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Antioxidant and antimicrobial constituents of *Crucianella maritima* L.

Jihan M. Badr*

Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

Abstract – Phytochemical further investigation of the chloroform extract of the aerial parts of *Crucianella maritima* L. (Rubiaceae) growing in Egypt resulted in the isolation of a new anthraquinone; 3-formyl-1-hydroxy-2-methoxy anthraquinone (**3**) along with the four known compounds isolated for the first time from the genus *Crucianella*; alizarin-1-methyl ether (**2**), 1,4-dihydroxy-2-methoxy anthraquinone (**5**), 1, 3, 6-trihydroxy-2-methoxy anthraquinone (**7**) and the flavonol kaempferol (**8**), beside four known compounds previously isolated from the same plant. The structures of the isolated compounds were established based on different spectroscopic data including UV, IR, EIMS, 1D and 2D-NMR. Moreover, the antioxidant and antimicrobial activities of the isolated compounds were evaluated.

Keywords – Rubiaceae, *Crucianella maritima* L., anthraquinones, spectroscopic techniques, antioxidant, antimicrobial activity.

Introduction

Plants belonging to different genera of the Family Rubiaceae are of much current biological and chemical interest. They are characterized by accumulation of bioactive metabolites including anthraquinones. Many of these anthraquinones exhibited a diversity of biological activities as antibacterial (Kalyoncu *et al*., 2006; Kariba, 2002), antifungal (Manojlovic *et al*., 2005; Hamza *et al*., 2006), antimalarial (Andrade-Neto *et al*., 2003), anti-feedant (Morimoto *et al*., 2002), antioxidant (Mavi *et al*., 2004; Lin *et al*., 2004), anti-inflammatory (Mandal *et al*., 2003; Lin *et al*., 2002), anticancer (Arpornsuwan *et al*., 2006; Gupta *et al*., 2004), antidiabetic (Jose *et al*., 2007), immunomodulatory (Palu *et al*., 2008; Yoshida *et al*., 1997), wound healing (Kumar *et al*., 2007), liver protective (Wang *et al*., 2008) and neuroprotective (Kim *et al*., 2001). Previous investigation of *Crucianella maritima* (Rubiaceae) has led to isolation of a number of compounds including anthraquinones, flavonoids, iridoids and sterols. Many of the isolated compounds revealed significant antibacterial activities (El-Lakany *et al*.,2004). In a continuation of this study, a phytochemical examination of the chloroform extract of the aerial parts of *C. maritima* was conducted. This work furnished a new compound; 3-formyl-1-hydroxy-2-methoxy anthraquinone (**3**). In addition, the four known compounds alizarin-1-methyl ether (**2**), 1, 4-dihydroxy-2-methoxy anthraquinone (**5**), 1, 3, 6-trihydroxy-2-methoxy anthraquinone (**7**) and kaempferol (**8**) were isolated for the first time from the genus *Crucianella.* Moreover, four compounds that were previously isolated from the same plant namely, 1-hydroxy-2,3-dimethoxy anthraquinone (**1**), 1,3-dihydroxy-2-methoxy anthraquinone (**4**), alizarin (**6**) and quercetin (**9**) were isolated and identified. The antioxidant activity of all the isolated compounds was examined using DPPH reagent and all the isolated compounds exhibited variable degrees of activities. In addition, the antimicrobial activities of compounds **3**, **5** and **7** were determined, where compound **3** showed significant activity against *Staphylococcus aureus.*

*Author for correspondence
Tel: +91-002-03-4249428; E-mail: gihan96@hotmail.com

Experimental

General experimental procedures – UV spectra were measured on a *Hitachi* 300 Spectrophotometer; λ_{max} (log ε). IR spectra were obtained on a Perkin Elmer RXIFT-IR system; ν_{max} (KBr). 1D- and 2D- NMR (HMQC and HMBC) spectra were recorded on a *Joel* spectrometer; at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. EIMS spectral data were obtained using *JEOL JMS-GC mate*; in m/z (rel. %). For column chromatography, silica gel (*Merck*, 70 - 230 mesh ASTM) and sephadex LH-20 (*Pharmacia*) were used. Pre-coated silica gel 60 F-254 plates (*Merck*) were used for TLC and PTLC. Spots were visualized by exposure to NH_3 vapour and UV radiation.

