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Swertiamarin Isolation From The Herbal Plant *Enicostemma Littorale* Blume Using Flash Chromatography – A Straightforward Approach

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ABSTRACT

Swertiamarin (SWMN) from herbal plant *Enicostemma littorale* Blume was isolated using Flash Chromatography as white crystals with a yield of 9.85% w/w and ~98.5% purity. Swertiamarin was characterized using melting point, TLC fingerprinting, HPTLC, MS, FT-IR, and 1H-NMR. All values were compared using the available information. Flash chromatography was used in this investigation to isolate SWMN quickly, easily, and efficiently.

Keywords: Flash chromatography, Swertiamarin (SWMN), Separation, Isolation, *Enicostemma littorale*.

1. Introduction

Anthocleista procera, Enicostemma littorale, Swertia chiraytiya, davidi, patens, mileensis, and pseudochinesis all contain swertiamarin (SWMN)[1,2] and portrayed as an *Enicostemma littorale* biological lead compound. The pharmacological actions of SWMN are diverse[2–18]. Isolation of SWMN was done by solvent fractionation[9], column chromatography using silica gel[8,10,19,20] and by centrifugal partition chromatography[21] from different *Enicostemma* and *Swertia* species with improved yield of SWMN. But, all methods were considerably time

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Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

consuming[21] for the separation and isolation of SWMN from different plants. Bioactive compounds from crude extracts or fractions must be separated, isolated, and purified. Flash chromatography separates natural product mixtures with intermediate resolution at cheap cost[22]. Chromatographically, it separates fast [23,24]. Pure phytoconstituents are separated via chromatography. However, combining one or more chromatography procedures may purify phytoconstituents[25].

In this study, SWMN was separated from *Enicostemma littorale* using flash chromatography for the first time. We demonstrate flash chromatography-based SWMN isolation from Mamejava (Enicostemma littorale Blume) alcoholic extract (CombiFlash Rf 200 TELEDYNE ISKO).

2. Materials and Procedures

2.1 Equipment's and Materials

TLC plates with a pre-coat (silica gel 60 F254 (E. Merck); p-anisaldehyde reagent (Spectrochem); UV-cabinet (CAMAG); silica gel (particle size 40-60 /230 – 400#) (Merck, Germany); melting point apparatus (VEEGO-VMP-PM); TLC chamber with two troughs (10x10), and high performance thin layer chromatography (HPTLC) (CAMAG Flash chromatography was conducted using analytical-grade solvents that were bought from Fisher Scientific. SWMN was separated using a method for automatic flash chromatography (CombiFlash $R_{f\ 200}$, Teledyne Isco, Lincoln, NE, USA).

2.2 Plant collection and authenticity

Enicostemma littorale Blume's whole plant was collected in March 2021 from Dharampur in the Valsad district; certified as authentic by a taxonomist; and selected after consulting the Gujarat Flora[26]. IGU/Botany/514 voucher specimen was kept at Indira Gandhi University at Meerpur, Haryana, India. Plant material was pulverised and kept for later use after being dried at 37°C in a hot air oven.

2.3 Fractionation of Enicostemma littorale Blume *alcoholic extract*

Powdered material (200 g) was extracted with absolute alcohol (5 x 300 ml) on a shaker at 70 rpm until no swertiamarin (SWMN) was detected in thin layer chromatography (TLC). Under reduced pressure, the solvent was vaporised in a rotating evaporator at 40 °C. Cold diethyl ether was

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

applied to the resultant alcoholic extract to create a precipitate that weighed 60 g. The precipitate was loaded onto a chromatographic column with a silica gel slurry (60-120#, E. Merck, Germany), eluted with petroleum ether, and then subjected to a gradient elution with ethyl acetate (0-80%). Co-thin layer chromatography (co-TLC) in fractions of ethyl acetate and petroleum ether (80:20 v/v) was then used to monitor SWMN. SWMN-containing fractions were concentrated to dryness. 500 mg of dried chemicals were separated by flash chromatography.

2.4 Sample Preparation, isolation and separation by Flash chromatography

The empty solid sample cartridge's bottom frit was checked before processing. Under decreased pressure, the sample mixture (500 mg) was dissolved in a binary solvent solution (chloroform and methanol) and adsorbed on silica gel (240-400#). The cartridge was tapped on the benchtop to settle the sample. The cartridge's frit was pressed against the mixture using a plastic plunger. Wiped and capped the cartridge's leftover powder. Loading the solid sample cartridge with cap on the sample injection port.

