Phenylpropanoid and phenylethanoid glycosides from Scrophularia xanthoglossa and their antioxidative and antiinflammatory activities

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ABSTRACT

Repeated column chromatography of the butanol extract of Scrophularia xanthoglossa yielded three phenylpropanoid glycosides identified as scropheanoside-I, scropheanoside-II, scropheanoside-III together with two phenylethanoid glycosides identified as, acetoside, and martynoside. Their structures were determined by spectroscopic analysis, chemical evidence as well as comparison with literature values. The biological activity was also studied where acetoside and martynoside showed significant antioxidative activity, while scropheanoside-III showed significant anti-inflammatory activity.

Key words: Scrophularia xanthoglossa, Scrophulariaceae, Scropheanosides I-III, acetosid and martynosid, antioxidative, anti-inflammatory.

INTRODUCTION

In our previous study [Abbas and Zayed, 2004], we reported the isolation and structure determination of five iridoid glycosides; aucubin, harpagid, 6-O-α-L-rhamnopyranosyl-aucubin, harpagoside and 6-O-α-L-rhamnopyranosyl-catalpol from Scrophularia xanthoglossa Bioss. A survey of literature showed that no phytochemical and pharmacological works have been reported on phenylpropanoid and phenylethanoid glycosides from Scrophularia xanthoglossa Bioss., a perennial plant growing in Yemen and southwestern Arabia [Miagahid,1989]. In continuation to our studies on the constituents of Scrophularia xanthoglossa, we investigated the butanol extract of the plant to furnish the isolation and identification of three phenylpropanoid glycosides scropheanosides I-III, and two phenylethanoid glycosides; acetoside and martynoside. The antioxidative activity of the five glycosides was evaluated against 2, 2'-diphenyl-1-picrylhydrazyl (DPPH). The five compounds isolated were also evaluated as anti-inflammatory, where scropheanoside-III was the most active among all compounds tested.

EXPERIMENTAL

General Experimental Procedures

Optical rotation were measured on a Zeiss polarimeter Model 53187 using a sodium lamp. UV spectra were obtained on a Varian CARY 2290 spectrophotometer in Me OH. IR spectra were recorded on a Perkin –Elmer Model 337 spectrometer in K Br discs. FABMS were recorded with an MS 2500 high-resolution spectrometer (Kratos, Manchester, UK) with 70 eV, an ion source temperature of 180 °C and a direct inlet using meta nitrobenzyl alcohol and/ or thioglycerin as matrix. NMR spectra were measured on CD3OD and pyridine-d5 and recorded at 300 MHz for 1H-NMR and 75 MHz for 13C-NMR using TMS as internal standard on a Varian XL300 (Darmstadt, Germany). Chemical shifts were expressed in δ values (1H) and ppm (13C-NMR). Precoated thin–layer chromatographic (TLC) plates coated with silica gel
60 F_{254}, 0.25 mm layer thickness and silica gel (60-120 mesh) for column chromatography were obtained from Merck (Darmstadt, Germany). The spots were visualized by spraying with 1% vanillin-H₂SO₄ solution followed by heating at 100 °C for 10 minutes. Solvent systems used for TLC were: I. CHCl₃-CH₃OH-H₂O (15:7.5:2.5, lower phase); II. Et OAc - CH₃OH - n-C₃H₇OH - H₂O (15:10:2:7, lower phase).

Plant Material

Scrophularia xanthoglossa Bioss. is a glabrous perennial plant (up to one meter high) that grows in Yemen and Southwestern Arabia [Miagahid, 1989]. The sample of aerial parts for the present study was collected from roads and hill sides in Sanaa region (Wadi Zahr) in Yemen in March 1997. Identity of the plant was confirmed by Professor Sultan Abdeen, Faculty of Pharmacy, King Saud University. A certified specimen has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, King Saud University, Saudi Arabia. The aerial parts of the plant were air-dried and powdered before extraction.

