International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 283-287 www.ijprajournal.com ISSN: 2249-7781

Review of HPLC techniques for isolating flavonoids from plants belonging to Solanum family

Swathy Lakshmi N[1], [2]Dr.Lal Prasanth M L, [3]Jisha Prems, [4]Tina Raju
[1,4]*Associate Professor, Dr.Moopen's College of Pharmacy, Wayanad, Kerala*
[2]*Principal, Dr.Moopen's College of Pharmacy, Wayanad, Kerala*
[3]*Professor, Dr.Moopen's College of Pharmacy, Wayanad, Kerala*

Submitted: 11-03-2024 Accepted: 21-03-2024

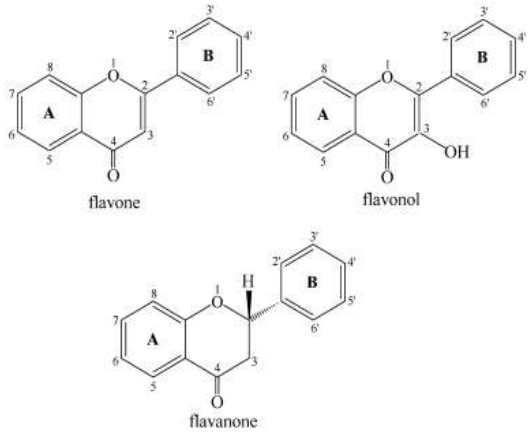
ABSTRACT: Solanum virginianum, a wild eggplant bearing spines, commonly known as "Kantakari" belongs to the family "Solanaceae." It comprises numerous remedial constituents such as glycosides, anthraquinones, flavonoids, sterols, saponins and alkaloids. This traditionally used medicinal herb has been reported to own cardio-protective, hepatoprotective, antidiabetic,34 anti-asthmatic, anti-inflammatory and anti-cancer properties.High-performance liquid chromatography methods are developed for qualitative and quantitative analyses of flavonoids in fruits and beverages, wine, honey, propolis, and, especially, in various plant materials using different detection systems. This technique is an emerging tool for isolation and analysis of flavonoids due to its high resolution, sensitivity and versatility. The current study gives enough idea about various flavonoid identification techniques with special emphasis on HPLC techniques of plants belonging to Solanum family.

KEYWORDS: Solanum virginianum, flavanids,HPLC,

I. INTRODUCTION

Flavonoids

Plants, fruits, and seeds contain large amounts of flavonoids, which are secondary metabolites that give them their distinctive colour, flavour, and scent. Flavonoids have a wide range of roles in plants, including controlling cell division, drawing pollinating insects, and providing defence against biotic and abiotic stressors[1]. Plant flavonoids, for example, have multiple physiological roles in drought, heat, and frost tolerance and can function as signal molecules, UV filters, and scavengers of reactive oxygen species (ROS)[2-4]. These substances bioactive qualities, which include anti-inflammatory, anti-cancer, anti-aging, cardio-protective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral effects, have been linked to a wide range of health advantages in people[5-7]. However, the bioavailability and biological activity of flavonoids in humans is influenced by their chemical structure, namely by the presence of hydroxy groups[8]. The fundamental flavone in flavonoids is a 15-carbon one. Based on the structure of the flavonoids, they can be classified into six major classes, flavan-3-ols, flavones, flavanol's, flavanones, isoflavones, and anthocyanins[9,10].



High Performance Liquid Chromatography

Numerous published reports describe the process of isolating and characterizing flavonoids from plant material using various techniques, mostly chromatographic and spectroscopic. High-performance liquid chromatography (HPLC) is accepted as the most practical technique available today for the identification and separation of flavonoids employing a variety of detection methods[11-16] Regarding the quantitative analysis, a large body of research has been published in the last few years attesting to the applicability of this method for the simultaneous determination of flavonoid compounds in different samples. UV diode array detectors are one of the detection systems used in high-performance liquid chromatography technologies that are created for

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 283-287 www.ijprajournal.com ISSN: 2249-7781

qualitative and quantitative analysis of flavonoids in fruits and drinks, wine, honey, propolis, and, especially, in diverse plant materials[15–18].

