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# Anti-leishmanial activities of some compounds of *Tarchonanthus camphoratus* leaf surface exudates

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**Abstract**
One sesquiterpene, (-)-parthenolide (1), and four known methoxylated flavonoids; 5,7,3´,4´-tetrahydroxy-3-methoxyflavone (2), 5,7,4´-trihydroxy-6-methoxyflavone (3), 5,7,3´,4´-tetrahydroxy-6-methoxyflavone (4) and 5-hydroxy-7,8-dimethoxyflavanone (5) were characterized from leaf surface exudates of *Tarchonanthus camphoratus.* The structures of the compounds were determined based on spectroscopic data analyses. Compound 2 exhibited moderate anti-leishmanial activities against *Leishmania donovani* with $IC_{50}$ value of 12.84 µg/mL. (*vs* 0.85 for pentamidine and 0.12 µg/mL for amphotericin B). Compound 4 and 5 also showed anti-leishmanial activities with $IC_{50}$ values of 26.24 and 23.15 µg/mL respectively. All compounds tested were not cytotoxic at 5 µg/mL.

**Keywords:** sesquiterpene, parthenolide, flavonoids, Pentamidine, amphotericin

## Introduction
### Background
*Tarchonanthus camphoratus* belongs to the family Asteraceae and grows to 2-9 m high. This plant has characteristic leaves that are grey green above and pale grey and felted underneath, with prominent venation on the underside [1]. It grows in semi-arid regions of Kenya and Ethiopia [1]. Studies have shown that plants growing in these xeric habitats exude relatively simple organic compounds onto the outer aerial surface to protect the internal tissues from the harsh environmental conditions [2]. These surface compounds have become the subject of study as promising plant and human disease-controlling agents [3].

**Fig 1:** *T. camphoratus*

### 1.2 Ethno-medical application of *T. camphoratus*
The leaves of this plant have a wide range of ethno-medical applications. When burnt and inhaled, the leaves cure blocked sinuses, asthma and headache (Pretorius, 2008). The boiled leave extract treats cough, toothache, abdominal pain, bronchitis. The highly scented leaves are also used for massaging the body as perfume [4]. The Maasai of Kenya and Tanzania, for example, use the leaves of this plant as a deodorant [5]. The plant also shows powerful insect repellent action [6].

## 2.0 Experimental
### 2.1 Plant material
The fresh aerial parts of *T. camphoratus* were collected from Narok County, near Narok town (about 200 km from University of Nairobi on 27th January 2015 and identified by Mr. Patrick

Mutiso, a Botanist of the University of Nairobi Herbarium, School of Biological Sciences (SBS), where a voucher specimen (Okemwa-27/January, 2017) is preserved.

### 2.2 Extraction and isolation of compounds from the leaves of *T. camphoratus*

The surface exudates of the fresh aerial parts (4 kg) of *T. camphoratus* were extracted by successively dipping into portions of ethyl acetate and acetone for short periods (≈15s) to avoid extraction of internal tissue compounds. The extracts were filtered under pressure and solvent removed by rotatory evaporator. This yielded 112 g of a black crude extract translating to 2.8% yield. An amount of 100 g of the extract was adsorbed onto 115 g of silica gel ($SiO_2$, Merck grade 9385, pore size 60 Å, 230-400 mesh particle size) under 2% ethyl acetate (EtOAc) in *n*-hexane. Separation was effected using gravity column chromatography where the adsorbed extract was loaded onto a 1 kg $SiO_2$ column (15 cm x 10 cm). Stepwise gradient elution with mixtures of EtOAc in *n*-hexane starting with 2% EtOAc in *n*-hexane up to 18% in increasing order of polarities was carried out leading to 272 fractions of 300 ml each. The fractions were combined based on their thin layer chromatography (TLC) profiles into 28 fractions. The last fraction eluted with 18% EtOAc in *n*-hexane yielded a mixture of three compounds. The mixture was purified on preparative TLC by developing severally using 2% methanol ($CH_3OH$) in $CH_2Cl_2$. The major band was carefully scratched from the plate, soaked in 4% MeOH in $CH_2Cl_2$ and concentrated *in vacuo* using rotary evaporator. Compound 2 crystallized from the seventh fraction eluted with 10% EtOAc in *n*-hexane while 1 crystallized from the fifth fraction eluted with 8% EtOAc in *n*-hexane as white crystals. 4 were obtained by purification using PTLC (3% MeOH in $CH_2Cl_2$) of the mother liquor of the fraction of the major column eluted with10% EtOAc in *n*-hexane. The fraction, eluted with 16% EtOAc in *n*-hexane was purified further using column chromatography eluting initially with 12% EtOAc in *n*-hexane upto 18% in increasing order of polarity. White crystals of 5 recrystallized from the first fraction and yellow ones of 3 from the third fraction of this minor column. Compounds were visualized by observing under UV light at 254 nm followed by spraying the plates with 1% vanillin-$H_2SO_4$ spray reagent and placing the plates in iodine tanks in order to view the compounds that were UV inactive. 1D and 2D NMR spectra were recorded in $CDCl_3$, acetone-$d_6$, MeOD and DMSO depending on solubility of the compound under analysis. Electronspray Ionization High-Resolution Mass Spectroscopy (EI-HRMS) spectra recorded on 70 ev, on SSQ 710 MAT mass spectrometer.