Plant material – The plant was collected in March 2005 from sandy districts of the Mediterranean coastal strip, 70 - 80 kilometers West of Alexandria. The plant was identified by comparison with a voucher sample deposited in the Department of Pharmacognosy, Faculty of Pharmacy, University of Alexandria, Egypt.

Extraction and isolation – The aerial parts of the plant were separated, air dried and finely powdered. The powder (5 Kg) was macerated with ethyl alcohol and the total alcoholic extract was concentrated under vacuum, fractionated with hexane, $CHCl_3$ and EtOAc; each of these extracts was concentrated. A portion of 10 g from the $CHCl_3$ extract was chromatographed on a silica gel column packed in hexane and eluted with a step gradient of hexane - $CHCl_3$ - CH_3OH. The four main fractions (A-D) which were eluted with hexane: $CHCl_3$ (50 : 50 to 30 : 70), $CHCl_3$ (100%), $CHCl_3$: CH_3OH (97 : 3 to 94 : 6) and $CHCl_3$: CH_3OH (92 : 8 to 88 : 12) respectively showed distinct spots. Sub-fraction A was purified over silica gel column using isochratic elution with hexane: $CHCl_3$ (1 : 3) to afford 4 mg of a yellow deposit designated as compound **1** (3 mg). Sub-fraction B was purified on column packed with sephadex and eluted by $CHCl_3$: CH_3OH (1 : 1) followed by further purification by PTLC using hexane : $CHCl_3$: CH_3OH (5 : 5 : 0.5) as a developing system for multiple development, two bands were scrapped off, eluted with $CHCl_3$ to afford yellowish - orange deposit and dark orange sandy crystals designated as compounds **2** (12 mg) and **3** (16 mg) respectively. Sub-fraction C was chromatographed on a silica gel column, equilibrated with $CHCl_3$ and elution was performed with a step gradient of $CHCl_3$: CH_3OH, where fractions eluted with 1% and 2% MeOH in $CHCl_3$ were combined and separated by PTLC using $CHCl_3$: CH_3OH (19.5 : 0.5) as a developing system for multiple development to afford orange sandy crystals, reddish - orange residue and reddish - purple residue designated as compounds **4** (20 mg), **5** (18 mg) and **6** (25 mg) respectively. Sub-fraction D was purified on column chromatography packed with silica gel and equilibrated with CH_2Cl_2. Gradient elution was performed using CH_2Cl_2: CH_3OH. Fractions eluted by 7% to 10% MeOH in CH_2Cl_2 were combined and finally purified on PTLC using CH_2Cl_2 : CH_3OH (9.5 : 0.5) as a developing system to give finally red residue and bright yellow deposit designated as compounds **7** (8 mg) and **8** (10 mg). The fraction eluted with 12% CH_3OH in CH_2Cl_2 afforded a readily crystalline bright yellow compound (**9**) (30 mg).

Alizarin-1-methyl ether (**2**): Yellowish-orange deposit. UV (λ_{max}, MeOH): 210, 270, 391, (λ_{max}, MeOH/KOH) 211, 244, 306, 467. EI-MS: 254 (70, $[M]^+$), 237 (10, $[M - OH]^+$), 236 (30, $[M - H_2O]^+$), 225 (4, $[M - COH]^+$), 211 (4, $[M - COCH_3]^+$), 152 (13), 43 (100).