The silica gel-impregnated mixture containing SWMN (500 mg) was flash chromatographed on a 40 g silica gel flash column (240-400#) TELEDYNE ISKO CombiFlash Rf_{200}. To eliminate air interruption, the instrument was started with a purging operation lasting up to 0.5 minutes to remove air from the tubing. The prepared sample of 200 mg was flash chromatographed using a binary solvent technique (chloroform and methanol). For fraction collection, two collection tube racks (60-5237-032) were employed. Prior to separations, the column was equilibrated with solvent A (chloroform). SWMN was separated in a gradient system of solvents A (chloroform) and B (methanol) for 20 minutes: 100% A for 3 minutes, 10% B for 4.5 minutes, 2.5 minutes, 20% B for 2 minutes, and 12 minutes. The flow rate was kept constant at 20 mL/min, and separate fractions (15 mL) were collected by measuring the eluting analytes at 242 nm (red colour) and 360 nm (purple colour), as indicated in the **(Fig. 1).** Kumar et al[10], stated that the wavelength of SWMN was 242 nm. Six SWMN-containing fractions (22-27) were pooled together and concentrated to dryness in a rotary evaporator under vacuum, and the yield was determined. The extraction, fractionation, and isolation procedures were observed using thin-layer chromatography (TLC) for SWMN (chloroform:methanol, 8.5:1.5 v/v).

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

2.5 Identification and purity determination of isolated SWMN

The isolated SWMN was identified by melting point, co-TLC fingerprinting with std. SWMN in the chloroform/methanol (8.5:1.5 v/v) mobile phase of the TLC[10] under UV 254 nm and derivatized by the alcoholic p-anisaldehyde reagent to establish purity. HPTLC analysis was performed using a CAMAG 140 LINOMAT 5 automated TLC spotter to deposit samples to pre-coated plates of silica gel 60 F_{254} (E. Merck; 139-Kieselgel 60F_{254}) (Switzerland). CAMAG TLC Scanner-3 and CAMAG 1.3.0 (WinCATS software) acquired UV spectra.

2.6 Characterization of isolated SWMN

Shimadzu Fourier-transform Infrared (FT-IR) spectrophotometer was used to record infrared spectrum; Perkin-Elmer API 165 mass spectrometer (LC/MS) was used to get molecular ion mass spectra; and 1H nuclear magnetic resonance (^{1}H-NMR)and ^{13}C-NMR spectra were recorded in MeOD on a Bruker Advance II (500 MHz) FT-NMR spectrometer.

3. Result and Discussion

SWMN was fractionated from *Enicostemma littorale* Blume and separated by flash chromatography. The yield of the isolated SWMN was (50.8 mg, 9.85% w/w) as colorless crystals from the fraction containing SWMN (500 mg).

3.1. Identification and purity profiling of isolated SWMN

Rf values of concentrated fractions (22-27) from flash chromatography verified the identification of SWMN (Fig. 1). TLC indicated a single spot of collected and concentrated fractions (22-27) (2nd lane) from flash chromatography with the same Rf value (0.30) as standard SWMN (1st lane) in flash chromatography. TLC fingerprinting of isolated and standard SWMN in mobile phase [chloroform/ methanol (8.5: 1.5 v/v)] showed that Rf (0.28) values were identical (Fig. 2). SWMN was identified by TLC.

ISSN PRINT 2319 1775 Online 2320 7876

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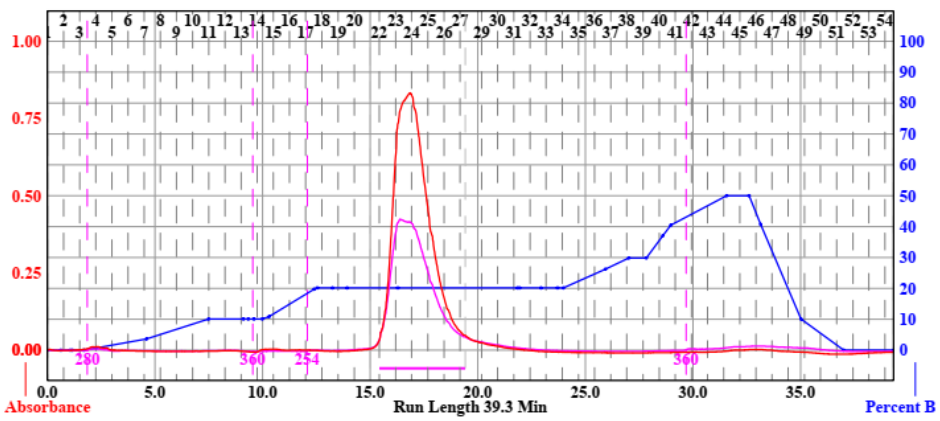