### 2.3 Bioactivities

#### 2.3.1 *In vitro* anti-leishmanial activity assay

The *in vitro* test was performed as described by Hoet *et al* [7]. Amphotericin B (a commercial anti-leishmaniasis drug) and pentamidine were used as positive controls in all experiments with an initial concentration of 1.0 µg/ml. First stock solutions of crude extracts and compounds were prepared in DMSO or in ethanol/water (2:1) for water extracts at 20 mg/ml. The solutions were further diluted in the medium to give 0.2 mg/ml stock solutions. Extracts and compounds were tested against standard strain *Leishmania donovani* in eight serial three-fold dilutions (final concentration range: 100–0.05 µg/ml) in 96-well microtiter plates.

#### 2.3.2 *In vitro* cytotoxicity analysis

Monkey kidney fibroblasts (VERO) were obtained from the American type culture collection (ATTC, Rockville, MD). The cell viability studies were done against the fibroblasts. The cells were seeded at a density of 25,000 cells/well and incubated for 24 h in 96-well microplates. Samples at different concentrations were added and plates were further incubated for 48 h. the number of viable cells were determined using neutral red according to [8] DMSO and Doxorubicin (98-102% purity assessed by HPLC) were used as positive and negative controls, respectively.

## 3.0 Results and Discussion

### 3.1 Structure elucidation

On extraction, the mass of the surface exudate extract was 9% yield /dry leaf weight from which the seven compounds. Structure elucidations of the compounds was accomplished through 1D and 2D NMR and mass spectrometric analyses, and also by comparison with standard published spectra.

#### 3.1.1 (-)-Parthenolide (1)

The compound had an $R_f$ of 0.40 in 60 % $CH_2Cl_2$ in n-hexane. Analyzing the spectral data showed it to be (-)-Parthenolide that was initially isolated from the same plant[4]. The $^{13}$C-NMR revealed the presence of thirteen carbon atoms in the structure. Both $^{13}$C-NMR and DEPT showed the compound has four quaternary carbons and the rest protonated. One of the quaternary carbons is $d_C$ 169.3. This chemical shift is typical for ketone group and was thus assigned to the carbonyl carbon in the skeletal structure. The remaining three quaternary carbons appearing at $d_C$ 134.6, 61.4 and 139.3 were caused by C−3, 7 and 11 respectively. C-7 is $sp^3$ hybridized but appeared lowfield because of being bonded to oxygen in the epoxide ring system. The C−3 and −11, which were $sp^2$ hybridized were far much downfield shifted due to deshielding by anisotropy found in unsaturated moieties. Protonated $sp^2$carbons, C−10 andC−14, were also observed at $d_C$ 125.2 and 121.2 respectively. Due to their diastereotopic nature, C-14 protons formed doublets at $d_H$ 6.31 ($J$=2.8) and 5.62 ($J$=2.8). The proton bonded to C-10 was a doublet at 5.21 ppm ($J$=9.6). The coupling constant indicated strong magnetic interaction with the axial proton on C−9. Methyl C−15 and 16 distinctively emerged at $d_C$ 16.9 and 17.3 in $^{13}$C-NMR. The corresponding protons caused singlets at $d_H$ 1.30 and 1.71 respectively, each having an integration of three protons. The $^{13}$C-NMR and DEPT showed four methylene C−8, 9, 12 and 13 at $d_C$ 36.3, 24.1, 41.2 and 30.5respectively, within their chemical shift ranges. Protons of these carbons formed multiplets in the range $d_H$ 1.21- 2.43. Two methine carbons, C-4 and -5 were also observed at $d_C$ 47.6 and 82.5 respectively. The low chemical shift for the latter is due to its direct attachment to heteroatomic and electronegative oxygen. A summary of $^1$H- and $^{13}$C-NMR chemical shift assignments is given in Table 1.

