

PHENOLICS OF *CYPERUS ALOPECUROIDES* ROTTB. INFLORESCENCES AND THEIR BIOLOGICAL ACTIVITIES

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تم في هذا البحث فصل ستة عشر مركبا فينوليا من خلاصة
الكحول الميثيلي لنورات نبات سيبرس الوبيكرويدس روتب. وقد تم
التعرف عليها كالاتي: سكوبوليتين () ايزوليكويريتيجينين -
اينثر () ليوتولين و - ثنائي ميثيل اينثر () ليوتولين و -
ثنائي ميثيل اينثر () اوريوسيدين - ميثيل اينثر () ايجينين ()
ليوتولين () حمض الفريوليك () ليوتولين -
جلوكوبيرانوزيد () ليوتولين - - جلوكوبيرانوزيد ()
كويرسيتين - - جلوكوبيرانوزيد () ايجينين -
نيوهسبيريدوزيد () كمفيرول - روتينوزيد () كويرسيتين
- روتينوزيد () كمفيرول - - زيلوبيرانوزيد -
الفا رامنوبيرانوزيد () كمفيرول - - جلوكوبيرانوزيد
- الفا رامنوبيرانوزيد () . المركبان (و) لأول مرة
من جنس السيبرس والمركبان (و) لأول مرة من العائلة
السعدية والمركبات الأخرى (ماعد و و) تفصل لأول مرة من
النبات.
وقد تم التعرف على التركيب الدقيق لهذه المركبات بدراسة
خواصها الطبيعية والكيميائية وباستخدام طرق التحليل الطيفي المختلفة
بالإضافة إلى مقارنتها بالمراجع المختلفة أو بمثيلاتها من العينات
القياسية.

وقد أظهرت بعض المركبات المفصولة تأثيرات فعالة كمضادات للأوكسدة وسامة على بعض الخلايا السرطانية ومثبطات لأنزيم الألفا أميليز.

Sixteen phenolic compounds, scopoletin (**1**), isoliquiritigenin 4'-methyl ether (**2**), luteolin 5,3'-dimethyl ether (**3**), luteolin 7,3'-dimethyl ether (**4**), aureusidin 4-methyl ether (**5**), apigenin (**6**), luteolin (**7**), trans-ferulic acid (**8**), luteolin 4'-O- β -D-glucopyranoside (**9**), luteolin 7-O- β -D-glucopyranoside (**10**), quercetin 3-O- β -D-glucopyranoside (**11**), apigenin 7-O-neohesperidoside (**12**), kaempferol 3-O-rutinoside (**13**), quercetin 3-O-rutinoside (**14**), kaempferol 3-O-[2-O-D-xylopyranosyl-6-O- α -L-rhamnopyranosyl]- β -D-glucopyranoside (**15**) and kaempferol 3-O-[2-O-D-glucopyranosyl-6-O- α -L-rhamnopyranosyl]- β -D-glucopyranoside (**16**) were isolated from the methanolic extract of the inflorescences of *Cyperus alopecuroides* Rottb. for the first time. Their structures have been established on the basis of physical, chemical and spectroscopic methods in addition to comparison with literature data and/or authentic samples. The antioxidant and cytotoxic activities in addition to α -amylase inhibitory activity of the isolated compounds have been studied.

INTRODUCTION

The sedge family, Cyperaceae comprises about 4000 species within 90 genera. *Cyperus* is the largest genus that includes about 550 species.¹

Cyperus alopecuroides Rottb. (Foxtail sedge; Samar) is a perennial stout leafy herb, reaching up to 150 cm high, having triangular culm, broad flat leaves, large numerous lanceolate inflorescences and widely distributed in tropical areas. In Egypt, it is naturally growing at the Nile Delta, the borders between fields and also cultivated in limited areas for

commercial manufacture of mat and traditional chair-making.²

Previous phytochemical studies on *Cyperus* species led to the isolation of quinones,³⁻⁵ flavonoids,⁶⁻⁹ coumarins,^{10&11} furanochromones,¹² sesquiterpenes,¹³⁻¹⁷ alkaloids,¹⁸ saponins,¹⁹ sterols,^{19&20} fatty acids²⁰ and phenolic acids.²¹

Many *Cyperus* species are used as foods. In folk medicine, they are used as spermatogenic, aphrodisiac, galactagogue, pectoral, emollient, anthelmintic, diaphoretic, astringent, digestive tonic, anti-inflammatory, antispasmodic, antirheumatic and hepatoprotective. Also, they are used

as diuretic and to promote menstruation.^{17,22-25}

The ethanolic extract of the overground parts of *Cyperus alopecuroides* Rottb. produced signs of pain and allergy on rabbit's skin.^{26&27} These results revealed that the extract contained histamine or histamine like substance.^{26&27} Also, the ethanolic and the ethereal extracts of the overground parts showed antimicrobial activity^{10&27} while the ethanolic extract of the inflorescences showed moderate estrogenic activity.²⁸

Concerning the diversity of chemical classes of *C. alopecuroides* Rottb., several previous reports were cited: sesquiterpenes and a diterpene from the essential oil of the tubers and rhizomes;^{15,29&30} quinones from the rhizomes and the inflorescences;^{3&28} coumarins from the aerial parts¹⁰ in addition to flavonoids from the leaves and inflorescences^{6&28} were isolated. Also, the carbohydrate, lipid, protein and amino acid contents from the overground parts of the plant were studied.²⁶ In the present paper, we wish to describe the isolation and characterization in addition to investigation of the biological activities of the phenolic constituents of the inflorescences of this plant.

EXPERIMENTAL

General procedures

The UV spectra were carried out in methanol (Merck) using a Perkin-Elmer Lambda 25 UV/VIS spectrophotometer. Electron impact mass spectra (EIMS) were recorded

on a Finnigan MAT TSQ 7000 mass spectrometer. Positive-ion electrospray ionization mass spectra (ESIMS) were performed on a Thermofinnigan LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped with a photodiode-array detector. Gas chromatographic (GC) analysis was carried out using an Agilent 6850 series gas chromatograph coupled to a flame ionization detector (FID) and HP-5 column (cross linked 5% PHM/Siloxan, 30 m × 250 µm diameter × 0.25 µm film thickness, Macherey und Nagel, Düren, Germany). ¹H- and ¹³C-NMR spectra were measured on Brüker DRX 500 spectrometer (Brüker, Rheinstetten, Germany). High performance liquid chromatography was performed on a HPLC system (Merck, Darmstadt, Germany) consisting of a Lachrom-Merck Hitachi L-7100 pump and an L-7400 UV detector using a C-18 column (250 × 8 mm i.d., prefilled with Eurospher 100, Knauer, Berlin, Germany), a flow rate 5.0 ml/ min, UV detection at λ 280 nm and HPLC gradient programm: 60:40 methanol/water at 0 and 5 min; 100:0 methanol/water at 38 and 45 min. Vacuum liquid chromatography (VLC) was carried out on silica gel 60 (0.04-0.063 mm, 500 g, Merck). Column chromatographic separations were performed over silica gel 60 (0.040-0.063 mm, Merck), Sephadex LH-20 (0.25-0.1 mm, Merck) and RP-18 columns (40-63 µm, Merck). TLC analyses were carried out on pre-coated silica gel F₂₅₄ aluminium

sheets and RP-18 F₂₅₄ s glass plates (Merck). Compounds were detected by UV absorption at λ 254 and 366 nm followed by spraying with anisaldehyde/H₂SO₄ reagent and heating at 110°C for 1-2 min. The solvent systems used for TLC analyses were: dichloromethane-methanol (9.5:0.5, solvent system I), dichloromethane-methanol (9:1, solvent system II), dichloromethane-methanol (8:2, solvent system III), dichloromethane-methanol (6:4, solvent system IV) and *n*-butanol-acetone-formic acid-water (60:17:8:15, solvent system V). Authentic flavonoids were obtained from the Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-University, Universitätsstraße 1, 40225 Düsseldorf, Germany. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), propyl gallate (PG) and reference sugars were purchased from Sigma Chemical Co. (Germany). The EnzCheck Amylase Assay Kit (E-11954) was purchased from Molecular Probes (GmbH, Germany). Acarbose (Ac) was purchased from Kohlpharma (GmbH, Germany).

Plant material

The inflorescences of *Cyperus alopecuroides* Rottb. were collected in July 2002 from plants growing on the margins of the ponds and banks of the Nile-River, Mankabad, Assiut, Egypt. The plant was kindly identified by Prof. Dr. Salah El-Nagar, Professor of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen

has been deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Extraction and isolation

The air-dried powdered inflorescences of *C. alopecuroides* Rottb. (2 kg) were extracted with methanol (6 L \times 4) at room temperature. The combined extract was concentrated under reduced pressure to afford a dark brownish green residue (130.0 g). The latter was suspended in distilled water (500 ml) then partitioned with *n*-hexane (500 ml \times 4), ethyl acetate (500 ml \times 4) and *n*-butanol (500 ml \times 3), successively. Each fraction was concentrated under reduced pressure to give *n*-hexane (36.5 g), ethyl acetate (31.8 g), *n*-butanol (28.5 g) and aqueous (33.0 g) residue.

