A new pregnane glycoside from *Gomphocarpus fruticosus* growing in Egypt

Amani M. Marzouka, Samir M. Osmanb and Ahmed A. Gohara

aFaculty of Pharmacy, Department of Pharmacognosy, Mansoura University, Mansoura, Egypt; bFaculty of Pharmacy, Department of Pharmacognosy, 6th of October University, 6th of October City, Egypt

1. Introduction

The former family Asclepiadaceae (now subfamily Asclepiadoideae of family Apocynaceae) is reputed for its content of cardenolides and pregnane glycosides (Hegnauer 1964). *Gomphocarpus fruticosus* (L.) Ait. (syn. *Asclepias fruticosa* L.), subfamily Asclepiadoideae of the family Apocynaceae, is a woody perennial shrub indigenous to south and tropical Africa, also known from north Africa, Arabian Peninsula and south Europe, where it is probably introduced (Goyder & Nicholas 2001). In Egypt, it grows in Nile Delta, the Mediterranean coastal region and South Sinai where it is known as herke (Muschler 1912; Tackholm 1974). *G. fruticosus* is used in the traditional medicine of tropical Africa to treat malaria, diabetes,
asthma, bronchitis, cardiac palpitations, as a diuretic and a treatment for anthrax in cattle (Burkill 1985). In the Arabian Peninsula it is used to treat tumors, skin diseases, scabies and itching (Mothana et al. 2014). G. species are reported to contain 5α-cardenolides with doubly linked sugars and pregnane glycosides (El-Askary et al. 1993). Several cardenolides were identified in G. fruticosus plants of different geographical origins. Uzarigenin, desglucouzarigenin, gomphotin, gomphotoxizin and gomphacil were reported from plants growing along the black sea (Chernobai & Komissarenko 1971; Komissarenko et al. 1995, 1997). Australian plants afforded gomphoside and afroside (Carman et al. 1964), while A. fruticosa L. (syn. G. fruticosus) cultivated in Japan further afforded uscharin and calactin derivatives and coroglaucigenin and corotoxigenin glycosides (Warashina & Noro 1994a). Pregnan glycosides of ikeemagenin, kidjolanin (Abe et al. 1994), lineolon and isolineolon aglycones (Warashina & Noro 1994a, 1994b) were also identified in this species.

Cardiac glycosides have been in clinical use for the treatment of heart failure for almost two centuries. Recent findings have highlighted potential new multi-therapeutic roles for compounds like digioxin, digitoxigenin, digitonin, ouabain, oleandrin, lanatoside C and proscillaridin A in various diseases. These included cancer, cystic fibrosis of lungs, stroke and heart ischaemia and neurodegenerative diseases (Prassas & Diamandis 2008). Cardenolides having doubly linked sugars, commonly found in G. species, also displayed cardiotonic action in addition to other various biological activities. Gomphoside is 10 times as potent as the cardiotonic compound digoxin and 5 times as potent as ouabain (Thomas et al. 1990). Calotropin is cytotoxic against various human cancer cell lines (Gupta et al. 2009); calactin is a potential anticancer agent for treating leukaemia (Lee et al. 2012) and uscharin is an active molluscicidal agent (Hussein et al. 1994). Pregnan glycosides isolated from different Asclepiadaceae plants similarly displayed various biological activities including digitalis-like (Melero et al. 2000); cytotoxic (Wang et al. 2008; Liu et al. 2014); antidiyslipidemic and antioxidant activities (Sethi et al. 2013); antitrypanosomal (Gurib-Fakim & Mahomoodally 2013); chondroprotective (Sanyacharernkul et al. 2009) and anti-obesity effects (Abdel-Mogib & Raghib 2013; Elsebai & Mohamed 2015). In our plan to discover cardioactive compounds from Egyptian natural resources, we reviewed the phytochemical investigation of G. fruticosus (L.) Ait. growing in Egypt. A single report for the identification of flavonoids of quercetin, kaempferol and isorhamnetin-type glycosides could be traced in the literature (Heneidak et al. 2006). So, it was deemed of interest to investigate the cardenolide and pregnane glycosides content of the plant.