Fig. 1(A)

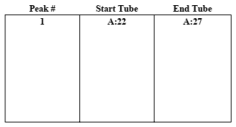

Fig. 1 (B)

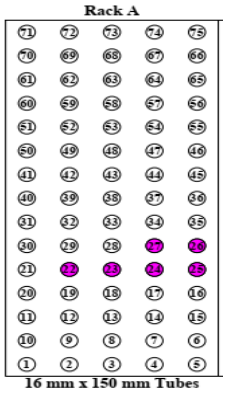

Fig. 1 (C)

Fig. 1 (a) Flash chromatogram of dry mass fraction (500 mg). Peaks obtained for each separation are coloured and numbered. Red and purple line graphs represent absorbance measured at 242 and 360 nm, respectively.; (b) shown the peak at wavelength 242 nm; (c) shown the collected fractions at wavelength 242 nm in test tubes.

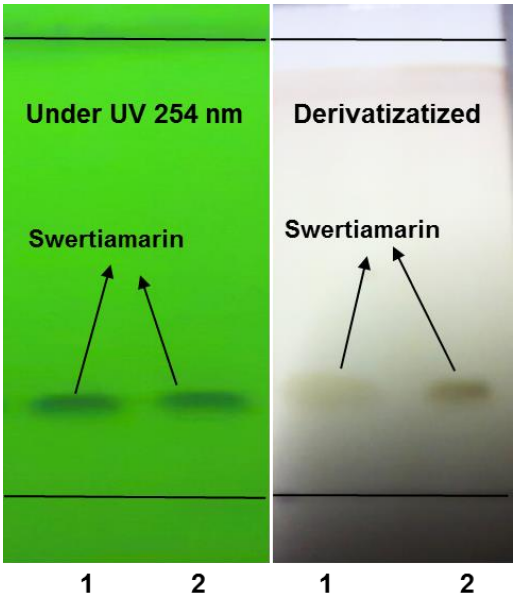

Fig. 2 Standard SWMN (1st lane) and isolated SWMN in gathered test tube fractions (22-27) (2nd lane) TLC fingerprint profiles in the TLC mobile phase [chloroform/methanol (8.5: 1.5)].TLC was observed under 254 nm and derivatized by the p-anisaldehyde reagent.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Co-eluting impurities like metabolites or isomers create peaks that are too close to the original substance's peak. The chromatogram reveals shoulder patterns if their concentrations are high. Beer's law states that a spectrochromatogram with two bilinear forms shows the simultaneous absorption of the primary substance and a co-eluting impurity[10,27]. Pure solid compounds could be tentatively identified by melting point. A small impurity lowers a compound's melting point by a few degrees and expands its melting-point temperature range[28]. The sharp melting point of isolated SWMN was 113-114°C, matching Merck Index data, 2013[29] results for pure substance.

Overlay UV absorption spectrum at start, middle, and end point of spot on TLC plate at wavelength 254 nm indicated purity of isolated SWMN. HPTLC and CAMAG 1.3.0 WinCATS software showed that the isolated SWMN was ~98.5% pure. HPTLC was used to acquire an overlay UV spectra of standard and isolated SWMN. The max of both was 242 nm as shown in (Fig. 3).

