Bioactive flavanoids from *Glycosmis arborea*

Mohammad Faheem Khan, Nisha Negi, Rajnikant Sharma and Devendra Singh Negi*

**Abstract**

**Background:** *Glycosmis* is a genus of evergreen glabrous shrub and distributed all over India. It possesses various medicinal properties and is used in indigenous medicine for cough, rheumatism, anemia, and jaundice. *Glycosmis arborea* is a rich source of alkaloids, terpenoids, coumarins, as well as flavonoids.

**Results:** The chemical investigation of methanol fraction of the leaves of *G. arborea* led to the isolation of one new flavone C-glycoside along with three known flavanoids, named as 5,7-dihydroxy-2-[4-hydroxy-3-(methoxy methyl) phenyl]-6-C-β-D-glucopyranosyl flavone (4), 5,7,4′-trihydroxy-3′-methoxy flavone (1), 5,4′-dihydroxy-3′-methoxy-7-O-β-D-glucopyranosyl flavanone (2), and 5,4′-dihydroxy-3′-methoxy-7-O-(a-L-rhamnosyl-(1″→6″))β-D-glucopyranosyl flavanone (3), respectively. The structures of all compounds were elucidated with the help of nuclear magnetic resonance spectrometry. Pure compounds and fractions were evaluated for pest antifeedant and antimicrobial activity.

**Conclusion:** Four compounds were isolated from the leaves of *G. arborea*. Among them, compound 4 showed significant antimicrobial activity.

**Keywords:** *Glycosmis arborea*, Rutaceae, Flavone C-glycoside, Antifeedant activity, Antimicrobial activity

**Background**

*Glycosmis* is a genus of evergreen glabrous shrub, distributed in warm and temperate regions of the world, and is a rich source of alkaloids and amide; however, terpenoids, coumarins, and flavonoids were also reported [1,2]. Previously, a new carbazole alkaloid, designated as glycoborinine, was isolated from the roots of *Glycosmis arborea*, along with two known alkaloids, carbazole glycozoline and glycozolidine, and two known quinoline alkaloids, viz. skimianine and 3-(3′,3″-dimethylallyl)-4,8-dimethoxy-N-methylquinolin-2-one [3]. There are about 60 species in the Indo-Malaysia region, and 7 are found in India. In Uttarakhand, *G. arborea* (Hindi-Ban Nimbh, Sanskrit-Ashvashokhta) grows commonly in Sal and miscellaneous forests of Tarai Bhabher at 600-m heights [4].

As a part of our ongoing studies aimed at the phytochemical and pharmacological characterization of this plant, we found that hexane and methanol fractions of the ethanol extract of *G. arborea* leaves showed significant antifeedant and antimicrobial activity. Herein, therefore, we decided to carry out a detailed study to investigate the chemical composition of *G. arborea*. In particular, we report the isolation and characterization of one new flavone C-glycoside (4) along with three known compounds (1 to 3) (Figure 1) with their antifeedant and antimicrobial activities. The known compounds were identified by using spectroscopic methods including infrared (IR), UV, mass, and 1D and 2D nuclear magnetic resonance (NMR) analysis and also by comparing data already reported in the literature. Methanol fraction yielded one new flavone C-glycoside (4) and three known compounds viz. 5,7,4′-trihydroxy-3′-methoxy flavone (1) [5], 5,5′-dihydroxy-4′-methoxy-7-O-β-D-glucopyranosyl flavanone (2) [6], and 5,5′-dihydroxy-4′-methoxy-7-O-(L-rhamnosyl-(1″→6″))-β-D-glucopyranosyl flavanone (3) [7]. Among these, compounds 1 and 2 have been reported for the first time from *G. arborea*.

**Methods**

**Plant material**

The *G. arborea* leaves were collected from Rajaji National Park, Rishikesh, Uttarakhand, India during the flowering season and identified by a taxonomist of the Botany Department of HNB Garhwal University, Rishikesh. A voucher specimen is deposited in the Department of Botany, HNB Garhwal University, Rishikesh.
Extraction and isolation

Parts of the dried leaves of *G. arborea* (1.8 kg) were air dried, grinded, and refluxed with 90% ethanol. The total ethanol extract was concentrated under reduced pressure at a temperature below 50°C to a dark green viscous mass coded as F001 (120 g) that was partitioned with hexane (F003) (21 g) and *n*-butanol F004 (88 g). The *n*-butanol soluble layer was then successfully fractionated into chloroform (4 g) (F005), ethyl acetate (19 g) (F006), and methanol soluble fraction (63 g) (F007). The methanol soluble fraction (F007) after removal of the solvent was chromatographed over silica gel (800 g) and eluted with mixtures of CHCl₃/MeOH as eluents to give four fractions A1 to A4 (9:1, 88:12, 85:15, 82:18). Fraction A2 (2.4 g) was rechromatographed on silica gel with CHCl₃/MeOH mixtures (9:1, 85:15) and yielded compounds 1 (70 mg), 2 (50 mg), 3 (46 mg), and 4 (36 mg).

