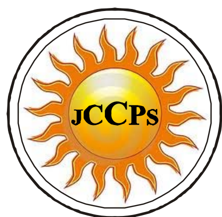

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ISOLATION OF LUTEOLIN 8-C-β-D-GLUCOPYRANOSIDE FROM THE ROOTS OF *SALVADORA PERSICA* (RUTACEAE)

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ABSTRACT

A flavone glucoside, luteolin 8-C-*β*-D-glucopyranoside was isolated from the ethyl acetate extract of the roots of *Salvadora persica* and separated using column chromatography techniques. The structure of the compound was established by UV, IR, ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, DEPT and MS studies. The compound has never been reported previously from this plant.

Key words: Flavone glucoside, *Salvadora persica*, Rutaceae, Spectroscopic data.

INTRODUCTION

Salvadora persica, belongs to the family Rutaceae, commonly known as Arak tree. According to Rastogi et al.[1], the species contain trimethylamine, chloride, fluoride in a large amounts, silica sulphur, vitamin C and small quantities of tannins, saponions and sterols. The fluoride content is known to be beneficial and the silica in many chewing sticks help to clean the teeth, acting as an abrasive. It is used for centuries as a natural toothbrush. Its fibrous branches have been promoted by the World Health Organization for oral hygiene use. Research suggests that it contains a number of medically beneficial properties including abrasives, antiseptics, astringent, detergents, enzyme inhibitors and fluoride. Chewing sticks derived from the Rutaceae contain alkaloids, which have a bacterial effect. Those obtained from *Aegles marmelos*, *Salvadora persica*, *Azadirachta indica* and somenative other plants contain essential oils and exert corminative, antiseptic and analgaesic action[2-6].

The chemical constituents of medicinal plants of Rutaceae family found in different countries especially in India, Negiria and Phillipine contains different kinds of flvonoids[7-10]. Here, we have reported the isolation and characterization of flavone glucoside from ethyl acetate extract of the roots of *Salvadora persica*.

EXPERIMENTAL

General procedures

Ultra-violet absorption spectrum was recorded on Perkin–Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed using the KBr disc method on Perkin-Elmer 1710 Infrared Fourier Transformation Spectrometer. NMR spectra were recorded on Bruker AVANCE DRX- 400 (400, 100 Hz).

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Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. FEBMS was recorded on JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried using silica gel (60-120 mesh). Chemicals are of analytical reagent grade and TLC Silica gel plates (0.25 mm), were purchased from E. - Merck.

Plant material

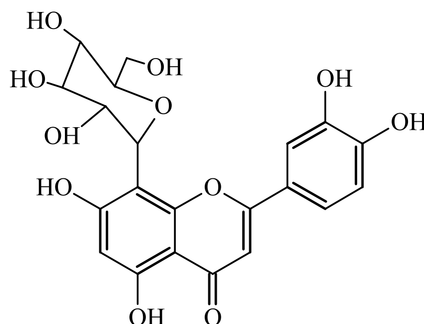
The roots of *Salvadora persica* used in this study were collected from the rural areas of Kingdom of Saudi Arabia. A voucher specimen was deposited at the Herbarium of Department of Biology, Taif University (RK 4482). The roots collected were further shelled and dried at room temperature. The dried roots were ground to a fine powder using Thomas-Willey Milling Machine.

Extraction and isolation

Dried, pulverized roots (2.5 Kg) of *Salvadora persica* first defatted with petroleum ether (3 l × 5 times) for the removal of apolar substances and then extracted with chloroform, ethyl acetate and methanol (3 l × 5 times each). The ethyl acetate extract was then evaporated under vacuum on rotatory evaporator below 50°C temperature to yield a brownish mass (68.0 g). A well stirred suspension of silica gel (100-150 g in petroleum ether 60-80°C) was poured into column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess of petroleum ether was allowed to pass through column. Slurry was made to this mass with silica gel in petroleum ether and was digested to well stirred column. The column was successively eluted with the petrol, chloroform, ethyl acetate and methanol and their mixtures of increasing polarity. Elution with chloform: methanol (2 : 8) afforded a yellow powder (1.43 g). Purified several times by recrystalisation using ethanol as a solvent and then checked the purity of the yield by determining the melting point.