Extraction and Isolation

The air-dried powdered plant material of Scrophularia xanthoglossa Bioss. (500g) was exhaustively extracted with ethanol (95%, 5 x 2 L) by cold maceration. The solvent was distilled off under reduced pressure, then the residue (60 g) was suspended in distilled water (500 ml), the water-insoluble material was removed by filtration. The aqueous solution was successively extracted with petroleum ether, ether, chloroform, ethyl acetate, and finally with butanol saturated with water (3 L each). The solvent in each case was distilled off under reduced pressure to afford (13, 7, 2, 11, 17 g respectively). TLC examination of the butanol extract using solvent system II revealed the presence of five spots having Rf values 0.56, 0.51, 0.47, 0.36 and 0.31.

About 10 g of the dried butanol extract were subjected to flash silica gel column (Merck, 500 g, 4.5x120 cm) using CHCl₃ – MeOH - H₂O gradient elution and repeated purification columns to afford the following compounds:

**Compound 1**

Obtained as a white amorphous powder; [α]D²⁵ -100° (CH₃OH, c=1) with R₁, 0.51 (system I) and 0.56 (system II). IR (K Br): νmax cm⁻¹ 3400, 2900, 1700, 1660, 1630, 1580, 1510, 1440, 1260, 1030. Positive FABMS: m/z 691 [M+Na]+, 669 [M+H]+, 323 [C₁₈H₁₆O₁₇]⁻. 1H-NMR (300 MHz, CD₃OD, δ, ppm, J=Hz): 4.9 (d, J=7 Hz, 1'-H), 6.3 (dd, J=6.5 & 2 Hz, 3'-H), 5.1 (dd, J=6.5 & 4 Hz, 4'-H), 2.8 (ddd, J=7.5, 5.5, 4 & 2 Hz, 5'-H), 4.4 (m, δ-H), 5.7 (ddd, J=2.2, 2 & 2 Hz, 7-H), 2.88 (dd, J=7.5 & 9 Hz, 9-H), 4.23 (d, J=16 Hz, 10a-H), 4.7 (d, J=8 Hz, 10b-H), 3.2 (d, J=9 & 8 Hz, 2'-H), 3.4 (dd, J=9 & 9 Hz, 3'-H), 3.26 - 3.34 (m, 4'-H), 3.6 (dd, J=12 & 6 Hz, 6'a-H), 3.83 (dd, J=2 & 3.5 Hz, 2'-H), 3.86 (dd, J=9.5 & 3.5 Hz, 3'-H), 5 (d, J=9.5 Hz, 4'-H), 3.9 (d, J=9 & 6.5 Hz, 5'-H), 1.2 (d, J=6.5 Hz, 6'-H), 7.09 (d, J=2 Hz, 2''-H), 6.9 (d, J=8 Hz, 5''-H), 7.06 (dd, J=8 & 2 Hz, 6''-H), 3.65 (d, J=16 Hz, 10'-H), 7.6 (d, J=16 Hz, 1'-H), 3.8 (s, OCH₃). 13C-NMR (75 MHz, CD₃OD, δ, ppm) see Table 1.

**Compound 2**

Obtained as white amorphous powder; [α]D²⁵ -180° (CH₃OH, c=1) with R₁, 0.51 (system I) and 0.56 (system II). IR (K Br): νmax cm⁻¹ 3400, 2900, 1700, 1660, 1600, 1580, 1500. Positive FABMS: m/z 685 [M+H]+ Calcd for the formula (C₃₁H₄₀O₁₇+H), 707 [M+Na]+, 323, 177. 1H-NMR (300 MHz, CD₃OD, δ, ppm, J=Hz): 5.1 (d, J=9.5 Hz, 1'-H), 6.37 (dd, J=6.5 & 2 Hz, 3'-H), 5 (dd, J=6.5 & 4 Hz, 4'-H), 2.8 (ddd, J=8, 8 & 2 Hz, 5'-H), 4 (d, J=8 & 2 Hz, 6'-H), 3.7 (d, J=2 Hz, 7-H), 2.7 (d, J=8 & 9.5 Hz, 9-H), 3.8 (d, J=13 Hz, 10'-H), 4.2 (d, J=13 Hz, 10'-H), 4.8 (d, J=8 Hz, 1''-H), 3.3 (dd, J=9 & 8 Hz, 2''-H), 3.4 (dd, J=9 & 9 Hz, 3''-H), 3.26 (d, J=9 & 8 Hz, 3''-H), 3.3 (m, 5'-H), 3.6 (dd, J=6 & 2 Hz, 6'-H), 3.9 (dd, J=12 & 6 Hz, 6''-H), 5 (d, J=2 Hz, 1''-H), 3.9 (d, J=4 & 2 Hz, 2''-H), 3.8 (dd, J=9.5 & 8 Hz, 3''-H), 5 (d, J=9.5 & 9.5 Hz, 4''-H), 3.89 (dd, J=6 & 9.5 Hz, 5''-H), 1.2 (d, J=6 Hz, 6''-H), 7 (d, J=2 Hz, 2''-H), 6.9 (d, J=8.5 Hz, 5''-H), 7 (dd, J=8.5 & 2 Hz, 6''-H), 6.4 (d, J=16 Hz, 10'-H), 7.6 (d, J=16 Hz, 1'-H), 3.9 (s, OCH₃). 13C-NMR (75 MHz, CD₃OD, δ, ppm) see Table 1.

