SUPPLEMENTARY MATERIAL

Phytochemical constituents and chemosystematic significance of *Chrozophora tinctoria* (L.) Raf.

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Twelve compounds were isolated from *Chrozophora tinctoria* (L.) Raf. They were identified as kaempferol, kaempferol 3-*O*- β -glucopyranoside, kaempferol 3-*O*-(6''- α -rhamnopyranosyl)- β -glucopyranoside, quercetin, quercetin 3-*O*- β -glucopyranoside, quercetin 3-*O*-(6''- α -rhamnopyranosyl)- β -glucopyranoside; rutin, apigenin, apigenin 7-*O*- β -glucopyranoside, acacetin, gallic acid, methyl gallate and β -sitosterol-3-*O*- β -glucopyranoside. Their structures were elucidated by chemical and spectral methods. Furthermore, chemosystematic of the isolated compounds is discussed briefly. It was indicated that *C. tinctoria* is the only species of *Chrozophora* that has the capability to synthesis kaempferol aglycone and their glycosides, and the finding is supported by its distinct morphological and anatomical aspects.

Experimental

General

NMR experiments were recorded on a Jeol EX-500 spectroscopy: 500 MHz (^1H NMR), 125 MHz (^{13}C NMR) and INOVA Mercury spectroscopy: 300 (^1H NMR). UV spectrophotometer (Shimadzu UV-240), EIMS: Finnigan-Mat SSQ 7000 spectrometer. CC Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) and Sephadex LH-20 (Pharmazia) using MeOH/H₂O as eluent. PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems 1) H₂O, 2) 15% HOAc (H₂O–HOAc 85:15), 3) BAW (*n*-BuOH–HOAc–H₂O 4:1:5, upper layer), 4) (C₆H₆–*n*-BuOH–H₂O–pyridine 1:5:3:3, upper layer). Solvents 3 and 4 were used for sugar analysis. Complete acid hydrolysis (2 N HCL, 2 h, 100°C) was carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties. Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC.

Plant material

The plant material was collected from costal Road, 30 km south of Shalatin in March 2004, and identified by Prof. Salwa Ali Kawashty. A voucher specimen (no. 403) was deposited in the herbarium of the National Research Center (CAIRC).

Extraction and isolation

The air-dried powdered whole plant (650 Kg) was extracted with 70% EtOH (3 x 3L). The solvent was evaporated under reduced pressure at 50°C. The dried 70% aqueous ethanol extract was defatted with petroleum ether. The defatted aqueous ethanol extract (148 g) was then subjected to Polyamide CC (5.5x125cm) eluted with H₂O and followed by H₂O/MeOH mixtures of decreasing polarity. A total 52 fractions were collected, each 300 ml; these were controlled by PC to give four main fractions (FA-FD). FA was chromatographed on Sephadex column using H₂O:MeOH (1:1) for elution yielding two sub fractions, each was subjected to PPC using BAW and followed by Sephadex column using methanol to yield compounds **3** (48 mg) and **6** (35 mg). CC of FB on polyamide column (2.5x45cm), gradient elution with H₂O/MeOH, gave two major sub fractions; FB1 and FB2. Repeated CC for each on Sephadex using MeOH: H₂O (1:1), followed by PPC using BAW then 15% HOAc, afforded compounds **8** (23 mg), **11** (36 mg) and **12** (24 mg) and compounds **2** (30 mg) and **10** (48 mg), respectively. FC was applied on PPC using BAW then H₂O double solvent yielded compounds **5** (10 mg) and **7** (28 mg). Compound **1** (16 mg), **4** (17 mg) and **10** (12 mg) were obtained from FD by PPC using 15% HOAc and followed by BAW two times.

