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Der Pharmacia Lettre, 2016, 8 (5):321-324
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Isolation and Characterization of flavonoids and flavone glycosides from the ethnic traditional medicinal plant *Cotoneaster bacillaris* Wall. Ex Lindl.

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ABSTRACT

Two flavonols namely 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (quercetin), 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (Kaemferol) and two flavone glycosides 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(((3S,4S,5S,6R)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one (quercetrin), Rutin followed by ursolic acid, scopoletin have been isolated from the methanolic extract of aerial part of traditional medicinal plant Cotoneaster bacillaris. The structures of the compounds were characterized by using Mass, Proton and 13C-NMR respectively.

Keywords: *Cotoneaster bacillaris*, Rosaceae, Flavonoids, Medicinal plants

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INTRODUCTION

Medicinal plants always flourish good source for the development of drug discovery. Natural abundance of valuable chemical entities in the traditional plant sources always gives attention to the chemical community for drug development [1]. Number of active principles like flavonoids, alkaloids, sterols, polyphenols, terpenoids and glycosides have been isolated from medicinal plant sources and they have successfully used for the treatment of various ailments [2]. Rosaceae is one of the important family showing rich in secondary metabolites with various biological activities [3]. There are number of bioactive compounds have been isolated and reported in the genus *cotoneaster* belongs to this family [4-7]. In the present study the two flavones and flavone glycosides have been isolated by the ethnic medicinal plant *Cotoneaster bacillaris* Wall. Ex Lindl followed by the isolation of the coumarin 'scopoletin' and the pentacyclic triterpenoid 'ursolic acid'. The structures of the compounds were confirmed by Mass, ^{1}H and ^{13}C NMR.

MATERIALS AND METHODS

General experimental procedure: All reagents were purchased from Sigma-Aldrich. TLC was monitored with silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were visualized in the ultraviolet light chamber, Iodine chamber, 5% MeOH-H_2SO_4 mixture. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHN Analyzer (Perkin elmer, series II 2400). ^{1}H NMR and ^{13}C NMR spectra were determined on a Bruker-300 NMR spectrometer and chemical shifts were expressed as part per million against

TMS as internal reference. Mass spectra were recorded on Agilent 1200 (Liquid Chromatography), Agilent 6320 (Quadrupole Mass Analyzer) spectrophotometer.

Plant material:2.3 kg of aerial part of C. bacillaris was collected from tropical evergreen forests of Western Ghats, India. The plant was authenticated by Dr. Jayendran, Department of Botany, Government Arts College, Ootacamund, India. A voucher specimen (JCB1508) was deposited in Government Arts College, Ootacamund, India. The collected plant material was shade dried and coarsely powdered for extraction. Extracts were prepared by soaking plant material in Methanol successively at room temperature for 24 h and repeated thrice with the residue. The extract was filtered through Whatman No.1 filter paper and concentrated by using Rotary evaporator (Buchi® Rotavap R-210).

Extraction and isolation: The methanol extract (12.3g) from *C. bacillaris* was taken for column chromatography with silica gel (60-120 mesh) (150 g). Elution was started with Hexane, followed by increasing ethyl acetate (EA)-hexane combinations (5,10,15,25, 50 and 80% EA in hexane) and finally with 100 % EA. Then the column was washed with MeOH. The column elution was monitored by TLC and fractions were pooled based on similar TLC profiles. In total, 15 fractions (CB1-CB15) were collected and concentrated Rotary vacuum evaporator. Fraction CB3 and CB4 had similar TLC profiles and pooled up both the test tubes. Often the precipitate formed was centrifuged and separated. Dried the white precipitate under vacuum and it yields ursolic acid (130 mg). Fractions (CB 6-8) were mixed according to the TLC pattern and the supernatant was further subjected to column chromatography with silica (100-200 mesh). Elution was done initially with chloroform followed by increasing methanol (5, 10, 20, 50 % MeOH in Chloroform). This column yielded 8 fractions. Fractions $CB_2$3 and $CB_2$7 yielded Scopoletin (53 mg). Fractions CB10 – CB15 were mixed and subjected to column chromatography with Silica gel (100-200 mesh). The column was packed initially with chloroform followed by increasing concentrations of Methanol-chloroform combinations (2, 5, 10, 15, 20, 40, and 50%) and finally with pure methanol. 7combined fractions were finally obtained. Further purifications of fractions $CB_3$1- $CB_3$3 yield quercetin (23 mg) and keamferol (27mg). Fractions $CB_3$4-6 yielded Quercetrin (21mg) and rutin (34mg)with further separation and purification. The compounds were carefully separated, evaporated to dryness and characterized for structural elucidation.

