

PHCOG MAG. Research Article

Caffeoyl Derivatives and Flavonoids from Three Compositae Species

Fathy Mohamed Soliman¹, Afaf Hassan Shehata¹, Amal El-Sayed Khaleel^{1*},
Shahira Mohamed Ezzat¹ and Amany Ameen Sleem²

¹Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr-El-Ainy, Cairo 11562, Egypt.

²Department of Pharmacology, National Research Center, Dokki, Giza, Egypt.

Author for correspondence: amaalkhaleel@yahoo.com

ABSTRACT

Four flavones; chrysoeriol (1), apigenin (3), luteolin (6), orientin (11), and three flavanones; naringenin (2), eriodictyol (4) and 3',4',5',7-pentahydroxy flavanone (5), five flavonols; rhamnetin (13), hyperoside (15), rhamnetin-3-O-β-D-glucoside (17), mearnsitin-3-O-β-D-glucoside (19) and isoquercitrin (20), an aurone; 3',4',5',6-tetrahydroxyaurone-4-O-glucoside (bracteol) (7), six caffeoyl derivatives of quinic acid; 3,4-dicaffeoylquinic acid-methyl ester (8), 3,5-dicaffeoylquinic acid-methyl ester (9), 3,5-dicaffeoylquinic acid (10), neochlorogenic acid (12), 1,4,5-tricaffeoylquinic acid (14) and 1,5-dicaffeoylquinic acid (16) and caffeic acid (18) were isolated from the flowers and the remaining aerial parts of *Helichrysum bracteatum* (Vent.) Andrews, *Gazania nivea* DC. and *Dimorphotheca ecklonis* DC. Compounds 8 and 9 were isolated for the first time from genus *Helichrysum*, compounds 10 and 12 from *H. bracteatum*, compounds 12-17 from genus *Gazania*, compounds 10, 12, 18 and 20 from genus *Dimorphotheca* and compound 19 from genus *Dimorphotheca* and family Compositae. The anti-inflammatory, analgesic and antipyretic effects of the flowers and the remaining aerial parts of the three studied plants as well as the major isolated compounds of the most potent extract were studied. Bracteol (7) was the most potent anti-inflammatory and antipyretic, while 3,5-dicaffeoylquinic acid (10) was the most potent analgesic. The flowers of the three plants showed a significant hepatoprotective effect.

KEY WORDS: flavonoids, caffeoyl quinic acid derivatives; *Helichrysum bracteatum*, *Gazania nivea*, *Dimorphotheca ecklonis*; analgesic, antipyretic, anti-inflammatory, hepatoprotective activities.

INTRODUCTION

Helichrysum bracteatum (Vent.) Andrews, *Gazania nivea* DC. and *Dimorphotheca ecklonis* DC. are three plants belonging to family Compositae and cultivated in Egypt for ornamental purposes. Many flavonoids were previously isolated from *H. bracteatum* growing in Germany and Spain (1-5). Phenolic acids such as caffeic, chlorogenic, neochlorogenic, as well as 1,3-, 1,4-, 1,5-, 3,5-, and 4,5 dicaffeoylquinic acids were isolated before from other *Helichrysum* species (6-8). Some reports were found concerning the anti-inflammatory, hepatoprotective, antimicrobial, anticancer and antioxidant effects of different *Helichrysum* species. (9-12) Meanwhile, no literature was traced concerning the phenolic acids content of *H. bracteatum*, the flavonoid content of the plant cultivated in Egypt or on the chemical constituents or the biological activity of *Gazania nivea*, and *D. ecklonis*. Therefore, the phenolic contents as well as some of the biological properties which may be

related to these constituents of the three plants cultivated in Egypt have been investigated in this study.

MATERIALS AND METHODS

General

Melting points were determined on electrothermal 9100 (UK). ¹H-, ¹H-¹H COSY and ¹³C- NMR analyses spectra were recorded on Varian Mercury (¹H-NMR, 300 MHz, ¹³C, 75 MHz), Jeol JNM ECA 500 instrument (¹H-NMR, 500 MHz, ¹³C, 125 MHz) and Jeol JHA-LAA 400 WB-FT (¹H, 400 MHz; ¹³C, 100 MHz) spectrometer. Thin-layer chromatography (TLC) was performed on silica gel GF₂₅₄ precoated plates (Machery Nagel, Germany) and silica gel G₆₀ (E-Merck) for preparative TLC. Silica gel 60 (Machery Nagel 230-400 mesh ASTM) and silica gel H (E-Merck) for VLC were used for column chromatography.

Materials for biological study

Paracetamol (Paramol)[®], Misr Co., Egypt; indomethac

-in (Indomethacin)[®], Eipico, Egyptian Int. Pharmaceutical Industries Co.; Dipyrone-metamizol sodium (Novalgin)[®] (Aventis, S.A.E., Egypt); Carrageenan (Sigma Co., USA); Brewer's yeast (MEPACO, Cairo, Egypt); Silymarin (Sedico Pharmaceutical Co., 6 October City, Egypt) and Carbon tetrachloride (analar, El-Gomhoreya Co., Cairo, Egypt). Transaminase Kits (Bio-Meriéux Co.): biochemical kits for assessment of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase enzymes. Adult male albino rats of Sprague Dawley Strain weighing 100-150 g and albino mice (20 - 25g). All animals were kept on standard laboratory diet and under hygienic conditions.

Plant material

The flowers and remaining aerial parts (after separation of the flowers) of *Helichrysum bracteatum* (Vent.) Andrews (the orange cultivar), *Gazania nivea* DC. and *Dimorphotheca ecklonis* DC. were supplied from the Experimental Research Station of Faculty of Pharmacy, Cairo University, Giza, Egypt. Identification of the plant materials was carried out by Prof. Dr. Moneir M. Abdel Ghani, Prof. of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Egypt. Voucher specimens were deposited at the Museum of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Extraction and Isolation:

The ethanolic extracts of each of the flowers (1 kg) and the remaining aerial parts (1.5 kg) of *Helichrysum bracteatum*, *Gazania nivea* and *Dimorphotheca ecklonis* were prepared by cold maceration with 95 % ethanol (7 X 10 L). Each extract was fractionated successively with petroleum ether (10 x 200 ml), chloroform (8 x 200 ml), ethyl acetate (10 x 200 ml) and *n*-butanol saturated with water (12 x 200 ml).) to yield 23.22, 17.3, 12 and 10 g. of the ethyl acetate fractions of *H. bracteatum* flowers, *H. bracteatum* remaining aerial parts, *G. nivea* flowers and *D. ecklonis* flowers and 53 g of the *n*-butanol fraction of *G. nivea* flowers. The fractions rich in phenolic compounds, based on their TLC investigation, were used for the isolation of these compounds.

Each of the investigated fractions was chromatographed over a vacuum liquid chromatography column (VLC, Si gel H, 70 g, 20 x 5 cm). Gradient elution was carried out using ethyl acetate-chloroform mixtures and ethyl acetate-methanol mixtures with increasing polarity. Fractions 200 ml each were collected and monitored by TLC.

Similar fractions were pooled together. Each of the pooled fractions was purified on several sephadex LH-20 columns (40 x 2 cm) using methanol and /or methanol-water (1:1 v/v) as an eluent.

