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Corniculatin A, a new flavonoidal glucoside from *Oxalis corniculata*

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Abstract: *Oxalis corniculata* L. (creeping wood sorrel) is medicinally important member of family Oxalidaceae, and is distributed in the tropical and subtropical regions of the world. It has been used in different systems of traditional medications for different diseases. Corniculatin A, a new flavonoid glucoside, was isolated from the ethyl acetate soluble fraction of the whole plant along with luteolin, luteolin-7-*O*-β-D-glucoside and β-sitosterol-3-*O*-β-D-glucoside, This is the first report of these compounds from this species. Their structures were elucidated on the basis of spectral data including mass and 2D NMR experiments.

Introduction

The genus *Oxalis* consists of over 900 species which are mostly distributed in sub-tropical regions of Asia, and is also present in some other parts of the world (Bhattacharjee, 1998). An important species of this genus is *Oxalis corniculata* L. (commonly known as creeping wood sorrel), which is common in Africa, China, Phillipines, India and Pakistan (Jafri, 1966). It is delicate in appearance and slow growing herbaceous plant. It is present nearly in all warmer parts of India and Pakistan, especially in the Himalayan regions up to 8,000 ft-cosmopolitan (Kirtikar & Basu, 1975). It has versatile medicinal uses like treatment for relieving the intoxication produced by *Datura*, as a refrigerant (Mohammad & Mir, 2000), a decoction of the roots is used to treat a range of health problems including giddiness, dysentery, diarrhea and worms (Kirtikar & Basu, 1975). The leaves are used for curing various diseases like cold, fever, cough, stomachache, stop bleeding from wounds and as anthelmintic (Mohammad & Mir, 2000). Recently its anticancer activity was reported (Kathiriya et al., 2010). *Oxalis corniculata* is a well known herb to have an acid taste due to the high content of oxalate in its leaves and stems. Phytochemical investigations of *O. corniculata* have revealed the presence of palmitic acid, tannins, and a mixture of oleic, linoleic, linolenic and stearic acids (Raghvendra et al., 2006). Methanolic and ethanolic extracts of this plant have carbohydrate, proteins, amino acids, volatile oil, glycosides, phytosterols, phenolic compounds and flavonoids. It also contains calcium, fibers and tannins. Leaves contain flavones, calcium oxalate, citric acids and tartaric acid etc. (Unni et al., 2009). It has been reported in the literature, the flavones and derivatives of cinnamic acids and benzoic acids are observed so far in *O. corniculata* (DellaGreca et al., 2007; 2009; Mizokami et al., 2008). The chemotaxanomic and ethnopharmacological importance of the genus *Oxalis* prompted us to carry out further studies on *O. corniculata*. Our research group is interested in searching for new flavonoidal glycosides from natural resources and the aim of this research was the isolation and identification of the antioxidant active compounds from the *O. corniculata* plant.

Materials and Methods

Plant material

Healthy, whole plant of *Oxalis corniculata* L., Oxalidaceae, identified by Dr. Surraiya Khatoon, plant taxonomist, Department of Botany, University of Karachi, where a voucher specimen (no. KU 112b) was deposited.

Extraction and isolation

The shade dried plant material (20 kg) was ground and extracted thrice with ethanol at room temperature. Solvents were evaporated through vacuum distillation to obtain the crude ethanolic extract (490 g). The crude ethanolic extract was first dissolved in water and defated with *n*-hexane, which gave *n*-hexane fraction

(80 g). The water soluble fraction was further fractionated into chloroform (30 g), ethyl acetate (90 g) and *n*-butanol soluble fractions (155 g).

Ethyl acetate fraction

The column chromatography of the EtOAc soluble sub-fraction (90 g) over silica gel was performed using *n*-hexane/EtOAc (in increasing order of polarity) as solvent system, which afforded six major sub-fractions A-F.

