

Chemical Constituents and Antioxidant Activity of *Citrus paradisi* (Star-ruby Red Grapefruit) and *Citrus sinensis* (Blood Sweet Orange) Egyptian Cultivars

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Eleven known coumarins: osthol, xanthotoxin, bergapten, isopimpinellin, scoparone, limettin, auraptene, scopoletin, bergaptol and umbelliferone were isolated for the first time from *n*-hexane fraction of peels of star ruby red grapefruit Egyptian cultivar, while only six coumarins: isopratorin, osthol, bergapten, scoparone, limettin and bergaptol were isolated from *n*-hexane fraction of peels of blood sweet orange Egyptian cultivar. In addition, two steroids: β -sitosterol and stigmasterol were isolated from both plants.

Three flavonoid glycosides were isolated from *n*-butanol fraction of star-ruby red grapefruit: poncirin, neohesperidin and naringin, whereas didymin, hesperidin and narirutin were isolated from blood sweet orange *n*-butanol fraction. Nobiletin and tangeretin were isolated from the dichloromethane fractions in both plants.

The volatile oils of peels of star-ruby red grapefruit and blood-sweet orange cultivars were analyzed by GC/Mass. Limonene was identified as the major constituent in both *Citrus* species: 46 and 92% respectively.

Study of the antioxidant activity of different extracts of both *Citrus* cultivars revealed that the alcoholic extracts of both cultivars showed the highest antioxidant activity.

Key Words: *Citrus paradisi*, *Citrus sinensis*, Chemical constituents, Antioxidant activity, Egyptian cultivar.

INTRODUCTION

Citrus fruits have been collected and used by man for centuries for medicinal, herbal and agricultural purposes^{1,2}. Their constituents of coumarins³⁻⁶, flavonoids⁷⁻¹¹ and terpenoids¹²⁻¹⁵ with their different biological and pharmacological properties^{3,16,17} make them of special importance. *Citrus paradisi* (grapefruit) and *Citrus sinensis* (sweet orange) are important crops in Egypt which are cultivated with different *Citrus* species giving different hybrid cultivars, where their constituents might vary in their ratios or even presence from the original unhybrid species².

The two cultivars star-ruby red grapefruit and blood-sweet orange Egyptian cultivars have not been studied before for their coumarin and flavonoid contents, which prompted the author to undertake a detailed investigation of their chemical constituents.

EXPERIMENTAL

Plant material

Fruits of *Citrus paradisi* (ruby-star red grapefruit) and *C. sinensis* (blood-sweet orange) were obtained from fruiting trees cultivated at the Agricultural Crops Research Institute, Giza, Egypt. The two plants were identified by Dr. Salama Eid Salem, the Chief Researcher Hort. Res. of Agricultural Crops Research Institute, Giza, Egypt.

Authentic reference compounds

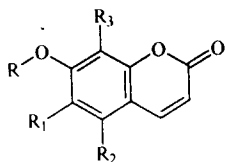
All the eleven coumarins, *e.g.*, auraptene, auraptene, bergapten, bergaptol, isoisopratorin, limettin, osthol isopimpinellin, xanthotoxin, scoparone, umbelliferone, and two steroids, *i.e.*, β -sitosterol and stigmasterol were available in the Department of Chemistry of Natural and Microbial Products, National Research Centre.

Flavonoid glycosides: Hesperidin, neohesperidin, poncirin, naringin, narirutin, didymin, nobilitin and tangeritin were obtained from the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

Special reagents: Iodine-potassium iodide spray reagent¹⁸: 0.2 g I₂ and 4 g KI were dissolved in 100 mL water for detection of coumarins.

Chlorosulphonic acid reagent¹⁹: 5 mL chlorosulphonic acid was added to 10 mL glacial acetic acid with cooling for detection of sterols.

Coumarins:




R = CH₃; R₁ = OCH₃; R₂ = R₃ = H

R = R₁ = R₂ = H; R₃ = OCH₃

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R = R₁ = R₂ = R₃ = H

R = CH₃; R₁ = R₂ = H; R₃ = CH₂CHC(CH₃)₂

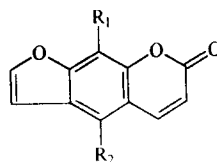
R = CH₃; R₁ = R₂ = H; R₃ = 

R₁ = R₃ = H; R₂ = OCH₃

R = CH₂CHC(CH₃)CH:CH:CHC(CH₃)₂

Scoparone
Scopoletin
Limettin
Umbelliferone
Osthol
Auraptene

Auraptene



R₁ = OCH₃; R₂ = H

R₁ = H; R₂ = OCH₃

R₁ = R₂ = OCH₃

R₁ = H; R₂ = OH

R₁ = H

R₂ = OCH₂CHC(CH₃)₂

Xanthotoxin

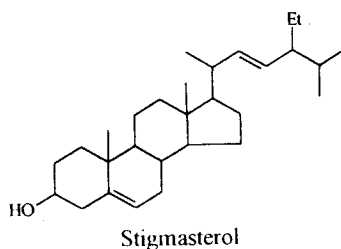
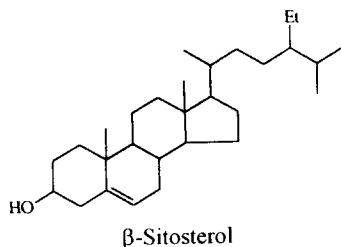
Bergapten

