Alkaloids of *Antizoma miersiana* (Menispermaceae). Biochem. Syst. Ecol. 33:799-807.

De Wet H, Van Wyk BE (2008). An ethnobotanical survey of southern African Menispermaceae. South Afr. J. Bot. 74:2-9.

Dictionary of Natural Products (1996). Release 4:2. Chapman and Hall, London (CD289 ROM).

Dictionary of Natural Products on CD-ROM (2006). Chapman Hall, CRC Press, Hampden Data Services, Ltd.

Dykman EJ (1891). Kook-Koek- en Resepte Boek. Paarl: Paarlse Drukpers Maatskappy.

Farthing MJ (2000). Diarrhoea: a significant worldwide problem. Inter. J. Antimicrob. Agent 14:65-69.

Freitas MR, Alencar JL, Da-Cunha EVL, Barbosa-filho JM, Gray AI (1995). Milonine, an 8,14-dihydromorphinandienone alkaloid from leaves of Cissampelos sympodialis. Phytochemistry 40(5):1553-1555.

Ganguly M, Borthakur MK, Devi N, Mahanta R (2007). Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female mice. J. Ethnopharm. 111:688-691.

Gessler MC, Msuya DE, Nkuyuna MHH, Mwasunmbi LB, Schar A, Heinrich M, Tanner M (1995). Traditional healers in Tanzania: The treatment of malaria with plant remedies. J. Ethnopharm. 48(3):131-144.

Graham JG, Quinn ML, Fabricant DS, Farnsworth NR (2000). Plants used against cancer – an extension of the work of Jonathan Hartwell. J. Ethnopharm. 73:347-377.

Guinaudeau H, Bruneton J (1993). Isoquinoline alkaloids. Alkaloids and Sulphur Compounds. Methods in Plant Biochemistry 8 Waterman, P. G., ed., London Academy Press. pp. 373-419.

Guinaudeau H, Lebouef M, Cavé A (1979). Aporphine Alkaloids II. J. Nat. Prod. 42(4):325-360.

Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango RM, Maganyi OW, Soejarto DD (2003). Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. J. Ethnopharm. 84:57-78.

Iwu MM (1993). Handbook of African Medicinal Plants. C.R.C. Press, Boca Raton, Florida. pp. 26-267.

Jittra S, Suwayd N, Steve WC, Douglas GH (2005). Extraction and physicochemical characterization of Krueo Ma Noy pectin. Food Hydrocol. 19:793-801.

Kaur K, Jain M, Kaur T, Jain R (2009). Antimalarials from nature. Bioorg. Med. Chem. 17:3229-3256.

Liu S, Babajide O, Charles DH, Alvie M (1990). 3-Methoxysampangine, a novel antifungal copyrine alkaloids fungi deistopholis pattern. Antimicrob. Agent Chemother. 34(4):529-533.

Mabry TJ, Markham KR, Thomas MB (1970). The Systematic Identification of Flavonoids. Springer Verlag., New York. P 2204.

Mander M (1998). Marketing of indigenous medicinal plants in south Africa. In: A Case study in Kwa-Zulu Natal. FAO, United Nations, Rome.

Markham KR, Chari VM (1982). Carbon-13 NMR spectrosocopy of flavonoids. In: Harborne JB, Mabry TJ (Eds.), The Flavonoids: Advances in Research. Chapman and Hall, London, UK. pp. 19-134.

McGaw LJ, Jager AK, van Staden J (2000). Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J. Ethnopharm. 72:247-263.

Mc Laughlin JL, Chang CJ, Smith DL (1991). Bench – top bioassay for the discovery of bioactive natural products. An update. Attamra (Ed), studies in natural product chemistry. Elservier Science Publishers B.V, Amsterdam. pp. 383-409.

Pillay P, Maharaj VJ, Smith PJ (2008). Investigating South African plants as a source of new antimalarial drugs. J. Ethnopharm. 119:438-454.

Rahman MM, Gibbons S, Gray AI (2007). Isoflavanones from Uraria picta and their antimicrobial activity. Phytochemistry 68:1692-1697.

Rahman MM, Gray AI (2005). A benzoisofuranone derivative and carbazole alkaloids from *Murraya koenigii* and their antimicrobial activity. Phytochemistry 66:1601-1606.

Ramirez I (2003). Cissampeloflavone, a chalcone-flavone dimer from *Cissampelos pareira*. Phytochemistry 64(2):645-647.

Rasoanaivo P, Ratsimamanga-Urverg S (1993). Biological Evaluation of Plants with Reference to the Malagasy Flora. *Napreca*, Madagascar. pp. 9-83.

Roitman JN, James LF (1985). Chemistry of toxic range plants. Highly oxygenated flavonol methyl ethers from *Gutierrezia microcephala*.

Phytochemistry 24(4):835-848.
Rood B (1994). *Uit die veldapteek*. Tafelberg, Cape Town.
Sanchez-Medina A, García-Sosa K, May-Pat F, Peña-Rodríguez LM (2001). Evaluation of biological activity of crude extracts from plants used in Yucatecan Traditional Medicine Part I. Antioxidant, antimicrobial and β-glucosidase inhibition activities. Phytomedicine 8(2):144-151.
Shai LJ, McGaw LJ, Aderogba MA, Mdee LK, Eloff JN (2008). Triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm.f) C.A. Sm. Leaves. J. Ethnopharmacol. 119:238-241.
Sloan A, Deborah LZ, Kenneth M, Joanne SP, Christine MB, Terry M, Robert B, Seef P, Dennis S, Donald T, Sheo BS (2007). Anthelmintic Activity of Aporphine Alkaloids from *Cissampelos capensis*. Planta Med. Lett. 73(3):296-297.
Smith CA (1966). Common Names of South African Plants. Memoirs of the botanical survey of South Africa, Vol. 35. Department of Agriculture & Technological Service. The Government Printer, Pretoria.
Ssegawa P, Kasenene JM (2007). Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. J. Ethnopharm. 113:521-540.
Stuart K L (1971). Morphinandienone alkaloids. Chem. Rev. 71:47-72.
Taylor L (2005). The Healing Power of Rainforest Herbs, Square One Publishers Inc., USA.
Van Wyk BE (2008). A review of Khoi-San and Cape Dutch medical ethnobotany. J. Ethnopharm. 119:331-341.
Van Wyk BE, De Wet H, Van Heerden FR, (2008). An ethnobotanical survey of medicinal plants in the south-eastern Karoo, South Africa. South Afr. J. Bot. 74:696-704.
Van Wyk BE, Gericke N (2000). People's Plants: A Guide to Useful Plants of Southern Africa. Briza Publications, 1st ed. Pretoria, South Africa, ISBN: 978 1 875093 19 9.
Van Wyk BE, Van Oudtshoorn B, Gericke N (1997). Medicinal Plants of South Africa 2nd improved impression, 2000. Briza Publications, Pretoria.
Vecchietti V, Casagrande C, Ferrari G, Danieli B, Palmisano G (1981). Alkaloids of *Ocotea acutangula.* J. Chem. Soc. Perkin Transact. 1:578-581.
Vlietinck AJ (1997). Biologically active substances from traditional drugs. In: Hostettman K, Lea PJ (Eds.), Proceeding of the Phytochemical Society of Europe on Biologically Active Natural Products. Oxford University Press, Oxford. pp. 33-47.
Wagner H, Bladt S (2001). Plant Drug Analysis: a thin layer chromatography Atlas, second edition. Springer, New York. pp. 349-364.
Watt JM, Breyer-Brandwijk MG (1962). Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Churchill Livingstone, Edinburgh – London. pp. 53-54, 325, 450-451.
Wheeler DMS, Kinstle TH, Rinehart KL (1968). Additions and corrections-mass spectral studies alkaloids related to morphine. J. Am. Chem. Soc. 90(21):5947-5947.
World Health Organisation (WHO) (2002). WHO Traditional Medicine Strategy 2002–2005.World Health Organisation, Geneva, WHO/EDM/TRM/2002.1.
World Health Organisation (WHO) (2005). World Malaria Report http://www.rbm.who.int/*wmr2005/pdf/ adv e.pdf*.
Wu SJ (2007). Tetrandrine inhibits proinflammatory cytokines, iNOS and COX-2 expression in human monocytic cells. Biol. Pharm. Bull. 30(1):59-62.

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Full Length Research Paper

In vivo TNF-α and IL-1β inhibitory activity of phenolics isolated from *Trachelospermum jasminoides* (Lindl.) Lem

Maha Salama[1]*, Seham El-Hawary[1], Ola Mousa[2], Noha El- Askary[2] and Ahmed Esmat[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Al-Ahram Canadian University, Egypt.
[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, Egypt.

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A bio-guided fractionation of the defatted ethanolic extract (DEE) of the aerial parts of *Trachelospermum jasminoides* and its fractions: ethyl acetate fraction (EAF), chloroform fraction (CF) and *n*-butanol fraction (BF) were carried out, using carragenan induced rat paw edema method, to evaluate the *in vivo* acute anti-inflammatory potential at a given dose of 100 mg/kg body weight compared to indomethacin (20 mg/kg body weight). The EAF revealed the highest anti-inflammatory activity (76.92%) relative to the DEE and the CF (63.82 and 48.75%, respectively) at the same tested dose, while the BF was significantly inactive. The EAF and its major isolated compounds were investigated to determine the level of pro-inflammatory cytokines using enzyme-linked immunosorbant assay (ELISA). Seven major compounds were isolated from the EAF, identified as: trachelogenin, nor-trachelogenin and tracheloside, in addition to apigenin, luteolin, quercetin and luteolin-7-O-*β*-D-glucopyranoside. Identification of the isolated compounds was achieved by their physico-chemical properties and spectral analysis (1D and 2D NMR). The EAF and the isolated compounds inhibited the excessive production of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β. Furthermore, the liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS) for the bioactive EAF was carried out to complete the phytochemical picture. The results of this study verified that the EAF and the major compounds exert their action through inhibition of TNF-α and IL-1 β.

Key words: *Trachelospermum jasminoides*, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS).

INTRODUCTION

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents (Nadkarni, 2000). The classic signs of inflammation are local redness, swelling, pain, heat and loss of function (Pervical, 1999). There are mainly two types of inflammation; acute inflammation which is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes while,

*Corresponding author. E-mail: maha.salama@pharma.cu.edu.eg.

chronic inflammation is usually associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation (angiogenesis) and fibrosis. Cytokines represent a group of multifunctional substances that are involved in the inflammatory response (Sacca et al., 1997). Pro-inflammatory cytokines as interleukin (IL-1*β*), tumour necrosis factor (TNF-α), IL-6 and IL-18 are involved in the initiation and amplification of inflammation (Dinarello, 2008). Overproduction of pro-inflammatory cytokines in macrophages is responsible for many inflammatory diseases, including rheumatoid arthritis, atherosclerosis, and hepatitis (Opal and DePalo, 2000; Srinivasan et al., 2011; Verma and Singh, 2008). Therefore, inhibiting their production may serve to prevent or suppress a variety of inflammatory diseases. Inflammation is usually treated by Nonsteroidal Anti-inflammatory Drugs (NSAIDs). Unfortunately, these drugs cause increased risk of blood clots resulting in heart attacks and strokes (Nadkarni, 2000). Only protein-based drugs are available for the clinical inhibition of TNF-α activity. TNF-α inhibitors from natural origins are being advanced for the treatment of inflammatory disorders. Natural products have been, and continue to be, a major source of pharmacologically active substances from which drugs can be developed (Paul et al., 2006). The screening and development of drugs for their anti-inflammatory activity is the need of hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants (Bingtao et al., 1995). Nearly 80% of people living in developing countries still depend on plant-based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plants (Verma and Singh, 2008).

Family Apocynaceae (Li et al., 1995) (Dogbane) is one of the largest plant families comprising over 424 genera and up to 1500 species. Plants belonging to this family are distributed in tropical and temperate areas. *Trachelospermum jasminoides* (Lindl.) Lem. is a member of family Apocynaceae. Many of which is a woody climber plant found in the tropics and subtropics. It is an evergreen woody liana growing to 50 cm in height. It is also known as Luoshi or Chinese jasmine (Endress and Bruyns, 2000; Nishibe et al., 2002).

Bioactive metabolites flavonoids, lignans, sterols and triterpenes, alkaloids were reported in the plant (Zhang et al., 2013; Zhu et al., 2013; Tan et al., 2010; Xing-qi et al., 2006; Tan et al., 2005; Jing et al., 2005; Atta-ur-Rahman et al., 1988; Fatima et al., 1987).

