Research Article

www.ijrap.net

ISOLATION AND CHARACTERIZATION OF QUERCETIN FROM AMARANTHACEAE FAMILY PLANTS

Goswami PK [1]*, Srivastava RS [2]

[1]PhD Research Scholar, Pacific University, Udaipur, India

[2]MET's Institute of Pharmacy, Bandra (W), Mumbai, Maharashtra, India

Received on: 07/05/17 Accepted on: 22/06/17

***Corresponding author**

E-mail: priyanka8408@gmail.com

DOI: 10.7897/2277-4343.084218

ABSTRACT

The two medicinal plants viz. *Nothosaerva brachiata* and *Gomphrena celosioides* belonging to amaranthaceae family were chosen to isolate flavonoid compounds since no scientific study reported so far. Quercetin was isolated from N. brachiata and G. celosioides. The soxhlet extraction was carried out for dried root powder of both the plants with petroleum ether, chloroform and methanol successively. The methanolic fraction so obtained was successively extracted with petroleum ether, diethyl ether and ethyl acetate. The ethyl acetate fraction was hydrolysed with 7% H2SO4, kept for hydrolysis for 7 hours to obtain quercetin. It was then extracted with ethyl acetate to obtain crude quercetin. The isolated compounds were subjected for characterization by TLC, HPTLC and HRLC-MS. The isolated compound GQ, N2Q were identified as "Quercetin". This is the first report of isolation of said phyto compounds from these plants.

Keywords: Amaranthaceae, *Nothosaerva brachiata*, *Gomphrena celosioides*, Quercetin

INTRODUCTION

Phyto chemistry is defined as the study of chemical composition of medicinal plants or phyto drugs. According to World Health Organization (WHO), phyto medicines are getting lots of popularity. Chemists started focusing on herbal supplements as bioactive phytochemicals due to discovery of opium and other pharmacologically active alkaloids[1]. Isolation of phyto constituents is the first step in the analysis of medicinal plants[2]. The characterization and study of the chemical structure of isolated phytoconstituents can be used as models for new synthetic compounds[3]. *Nothosaerva brachiata* is belonging to the family amaranthaceae and is categorized as pashanabheda in Ayurveda. It is distributed in tropical Africa and Asia. In India, it's distributed throughout. It is used for its astringent, antiseptic, diuretic, lithotrophic and pain killer properties. *Gomphrena celosioides* is also member of the family amaranthaceae. It is distributed in South America, Paraguay and India. The decoction of whole plant is given in fertility regulating, while roots and shoots together given for piles and roots chewed in cough[4-6]. Phyto compounds like 3-(4- hydroxyphenyl) methylpropenoate, Aurantiamides, Aurantiamide acetate and Ecdysterone were previously isolated from the plant *Gomphrena celosioides* while one methoxy derivative of kaempferol was identified in *Nothosaerva brachiata*[7-10].

The present study deals with the isolation and characterization of the flavonoid Quercetin from *N. brachiata* & *G. celosioides* and flavonoid Dihydromyricetin from *N. brachiata*. Flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating properties[11]. Flavonoids contributes towards biological activities like antibacterial, anticancer, anti-inflammatory, antiviral and hepatoprotective[12]. Quercetin is a plant pigment, abundantly occurs in many ethnic plants, especially onion and tea, therefore, a sufficient amount may be consumed daily. Quercetin does have ethnopharmacology property as an anticancer, antioxidant and neuroprotective. Phase-I clinical trials reported inhibition on tyrosine kinase by Quercetin which proposes it's use in tumours[13].

MATERIALS AND METHODS

Chemicals

Quercetin standard was procured from Yucca Enterprises (Mumbai). Methanol was obtained from HiMedia (India).

Plant material

Roots of *Nothosaerva brachiata* and *Gomphrena celosioides* were collected from vicinity of Tirunelveli of Tamil Nadu during November 2015. The plant material was authenticated by V. Chelladurai, Retired Research officer, Botany, CCRAS, Govt. of India, Tirunelveli, Tamil Nadu. The both roots were tray dried at 40˚C. The dried roots were powdered using a dry grinder to get coarse powder. The powder was kept in close-fitting container.

