Investigational
Medicinal Chemistry & Pharmacology

Research Article Open Access

Anti-leishmanial activity of some surface compounds of *Tarchonanthus camphoratus*

Okemwa Evans Kenanda[1]* and Leonidah Kerubo Omosa[2]

Abstract

Background: The World Health Organization recognizes leishmaniasis as a major tropical disease with no effective vaccine against it. Chemotherapy, the only effective way to treat all forms of the disease, is toxic and expensive. Hence, there is need for scientists to scale up the search for new anti-leishmanial agents. The present study investigated the *in vitro* anti-leishmanial activity of five surface compounds of *Tarchonanthus camphoratus.*

Methods: The surface exudates were obtained by rinsing the aerial parts of the plant with 100% ethyl acetate and acetone. Five compounds were isolated from the exudates and purified by column (CC) and Thin Layer Chromatographic (TLC) techniques, respectively. The structures of the compounds were elucidated by use of Nuclear Magnetic Resonance spectroscopy (NMR) and Electrospray Ionization High-Resolution Mass Spectroscopy (EIHRMS). The identified compounds were then screened for anti-leishmanial activity using *Leishmania donovani* as the standard strain.

Results: The five compounds were a known 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**). Compound **2** exhibited moderate anti-leishmanial activities against *Leishmania donovani* with IC_{50} value of 12.84 ± 0.62 µg/mL. (*vs* 0.85 ± 0.04 for pentamidine and 0.12 ± 0.02 µg/mL for amphotericin B). Compound **4** and **5** also showed anti-leishmanial activities with IC_{50} values of 26.24 ± 0.14 and 23.15 ± 0.84 µg/mL, respectively. Compound **1** and **3** were inactive at the tested concentration as they inhibited <70% of growth of *L. donovani* standard drug.

Conclusion: Compounds **2**, **4** and **5** were active against standard strain and can be targets for synthetic modification for activity optimization.

Keywords: sesquiterpene; parthenolide; flavonoids; *Leishmania donovani.*

*Correspondence: Phone: +254 717 976 211; Email: ekenanda@kisiiuniversity.ac.ke (Okemwa Evans Kenanda)
[1]Department of Research and Extension, Kisii University, P.O. Box 408-40200, Kisii, Kenya
[2]Department of Chemistry, College of Physical and Biological Sciences, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya.

Citation on this article: Kenanda OE and Omosa LK. Anti-leishmanial activity of some surface compounds of Tarchonanthus camphoratus. Investigational Medicinal Chemistry and Pharmacology (2019) 2(2):30; Doi: https://dx.doi.org/10.31183/imcp.2019.00030.

Background

Leishmaniasis is an infectious disease caused by parasites of the Leishmania type. It is spread by the bite of certain types of sand flies. The World Health Organization has classified leishmaniasis as a major tropical disease [1]. It constitutes a major health problem, especially in Africa. As is the case for any parasitic diseases, there is no effective vaccine against leishmaniasis. Chemotherapy, the only effective way to treat all forms of the disease is toxic and expensive [2]. Consequently, there is need for scientists to scale up the search for new anti-leishmanial agents.

Medicinal plant extracts possess several pharmacological properties such as anti-bacterial, anti-oxidant, anti-tumor, anti-inflammatory and anti-leishmanial activities [3]. These activities are attributed to the presence of a diversity of functional compounds that possess biological properties [4]. Hence, the current research focused on isolation of compounds of *Tarchonanthus camphorates* (a medicinal plant) and evaluation of their anti-leishmanial activity.

Tarchonanthus camphoratus belongs to the family *Asteraceae.* This plant has characteristic leaves that are grey green above and pale grey and felted underneath, with prominent venation on the underside. It grows in semi-arid regions of Kenya and Ethiopia [5-6]. Studies have revealed that plants growing under stress drought conditions have the concentrations of their secondary metabolites (bioactive phytochemicals) significantly enhanced [7]. This is because all metabolic processes are pushed towards the synthesis of highly reduced compounds, such as isoprenoids, phenols, or alkaloids that play a major role in the adaptation of the plants to the environment [8]. These compounds have become the subject of study as promising human disease-controlling agents [9].