**Compound 3**

Obtained as white amorphous powder; [α]D²⁵ -89° (CH₃OH, c=1) with R₁, 0.43 (system I) and
o.47 (system II). IR (KBr): $\nu_{\text{max}}$ cm$^{-1}$ 3400, 1745, 1715, 1630, 1575. Positive FABMS: $m/z$ 740 [M + NH$_4$]$^+$, 745 [M + Na]$^+$. Calcd for the formula (C$_{34}$H$_{42}$O$_{17}$), 361 (M - C$_{15}$H$_{12}$O$_{10}$) . 1H-NMR (300 MHz, CD$_3$OD, $\delta$, ppm, $J_{\text{Hz}}$): of the catalpol nucleus and the glucose moiety are in good agreement with those of compound 2 isolated from the same plant, 5.1 (d, $J=2$ Hz, $\alpha$-H), 5.3 (dd, $J=4$ & 2 Hz, 2$^\text{H}$-H), 5.4 (dd, $J=9.5$ & 9.5 Hz, 3$^\text{H}$-H), 5 (dd, $J=9.5$ & 9.5 Hz, 4$^\text{H}$-H), 4 (dd, $J=6$ & 8 Hz, 5$^\text{H}$-H), 1.2 (d, $J=6$ Hz, 6$^\text{H}$-H), 7.4 (m, 2$^\text{H}$-H), 7.6 (m, 3$^\text{H}$-H), 7.4 (m, 4$^\text{H}$-H), 7.6 (m, 5$^\text{H}$-H), 6.4 (d, $J=16$ Hz, $\beta$-H), 7.6 (d, $J=16$ Hz, $\alpha$-H), 2 (s, COCH$_3$), 2.1 (s, CO-CH$_3$) . 13C-NMR (75 MHz, CD$_3$OD, $\delta$, ppm) see Table 1.

**Compound 4**

Obtained as yellowish-white amorphous powder; [$\alpha$]$_{D}^{20}$-31$^\circ$ (CH$_3$OH, $c=1$) with $R_{f}$ 0.33 (system I) and 0.36 (system II). IR (KBr): $\nu_{\text{max}}$ cm$^{-1}$ 3380, 2920, 1795, 1630, 1600, 1510. Positive FABMS: $m/z$ 625 [M + H]$^+$, 642 [M + NH$_4$]$^+$, 647 [M + Na]$^+$. Calcd for the formula (C$_{29}$H$_{36}$O$_{15}$). The UV (MeOH, $\lambda_{\text{max}}$, nm): 328, 288. $^1$H-NMR (300 MHz, CD$_3$OD, $\delta$, ppm, $J_{\text{Hz}}$) of the compound showed close similarity to the $^1$H-NMR of compound 4 isolated from the same plant, except for the presence of two methoxyl groups [$\delta$ ($\alpha$, 4- OCH$_3$) and $\delta$ ($\beta$, 3'- OCH$_3$)]. $^{13}$C-NMR (75MHz, CD$_3$OD, $\delta$, ppm): 132 (C-1), 116 (C-2), 147 (C-3), 147 (C-4), 116 (C-5), 121 (C-6), 72 (C-$\alpha$), 56 (4- OCH$_3$), 104 (C-$\beta$), 76 (C-$\gamma$), 81 (C-$\delta$), 70 (C-$\varepsilon$), 76 (C-$\zeta$), 62 (C-$\kappa$), 115 (C-$\lambda$), 147 (C-$\mu$), 168 (C-0), 127 (C-$\nu$), 111 (C-$\xi$), 150 (C-$\psi$), 149 (C-$\tau$), 116 (C-$\sigma$), 124 (C-$\nu$), 56 (3'- OCH$_3$), 102 (C-$\lambda$), 72 (C-$\kappa$), 72 (C-$\mu$), 73 (C-$\nu$), 70 (C-$\xi$), 70 (C-$\psi$), 18 (C-$\sigma$)