Isopimpinellin

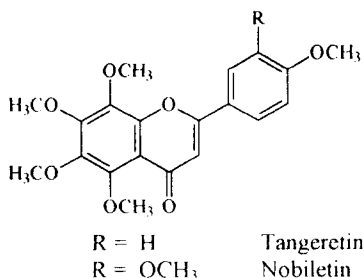
Bergaptol

Isoisopratorin

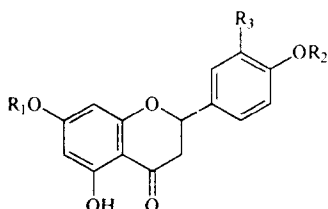
Sterols:



Polymethoxylated flavonoids:



Flavonoid Glycosides:



- | | |
|--|---------------|
| $R_1 =$ rhamnoglucosyl (neohesperidoside); $R_2 = CH_3$; $R_3 = H$ | Poncirin |
| $R_1 =$ rhamnoglucosyl (rutinoside); $R_2 = CH_3$; $R_3 = H$ | Didymin |
| $R_1 =$ rhamnoglucosyl (neohesperidoside); $R_2 = CH_3$; $R_3 = OH$ | Neohesperidin |
| $R_1 =$ rhamnoglucosyl (rutinoside); $R_2 = CH_3$; $R_3 = OH$ | Hesperidin |
| $R_1 =$ rhamnoglucosyl (neohesperidoside); $R_2 = R_3 = H$ | Naringin |
| $R_1 =$ rhamnoglucosyl (rutinoside); $R_2 = R_3 = H$ | Narirutin |

Melting points were determined on Buchi 510 melting point apparatus. UV spectra were done on a Shimadzu UV-Vis spectrophotometer. 1H -NMR spectra were recorded on a Jeol-EX 270 in DMSO- D_6 . The chemical shifts are referred to internal standard TMS.

GLC for isolation and identification of sterol was carried out using Hewlett Packard (HP) 6890 Series GC system; capillary column HP-1 (30 m \times 250 μ m and 0.25 μ m film thickness); temp. program 70–280°C, 8 min; N_2 as carrier gas at a flow rate of 30 mL/min; chart speed 1.5 mL/min; FID detector.

GC/MS of the volatile oil was performed on a Varian 3400 GC system interfaced with a Finnigan SSQ 7000 mass spectrometer detector and equipped with DB-5 capillary column (30 m \times 0.23 mm and 0.25 μ m of film thickness). Oven temperature maintained at 40°C for 5 min, then raised to 260°C at a rate of 37 min with final hold time of 10 min; injector temperature 220°C; ion source temperature 180°C; carrier gas He at a flow rate of 1 mL/min; chart speed 0.5 cm/min; ionization voltage 70 eV.

Extraction and fractionation

2 kg of powder peels of both star-ruby red grapefruit and blood-sweet orange were extracted with aqueous ethanol (95% followed by 70%). The combined extracts were concentrated under reduced pressure to give two aqueous syrups from both *Citrus* cultivars which were successively extracted with *n*-hexane to yield 19 and 8.5 g residues respectively; methylene chloride (5.8 and 12.5 g),

ethyl acetate (5.2 and 3 g) and *n*-butanol (27 and 20 g) respectively. Two-dimensional paper chromatographic technique²⁰ using the solvent systems [BuOH : HOAc : H₂O, BAW (4 : 1 : 5)] and HOAc (15%) showed that the *n*-hexane fraction contained non-polar chlorophyll, sterols and coumarins; the methylene chloride fractions contained polymethoxylated flavonoids while the ethyl acetate and *n*-butanol fractions contained flavonoid glycosides; all were visualized under UV at 365 nm before and after exposure to NH₃ vapours.

Isolation and identification of coumarins and sterols

The *n*-hexane fraction (19, 8.5 g) of star-ruby red grapefruit and blood-sweet orange Egyptian cultivars were used for the separation of coumarins and sterols on silica gel columns (G60, Merck) eluted with *n*-hexane and benzene in the proportion (4 : 1; 1 : 1 and 1 : 4), benzene and 0.5, 1 and 2% methanol in benzene. Fractions with similar TLC patterns (on pre-coated aluminium-packed silica gel 60F₂₅₄ EM Darmstadt, Germany) were combined together; chromatoplates were visualized under UV at 258nm using benzene : ethyl acetate (8 : 2) as a solvent system, I₂/KI as a spray reagent for the identification of coumarins and chlorosulphonic acid reagent with heating at 110°C for 2 min for identification of sterols, which were eluted as a mixture with 0.5% methanol in benzene and they were analyzed and identified by GLC under the aforementioned conditions.