2. Results and discussion

Chromatographic fractionation of a CH₂Cl₂ extract of the air-dried aerial parts of G. fruticosus (L.) Ait. growing in Egypt afforded a new pregnane glycoside (5) identified as lineolon-3-O-β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranosyl-(1–4)-β-D-cymaropyranoside, along with six known compounds (1–4, 6–7). These included triterpenoids, cardenolide aglycone and glycosides (Figure 1). The known compounds were identified by comparing their physical and spectroscopic data to those reported in literature (supplementary data, Tables S1 and S2). The triterpenoids 3β-taraxerol acetate (1) (Sasaki et al. 1965); 13α-methyl, 27-norolean-14-en, 3β-ol (3β-taraxerol) (2) (Mahato & Kundu 1994; Prachi & Pradeep 2014) and betulinic acid (3) (Mahato & Kundu 1994; Marzouk 2009) are identified in this work for the first time from G. fruticosus. The cardenolide aglycone uzarigenin (4) (El Gamal et al. 1999; Pauli et al. 1999;
The cardenolide glycosides gomphoside (6) (Carman et al. 1964; Lichtenthaler et al. 2005) and calotropin (7) (El-Askary et al. 1993) were also isolated in this work. The former was identified earlier in G. fruticosus of Australian origin (Carman et al. 1964), while the later was isolated from plants cultivated in Japan (Abe et al. 1994; Warashina & Noro 1994b) and from Gomphocarpus sinaicus of Egyptian origin (El-Askary et al. 1993).

Compound (5) was obtained as an amorphous solid, m.p 160–162 °C. It gave positive Molisch’s test, indicating its glycosidic nature. HRFAB MS spectrum displayed a quasi molecular ion at m/z 819.4511 [M + Na]+, consistent with a molecular formula of C_{42}H_{68}O_{14}Na. The ¹H- and ¹³C-NMR data (Experimental) indicated a polyoxygenated preg-5-en-20-one skeleton. ¹H-NMR spectrum showed three methyl singlets (δ 1.07, 1.21 and 2.18) and an olefinic proton signal at δ 5.27 (s). Two secondary hydroxyl protons at δ 3.16 (dd, J = 11.4, 4.1 Hz) and 3.80 (m), which were corporate by HSQC experiment to carbon signals at δ 68.5 and 78.0, suggested hydroxylation at the corresponding positions and could be assigned to C-12 and C-3, respectively. Three anomeric sugar protons were also observed in ¹H-NMR spectrum at δ 4.43 (dd, J = 9.6, 2.0 Hz), 4.47 (dd, J = 9.2, 2.0 Hz) and 4.68 (dd, J = 9.6, 2.0 Hz) and indicated that compound (5) is a triglycoside. The structure of the aglycone part was deduced to be lineolon from HMBC correlation peaks (Figure S7) observed between proton signal at δ 1.21 (s, CH₃-18) and each of the carbon resonances at δ 55.9 (C-13), 60.7 (C-17),

Figure 1. Structures of compounds 1–7.
68.5 (C-12) and 85.7 (C-14) and between the proton signal at δ 1.07 (s, CH₃-19) and carbon resonances at 37.1 (C-10), 38.7 (C-1), 44.1 (C-9) and 141.2 (C-5). Also HMBC correlation peaks between H-6 (δ 5.27, s), H-9 (δ 1.40, dd, J = 11.0, 4.0 Hz) and the quaternary carbon resonance at 74.6, confirmed hydroxylation at C-8. The NMR data of the aglycone part (Experimental) were in good agreement with previously reported values for lineolon (Abe & Yamauchi 2000; Warashina & Noro 2000). In ¹³C-NMR spectrum, shielding of C-2 and C-4 by 4.7 and 4.0 ppm, respectively, as well as deshielding of C-3 by 6.1 ppm (Experimental) compared to the same positions in pregnane aglycones run in the same solvent (Abe et al. 1999) indicated glycosidation at C-3 hydroxyl. The presence of three methyl doublets in the aliphatic region of the ¹H-NMR spectrum (δ 1.15, 1.16 and 1.25) and three methoxyl singlets (δ 3.32 and 3.41, integrated for six protons) indicated the presence of 2,6-dideoxyhexopyranoses commonly found in Asclepias glycosides (Araya et al. 2012). Using HMBC and HSQC experiments and comparing the ¹H-APT and ¹³C-NMR data (Experimental) to literature values (Abe et al. 1994; Abe & Yamauchi 2000; Warashina & Noro 2000; Araya et al. 2012), the structures of the sugars were elucidated as two units of cymarose and a terminal unit of oleandrose. The connectivity of the sugars to the aglycone and the interglycosidic linkages were established as follows: a correlation peak in HMBC spectrum (Figure S7) between the anomeric proton (δ 4.68, dd, J = 9.6, 2.0 Hz, H-1’) and C-3 (δ 78.0) identified one of the cymarose units as the internal sugar. Another anomeric proton signal (δ 4.47, dd, J = 9.2, 2.0 Hz, H-1”) showed HMBC correlation to C-4’ of the internal cymarose (δ 82.4) to identify the middle sugar as cymarose as well. The third anomeric proton (δ 4.43, dd, J = 9.6, 2.0 Hz, H-1””) showed HMBC correlation to C-4” of the middle cymarose unit (δ 82.6). The β-linkages of the sugars were established by the large coupling constants (J = 9.2–9.6 Hz) observed for their anomeric protons. Thus, the structure of (5) was determined to be lineolon-3-O-[β-D-oleandropyranosyl-(1–4)-β-D-cymaropyranosyl-(1–4)-β-D-cymaropyranoside], which is a new natural product identified for the first time in this work from G. fruticosus and named gomphofruticososide.