The *T.camphoratus* has wide range of ethno-medical applications. When burnt and inhaled, the leaves cure blocked sinuses, asthma and headache. The boiled leave extract treats cough, toothache, abdominal pain, bronchitis. The highly scented leaves are also used for massaging the body as perfume [9]. The Maasai of Kenya, for example, use the leaves of this plant as a deodorant [10]. The plant also shows powerful insect repellent action [11]. Problems such as blocked sinuses and headache can be healed by inhaling the smoke from the burning green leaves. Drinking boiled mixture of leaves can help to treat coughing, toothache and bronchitis [12].

The reported pharmacological activities of this plant include decongestant and anti-spasmodic effects [13]. The aqueous extracts of its leaves have demonstrated *in vivo* analgesic and antipyretic activity. It is also effective in treating fever induced by lipopolysaccharides in rats [13]. In general, the plant has wide range of both ethno-medical applications and pharmacological profile making it a suitable target for investigation.

Methods

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.

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 9.8% 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 up to 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. Electrospray Ionization High-Resolution Mass Spectroscopy (EI-HRMS) spectra recorded on 70 ev, on SSQ 710 MAT mass spectrometer (Table 1).

Bioactivities

In vitro anti-leishmanial activity assay

The *in vitro* test was performed as described by Hoet *et al*. [14]. Amphotericin B (a commercial anti-leishmaniasis drug) and pentamidine, obtained from American Type Culture Collection, ATCC (Manassas, VA) 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 dimethyl Sulphoxide DMSO or in ethanol/water (2:1) for water extracts at 20 µg/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*, obtained from American Type Culture Collection, ATCC (Manassas, VA), in eight serial three-fold dilutions (final concentration range: 100–0.045 µg/mL) in 96-well microtiter plates. The samples were tested in triplicate and results recorded in Table 2.

Results and Discussion

Structure elucidation

On extraction, the mass of the surface exudate extract was 9.8 % yield/dry leaf weight from which the five compounds were isolated. Structure elucidation of the compound was accomplished through 1D-and 2D-NMR and mass spectrometric analyses.

*(-)-Parthenolide (**1**)*

The compound had a retention factor (R_f) of 0.40 in 60 % CH_2Cl_2 in n-hexane. Analyzing the spectral data showed it to be (-)-Parthenolide (see Figure 1) that was initially isolated from the same plant [9]. The Carbon-13 Nuclear Magnetic Resonance spectroscopy (^{13}C-NMR) revealed the presence of thirteen carbon atoms in the structure. Both ^{13}C-NMR and Distortionless Enhancement of Polarisation Transfer NMR (DEPT) showed the compound has four quaternary carbons and the rest were protonated. One of the quaternary carbons was δ_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 δ_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 C−11, which were sp^2 hybridized were downfield shifted due to deshielding by anisotropy found in unsaturated moieties. Protonated sp^2 carbons, C−10 and C−14, were also observed at δ_C 125.2 and 121.2, respectively. Due to their diastereotopic nature, C-14 protons appeared as doublets at δ_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 resonated at δ_C 16.9 and 17.3 in ^{13}C-NMR spectrum. The corresponding protons appeared as singlets at δ_H 1.30 and 1.71, respectively, each having an integration of three protons. The ^{13}C-NMR and DEPT showed four methylenes C−8, 9, 12 and 13 at δ_C 36.3, 24.1, 41.2 and 30.5, respectively, within their chemical shift ranges. Protons of these carbons appeared as multiplets in the range δ_H 1.21- 2.43. Two methine carbons, C-4 and -5 were also observed at δ_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.

