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# FLAVONOID GLYCOSIDES FROM THE LEAVES OF *SALVADORA PERSICA* L.

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تم فصل والتعرف على الجليكوزيدات الفلافونيدية لأوراق نبات السواك حيث أمكن بإستخدام كروماتوجرافيا السائل ذو الكفاءة العالية فصل أحدى عشر مركبا ، وجد أن سبعة مركبات منهم: أيزورامنتين-٣-أ-روبينوبيوزيد ، كامبيفيرول-٣-أ-روبينوبيوزيد ، نارسيسين ، كامبيفيرول-٣-أ-روتينوزيد ، أيزورامنتين-٣-أ-ب-د-جالاكتوزيد ، أستراجلين وأيزورامنتين-٣-أ-ب-د-جلوكوزيد يتم فصلهم لأول مرة من جنس السلفادورا والأربعة الباقيين أيزورامنتين-٣-(٢، ٦-داى رامنوبيرانوزيل جالاكتوبيرانوزيد) ، موريتيانين ، أيزورامنتين-٣-أ-(٢-جلوكو رامنوزيل ريتينوزيد) وكامبيفيرول-٣-أ-(٢-جلوكو رامنوزيل ريتينوزيد) يفصلوا لأول مرة من العائلة السلفادورية. وقد تم التعرف على هذه المركبات عن طريق التحاليل الطيفية المختلفة ودراسة خواصها الكروماتوجرافية ودرجة أنصهارها ومقارنتها بعينات قياسية.

*From the leaves of Salvadora persica L. eleven flavonoidal glycosides are isolated and identified. Seven of them: Isorhamnetin-3-O-robinobioside, Kaempferol-3-O-robinobioside, Narcissin, Kaempferol-3-O-rutinoside, Isorhamnetin-3-O-β-galactoside, Astragalin and Isorhamnetin-3-O-β-D-glucoside are isolated for the first time from the genus Salvadora. While the others four are identified as: Isorhamnetin-3-(2,6-di-rhamnopyranosyl-galactopyranoside), Mauritianin, Isorhamnetin-3-O-(2-Glc-rhamnosylrutinoside) and kaempferol-3-O-(2 Glc-rhamnosylrutinoside) and reported for the first time in family Salvadoraceae.*

## INTRODUCTION

*Salvadora persica* L. (*Salvadoraceae*) is a native plant to Egypt, Saudi Arabia, India and Palestine.[1] The plant has been used in Folk medicine as antirheumatic, stimulant and as a remedy for diabetes, gastritis and gonorrhoea. In addition, the roots of the plant are used as toothbrush.[2-4]

Abdel-Wahab *et al.* reported the isolation and identification of kaempferol, quercetin, quercetrin, rutin and quercetin 7-O-glucoside from the roots of Egyptian *Salvadora persica* L.[5]

In a previous communication, we reported the isolation and identification of two new phenolic glycosides; viz, salvadoside and salvadoraside from the stems of *Salvadora persica* L.[6]

## EXPERIMENTAL

**Plant material**: was collected in March 1988 at the flowering stage from Wady Ghadier, Eastern desert of Egypt. The plant was identified by Prof. Dr. N. El-Hadidy, Cairo University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

### Methods

All melting points are uncorrected. $^1$H-NMR spectra were recorded at 400 MHz and $^{13}$C-NMR at 100 MHz using (TMS) as internal standard and DMSO as solvent. TLC was carried out on precoated silica gel plates (Kieselgel 60 F254, Merck). For column chromatography, silica gel G (E. Merck), Lichroprep RP-8 (40-63 $\mu$m, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used.

For preparative HPLC: Tosoh HPLC 803 D pump, Tosoh UV-8 at 254 detector, using columns: Amide-80 (21.5 mm i-d. X30 cc) with

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flow rate 6 ml/min and Hydroxyapatite (10 mm i-d X40 cm), flow rate: 1.5 ml/min, chart speed 2 mm/min.
For MS Jeol JMS-SX 102 spectrometer apparatus. The solvent systems were:

System I $CHCl_3$:MeOH:$H_2O$ (65:35:5)
System II $CHCl_3$:MeOH:$H_2O$ (75:25:3)
System III Pet. ether:EtOAc (9:1)
System IV EtOAc:MeOH:$H_2O$ (85:15:1)

**Spray reagent**

1- 1% Aluminium chloride in methanol.
2- 10% $H_2SO_4$, then heated at 120°C for 5 minutes.