5,7,4'-trihydroxy-3'-methoxy flavone (1, C₁₆H₁₂O₆)

M.p. 264°C; UV (MeOH) λ_max nm: 295, 328. IR (KBr) ν_max cm⁻¹: 3423, 1620, 1594, 1382, 1351, 1078, 770. ESIMS (% int.) (C₁₆H₁₂O₆) m/z: 300 [M]+, 285 [M-CH₃]+, 272 [M-CO]+, 257 [M-CH₃]+, 241, 242, 215, 204, 193, 176, 152 [A₁]+, 148 [B₁]+, 136, 124 [A₁-28]+ 107, 105. ¹H NMR (400 MHz, DMSO-d₆): δ ppm 6.62 (1H, s, H-3) 6.05 (1H, d, J = 2.0 Hz, H-6), 6.38 (1H, d, J = 2.0 Hz, H-8), 7.63 (1H, d, J = 2.4 Hz, H-2) 6.87 (1H, d, J = 9.0 Hz, H-5), 7.78 (1H, dd, J = 2.4, 9.0 Hz, H-6) 3.86 (3H, brd s, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 161.02 (C-2) 106.32 (C-3), 182.2 (C-4) 160.9 (C-5), 98.7 (C-6), 164.2 (C-7), 93.9 (C-8), 103.6 (C-4a), 157.1 (C-8a), 124.1 (C-1), 115.6 (C-2'), 148.4 (C-3'), 145.2 (C-4'), 117.0 (C-5'), 122.3 (C-6'), 56.2 (OCH₃).

5,4'-dihydroxy-3'-methoxy-7-O-[α-L-rhamnosyl-(1‴→6″)]-β-D-glucopyranosyl flavanone (2, C₂₃H₂₅O₁₅)

UV (MeOH) λ_max nm: 293, 326. IR (KBr) ν_max cm⁻¹: 3472 (OH), 1630, 1602 (aromatic), 1521, 1352, 1092, 815. ESIMS (C₂₃H₂₅O₁₅) (% int.) m/z: 610 [M]+, 633 [M + Na]+ (54), 595 (2), 551 (5), 507 (7), 463 (7), 388 (77), 364 (93), 338 (70), 306 (50), 233 (35), 179 (43), 151 (16). ¹H NMR (400 MHz, C₅H₅N-d₅): δ ppm 12.24 (1H, s, OH-5), 8.84 (1H, s, OH-5′), 7.63 (1H, d, J = 2.4 Hz, H-2) 6.87 (1H, d, J = 9.0 Hz, H-5), 7.78 (1H, dd, J = 2.4, 9.0 Hz, H-6) 3.86 (3H, brd s, OCH₃). ¹³C NMR (100 MHz, DMSO-d₆): δ ppm 161.02 (C-2) 106.32 (C-3), 182.2 (C-4) 160.9 (C-5), 98.7 (C-6), 164.2 (C-7), 93.9 (C-8), 103.6 (C-4a), 157.1 (C-8a), 124.1 (C-1), 115.6 (C-2'), 148.4 (C-3'), 145.2 (C-4'), 117.0 (C-5'), 122.3 (C-6'), 56.2 (OCH₃).
6.3 Hz, H-2), 6.96 (1H, d, J = 6.3 Hz, H-3′), 7.51 (1H, d, J = 2.1 Hz, H-6), 5.69 (1H, d, J = 7.6 Hz, H-1′), 4.26 to 4.66 (5H, m, H-2″-H-6″), 5.43 (1H, d, J = 3.0 Hz, H-1″), 4.14 to 4.60 (4H, m, H-2″-H-5″′), 1.57 (3H, d, J = 5.7 Hz, H-6″″), 3.71 (3H, brd s, OCH3). 13C NMR (100 MHz, C6H5D6): δ ppm 79.50 (C-2), 43.16 (C-3), 197.1 (C-4), 166.48 (C-5), 96.44 (C-6), 164.08 (C-7), 97.32 (C-8), 104.34 (C-4a), 163.49 (C-8a), 112.15 (C-1′), 118.49 (C-2′), 112.31 (C-3′), 148.43 (C-4′), 149.12 (C-5′), 115.32 (C-6′), 102.51 (C-1″), 72.79 to 78.45 (C-2″, C-5′), 67.37 (C-6″), 101.55 (C-1″′), 69.83 to 77.24 (C-2″′, C-5″′), 18.61 (C-6″′, methyl), 55.89 (OCH3).