Structure elucidation of the isolated compounds

The identification of compounds were determined by R_f values, colour reactions, acid hydrolysis, EIMS spectrometry, UV spectrophotometry (Mabry et al., 1970; Markham, 1982), ¹H- and ¹³C-NMR spectroscopy and Co-PC with reference samples, then comparison of their spectroscopic data with previously reported values (Agrawal, 1989; Markham and Geiger, 1994; Tabussam et al., 2013; Subramain et al., 2013). The present work deals with the isolation and characterization of twelve compounds. They were identified as kaempferol (**1**), kaempferol 3-*O*-β-glucopyranoside (**2**), kaempferol 3-*O*-(6''-α-rhamnopyranosyl)-β-glucopyranoside (**3**), quercetin (**4**), quercetin 3-*O*-β-glucopyranoside (**5**), quercetin 3-

O-(6''- α -rhamnopyranosyl)- β -glucopyranoside; rutin (**6**), apigenin (**7**), apigenin 7-*O*- β -glucopyranoside (**8**), apigenin 4'-OCH₃; Acacetin (**9**), gallic acid (**10**), methyl gallate (**11**) and β -sitosterol-3-*O*- β -glucopyranoside (**12**).

Kaempferol (**1**)

Yellow amorphous powder, *R_f* = 0.74 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 266, 292sh, 319sh, 366; (+NaOMe) 276, 320sh, 411; (+AlCl₃) 269, 305, 350, 423; (+AlCl₃/HCl) 267, 305, 350, 424; (+NaOAc) 274, 306, 378; (+NaOAc/H₃BO₃) 265, 294, 319, 369. ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S1): 8.03 (2H, d, *J* = 8.5, H-2', 6'); 6.93 (2H, d, *J* = 8.5, H-3', 5'); 6.41 (1H, d, *J* = 2.0, H-8); 6.17 (1H, d, *J* = 2.0, H-6). EIMS (Figure S2); *m/z* 286.1

Kaempferol 3-*O*- β -glucopyranoside (**2**)

Yellow amorphous powders, *R_f* 0.68 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 266, 346; (+NaOMe) 274, 327sh, 401; (+AlCl₃) 274, 304, 349, 396; (+AlCl₃/HCl) 274, 345, 394; (+NaOAc) 274, 305, 393; (+NaOAc/H₃BO₃) 267, 352. ¹H NMR (500 MHz, CD₃OD, δ , ppm, *J*/Hz) (Figure S3): 8.02 (2H, d, *J* = 8.5, H-2', 6'); 6.85 (2H, d, *J* = 8.5, H-3', 5'); 6.37 (1H, d, *J* = 2.0, H-8); 6.17 (1H, d, *J* = 2.0, H-6); 5.24 (1H, d, *J* = 7.2, H-1''); 3-3.8 (5H, m, overlapped with -OH signals, H-2''- H-6''). ¹³C NMR (125 MHz, CD₃OD, ppm) (Figure S4): 178.2 (C-4), 164.8 (C-7), 161.7 (C-5), 160.2 (C-4'), 157.7 (C-2), 157.1 (C-9), 134.1 (C-3), 130.9 (C-2'), 130.9 (C-6'), 121.4 (C-1'), 114.7 (C-3'), 114.7 (C-5'), 104.2 (C-10), 102.7 (C-1''), 98.6 (C-6), 93.4 (C-8), 77.1 (C-5''), 76.7 (C-3''), 74 (C-2''), 70 (C-4''), 61.2 (C-6'')

Kaempferol 3-*O*-(6''- α -rhamnopyranosyl)- β -glucopyranoside (**3**)

A pale yellow powder, *R_f* 0.47 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 267, 349; (+NaOMe) 273, 324, 395; (+AlCl₃) 272, 300, 354, 395; (+AlCl₃/HCl) 271, 301, 356, 394; (+NaOAc) 273, 310, 385; (+NaOAc/H₃BO₃) 266, 355. ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S5): 7.92 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.82 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.44 (1H, s (br), H-8), 6.18 (1H, s (br), H-6), 5.23 (1H, d, *J* = 7.5 Hz, H-1''), 4.37 (1H, s (br), H-1'''), 3.2-3.9 (9 H, hidden under water signals, H-2'' $\rightarrow$ H-6'' & H-2''' $\rightarrow$ H-5'''), 1.01 (1H, d, *J* = 6.0 Hz, H-6'''). ¹³C NMR (125 MHz, DMSO-*d*₆, ppm) (Figure S6): 178.2 (C-4), 164.9 (C-7), 161.8 (C-4'), 161.5 (C-5),