The plant is reputed for its anti-inflammatory, analgesic activities and its anti cancer activities (Li et al., 2003; Nishibe and Han, 2002; Nishibe et al., 1987). The ethanol extract of *T. jasminoides* showed potent inhibitory activities against both COX-1 and PLA_2 (Lai et al., 2003). Moreover, its lignans content significantly inhibited lipid peroxidation (Fujimoto et al., 1992) evidencing its anti oxidant activity. Also, the lignans exhibited relaxation effects on histamine-induced contraction of tracheal muscles (Fujimoto et al., 1992).

Herein, a bio-guided fractionation for the defatted ethanolic extract (DEE) of the aerial parts of *T. jasminoides* was carried out to investigate the *in vivo* acute anti-inflammatory activity with the aim of determining the most active fraction. Isolation of the major compounds from the bioactive fraction as well as their anti-inflammatory activity has been studied. Furthermore, a qualitative analysis applying LC/ESI-MS technique was achieved for the most active fraction to identify other major compounds present in the bioactive fraction and give a clear phytochemical picture.

MATERIALS AND METHODS

Plant

Samples of *T. jasminoides* (Lindl.) Lem. used in this study were collected since May 2008 from El-Orman Botanical Garden, Giza, Egypt. Plant identity was kindly authenticated by Dr. Mohamed El Gebaly, Botany Taxonomist, Cairo University and Engineer Therese Labib, senior specialist of plant identification at El-Orman Botanical Garden, Giza, Egypt. A voucher sample (TS-002008), has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Chemicals and equipments

Inflammatory-grade carrageenan was purchased from FMC (Rockland, ME, USA). All extracts were dissolved in carboxymethylcellulose before being injected into the animals. Indomethacin was purchased from Sigma-aldrich, USA. TNF-α and IL-1β were quantified using enzyme- linked immunosorbent assay kits (Boster Biological Technology Co., Inc, CA, USA). All other chemicals were of the highest available commercial grade. Silica gel H (Merck, Darmstadt, Germany) for vacuum liquid chromatography (VLC), silica gel 60 (70 to 230 mesh ASTM, Fluka, Steinheim, Germany) and Sephadex LH 20 (Pharmacia, Stockholm, Sweden) were used for column chromatography. Thin-layer chromatography (TLC) was performed on silica gel GF254 precoated plates (Fluka) using the following solvent systems; S_1 (*n*-hexane/ethyl acetate, 80:20 v/v), S_2 (chloroform/methanol, 9:1) S_3 (chloroform/methanol/formic acid, 90:10:2). The chromatograms were visualized under UV (at 254 and 366 nm) before and after exposure to ammonia vapour and spraying with $AlCl_3$, as well as after spraying with anisaldehyde/sulphuric acid reagent. Melting points (uncorrected) were determined on an Electrothermal 9100 (Ontario, Canada). Shimadzu-IR-435 Infrared spectrophotometer was used for measuring IR spectra. UV spectra were measured using a Shimadzu UV 240 (P/N 204-58000) spectrophotometer (Kyoto, Japan). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were measured on a Varian Mercury-VX-300 instrument (Palo Alto, CA, USA). The NMR spectra were recorded in $CDCl_3$ and DMSO-d6 and chemical shifts were given in *d* (ppm) relative to TMS as internal standard.

Animals

Throughout the experiments, adult male Sprague–Dawley rats weighing 180 to 200 g were used for evaluating the anti- inflammatory

activity and 25 to 30 g male albino mice for LD_{50}; all animals were supplied by Pharmacology Department, Faculty of Pharmacy, Ain Shams University. They were housed at a temperature of 23 ± 2°C with free access to water and standard food pellets. Rats were acclimatized in our animal facility for at least 1 week prior to the experiment. All animal procedures were conducted in accordance with internationally accepted principles for laboratory animal use and care, and had been approved by the Ethics Committee of the National Research Centre (No. 9 to 031) in accordance with recommendations for the proper care and use of laboratory animals (NIH Publication No. 80 to 23; revised 1978).

Extraction

Two kilograms of the air-dried powdered aerial parts of *T. jasminoides* (Lindl.) Lem were exhaustively defatted with *n*-hexane on cold (2×3 L). The defatted powder was dried then extracted with ethanol 90% (cold maceration) (2×4 L). The defatted ethanolic extract (DEE) was evaporated (60°C) under reduced pressure to yield 302.9 g of dry residue. The dry residue was then suspended in water (600 ml) and partitioned successively with chloroform (1.5 L) followed by ethyl acetate (1.5 L) and *n*-butanol (1.2 L). The solvents were evaporated under reduced pressure to yield chloroform fraction (CF) (9 g), ethyl acetate fraction (EAF) (13.3 g) and *n*-butanol fraction (BF) (2.032 g).

Determination of median lethal dose (LD_{50})

The LD_{50} of the DEE of the aerial parts of *T. jasminoides* was carried out according to Karber (1931).

Screening for the acute anti-inflammatory activity

The DEE and its sub-fractions (CF, EAF and BF) were investigated for their acute *in vivo* anti-inflammatory activities according to the carrageenan induced rat paw edema method (Winter et al., 1962). Six groups of 6 animals each of adult male albino rats were used. The rats were kept under the same hygienic conditions, well balanced diet and water supplied *ad libitum*. The first group received 1 ml saline orally (negative control). The second group was given indomethacin orally in a dose of 20 mg/kg body weight (positive control). The other four groups received the DEE, CF, EAF and BF fractions of the aerial parts each in a dose of 100 mg/kg body weight. One hour later, edema was induced by a sub planter injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. Three hours after the induction of inflammation, the rats were sacrificed. The right hind paw weight was measured immediately after carrageenan injection by water displacement using a plethysmometer (model 7140, Ugo Basile, Comerio, Italy). The paw weight was re-measured 1, 2 and 3 h after carrageenan injection. The mean response for increase in the paw edema after acute inflammation was calculated.

$$\text{Edema (\%=)} \quad \frac{\text{Weight of the right paw - Weight of the left paw}}{\text{Weight of the left paw}} \times 100$$

The percentage of inhibition in the mean of the treated group in comparison with the control non-treated group was estimated and calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Paw edema of control - Paw edema of treated}}{\text{Paw edema of control}} \times 100$$

Isolation of compounds from the bioactive EAF

The bioactive EAF of the aerial parts of *T. jasminoides* was subjected to purification as follows.

Ten grams of the EAF were chromatographed on a VLC (7×12.5 cm), packed with 270 g silica gel 60. Gradient elution was performed starting with chloroform and increasing the polarity by 2.5% stepwise addition of ethyl acetate till 100%, followed by increasing the polarity with methanol in 5% increment till 50% MeOH/EtOAC. Fractions were collected (200 ml each) and monitored by TLC using solvent systems S_1-S_3, the spots were visualized under UV light before and after exposure to ammonia vapour and by using aluminium chloride and *p*-anisaldehyde as spray reagents yielding collective fractions designated as fractions I to V. Fraction I (970 mg) was further purified on a column silica gel 60 (30 cm L × 3 cm D), eluted using 5 to 50% EtOAc/CH_2Cl_2, in fractions of 5 ml, to afford two main sub-fractions (Sub-fractions Ia and Ib). Sub-fraction Ia: 400 mg, purified on Si gel column (17 × 1 cm D) eluted with EtOAc:MeOH:H_2O (100:16:11) v/v, then purified over Rp-18 column with system MeOH: H_2O (50:50) v/v to yield compound C_1 (190 mg). Sub-fraction Ib: (225 mg) purified on Si gel column (17 ×1 cm D) eluted with $CHCl_3$, then purified over Rp-18 column with system MeOH:H_2O (50:50) v/v to yield compound C_2 (120 mg). Fraction II (410 mg) was purified on a sephadex LH-20 column (28×1.8 cm) using methanol for elution; then it was repeatedly purified on Sephadex LH-20 column using MeOH:H_2O (80:20) v/v to give two yellow coloured compounds; compound C_3 (11 mg) and compound C_4 (16 mg). Fraction III (200 mg) was chromatographed repeatedly on a sephadex LH-20 column (28×1.8 cm) using methanol: water (90:10 v/v) to give pale yellow powder of compound C_5 (97 mg) and 11 mg of yellow powder compound C_6. Fraction IV (0.6 g) chromatographed on a sephadex LH-20 column (28 × 1.8 cm) using methanol:water (80:20) v/v for elution, then chromatographed repeatedly over Rp-18 column using methanol:water (80:20) v/v for elution, to yield one major spot, dried and purified over Rp-18 column with methanol:water (50:50) v/v to give 210 mg of a white amorphous powder compound C_7. Each purified compound was subjected to detailed studies to elucidate its structure.

***In vivo* anti inflammatory activity for the bioactive fractions and the major isolated compounds**

Carrageenan induced rat paw edema method was applied for the bioactive EAF and the major isolated compounds as mentioned earlier. The animals were equally divided into 8 groups (1 to 8), 6 animals per group. Using an intragastric tube, groups 1 and 2 were given the vehicle (0.5% carboxymethylcellulose). Animals in group 3 received indomethacin (10 mg/kg) as a standard anti-inflammatory drug suspended in aqueous carboxymethylcellulose (0.5% w/v), whereas remaining groups were treated by: EAF and the major isolated compounds: C_1, C_2, C_5 and C_7 at two dose levels (25 and 50 mg/kg). The dosing volume was kept constant (10 ml/kg) for all

Table 1. Acute *in Vivo* anti-inflammatory activity of the DEE and the different fractions of the aerial parts of *Trachelospermum jasminoides* (Lindl.)Lem.

Group	% Edema (Mean ±S.E)	Inhibition (%)	Potency (%)
Control (un-treated)(1 ml saline)	59.7±1.8	0	-
DEE (100 mg/kg body weight)	36.4±1.3*	39.02	63.82
EAF (100 mg/kg body weight)	31.5±1.1*	47.23	76.92
CF (100 mg/kg body weight)	41.9±1.6*	29.81	48.75
BF(100 mg/kg body weight)	68±1.9	0	-
Indomethacin (20 mg/kg body weight)	23.2±0.4*	61.14	100

*Significantly different from control group at P<0.01. DEE: Defatted ethanolic extract, EAF: ethyl acetate fraction, CF: chloroform fraction,
BF: *n*-Butanol fractions. Data are presented as mean ± SEM. n=6.

the orally treated groups. Edema was introduced as mentioned earlier.

Measurement of TNF-α and IL-1β levels in the rat paw

Right hind paws were removed. A volume of 0.1 ml of saline containing 10 µM indomethacin was injected to aid removal of the eicosanoid-containing fluid and to stop further production of TNF-α and IL-1β. Paws were incised with a scalpel and suspended off the bottom of polypropylene tubes with Eppendorf pipette tips to facilitate drainage of the inflammatory exudates. For the purpose of the removal of the inflammatory exudates, paws were centrifuged at 1,800 g for 15 min. TNF-α and IL-1β were quantified in the collected exudates using enzyme-linked immunosorbent assay kits (Boster Biological Technology Co., Inc, CA, USA) according to the manufacturer instructions. Both assays are based on the sandwich technique in which specific antibodies to TNF-α or IL-1*β* were pre-coated on to 96-well plate. The specific detection antibodies were biotinylated. The test samples and biotinylated detection antibodies were added sequentially followed by washing. Avidin-biotin-Peroxidase Complex was added and unbound conjugates were washed. A substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped, and the absorbance is read at 450 nm using an ELISA microplate reader (ChroMate-4300, FL, USA). The intensity of the color is directly proportional to the concentration of TNF-α or IL-1β in the sample. All biological experiments were done in triplicates

Statistical analysis

Data were expressed as the means ± standard error of mean (SEM). The differences between groups were tested by one-way analyses of variance (ANOVA) followed by the Tukey post-hoc test. All statistical analyses were performed using Graph Pad Instat software version 3 (ISI_ software, CA, USA). The probability of P<0.05 was considered statistically significant.

Apparatus and chromatographic conditions

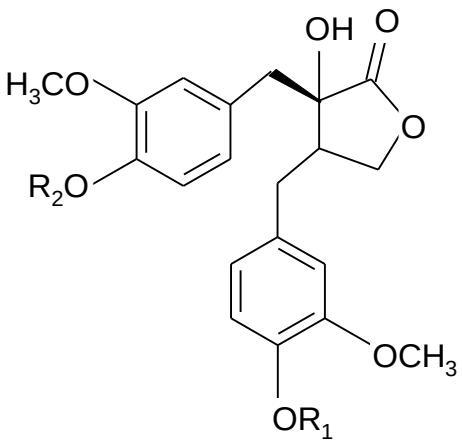
LC analysis was performed using Agilent 1100 (Helwet Packard) HPLC system coupled to a UV detector and an Esquire 3000 plus ion trap mass spectrometer (Bruker Daltonic GmbH, Bremen, Germany) equipped with an electrospray ionization (ESI). Gradient elution of analytes was achieved using water and acetonitrile, both containing 0.1% HCOOH at a constant flow rate of 600 µl min^{-1}, was used. Chromatographic separation of the EAF was performed on RP-C_{18} column; 250×4 mm ID, 5 µm (Merck, Darmstadt, Germany) at 30°C, connected to a guard column; 10 × 4 mm ID, 5 µm (Phenomenex, Torrance, CA). The mobile phase consisting of acetonitrile/0.1% formic acid (solvent system A) and water/0.1% formic acid (solvent system B) was eluted with a gradient system as solution A-B: by 0 min (100% A), 2 min (95% A), 5 min (70% A), 8 min (66% A), 11 min (45% A) and 17 to 20 min (100% A) v/v). The flow rate was set at 0.1 µl min^{-1} and the sample injection volume was 15 µl. The UV detection wavelength was set at 190 to 600 nm. Mass spectroscopic conditions were used as follows: the extract was ionized in the negative ESI interface of the mass spectrometer. The temperature and the voltage of the heated capillary were 300°C and 25 V, respectively. The sheath gas-nitrogen (61/min); (-) 4KV was used. Mass range was set to 200 to 1000 m/z.