Identification of the isolated coumarins and sterols was done by direct comparisons with authentic samples including Co-TLC spots and mixed m.p.s (Table 1). Further confirmations of the structures of the isolated compounds were carried out by MS and NMR spectra and compared with the reported data²¹.

TABLE-1
PERCENTAGE YIELDS OF COUMARINS ISOLATED FROM
n-HEXANE EXTRACT OF STAR-RUBY RED GRAPEFRUIT AND
BLOOD-SWEET ORANGE EGYPTIAN CULTIVARS.

| R _f | Fluorescence under UV | Colour with I ₂ /KI | Isolated compounds | m.p. (°C) | Yield (%) | |
|----------------|-----------------------|--------------------------------|--------------------|-----------|----------------------------|--------------------|
| | | | | | Star-ruby red grape fruits | Blood-sweet orange |
| 0.75 | Yellowish green | Brownish orange | Isoimpratorin | 97 | — | 0.12 |
| 0.68 | Blue | Bluish violet | Osthol | 84 | 0.08 | 0.02 |
| 0.65 | Yellowish green | Greyish brown | Bergapten | 188 | 0.28 | 0.13 |
| 0.60 | Yellowish green | Brick red | Xanthotoxin | 145 | 0.19 | — |
| 0.57 | Dark yellow | Intense violet | Isopimpinellin | 148 | 0.16 | — |
| 0.55 | Shining blue | Dark blue | Scoparone | 142 | 0.11 | 0.11 |
| 0.53 | Sky blue | Sky blue | Limettin | 146 | 0.12 | 0.22 |
| 0.50 | Blue | Colourless | Aurapten | 68 | 0.32 | — |
| 0.47 | Blue | Colourless | Auraptene | 98 | 0.64 | — |
| 0.40 | Blue | Brown | Scopoletin | 204 | 0.11 | — |
| 0.35 | Yellowish brown | Dark green | Bergaptol | 276 | 0.12 | 0.31 |
| 0.33 | Blue | Colourless | Umbelliferone | 223 | 0.10 | — |

Isolation and identification of the flavonoids

The dichloromethane fractions (5.8 and 12.5 g) residues of star-ruby red grapefruit and blood-sweet orange Egyptian cultivars, respectively, were used for separation of the polymethoxylated flavonoids on silica gel columns eluted with dichloromethane increasing the polarity gradually with 1% methanol.

Fractions with similar TLC patterns using pre-coated aluminium-packed silica gel 60F₂₅₄-plates and BAW (4 : 1 : 5) as a solvent system and the plates which were visualized under UV at 365 nm before and after exposure to NH₃ vapours, were combined together.

Crystallization from methanol gave two pure compounds; the identification of each compound was done using m.p., UV, NMR and MS spectral data and also by direct comparison with authentic samples (Tables 2, 3).

TABLE-2
PERCENTAGE YIELDS OF POLYMETHOXYLATED FLAVONOIDS ISOLATED BY COLUMN CHROMATOGRAPHY FROM METHYLENE CHLORIDE PEEL EXTRACT OF STAR-RUBY RED GRAPEFRUIT AND BLOOD-SWEET ORANGE (THEIR UV AND m.p. DATA)

| Compound | R _f BAW | Fluorescence | | UV spectra | m.p. (°C) | Yield (%) | |
|------------|-----------------------|--------------|--------------------|---------------------|--------------|-----------------------------|-----------------------|
| | | UV | UV/NH ₃ | | | Star-ruby red grapefruit | Blood-sweet orange |
| Tangeretin | 0.66 | Light blue | Yellow | 270, 320 | 151 | 0.09 | 0.05 |
| Nobiletin | 0.58 | Light blue | Yellow | 248, 277, 332 | 124 | 0.06 | 0.04 |

TABLE-3
¹H-NMR DATA OF THE ISOLATED POLYMETHOXYLATED FLAVONOIDS (DMSO-d₆)

| | Tangeretin | Nobiletin |
|----------------------|----------------------|---------------------------|
| OCH ₃ -5 | 3.78 (3H, s) | 3.79(3H, s) |
| OCH ₃ -6 | 3.80 (3H, s) | 3.81 (3H, s) |
| OCH ₃ -7 | 3.84 (3H,s) | 3.84 (3H, s) |
| OCH ₃ -8 | 3.91 (3H, s) | 3.86 (3H, s) |
| OCH ₃ -3' | — | 3.93 (3H, s) |
| OCH ₃ -4' | 4.02 (3H, s) | 4.03 (3H, s) |
| H-3 | 6.76 (1H, s) | 6.85 (3H, s) |
| H-3' and H-6' | 7.14 (2H, d, 9.5 Hz) | — |
| H-5' | — | 7.16 (1H, d, 8.5 Hz) |
| H-2' | — | 7.54 (1H, d, 2.1 Hz) |
| H-6' | — | 7.61 (1H,dd, 8.5, 2.1 Hz) |
| H-2' and H-6' | 8.0 (2H, d, 9.5, Hz) | — |