**Extraction and isolation**

The powder of air-dried leaves (250 g.) was extracted with ethanol 70% by maceration at room temperature. The alcoholic extract was concentrated under reduced pressure till syrupy in consistency (50 g.), then diluted with water and defatted with diethylether. The defatted aqueous layer was exhaustively extracted with ethyl acetate and then subjected to column chromatography (50x10 cm) on Diaion HP-20; elution was carried out with water, methanol and acetone. The flavonoid glycosides were detected only in the MeOH eluate. TLC of the ethyl acetate extract revealed the presence of five spots with $R_F$ values 0.88, 0.83, 0.79, 0.57 and 0.38 (system I). MeOH eluate showed a single spot with $R_F$ 0.13 (system I).

**Column chromatographic fractionation of EtOAc extract**

The residue left after evaporation of the EtOAc extract (20 g.) was dissolved in a small amount of MeOH and transferred to a column of Lichroprep RP-8. Elution was performed using 50% MeOH. The effluent was collected in fractions (50 ml) each, concentrated and screened by TLC using solvent system I. Fraction I (0-5) with $R_F$ 0.38, Fraction II (8-10) with $R_F$ 0.57 and fraction III with $R_F$ 0.79, 0.83 and 0.88. Group I was subjected to HPLC using amide-80 column and eluted with 93% acetonitrile to give three compounds SF-1, SF-2 and SF-3. Compound SF-2 was a mixture of two compounds, consequently it was subjected to further HPLC using Hydroxyapatite column and eluted with 88% acetonitrile to produce two compounds SF-2(a) and SF-2(b). Group II was subjected to HPLC using Amide-80 column and 93% acetonitrile to give three compounds SF-4, SF-5 and SF-6.

**Isolation of flavonoid glycosides of MeOH eluate**

MeOH eluate was evaporated, yielded residue (30 g.), dissolved in a small amount of methanol and subjected to HPLC using Amide-80 column. Elution was performed with 85% acetonitrile affording three compounds SF-7, SF-8 and SF-9. Compound SF-8 was then subjected to HPLC using Hydroxyapatite column and 85% acetonitrile to give two compounds SF-8(a) and SF-8(b).

**Identification of the isolated compounds**

**Compound SF-1:** (20 mg) yellow needles from MeOH (m.p. 185-187°C), $[\alpha]_D^{20}$ -65° (c= 0.10, MeOH), with $R_F$= 0.19 (system II). FAB-MS spectrum revealed two peaks: m/z 623 $[M-H]^-$ and m/z 315 $[M-H-Gal-Rha]^-$. The acid hydrolysis with 2N HCl yielded D-galactose and L-rhamnose as a sugar moiety and isorhamnetin as aglycone.[7] $^{13}C$ and $^1H$ NMR data are similar to those reported for isorhamentin-3-O-robinobioside.[8]

**Compound SF-2(a):** (7 mg) was obtained as yellow powder from MeOH, $[\alpha]_D^{20}$ -10° (c= 0.025, MeOH), with $R_F$= 0.16 (system II). FAB-MS spectrum showed two peaks: m/z 593 $[M-H]^-$ and m/z 285 $[M-H-Gal-Rha]^-$. The acid hydrolysis revealed the presence of D-galactose and L-rhamnose in addition to kaempferol as aglycone[8] ($R_F$= 0.83 system III).