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

This flavone was isolated from the surface exudates of the aerial parts of *Tarchonanthus camphoratus* amorphous as white solid with an R_f value of 0.41 in CH_2Cl_2 *n*-hexane. It was identified as 5,7,3´,4´-tetrahydroxy-3-methoxychalcone, a known chalcone. Its ^{13}C-NMR spectrum revealed the presence of sixteen carbons atoms with the carbonyl carbon of the ketone group appearing at δ_C 182.6. The peaks appearing δ_C 129.2 and 128.4 were assigned to C-2 and C-3, respectively. The methoxy carbon was downfield shifted typically appearing at δ_C 59.8 and the corresponding protons at δ_H 3.87(*s*).

Aromatic carbons of ring A, with oxygen substitution, appeared in their expected chemical shift ranges. C-5 was assigned to δ_C 156.7. The phenolic proton of the hydroxy group bonded to this carbon was downfield shifted to appear at δ_H 13.23 in the low field 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 the carbonyl carbon, C-7 was most deshielded as a result appeared at δ_C 164.4. As a consequence of electron withdrawing effect of heteroatomic oxygen C-9 was also observed at δ_C 153.1 ppm in the downfield region of ^{13}C-NMR spectrum. Non-substituted aromatic carbon (ArC), C-6 and C-8, appeared at δ_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 δ_C 104.8 of a quaternary aromatic carbon was certainly due to C-10.

Hydroxy substituted carbons of ring B gave rise to signals δ142.4 and 145.6 in *ortho* orientation with respect to each other and the chemical shifts are typical of this type of carbons. The protonated carbons of the aromatic ring were assigned to δ_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 δ_C 7.47- 7.51. The chemicals shifts of this compound and their assignments are recorded in Table 1.

*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.* [9].

The ^{13}C-NMR spectrum revealed that it had 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 AA'BB' 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 double the intensity were assigned to signals at δ_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 δ_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 δ_C 164.4 and 102.7 were assigned to C-2 and C-3. C-2 was so downfield shifted because it was a sp^2 and bonded to an electronegative heteroatomic oxygen in a six-membered ring system. DEPT indicated that C-3 was protonated. The quaternary carbon appearing at δ_C 104.5 was undoubtedly assigned to C-10. It is usual for quaternary ArC between 1,3-*diortho* oxygen substituted aromatic carbon (ArC) to resonate at approximately δ_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 δ_C 164.4 and δ_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 1.

*6,7,3´4´-Tetrahydroxy-6-methoxyflavone (**4**)*

6,7,3´4´-Tetrahydroxy-6-methoxyflavone (**4**) (Figure 1) 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 δ_H 6.55 suggesting a 1,2,3,4,5-pentasubstituted benzene skeleton. This helped formulate ring A. There was another singlet at δ_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 was 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 δ_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 δ_C 164.5. The signal at δ_C 102.8 of a protonated carbon was assigned to C-3. Its proton, as mentioned earlier, was observed at δ_H 6.61.

For ring A, three oxygenated carbons were observed within their expected chemical shift ranges. The signals δ_C 153.1, 157.7 and 152.8 were assigned to C-5 C-7 and C-9, respectively. However, the methoxylated C-6 was downfield shifted to appear at δ_C 131.8 due to strong shielding from hydroxyl groups in both *ortho* positions. The non-substituted ArC, C-8, was responsible for the peak at δ_C 94.6 with its corresponding proton appearing as a singlet at δ_H 6.55. From DEPT spectrum, the signal at δ_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 the hydroxyl group on *ortho* carbons, C-2' and C-5' were assigned to relatively upfield chemical shifts δ_C 113.7 and 116.5, respectively with non-substituted C-6' in the *meta* position appearing slightly lowfield at δ_C 119.4. The quaternary C'-1 of the ring was assigned to chemical shift at δ_C 122.0. Aromatic protons in this ring system appeared between δ_H 6.88-7.38. Based on these spectral data found to nepetin which was isolated from this plant by Van Wyk *et al*. [13]. Its NMR chemical shift assignments are recorded in Table 1.