Our results for G. fruticosus lies in partial agreement with those of Seiber et al. (1983), who reported that gomphoside and afroside (the 15-OH derivative of gomphoside) are typical for this species. However, tracing of recent literature revealed that afroside (but not gomphoside) is present in other Asclepias species. Afroside was not isolated in this study, nor were the thiazoline and thiazolidine cardenolides (e.g. uscharin and uscharidin) commonly occurring in Asclepias (Cheung et al. 1983). Whether these cardenolides are totally absent or present as minor constituents, is still not clear. Further investigation of the more polar fractions of the MeOH extract is in progress as TLC screening of the methanolic mother liquor (data not shown) revealed several spots giving the same colour reactions of the isolated compounds. The other isolated cardenolides viz. uzarigenin and calotropin are of wide occurrence in the genus and family. The triterpenoids isolated in this work are reported from G. fruticosus for the first time and are also common in other species of the Asclepiadaceae (Moulisha et al. 2009; Karthikeyan & Balusubramanian 2014; Maldonado et al. 2015). Betulinic acid is an exception, as it could not be traced in any other Asclepias species and hence might be considered as another hallmark for G. fruticosus.

In conclusion: this study resulted in the isolation and identification of a new pregnane glycoside, three cardenolides and three triterpenoids, previously unreported from G. fruticosus; data which warrants more pharmacological studies of the plant extracts.
3. Experimental

3.1. Plant material

G. fruticosus (L.) Ait. was collected from South Sinai. The identity of the plant was confirmed by Professor I. Mashaly, Dept. of Botany, Faculty of Science, Mansoura University. A voucher (1504) specimen was deposited in Department of Pharmacognosy, Faculty of Pharmacy at Mansoura University.

3.2. General experimental procedures

Melting points (uncorr.) were recorded on Yamagimoto micro-melting point apparatus MP-500D (Japan). Optical rotation was measured using A. Krüss optronics digital polarimeter P 8000 (Germany). IR spectra were recorded on a Shimadzu FTIR-8100 spectrometer (Japan). 1H, 13C-NMR, APT, HMBC and HSQC spectra were obtained with JEOL JNM-LA 400 (Japan) and Bruker dpx 500 (USA) high-field spectrometers, operating at 400 and 500 MHz (1H) and 100 and 125 MHz (13C). El mass spectra (70 eV) were recorded on a Shimadzu Qp-2010 plus mass spectrometer (Shimadzu, Japan). HRFAB mass spectra were taken on a JEOL JMS600 mass spectrometer (JEOL Instruments, Japan). Column chromatography was performed on silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) and silica gel 60 Rp-18 (40–63 μm, Merck, Darmstadt, Germany). TLC was carried out on precoated Si gel 60 GF254 and Rp-18 (0.25 mm, Merck, Darmstadt, Germany) plates. Developed chromatograms were visualised by spraying with 0.01% vanillin/H2SO4, followed by heating until maximum development of the spots colour. Solvents were reagent grade.

