Arabian Journal of Chemistry (2017) **10**, 109–113

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Chemical constituents of *Carissa edulis* Vahl

Hanan M. Al-Youssef [a,*], **Wafaa H.B. Hassan** [a,b]

[a] *King Saud University, Faculty of Pharmacy, Pharmacognosy Department, Saudi Arabia*
[b] *Zagazig University, Faculty of Pharmacy, Pharmacognosy Department, Zagazig, Egypt*

Received 28 April 2012; accepted 5 January 2014
Available online 10 January 2014

KEYWORDS

Carissa edulis;
3-*O*-acetyl chlorogenic acid;
Flavonoids;
Caredulis;
Alkyl rhamnose

Abstract Reinvestigation of the biologically active ethyl acetate and butanol extracts of the aerial parts of *Carissa edulis* afforded 3-*O*-acetyl chlorogenic acid (**I**), along with four known flavonol glucosides including, kaempferol 3-*O*-*β*-D glucopyranoside (**II**), quercetin-3-*O*-*β*-D glucopyranoside (**III**), rhamnetin-3-*O*-*β*-D glucopyranoside (**IV**) and isorhamnetin-3-*O*-*β*-D-glucopyranoside (**V**) from ethyl acetate fraction. Isorhamnetin-3-*O*-*β*-D-glucopyranoside-(2″→1‴)-rhamnopyranoside (**VI**), Caredulis, 1-{1-[2-(2 hydroxypropoxy) propoxy] propan-2-yloxy} propan-2-ol (**VII**) and (+) butyl-*O*-α-L-rhamnoside (**VIII**) were isolated from butanol fraction. Characterization of these compounds was achieved by various spectroscopic methods (UV, MS, ^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC) and through comparison with published data. Compounds **I**–**VIII** were isolated from *C. edulis* for the first time, while compounds **VII** and **VIII** were isolated for the first time from nature.

1. Introduction

Family Apocyanaceae consists of 250 genera and 200 species (Atiqur Rahman et al., 2003, 2004; Burkill, 1985; Hutchinoson and Dalziel, 1963; Irvine, 1961). It is represented in Saudi Arabia by seven genera among them is genus *Carissa* (Atiqur Rahman et al., 2003, 2004). This genus is a rich source of different natural classes of compounds such as sesquiterpenes, cardiac glycosides, phenolic compounds, flavonoids, lignans, chlorogenic acid derivatives (Al-Youssef and Hassan, 2010; Kirara et al., 2006; Pal et al., 1975; Wangteeraprasert and Likhitwita Yawuid, 2009). A literature search for the phytoconstituents of *Carissa edulis* indicated the isolation of flavonoids, phenolic compounds and chlorogenic acid derivatives (Al-Youssef and Hassan, 2010), Lignans (Achenbach et al., 1983,1985; Pal et al., 1975; Wangteeraprasert and Likhitwita Yawuid, 2009), sesquiterpene (Wangteeraprasert and Likhitwita Yawuid, 2009), in addition to 2-hydroxacetophenone (Bently et al., 1984). *C. edulis* is used as a folkloric remedy for treatment of headache, chest complains, rheumatism, oedema, gonorrhoea, syphilis, rabies and it is also used as a remedy for fever, sickle cell anaemia, cough, ulcer, toothache, and worm infestation (Al-Youssef and Hassan, 2010). In pharmacological studies *C. edulis* exhibited antiviral (Tolo et al., 2006, 2010), anticonvulsant (Jawaid et al., 2011; Ya'u Yaro et al., 2008), antiplasmodial (Kebenei et al., 2011; Koch et al., 2005; Kiriri et al., 2006), antimicrobial (Ibrahim et al., 2005), analgesic (Ibrahim et al., 2007), diuretic (Mekaconnen and Urga, 2004), as well as hypoglycaemic activity (El-Fiky

* Corresponding author. Tel.: +966 0555287629.
E-mail address: h_alyoussef@yahoo.com (H.M. Al-Youssef).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.01.004

et al., 1996). In continuation of our phytochemical investigation of *C. edulis* which grows in Saudi Arabia we report here for the first time the isolation and structure elucidation of two new compounds, **VII** and **VIII**, and five known flavonoid glycosides **II**–**VI**, as well as chlorogenic acid derivative **I** from the polar fraction of this plant.

