FABAD J. Pharm. Sci., 48, 2, 279-284, 2023
Doi: 10.55262/fabadeczacilik.1192521
RESEARCH ARTICLE

Phytochemical and Antimicrobial Study of *Glycosmis mauritiana* (Lam.) Tanaka

Arifa MEHREEN[*], Shagufta KAMAL[**], Sevinj MUSAYEVA VAGİF[***], Abdur Rahman SIDDIQUE[****], Rida JABBAR[*****], Sumia URAİNAB[******°]

Phytochemical and Antimicrobial Study of Glycosmis mauritiana (Lam.) Tanaka

SUMMARY

We have successfully extracted a novel geranyl flavanone from the root barks of Glycosmis mauritiana. The structure was elicudated as 6-geranyl-5-hydroxy-3'-methoxy-7, 8- (2", 2"-dimethyl pyrano) flavanone- 4'-O-D-glucopyranoside (GM-1), along with 3 known compounds;1-hydroxy-2,3-dimethoxy-10-methylacridin-9-one (GM-2), 1,3,5-trihydroxy-10-methyl-4-(3-methylbut-2-enyl) acridin-9-one (GM-3), 6,11-dihydroxy-3,3-dimethyl-12H-pyrano[2,3-c]acridin-7-one (GM-4), was separated and identified by a variety of spectroscopic techniques. The range of inhibition against four bacteria examined was shown to be 4-25 mm; the GM-1 revealed a significant inhibition zone.

Key Words: *Rutaceae, Glycosmis mauritiana, Flavanone*

Glycosmis mauritiana (Lam.) Tanaka'nın Fitokimyasal ve Antimikrobiyal Çalışması

ÖZ

Glycosmis mauritiana'nın kök kabuklarından başarılı bir şekilde yeni bir geranil flavanon elde ettik. Bu bileşiğin yapısı, 6-geranil-5-hidroksi-3'-metoksi-7,8-(2",2"-dimetil piran)flavanon-4'-O-D-glukopiranozit (GM-1) olarak aydınlatılmıştır. Ayrıca, çeşitli spektroskopik tekniklerle yapıları tanımlanmış olan 1-hidroksi-2,3-dimetoksi-10-metilakridin-9-on (GM-2), 1,3,5-trihidroksi-10-metil-4-(3-metilbut-2-enil)akridin-9-on (GM-3), 6,11-dihidroksi-3,3-dimetil-12H-pirano[2,3-c]akridin-7-on (GM-4) adlı üç bilinen bileşik bulunmuştur. Dört farklı bakteriye karşı inhibisyon aralığı 4-25 mm olarak gösterilmiş olup, GM-1 önemli bir inhibisyon bölgesi göstermiştir.

Anahtar Kelimeler: *Rutaceae, Glycosmis mauritiana, Flavanon*

Received: 21.10.2022
Revised: 09.07.2023
Accepted: 10.07.2023

[*] ORCID: 0000-0001-5899-8027,Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan
[**] ORCID: 0000-0003-4073-2583, Department of Biochemistry, Government College University, Faisalabad, Pakistan
[***] ORCID: 0000-0002-3957-8825, Institute of Molecular Biology and Biotechnologies, Baku, Azerbaijan
[****] ORCID: 0000-0003-4517-3725, Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan
[*****] ORCID: 0000-0002-5074-1478, Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan
[******] ORCID: 0000-0003-1066-9894, Department of Biochemistry, Government College University, Faisalabad, Pakistan

[°] Corresponding Author; Sumia Urainab
E-mail: sumiaurainab23@gmail.com, Phone: 00923204222032

INTRODUCTION

In the Rutaceae family, *Glycosmis mauritiana* (syn. *Limonia pentaphylla* Auct., *Glycosmis pentaphylla* Auct.), often called Ash-sheora, Orange Berry, Rum Berry, and Gin Berry. The *Glycosmis maurtiana* found in its natural habitats in India, Pakistan, Malaysia, China, Sri Lanka, Myanmar, Thailand, Indonesia, and Malaya. Plants in this genus have a long history of usage as traditional medicine for treating a wide range of medical conditions (Khare, 2011). As previous research has shown, the *Glycosmis* genus contains quinazolines, furoquinolines (Yasir et al., 2019), carbazoles (Chakraborty, 2022), coumarins (Blanco Carcache et al., 2022), sulphur-containing amides (Hofer and Greger, 2000), quinolones, flavonoids and acridone-type alkaloids (Intekhab, et al., 2011), which have demonstrated to have broad-spectrum activity. The examination of pertinent scholarly works suggests that further investigation is required to explore the phytochemicals in *Glycosmis mauritiana*. As a result, our research endeavors were directed toward examining phytochemicals and their antimicrobial properties with the *Glycosmis mauritiana*.

