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Leishmanicidal activity of onopordopicrin isolated from the leaves of *Brachylaena discolor*

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ABSTRACT

Brachylaena discolor (Asteraceae) is a medicinal plant used in Mozambique to treat stomachache, tuberculosis, and diabetes. To discover new lead compounds with leishmanicidal activity, a methanolic extract of the leaves of this plant was investigated. Through fractionation, employing several chromatographic methods, two sesquiterpene lactones onopordopicrin (**1**) and its germacronolide epoxide derivative (**2**), together with other ten know phenolic compounds derivatives (**3-12**) were isolated. The structures of the isolated compounds were determined by spectroscopic methods, mainly 1D and 2D NMR experiments, as well as by comparing their spectroscopic data as reported in the literature. Compounds **1** and **2** were evaluated for their leishmanicidal activity using the Colorimetric method-XXT. Compound **1** showed a significant leishmanicidal activity against *Leishmania amazonensis* and *L. braziliensis* (IC_{50} values 39.6 and 27.9 µM, respectively) as compared to miltefosine (12.5 and 12.0 µM, respectively), a currently used agent to treat leishmaniosis. While compound **2** was inactive against both stains tested with IC_{50} values >50µM compared with the same agent. Compounds **3-12** were not assayed for leishmanicidal activity. This is the first study reporting the above-mentioned activity of onopordopicrin (**1**). The results could suggest that compound **1** is a promising lead structure to treat leishmaniosis.

Keywords: Asteraceae, *Brachylaena discolor*, Onopordopicrin, Germanocronolides, Antileishmanial activity.

Introduction

Out of more than 5,500 plant species recorded in Mozambique, about 10% are used for medicinal purposes, including the treatment of infections and other diseases.[1,2] Traditional medicine has remained as the most affordable and available source to solve health problems in rural communities all over the world. According to WHO, 80 % of the world's population still rely on such remedies for their basic healthcare needs. In addition, the knowledge provided by the traditional uses of plants to treat different illnesses has been helpful in the search of new biologically active metabolites.[3,4] Based on such knowledge, phytochemical studies on *Brachylaena* (Asteraceae) species has led to the identification of a large number of sesquiterpene lactones. The sesquiterpene lactones are considered the characteristic secondary metabolites of the genus, exhibiting mainly antibacterial activity.[5–9] The genus *Brachylaena* is composed of 13 to 20 plant species, mostly distributed in the Southern part of Africa.[8,10] *Brachylaena discolor D.C.* (var. *discolor*) is an evergreen shrub or small tree usually 4 to 10 m in height. In Mozambique, the plant is found in the Southern part of the country and grows in coastal woodland and bushland, and on the margins of evergreen forests. The roots and leaves have been used for medicinal purposes for the treatment of stomachache, tuberculosis, and diabetes.[4,11–13]

Previous phytochemical investigation on the aerial parts of *B. discolor* (var. *discolor*) led to the isolation of a sesquiterpene lactone, onopordopicrin (**1**).[6] In a continuing study of phytochemical constituents derived from Mozambican traditional medicinal plants, an extract of the leaves of *B. discolor* was investigated. In this paper, we wish to report the isolation and leishmanicidal activity of compound **1** and **2** assayed against *Leishmania amazonensis* and *L. braziliensis*, which is the first time compounds **1** and **2** are assayed for antileishmanial activity. The structure of compound (**1**) was established by high resolution NMR and MS experiments, and confirmed by the comparison of its experimental spectroscopic and physical data with those reported in the literature.[14] Metabolite (**1**) has been found in larger amounts in various plants of the Asteraceae family[15–18] and has been reported to possess cytotoxic and antibacterial activities.[14,19]