About 30.8 g of the ethyl acetate fraction was subjected to VLC using *n*-hexane-ethyl acetate and ethyl acetate-methanol gradients to obtain seven group fractions: fraction E-1 (4.3 g, eluted with *n*-hexane-ethyl acetate 75:25), fraction E-2 (5.3 g, eluted with *n*-hexane-ethyl acetate 50:50), fraction E-3 (3.0 g, eluted with *n*-hexane-ethyl acetate 25:75), fraction E-4 (3.7 g, eluted with ethyl acetate), fraction E-5 (3.0 g, eluted with ethyl acetate-methanol 75:25), fraction E-6 (4.0 g, eluted with ethyl acetate-methanol 50:50) and fraction E-7 (6.0 g, eluted with methanol). About 500 mg of fraction E-2 was submitted to HPLC to yield pure compounds **1** (8 mg), **2** (3 mg), **3** (9

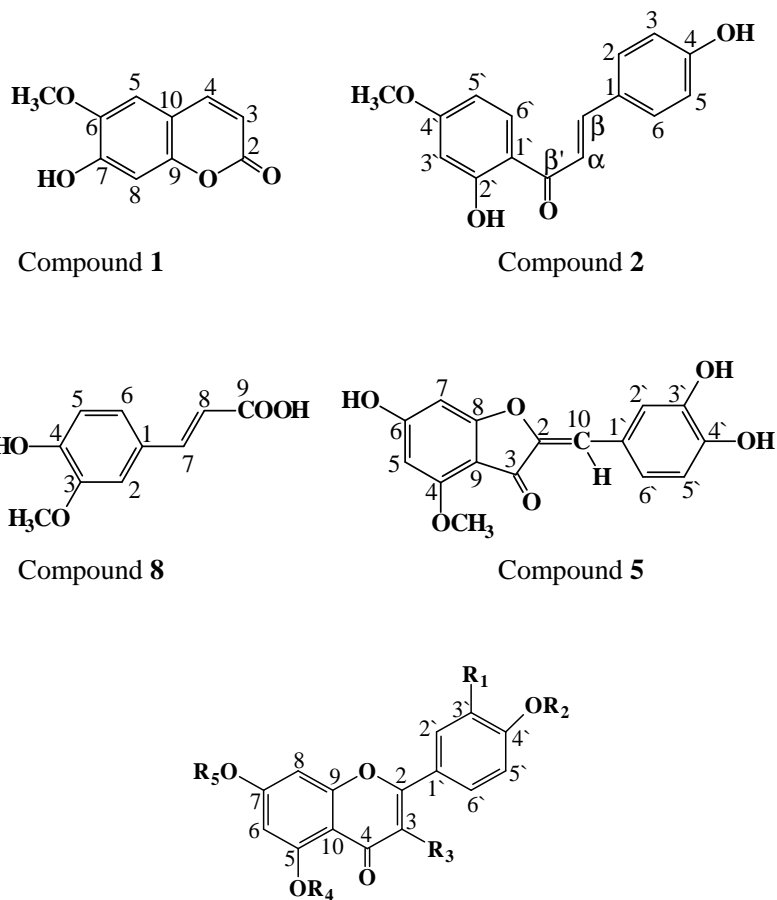
mg), **4** (5 mg) and **5** (9 mg). Fraction E-3 was subjected to silica gel column chromatography (90.0 g, 50 × 3 cm) using dichloromethane-methanol 94:6 to afford pure compounds **6** (11 mg), **7** (15 mg) and **8** (11 mg). About 2.0 g of fraction E-6 was chromatographed over Sephadex LH-20 column (100 g, 60 × 2 cm) using methanol as an eluent to obtain two main subfractions A (500 mg) and B (1.0 g). Repeated crystallization of subfraction E-6-A from methanol yielded pure compound **9** (17 mg). The subfraction E-6-B was rechromatographed over RP-18 column (100 g, 30 × 1.5 cm) using methanol-water 2:3 to afford pure compounds **10** (16 mg) and **11** (12 mg).

About 27.5 g of the *n*-butanol fraction was submitted to VLC using gradient dichloromethane-methanol to get four group fractions: fraction B-1 (4.0 g, eluted with dichloromethane-methanol 75:25), fraction B-2 (7.2 g, eluted with dichloromethane-methanol 50:50), fraction B-3 (8.8 g, eluted with dichloromethane-methanol 25:75) and fraction B-4 (6.0 g, eluted with methanol). About 2.0 g of fraction B-2 was chromatographed on Sephadex LH-20 column (100 g, 60 × 2 cm) using methanol to give two main subfractions B-2-I (700 mg) and B-2-II (1.0 g). Rechromatography of the subfraction B-2-I on RP-18 column (100 g, 30 × 1.5 cm) using methanol-water 30:70 afforded pure compounds **12** (18 mg) and **13** (12 mg). Purification of the subfraction B-2-II on silica gel column (30 g, 30

× 1.5 cm) using dichloromethane-methanol (70:30) yielded pure compound **14** (21 mg). About 2 g of fraction B-3 was purified by Sephadex LH-20 column chromatography (100 g, 60 × 2 cm) using methanol followed by rechromatography on RP-18 column (100 g, 30 × 1.5 cm) using methanol-water 30:70 to give pure compounds **15** (26 mg) and **16** (13 mg).

Compound 1: Colourless prisms (MeOH); mp 206-208°; $R_f = 0.54$ (solvent system I); UV λ_{\max} (MeOH) nm: 229, 345. $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 7.60 (1H, d, $J = 9.5$ Hz, H-4), 6.91 (1H, s, H-8), 6.84 (1H, s, H-5), 6.27 (1H, d, $J = 9.5$ Hz, H-3), 6.21 (1H, s, 7-OH) and 3.95 (3H, s, 6-OCH₃). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3): δ 161.47 (s, C-2), 150.22 (s, C-7), 149.69 (s, C-9), 144.01 (s, C-6), 143.31 (d, C-4), 113.36 (d, C-3), 111.47 (s, C-10), 107.48 (d, C-5), 103.17 (d, C-8), 56.39 (q, 6-OCH₃). EI-MS m/z (rel. int.%): 192 [M]⁺ C₁₀H₈O₄ (100), 177 (65), 164 (31), 149 (60), 129 (51), 121 (19), 111 (13), 97 (7), 83 (13), 69 (29), 57 (19), 55 (21), 44 (19).

Compound 2: Yellow powder; $R_f = 0.52$ (solvent system I); UV λ_{\max} (MeOH) nm: 240, 373. $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ 13.53 (1H, s, 2'-OH), 7.85 (1H, d, $J = 15.5$ Hz, H- β), 7.83 (1H, d, $J = 9.2$ Hz, H-6'), 7.58 (2H, d, $J = 8.8$ Hz, H-2/6), 7.46 (1H, d, $J = 15.5$ Hz, H- α), 6.96 (1H, br s, H-3'), 6.89 (2H, d, $J = 8.8$ Hz, H-3/5),



- Compound 3 $R^1 = \text{OCH}_3$, $R^4 = \text{CH}_3$, $R^2 = R^3 = R^5 = \text{H}$
 Compound 4 $R^1 = \text{OCH}_3$, $R^5 = \text{CH}_3$, $R^2 = R^3 = R^4 = \text{H}$
 Compound 6 $R^1 = R^2 = R^3 = R^4 = R^5 = \text{H}$
 Compound 7 $R^1 = \text{OH}$, $R^2 = R^3 = R^4 = R^5 = \text{H}$
 Compound 9 $R^1 = \text{OH}$, $R^3 = R^4 = R^5 = \text{H}$, $R^2 = \text{Glc}$
 Compound 10 $R^1 = \text{OH}$, $R^2 = R^3 = R^4 = \text{H}$, $R^5 = \text{Glc}$
 Compound 11 $R^1 = \text{OH}$, $R^2 = R^4 = R^5 = \text{H}$, $R^3 = O \text{ Glc}$
 Compound 12 $R^1 = R^2 = R^3 = R^4 = \text{H}$, $R^5 = \text{Glc}$ ($2'' \rightarrow 1'''$) rha
 Compound 13 $R^1 = R^2 = R^4 = R^5 = \text{H}$, $R^3 = O\text{-Glc}$ ($6'' \rightarrow 1'''$) rha
 Compound 14 $R^1 = \text{OH}$, $R^2 = R^4 = R^5 = \text{H}$, $R^3 = O\text{-Glc}$ ($6'' \rightarrow 1'''$) rha
 Compound 15 $R^1 = R^2 = R^4 = R^5 = \text{H}$, $R^3 = O\text{-}2^G\text{-xylosylrutinose}$
 Compound 16 $R^1 = R^2 = R^4 = R^5 = \text{H}$, $R^3 = O\text{-}2^G\text{-glucosylrutinose}$

Fig. 1: Structures of the isolated compounds.

6.48 (1H, dd, $J = 9.2, 1.6$ Hz, H-5'), 3.86 (3H, s, 4'-OCH₃). Positive ESI-MS m/z (rel. int.%): 271 [M+H]⁺ C₁₆H₁₄O₄ (100), 151 (15) and 147 (23).

Compound 3: Yellow powder; $R_f = 0.70$ (solvent system II); UV λ_{\max} (MeOH) nm: 243, 249 sh, 268, 340; +NaOMe: 268, 275 sh, 329 sh, 403; +AlCl₃: 243, 249 sh, 269, 340; +AlCl₃/HCl: 243, 249 sh, 269, 340; +NaOAc: 263, 321, 394; +NaOAc/H₃BO₃: 268, 347. ¹H-NMR (500 MHz, CD₃OD): δ 7.47 (1H, dd, $J = 8.5, 2.2$ Hz, H-6'), 7.45 (1H, d, $J = 2.2$ Hz, H-2'), 6.92 (1H, d, $J = 8.5$ Hz, H-5'), 6.55 (1H, s, H-3), 6.54 (1H, d, $J = 2.2$ Hz, H-8), 6.41 (1H, d, $J = 2.2$ Hz, H-6), 3.95 (3H, s, 3'-OCH₃), 3.88 (3H, s, 5-OCH₃). Positive-ion ESI-MS m/z (rel. int.%): 315 [M+H]⁺ C₁₇H₁₄O₆ (100).

