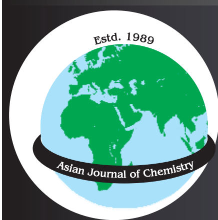
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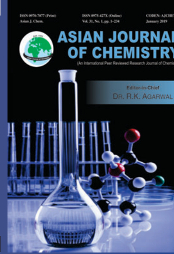

# Antibacterial Activity of a New Flavone Glycoside from the Stems of *Holmskioldia sanguinea* Retz.

R.N. YADAVA* and JYOTSNA RAGHUVANSI

Department of Chemistry, Dr. H.S. Gour Vishwavidyalaya (A Central University), Sagar-470003, India

*Corresponding author: E-mail: rnyadava@rediffmail.com; sjyotsna0@gmail.com

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A new flavone glycoside was isolated from ethanolic extract of stem parts of *Holmskioldia sanguinea* Retz. Its structure was characterized as 3,4′-dihydroxy-5,7-dimethoxyflavone-3-O-β-D-galactopyranosyl(1→4)-O-β-D-arabinopyranosyl-4′-O-α-L-rhamnopyranoside (**1**) by colour reactions, chemical degradation and spectroscopic analysis. Antibacterial activity of compound **1** was evaluated against various Gram positive and Gram negative bacteria showing a significant effects.

**Keywords: *Holmskioldia sanguinea* Retz., Flavone glycoside, Antibacterial activity, Verbenaceae.**

## INTRODUCTION

*Holmskioldia sanguinea* Retz. belongs to Verbenaceae family which is commonly known as Kapni or Rithoul in Hindi [1,2]. It is distributed in Punjab eastward in hills ascending upto 1500 m and subtropical and Himalayan regions from Kumaon to Bhutan. It's shrub is 10-30 feet in height. The crushed fresh leaves and shoots are applied in rheumatism and rheumatoid arthritis. Its extracts of leaves and stembark are used in the treatment of dysentery. Various pharmacological activities like analgesic, anticancer, diuretic, anti-inflammatory, CNS depressive and antimicrobial activities have been reported from aerial parts of plant [3-8]. A large number of phytochemical compounds isolated from plant have been reported by earlier workers [9-11].

In the present work, the isolation and structural characterization of a new compound from ethanolic extract of stems parts of this plant which is reported as 3,4′-dihydroxy-5,7-dimethoxyflavone-3-O-β-D-galactopyranosyl(1→4)-O-β-D-arabinopyranosyl-4′-O-α-L-rhamnopyranoside (**1**, Fig. 1) which showed a significant antibacterial activity.

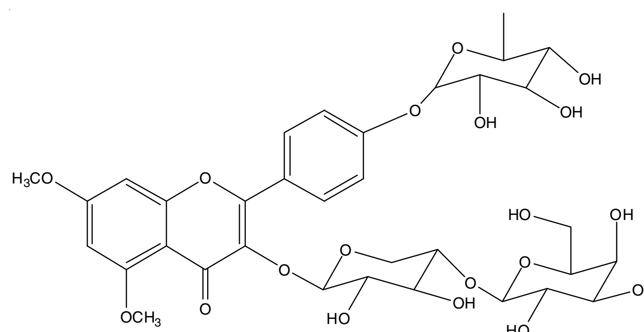


Fig. 1. Structure of 3,4′-dihydroxy-5,7-dimethoxyflavone-3-O-β-D-galactopyranosyl(1→4)-O-β-D-arabinopyranosyl-4′-O-α-L-rhamnopyranoside (**1**)

## EXPERIMENTAL

All the melting points were determined on a thermoelectrically melting point apparatus and are uncorrected. The IR spectra were recorded at Shimadzu FT-IR 8400S in KBr disc. UV spectra were determined on Shimadzu-120 double beam spectrometer in MeOH. $^1H$ NMR spectra were recorded on Bruker DRX 300 MHz spectrometer in $CDCl_3$ using TMS as internal standard. $^{13}C$ NMR spectra were recorded on Bruker DRX 75 MHz spectrometer using $CDCl_3$. The chemical shift values are reported in ppm (δ) units and coupling constant (J) in Hz. The FAB mass spectra were recorded on Jeol-SX (102) mass spectrometer.