#### 3.1.2 5,7,3´,4´-Tetrahydroxy-3-methoxychalcone (2)

This flavone was isolated from the surface exudates of the aerial parts of *Tarchonanthus camphoratus* amorphous white solid with an $R_f$ value of 0.41 in $CH_2Cl_2$ in- hexane. It was identified as 5,7,3´,4´-Tetrahydroxy-3-methoxychalcone, a known chalcone ((Hegaz *et al.*, 2015). Its $^{13}$C-NMR spectrum revealed the presence of sixteen carbons atoms with the carbonyl carbon of the ketone group appearing at $d_C$ 182.6. The peaks appearing $d_C$ 129.2 and 128.4 were assigned to C-2 and C-3 respectively. The methoxy carbon was downfield

shifted typically appearing at $d_C$ 59.8 and the corresponding protons at $d_H$ 3.87(*s*).

Aromatic carbons of ring A, with oxygen substitution, appeared in their expected chemical shift ranges. C-5 was assigned to $d_C$ 156.7. The phenolic proton of hydroxy group bonded to this carbon was downfield shifted to appear at $d_H$ 13.23 in the lowfield region of $^1$H-NMR spectrum due to hydrogen bonding with carbonyl carbon that lengthens the O−H bond and deshields the proton. With the exception of carbonyl carbon, C-7 is most deshielded as a result appeared at $d_C$ 164.4. As a consequence of electron withdrawing effect of heteroatomic oxygen C-9 was also observed at $d_C$ 153.1 ppm in the downfield region of $^{13}$C-NMR spectrum. Non-substituted ArC, C-6 and C-8, appeared at $d_C$ 93.8 and 102.7. These are ArCs between oxygenated ArCs and experience strong shielding impacted by OH groups on the contiguous carbon atoms. The signal at $d_C$ 104.8 of a quaternary aromatic carbon was certainly due to C-10.

Hydroxy substituted carbons of ring B gave rise to signals d 142.4 and 145.6 in *ortho* orientation with respect to each other and the chemical shifts are typical to this type of carbons. The protonated carbons of the aromatic ring were assigned to $d_C$ 113.2 (C-2), 115.7(C-5) and 119.2 (C-6) in the upfield end of the aromatic region. The corresponding protons were observed in the range of $d_C$ 7.47- 7.51. The chemicals shifts of this compound and their assignments are recorded in table 1.

### 3.1.3 5,7,4´-Trihydroxy-6-methoxyflavone (3)

This compound was successfully isolated from surface exudates of *Tarchonanthus camphoratus.* It was isolated as yellow crystals with $R_f$ 0.46 in 2:5 EtOAc: n-hexane. Its structure was elucidated from NMR spectroscopy and comparison with spectral data of related compounds and was identified as hispidulin previously isolated from the same plant by Van Wyk *et al* [4].

The $^{13}$C-NMR spectrum revealed that it has sixteen carbon atoms. From DEPT spectral analysis, the compound has nine quaternary carbons and the rest being protonated. The $^1$H-NMR spectrum revealed two sets of protons exhibiting AABB spin system. This implicated a *para*- disubstituted benzene moiety. They were doublets at 6.90 ($J$=6.8) and 7.84 ($J$=6.4) ppm. The corresponding symmetric carbons of twice intensity were assigned to signals at $d_C$ 116.3 and 128.8 with C-3'/5'. They were upfield shifted due to the strong shielding effect from OH group on C-4'. This explains the existence of ring B with substitution at the *para* position.

For ring C, the chemical shift at $d_C$ 182.2 was typical for carbonyl carbon of either ketone or aldehyde and was assigned to C-4. From $^{13}$C-NMR spectrum, the signals at $d_C$ 164.4 and 102.7 were assigned to C-2 and C-3. C-2 was so downfield shifted because it is a $sp^2$ and bonded to an electronegative heteroatomic oxygen in a six-membered ring system. DEPT indicated that C-3 is protonated. The quaternary carbon appearing at $d_C$ 104.5 is undoubtedly assigned to C-10. It is usual for quaternary ArC between 1,3-*diortho* oxygen substituted ArC to resonate at approximately $d_C$ 100.0.