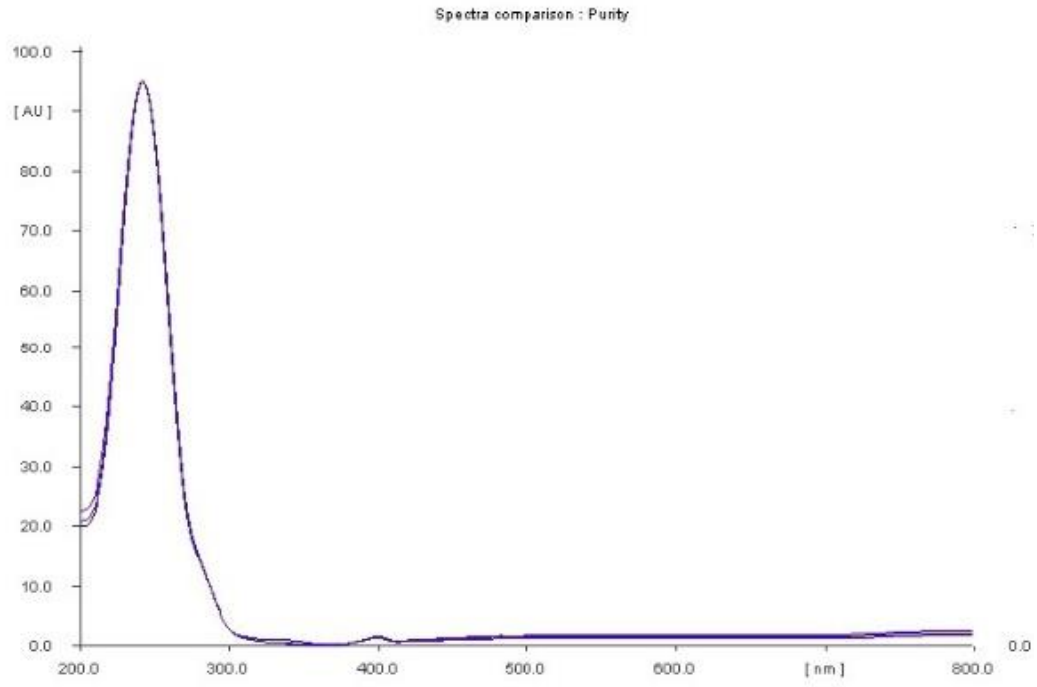

Fig. 3 overlay UV chromatogram of the isolated SWMN separated on TLC at (254 nm) wavelength (start, middle, and end locations of the band).

ISSN PRINT 2319 1775 Online 2320 7876

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3.2 Characterization of isolated SWMN by MS, FTIR, and NMR

The mass spectrum of isolated SWMN in (-)ve mode at 373.7 m/z and (+)ve mode at 375.2 m/z[30] corresponds to the (C16H22O10 and 374) molecular formula and molecular weight, respectively **(**Fig. 4**).**

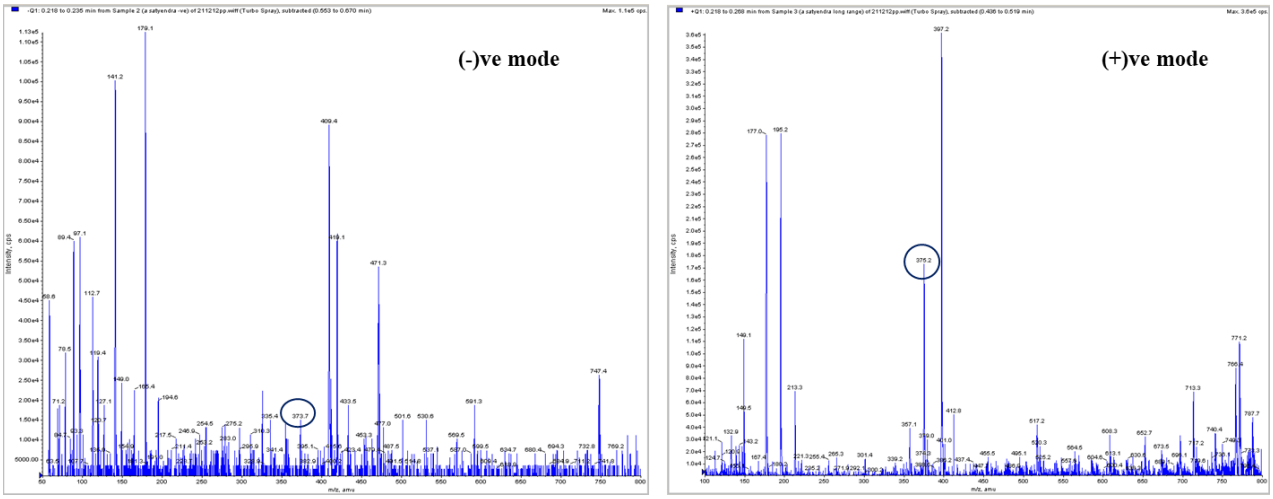

Fig. 4 Mass spectrum of isolated SWMN in (-)ve mode at 373 m/z and (+)ve mode 375 m/z.