2. Experimental

2.1. Plant material

The aerial parts of *C. edulis* were collected in 2008 from Aqubat Tanouma Baljorashi, southern region of Saudi Arabia. The plant was identified by Dr. M. Atiqur Rahman, Prof. of Taxonomy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#14151) was kept in the herbarium of the Research Center for Medicinal, Aromatic and Poisonous Plants of the same college.

2.2. Apparatus

The positive FAB mass spectra were recorded on a Finnigan MAT 300 mass spectrometer. EIMS spectra were measured using an EI finnigan model 4600 quadrupole system or shimadozu QP 500 GC/MS spectrometer. Ultraviolet spectra were obtained using a Hewlett–Packard HP-845-UV–Vis spectrometer, Ultraviolet used in visualizing TLC plates was a Mineralight device, multiband UV-254/366 obtained from UVP, Inc., USA. Melting points were determined on a Mettler FP 80 Central processor supplied with a Mettler FP 81 MBC cell apparatus and were uncorrected. Specific rotation was measured as a solution in methanol on a Perkin–Elmer 241 MC polarimeter using one decimeter tube. The ^{1}H (500 MHz) and ^{13}C & DEPT 90 and 135 NMR (125 MHz) and 2D NMR (COSY, HSQC, HMBC) were recorded on Brucker DRX 500 spectrometer in MeOD using TMS as internal standard reference, chemical shift in δ (ppm) and coupling constants (J values) are in Hz. Column chromatography was performed using silica gel G (60–230 meshes, Merck) as an adsorbent, RP-18 silica gel F_{254} "Whatmann" for column chromatography and Sephadex LH-20, (15–100 cm). TLC was performed on silica gel 60 F_{254} Merk plates. Visualization of the plates was performed using visible light, UV fluorescence and/or spraying with $FeCl_3$ or ammonia. Chromatographic systems used were EtOAc: MeOH: H_2O (30:5:4 and 30:5:1). The solvents used were ethanol 95%, acetone, petroleum ether 40–60 °C, chloroform, methanol, butanol and ethyl acetate, which were distilled prior to use. Analytical grade solvents were used for chromatography and crystallization while those used for extraction processes were general purpose reagents (GPR). Spectroscopic grade and deuterated solvents were used for spectral analyses.

2.3. Extraction and isolation

The air dried powdered aerial parts of *C. edulis* Vahl (1.2 kg) were exhaustively extracted with 95% ethyl alcohol at room temperature. The ethanolic extract was evaporated under vacuum to yield 150 g of dark green residue. The residue was suspended in 1 L water/methanol (8:2). The solution was successively extracted with petroleum ether 40–60 °C (3×0.5L), $CHCl_3$ (3×0.5 L), EtOAc (3×0.5 L) and *n*-butanol (4×0.5 L). The aqueous layer after extraction with *n*-butanol was lyophilized to produce 114.0 g residue.

The ethyl acetate fraction (16.0 g) was chromatographed over silica gel column (250 g, 2.5 cm), elution was started with hexane and ethyl acetate mixture (8:2), and the polarity was increased until 100% ethyl acetate, followed by methanol in a gradient elution technique. A total of 150 fractions of 200 ml each were collected, screened by TLC and similar fractions were pooled to give three main fractions: Fraction A, fractions 6–20 (2.0 g), Fraction B, fractions 25–60 (5.0 g) and Fraction C, fractions 70–110 (5.8 g). Fraction B (5.0 g) was chromatographed over silica gel column (150 g, 2 cm), eluting with hexane and ethyl acetate mixture (6:4) and the polarity was increased until 100% ethyl acetate, followed by methanol in a gradient elution technique. A total of 90 fractions of 100 ml each were collected and similar fractions were pooled. The important fractions were further purified by re-chromatography on silica gel column. Fractions 5–19 (90 mg), eluted with hexane/ethyl acetate (4:6) were crystallized from methanol to give 20 mg of **I**. Fractions 45–57 (65 mg), eluted with ethyl acetate/methanol (8:2) were crystallized from methanol to give 16 mg of pure **II**. Fractions 75–87 (88 mg), eluted with ethyl acetate/methanol (6:4) were crystallized from methanol to afford 20 mg of compound **III**. Fractions 20–27 (100 mg), eluted with hexane/ethyl acetate (1:9) were crystallized from methanol to give 15 mg of **IV**. Fractions 30–40 (50 mg), eluted with ethyl acetate only were crystallized from methanol to give 20 mg of **V**.