157.8 (C-9), 157.4 (C-2), 133.7 (C-3), 131.7 (C-2'), 131.7 (C-6'), 121.3 (C-1'), 115.8 (C-3'), 115.8 (C-5'), 104.4 (C-10), 101.5 (C-1'''), 100.9 (C-1''), 99.4 (C-6), 94.3 (C-8), 76.9 (C-3''), 76.4 (C-5''), 74.59 (C-2''), 72.36 (C-4'''), 71.1 (C-3'''), 70.9 (C-2'''), 70.5 (C-4''), 68.7 (C-5'''), 67.5 (C-6''), 18.4 (C-6''')

Quercetin (4)

Yellow amorphous powder, *R_f* 0.66 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 256, 295, 370 (+NaOMe) 269, 331, 428; (+AlCl₃) 271, 302sh, 349, 442; (+AlCl₃/HCl) 267, 302sh, 358, 428; (+NaOAc) 276, 319sh, 380; (+NaOAc/H₃BO₃) 260, 301, 383. ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S7): 7.69 (1H, d, *J* = 2.0, H-2'); 7.52 (1H, dd, *J* = 2.0, 8.5, H-6'); 6.91 (1H, d, *J* = 8.5, H-3', 5'); 6.42 (1H, d, *J* = 2.0, H-8); 6.19 (1H, d, *J* = 2.0, H-6). The EI-MS (Figure S8); *m/z* 301.98

Quercetin 3-*O*- β -glucopyranoside (5)

Yellow amorphous powder, *R_f* 0.58 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 257, 358 (+NaOMe) 272, 329sh, 411; (+AlCl₃) 272, 324, 385, 396; (+AlCl₃/HCl) 269, 297sh, 362, 399; (+NaOAc) 272, 326, 375; (+NaOAc/H₃BO₃) 261, 378. ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S9): 7.52 (2H, m, *J* = 2.0, H-2', 6'); 6.79 (1H, d, *J* = 8.5, H-5'); 6.36 (1H, d, *J* = 2.0, H-8); 6.15 (1H, d, *J* = 2.0, H-6), 5.43 (1H, d, *J* = 7.2, H-1''); 3-4 (5H, hidden under water signals, H-2''-H-6''). ¹³C NMR (125 MHz, DMSO-*d*₆, ppm) (Figure S10): 177.5 (C-4), 164.2 (C-7), 161.6 (C-5), 157.1 (C-9), 156.3 (C-2), 149.7 (C-4'), 145.4 (C-3'); 133.6 (C-3), 122.7 (C-6'), 121.1 (C-1'), 116.2 (C-5'), 115.8 (C-2'), 103.5 (C-10), 101.6 (C-1''), 99.9 (C-6), 94.4 (C-8), 78.1 (C-4''), 77.1 (C-3''), 74.6 (C-2''), 71.8 (C-5''), 61.5 (C-6'').

Quercetin 3-*O*-(6''- α -rhamnopyranosyl)- β -glucopyranoside; rutin (6)

Yellow amorphous powder, *R_f* 0.45 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 258, 358; (+NaOMe) 272, 329sh, 410; (+AlCl₃) 274, 338, 433; (+AlCl₃/HCl) 269, 297sh, 362, 399; (+NaOAc) 272, 324, 385; (+NaOAc/H₃BO₃) 261, 378. ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S11): 7.5 (1H, dd, *J* = 8.5, *J* = 2.0, H-6'), 7.4 (1H, d, *J* = 2.0, H-2'), 6.7 (1H, d, *J* = 8.5 Hz, H-5'), 6.2 (1H, d, *J* = 2.0, H-8), 6.0 (1H, d, *J* = 2.0, H-6), 5.23 (1H, d, *J* = 8 Hz, H-1''), 4.37 (1H, broad s, H-1''), 3.2-3.9 ((9 H, hidden under water signals, H-2'' $\rightarrow$ H-6'' & H-2''' $\rightarrow$ H-5'''), 1.02 (1H, d, *J* = 6.0 Hz, H-6'''). ¹³C NMR (125 MHz, DMSO-*d*₆, ppm) (Figure S12): 177.8 (C-4), 164.6 (C-