Sub-fraction D

It was subjected to further column chromatography over silica gel using *n*-hexane-EtOAc (3.5:6.5) and *n*-hexane-EtOAc (4:6) solvent systems which afforded compound **2** (6 mg) and compound **4** (21 mg) respectively.

Sub-fraction E

Further column chromatography of this fraction over silica gel eluting with dichloromethane/methanol solvent system was carried out. It afforded two pure compounds. Compound **3** (8 mg) was obtained from solvent system dichloromethane/methanol (9.5:0.5). Similarly dichloromethane/methanol (9:1) solvent system gave compound **1** (25 mg).

Instruments and materials

The UV and IR spectra were recorded on a Hitachi UV-3200 and JASCO 302-A spectrometers. ^{1}H NMR and ^{13}C NMR and two-dimensional COSY, NOSEY, HMQC, and HMBC, were recorded on the Bruker AV-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in C5D5N with TMS as internal stander. Chemical shifts δ are shown in ppm relative to TMS as internal standard and scalar coupling are reported in Hz. The HR-FAB-MS were recorded on a JEOL JMS-HX-110 mass spectrometer. Analytical and preparative TLC were carried out on pre-coated Silica gel 60 F254 plates (E. Merck, Darmstadt, Germany), and visualized under UV radiation light and by spraying with the ceric sulfate solution. Silica gel (230-400 mesh, E. Merck) was used for column chromatography. Melting points were determined on a Gallenkemp apparatus and are uncorrected.

Results and Discussion

The ethanolic extract of *Oxalis corniculata* was suspended in water and fractioned into *n*-hexane, chloroform, ethyl acetate and *n*-butanol soluble fractions. Column chromatography of the ethyl acetate fraction provided compounds **1-4**, respectively.

Corniculatin A (**1**) was obtained as a yellowish gummy solid which gave violet coloration with $FeCl_3$ for a phenol and males test for glycoside (Shinoda, 1928). The IR spectrum showed the presence of hydroxyl groups (3315-3420 cm^{-1}), ester carbonyl (1695 cm^{-1}), conjugated carbonyl (1671 cm^{-1}), olefinic bond (1630 cm^{-1}) and aromatic moiety (1541 and 1510 cm^{-1}). The UV spectrum was characteristic of flavonoid glycoside showing the absorption maxima 208, 265 and 323 nm (Mabry et al., 1970). The addition of $AlCl_3$ and HCl, the band II showed a bathochromic shift of 28 nm revealing the presence of the chelated hydroxyl group at C-5 of a flavonoid (Mabry et al., 1970). The molecular formula was deduced from the HR-FAB-MS (positive mode), showing a quasi-molecular ion peak at *m/z* 595.1459 consistent with the molecular formula $C_{30}H_{26}O_{13}$. EI-MS frangmentation pattern gave further clue about the structure, important fragments in EI-MS were at *m/z* 486 [M-*p*-hydoxycinnamoyl]$^{+}$ and 286 [M-*p*-hydroxycinnamoyl-glucose]$^{+}$. In addition, the presence of fragments at *m/z* 152 and 442 were due to Diels Alder fragmentation confirming the presence of two hydroxyl groups in ring A and the remaining subsitituents in ring B. It was further confirmed by the broadband and DEPT ^{13}C-NMR.

The ^{1}H-NMR spectrum of **1** showed a characteristic downfield signal at δ 12.81 assignable to a chelated hydroxyl group at C-5. Two *meta* coupled hydrogens of ring A were observed as doublets (*J*=2.0 Hz) at δ 6.25 and 6.53. The three aromatic protons of the tri-substituted ring B resonated at δ 7.81 (1H d, *J*=2.3 Hz), 7.81 (1H d, *J*=8.2 Hz) and 7.45 (1H dd, *J*=8.3, 2.3 Hz). It also showed the presence of (*E*)-*p*-hydroxycinnamoyl moiety [trains olefinic protons at δ 6.34 and 7.62 (each 1H, d, *J*=15.3 Hz) and *p*-hydroxyphenyl ring showing AA′BB′ pattern at δ 7.48 (d, *J*=8. 4 Hz) and 7.28 (d, *J*=8. 4 Hz)]. The anomeric proton of the hexose moiety was observed at δ 5.21 (d, *J*=7.3Hz) confirming its β-linkage. Further signals of oxymethine and oxymethylene protons were observed in the range of δ 4.90-3.19 (Table 1).