Compound 4: Yellow powder; $R_f = 0.65$ (solvent system II); UV λ_{\max} (MeOH) nm: 245 sh, 252, 267, 347; +NaOMe: 262, 292 sh, 402; +AlCl₃: 267 sh, 274, 296, 369 sh, 392; +AlCl₃/HCl: 262, 276, 291 sh, 355, 390; +NaOAc: 254, 263 sh, 288, 350, 406; +NaOAc/H₃BO₃: 250, 267, 348. ¹H-NMR (500 MHz, CDCl₃): δ 12.79 (1H, s, 5-OH), 7.49 (1H, dd, $J = 7.5, 2.2$ Hz, H-6'), 7.33 (1H, d, $J = 2.2$ Hz, H-2'), 7.04 (1H, d, $J = 7.5$ Hz, H-5'), 6.57 (1H, s, H-3), 6.49 (1H, d, $J = 2.2$ Hz, H-8), 6.38 (1H, d, $J = 2.2$ Hz, H-6), 4.01 (3H, s, 3'-OCH₃), 3.89 (3H, s, 7-OCH₃). Positive-ion ESI-

MS m/z (rel. int.%): 315 [M+H]⁺ C₁₇H₁₄O₆ (100).

Compound 5: Yellow powder; $R_f = 0.59$ (solvent system II); UV λ_{\max} (MeOH) nm: 254 sh, 270 sh, 330 sh, 400; +NaOMe: 253, 273 sh, 383 sh, 402, 465; +AlCl₃: 259 sh, 287, 342, 448; +AlCl₃/HCl: 255 sh, 270 sh, 329 sh, 404; +NaOAc: 268, 316 sh, 384 sh, 420; +NaOAc/H₃BO₃: 264, 280, 346 sh, 435. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.38 (1H, d, $J = 1.9$ Hz, H-2'), 7.16 (1H, dd, $J = 8.2, 1.9$ Hz, H-6'), 6.80 (1H, d, $J = 8.2$ Hz, H-5'), 6.46 (1H, s, H-10), 6.29 (1H, d, $J = 1.6$ Hz, H-7), 6.14 (1H, d, $J = 1.6$ Hz, H-5), 3.82 (3H, s, 4-OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 178.40 (s, C-3), 168.40 (s, C-6), 167.76 (s, C-8), 159.28 (s, C-4), 147.57 (s, C-4'), 145.76 (s, C-2), 145.46 (s, C-3'), 124.02 (d, C-6'), 123.53 (s, C-1'), 117.57 (d, C-2'), 115.95 (d, C-5'), 110.01 (d, C-10), 102.86 (s, C-9), 94.66 (d, C-5), 91.30 (d, C-7), 55.76 (q, 4-OCH₃). Positive-ion ESI-MS m/z (rel. int.%): 301 [M+H]⁺ C₁₆H₁₂O₆ (100).

Compound 6: Yellow powder; $R_f = 0.58$ (solvent system II); UV λ_{\max} (MeOH) nm: 268, 337; +NaOMe: 275, 392; +AlCl₃: 276, 300, 345, 384; +AlCl₃/HCl: 276, 298, 341, 380; +NaOAc: 275, 300, 375; +NaOAc/H₃BO₃: 268, 338. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.95 (1H, s, 5-OH), 7.91 (2H, d, $J = 8.8$ Hz, H-2'/6'), 6.91 (2H, d, $J = 8.8$ Hz, H-3'/5'), 6.76 (1H, s, H-3), 6.46 (1H, d,

$J = 1.9$ Hz, H-8), 6.18 (1H, d, $J = 1.9$ Hz, H-6). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6): δ 181.69 (s, C-4), 164.39 (s, C-2), 163.68 (s, C-7), 161.42 (s, C-5), 161.19 (s, C-9), 157.30 (s, C-4'), 128.45 (d, C-2'/6'), 121.12 (s, C-1'), 115.94 (d, C-3'/5'), 103.58 (s, C-10), 102.79 (d, C-3), 98.87 (d, C-6), 93.98 (d, C-8). Positive-ion ESI-MS m/z (rel. int.%): 271 $[\text{M}+\text{H}]^+$ $\text{C}_{15}\text{H}_{10}\text{O}_5$ (100).

Compound 7: Yellow powder; $R_f = 0.51$ (solvent system II); UV λ_{max} (MeOH) nm: 242 sh, 255, 267, 290 sh, 350; +NaOMe: 267, 329, 402; + AlCl_3 : 274, 300 sh, 328, 427; + AlCl_3/HCl : 266 sh, 276, 294 sh, 355, 385; +NaOAc: 270, 326 sh, 384; +NaOAc/ H_3BO_3 : 260, 300 sh, 370, 430 sh. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ 12.97 (1H, s, 5-OH), 7.40 (1H, dd, $J = 8.2, 2.2$ Hz, H-6'), 7.39 (1H, d, $J = 2.2$ Hz, H-2'), 6.88 (1H, d, $J = 8.2$ Hz, H-5'), 6.66 (1H, s, H-3), 6.43 (1H, d, $J = 2.2$ Hz, H-8), 6.18 (1H, d, $J = 2.2$ Hz, H-6). Positive-ion ESI-MS m/z (rel. int.%): 287 $[\text{M}+\text{H}]^+$ $\text{C}_{15}\text{H}_{10}\text{O}_6$ (100).

Compound 8: White needles (MeOH); mp 169-171°; $R_f = 0.21$ (solvent system I); UV λ_{max} (MeOH) nm: 244, 296, 323. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ 12.19 (1H, s, 9-COOH), 9.17 (1H, s, 4-OH), 7.43 (1H, d, $J = 16.0$ Hz, H-7), 7.08 (1H, dd, $J = 8.2, 1.9$ Hz, H-6), 7.06 (1H, d, $J = 1.9$ Hz, H-2), 6.93 (1H, d, $J = 8.2$ Hz, H-5), 6.23 (1H, d, $J = 16.0$ Hz, H-8), 3.79 (3H, s, 3-OCH $_3$). EI-MS

m/z (rel. int.%): 194 $[\text{M}]^+$ $\text{C}_{10}\text{H}_{10}\text{O}_4$ (100), 179 (42), 161 (7), 151 (10), 149 (9), 133 (31), 123 (7), 117 (5), 105 (7), 95 (5), 89 (6), 77 (10), 67 (3), 51 (4), 43 (3), 40 (3).

Compound 9: Yellow powder; $R_f = 0.60$ (solvent system III); UV λ_{max} (MeOH) nm: 250 sh, 268, 291 sh, 340; +NaOMe: 270, 303 sh, 361; + AlCl_3 : 267 sh, 273, 296, 362, 390; + AlCl_3/HCl : 264 sh, 276, 295, 351, 382; +NaOAc: 276, 323, 366; +NaOAc/ H_3BO_3 : 253 sh, 268, 347. $^1\text{H-NMR}$ (500 MHz, DMSO- d_6): δ 12.89 (1H, s, 5-OH), 7.50 (1H, dd, $J = 8.5, 2.2$ Hz, H-6'), 7.49 (1H, br s, H-2'), 7.23 (1H, d, $J = 8.5$ Hz, H-5'), 6.80 (1H, s, H-3), 6.49 (1H, d, $J = 1.9$ Hz, H-8), 6.19 (1H, d, $J = 1.9$ Hz, H-6), 4.89 (1H, d, $J = 7.3$ Hz, H-1''), 3.73-3.19 (m, sugar protons). $^{13}\text{C-NMR}$ (125 MHz, DMSO- d_6): δ 181.79 (s, C-4), 164.45 (s, C-2), 163.20 (s, C-7), 161.47 (s, C-5), 157.39 (s, C-9), 148.58 (s, C-4'), 146.98 (s, C-3'), 124.76 (s, C-1'), 118.54 (d, C-6'), 116.03 (d, C-5'), 113.64 (d, C-2'), 104.00 (d, C-3), 103.80 (s, C-10), 101.24 (d, C-1''), 99.00 (d, C-6), 94.09 (d, C-8), 77.33 (d, C-5''), 75.89 (d, C-3''), 73.27 (d, C-2''), 69.80 (d, C-4''), 60.74 (t, C-6''). Positive-ion ESI-MS m/z (rel. int.%): 449 $[\text{M}+\text{H}]^+$ $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ (100), 287 $[\text{M-Glc}+\text{H}]^+$ (23).

Compound 10: Yellow powder; $R_f = 0.57$ (solvent system III); UV λ_{max} (MeOH) nm: 255, 268, 291 sh, 349; +NaOMe: 263, 300 sh, 395; + AlCl_3 :

274, 298 sh, 329, 433; +AlCl₃/HCl: 273, 294 sh, 358, 388; +NaOAc: 259, 266, 367 sh, 404; +NaOAc/H₃BO₃: 259, 373. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.98 (1H, s, 5-OH), 7.44 (1H, dd, *J* = 8.2, 2.2 Hz, H-6'), 7.41 (1H, d, *J* = 2.2 Hz, H-2'), 6.89 (1H, d, *J* = 8.2 Hz, H-5'), 6.78 (1H, d, *J* = 2.2 Hz, H-8), 6.74 (1H, s, H-3), 6.43 (1H, d, *J* = 2.2 Hz, H-6), 5.08 (1H, d, *J* = 7.6 Hz, H-1''), 3.71-3.15 (m, sugar protons). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 181.90 (s, C-4), 164.47 (s, C-2), 162.94 (s, C-7), 161.13 (s, C-5), 156.94 (s, C-9), 149.92 (s, C-4'), 145.78 (s, C-3'), 121.38 (s, C-1'), 119.18 (d, C-6'), 116.00 (d, C-5'), 113.56 (d, C-2'), 105.34 (s, C-10), 103.17 (d, C-3), 99.86 (d, C-1''), 99.53 (d, C-6), 94.71 (d, C-8), 77.16 (d, C-5''), 76.39 (d, C-3''), 73.12 (d, C-2''), 69.53 (d, C-4''), 60.60 (t, C-6''). Positive-ion ESI-MS *m/z* (rel. int.%): 449 [M+H]⁺ C₂₁H₂₀O₁₁ (100), 287 [M-Glc+H]⁺ (35).