Extraction and isolation of the compounds

The successive soxhlet extraction method was used with powder: solvent ration as (1:10) and extraction period as 24 hours for each of the solvent. The first solvent used was Petroleum ether to de-fat the powder material. The marc was collected and dried and further extracted with chloroform and methanol respectively. The methanol extract fraction was collected and concentrated to get semisolid consistency. The obtained methanol fraction was further extracted with dissolution method with petroleum ether, diethyl ether and finally with ethyl acetate. The ethyl acetate extraction process was repeated three times to ensure complete extraction which was further used to isolation of quercetin.

Isolation of Quercetin

Acid hydrolysis of the ethyl acetate fraction was done by adding 7% H_2SO_4 for period of five hours. The hydrolysed fraction was

extracted with ethyl acetate three times using separating funnel. After separation, ethyl acetate part was concentrated. The concentrated fraction was recrystallized with ethanol to get pure Quercetin. Quercetin was subjected to characterization by TLC, HPTLC and HRLC-MS studies[14].

Characterization of Compounds
Thin Layer Chromatography (TLC)

The pre-coated alumina TLC plate (3.0x8.0 cm) were used for the study. The isolated and standard Quercetin was dissolved in ethanol and was applied 1 cm above the edge of the plate. This plate was developed in an air tight chromatography chamber containing about 8.5 ml of solvent mixture of ethyl acetate, toluene, formic acid (4:3.5:0.5). The developed plates were air dried and visualized under UV.

High Performance Thin Layer Chromatography (HPTLC)

The isolated and standard Quercetin was subjected to HPTLC studies. The HPTLC studies were carried out at Anchrom Enterprises (l) Pvt. Ltd., Mumbai, India. Optimized parameters for HPTLC studies are as follows.

Table 1: HPTLC instrument optimization

Parameters	Description
Instrument	*CAMAG* HPTLC
Stationary Phase	Silica gel 60 F254 HPTLC pre-coated plates
Mobile Phase	Ethyl acetate: Formic acid: Glacial acetic acid: Water (10: 0.5: 0.5: 1.3)
Derivatization solution	Anisaldehyde Sulphuric acid + Heating at 120° C for 5 minutes
Sample Applicator	*CAMAG* LINOMAT V
Band Width	8.0mm
Syringe	CAMAG Linomat Syringe (100µL capacity)
Volume of Mobile Phase	11.0ml
Development Mode	CAMAG Twin Trough Chamber.
Development Distance	70 mm.
Chamber Saturation Time	20 mins.
Densitometer	Scanner 4
Scanning Wavelength	254nm, 366nm, 430nm.
Software	Win CATS.(version 1.4.6)
Lamp	D2,Hg,W.
Measurements Mode	Absorbance & Florescence & visible
Photo Documentation	CAMAG TLC Visualizer

Table 2: HPTLC program procedure for Quercetin fingerprinting

Sr. No.	Track No.	Sample applied	Volume
1	1	GQ	10 µl
2	2	GQ	20 µl
3	3	NQ	10 µl
4	4	NQ	20 µl
5	5	Quercetin	1 µl
6	6	Quercetin	2 µl
7	7	Kaempferol	1 µl
8	8	Kaempferol	2 µl
9	9	N2Q	10 µl
10	10	N2Q	20 µl

All extracts, isolated quercetin and standard were applied in duplicate having 8 mm bandwidth on a pre-coated silica gel 60 F254 TLC plate, with Linomat V applicator using a Hamilton syringe. No pre-washing of the plates was done. Chamber saturation time was 10 minutes for all application. The TLC plates were kept for development for a migration distance of 70 mm for all extracts and standard. The developed plates were dried with blow dryer and scanned at wavelengths 254nm, 366 nm and 425nm and source of radiation were deuterium, mercury and tungsten lamps respectively. The Rf and peak area of the extracts were interpreted by using the software WinCATS. The developed plates were photo documented under 254 nm, 366 nm and visible light, using Camag visualizer. Similar procedures were followed after derivatization of TLC plates.

