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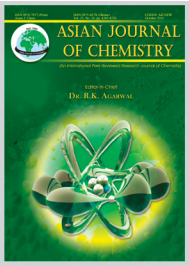

Constituents of *Cressa cretica* L., A Halophytic Plant

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Three coumarins (**1-3**), four flavonoids (**4-7**) along with two phytosterols (**8-9**) were isolated from a halophytic plant *Cressa cretica*. Their structure established as coumarin (**1**), umbelliferone (**2**), daphnetin (**3**), quercetin (**4**), kaempferol (**5**), quercetin 3-O-β-D-glucoside (**6**), quercetin-3-O-α-L-rhamno-(1→6)-β-D-glucoside (**7**), stigmasterol (**8**) and β-sitosterol (**9**) on the basis of their comprehensive spectroscopic analysis including UV, IR, ^{1}H NMR, ^{13}C NMR, DART mass data.

Key Words: *Cressa cretica*, Halophytic, Convolvulaceae, Glycosides.

INTRODUCTION

Cressa cretica L. (Convolulaceae), popularly known as Rudanti in Hindi and is widely grown halophytic plant. Different parts of the plant have been claimed to be valuable in a wide spectrum of diseases[1-3]. In earlier studies *Cressa cretica* L. flowers exhibited cytotoxic and antiinflammatory activity *in vitro*[4]. *Cressa cretica* is reported to be antibilous, antituberculosis and expectorant[2,3,5]. It is also reported the fruits of *Cressa cretica* is a potential source of edible oil. The oil of *Cressa cretica* was free from any undesirable components and could safely be recommended for human consumption[6]. Previously we reported the antitussive activity of the plant in rhodents[7].

EXPERIMENTAL

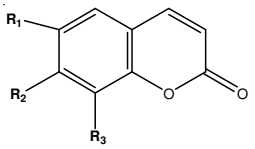
The plants (whole part of *Cressa cretica*, 10 kg) were collected from Nalban Island of Chilika lake, Orissa, India and was preliminarily identified by Dr. M. Brahmam, Senior Scientist, Natural product division, Institute of Mineral and Material Technology, (formerly known as Regional Research laboratory, Bhubaneswar) India and which was later on authenticated from botanical survey of India, Howrah, West Bengal, India vide access no. (CNH/I-I/32/2010/Tech.II/237-3). A voucher specimen has been kept in our laboratory for future reference.

Preparation of extract: The aerial parts were air-dried pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with methanol. The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40 ºC through rota-vapour (Rotavapour RII, Buchi Labortechnik AG, Switzerland) to obtain dry extract (16.73 % w/w). *Cressa cretica* methanolic extract (CME) was adsorbed on to the 250 g of silica gel of 60-120 mesh size and fractionated using solvents of increasing polarity such as hexane (Fr-He), ethylacetate (Fr-Et) and methanol (Fr-Me).

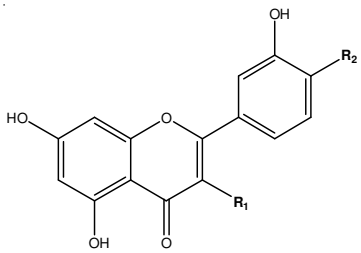
Isolation of phytoconstituents from Fr-Et and Fr-Me: Ethyl acetate fraction (Fr-Et) was repeatedly subjected to silica gel column chromatography, respectively, eluting with gradient hexane: chloroform: methanol to give three fractions (A, B, C) (3:4:0, 1:7:2, 0:1:1). The fraction A was subjected to further silica gel chromatography and followed by preparative TLC (PTLC) using hexane:CH_2Cl_2:MeOH (1:3:2) gave two compounds, compound **1** (27.1 mg) and compound **2** (32.4 mg), compound **3** (16.6 mg) fraction B by eluting column and subsequent PTLC using $CHCl_3$:MeOH:acetic acid (1:1:0.5) gave compound **4** (18.9) mg and fraction C gave compound **5** (21.7) mg using $CHCl_3$:MeOH:acetic acid (5:2:1)as solvent system for PTLC.