*5-Hydroxy-7,8-dimethoxyflavone (**5**)*

This compound was isolated from the internal tissue extract. It crystallized as a yellow compound 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 (one dimensional) and 2D (two dimensional) 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 δ_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 δ_C 190.4 and the more shielded methine carbon at δ_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 δ_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 δ_H 3.31-3.35. The proton appeared downfield of methylene protons due to its close proximity to the 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' (δ_C 125.7) of ring B. It also showed ^2J-Heteronuclear Multiple-Bond Correlation (HMBC) with methylene carbon, which resonated at δ_C 44.9 and the quaternary carbon (δ_C 139.5) of ring B. Correlation spectroscopy (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 Heteronuclear Multiple – Quantum Correlation (HMQC), the corresponding proton was a singlet at δ_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 δ_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 C−7 appearing at δ_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 δ_C 125.7 and δ 128.3. C'-4 of this ring was assigned the chemical shift at δ_C 128.2. From HMQC, The corresponding protons to these carbons appeared in the region of δ_H 7.34-7.53 as multiplets integrating for five protons. Table 1 shows the ^{1}H- and ^{13}C-NMR chemical shift assignments. Based on the these spectral analyses, the compound was identified as 5-hydroxy-7,8-dimethoxyflavone (**5**) (Figure 1).

Bioactivities

All the five compounds were evaluated for their *in vitro* anti-leishmanial activity (see Table 2). Compound **2** exhibited anti-leishmanial activity against *Leishmania donovani* with IC_{50} values of 12.84 ± 0.62 µg/mL. These activity was lower than the standard drugs, pentamidine (IC_{50} =0.85 ± 0.04 µg/mL) and amphotericin B (IC_{50} =0.12 ± 0.02 µg/mL). Compounds **4** and **5** also showed anti-leishmanial activities with an IC_{50} values of 26.24 ± 0.14 and 23.15 ± 0.84 µg/mL, respectively. In general, the three active compounds exhibited moderate anti-leishmanial activity as their IC_{50}values were within the range $10<IC_{50}<50$ µg/mL [15]. Compound **1** and **3** were inactive at the tested concentration as they inhibited <70% of growth of *L. donovani.*

The activity of compounds **2** and **4** could be attributed to hydroxylation at position 4. Studies have shown that anti-leishmanial activity is associated with less lipophilic flavonoids, in particular those with 4′-hydroxyl-substituted B rings and hetero/polyaromatic A rings [16]. Compounds **2** and **4** had similar substitution pattern in ring B but differed in rings A and C. The placement of the methoxy group in ring C at C-3 in compound **2** seemed to contribute to increased anti-leishmanial activity by two fold as compared to its placement in ring A at C-6 position in compound **4**.

Methoxylated ring A of flavonoids has also been found to play a significant contribution to anti-leishmanial activity of flavonoids [16]. However, the observations of the current study were contrary to previous results as the two compound with methoxylation at ring A (**3** and **4**) exhibited substantially lower anti-leishmanial activities as compared with compounds **2** with methoxylation at ring C. Previous studies on compounds of this class with structural similarity have shown positive promising results. For instance, an isoflavone biochanin A (Figure 2) showed 50% effective concentration (EC_{50}) value of 18.96 µg/mL against promastigotes of *Leishmania* (L.) *chagasi* [17], quercitrin (Figure 2) demonstrated to be a potent anti-leishmanial compound ($IC_{50} \approx 1$ µg/mL) [18] and luteolin (Figure 2), had already been described as a promising anti-leishmanial drug [19].