The ethyl acetate fraction (20 g) of the flowers of *H. bracteatum* yielded six main fractions (A_{HF}-F_{HF}). Rechromatography of fraction A_{HF} (10-25% ethyl acetate / chloroform, 3 g) on sephadex LH-20 yielded two main subfractions. Fraction A_{HF}-1 was purified using preparative TLC technique developed with (CHCl₃- MeOH 9.5: 0.5), double run to yield compounds 1 (yellow powder, 15 mg) and 2 (orange yellow powder, 12 mg). Fraction A_{HF}-2 was evaporated to give yellow powder of compound 3 (20 mg). Fraction B_{HF} (30-45% ethyl acetate / chloroform, 2.9 g) upon purification on sephadex LH-20 gave two main subfractions. Fraction B_{HF}-1 was rechromatographed on a Si gel column (30g, 25 x 1.5 cm) eluted with *n*-hexane-ethyl acetate (8:2 v/v) to give orange yellow powder of compound 4 (110 mg) and orange yellow powder of compound 5 (40 mg). Fraction B_{HF}-2 was evaporated to yield yellow powder of compound 6 (170 mg). Fraction F_{HF} (25-30% methanol / ethyl acetate, 3.9 g) afforded compound 7 (orange powder, 200 mg). Fractions C_{HF}, E_{HF} (50 % ethyl acetate / chloroform - 20% methanol / ethyl acetate) contained major compounds 8, 9, 10 and 11 which were found in larger proportions and isolated from the ethyl acetate extract of the remaining aerial parts of the plant .

The ethyl acetate fraction (15 g) of the remaining aerial parts of *H. bracteatum* yielded five major fractions (A_{HA}-E_{HA}). Fraction A_{HA} (30-45% ethyl acetate / chloroform, 2.5 g) afforded 54 mg yellow powder of compound 6. Rechromatography of fraction B_{HA} (50-90% ethyl acetate / chloroform, 2.8 g) on a Si gel column (30g, 25 x 1.5 cm) using chloroform-methanol (9.2:0.8 v/v) as eluting solvent yielded, 23 mg brownish yellow powder of compound 8 and 30 mg brownish yellow powder of compound 9. Fraction C_{HA} (95 % ethyl acetate /chloroform-5% methanol /ethyl acetate, 1.2 g) afforded brownish yellow powder of compound 10 (20 mg). Fraction D_{HA} (10-20 % methanol / ethyl acetate, 3 g) gave 50 mg of compound 10 and 108 mg of dark yellow powder of compound 11 and finally compound 12 was obtained from fraction E_{HA} (25-50 % methanol / ethyl acetate, 1.8 g) as yellowish white powder (105 mg).

The ethyl acetate fraction (10 g) of the flowers of *G. nivea* yielded three main fractions (A_{GF}-C_{GF}). Compound 13 was obtained as yellow powder (20 mg) from fraction A_{GF} (55-65% ethyl acetate / chloroform,

1.8 g), compound **14** as brownish yellow powder (115 mg) from fraction B_{GF} (85% ethyl acetate / chloroform-5% methanol / chloroform, 2.5 g) and compound **15** as yellow powder (30 mg) from fraction C_{GF} (10-20% methanol / ethyl acetate, 1.2 g).

n-Butanol fraction (10 g) of the flowers of *G. nivea* yielded three main fractions (D_{GF} - F_{GF}). Fraction D_{GF} (5-10% methanol / ethyl acetate, 1.5 g) gave brownish yellow powder of compound **16** (110 mg). Fraction E_{GF} (15-20% methanol / ethyl acetate, 1.9 g) was further purified on reversed phase C_{-18} column eluted with methanol-water (30:70 v/v) to give compounds **17** (yellow powder, 29 mg) and **15** (yellow powder, 10 mg). Compound **12** (120 mg) was obtained from fraction F_{GF} (25-50% methanol / ethyl acetate, 2 g) as a yellowish white powder.

The ethyl acetate fraction (10 g) of the flowers of *D. ecklonis* yielded four main fractions (A_{DF} - D_{DF}). Fraction A_{DF} (40-50% ethyl acetate / chloroform, 0.9 g) afforded white powder of compound **18** (15 mg). Further purification of fraction B_{DF} (55-100 % ethyl acetate / chloroform, 2 g) on a Si gel column (25 × 1.5, 30 g) using a mixture of chloroform-methanol (9.6:0.4 v/v) as an eluent gave brownish yellow powder of compound **10** (40 mg). Fraction C_{DF} (5-10% methanol in ethyl acetate, 1.4 g) yielded compounds **19** (yellow powder, 16 mg) and **20** (yellow powder, 18 mg). Finally, compound **12** was obtained as brownish yellow powder (109 mg) from fraction D_{DF} (25-50% methanol / ethyl acetate, 2.3 g).

The yield of the isolated compounds in mg/g extract from the plants under investigation is given in Table (1).

Compound 7

1H -NMR: δ ppm [300 MHz, DMSO] 5.08 (1H, d, $J=7.2$, H-1'), 6.29 (1H, s, H-5), 6.33 (1H, s, H-7), 6.38 (1H, s, benzylic proton =CH-), 6.9 (2H, s, H-2', 6'). ^{13}C -NMR: δ ppm [75 MHz, DMSO] 92.9 (C-5), 98.04 (C-7), 103.71 (C-3a), 111.19 (C-2', 6'), 112.16 (=CH), 122.6 (C-1'), 146.22 (C-2), 146.52 (C-3', 4', 5'), 157.15 (C-4), 167.89 (C-6), 168 (C-7a) and 179.52 (C-3). The sugar moiety: 60.80 (C-6''), 69.82 (C-4''), 73.26 (C-2''), 76.55 (C-3''), 77.33 (C-5'') and 99.95 (C-1'').

Analgesic, antipyretic and anti-inflammatory effects

The aqueous and ethanolic extracts (100 mg/kg body weight) of *H. bracteatum*, *G. nivea* and *D. ecklonis* flowers and the remaining aerial parts, the petroleum ether and ethyl acetate fractions of the ethanolic extract of *H. bracteatum* flowers as well as bractein (**7**), 3,4-dicaffeoyl quinic acid methyl ester (**8**), 3,5-dicaffeoyl quinic acid methyl ester (**9**) and 3,5-

dicaffeoyl quinic acid (**10**) at a dose of 50 mg/kg. body weight were tested for their analgesic, antipyretic and anti-inflammatory activity. In addition, eriodictyol (**4**), luteolin (**6**) and orientin (**11**) were tested for their analgesic and antipyretic properties at a dose of 50 mg/kg body weight.