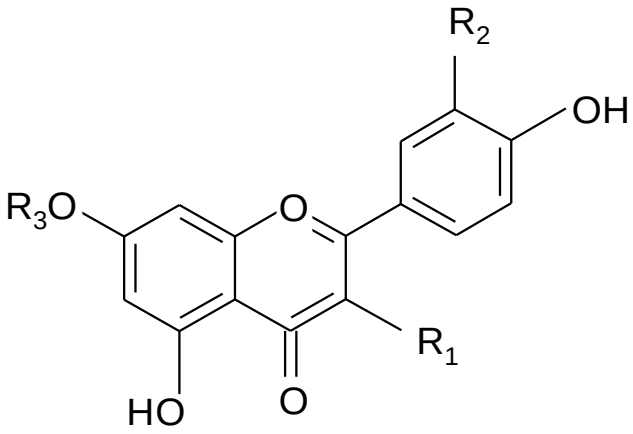
Sample preparation

Two milligrams of the EAF were dissolved in 5 ml of methanol in a volumetric flask and ultrasonicated. Purification was performed using solid phase extraction (SPE) cartridges (LiChrolut RP-18; Merck), which were activated and pre-conditioned using 3 × 1 ml of methanol followed by 3 × 1 ml of water. Purification was then carried out by applying the dissolved extract onto the SPE cartridge, followed by elution with H_2O: MeOH (1:4). The purified fraction was then separately subjected to LC/ESI-MS analysis. Identification of the phenolic compounds was carried out by comparing MS^1 and MS^2 of each peak with the reference data. For more confirmation selective ion monitoring (SIM) technique was applied.

RESULTS

The LD_{50} of the DEE of the aerial parts was safe up to 5000 mg/kg body weight. The data (Table 1) showed that the EAF of the aerial parts was the most bioactive fraction revealed by its high potency (76.92%) at the given dose of 100 mg/kg body weight compared to indomethacin (20 mg/kg body weight). Consequently, the bioactive EAF was chosen for phytochemical study and seven compounds (1 to 7) (Figure 1) were isolated. The compounds were identified based on their spectral data analyses. Moreover, the aim of this study was to measure





(C_1) $R_1 = CH_3$ $R_2 = H$
(C_2) $R_1 = H$ $R_2 = H$
(C_7) $R_1 = CH_3$ $R_2 = Glc.$
Glc.= β-D-glucopyranosyl

(C_3) $R_1 = H$ $R_2 = H$ $R_3 = H$
(C_4) $R_1 = H$ $R_2 = OH$ $R_3 = H$
(C_5) $R_1 = OH$ $R_2 = OH$ $R_3 = H$
(C_6) $R_1 = H$ $R_2 = OH$ $R_3 = Glc.$
Glc.= β-D-glucopyranosyl

Figure 1. Structure of the isolated compounds from the EAF of the aerial parts of *T. jasminoides*.

the activity of the EAF alongside with the isolated compounds at lower doses (50 and 25 mg/kg body weight) to determine the least dose responsible for the activity in addition to determining their mechanism of action.

Compound 1

White amorphous powder (MeOH, 190 mg), M.P: 167-170°C, EI-MS: m/z (rel. int. %): 387.1(95%), 357 (60%), 339 (50%), 329 (100%), 249 (20%), 193 (18%), (49%), 195 (100%), IR: 3412, 1770, 1590, 1515, and 1465 cm^{-1}. ^{1}H-NMR δ ppm(400 MHz, DMSO), δ_H 6.58 (s, 1H, 8'-OH), 3.04, 2.95 (d, each 1H, J = 12 Hz, H-7'), δ_H 2.77 (dd, 1H, J = 12.4, 3.2 Hz, Ha-7), 2.53 (m, 1H, Hb-7), δ_H 4.00 (dd, 1H, J = 7.2, 6.4 Hz, Ha-9), δ_H 3.98 (dd, 1H, J = 7.2, 9.2 Hz, Hb-9), 3.72, 3.65 and 3.63 Hz (each s, 3H, 4-OCH_3, 3-OCH_3, 3`-$OCH_3$), $\delta_H$ 6.85 (d, 1H, J = 1.7 Hz, H-2'), 6.82 (dd, 1H, J = 1.7, 8 Hz, H-6'), 6.80 (d, 1H, J = 8 Hz, H-5'), a singlet signal at $\delta_H$ 6.67 (2H, H-2, H-6). $^{13}$C-NMR δ ppm (100 MHz, DMSO); δc 178.4(C-9`), 70.1 (C-8`), 55.9 (4-OCH3), 55.8 (3-$OCH_3$), 55.82 (3`-OCH_3), 42.7 (C-8).

Compound 2

White amorphous powder (MeOH, 120 mg), M.P: 170-175°C, EI-MS: m/z (rel. int. %): 373.3 (95%), 355.1 (97%), 339 (50%), 327 (100%), 235 (22%), 223 (20%). IR: 3414, 1765, 1589, 1511, and 1458 cm^{-1}. ^{1}H-NMR δ ppm (400 MHz, DMSO); δ_H 6.59 (s, 1H, 8′-OH), 3.10, 2.96 (d, each 1H, *J* = 12 Hz, H-7'), 2.51 (dd, 1H, *J* = 12.4,3.2 Hz, H_a-7), 2.51 (m, 1H, H_b-7), at δ_H 4.1 (dd, 1H, *J* = 7.2, 6.4 Hz,H_a-9), 3.99 (dd, 1H, *J* = 7.2, 9.2 Hz, H_b-9), 3.69 and 3.63 Hz (s, 3H, 3-OCH_3, 3`-$OCH_3$), 6.82 (d, 1H, *J* = 1.7 Hz, H-2'), 6.8 (dd, 1H, *J* = 1.7, 8 Hz, H-6'), 6.77 (d, 1H, *J* = 8 Hz, H-5'), singlet signal at $\delta_H$ 6.68 (2H, H-2,H-6). $^{13}$C-NMR δ ppm (100 MHz, DMSO); $\delta_c$ 178.4 (C-9`), 70.1(C-8`), 55.8 (3-$OCH_3$), 55.8 (3`-OCH_3), 42.7 (C-8).

Compound 3

Seven milligrams of pale yellow powder, M.P. 343-346°C, UV λ_{max} nm (MeOH): 268, 298(sh.), 338; NaOMe: 276, 300(sh.), 348, 392; $AlCl_3$: 276, 300, 348, 384; $AlCl_3$/HCl: 278, 298(sh.), 342, 382; NaOAC: 278, 336, 386; NaOAC/H_3BO_3: 276, 298, 338. ^{1}HNMR δ ppm (300 MHz, DMSO); δ_H 7.91 (1H, br.d, H-6`), 7.89 (1H, br.s, H-2`), 6.94 (1H, d, *J* =9, H-5`), 6.91 (1H, d, *J* =9, H-3`), 6.46 (1H, s, H-3), 6.45 (1H, d, *J*=2.1 Hz, H-8), 6.18 (1H, d, *J*= 9 Hz, H-6).

Compound 4

Thirty-five milligrams of yellow powder, M.P. 223-225°C, UV λ_{max} nm (MeOH): 267, 290(sh.), 345; NaOMe: 274, 324 (sh.), 406; $AlCl_3$: 278, 296(sh.), 346, 420; $AlCl_3$/HCl: 276, 296(sh.), 358, 390; NaOAC: 276, 320(sh.), 404; NaOAC/H_3BO_3: 276, 294(sh.), 370. ^{1}HNMR δ ppm (300

Table 2. The effect of EAF and the isolated compounds on rat paw volume in carrageenan-induced rat paw edema model.

Group	1 h		2 h		3 h	
	Paw volume (ml)	% Edema inhibition	Paw volume (ml)	% Edema inhibition	Paw volume (ml)	% Edema inhibition
Control	0.66 ± 0.07	-	0.68 ± 0.05	-	0.65 ± 0.04	-
Carrageenan	0.718 ± 0.144	-	0.70[a] ± 0.10	-	0.81[a] ± 0.10	-
Indomethacin (10 mg/kg)	0.695 ± 0.04	40.0	0.59 ± 0.08	50.77	0.56[b] ± 0.04	68.49
EAF (25 mg/kg)	0.711 ± 0.02	11.43	0.65 ± 0.04	23.85	0.65[a,b]± 0.02	43.84
EAF (50 mg/kg)	0.708 ± 0.03	17.14	0.63 ± 0.05	31.54	0.59[b] ± 0.03	61.19
C_1 (25 mg/kg)	0.715 ± 0.07	5.71	0.67 ± 0.04	11.54	0.69[a] ± 0.09	34.70
C_1 (50 mg/kg)	0.713 ± 0.03	8.57	0.64[a] ± 0.05	25.38	0.6[a,b] ± 0.05	59.82
C_2 (25 mg/kg)	0.703 ± 0.09	25.712	0.63 ± 0.06	32.31	0.63[a,b] ± 0.12	51.60
C_2 (50 mg/kg)	0.705 ± 0.03	22.86	0.58 ± 0.04	56.92	0.56[b] ± 0.04	69.86
C_5 (25mg/kg)	0.713 ± 0.06	8.57	0.66[a] ± 0.05	18.46	0.72[a] ± 0.01	26.03
C_5 (50 mg/kg)	0.715 ± 0.03	5.71	0.65[a] ± 0.07	21.54	0.67[a,b] ± 0.04	39.73
C_7 (25 mg/kg)	0.715 ± 0.03	5.718	0.67[a] ± 0.05	11.54	0.71[a] ± 0.05	29.22
C_7 (50 mg/kg)	0.713 ± 0.03	8.57	0.66[a] ± 0.03	16.15	0.67[a,b] ± 0.02	40.18

EAF: Ethyl acetate fraction; C_1, C_2, C_5 and C_7: The major isolated compounds from the ethyl acetate fraction tested at two dose levels (25 and 50 mg); Carrageenan: untreated group. Indomethacin (10mg/kg): treated group; Data are presented as mean ± SEM; n=6. [a]Statistically different from the corresponding control group at $p < 0.05$. [b]Statistically different from the corresponding carrageenan-treated group at $p < 0.05$.

MHz, DMSO) δ_H 7.37 (1H, br.d, H-6`), 7.32 (1H, br.s, H-2`), 6.91 (1H, d, *J* =8.7,H-5`), 6.53 (1H, s, H-3), 6.44 (1H, d, *J*=2.1 Hz, H-8), 6.21 (1H, d, *J*= 1.8 Hz, H-6).

Compound 5

Ninety seven milligrams of yellow powder, M.P. 315-317°C, UV λmax nm (MeOH): 256, 301 sh, 372; NaOMe: 247 sh, 330, 406; $AlCl_3$: 269, 457; $AlCl_3$/HCl: 267, 303 sh, 352 sh, 429; NaOAC: 268, 329 sh, 390; NaOAC/H_3BO_3: 259, 386. ^{1}HNMR δ ppm (300 MHz, DMSO) δ_H 6.17 (1H, *d*, *J*= 2 Hz, H-6) and 6.37 (1H, *d*, *J*=2.1 Hz, H-8), 6.89 (1H, *d*, *J* =11.2, H-5`), 7.72 (1H, *br.s*, H-2`) and 7.64 (1H, *br.d*, H-6`).

