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DETECTION AND ESTIMATION OF FREE RADICAL SCAVENGERS QUERCETIN, RUTIN, GALLIC ACID AND CAFFEIC ACID IN SAFFRON BY HPTLC TECHNIQUE

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ABSTRACT

Saffron is one of the most widely used spice in all over the world for its antioxidant property. The present study mainly aimed to detect the presence of flavonoids Quercetin, Rutin, Gallic acid and Caffeic acid in three marketed products of Saffron by HPTLC. The HPTLC method was performed using HPTLC aluminium sheets precoated with Silica Gel 60 GF_{254} as stationary phase and Ethyl acetate: Methanol: Water: Acetic acid (6.6: 2.3: 1.1: 0.7) as the mobile phase. The developed chromatogram was scanned at 254nm using Camag Scanner III. The Rf value of the standard Quercetin, Rutin, Gallic acid and Caffeic acid were found to be 0.84, 0.18, 0.76 and 0.85 The analysis outcomes confirm 0.12% of Quercetin in MEGSSC-2, 0.52% of Quercetin rationally more in MEMBSK-3 and 0.12% of Caffeic acid in MEKMS-1 among the three marketed products of Saffron tested. The developed HPTLC method can be laboring for the routine investigations of flavonoids and phenolic acids. Among the three marketed product Kesaritatva was found to have more number of flavonoids.

KEYWORDS: HPTLC, Saffron, Flavonoids, Antioxidant properties.

INTRODUCTION

Saffron (*Crocus sativus*) that belongs to the family Iridaceae is the most expensive spice in the world, which is a tiny part of a fragrant flower. The vivid red-coloured three filament 'stigma' of this flower is dried to make the spice saffron.[1] Saffron is composed of four major active ingredients which include crocin, crocetin, picrocrocin and saffranal. Among more

than 150 chemicals of saffron, the most biologically active components are two carotenoids including crocin and crocetin.[2] These compounds are responsible for many medicinal properties, where the important include strong antioxidant and radical scavenger properties against a variety of pro-inflammatory cytokines.[3] The other constituents present in saffron are Kaempferol Glycoside, Quercetin, Isorhametin Glycoside as Mono, Di, Tri, Delphiidin 3-O-Glucoside, Delphinidin 3 (Anthocyanins) Lutein –Diesters (Xanthophyll) Reservertrol, Caffeic Acid, Vanillic Acid, 4 - Hydroxy Benzoic Acid (Phenolics) Oleuropein (Polyphenolic Compound), Gallic Acid (Phenolic Acid) and Trace Amounts of Vitamin B_1 and B_2.[4] Crocin is responsible for colour of the saffron, picrocrocin for bitter taste and saffranal for aroma.[5] The lambda max of the coloured compounds include picrocrocin – 250nm, saffranal – 310nm, crocin – 440nm.[6][7] In cosmetics, saffron is used as Anti-UV Agent, treatment of Redness of Dark Spots, Anti-Aging Effect and Diseases of the Skin, Perfumery, Natural Pigments. From ancient times saffron contains many pharmacological activity such as anti-depressant, Treating Sexual Dysfunction, Antioxidant, anti-carcinogenic, Antispasmodic and Digestive Tonic, Anti-Inflammatory and Analgesic Effect Healing of Second-Degree Burns, Effect on Blood Glucose and Insulin Resistance, Cholesterol Levels and Eyes.[8] In foods, saffron is used as a spice, yellow food colouring, and as a flavouring agent. High-performance thin layer chromatography (HPTLC) is one of the sophisticated instrumental techniques. In the recent years it is used extensively for fingerprinting of medicinal plants, products and for screening lichen substances, quantification of active ingredients and herbal drugs, phytochemical and biomedical analysis and for detection ofadulterants in the formulation.[9]

MATERIALS AND METHODS

Collection of saffron for HPTLC screening

Three marketed products of saffron were procured from the market. The products were saffron purchased from Kulumanali, Saffron purchased from Grocery shop (Coimbatore) and market brand saffron Kesari tatva.

Equipment

A Camag HPTLC system comprising of Linomat 5 applicator and Camag TLC scanner and single pan balance of Shimadzu model was used for weighing the samples.

Chemicals and solvents

Rutin, Quercetin and Gallic acid were procured from Sigma chemical Company Inc., USA. Caffeic acid was procured from Yucca Enterprises, Mumbai. Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. HPTLC was carried out using Merck aluminium sheet coated with Silica gel GF_{254} (0.2mm).