RESULTS AND DISCUSSION

Figure 1 clearly represents the structures of the molecules isolated from the aerial part of *C. bacillaris*. Those isolated compounds have been characterized by using ^{1}H and ^{13}C NMR which is discussed below.

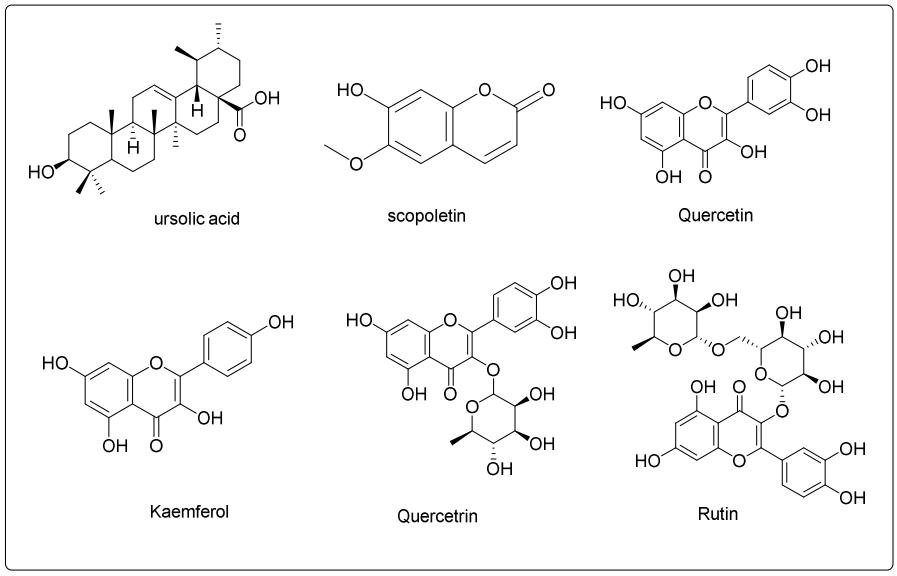

Figure 1. Structure of the compounds isolated from *C. bacillaris*

Ursolic acid (**1**):Molecular formula: $C_{30}H_{48}O_3$. ^{1}H-NMR (DMSO-d6, δ ppm): 11.94 (s, 1H), 5.12 (s, 1H), 4.29 (s, 1H), 3.02 (t, J=8.7Hz, 1H), 2.10 (d, J=10.8Hz, 1H), 1.97-1.75 (m, 3H), 1.60-1.5 (m, 10H), 1.35-1.23 (m, 6H), 1.14 (s, 1H), 1.04 (s, 3H), 0.963-0.866 (m, 11H), 0.811 (D, J=6.3Hz, 3H), 0.748 (S, 2H), 0.695 (d, J=12Hz, 4H), ^{13}C NMR (DMSO-d6, δ ppm): 38.3 (C-1), 28.1(C-2), 78.2 (C-3), 38.3 (C-4), 54.9 (C-5), 17.9 (C-6), 32.9 (C-7), 39.5 (C-8), 48.4 (C-9), 36.9 (C-10), 23.5 (C-11), 126 (C-12), 140 (C-13), 42.7 (C-14), 29 (C-15), 24.7 (C-16), 48 (C-17), 53.8 (C-18), 39.7 (C-19), 39 (C-20), 31 (C-21), 37 (C-22), 28.7 (C-23), 15.6 (C-24), 16.6 (C-25), 17.4 (C-26), 24 (C-27),18 (C-28),17.3 (C-29), 21.2 (C-30), m/z = 456.