High Resolution Liquid Chromatography Mass Spectroscopy (HRLC-MS)

The isolated Quercetin from *N. brachiata* & *G. celosioides* and crystalized compound Dihydromyricetin from N. brachiata were isolated and characterized by HR-LCMS. The analysis was performed at SAIF Laboratory, IIT, Powai.

Instrument Details

Model- 6550 iFunnel QTOF LCMS/MS coupled with 1290 infinity UHPLC binary pump.
Make- Agilent technologies.
Column Details- Zorbax SB C18, 2.1 x 100mm, 1.8 micron
Mobile Phase-
Solvent A- 100% MilliQ water +0.1% Formic Acid
Solvent B- 100% Acetonitrile

Sample preparation

Small quantity of sample was taken and dissolved in methanol and then it was filtered through syringe filter using Dura pore GVWP 0.22 micron 13mm filter paper. And this filtrate was injected into the system.

RESULTS

Thin Layer Chromatography (TLC)

Short UV **Long UV** **White Light**

Figure 1: TLC of Isolated Quercetin

High Performance Thin Layer Chromatography (HPTLC)

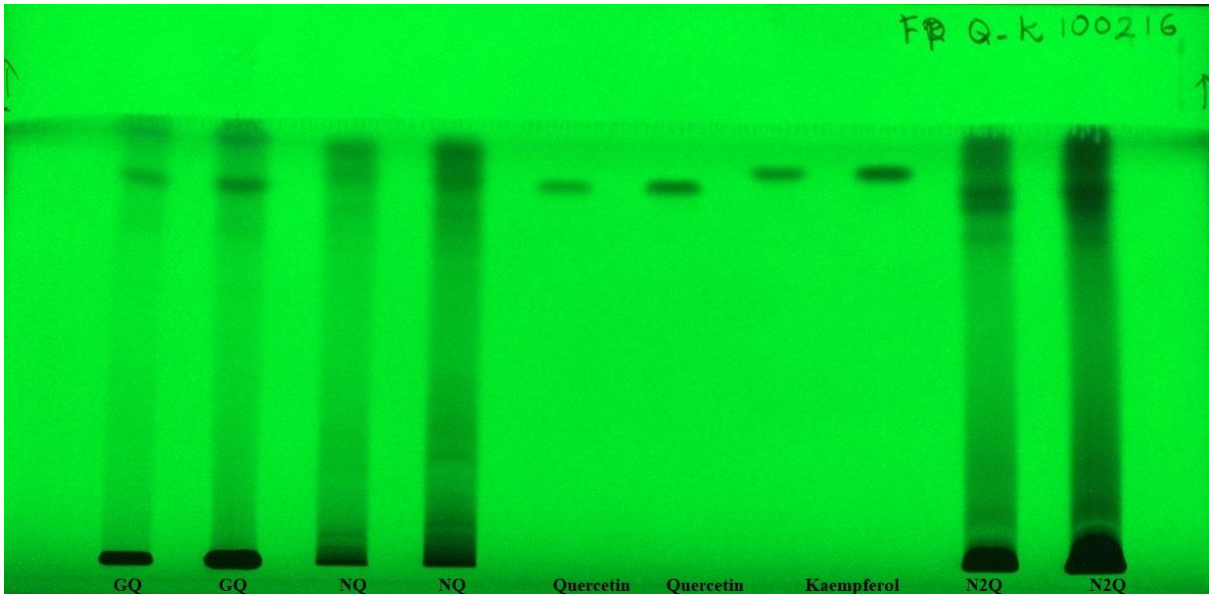


Figure 2: HPTLC fingerprinting of Isolated Quercetin at 254 nm

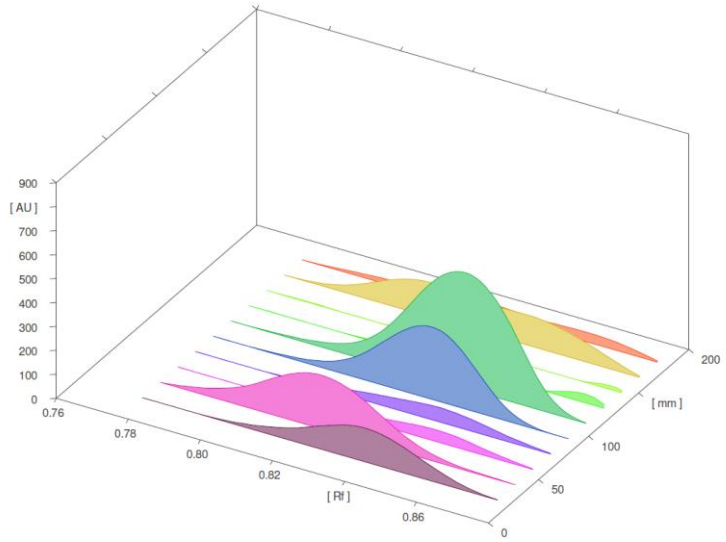
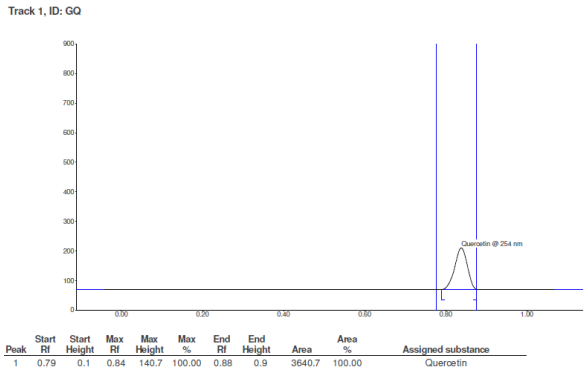
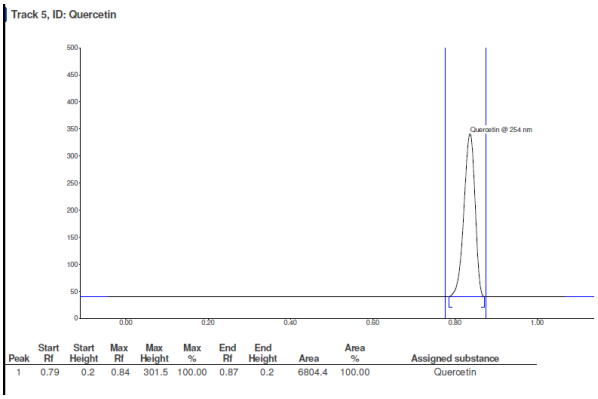


Figure 3: HPTLC fingerprinting Quercetin, all tracks at Sc4 wave length



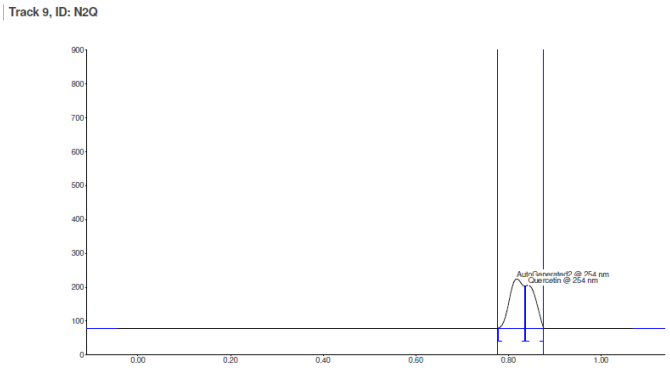
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.79	0.1	0.84	140.7	100.00	0.88	0.9	3640.7	100.00	Quercetin

Figure 4: HPTLC chromatogram & Rf value of GQ



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.79	0.2	0.84	301.5	100.00	0.87	0.2	6804.4	100.00	Quercetin

Figure 5: HPTLC chromatogram & Rf value of Standard Quercetin



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.78	0.2	0.82	145.3	53.56	0.84	123.9	3691.5	60.80	AutoGenerated2
2	0.84	124.2	0.84	126.0	46.44	0.88	5.5	2380.3	39.20	Quercetin

