A COMPARATIVE STUDY OF THE FLAVONOIDS AND SOME BIOLOGICAL ACTIVITIES OF TWO *Chenopodium* SPECIES

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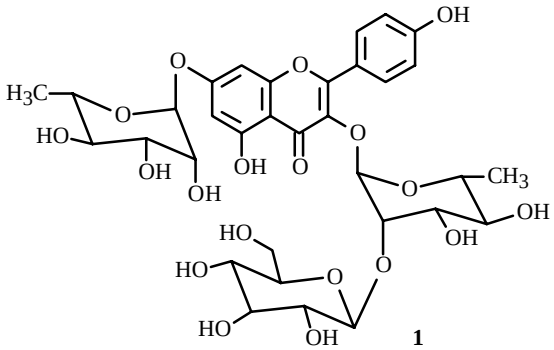
*A new kaempferol 3-*O*-(2-*β*-D-glucopyranosyl)-*α*-L-rhamnopyranoside-7-*O*-*α*-L-rhamnopyranoside and eight known flavonoid compounds were isolated and identified. A biological study includes determination of LD_{50}, anti-inflammatory effect, analgesic effect, ulcerogenic effect, diuretic effect, and toxicity effect.*

Key words*: Chenopodiaceae, chenopodium,* flavonoids, biological activity.

The family *Chenopodiaceae* is a large family comprising about 100 genera and 1500 species [1–3]. Plants of the genus *Chenopodium* have been reported to have therapeutic and edible properties [4–6]. *Chenopodium album* L. and *Chenopodium murale* L. are widely used in folk medicine as a potherb, spinach, anthelmintic, and laxative [7]. In general, plants of the family *Chenopodiaceae* contain different groups of secondary metabolites of which the most important are alkaloids [8], lipids [9], carbohydrates [10], phenolics, and saponins [11]. It was, thus, deemed of interest to study the phytoconstituents of two members of *Chenopodium* species indigenous to Egypt and to evaluate the toxicity and certain biological activities of the ethanolic (70%) extract prepared from them.

The crude extract of *Chenopodium album* L. (I) and *C. murale* L. (II) were fractionated and chromatographed. Eight flavonoid compounds were isolated from each species: kaempferol-3-*O*-(4-*β*-D-xylopyranosyl)-*α*-L-rhamnopyranoside-7-*O*-*α*-L-rhamno-pyranoside, 3-*O*-(4-*β*-D-apiofuranosyl)-*α*-L-rhamnopyranoside-7-*O*-*α*-L-rhamnopyranoside,3,7-di-*O*-*α*-L-rhamnopyranoside, 3-*O*-glucopyranoside and quercetin 3,7-di-*O*-*β*-D-glucopyranoside, 3-*O*-glucosylglucuronide, 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside, 3-*O*-*β*-D-glucopyranoside (isolated from *Chenopodium album* L.), kaempferol 3-*O*-(4-*β*-D–xylopyranosyl)-*α*-L-rhamnopyranoside-7-*O*-*α*-L-rhamnopyranoside, 3-*O*-(4-*β*-D-apiofuranosyl)-*α*-L-rhamnopyranoside-7-*O*-*α*-L-rhamnopyranoside,3-*O*-(2-*β*-D-glucopyranosyl)-*α*-L-rhamnopyranoside-7-*O*-*α*-L-rhamnopyranoside, 3,7-di-*O*-*α*-L-rhamnopyranoside, 3-*O*-*β*-D-glucopyranoside-7-*O*-*α*-L-rhamnopyranoside, kaempferol aglycone and quercetin 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside, and 3-*O*-*β*-D-glucopyranoside (isolated from *Chenopodium murale* L.).

The structure of the new flavonoid **1** was confirmed through chemical and physical investigations.