**Plant material:** The stems of the plant was collected locally around Sagar region and identified by taxonomist, Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar, India. A voucher specimen (Bot/H/05/12/06) has been deposited in Natural Products Laboratory, Department of Chemistry of this university.

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**Extraction and isolation:** The air dried and powdered stems (5 Kg) of the plant were extracted with ethanol in Soxhlet extractor for consecutive 7 days . The total ethanolic extract was concenterated under reduced pressure and successively partitioned with petroleum ether, chloroform, ethyl acetate, acetone and methanol. The methanol soluble fraction of the plant was concenterated under reduced pressure in the rotatory evaporator to yield a light brown viscous mass which was subjected to TLC examination over silica gel-G using nBAW (4:1:5) as solvent and iodine as visualizing agent and showed two spots on TLC. The compound was separated and purified by column chromatography over silica gel using $CHCl_3$:MeOH (6:4) as eluent.The two compounds were referred as **1** and **2**. Compound **2** was obtained in small amount hence rejected and further characterization of compound **1** was carried out. The solvent was removed and crystallized from ether, which yielded compound **1** (1.92 g).

**Spectral data of compound 1:** m.f. $C_{34}H_{42}O_{19}$, [$M^+$] *m/z* 754, m.p. 266-267 ºC . Elemental analysis of $C_{34}H_{42}O_{19}$ calcd. (found) (%): C 53.84 (53.441), H 6.08 (6.03), O 40.07 (39.83). UV (MeOH) $\lambda_{max}$: 265, 312, 354 nm. IR (KBr, $\lambda_{max}$, $cm^{-1}$): 3397, 1658, 1600, 1558, 1512. $^1$H NMR (300 MHz, $CDCl_3$): δ 3.78 (3H, s, $C_5$-$OCH_3$), 3.76 (3H, s, $C_7$-$OCH_3$), 6.07 (1H, s, H-6), 6.05 (1H, s, H-8), 7.19 (1H, d, *J* = 8.7 Hz, H-2′, H-6′), 6.72 (1H, d, *J* = 8.7 Hz, H-3′, H-5′), 5.88 (1H, d, *J* = 2.1 Hz, H-1″), 4.18 (1H, dd, *J* = 7.2, 9.2 Hz, H-2″), 3.85 (1H, t, *J* = 9.0 Hz, H-3″), 3.59 (1H, m, H-4″), 3.21 (1H, m, H-5″), 1.21 (3H, d, *J* = 5.8 Hz, $CH_3$), 5.68 (1H, d, *J* = 7.1 Hz, H-1‴), 3.73 (1H, dd, *J* = 7.8, 2.6 Hz, H-2‴), 3.71 (1H, dd, *J* = 7.9, 2.3 Hz, H-3‴), 3.65 (1H, m, H-4‴), 3.32 (1H, dd, *J* = 11.7, 2.1 Hz, $H\text{-}5_a$‴), 3.28 (1H, dd, *J* = 11.5, 2.3 Hz, $H\text{-}5_b$‴), 5.03 (1H, d, *J* = 7.3 Hz, H-1⁗), 3.66 (1H, dd, *J* = 7.6, 2.9 Hz, H-2⁗), 3.49 (1H, dd, *J* = 7.7, 2.5 Hz, H-3⁗), 3.40 (1H, m, H-4⁗), 3.36 (1H, m, H-5⁗), 3.31 (1H, dd, *J* = 12.4, 2.3 Hz, $H\text{-}6_a$⁗), 3.25 (1H, dd, *J* = 12.2, 2.5 Hz, $H\text{-}6_b$⁗). $^{13}$C NMR (75 MHz, $CDCl_3$) 156.62 (C-2), 148.20 (C-3), 187.0 (C-4), 164.9 (C-5), 94.4 (C-6), 169.5 (C-7), 95.4 (C-8), 159.9 (C-9), 103.1 (C-10), 56.0 (C-5, C-7- OMe), 126.5 (C-1′), 126.8 (C-2′), 114.1 (C-3′), 158.0 (C-4′), 114.1 (C-5′), 126.8 (C-6′), 100.9 (C-1″), 68.7 (C-2″), 69.7 (C-3″), 72.3 (C-4″), 73.4 (C-5″), 11.9 ($CH_3$), 99.9 (C-1‴), 67.5 (C-2‴), 71.4 (C-3‴), 68.6 (C-4‴), 73.9 (C-5‴), 97.3 (C-1⁗), 75.1 (C-2⁗), 70.0 (C-3⁗), 69.1 (C-4⁗), 71.1 (C-5⁗), 62.5 (C-6⁗).