3.3. Extraction and isolation

The air-dried powdered aerial parts of G. fruticosus (1.4 kg) were extracted with MeOH (10 L × 5) at room temperature. Removal of the solvent under reduced pressure at 45 °C gave 171.0 g (12.21%w/w) of solid residue, which was suspended in 200 ml of MeOH, diluted to 500 ml with dist. H2O and defatted with pet ether and then extracted with CH2Cl2 (1 L × 5). Evaporation of the solvents gave 41.0 g (2.93% w/w) of pet ether extract and 45.0 g (3.21%w/w) of CH2Cl2 extract, respectively. The CH2Cl2 extract was purified by passing over activated charcoal and eluted with MeOH (2 L) to yield 21.5 g of resinous solid. The partially purified CH2Cl2 extract was chromatographed on silica gel column (3.5 x 70 cm) eluted with mixtures of pet ether and EtOAc. Hundred millilitre fractions were collected and fractions having similar TLC pattern (using vanillin/H2SO4 as spray reagent) were pooled. Fractions (1.73 g) eluted with 5% EtOAc in pet ether afforded 1 as colourless needle crystals (42.0 mg) by crystallisation from the same solvent. Further fractions eluted with 5% EtOAc in pet ether (920 mg) contained one major spot and several minor spots. They were rechromatographed on silica gel column (1.5 × 70.0 cm) and eluted with mixtures of EtOAc in pet ether to yield crude 2 (290 mg). Purification on a silica gel column (1.0 × 60.0 cm) eluted with 100% CH2Cl2 afforded pure 2 as colourless needle crystals (112.0 mg). Fractions (1.44 g) eluted with 10% EtOAc in pet ether contained one major spot in addition to several minor ones. It was rechromatographed on silica gel column (1.5 × 90 cm) and eluted with the same solvent system to yield crude 3 (400 mg). Purification on a silica gel column (1.0 × 70.0 cm) eluted with mixtures of CH2Cl2 and MeOH afforded pure 3 as colourless needle crystals (90.0 mg). Fractions
(4.23 g) eluted with 40–50% EtOAc in pet ether contained several spots. A portion of which (1.0 g) was fractionated on reversed phase silica Rp-18 open column (2.5 x 40 cm) eluted with mixtures of MeOH–H2O and collecting 10-ml fractions. Subfractions (210.0 mg) eluted with 60% MeOH were purified on silica gel column eluted with mixtures of CH2Cl2–EtOAc (50:50) containing increasing proportions of MeOH to yield pure 4 as colourless needles (42.0 mg). Another portion (1.12 g) of fractions eluted with 40–50% EtOAc in pet ether was rechromatographed on silica gel column eluted with increasing proportions of pet ether in EtOAc. Subfractions (140.0 mg) eluted with 35% pet ether in EtOAc were further purified on silica gel column eluted with 50:50 mixture of pet ether–CH2Cl2 containing increasing proportions of MeOH to yield 5 as a white amorphous solid (23.0 mg) in fractions eluted with 10% MeOH and 6 and 7 as crystalline solids (13.0 and 30.0 mg) in subsequent fractions eluted with 15% MeOH.