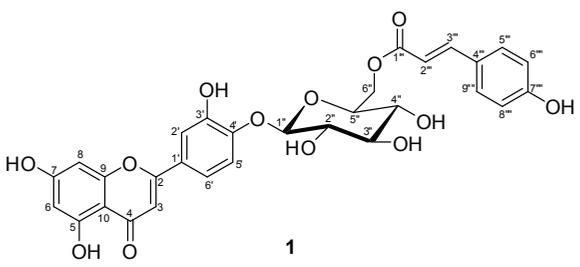
The broadband and DEPT ^{13}C-NMR spectra showed thirty signals comprising one methylene, seventeen methine and twelve quaternary carbons (Table 1). The signals at δ 158.4, 106.1, 178.2, 161.6, and 104.2 were typical of C-2, C-3, C-4, C-9 and C-10 of a flavone moiety (Imperato et al., 1997). It also showed signals for an ester carbonyl at δ 169.1 and olefinic carbons at δ 117.7 and 144.4. Three oxygenated aromatic carbons resonated at δ 164.8, 157.2 and 149.2. The anomeric carbon of a hexose moiety was observed at δ 100.3 while its oxymethine and oxymethylene carbons were observed in the range of δ 75.3-62.3.

In flavonoids having glucose moiety at C-3′, C-3′ and C-4′ resonates at δ 145.4 and δ 150.2 respectively (Agrawal, 1989). While in flavonoids having glucose

Table 1. ^{1}H (400 MHz), ^{13}C (100 MHz) NMR and HMBC correlations of compound **1** (C_5D_5N).

Position	^{13}C-NMR	^{1}H-NMR	HMBC
2	158.4	-	-
3	106.1	6.93	1′,4,2,10
4	178.2	-	-
5	157.2	-	-
6	99.9	6.26 (1H, d, *J* =2.0Hz)	5, 7, 8, 10
7	164.8	-	-
8	94.3	6.53 (1H, d, *J* =2.0Hz)	6, 7, 9, 10
9	161.6	-	-
10	104.2	-	-
1′	125.5	-	-
2′	123.9	7.81 (1H, d, *J*= 2.3Hz)	3′, 4′, 5′
3′	148.5		
4′	149.2		
5′	119.01	7.93 (1H, d, *J*= 8.2Hz)	4′, 6′
6′	129.3	7.45 (1H, dd, *J*= 2.3,8.2 Hz)	1′,5′,2′
5-OH	-	12.81	-
1″	100.3	5.21 (d, *J* = 7.3Hz)	4′, 2″,5″
2″	75.3	3.51 (1H,m)	1″, 2″, 4″
3″	71.6	3.62 (1H,m)	1″, 5″
4″	72.1	3.19 (1H,m)	2″,3″
5″	77.2	4.02 (1H,m)	4″,3″
6″	68.3	4.90 (1H, dd, *J* =5.3, 11.4 Hz) 4.42 (1H, dd, *J* =5.5, 11.4 Hz)	5″
1‴	169.1	-	-
2‴	117.7	6.37 (1H, d, *J* =15.3Hz)	3‴, 4‴
3‴	144.5	7.62 (1H, d, *J* =15.3Hz)	1‴, 5‴, 9‴
4‴	125.5	-	-
5‴, 9‴	132.1	7.48 (1H, dd, *J* =2.4, 8.4 Hz)	4‴, 6‴, 7‴
6‴, 8‴	115.7	7.28 (1H, dd, *J* =2.4, 8.4 Hz)	7‴, 5‴, 9‴
7‴	161.8	-	-