MATERIAL AND METHODS

Instrumental

Ultraviolet (UV) spectrometer Perkin-Elmer Lambda Bio 20 (Perkin Elmer, USA) was used to record ultraviolet absorption spectrum. On Perkin-Elmer 1710 Fourier transform spectrometer (Perkin Elmer, USA), infrared (IR) spectroscopy was carried out utilising the KBr disc. As an internal standard, tetramethylsilane (TMS) is used to calculate δ values (ppm). The FEBMS were recorded using the JEOLSX 1021/DA-6000 mass spectrometer (JEOL Ltd. Japan). The Bruker AVANCE DRX-400 (German) was used to record NMR spectra (400, 100 MHz). Silica gel (60–120 mesh) was used for column chromatography. Chemicals and reagents used in this experiment were AR quality from E-Merck (Pakistan).

Plant material

In June 2021, root barks of *Glycosmis mauritiana* were picked throughout remote areas of the Faisalabad District. The herbarium specimens kept in the faculty of botany's herbarium at Agriculture University Faisalabad, Pakistan were compared to ensure validity. Secondary plant components can be extracted from both fresh and dried plant material. It is preferable to utilize newly collected and dried material, as dried substance that has been stored pro a longer duration may lose a significant amount of its original qualities.

Extraction

Air-dried roots of *Glycosmis mauritiana* were first defatted with petrol-ether (3L x 5 times) to obtain 80 g of petrol-extract on distillation under reduced pressure. The marc was then extracted with chloroform, methanol and ethyl acetate (3L x 5 times each). On standing at room temperature, the petrol-ether extract produced a yellow precipitate, which on re-crystallization with chloroform, produced yellow crystals of compound **GM-4**. Similarly, the chloroform extract was subjected to column chromatography. The column was uninterruptedly eluted with the solvents and solvent mixtures of increasing polarity. Elution with $CHCl_3$: EtOAc (8:2) yielded compound **GM-3**.

The alcoholic extract thus obtained was decanted in 500 ml distilled water to get water soluble and insoluble portions. After partitioning with benzene, the water-insoluble part (ppt.) was dissolved in methanol to provide 15 g of methanolic extract. The slurry was made to methanolic extract with 5 g of silica gel in pet-ether and was digested to well settle column. The column was uninterruptedly eluted with the solvents and solvent mixtures of increasing polarity. Elution with $CHCl_3$: MeOH (7:1) afforded **GM-2**.

The ethyl acetate extract was evaporated under a vacuum on a rotatory evaporator below 50 ^{O}C temperature to yield 35 g of ethyl acetate extract. A well-stirred silica gel suspension (100 -150 g in pet-ether 60-80^{0}) was poured into a column (150 cm long and

50 mm diameter). When the absorbent was well settled, the excess petrol was allowed to pass through the column. The slurry was made of 5 g of silica gel in ethyl acetate and was digested to well resolve column. The column was eluted with the solvents and solvent mixtures of increasing polarity. Elution with $CHCl_3$: MeOH (6:4) yielded compound **GM-1**.

RESULTS AND DISCUSSION

GM-1 was isolated as a yellow powder from the ethyl acetate extract by eluting the column with $CHCl_3$: MeOH (6: 4). The compound exhibits a purple spot on the thin layer chromatography plate when evaluated under UV light. The compound showed a positive chemical test for flavonoid glycoside (Mabry, 1970). This compound is a flavanone containing at least one hydroxyl group that was identified by UV spectral investigations, which revealed a dark blue color in Gibb's test, green with $FeCl_3$, and a pink color with magnesium-HCl (conc.) acid (Mabry, 1970). The UV spectra of the compound revealed bands with maximum wavelengths at 218 and 291 nm, typical of a flavanone skeleton (Mabry, 1970) (Figure 1).