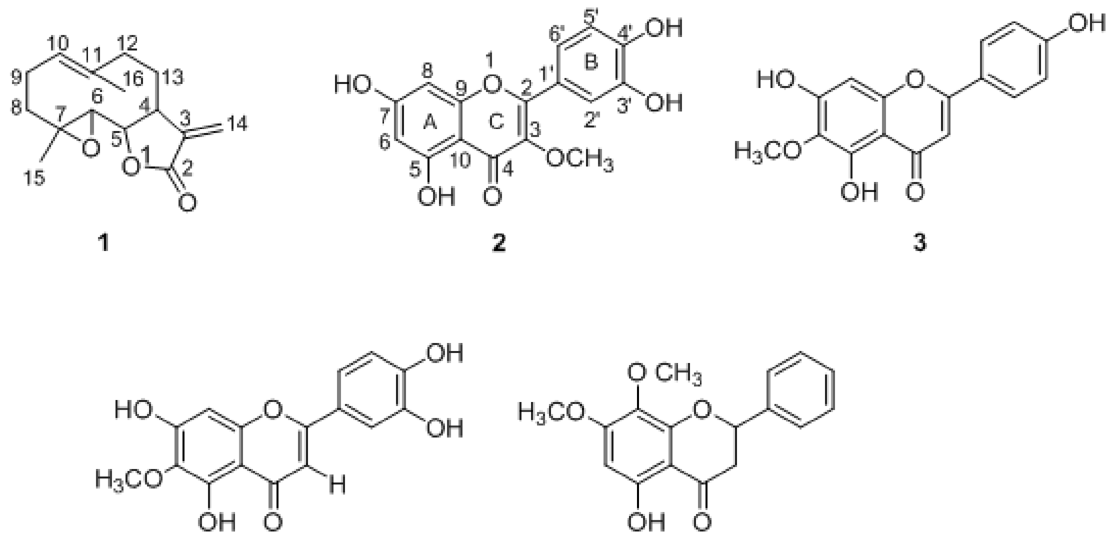

Figure 1. Compounds isolated from surface exudates of *Tarchonanthus camphoratus*

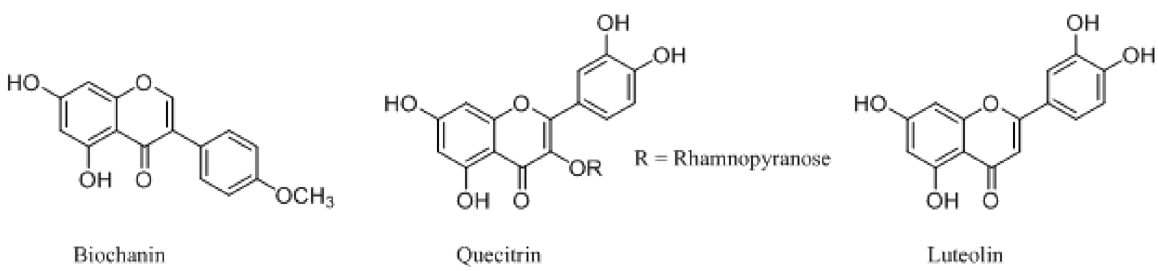

Figure 2. Reported flavonoids with anti-leishmanial activity

Table 1. Natural compounds isolated from *Tarchonanthus camphorates*

1(**Acetone-d$_6$**)			2(**Acetone-d$_6$**)			**3 (acetone -d$_6$)**		
PS	**δ_C (Hz)**	**δ_H (Hz)**	**PS**	**δ_C (Hz)**	**δ_H (Hz)**	**PS**	**δ_C (Hz)**	**δ_H (Hz)**
						2	164.4	
2	169.3		2	149.2		3	102.7 (1C, CH, sp^2C)	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 1: Natural compounds isolated from *Tarchonanthus camphoratus (cont.)*