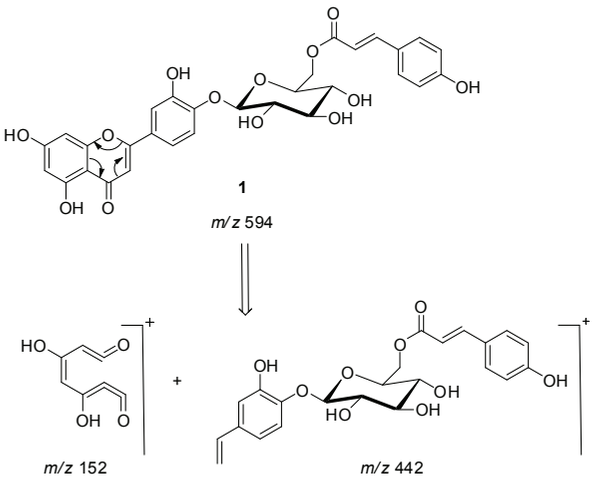
moiety at C-4′, C-3′ and C-4′ resonates at δ 147.2 and δ 148.7 respectively (Agrawal, 1989). In our compound C-3′ and C-4′ resonates at δ 148.5 and δ 149.2, respectively, which confirmed the presence of glucose moiety at C-4′. The presence of glucose moiety at C-4′ was further confirmed by the HMBC experiments which showed 3J correlation of the anomeric proton at δ 5.21 with C-4′ (149.2), C-3″ (71.6), C-5″ (77.2) and ^{2}J correlations with C-2″ (75.3). Simmilarly the (*E*)-p-hydroxycinnamoyl moiety was located to be present at C-6″ with the help of HMBC correlations between the H-6″ protons at δ 4.90 and δ 4.42 with C-1‴ (169.1). The other HMBC correlations were in complete agreement with the assigned structure of corniculatin A as luteolin-6″-(*E*-*p*-hydroxycinnamoyl) 4′-*O*-β-D-glucopyranoside (**1**).



Corniculatin A

Yellow gummy solid: [α]D^{20}+51.7: UV λ_{max} MeOH (log ε) nm: 210 (2.9), 264 (2.2), 323 (4.5); IR ν_{max} KBr cm-1: 3315-3420 (OH), 1695 (ester CO), 1671 (CO), 1630 (C-C), 1541, 1510 (aromatic moieties); EI-MS *m/z*

(rel. int. %): 486, 286, 152 and 442 HR-FAB-MS (positive mode): *m/z* 595.1459 $[M+H]^+$ (calcd. for $C_{30}H_{27}O_{13}$, 595.1450); For ^1H and ^{13}C NMR data (Scheme 1).



Scheme-1. Retro-Diels-Alder fragmentation of **1**.

Acid hydrolysis

Compound **1** (8 mg) was dissolved in MeOH (10 mL), added 10 % HCl (3 mL) and refluxed for 35 min. After cooling, it was extracted in dichloromethane. The residue from the organic phase was subjected to column chromatography over silica gel using hexanes-acetone (4:6) to afford luteolin, m.p. 330-333 °C. Increasing the polarity with *n*-hexane-acetone [(3:7) furnished (*E*)-*p*-hydroxycinnamic acid, m.p. 137 °C. These were identified through comparison of physical and spectral data with those reported in the literature (Awaad et al., 2006). The sugar from the aqueous phase could be identified as D-glucose through optical rotation and Co-TLC with an authentic sample.

The key structure of corniculatin A (**1**) was provided by acid hydrolysis, besides the sugar moiety, the aglycones identified as (*E*)-*p*-hydroxycinnamic acid and luteolin, respectively, through comparison of physical and spectral data with those reported in literature (Haruna et al., 1982; Mitscher et al., 1985).

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Authors' contributions

MI contributed in collecting the plant sample, running the laboratory work, analysis and drafting of the data. MI, TM and NH contributed to the analysis of the spectroscopic data and writing of the manuscript. IH designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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