3-formyl-1-hydroxy-2-methoxy anthraquinone (**3**): Dark orange sandy crystals. UV (λ_{max}, MeOH): 210, 283, 312 (sh), 425, (λ_{max}, MeOH / KOH): 213, 243 (sh), 311, 360, 502. IR (ν_{max}, KBr): 3413, 2921, 1700, 1667, 1632, 1586, 1452, 1123, 1033, 871. EI–MS: 282 (4, M^+), 254 (58, $[M - CO]^+$), 253 (4, $[M - COH]^+$), 237 (18, $[M - CO - OH]^+$), 225 (8, $[M - CO - COH]^+$), 197 (13), 169 (6), 115 (18), 92 (14), 43 (100).

1,4-dihydroxy-2-methoxy anthraquinone (**5**): Reddish - orange residue. UV (λ_{max}, MeOH): 210, 248, 281, 320 (sh.), 480, (λ_{max}, MeOH / KOH): 212, 250, 316, 502, 542, 560 (sh.). IR (ν_{max}, KBr): 3437, 2920, 1640, 1631, 1587, 1450, 1121, 894. EI-MS: 270 (21, M^+), 252 (27, $[M - H_2O]^+$); 241 (18, $[M - COH]^+$); 227 (9, $[M - COCH_3]^+$); 225 (52, $[M - CO - OH]^+$); 208 (10, $[M - CO - 2OH]^+$), 168 (16), 152 (18), 126 (12), 76 (37), 55 (53), 43 (100).

1, 3, 6-trihydroxy-2-methoxy anthraquinone (**7**): Red residue. UV (λ_{max}, MeOH): 220, 268, 411, (λ_{max}, MeOH / KOH): 210, 264, 411, 503. EI-MS: 286 (8, $[M]^+$), 268 (5, $[M-H_2O]^+$), 243 (3, $[M-COCH_3]^+$), 219 (9), 137 (100), 103 (8).

NMR data were compiled: Tables 1 and 2

Antioxidant activities – Each of the pure compounds was dissolved in methanol at a concentration of 1 mg /ml and the flavonoid rutin was prepared at a similar concentration and used as a positive control (Nijveldet *et al*., 2001). Six ml of each compound were applied in the form of a spot, 4 mm in diameter. The radical scavenging effects were detected on the TLC plates, using a spray reagent composed of 0.2% (w/v) solution of 1,1-diphenyl-2- picrylhydrazyl radical (DPPH) in methanol. Plates were observed 30 min after spraying. Active compounds were detected as yellow spots against a purple background (Takamatsu *et al*., 2003).

Antimicrobial activities – The antimicrobial activities of compounds **3**, **5** and **7** were tested against the organisms; *Staphylococcus aureus* (ATCC 6538P), *Pseudomonas aeroginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739) and *Candida albicans* (ATCC 2091) using agar diffusion method. Accurately weighed 1 mg of each compound was dissolved in 1 mL DMF and 100 μL of each solution were inserted in the cups then incubated at 37 °C for 24 hours. The inhibition zones were measured and compared with the reference antibiotics and antifungal drugs; ampicillin (10 μg/disc), imipenam (10 μg/disc) and clotrimazole (10 mg/mL). The results of the antimicrobial activities are presented in Table 3.

Table 1. ^{1}H-NMR data of compounds **2**, **3**, **5** ($CDCl_3$) and **7** (DMSO. d_6)

	δ_H [mult., *J* (Hz)]			
Position	**2**	**3**	**5**	**7**
1-*OH*	–	13.17 (s)	13.30 (s)	12.90 (s)
1-*OCH*$_3$	3.90 (s)	–	–	–
2-*OCH*$_3$	–	3.96 (s)	4.06 (s)	3.75 (s)
3	7.11 (d, 8.4)	–	7.69 (s)	–
3-*CHO*	–	9.80 (s)	–	–
4	7.74 (d, 8.4)	7.14 (s)	–	7.45 (s)
4-*OH*	–	–	12.09 (s)	–
5	8.28 (m)	8.25 - 8.27 (m)	8.29 - 8.35 (m)	7.35 (brs)
6	7.84 (m)	7.74 - 7.77 (m)	7.80 - 7.84 (m)	–
7	7.84 (m)	7.74 - 7.77 (m)	7.80 - 7.84 (m)	7.31 (brd, 8.4)
8	8.28 (m)	8.25 - 8.27 (m)	8.29 - 8.35 (m)	7.90 (d, 8.4)