Materials and Methods

General Experimental Procedures

1D and 2D NMR spectra were recorded at room temperature with a Bruker Advance II 400 spectrometer. The chemical shifts (δ) are reported in ppm relative to solvent residual signals (δ_H 7.26 and δ_C 77.0 for $CDCl_3$; δ_H 3.31 and δ_C 47.0 for CD_3OD; δ_H 2.50 and δ_C 39.5 for DMSO-d_6), while the coupling constants (J) are expressed in Hz. HRES-MS were performed with a Waters Acquity UPLC + Waters XEVO-G2 system spectrometer. The IR spectra data were recorded on a Bruker Alpha-P ART-IR spectrophotometer. The melting point measurements were carried out on Gallenkamp instrument. The column chromatography (CC) was performed using silica gel 60 (230-400 mesh, Merck) and gel permeation on Sephadex LH-20 (GE-Healthcare)

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Analytical TLC plates were visualized under UV lamp at 254 nm and spraying with vanillin followed by heating. All solvents used were of analytical grade.

Leishmanicidal assay

The activity was measured on in vitro cultures of Leishmania parasite in promastigote forms of complex L. amazonensis (clon 1: Lma, MHOM/BR/76/LTB-012) and complex L. braziliensis (strand M2904 C192 RJA), cultivated at 26°C in Schneider medium (pH 6.8) supplemented with inactivated (56°C x 30min) calf bovine serum (10%). Parasites in logarithmic phase of growth, at a concentration of 1x106parasites/mL, were distributed on 96 micro well plates and different concentration of compounds **1** and **2** (100, 50, 25, 12.5, 6.2, 3.1, 1.5 µM) were added. The micro well plates were incubated for 72hrs at 26 °C after which a solution of XTT (1mg/mL) in PBS (pH 7.0 at 37°C) with PMS (Sigma Aldrich, 0,06mg/mL), was added (50µL/well) and the incubation continued for 4hrs at 26 °C. All assays were carried out as triplicates. DMSO (1%) and miltefosine were used as negative and positive control. The optical density of each well was determined with a Synergy HT microplate reader, at λ 200-450nm. The IC_{50} values were calculated using the Gen5 program (BioTek).[20]

Plant material

The leaves of B. discolor were collected in Magude District, Maputo Province, Mozambique, in August 2014. The plant was identified locally and a voucher specimen under accession number 225 is kept at the Herbarium of the Botanical Garden of Eduardo Mondlane University.

Extraction and isolation of compounds

The air-dried and powdered leaves of B. discolour (350g) were extracted 3 times for 24h with 200ml MeOH. The combined extracts were evaporated to dryness under reduced pressure to give a crude MeOH extract (22.5g). This was redissolved in a mixture of MeOH:H_2O (10:90) and then extracted with n-heptane, $CHCl_3$, and EtOAc to yield 10.5g, 1.1g, and 2.5g, respectively, of organic subfractions.

The EtOAc subfraction (2.3g) was subjected to column chromatography (CC) on silica gel eluted with DCM:EtOAc mixtures in different ratios (0-100%) and then EtOAc:MeOH (95:5) to yield 5 fractions (A-E). Fraction D (697mg) was subjected to Sephadex LH-20 chromatography eluted with a mixture of MeOH:$CHCl_3$ (1:1) to afford 5 subfractions (D1-D5). Subfraction D2 (304mg) was passed through Sephadex LH-20 using the same solvent system as previously employed to afford two main subfractions (D21 and D22). The subfraction D22 (95.0mg) was chromatographed on Sephadex LH-20 (100% MeOH) giving hydroxytyrosol (22.7mg), dihydroxysinapic acid (31.7mg) and 6''-O-acetyl homoplantaginin (26.7mg). After passing the fraction D5 (180.1mg) through Sephadex LH-20 (100% MeOH), quercetin-7-O-galactopyranoside (24.2mg), quercetin-3-O-glucoside-7,3',4'-trimethyl ether (13.9mg), quercetin-3-*O*-β-D-galactopyranoside (9.0mg), and eupafolin (7.0mg) were isolated. Fraction E (300 mg) was submitted to Sephadex LH-20 MeOH:$CHCl_3$ (1:1) to afford subfractions E1-E4. The Sephadex LH-20 (100%) fractionation carried out with subfraction E3 (145.2mg) resulted in the isolation of onoporidin (10.2mg). Fraction E4 (48.0mg) was subjected to Sephadex LH-20 fractionation with a mixture of MeOH:$CHCl_3$ (1:1) to yield 3'-hydroxygenkwanin (11.0mg), and luteolin (17.0mg). Fraction B (198.7 mg) was submitted to silica gel in a mixture of DCM:EtOAc (0-100%) to give **1** (88.9 mg) and **2** (68.6mg) as the main components.