The butanol extract (3.5 g) was chromatographed on a Sephadex LH-20 column, using methanol as an eluent. Forty-five fractions of 20 ml each were collected and the similar fractions were pooled together. Two main fractions containing three major spots were subjected to further column chromatography on the RP-18 silica gel column, using water only and the polarity was decreased until 100% methanol to give 15 mg yellow crystals of compound (**VI**), compound (**VII**) as light brown amorphous solid (40 mg) and 15 mg of compound (**VIII**).

2.4. Acid hydrolysis of II–VI and VIII

About 3 mg of each compound (**II**–**VI** and **VIII**), in a mixture of 5% HCl (2 ml) and MeOH (2 ml) was heated separately under reflux for 1.5 h. The reaction mixture was cooled and diluted with 2 ml water, and neutralized with 5% Na_2CO_3. The neutralized products were concentrated and subjected to TLC analysis (EtOAc: MeOH: H_2O: HOAc, 6:2:1:1). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating. The sugars were identified as D-glucose and L-rhamnose after comparison with authentic samples.

Compound **I** Fig. 1: Yellow powder; UV absorbance at λ_{max} (MeOH) 330 and 310 nm; $[\alpha]_D$ 3.36 (c = 0.011, MeOH); FABMS m/z 397 $[M^+ + H]$ for $C_{18}H_{20}O_{10}$. It was identified as 3-*O*-acetyl chlorogenic acid by comparison of its UV, FAB-MS, ^{1}H NMR and ^{13}C NMR data with literatures (Al-Youssef and Hassan, 2010; Antognoni et al., 2011; Haribal et al., 1998; Kim et al., 2011; Whitaker and Stammel, 2003).

Compound **II** (Fig. 1): Yellow needles, mp. 163-164 °C (EtOH), R_f 0.45 [EtOAc: MeOH: H_2O, 30: 5: 1], acid hydrolysis of **II** yielded glucose and kaempferol, FABMS m/z 449

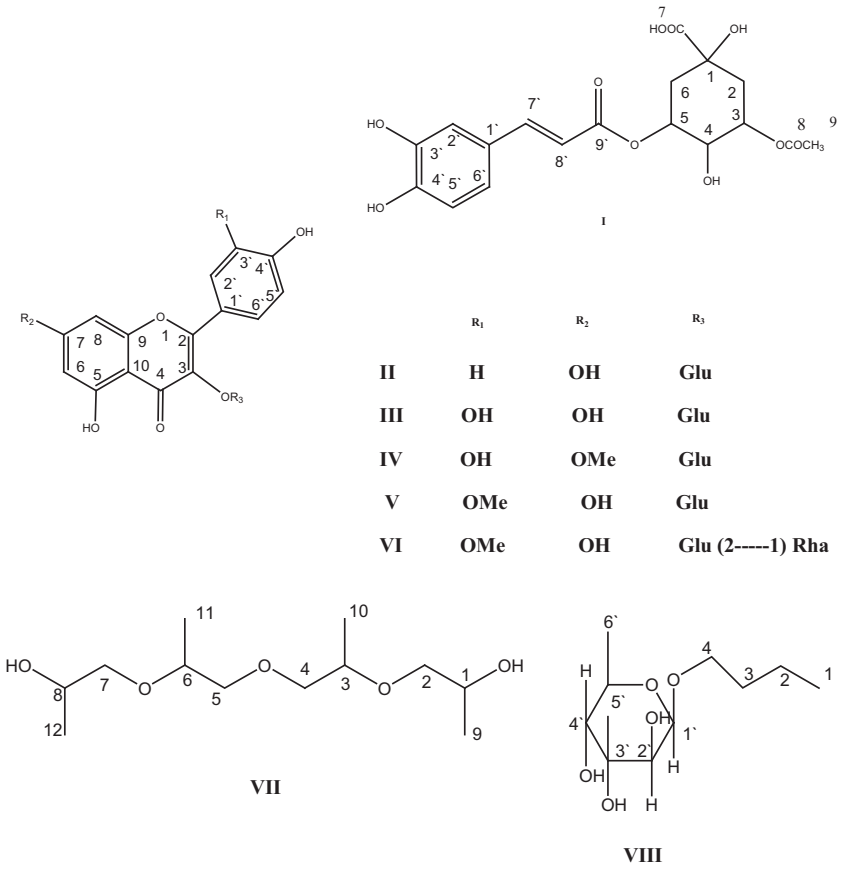

Figure 1 Structure of compounds **I–VIII** isolated from *Carissa edulis*.