Table 2. ^{13}C- NMR data of compounds **3**, **5** ($CDCl_3$) and **7** (DMSO. d_6)

	δ_C (mult.)		
Position	**3**	**5**	**7**
1	156.0 (C)	155.5 (C)	157.4 (C)
2	147.4 (C)	150.5 (C)	143.6 (C)
2-*OCH*$_3$	56.5 (CH_3)	56.6 (CH_3)	61.2 (CH_3)
3	129.1(C)	108.4 (C)	158.6 (C)
3-*CHO*	190.9 (CH)	–	–
4	106.7 (CH)	159.1 (C)	110.3 (CH)
5	127.4 (CH)	127.0 (CH)	113.1 (CH)
6	134.4 (CH)	134.9 (CH)	163.8 (C)
7	134.1 (CH)	134.7 (CH)	121.1 (CH)
8	126.8 (CH)	127.5 (CH)	129.0 (CH)
9	187.8 (C)	187.5 (C)	187.0 (C)
10	181.8 (C)	186.6 (C)	183.5 (C)
11	134.5* (C)	134.5* (C)	135.2 (C)
12	138.9* (C)	135.2* (C)	128.8 (C)
13	111.4 (C)	112.4 (C)	111.1 (C)
14	140.5 (C)	126.3 (C)	130.8 (C)

* Exchangeable values within the same column.

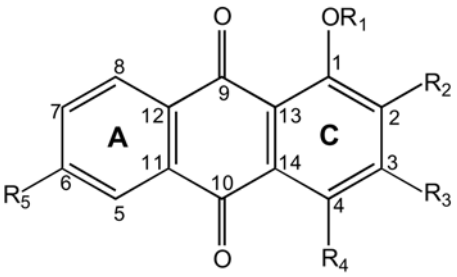
Results and discussion

The anthraquinones **1**, **4**, **6,** and the flavonols **8**, **9** were readily identified as 1-hydroxy-2, 3-dimethoxy anthraquinone, 1, 3-dihydroxy-2-methoxy anthraquinone, alizarin, kaempferol and quercetin respectively based on both ^{1}H-NMR and UV data as well as direct comparison with reference samples through co-chromatography and 2D-TLC. NMR spectra of compounds **2, 3**, **5** and **7** indicated the presence of two aromatic rings and two doubly conjugated carbonyl carbons in all these compounds thus

Table 3. Antimicrobial activity of compounds 3, 5 and 7 against the tested microorganisms

	Inhibition zone (mm)			
Compound	*S. aureus*	*P. aeroginosa*	*E. coli*	*C. albicans*
3	25	3	NI	3
5	17	3	NI	4
7	NI	3	NI	1
Imipenam	30	30	26	--
Ampicillin	30	--	--	--
Clotrimazole	--	--	--	40