II. MATERIALS & METHODS

Pravin Morankar et al developed anHPLC method for quantifying flavonoid compounds The methanolic extract of S. virginianum under inquiry was employed as a preliminary evaluation of different chemicals for HPLC investigation of flavonoid compounds. Waters Ace software was used to evaluate the data produced by the HPLC system, which consisted of a waters fitted with a UV dual detector. Application of a Thermo C18 column (250X4.6mm, 5µm) was made for chromatographic separation. A 50:50 v/v acetonitrile:methanol mobile phase was used for the chromatographic analysis, which was carried out at room temperatureon an RP-C18 analytical column. The sample was isocratically eluted at a flow rate of 1 mL/min. For every sample run, a 20 µL little sample volume was fed into the HPLC apparatus[19-23]

Figure 1 displays the standard quercetin and hydroalcoholic extract HPLC chromatograms, with values reported in parts per million. Figure 2 displays HPLC chromatograms of methanolic extract. It was discovered that the retention times for the standard and extracts were, respectively, 2.623 and 2.606 minutes. Table 1 presented the characteristics parameters for standard quercetin, whereas Table 2 presented the findings of the quantitative estimation of quercetin in methanolic extracts.

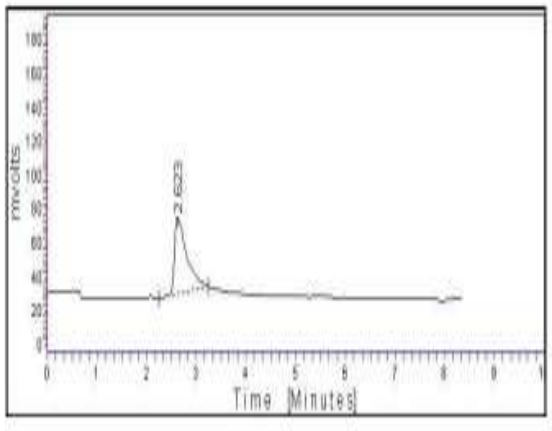


Figure 1 (Standard quercetin)

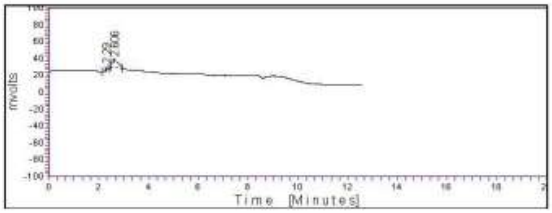


Figure 2 (Methanolic extract)

Compound	Linearity range µg/ml	Correlation co-efficient	Slope	Intercept
Quercetin	5-25	0.999	25.25	-1.451

Table 1 (Parameters for calibration of standard quercetin)

Extract	RT	Area	% Assay
C. heynei	2.606	177.165	0.820
S. virginianum	2.606	157.145	0.798

Table 2(quantitative estimation of quercetin in methanolic extracts)

RifkiHusnulKhuluk et al founded out eight flavonoids that may be simultaneously determined in Sonchus arvensis using a high-performance liquid chromatography diode array detector (HPLC-DAD). The flavonoids were orientin, hyperoside, rutin, myricetin, luteolin, quercetin, kaempferol, and apigenin This approach is easy to use and highly effective.

This study aimed to develop a method for the simultaneous identification and quantification of flavonoids in S. arvensis, namely orientin, hyperoside, rutin, myricetin, luteolin, quercetin, kaempferol, and apigenin. We also applied the developed method for discrimination of geographical origin and plant parts used from S. arvensis. Thus, in the present study, a combination of chromatographic fingerprint and chemometric analysis was used as a powerful tool for discriminating between plant parts used to prepare the samples and identify the S. arvensis sample's geographical origin.

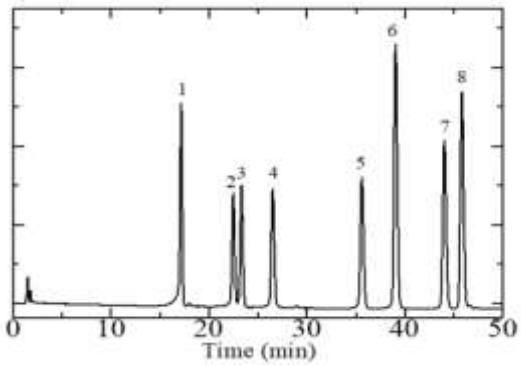
Using this technique, the samples were separated according to their geographical origin and the plant parts (leaves, stems, and roots). On a reverse-phase C18 column, the chromatographic separation was performed by eluting using a gradient containing methanol and 0.2% aqueous formic acid at a flow rate of 1 mL/min. The established method's system applicability has satisfied the requirements of good separation under ideal circumstances. With a correlation value (r2) > 0.9990, the calibration curve demonstrates a linear

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 283-287 www.ijprajournal.com ISSN: 2249-7781

connection between the peak area and analyte concentration.

Investigations into the effects of mobile phase composition were conducted in order to achieve the optimal separation conditions for quantitative analysis and a chromatographic fingerprint. Separations 2021, 8, 12 4 of 10, and the detection wavelength were tuned.