The ethyl acetate and *n*-butanol fractions were added together for each individual *Citrus* cultivar (32.2 g for star-ruby red grapefruit and 23 g for blood-sweet orange) and they were used for separation of the flavonoid glycosides on cellulose column (E. Merck, Darmstadt, Germany) and eluted with water methanol step gradient; further isolation and purification of the compounds was carried out on Sephadex LH-20 columns. The isolated compounds were hydrolyzed and the resulting aglycons and sugars were characterized by standard procedure²⁰. The identification of the compounds was carried out by m.p. and UV, NMR and MS spectral data (Tables 4–6).

TABLE-4
PERCENTAGE YIELDS OF FLAVONOID GLYCOSIDES ISOLATED BY
COLUMN CHROMATOGRAPHY FROM EtOAc/*n*-BUTANOL PEEL EXTRACT OF
STAR-RUBY RED GRAPEFRUIT AND BLOOD-SWEET ORANGE

| Isolated compounds | R _f | | Fluorescence | | m.p. (°C) | Yield (%) | |
|--------------------|----------------|------|--------------|--------------------|-----------|--------------------------|--------------------|
| | BAW | HOAc | UV | UV/NH ₃ | | Star-ruby red grapefruit | Blood-sweet orange |
| Naringin | 0.49 | 0.81 | Purple | Light blue | 171 | 3.10 | — |
| Neohesperidin | 0.50 | 0.77 | Purple | Light blue | 262 | 0.25 | — |
| Poncirin | 0.54 | 0.73 | Purple | Light blue | 212 | 0.14 | — |
| Narirutin | 0.46 | 0.80 | Purple | Light blue | 181 | — | 0.09 |
| Hesperidin | 0.51 | 0.78 | Purple | Light blue | 244 | — | 1.15 |
| Didymin | 0.53 | 0.76 | Purple | Light blue | 208 | — | 0.08 |

TABLE-5
UV SPECTRAL DATA OF THE ISOLATED FLAVONOID GLYCOSIDES

| Flavonoid | MeOH | NaOMe | AlCl ₃ | AlCl ₃ + HCl | NaOAc | NaOAc/H ₃ BO ₃ |
|---------------|-------------|----------|-------------------|-------------------------|----------|--------------------------------------|
| Naringin | 283,333 sh | 283, 386 | 301, 383 | 301, 383 | 283, 333 | 283, 333 |
| Neohesperidin | 283, 324 | 284, 350 | 307, 373 | 306, 373 | 285, 337 | 282, 337 |
| Poncirin | 283, 330 sh | 283, 358 | 309, 373 | 309, 373 | 283, 332 | 283, 332 |
| Narirutin | 284, 328 sh | 283, 385 | 308, 383 | 308, 382 | 283, 330 | 285, 330 |
| Hesperidin | 285, 330 | 287, 354 | 308, 377 | 308, 377 | 285, 330 | 285, 332 |
| Didymin | 283, 330 | 283, 360 | 308, 372 | 308, 372 | 283, 330 | 283, 330 |

Preparation and analysis of the oil

Fresh peels 500 g of each star-ruby red grapefruit and blood-sweet orange were submitted to hydro-distillation in a modified distillation extraction head²², which allowed simultaneous extraction of the volatile vapours in an organic solvent (*n*-pentane), dried over anhydrous sodium sulphate and the solvent was removed by a steam of nitrogen on its surface. The obtained oils, 1.5 and 5% (v/w), of fresh peels of star-ruby red grapefruit and blood-sweet orange, respectively, were