Anti-inflammatory activity

The tested extracts as well as the isolated compounds were tested for the anti-inflammatory activity and compared with that of indomethacin (20 mg/kg body weight) as a standard using carrageenan-induced rat hind paw oedema test on male rats of albino Sprague Dawely strain (130-140 g) according to the method of Winter *et al.* ⁽¹³⁾ Results are shown in Table (2). One hour after the administration of a single dose of the tested extracts, inflammation was induced by sub planter injection of 0.1 ml of 1% carrageenan solution in saline in the right hind paw while 0.1 ml saline was injected in the left hind paw. Four hours after oral administration of the different extracts, both hind paws were separately weighed to calculate the weight of oedema. The percentage of oedema (inflammation) was calculated according to the following equation:

$$\frac{\text{Weight of right paw} - \text{weight of left paw}}{\text{weight of left paw}} \times 100$$

Analgesic activity

The tested extracts as well as the isolated compounds were evaluated on male rats of albino Sprague Dawely strain (130-140 g) and compared with that of novalgin (50 mg/ kg. body weight) as standard, using electric current as anxious stimulus as described by Charlier *et al.* ⁽¹⁴⁾

Animals were received the tested extracts orally in a dose of 100-mg/kg b. wt., the isolated compounds in a dose of 50 mg/kg b. wt. or 50 mg/kg novalgin to serve as a positive control. Electrical stimulation was applied to the rat tail by means of 515 Master shocker using alternative current of 50 cycle / second for 0.2 second. The minimum voltage required for the animal to emit a cry was recorded before treatment at zero time v_o , as well as treated groups v_t . The results (Table 3) were measured after 1 and 2 hours from zero time. The percentage of change was calculated according the following equation:

$$\text{Percentage of change} = (v_t - v_o) \times 100 / v_o$$

Antipyretic activity

The activity of the tested extracts and the isolated compounds were evaluated on male rats of albino Sprague Dawely strain (130-140 g) and compared with that of paracetamol (100 mg/kg. body weight) as standard, using yeast-induced hyperthermia method as

described by Tomazetti *et al* ⁽¹⁵⁾ Pyrexia was induced to each animal by injecting a dose of 1ml/100 g b. wt. of yeast suspension (44%) intramuscularly. After 18 hours, the rectal temperature of each animal was recorded and the obtained temperature represented the base line of elevated body temperature to which the antipyretic activity will be compared. The animals were given the plant extracts orally in a dose of 100 mg/kg b. wt., the isolated compounds in a dose of 50 mg/kg.b.wt. and paracetamol (100 mg/kg b. wt.) to serve as a positive control. The induced raise in temperature was recorded before treatment at zero time T_0 and in treated groups T_t . Results were measured after one and two hours. Percentage of change was calculated according the following equation:

$$\text{Percentage of change} = (T_0 - T_t) \times 100 / T_0$$

Results are recorded in Table (4).

Hepatoprotective activity

The aqueous and ethanolic extracts (100 mg/kg body weight) of *H. bracteatum*, *G. nivea* and *D. ecklonis* flowers as well as the petroleum ether and ethyl acetate fractions of the ethanolic extract of *H. bracteatum* were tested for their hepatoprotective activity. The tested extracts were administered at a daily dose of 100 mg/kg body weight for one month before induction of liver damage by intraperitoneal injection of 5ml / kg of 25% carbon tetrachloride (CCl_4) in liquid paraffin according to the method of Klassan and Plaa (1969)(16) using silymarin 25 mg / kg body weight as a reference drug. The extracts as well as the reference drug was continued to be administered to the rats for another month after liver damage. The levels of aspartate aminotransferase (AST)(17), alanine aminotransferase (ALT)(17) and alkaline phosphatase (ALP)(18) enzymes were measured in the blood of each group at zero time, after one month of receiving the tested drug, 72 hours after induction of liver damage and after one month of treatment with the tested samples. Results are shown in Table (5).

The LD_{50}

The LD_{50} of the ethanolic and aqueous extracts of the flowers and the remaining aerial parts of *H. bracteatum* *G. nivea* and *D. ecklonis* and the most active extracts were estimated following Karber, G. (1931) procedure (19).

RESULTS AND DISCUSSION

Twelve compounds were isolated from *H. bracteatum*; compounds 1-7 from the flowers and compounds 6 & 8-12 from the remaining aerial parts. On the other

hand, six compounds (12-17) were isolated from *G. nivea* flowers and five compounds (10, 12 and 18-20) from *D. ecklonis* flowers.

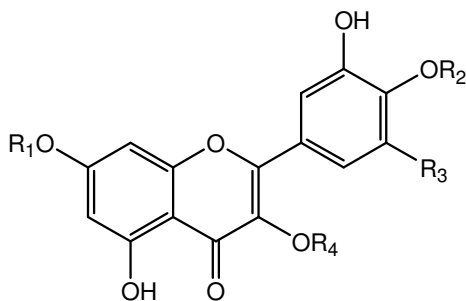
Compounds 1, 3, 6 and 11 were identified as chrysoeriol, (20, 21) apigenin (22), luteolin (22-24) and orientin (23) flavones. Compounds 1 and 3 were isolated before from the ivory white flowers of *H. bracteatum* growing in Spain, (4) while compounds 6 and 11 were isolated before from the highly coloured involucre bracts and the leaves respectively of *H. bracteatum* growing in Germany. (1)

The UV spectral data of 2, 4 and 5 showed their flavanone nature. (22,25) Compounds 2, 4 and 5 were identified as naringenin, (20, 21) eriodictyol (26) and 3',4',5',7-pentahydroxy flavanone (3) which were isolated before from the bracts of *H. bracteatum* growing in Germany. (3)

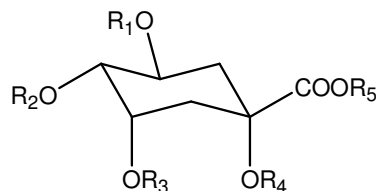
The UV spectral data of 7 in methanol with λ_{max} 405 nm indicated that it is an aurone and it was comparable to that of bractein.(20) No change in band II on the addition of AlCl_3 , which suggested that compound 7 is a 4-O-glycoside.(27) $^1\text{H-NMR}$ spectrum of 7 showed the presence of four aromatic protons suggested a pentasubstituted aurone.(22,25,27) A singlet at δ 6.90 ppm integrated as two protons assigned to H-2' and H-6', two singlets at δ 6.33 and 6.29 ppm, each integrated as one proton assigned to H-7 and H-5, respectively and a singlet at δ 6.38 ppm integrated as one proton and assigned to the benzylic proton (=CH-) were displayed in $^1\text{H-NMR}$ of the compound. In addition, it showed a doublet at δ 5.08 ppm with a coupling constant 7.2 Hz, assigned to the anomeric proton and indicated a β -linked sugar. $^{13}\text{C-NMR}$ data of the compound are in accordance with the data published for a pentasubstituted aurone with glucose as a sugar moiety (24). From the above data compound 7 was identified as 3',4',5',6-tetrahydroxyaurone-4-O-glucoside [bractein]. This compound was isolated before from the leaves and flowers of six variously coloured cultivars of *H. bracteatum* growing in Germany. (3) We report here the NMR data of the compound due to lack of literature.

All the previous compounds are reported for the first time from the plant cultivated in Egypt.

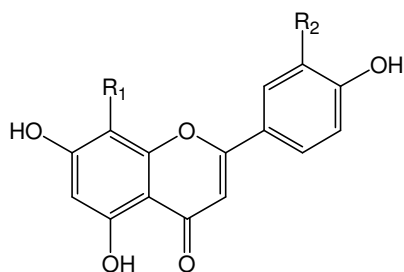
Compound 13, 15, 17 and 20 were identified as rhamnetin, (22) hyperoside (28), rhamnetin-3-O- β -D-glucoside (29) and isoquercitrin (28). This is the first report for the isolation of these compounds from genus *Gazania*.