-- = not tested
NI = no inhibition

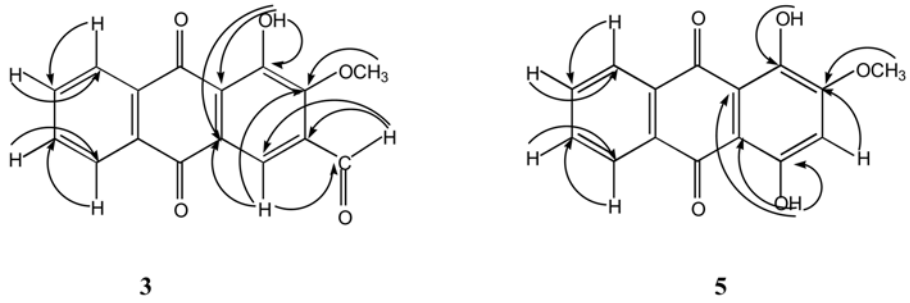


	R_1	R_2	R_3	R_4	R_5
1	H	OCH_3	OCH_3	H	H
2	CH_3	OH	H	H	H
3	H	OCH_3	COH	H	H
4	H	OCH_3	OH	H	H
5	H	OCH_3	H	OH	H
6	H	OH	H	H	H
7	H	OCH_3	OH	H	OH

Fig. 1. Structures of the isolated compounds **1 - 7**.

supported their anthraquinone skeleton.

Compound **2** was isolated as yellowish - orange deposit. ^{1}H-NMR spectrum revealed two multiplets centered at δ_H 8.28 and 7.84 each integrated for two protons confirming a non substituted ring A. The two doublets observed at δ_H 7.74 (1 H, *J* = 8.4 Hz, H - C (4)) and 7.11 (1 H, *J* = 8.4 Hz, H - C (3)) supported 1, 2 ortho di-substituted ring C. Combined spectral data including UV, ^{1}H-NMR and EIMS allowed the assignment of the two substituents as a hydroxyl and a methoxyl functionalities. The absence of a downfield singlet for a peri-hydroxyl group supported the location of the hydroxyl group at C-2 while the singlet resonating at δ_H 3.90, integrated for three protons was attributed to a methoxyl group that should be cited at C-1. The UV, ^{1}H-NMR and EIMS data of compound **2** were reminiscent of those previously reported for 2-hydroxy-1-methoxy anthraquinone



3 **5**

Fig. 2. HMBC correlations of compounds **3** and **5**.

(alizarin-1-methylether) (Kuiper *et al*., 1981).

Compound **3** was isolated as dark orange sandy crystals. Its IR spectrum revealed the characteristic bands at 3413, 1667 and 1632 cm^{-1} for a free hydroxyl group, unchelated and chelated carbonyl groups respectively. The EIMS together with different NMR data of compound **3** suggested its molecular formula to be $C_{16}H_{10}O_5$. ^{13}C-NMR spectrum revealed sixteen resolved signals of which two resonated at δ_C 187.8 and 181.8 for a chelated (C-9) and non chelated (C-10) carbonyl groups respectively. The two signals at δ_C 156.0 and 147.4 confirmed the presence of two oxygenated aromatic carbons. In the ^{1}H-NMR spectrum, the two multiplets at δ_H 8.25 - 8.27 and δ_H 7.74 - 7.77 each integrated for two protons confirmed a non-substituted ring A, while the singlet resonating at δ_H 7.14 proved a tri-substituted ring C. Another three singlets were detected in the ^{1}H-NMR spectrum; a downfield shifted singlet at δ_H 13.17 supported the presence of a peri hydroxyl group at C-1, a singlet resonating at δ_H 9.80 together with its carbon at δ_C 190.9 for an aldehyde group and the singlet detected at δ_H 3.96 alongside with its carbon at δ_C 56.5 confirmed the presence of a methoxyl functionality. The location of the aldehyde group at C-3 was confirmed from the HMBC correlations that revealed cross peaks between H-4 with the carbon at δ_C 190.9. Also, the aldehydic proton at δ_H 9.80 was strongly correlated with C-4 at δ_C 106.7 (Fig. 2). The position of the formyl moiety at C-3 was also confirmed from the chemical shift of the aldehydic proton in the ^{1}H-NMR spectrum that differs form the reported data for the same group when flanged between a hydroxyl and a methoxyl moieties (Zhou *et al*., 1994; Koumaglo *et al*., 1992). Accordingly, the methoxyl group should be cited at C-2; a situation that was confirmed from the chemical shift of C-2 resonating at δ_C 147.4 in ^{13}C NMR indicating an ortho-position of the methoxyl with the peri-hydroxyl group (Koyama *et al*.,1993; El-Gamal *et al*.,1995). The exact assignment of all the carbons was made possible through HMQC and HMBC data. According to the available literature, compound **3** is a new anthraquinone assigned as 3-formyl-1-hydroxy-2-methoxy anthraquinone.