**Compound SF-2(b):** (6 mg) yellow needles from MeOH (m.p. 179-181°C), $[\alpha]_D^{22}$ -52° (c= 0.12, MeOH), $R_F$= 0.16 (system II). FAB-MS spectrum showed three peaks: 623 $[M-H]^-$, m/z 477 $[M-H-Rha]^-$ and m/z 315 $[M-H-Rha-Glc]^-$. The acid hydrolysis gave D-glucose and L-

rhamnose in addition to isorhamnetin as aglycone.[7]

**Compound SF-3:** (40 mg) yellow needles from MeOH (m.p. 158-160°C), $[\alpha]_D^{23}$ -18° (c= 0.025, MeOH), $R_F$= 0.16 (system II). FAB-MS spectrum showed three peaks; m/z 593 $[M-H]^-$, m/z 447 [M-H-Rha]$^-$ and m/z 285 [M-H-Rha-Glc]$^-$. The acid hydrolysis yielded D-glucose and L-rhamnose as sugars and kaempferol as aglycone.[9]

**Compound SF-4:** (5 mg) yellow powder from MeOH, $[\alpha]_D^{23}$ -28° (c= 0.025, MeOH), $R_F$= 0.25 (system IV). FAB-MS spectrum revealed two peaks: m/z 477 $[M-H]^-$ and m/z 315 [M-H-Gal]$^-$. The acid hydrolysis produced isorhamnetin and D-galactose.

**Compound SF-5:** (20 mg) was obtained as yellow powder from MeOH, $[\alpha]_D^{23}$ -14° (c= 0.025, MeOH), $R_F$= 0.24 (system IV). FAB-MS spectrum showed two peaks: m/z 447 [M-H-Rha]$^-$ and m/z 285 [M-H-Glc]$^-$. The acid hydrolysis yielded kaempferol and D-glucose.

**Compound SF-7:** (30 mg) yellow powder from MeOH, $[\alpha]_D^{23}$ -14° (c= 0.025, MeOH), $R_F$= 0.17 (system I). FAB-MS spectrum showed three peaks: m/z 769 $[M-H]^-$, m/z 623 [M-H-Rha]$^-$ and m/z 315 [M-H-2Rha-Gal]$^-$. The acid hydrolysis afforded isorhamnetin in addition to D-galactose and L-rhamnose.

**Compound SF-8(a):** (8 mg) was obtained as yellow powder from MeOH, $[\alpha]_D^{23}$ -56° (c= 0.025, MeOH), $R_F$= 0.15 (system I). $^{13}$C-NMR spectral analysis revealed the presence of three sugars from the signals at 99.0, 100.6 and 100.1 ppm corresponding to the anomeric carbons of the sugars. The downfield shift of C-2 and C-6 galactose indicates the attachment of rhamnosyl units to these positions. The other signals are similar to those reported for 3-substituted kaempferol.[9] FAB-MS spectrum showed two peaks: m/z 739 $[M-H]^-$, m/z 285 [M-H-2Rha-Gal]$^-$. EI-MS spectrum of the acetate derivative of SF-8(a) revealed two peaks: m/z 791 [Gal-2Rha-acetate+H]$^+$ and m/z 273 [terminal Rha acetate+H]$^+$. EI-MS spectrum confirmed the attachment of rhamnosyl units to C-2 and C-6 galactose from the peaks m/z 273 and m/z 791.[10]

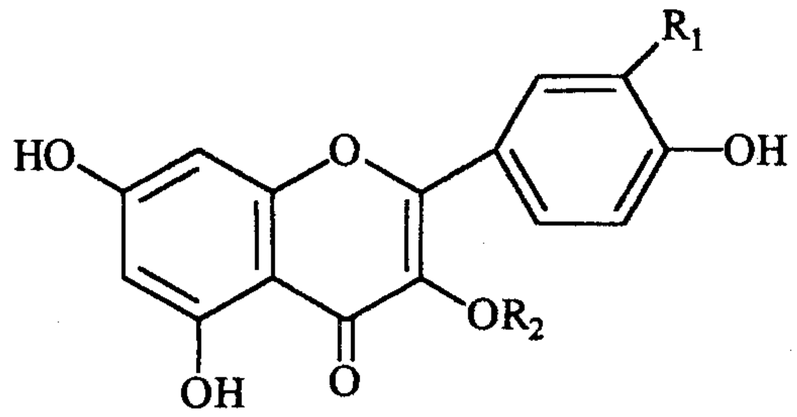
**Compound SF-8(b):** (10 mg) was isolated as yellow needles from MeOH (m.p. 193-195°C), $[\alpha]_D^{23}$ -15° (c= 0.025, MeOH), $R_F$= 0.15 (system I). FAB-MS spectrum showed three peaks: m/z 769 $[M-H]^-$, m/z 623 [M-H-Rha]$^-$ and m/z 315 [M-H-2Rha-Glc]$^-$. The acid hydrolysis afforded isorhamnetin in addition to D-glucose and L-rhamnose.