Results and Discussion

The leaves of *B. discolor* were air-dried at room temperature, and the dried leaves were macerated with MeOH. The crude methanolic extract was then fractionated between *n*-heptane, $CHCl_3$, and EtOAc. Repeated chromatographic purifications of the EtOAc extract yielded twelve known metabolites. Besides the isolation of compound (**1**), the sesquiterpene lactone germacronolide epoxy derivative (**2**),[5] was isolated, along with the following phenolic compounds: hydroxytyrosol (**3**),[21] dihydroxysinapic acid (**4**),[22] 6''-*O*-acetyl homoplantaginin (**5**),[23] onoporidin (**6**),[24] 3'-hydroxygenkwanin (**7**),[25] luteolin (**8**),[26] quercetin 3-*O*-glucoside-7,3',4'-trimethyl ether (**9**),[27] quercetin-3-*O*-β-*D*-galactopyranoside (**10**),[28] eupafolin (**11**),[29] quercetin-7-galactopyranoside (**12**).[30] The structures of the isolated compound are presented in Figure 1.

Compounds **1** and **2** were evaluated for their antileishmanial activity. Compound **1** showed a promising antileishmanial activity against *L. amazonensis* and *L. braziliensis* strains with IC_{50} values of 39.6 and 27.9µM, respectively. Miltefosine used to treat leishmaniosis, with IC_{50} values 12.5 and 12.0µM, respectively, was used as positive control. Interestingly, compound **2** was inactive against both strains tested according to table 1. The difference in activity of these two metabolites could be attributed to the presence of two *α*, *β*-unsaturated carbonyl functionality in **1**, while **1** has two of these functionalities **2** has only one, in addition to an epoxide moiety. The presence of a second *α,β*-unsaturated carbonyl functionality in **1** could influence the activity, as such activated methylene groups are commonly identified as responsible for many biological effects as a consequence of their electrophilic reactivity as Michael acceptors, which can react with, for example, the thiol groups present in proteins.[16,31] This is also supported by the fact that dehydrozaluzanin C, another sesquiterpene lactone isolated from *Mummocia maronii* (Asteraceae) with two *α,β*-unsaturated carbonyl groups, inhibits the growth of leishmanicidal promastigotes at concentrations ranging between 10.0 to 44.6µM.[32,33] The 2-(hydroxymethyl)acrylate side chain in **1**, as well as the number of *α,β*-unsaturated carbonyl groups may be implicated for other biological activities. For example, in comparison of antitrypanosomal activity the lack of the side chain of **1** drastically lowers its activity.[19]

Sesquiterpene lactones are bioactive secondary metabolites, which are found in a variety of plants. The biological activities they possess could be useful for a range of conditions, from skin ulcer to atherosclerosis, neurodegeneration, and even cancer. They have also been proposed as lead compounds for the design of new anti-inflammatory drugs.[16,34] This class comprises a large group with more than 5000 known metabolites, mostly found in the family Asteraceae.[31] Sesquiterpene lactones are derived from the mevalonic acid pathway, and metabolites **1** and **2** have a germacronolide terpenoid skeleton.[35] Compounds **1** and **2** could therefore share the same biosynthetic pathway, possessing an additional and unusual four carbon atom unit. However, the detailed biosynthetic mechanism is still not understood.