Compound 11: Yellow powder; R_f = 0.55 (solvent system III); UV λ_{max} (MeOH) nm: 257, 266 sh, 296 sh, 357; +NaOMe: 271, 327, 400; +AlCl₃: 275, 304 sh, 332, 437; +AlCl₃/HCl: 271, 303 sh, 353, 402; +NaOAc: 273, 325 sh, 379; +NaOAc/H₃BO₃: 261, 298 sh, 374. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.63 (1H, s, 5-OH), 7.57 (1H, dd, *J* = 8.8, 2.2 Hz, H-6'), 7.56 (1H, br s, H-2'), 6.83 (1H, d, *J* = 8.8 Hz, H-5'), 6.40 (1H, d, br s, H-8), 6.19 (1H, d, br s, H-6), 5.46 (1H, d, *J* = 7.3 Hz, H-

1''), 3.58-3.08 (m, sugar protons). Positive-ion ESI-MS *m/z* (rel. int.%): 465 [M+H]⁺ C₂₁H₂₀O₁₂ (100), 303 [M-Glc+H]⁺ (62).

Compound 12: Yellowish white powder; R_f = 0.32 (solvent system III); UV λ_{max} (MeOH) nm: 267, 339; +NaOMe: 245 sh, 266, 392; +AlCl₃: 274, 300, 348, 388; +AlCl₃/HCl: 275, 299, 340, 384; +NaOAc: 268, 354, 393; +NaOAc/H₃BO₃: 268, 347. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.96 (1H, s, 5-OH), 7.92 (2H, d, *J* = 8.8 Hz, H-2'/6'), 6.93 (2H, d, *J* = 8.8 Hz, H-3'/5'), 6.85 (1H, s, H-3), 6.78 (1H, d, *J* = 1.9 Hz, H-8), 6.36 (1H, d, *J* = 1.9 Hz, H-6), 5.23 (1H, d, *J* = 7.3 Hz, H-1''), 5.12 (1H, s, H-1'''), 5.37-3.21 (m, sugar protons), 1.20 (3H, d, *J* = 6.0 Hz H-6'''). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 182.05 (C-4), 164.34 (C-2), 162.59 (C-7), 161.43 (C-5), 161.17 (C-4'), 157.04 (C-9), 128.65 (C-2'/6'), 121.05 (C-1'), 116.10 (C-3'/5'), 105.49 (C-10), 103.24 (C-3), 100.54 (C-1'''), 99.38 (C-6), 97.85 (C-1''), 94.57 (C-8), 77.28 (C-5''), 77.07 (C-2''), 76.35 (C-3''), 71.93 (C-4'''), 70.55 (C-3'''), 70.47 (C-2'''), 69.70 (C-4''), 68.43 (C-5'''), 60.54 (C-6''), 18.15 (C-6'''). Positive-ion ESI-MS *m/z* (rel. int.%): 579 [M+H]⁺ C₂₇H₃₀O₁₄ (100), 271 [M-Rha-Glc+H]⁺ (20).

Compound 13: Yellow powder; R_f = 0.28 (solvent system III); UV λ_{max} (MeOH) nm: 258 sh, 266, 298, 347; +NaOMe: 275, 325, 401; +AlCl₃: 257

sh, 275, 304, 354, 400; +AlCl₃/HCl: 257 sh, 276, 303, 349, 398; +NaOAc: 274, 316, 390; +NaOAc/H₃BO₃: 266, 298 sh, 352. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.52 (1H, s, 5-OH), 7.96 (2H, d, *J* = 8.8 Hz, H-2'/6'), 6.86 (2H, d, *J* = 8.8 Hz, H-3'/5'), 6.34 (1H, d, *J* = 1.5 Hz, H-8), 6.14 (1H, d, *J* = 1.5 Hz, H-6), 5.28 (1H, d, *J* = 7.6 Hz, H-1''), 4.37 (1H, s, H-1'''), 3.88-3.05 (m, sugar protons), 1.20 (3H, d, *J* = 6.0 Hz, H-6'''). Positive-ion ESI-MS *m/z* (rel. int.%): 595 [M+H]⁺ C₂₇H₃₀O₁₅ (100), 449 [M-Rha+H]⁺ (43), 287 [M-Rha-Glc+H]⁺ (45).

Compound 14: Yellow powder; R_f = 0.21 (solvent system III); UV λ_{max} (MeOH) nm: 256, 266 sh, 299 sh, 357; +NaOMe: 272, 327, 400; +AlCl₃: 275, 304 sh, 335, 433; +AlCl₃/HCl: 273, 300 sh, 355, 404; +NaOAc: 272, 325 sh, 380; +NaOAc/H₃BO₃: 260, 300 sh, 375. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.59 (1H, s, 5-OH), 7.53 (1H, dd, *J* = 8.5, 2.2 Hz, H-6'), 7.52 (1H, br s H-2'), 6.83 (1H, d, *J* = 8.5 Hz, H-5'), 6.38 (1H, d, *J* = 2.2 Hz, H-8), 6.19 (1H, d, *J* = 2.2 Hz, H-6), 5.34 (1H, d, *J* = 7.6 Hz, H-1''), 4.37 (1H, d, *J* = 0.6 Hz, H-1'''), 0.98 (3H, d, *J* = 6.3 Hz, H-6'''), 5.33-3.05 (m, sugar protons). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 177.44 (s, C-4), 164.14 (s, C-7), 161.28 (s, C-5), 156.69 (s, C-9), 156.49 (s, C-2), 148.47 (s, C-4'), 144.81 (s, C-3'), 133.36 (s, C-3), 121.66 (d, C-6'), 121.25 (s, C-1'), 116.34 (d, C-5'), 115.30 (d, C-2'),

104.04 (s, C-10), 101.24 (d, C-1''), 100.81 (d, C-1'''), 98.75 (d, C-6), 93.66 (d, C-8), 76.50 (d, C-5''), 75.96 (d, C-3''), 74.13 (d, C-2''), 71.90 (d, C-4'''), 70.61 (d, C-3'''), 70.44 (d, C-2'''), 70.06 (d, C-4''), 68.31 (d, C-5'''), 67.06 (t, C-6''), 17.80 (q, C-6'''). Positive-ion ESI-MS *m/z* (rel. int.%): 611 [M+H]⁺ C₂₇H₃₀O₁₆ (100), 465 [M-Rha+H]⁺ (25), 303 [M-Rha-Glc+H]⁺ (35).

Compound 15: Yellowish brown powder; R_f = 0.84 (solvent system IV); UV λ_{max} (MeOH) nm: 258 sh, 266, 295, 345; +NaOMe: 275, 325, 398; +AlCl₃: 257 sh, 274, 304, 350, 395; +AlCl₃/HCl: 256 sh, 275, 303, 349, 394; +NaOAc: 275, 315, 390; +NaOAc/H₃BO₃: 265, 298 sh, 348. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 12.62 (1H, s, 5-OH), 8.01 (2H, d, *J* = 8.8 Hz, H-2'/6'), 6.88 (2H, d, *J* = 8.8 Hz, H-3'/5'), 6.36 (1H, br s, H-8), 6.16 (1H, br s, H-6), 5.57 (1H, d, *J* = 7.3 Hz, 3-Glc H-1), 4.59 (1H, d, *J* = 7.3 Hz, 2''-Xyl H-1), 4.34 (1H, s, Rha H-1), 3.74-3.03 (m, sugar protons), 0.94 (3H, d, *J* = 6.0 Hz, H-6'''). ¹³C-NMR (125 MHz, DMSO-*d*₆): see Table 1. Positive-ion ESI-MS *m/z* (rel. int.%): 727 [M+H]⁺ C₃₂H₃₈O₁₉ (100), 595 [M-Xyl+H]⁺ (30), 449 [M-Xyl-Rha+H]⁺ (28), 287 [M-Xyl-Rha-Glc+H]⁺ (55).

Compound 16: Yellowish brown powder; R_f = 0.82 (solvent system IV); UV λ_{max} (MeOH) nm: 266, 296, 346; +NaOMe: 274, 325, 399; +AlCl₃: 257 sh, 275, 302, 354, 398;

Table 1: ^{13}C -NMR data for compounds **15** and **16** (125 MHz in $\text{DMSO-}d_6$).

Carbon	Compd. 15	Compd. 16
Aglycone		
2	155.93	155.50
3	132.87	132.40
4	177.41	176.65
5	161.34	161.06
6	99.04	100.42
7	165.01	165.17
8	93.94	94.25
9	156.62	156.76
10	103.72	103.74
1'	121.06	121.04
2', 6'	131.09	130.80
4'	160.04	159.84
3', 5'	115.33	115.18
Sugars		
3-Glc		
1''	98.33	98.33
2''	81.66	81.93
3''	76.83	76.51 ^a
4''	69.65	69.51
5''	75.84	75.47 ^a
6''	66.23	66.11
2''-Glucose		
1		103.74
2		74.17
3		76.33 ^b
4		70.53
5		77.03 ^b
6		60.80
2''-Xylose		
1	104.49	
2	73.88	
3	76.16	
4	69.54	
5	65.76	
6''-Rhamnose		
1	100.54	100.42
2	70.44 ^a	70.26 ^c
3	70.71 ^a	69.66 ^c
4	71.96	71.80
5	68.34	68.18
6	17.78	17.66

^{a-c} Interchangeable assignments within the spectrum

+ AlCl_3/HCl : 256 sh, 274, 300, 349, 396; + NaOAc : 275, 316, 390; + $\text{NaOAc}/\text{H}_3\text{BO}_3$: 266, 298 sh, 347. $^1\text{H-NMR}$ (500 MHz, $\text{DMSO-}d_6$): δ 12.56 (1H, s, 5-OH), 7.94 (2H, d, $J = 8.8$ Hz, H-2'/6'), 6.87 (2H, d, $J = 8.8$ Hz, H-3'/5'), 6.19 (1H, br s, H-8), 6.00 (1H, br s, H-6), 5.50 (1H, d, $J = 7.0$ Hz, 3-Glc H-1), 4.58 (1H, d, $J = 7.6$ Hz, 2''-Glc H-1), 4.29 (1H, br s, Rha H-1), 5.42-3.04 (m, sugar protons), 0.95 (3H, d, $J = 6.3$ Hz, H-6'''). $^{13}\text{C-NMR}$ (125 MHz, $\text{DMSO-}d_6$): see Table 1. Positive-ion ESI-MS m/z (rel. int.%): 757 $[\text{M}+\text{H}]^+$ $\text{C}_{33}\text{H}_{40}\text{O}_{20}$ (100), 595 $[\text{M-Glc}+\text{H}]^+$ (15), 449 $[\text{M-Glc-Rha}+\text{H}]^+$ (20), 287 $[\text{M-Glc-Rha-Glc}+\text{H}]^+$ (40).