4(**DMSO**)			5(**CDCl$_3$**)		
PS	**δ_C (Hz)**	**δ_H (Hz)**	**PS**	**δ_C (Hz)**	**δ_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}$= 12.0, J_{gem}=4.0)
					2.95-3.03 (*dd*, 1H, CH$_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 2. *In vitro* anti-leishmanial activity of surface compounds of *T. camphoratus* (IC_{50} and IC_{90} ± SD µg/mL) against *L. donovani* standard strain. (Values are means ± standard deviation of three determinations)

Sample/compound	***L. donovani* IC_{50} µg/mL***	***L. donovani* IC_{90} µg/mL****
Pentamidine	0.85± 0.04	1.75± 0.0.06
Amphotericin B	0.12±0.02	0.15±0.03
1	NA	NA
2	12.84±0.62	26.17±1.12
3	NA	NA
4	26.24±0.14	39.25±0.71
5	23.15±0.84	33.69±1.16

*The concentration (µg/ml) that affords 50% inhibition of growth; ** The concentration (µg/ml) that affords 90% inhibition of growth; NA = not active

Conclusions

The current investigation reveals that three surface compounds of *T. camphorates.* Compounds, namely 5,7,3´,4´-tetrahydroxy-3-methoxychalcone (**2**), 6,7,3´4´-tetrahydroxy-6-methoxyflavone (**4**) and 5-hydroxy-7,8-dimethoxyflavone (**5**), possess activity against *Leishmania donovani* standard strain and should be isolated and made targets for activity optimization through synthetic modification. These compounds belong to a class of compounds, flavonoids, with a wide spectrum of biological activities, but few studies have been devoted to their anti-leishmanial activity. In the studies, these natural polyphenols demonstrated activity against anti-leishmanial strains and should be subjects for future investigations.

Abbreviations

ArC - Aromatic Carbon
ATCC - American Type Culture Collection, ATCC
CC - Column chromatography
TLC - Thin layer column chromatography
COSY - Correlation Spectroscopy
DEPT - Distortionless Enhancement of Polarisation Transfer
ETOAc - Ethyl Acetate
HMBC - Heteronuclear Multiple-Quantum Correlation
HMQC - Heteronuclear Multiple-Quantum Correlation
ISP - International Science Programme (ISP)
J_{gem} - Coupling constant for germinal protons
J_{vin} - Coupling constant for germinal protons
NA - Not Active
NACOSTI - National Commission for Science and Technology and Innovation
NMR - Nuclear Magnetic Resonance spectroscopy
ArC - Aromatic carbon

Authors' Contribution

OEK did isolation and purification, structure elucidation of compounds and drafted the manuscript of this publication. LKO interpreted bioassay data, assisted in structure elucidation and editing of the manuscript. The two authors also proof- read and approved the final version of the script.

Acknowledgments

The authors wish to acknowledge to Mr. Patrick C. Mutiso for identification and collection of plant materials. The authors also wish to thank the National Commission for Science and Technology and Innovation (NACOSTI), Kenya and International Science Programme (ISP), Uppsalla University, through KEN-02 project for providing grants that supported this research. ID and 2D NMR, MS and anti-leishmanial assay were supported by the USDA ARS specific Cooperative Agreement No. 58-6408-1-603 and NIH, NIAID, Division of AIDS, Grant No. AI 27094, respectively. Dr. Evans Okemwa Kenanda is grateful to Muhammad Illias for providing the materials and carrying out the bioassay experiments.

Conflict of interest

The authors declare that they have no competing interests.