TABLE-6
¹H-NMR DATA OF THE ISOLATED FLAVONOIDS GLYCOSIDES

| | Naringin | Neohesperidin | Poncirin | Narirutin | Hesperidin | Didymin |
|--|-----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|-----------------------------------|-----------------------------------|
| CH ₃ rham | 1.13 (d, 6.12 Hz) | 1.12 (d, 6.2 Hz) | 1.13 (d, 6.12 Hz) | 1.0 (d, 6.2 Hz) | 0.98 (d, 6.23 Hz) | 0.96 (d, 6.12 Hz) |
| H-3 | 2.71 (dd, dd, 2.9, 12.7, 17.0 Hz) | 2.70 (dd, dd, 2.9, 12.7, 17.0 Hz) | 2.76 (dd, dd, 2.9, 12.7, 17.0 Hz) | 2.77 (dd, dd, 2.9, 12.7, 17.05 Hz) | 2.76 (dd, dd, 2.9, 12.7, 17.0 Hz) | 2.77 (dd, dd, 2.9, 12.7, 17.0 Hz) |
| 10H-rhamnoglucosyl OCH ₃ -4' | 3.12-3.75 — | 3.12-3.75 3.76 (s) | 3.12-3.75 3.75 (s) | 3.12-3.75 — | 3.12-3.75 3.78 (s) | 3.12-3.75 3.77 (s) |
| H'' | 5.14 (d, 7.3 Hz) | 5.13 (d, 7.5 Hz) | 5.12 (d, 6.12 Hz) | 5.12 (d, 7.31 Hz) | 5.14 (d, 7.48 Hz) | 5.13 (d, 6.1 Hz) |
| H''' | 4.98 (d, 1.17 Hz) | 5.1 (d, 1.14 Hz) | 5.1 (d, 1.14 Hz) | 4.38 (d, 1.14 Hz) | 4.4 (d, 1.14 Hz) | 4.5 (d, 1.14 Hz) |
| H-2 | 5.4 (dd, 2.9, 12.8 Hz) | 5.5 (dd, 2.9, 12.8 Hz) | 5.6 (dd, 2.9, 12.8 Hz) | 5.49 (dd, 2.9, 12.8 Hz) | 5.5 (dd, 2.9, 12.8 Hz) | 5.6 (dd, 2.9, 12.8 Hz) |
| H-6 | 6.06 (d, 2.2 Hz) | 6.09 (d, 2.2 Hz) | 6.01 (d, 2.2 Hz) | 6.01 (d, 2.2 Hz) | 6.02 (d, 2.2 Hz) | 6.02 (d, 2.2 Hz) |
| H-8 | 6.1 (d, 2.2 Hz) | 6.1 (d, 2.2 Hz) | 6.09 (d, 2.2 Hz) | 6.1 (d, 2.2 Hz) | 6.1 (d, 2.2 Hz) | 6.12 (d, 2.2 Hz) |
| H-3' and H-5' | 6.7 (d, 8.8 Hz) | — | 7.3 (d, 9.5 Hz) | 6.5 (d, 8.8 Hz) | — | 6.9 (d, 9.5 Hz) |
| H-2' and H-6' | 7.3 (d, 8.8 Hz) | — | 7.5 (d, 9.5 Hz) | 7.1 (d, 8.8 Hz) | — | 7.4 (d, 9.5 Hz) |
| H-2', H-5' and H-6' | — | 6.94 (m) | — | — | 6.94 (m) | — |

subjected to GC/MS analysis. The identification of the compounds was carried out by comparison of their retention times and mass spectra with those of literature and a computerized MS data bank²³.

Percentage yield of identified components of star-ruby red grapefruit are shown in Table-7.

Antioxidant activity

Antioxidant activity was tested using α,α -diphenyl- β -picryl-hydrazyl (DPPH)²⁴, on the oils, alcohol and alcohol/water extracts of both *Citrus* species.

RESULTS AND DISCUSSION

Seven coumarins: osthol, auraptene, limettin, scoparone, aureptene, scopoletin and umbelliferone in addition to four psoralens: bergaptene, bergaptol, xanthotoxin and isopimpinellin were isolated from *n*-hexane peel concentrate of star-ruby grapefruit Egyptian cultivar. Xanthotoxin and isopimpinellin were isolated for the first time from *C. paradisi* species; the other compounds were detected in peel oil of grapefruit²⁵⁻²⁸.

Three coumarins: osthol, limettin and scoparone were isolated from the *n*-hexane peel extract of blood-sweet orange in addition to three psoralens: isoimpratorin, bergaptene and bergaptol. However, these compounds were detected either in peel oil^{9, 13, 25, 27, 29} or leaf extract³⁰ of sweet orange. They had not been isolated before from peel extract of blood-sweet orange Egyptian cultivar.

Table-1 shows that the major coumarin obtained from the *n*-hexane extract of star-ruby red grapefruit was auraptene 0.64% followed by auraptene 0.32% which differs from the reported data of peel oil where auraptene was in higher concentration than auraptene (0.72 and 0.49% respectively)³¹. The major compound obtained from blood sweet orange was bergaptol.

Two known sterols which were analyzed by GLC and were identified as β -sitosterol and stigmasterol with higher yield from blood-sweet orange 0.56 and 0.52%, respectively than from star-ruby red grapefruit 0.22 and 0.21% respectively.