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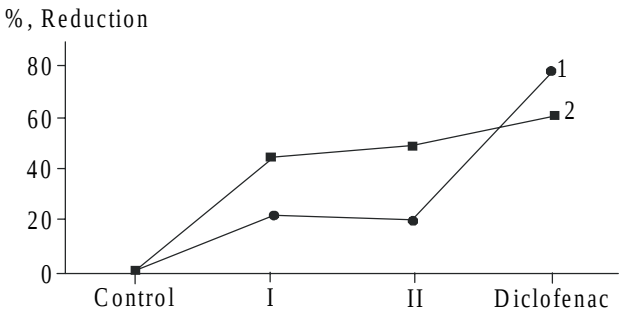

Fig. 1

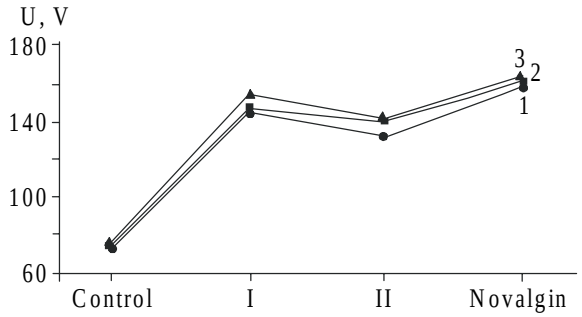

Fig. 2

Fig. 1. Effect of acute and chronic inflammation: 1 - % Acute reduction; 2 - % Chronic reduction
Fig. 2. Analgesic activity: 1 – 60 min; 2 – 120 min; 3 – 180 min.

The color reaction on paper chromatography of **1** with ammonia vapor changed from brown to yellow, indicating that the compound has a flavonoid chromophore. The complete acid hydrolysis gave kaempferol as agylcone and glucose and rhamnose as the sugar moiety. UV spectrophotometry showed no bathochromic shift in band II with sodium acetate reagent compared to the methanol spectrum, indicating that position 7 is occupied, and the spectrum of methanol was changed by adding a few drops of NaOMe and exhibited a bathochromic shift in Band I ($\Delta\lambda$ 45) compared to the methanol spectrum as well as an increase in intensity, indicating a free OH group at C-4′; the stability of the spectrum in the presence of NaOMe indicates that position 3 is occupied [12].

The ^{1}H NMR spectrum **1** in deuterated DMSO showed signals consistent with the presence of glucose, rhamnose, and kaempferol. The spectrum revealed the signals of kaempferol aglycone, a doublet at δ 7.8 with coupling constant 8 Hz assigned for H-2′,6′, and this upfield chemical shift confirmed that the rhamnose unit is directly attached to C-3 of kaempferol [13] and the doublet at δ 6.9 with coupling constant 8 Hz assigned to H-3′,5′. The spectrum also showed the two meta coupling protons resonating at δ 6.4 and 6.65 with coupling constant 2 Hz assigned to H-6 and H-8, respectively, and this downfield chemical shift confirms that C-7 is occupied.

The spectrum showed three anomeric proton signals, two of them resonating at δ 5.55 and 5.25 with coupling constant 1.1 Hz, indicating that there are two rhamnosyl sugar moeities with the α-configuration, and the upfield location of the two rhamnosyl moieties indicates that they are directly attached to the kaempferol ring at C-7 and C-3, while the third anomeric proton resonated at 4.35 with coupling constant 7 Hz, indicating the β-configuration of glucose, and its upfield location indicated that the glucose moiety is terminal to one of the rhamnose-glucose units.

Enzymatic hydrolysis using β-glucosidase yielded an intermediate, identified as kaempferol 3,7 di-*O*-α-L-rhamnopyranoside (detected by Co-chromatography with an authentic sample), and fast partial hydrolysis afforded an intermediate identified as kaempferol 3-*O*-rhamnose-*O*-glucose (detected by Co-chromatography with an authentic sample); both hydrolysis indicated that the rhamnose–glucose was located at C-3. Finally the structure of the compound was confirmed by ^{13}C NMR spectroscopy, which indicated that the interglycosidic linkage in the rhamnose-glucose unit at the C-3 position was at the C-2″ of rhamnose as evidenced by the downfield shift of the C-2″ of the rhamnose moiety from δ 72.0 to 81.8 and upfield shift of the two adjacent carbons C-1″ from δ 104 to 101.4 and C-3″ from δ 76 to 72.7 [14]. Thus the compound is identified as kaempferol 3-*O*-(2-β-D-glucopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside, a new flavonoid glycoside **1** which has not been reported before to occur in nature.