Onopordopicrin (**1**): Colorless oil; $[\alpha]_D^{25}$ + 17.2° (*c* 1.00, $CHCl_3$); m.p. 57-59°C; HR-ESMS *m/z*: 349.1651 $[M+H]^+$ (calc. for $C_{19}H_{24}O_6$, 349.1650); IR film (cm^{-1}) 3450 (OH), 2925 (methylene), 1755 (C=O, γ-lactone), 1705 (C=O, unsaturated ester); ^{1}H-NMR (400 MHz, $CDCl_3$): 5.02 (1H, *dd*, *J* = 11.1, 4.0 Hz, H-1), 2.24 (1H, *m*, H-2α), 2.00 (1H, *m*, H-2β), 2.24 (1H, *m*, H-3α), 2.62 (1H, *m*, H-3β), 4.85 (1H, *d*, *J* = 10.0 Hz, H-5), 5.14 (1H, *dd*, *J* = 10.0, 10.0 Hz, H-6), 3.11 (1H, *m*, H-7), 5.19 (1H, *dd*, *J* = 9.4, 10.0 Hz, H-8), 2.62 (1H, *m*, H-9α), 2.00 (1H, *m*, H-9β), 6.29 (1H, *d*, *J* = 3.5 Hz, H-13α), 5.81 (1H, *d*, *J* = 3.5 Hz, H-13β), 1.51 (3H, *s*, H-14), 4.23 (1H, br *d*, H-15α), 4.07 (1H, br *d*, H-15β), 4.31 (2H, *s*, H-3'), 6.30 (1H, br *d*, H-4'α), 6.00 (1H, br *d*, H-4'β). ^{13}C-NMR (100 MHz, $CDCl_3$): 129.7 (C-1), 26.0 (C-2), 34.5 (C-3), 144.5 (C-4), 127.9 (C-5), 77.3 (C-6), 52.8 (C-7), 72.9 (C-8), 48.8 (C-9), 139.6 (C-10), 132.1 (C-11), 165.1 (C-12), 125.6 (C-13), 16.6 (C-14), 60.5 (C-15), 170.5 (C-1'), 135.2 (C-2'), 60.9 (C-3'), 125.7 (C-4').

Salonitelonide-8-O-2',3'-isobutyrate (**2**): Colorless oil; $[\alpha]_D^{25}$ + 16.4° (*c* 1.00, $CHCl_3$); m.p. 69-71°C; HR-ESMS *m/z*: 348.1570 $[M]^+$ (calc. for $C_{19}H_{24}O_6$, 348.1569); IR film (cm^{-1}): 3400 (OH), 1780 (C=O, γ-lactone), 1740 (C=O), ^{1}H-NMR (400 MHz, $CDCl_3$): 4.89 (1H, *dd*, *J* = 11.0, 4.2 Hz, H-1), 1.97 (1H, *m*, H-2α), 2.24 (1H, *m*, H-2β), 2.59 (1H, *m*, H-3α), 2.25 (1H, *m*, H-3β), 4.75 (1H, *d*, *J* = 10.0 Hz, H-5), 5.09 (1H, *d*, *J* = 10.0, 10.0 Hz, H-6), 3.01 (1H, *m*, H-7), 5.16 (1H, *dd*, *J* = 10.0, 9.0 Hz, H-8), 2.53 (1H, *d*, *J* = 13.1 Hz, H-9α), 2.44 (1H, *dd*, *J* = 13.1, 10.0 Hz, H-9β), 6.37 (1H, *d*, *J* = 3.6 Hz, H-13α), 6.10 (1H, *d*, *J* = 3.6 Hz, H-13β), 1.49 (3H, *s*, H-14), 4.30 (1H, br *d*, H-15α), 4.08 (1H, br *d*, H-15β), 3.11 (1H, *d*, *J* = 6.2 Hz, H-3'α), 2.80 (1H, *d*, *J* = 6.2 Hz, H-3'β), 1.57 (1H, *s*, H-4). ^{13}C-NMR (100 MHz, $CDCl_3$): 127.1 (C-1), 28.3 (C-2), 31.6 (C-3), 143.1 (C-4), 127.5 (C-5), 76.1 (C-6), 50.6 (C-7), 75.3 (C-8), 40.8 (C-9), 133.7 (C-10), 137.5 (C-11), 171.0 (C-12), 123.0 (C-13), 16.7 (C-14), 61.9 (C-15), 168.3 (C-1'), 55.2 (C-2'), 55.1 (C-3'), 19.0 (C-4').