**Acid hydrolysis of compound 1:** Compound **1** (150 mg) was dissolved in 20 mL methanol and refluxed with 10 % $H_2SO_4$ (10 mL) on water bath for 6.5 h. The contents so obtained were concentrated and cooled, further residue was extracted with diethyl ether. The ethereal layer was washed with water and the residue was chromatographed over silica gel using a mixture $CHCl_3$ and MeOH as solvent to give aglycone **1A** which was identified as 5,7-dimethoxy-3,4′-dihydroxy flavone (Fig. 2).

**Permethylation of compound 1:** Compound **1** (35 mg) was dissolved in 30 mL DMF and treated with methyl iodide (5 mL) and $Ag_2O$ (15 mg) in a round bottom flask fitted with air condenser and refluxed for 2 days. The reaction mixture was filtered and washed with DMF. The filtrate was concentrated under reduced pressure and hydrolyzed with 10 % $H_2SO_4$ to give methylated aglycone identified as 5,7-dimethoxy-3,4′-dihydroxy flavone. The aqueous hydrolyzate obtained after

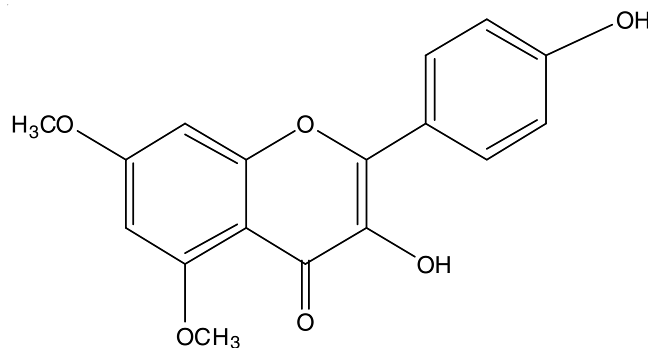

Fig. 2. Structure of compound **1A**

the removal of aglycone was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filterate was concenterated under reduced pressure and subjected to paper chromatography examination using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying agent. The methylated sugars were identified as , 2,3,4-tri-O-methyl-L-rhamnose ($R_G$ 1.01) 2,3,4,6-tetra-O-methyl-D-galactose ($R_G$ 0.78) and 2,3-di-methoxy-D-arabinose ($R_G$ 0.64) .

**Enzymatic hydrolysis of compound 1:** Compound **1** (30 mg) was dissolved in MeOH (20 mL) and hydrolyzed with equal volume of takadiastase enzyme. The contents were allowed at room temperature for 3 days and filtered. The hydrolyzate was concenterated and subjected to paper chromatography using nBAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying agent which showed the presence of L-rhamnose ($R_f$ 0.37) . The proaglycone was dissolved in MeOH (20 mL) and further hydrolysed with almond emulsion enzyme at room temperature yielded aglycone identified as 5,7-dimethoxy-3,4′-dihydroxy flavone and sugars were identified as D-arabinose ($R_f$ 0.21) and D-galactose ($R_f$ 0.16).

**Spectral data of compound 1A:** m.f. $C_{17}H_{14}O_6$, m.p. 224-225 ºC, [M+] *m/z* 314. Elemental analysis of $C_{17}H_{14}O_6$ calcd. (found) (%): C 65.04 (65.02), H 4.87 (4.89), O 30.54 (30.57). UV (MeOH) $\lambda_{max}$: 273, 323, 379 nm. IR (KBr, $\lambda_{max}$, $cm^{-1}$): 3450 (-OH), 1652, 1600, 1582, 1514. $^1$H NMR (300 MHz, $CDCl_3$): δ 14.91 (1H, s, 3-OH), 5.2 (1H, s, 4′-OH), 3.78 (3H, s, $C_5$-$OCH_3$), 3.76 (3H, s, $C_7$-$OCH_3$), 6.07 (1H, s, H-6), 6.05 (1H, s, H-8), 7.19 (1H, d, *J* = 8.7 Hz, H-2′, H-6), 6.72 (1H, d, *J* = 8.7 Hz, H-3′, H-5′). $^{13}$C NMR (75 MHz, $CDCl_3$) 156.62 (C-2), 148.20 (C-3), 187.0 (C-4 ), 164.9 (C-5), 94.4 (C-6), 169.5 (C-7), 95.4 (C-8), 159.9 (C-9), 103.1 (C-10), 56.0 (C-5, C-7- OMe), 126.5 (C-1′), 126.8 (C-2′), 114.1 (C-3′), 158.0 (C-4′), 114.1 (C-5′), 126.8 (C-6′).