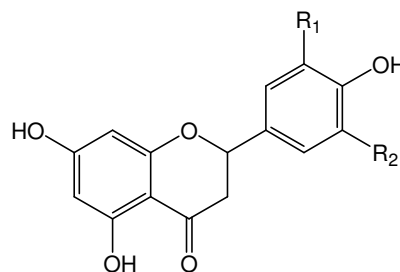
	R ₁	R ₂	R ₃	R ₄
13	CH ₃	H	H	H
15	H	H	H	Galactose
17	CH ₃	H	H	Glucose
19	H	CH ₃	OH	Glucose
20	H	H	H	Glucose



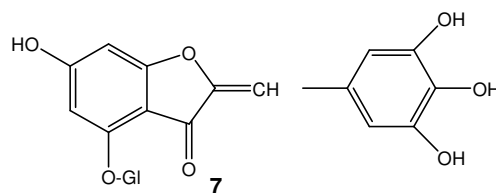
	R ₁	R ₂	R ₃	R ₄	R ₅
8	H	Caf	Caf	H	CH ₃
9	Caf	H	Caf	H	CH ₃
10	Caf	H	Caf	H	H
12	H	H	Caf	H	H
14	Caf	Caf	H	Caf	H
16	Caf	H	H	Caf	H



	R ₁	R ₂
1	H	OCH ₃
3	H	H
6	H	OH
11	Glucose	OH



	R ₁	R ₂
2	H	H
4	OH	H
5	OH	OH



The UV spectral data and ¹H-NMR spectrum of **19** displayed the characteristic protons of mearnsetin aglycone (30). PC analysis of the acid hydrolysate of the compound showed the presence of D-glucose. Therefore, compound **19** was identified as **mearnsetin-3-O-β-D-glucoside**. This is a rare flavonoid isolated only before from *Licania heteromorpha* (31). Therefore, it is the first report for the isolation of this compound from genus *Dimorphotheca* and from family Compositae.

The UV spectral data of compounds **8**, **9**, **10**, **12**, **14**,

16 and **18** showed the characteristic features of hydroxycinnamic acids in methanol (32).

¹H-NMR spectrum of compound **12** showed the presence of one caffeic and one quinic acid moieties. By comparing with the published data, (33) compound **12** was identified as **3-caffeoylquinic acid [neochorogenic acid]**. This is the first report for the isolation of this compound from *H. bracteatum*, genus *Gazania* and genus *Dimorphotheca*.

¹H-NMR spectrum of **8**, **9**, **10** and **16** showed the characteristic signals for a quinic acid and two caffeic acid moieties (32-34). The determination of the sites

of acylation in quinic acid moiety was based on their ^1H and ^{13}C -NMR data. Compounds **8**, **9**, **10** and **16** were identified as **3,4-dicaffeoylquinic acid-methyl ester**, (35) **3,5-dicaffeoylquinic acid-methyl ester**, (32) **3,5-dicaffeoylquinic acid** (32) and **1,5-dicaffeoylquinic acid** (36). This is the first report for the isolation of compounds **8** and **9** from genus *Helichrysum*, compound **10** from *H. bracteatum* and from genus *Dimorphotheca* and compound **16** from genus *Gazania*.

^1H -NMR spectrum of **14** showed three caffeic acid and a quinic acid moieties. The downfield shift of H-4 and H-5 showed that the acylation of the quinic acid by two caffeic acid molecules was on the hydroxyl groups at C-4 and C-5, and the third caffeic acid molecule acylated the OH on C-1. So, compound **14** was identified as **1,4,5-tricaffeoylquinic acid**. This is the first report for the isolation of this compound from genus *Gazania*. This is a rare phenolic acid which was isolated only from the flowers of *Arnica Montana* L. and *A. chamissonis* Less. (37)

The assignment of the protons of the quinic acid moiety in compounds **8**, **9**, **10**, **12**, **14** and **16** was determined using ^1H - ^1H COSY.

Compound **18** was identified as **caffeic acid** (32). This is the first report for the isolation of this compound from genus *Dimorphotheca*

The absence of flavonols and the presence of the flavones, aurones and flavanones in *H. bracteatum*, in addition to C-glycosyl flavones, which appear to be rare in the tribe Inulae but only reported in *H. bracteatum* was in agree with the reported chemotaxonomy of the tribe Inulae. (38)

Too little is known yet regarding the distribution of flavonoids in tribes Calenduleae and Arctotideae to make use of them as taxonomic markers. Meanwhile, we could notice that only flavonols could be detected in *G. nivea* (tribe Arctotideae) and *D. ecklonis* (tribe Calenduleae) but other classes of flavonoids were absent, which may be of chemotaxonomic interest. The presence of the rare flavonol mearnsetin-3-O- β -D-glucoside is in contrary to that previously reported which was the absence of flavonols with trihydroxylated B-ring pattern in the family Compositae. (38) Concerning the phenolic acids, tricaffeoyl quinic acid was isolated only from *G. nivea* which belongs to tribe Arctotideae, while the mono and dicaffeoyl quinic acids were isolated from the three plants under investigation.

The analgesic, antipyretic and anti-inflammatory effects: The observed data revealed that all the

tested extracts showed significant analgesic, antipyretic and anti-inflammatory effects when compared to control group as shown in Tables (2-4). The most potent extract was the ethanolic extract of the flowers of *H. bracteatum*. So, the petroleum ether and ethyl acetate fractions of this extract were evaluated for these activities. The ethyl acetate fraction showed a higher activity than the petroleum ether fraction. While many flavonoids isolated from the ethyl acetate fraction were reported to exhibit anti-inflammatory activity such as naringenin (**2**), apigenin (**3**), eriodictyol (**4**), luteolin (**6**) and orientin (**11**) (39), no studies were found concerning the anti-inflammatory activity of the aurone; bractein (**7**) and the phenolic acids; 3,4-dicaffeoyl quinic acid methyl ester (**8**), 3,5-dicaffeoyl quinic acid methyl ester (**9**) and 3,5-dicaffeoyl quinic acid (**10**) which were isolated from the ethyl acetate fraction of the flowers of *H. bracteatum* in considerable amounts. Thus, these compounds were tested for the anti-inflammatory activity and it was found that all had significant anti-inflammatory effect at a dose of 50 mg/kg, when compared to the control group as shown in Table (2). Bractein (**7**) was the most potent (85.14% of that of indomethacin).

On the other hand, the major flavonoids and phenolic acids isolated from the ethyl acetate of the flowers of *H. bracteatum*; luteolin (**6**), eriodictyol (**4**), bractein (**7**), orientin (**11**), 3,4-dicaffeoyl quinic acid methyl ester (**8**), 3,5-dicaffeoyl quinic acid methyl ester (**9**) and 3,5-dicaffeoyl quinic acid (**10**) were not tested before for the analgesic and the antipyretic activity. They exhibited significant increase in the voltage needed to emit a cry after 1 and 2 hours. 3,5-dicaffeoyl quinic acid (**10**) was the most active compound whose potency was 69.71 % of that of novalgin. Also, all the tested compounds showed significant decrease in the rectal temperature of the yeast-induced hyperthermic rats, but the aurone bractein (**7**) was the most potent and its potency was even higher than that of paracetamol.