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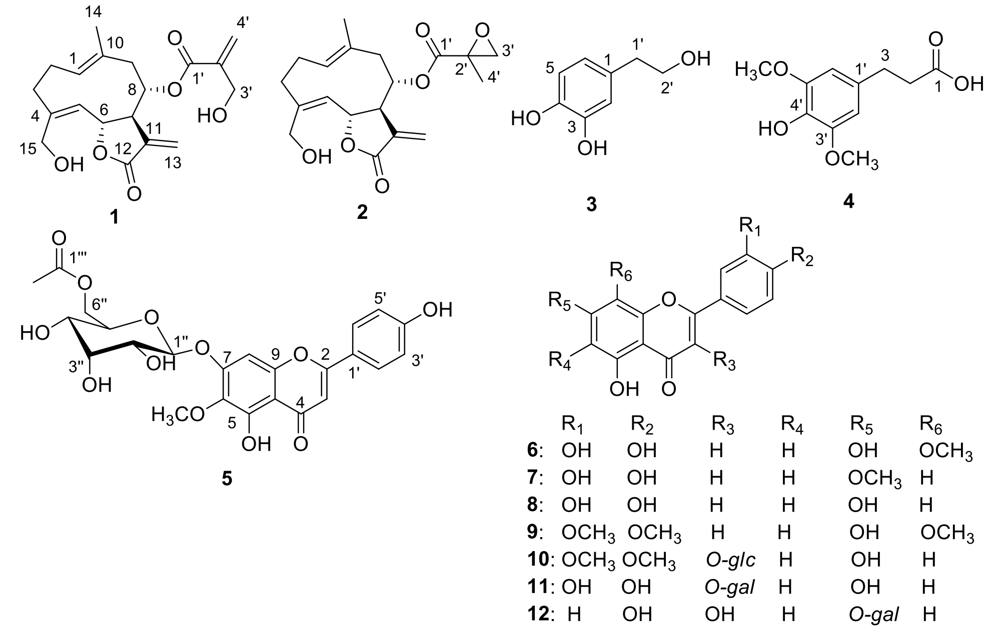

Figure 1: Structures of compounds **1-12** isolated from the methanolic extract of *B. discolor* leaves.

Table 1: IC_{50} values in μM of tested compounds.

Sample	***L. amazonensis clon 1***	***L. braziliensis***
Compound **1**	39.6	27.9
Compound **2**	>50	>50
Miltefone	12.5	12.0

Conclusion

In this study, compound **1** showed remarkable leishmanicidal activity as compared to the miltefone, a currently used agent in leishmaniosis treatment. Thus, contributing for the suggestion of *B. discolor* as a medicinal plant to treat leishmaniosis ailment, besides the known traditional uses.