Compound 6

Fourteen milligrams of yellow powder, M.P. 255-258°C, R_f=0.4 in S_3, it gave +ve Molisch's test indicating its glycosidic nature. UVλ_{max} nm (MeOH): 258, 268sh, 345; NaOMe: 268, 386; $AlCl_3$: 274, 298sh, 404; $AlCl_3$/HCl: 268, 296sh, 356sh, 388; NaOAC: 268, 348, 398; NaOAC/H_3BO_3: 266, 370. ^{1}HNMR δ ppm (300 MHz, DMSO) aglycone:δ_H 7.86 (1H, br.d, H-6`), 7.39 (1H, br.s, H-2`), 6.93 (1H, d, *J* =8.7, H-5`), 6.89 (1H, s, H-3), 6.92 (1H, d, *J*=2.1 Hz, H-8), 6.77 (1H, d, *J*= 1.8 Hz, H-6), Sugar: δ_H 3.96 (1H, d, *J*= 10 Hz, H-1``), 3.51 ( 1H, d, *J*= 10 Hz, H-4``).^{13}C-NMR δ ppm (75 MHz, DMSO); δ_C 183.8 (C-4), 166.5 (C-2), 164.5 (C-7), 162.6 (C-5), 158.7 (C-9), 150.9 (C-4`), 146.3 (C-3`), 122.8 (C-1`), 120.3 (C-6`), 116.9 (C-5`), 116.6 (C-2`), 107.1 (C-10), 104.04 (C-3), 101.4(C-1``), 101.03 (C-6), 95.9 (C-8).

Compound 7

210 mg of white powder, M.P. 167-170°C, R_f 0.38 in S_3, it gave +ve Molisch's test indicating its glycosidic nature. EI-MS: m/z (rel. int. %): 549.5 (97%), 387.5 (100%), 357 (60%), 339 (50%), 329 (80%), 249 (20%), 193 (18%); IR: 3414, 1768, 1592, 1512, and 1460cm^{-1}. ^{1}HNMR δ ppm (400 MHz, DMSO) aglycone: δ_H 6.65 (s, 1H, 8′-OH), 3.01, 2.87 (d, each 1H, *J* = 13.5 Hz, H-7'), 2.83 (dd, 1H, *J* = 12.4, 3.2 Hz, H_a-7), 2.64 (m, 1H, H_b-7), 3.97 (dd, 1H, *J* = 8.4, 6.4 Hz, H_a-9), 3.94 (dd, 1H, *J* = 8.4, 9.2 Hz, H_b-9), δ_H 3.71, 3.69 and 3.63 Hz (each s, 3H, 4-OCH_3, 3-OCH_3, 3`-$OCH_3$) $\delta_H$ 6.97 (d, 1H, *J* = 1.7 Hz, H-2'), 6.84 (dd, 1H, *J* = 1.7, 8 Hz, H-6'), 6.81 (d, 1H, *J* = 8 Hz, H-5'), $\delta_H$ 6.68 (2H, H-2,H-6), sugar: $\delta_H$ 4.84 (d, 1H, *J* = 6 Hz). $^{13}$C-NMR δ ppm (100 MHz, DMSO); $\delta_C$177.8 (C-9`), 100.1 (C-1``), 75.3 (C-8`), 55.6 (4-$OCH_3$), 55.4 (3-$OCH_3$), 55.3 (3`-OCH_3), 42.7 (C-8).

As shown in Table 2, intraplantar injection of carrageenan into rats resulted in severe discernible inflammation and significant increase in the mean volume of the challenged paw compared to that of the untreated paws (after 2 to 3 h of carrageenan injection). Pre-treatment of rats with EAF and the major isolated compounds: C_1, C_2, C_5 and C_7, at dose levels of 25 and 50 mg/kg body weight did not significantly inhibit the carrageenan-induced increase in the edema volume of rat paws after 1 and 2 h of carrageenan injection, except for C_2 at 50 mg/kg (56.92% inhibition). Nevertheless, their

Table 3. Effect of oral administration of EAF and the isolated compounds on TNF-α level in carrageenan-induced rat paw edema model.

Group	TNF-α level conc. (pg/ml) Mean ± SEM
Control	4.69 ± 0.63
Carrageenan	84.90[a] ± 3.77
indomethacin (10 mg/kg)	12.60[b] ± 1.30
EAF (25 mg/kg)	26.15[a,b] ± 3.44
EAF(50 mg/kg)	11.35[b] ±1.44
C_1 (25 mg/kg)	23.44[a,b] ± 2.86
C_1 (50 mg/kg)	6.98[b] ± 1.57
C_2 (25 mg/kg)	22.60[a,b] ± 3.77
C_2 (50 mg/kg)	6.98[b] ± 1.80
C_5 (25 mg/kg)	55.73[a,b] ± 8.13
C_5 (50 mg/kg)	22.40[a,b] ± 2.95
C_7 (25 mg/kg)	44.90[a,b] ± 4.39
C_7 (50 mg/kg)	15.31[a,b] ± 1.25

Table 4. Effect of oral administration of EAF and the major isolated compounds on IL-1β concentration in carrageenan-induced rat paw edema model.

Treatment	IL-1β concentration (pg/ml) Mean ±SE
Control	29. 24 ± 2.91
Carragenan	118.14[a] ± 11.12
Indomethacin (10 mg/kg)	52.91[b] ± 5.2
EAF (25 mg/kg)	20.2[b] ± 1.87
EAF (50 mg/kg)	6.76[b] ± 0.63
C_1 (25 mg/kg)	33.57[b] ± 3.08
C_1 (50 mg/kg)	24.84[b] ± 2.36
C_2 (25 mg/kg)	77.27[b] ± 7.65
C_2 (50 mg/kg)	39.65[b] ± 3.87
C_5 (25 mg/kg)	27.97[b] ± 2.56
C_5 (50 mg/kg)	9.56[b] ± 0.87
C_7 (25 mg/kg)	32.37[b] ± 2.89
C_7 (50 mg/kg)	12.76[b] ± 1.17

EAF: Ethyl acetate fraction; C_1-C_7: The major isolated compounds from the ethyl acetate fraction tested at two dose levels (25 and 50 mg);
Carrageenan: untreated group. Indomethacin (10 mg/kg): treated group. Data are expressed as mean ± SEM of six rats; n=6. [a]$p<0.001$ compared to control group. [b]$p<0.001$ compared to carrageenan-treated group.

inhibitory effect on carrageenan-induced edema was significantly improved after 3 h at the two tested doses (in a dose dependent manner) except for the compounds C_5 and C_7 exhibited weak activity at the two tested doses as regards to the standard drug. As illustrated in Tables 3 and 4, injection of carrageenan into the rat hind paw induced a significant increase in the hind paw TNF-α and IL-1β concentrations, 3 h after injection, 84.9 and 118.14 pg/ml, respectively, compared to the control (4.69 and 29.24 pg/ml), respectively. Treatment of rats with EAF and the major isolated compounds at both dose levels caused a significant reduction of increased TNF-α generation by carrageenan ($P<0.05$). The two lignan compounds $C_1$and C_2 showed the highest decrease in the TNF-α level at the two tested doses; 25 and 50 mg/kg by 23 and 6.8 pg/ml, respectively as regards to C_1 and 22 and 6.9 pg/ml, respectively as regards to C_2. The EAF showed a lower activity than the two compounds at the

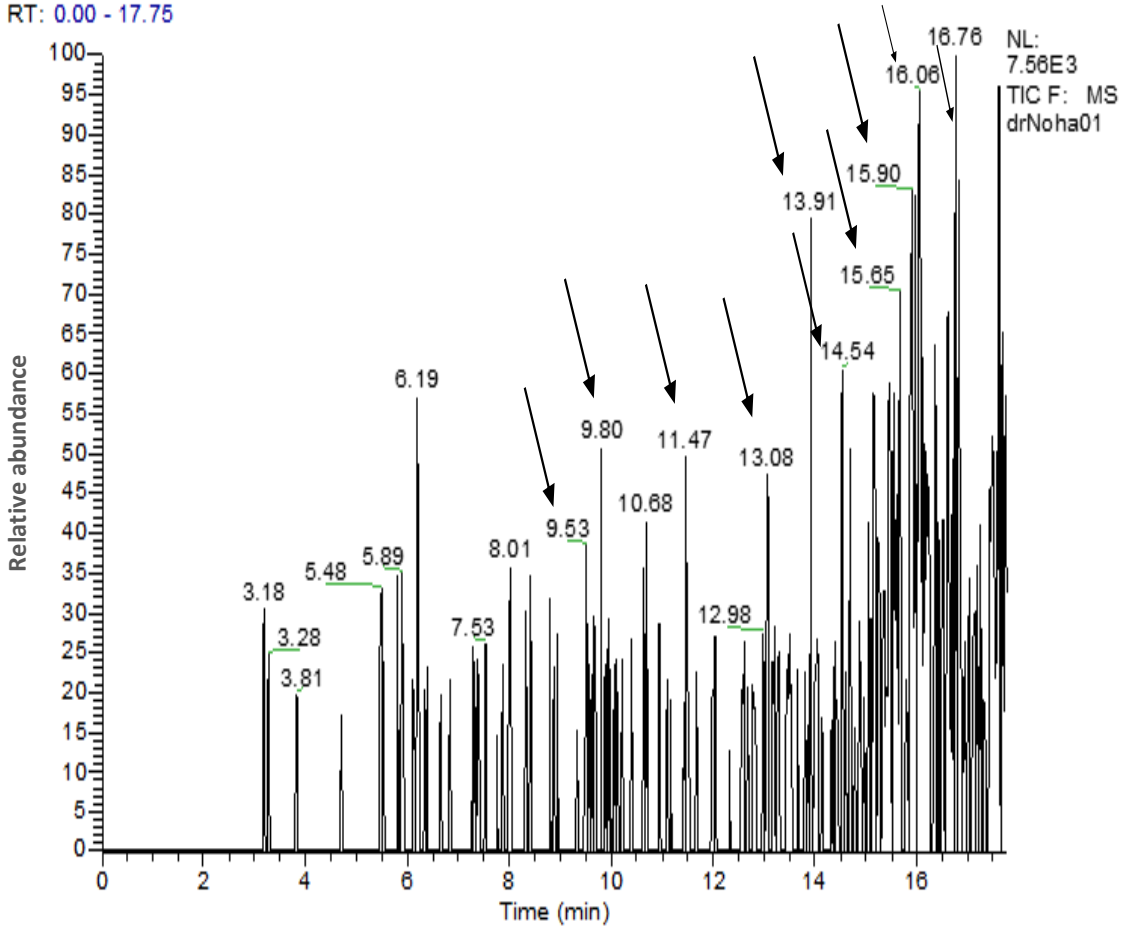


Figure 2. Chromatogram showing the separated phenolic compounds from EAF of *T. jasminoides*. Arrows indicating the peaks of interest.

two tested doses 25 and 50 mg/kg by decreasing TNF-α level to 26 and 11 pg/ml, respectively. On the other hand, C_5 and C_7 were less active than the EAF and C_1 and C_2 at the two tested doses being more potent at the higher dose (50 mg/kg). A similar pattern of activity was obtained with IL-1β concentration. Although C_1 and C_2 were the most active regarding TNF-α inhibition; this activity was reversed in case of measuring the level of IL-1β. Where C_5 and C_7 showed a decrease in the IL-1β level more than C_1 and C_2; C_5 decreased IL-1β level by 27.97 and 9.76 pg/ml at 25 and 50 mg/kg, respectively, while C_7 caused a decrease by 32.37 and 12.76 pg/ml at 25 and 50 mg/kg, respectively. Also, the EAF was the most active by decreasing IL-1β level to 20.2 and 6.76 pg/ml at 25 and 50 mg/kg, respectively. The increase of both TNF-α and IL-1β levels was also significantly prevented by indomethacin (10 mg/kg, i.p., $P<0.05$). It is noteworthy to mention that the EAF, C_1, C_5 and C_7 decreased the IL-1β levels more than that of indomethacin at the two tested doses.

Based on the aforementioned results, it was of interest to analyze the EAF using LC/ESI-MS analysis applying "selective ion monitoring (SIM)" technique to identify other compounds, which were not isolated, that might also contribute to the observed bioactivity and identify the major fingerprint ions. Figure 2 showed the ion chromatogram of the separated phenolics from EAF obtained from the LC-MS, their relative abundance as well as their retention times. Only the compounds of interest according to the available literature were selected for further identification (MS^2). Table 5 revealed the detection of the phenolic compounds of interest and the results of MS^2 for each compound.

DISCUSSION

Recently, the popularity of remedies consisting of natural products found in foods, roots and herbs has increased in both the domestic and global healthcare markets. The

Table 5. Results of MS^2 of the identified phenolic compounds from the EAF of the aerial parts of *Trachelospermum jasminoides* (Lindl.)Lem.