The methanol fraction was steeped in water pH 3 and partitioned with EtOAc (1:1) to yield organic and aqueous extracts fractions. The aqueous extract fraction was adjusted to pH 12 and partitioned with EtOAc (1:1 v/v) to yield basic fraction (Fr. A_1 0.421 g). Organic extracts were partitioned with 5 % $NaHCO_3$ (pH 9) to yield organic and aqueous extracts. The aqueous extract was adjusted to pH 3 and partitioned with EtOAc (1:1) to yield acidic fraction (Fr. B1 0.286 g). While, the organic extracts were dried with Na_2SO_4 and partitioned with 5 % Na_2CO_3 (pH 12) to yield organic and aqueous extracts. The aqueous extract was adjusted to pH 6 and partitioned with

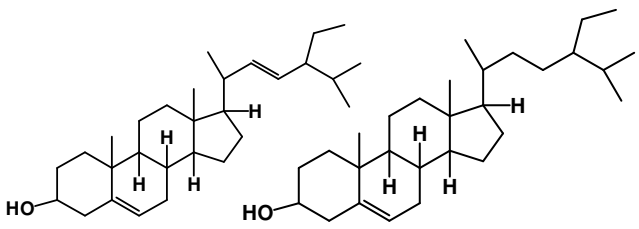
EtOAc (1:1) to yield phenolic fraction (Fr. C_1 0.354 g). Finally, the organic extracts fraction was dried on Na_2SO_4 and evaporated in rotary evaporator to yield neutral fraction (Fr. D_1 1.154 g). The phenolics fraction was subjected to silica gel column and eluted with hexane: EtOAc: MeOH (1:7:2). That was chromatographyed on silica gel column and followed by preparative TLC (PTLC) plates silica gel 60 F_{254} (Merck Ltd.) using chloroform:MeOH (2:1) as solvent system to give compound **6** (29.5 mg) and compound **7** (23.1 mg). The neutral fraction (Fr. D_1) was subjected to silica gel column and eluted with hexane: EtOAc: acetone to yield fraction at 8.5:1.5:0 (fraction D_1-1). Fraction (D_1-1) was chromatographyed on a silica gel column and eluted with hexane:EtOAc (2:1) and followed by preparative TLC (PTLC) plates silica gel 60 F_{254} (Merck Ltd.) using ether: hexane: MeOH (5:1:0.1) and pet ether: ethylacetate (9:1) as a solvent system to give two compounds **8** (36.3 mg) and compound **9** (19.7 mg). The structures are represented in Fig. 1.



	R_1	R_2	R_3
Coumarin (**1**)	H	H	H
Umbelliferone (**2**)	H	OH	H
Daphnetin (**3**)	H	OH	OH



	R_1	R_2
Quercetin (**4**)	OH	OH
Kampferol (**5**)	OH	H
Quercetin-3-O-glucoside (**6**)	O-gluc	OH
Rutin (**7**)	O-rutinosyl	OH



Stigmasterol (**8**) β-sitosterol (**9**)

Fig. 1. Structures of the isolated compounds from *Cressa cretica*

RESULTS AND DISCUSSION

Compound **1**, obtained as white crystalline powder, m.p. 69 ºC, has a UV spectrum (MeOH λ_{max}, nm): 310 and 273, showed a green fluorescence. IR (KBr, γ, cm^{-1}): 1710, 1683, 1610, 1558, 1402, 1220. Band at 1710 cm^{-1}, a typical peak if coumarin lactone another band at1683 cm^{-1} attributed to a carbonyl absorption. ^{1}H NMR (400 MHz, DMSO, δ, ppm, J/Hz): 7.96 (1H, d, *J* = 9.5 Hz, H-4), 7.64 (1H, d, *J* = 8.4 Hz, H-5), 7.29 (2H, dd, *J* = 8.4,2.2 Hz, H-6, H-7), 6.70 (1H, H-8). ^{13}C NMR (100 MHz, DMSO, δ, ppm): 159.89 (C-2), 116.14 (C-3), 144.42 (C-4), 131.83 (C-5), 124.37 (C-6), 128.35 (C-7), 116.19 (C-8), 153.43 (C-9). DART- MS m/z: 147 $[M]^+$ (100). Mol formula was found to be $C_9H_6O_2$. Identified as simple coumarin, isolated for first time from *Cressa cretica*.

Compound **2**, yellowish white crystalline powder, m.p. 224.4 ºC, UV spectrum (MeOH λ_{max}, nm): 253, 324. IR (KBr, γ, cm^{-1}): 3161, 1680, 1600, 1579, 1400, 1385, 1238, 1139. ^{1}H NMR (400 MHz, DMSO, δ, ppm, J/Hz) 10.567(1H, s H-7), 7.89 (1H, d, *J* = 9.5 Hz, H-4), 7.59 (1H, d, *J* = 8.4 Hz, H-5), 6.78 (1 H, dd, *J* = 8.4,2.2 Hz, H-6), 6.70 (1H, d, *J* = 2.2 Hz, H-8). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 160.53 (C-2), 112.13 (C-3), 154.58 (C-4), 129.77 (C-5), 113.13 (C-6), 161.35 (C-7), 102.23 (C-8). DART- MS m/z: 163.06 $[M]^+$ (100), Mol formula was found to be $C_9H_6O_2$. Identified as umbelliferone[8].