FTIR spectra of isolated (SWMN) exhibited multiple strong peaks in between 4000-400 cm^{-1}. IR (KBr, cm^{-1}) ν: 3347 O-H (stretch., hydroxyl), C-H (stretch., C-H stretching of alkanes) 2923, C=O (stretch.) 1696, C=C (stretch., aromatic alkene) 1617, C-O-C (stretch., C-O vibrations of alcohols) 1408, C=CH2 (stretch. terminal alkene) 846 **(**Fig. 5**)**[10,30].

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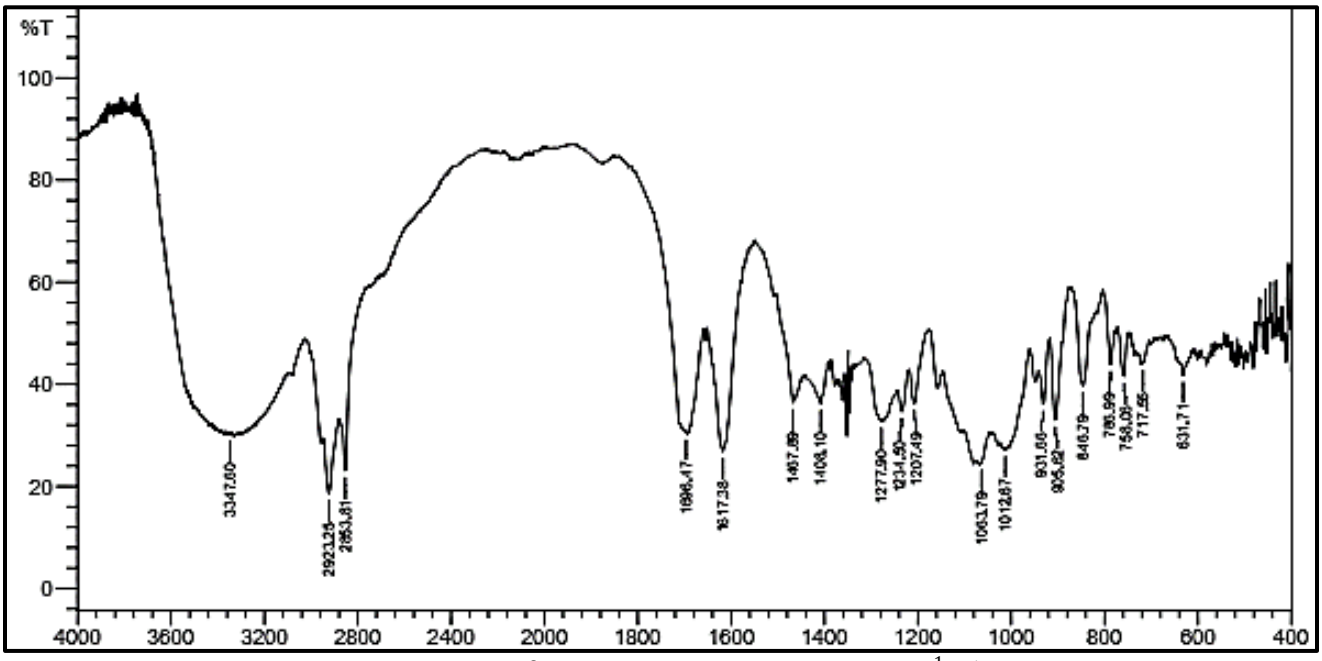

Fig. 5 IR spectrum of the isolated SWMN (KBr, cm^{-1}, ν).

^{1}H-NMR signals of isolated SWMN (400 MHz, MeOD); chemical shift values (δ): 1.28 (ddd; J = 13, 5; 11 Hz; H-6α), 1.67 (ddd; J = 13; 2; 3 Hz; H-6β), 2.95 (dd, J = 1; 9.5 Hz; H-9), 3.81 (dd; J = 12; 2 Hz; H-7β), 4.26 (dd; J = 4; 1.5 Hz; H-7α), 5.63 (d; J = 1.7 Hz; H-1), 7.54 (s; H-3) and other protons indicated their presence at 3.56 (m; H - 2', 3', 4', 5'); 3.81 (dd; J = 5; 2 Hz, H-11α), 4.45 (dd; J = 11, 3 Hz; H-11β); 4.66 (dd; J = 12, 5, 11 Hz; H-10)[10,30,31] as shown in **(**Fig. 6**).**