**Antibacterial activity:** Antibacterial activity of compound **1** was carried out by agar disc diffusion method [12,13]. Stock solution of the compound was prepared in water, which was further diluted to obtain desired concentrations (50, 100, 150, 200 µg/mL). Ofloxacin was used as standard antibacterial agent. Inoculation of bacterial strains was done in nutrient broth media and incubated for 6 h to maintain standard turbidity ($10^6$ CFU/mL). Inoculum of bacterial strains (1 mL) was seeded in Muller Hinton agar plate. Disc (6 mm) was dipped in different concentrations (50, 100, 150, 200 µg/mL) of compound **1** and allowed to dry and further impregnated on seeded agar plates. The plates were incubated at 37 ºC for 24 h. The antibacterial activity was done by measuring the diameter of the zone of

inhibition (mm) formed around the disc. All experiments were done in triplicates. Data was expressed as ± SD .

**Minimum inhibitory concentration (MIC):** MIC for the compound **1** was determined by using broth dilution technique [14]. The microbial suspension in nutrient broth media was prepared and incubated for 24 h and 37 ºC and turbidity was verified spectrophotometrically ($1 \times 10^8$ CFU/mL) to find optical density at 600 nm . In each test tube, sterile nutrient broth media were added and extract was added in the first test tube to get concentration of 100 µg/mL, from which serial dilution (two-folds) were made to desired concentration (50-0.09 µg/ mL). Now the bacterial culture were added to each test tube (0.5 mL) and incubated at 37 ºC for 24 h. The lowest concentration of compound **1** preventing the visible growth of microbes (no turbidity) were taken as MIC values. All MIC values were taken in triplicates.

## RESULTS AND DISCUSSION

Compound **1** showed Molisch and Shinoda [15,16] tests which confirmed the presence of flavonoidal glycoside. Its UV (MeOH) absorbtion bands observed at 265, 312, 354 nm. IR spectra showed the peaks at 3397 (-OH), 1653 (α,β-unsaturated -C=O), 1600, 1581, 1512 corresponds to aromatic ring system. In $^1$H NMR spectrum of compound **1**, two singlets at δ 6.07 (1H, s) and δ 6.05 (1H, s) were assigned for H-6 and H-8 in ring A. Doublets at 7.19 (1H, d, $J$ = 8.7 Hz) for H-2′ and H-6′ and at 6.72 (1H, d, $J$ = 8.7 Hz) for H-3′ and H-5′ in ring B. A singlet was assigned at δ 3.78, δ 3.76 (-$OCH_3$) group at C-5 and C-7 in ring A. The anomeric proton signals at δ 5.88 (1H, d, $J$ = 2.1 Hz), δ 5.68 (1H, d, $J$ = 7.1 Hz) and δ 5.03 (1H, d, $J$ = 7.3 Hz) were assigned to H-1″ of L-rhamnose, H-1‴ of D-arabinose and H-1⁗ of D-galactose, respectively. Two coupling constants at ($J$ = 7.1 Hz) and ($J$ = 7.3 Hz) confirmed the β-configuration for the D-arabinose and D-galactose at H-1‴ and H-1⁗ and ($J$ = 2.1 Hz) corresponds to α-configuration of L-rhamnose, respectively [17-19]. In the mass spectrum of compound **1**, characteristic ion peaks at $m/z$ 754 [$M^+$], 608 [$M^+$-L-rhamnose], 446 [$M^+$-D-galactose], 314 [$M^+$-D-arabinose, aglycone] were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, D-galactose and D-arabinose hence indicating L-rhamnose to be linked at C-4′ position of aglycone and D-arabinose and D-galactose were linked to aglycone at C-3 position. Compound **1** on acidic hydrolysis with 10 % ethanolic $H_2SO_4$ yielded compound (**1A**) having m.f. $C_{17}H_{14}O_6$, m.p. 224-225 ºC, [ $M^+$] 314 (FABMS), which was identified as 5,7-dimethoxy-3,4′-dihydroxy flavone.