The parameters employed to optimize the chromatographic settings were the overall analysis duration and the resolution of each analyte. Table reports representative chromatograms of the flavonoid standard solutions. Gradient elution of phase A, methanol, and an elution of phase B, 0.2% formic acid in the water, were used to obtain the best chromatographic separation.



An analytical performance test is a method used to demonstrate that a particular analytical procedure yields findings that meet the procedure's stated goals and objectives. Information about the goodness, quality, consistency, and dependability of the analytical results can be obtained from this evaluation. The parameters of system appropriateness, linearity, LOD, LOQ, accuracy, and stability define the result of the analytical performance test

Five replicate analyses of a mixed standard solution containing orientin, hyperoside, rutin, myricetin, luteolin, quercetin, kaempferol, and apigenin were used to evaluate the appropriateness of the system. To assess the system adequacy, the relative standard deviation (RSD) values of the retention duration, peak area, capacity factor, tailing factor, and theoretical plate number were computed (Table 1). The confidence intervals (RSD) for each parameter measured during the system suitability tests

System suitability test parameters separation and quantification of flavonoids in a mixed standard solution of orientin (OR), hyperoside(HP), rutin (RT), myricetin (MR), luteolin (LT),quercetin (QR), kaempferol (KM), and apigenin (AG).

Analyte[a]	OR	HP	RT	MR	LT	QR	KM	AG
Retention time (min)								
Mean	16.912	21.952	22.996	26.224	35.057	38.451	43.334	45.136
RSD (%)	0.03	0.21	0.05	0.24	0.03	0.05	0.1	0.06
Peak area								
Mean	276,087	1,769,838	275,124	246,515	247,129	454,891	331,047	373,706
RSD (%)	0.31	0.42	0.22	0.34	0.62	0.41	0.13	0.46
Capacity factor								
Mean	10.956	14.684	15.258	14.453	23.779	26.197	39.685	32.605
RSD (%)	0.14	0.17	0.07	0.44	0.12	0.04	0.13	0.51
Tailing factor								
Mean	0.819	1.036	1.014	1.341	0.972	0.934	1.291	1.125
RSD (%)	0.65	0.75	0.63	0.93	0.56	0.75	0.42	0.36
Theoretical plate number								
Mean	21,383	23,821	27,756	27,336	42,393	52,963	63,706	73,598
RSD (%)	0.38	0.32	1.83	1.92	1.22	2.17	1.78	2.01

Chemical composition, anti-bacterial and antioxidant potential of S. virginianum. Preliminary screening of phytochemicals revealed the presence of alkaloids, phenols, flavonoids, glycosides, tannins, terpenoids, and saponins. Furthermore, FTIR spectrum displays various functional groups which might be responsible for medicinal importance of plant. Quantitative analysis of phytocompounds showed that ME exhibited highest amount of total phenol and flavonoids.

III. DISCUSSION &CONCLUSION

Natural products are a well-researched avenue for the discovery and development of new drugs since they are abundant in physiologically active chemicals and a prime example of molecular diversity. Antioxidants like polyphenols, which are abundant in medicinal plants, can be useful in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and breaking down peroxides. Numerous of these phytochemicals have strong antioxidant properties that are linked to a decreased risk of developing and dying from a number of diseases in humans.

S. virginianum is a promising candidate for a novel phytomedicine with multiple therapeutic applications. Subsequent research will focus on identifying bioactive components and quality requirements in order to build a possible medication. By this type of flavonoid isolation approaches like

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 2 Mar-Apr 2024, pp: 283-287 www.ijprajournal.com ISSN: 2249-7781

HPLC, providing valuable insights to the researchers and future pharmacologists without any doubt.

The problems facing all modern analysts are to develop more advanced instruments and devices for faster and more efficient sample preparation in addition to improving the properties of the stationary phases. The "going back to nature" movement and the discovery of flavonoids' positive health effects have spurred the development of quicker and more effective methods for identifying and quantifying them; HPLC is still the most effective method for separating them from complex mixtures.

REFERENCE

[1]. De Luna S.L., Ramírez-Garza R.E., Saldívar S.O.S. Environmentally friendly methods for flavonoid extraction from plant material: Impact of their operating conditions on yield and antioxidant properties. Sci. World J. 2020;

[2]. Ferdinando M.D., Brunetti C., Fini A., Tattini M. Flavonoids as antioxidants in plants under abiotic stresses. In: Ahmad P., Prasad M.N.V., editors. Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability.