Compound **3**, isolated as off white amorphous solid, m.p. 79 ºC, DART- MS m/z 178.1 $[M]^+$. Its UV spectrum (MeOH λ_{max}, nm): 266, 322 was characteristic of a coumarin derivative. The IR spectrum showed the presence of an ester function and an α-β unsaturated lactone (1735 and 1720 cm^{-1}). The ^{1}H NMR spectrum of **1** showed two proton doublets at δ 7.90 and 6.10 (*J* = 9.5 Hz) characteristic for the H-3 and H-4 of coumarins[9]. The presence of further two proton doublets at δ 7.15 and 7.07 (*J* = 8.7Hz) in the ^{1}H NMR spectrum and resonance of carbons bearing two oxygen moieties at 149.4 and 135.7 indicated a 7, 8-disubstituted coumarin. IR (KBr, γ, cm^{-1}): 3458, 1735, 1720, 1690, 1620, 1500, 1290, 1200 and 1100. ^{1}H NMR (400 MHz, DMSO, δ, ppm, J/Hz): 7.90 (1H, d, *J* = 9.5Hz, H-4), 7.35 (1H, d, *J* = 8.5Hz, H-5) 6.65 (1H, d, 8.5 Hz, H-6), 6.12 (1H, d, *J* = 9.5Hz, H-3), 5.0 (2H, d, *J* = 8.0 Hz, H-7,H-8). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 160.9 (C-2), 114.6 (C-3), 146.0 (C-4), 119.4 (C-5), 114.0 (C-6), 149.4 (C-7), 135.7 (C-8), 116.4 (C-9), 144.3 (C-10). Identified as daphnetin, isolated for the first time from this plant [10].

Compound **4**, obtained as yellow needles, m.p. 311 ºC; IR (KBr, γ, cm^{-1}): 3350, 1685, 1615; UV (MeOH λ_{max}, nm): 255, 370. ^{1}H NMR (400 MHz, DMSO, δ, ppm, J/Hz) δ 6.191 (1H, s, H-6), 6.35 (1H, s, H-8), 7.68 (1H, *J* =- 2.0 Hz, H-2'), 6.90 (1H, d, *J* = 8.5 Hz, H-5´), 7.40 (1H, d, *J* =- 8.5 Hz, H-6´); ^{13}C NMR (100 MHz, DMSO, δ, ppm) 175.9 (C-3), 147.76 (C- 2), 160.79 (C-5), 98.267 (C-6), 163.94 (C-7), 156.21 (C-9), 122.05 (C-1´), 115.14 (C-2´), 146.8 (C-3´), 145.12 (C-4´), 115.68 (C-5´). DART-MS m/z 303 $[MH]^+$, 302. Identified as quercetin[11].

Compound **5**, isolated as yellowish green amorphous powder, m.p. 282 ºC. UV (MeOH λ_{max}, nm): 266, 324, 365. IR (KBr, γ, cm^{-1}): 3321, 2816, 1662, 1654, 1608, 1502, ^{1}H NMR spectrum(400 MHz, DMSO, δ, ppm, J/Hz) 6.19 (1H, d, *J* = 2.0 Hz, H-6), 6.44 (1H, d, *J* = 2.0 H-8), δ 6.92 (2H, d, *J* = 8.0 Hz, H-8), 8.03 (2H, d, *J* = 8.0 Hz, H-3´-5´). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 179.40 (C-4), 163.90 (C-7), 162.99 (C- 5), 161.60 (C-4'), 156.19 (C-9), 135.68 (C-3), 122.08 (C-1´), 116.20 (C-3´, C-5´), 104.70 (C-10). DART-MS m/z 287. Identified as kampferol[12].

Compound **6**, isolated as amorphous yellow solid with molecular formula as $C_{21}H_{20}O_{12}$ determined from DART-MS and NMR data. Its UV (MeOH λ_{max}, nm) 358, 256. IR (KBr, γ,

cm^{-1}): 3290,1658,1606,1060. ^1H NMR spectrum (400 MHz, DMSO, δ, ppm, J/Hz) 7.70 (1H, d, *J* = 2.1 Hz, H-2´), 7.59 (1H dd, *J* = 8.4, 2.1 Hz, H-6´), 6.89 (1H d, *J* = 8.4 Hz, H-5´), 6.83 (1H, d, *J* = 2.3 Hz, H-8), 6.40 (1H, d, *J* = 2.1 Hz, H-6), 5.12 (1H, d, *J* = 7.5 Hz, H-1´´), 3.70-3.20 (5H, sugar). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 158.40 (C-2), 135.60 (C-3), 179.40 (C-4), 161.00 (C-5), 123.10 (C-1´), 117.30 (C-2´), 145.90 (C-3´), 149.20 (C-4´), 116.00 (C-5´), 123.20 (C-6´), 104.30 (C-1´´), 78.10 (C-2´´), 75.70 (C-3´´) DART-MS m/z $[M]^-$ 463 (100) and 303. The NMR spectra indicated the presence of a quercetin moiety and sugar unit[13]. Identified as quercetin-3-O-β-D-glucoside. The doublet at δ 5.12 (diaxial coupling *J* = 7.5 Hz) was assigned to the anomeric proton of hexose and suggested a IR γ at 1060 cm^{-1} suggested a glycosidic linkage. Acid hydrolysis of compound **6** gave glucose and quercetin, which were identified by co-chromatography with an authentic sample[14].