5,7-dihydroxy-2-(4-hydroxy-3-(methoxymethyl)phenyl)-6-C-β-D-glucopyranosyl flavone (4, C23H24O11)

UV (MeOH) λmax nm: 208, 306. IR (KBr) νmax cm⁻¹: 3437, 1660, 1590, 1350, 1074, 901, 836. EIMS (C23H24O11) % int. m/z: 493 [M + OH]⁺ (18), 478 [M + 2H]⁺ (11), 448 [M-CO]⁺ (14), 455 (94), 444 (11), 433 (9), 413 (22), 301 (4), 260 (3), 203 (2), 136 (2). 1H NMR (400 MHz, DMSO-d6) and 13C NMR (100 MHz, DMSO-d6) δ (see Table 1).

### Antifeedant assay

The antifeedant activity of the extracts against the polyphagous pest Spodoptera litura was tested using the leaf dip method [7]. Five percent concentrations of each extract were prepared out of castor leaf (Ricinus communis L.) and were diluted in water containing 0.05% Triton X-100. The leaf discs of about 5 cm² were used as controls. The leaf discs dipped only in water containing 0.05% Triton X-100 were used as controls. The leaf discs were dipped for 30 s in an extract or compound separately. The leaf discs dipped only in water containing 0.05% Triton X-100 were used as controls. The leaf discs were air dried, and on each treated leaf disc, 10 larvae of S. litura (1 day old) were released. Three replications were maintained for each extract. Larval weight was taken after 4 days of treatment. Antifeedant activity of fractions and the purified compounds were tested against the polyphagous crop pest S. litura (Table 2).

### Antibacterial assay

The in vitro antibacterial activity was tested by the disc diffusion method [8] using pathogenic strains of Agrobacterium tumefaciens, Pseudomonas syringae, and Pectobacterium carotovorum. Concentrations of 200 and 500 μg/disc of compounds were impregnated on the discs. These discs were placed on the surface of the agar plates already inoculated with pathogenic bacteria. The plates were incubated at 37°C and examined at 48 h for zone of inhibition, if any, around the discs. Gentamicin was used in the assay as a standard control drug. An additional control disc without any sample but impregnated with an equivalent amount of solvent (DMSO) was also used in the assay. The result of antibacterial activity indicated that methanol fraction and compound 4 exhibited a mild to moderate activity (Table 3).

### Results and discussion

Chemistry

The repeated chromatography of methanol fraction of the leaves of G. arborea led to the isolation of four flavonoids by gradient elution with the CHCl3/MeOH mixture of increasing polarity. Compound 4 was isolated as a yellow solid which was further crystalized in acetone.

### Table 1 NMR spectroscopic (400 MHz) data of compound 4 in DMSO-d6

| Positions | 1H (in Hz) | 13C | HSQC | HMBC |
|-----------|------------|-----|------|------|
| 2         | -          | 161.51 | qC  |      |
| 3         | 6.77 (s)   | 103.46 | CH  | C-4  |
| 4         | -          | 182.47 | qC  |      |
| 5         | -          | 162.97 | qC  |      |
| 6         | -          | 104.97 | qC  |      |
| 7         | -          | 156.36 | qC  |      |
| 8         | 6.26 (s)   | 98.50  | CH  | C-8a |
| 9a        | -          | 104.38 | qC  |      |
| 1″        | -          | 121.98 | qC  |      |
| 2″        | 8.02 (d, J = 2.4, 8.4) | 129.34 | C-5′′, C-8a |
| 3″        | 6.88 (d, J = 8.4) | 116.17 | C-1″  |      |
| 4″        | -          | 151.42 | qC  |      |
| 5″        | -          | 164.30 | qC  |      |
| 6″        | 6.91 (d, J = 2.4) | 116.17 | C-1″  |      |
| 1″′       | 4.65 (d, J = 9.9) | 73.74  | CH  | C-3′ |
| 2″′       | 3.82 (dd, J = 9.2, 9.6) | 70.86  | CH  | C-1″ |
| 3″′       | 3.24 (t, J = 9.2) | 79.01  | CH  |      |
| 4″′       | 3.34 (t, J = 9.2) | 71.18  | CH  |      |
| 5″′       | 3.22 (dd, J = 5.0, 9.2) | 82.22  | CH  | C-1″ |
| 6″′       | 3.75 (d, J = 11.6) | 61.65  | CH3 | C-5′ |
| 5″′       | 3.52 (dd, J = 6.0,12.0) | 13CNMR δ (C-6, methyl) 55.89 (OCH3). 13C NMR (100 MHz, DMSO-d6) δ (see Table 1).