$[M^+ + H]$ for C_{21} H_{21} O_{11}, *m/z* 286 $[M^+$-glucose]. It was identified as kaempferol-3-O-*β*-D-glucopyranoside by comparison of its UV, FAB-MS, ^1H NMR and ^{13}C NMR data with literature (Whitaker and Stammel, 2003; Amer et al., 2001; Mabry et al., 1970; Harborne et al., 1975; Markham, 1978).

Compound **III** (Fig. 1): Yellow powder, mp. 240–241 °C (EtOH), R_f, 0.35 [EtOAc: MeOH: H_2O, 30: 5: 1], acid hydrolysis of **III** yielded glucose and quercetin, FABMS *m/z* 465 $[M^+ + H]$ for C_{21} H_{21} O_{12}, *m/z* 302 $[M^+$-glucose]. It was identified as quercetin-3-*O*-*β*-D glucopyranoside by comparison of its UV, FAB-MS, ^1H NMR and ^{13}C NMR data with literatures (Amer et al., 2001; Harborne et al., 1975; Mabry et al., 1970; Markham, 1978; Whitaker and Stammel, 2003).

Compound **IV** (Fig. 1): Yellow powder, R_f, 0.60 [EtOAc: MeOH: H_2O, 30: 5: 4], acid hydrolysis of **IV** yielded glucose and rhamnetin, FABMS *m/z* 479 $[M^+ + H]$ for C_{22} H_{23} O_{12}, *m/z* 316 $[M^+$-glucose]. It was identified as rhamnetin-3-*O*-*β*-D glucopyranoside by comparison of its UV, FAB-MS, ^1H NMR and ^{13}C NMR data with literatures (Amer et al., 2001; Harborne et al., 1975; Mabry et al., 1970; ; Markham, 1978; Whitaker and Stammel, 2003).

Compound **V** (Fig. 1): Yellow powder, mp. 215–217 °C (EtOH), R_f, 0.60 [EtOAc: MeOH: H_2O, 30: 5: 4], acid hydrolysis of **V** yielded glucose and isorhamnetin, FABMS *m/z* 479 $[M+H]^+$ for C_{22} H_{23} O_{12}, *m/z* 316 $[M^+$-glucose]. It was identified as isorhamnetin-3-*O*-*β*-D-glucopyranoside by comparison of its UV, FAB-MS, ^1H NMR and ^{13}C NMR data with literatures (Amer et al., 2001; Harborne et al., 1975; Mabry et al., 1970; Markham, 1978; Whitaker and Stammel, 2003).

Compound **VI** (Fig. 1): Yellow powder, R_f, 0.30 [EtOAc: MeOH: H_2O, 30: 5: 4], acid hydrolysis of **VI** yielded glucose, rhamnose and rhamnetin, FABMS *m/z* 625 $[M+H]^+$ for C_{28} H_{33} O_{16}, *m/z* 479 $[M^+$-rhamnose], *m/z* 317 $[M^+$-glucose]. It was identified as **i**sorhamnetin-3-*O*-*β*-D-glucopyranoside-(2″→1‴)-L-rhamnoside by comparison of its UV, FAB-MS, ^1H NMR and ^{13}C NMR data with literatures (Braca et al., 1999; Cimanga et al., 1997).

Compound (**VII**) Caredulis, 1-{1-[2-(2 hydroxypropoxy) propoxy] propan-2-yloxy} propan-2-ol, Fig. 1: A brown amorphous solid, R_f 0.80 (CHCl: MeOH (9:1)). EIMS $[M]^+$ at *m/z* 250 for the molecular formula $C_{12}H_{26}O_5$. For NMR data see (Table 1), HMBC correlations Fig. 2.

Compound **(VIII)** (+) butyl-*O*-α-L-rhamnoside, Fig. 1: A dark yellow amorphous solid, R_f 0.73 (CHCl: MeOH (9:1)). $[\alpha]^{25}_D$ is, + 56.3° (*c* = 0.0035, MeOH);. EI-MS *m/z* 220 for the molecular formula C_{10} $H_{20}O_5$. For NMR data see (Table 2), HMBC correlations Fig. 2.