In IR spectra, absorption bands appeared at 1645 cm^{-1} and 3327^{-1} of the molecule were ascribed to conjugated ketone and hydroxyl groups, respectively. Thus, the UV and IR of the compound show absorption bands, indicating that it belongs to the flavanone class (Mabry, 1970). When the ^{1}H NMR spectrum of the molecule was examined, it was evident that the signals of aromatic protons and signals from the pyran ring, the glucosyl moiety, and the geranyl group were present.

In the ^{1}H NMR spectra of the compound, signals were observed at 5.57 (1H, dd, J= 12.5, 3.2 Hz), 2.98 (1H, dd, J= 17.6, 3.2 Hz), and 2.81 (1H, dd, J= 17.6, 12.5 Hz). These protons were allocated to the flavanone C-ring protons. These protons are found in flavanones in the H-2, H-3_{ax}, and H-3_{eq} configurations. In ^{1}H NMR, a signal was found at δ_H 12.30, which corresponding to the C-5 hydroxyl group. Three protons signals were also seen at δ_H 7.14 (1H, d, J = 2.3 Hz, H-2′), 7.09 (1H, d, J= 8.3 Hz, H-5′), and 6.99 (1H, dd, J= 8.3, 2.3 Hz, H-6′) attributed to the aromatic protons of the 3′ and 4′ disubstituted B- ring (Andersen, 2006). Aside from that, two proton doublets of the AB pattern were observed at δ 5.39 (1H, d, J = 9.8 Hz) and 6.48 (1H, d, J = 9.8 Hz), which were attributed to the chromen protons H-3" and H-4", respectively (site C-7 /C-8) of the flavanone (Mabry, 1970). In order to account for the two methyl groups at C-2", the sharp singlets were observed and assigned to δ_H 1.43 and 1.45, each for three protons. Furthermore, the ^{1}H NMR singlets detected at δ_H 1.54, 1.63, and 1.67 were assigned to three vinylic methyl protons, consistent with the literature. Additionally, this flavanone allocated signals at δ_H 3.35, 2.09, and 1.98 for the three methylene protons. Additionally, signals were seen at δ_H 5.16 (H-6‴) and 5.27 (H-2‴) assigned for two methine protons, ascribed to the presence of a geranyl substituent in the compound (Andersen, 2006; Harborne, 1986; Phillips, 1996; Smejkal, 2007). According to the findings, the ^{1}H NMR also revealed a signal at δ_H 3.87 ascribed to a methoxyl group in ring B. Moreover, a one proton singlet detected at δ_H 5.43 (1H, d, J= 8.4 Hz) is assignable for an anomeric proton (H-1⁗), indicative that this sugar has β- arrangement. Additionally, signals found in the range δ_H 3.35–4.46 are assignable to different protons of sugar molecules (Mabry, 1970; Harborne, 1986). The compound was hydrolyzed with 2N HCl and treated with the standard workup to form glucose. A comparison of Co-PC's results with authentic samples revealed that the sugar is D-glucose. Ring A comprises the geranyl and 2, 2-dimethyl chromen groups. There is substantial evidence that the C-6 geranyl group can be attached to the C-6 in the compound as the chemical shift δ_C 113.12 (Agrawal, 1989; Markham, 1982). The geranyl moiety was proven to be at C-6 of ring A using HMBC correlations between H-1‴ at δ_H 3.35 and C-6 (108.23), C-5 (160.21) and C-7 (159.33). Moreover, connection at δ_H 5.43 of H-1⁗ with C-4′ (146.26) was used to determine the position of the β-D-glucose moiety at C-4′. The ^{13}C NMR spectrum

indicates that the molecule contains a 4'-O-D-glucopyranoside linkage, as evidenced by the presence of a signal at δc 102.57, which is attributed to the anomeric carbon. (Agrawal, 1989; Markham, 1982). These data indicated the presence of angular chromen ring with a geranyl group at C-6. Additionally, the protons at δ 3.87 correlated to C-3′ (149.31), designated that the methoxyl group positioned at C-3′ (Agrawal, 1989; Markham, 1982). Spectral studies ultimately identified the compound as 6-geranyl-5-hydroxy-3′ -methoxy- 7, 8- (2″, 2″ -dimethyl pyrano) flavanone-4'-*O*-β-D-glucopyranoside, a new flavanone glycoside from this plant.