Peak No.	Compound	Rt	$[M-H]^-$	MS^2	Formula
			Flavones and isoflavone glycosides		
1	Apigenin7-*O*-*β*-D-glucoside	9.51	431.3	431.3 (100%), 269	$C_{21}H_{20}O_{10}$
2	Diadzin	9.81	415.3	415.3 (100%), 253	$C_{12}H_{20}O_{10}$
			Favones and flavonol aglycones		
3	Quercetin	11.47	301.2	301.2, 155 (100%)	$C_{15}H_{10}O_7$
4	Luteolin	13.08	285.2	285.2 (100%), 217, 199, 175, 155, 133, 107	$C_{15}H_{10}O_6$
5	Apigenin	13.91	269.2	269.2 (100%), 151	$C_{15}H_{10}O_5$
6	Chrysoeriol	14.54	299.2	299.2 (100%), 285	$C_{16}H_{12}O_6$
			Lignans		
7	Arctigenin	15.65	371.1	371.1, 356 (100%), 312, 295, 209	$C_{21}H_{24}O_6$
8	Matairesinol	15.90	357.1	357.1, 342, 313 (100%), 298, 209	$C_{20}H_{22}O_6$
9	Trachelogenin	16.06	387.1	387.1, 357, 339, 329 (100%), 249, 193	$C_{21}H_{24}O_7$
10	Nor-Trachelogenin	16.76	373.3	373.3, 355, 327(100%), 235, 223	$C_{20}H_{22}O_7$

EAF: Ethyl acetate fraction Rt: Retention time; $[M-H]^-$ = molecular ion peak; MS^2=MS/MS.

attention of pharmacologist throughout the world has been focused on finding out more safe and potent anti-inflammatory drug. Biological results revealed that, the LD_{50} of the tested extract was safe and non-toxic (Buck et al., 1976). The EAF of the aerial parts was the most bioactive fraction evidenced by its high potency (76.92% relative to indomethacin) at a given dose of 100 mg/kg body weight relative to the other tested extracts and fractions and compared to indomethacin (20 mg/kg body weight). Based on the given results, seven compounds have been isolated from the bioactive EAF; three lignans: trachelogenin (C_1), nor-trachelogenin (C_2) and tracheloside (C_7), in addition to four flavonoids; apigenin (C_3), luteolin (C_4), quercetin (C_5) and luteolin-7-O-*β*-D-glucopyranoside (C_6). Identification of the isolated compounds was achieved by analysis of their physico-chemical properties and spectral data (1D & 2D NMR) and by comparison with reported data (Tan et al., 2005; Nishibe, 1994)

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation (Vinegar et al., 1969). It is a biphasic response; the first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peek within 3 h. The second phase of edema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin. Indomethacin is a cycloxygenase inhibitor, the EAF and the major isolated tested compounds showed a significant anti-inflammatory activity against carrageenan induced paw edema which is comparable to indomethacin. In that view, it might have the same mode of action to inhibit the cycloxygenase enzyme (COX) (Kumari et al., 2012). It was also reported that the ethanol extract of *T. jasminoides* L. showed potent inhibitory activities against both COX-1 and PLA_2 (Lai et al., 2003). Moreover, COX-2 inhibitory activity of flavonoids and lignans has been reported (D'Mello et al., 2011; Zhao et al., 2009).

One of the major side effects of indomethacin is that it causes gastric erosion (Maciel et al., 2004; Yoshikawa et al., 1993; whittle, 1977). In our study, the isolated compounds that exhibited anti inflammatory activity by different mechanisms are reported for their gastroprotective and anti ulcer activities; flavonoids display gastroprotective effects acting as anti-secretory, cytoprotective and antioxidant agents. Flavonoids also act in healing of gastric ulcers, that is, they can be new alternatives for suppression or modulation of peptic ulcers associated with *H. pylori* (Farzaei et al., 2013). Other studies (Zayachkivska et al., 2005) reported that plant-originated flavonoid substances are highly gastroprotective due to enhancement of the expression of NOS and release of NO and neuropeptides, such as calcitonin gene related peptide (CGRP) released from sensory afferent nerves increasing gastric micro-circulation. Moreover, it was reported that some lignan derivatives possess anti-ulcerogenic activity (Gurbuz et al., 2004).

Many diseases as arteriosclerosis, chronic hepatitis and pulmonary fibrosis, involve the overproduction of inflammatory mediators (Isomaki and Punnone, 1997; Libby et al., 2002; Tilg et al., 1992; Coker et al., 1998) and by inhibiting their production might serve to prevent or suppress a variety of inflammatory diseases. Pro-inflammatory cytokines, IL-1β, IL-6, and TNF-α, have

attracted more attention in that they can be localized to the infected tissue, manifested systemically throughout the body, and cause vasodilation as well as symptoms of inflammation and associated with the development of diabetes, septic shock, tumorigenesis, rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease (Habtemariam, 2000). TNF-α and IL-1β inhibitors from natural origins are being advanced for the treatment of inflammatory disorders. Flavonoids and lignans were previously reported to possess anti-inflammatory action and their mechanism of action has been discussed (Liu et al., 2009; Gerritsen et al., 1995; Middleton, 1998; Kotanidou, 2002; Cho et al., 1998; Lee et al., 2011; Zhoa et al., 2009).

The EAF and the major tested compounds dose dependently decreased the production of the most prominent pro-inflammatory cytokines; IL-1β and TNF-α. Furthermore, our findings provide evidence of the bioactivity of the EAF and the tested compounds in inflammatory diseases and suggest that they may exert anti-inflammatory effect by inhibiting the pro-inflammatory mediators. Our results are in accordance with the previously reported results for the mechanism of action of trachelogenin, nor-trachelogenin and tracheloside, apigenin, quercetin and luteolin for exerting their anti-inflammatory activities through modulation of the cytokine system (Jia et al., 2014; Seo et al., 2014; Zhu et al., 2013; Harrison and Cooper, 2008; Chen et al., 2004; Ueda, 2004; Xagorari et al., 2001; Habtemariam et al., 2000).

As a result of the renewed focus on natural remedies, efficient identification and analysis of active compounds is a growing area of method development. LC analysis of the EAF resulted in the identification of 10 phenolic compounds. The compounds were identified by their molecular ion peaks in negative mode and by comparing the reported data (Sanchez et al., 2003; Eklund et al., 2008; El- Helal et al., 2010).

The peak number 3, Rt. 11.47 min had absorption maxima at 365 nm, indicating its flavonol nature (Table 5). MS fragmentation ions at *m*/*z* 301 in negative representing [M-H], and MS/MS fragmentation matched that of authentic standard quercetin (*m*/*z* 179 and 151). The *m/z* at 151 indicates the cleavage of ring-C (El- Helal et al., 2010). The peak 2 Rt. 9.81 min had absorption maxima at 255 and 300 nm which are characteristics for an isoflavone structure (Kale and Laddha, 2012). With a base peak at *m/z* 415.3, its MS/MS fragmentation showed a peak at m/z 253 with the loss 162 amu (corresponding to O-glucose) Peak 2 was identified as Diadzin. The peak number 1, Rt. 9.51 min and number 5, Rt. 13.91 min had the UV spectra that showed maxima at 272 and 345 nm corresponding to bands II and I, respectively, and characteristic of a flavone. MS/MS spectra of peak 1 showed a base peak at *m/z* 431.3 and a peak at 269 with the loss of 162 amu corresponding to the loss of an O-hexose. Peak 1 was thus identified as apigenin 7-*O*-*β*-D-glucoside. Peak 5 with the base peak at 269.2 corresponding to [M-H] and MS/MS fragmentation matched that of apigenin (*m*/*z* 179 and 151) (El- Helal et al., 2010). Peaks 4, Rt. 13.08 min and 6, Rt. 14.54 min showed a base peak at m/z 285 and 299, respectively. The MS/MS fragmentation of the compound represented by peak 4 matched that of luteolin (*m/z* 217, 199, 175, 155, 133, 107). MS/MS of the compound represented by peak 6 showed the base peak at m/z 285 with the loss of 14 amu corresponding to loss of a CH_3 group. This was in accordance with the fragmentation of luteolin-3`O-methyl ether (chryseriol) (El- Helal et al., 2010). Peak 7, Rt 15.65 min with molecular ion peak at m/z 371 corresponds to $[M-H]^-$, MS/MS of the compound showed *m/z* 356 with the loss of 15 amu corresponding to loss of a methyl group, m/z 312 indicated a loss of 44 amu loss of CO_2 characteristic for the butyrolactone moiety. The loss of an OH group could be indicated from the presence of m/z 295 [312-17]. The presence of a peak 209 indicates the loss 86 amu. This fragmentation was in accordance with that of Arctigenin (Eklund et al., 2008). Peak 8, Rt. 15.9 min with a molecular ion peak *m/z* 357.1 corresponding to [M-H] showed the same fragmentation pattern as that of Arctigenin except for the lack of a methyl group. This is in accordance with that of matairesinol (Eklund et al., 2008). Peak 9, Rt. 16.06 min and molecular ion *m/z* 387.1 [M-H], its MS/MS pattern showed m/z 357 with the loss of 30 amu, corresponding to the loss of 2 methyls, m/z 339 corresponds to loss of H_2O from 357, 329 (100%), 249, 193. This fragmentation is in accordance with that of trachelogenin (Eklund et al., 2008). Peak 10, Rt. 16.76 min showed a molecular ion peak at m/z 373.3 corresponding to [M-H] with the same fragmentation pattern as trachelogenin devoid of a methyl group. This was in accordance with that of Nor-trachelogenin (Eklund et al., 2008).

By the aid of LC-ESI/MS analysis, the picture has been completed confirming the presence of these phenolic compounds which were isolated from the EAF in addition to other compounds.

Conclusion

Applying bio-guided fractionation, seven compounds in the bioactive EAF of the aerial parts of *T. jasminoides* cultivated in Egypt were isolated; three lignans and four flavonoids. The bioactive fraction together with the major isolated compounds; trachelogenin, nor-trachelogenin, tracheloside and quercetin revealed significant anti-inflammatory effect by inhibiting pro-inflammatory cytokines. Therefore, an extract that could be used as an anti-inflammatory remedy that may serve as model for anti-inflammatory drug development was proposed. Another important point is that the potent anti-inflammatory activity together with the anti ulcerogenic

effect of the phenolic compounds from *T. jasminoides* should be further evaluated to develop safe agents to introduce in modern therapy.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Atta-ur-Rahman, Fatima T, Crank G, Wasti S (1988). Alkaloids from *Trachelospermum jasminoides*. Planta Med. 54:364.

Bingtao L, Leeuwenberg AJM, Middleton DJ (1995). Apocynaceae. Flora of China. 16:143-88.

Buck WB, Osweiter GD, Van Gelder AG (1976). Clinical and Diagnostic Veterinary Toxicology. 2nd Ed., 52011, Kendall/ Hund Publishing Company, Iowa.

Chen CC, Chow MP, Huang WC, Lin YC, Chang YJ (2004). Flavonoids inhibit tumor necrosis factor-alpha-induced up-regulation of intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells through activator protein-1 and nuclear factor-kappaB: structure-activity relationships. Mol. Pharmacol. 66(3):683-93.

Cho JY, Park J, Yoo ES, Yoshikawa K, Baik KU, Lee J, Park MH (1988). Inhibitory effect of lignans from the rhizomes of *Coptis japonica* var. *dissecta* on tumor necrosis factor-alpha production in lipopolysaccharide-stimulated RAW264.7 cells. Arch. Pharm. Res. 21:12-6.

Coker R, Laurent GJ (1998). Pulmonary fibrosis: cytokines in the balance. Eur. Respir. J. 11:1218-1221.

D' Mello P, Gadhwal M K, Joshi U, Shetgir P (2011). Modeling of Cox-2 inhibotory activity of flavonoids. Int. J. Pharm. Pharm. Sci. 3:33-40.

Dinarello CA (2000). Pro-inflammatory cytokines. Chest 118:503-508.

Eklund P, Joseph M, Kronberg L, Smeds A Sjoholm R (2008). Identification of lignans by liquid chromatography-electrospray ionization ion-trap mass spectrometry. J. Mass Spectrom. 43:97-107.

El-Helal A, Al-Amier H, Ibrahim T (2010). Comparative study of the flavonoids of some verbena species cultivated in Egypt by using high-performance liquid chromatography coupled with ultraviolet spectroscopy and atmospheric pressure chemical ionization mass spectrometry. J. Chromatogr. A. 1217:6388-93.

Endress M, Bruyns P (2000). A revised classification of the Apocynaceae. Bot. Rev. 66:1-56.

Farzaei MH, Khazaei M, Abbasabadei Z, Maryam Feyzmahdavi M, Gholam Reza Mohseni GR (2013). Protective Effect of *Tragopogon graminifolius* DC Against Ethanol Induced Gastric Ulcer. Iran Red Crescent Med. J. 15:813-16.

Fatima T, Ijaz S, Crank G, Wasti S (1987). Indole Alkaloids from *Trachelospermum jasminoides*. Planta Med. 53:57-59.

Fujimoto T, Nose M, Takeda T, Ogihara Y, Nishibe S, Minam M (1992). Studies on the Chinese crude drug "Luoshiteng" (II). On the biologically active components in the stem part of luoshiteng originating from *Trachelospermum jasminoides*". Shoyakugaku Zasshi 46:224-229.