Acute toxicity experiments were carried out on the ethanolic extract of the plants. Extracts I (*Chenopodium murale*, 8 gm/kg b.wt) and II (*Chenopodium album* 7.13 gm/kg b.wt) were administered in mice [15]. The toxic symptoms and mortality rate were recorded after 24 h of treatment. The data shown in fig. 1 revealed that alcoholic extracts of I and II in a dose of 80 and 71.3 mg/kg b.wt, respectively, significantly reduced carrageenan edema volume by 20%. In the case of chronic inflammation, the extracts decreased the cotton pellet weight by 45 and 50%, respectively, which is considered as good when compared with diclofenac (1 g/kg b.wt) as the control.

Data represented in fig. 2 show that the untreated rats responded to the electric shock at about 73 volts. The extract – treated rats (I and II) gave a response at about 150 and 140 volts after 3 h. Compared with the analgesic effect of novalgin (5 mg/kg b.wt.) on rats, the extracts are considered to have a significant analgesic activity.

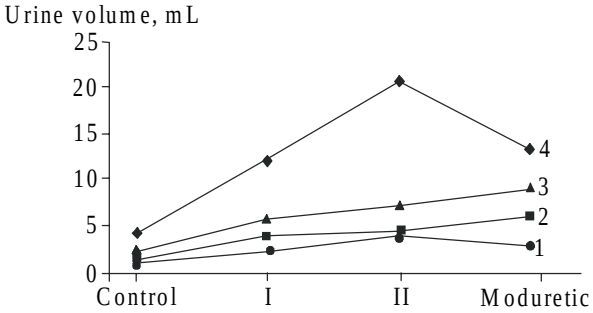

Fig. 3. Diuretic activity: 1 – 2 hrs; 2 – 3 hrs; 3 – 4 hrs; 4 – 24 hrs.

As shown in Fig. 3, alcoholic extracts of I and II have a significant diuretic effect throughout the 24 hrs after administration, where the volume of urine increased from 4 mL to 12 and 20 mL, respectively, compared to the effect of Moduretic (1.1 mg/l00 g b.wt) on urine volume, where the volume increased from 4 to 13 mL. Concerning the concentration of Na^+ and K^+ in urine, extract II as a diuretic agent has a less adverse effect on serum potassium ion level than Moduretic. Concerning the ulcerogenic effect, the alcoholic extract of plants I and II in doses of 80 and 71.3 mg/kg b.wt, respectively, has no ulcerogenic effect on the stomach of treated rats and no irritation was detected in the stomach.

Since the dose used in the examination reached 1600 mg dry extract /kg b.wt and no signs of toxicity appeared, the studied plant extracts were considered nontoxic.

The observed anti-inflammatory and analgesic activity may be attributed to the presence of phenolic compounds, as many flavonoid compounds are reported to have anti-inflammatory activity [16–18]. The mechanism of action of flavonoids against inflammation involves the inhibition of cyclooxygenase enzymes, and therefore, prostaglandin synthesis [19], thus preventing the generation of free radicals, which cause tissue damage during inflammation [20].