### Table 2 Pest antifeedant activity of G. arborea fractions against S. litura L

| Fractions | Percent feeding index (PFI) (μg/cm²) |
|-----------|-------------------------------------|
| Hexane    | 46.71 ± 4.07                        |
| Methanol  | 50.21 ± 5.21                        |

A concentration of 0.05% Triton X-100 was used as control.
It had the composition C_{23}H_{24}O_{11} (m/z = 476) as derived from the positive mode of electrospray ionization mass spectrometry (ESIMS) analysis. A positive Shinoda test and color reaction with ferric chloride suggested the presence of free phenolic hydroxyl groups. The UV spectrum exhibited absorption maxima at 208 and 306 nm which are characteristic of a flavone skeleton [9]. Its IR spectrum showed the presence of a hydroxyl group at 3,437 cm$^{-1}$ and carbonyl [M+-CO] and further at 433 m/z $[M^{+}+2H]$. Fragment at 445 m/z was due to loss of carbonyl [M$^{+}$-CO] and further at 433 m/z was due to loss of the methyl group [M$^{+}$-CO-CH$_3$]. A higher percentage of fragmentation also appeared at 455 m/z $[M^{+}$-H$_2$O-3H$]$. The remaining HMBC correlations are given in Table 1.

The structure was supported by the mass spectroscopic studies, which showed molecular ion peak at 478 m/z [M$^{+}$+2H], Fragment at 445 m/z was due to loss of carbonyl [M$^{+}$-CO] and further at 433 m/z was due to loss of the methyl group [M$^{+}$-CO-CH$_3$]. A higher percentage of fragmentation also appeared at 455 m/z $[M^{+}$-H$_2$O-3H$]$. Thus, compound 4 was unambiguously identified as 5,7-dihydroxy-2-[4-hydroxy-3-(methoxymethyl) phenyl]-6-C-β-D-glucopyranosyl flavone, a new flavone C-glycoside named as Arboreaside.

**Biological studies**

**Antifeedant activity**

All the fractions and compounds were tested for antifeedant activity. Among them, the hexane and methanol fractions showed significant antifeedant action against S. litura L. In a dual-choice leaf disc method, hexane and methanol fractions were tested for pesticidal potential.

**Table 3 Antibacterial activity of G. arborea fraction and isolated compound against plant bacterial pathogens**

| Particular          | Concentration (μg/disc) | Zone of inhibition (in mm) | Agrobacterium tumifaciens | Pseudomonas syringae | Pectobacterium carotovorum |
|---------------------|-------------------------|-----------------------------|---------------------------|---------------------|---------------------------|
| Methanol fraction   | 200                     | 12                          | -                         | 14                  | -                         |
|                     | 500                     | 16                          | 6                         | 18                  | -                         |
| Compound 4          | 200                     | 9                           | -                         | -                   | -                         |
|                     | 500                     | 11                          | -                         | -                   | -                         |

Gentamicin was used as a standard control drug.
The hexane fraction showed a percent feeding index (PFI) of 46.71 ± 4.07, while the methanol fraction showed a PFI of 50.21 ± 5.01 as given in Table 2.

**Antibacterial activity**

All the fractions and compounds were also tested for antibacterial activity. The methanol fraction and compound 4 showed antimicrobial activity against the plant bacterial pathogens *A. tumifaciens* and *P. syringae*, and *P. carotovorum*. It was found that moderate inhibitory activities were observed against *A. tumifaciens* and *P. carotovorum* at a concentration of 200 μg, whereas *P. syringae* was found to be fatal at 500 μg. Compound 4 showed moderate inhibition against *A. tumifaciens* as shown in the Table 3.