Gerritsen ME, Carley WW, Ranges GE, Shen CP, Phan SA, Ligon GF, Perry CA (1995). Flavonoids inhibit cytokine-induced endothelial cell adhesion protein gene expression. Am. J. Pathol. 147:278-92.

Gurbuz I, Erdemoglu N, Yesilada E, Sener B (2004). Anti-ulcerogenic Lignans from *Taxus baccata* L. Z. Naturforsch 59c:233-236.

Habtemariam S (2000). Natural inhibitors of tumour necrosis factor-α-production, secretion and function. Planta Med. 66:303-313.

Harrison AP, Cooper RG (2008). Quercetin: health benefits with relevance to TNF-α-linked inflammatory diseases. J. Pre-Clin. Clin. Res. 2:135-139.

Isomaki P, Punnonen J (1997). Pro- and anti-inflammatory cytokines in rheumatoid arthritis. Ann. Med. 29(6):499-507.

Jia Z, Nallasamy P, Liu D, Shah H, Li JZ, Chitrakar R, Si H, McCormick J, Zhu H, Zhen W, Li Y (2014). Luteolin protects against vascular inflammation in mice and TNF-alpha-induced monocyte adhesion to endothelial cells via suppressing IKBα/NF-κB signaling pathway. J. Nutr. Biochem. Article in press.

Jing L, Jiang YN , Shan LY, Fu L, Min Zhao Y (2011). Novel lignans from the stems and leaves of *Trachelospermum jasminoides*. Chin. Chem. Lett. 22:1075-77.

Kale MS, Laddha KS (2012). Isolation, Characterization and Quantification of Isoflavone in *Momordica dioica* Roxb. Ex Wild (Cucurbitaceae) Fruits. Int. J. Appl. Res. Natl. Prod. 5:28-36.

Karber G (1931). Determination of median leathal dose. Arch. Exp. Pathol. Pharmacol. 162:480-487.

Kotanidou A, Xagorari A, Bagli E, Kitsanta P, Fotsis T, Papapetropoulos A, Roussos C (2002). Luteolin reduces lipopolysaccharide-induced lethal toxicity and expression of proinflammatory molecules in mice. Am. J. Respir. Crit. Care Med. 165:818-23.

Kumari STK, Lincy MP, Muthukumarasamy S, Mohan VR (2012). Anti-inflammatory activity of *Sarcostemma secamone* (L.) Bennet whole plant against carrageenan induced paw edema. Biosci. Discov. 3:288-291.

Lai P, Fan C, Li A (2003). Comparative study on the anti-inflammatory and analgesic effects of *Trachelospermum jasminoides* (Apocynaceae) and *Ficus pumila* L. (Moraceae). Chin. Arch. Tradit. Chin. Med. 21(1):154-155.

Lee S, Shin S, Kim H, Han S, Kim K, Kwon J, Kwak JH, Lee CK, Ha NJ, Yim D , Kim K (2011). Anti-inflammatory function of arctiin by inhibiting COX-2 expression via NF-κB pathways. J. Inflamm. 8:16.

Li PT, Leeuwenberg AJM, Middleton DJ (1995). Flora of China. 16:143-188.

Libby P, Ridker P, Maseri A (2002). Inflammation and atherosclerosis. Am. Heart Assoc. 150:1135-1143.

Liu MH, Lin YS, Sheu SY, Sun JS (2009). Anti-inflammatory effects of daidzein on primary astroglial cell culture. Nutr. Neurosci. 12:123-34.

Maciel HP, Cardoso LG, Ferreira LR, Perazzo FF, Carvalho JC (2004). Anti-inflammatory and ulcerogenic effects of indomethacin and tenoxicam in combination with cimetidine. Inflammopharmacology 12:203-10.

Middleton E Jr (1998). Effect of plant flavonoids on immune and inflammatory cell function. Adv. Exp. Med. Biol. 439:175-82.

Nadkarni AK (2000). Indian Materia Medica. Popular Press Bldg. 3rd ed.

Nishibe S (1994). Bioactive phenolic compounds in traditional medicines. Pure Appl. Chem. 66:2263-66.

Nishibe S, Han Y, Noguchi Y, Sakai E, Tanaka T (2002). Studies on the Chinese crude drug Luoshiteng, the plant origins on the market and their identification. Natl. Med. 56:40-46.

Nishibe S, Han YM (2002). Chemical constituents from *Trachelospermum jasminoides* and its anticancer activity. World Phytomed. 17:57-8.

Nishibe S, Sakushima, Noro T, Fukushima S (1987). Studies on the Chinese drug Luoshiteng (I). Xanthine oxidase inhibitors from the leaf part of Luoshiteng originating from *Trachelospermum jasminoides*. Shoyakugaku Zasshi 41:116-20.

Opal SM, DePalo VA (1999). Anti-inflammatory cytokines. Chest 117:1162-1172.

Paul AT, Gohil VM, Bhutani KK (2006). Modulating TNF-alpha signaling with natural products. Drug Discov. Today 11(15-16):725-32.

Pervical M (1999). Understanding the natural management of pain and inflammation. Clin. Nutr. Insights 4:1-5.

Sacca R, Cuff CA, Ruddle NH (1997). Mediators of inflammation. Curr. Opin. Immunol. 9:851-857.

Sanchez F, Jauregui O, Casals I, Andres C, Izquierdo M, Lamuelo R (2003). Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cacao. J. Mass Spectrom. 38:35-42.

Seo HS, Sikder MA, Lee HJ, Ryu J, Lee CJ (2014). Apigenin Inhibits Tumor Necrosis Factor-α-Induced Production and Gene Expression of Mucin through Regulating Nuclear Factor-Kappa B Signaling Pathway in Airway Epithelial Cells. Biomol. Ther. 22:525-531.

Srinivasan K, Muruganandan S, Lal J, Chandra S, Tandan SK, Ravi PV (2011). Evaluation of anti-inflammatory activity of *Pongamia pinnata* in rats. J. Ethnopharmacol. 78:151-157.

Tan X, Chen H, Liu R, Tan C, Xu C, Xuan W, Zhang W (2005). Lignans

from *Trachelospermum jasminoides*. Planta Med. 71:93-95.
Tan X, Guo L, Chen H, Wu L, Kong F (2010). Study on the flavonoids constituents of *Trachelospermum jasminoides*. J. Chin. Med. Mater. 33:58-60.
Tan X, Guo L, Qiu Y, Chen H, Tan C (2010). Chemical constituents of *Trachelospermum jasminoides*. Nat. Prod. Res. 24:1248-52.
Tilg H, Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G (1992). Serum levels of cytokines in chronic liver diseases. Gastroenterology 103:264-274.
Ueda H, Yamazaki C, Yamazaki M (2004). A Hydroxyl group of flavonoids affects oral anti-inflammatory activity and inhibition of systemic tumor necrosis factor-α production. Biosci. Biotechnol. Biochem. 68:119-125.
Verma S, Singh S (2008). Current and future status of herbal medicine. Vet. World 11:347-50.
Vinegar R, Schreiber W, Hugo R (1969). Biphasic development of carrageenan edema on rats. J. Pharmacol. Exp. Ther. 66:96-10.
Whittle BJR (1977). Mechanisms underlying Gastric Mucosal Damage Induced by Indomethacin and Bile-Salts, and the actions of Prostaglandins. Br. J. Pharm. 60:455-460.
Winter GA, Risley EA, Nuss GW (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111:544-47.
Xagorari A, Papapetropoulos A, Mauromatis A, Economou M, Fotsis T, Roussos C (2001). Luteolin inhibits an endotoxin-stimulated phosphortlation cascade and proinflammatory cytokine production in macrophages. J. Pharmacol. Exp. Ther. 296:181-7.
Xing-qi T, Hai-sheng C, Mi Z, Yue Z (2006). Triterpenoids from canes with leaves of *Trachelospermum jasminoides.* Chin. Tradit. Herbal Drugs 37:171-174.
Yoshikawa T, Naito Y, Kishi A, Tomii T, Kaneko T, Linuma S, Ichikawa H, Yasuda M, Takahashi S, Kondo M (1993). Role of active oxygen, lipid peroxidation, and anti-oxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut 34:732-737.
Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR (2005). Gastroprotective effects of flavonoids in plant extracts. J. Physiol. Pharmacol. 1:219-31.
Zhang J, Yin Z, Liang J (2013). A new isoflavonoid glycoside from the aerial parts of *Trachelospermum jasminoides*. Chin. J. Nat. Med. 11:274-276 .
Zhao F, Lu Wang L, Liu K (2009). *In vitro* anti-inflammatory effects of arctigenin, a lignan from *Arctium lappa* L., through inhibition on iNOS pathway. J. Ethnopharmacol. 122:457-462.
Zhu C, Jing L , Yub N, Yang X, Yimin Zhao Y (2013). A new lignin and active compounds inhibiting NF-jB signaling pathway from *Caulis Trachelospermi*. Acta Pharm. Sin. 3:109-112.

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Full Length Research Paper

Antifungal activity of the aqueous extract of *Stachytarpheta cayennensis*, (Rich.) Vahl. (Verbenaceae), on oral candida species

Sideney Becker Onofre[1]*, Zípora Morgana Quinteiro dos Santos[2], Francini Yumi Kagimura[3] and Shaiana Paula Mattiello[4]

[1]Center of Exact and Environmental Sciences - Postgraduate Program in Technology and Innovation Management - PPGTI - Universidade Comunitária da Região de Chapecó - UNOCHAPECÓ - Chapecó - Santa Catarina - Brazil.
[2]Laboratory of Chemistry and Biochemistry of the Universidade Paranaense - UNIPAR - Francisco Beltrão Campus - Paraná - Brazil.
[3]Department of Biochemistry and Biotechnology - Postgraduate Program in Chemical and Biochemical Processes of the Universidade Tecnológica Federal do Paraná - UTFPR - Pato Branco - Paraná - Brazil.
[4]Molecular Biology Laboratory - Postgraduate Program in Cellular and Molecular Biology of the Pontifícia Universidade Católica do Rio Grande do Sul - PUCRS - Porto Alegre - Rio Grande do Sul - Brazil.

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The objective of this study was to evaluate the antifungal activity of the aqueous extract of the leaves of the "verbena" (*gervão roxo*) *Stachytarpheta cayennensis,* (Rich.) Vahl. (Verbenaceae), by determining the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) on different species and strains of the genus Candida. In this study, the species *Candida albicans, Candida tropicalis, Candida glabrata, Candida stellatoidea, Candida dubliniensis* and *Candida krusei* were included. Four strains of *C. albicans* and three of *C. tropicalis* were used, while for the other species only one strain was tested. These yeasts were used in this study because of their importance and frequency in the oral cavity. The yeasts were subjected to treatments with concentrations of the aqueous extract ranging from 0.09 to 25 mg mL^{-1}. The results indicated, however, that concentrations of less than 12.5 mg mL^{-1} were insufficient to show a fungistatic or fungicidal effect. The concentration of 12.5 mg mL^{-1} showed a fungistatic effect on most tested strains and species, *C. albicans* CαFB-12 and ATCC-44858; *C. tropicalis* CTFB-22 and CTFB29. The fungicidal effect (MFC) was observed only on the species *C. krusei* for the concentration of 12.5 mg mL^{-1}. Based on the employed methodology, it is concluded that the aqueous extract of *S. cayennensis* showed an antifungal, mainly fungistatic, effect on oral *Candida* species.

Key words: Yeasts, candidiasis, metabolites, *Stachytarpheta cayennensis.*

INTRODUCTION

Using plants for the treatment and cure of diseases is as old as the human species itself, with popular knowledge making a great contribution to the dissemination of the therapeutic virtues of these plants. This knowledge has

*Corresponding author. E-mail: beckerside@unochepeco.edu.br. Tel: +55 (46) 3055-6465/+55 (46) 99739131.

often represented a therapeutic resource for many communities and ethnic groups (Maciel et al., 2002) who do not have access to other forms of treatments offered by medicine, or who prefer it in relation to traditional medicine because of cultural issues.

This way, users of medicinal plants from various parts of the world have maintained the practice of phytotherapy and validated the therapeutic information that has been accumulated over centuries. Indirectly, popular medicine awakens the interest of researchers in multidisciplinary studies that enrich the inexhaustible knowledge regarding the therapeutic use of medicinal plants. It is estimated that in continents such as Africa, up to 80% of the population uses medication from plant origins. In Germany and France, this is 75%, in Canada 70%, and in the US 42% (Gregorio, 2006).

The genus *Stachytarpheta* (Verbenaceae) has 133 species. It is distributed throughout Brazil. The species of this genus are generally shrubs, branching subshrubs or, in rare cases, herbs that range from 0.5 to 1.5 m in height, although certain species may reach up to 4 m (Salimena-Pires and Giulietti, 1998).