**Acid Hydrolysis**

Compounds isolated (15 mg each) were refluxed, separately, with 0.1 N HCl (30 ml) for 6 hr. Then H$_2$O was added and the mixtures were extracted with CHCl$_3$. The aqueous layer, in each case was neutralized with Ag$_2$CO$_3$ and filtered. The filtrate was evaporated in vacuo and the residue was examined by comparison with authentic sugar samples using silica gel $F_{254}$ plate, n- BuOH - PrOH - H$_2$SO$_4$ for detection. All compounds gave glucose ($R_{0.3}$) and rhamnose ($R_{0.55}$).

**Measurement of DPPH radical scavenging activity**

Each ETOH solution (100µl) of compounds 1-5 at various concentrations was added to 1.5x10$^{-5}$ DPPH/ETOH solution. The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 530 nm after 30 min. The radical scavenging activity was determined by subtracting the absorbance with that of blank (100%) containing only DPPH and solvent. The samples were prepared using the same dilution procedures and dl -tocopherol was used as standard [Abourashed, 2005].

**Experimental animals**

Wister rats of either sex weighing 200-250 g were used to determine anti-inflammatory activity. The animals were maintained at 23±2°C with a 12h light and dark cycle, fed a Purina rat chow diet supplied by Grain Silos and Flour Mills Organization, Riyadh, Saudi Arabia, and had free access to food and water.
Determination of Anti-inflammatory Activity

Six rats each were allotted to different treatment groups. Edema was induced in the rats by injecting carrageenin (0.05ml, 1% w/v in normal saline) into the subplantar tissue of the right hind paw [Winter et al., 1962]. Paw volume (mm) was measured with a plethysmometer (7140, Ugo Basile) before carrageenin injection and 0, 1, 2 and 3h thereafter. The edema was reported as the difference between the initial and the final volume. The anti-inflammatory effect was expressed as the percentage inhibition compared with vehicle-treated animals with respect to a reference group treated with phenylbutazone (100 mg/kg). The test compounds (10mg/kg) with distilled water (0.1ml/100g rat) were administered orally 1h before injection of the phlogistic agent.

RESULTS AND DISCUSSION

Compound 1, Scropheanoside-I, with formula C_{31}H_{40}O_{16} through the positive FABMS, with ion beak at m/z 669 [M+H]^+, 691 [M +Na]^+, 323 [C_{16}H_{18}O_{7}] for isoferuloyl-rhamnose fragment and m/z 177 [C_{10}H_{9}O_{3}] for isoferuloyl fragment. The IR spectrum of the compound showed absorption bands at 3400 cm\(^{-1}\) (hydroxyl groups), 1700 cm\(^{-1}\) (\(\alpha,\beta\)-unsaturated ester), and 1630 cm\(^{-1}\) (conjugated double bond ). \(^1\)H-NMR data indicated an olefinic proton at C-3 at \(\delta 6.3\) (1 H, dd) with vicinal coupling \(J_{3,4}=6.5\) with H-4 (\(\delta 5.1\) ppm ) and an allylic coupling \(J_{3,5}=2\) Hz with H-5 ( \(\delta 2.8\) ppm ). The presence of H-7 at \(\delta 5.7\) ppm and the three homoallylic coupling of H-6 with protons at C-9 and C-10 indicated the presence of carbon, carbon –double bond between C-7 and C-8 of the iridoid nucleus of the compound. The \(^1\)H-NMR data showed full agreement with the reported data of aucubin [Abbas and Zayed, 2004, and Pachaly et al., 1994]. The \(^1\)H-NMR spectrum exhibited signals for three aromatic protons (\(\delta_H 7.09, d, J = 2\) Hz, 6.9, d, \(J = 8\) Hz and 7.06, dd , \(J = 8 \& 2\) Hz) and two olefinic protons ( \(\delta_H 6.35, d, \) and 7.6, d, AB system, \(J_{ab}=16\) Hz ) substantiating the presence of trans isoferuloyl moiety , one methoxy signal (\(\delta_H 3.8, s\) ), one methyl signal (\(\delta_H 1.2, d, J = 6.5\) Hz ) and two anomic protons ( \(\delta_H 4.7, d, J = 8\) Hz, 4.8, d , \(J =2\) Hz ) indicating the presence of two sugar moieties in the molecule.
On acid hydrolysis, the compound afforded D-glucose and L-rhamnose as sugar moieties. The chemical shifts of protons and carbon atoms were compared with the reported data [Pachaly et al., 1994] which indicated the presence of β-D-glucose and α-L- rhamnose, respectively. The H-1 of the aglycone exhibited long range coupling in the heteronuclear multiple bond connectivity (HMBC) spectrum with C-1' of the glucose unit, H-6 correlated with C-1" of the rhamnose moiety which indicated that glucose is connected with ether linkage at position 1 and rhamnose with position 6.