**Compound SF-9:** (20 mg) was obtained as yellow needles from MeOH (m.p. 189-191°C), $[\alpha]_D^{23}$ -31° (c= 0.025, MeOH), $R_F$= 0.14 (system I). $^{13}$C-NMR spectrum revealed the downfield shift of C-2 and C-6 glucose due to substitution with rhamnose in these positions. FAB-MS spectrum showed three peaks: m/z 739 $[M-H]^-$, m/z 593 [M-H-Rha]$^-$ and m/z 285 [M-H-2Rha-Glc]$^-$. EI-MS spectrum of SF-9 acetate revealed two peaks: m/z 791 [Glc-2Rha-acetate+H]$^+$ and m/z 273 [Rha acetate+H]$^+$. The acid hydrolysis yielded kaempferol in addition to D-glucose and L-rhamnose.

The identification of the isolated aglycones from each of the isolated glycosides was based on Co-chromatography alongside authentic samples, spectral analysis $^1$H-NMR, $^{13}$C-NMR, FAB-MS and EI-MS of acetate derivatives and comparison with reported data.[9]

## RESULTS AND DISCUSSION

Inspection of $^{13}$C-NMR spectra of compounds 2,4,6 and 9 (Table 1) revealed their similarity to those reported for 3-O-substituted kaempferol while the spectra of compounds 1,3,5,7,8 and 10 were coincident with those of 3-O-substituted isorhamnetin.[9] Acid hydrolysis of compounds 6 and 7 afforded D-glucose, compound 5 yielded D-galactose, compounds 3,4,10 and 11 gave D-glucose and L-rhamnose while compounds 1,2,8 and 9 afforded D-galactose and L-rhamnose. For this reason, compounds 5,6 and 7 have been identified as:



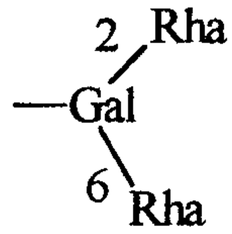
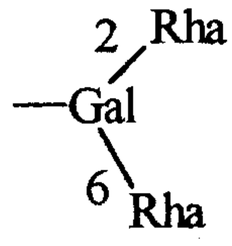
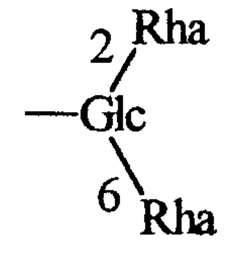
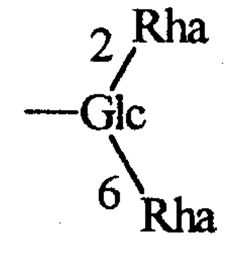

| | $R_1$ | $R_2$ |
|---|---|---|
| 1- Isorhamnetin-3-O-robinobioside | $OCH_3$ | −β−D−Gal$^{6}$−α−L−Rha |
| 2- Kaempferol-3-O-robinobioside | H | −β−D−Gal$^{6}$−α−L−Rha |
| 3- Narcissin | $OCH_3$ | −β−D−Glc$^{6}$−α−L−Rha |
| 4- Kaempferol-3-O-rutinoside | H | −β−D−Glc$^{6}$−α−L−Rha |
| 5- Isorhamnetin-3-O-β-D-galactoside | $OCH_3$ | −β−D−Gal |
| 6- Astragalin | H | −β−D−Glc |
| 7- Isorhamnetin-3-O-β-D-glucoside | $OCH_3$ | −β−D−Glc |
| 8- Isorhamnetin-3-(2,6-dirhamno-pyranosylgalactopyranoside | $OCH_3$ |  |
| 9- Mauritianin | H |  |
| 10- Isorhamnetin-3-O-(2-Glc-rhamnosyl-rutinoside) | $OCH_3$ |  |
| 11- Kaempferol-3-O-(2-Glc-rhamnosyl-rutinoside | H |  |