In $^1$H-NMR, the presence of a singlet at 6.55 ppm, in the aromatic region, revealed the existence of a 1,2,3,4,5-pentasubstituted benzene ring. This proton was attached to C-8 of ring A. Another singlet appeared in this region (at 6.65 ppm) but this was due to the proton bonded to C-3. Furthermore, the $^{13}$C-NMR spectrum showed peaks at $d_C$ 164.4 and $d_C$ 182.5 assigned to C-2 and C-4 respectively. These peaks were downfield shifted due to oxygenation. Their exact chemical shifts are given in Table 2.

### 3.1.4 6,7,3´4´-Tetrahydroxy-6-methoxyflavone (4)

This a flavone that was isolated from the surface extract of *Tarchonanthus camphoratus* aerial parts. It is a yellow compound with $R_f$ of 0.43 in 1:1 EtOAc in n-hexane.

The $^{13}$C-NMR spectrum exhibited 16 signals which were consistent with the proposed structure. The $^{13}$C NMR spectrum showed no overlapping of signals; all peaks were almost of equal intensity. The 1h-nmr spectrum showed a singlet at $d_H$ 6.55 suggesting a 1,2,3,4,5-pentasubstituted benzene skeleton. This helped formulate ring A. There was another singlet at $d_H$ 6.61 corresponding to C-3 of ring C. The DEPT spectrum indicated ten quaternary carbons with ring A and C accounting for seven of them. The remaining three carbons are C-1', -3' and -4'. Both $^1$H-NMR and $^{13}$C-NMR revealed no symmetric substitution in the structure (no overlapping of signals). Hence, to avoid symmetry, the OH groups were attached to C-3' and C-4'.

From $13^C$-NMR spectrum, the signal at $d_C$ 182.5 was assigned to C=O moiety of a ketone which typically appears at this chemical shift value. Therefore, the chemical shift was undoubtedly due to C-4. C-2, a $sp^2$ quaternary carbon bonded to heteroatomic oxygen in a six-membered ring system was observed at $d_C$ 164.5. The signal at $d_C$ 102.8 of a protonated carbon was assigned to C-3. Its proton, as mentioned earlier, was observed at $d_H$ 6.61.

For ring A, three oxygenated carbons were observed within their expected chemical shift ranges. The signals $d_C$ 153.1, 157.7 and 152.8 were assigned to C-5 C-7 and C-9 respectively. However, methoxylated C-6 was downfield shifted to appear at $d_C$ 131.8 due to strong shielding from hydroxy groups in both *ortho* positions. The non-substituted ArC, C-8, was responsible for the peak at $d_C$ 94.6 with its corresponding proton appearing as a singlet at $d_H$ 6.55. From DEPT spectrum, the signal at $d_C$ 104.5 was due to a quaternary carbon and is typical for a ArC between 1,3-*diortho* oxygen substituted ArCs. This was certainly due to C-10.

In ring C, due to asymmetric substitution, none of the six carbons overlapped. As result of strong shielding effect of hydroxyl group on *ortho* carbons, C-2' and C-5' were assigned to relatively upfield chemical shifts $d_C$ 113.7 and 116.5 respectively with non-substituted C-6' in the *meta* position appearing slightly lowfield at $d_C$ 119.4. The quaternary C'-1 of the ring was assigned to chemical shift at $d_C$ 122.0. Aromatic protons in this ring system appeared between $d_H$ 6.88-7.38. it was found to ne nepetin which was isolated from this plant by Van Wyk *et al*. (1997). Its NMR chemical shift assignments are recorded in Table 2.

### 3.1.5 5-Hydroxy-7,8-dimethoxyflavone (5)

This compound was isolated from the internal tissue extract. It crystallized as a yellow compound that crystallized from MeOH in $CH_2Cl_2$ with an $R_f$ of 0.34 in 30% EtOAc in n-hexane.