The assignments of glucosyl and rhamnosyl protons were also made with the help of 1H-1H – COSY experiments starting with their anomeric protons (δH 4.7, d, J = 8 Hz, glucosyl H-1, δH 4.8, d, J = 2 Hz, rhamnosyl H-1) and methylene protons (δH 3.6, dd, J = 12 & 6 Hz, 6'-H; 3.8, dd, J = 12 & 2 Hz, 6''-H) in case of glucose and methyl proton.

Table 1: 13C- NMR Spectral Data of Scropheanoside I-III (75 MHz, CD₃OD)

| Position | Scropheanoside I | Scropheanoside II | Scropheanoside III |
|----------|------------------|-------------------|-------------------|
| 1        | 98               | 95                | 95                |
| 3        | 142              | 142               | 142               |
| 4        | 105              | 103               | 103               |
| 5        | 44               | 37                | 37                |
| 6        | 89               | 84                | 84                |
| 7        | 127              | 59                | 59                |
| 8        | 149              | 67                | 66                |
| 9        | 49               | 43                | 43                |
| 10       | 61               | 61                | 61                |
| 1'       | 100              | 100               | 100               |
| 2'       | 75               | 75                | 75                |
| 3'       | 78               | 78                | 78                |
| 4'       | 71               | 71                | 71                |
| 5'       | 78               | 78                | 78                |
| 6'       | 62               | 62                | 62                |
| 1"       | 101              | 101               | 98                |
| 2"       | 72               | 72                | 71                |
| 3"       | 70               | 70                | 70                |
| 4"       | 75               | 75                | 72                |
| 5"       | 68               | 68                | 68                |
| 6"       | 18               | 18                | 18                |
| 1'"      | 128              | 128               | 135               |
| 2'"      | 114              | 114               | 129               |
| 3'"      | 147              | 148               | 130               |
| 4'"      | 151              | 151               | 131               |
| 5'"      | 112              | 112               | 129               |
| 6'"      | 127              | 123               | 129               |
| α        | 116              | 116               | 117               |
| β        | 146              | 146               | 147               |
| C=O      | 168              | 168               | 167               |
| OCH₃      | 56               | 56                |                   |
| CO-CH₃    | 171              |                   |                   |
| CO-CH₃    | 172              |                   |                   |
| COCH₃     | 21               |                   |                   |
| COCH₃     | 21               |                   |                   |
(δ 1.2, d, J = 6.5 Hz, Me-6") in case of rhamnose. The long rang couplings in the HMBC spectrum also confirmed the above assignments. The 13C-NMR (75 MHz, CD3OD, Table 1) and DEPT spectra of the compound showed 31 carbon atoms for the molecule consisting of two methyls, two methylenes, twenty two methines, four quaternary and one carbonyl carbon atom (in total C31H40). The sequential assignments of protons and carbon atoms were made with the help of 1H-1H-COSY and HETCOR spectra. The spectral data of the compound were also compared with those of scropheanoside-I, isolated from Scrophularia koraiensis [Pachaly et al., 1994] which showed a close resemblance. Unequivocally, the structure of the compound was elucidated to be 6-O-α-L-(4-O-isoferuloyl)-rhamnopyranosyl aucubin.