Compound **5** was isolated as reddish- orange residue. Its molecular formula was deduced as $C_{15}H_{10}O_5$ from combined spectral data (EIMS, ^{1}H- and ^{13}C- NMR). The presence of two chelated carbonyls was suggested from the IR spectrum that showed two bands at 1640 and 1631 cm^{-1}. The ^{1}H-NMR spectrum confirmed a non- substituted ring A through the two multiplets detected at δ_H 8.29-8.35 and δ_H 7.80 - 7.84 each integrated for two protons (H-C (5) & H-C (8) and H-C (6) & H-C (7) respectively). It also declared a trisubstituted ring C through the singlet detected at δ_H 7.69 along with its corresponding carbon at δ_C 108.4. The location of the two hydroxyl groups at C-1 and C-4 was confirmed from the two downfield singlets resonating at δ_H 12.09 and 13.3 as well as the two chelated carbonyl signals detected at δ_C 187.5 and 186.6 in the ^{13}C-NMR spectrum. The remaining substituent at C-2 was easily assigned as a methoxyl functionality that resonated as a singlet at δ_H 4.06 integrated for three protons alongside with the carbon at δ_C 56.6. HMQC spectrum was used to correlate the protons to their attached carbons, while connectivities deduced from HMBC correlations (Fig. 2) readily established the exact assignment of the carbons and confirmed the structure as 1,4-dihydroxy-2-methoxy anthraquinone.

Compound **7** was isolated as red residue. Its molecular formula was determined as $C_{15}H_{10}O_6$ from EIMS and different NMR spectral data. In the ^{1}H-NMR spectrum of compound **7** the singlet detected at δ_H 7.45 along with its corresponding carbon at δ_C 110.3 supported a trisubstituted ring C while the three signals at δ_H 7.35 (brs), 7.90 (d, J = 8.4 Hz) and 7.31 (brd), each integrated for one proton, confirmed a mono substituted ring A. The natures of the four substituents were predicted from the ^{13}C-NMR spectrum that revealed fifteen resolved signals of which four resonated at δ_C 163.8, 158.6, 157.4 and 143.6 pointing to the presence of four oxygenated aromatic carbons. The carbon signal at δ_C 61.2 together with its

corresponding singlet at δ_H 3.75, integrated for three protons proved the presence of a methoxyl group. The chelated carbonyl signal resonating at δ 187.0 and the downfield shifted proton singlet detected at δ_H 12.9 confirmed the location of hydroxyl functionality at C-1. The two other substituents were predicted as hydroxyl groups from both ^{13}C-NMR and MS data, while the position of all the substituents revealed themselves by comparison with the previously reported spectroscopic assignment of 1, 3, 6-trihydroxy-2- methoxy anthraquinone which was first isolated from *Morinda citrifolia* (Pawlus *et al*., 2005) and this is the second report for its isolation from a natural source.

All the pure isolated compounds were examined for their antioxidant activities using TLC autographic assay for DPPH radical scavenging effect. The flavonoid rutin was used as a positive control. Both the flavonoids kaempferol (**8**) and quecetin (**9**) gave a highly bright yellow coloured spot against a purple background immediately after spraying, indicating a powerful antioxidant activity similar to that of rutin. The anthraquinones (**3**, **4**, **6**, and **7**) also revealed similar results as rutin while anthraquinones **1, 2** and **5** produced a less bright yellow colour compared to rutin suggesting a moderate antioxidant activity. The variation in the antioxidant activities of the tested anthraquinones could be attributed to the number and location of the hydroxyl groups on the anthraquinone skeleton. These results coincide with previous studies which concluded that two hydroxyl groups arranged at either the meta or ortho positions are required for an anthraquinone to inhibit lipid peroxidation in rat heart mitochondrial system (Jasril *et al*., 2003). The significant activity exhibited by compound **3** may be attributed to the formyl functionality at C-3 as well as the hydroxyl at C-1. On the other hand, none of the tested compounds (**3**, **5** and **7**) revealed activity against *Escherichia coli* while a remarkable activity was exerted by compound **3** towards *Staphylococcus aureus* and a moderate activity was exhibited by compound **5** against the same microorganism. All the tested compounds showed a very weak activity against both *Candida albicans* and *Pseudomonas aeroginosa.*