It is worth mentioning that the aqueous extracts for each of the alcoholic extracts showed anti- inflammatory and analgesic effect without any adverse ulcerogenic effect, but most of the non-steroidal anti-inflammatory drugs produce gastrointestinal erosions, pre-ulcerous changes, and bleeding [19]. The presented work also revealed that the examined alcoholic extracts showed diuretic activity concerning the concentration of Na^+ and K^+ in urine; diuretics generally increase urine excretion and output of sodium ions, but potassium depletion (hypokalemia) is considered the most dangerous adverse effect of diuretics [21]. moduretic was a typical example, where the concentration of K^+ in urine was raised from 48 to 61 mMol, causing severe hypokalemia, but potassium ion concentration in urine of *Chenopodium album* extract treated rats was 45 mMol, causing mild hypokalemia. Hypokalemia, by interfering with neuromuscular transmission, causes muscular weakness and hypotonia in addition to cardiac arrthymias (139, 140). Accordingly, extract II can be considered a diuretic with slight adverse effect on K^+ level. In conclusion, *Chenopodium album* and *Chenopodium murale* were found to be highly safe as a drug plant with no serious adverse effect and can be used for curing inflammation and relieving pain.

EXPERIMENTAL

Plant Materials. Fresh samples of *Chenopodium album* L. (I) were collected from Wadi Al Rhaha south Sinai and *Chenopodium murale* L. Samples of (II) were collected from Bahariya Oases. Samples were identified by Dr. Salwa kawashty.

Isolation and Identification of Flavonoid Constituents. Three kilograms of the dried aerial parts of both *Chenopodium* species were defatted with petroleum ether and macerated separately for three days using 70% ethanol/water at room temperature. Each extract was evaporated under reduced pressure and temperature to afford two residues (113 g for *Chenopodium album*, 105 g for *C. murale*). Each extract was subjected to a polyamide column starting with water as eluent then decreasing the polarity by increasing the concentration of EtOH. Purification was achieved by a combination of paper chromatography using water, AcOH: water 3: 17, BAW (*n*-BuOH–AcOH–water 4:1:5, upper phase), and silica gel thin layer chromatography using chloroform–methanol–water 65:45:12 as well as benzene–ethyl acaetate–acetic acid 8:5:2, and final purification was performed on Sephadex LH-20 Column for several times to confirm the purification of the separated flavonoid compounds. The structure elucidation and identification of the pure isolated flavonoid compounds were carried out through chemical investigation (complete and mild acid hydrolysis, hydrogen peroxide oxidation, and enzymatic hydrolysis) as well as

physical investigation using UV, ^{1}H NMR, ^{13}C NMR, and FAB mass Spectroscopic methods and comparing the obtained data with that available in the current literature.

Kaempferol-3-*O*-(2-β-D-glucopyranosyl)-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (1). Pale yellow crystal, m.p. (242–247°C). UV (λ_{max}, MeOH): 265, 342, (NaOMe) 266, 387; ($AlCl_3$) 275, 299, 344, 393; ($AlCl_3$/HCl) 275, 341, 393; (NaOAc) 265, 380; (NaOAc/H_3BO_3) 265, 345, ^{1}H NMR (δ, DMSO-d_6, J/Hz): 67.8 (2H, d, J = 8.5, H-2′,6′), 6.9 (2H, d, J = 8.5, H-3′,5′), 6.65 (1H, d, J = 2.5, H-8), 6.4 (1H, d, J = 2.5, H-6), 5.55 (1H, s, H-1″), 5.25 (1H, s, H-1‴), 4.35 (1H, d, J = 7, H-1⁗), 3.40–4.00 (m, sugar protons hidden by -OH-groups), 1.1 (3H, d, J = 6, CH_3-rhamnose), 0.85 (3H, d, J = 6, CH_3-rhamnose). ^{13}C NMR (δ, DMSO-d_6): 177.5 (C-4), 163.8 (C-7), 162.2 (C-5), 161 (C-4′), 158.3 (C-2), 156.6 (C-9), 133.2 (C-3), 131.1 (C-2′), 131.1 (C-6′), 121 (C-1′), 115.2 (C-3′), 115.2 (C-5′), 108.7 (C-1‴), 105.3 (C-10), 101.4 (C-1″), 99.8 (C-1⁗), 98.8 (C-6), 94.6 (C-8), 81.8 (C-2″), 78.4, (C-5‴), 77.6 (C-3‴), 76.2 (C-2‴), 74.5 (C-4″), 73.8 (C-4‴), 72.7 (C-3″), 72 (C-5″), 71 (C4⁗), 70.8 (C-3⁗), 70 (C-2⁗), 69.6 (C-5⁗), 61.4 (C-6‴), 18 (C-6″), 17.4 (C-6⁗).