3.4. Lineolon-3-O-[β-D-oleanandropanosyl-(1–4)-β-D-cymaropyranosyl-(1–4)-β-D-cymaropyranoside] (5)

Amorphous solid, m.p. 160–162 °C. [α]D25−2.6 (c 0.1, CHCl3). Rf : 0.20 (pet ether/CH2Cl2, 50:50 containing 5% MeOH). IR (KBr) νmax: 3450, (–OH), 2920 (cH), 1780 (c=O ketone), 1705 (c=c), 1180 (c–O), 1100 cm−1. 1H NMR (400 MHz, CDCl3–CD3OD, 4:1): δ 0.99 (m, H-1), 1.51 (m, H-2a), 1.78 (m, H-2b), 3.80 (m, H-3), 2.12 (m, H-4a) 2.31 (m, H-4b), 5.27 (s, H-6), 2.15 (m, H-7), 1.40 (dd, J = 4.0, 11.0 Hz, H-9), 1.69 (m, H-11a), 1.86 (m, H-11b), 3.16 (dd, J = 11.4, 4.1 Hz, H-12), 1.65 (m, H-15a) 1.73 (m, H-15b), 1.85 (m, H-16a), 1.95 (m, H-16b), 3.35 (m, H-17), 1.21 (s, CH3-18), 1.07 (s, CH3-19), 2.18 (s, CH3-21), 4.68 (dd, J = 9.6, 2.0 Hz, H-1’), 2.02 (m, H-2’), 3.07 (m, H-3’), 3.15 (m, H-4’), 3.61 (m, H-5’), 1.15 (d, J = 6.5 Hz, CH3-6’), 3.41 (s, OMe), 4.47 (dd, J = 9.2, 2.0 Hz, H-1”), 2.12 (m, H-2”), 3.09 (m, H-3”), 3.14 (m, H-4”), 3.80 (m, H-5”), 1.16 (d, J = 6.0 Hz, CH3-6”), 3.41 (s, OMe), 4.43 (dd, J = 9.6, 2.0 Hz, H1’’), 2.25 (m, H-2’’), 3.49 (m, H-3’’), 3.10 (m, H-4’’), 3.21 (m, H-5’’), 1.25 (d, J = 6.0 Hz, CH3-6’”), 3.32 (s, OMe). 13CNMR (100 MHz, CDCl3–CD3OD, 4:1): δ 38.7 (C-1), 27.2 (C-2), 78.0 (C-3), 38.8 (C-4), 141.2 (C-5), 117.4 (C-6), 34.2 (C-7), 74.6 (C-8), 44.1 (C-9), 37.1 (C-10), 28.9 (C-11), 68.5 (C-12), 55.9 (C-13), 85.7 (C-14), 33.1 (C-15), 23.2 (C-16), 60.7 (C-17), 13.0 (C-18), 18.9 (C-19), 214.8 (C-20), 31.8 (C-21), 96.1 (C-1’), 34.3 (C-2’), 78.0 (C-3’), 82.4 (C-4’), 68.3 (C-5’), 18.2 (CH3-6’), 58.0 (OMe), 99.6 (C-1”’), 34.4 (C-2”’), 75.3 (C-3”’), 82.6 (C-4”’), 68.3 (C-5”’), 18.23 (CH3-6”’), 58.2 (OMe), 101.4 (C-1””), 37.1 (C-2””), 80.6 (C-3””), 75.3 (C-4””), 71.5 (C-5””), 18.0 (CH3-6””), 56.3 (OMe). HRFABMS (positive ion mode) m/z (rel. int.) 819.4511 (100) [M + Na]+ (calc. 819.4507), 796.4613 (26) [M]+ (calc. 796.4609). El-MS 70 eV, m/z (rel. int.): 798 (4) [M + 2]+, 797 (2.5) [M + 1]+, 778 (2.0) [M–H2O]+, 753 (1.5) [M–CH3C=O]+, 608 (9) [M–CH3C=O–Sugars]+, 429 (57) [M–CH3C=O–2 Sugars–2H2O]+, 321(29) [M–CH3C=O–3 Sugars]+, 267 (20), 167 (31), 83 (50), 69 (100).

Acknowledgement

The authors acknowledge the kind help of Dr Mohamad El Raey, National Research Centre, Dokki, Egypt, in carrying out the optical rotation and El mass measurements of the new compound.