Compound

Orientin (Luteolin-8-*C*-glucoside; Lutexin) $C_{21}H_{20}O_{11}$ was obtained as yellow powder, UV $\lambda_{nm:}$ (MeOH) 350, 269, 257, (MeOH-NaOMe) 268, 277, 405, (MeOH-NaOAc) 272, 277, 327, 393), (MeOH-NaOAc-H_3BO_3) 267, 303, 375, 422, (MeOH-$AlCl_3$) 274, 333, 418, (MeOH-$AlCl_3$-HCl) 267, 276, 313, 357, 384. IR (KBr, cm^{-1}): 3410 (-OH), 1655 (α, β-unsaturated carbonyl group), 1613 (aromatic C=C). ESIMS: 448 $(M)^+$, 430 $(M\text{-}H_2O)^+$, 314 $(M\text{-}C_8H_6O_2)^+$, 299 $(M\text{-aglycone-}CH_2)^+$, 286 $(M\text{-}162)^+$, 134 $(M\text{-}314)^+$, 69 $(M\text{-}379)^+$. ^1H NMR (400 MHz, DMSO-d_6) δ: 13.15 (1H, s, 5-OH), 10.82-9.15 (3H, s, 3′, 4′, 7′-OH), 7.52 (1H, dd, J = 2.2, 8.3 Hz, H-6′), 7.45 (1H, d, J = 2.2 Hz, H-2′), 6.90 (1H, d, J = 8.3 Hz, H-5′), 6.65 (1H, s, H-3), 6.56 (1H, s, H-6), 5.05 (1H, d, J = 7.0 Hz), 3.30-3.90 (sugar protons, *m*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.16 (C-2), 102.41 (C-3), 182.03 (C-4), 160.47 (C-5), 98.33 (C-6), 162.80 (C-7), 104.65 (C-8), 156.00 (C-9), 104.00 (C-10), 121.97 (C-1′), 114.07 (C-2′), 145.95 (C-3′), 149.90 (C-4′), 115.78 (C-5′), 119.45 (C-6′), 73.50 (C-1″), 70.90 (C-2″), 78.88 (C-3″), 70.83 (C-4″), 82.04 (C-5″), 61.76 (C-6″). R_f: 0.58: EtOAc:HOAc:HCO_2H:H_2O (10 : 1 : 1 : 2.5), 0.67: EtOAc : $CH_3CH_2COCH_3$: HCO_2H : H_2O (5 : 3 : 1 : 1); elemental analysis: Calcd. % C = 54.82, % H = 4.55, % O = 43.64. Found: % C = 54.96, % H = 4.48, % O = 43.75.



Luteolin-8-C-gkycoside

RESULTS AND DISCUSSION

The compound was obtained as a yellow powder, exhibited a molecular ion peak at *m/z* 448 $(M + H)^+$ in its electro spray mass spectrum corresponded to the molecular formula $C_{21}H_{20}O_{11}$. The compound showed positive ferric chloride and Shinoda tests for flavonoids, suggested that the compound may be a flavonoid[11,12]. These results also suggested that compound is a flavonoid derivative with a free hydroxyl group at C-5[11].

The UV spectrum in MeOH gave maxima at 350 and 269 nm, respectively, indicating that the compound belongs to the flavone groups. 3′, 4′- or 3′, 4′, 5′-oxygenated flavones usually exhibit two absorption peaks (one maximum with a shoulder) between 250 and 275 nm, while the 4′-oxygenated equivalents have only one peak in this range[12]. The methanol UV spectra of this compound showed two peaks at 257 and 269 nm, respectively; indicating that the B-ring contain 3′, 4′- or 3′, 4′, 5′-OH groups. The NaOMe spectrum of the compound was stable for 5 min confirming the absence of 3-OH group. The NaOAc/H_3BO_3 spectrum also indicated for the presence or absence of an ortho-dihydroxy group at all locations of the flavonoid nucleus[13-16].