Figure 6: HPTLC chromatogram & Rf value of N2Q

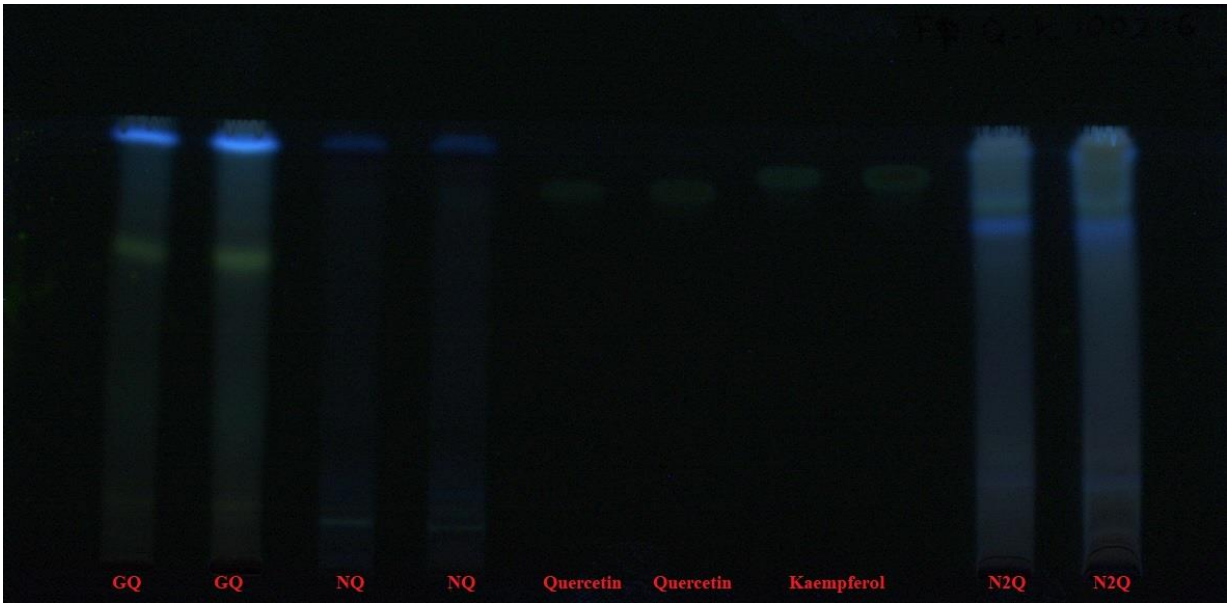


Figure 7: HPTLC fingerprinting of Isolated Quercetin at 366 nm

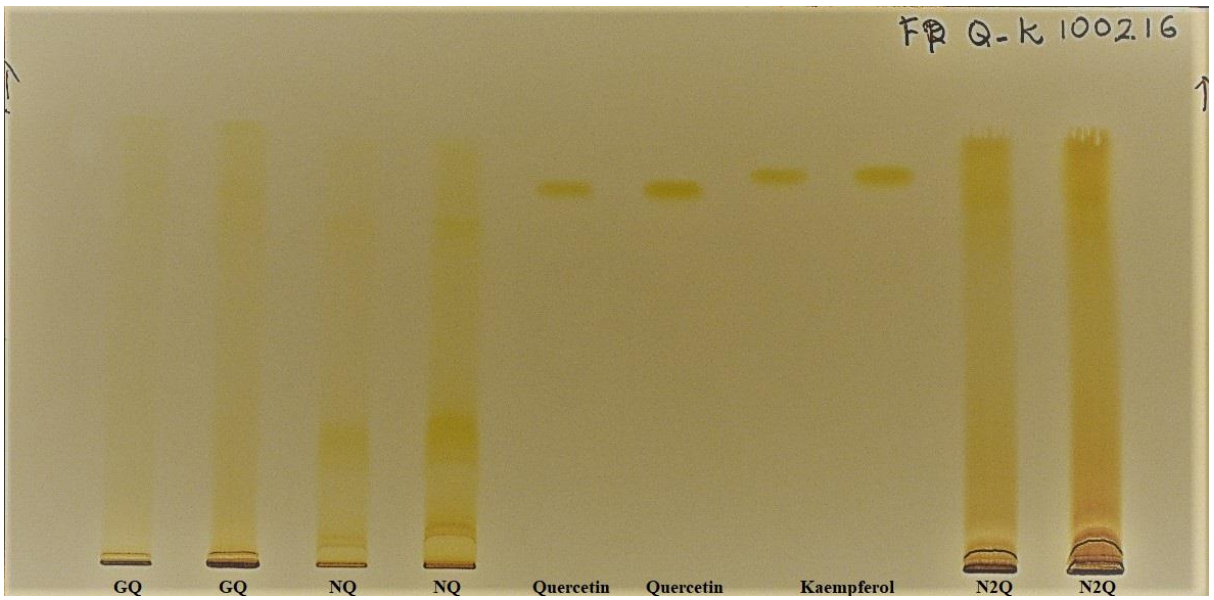
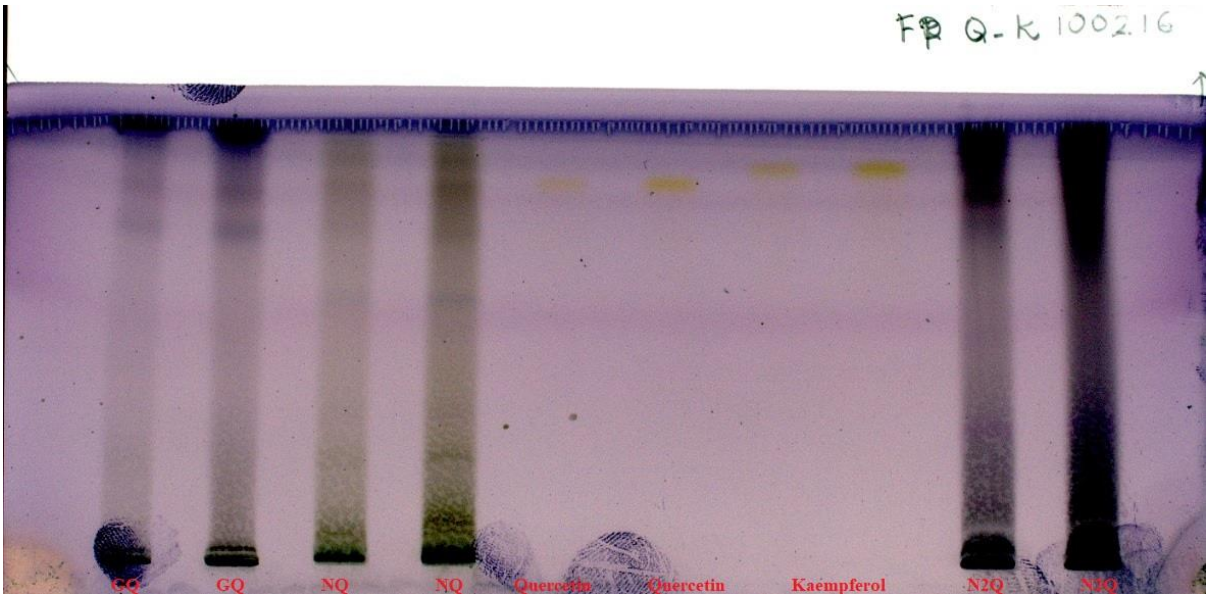


Figure 8: HPTLC fingerprinting of Isolated Quercetin in Visible light



Where, T = Track no.
T1, T2: GQ, T3, T4: NQ, T5, T6: Standard Quercetin, T7, T8: Kaempferol, T9, T10: N2Q

Figure 9: HPTLC fingerprinting of Isolated Quercetin at 366 nm after derivatization