Compound 2, Scropheanoside-II, showed positive FABMS at m/z 685 [M+H]+, 707 [M+Na]+, for molecular formula C31H40O17. The fragment ion peaks at m/z 323 and 177 due to isoferuloyl-rhamnose residue similar to compound 1. Acid hydrolysis of compound 2 gave D-glucose and L-rhamnose. Interpretation of 1H and 13C-NMR indicated an iridoid nucleus with C-9 aglycone of catalpol derivative [Pachaly et al., 1994]. The 1HNMR showed absorption at δ 6.37 (1 H, dd, J = 6.5 & 2 Hz) of olefinic H-3 for iridoid nucleus. The resonance of H-7 at δ3.7 ppm indicated the epoxy-function between C-7 and C-8. The 1H-NMR also showed signals for β-glucose, α-L-rhamnose and isoferuloyl residues similar to compound 1. Analysis of the 13C-NMR spectrum, which showed 31 carbon signals, nine corresponding to aglycone, twelve for sugar moieties and ten for isoferuloyl moiety. Full assignments of the 1H and 13C-NMR signals were accomplished using 1H-1H-COSY and H-C correlation. The absorption at δ c 95 and 84 ppm were assigned to C-1 and C-6, respectively, indicating glycosidation at positions 1 and 6 of the iridoid skeleton.

The 13C-NMR data also showed two olefinic carbons of the iridoid skeleton at C-3 (δ c 142) and C-4 (δ c 103). The diglycosidic structure was confirmed by the 13C-NMR spectrum of the compound, where two anomeric carbons at δ c 100 and 101 were observed. All protons of the two sugar units were assigned unambiguously from the shift correlation spectroscopy (COSY) spectrum, and the two sugars were found to be β-glucose and α-L-rhamnose. The significant deshielding of C-4" of rhamnose (δ c 75) confirmed the placement of isoferloyl residue to C-4" of rhamnose. 13C-NMR also showed signals corresponding to trans isoferuloyl at δ c 116 (C-α), 146 (C-β), 168 (C=O), signals for six aromatic carbons (δ c 128, 114, 148, 151, 112 & 122) and signals for methoxy function at 56. Finally the compound was proved to be scropheanoside-II by comparing 1H and 13C-NMR spectral data with those of previously reported data [Pachaly et al., 1994].

Compound 3, Scropheanoside-III, with molecular formula C34H42O17, positive FABMS at m/z...
The fragment ion peak at m/z 361 [M-C_{15}H_{12}O_{10}]^{+} for acetyl cinnamoyl rhamnosyl residue. The $^{13}$C-NMR spectrum showed 34 carbon atoms for the molecule containing three methyls, two methylenes, 24 methines, two quaternary and three carbonyl carbon atoms (in total C_{34}H_{42}). The sequential assignments of protons and carbon atoms were made with the help of $^{1}H$-$^{1}H$ (COSY) and HETCOR experiments starting with the easily distinguishable acetal methin proton at $\delta$ 5.1 assigned to position 1 ($\delta_c$ 95), H-9 ($\delta_H$ 2.7, $\delta_c$ 43) and H-5 ($\delta_H$ 2.4, $\delta_c$ 37).

The $^{1}H$- and $^{13}$C-NMR spectra exhibited signals for five aromatic protons substantiating the presence of a trans-cinnamoyl moiety, two acetoxy signals ($\delta_H$ 2.03, s; 2.15, s; 2 x Me; $\delta_c$ 171.5, 171.8, 2 C=O) and two anemic protons $\delta_H$ 4.8, d, J = 8 Hz, $\delta_c$ 100; $\delta_H$ 5.1, d, J = 2 Hz, $\delta_c$ 98), indicating the presence of two sugar moieties in the molecule. The chemical shifts of proton and carbon atoms were compared with the values reported in the literature [Pachaly et al., 1994, Agarwal, 1992, and Miyase and Mimatsu, 1999].

The spectral data of the compound were also compared with those of scropolioside-A and scropolioside-D, previously isolated from Scrophularia scopolii and Scrophularia ilwensis, respectively [Calis et al., 1988, and 1993] which showed a close resemblance.