Flavones containing ortho-dihydroxy group in ring-B shows a consistent 12-30 nm bathochromic shift of band I in the presence of NaOAc/H_3BO_3, diagnostic for the presence of an ortho-dihydroxy group (at C-6, 7 or C-7, 8) in the A-ring. This compound produced a significant bathochromic shift (350-375 nm), which is diagnostic for the presence of an ortho-dihydroxy group (C-3′, 4′ or C-4′, 5′) in the B-ring.

The presence of an ortho-dihydroxy group in the B-ring of flavones can be detected by comparison of the spectrum of the flavonoid in the presence of $AlCl_3$ with that obtained in $AlCl_3$/HCl. A hypsochromic shift of 30-40 nm was observed in band I of the $AlCl_3$ spectrum on the addition of acid concluded the presence of ortho-dihydroxy group.

The bathochromic shift with $AlCl_3$, i.e. the band I shift from 350 nm, splitting into two bands with peaks at 333 and 418 nm, indicated the presence of an OH group in position 5 (Markham, 2001; Harborne and Baxter, 1999). After adding the NaOAc, the band shift II by 8 nm (277-269 nm), indicated the presence of OH group at position 7. After the addition of NaOAc + H_3BO_3 the shift of band I by 25 nm (375-350 nm), indicated the presence of OH group in positions 3′ and 4′[13-17]. The IR spectra showed absorption bands at 3410 (-OH), 1655 (α, β-unsaturated carbonyl group) and 1613 (aromatic C=C) cm^{-1} functionalities. The ^1H NMR spectrum of the compound exhibited signal at δ 13.15 (1H, s) attributed a chelated hydroxyl group. Further four signals observed at δ 10.82-9.15 were due to a phenolic hydroxyl groups. The ^1H NMR also demonstrated two one proton doublets at δ 7.45 (1H, d, $J = 2.2$ Hz) and δ 6.90 (1H, d, $J = 8.3$ Hz) and one doublet of doublet at δ 7.52 (1H, dd, $J = 2.2$, 8.3 Hz) assignable to H-2′, H-5′ and H-6′ protons, respectively. The appearance of two doublets and their coupling constant values are in further agreement with the hydroxy groups at C-3′ and C-4′ i.e., a luteolin moiety as a basic skeleton[15-19].

The ^1H NMR displayed one proton singlet at δ 6.65 could be assigned to H-3 proton[17]. In addition, the methine carbon signal at δ 102.41 was attributed to C-3 in the ^{13}C NMR spectrum, indicating a 5, 7-dihydroxy-flavone. In ^1H NMR a one proton singlet appearing at δ 6.56 was attributed for H-6 proton. The resonances of the anomeric proton observed in the low-field region in the ^1H NMR spectra at δ 5.05 (1H, d, $J = 7.0$ Hz) of the compound, implied that compound was luteolin glucoside. No sugar was released, when the compound was heated along with acid, confirmed the presence of C-glycosylation. The $(M-18)^+$ peaks in mass spectrum also supported the presence of C-glycosylation[18].

The structure was further supported by its ^{13}C NMR spectrum[19,20], which demonstrated a downfield signal at δ 182.03 assigned to carbonyl carbon C-4. The three downfield signals appearing at δ 145. 95,

149.90, 160.47 and 162.80 were assigned to C-3′, C-4′, C-5 and C-7, bearing hydroxyl group, respectively. Further, a signal at δ 98.33 assigned to C-6 further supported presence of hydroxyl group at C-5 and C-7. The anomeric carbon signal at δ 73.50 in its ^{13}C NMR spectrum indicates the 8-C-β-D-glucopyranoside structure of the compound[21]. The position of sugar was concluded to be at C-8-OH based on comparison of ^{1}H and ^{13}C NMR spectra with those reported in the literature of known compound orientin (luteolin 8-C-β-D-glucopyranoside[21].

CONCLUSION

From the survey of the literature to the best of our knowledge luteolin 8-C-β-D-glucopyranoside was previously unknown from *Salvadora persica* and further examination of the constituents of this plant is currently in progress.

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