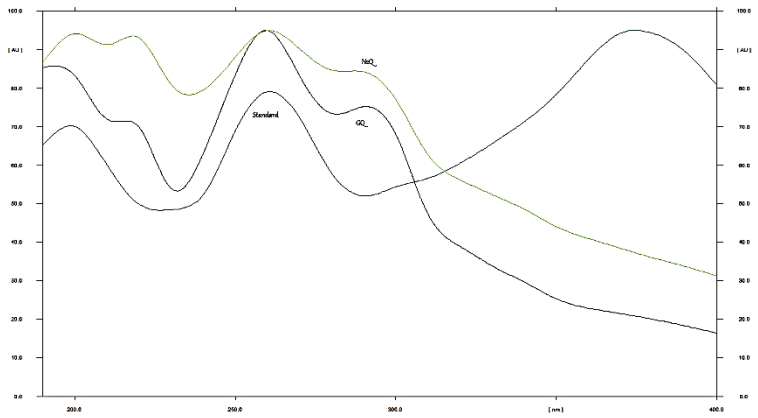
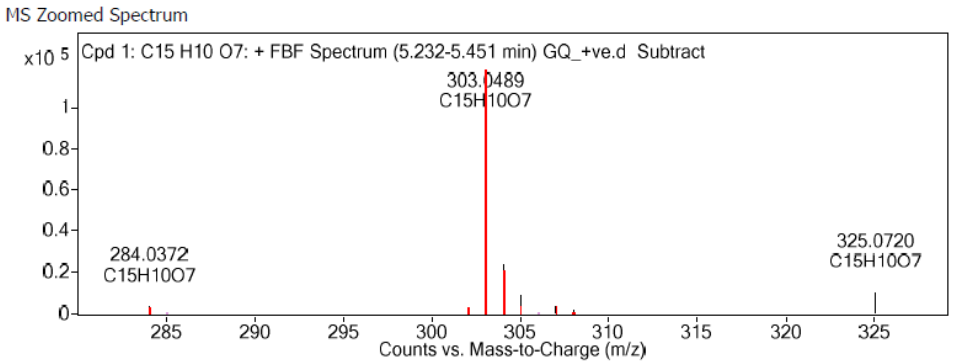


Figure 10: HPTLC fingerprinting of Isolated Quercetin spectrum pattern

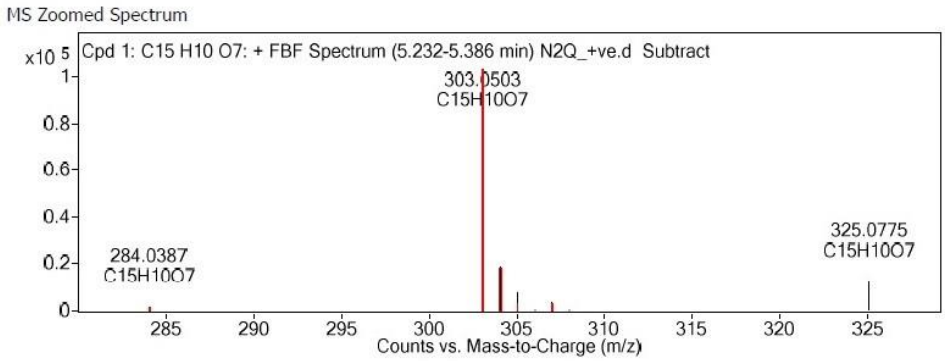
High Resolution Liquid Chromatography Mass Spectroscopy (HRLC-MS)



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
284.0372	1	2924.14	C15H10O7	M+[-H2O]
302.0604	1	2711.77	C15H10O7	M+
303.0489	1	118037.28	C15H10O7	(M+H)+
304.0529	1	23830.08	C15H10O7	(M+H)+
305.0551	1	8350.05	C15H10O7	(M+H)+
306.9946	1	3020.19	C15H10O7	(M+Na)+[-H2O]
308.0042	1	1110.44	C15H10O7	(M+Na)+[-H2O]
325.072	1	9734.5	C15H10O7	(M+Na)+

Figure 11: HR-LCMS Spectra: Compound 1 GQ



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
284.0387	1	1196.12	C15H10O7	M+[-H2O]
303.0503	1	103439.23	C15H10O7	(M+H)+
304.0542	1	18623.14	C15H10O7	(M+H)+
305.0565	1	7674.16	C15H10O7	(M+H)+
306.9969	1	3459.48	C15H10O7	(M+Na)+[-H2O]
325.0775	1	12078.71	C15H10O7	(M+Na)+