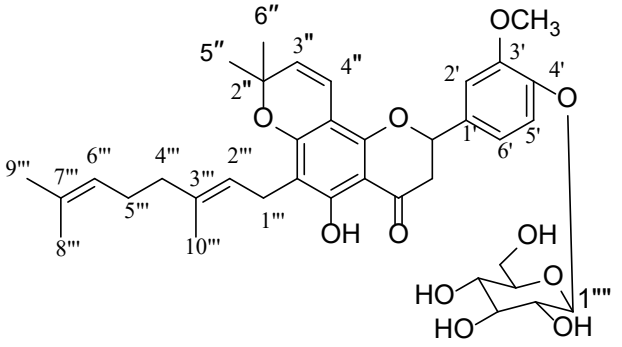
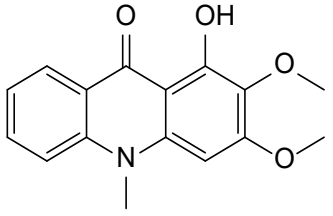
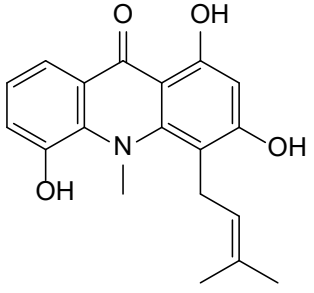


Figure 1. GM-1

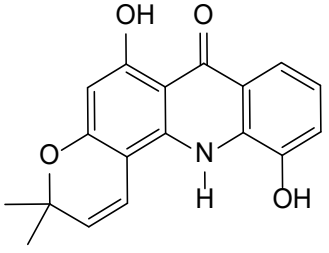
Three known compounds were isolated and identified as 1-hydroxy-2,3-dimethoxy-10-methylacridin-9-one (arborinine GM-2), 1,3,5-trihydroxy-10-methyl-4-(3-methylbut-2-enyl)acridin-9-one (GM-3), 6,11-dihydroxy-3,3-dimethyl-12*H*-pyrano[2,3-c]acridin-7-one (GM-4) through matching their NMR values to one previously published (Figure 2).



GM-2



GM-3



GM-4

Figure 2. The chemical formulas of GM-2, 3 and 4.

Antimicrobial activity

Nutritional agar media preparation

Nutrient agar, 28 g, mixed with 1 L of deionised water, autoclaved for 15 minutes at 121°C, then chilled to 47°C before being placed into Petri dishes.

Testing for antibacterial activity

The antibacterial activity of the compounds was evaluated using the cup-plate agar diffusion method. During the experiment, 100 ml of molten nutritional agar was mixed thoroughly with one milliliter of the standard bacterial stock solution (108-109 CFU/ml). In sterile Petri plates, 20 ml aliquots of the infected nutrient agar were delivered to each recipient. The agars were allowed to be set before 5 cups (10mm in diameter) were cut out of each plate using a sterile cork borer (No. 5), and the agar disc was carefully stood apart. On alternating days, a 0.1ml sample of each compound dilution in methanol was pipetted into alternate cups using an automated microliter pipette. The cups were permitted to diffuse for two hours at room temperature. The plates were then incubated for 18 hours in an upright position at 37°C to finish the procedure. Each compound was tested against each test organism three times, with three duplicates for each extract. When the growth inhibition zones formed during the incubation process were measured, the diameters were averaged, and the mean values were calculated (Table 1).

Table 1. Zones of inhibitions of different extracts of selected medicinal plant Concentration of extracts

Compound/ Bacterial organisms	Concentration in mg/ml	*Bacillus subtitles*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas arginosa*
GM-1	100	25	21	19	15
	50	24	17	18	10
	25	23	14	17	8
	12.5	22	12	12	6
GM-2	100	22	18	17	9
	50	20	17	16	7
	25	19	15	15	6
	12.5	18	14	11	4
GM-3	100	20	8	9	11
	50	19	7	8	10
	25	17	5	7	8
	12.5	15	2	4	7
GM-4	100	12	20	17	10
	50	11	19	15	9
	25	9	17	14	8
	12.5	7	16	11	5

The extract of *Glycosmis mauritiana* was tested for antibacterial activity against four bacterial strains and one fungus at different dosages using the cup plate agar diffusion technique, and the inhibition zone was determined in millimeters (mm). The inhibition range was 4-25 mm, against four bacteria tested; the GM-1 demonstrated a substantial inhibition zone.