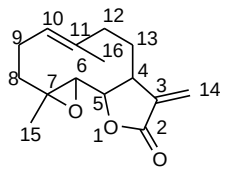
The structure of this compound was determined by 1D and 2D NMR spectroscopy. From $^{13}$C-NMR revealed the presence of seventeen carbon atoms which was consistent with the proposed structure. In $^1$H-NMR spectrum, the methylene and methine protons of ring C exhibited a typical ABX spin system. As a consequence of diastereotopic nature of the methylene protons in the Azole ring, they were observed as doublet of doublets in the ranges of $d_H$ 2.71-2.76 ( $J_{vic}$= 12.0,

$J_{gem}$=4.0), and 2.95-3.03 (*dd*, 1H, $CH_2$ $J_{vic}$= 12.0, $J_{gem}$=4.0). The coupling constants indicated strong vicinal and geminal coupling. Furthermore, they had long range connectivities to carbonyl carbon (C=O) at $\delta_C$ 190.4 and the more shielded methine carbon at $\delta_C$ 79.1 which was downfield shifted due to its attachment to heteroatomic oxygen. This is expected for methine carbons of in a five-membered heterocyclic ring which resonate in the region of $d_C$ 77-110.

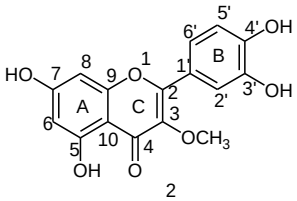
The methine proton, due to coupling with both axial (*J*=12.0) and equatorial (*J*=4.0) methylene protons, also appeared as a doublet of doublets in the region of $d_H$ 3.31-3.35. The proton appeared downfield of methylene protons due to its close proximity to a benzene ring and the heteroatom oxygen. Long range connectivities ($^3J$) were observed between the proton and carbonyl carbon and the non-substituted carbons, C-2′/6′ ($\delta_C$ 125.7) of ring B. It also showed $^2J$ HMBC with methylene carbon, which resonated at $\delta_C$ 44.9 and the quaternary carbon ($\delta_C$ 139.5) of ring B. COSY spectrum, also indicated its correlation with the methylene protons.

The $^{13}$C NMR signal for the non-substituted aromatic carbon on ring A was typically observed at δ 92.8. From HMQC correlation, the corresponding proton was a singlet at $d_C$ 6.15 in the aromatic region of $^1$H-NMR spectrum. Furthermore, HMBC experiment clearly indicated its $^3J_{HC}$ connectivity to the methoxy substituted carbon (C-8) and quaternary carbon (C-10) appearing at $d_C$ 129.3, and 104.2 respectively. There was also HMBC correlation of this proton with the 1,3-*diortho* oxygenated aromatic carbons, C−9 and −7 appearing at $d_C$ 156.8 and 158.0 respectively.

The intense signals of the two pairs of equivalent carbons, C-2′/6′ and 3′/5′, on ring B appeared at $\delta_C$ 125.7 and δ 128.3. C′-4 of this ring was assigned the chemical shift at $\delta_C$ 128.2. From HMQC, The corresponding protons to these carbons appeared in the region of $\delta_H$ 7.34-7.53 as multiplets integrating for five protons. Table 2 shows the $^1$H- and $^{13}$C-NMR chemical shift assignments.




1

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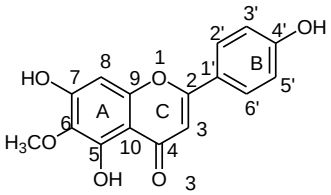


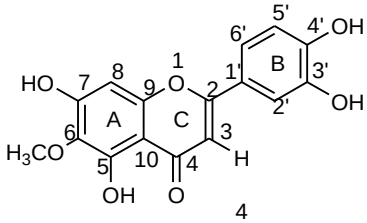


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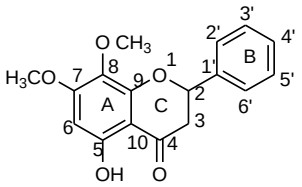


5

### 3.2 Bioactivities

All the five compounds were evaluated for their *in vitro* anti-leishmanial activity. Compound 2 exhibited moderate anti-leishmanial activities and cytotoxicity against *Leishmania donovani* and Vero cells with $IC_{50}$ values of 12.84 ± 0.3 µg/mL. These activity was relatively lower than the standard drugs, pentamidine ($IC_{50}$ = 0.85 µg/mL) and amphotericin B ($IC_{50}$ = 0.12 µg/mL). Compounds 4 and 5 also showed anti-leishmanial activities with an $IC_{50}$ value of 26.24 ± 0.4 and 23.15 ± 0.4 µg/mL respectively. All compounds were not cytotoxic upto the maximum concentration tested (5 mg/mL).