[3]. Panche A.N., Diwan A.D., Chandra S.R. Flavonoids: An overview. J. Nutr. Sci. 2016;

[4]. Dias M.C., Pinto D.C.G.A., Freitas H., Santos C., Silva A.M.S. The antioxidant system in Olea europaea to enhanced UV-B radiation also depends on flavonoids and secoiridoids. Phytochemistry. 2020;

[5]. Saini N., Gahlawat S.K., Lather V. Flavonoids: A nutraceutical and its role as anti-inflammatory and anticancer agent. In: Gahlawat S., Salar R., Siwach P., Duhan J., Kumar S., Kaur P., editors. Plant Biotechnology: Recent Advancements and Developments;2017.

[6]. Jucá M.M., Filho F.M.S.C., de Almeida J.C., Mesquita D.S., Barriga J.R.M., Dias K.C.F., Barbosa T.M., Vasconcelos L.C., Leal L.K.A.M., Ribeiro J.R., et al. Flavonoids: Biological activities and therapeutic potential. Nat. Prod. Res. 2020;5(1):692–705.

[7]. Fraga C.G., Croft K.D., Kennedy D.O., Tomás-Barberán F.A. The effects of polyphenols and other bioactives on human health. Food Funct. 2019;10(1):514–528.

[8]. Maleki S.J., Crespo J.F., Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem. 2019;2(9):125124.

[9]. D'Amelia V., Aversano R., Chiaiese P., Carputo D. The antioxidant properties of plant flavonoids: Their exploitation by molecular plant breeding. Phytochem. Rev. 2018;17(4):611–625.

[10]. Šamec D., Karalija E., Šola I., Bok V.V., Salopek-Sondi B. The role of polyphenols in abiotic stress response: The influence of molecular
structure. Plants. 2021;10(1):118.

[11]. Bravo L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev. 1998;56(11):317–33. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x

[12]. Proestos C, Boziaris IS, Nychas GJE, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: In-vestigation of their antioxidant capacity and antimicrobial activity. Food Chem. 2006;95(4):664–71.
https://doi.org/10.1016/j.foodchem.2005.01.049

[13]. Shahidi F, Yeo JD. Insoluble-bound phenolics in food. Molecules. 2016;21(9):1216.https://doi.org/10.3390/molecules21091216

[14]. Pérez-Gregorio RM, García-Falcón MS, Simal-Gándara J, Rodrigues AS, Almeida DPF. Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. J Food Compos Anal. 2010;23(6):592–8.

[15]. https://doi.org/10.1016/j.jfca.2009.08.013

[16]. Chen JH, Ho CT. Antioxidant activities of caffeic acids and its related hydrocinnamic acid compounds. J Agric Food Chem. 1997; 45:2374-2378.

[17]. Cuyckens F, Claeys M. Mass spectrometry in the structural analysis of flavonoids. J Mass Spectro. 2004; 39:1-15.

[18]. Khosh F, Khosh M. Natural approach to hypertension. Altern Med Rev. 2001; 6(6):590-600.

[19]. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Biochemical and molecular action of nutrients. J Nutri 2001; 131:2837-2842.

[20]. Kavishankar GB, Lakshmidevi N, Murthy M, Prakash HS, Niranjana SR. Diabetes and medicinal plants-A review. Int J Pharm Biomed Sci. 2011; 2(3):65-80.
[21]. Holm, L.; Doll, J.; Holm, E.; Pancho, J.; Herberger, J. World Weeds: Natural Histories and Distribution; John Wiley and Sons Inc.:
[22]. Li, X.M.; Yang, P.L. Research progress of Sonchus species. Int. J. Food Prop. 2018, 21, 147–157.
[23]. Ministry of Health Republic of Indonesia. Indonesia Herbal Pharmacopoeia, 1st ed.; Ministry of Health Republic of Indonesia: Jakarta, Indonesia, 2010; pp. 138–140.
[24]. Manoi, F. Effect fineness extraction of materials and old quality extract (Sonchus arvensis L.). J. Appl. Agric. Res. **2015**, 15, 156–161. Separations **2021**, 8, 12 10 of 10.
[25]. Milbury, P.E. Analysis of complex mixtures of flavonoids and polyphenols by high-performance liquid chromatography electrochemical detection methods. Methods Enzymol.2001, 335, 15–26.
[26]. Hofseth LJ. Nitric oxide as a target of complementary and alternative medicines to prevent and treat inflammation and cancer. Cancer Lett. 2008; 268:10-30.
[27]. Tarling CA, Woods K, Zhang R et al. The search for novel human pancreatic. α-amylase inhibitors: high-throughput screening of terrestrial and marine natural product extracts. Chem Biol Chem. 2008; 9:433-438.
[28]. Shobana S, Sreerama YN, Malleshi N. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. Food Chem. 2009; 115:1268-1273.