3. Results and discussion

Compound (VII) was isolated as a brown amorphous solid in EtOAc:MeOH (8:2); $[\alpha]^{25}_D$ is 0.128 (*c* = 0.8 g/L MeOH). The EIMS of this compound showed a molecular ion peak at *m/z* 250 $[M]^+$ corresponding to the molecular formula $C_{12}H_{26}O_5$ and a peak at *m/z* 249 $[M–H]^+$ indicating its alcoholic nature. It is freely soluble in methanol, ethyl acetate and insoluble in petroleum ether. It is UV inactive; it gave no colour change

Table 1 ^{1}H & ^{13}C NMR (δ_C DEPT) NMR data of compound (**VII**).

Position	δ_H, mult. J (Hz)	δ_C, mult.	COSY	HMBC
9, 10, 11, 12 (CH_3)	1.16 d, J = 7.5	17.8 *q*	CH_2, CH	CH_2, CH
2, 4, 5, 7 (CH_2)	3.48–3.55 *m*	74.0–74.5 *t*	CH_3	CH_3
1, 3, 6, 8 (CH)	3.60–3.62 *m*	76.5–76.8 *d*	CH_3	CH_3

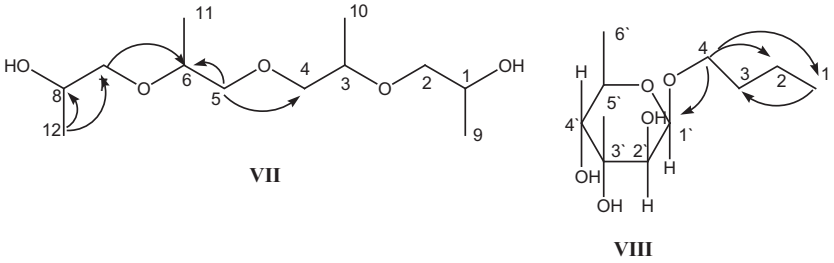

Figure 2 Important HMBC correlations of compounds **VII** and **VIII**.

Table 2 ^{1}H & ^{13}C NMR (δ_C DEPT) NMR data of compound (**VIII**).

Position	δ_H, mult. J (Hz)	δ_C, mult.	COSY	HMBC
1	0.95 *t*, J = 7.0	14.3 *q*	2	2, 3
2	1.40 *m*	20.4 *t*	1, 3	1, 2
3	1.57 *m*	32.9 *t*	2, 4	1, 2
4	3.50 *m* 3.65 *m*	62.3 *t*	3	1, 1‘, 2, 3
1‘	4.90 *d*, J = 3.0	103.7 *d*	2‘	4
2‘	3.91 *m*	71.4 *d*	1‘	-
3‘	3.75 *m*	70.7 *d*	-	2‘, 6‘
4‘	3.39 *m*	72.2 *d*	-	2
5‘	3.59 *m*	71.2 *s*	-	3‘
6‘	1.14 *d*, J = 5.0	19.7 *q*	5‘	4‘, 5‘

with $FeCl_3$ solution. It appeared as a black spot after spray with *p*-anisaldehyde. The ^{1}H NMR and ^{13}C NMR spectral data of compound **VII** in CD_3OD are presented in Table 1. ^{1}H NMR spectrum showed a sharp doublet at δ_H 1.16 integrated for 12 protons indicating four identical methyl groups. Furthermore, the signals at a range of δ_H 3.48–3.55 integrated for eight protons for four oxygenated methylene groups, as well as the signals at δ_H 3.60–3.62 appeared as multiplet integrated for four protons assigned to four oxygenated methine protons. ^{13}C NMR and DEPT spectra exhibited resonances for methyl carbon appeared as one signal, which are account for four methyles at δ_C 17.8 (C-9, C-10, C-11 and C-12), four oxygenated methylene carbons at δ_C 74.0, 74.1, 74.4 and 74.5 for C-2, C-4, C-5 and C-7 respectively. As well as four signals at δ 76.5, 76.7, 76.7 and 76.8 for oxygenated methine carbons C-1, C-3, C-6 and C-8 respectively**.** H-H COSY experiments supported the above signal assignments. It showed a relationship between the methyl protons and both oxygenated methylene and methine protons and vise versa. HSQC experiment of compound **VII** is helpful in correlating each proton to the corresponding carbon. The exact location of the methyl groups was deduced based on HMBC correlations, Fig. 2, where the four methyl protons at δ_H 1.16 showed correlations with both oxygenated methylene carbon absorptions at δ_c 74.0, 74.1, 74.4 and 74.5 as well as oxygenated methine carbon absorptions at δ_c 76.5, 76.7, 76.7 and 76.8. Also, the four oxygenated methylene carbons showed a correlation with both oxygenated methylene and methine protons. Based on the above data compound **VII** is identified as 1-{1-[2-(2 hydroxypropoxy) propoxy] propan-2-yloxy} propan-2-ol, Fig. 1, Caredulis. This is the first time to isolate Caredulis from nature.