Two compounds were isolated from methylene chloride extracts of both *Citrus* species and they were identified by UV spectra showing peaks of absorption at λ_{\max} 270 and 320 nm and λ_{\max} 248, 277 and 332 nm (Table-2), where the spectra have not been changed by the addition of different shift reagents identifying that both compounds have no free hydroxyl groups. ¹H-NMR spectra showing different δ values of H-2' H-5' and H-6' with different J-values and the additional singlet peak at δ 3.93 methoxy group at C-3' as shown in Table-3, confirming the structural difference between the two isolated polymethoxylated flavonoids which were identified as tangeritin and nobiletin. They were reported in grapefruit peel oil in lower yield (0.06 and 0.04%, respectively)³¹ together with other polymethoxylated flavonoids in peel oils^{9, 13, 31, 32} and leaf extract^{33, 34} of sweet orange.

Chromatographic separation of ethyl acetate/*n*-butanol extract of the peels of both *Citrus* species afforded six compounds. From chromatographic and hydrolytic data, colour reaction as well as UV spectral analysis in different reagents

(Tables 4, 5), it could be concluded that the isolated compounds are flavonoid glycosides. Acid hydrolysis of these compounds yielded glucose and rhamnose as sugar moieties whereas the aglycones were identified as naringenin, hesperidin and isosakurantin by comparing PC with authentic samples. UV data showed no bathochromic shift in band II with NaOAc indicating the absence of free 7-OH in all isolated compounds confirming the glycosidation at 7-position. The unchanged bathochromic shift of band II on the addition of HCl to the $AlCl_3$ spectra indicated the presence of acid-stable free 5-OH. The absence of hypochromic shift of bands I and II by the addition of boric acid to the NaOAc (compared to the methanol spectra) confirming the absence of two *ortho* free —OH groups in all compounds.

The 1H -NMR of the isolated compounds showed a *dd*, *dd* peak at δ 2.71 and *dd* peaks at 5.5 ppm belonging to H-3, H-2 respectively confirming the flavanone identity of the isolated compounds. The anomeric proton of rhamnose (H-1'') appears as doublet (with small *J* value 1.17 Hz) at δ 4.98–5.10 ppm in neohesperidosides (rhamnosyl α -1 \rightarrow 2-glucose)²⁰ as in naringin, neohesperidin and poncirin which were isolated from ethyl acetate/*n*-butanol extract of star-ruby red grapefruit, while it appears at δ 4.3–4.5 ppm in rutinosides (rhamnosyl α -1 \rightarrow 6-glucose) as narirutin, hesperidin and didymin which isolated from ethyl acetate/*n*-butanol extract of blood-sweet orange. Also, the protons of methyl rhamnose group appear as a doublet with large *J* value (6.12 Hz) at δ 0.96–0.10 ppm in rutinosides while it appears at δ 1.12–1.13 ppm in neohesperidosides. The multiplet peak at δ 6.94 ppm that belongs to 2', 5' and 6' protons confirming the disubstitution of ring B at 3', 4' as shown in the compounds neohesperidin and hesperidin (Table-6).

These data were confirmed by CI^+ -MS which shows a 50% base peak at m/z 581 (M^+), and a 100% peak at m/z 273 (M^+ -308) of the aglycone (naringenin as it loses sugar moiety, 34% of m/z 435 (M^+ -146) and at m/z 419 (M^+ -162) as shown in case of naringin and narirutin, whereas in case of neohesperidin and hesperidin they show 100% base peak at m/z 611 (M^+), 45% at m/z 303 (M^+ -308) of the aglycone (hesperidin as the compound loses the rhamnoglucosyl group), at m/z 465 [M^+ -146 (M-rhamnose)] and m/z 449 [M^+ -162 (M-glucose)], while in case of poncirin and didymin they show 100% base peak at m/z 595 (M^+), 75% m/z 287 of the aglycone isosakurantin, at m/z 448 (M^+ -rhamnose) and at m/z 433 (M^+ -glucose).

The data is in accordance with previously reported data of the structure-bitter taste relationship^{2, 8} as the presence of the neohesperidosyl group in the flavanone nucleus is responsible for the bitter taste as in naringin, neohesperidin and poncirin of star-ruby red grapefruit while its replacement with the rutinosyl group nullifies its bitter taste as in hesperidin, narirutin and didymin of blood-sweet orange. Also this data is in accordance with previously reported classification of *Citrus* being categorized as predominantly containing either rutinosides or neohesperidoside³⁵.