Figure 12: HR-LCMS Spectra: Compound 2 N2Q

DISCUSSION

TLC study revealed the matching of Rf value GQ and N2Q samples along with standard Quercetin. HPTLC program was run according to Table 1 & 2. HPTLC was run for isolated Quercetin sample GQ, NQ, N2Q along with standard Quercetin and Phytosterol. As shown in Figure 2,7,8,9 HPTLC plate was scanned under 254 nm, 366nm and visible light before and after derivatization. It showed the matching bands of GQ, N2Q with the standard Quercetin. The same max Rf value as 0.84 was obtained for GQ, N2Q and standard Quercetin as shown in the Figure 4-6. The spectrum pattern of GQ and N2Q seemed to be similar to standard Quercetin as shown in Figure 10. As shown in Figure 11, HRLCMS spectrum of GQ showed the peak at molecular mass 302.0439 and m/z 303.0489. The molecular formula for compound GQ was found to be C15H10O7 at retention time 5.232 minute. As shown in Figure 12, HRLCMS spectrum of N2Q showed peak at molecular mass 302.0463 and m/z 303.0503. The molecular formula for compound N2Q was found to be C15H10O7 at retention time 5.232 minute. So, isolated sample GQ and N2Q were confirmed as Quercetin by molecular formula obtained by HRLCMS.

CONCLUSION

Quercetin was isolated and confirmed by HPTLC and HRLCMS studies. This is the first report of isolation and characterization for the said compounds from the selected plants.

REFERENCES

1. Singh A. Herbalism, Phytochemistry and Ethnopharmacology. New Delhi: CRC Press; 2011. p. 33.
2. Mandal SC et al. Essentials of Botanical Extraction: Principles and Applications. Academic Press; 2015.
3. Raaman N. Phytochemical techniques. New India Publishing, 2016. p.2.
4. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New Delhi: Springer Science & Business Media; 2008. p. 440.
5. Umberto Quattrocchi. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology (5 Volume Set). New Delhi: CRC Press; 2016. p. 1872, 2642.
6. Dagmar Wujastyk. Modern and Global Ayurveda: Pluralism and Paradigms. New York: Sunny Press; 2013. p. 145.
7. Dosumu OO, Idowu PA, Onocha PA, Ekundayo O. Isolation of 3-(4-Hydroxyphenyl) Methylpropenoate and bioavailability evaluation of *Gomphrena celosioides* extracts. EXCLI J 2010; 9: 173-180.
8. Dosumu OO, Onocha P, Ekundayo O and Ali M. Isolation of Aurantiamides from *Gomphrena celosioides* C. Mart. Iran J Pharm Res 2014; 13(1): 143-147.
9. Banerji A, Chintalwar GJ, Joshi NK, Chadha MS. Isolation of ecdysterone from Indian plants. Phytochem 1971; 10(9): 2225-2226.
10. Mammen D, Daniel M, Sane RT. Identification of Pharmacognostical and Phytochemical Biomarkers to distinguish between *Aerva lanata* Juss ex Schultes and its substitute, *Nothosaerva brachiata* (L.). W. & A. Int J Pharm Res 2012; 4(3): 116-119.
11. Heim KE, Tagliaferro AR, Bobiliya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The J Nutri Biochem 2002; 13(10): 572-584.
12. Kumar S, Pandey AK. Chemistry and Biological activities of Flavonoids: An overview. The Scientific World Journal 2013; Article ID 162750, 16 pages.
13. Maalik A, Khan FA, Mumtaz A, Mehmood A et al. Pharmacological application of quercetin and its derivatives: A short review. Trop J Pharm Res 2014; 13(9): 1561-1566.
14. Sanghavi N, Srivastava R, Malode Y. Isolation and identification of the flavonoid "Quercetin" from *Tridax procumbens* Linn. Int J Pharm Sci Res 2014; 5(4): 1454-1459.

Cite this article as:

Goswami PK and Srivastava RS. Isolation and characterization of quercetin from Amaranthaceae family plants. Int. J. Res. Ayurveda Pharm. 2017;8(4):70-76 http://dx.doi.org/ 10.7897/2277-4343.084218

Source of support: Nil, Conflict of interest: None Declared