The hepatoprotective activity

Early studies of flavonoids investigated their significant hepatoprotective effect.(40) In addition, phenolic acids especially caffeoyl quinic acids were reported to exhibit significant hepatoprotective activity.(35)The chemical analysis revealed that the flowers of the three plants were more rich (TLC investigation) in phenolic compounds than the

Table 1. The yield of the isolated compounds from *H. bracteatum*, *G. nivea* and *D. ecklonis*.

Isolated compounds	Amount isolated in mg/g extract.				
	<i>H. bracteatum</i> flowers	<i>H. bracteatum</i> remaining aerial parts	<i>G. nivea</i> flowers ethyl acetate	<i>G. nivea</i> flowers butanol	<i>D. ecklonis</i> flowers
Chrysoeriol (1)	0.75	-	-	-	-
Naringenin (2)	0.6	-	-	-	-
Apigenin (3)	1	-	-	-	-
Eriodictyol (4)	5.5	-	-	-	-
3',4',5,5',7-Pentahydroxy flavanone (5)	2	-	-	-	-
Luteolin (6)	8.5	3.6	-	-	-
Bractein (7)	10	-	-	-	-
3,4-Dicaffeoylquinic acid-methyl ester (8)	-	1.53	-	-	-
3,5-Dicaffeoylquinic acid-methyl ester (9)	-	2	-	-	-
3,5-Dicaffeoylquinic acid (10)	-	4.7	-	-	4
Orientin (11)	-	7.2	-	-	-
Neochlorogenic acid (12)	-	7	-	12	10.9
Rhamnetin (13)	-	-	2	-	-
1,4,5-tricaffeoylquinic acid (14) hyperoside (15)	-	-	1.1 3	- 1	- -
1,5-Dicaffeoylquinic acid (16)	-	-	-	11	-
Rhamnetin-3-O- β -D-glucoside (17)	-	-	-	2.9	-
Caffeic acid (18)	-	-	-	-	1.5
Mearnsitin-3-O- β -D-glucoside (19)	-	-	-	-	1.6
Isoquercitrin (20)	-	-	-	-	1.8

Table 2. Acute anti-inflammatory activity of *H. bracteatum*, *G. nivea* and *D. ecklonis*.

Group	Type of extract.	% Oedema	
		Mean $\pm$ S.E.	% of change
Control	-	61.3 $\pm$ 2.1	-
<i>H. bracteatum</i> flowers	Aqueous	26.3 $\pm$ 0.5*	57.1
	Ethanollic	25.5 $\pm$ 0.4*	58.4
	Pet. Ether fr.	37.3 $\pm$ 1.1*	39.2
	Ethyl acetate fr.	25.7 $\pm$ 0.8*	58.1
	Compound (7)	26.8 $\pm$ 0.5*	56.3
	Compound (8)	27.4 $\pm$ 0.8*	55.3
	Compound (9)	28 $\pm$ 0.4*	54.3
	Compound (10)	30.1 $\pm$ 0.9*	50.9
<i>H. bracteatum</i> remaining aerial parts	Aqueous	29.7 $\pm$ 0.6*	51.6
	Ethanollic	28.5 $\pm$ 0.7*	53.5
<i>G. nivea</i> flowers	Aqueous	32.2 $\pm$ 0.8*	47.5
	Ethanollic	31.3 $\pm$ 0.4*	48.9
<i>G. nivea</i> remaining aerial parts	Aqueous	45.6 $\pm$ 0.9*	25.6
	Ethanollic	41.2 $\pm$ 0.6*	32.8
<i>D. ecklonis</i> flowers	Aqueous	36.4 $\pm$ 0.9*	40.6
	Ethanollic	35.6 $\pm$ 0.7*	41.9
<i>D. ecklonis</i> remaining aerial parts	Aqueous	39.7 $\pm$ 1.1*	35.2
	Ethanollic	38.1 $\pm$ 1.2*	37.9
Indomethacin	-	21.4 $\pm$ 0.5*	66.1

The extracts were given at a dose of 100 mg/kg body weight. Indomethacin was give at a dose of 20 mg/kg body weight. fr., fraction. ; Significantly different from control group at $p < 0.01$

Table 3. Analgesic activity of *H. bracteatum*, *G. nivea* and *D. ecklonis*

Group	Type of extract	Volts needed before treatment (zero time)	Volts needed after single oral dose			
			One hour		Two hours	
			Mean $\pm$ S.E.	% of change	Mean $\pm$ S.E.	% of change
Control	-	73.6 $\pm$ 1.7	74.1 $\pm$ 1.2	0.7	74.5 $\pm$ 1.4*	1.2
<i>H. bracteatum</i> flowers	Aqueous	77.5 $\pm$ 1.5	131.2 $\pm$ 6.1*	69.3	151.6 $\pm$ 7.3*	95.6
	Ethanollic	78.4 $\pm$ 1.7	138.1 $\pm$ 5.9*	76.2	141.5 $\pm$ 5.7*	80.5
	Pet. Ether fr.	79.4 $\pm$ 1.6	104.9 $\pm$ 4.7*	32.1	118.2 $\pm$ 6.2*	48.9
	Ethyl acetate fr.	77.8 $\pm$ 1.3	121 $\pm$ 5.2*	55.5	158.4 $\pm$ 6.8*	72.5
	Compound (4)	76.4 $\pm$ 6.8	107.2 $\pm$ 4.9*	40.3	129.8 $\pm$ 4.9*	69.9
	Compound (6)	78.9 $\pm$ 1.4	113.4 $\pm$ 5.1*	43.7	131.4 $\pm$ 4.2*	66.5
	Compound (7)	79.1 $\pm$ 1.6	126.8 $\pm$ 5.4*	60.3	139.2 $\pm$ 5.6*	75.9
	Compound (8)	72.9 $\pm$ 1.5	121.3 $\pm$ 5.2*	66.4	145.3 $\pm$ 5.8*	99.31
	Compound (9)	73.1 $\pm$ 1.4	121.5 $\pm$ 3.9*	66.2	144.3 $\pm$ 4.8*	97.4
	Compound (10)	75.1 $\pm$ 1.7	124.6 $\pm$ 4.9*	65.9	150.2 $\pm$ 6.2*	100
	Compound (11)	77.6 $\pm$ 2.1	115.2 $\pm$ 6.1*	48.4	141.5 $\pm$ 6.1*	82.3
<i>H. bracteatum</i> remaining aerial parts .	Ethanollic	79.1 $\pm$ 1.8	143.7 $\pm$ 6.4*	81.7	165.4 $\pm$ 5.8*	109.1
	Aqueous	77.2 $\pm$ 2.1	135.4 $\pm$ 5.7*	75.4	132.3 $\pm$ 4.9*	71.3
<i>G. nivea</i> flowers	Aqueous	76.1 $\pm$ 1.3	117.3 $\pm$ 4.6*	54.1	123.1 $\pm$ 5.1*	61.8
	Ethanollic	74.3 $\pm$ 1.2	128.6 $\pm$ 4.3*	73.1	136.2 $\pm$ 5.2*	83.3
<i>G. nivea</i> remaining aerial parts	Aqueous	75.2 $\pm$ 1.8	104.2 $\pm$ 3.8*	38.6	112.5 $\pm$ 5.7*	49.6
	Ethanollic	77.6 $\pm$ 1.7	121.5 $\pm$ 3.9*	43.7	120.2 $\pm$ 6.3*	54.9
<i>D. ecklonis</i> flowers	Aqueous	76.4 $\pm$ 2.1	102.7 $\pm$ 3.5*	34.4	115.1 $\pm$ 4.3*	50.7
	Ethanollic	74.8 $\pm$ 1.9	108.9 $\pm$ 4.1*	45.6	119.2 $\pm$ 5.2*	59.4
<i>D. ecklonis</i> remaining aerial parts	Aqueous	75.9 $\pm$ 1.6	112.4 $\pm$ 5.2*	48.1	113.9 $\pm$ 5.9*	50.1
	Ethanollic	76.8 $\pm$ 1.4	116.5 $\pm$ 6.1*	51.7	118.6 $\pm$ 4.9*	54.4
Novalgin	-	74.9 $\pm$ 1.6	158.4 $\pm$ 5.7*	111.5	182.3 $\pm$ 6.4*	143.4

The extracts were given at a dose of 100 mg/kg body weight. Novalgin was give at a dose of 50 mg/kg body weight. fr., fraction.