Acid hydrolysis³¹

A solution of the isolated glycoside (5 mg in 10 ml methanol) was treated with 3% H_2SO_4 (1.5 ml) and heated at 100 °C for 1 hr. The aglycone was extracted with EtOAc, concentrated under reduced pressure, purified on Sephadex LH-20 column using methanol and identified by co-TLC with an authentic sample using solvent system II. The sugars in the aqueous layer were identified by co-TLC with authentic materials using solvent system V.

Acid hydrolysis and GC analysis³²

A solution of compound **15** (5 mg in 25 ml methanol) was treated with 3N HCl (15 ml) and stirred at 80° for about 5 h. Upon drying with a flow of nitrogen, the residue was dissolved in (-)-2-butanol (0.5 ml) and one drop

of trifluoroacetic acid. The solution was transferred to an ampoule which was sealed and heated at 130° in an oven overnight until complete butanolysis. After being taken to dryness, the resulting residue was reacted with hexamethyldisilazane / chlorotrimethylsilane / pyridine (1:1:5, 0.1 ml) for 30 min at room temperature. The solution was then centrifuged and the supernatant (1 µl) was analysed by an Agilent 6850 gas chromatograph. A temperature gradient from 135° to 200° at 1°/min was applied. The injection volume was 1 µl. The carrier gas used was nitrogen with a split ratio: 50:1 and 1 ml/min constant flow rate. The injection port and detector temperatures were set at 200° and 220°, respectively. For the hydrolysate, four peaks were detected at 42.9, 48.6, 27.0 and 31.7 min. Authentic standards prepared in a similar manner from commercially available L-glucose, D-glucose and D-xylose, gave peaks at: 42.9 and 48.6 min for D-glucose; 41.7 and 48.4 min for L-glucose; 27.0 and 31.7 min for D-xylose.

Antioxidant activity³³

The antioxidant activity was quantified by the decrease in the absorption of each of the isolated compounds or soluble fractions in 118×10⁻⁵ % DPPH solution (final concentration of the sample in the cuvette was 20 µM for pure compounds and 0.5 and 1.0 mg/ml for soluble fractions) monitored at 517

nm using a spectrophotometer. The absorbance of DPPH in methanol (with or without compounds) was measured after 2 min. The antioxidant activity of each compound was measured in relation to propyl gallate (a known synthetic antioxidant) set as 100% antioxidant activity. Determinations were performed in triplicate. The antioxidant activity was calculated using the following equation:³⁴

Antioxidant activity = 100 x

$$\left(1 - \frac{\text{absorbance with compound}}{\text{absorbance of the blank}}\right)$$

Cytotoxic activity³⁵

L5178Y mouse lymphoma cells were grown in Eagle's minimal essential medium supplemented with 10% horse serum in roller tube culture. For the dose-response experiments, 5 ml cultures were initiated by inoculation of 5×10³ cells/ml and incubated at 37° for 72 h. Controls showed a population doubling time of 10.5 h. Cell growth was determined by a cell count with a Cytocomp counter (128-channel counter, system Michaelis, Mainz, Germany) incorporating a 32-channel size-distribution plotter.

α-amylase inhibitory activity³⁶

The method is based on assay of α-amylase by EnzCheck® Amylase Assay Kit (E-11954). The provided stock solution of DQ starch and α-amylase enzyme were diluted with the reaction buffer (pH 6.9) according to

the reported protocol.³⁶ To the microplate wells, 50 µg/10 µl of the tested compound in DMSO, 50 µl of the diluted enzyme and 40 µl of the reaction buffer were added and allowed to stand for 5 min at room temperature then 100 µl of DQ starch was added. The fluorescence intensity of the digestion products from the DQ starch (with or without compounds) was measured using a kinetic assay program in the Tecan Genios microplate reader at λ_{max} 485±10 nm starting from zero min to 60 min at 10 min intervals. All determinations were performed in triplicate. The α -amylase inhibitory activity of each test compound was measured in relation to acarbose (Ac) set as 100% α -amylase inhibitory activity. The percentage of α -amylase activity and α -amylase inhibition were calculated using the following equations:

$$\% \alpha\text{-amylase activity} = 100 \times \frac{(F_s^{60} - F_s^0)}{(F_c^{60} - F_c^0)}$$

where F_s^{60} : Fluorescence with the sample at 60 min, F_s^0 : Fluorescence with the sample at 0 min, F_c^{60} : Fluorescence of the control at 60 min, F_c^0 : Fluorescence of the control at 0 min.

$$\% \alpha\text{-amylase inhibition} = 100 - \% \alpha\text{-amylase activity}$$

RESULTS AND DISCUSSION

The methanolic extract of *C. alopecuroides* Rottb. inflorescences

was successively partitioned between water and *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate and *n*-butanol soluble fractions through series of different chromatographic fractionation techniques, afforded sixteen phenolic compounds **1-16**.

Compound **1** was assigned a molecular formula of $C_{10}H_8O_4$ by EIMS analysis that appeared $[M]^+$ at m/z 192 (100%) in addition to mass fragment peaks at m/z 177 (65%), 164 (30%) and 149 (60%). The $^1\text{H-NMR}$ spectrum revealed signals of two olefinic protons with *cis*-coupling at δ 6.27 and 7.60 (each 1H, d, $J=9.5$ Hz) indicative of the H-3 and H-4 of the coumarin nucleus;³⁷ two aromatic protons (*para* to each others) at δ 6.84 and 6.91 (each 1H, s, H-5, H-8) and a singlet for one methoxyl group at δ 3.95 (3H). $^{13}\text{C-NMR}$ spectrum exhibited signals for 10 carbon atoms including a signal for δ -lactone function at δ 161.47. These spectral data showed good agreement with those reported for scopoletin.^{37&38} Thus, compound **1** was established as scopoletin.

Compound **2** possessed the molecular formula $C_{16}H_{14}O_4$ by ESIMS that showed $[M+H]^+$ at m/z 271, together with mass fragment peaks at m/z 151 (15%) and 147 (23%). The UV spectrum exhibited two absorption bands at λ_{max} 240 and 373 nm for chalcones.³⁹ This was supported by the $^1\text{H-NMR}$ spectrum which showed signals for two *trans*-olefinic protons at δ 7.46 and 7.85 (each 1H, d, $J=15.5$ Hz, H- α and β);

two sets of *ortho*-coupled aromatic protons of B-ring at δ 6.89 (2H, d, $J=8.8$ Hz, H-3/5) and 7.58 (2H, d, $J=8.8$ Hz, H-2/6); three aromatic protons of A-ring at δ 6.48 (1H, dd, $J=9.2, 1.6$ Hz, H-5'), 6.96 (1H, br s, H-3') and 7.83 (1H, d, $J=9.2$ Hz, H-6') in addition to a singlet at δ 3.86 (3H) for one methoxyl group.⁴⁰ The dominant mass fragment peaks at m/z 147 and 151 indicated the presence of one hydroxyl group in the B-ring in addition to one hydroxyl and one methoxyl group in the A-ring.⁴¹ The placement of the methoxyl group at position 4' was supported by the HMBC spectrum that displayed a correlation between the methoxyl protons (δ 3.86) and C-4' (δ 166.4).⁴¹ Therefore, compound **2** was concluded to have the structure 4,2'-dihydroxy-4'-methoxychalcone (isoliquiritigenin 4'-methyl ether).

ESIMS of compound **5** showed $[M+H]^+$ at m/z 301 for $C_{16}H_{12}O_6$. It had a UV spectrum typical of an aurone having *ortho*-dihydroxyl groups on the B-ring and lacking a free hydroxyl group at C-4 since the complex formed with $AlCl_3$ was acid labile.³⁹ The 1H -NMR spectrum exhibited the pattern of aurones of the aureusidin type⁴⁰ by the appearance of one singlet at δ 6.46 (1H, s, H-10); three protons with the shifts and splitting of a catechol B-ring at δ 6.80 (1H, d, $J=8.2$ Hz, H-5'), 7.16 (1H, dd, $J=8.2, 1.9$ Hz, H-6') and 7.38 (1H, d, $J=1.9$ Hz, H-2'); two doublets at δ 6.14 and 6.29 (each 1H, $J=1.6$ Hz) characteristic for *meta*-protons on

A-ring and a singlet at δ 3.82 (3H) for a methoxyl group. The ^{13}C -NMR spectrum revealed the presence of 16 carbon atoms were attributed to aureusidin derivative.⁴² The methoxyl group was located at the 4-position according to long-range HMBC correlation between the methoxyl protons at δ 3.82 and C-4 at δ 159.28. On the basis of these data, compound **5** was shown to be aureusidin 4-methyl ether (rengasin).