**Experimental**

All melting points are uncorrected and were taken in open capillaries. The UV spectra were recorded on a PerkinElmer Lambda 15 UV/VIS spectrophotometer (PerkinElmer, Waltham, MA, USA) in methanol as blank. IR spectra were recorded on a PerkinElmer Infrared 15 in KBr pellets and are expressed per centimeter. The ¹H and ¹³C NMR were scanned on a Bruker AVANCE 400 MHz (Bruker Corporation, Billerica, MA, USA) at C5D5N-d5, DMSO-d6, CD3OD, and CDCl3 at 400, 300, and 100 MHz with TMS as internal reference. Proton-detected heteronuclear correlations were measured using HMQC (optimized for JHC = 14.5 Hz) and HMBC (optimized for JHC = 7 Hz). Mass spectra were recorded on a Micromass Quattro II (Micromass UK Ltd., Manchester, UK) at 70 eV for ESIMS. Column chromatography was carried out using silica gel (60 to 120 mesh, Qualigen (Carlsbad, CA, USA)/Merck (Whitehouse Station, NJ, USA)). Thin layer chromatography was carried out over plates made of silica gel G of Qualigen/Merck.

**Conclusion**

In conclusion, the present paper has shown the isolation and structure elucidation of one new flavone C-glycoside (4) along with three known compounds (1 to 3) from methanol fraction of *G. arborea* leaves. With regard to bioactivity, all fractions and isolated compounds were evaluated for antifeedant and antibacterial activity. Among them, hexane and methanol fractions showed antifeedant activity, whereas methanol fraction and compound 4 showed significant antibacterial activity.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgment**

The authors are extremely grateful to Prof. A.D. Kinghorn, USA for the NMR and ESIMS experiments and Dr. S. Narasimhan, AHRF, Chennai for the antifeedant and antibacterial activity. This investigation received financial assistance from CSIR, New Delhi in the form of a network project [02(3286)/12].

**References**

1. Ito C, Itoigawa M, Sato A, Hasan CM, Rashid NA, Tokuda H, Mukainoka T, Nishino H, Furukawa H (2004) Chemical constituents of Glycosmis arborea: three new carboaldehyde and their biological activity. J Nat Prod 67:1488–1491
2. Pacher T, Bacher M, Hofer O, Greger H (2001) Stress induced carboaldehyde phytoalexins in Glycosmis species. Phytochemistry 58:129–135
3. Chattavarty AK, Sarkar T, Masuda K, Shinjima K (1999) Carboaldehyde from roots of Glycosmis arborea. Phytochemistry 50:1263–1266
4. Gaur RD (1999) Flora of District Garhwal North West Himalaya. Transmedia Publication, Srinagar, p 380
5. Yahagi T, Daikovna A, Kitanaka S (2012) Flavonol acyl glycosides from flower of *Abelia julibrissin* and their inhibitory effects on lipid accumulation in 3T3-L1 cells. Chem Pharm Bull 60:129–136
6. Yu-Jen K, Yu-Ching Y, Li-Jie Z, Ming-Der W, Li-Ming YK, Yuh-Chi K, Syh-Yuan H, Cheng-Jen C, Kuo-Hsiung L, Hsiu-O H, Yao-Haur K (2010) Flavanone and diphenylpropene glycosides and glycosidic acyl esters from *Viscum articulatum*. J Nat Prod 73:109–114
7. Negi DS, Kumar A, Sharma RK, Shukla N, Negi N, Tamta ML, Bansal Y, Prasert P, Cairns JRK (2011) Structure confirmation of rare conjugate glycosides from Glycosmis arborea (Roxb) with the action of β-glucosidases. Res J Phytochem 5:32–40
8. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH (1999) Manual of clinical microbiology, 4th edn. ASM, Washington, p 1527
9. Markham KR (1982) Techniques of flavonoids identification, vol 3. Academic, London, pp 36–51
10. Fang N, Leidig M, Mabry TJ, Munekazu I (1985) Six 2hydroxyflavonols from *Gustavia microphloea*. Phytochemistry 24:3029–3034
11. Agrawal PK (1989) Carbon-13 NMR of flavonoids. Elsevier Science, Amsterdam
12. Miserez F, Potterat O, Marston A, Mangai GM, Hostettmann K (1996) Flavonol glycosides from *Vernonia galamensis* sp. nanobrissus. Phytochemistry 43:283–286
13. Iheyanga Y, Sugarn K, Marumo M (1994) Chemical constituents from roots of *Polygala japonica*. Chem Pharm Bull 42:2305–2308

doi:10.1186/2191-2858-3-4
Cite this article as: Khan et al.: Bioactive flavanoids from *Glycosmis arborea*. Organic and Medicinal Chemistry Letters 2013 3:4.