Article history:

Received: 11 July 2019
Received in revised form: 29 July 2019
Accepted: 30 July 2019
Available online: 30 July 2019

References

1. Lianet M. 2009. Current treatment of leishmaniasis: A Review. *The Open Antimicrobial Agents Journal*. 1:9-19
2. Coombs GH, Croft SL. 2009. Leishmaniasis– current chemotherapy and recent advances in the search for novel drugs. *Trends in Parasitology*. 9(11)
3. Bouyahya A, Et-Touys A, Nadia D, Hajiba , Jamal .2018. Antileishmanial potential of medicinal plant extracts from the North-West of Morocco. *Beni-Suef University Journal of basic and applied sciences.* 7:50-54
4. Lahlou M. 2013. The Success of Natural Products in Drug Discovery. *Pharmacology and Pharmacy*, *04*(03):17–31
5. Costa R, d'Acampora ZB, Crupi ML, Fina MRD, Valentino MR., Dugo P, Mondello L. 2008. GC–MS, GC–O and enantio–GC investigation of the essential oil of *Tarchonanthus camphoratus* L. *Flavour and Fragrance Journal*. *23*(1): 40–48.
6. Modak B, Leonor CM, González-Nilo F, Torres R. 2005. Structure–antioxidant activity relationships of flavonoids isolated from the resinous exudate of *Heliotropium sinuatum*. *Bioorganic & Medicinal Chemistry Letters*. 15(2): 309–312.
7. Kleinwächter M, Selmar D. 2015. New insights explain that drought stress enhances the quality of spice and medicinal plants: potential applications. *Agronomy for Sustainable Development*. 35(1):121–131
8. González-Lamothe R, Mitchell, G, Gattuso M, Diarra, M, Malouin F, Bouarab, K. 2009. Plant antimicrobial agents and their effects on plant and human pathogens. *International Journal of Molecular Sciences*. 10(8):3400–3419
9. Van Wyk BE, de Wet H,Van Heerden FR. 2008. An ethnobotanical survey of medicinal plants in the South Eastern Karoo, South Africa. *South African Journal of Botany*. 74(4):696–704.
10. Young TP, Francombe C. 1991. Growth and yield estimates in natural stands of leleshwa (*Tarconanthus camphoratus*). *Forest Ecology and Management*. 41(3–4):309–321.
11. Omolo MO, Okinyo D, Ndiege IO, Lwande W, Hassanali A. 2004. Repellency of essential oils of some Kenyan plants against Anopheles gambiae. *Phytochemistry*. 65(20), 2797–2802.
12. Wafaa A T, Mohamed-Elamir FH, Emad MH, Tarik AM, Hassan AA., Abdessamad D, 2015. Sesquiterpene Acid Derivatives from *Tarchonanthus camphoratus. Research Journal of Pharmaceutical, Biological and Chemical Sciences*.**6**(1):659
13. Van Wyk, BE, Van Oudtshoorn B, Gericke N. 1997. Medicinal plants of South Africa.*Briza Publications*, Pretoria, South Africa: 304.
14. Quetin-Leclercq J, Hoet S, Block S, Wautier MC, Stévigny C. 2004. Studies on *Cassytha filiformis* from Benin: isolation, biological activities and quantification of aporphines. 27.
15. Kuete V, Efferth T. 2015. African flora has the potential to fight multidrug resistance of cancer. *Biomedical Research International.* 2015:914813.
16. Mei L, Prapon W, Simon LC, Lay-Choo A, Mei-Lin TG. 2003. Structure–activity relationships ofantileishmanial and antimalarial chalcones. *Bioorganic Chemistry*.11:2729–2738].
17. Sartorelli P, Camila SC, Reimão JQ, Marcelo JPF, Tempone AG. 2009. Antiparasitic activity of biochanin A, an isolated isoflavone from fruits of *Cassia fistula* (Leguminosae). *Parasitology Research*. 104(2):311–314
18. Michelle F, Elaine AC, Ana PASilvia AG. Da S , Carlos RK, GuetteC, Bartira R-B, Sônia SC. 2006. Quercitrin: An antileishmanial flavonoid glycoside from *Kalanchoe pinnata. Planta Medica*. 72(1): 81-83
19. Heman.ta KM, Bidyottam MA , Saha ARC, ChiranjibP, Suparna M, Sibabrata M, Santu B. 2000. Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Molecular Medicine*.6(6): 527–541