The EI mass spectrum of compound **8** showed $[M]^+$ at m/z 194 (100%) corresponding to $C_{10}H_{10}O_4$, in addition to mass fragment peaks at m/z 179 (42%), 151 (10%), 149 (9%), 133 (31%) and 77 (10%). The 1H -NMR spectrum showed signals of *trans*-3,4-disubstituted cinnamic acid: two olefinic protons with *trans*-coupling at δ 6.23 and 7.43 (each 1H, d, $J=16.0$ Hz); three aromatic protons at δ 6.93 (1H, d, $J=8.2$ Hz), 7.06 (1H, d, $J=1.9$ Hz) and 7.08 (1H, dd, $J=8.2, 1.9$ Hz) in addition to a signal of one methoxyl group at δ 3.79 (3H, s). By comparison of these data with those published,^{43&44} compound **8** was characterized as *trans*-4-hydroxy-3-methoxycinnamic acid (*trans*-ferulic acid).

Compounds **3**, **4**, **6**, **7**, **9**, **10** and **12** displayed characteristic UV absorption maxima for flavone skeleton.³⁹ The 1H -NMR spectral data for these compounds also confirmed the presence of flavone nucleus in these molecules.⁴⁰

The ESI mass spectrum of compound **3** showed $[M+H]^+$ at m/z

315 was consistent with the molecular formula $C_{17}H_{14}O_6$. It had UV spectrum of a flavone having free hydroxyl groups at C-4' and C-7 and lacking a free hydroxyl group at C-5.³⁹ The 1H -NMR spectrum exhibited the pattern of luteolin derivative⁴⁰ by the appearance of a singlet aromatic proton at δ 6.55 (1H, H-3); two *meta*-coupled protons in the A-ring at δ 6.41, 6.54 (each 1H, d, $J=2.2$ Hz, H-6 and H-8); three aromatic protons in the 3',4'-disubstituted B-ring at δ 6.92 (1H, d, $J=8.2$ Hz, H-5'), 7.45 (1H, d, $J=2.2$ Hz, H-2') and 7.47 (1H, dd, $J=8.2, 2.2$ Hz, H-6') in addition to two singlets of two methoxyl groups at δ 3.88 and 3.95 (each 3H). Considering the UV results, the two methoxyl groups could be placed at C-5 and C-3'. These placements are supported by the HMBC spectrum, the protons of the methoxyl groups at δ 3.88 and 3.95 showed correlations with the carbons at position 5 (δ 161.5) and 3' (δ 148.6), respectively. By comparison of these spectral data with those in literature,⁴⁵ compound **3** was deduced as luteolin 5,3'-dimethyl ether.

Compound **4** deduced the molecular formula $C_{17}H_{14}O_6$ by ESIMS analysis ($[M+H]^+$ at m/z 315). The UV spectral data suggested its structure to be a flavone having free hydroxyl groups at the 5- and 4'-positions and lacking a free hydroxyl group at the 7-position.³⁹ Its 1H -NMR spectral data were similar to those of compound **3**. According to the UV spectral data, the methoxyl groups

could be placed at positions 7 and 3'. On the basis of these spectral data and by comparison with the reported data,⁴⁶ compound **4** was concluded to be luteolin 7,3'-dimethyl ether (velutin).⁴⁶

The ESI mass spectra of compounds **6** and **7** showed $[M+H]^+$ at m/z 271 and 287 corresponding to the molecular formulae $C_{15}H_{10}O_5$ and $C_{15}H_{10}O_6$, respectively. They were characterized as apigenin and luteolin by comparison of their spectral data with published data.^{39,40,42&47}

ESIMS of compound **9** showed $[M+H]^+$ at m/z 449 corresponding to the molecular formula $C_{21}H_{20}O_{11}$ in addition to a prominent fragment peak at m/z 287 $[M-162$ (hexose unit) $+H]^+$ indicating its monoside nature. The UV spectral data suggested its structure to be 5,7-dihydroxyflavone derivative.³⁹ In the 1H - and ^{13}C -NMR spectra, there were signals for luteolin nucleus^{40&47} and a β -glucose moiety.⁴⁸ In the HMBC spectrum the correlation from the glucose anomeric proton (H-1'', δ 4.89) to C-4' (δ 148.58) confirmed the *O*-glycosidation at C-4'. Acid hydrolysis³¹ of compound **9** yielded luteolin and glucose (co-TLC with authentic samples). On the basis of these results and by comparing the spectral data with literature,⁴⁹ compound **9** could be identified as luteolin 4'-*O*- β -D-glucopyranoside.

Compound **10** had the same ESI mass spectrum of compound **9**. Its UV spectra showed absorption pattern of 5,3',4'-trihydroxy flavone

derivative.³⁹ The ¹H- and ¹³C-NMR spectra were similar to those of compound **9**. The HMBC spectrum showed correlation between the anomeric proton signal (δ 5.08, H-1'') to C-7 (δ 162.94) confirmed the *O*-glycosidation at C-7. Acid hydrolysis³¹ of compound **10** gave luteolin and glucose (co-TLC with authentic samples). From these results and comparison with literature data,^{39,40&47} the structure of compound **10** was assigned as luteolin 7-*O*- β -D-glucopyranoside.

ESIMS of compound **12** showed [M+H]⁺ at *m/z* 579 for C₂₇H₃₀O₁₄ in addition to a significant fragment peak at *m/z* 271 [M-146 (methyl pentose unit)-162 (hexose unit)+H]⁺ indicating its bioside nature. UV spectral data of **12** suggested its structure to be 5,4'-dihydroxyflavone derivative.³⁹ The ¹H- and ¹³C-NMR spectra displayed signals owing to apigenin,^{40&47} one β -D-glucopyranosyl and one α -L-rhamnopyranosyl⁴⁸ units. The significant deshielding of the ¹³C signal associated with C-2'' of the glucose unit indicated further glucosylation at C-2''. In the HMBC spectrum, the 1''' \rightarrow 2'' link between the two sugars was confirmed by a ³J correlation from the anomeric proton (δ 5.12) of the rhamnose unit to 2'' (δ 77.07) of the glucose unit. Also, the *O*-diglycosidic linkage at the 7-position was supported from the correlation between the anomeric proton (δ 5.23) of the glucose unit and C-7 (δ 162.59). Acid hydrolysis³¹ of compound **12** liberated apigenin,

glucose and rhamnose (co-TLC with authentic samples). From these results and by comparison with the reported data,^{39,40&47} compound **12** was identified as apigenin 7-*O*-neohesperidoside.

The UV spectral data of compounds **11** and **14** suggested their structures to be flavonols with a substituted 3-hydroxyl group and free hydroxyl groups at positions 5, 7, 3' and 4'.³⁹ By comparison of their ¹H-NMR and ¹³C-NMR spectral data with those reported in literature,^{40&47} compounds **11** and **14** were identified as quercetin 3-*O*- β -D-glucopyranoside and quercetin 3-*O*-rutinoside (rutin), respectively.

The UV spectra of compounds **13**, **15** and **16** exhibited characteristic absorption bands of flavonols with a substituted 3-hydroxyl group and free hydroxyl groups at the 5-, 7- and 4'-positions.³⁹ The ¹H-NMR spectral data for these compounds supported their structures as kaempferol derivatives.⁴⁰

Compound **13** showed [M+H]⁺ at *m/z* 595 in its ESI mass spectrum of corresponding to the molecular formula C₂₇H₃₀O₁₅. Also, the spectrum revealed two prominent fragment peaks at *m/z* 449 [M-146 (methyl pentose unit)+H]⁺ and 287 [M-146-162 (hexose unit)+H]⁺ indicating compound **13** as kaempferol 3-*O*-bioside. Its ¹H-NMR spectrum displayed signals due to kaempferol skeleton⁴⁰ together with two anomeric protons [δ 5.28 (1H, d, *J* = 7.6 Hz, Glc H-1) and 4.37 (1H, s,

Rha H-1)].⁴⁸ Acid hydrolysis³¹ of compound **13** gave kaempferol, glucose and rhamnose (co-TLC with authentic samples). Thus, compound **13** was identified as kaempferol 3-*O*-rutinoside.

The molecular formula of compound **15** was established as C₃₂H₃₈O₁₉ by ESI mass analysis that showed [M+H]⁺ at *m/z* 727. The three significant fragment peaks at *m/z* 595 [M-132 (pentose unit)+H]⁺, 449 [M-132-146 (methyl pentose unit)+H]⁺ and 287 [M-132-146-162 (hexose unit)+H]⁺ indicated compound **15** as kaempferol 3-*O*-triglycoside. The ¹H-NMR spectrum exhibited signals of kaempferol⁴⁰ in addition to three anomeric signals [δ 5.57 (1H, d, *J*= 7.3 Hz, Glc H-1), 4.59 (1H, d, *J*= 7.3 Hz, Xyl H-1) and 4.34 (1H, s, Rha H-1)].⁴⁸ The high field position of H-1 of β-xylose (δ 4.59) indicated that it was a terminal sugar. Because the H-1 signal of the C-3 linked glucose was shifted downfield (Δδ = 0.29 ppm) compared to that of rutinoyl unit in compound **13** it is suggested that xylose is attached to C-2".⁵⁰ The ¹³C-NMR spectrum displayed thirteen carbon signals were attributed to kaempferol nucleus^{42&47} and seventeen carbon signals in the region of sugars including three anomeric signals at δ 104.49, 100.54 and 98.33 (see Table 1). The ¹³C-NMR shifts of the three sugars are consistent with those corresponding to one β-D-glucopyranosyl, one β-D-xylopyranosyl and one α-L-rhamnopyranosyl

moiety.⁴⁸ The deshielding of the ¹³C signals associated with C-2" (δ 81.66) and C-6" (δ 66.23) of the glucose unit indicated the glycosylation at C-2" and C-6". The identity of the three sugars and their sequence were determined by the exhaustive analysis of NMR spectral data (HMQC and HMBC). In the HMBC spectrum the anomeric proton signal at δ 5.57 (Glc H-1) showed correlation with the carbon signal at δ 132.87 (C-3) indicating the *O*-triglycosidic linkage at the 3-position of kaempferol. Also, the anomeric proton signal at δ 4.59 (Xyl H-1) showed correlation with the carbon signal at δ 81.66 (C-2") confirmed the interglycosidic linkage xylose (1→2) glucose. Furthermore, the correlation between the anomeric proton signal at δ 4.34 (Rha H-1) and the carbon signal at δ 66.23 (C-6") confirmed the interglycosidic linkage rhamnose (1→6) glucose. To confirm the nature and the absolute stereochemistry of the sugar moieties, an aliquot of **15** was hydrolysed and reacted with (-)-2-butanol.³² GC analysis revealed the presence of D-glucose and D-xylose by comparison of their retention time data with those of the relevant standards. From the above evidences and by comparison with literature data,^{31&50} compound **15** was established as kaempferol 3-*O*-[2-*O*-D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside³¹ or kaempferol 3-[2^G-xylosyl-rutinoside]⁵⁰ which is isolated here for the first time from family Cyperaceae. It was previously reported

from the leaves of *Hosta ventricosa* (Salisb) Stearn (Liliaceae)⁵⁰ and seed cake of *Camellia sinensis* O. Kuntze (Theaceae).³¹