Disclosure statement

No potential conflict of interest was reported by the authors.
References

Abdel-Megib M, Raghib H. 2013. Two new pregnane glycoside diesters from Caralluma russeliana. Nat Prod Res. 27:1287–1292.
Abe F, Fujishima H, Iwase Y, Yamauchi T, Kinjo K, Yaga S. 1999. Pregnanes and pregnane glycosides from Hoya carnosa. Chem Pharm Bull. 47:1128–1133.
Abe F, Mori Y, Okabe H, Yamauchi T. 1994. Steroidal constituents from the roots and stems of Asclepias fruticosa. Chem Pharm Bull. 42:1777–1783.
Abe F, Yamauchi T. 2000. Pregnane glycosides from the roots of Asclepias tuberosa. Chem Pharm Bull. 48:1017–1022.
Araya J, Binns F, Kindscher K, Timmermann Barbara N. 2012. Verticillosides A-M: polyoxygenated pregnane glycosides from Asclepias verticillata L. Phytochemistry 78:179–189.
Burkill H. 1985. The Useful Plants of West Tropical Africa, Vol. 1, families A-D. 2nd ed. Kew: Royal Bot Gardens.
Carman R, Coombe R, Watson T. 1964. The cardiac glycosides of Gomphocarpus fruticosus (R.Br.). IV. The nuclear magnetic resonance spectrum of gomphoside. Aust J Chem. 17:573–577.
Chernobai V, Komissarenko N. 1971. Cardenolides of Gomphocarpus fruticosus and the partial synthesis of uzarigenin glycosides. Chem Nat Compd. 7:421–424.
Cheung A, Chiu F, Watson T, Wells R. 1983. Cardenolide glycosides of the asclepiadaceae. New glycosides from Asclepias fruticosa and the stereochemistry of uscharin, voruscharin and calotoxin. J Chem Soc Perkin Trans. 1:2827–2835.
El-Askary H, Holzl J, Hilal S, El-kashourey E. 1993. Cardenolide glycosides with doubly linked sugars from Gomphocarpus sinaicus. Phytochemistry 34:1399–1402.
Elgamal M, Hanna A, Morsy N, Duddeck H, Simon A, Gáti T, Tóth G. 1999. Complete 1H and 13C signal assignments of 5α-cardenolides isolated from Calotropis procera R. BR. J Mol Struct. 477:201–208.
Elsebai M, Mohamed I. 2015. New pregnane glycoside derivative from Caralluma retrosiciens (Ehrenb.). Nat Prod Res. 29:1426–1431.
Gohar A, El-Olemy M, Abdel-Sattar E. 2000. Cardenolides and β-sitosterol glycoside from Pergularia tomentosa. Nat Prod Sci. 6:142–146.
Goyder D, Nicholas A. 2001. A revision of Gomphocarpus R. Br. (Apocynaceae: Asclepiadeae). Kew Bull. 56:769–836.
Gupta S, Namdeo P, Upmanyu N, Garg G. 2009. Phytochemical and pharmacological activity of Calotropis Gigantea as a potential medicinal plant: an overview. Pharmacologyonline 3:757–769.
Gurib-Fakim A, Mahomoodally M. 2013. African flora as potential sources of medicinal plants: towards the chemotherapy of major parasitic and other infectious diseases-a review. Jordan J Biol Sci. 6:77–84.
Hegnauer R. 1964. Chemotaxonomie der Pflanzen [Chemotaxonomy of Plants]. Vol. 3. Switzerland: Birkhauser Verlag; p. 199–223.
Heneidak S, Grayer R, Kite G, Simmonds M. 2006. Flavonoid glycosides from Egyptian species of the tribe Asclepiadeae (Apocynaceae, subfamily Asclepiadoideae). Biochem Syst Ecol. 34:575–584.
Hussein H, Kamel A, Abou Zeid M, Abdel Khalek S, El Sebae H, Saleh M. 1994. Uscharin the most potent molluscicidal compound tested against land snails. J Chem Ecol. 20:135–140.
Kar thikeyan M, Balasubramanian T. 2014. Phytochemical analysis of Cynanchum callilatatum through GCMS and LCMS. J Homeop Ayurv Med. 3:143–148. doi:10.4172/2167-1206.1000143
Komissarenko N, Chernobai V, Komissarenko A. 1995. New cardenolides from the leaves of Gomphocarpus fruticosus. Khim Prir Soed. 6:824–832. Russian. Through CARPLUS database.
Komissarenko N, Chernobai V, Komissarenko A. 1997. Gomphacil a cardenolide glycoside of Gomphocarpus fruticosus. Chem Nat Prod. 33:55–56.
Lee C, Lin Y, Chang W, Wu Y, Chang J. 2012. The small molecule calactin induces DNA damage and apoptosis in human leukemia cells. Eur J Cancer Prev. 21:467–473.
Lichtenthaler F, Cuny E, Sakanaka Q. 2005. A concise and general method for doubly attaching 2-ketosugars to aglycone diols: synthesis of the gomphosides and spectinomycin. Angew Chem Int Ed. 44:4949–4948.