**Table 1:** $^{13}$C-NMR spectral data of the isolated flavonoid glycosides (100 MHz, DMSO).

| C | SF-1 | SF-2-a | SF-2-b | SF-3 | SF-4 | SF-5 | SF-6 | SF-8-a | SF-7 | SF-9 | SF-8-b |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 156.4[a] | 156.4[a] | 156.5[a] | 156.5[a] | 156.3[a] | 156.4[a] | 156.4[a] | 156.4[a] | 156.3[a] | 156.4[a] | 156.3[a] |
| 3 | 133.1 | 133.3 | 133.0 | 133.2 | 133.3 | 133.2 | 133.0 | 132.6 | 132.6 | 133.2 | 132.4 |
| 4 | 177.4 | 177.2 | 177.3 | 177.4 | 177.3 | 177.5 | 177.4 | 177.3 | 177.2 | 177.2 | 177.1 |
| 5 | 161.2 | 161.0 | 161.2 | 161.2 | 161.2 | 161.2 | 161.2 | 161.2 | 161.2 | 161.2 | 161.1 |
| 6 | 98.8 | 98.7 | 98.7 | 98.8 | 98.7 | 98.8 | 98.8 | 98.7 | 98.7 | 98.4 | 98.8 |
| 7 | 164.5 | 164.5 | 164.2 | 164.3 | 164.1 | 164.3 | 164.2 | 164.1 | 164.2 | 164.3 | 164.2 |
| 8 | 93.8 | 93.6 | 93.8 | 93.8 | 93.8 | 93.7 | 93.7 | 93.7 | 93.7 | 93.8 | obscured |
| 9 | 156.3[a] | 156.3[a] | 156.5[a] | 156.9[a] | 156.4[a] | 156.4[a] | 156.3[a] | 156.4[a] | 156.1[a] | 156.8[a] | 156.4[a] |
| 10 | 103.9 | 103.6 | 104.0 | 104.0 | 104.0 | 104.1 | 104.1 | 103.9 | 103.9 | 103.9 | 103.9 |
| '1 | 122.0 | 120.7 | 122.3 | 120.9 | 121.9 | 120.9 | 122.1 | 120.9 | 121.7 | 121.0 | 122.0 |
| '2 | 113.5[b] | 130.7 | 113.3[b] | 130.9 | 113.6[b] | 130.9 | 113.5[b] | 130.8 | 113.4[b] | 130.7 | 113.2[b] |
| '3 | 149.5 | 114.9 | 149.4 | 115.1 | 149.5 | 115.1 | 149.4 | 115.1 | 149.3 | 115.1 | 149.3 |
| '4 | 147.0 | 159.8 | 146.9 | 159.9 | 146.9 | 160.0 | 146.9 | 159.8 | 146.9 | 159.8 | 146.8 |
| '5 | 115.2[b] | 114.9 | 115.2[b] | 115.1 | 115.0[b] | 115.1 | 115.2[b] | 115.1 | 115.1[b] | 115.1 | 115.1[b] |
| '6 | 121.0 | 130.7 | 121.0 | 130.9 | 120.9 | 130.9 | 121.1 | 130.8 | 121.0 | 130.7 | 121.0 |
| $OCH_3$ | 55.9 | - | 55.7 | - | 55.9 | - | 55.7 | - | 55.9 | - | 55.6 |
| ß-D-glucose | | | | | | | | | | | |
| 1 | | | 101.2 | 101.4 | | 100.9 | 100.8 | | | 98.6 | 98.6 |
| 2 | | | 74.3 | 74.2 | | 74.2 | 74.4 | | | 77.5 | 77.5 |
| 3 | | | 76.4 | 76.4 | | 77.5 | 77.5 | | | 77.3 | 77.0 |
| 4 | | | 70.6 | 70.6 | | 69.9 | 69.8 | | | 70.6 | 70.5 |
| 5 | | | 75.9 | 75.7 | | 76.4 | 76.4 | | | 75.5 | 75.8 |
| 6 | | | 66.8 | 66.9 | | 60.9 | 60.6 | | | 66.7 | 66.8 |
| ß-D-galactose | | | | | | | | | | | |
| 1 | 101.8 | 102.1 | | | 101.8 | | | 99.0 | 99.0 | | |
| 2 | 71.2 | 71.0 | | | 71.2 | | | 74.9 | 75.1 | | |
| 3 | 73.0 | 73.0 | | | 73.2 | | | 73.8 | 73.4 | | |
| 4 | 68.0 | 68.1 | | | 67.9 | | | 68.5 | 68.1 | | |
| 5 | 73.6 | 73.5 | | | 75.9 | | | 73.3 | 73.6 | | |
| 6 | 65.2 | 65.3 | | | 60.1 | | | 65.1 | 65.1 | | |
| α-L-rhamnose | | | | | | | | | | | |
| 1 | 100.1 | 100.0 | 100.9 | 100.8 | | | | 100.6,100.1 | 100.8,100.1 | 100.8,100.6 | 100.7,100.9 |
| 2 | 70.6 | 70.5 | 70.3 | 70.3 | | | | 70.7, 70.6 | 70.6, 70.6 | 70.6, 70.5 | 70.5, 70.5 |
| 3 | 70.4 | 70.3 | 70.1 | 69.9 | | | | 70.4, 70.4 | 70.6, 70.4 | 70.3, 70.2 | 70.4, 70.3 |
| 4 | 71.9 | 71.8 | 71.8 | 71.8 | | | | 71.9, 71.9 | 71.7, 71.8 | 71.8, 71.8 | 71.7, 71.7 |
| 5 | 68.3 | 68.0 | 68.3 | 68.3 | | | | 68.3, 68.2 | 68.5, 68.3 | 68.3, 68.2 | 68.2, 68.2 |
| 6 | 17.9 | 17.9 | 17.7 | 17.7 | | | | 17.9 17.2 | 17.9, 17.0 | 17.7, 17.3 | 17.6, 17.0 |