References

Andrade-Neto, V.F., Brandão, M.G., Stehmann, J.R., Oliveira, L.A., and Krettli, A.U., Antimalarial activity of Cinchona-like plants used to treat fever and malaria in Brazil. *J Ethnopharmacol.* **87**, 253-256 (2003).

Arpornsuwan , T. and *Punjanon,* T., Tumor cell-selective antiproliferative effect of the extract from *M. citrifolia* fruits. *Phytother Res*. **20**, 515-517 (2006).

El-Lakany, A.M., Aboul-Ela, M.A., Abdel-Kader, M.S., Badr, J.M., Sabri, N.N., and Goher, Y., Anthraquinones with antibacterial activities from *C. maritima* L. growing in Egypt. *Nat. Prod. Sciences,* **10**, 63-68 (2004).

El-Gamal, A.A., Takeya, K., Itokawa, H., Halim, A.F., Amer, M.M., Saad, H.E. A. and Awad, S.A., Anthraquinones from *G. sinaicum*. *Phytochemistry* **40**, 245-251 (1995).

Gupta, S., Zhang, D., Yi, J., and Shao, J., Anticancer activities of *O. diffusa*. *J. Herb. Pharmacother*. **4**, 21-33 (2004).

Hamza, O.J.M., van den Bout-van den Beukel, C.J.P., Matee, M.I.N., Moshi, M.J., Mikx, F.H.M., Selemani, H.O., Mbwambo, Z.H., and Verweij, P.E., Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. *J Ethnopharmacol.* **108**, 124-132 (2006).

Jasril, N.H., Lajis, L.Y., Mooi, L.Y., Abdullah, M.A., Sukari, M.A., and Ali, A.M., Antitumour promoting and antioxidant activities of anthraquinones isolated from the cell suspension culture of *M. elliptica*. *As. Pac. J. Mol. Biol. Biotechnol*. **11**, 3-7 (2003).

Jose, G.A., Omar, M.C., Fernando, B., Robert, B., Jose, P.C., Andres, N., and Rachel, M., Antidiabetic properties of selected Mexican copalchis of the Rubiaceae family. *Phytochemistry* **68**, 2087-2095 (2007).

Kalyoncu, F., Cetin, B., and Saglam, H., Antimicrobial activity of common madder (*R. tinctorum* L.). *Phytother Res.* **20**, 490-492 (2006).

Kariba, R.M., Antimicrobial activity of *H. parvifolium*. *Fitoterapia* **73**, 523-525 (2002).

Kim, Y., Park, E.J., Kim, J., Kim, Y., Kim, S.R., and Kim, Y.Y., Neuroprotective constituents from *H. diffusa*. *J. Nat. Prod*. **64**, 75-78 (2001).

Koumaglo, K., Gbeassor, M., Nikabu, O., de Souza, and Werner, C.W., Effects of three compounds extracted from *M. lucida* on *Plasmodium falciparum*. *Planta Med*. **58**, 533-534 (1992).

Koyama, J., Ogura, T., and Tagahara, K., Anthraquinones of *G. spurium*. *Phytochemistry* **33**, 1540-1542 (1993).

Kuiper, J. and Labadie, R.P., Polyploid complexes within the genus *Galium*. *Planta Med*. **42**, 390-399 (1981).