The replacement of the secondary Michael acceptor group at C-8 on compound **1** by an epoxide group at same carbon atom on compound **2** seems to be responsible for the leishmanicidal activity in both strains tested. However, further studies are required to clearly define the molecular mechanism of action underlying the leishmanicidal activity in compound **1** and not observed in compound **2** in both *L. amazonensis* and *L. braziliensis* strains assayed.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Bandeira SO, Gaspar F, Pagula FP. Ethnobotany and healthcare in Mozambique. Pharm Biol. 2001; 39(1):70–73.
2. Bruschi P, Morganti M, Mancini M, Signorini MA. Traditional Healers and Laypeople: A qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). J Ethnopharmacol. 2011; 138(2):543–563.
3. Hostettmann, K; Marston, A; Ndjoko, K; Wolfender, JL. The potential of African plants as a source of drugs. Curr Org Chem. 2000; 4(10):973–1010.
4. Maroyi A. Traditional use of medicinal plants in South-Central Zimbabwe: Review and perspectives. J Ethnobiol Ethnomed. 2013; 9(1):31–48.
5. Bohlmann F and Zdero C. Sesqueterpene lactones from *Brachylaena* species. Phytochemistry 1982; 21(3):647–651.
6. Zdero, C; Bohlmann, F. Sesqueterpene lactones from the genus *Brachylaena*. *Phytochemistry* 1987, *26* (9), 2597–2601.
7. Zdero, C; Bohlmann, F; Wasshausen, DC. Guaianolides from *Brachylaena* species. *Phytochemistry* 1991, *30* (11), 3810–3811.
8. Chaturvedula, VSP; Schilling, JK; Miller, JS; Andriantsiferana, R; Rasamison, VE; Kingston, DGI. Two new triterpene esters from the twigs of *Brachylaena ramiflora* from the Madagascar rainforest. *J. Nat. Prod.* 2002, *65* (8), 1222–1224.
9. Vieira, PC; Himejima, M; Kubo, I. Sesquiterpenoids from *Brachylaena hutchisii*. *J. Nat. Prod.* 1991, *45* (2), 416–420.
10. Beentje, HT. The genus *Brachylaena* (Compositae: Mutisieae). *Kew Bull.* 2000, *55* (1), 1–41.
11. Deutschländer, MS; Lall, N; van de Venter, M. Plant species used in the treatment of diabetes by South African traditional healers: An Inventory. *Pharm. Biol.* 2009, *47* (4), 348–365.
12. Jansen, PCM; Mendes, O. *Plantas medicinais - Seu uso tradicional em Moçambique*, T4 ed.; Partido, I. do, Ed.; Ministério da Saúde Gabinete de estudos de Medicina Tradicional: Maputo, Mozambique, 1991.
13. Mellem, J. Effect of the methanolic extract of *Brachylaena discolor* in streptozotocin-induced diabetic rat model. *African J. Pharm. Pharmacol.* 2013, *7* (12), 636–642.
14. Lonergan, G; Routsi, E; Georgiardis, T; Agelis, G; Hondrelis, J; Matsoukas, J; Larsen, LK; Caplan, FR. Isolation, NMR studies, and biological activities of onopordopicrin from *Centaurea sonchzfola*. *J. Nat. Prod.* 1992, *55* (5), 225–228.
15. Bach, SM; Fortuna, MA; Attarian, R; Trimarco, JT; Catalán,

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

CAN; Av-Gay, Y; Bach, H. Antibacterial and cytotoxic activities of the sesquiterpene lactones cnicin and onopordopicrin. *Nat. Prod. Comm.* 2011, *6* (11), 163–166.