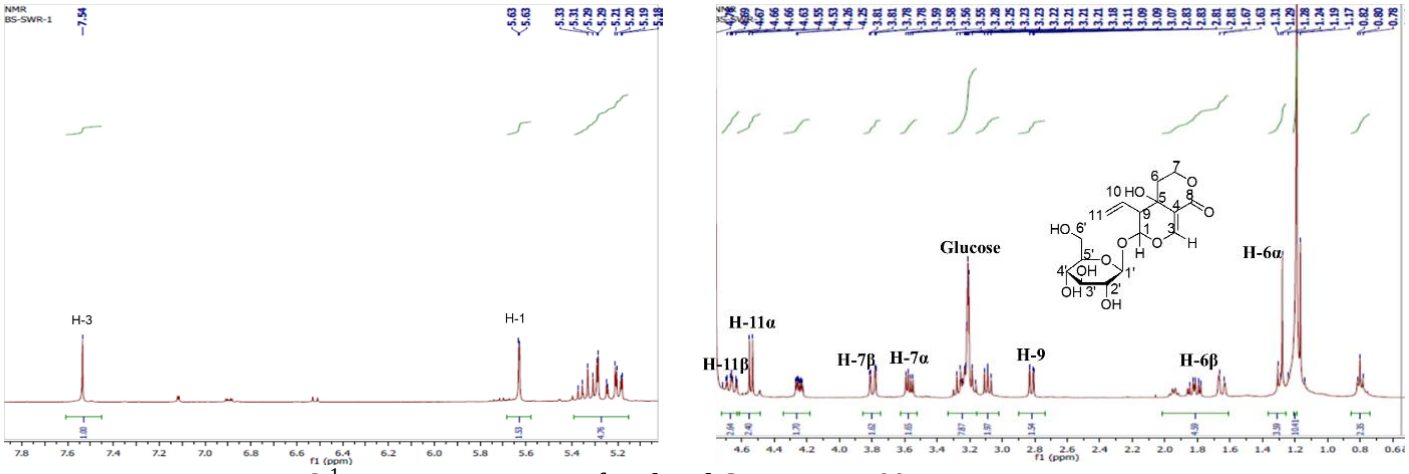

Fig. 6 ^{1}H-NMR spectrum of isolated SWMN at 400MHz.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Spectral methods including MS, FTIR, and ^{1}H-NMR were used to analyse the structure of isolated SWMN **(**Fig. 7**)**[10,29,31].

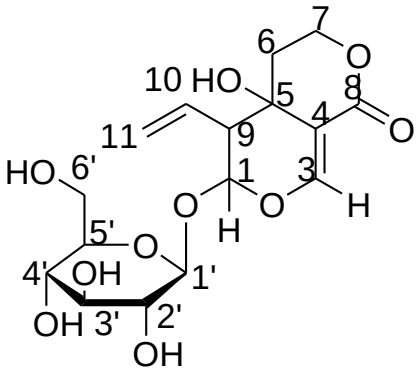

Fig. 7 Structural representation of isolated SWMN ($C_{16}H_{22}O_{10}$).

Conclusion

Flash chromatography separated SWMN from *Enicostemma littorale* Blume alcoholic extract fractions in high yield and purity. This rapid, easy, and effective approach can isolate this chemical for biological activity investigations.

Acknowledgement

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Conflicts of interest

The authors have declared that there are no conflicts of interest.

References:

[1] P. Abirami, M. Gomathinayagam, A REVIEW ON ENICOSTEMMA LITTORALE, Pharmacologyonline. 1 (2011) 75–83.

[2] A. Singh, Phytochemicals of Gentianaceae : A Review of Pharmacological Properties, J. Liq. Chromatogr. (2005) 33–36.

[3] M.M. Shabi, S. Uthrapathy, C.D. Raj, G. Krishnamoorthy, D. Ravindhran, J. Joseph, V.G. Rajamanickam, Analgesic and Anti-Arthritic Effect of Enicostemma littorale Blume, (2014) 1018–1024.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

[4] C.-M. Lu, L.-C. Lin, T.-H. Tsai, Determination and Pharmacokinetic Study of Gentiopicroside, Geniposide, Baicalin, and Swertiamarin in Chinese Herbal Formulae after Oral Administration in Rats by LC-MS/MS, Molecules. 19 (2014) 21560–21578. doi:10.3390/molecules191221560.