Table-4 shows that the highest percentage of the flavonoid obtained from EtOAc/*n*-butanol extract of star-ruby red grape fruit Egyptian cultivar was that of naringin (3.10%) while hesperidin was the major flavonoid obtained from

blood-sweet orange (1.15%) which is also in accordance with reported data of grapefruit and sweet orange species being the major obtained flavonoids from their fruit juices and peels^{11, 36} as well as from their leaves^{34, 35}

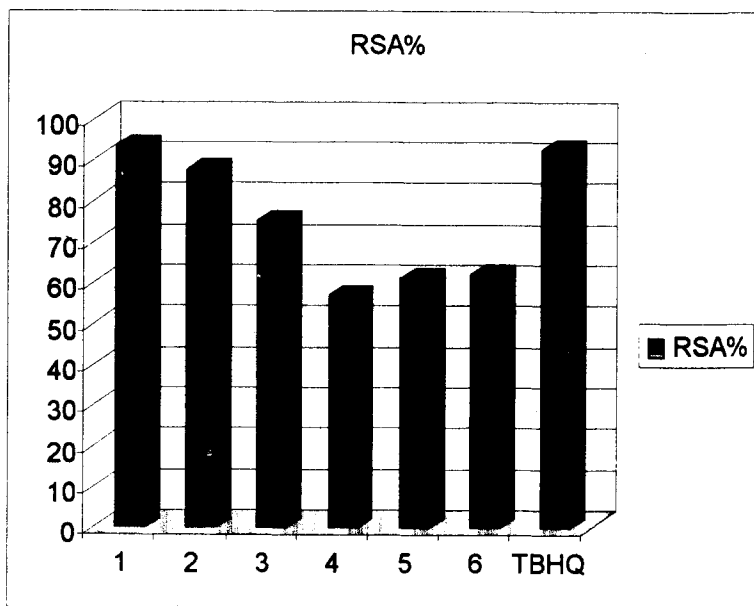
The percentage yield of volatile oils obtained from peels of star-ruby red grape fruit and blood-sweet orange Egyptian cultivars which were 1.5 and 5% v/w fresh weight respectively, were in accordance with the reported data^{37, 38}. GC/MS analysis (Table-7) shows that limonene represents the main constituent of peel oil of star-ruby red grape fruit 46.22% and all other identified constituents represent 40.77% of the peel oil concentrate. However, this is the first report of their percentages.

TABLE-7
GC/MS ANALYSIS OF PEEL OIL OF STAR-RUBY RED
GRAPEFRUIT EGYPTIAN CULTIVAR

| Compound | M ⁺ | R _t min | % |
|------------------------------|----------------|--------------------|-------|
| α-Pinene | 136 | 7.38 | 1.23 |
| Myrcene | 136 | 9.46 | 2.24 |
| Limonene | 136 | 14.22 | 46.22 |
| <i>n</i> -Undecane | 156 | 14.59 | 9.59 |
| <i>trans</i> -Linalool oxide | 170 | 15.25 | 4.43 |
| Linalool | 154 | 15.47 | 3.08 |
| 4-Terpineol | 154 | 18.53 | 0.68 |
| α-Terpineol | 154 | 19.45 | 2.26 |
| <i>n</i> -Decanal | 156 | 20.07 | 2.62 |
| Nerol | 154 | 20.56 | 0.85 |
| Neral | 152 | 21.31 | 1.06 |
| Geraniol | 154 | 22.10 | 1.20 |
| <i>n</i> -Decanol | 158 | 23.06 | 1.58 |
| α-Copaene | 204 | 27.29 | 1.15 |
| Geranyl acetate | 196 | 27.40 | 1.09 |
| β-Cubebene | 204 | 27.59 | 0.93 |
| Caryophyllene | 204 | 29.31 | 3.26 |
| α-Humulene | 204 | 30.53 | 0.62 |
| γ-Cadinene | 204 | 31.59 | 1.92 |
| δ-Cadinene | 240 | 33.34 | 1.92 |

Also limonene is the main constituent of peel oil of blood-sweet orange Egyptian cultivar (92%) which differs from the previously reported (46.07%) in addition to minor unidentified concentrates.

Peel alcoholic extracts of both *C. paradisi* and *C. sinensis* Egyptian cultivars were found to have higher activity as radical scavengers (93.4 and 87.4%, respectively) than the other extracts of both species. Their activity may be attributed to their flavonoid contents according to the reported data^{16, 39, 40} (Fig. 1).



1 = alc. ext. of *C. paradisi*, 2 = alc. ext. of *C. sinensis*
 3 = alc-water ext. of *C. paradisi*, 4 = alc-water ext. of *C. sinensis*
 5 = oil of *C. paradisi* 6 = oil of *C. sinensis*
 RSA (%) = radical scavenging activity TBHQ = tert. butyl hydroquinone

Fig. 1. Antioxidant activity of different extracts of *C. paradisi* and *C. sinensis* Egyptian cultivars

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REFERENCES

1. El-Antaki Dawood, Tazkret Oli El-Albab, El-Baby El-Halaby & Co., Cairo, Vol I (1952).
2. P. G. Waterman and M.F. Grondon, Chemistry and Chemical Taxonomy of the Rutales, Academic Press Inc., New York (1983).
3. R.D.H. Murray, J. Mendez, and S.A. Brown, The Natural Coumarins: Occurrence, Chemistry and Biochemistry, John Wiley & Sons, New York (1983).
4. M.A. Abdel-Alim, and E.A.M. El-Khrisy, *Bull. NRC*, **16**, 81 (1991).
5. D. McHale, P.P. Khopkar and J.B. Sheridan, *Phytochemistry*, **26**, 2547 (1987).
6. A.I. Gray, and P.G. Waterman, *Phytochemistry*, **17**, 845 (1978).
7. J.B. Harborne, T.J. Mabry, and H. Mabry, The Flavonoids, Chapman & Hall, London (1975).