7), 161.7 (C-5), 157.1 (C-9), 156.9 (C-2), 149.0 (C-4'), 145.3 (C-3'), 133.8 (C-3), 122.3 (C-6'), 121.6 (C-1'), 116.7 (C-5'), 115.7 (C-2'), 104.4 (C-10), 101.7(C-1'''), 101.2(C-1''), 98.7 (C-6), 93.9 (C-8), 76.9(C-3''), 76.4(C-5''), 74.59(C-2''), 72.36(C-4'''), 71.1(C-3'''), 70.9(C-2'''), 70.5(C-4''), 68.7(C-5'''), 67.5(C-6''), 18.4(C6''').

Apigenin (7)

Yellow amorphous powder, *R_f* 0.72 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 272, 331; (+NaOMe) 268, 332, 400; (+AlCl₃) 279,306, 351, 387; (+AlCl₃/HCl) 279, 304, 346, 386; (+NaOAc) 281, 306, 387; (+NaOAc/ H₃BO₃) 268,342. ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S13): δ 7.9 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.9 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.7 (1H, s, H-3), 6.5 (1H, d, *J* = 2.5 Hz, H-8), 6.2 (1H, d, *J* = 2.5 Hz, H-6). EIMS (Figure S14) *m/z*: 270.1

Apigenin 7-*O*- β -glucopyranoside (8)

Yellow amorphous powder, *R_f* 0.62 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 273, 332; (+MeOH/ NaOMe) 279, 342, 394; (+AlCl₃) 279,304, 344, 385; (+AlCl₃/HCl) 280, 304, 345, 390; (+NaOAc) 275,300, 385; (+NaOAc/ H₃BO₃) 273,334. ¹H-NMR, 500 MHz in DMSO-*d*₆ (Figure S15): δ 7.96 (2H, d, *J* = 8.7 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 8.7 Hz, H-3',5'), 6.85 (1H, s, H-3), 6.82 (1H, d, *J* = 2.0 Hz, H-8), 6.44 (1H, d, *J* = 2.0 Hz, H-6), 5.04 (1H, d, *J* = 7.2 Hz H-1''), 3-4 (5H, m, H-2''-H-6'').

Apigenin 4'-OCH₃; Acacetin (9)

Yellow amorphous powder, *R_f* 0.7 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 269, 303sh, 326; (+NaOMe) 276, 295sh, 375; (+AlCl₃) 276,303, 346, 378; (+AlCl₃/HCl) 278, 301, 338, 377; (+NaOAc) 276, 300, 359; (+NaOAc/ H₃BO₃) 272, 301, 342. ¹H NMR (400 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S16): δ 8.0 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 7.09 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.81 (1H, s, H-3), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.12 (1H, d, *J* = 2.0 Hz, H-6), 3.86 (1H, s, OCH₃)

Gallic acid (10)

White amorphous crystals. UV spectral data, λ_{max} (nm): (MeOH) 215, 271; ¹H NMR (500 MHz, DMSO-*d*₆, δ , ppm) (Figure S17); δ 6.9 (2H, s, H-2, H-6); EIMS (Figure S18), *m/z* 170

Methyl gallate (**11**)

White amorphous powder. UV spectral data, λ_{max} (nm): (MeOH) 219, 375, (+NaOMe), 208.6, 234sh, 318.4; ^1H NMR (500 MHz, DMSO-*d*₆, δ , ppm) (Figure S19); 6.89 (2H, s, H-2, H-6); 3.7 (3H, s, OCH₃). EI-MS (Figure S20): m/z 184.11

β -sitosterol-3-*O*- β -glucopyranoside (**12**)