[5] H. Vaidya, M. Rajani, V. Sudarsanam, H. Padh, R. Goyal, Swertiamarin: A lead from Enicostemma littorale Blume. for anti-hyperlipidaemic effect, Eur. J. Pharmacol. 617 (2009) 108–112. doi:10.1016/j.ejphar.2009.06.053.

[6] R.D. Sonawane, S.L. Vishwakarma, S. Lakshmi, M. Rajani, H. Padh, R.K. Goyal, Amelioration of STZ-induced type 1 diabetic nephropathy by aqueous extract of Enicostemma littorale Blume and swertiamarin in rats, Mol. Cell. Biochem. 340 (2010) 1–6. doi:10.1007/s11010-010-0393-x.

[7] J. Vaijanathappa, S. Badami, Antiedematogenic and free radical scavenging activity of swertiamarin isolated from Enicostemma axillare., Planta Med. 75 (2009) 12–7. doi:10.1055/s-0028-1088333.

[8] V. Jaishree, S. Badami, M. Rupesh Kumar, T. Tamizhmani, Antinociceptive activity of swertiamarin isolated from Enicostemma axillare, Phytomedicine. 16 (2009) 227–232. doi:10.1016/j.phymed.2008.09.010.

[9] T.P. Patel, S. Soni, P. Parikh, J. Gosai, R. Chruvattil, S. Gupta, Swertiamarin: An active lead from enicostemma littorale regulates hepatic and adipose tissue gene expression by targeting PPAR-γ and improves insulin sensitivity in experimental niddm rat model, Evidence-Based Complement. Altern. Med. 2013 (2013) 1–11. doi:10.1155/2013/358673.

[10] S. Kumar, V. Jairaj, An Effective Method for Isolation of Pure Swertiamarin from Enicostemma littorale Blume, Indo Glob. J. Pharm. Sci. 08 (2018) 01–08. doi:10.35652/igjps.2018.0108.

[11] M. Rajani, N.S. Kanaki, Phytochemical Standardization of Herbal Drugs and Polyherbal Formulations, Bi[1] M. Rajani, N.S. Kanaki, Phytochem. Stand. Herb. Drugs Polyherbal

ISSN PRINT 2319 1775 Online 2320 7876

Research paper © 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Formul. Bioact. Mol. Med. Plants. 349–369. Doi10.1007/978-3-540-74603-4_19.Oactive Mol. Med. Plants. (2008) 349–369. doi:10.1007/978-3-540-74603-4_19.

[12] L. Yang, Y. Wang, L. Wang, H. Xiao, Z. Wang, Z. Hu, Rapid quantification of iridoid glycosides analogues in the formulated Chinese medicine Longdan Xiegan Decoction using high-performance liquid chromatography coupled with mass spectromentry, J. Chromatogr. A. 1216 (2009) 2098–2103. doi:10.1016/j.chroma.2008.06.043.

[13] B. Dinda, D.R. Chowdhury, B.C. Mohanta, Naturally occurring iridoids, secoiridoids and their bioactivity. An updated review, part 3., Chem. Pharm. Bull. (Tokyo). 57 (2009) 765–796. doi:10.1248/cpb.57.765.

[14] K. Chiba, M. Yamazaki, M. Kikuchi, R. Kakuda, M. Kikuchi, New physiological function of secoiridoids: neuritogenic activity in PC12h cells., J. Nat. Med. 65 (2011) 186–90. doi:10.1007/s11418-010-0449-y.

[15] V. Jaishree, S. Badami, P.T. Krishnamurthy, Antioxidant and hepatoprotective effect of the ethyl acetate extract of Enicostemma axillare (Lam). Raynal against CCl4-induced liver injury in rats, Indian J. Exp. Biol. 48 (2010) 896–904.

[16] H. Vaidya, R.K. Goyal, S.K. Cheema, Anti-diabetic Activity of Swertiamarin is due to an Active Metabolite, Gentianine, that Up regulates PPAR-γ Gene Expression in 3T3-L1 cells, Phyther. Res. 4 (2012) 624–7. doi:10.1002/ptr 47/63.