* Significantly different from zero time at $p < 0.01$. % of change calculated as regard zero time.

Table 4. Antipyretic activity of *H. bracteatum*, *G. nivea* and *D. ecklonis*

Group	Type of extract	Induced rise in temperature	Body Temperature change			
			One hour		Two hours	
			Mean $\pm$ S.E.	% of change	Mean $\pm$ S.E.	% of change
Control	-	38.6 $\pm$ 0.2	38.9 $\pm$ 0.3	0.8	39.1 $\pm$ 0.4	1.3
<i>H. bracteatum</i> flowers	Aqueous	39.8 $\pm$ 0.2	38.1 $\pm$ 0.2*	4.3	36.9 $\pm$ 0.3*	6.0
	Ethanollic	39.6 $\pm$ 0.3	37.6 $\pm$ 0.1*	5.8	37.5 $\pm$ 0.1*	6.8
	Pet. ether	39.0 $\pm$ 0.4	38.9 $\pm$ 0.3*	0.3	38.6 $\pm$ 0.2*	1.02
	Ethyl acetate	39.3 $\pm$ 0.2	38.4 $\pm$ 0.3*	2.3	37.1 $\pm$ 0.1*	5.6
	Compound (4)	38.9 $\pm$ 0.2	38.2 $\pm$ 0.2*	1.8	37.1 $\pm$ 0.1*	4.6
	Compound (6)	39.1 $\pm$ 0.3	38.1 $\pm$ 0.2*	2.6	37.2 $\pm$ 0.1*	4.9
	Compound (7)	40.2 $\pm$ 0.4	38.6 $\pm$ 0.4*	4	36.9 $\pm$ 0.1*	8.2
	Compound (8)	38.7 $\pm$ 0.2	38.1 $\pm$ 0.2*	1.6	37.6 $\pm$ 0.1*	3.3
	Compound (9)	38.9 $\pm$ 0.1	38.1 $\pm$ 0.2*	2.1	37.5 $\pm$ 0.1*	3.6
	Compound (10)	39.6 $\pm$ 0.4	38.2 $\pm$ 0.3*	3.5	36.9 $\pm$ 0.04*	6.8
	Compound (11)	39.4 $\pm$ 0.4	38.6 $\pm$ 0.2*	2	37.3 $\pm$ 0.1*	5.3
<i>H. bracteatum</i> remaining aerial parts .	Aqueous	39.1 $\pm$ 0.4	37.9 $\pm$ 0.1*	3.1	37.3 $\pm$ 0.1*	4.6
	Ethanollic	39.5 $\pm$ 0.2	38.4 $\pm$ 0.2*	2.8	37.8 $\pm$ 0.1*	4.8
<i>G. nivea</i> flowers	Aqueous	38.7 $\pm$ 0.3	38.1 $\pm$ 0.1*	1.6	37.9 $\pm$ 0.2*	2.1
	Ethanollic	39.4 $\pm$ 0.3	38.4 $\pm$ 0.3*	2.5	38.1 $\pm$ 0.3*	3.3
<i>G. nivea</i> remaining aerial parts	Aqueous	39.3 $\pm$ 0.4	38.6 $\pm$ 0.1*	1.8	38.1 $\pm$ 0.2*	3.1
	Ethanollic	38.8 $\pm$ 0.3	38.5 $\pm$ 0.2*	0.8	37.9 $\pm$ 0.1*	2.3
<i>D. ecklonis</i> flowers.	Aqueous	38.9 $\pm$ 0.1	38.1 $\pm$ 0.2*	2.1	37.5 $\pm$ 0.1*	3.6
	Ethanollic	39.1 $\pm$ 0.2	38.2 $\pm$ 0.1*	2.3	37.6 $\pm$ 0.1*	3.8
<i>D. ecklonis</i> remaining aerial parts	Aqueous	39.5 $\pm$ 0.4	38.1 $\pm$ 0.3*	3.5	37.8 $\pm$ 0.2*	4.3
	Ethanollic	39.2 $\pm$ 0.4	38.2 $\pm$ 0.1*	2.6	37.2 $\pm$ 0.2*	3.3
Paracetamol	-	39.4 $\pm$ 0.3	37.2 $\pm$ 0.1*	5.6	36.5 $\pm$ 0.03*	7.3

The extracts were given at a dose of 100 mg/kg body weight. Paracetamol was give at a dose of 100 mg/kg body weight. fr., fraction. * Significantly different from zero time at $p < 0.01$.; % of change calculated as regard zero time.

Table 5. Effect of extracts of the flowers of *H. bracteatum*, *G. nivea* and *D. ecklonis* on the serum AST, TLT and ALP level