[a] and [b]: values interchangeable in each column.

Isorhamnetin-3-O-ß-D-galactoside, Astragalin and Isorhamnetin-3-O-ß-D-glucoside, respectively compound 2 was assigned as kaempferol-3-O-robinobioside in which the attachment of the α-rhamnosyl unit to C-6 of the ß-galactopyranosyl unit was indicated from its downfield shift (δ 65.3 ppm).[11]

Similarly, the attachment of the α-rhamnopyranosyl unit to C-6 Glc in compound 4 was deduced from its downfield shift (δ 66.9 ppm) in the $^{13}$C-NMR spectum,[11] confirming that compound 4 is kaempferol 3-O-rutinoside. $^{13}$C-NMR spectrum of compound 9 was almost similar to that of 2 except the downfield shift of C-2 of the ß-galactopyranosyl unit (δ 75.1 ppm) indicating the attachement of the second α-rhamnopyranosyl unit to this position. Consequently, compound 9 was assigned as Mauritianin. Also, compound 11 was superimposalbe to 4 in its $^{13}$C-NMR spectral analysis except the downfield shift of C-2 of the ß-glucopyranosyl unit (δ 77.5 ppm) proving its substitution with α-rhamnopyranosyl unit. Thus, compound 11 was identified as kaempferol-3-O-(2-Glc-rhamnosyl)-rutinoside. On the other hand, the carbon signals of the sugar moities of compounds 1,3,8 and 10 were coincident with those of 2,4,9 and 11 respectively. For this reason, the structures of compounds 1,3,8 and 10 have been assigned as: Isorhamnetin-3-O-robinobioside, Narcissin, Isorhamnetin-3-(2,6-dirhamnopyranosyl galactopyranoside) and Isorhamnetin-3-O (2-Glc-rhamnosylrutinoside) respectively.

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