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**Table 1:** Natural compounds isolated from *Tarchonanthus camphoratus*

| 1( **Acetone-$d_6$**) | | | 2( **Acetone-$d_6$**) | | | **3 (acetone -$d_6$)** | | |
|---|---|---|---|---|---|---|---|---|
| **PS** | **$d_C$ (Hz)** | **$d_H$ (Hz)** | **PS** | **$d_C$ (Hz)** | **$d_H$ (Hz)** | **PS** | **$d_C$ (Hz)** | **$d_H$ (Hz)** |
| | | | | | | 2 | 164.4 | |
| 2 | 169.3 | | 2 | 149.2 | | 3 | 102.7 (1C, CH, $sp^2$C) | 6.70 (*s*, 1H, CH) |
| 3 | 134.6 | | 3 | 128.4 | | 4 | 182.5 (1C, *q*, C=O) | |
| 4 | 47.6 | 2.35-2.43 (*m*) | 4 | 182.6 | | 5 | 153.1 (1C, *q*, ArC-OH) | 12.97 (*s*, 1H, ArOH) |
| 5 | 82.5 | 3.84 (*t*, 1H, CH, *J*=6.8) | 5 | 156.7 | 13.24 (*s*, 1H, ArOH) | 6 | 131.8 (1C, *q*, ArC-$OCH_3$) | |
| 6 | 66.4 | 2.80 (*d*, 1H, CH, *J*=2.4) | 6 | 102. 7 | 6.59 (*s*, 1H, CH, ArH) | 7 | 157.6 (1C, *q*, ArC-OH) | 5.51 (*s*, 1H, ArOH) |
| 7 | 61.4 | | 7 | 164.4 | | 8 | 94.7 (1C, CH, ArC) | 6.58 (*s*, 1H, CH, ArH) |
| 8 | 36.3 | 1.70-1.76 (*m*, 2H, $CH_2$) | 8 | 93.8 | 6.60 (*s*, 1H, CH, ArH) | 9 | 152.8 (1C, *q*, ArC-O) | |
| 9 | 24.1 | 2.13-2.00 (*m*, 2H, $CH_2$) | 9 | 153.1 | | 10 | 104.5 (1C, *q*, ArC) | |
| 10 | 125.2 | 5.21 (*d*, 1H, CH, *J*=9.6) | 10 | 104.8 | | 1' | 121.7 (1C, *q*, ArC) | |
| 11 | 139.3 | | 1' | 122.8 | | 2'/6' | 129.8 (2C, CH, ArC) | 6.92 (*d*, 2H, ArH, *J*=6.8) |
| 12 | 41.2 | 2.13-2.00 (*m*, 2H, $CH_2$) | 2' | 113.2 | 7.47 (*d*, CH, ArH, *J*=8.0) | 3'/5' | 116.4 (2C, CH, ArC) | 6.88 (*d*, 2H, ArH, *J*=6.8) |
| 13 | 30.5 | 1.21-1.27 (*m*, 2H, $CH_2$) | 3' | 145.6 | 3.14, 9.50 (*s*, (*broad*), 1H, ArH) | 4' | 161.5 (1C, q, ArC-OH | 6.79 (*s*, 1H, ArOH) |
| 14 | 121.2 | 6.31 (*d*, 1H, $CH_2$, *J*=2.8) 5.62 (*d*, 1H, $CH_2$, *J*=2.8) | 4' | 142.4 | | 6-$OCH_3$ | 60.4 (1C, $CH_3$, $OCH_3$) | 3.73 (*s*, 3H, $CH_3$) |
| 15 | 17.3 | 1.30 (*s*, 3H, $CH_3$) | 5' | 115.7 | 7.00 (*d*, CH, ArH, *J*=8.0) | | | |
| 16 | 16.9 | 1.71 (*s*, 3H, $CH_3$) | 6' | 119.2 | 7.51 (*d*, 1H, ArH, *J*=4.0 | | | |
| | | | 3-$OCH_3$ | 59.8 | 3.87 (*s*, 3H, $CH_3$) | | | |