White amorphous powder; *R_f* 0.42 (CHCl₃/H₂O, 4:1) ^1H NMR (500 MHz, DMSO-*d*₆, δ , ppm, *J*/Hz) (Figure S21): 5.29 (1H, bs, H-6), 4.17 (1H, d, *J* = 7.0 Hz H-1'), 3.61 (1H, m, H-3), 3.36 (1H, m, H-4'), 3.08 (1H, m, H-3'), 3.03 (1H, m, H-2'), 2.92 (1H, m, H-5'), 2.34 (2H, m, H-25), 2.31 (2H, m, H-4), 1.93 (2H, m, H-16), 1.91 (1H, m, H-17), 1.9 (2H, m, H-15), 1.77 (2H, m, H-7), 1.77 (2H, m, H-22), 1.77 (2H, m, H-23), 1.44 (2H, m, H-12), 1.41 (2H, m, H-1), 1.35 (2H, m, H-2), 1.35 (2H, m, H-11), 1.35 (2H, m, H-28), 1.32 (1H, m, H-20), 1.22 (1H, m, H-9), 1.22 (1H, m, H-8), 1.22 (1H, m, H-14), 1.11 (1H, m, H-24), 0.92 (3H, s, CH₃-19), 0.85 (3H, d, *J* = 6.2 Hz, CH₃-21), 0.79 (3H, t, *J* = 7.8 Hz, CH₃-29), 0.77 (3H, d, *J* = 6.6 Hz, CH₃-27), 0.76 (3H, d, *J* = 6.3 Hz, CH₃-26), 0.61 (3H, s, CH₃-18). ^{13}C NMR (125 MHz, DMSO-*d*₆, ppm) (Figure S22): 140.8 (C-5), 121.8 (C-6), 101.3 (C-1'), 77.4 (C-3'), 77.3 (C-5'), 77.3 (C-3), 73.9 (C-2'), 70.6 (C-4'), 61.6 (C-6'), 56.7 (C-14), 55.9 (C-17), 50.1 (C-9), 45.6, (C-24), 42.5 (C-13), 39.2 (C-4), 37.8 (C-12), 36.7 (C-1), 35.9 (C-10), 34.6 (C-20), 32.2 (C-22), 32.1 (C-7), 29.9 (C-8), 29.9 (C-2), 28.7 (C-25), 27.9 (C-16), 26.1 (C-23), 24.6 (C-15), 23.2 (C-28), 21.4 (C-11), 20.2 (C-26), 19.6 (C-19), 19.5 (C-27), 19.2 (C-21), 12.3 (C-29), 12.2 (C-18)

Figure S1. ¹H NMR spectrum of (1), in DMSO-*d*₆, 500 MHz.

Figure S2. EI-MS spectrum of (1).

Figure S3. ¹H NMR spectrum of (2), in CD₃OD, 500 MHz.

Figure S4. ¹³C NMR spectrum of (2), in CD₃OD, 125 MHz.

Figure S5. ^1H NMR spectrum of (3), in $\text{DMSO-}d_6$, 500 MHz.

Figure S6. ^{13}C NMR spectrum of (3), in $\text{DMSO-}d_6$, 125 MHz.

Figure S7. ^1H NMR spectrum of (4), in $\text{DMSO-}d_6$, 500 MHz.

Figure S8. EI-MS spectrum of (4).

Figure S9. ^1H NMR spectrum of (5), in $\text{DMSO}-d_6$, 500 MHz.

Figure S10. ^{13}C NMR spectrum of (5), in $\text{DMSO}-d_6$, 125 MHz.

Figure S11. ¹H NMR spectrum of (6), in DMSO-*d*₆, 500 MHz.

Figure S12. ¹³C NMR spectrum of (6), in DMSO-*d*₆, 125 MHz.

Figure S13. ^1H NMR spectrum of (7), in $\text{DMSO}-d_6$, 500 MHz.

Figure S14. EI-MS spectrum of (7).

Figure S15. ^1H NMR spectrum of (8), in $\text{DMSO-}d_6$, 500 MHz.

Figure S16. ^1H NMR spectrum of (9), in $\text{DMSO-}d_6$, 500 MHz.

Figure S17. ¹H NMR spectrum of (10), in DMSO-*d*₆, 300 MHz.

Figure S18. EI-MS spectrum of (10).

Figure S19. ^1H NMR spectrum of (11), in $\text{DMSO-}d_6$, 500 MHz.

Figure S20. EI-MS spectrum of (11).

Figure S21. ^1H NMR spectrum of (12), in $\text{DMSO}-d_6$, 500 MHz.

Figure S22. ^{13}C NMR spectrum of (12), in $\text{DMSO}-d_6$, 125 MHz.