The ESI mass spectrum of compound **16** showed $[M+H]^+$ at m/z 757 corresponding to the molecular formula $C_{33}H_{40}O_{20}$. Also, three prominent fragment peaks appeared at 595 $[M-162$ (hexose unit) $+H]^+$, 449 $[M-162-146$ (methyl pentose unit) $+H]^+$ and 287 $[M-162-146-162$ (hexose unit) $+H]^+$ indicating compound **16** as kaempferol 3-*O*-triglycoside. Its ¹H-NMR spectrum exhibited signals owing to kaempferol⁴⁰ and three sugar units that were indicated by the appearance of three anomeric signals at δ 5.50 (1H, d, $J = 7.0$ Hz, Glc H-1), 4.58 (1H, d, $J = 7.6$ Hz, Glc H-1) and 4.29 (1H, s, Rha H-1).⁴⁸ The strongest deshielded sugar doublet at δ 5.50 apparently corresponding to the anomeric proton of glucose directly attached to C-3 position of the aglycone.⁵⁰ The ¹³C-NMR spectrum displayed 30 carbon signals (see Table 1) were attributed to kaempferol,^{42&47} two β -glucopyranosyl units and one α -rhamnopyranosyl unit that were indicated by the appearance of three anomeric signals at δ 98.33, 103.74 and 100.42, respectively.⁴⁸ The interglycosidic linkages at C-2'' and C-6'' were identified by the downfield shifts of C-2'' (δ 81.93) and C-6'' (δ 66.11). The HMQC and HMBC spectra identified the three sugars and their sequence. The HMBC spectrum

showed a cross peak between the anomeric proton signal at δ 4.58 (2''-Glc H-1) and the carbon signal at δ 81.93 (3-Glc C-2''), this confirmed the interglycosidic linkage glucose (1 \rightarrow 2) glucose. Also, the anomeric proton signal at δ 4.29 (6''-Rha H-1) showed a cross peak with the carbon signal at δ 66.11 (3-Glc C-6''), this supported the interglycosidic linkage rhamnose (1 \rightarrow 6) glucose. Acid hydrolysis³¹ of compound **16** yielded kaempferol, glucose and rhamnose (co-TLC). The aforementioned data showed good agreement with those reported for kaempferol 3-*O*-[2-*O*-D-glucopyrano-syl-6-*O*- α -L-rhamnopyranosyl]- β -D-glucopyranoside or kaempferol 3-[2^G-glucosylrutinoside]⁵⁰ which is isolated for the first time from the family Cyperaceae. It was previously reported from the leaves of *Hosta ventricosa* (Salisb) Stearn (Liliaceae).⁵⁰

In conclusion, luteolin (**7**), luteolin 7-*O*- β -D-glucopyranoside (**10**) and quercetin 3-*O*-rutinoside (**14**) were previously reported from the leaves of the studied plant⁶ while the others are isolated for the first time from *C. alpoecuroides* Rottb. To the best of our knowledge, compounds **2** and **5** are first report from the genus *Cyperus* while compounds **15** and **16** are first report from the family Cyperaceae.

Flavonoids have the property of inhibiting autoxidation reactions and scavenging of free radicals but the relation between their structure and activity remains unclear.⁵¹ There are

three functional groups that have been attributed to increase the antiradical activities among the flavonoids: the presence of an *ortho*-dihydroxylation of the B-ring of the flavonoid molecule; the C2–C3 double bond in concert with 4-oxo functionality of the C-ring; and the additional presence of both a 3- and a 5-hydroxyl moiety of the C and A rings, respectively.^{51&52} Figure 2 shows the results obtained for antioxidant activity of fourteen flavonoids isolated from *C. alopecuroides* Rottb. Flavones and flavonols with a substituted hydroxyl group at the C-3 position, which have only a C-4' hydroxyl group in the B-ring showed low antioxidant activity falls within the range of 1.0-21.1%. A strong antioxidant activity was shown by compounds with an *ortho*-dihydroxy system in the B-ring: quercetin 3-*O*- β -D-rutinoside (79.2%), luteolin (75.0%), luteolin 7-*O*- β -D-glucopyranoside (71.9%) and quercetin 3-*O*- β -D-glucopyranoside (70.7%), aureusidin 4-methyl ether (46.5%). It appears that the reason for their high reactivity is the strong effect of the C-3' hydroxyl group on the reactivity of the hydroxyl at C-4'. This is in agreement with conclusions that the *ortho*-dihydroxy system in the B-ring of the flavonoids is highly effective against free radicals.^{34&51} The synergistic effects of flavonoid mixtures may be responsible for high antioxidant activity of the ethyl acetate, *n*-butanol and aqueous extracts⁵³ as shown in Figure 3.

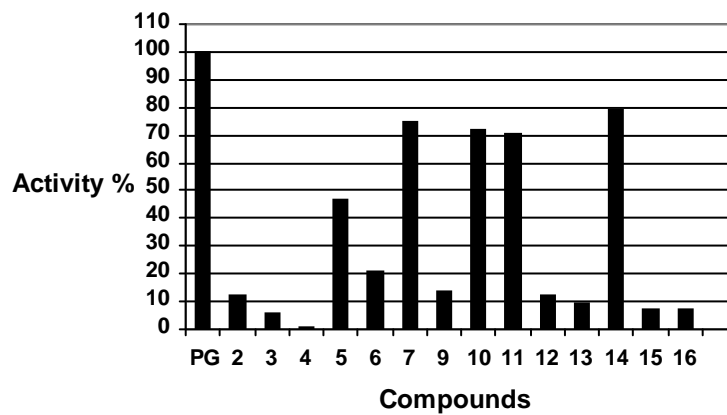
The cytotoxicity of nine phenolic compounds isolated from *C. alopecuroides* Rottb. was assessed *in vitro* with a mouse lymphoma cell line using the microculture tetrazolium (MTT) assay.⁵⁴ The compounds were tested for their cytotoxic activities at a range of concentrations of (3–10 μ g/ml). All compounds were found to be active against the cell line chosen (Table 2). Luteolin 5,3'-dimethyl ether and luteolin 7,3'-dimethyl ether are the most active compounds in this study with ED₅₀ of 2.7 and 3.2 μ g/ml, respectively.

Twelve flavonoids were evaluated for α -amylase inhibitory activity. As shown in Figure 4, luteolin (99.6%), aureusidin 4-methyl ether (90.5%), luteolin 5,3'-dimethyl ether (85.0%) and luteolin 4'-*O*- β -D-glucopyranoside (83.8%) showed strong inhibitory activity of α -amylase. The α -amylase inhibitory activity of luteolin (IC₅₀ 50-125 μ g/ml) was similar to acarbose (IC₅₀ 50-120 μ g/ml). Apparently, the α -amylase inhibitory activity increased with the presence of hydroxyl groups at 3' and 4'-position of the B-ring.⁵⁵

In conclusion, the present study revealed that the titled plant contains a large number of phenolics with strong antioxidant, cytotoxic activities and an inhibitory activity of α -amylase. Thus, the plant could be considered of potential impact on human health.

Table 2: Cytotoxic activity of some phenolic compounds isolated from the inflorescences of *C. alopecuroides* Rottb.

Compound	ED ₅₀ (μg/ml)
Luteolin 5,3'-dimethyl ether (3)	2.7
Luteolin 7,3'-dimethyl ether (4)	3.2
Aureusidin 4-methyl ether (5)	7.2
<i>Trans</i> -ferulic acid (8)	>10.0
Luteolin 4'- <i>O</i> -β-D-glucopyranoside (9)	>10.0
Luteolin 7- <i>O</i> -β-D-glucopyranoside (10)	> 3.0
Kaempferol 3- <i>O</i> -β-D-rutinoside (13)	8.8
Kaempferol 3-[2 ^G -xylosylrutinoside] (15)	>10.0
Kaempferol 3-[2 ^G -glucosylrutinoside] (16)	>10.0

**Fig. 2:** Antioxidant activity of some flavonoids isolated from the inflorescences of *C. alopecuroides* Rottb.

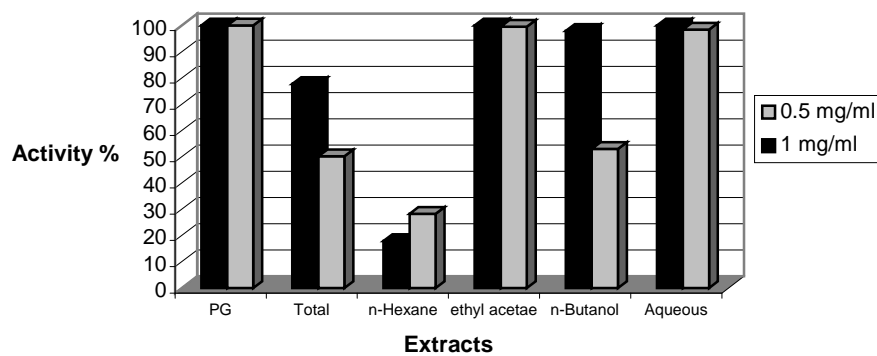


Fig. 3: Antioxidant activity of the different extracts from the inflorescences of *C. alopecuroides* Rottb.