CONCLUSION

A literature survey indicated that 6-geranyl-5-hydroxy-3′ -methoxy-7, 8- (2", 2"-dimethyl pyrano) flavanone- 4'-O-D-glucopyranoside is a novel flavanone.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

SK: Conceptualization, Analysis and/or Interpretation

AM: Conceptualization , Reviewing the article before submission, Materials, Supervision, Design

SMV, RJ: Literature Review, Data collection

ARS, RJ: Data Collection

SU: Taking responsibility in the construction of the whole manuscript

REFERENCES

Agrawal, P. (1989). Carbon-13 NMR of Flavonoids. Amsterdam: Elsevier Science.

Andersen, O. M., Markham, K. R. (2006) Flavonoids Chemistry, Biochemistry and Applications, CRC Press, Taylor and Francis, Boca Raton. https://doi.org/10.1201/9781420039443

Blanco, C. P., Anaya, E. G., Ninh, T., Moore, C., Rivera, C. J., & Ren, Y. (2022). Cytotoxic constituents of *Glycosmis ovoidea* collected in Vietnam. *Fitoterapia*, *162*, 105265. https://doi.org/10.1016/j.fitote.2022.105265

Chakraborty, M. (2022). Identification of Naturally Occurring Carbazole Alkaloids Isolated from *Murraya koenigii* & *Glycosmis pentaphylla* by the Preparation of HPLC Fingerprint. *Journal of Scientific Research*, *14*(1), 289-300. https://doi.org/10.3329/jsr.v14i1.53526

Harborne, J. (1986) The Flavonoids Advances in Research Since 1986. Routledge

Hofer, O., Greger, H. (2000). Sulfur-Containing Amides from *Glycosmis* Species (Rutaceae). In: Herz, W., Falk, H., Kirby, G. W., Moore, RE. (Editors) Fortschritte der Chemie organischer Naturstoffe / Progress in the Chemistry of Organic Natural Products, vol 80. Springer, Vienna. https://doi.org/10.1007/978-3-7091-6331-3_2

Intekhab, J., Aslam, M., & Khalid, H. (2011). Phytochemical Study of *Glycosmis mauritiana American Journal of Plant Sciences*, *02*(05), 657-659. https://doi.org/10.4236/ajps.2011.25078

Khare, C. P. (2011). Indian Medicinal Plants: An Illustrated Dictionary. Springer-Verlag Berlin Heidelberg.

Mabry, T. J., Markham, K. R., Thomas, MB. (1970) The Systematic Identification of Flavonoids, Springer, New York. https://doi.org/10.1007/978-3-642-88458-0

Markham, K.R., Chari, V.M. (1982). Carbon-13 NMR Spectroscopy of Flavonoids. In: Harborne, J.B., Mabry, T.J. (Eds.) The Flavonoids. Springer, Boston, MA. Pp.19-134. https://doi.org/10.1007/978-1-4899-2915-0_2

Phillips, W. R., Baj, N. J., & Gunatilaka, Kingston, A. L. (1996) C Geranyl compounds from *Mimulus clevelandii*. *J. Nat. Prod*. 59, 495–497. https://doi.org/10.1021/np960240l

Smejkal, K., Grycova, L., Marek, R., Lemie, Re. F., Jankovska, D., Forejtni, K. H., Vanco, J., & Suchy, V. (2007) C-Geranyl compounds from *Paulownia tomentosa* fruits. *J. Nat. Prod*. 70, 1244–1248. https://doi.org/10.1055/s-2008-1081339

Yasir, M., Tripathi, M., Singh, P., & Shrivastava, R. (2019). The Genus Glycosmis [Rutaceae]: A Comprehensive Review on its Phytochemical and Pharmacological Perspectives. *The Natural Products Journal*, 9(2), 98-124. https://doi.org/10.2174/2210315508666180622121212