Liu C, Liao Z, Liu S, Sun J, Yao G, Wang H. 2014. A new pregnane glycoside from Rubus phoenicolasius and its antiproliferative activity. Nat Prod Res. 28:1843–1846.

Mahato S, Kundu A. 1994. 13C NMR spectra of pentacyclic triterpenoids—a compilation and some salient features. Phytochemistry 37:1517–1575.

Maldonado E, Amador S, Juárez-Jaimes V. 2015. Secondary metabolites from Asclepias otarioides. J Mex Chem Soc. 59:50–52.

Marzouk A. 2009. Hepatoprotective triterpenes from hairy root cultures of Ocimum basilicum L. Z Naturforsch. 64c: 201–209.

Melero C, Medarde M, San Feliciano A. 2000. A short review on cardiotonic steroids and their aminoguanidine analogues. Molecules 5:51–81.

Mothana R, Al-Musayeib N, Al-Ajmi M, Cos P, Maes L. 2014. Evaluation of the in vitro antiplasmodial activity of medicinal plants used in Saudi and Yemeni traditional medicine. Evid Based Complement Altern Med. 2014:1–7.

Moulisha B, Bikash M, Palit Partha P, Kumar G, Sukdeb B, Kanti H. 2009. In vitro nti-leishmanial and anti-tumour activities of a pentacyclic triterpenoid compound isolated from the fruits of Dregea volubilis Benth Asclepiadaceae. Trop J Pharm Res. 8:127–131.

Muschler R. 1912. Manual flora of Egypt. Berlin: Friedlander R Publisher; p. 83.

Pauli G, Matthiesen U, Fronczek F. 1999. Sulfates as novel steroid metabolites in higher plants. Phytochemistry 52:1075–1084.

Prachi S, Pradeep T. 2014. 13α-methyl-27-norolean-14-en-3β-ol, a triterpene isolated from the stem of Euphorbia hirta (Linn.) possess an anti-asthmatic properties. Res J Chem Sci. 4:21–26.

Prassas I, Diamandis E. 2008. Novel therapeutic applications of cardiac glycosides. Nat Rev Drug Discovery. 7:926–935.

Sanyacharernkul S, Itghiarbha A, Kongtawelert P, Meepowpan P, Nuntasaen N, Pompimon W. 2009. A new polyoxy pregnane glycoside from the roots of Dregea volubilis (L.f) Benth. ex Hook. f and its chondroprotective effect. Am J Biochem Biotechnol. 5:202–209.

Sasaki S, Aoyagi S, Hsii H. 1965. The isolation of taraxerol, taraxeryl acetate and taraxerone from Crossostephium chinence Makino (Compositae). Chem Pharm Bull. 13:87–88.

Seiber JN, Lee SM, Benson JM. 1983. Cardiac glycosides (cardenolides) in species of Asclepias (Asclepiadaceae). In: Keeler RF, Tu AT, editors. Handbook of natural toxins. Vol. 1. New York: Marcel Dekker Inc; p. 43–84.

Sethi A, Bhatia A, Maurya A, Panday A, Bhatia G, Shrivastava A, Singh R, Prakash R. 2013. Proficient synthesis of biologically active pregnane derivatives and its glycoside – experimental and theoretical approach. J Mol Struct. 1052:112–124.

Tackholm V. 1974. Student flora of Egypt. 2nd ed. Beirut: Cairo University – Cooperative Printing.

Thomas R, Gray P, Andrews J. 1990. Digitalis: its mode of action, receptor and structure activity relationships. In: Testa B, editor. Advances in Drug Research. Vol. 19. London: Academic Press Ltd; p. 311–562.

Wang Z, Wang M, Mei W, Han Z, Dai H. 2008. A new cytotoxic pregnanone from Calotropis gigantea. Molecules. 13:3033–3039.

Warashina T, Noro T. 1994a. Steroidal glycosides and cardenolide glycosides from Asclepias fruticosa. Phytochemistry. 37:217–226.

Warashina T, Noro T. 1994b. Steroidal glycosides from Asclepias fruticosa L. Chem Pharm Bull. 42:322–326.

Warashina T, Noro T. 2000. Steroidal glycosides from the aerial parts of Asclepias incarnata L. II. Chem Pharm Bull. 48:99–107.