8. R.M. Horowitz and B. Shaw, in: S. Nagy, P.E. Shaw and M.K. Veldhuis (Eds.), *Citrus Science and Technology*, Vol. I, Avi Publishing Co., Inc., Westport, Connecticut (1977).
9. T.H. Tatum and R.E. Berry, *Phytochemistry*, **11**, 2283 (1972).
10. S.M.I. Mustafa, I.A. Attia and N.A. Hassan, *Fitoterapia*, **55**, 295 (1984).
11. F.J. Arriaga and A. Rumbero, *Fitoterapia*, **61**, 31 (1990).
12. M.G. Moshonas and P.E. Shaw, *J. Agric. Food Chem.*, **26**, 1288 (1978).
13. P. Dugo, L. Mandello, E. Cogliandro, A. Verzera and G. Dugo, *J. Agric. Food Chem.*, **44**, 544 (1996).
14. D. Chouchi, D. Barth, E. Reverchon and G.P. Delia, *J. Agric. Food Chem.*, **44**, 1100 (1996).
15. M. Gabor, *Handbook of Experimental Pharmacology*, Verlag, New York (1979).
16. J.A. Manthey and K. Grohmann, *Flavonoids in the Living System*, Plenum Press, New York (1998).
17. E. Middleton (Jr.), C. Kandaswami and T.C. Theoharides, *Pharmacol. Rev.*, **52**, 673 (2000).
18. N.R. Fransworth, *J. Pharm. Sci.*, **5**, 255 (1966).
19. E. Stahl, *Thin Layer Chromatography*, Springer-Verlag, New York, 2nd Edn. (1969).
20. T.J. Mabry, K.R. Markham, and M.B. Thomas, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York-Heidelberg-Berlin (1970).
21. B.E. Nielsen, in: V.H. Haywood (Ed.), *The Biology and Chemistry of Umbelliferae*, Academic Press, London (1971).
22. T.H. Schultz, R.A. Flath, T. Richard Man, S.B. Egging and R. Teranishi, *J. Agric. Food Chem.*, **25**, 448 (1977).
23. R.P. Adams, *Identification of Essential Oils by Ion Trap Mass Spectrometry*, Academic Press (1989).
24. N. Nenadis and M. Tsimidou, *J. Am. Oil Chem. Soc.*, **79**, 1191 (2002).
25. W.L. Stanley and L. Jurd, *J. Agric. Food Chem.*, **19**, 1106 (1971).
26. J.H. Tatum and R.E. Berry, *Phytochemistry*, **16**, 1091 (1977).
27. J.F. Fisher and L.A. Trama, *J. Agric. Food Chem.*, **27**, 1334 (1979).
28. J.F. Kefford and B. V. Chandler, *The Chemical Constituents of Citrus Fruits*, Academic Press, New York, pp. 105–111 (1970).
29. G. D'Amore and R. Calapaj, *Rassegna Chimica*, **6**, 264 (1965).
30. E.G. Haggag, I.I. Mohmoud, E.A. Abu-Mostafa and T.J. Mabry, *Asian J. Chem.*, **11**, 784 (1999).
31. D. McHale and J.B. Sheridan, *J. Ess. Oil Res.*, **1**, 139 (1989).
32. E.M. Gaydou, J.P. Bianchini and R.P. Randriamiharisoa, *J. Agric. Food Chem.*, **35**, 525 (1987).
33. M. Piatelli and G. Impellizzeri, *Phytochemistry*, **10**, 2657 (1971).
34. F.G. Haggag, I.I. Mahmoud, E.A. Abu-Mostafa, and T.J. Mabry, *Asian J. Chem.*, **11**, 707 (1999).
35. K. Kanes, B. Tisserat, M. Berhow and C. Vandercook, *Phytochemistry*, **32**, 967 (1993).
36. W.C. Oogh, S.J. Oogh, C.M. Detavernier and A. Huyghebaert, *J. Agric. Food Chem.*, **42**, 2183 (1994).
37. E.P. Claus and V.E. Tyler (Jr.), *Pharmacognosy*, 5th Edn., Henry Krimpton, London (1965).
38. M.S. Karaway, S.I. Balbaa and M.S. Hifhawy, *J. Pharm. Sci.*, **60**, 381 (1971).
39. C. Armando, S. Mathye and N.P. Beatriz, *J. Sci. Food Agric.*, **77**, 463 (1998).
40. B. Tirillini, *Fitoterapia*, **71**, S29 (2000).