**Compound 4, Acetoside** showed UV at $\lambda_{\text{max}}$ 320, 280 and 230 nm. The molecular formula C_{29}H_{36}O_{15} established by positive FABMS at m/z 625 [M+H]$^+$, m/z 642 [M+NH_{4}]$^+$ and m/z 647 [M+Na]$^+$. $^{13}$C-NMR experiments showed three methylene, eighteen methane, one methyl and seven quaternary carbons with two characteristic anemic carbons at $\delta_c$ 104 and 102. The $^{1}H$-NMR spectrum showed the presence of (E)-caffeic acid and 3,4-dihydroxyphenyl ethanol moieties confirmed by the six aromatic proton signals between $\delta$ 6.6-7.0 for 2ABX system, two olefinic protons (AB system, d, J_{AB} = 16 Hz) at $\delta$ 6.3 and 7.6, a benzylic methylene at $\delta$ 2.8 (2H, dd, J = 7 & 7 Hz) and two non-equivalent protons at $\delta$ 3.7 and 4.0 (each 1H, m). Additionally, two doublets of anomic protons were observed at $\delta$ 4.4 (d, J = 8 Hz) and at $\delta$ 5.2 (d, J = 2 Hz) indicating diglycosidic structure. The significant deshielding of C-4' of the glucose at $\delta_c$ (70.) confirmed that caffeoyl residue was attached to C-4' of glucose. A downfield shift of C-3' of glucose $\delta_c$ (81) indicated that rhamnose unit was terminal and attached to C-3' of glucose. The rest of $^{1}H$ and $^{13}$C-NMR data were in full agreement with those reported for acetoside [Pachaly et al., 1994, Li et al., 2000, and Akdemir et al., 1991].

**Compound 5, Martynoside** with molecular formula (C_{31}H_{40}O_{15}) established by positive FABMS m/z 653 [M+H]$^+$ and m/z 670 [M+NH_{4}]$^+$. The $^{13}$C-NMR spectrum of the compound exhibited 31 carbon resonances. The $^{1}H$-NMR exhibited six aromatic for one ferulic acid and phenylethyl aglycon part; two olefinic protons (d, each, H- $\alpha'$ and $\beta'$) with $J_{AB}$ = 16 Hz for a trans ferulic acid unit; two methoxy groups; ethylene protons and one secondary methyl group as typically found for rhamnose were assigned. Additionally, two anemic protons were observed at $\delta$ 4.4 (d, J = 8 Hz) and $\delta$ 5.2 (d, J = 2 Hz) which was consistent with $\beta$-glucose unit and $\alpha'$-rhamnose unit, respectively. The $^{1}H$-NMR spectrum also confirmed placement of the feruloyl moiety at C-4' of the glucose moiety (deshielding of H-4' glucose resonances at $\delta$ 4.8, t, J = 9.6 Hz). The signals attributed to the aglycone and an acyl moiety was consistent with the presence of 3-hydroxy -4 methoxy phenylethanol and ferulic acid, respectively. Because no substituent chemical shift was observed for the rhamnose unit, the deoxy sugar was proven to be terminally linked to the glucose moiety. The complete assignment of most protons and carbons were based on the $^{1}H$-$^{1}H$ COSY, HMQ and HMBC experiments. The structure of the compound was confirmed to be martynoside by comparison with published data [Pachaly et al., 1994].
α-tocopherol as natural antioxidant. Their IC\textsubscript{50} values are as follows: Martynoside 3.6x10^{-4} M; acetoside 2.4x10^{-4} M and dl-α- tocopherol 4.8x10^{-4} M. Inhibitory activity of acetoside was higher than that of martynoside.

**Anti-inflammatory activity**

The results are presented in Table 2. The phenylpropanoid glycosides decreased edema in the range of 22-31 % at a dose of 10 mg/kg after 3 h with respect to the control group treated only with carrageenin, against phenylbutazone (60 % decrease) at a dose of 100 mg/kg, which indicated that the test compounds had moderate anti-inflammatory activity. Scropheanoside- III (31% decrease) and Scropheanoside- I (26 % decrease). It may be concluded that compound containing a cinnamoyl moiety as found in Scropheanoside- III has significant activity.

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