16. Formisano, C; Sanna, C; Ballero, M; Chianese, G; Sirignano, C; Rigano, D; Millán, E; Muñoz, E; Taglialatela-Scafati, O. Anti-inflmmatory sesquiterpene lactones from *Onopordum illyricum* L . (Asteraceae), an Italian Medicinal Plant. *Fitoterapia* 2017, *116*, 61–65.
17. Onguéné, A; Ntie-Kang, P; Lifongo, F. The potential of antimalarial compounds derived from African medicinal plants, Part I: A pharmacological evaluation of alkaloids and terpenoids. *Malar. J.* 2013, *12* (1), 4–25.
18. Novello, CR; Teresinha, I; Schuquel, A; Sakuragui, CM; Luftmann, H; Ueda-Nakamura, T; Nakamura, CV; Mello, JCP. Evaluation of the antiproliferative activity of the leaves from *ArctiumlLappa* by a bioassay-guided fractionation. *Molecules* 2012, *17*, 1852–1859.
19. Zimmermann, S; Fouché, G; De Mieri, M; Yoshimoto, Y; Usuki, T; Nthambeleni, R; Parkinson, CJ; Van Der Westhuyzen, C; Kaiser, M; Hamburger, M. Structure-Activity Relationship Study of sesquiterpene lactones and their semi-synthetic amino derivatives as potential antitrypanosomal products. *Molecules* 2014, *19* (3), 3523–3538.
20. Capusiri, ES; Pinell, GR; Huallpara, JCT; Turba, AG. Colorimetric method - XTT: As a tool for high performance evaluation of substances with leishmanicidal activity. *Biofabro* 2008, *16*, 21–27.
21. Park, CH; Kim, KH; Lee, IK; Lee, SY; Choi, SU; Lee, JH; Lee, KR. Phenolic onstituents of *Acorus gramineus*. *Arch. Pharm. Res.* 2011, *34* (8), 1289–1296.
22. Lee, Y-M; Kwon, M-J; Kim, J-K; Suh, H; Choi, J-S; Song, Y-O. Isolation and identification of active principle in Chinese cabage Kimchi responsable for antioxidant effect. *Korean J. Food Sci. Technol.* 2004, *36* (1), 129–133.
23. Jin, MR; Xu, H; Duan, CH; Chou, GX. Two new flavones from *Salvia plebeia*. *Nat. Prod. Res.* 2015, *29* (14), 1315–1322.
24. Wollenweber, E; Dörr, M; Roitman, JN; Schilling, E. External flavonoids of three species of *Viguiera* (Asteraceae). *Z. Naturforsch.* 1995, *50c*, 588–590.
25. Park, S; Cui, X; Ahn, D; Lee, EB; Cha, DS; Jeon, H; Zee, OP; Kim, Y; Kim, DK. Anti-oxidative activities of 3'-hydroxygenkwanin from the flower buds of *Daphne genkwa*. *Nat. Prod. Sci.* 2014, *20* (2), 80–85.
26. Miyazawa, M; Hisama, M. Antimutagenic activity of flavonoids from *Chrysanthemum morifolium*. *Biosci. Biotechnol. Biochem.* 2003, *67* (10), 2091–2099.
27. Al-Youssef, H; Hassan, WHB. Phytochemical and biological studies of the aerial parts of *Carissa edulis* growing in Saudi Arabia. *Biomed. Pharmacol. J.* 2012, *5* (1), 9–18.
28. Kunert, O; Haslinger, E; Schmid, MG; Reiner, J; Bucar, F; Mulatu, E; Abebe, D; Debella, A. Three saponins, a steroid, and a flavanolglycoside from *Achyrantes aspera*. *Monatshefte für Chemie* 2000, *131* (2), 195–204.
29. Wei, X; Huang, H; Wu, P; Cao, H; Ye, W. Phenolic constituents from *Mikania micrantha*. *Biochem. Syst. Ecol.* 2004, *32* (11), 1091–1096.
30. Güvenalp, Z; Demirezer, LO. Flavonol glycosides from *Asperula arvensis* L. *Turk. J. Chem.* 2005, *29*, 163–169.
31. Chadwick, M; Trewin, H; Gawthrop, F; Wagstaff, C. Sesquiterpenoids lactones: Benefits to plants and people. *Int. J. Mol. Sci.* 2013, *14* (6), 12780–12805.
32. Chan-Bacab, MJ; Peña-rodríguez, LM. Plant natural products with leishmanicidal activity. *Nat. Prod. Rep.* 2001, *18*, 674–688.
33. Fournet, A; Muñoz, V; Roblot, F; Hocquemiller, R. Antiprotozoal activity of dehydrozaluzanin C, a sesquiterpene lactone isolated from *Munnozia maronii* (Asteraceae). *Phytother. Res.* 1993, *7*, 111–115.
34. Sinisi, A; Millán, E; Abay, SM; Habluetzel, A; Appendino, G; Muñoz, E; Taglialatela-Scafati, O. Poly-electrophilic sesquiterpene lactones from *Vernonia amygdalina*: New members and differences in their mechanism of thiol trapping and in bioactivity. *J. Nat. Prod.* 2015, *24*, 1618–1623.
35. Majd M, Liu Q, Karimzadeh G, Malboobi MA, Beekwilder J, Cankar K, Vos R, Todorović S, Simonović A, Bouwmeester H. Biosynthesis and localization of parthenolide in glandular trichomes of feverfew (*Tanacetum Parthenium* L. Schulz Bip.). Phytochemistry 2011; 72:1739–1750.