Enzyme	Time	Control	Silymarin	<i>H. bracteatum</i> Vent.			<i>G. nivea</i> DC.			<i>D. ecklonis</i> DC.			
				Aqueous	Ethanolic	Ethyl acetate fr.	Aqueous	Ethanolic	Ethyl acetate fr.	<i>n</i> -Butanol fr.	Aqueous	Ethanolic	Ethyl acetate fr.
AST (U/L)	Zero	28.3±1.2	28.9±0.8	27.8±0.3	29.3±0.7	26.9±0.4	29.4±0.4	30.1±1.1	27.6±0.8	29.8±0.6	29.1±1.1	28.6±0.8	28.9±0.7
	30 day	27.4±0.6	26.7±1.2	25.3±0.6	26.2±0.9	27.1±0.6	29.2±0.7	29.4±0.6	27.1±0.9	29.4±0.5	27.5±0.8	27.8±0.9	28.1±0.6
	72 h. after liver damage	106.5±2.9	38.5±1.4*	49.8±0.7*	45.6±1.4*	47.3±2.8*	65.6±2.1*	58.9±1.4*	47.2±1.4*	63.4±1.2*	74.5±2.3*	69.3±2.4*	56.2±1.3*
	30 day after liver damage	138.1±4.5	25.3±0.5*	37.6±0.9*	32.4±1.1*	38.7±1.2*	48.3±1.4*	41.2±1.3*	32.6±1.1*	39.1±1.4*	46.2±1.5*	53.8±1.2*	40.2±1.5*
ALT (U/L)	Zero	29.2±0.8	27.6±0.6	32.4±1.2	31.6±1.1	29.4±0.3	31.2±1.1	29.8±0.5	32.9±1.1	30.4±1.3	26.9±0.6	28.8±0.7	30.9±0.8
	30 day	28.6±0.7	27.3±0.6	29.9±0.8	30.9±1.3	29.6±0.7	30.4±0.6	28.3±0.9	33.5±1.2	29.6±0.5	27.1±0.2	27.9±1.5	31.1±1.1
	72 h. after liver damage	112.8±5.1	39.1±0.8*	46.8±1.3*	52.1±1.6*	51.2±1.8*	67.2±2.3*	63.4±2.1*	49.8±1.6*	51.2±1.7*	76.1±2.3*	74.5±2.6*	62.3±2.8*
	30 day after liver damage	124.7±4.3	23.4±0.3*	38.2±1.2*	35.4±0.9*	32.9±1.2*	38.9±1.2*	43.2±1.7*	32.1±0.9*	35.1±1.1*	48.2±1.4*	45.1±2.3	39.4±1.6
ALP (U/L)	Zero	7.5±0.3	7.3±0.1	7.9±0.1	8.1±0.2	7.2±0.2	7.7±1.2	8.4±0.1	7.4±0.1	6.9±0.1	7.2±0.4	7.5±0.9	7.1±0.1
	30 day	7.6±0.2	7.1±0.2	7.6±0.1	7.8±0.2	7.1±0.3	7.5±0.3	8.2±0.2	7.5±0.2	7.1±0.2	7.1±0.6	7.5±0.1	7.2±0.1
	72 h. after liver damage	30.2±0.6	10.2±0.4*	14.1±0.3*	16.2±0.9*	15.6±0.6*	18.9±0.8*	18.6±0.2*	17.2±0.8*	16.8±0.9*	19.5±0.4*	22.3±1.2*	19.7±1.2*
	30 day after liver damage	32.1±0.9	6.4±0.2*	8.2±0.2*	9.3±0.4*	9.2±0.5*	15.2±0.9*	14.6±1.2*	12.8±0.9*	11.9±0.8*	16.1±1.2*	18.8±1.1*	13.5±1.1*

*Statistically significant different from control at $p<0.01$

the remaining aerial parts. So, the aqueous and ethanolic extracts of the flowers of the investigated plants as well as the ethyl acetate and *n*-butanol fractions which were found to be rich in these phenolic compounds were tested for the hepatoprotective activity. Liver damage by 25% CCl₄ (5 ml / kg) led to a significant rise in AST, ALT and ALP. A daily dose of all the tested extracts (100 mg/kg b. wt.) showed no significant change in AST, ALT and ALP levels after one month administration. Administration of these extracts for another month after induction of liver damage leads to a significant decrease in the liver enzymes levels from the control group. It is obvious (Table 5) that the ethanolic extract of the flowers of *H. bracteatum* was the most active. The ethyl acetate fractions of the ethanolic extracts of the flowers of *G. nivea* and *D. ecklonis* as well as *n*-butanol fraction of the ethanolic extract of the flowers of *G. nivea* were more active than the aqueous and ethanolic extracts of the same plants. Rhamnetin(39) and hyperoside(41) were reported to possess hepatoprotective activity, therefore, they may participate in the potent hepatoprotective activity of the ethyl acetate and *n*-butanol fractions of the ethanolic extracts of the flowers of *G. nivea* DC. On the other hand, the activity of the ethyl acetate fraction of the ethanolic extract of *D. ecklonis* DC. may be due to the presence of caffeic acid (42) and 3,5-dicaffeoyl quinic acid (35). Meanwhile, 3,5-dicaffeoyl quinic acid (35) may be responsible also with naringenin and luteolin⁽³⁹⁾, for the hepatoprotective activity of the ethanolic extract of *H. bracteatum*.

Determination of the LD₅₀

The LD₅₀ was found to be 8.9, 7.8, 8.1 and 8.7 g/kg for the alcoholic and aqueous extracts of the flowers and remaining aerial parts of *H. bracteatum*, respectively, 6.8, 6.5, 7.6 and 8.2 g/kg for the alcoholic and aqueous extracts of the flowers and remaining aerial parts of *G. nivea*, respectively, 6.3, 6.7, 6.5 and 6.8 g/kg, for the alcoholic and aqueous extracts of the flowers and remaining aerial parts of *D. ecklonis*, respectively, 8.5, 6.4 and 6.1 g/kg for the ethyl acetate fraction of the ethanolic extract of the flowers of *H. bracteatum*, *G. nivea* and *D. ecklonis*, respectively. Therefore, the tested extracts were found to be of high safety margin at the tested dose level.

REFERENCES

1. H. Rimpler, L. Langhammer, and H. J. Frenzel. Pigments of *Helichrysum bracteatum*. Distribution of the C₁₅ bodies within the plants. *Planta Med.* **11**: 325-31 (1963) [60: 5892 (1963)].
2. H. P. Kaufmann and A. El-Baya. Pro- and antioxidants in the field. XXIV. Their Effect on the biosynthesis of aurones in the blossom. *Fette, Seifen, Anstrichm.* **71** (1): 25-8 (1969) [70: 103684 a (1970)].
3. G. Forkmann. 5,7,3',4',5'-Pentahydroxyflavanone in the bracts of *Helichrysum bracteatum*. *Biosci.* **38 c** (11-12): 891-3 (1983) [100: 82762 v (1984)].
4. R. Gunasegaran, A. Ubeda, M. J., Alcaraz, R. Jayaprakasan, and A.G Nair. Acylated flavonol glucosides and other phenolics of *Helichrysum bracteatum*. *Pharmazie* **48** (3): 230-1 (1993).
5. R. Sivakumar, A.G. R Nair, and K. P. Madhusudhanan. Polyphenolic components of flowers of *Helichrysum bracteatum*, *Curr. Sci.* **69** (1): 23-4 (1995) [123: 280782 (1995)].
6. K. S. Cholponaev, V. E. Ruchkin, N. A. Tyukevkina, G. G. Zapesochnaya, V. A. Kurkin, and E. S. Matyev. Caffeoylquinic acids in aqueous extracts of *H. italicum* flowers. *Zdravookhr Kirg.* **2**: 29-30 (1990). [113: 129374 f (1990)].
7. G. G. Zapesochnaya, T. V. Dzyadevich and B. S. Karasartov. Phenols of *H. italicum*. *Khim. Pirir. Soedin.* **3**: 409-10 (1990). [114: 98154 y (1991)].
8. G. G. Zapesochnaya, V. A. Kurkin, T. V. Kudryavtseva, B. S. Karasatov, K. S. Choponbaev, N. A. Tyukevkina, and V. E. Ruchkin. Dicafeoylquinic acids from *H. italicum* and *Achillea cartilaginea*. *Khim. Pirir. Soedin.* **1**: 50-5 (1992). [135: 329232 z (2001)].
9. A. V. Martynov, M. V. Smilyanskaya, N. V. Popova, V. S. Kislichenko, I. O. Kozhukh, O. O. Volkovich, and O. Y. Tkachenko. Protein from *Helichrysum* has antiviral against vesicular stomatitis virus. *Farmatsevtichnii Zhurnal* **4**: 74-7 (2001) [136: 177512 w (2002)].
10. A. Sala, M. Del Carmen Recio R. M. Giner, S. Manes, H. Tournier, G. Schinella, and J. L. Rios. Anti-inflammatory and antioxidant properties of *H. italicum*. *J. Pharm. and Pharmacol.* **54** (3): 365-71 (2002).
11. V. Leko. Silymarin-herbal composition for protection and regeneration of structure and function of the liver. *PCT Int. Appl. Wo* 03 35, 087 (Cl. A61 k35/78) 1 May 2003, HR Appl. 20, 020, 361, 25 Apr. 2002 [Through C.A. 138: 343909 u (2003)].
12. A. V. Martynov, M. V. Smelyans'ka, I. O. Kozhukh, O. O. Volkovich, N. V. Popova, and V. S. Kislichenko. The antineoplastic action of glycoproteins similar to Ig G. *Farm. Zh.* **5**: 78-81 (2000) [134: 216930 m (2001)].
13. G. A. Winter, E. A. Risley and G. W. Nuss. Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* **3**: 1544-547, (1962).
14. R. Charlier, H. Prost, F. Binon, and G. Dellous. Pharmacology of an antitussive, 1-phenethyl-4-(2-propenyl)-4-propinoxypipridine acid fumarate. *Arch. Intern. Pharmacodynamic.* **134**: 306-327, (1961).