The aqueous hydrolyzate was neutralized with $BaCO_3$ and $BaSO_4$ was filtered off. On concentrating the filtrate, it was subjected to paper chromatography examination and showed the presence of D-galactose ($R_f$ 0.16), D-arabinose ($R_f$ 0.21) and L-rhamnose ($R_f$ 0.37). Compound **1** on periodate oxidation confirmed that all the sugars were present in pyranose form [20]. Permethylation [21] of compound **1** confirmed the position of sugar moieties, followed by acid-hydrolysis, which yielded methylated aglycone (**1A**) and methylated sugars. The methylated aglycone was identified as 5,7-dimethoxy-3,4′-dihydroxy flavone which confirmed glycosidation was involved at C-3-OH and C-4′-OH positions of aglycone. The methylated sugars were identified as 2,3,4,-tri-O-methyl-L-rhamnose ($R_G$ 1.01), 2,3,4,6-tetra-O-methyl-D-galactose ($R_G$ 0.78) and 2,3-di-methoxy-D-arabinose ($R_G$ 0.64) by paper chromatography . Therefore it was concluded that C-1‴-OH of D-arabinose was attached with OH group at C-3 position of aglycone, C-1⁗-OH of D-galactose was linked with C-4‴-OH of D-arabinose and C-1″ of L-rhamnose was attached with OH group at C-4′ position of aglycone. Thus interglycosidic linkage (1→4) was found between D-arabinose and D-galactose. Takadiastase enzyme was used for enzymatic hydrolysis [22] of compound **1** which liberated L-rhamnose and 3,4′-dihydroxy-5,7-dimethoxyflavone-3-O-β-D-galactopyranosyl(1→4)-O-β-D-arabinopyranoside as proaglycone. Proaglycone on further hydrolysis with almond emulsion enzyme liberated D-galactose first followed by D-arabinose and aglycone. Thus, the compound was identified as 3,4′-dihydroxy-5,7-dimethoxy-flavone-3-O-β-D-galactopyranosyl(1→4)-O-β-D-arabinopyranosyl-4′-O-α-L-rhamnopyranoside.

Moreover, compound **1** showed significant antibacterial activity, therefore it may be used as a potent antibacterial agent against diseases caused by these bacteria (Table-1).

### Conclusion

On the basis of physico-chemical tests and characterization techniques, the structure of compound **1** was established as 3,4′-dihydroxy-5,7-dimethoxyflavone-3-O-β-D-galactopyranosyl(1→4)-O-β-D-arabinopyranosyl-4′-O-α-L-rhamnopyranoside from the ethanolic extract of stems part of the plant.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUND **1**

| Organisms | Zone of inhibition (mm) | | | | Ofloxacin | MIC |
|---|---|---|---|---|---|---|
| | 50 µg/mL | 100 µg/mL | 150 µg/mL | 200 µg/mL | 10 µg/mL | (mg/mL) |
| *Bacillus subtilis* | 8.50 ± 0.07* | 11.32 ± 0.14** | 13.25 ± 0.10** | 16.00 ± 0.32** | 23.75 ± 0.43** | 0.28 |
| *Actinomyces israelii* | 6.50 ± 0.02* | 9.25 ± 0.16** | 11.25 ± 0.14** | 15.50 ± 0.28** | 27.50 ± 0.30** | 0.30 |
| *Proteus vulgaris* | 10.23 ± 0.05* | 12.25 ± 0.26** | 15.43 ± 0.07* | 18.75 ± 0.18** | 24.50 ± 0.23** | 0.23 |
| *Salmonella typhimurium* | 10.27 ± 0.08* | 12.35 ± 0.23** | 16.01 ± 0.07* | 19.75 ± 0.20** | 23.01 ± 0.15** | 0.21 |