Compound (VIII) was isolated as a dark yellow amorphous solid in EtOAc:MeOH (6:4), freely soluble in methanol, insoluble in chloroform. It is UV inactive. $[\alpha]^{25}{}_D$ is, + 56.3̊ (c = 0.0035, MeOH). It gave a positive Molisch's test and on acidic hydrolysis yielded sugar part which was identified by TLC comparison with reference sugar samples as rhamnose. The EI-MS showed molecular ion peak at m/z 220 [M^+] in correspondence with the molecular formula $C_{10}H_{20}O_5$, Fig. 1. All the carbons were clear in the ^{13}C NMR data (Table 2). ^{1}H NMR data (Table 2) revealed the presence of a rhamnosyl moiety as indicated by the anomeric proton signal at δ_H 4.90 (*d*, J = 3.0 Hz), methyl doublet at δ_H 1.14 (*d*, J = 5.0 Hz), as well as four oxygenated methine proton signals at δ_H 3.91, 3.75, 3.39 and 3.59, the assignment of sugar protons was based on COSY experiment. In the HSQC experiment of compound **VIII**, the methyl doublet at δ_H 1.14 was correlated to carbon absorption at δ_c 19.7 and the anomeric proton doublet at δ_H 4.90 was correlated to carbon absorption at δ_H 103.7. The ^{1}H NMR of compound **VIII** showed additional signals for three methylene protons that appeared as downfield multiplet at δ_H 3.50 and 3.65 correlated in HSQC to carbon resonance at δ_C 62.3. The other two methylene protons at s_H 1.40 and 1.57 showed correlation to carbon absorption at δ_C 20.4 and 32.9 respectively. Also, the methyl proton triplet at δ_H 0.95 correlated to carbon absorption at δ_c 14.3. These data were assigned for the butyl group. In HMBC a correlation, Fig. 2, was observed between anomeric proton of rhamnose and the methylene carbon absorption at δ_C 62.3. Also the correlations of the two methylene protons at δ 3.50 and 3.65 with the anomeric carbon at δ_c 103.7, Fig. 2. As a result of discussion, compound **VIII** is confirmed to be (+) butyl-*O*-α-L-rhamnoside. Literature survey indicated that this compound is a new alkylated rhamnoside and it is reported for the first time as natural product. The (−)

butyl-*O*-α-L-rhamnose was previously isolated from *lotus* lalambensis (Al-Youssef et al., 2008).

Acknowledgment

This research project was supported by a grant from the ''Research Center of the Center for Female Scientific and Medical Colleges'', Deanship of Scientific Research, King Saud University. Our sincere thanks to Mr. Maher El Gabal, central Lab. in King Saud University for carrying out the NMR analysis.