Its flowers are arranged in a spiral along the axis of the inflorescence in a very compact way, reaching up to 60 cm in length. Its corrolas are quite striking and easily located at a distance in the field. Usually they are blue, but they can have several colors depending on the species, such as red, violet, orange, white or black (Costa, 1960).

Stachytarpheta cayennensis (L.C. Rich) Vahl (Figure 1) is an erect, perennial, branching, somewhat angular, fibrous subshrub that is very resistant to traction. It usually has opposite, ovate leaves with a distinct petiole and serrated and indented edges, an acute or subacute apex, a slightly wrinkled appearance, green color, terminal inflorescence with linear stalks, sessile flower with a gamosepalous calyx, pilose on the dorsum, a corolla with five petals welded at the base, of a lilac coloring, with an androecium with two fertile stamens and two staminodes.

S. cayennensis (Rich.) Vahl, popularly known as verbena, belongs to the family Verbenaceae (Pio Correa, 1984). This species is found in the tropical and subtropical Americas, from Mexico to Brazil (Lopes, 1977; Troncoso, 1979), and it has been used in traditional medicine as an anti-inflammatory, analgesic, antipyretic, hepatoprotective and laxative agent, and in the treatment of gastric disorders (Mathias and Emily, 1993; Mesia-Vela et al., 2004). Crushed leaves and roots have also been applied in the treatment of skin lesions (Caribe and Campos, 1991), including ulcerated lesions caused by *Leishmania* species (Moreira et al., 1998, 2002). Some of the effects suggested by the population have already been demonstrated experimentally, such as the anti-inflammatory, analgesic, gastroprotective, antibacterial and antifungal activity (Schapoval et al., 1998; Mesia-Vela et al., 2004; Duarte et al., 2004; Falcão et al., 2005; Okoye et al., 2010; Oliveira et al., 2011; Trabulsi Filho et al., 2013; Neiva et al., 2014).

Its chemical composition includes alkaloids, glycosides (verbenalin and verbenin), tannins, saponins, flavonoids, steroids, quinones, phenolic compounds and gluconic acid (Mathias and Emily, 1993).

The objective of this study was to evaluate the antifungal effect of the aqueous extract of the leaves of the *S. cayennensis* (Rich.) Vahl. (Verbenaceae), collected in the Southwest region of Paraná - Brazil, by determining its MIC and MFC on different species and strains of the genus *Candida.*

MATERIALS AND METHODS

Collection and preparation of the raw material

The aerial parts of the plant (*gervão roxo*) [*S. cayennensis* (Rich.) Vahl.], belonging to family Verbenaceae, were collected in the municipality of Francisco Beltrão - Paraná - Brazil, during its flowering period (Spring). The entire collection was performed on the same day, in the month of November, 2013. The plants were exsiccated in the Laboratory of Chemistry and Biochemistry, Universidade Paranaense - UNIPAR - Francisco Beltrão Campus - Paraná - Brazil. One voucher specimen was deposited in the herbarium of UNIPAR under the number 12,643.

The plants were then stored in a dehumidification chamber at a temperature of 24°C during 45 days for drying. After this period, the leaves were separated and crushed, obtaining the plant biomass (powder) for the preparation of the aqueous extract.

The preparation of the Aqueous Extract of *S. cayennensis* (Rich.) Vahl. was carried out in the Laboratory of Chemistry and Phytochemistry, União de Ensino do Sudoeste do Paraná - Unisep - Dois Vizinhos - Paraná - Brazil. The isolation and growth of the yeasts, in addition to the microbiological tests, were performed at the Laboratory of Microbiology, Universidade Paranaense - Unipar - Francisco Beltrão Campus - Paraná - Brazil.

Preparation of the aqueous extract

Twenty grams of dry powder of the leaves of *S. cayennensis* (Rich.) Vahl. were used for 1000 ml of distilled water, using 5 stages of preparation to obtain the sterile aqueous extract: (1) 20 g of dry vegetable matter (powder) were suspended in 300 ml of distilled water at 70°C, remaining in infusion for 24 h, duly closed and protected from light; 2) After this period, the suspension was filtered, and 350 ml of distilled water was again added to the filtration plant residue at a temperature of 70°C, with this infusion being maintained for more than 24 h in similar conditions as the first step; 3) The extract obtained in this second step was filtered and 350 ml of distilled water at 70°C was added to the resulting residue. This infusion was maintained over 24 h, completing the extraction process; 4) After the last filtering, three crude aqueous extracts were obtained, which were mixed to produce one single extract; 5) The final extract was subjected to vacuum filtration, with the volume of extract to be used in the experiments being previously submitted to microbiological filtration with a Millipore® membrane of 0.22 μm. This solution was put into sterile glass tubes and kept at 4°C and protected from light.

Isolation of microorganisms

Pure collection strains (ATCC-44858) and three different strains of clinical isolates of *Candida albicans*, *Candida tropicalis* and

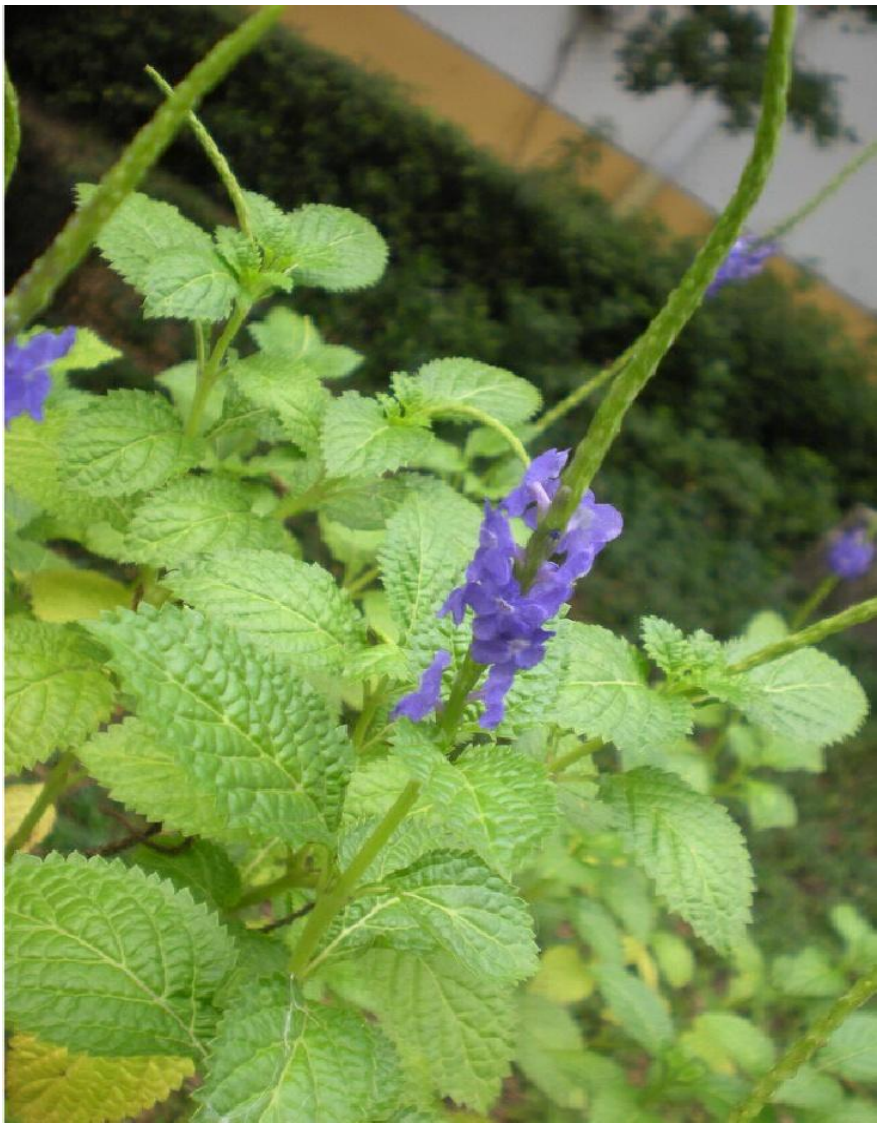

Figure 1. Botanical characteristics of verbena - *Stachytarpheta cayennensis* (L. C. Rich) Vahl.

Candida krusei, obtained from the Microbiology Laboratory, Universidade Paranaense - Unipar - Francisco Beltrão Campus - Paraná - Brazil, were used, which were collected from children between four and eight years of age. Strains of *C. albicans* (4 strains), *C. tropicalis* (3), *Candida glabrata* (1), *Candida stellatoidea* (1), *Candida dubliniensis* (1) and *C. krusei* (1) were used, totaling 11 fungal samples.

Preparation of the inoculum

The different yeast strains were activated in a Sabouraud dextrose agar medium, being planted and incubated for 24 h at 36°C. The cultures were suspended in 5 ml of sterile saline solution 0.85% (0.145 mol L^{-1}; 8.5 g L^{-1} NaCl). The resulting suspension was homogenized in a tube shaker for 15 s and the cell density was adjusted visually to the turbidity equivalent to 1.0 on the McFarland scale.

MIC determination

The antifungal activity tests were performed with the broth microdilution technique (BM), in accordance with the reference document M27A2 (NCCLS/CLSI, 2005). This methodology was used to test the yeasts in the aqueous extract of the leaves of *S. cayennensis,* (Rich.) Vahl., with the technique being adapted for this. The concentrations of the aqueous extract tested regarding the different species and strains of *Candida* varied from 0.09 to 25 mg mL^{-1}. A synthetic RPMI 1640 medium (with glutamine, without bicarbonate and with red phenol indicator) was used, buffered in 3-(N-morpholino) propanesulfonic acid (MOPSO), in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI) (NCCLS/CLSI, 2005).

Positive controls, which were characterized by 100 µl of the RPMI culture medium and 100 µl of inoculum solution, and negative controls, consisting only of 200 µl of the liquid RPMI culture medium, were used for all tests. The dishes were incubated at 36°C

Table 1. Effect of the aqueous extract of *Stachytarpheta cayennensis,* (Rich.) Vahl., on the different species and strains of the genus *Candida*.

Fungus		MIC	MFC
Species	**Strain**	**(mg mL^{-1})**	**(mg mL^{-1})**
Candida albicans	CαFB-01	R	R
C. albicans	CαFB-01	R	R
C. albicans	CαFB-01	12.50	R
C. albicans	ATCC-44858	12.50	R
C. tropicalis	CTFB-03	R	R
C. tropicalis	CTFB-22	25.00	R
C. tropicalis	CTFB-29	12.50	R
C. glabrata	CGFB-2x	12.50	R
C. stellatoideia	CSFB-1Ɛ	12.50	R
C. dubliniensis	CDFB-4β	12.50	R
C. krusei	CKFB-0α	12.50	12.50

MIC: Minimum inhibitory concentration; MFC: minimum fungicidal concentration; R: Did not have effect on the microorganisms tested - Resistant.

for 72 h, with readings taken every 24 h.

For the reading of the tests, the yeast growth that occurred in the dishes related to the different concentrations tested, was compared with its growth in the positive control dish. The lowest concentration capable of producing inhibition of yeast growth in relation to the positive control dish was identified as the minimum inhibitory concentration (MIC) of the plant extract for this sample. All dilutions of the plant extract and control groups were tested in triplicate.

MFC determination

The MFC was determined based on the results obtained for MIC. An aliquot of 50 μl of the dishes that showed inhibition was placed on the surface of a dish containing Sabouraud dextrose agar and incubated at 36°C for 24 h. MFC was considered to be the lowest concentration of the crude aqueous extract of *S. cayennensis,* (Rich.) Vahl., that did not demonstrate any fungal growth on the surface of the culture medium after incubation. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Table 1 shows the effect of the S. *cayennensis*, (Rich.) Vahl., extract on the different species and strains of the genus *Candida*. By analyzing the obtained results, one can see that the extract had a MIC of 25 mg mL^{-1} on the clinical isolate of *C. tropicalis,* and of 12.5 mg mL^{-1} on some isolates and on the ATCC-44858 strain of *C. albicans*, as well as on the isolates of *C. tropicalis*, C. *glabrata*, C. *stellatoidea*, *C. dubliniensis* and *C. Krusei*. The extract did not inhibit the growth of an isolate *of C. tropicalis* and two of *C. albicans.* However, with respect to the fungicidal activity, the minimum fungicidal concentration (MFC) of the extract was 12.5 mg mL^{-1} for the isolate of *C. krusei*. For all other strains and species, the extract did not have a MFC.