Kumar, B., Vijayakumar, M., Govindarajan, R., and Pushpangadan, P., Ethnopharmacological approaches to wound healing-exploring medicinal plants of India. *J Ethnopharmacol.* **114**, 103-113 (2007).

Lin, C.C., Ng, L.T., and Yang J.J., Antioxidant activity of extracts of peh-hue-juwa- chi-cao in a cell free system. *A.J.C.M.* **32**, 339-349 (2004).

Lin, C.C., Ng, L.T., Yang J.J., and Hsu, Y.F., Anti-inflammatory and hepatoprotective activity of extracts of peh-hue-juwa- chi-cao in male rats. *A.J.C.M.* **30**, 225-234 (2002).

Mandal, S.C., Mohana Lakshmi, S., Ashok Kumar, C.K., Sur, T.K., and Boominathan, R., Evaluation of anti-inflammatory potential of *P. indica* Linn. leaf extract (family: Rubiaceae) in rats. *Phytother. Res.* **17**, 817-820 (2003).

Manojlovic, N.T., Solujic, S., Sukdolak, S., and Milosev, M., Antifungal activity of *R. tinctorum*, *Rhamnus frangula* and *Caloplaca cerina*. *Fitoterapia* **76**, 244-246 (2005).

Mavi, A., Terzi, Z., Ozgen, U., Yildirim, A., and Coskun, M., Antioxidant properties of some medicinal plants: *P. ferulacea* (Apiaceae), *S. sempervivoides* (Crassulaceae), *M. neglecta* (Malvaceae), *C. taurica* (Rubiaceae), *R. pimpinellifolia* (Rosaceae), *G. verum* subsp. verum (Rubiaceae), *U. dioica* (Urticaceae). *Biol. Pharm. Bull.* **27**, 702-705 (2004).

Morimoto, M., Tanimoto, K., Sakatani, A., and Komai, K., Antifeedant activity of an anthraquinone aldehyde in *G. aparine* L. against *Spodoptera litura* F. *Phytochemistry* **60**, 163-166 (2002).

Nijveldt, R.J., Nood, E., Hoorn, D.E.C., Boelens, P.G., Norren, K., and Leeuwen, P.A.M., Flavonoids: a review of probable mechanism of

action and potent applications. *Am. J. Clin. Nutr.,* **74**, 418-425 (2001).
Palu , A.K., Kim, A.H., West, B.J., Deng, S., Jensen, J., and White, L., The effects of *M. citrifolia* L. (noni) on the immune system: its molecular mechanisms of action. *J Ethnopharmacol.* **115**, 502-506 (2008).
Pawlus, A.D., Su, B.N., Keller, W.J., and Kinghorn, A.D., An anthraquinone with potent quinine reductase-inducing activity and other constituents of the fruits of *M. citrifolia* (Noni). *J. Nat. Prod*. **68**, 1720-1722 (2005).
Takamatsu, S., Hodges, T.W., Rajbhandari, I., Gerwick, W.H., Hamann, M.T., and Nagle, D.G., Marine natural products as novel antioxidant prototypes. *J. Nat. Prod*, **66**, 605-608 (2003).
Wang, M.Y., Nowicki, D., Anderson, G., Jensen, J., and West, B., Liver Protective Effects of *M. citrifolia* (Noni). *Plant Foods Hum Nutr*. **63**, 59-63 (2008).
Yoshida, Y., Wang, M.Q., Liu, J.N., Shan, B.E., and Yamashita, U., Immunomodulating activity of Chinese medicinal herbs and *O. diffusa* in particular. *Int. J. Immunopharmacol*. **19**, 359-370 (1997).
Zhou, Z., Jiang, S.H., Zhu, D.Y., Lin, L.Z., and Cordell, G.A., Anthraquinones from *K. valerianoides. Phytochemistry* **36**, 765-768 (1994).

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