Scopoletin (**2**):Molecular formula: $C_{10}H_8O_4$. ^{1}H-NMR (DMSO-d6, δ ppm): 10.32 (s, 1H), 7.91 (d, *J*=9.3Hz, 1H), 7.22 (s, 1H), 6.78 (s, 1H), 6.23 (d, J=9.3Hz, 1H), 3.82 (s, 3H), ^{13}C NMR (DMSO-d6, δppm): 160 (C-2), 151(C-9), 149.4 (C-3), 145 (C-6), 144.4 (C-4), 111.6(C-5), 110.4 (C-7), 109.5 (C-10), 102.7 (C-8), 55.94 (-O-CH_3), m/z = 192.
Quercetin (**3**): Molecular formula: $C_{15}H_{10}O_7$. ^{1}H-NMR (DMSO-d6, δ ppm): 12.5 (s, 1H), 10.8 (s, 1H), 9.62 (s, 1H), 9.51 (d, J=16.8Hz, 2H), 7.68 (d, J=2.1Hz, 1H), 7.55(q, J=8.4Hz,8.7Hz, 1H), 6.89 (d, J=8.4Hz, 1H), 6.41(d, J=2.1Hz, 1H), 6.19 (d, J=1.8Hz, 1H), ^{13}C NMR (DMSO-d6, δ ppm): 175.7 (C-4), 163.8 (C-7), 160.6 (C-5), 156(C-9), 147 (C-2), 146 (C-4'),144 (C-5'),135 (C-3), 121.8 (C-1'), 119.9 (C-2'), 115.5 (C-3'), 114.9 (C-6'), 102.9 (C-10), 98.1 (C-6), 93.2 (C-8), m/z = 302.

Kaemferol (**4**): Molecular formula: $C_{15}H_{10}O_6$. ^{1}H-NMR (DMSO-d6, δ ppm): 12.48 (s, 1H), 10.14 (d, J=4.5Hz, 1H), 9.43 (s, 1H), 8.04 (q, J=9Hz, 9.9Hz, 2H), 6.95-6.90 (m, 2H), 6.43 (d, J=2.1Hz, 1H), 6.18 (d, J=2.1Hz, 1H), m/z = 286.

Quercetrin (**5**): Molecular formula: $C_{21}H_{20}O_{11.}$ ^{1}H-NMR (DMSO-d6, δ ppm):12.66 (s, 1H), 10.83 (s, 1H), 9.5 (s, 1H), 7.38-7.2 (m, 2H), 6.87 (d, J=8.4Hz, 1H), 6.39 (d, J=1.8Hz, 1H), 6.21(d, J=1.8Hz, 1H), 5.25 (d, J=1.2Hz, 1H), 4.96 (s, 1H), 4.73 (s, 2H),3.97 (s, 1H), 3.51 (q, J=8.7Hz, 1H), 3.37-3.11 (m, 2H), 0.80 (t, J=7.5Hz, 3H), ^{13}C NMR (DMSO-d6, δ ppm):177.6 (C-4), 164.2 (C-7), 161.2 (C-5), 157.2 (C-9), 156.4 (C-2), 148.4 (C-4'), 145.1 (C-5'), 134.1(C-3), 121.06 (C-1'), 120(C-2'), 115.6 (C-3'), 115.4 (C-6'), 104 (C-10), 98.6 (C-6), 93.5 (C-8), 71.1(C-1"), 70.5 (C-2"), 70.3 (C-3"), 70 (C-4'), 17.2 (C-5"), m/z = 448.

Rutin (**6**):Molecular formula: $C_{27}H_{30}O_{16}$. ^{1}H-NMR (DMSO-d6, δ ppm):12.60 (s, 1H), 10.84 (s, 1H), 9.68 (s, 1H), 9.20 (s, 1H), 7.55 (t, J=2.1Hz, 7.5Hz, 2H), 6.83 (d, J=8.7Hz, 1H), 6.38 (d, J=2.1Hz, 1H), 6.19 (d, J=2.1Hz, 1H), 5.30 (t, J=7.2Hz, 9.9Hz, 1H), 5.09 (t, J=7.8Hz,5.4Hz, 3H), 4.54 (s, 1H), 4.38 (d, J=1.2Hz, 3H), 3.70 (d, J=9.9Hz, 1H), 3.29-3.21 (m, 7H), 3.06 (t, J=9.3Hz, 9Hz, 2H), 0.99 (d, J=6.3Hz, 3H), m/z = 610.

According to the NMR results, Compound 1 shows peak at 11.94 ppm and 5.12ppm represents the carboxylic acid proton and –OH protons. Peaks at 1.97 to1.75ppm and1.35-1.23 shows 9 protons of methyl group present in the structure. Since the compound 1 is known we have confirmed the structure of the compound as ursolic acid with proton-NMR [8].