15. J. Tomazetti, D. S. Avila, A. P. Ferreira, J. S. Martins, F. R. Souza, C. Royer, M. A. Rubin, M. R. Oliveira, H. G. Bonacorso, M. A. Martins, N. Zanatta, C. F. Mello. Baker yeast-induced fever in young rats: characterization and validation of an animal model for antipyretics screening. *J. Neurosci Methods*. **147**(1): 29-35 (2005).
16. C. D. Klassan and G. L. Plaa. Comparison of the biochemical alteration elicited in liver of rats treated with CCl₄ and CHCl₃. *Toxic. Appl. Pharmacol.* **18**: 2019 (1969).
17. W. Thewfweld. Enzymatic method for determination of serum AST and ALT. *Dtsch. Med.* **99**: 343 (1974).
18. P. R. Kind and E. G. King. A colourimetric method for the determination of serum alkaline phosphatase. *J. Clin. Path.* **7**: 322 (1954).
19. G. Karber. Determination of LD₅₀. *Arch. Exp. Pathol. Pharma.* **162**: 480, (1931).
20. K.R. Markham and V.M. Chari, The Flavonoids: Advances in Research, eds: J.B. Harborne and T.J. Mabry, Chapman and Hall, London (1982).
21. K.R. Markham and H. Geiger, The Flavonoids: Advances in Research since 1986, ed: J.B. Harborne, Chapman and Hill, London (1994).
22. J. T. Mabry, K. R., Markham and M. B. Thomas. *The Systemic Identification of Flavonoids*. Vol. 1 & 2, (Springer-Verlage, New York, 1970).
23. B. H. Koeppen. C-Glycosylflavonoids. The chemistry of orientin and iso-orientin. *Biochem J.* **97**(2): 444-448 (1965).
24. P. K. Agrawal, ed. *Carbon-13 NMR of Flavonoids*, Elsevier, Amsterdam, Oxford, New York and Tokyo, (1989).
25. J. B. Harborne, T. J. Mabry, H. Mabry ed., *The Flavonoids*. vol. 1, (Academic Press, New York, 1975).
26. C. Marais, W. J. Rensburg, D. Ferreira, and J. A. Steenkamp. (S)- and (R)-Eriodictyol-6- β -glucopyranoside, novel keys to the fermentation of rooibos (*Aspalathus linearis*). *Phytochemistry*, **55** (1): 43-9 (2000).
27. A. N. Kesari, R. K. Gupta, and G. Watal. Two aurone glycosides from heartwood of *Pterocarpus santalinus*. *Phytochemistry*, **65**: 3125-29 (2004).
28. H. El-Askary. Flavonoids of *Scorzonera tortuosissima* and its spectrophotometric determination. *Bull. Fac. Pharm. Cairo Univ.* **37** (1): 69-73 (1999).
29. S. M. Ezzat. *Pharmacognostical study of certain Foeniculum species growing in Egypt*. M.Sci. Thesis, Cairo University, Cairo, Egypt (2003).
30. A. Braca, N. Tommasi, J. Mendez, I. Moreli, and C. Pizza. Three flavonoids from *Licania heteromorpha*. *Phytochemistry*. **51**(8): 1121-24 (1999).
31. A. Braca, N. De Tommasi, J. Mendez, and I. Morelli. Flavonoids and triterpenoids from *Licania heteromorpha* (Chrysobalanaceae). *Biochem. Syst. Ecol.* **27**(5): 527-30 (1999).
32. E. Abdel-Sattar, M. A., Kohiel, I. A. Shehata, O. A. Rashwan, and H. El-Askary. Phenolic constituents of *Gymnarrhena micrantha* Desf. *Bull. Fac. Pharm. Cairo Univ.* **38**(1):137-140 (2000).
33. N. Nakatani, S. Kayano, H. Kikuzaki, K. Sumino, K. Katagiri, and T. Mitani. Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in Prune (*Prunus domestica* L.). *J. Agric. Food Chem.* **48** (11): 5512-16(2000).
34. S. Papajewski, B. Vogler, J. Conrad, I. Klaiber, G. Roos, C.U. Walter, R. Suzmuth, and W. Kraus. Isolation from *Cussonia barteri* of 1'-O-chlorogenoylchlorogenic acid and 1'-O-chlorogenoylneochlorogenic acid, a new type of quinic acid esters. *Plant. Med.* **67**: 732-36 (2001).
35. P. Basnet, K. Matsushige, K. Hase, S. Kadota. Potent antihepatotoxic activity of dicaffeoylquinic acids from propolis, *Biol. Pharm. Bull.* **19** (1996) 1479-1484.
36. E. Ohkoshi, S. Kamo, M. Makino and Y. Fujimoto. ent-Kaurenoic acids from *Mikania hirsutissima* (Compositae). *Phytochemistry* **65** (7):885-90 (2004).
37. I. Merfort. 1,4,5-Tricaffeoylquinic acid and new flavonoid glycosides from arnica flos DAB 9. *Planta Medica.* **55**: 608 (1989).
38. V. H. Heywood, J. B. Harborne, and B. L. Turner. *The Biology and Chemistry of the Compositae*, (Academic Press, London, New York, San Francisco, A Subsidiary of Harcourt Brace Jovanovich Publishers, 1977).
39. J. Duke. Dr. Duke's Phytochemical and Ethnobotanical Databases. <http://www.ars-grin.gov/duke/>
40. K. Horvathova, A. Vachalkova, and L. Novotny. Flavonoids as chemoprotective agents in civilization diseases. *Neoplasma.* **48**(6): 435-41 (2001).
41. I. Mayumi. Hepatoprotective compounds from *Canarium album* and *Euphorbia nematocarpa*. *Chem. Pharm. Bull.* **38**(8): 2201-2203, (1990).
42. H. M. Chang, H. W. Yeung, W. W. Tso, and A. Koo. *Advances in Chinese Medicinal Materials*, (World Scientific Publishing Co., Philadelphia Pa., 1985) pp. 210. [through <http://www.ars-grin.gov/cgi-bin/duke/>]