Data are expressed as Mean ± SEM; Experiments were conducted in triplicates; P value indicates calculated probability; *p < 0.05, *< 0.01 considered as significant; *p indicates when p is less than 0.05 showed statistically significant values; **p indicates that when p is less than 0.01 showed statistically highly significant values

## REFERENCES


1. R.P. Rastogi and B.N. Mehrotra, Compendium of Indian Medicinal Plants, vol. 3, CDRI Lucknow and National Institute of Science Communication, New Delhi, p. 346 (1980-1984).
2. L.V. Asolkar, K.K. Kakkar and O.J. Chakre, Second Supplement to – A Glossary of Indian Medicinal Plants with Active Principles Part-I (A-K), National Institute of Science Communication (CSIR), p. 357 (1965-1981).
3. P. Singh, S. Jain, M. Bhala, R.B. Goyal, D. Jayaprakash and N.K. Lohiya, *Phytother. Res.*, **4**, 86 (1990); https://doi.org/10.1002/ptr.2650040303.
4. M. Pal, M. Singh, P.K. Chaudhuri, R.P. Sharma and N. Singh, *Phytother. Res.*, **10**, 357 (1996); https://doi.org/10.1002/(SICI)1099-1573(199606)10:4<357::AID-PTR840>3.0.CO;2-L.
5. M. Pal, H. Joshi, V.P. Kapoor, P. Pushpangadan and L. Chaurasia, *Phytother. Res.*, **17**, 1215 (2003); https://doi.org/10.1002/ptr.1327.
6. P.K. Chaudhuri, R. Srivastava, S. Kumar and S. Kumar, *Phytother. Res.*, **18**, 114 (2004); https://doi.org/10.1002/ptr.1278.
7. M. Pal, S.K. Tewari and Q.S. Zhao, *Int. J. Pharm. Pharm. Sci.*, **3**, 418 (2011).
8. M. Ajaib, N. Javed and E.H. Siddiqi, *Pharmacologyonline*, **1**, 135 (2013).
9. C.K. Joshi, P. Singh and L.C. Singhi, *J. Indian Chem. Soc.*, **60**, 905 (1983).
10. E. Helfrich and H. Rimpler, *Phytochemistry*, **50**, 619 (1999); https://doi.org/10.1016/S0031-9422(98)00559-7.
11. P.K. Chaudhari, M. Sing, M. Pal, R.P. Sharma and S.P. Jain, *Indian J. Chem.*, **38B**, 632 (1999) .
12. I.E. Oboh, J.O. Akerele and O. Obasuyi, *Tropical J. Pharm. Res.*, **6**, 809 (2007); https://doi.org/10.4314/tjpr.v6i4.3.
13. C. Perez, M. Pauli and P. Bazerque, *Acta Biol. Med. Exp.*, **15**, 113 (1990).
14. I. Wiegand, K. Hilpert and R.E.W. Hancock, *Nat. Protoc.*, **3**, 163 (2008); https://doi.org/10.1038/nprot.2007.521.
15. J. Shinoda, *J. Pharm. Soc. Japan*, **48**, 218 (1928).
16. T.J. Mabry, K.R. Markham and M.B. Thomas, The Systematic Identification of Flavonoids, Springer-Verlag: Berlin (1970).
17. W.A. Bubb, *Concepts Magn. Reson. Part A*, **19A**, 1 (2003); https://doi.org/10.1002/cmr.a.10080.
18. J.Ø. Duus, C.H. Gotfredsen and K. Bock, *Chem. Rev.*, **100**, 4589 (2000); https://doi.org/10.1021/cr990302n.
19. R.N. Yadava and P. Yadav, *Am. J. Phytomed. Clin. Therap.*, **1**, 672 (2013).
20. J.M. Bobbitt, *Adv. Carbohydrate Chem.*, **11**, 1 (1956).
21. N.P.J. Price, *Appl. Biochem. Biotechnol.*, **148**, 271 (2008); https://doi.org/10.1007/s12010-007-8044-8.
22. J.B. Harborne, *Phytochemistry*, **4**, 107 (1965); https://doi.org/10.1016/S0031-9422(00)86152-X.