Compound 2 shows -OH peak at 10.32ppm and methoxy peaks at 3.82ppm. Two aromatic protons at 6.78 and 7.2 confirm the aromatic moiety with phenolic and methoxy groups present in the structure. Further two doublet protons with the coupling constant of 7.91ppm and 6.23ppm reveals the double bond present in the coumarin structure. Additionally from the carbon spectrum of the compound 2, -C=O groups shows peaks at 160ppm and the peak at 55.94 ppm confirms the structure of the compound as 'scopoletin' [9].

In the ^{1}H-NMR spectrum of compound 3, the aromatic region exhibited at δ 7.68 (1H, d, J = 2.1 Hz), 7.62 (1H, q, J = 8.4 and 8.7 Hz), and 6.89 (1H, d, J = 8.4 Hz) due to a 30, 40 disubstitution of ring B and a typical meta-coupled pattern for H-6 and H-8 protons (δ 6.19 and 6.41, d, J = 2.1 Hz). The 13C–NMR spectrum of compound 3 showed that the presence of 15 aromatic carbon signals. Based on the NMR data and comparison of the data given in the literature, the structure of compound 3 was obtained as yellow powder and identified as quercetin.

Compound 4 was obtained as a yellow powder, the Mass spectral studies of the compound yielded a molecular mass of the compound as m/z 286. The UV spectrum showed λ_{max} at 265 and 366 nm. The^{1}H-NMR spectrum showed 2 peaks at δ 6.18 (1H, d, *J* = 2.1 Hz) and 6.43 ppm (1H, d, *J* = 2.1 Hz) consistent with the Meta protons H-6 and H-8 on A-ring and an AA'BB' system at 8.04 (q, J=9, 9.9, 2H) and 6.95-6.90 (m, 2H) corresponding to the protons on B-ring. The MS and^1 H-NMR data were compatible with the literatures of kaempferol.

Compound 5 was obtained as bright yellow precipitate and the ^{1}H NMR (300 MHz, DMSO-d6): 0.80 (3H, t, rhamnose-CH3), 3.37 - 3.11ppm multiplet (rhamnosyl Hs), 5.25 (1H, rhamnosyl H-1), meta coupling related protons at d 6.21 (1H, d, J = 1.8 Hz) and 6.39 (1H, d, J = 1.8 Hz) depicting substitution pattern of ring A. Ring B protons appeared at 6.87 (1H, d, J=8.4 Hz), 7.38-7.2 (2H, m). The presence of 5-OH was evident from the peak at 12.66 ppm. 13C NMR spectra data are in agreement with the literature and the compound was identified as quercetin-3-Orhamnoside (quercetrin).

^{1}H NMR spectrum of the isolated compound 6 exhibited a characteristic proton signal at 12.6ppm corresponding to a chelated hydroxyl group at C-5. In addition to this, the presence of five aromatic protons were seen in the ^{1}H NMR spectrum; two ortho-coupling protons assignable to H-6' at7.55 (t, J=2.1, 7.5, 2H) and H-5' at 6.83 (d, J=8.7, 1H),two-Meta coupling protons at 6.19 ppm (1H, d, J =2.1 Hz) and 6.38 (1H, d, J = 2.1 Hz). The 1H NMR spectrum also supported the presence of rhamnose and glucose moieties with the rhamnose anomeric proton signal at 4.38ppm and glucose signal at 5.33ppm. A doublet of methyl group of rhamnose was observed at high field at 0.99 (3H, d, J = 6.3 Hz). The rest of protons in the sugar moiety resonated between 3.32 and 3.81 ppm [10, 11].

From all the above data, the structure of the compound have been identified and confirmed as Ursolic acid (1), Scopoletin (2), Quercetin (3), Kaemferol (4), Quercetrin (5) and Rutin (6) with respect to the previous reports.

CONCLUSION

In conclude the present work, two flavonols namely quercetin, kaemferol and two flavone glycosides namely rutin and quercetrin have been isolated and characterized, followed by the isolation of ursolic acid and scopoletin from the methanolic extract of the ethnic medicinal plant *C. bacillaris.* This kind of studies useful in the future for the investigation of bioactive secondary metabolites present in the rare medicinal plants.

Acknowledgement
Authors sincerely thanks to SASTRA University for providing Infrastructure and financial support.

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