According to this study, concentrations of less than 12.5 mg mL^{-1} were insufficient for the aqueous extract of *S. cayennensis,* (Rich.) Vahl., to have a fungistatic or fungicidal effect *in vitro*. The concentration of 12.5 mg mL^{-1} , however, had a fungistatic effect on most tested strains and species (*C. albicans*, C. *tropicalis*, C. *glabrata*, C. *stellatoideia*, *C. dubliniensis* and *C. krusei),* with a more pronounced antifungal behavior being observed for the strains of *C. albicans* and *C. tropicalis*, and a fungicidal effect being observed only for the species *C. krusei.*

The species *S. cayennensis,* (Rich.) Vahl., evaluated in this study therefore shows satisfactory antimicrobial activity in the biological assays of antimicrobial activity on such pathogenic bacteria as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (Onofre et al., 2014).

According to Vargas (2006), both the root and leaves of *S. cayennensis* contain several substances with therapeutic potential, such as essential oils, polyphenols, an antibiotic compound similar to penicillin, vitamins B and C, calcium, phosphorus and iron.

The pharmacological effects suggested by the population have already been demonstrated experimentally, such as the anti-inflammatory, analgesic, gastroprotective, antibacterial and antifungal activity (Costa et al., 1960; Schapoval et al., 1998; Mesia-Vela et al., 2004; Duarte et al., 2004; Falcão et al., 2005).

Studies conducted by Robinson et al. (1990) and Vargas (2006) indicate that the triterpenoids obtained from the *S. cayennesis* have anti-inflammatory, antimicrobial, antiviral and analgesic effects. An *in vivo* experiment in rats has confirmed the analgesic and anti-inflammatory activity. The isolation in the extract of *S. cayennensis* of a glycosylated phenylpropanoid with anti-histaminic activity and of an iridoid, ipolamiide, with anti-histaminic and anti-bradykinin effect, contributed to

confirming the aforementioned effects.

Studies with the aqueous extract of *S. cayennensis* have demonstrated its antiulcerogenic effect and its inhibition of the secretion of gastric acid. This last effect is a consequence of the inhibition of the activity of the kinase protein dependent of AMPc (PKA). The anti-ulcer effect seems to involve the activation of defense mechanisms of the gastric mucosa, which is independent of the effect that inhibits the secretion of gastric acid (Vella, 1999; Penido et al., 2006).

In studies similar to this one that used the aqueous extract of *S. cayennensis*, the activity against strains of *Staphylococcus aureus*, *Micrococcus luteus, Bacillus subtilis, E. coli, P. aeruginosa* and *Salmonella typhimurium* has been observed (Duarte et al., 2004; Onofre et al., 2014).

In other studies with the aqueous extract of the leaves of *S. cayennensis* that sought to establish the intestinal transport of water and the effect on gastrointestinal propulsion in mice, a reduction in intestinal transit in relation to the control group was shown. This same work showed that the extracts of the leaves of *S. cayenensis* have a potential anti-diarrheal effect in infections by enteropathogens, highlighting the weak-to-moderate antibacterial activity *in vitro* on Gram-positive and Gram-negative bacteria (Rosas, 2004).

The bioautography assays revealed antimicrobial substances in the hexane fraction of the hydroalcoholic extract. In other studies, Vargas (2006), Okoye et al. (2010) and Okoye et al. (2014) observed that extract of the leaves of S. *cayennensis* proved to be effective in the treatment of *C. albicans* and other micro-organisms in oral cavity infections, inhibiting microbial growth.

The results of this study demonstrate the different effect of the extract of *S. cayenensis* on different species and strains of *Candida,* indicating that the aqueous extract of this plant has a considerable effect on the growth of these microorganisms. Studies in this sense were also carried out by Okoye et al. (2014). Their results showed that the components of the leaf extracts of *S. cayennensis* have immunomodulatory and antifungal activity.

The occurrence of candidiasis in Brazil and the clinical importance in immunocompromised patients justify this study with different strains of *Candida*. In this study, the antifungal effect of the crude aqueous extract of the leaves of *S. cayenensis* on one ATCC-44858 strain of *C. albicans*, and on three isolates of this species from the oral cavity, and on 7 strains of isolates of non-albicans *Candida* species, were evaluated.

The different behavior obtained for strains of the same species could be explained by the existence of genetic variability between the different strains, as can be seen in the results for the species *C. albicans* and *C. tropicalis*. In any research involving medicinal plants, and before attempting to extrapolate the results obtained, it is important to consider the environmental factors at the time of the collection of the plant, such as seasonality, climate, type of soil, and air temperature.

According to Freitas et al. (2004), the production of secondary metabolites by the plant occurs as a function of the plant-environment interaction in response to chemical and biological factors. This fact may explain the divergent results of extracts of the same species, but which were collected in different locations and time periods. The aqueous extract of *S. cayennensis* tested in this study was obtained from plants harvested in a single season (Spring), in a single month (November 2013) and in the same place of the municipality Francisco Beltrão - Paraná - Brazil.

The fungicidal effect of the aqueous extract of *S. cayennensis,* on the other hand, was only observed in one of the tested species. The concentration of 12.5 mg mL^{-1} was sufficient to inhibit the species *C. krusei*. However, even at the highest concentrations tested, no effect was observed on the other species, indicating the need for other tests involving concentrations exceeding 25 mg mL^{-1} of the aqueous extract of *S. cayennensis.*

Conclusion

The aqueous extract of the verbena (*gervão roxo*), *S. cayennensis* has an *in vitro* fungistatic effect on oral *Candida* species isolated from children between 4 and 8 years of age. The results allow the conclusion to be drawn that concentrations of less than 12.5 mg mL^{-1} were insufficient to show a fungistatic or fungicidal effect. The concentration of 12.5 mg mL^{-1} showed a fungistatic effect on the strains of *C. albicans* CαFB-12 and ATCC-44858; on *C. tropicalis,* strains CTFB-22 and CTFB29. The fungicidal effect was observed only on the species *C. krusei* (MFC = 12.5 mg mL^{-1}).

Conflicts of interest

The authors declare there are no ethical, publishing-related or financial conflicts of interest regarding the data of this study.

REFERENCES

Caribe J, Campos JM (1991). Plantas que ajudam o homem. Guia Prático para Época. Atual. Editora Pensamento.

Costa JML, Vale KC, França F, Saldanha ACR, Silva JO, Lago EL, Marsden PD, Magalhães AV, Silva CMP, Netto AS, Galvão CES (1990). Cura espontânea da leishmaniose causada por *Leishmania (Viannia) braziliensis* em lesões cutâneas. Rev. Soc. Bras. Med. Trop. 23:205-208.

Costa OA (1960). Estudo farmacognóstico do gervão. Rev. Bras. Farm. 41(11/12):615-650.

Duarte MCT, Figueira GM, Pereira B, Magalhães PM, Delarmelina C (2004). Atividade antimicrobina de extratos hidroalcoólicos de espécies da coleção de plantas medicinais CPQBA/UNICAMP. Rev. Bras. Farmacogn. 14(1):6-8.

Falcão HS, Lima IO, Santos VL, Dantas HF, Diniz MFFM, Barbosa-Filho JM, Batista LM (2005). Review of the plants with anti-inflammatory activity studied in Brazil. Rev. Bras. Farmacogn. 15:381-39.

Freitas MSM, Matins MA, Carvalho AJC, Carneiro RFV (2004). Crescimento e produção de fenóis totais em carqueja [*Baccharis trimera* (Less.) D.C.] em resposta à inoculação com fungos micorrízicos arbusculares, na presença e na ausência de adubação mineral. Rev. Bras. Plant Med. 6(3):30-34.

Gregorio G (2006). Nova legislação de fitomedicamentos inclui plantas brasileiras. Phytomédica 1(1):5-6.

Lopes SP (1977). Flora de Venezuela -Verbenaceae. Ed. Universidad de Los Andes, Venezuela.

Maciel MAM, Pinto CA, Veig JVF (2002). Plantas medicinais: a necessidade de estudos multidisciplinares. Quím. Nova 25(3):429-38.

Mathias LA, Emily A (1993). Tapping and Amazonian plethora: four medicinal plants of Marajó Island, Pará-Brazil. J. Ethnopharmacol. 40:53-75.

Mesia-Vela S, Souccar C, Lima-Landman MT, Lapa AJ (2004). Pharmacological study of *Stachytarpheta cayennensis* Vahl in rodents. Phytomedicine 11:616-624.

Moreira RCR, Costa JML, Saldanha AC, Silva AR (1998). Projeto Buriticupu Maranhão II. Plantas usadas como terapêutica da leishmaniose tegumentar americana na região de Buriticupu-Maranhão. Rev. Soc. Bras. Med. Trop. 31(1):248-256.

Moreira RCR, Rebêlo JMM, Gama MEA, Costa JML (2002). Nível de conhecimento sobre Leishmaniose Tegumentar Americana (LTA) e uso de terapias alternativas por populações de uma área endêmica da Amazônia do Maranhão, Brasil. Cad. Saúde Pública 18:187-195.

NCCLS/CLSI. (2005). Método de Referência para testes de diluição em caldo para determinação da sensibilidade de leveduras à terapia antifúngica: Norma aprovada – 2.ed. Brasília: Agência Nacional de Vigilância Sanitária. 45p.

Neiva VA, Ribeiro MNS, Nascimento FRF, Cartágenes MSS, Coutinho-Moraes DF, Amaral FMM (2014). Plant species used in giardiasis treatment: ethnopharmacology and *in vitro* evaluation of anti-Giardia activity. Braz. J. Pharmacogn. 24(2):214-224.

Okoye TC, Akah PA, Ezike AC, Uzor PF, Odoh UE, Igboeme SO, Onwuka UB, Okafor SN (2014). Immunomodulatory effects of *Stachytarpheta cayennensis* leaf extract and its synergistic effect with artesunate. BMC Complemet. Altern. Med. 14:376-383.

Okoye TC, Akah PA, Okoli CO, Ezike AC, Mbaoji FN (2010). Antimicrobial and antispasmodic activity of leaf extract and fractions of *Stachytarpheta cayennensis*. Asian Pacific J. Trop. Med. 3(3):189-192.

Oliveira DR, Leitão GG, Coelho TS, Silva PEA, Lourenço MCS, Arqmo SG (2011). Ethnopharmacological versus random plant selection methods for the evaluation of the antimycobacterial activity. Rev. Bras. Farmacogn. 21:793-806.

Penido C, Costa KA, Futuro DO, Paiva SR, Kaplan MA, Figueiredo MR, Henriques MG (2006). Anti-inflammatory and anti-ulcerogenic properties of *Stachytarpheta cayennensis* (L. C. Rich) Vahl. J. Ethnopharmacol. 104(1-2):225-233.

Pio Correa M (1984). Dicionário das plantas úteis do Brasil. v. III. Rio de Janeiro: Impressa Nacional. P 646.

Robinson RD, Williams LA, Lindo JF, Terry SI, Mansignh A (1990). Investigations of *Strongyloides stercoralis filariform* larvae *in vitro* by six commercial Jamaican plant extracts and tree anthelmintics. West Indian Med. J. 39(4):213-217.

Rosas LS (2004). Atividade antibacteriana de extratos de partes aéreas de *Stachytarpheta cayennensis* (Rich.) Vahl (Verbenaceae) frente a cepas Gram positivas e Gram negativas. Dissertacao (Mestrado em Saúde e Ambiente) - Universidade Federal do Maranhao.

Salimena-Pires FR, Giulietti AM (1998). Flora da Serra do Cipó, Minas Gerais: Verbenaceae. Bolet. Bot. Univ. 17:155-186.

Schapoval EES, Winter DE, Vargas MR, Chaves CG, Raquel BJA, Zuanazzi ATH (1998). Antiinflammatory and antinoceptive activities of extracts and isolated compounds from *Stachytarpheta* cayennensis. J. Ethnopharmacol. 60:53-59.

Trabulsi Filho FA, Andrade KCS, Silva EC, Castro ATO, Batista MCA, Ribeiro MNS, Amaral FMM (2013). Estudo de padronização de extratos de *Anacardium occidentale* L. na pesquisa e desenvolvimento de fitoterápicos giardicidas. Cad. Pesq. 20:7-15.

Troncoso NS (1979). Verbenaceae. In: Burkart, A. (Org.) Flora Ilustrada de Entre Rios, pt. 5. INTA, Buenos Aires. 6:229-294.

Vargas MRW (2006). *Stachytarpheta cayennensis* (L.C. Rich) Vahl: isolamento de constituintes químicos monitorados por ensaios farmacológicos. Dissertação de Mestrado. Universidade Federal do Rio Grande do Sul - Ciencias Farmaceuticas.

Vella SM (1995). Estudo farmacologico da *Stachytarpheta cayennensis* SCHAU (Gervao-Roxo), planta medicinal utilizada como antisecretora acida, antiulcera e analgesica. Mestrado. Universidade Federal de São Paulo – Farmacologia. 90p.

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