KEY: PS-position,

**Table 2:** Natural compounds isolated from *Tarchonanthus camphoratus (cont.)*

| 4( **DMSO**) | | | 5( **$CDCl_3$**) | | |
|---|---|---|---|---|---|
| **PS** | **$d_C$ (Hz)** | **$d_H$ (Hz)** | **PS** | **$d_C$ (Hz)** | **$d_H$ (Hz)** |
| 2 | 164.5 (1C, *q*, C-O) | | 2 | 79.1 (1C, CH) | 3.31-3.35 (1dd, $CH_2$ $J_{ax}$= 12.0, $J_{eq}$=4.0) |
| 3 | 102.8 (1C, $sp^2$ CH) | 6.61 (*s*, 1H, CH) | 3 | 47.0 (1C, $CH_2$) | 2.71-2.76 (*dd*, 1H, $CH_2$ $J_{vic}$= 12.0, $J_{gem}$=4.0)<br>2.95-3.03 (*dd*, 1H, $CH_2$ $J_{vic}$= 12.0, $J_{gem}$=4.0) |
| 4 | 182.5 (1C, *q*, C=O) | | 4 | 190.4 (1C, *q*, C=O) | |
| 5 | 153.1 (1C, *q*, ArC-OH) | 12.98 (*s*, 1H, CH, ArOH) | 5 | 157.9 (1C, *q*, ARC-OH) | 5.47 (*d* (*seudo*), 1H, ArOH) |
| 6 | 131. 8(1C, *q*, ArC-$OCH_3$) | | 6 | 92.6 (1C, CH, ArC) | 6.15 (*s*,1H, CH, ArH) |
| 7 | 157.7 (1C, *q*, ArC-OH) | | 7 | 158.0 (1C, *q*, ArC−$OCH_3$) | |
| 8 | 94.6 (1C,CH, ArC) | 6.55 (*s*, 1H, ArH) | 8 | 129.3 (1C, *q*, ArC−$OCH_3$) | |
| 9 | 152.8 (1C, *q*, ArC-O-) | | 9 | 156.8 (1C, *q*, ArC—O) | |
| 10 | 104.5 (1C, *q*, ArC) | | 10 | 104.2 (1C, *q*, ArC) | |
| 1' | 122.0 (1C, *q*, ArC) | | 1' | 139.1 (1C, *q*, ArC) | |
| 2' | 113.7 (1C, CH, ArC) | 6.88-7.38 (*m*, 3H, CH, ArH) | 2'/6' | 125.7 (2C, CH, ArC) | 7.34-7.53 (*m*, 5H, CH, ArHs) |
| 5' | 116.5 (1C, CH, ArC) | | 3'/5' | 128.3 (2C, CH, ArC) | |
| 3' | 150.1 (1C, *q*, ArC-OH) | 3.47 (*s*, 1H, CH, ArOH) | 4' | 128.2 (1C, CH, ArC) | |
| 4' | 146.1 (1C, *q*, ArC-OH) | 3.82 (*s*, 1H, CH, ArOH) | 7-$OCH_3$ | 54.8 (1C, $CH_3$, $OCH_3$) | 3.36, 3.79 (*s*, 6H, $CH_3$) |
| 6' | 119.4 (1C, CH, ArC) | | | | |
| 6-$OCH_3$ | 60.4 (1C, $CH_3$, $OCH_3$) | 3.73 (*s*, 3H, $CH_3$) | | | |
| | | | 8-OCH3 | 60.1 (1C, $CH_3$, $OCH_3$ | |

**Table 1:** Anti-leishmanial activity assay data

| **Sample/compound** | ***L. donovani* $IC_{50}$ µg/mL*** | ***L. donovani* $IC_{90}$ µg/mL**** |
|---|---|---|
| Pentamidine | 0.85 | 1.75 |
| Amphotericin B | 0.12 | 0.15 |
| 1 | NA | NA |
| 2 | 12.84 | 26.17 |
| 3 | NA | NA |
| 4 | 26.24 | 39.25 |
| 5 | 23.15 | 33.69 |
| LA | 11.75 | 24.91 |
| DHA | 14.37 | 28.59 |

*The concentration (µg/ml) that affords 50% inhibition of growth

**The concentration (µg/ml) that affords 90% inhibition of growth

NA = not active

EL = Ethyl linoleate

LA = Linoleic acid

DHA =*cis*-4,7,10,13,16,19-Docosahexaenoic acid ethyl ester

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