References

Achenbach, H., Weibal, R., Addac-mensah, I., 1983. Phytochemistry 24, 2325–2328.
Achenbach, H., Waible, R., Addae-Mensah, I., 1985. Phytochemistry 24, 749–753.
Al-yuossef, H.M., Hassan, W.H., 2010. Biosci. Biotechnol. Res. Asia 7, 635–646.
Al-Youssef, H.M., Murphy, B.T., Amer, M.E., Abdel-Kader, M.S., Kingston, D.J.I., 2008. Saudi Pharm. J. 16 (2), 122–134.
Amer, M.E., Kassem, F.F., Abdel-Kader, M., Alex, J., 2001. Pharm. Sci. 15, 99–102.
Antognoni, F., Perellino, N.C., Crippa, S., Dal Toso, R., Danieli, B., Poli, F., Pressi, G., 2011. Fitoterapia 82, 950–954.
Atiqur Rahman, M., Mossa, J.S., Al-Said, M.S., Al-Yahya, M.A., 2003. Saudi J. Biol. Sci. 10, 158–161.
Atiqur Rahman, M., Mossa, J.S., Al-Said, M.S., Al-Yahya, M.A., 2004. Fitoterapia 75, 149–161.
Bently, M.D., Brachett, S.R., Chapya, A., 1984. J. Nat. Prod. 47, 1056–1057.
Braca, A., Billia, R.A., Mendez, J., Morelli, I., 1999. Phytochemistry 51 (8), 1125–1128.
Burkill, H.M., The Useful Plants of West Africa, 2nd Ed vol. I. Families A-D Royal Botanical Gardens. Kew. 1985, Pp-145-146.
Cimanga, K., de Bruyne, T., Van Poel, B., Ma, Y., Bakana, P., Vanden-Berghe, D., Vlientlik, A.J., 1997. Planta Med. 63, 220–223.
El-Fiky, F.K., Abo-Karam, M.A., Afify, E.A., 1996. J. Ethnopharmacol. 50, 43–47.
Harborne, J.B., Mabry, T.J., Mabry, H., 1975. The flavonoids. Academic press, New York San Francisco, A subsidiary of Harcourt Brace Jovanovich, Publishers, 46–77.
Haribal, M., Feeny, P., Lester, C.C., 1998. Phytochemistry 49, 103–108.
Hutchinson, J.; Dalziel, J. M., Flora of West Africa, vol. II Crown Agents for Over sea Governments and Administration, Milbank, London S.W.I. 1963, Pp 51–54.
Ibrahim, H., Bolagi, R.O., Abdurahman, E.M., Shok, M., Ilyas, N., Habib, A.G., 2005. ChemClass J. 2, 15–18.
Ibrahim, H., Bolagi, R.O., Abdurahman, E.M., Shok, M., Ilyas, N., Musa, K.Y., Ukanda, I., 2007. Afr. J Biotechnol 6, 1233–1235.
Irvine, F.R., 1961. Woody Plants of Ghana. Oxford University Press, London, pp 616–618.
Jawaid, T., Argal, S., Singh, S., 2011. J. Pharm. Res. 4, 1138–1140.
Kebenei, J.S., Naalut, P.K., Sabah, A.O., 2011. Int. J. Appl. Res. Nat. Prod. 4, 1–5.
Kim, J.Y., Cho, J.Y., Ma, Y.K., Park, K.Y., Lee, S.H., Ham, K.S., Lee, H.J., Park, K.H., Moon, J.H., 2011. Food Chem. 125, 55–62.
Kirira, P.G., Rukungo, G.M., Wanyonyi, A.W., 2006. J. Ethnopharmacol. 106, 403–407.
Koch, A., Amez, P.T., Pezzuto, J., Soejarto, D., 2005. J. Ethnopharmacol. 101, 95–99.
Mabry, T.J., Markham, K.R., Thomas, M.B., The systematic identification of flavonoids; Springer-Verlag, New York, Heidelberg – Berlin, 1970.
Markham, K.R., 1978. Tetrahedron 34, 1389–1397.
Mekaconnen, N., Urga, K., 2004. J. Ethnopharmacol. 95, 57–61.
Pal, D.R., Kulshreshtha, R.P., Rastoqi, A., 1975. Phytochemistry 14, 2302–2303.
Tolo, F.M., Rukungo, G.M., Muli, F.W., 2006. J. Ethnopharmacol. 104, 92–99.
Nedi Tolo, F.M., Rukungo, G.M., Muli, F.W., Ochora, J.M., Irungu, B.N., Muthauru, C.N., Wanjiku, C.K., Mungai, G.M., Quang, N., Hashimoto, K., Asakaw, Y., 2010. J Med. Plants Res. 4, 1517–1522.
Wangteeraprasert, R., Likhitwita Yawuid, K., 2009. Chemica Acta 92, 1217–1223.
Whitaker, B.D., Stammel, J.R., 2003. J. Agri. Food Chem. 51, 3448–3454.
Ya'u Yaro, A.H., Abubakar, M.S., Hussaini, I.M., 2008. J. Ethnopharmacol. 120, 255–258.