[17] S. Saravanan, P. Pandikumar, N.P. Babu, V.I.H. Islam, K. Thirugnanasambantham, M.G. Paulraj, K. Balakrishna, S. Ignacimuthu, In Vivo and In Vitro Immunomodulatory Potential of Swertiamarin Isolated from Enicostema axillare (Lam.) A. Raynal That Acts as an Anti-inflammatory Agent, Inflammation. (2014). doi:10.1007/s10753-014-9862-9.

[18] S. Saravanan, V.I. Hairul Islam, K. Thirugnanasambantham, N. Pazhanivel, N. Raghuraman, M. Gabriel Paulraj, S. Ignacimuthu, Swertiamarin ameliorates inflammation and osteoclastogenesis intermediates in IL-1β induced rat fibroblast-like synoviocytes, Inflamm. Res. 63 (2014) 451–462. doi:10.1007/s00011-014-0717-5.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

[19] S. Vishwakarma, M. Rajani, M. Bagul, R. Goyal, A Rapid Method for the Isolation of Swertiamarin from Enicostemma littorale, Pharm. Biol. 42 (2004) 400–403. doi:10.1080/13880200490885095.

[20] H.B. Magora, M.M. Rahman, A.I. Gray, M.D. Cole, Swertiamarin from Enicostemma axillare subsp. axillare (Gentianaceae), Biochem. Syst. Ecol. 31 (2003) 553–555. doi:10.1016/S0305-1978(02)00200-4.

[21] V.S. Rana, Separation and Identification of Swertiamarin from Enicostemma axillare Lam. Raynal by Centrifugal Partition Chromatography and Nuclear Magnetic Resonance-Mass Spectrometry*, Pharm. Sci. Emerg. Drugs. 2 (2013) 1–5.

[22] P. Weber, M. Hamburger, N. Schafroth, O. Potterat, Flash chromatography on cartridges for the separation of plant extracts: Rules for the selection of chromatographic conditions and comparison with medium pressure liquid chromatography, Fitoterapia. 82 (2011) 155–161. doi:10.1016/j.fitote.2010.08.013.

[23] F. Bucar, A. Wube, M. Schmid, Natural product isolation-how to get from biological material to pure compounds, Nat. Prod. Rep. 30 (2013) 525–545. doi:10.1039/c3np20106f.

[24] X.X. Yu, Q.W. Wang, X.J. Xu, W.J. Lv, M.Q. Zhao, Z.K. Liang, Preparative isolation of Heteroclitin D from Kadsurae Caulis using normal-phase flash chromatography, J. Pharm. Anal. 3 (2013) 456–459. doi:10.1016/j.jpha.2013.07.004.

[25] Buchi, Isolation of curcumin from a natural extract by flash chromatography and prepHPLC, (2014) 1. https://static1.buchi.com/sites/default/files/shortnotes/SN_2014_150.pdf.

[26] G.L. Shah, Flora of Gujarat State, Volume 1 & 2, Sardar Patel University, Vallabh Vidyanagar, 1978. http://books.google.co.in/books/about/Flora_of_Gujarat_State.html?id=W_sLAAAAMAAJ&pgis=1 (accessed April 25, 2015).

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

[27] C. Ritter, J. Gilliard, ImpuR: A Collection of Diagnostic Tools Developed in R in the Context of Peak Impurity Detection in HPLC-DAD but Potentially Useful with Other Types of Time–Intensity Matrices, J. Stat. Softw. 18 (2007) 1–18. http://www.jstatsoft.org/v18/i09/paper (accessed July 15, 2015).

[28] J. Gilbert, S. Martin, Experimental Organic Chemistry: A Miniscale and Microscale Approach, Cengage Learning, 2010. https://books.google.com/books?id=8wIQwCmWz9EC&pgis=1 (accessed July 15, 2015).

[29] Buadavari S, Swertiamarin | The Merck Index, 15TH ed., 2013. https://www.rsc.org/Merck-Index/monograph/m10410/swertiamarin?q=unauthorize (accessed April 25, 2015).

[30] M. Anwar, M. Agmad, M. Aslam, K. Aftab, Enicostemma littorale: A new source of swertiamarin, Pak. J. Pharm. Sci. 9 (1996) 29–35.

[31] S.P. Tondon N, Phytochemical reference standards of selected Indian medicinal palnts. Vol 1, The Indian Council of Medical Research (ICMR), New Delhi, 2010. http://www.icmr.nic.in/pricepubl/phyto/phyto_vol_1.htm (accessed April 25, 2015).