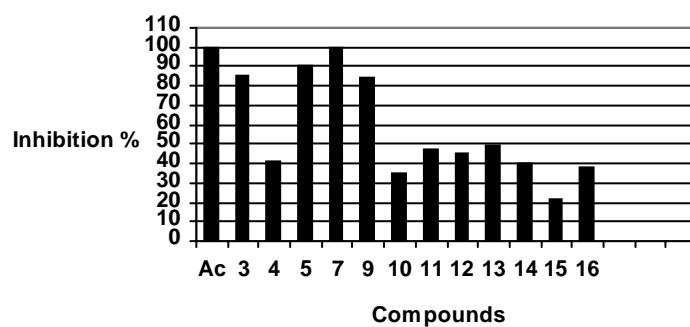


Fig. 4: α -Amylase inhibitory activity of some flavonoids isolated from the inflorescences of *C. alopecuroides* Rottb.

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REFERENCES

- 1- W. C. Evans, "Trease and Evans Pharmacognosy", 15th Ed., W. B. Saunders, London, 2002, p. 38.
- 2- L. Boulos and M. N. El-Hadidi, "The Weed Flora of Egypt", The American University in Cairo Press, Cairo, 1984, p. 58.
- 3- R. D. Allan, R. J. Wells, R. L. Correll and J. K. Macleod, *Phytochemistry*, 17, 263 (1978).
- 4- M. Morimoto, Y. Fujii and K. Komai, *ibid.*, 51, 605 (1999).
- 5- A. C. Alves, M. M. Moreira, M. I. Paul and M. A. C. Costa, *ibid.*, 31, 2825 (1992).
- 6- I. El-Habashy, R. M. A. Mansour, M. A. Zahran, M. N. El-Hadidi and N. A. M. Saleh, *Biochemical Systematics and Ecology*, 17, 191 (1989).
- 7- J. B. Harborne, C. A. Williams and K. L. Wilson, *Phytochemistry*, 21, 2491 (1982).
- 8- M. Abdel-Mogib, S. A. Basaif and S. T. Ezmirly, *Pharmazie*, 55, 693 (2000).
- 9- R. M. Seabra, A. M. S. Silva, P. B. Andrade and M. M. Moreira, *Phytochemistry*, 48, 1429 (1998).
- 10- A. S. Awaad and M. E. Zain, *Egypt. J. Pharm. Sci.*, 40, 107 (1999).
- 11- A. Dini, E. Ramundo, P. Saturnino, A. Scimone, I. Stagno d'Alcontres, *Boll. Soc. Ital. Biol. Sper.*, 68, 453 (1992).
- 12- H. M. Sayed, M. H. Mohamed, S. F. Farag and G. A. Mohamed, *Bull. Fac. Pharm. Cairo Univ.*, 39, 195 (2001).
- 13- H. Hikino and K. Aota, *Phytochemistry*, 15, 1265 (1976).
- 14- B. Nyasse, R. G. Tih, B. L. Sondengam, M. T. Martin and B. Bodo, *ibid.*, 27, 179 (1988).
- 15 - M. M. Sonwa, W. A. König, K. Kubeczka and O. Motl, *ibid.*, 45, 1435 (1997).
- 16- B. M. Fraga, *Natural Product Reports*, 9, 217 (1992).
- 17- F. Xu, T. Morikawa, H. Matsuda, K. Ninomiya and M. Yoshikawa, *J. Nat. Prod.*, 67, 569 (2004).
- 18- S. Jeong, T. Miyamoto, M. Inagaki, Y. Kim and R. Higuchi, *ibid.*, 65, 673 (2000).
- 19- P. N. Singh and S. B. Singh, *Phytochemistry*, 19, 2056 (1980).
- 20- A. Kar and C. I. Okpaleke, *Journal of African Medicinal Plants*, 5, 189 (1982).
- 21- K. Koichiro and U. Kunikazu, *Shokubutsu No Kagaku Chosetsu*, 16, 32 (1981). *Through C. A.*, 97 (15) 123942u (1982).
- 22- L. Boulos, "Medicinal Plants of North Africa", Reference

- Publications, Algonac, Michigan, 1983, pp. 80-2.
- 23- B. Oliver-Bever, "Medicinal Plants in Tropical West Africa", Cambridge University Press, Cambridge, 1986, pp. 144- 217.
 - 24- L. M. Perry and J. Metzger, "Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses", MIT Press, London, 1980, pp. 121-22.
 - 25- A. Chevallier, "The Encyclopedia of Medicinal Plants", Dorling Kindersley Ltd., London, 1996, p. 197.
 - 26- M. S. Hifnawy, Y. Y. El-Hyatmy, S. A. Kenawy, A. K. Yossef and A. S. Awaad, Bull. Fac. Pharm. Cairo Univ., 37, 99 (1999).
 - 27- M. S. Hifnawy, H. H. Ammar, S. K. Kenawy, M. E. Zaki, A. K. Yossef and A. S. Awaad, *ibid.*, 37, 107 (1999).
 - 28- M. I. Nassar, A. F. Abdel-Razik, E. A. M. El-Khrisy, A. M. Dawidar, A. Bystrom and T. J. Mabry, *Phytochemistry*, 60, 385 (2002).
 - 29- H. M. A. El-Gohary, Bull. Fac. Pharm. Cairo Univ., 42, 157 (2004).
 - 30- M. M. Sonwa and W. A. König, *Phytochemistry*, 56, 321 (2001).
 - 31- T. Sekine, J. Arita, A. Yamaguchi, K. Saito, S. Okonogi, N. Morisaki, S. Jwasaki and I. Murakoshi, *ibid.*, 30, 991 (1991).
 - 32- M. Fouad, K. Al-Trabeen, M. Badran, V. Wray, R. A. Edrada, P. Proksch and R. Ebel, *Arkivoc*, xiii, 17 (2004).
 - 33- B. Steffan, W. Wätjen, G. Michels, P. Niering, V. Wray, R. Ebel, R. A. Edrada, R. Kahl and P. Proksch, *J. Pharm. Pharmacol.*, 57, 233 (2005).
 - 34- M. Joyeux. A. Lobstein, R. Anton and F. Mortier, *Planta Med.*, 61, 126 (1995).
 - 35- R. A. Edrada, P. Proksch, V. Wray, L. Witte, W. E. Müller and R. W. M. Van Soest, *J. Nat. Prod.*, 59, 1056 (1996).
 - 36- Molecular Probes website: www.probes.com.
 - 37- S. Öksüz, A. Ulubelen, A. Barla and W. Voelter, *Turk. J. Chem.*, 26, 457 (2002).
 - 38- J. M. J. Vasconcelos, A. M. S. Silva and J. A. S. Cavaleiro, *Phytochemistry*, 49, 1421 (1998).
 - 39- T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids", Springer, New York (1970).
 - 40- J. B. Harborne, "The Flavonoids: Advances in Research Since 1986", Chapman and Hall, London (1994).
 - 41- V. Seidel, F. Bailleul and P. G. Waterman, *Phytochemistry*, 55, 439 (2000).
 - 42- P. K. Agrawal, "Carbon-13 NMR of Flavonoids", Elsevier Science, New York, Tokyo (1989).
 - 43- H. Tominaga, Y. Kobayashi, T. Goto, K. Kasemura and M. Nomura, *Yakugaku Zasshi*, 125, 371 (2005).

- 44- A. M. Dawidar, S. T. Ezmirly, M. Abdel-Mogib, Y. El-Dessouki and R. F. Angawi, *Pharmazie*, 55, 848 (2000).
- 45- H. Achenbach, M. Stöcker and M. A. Constenla, *Phytochemistry*, 27, 1835 (1988).
- 46- M. Sakakibara, D. Jr. Difeo, N. Nakatani, B. Timmermann and T. J. Mabry, *ibid.*, 15, 727 (1976).
- 47- J. B. Harborne and T. J. Mabry, "The Flavonoids: Advances in Research", Chapman and Hall, London (1982).
- 48- P. K. Agrawal, *Phytochemistry*, 31, 3307 (1992).
- 49- M. Yoshizaki, H. Fujino, M. Masuyama, M. Arisawa and N. Morita, *ibid.*, 26, 2557 (1987).
- 50- J. Budzianowski, *ibid.*, 29, 3643 (1990).
- 51- S. Burda and W. Oleszek, *J. Agric. Food Chem.*, 49, 2774 (2001).
- 52- A. J. Jr. Dugas, J. Castaneda-Acosta, G. C. Bonin, K. L. Price, N. H. Fischer and G.W. Winston, *J. Nat. Prod.*, 63, 327 (2000).
- 53- J. B. Harborne and C. A. Williams, *Phytochemistry*, 55, 481 (2000).
- 54- M. H. Kreuter, A. Robitzki, S. Chang, R. Steffen, M. Michaelis, Z. Kljajic, M. Bachmann, H. C. Schröder and W. E. G. Müller, *Comp. Biochem. Physiol.*, 101 C, 183 (1992).
- 55- T. Matsui, M. Kobayashi, S. Hayashida and K. Matsumoto, *Biosci. Biotechnol. Biochem.*, 66, 689 (2002).