Kaempferol-3-*O*-(4-β-D-xylopyranosyl)-α-L-rhamnopyranoside–7-*O*-α-L-rhamnopyranoside: UV (λ_{max}, MeOH): 344, 265 (NaOH) 388, 266 ($AlCl_3$) 394, 347, 299, 274 ($AlCl_3$/HCl) 394, 340, 274 (NaOAc) 352, 265 (NaOAc/H_3BO_3) 346, 265. ^{1}H NMR (δ, DMSO-d_6, J/Hz): 7.8 (2H, d, J = 8.5, H-2, H-6), 6.95 (2H, d, J = 8.5, H2, H-3′, H-5′), 6.75 (IH, d, J =2, H-8), 6.45 (IH, d, J = 2, H-6), 5.55 (1H, s, H-1‴), 4.19 (1H, d, J = 8, H-1⁗), 3.50–4.00 (m, sugars protons hidden by -OH-groups), 1.1 (3H, d, J = 5, CH_3-rhamnose), 0.85 (3H, d, J = 5, CH_3-rhamnose). ^{13}C NMR (δ, DMSO-d_6): 179 (C-4), 163 (C-7), 162.9 (C-5), 161.8 (C-4′), 159.5 (C-9), 157.9 (C-2), 134.5 (C-3), 131.9 (C-2′), 131.9 (C-6′), 121.2 (C-1′), 116.1 (C-3′), 116.1 (C-5′), 108.2 (C-1⁗), 106.1 (C-10), 101 (C-1″), 100.5 (C-6) 99.8 (C-1⁗), 94.1 (C-8), 80.9 (C-4″), 77.2 (C-3⁗), 76.2 (C-2⁗), 73.9 (C-2″), 73.5 (C-4‴), 72 (C-2‴), 71.8 (C-3″), 71.6 (C-5″), 71.5 (C-3‴), 71.2 (C-5‴), 70.4 (C-4⁗), 67.5 (C-5⁗), 18.1 (C-6‴), 17.7 (C-6″).

Material and Methods. Drug and chemicals. Moduretic (El-kahera–Co.), (1.1 mg/100 g b.wt); Novalgin (Hoechst–Co.) (0.5 mg/100 g b.wt); Diclofenac (Swiss pharma) (0.1 mg/100 g b.wt); Aspocid (Cid-Co.) (0.2 mg/100 g b.wt); Carrageenan (BDH) (0.05 mL (1% w/v).

Animals Used. Mice (20–25 g) of both sexes and adult male Sprague–Dawley albino rats (120–150 g) were obtained from the animal house of the National Research Center, Cairo, Egypt. The animals were housed in groups, each group consisting of 10 animals. They were kept in polyethylene cage under hygienic standard condition, temperature 25±3°C, relative humidity 40±10%, and fed with standard commercial diet and water ad libitum.

Anti-inflammatory Activity. The anti-inflammatory activity of the ethanolic extract of the two *Chenopodium* species was studied using the rat paw edema method [22] and the cotton pellet method [23]. In the first method, edema was induced by the injection of 0.05mL of 1% (w/v) carrageenan in distilled water into the subplantar area of the right hind paw. Diclofenac sodium (1 mg/kg b.wt.) was used as a reference drug.

Analgesic Activity. A noxious stimulus was aplied by electric current [24].

Diuretic Effect Determined by the Method [25]. Sodium and potassium ions were estimated by using a flame photometer [26] and compared with that of the control group.

Ulcerogenic Effect. The acute ulcerogenic effect of the tested ethanol extract was examined by the method of [27].

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