Compound **7**, isolated as crystalline yellow powder of m.p. 192 ºC with molecular formula of $C_{27}H_{30}O_{16}$. UV (MeOH λ_{max}, nm): 360, 257. IR (KBr, γ, cm^{-1}): 3348, 3419, 1653, 1600, 1458, 1058. ^1H NMR spectrum (400 MHz, DMSO, δ, ppm, J/Hz): 7.69 (1H, d, *J* = 2.1 Hz, H-2´), 7.59 (1H, dd, *J* = 8.4, 2.1 Hz, H-6´), 6.91 (1H, d, *J* = 8.4 Hz, H-5´), 3.09-3.46 (10 H, sugar), 1.10 (3H, d, *J* = 4 Hz, $-CH_3$ of rhamnose). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 177.46 (C-4), 164.14 (C-7), 161.30 (C-2), 156.71 (C-9), 148.49 (C-3´), 144.83 (C-4´), 133.38 (C-3), 121.68 (C-1´), 115.32 (C-2´), 104.06 (C-10), 101.26 (C-1´´´), 100.83 (C- 2´´), 93.69 (C-8), 76.52 (C-3´´), 75.97 (C- 5´´), 74.15 (C- 5´´´), 71.92 (C-4´´´), 70.64 (C-4´´). DART-MS m/z $[M]^+$ 610. Identified as quercetin-3-O- α-L-rhamno-(1→6)-β-D-glucoside. Acid hydrolysis of compound **7** gave glucose, rhamnose and quercetin, which were identified by co-chromatography with an authentic sample[15].

Compound **8**, isolated as white needles, m.p. 163 ºC. IR (KBr, γ, cm^{-1}): 3329, 2953, 2864, 1666, 1741, 1462, 1226, 1193. ^1H NMR (400 MHz, DMSO, δ, ppm, J/Hz) 5.14 (2H, m, H-22, H-23), 3.35 (1H, s, H-23), 0.91 (3H, d, *J* = 6.5 Hz, H-21), 0.87 (3H, d, *J* = 6.6 Hz, H-26), 0.83 (3H, d, *J* = 7.0Hz, H-29), 0.81 (3H, d, *J* = 6.5 Hz, H-27), 0.79 (3H, s, H-19), 0.65 (3H, s, H-18). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 37.3 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.9 (C-14), 24.4 (C-15), 28.4 (C-16), 56.1 (C-17), 11 (C-18), 21.1 (C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.2 (C-24), 31.9 (C-25), 21.2 (C-26), 19 (C-27), 25.4 (C-28), 12.1 (C-29). DART-MS m/z 412$[M^+]$, 397, 394. having molecular formula of $C_{29}H_{48}O$. Identified as stigmasterol[16].

Compound **9**, it is reported for the first time from *Cressa cretica* with molecular formula of $C_{29}H_{50}O$, m.p. 141 ºC IR (KBr, γ, cm^{-1}): 3319, 2946, 2854, 1640, 1470, 1460, 1189, 1060, 870, 720, 670. ^1H NMR spectrum (400 MHz, DMSO, δ, ppm, J/Hz) 5.31 (1H, d, *J* = 2.1 Hz, H-6), 3.25 (1H, tdd, *J* = 4.5 Hz, H-6), 0.91 (3H, s, H-19), 0.90 (3H, s). ^{13}C NMR (100 MHz, DMSO, δ, ppm) 37.3 (C-1), 31.6 (C-2), 71.8 (C-3), 42.2 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 51.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 11.9 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23, 45.9 (C-24), 29.2 (C-25), 19.8 (C-26), 19.3 (C-27), 23.1 (C-28), 12.2 (C-29). DART-MS m/z 414 $[M^+]$, 367, 329. Identified as β sitosterol[16].

Compound **1**, **3**, **5**, **9** are being reported for the first time from *Cressa cretica*. This paper deals with the isolation and structure elucidation of compounds **1-9** by comprehensive spectroscopic analysis, including NMR (^1H, ^{13}C), DART-Ms and comparison of their spectral data with literature. The structures elucidated are represented in Fig. 1.

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