Preparation of standards and extracts

1gm of each marketed formulations saffron which was purchased was taken and sonicated with 10ml of methanol and named as Methanolic extract of Kulumanali saffron (MEKMS-1), Methanolic extract of Saffron purchased from Grocery shop, Coimbatore (MEGSSC-2), Methanolic Extract of Market brand Kesari tatva (MEMBSK-3). The solution was filtered using Whatmann No. 1 filter paper and the filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml.

Application of sample

The sample solutions were spotted in the form of bands of width 6 mm with a Hamilton 500µl syringe on aluminium sheet pre coated plate 60 F_{254} (10 cm × 10 cm with 0.2 mm thickness, E. Merck) using a CamagLinomat V applicator. The slit dimension was kept 6 × 0.45mm. 10µl of each sample and 5 µl of standard solutions were applied on to the plate.

Development

The chromatogram was developed in Camag glass twin -through chamber (10-10 cm) previously saturated with the optimized mobile phase Ethyl acetate: Methanol: Water: Acetic acid (6.6: 2.3: 1.1: 0.7)[10][11] for 10 minutes with conditions of temperature 25°C, relative humidity 40%. The migration distance was 8cm. TLC plates were air dried with air dryer.

Detection

Densitometry scanning was performed using Camag TLC Scanner -III at 254 nm and 366 nm operated by a Wincats software (version 1:4.4). The plate was scanned at UV 254 nm using Camag TLC Scanner-3. Rf value of each compound which were separated on plate and data of peak area of each band was recorded.

RESULTS

The following different solvent compositions were tried for monitor the elution of flavonoids in the purchased saffron.[12][13] Ethyl acetate: glacial acetic acid formic acid: water

(100:3:3:28), Ethyl Acetate: Methanol: Water Toluene (100:13:10:13), Chloroform: ethyl acetate:methanol(6:4:0.3), Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4), Toluene: ethyl acetate (93:7) and chloroform: methanol (97:3). There was good elution of the formulation in the mobile phase of Ethyl acetate: Methanol: Water: Acetic acid (6.6: 2.3: 1.1: 0.7). The optimized chamber saturation time for mobile phase was 10 min at room temperature (25 ± 1°C). The densitometry analysis was performed at 254 nm in reflectance mode. The results were tabulated by considering each Rf value for one ingredients of formulation in it. Therefore, the obtained Rf value were compared with Rf value of the standard and well-known free radical scavengers rutin, quercetin, gallic acid and caffeic acid. For identifying these free radical scavengers Quercetin, Rutin, Gallic acid and Caffeic acid, we used UV light at 254 nm. The Rf values of these free radical scavengers Quercetin, Rutin, Gallic acid And Caffeic acid were found to be 0.84, 0.18, 0.76 and 0.85 respectively (Table 1). The percentage of markers were calculated andillustrated in bar chart in "Fig. 3".

DISCUSSION

The HPTLC of Saffron purchased from Kulumanali found to contain 0.1174% of caffeic acid, Saffron purchased from grocery shop found to contain 0.1192% of Quercetin and Kesaritatva found to contain 0.5187% of Quercetin, 0.0228% of Rutin, 0.1226% of Gallic acid (Table 1). The mobile phase used was Ethyl acetate: Methanol: Water: Acetic acid (6.6: 2.3: 1.1: 0.7). Flavonoids and phenolic acids which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicines.[14] The anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge freeradicals. Phenol and phenolic compound such as flavonoids have shown free-radical scavenging activity and protection against oxidative stress. These secondary metabolite in plant possess potent antioxidant activity in terms of its radical scavenging activity. The antioxidant activity of phenols is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers. Flavonoids ability of scavenging hydroxyl radicals and lipid peroxy radicals is important for prevention of diseases associated with oxidative damage of membranes, proteins and DNA. Gallic acid could autoxidate to produce significant levels of H_2O_2 and O_2 in malignant cells. These increased ROS levels could cause mitochondrial potential loss, cytochrome c release, and activation of caspases 3, 8, and 9. Therefore, Gallic acid could effectively kill cancer cells through apoptosis.[15] Caffeic acid is also an antioxidant that can reduce the oxidative stress that is formed in the body due to the effect of free radicals. The protective effect of caffeic acid as an antioxidant on α-tocopherol in low-

density lipoproteins (LDL) has previously been shown. Caffeic acid exhibits pro-oxidative properties in cancer cells that are associated with oxidative DNA (deoxyribonucleic acid) damage and, followed by its subsequent signaling, the induction of death in apoptotic cancer cells.[16]

Table 1: Estimation of free radical scavengers Quercetin, Rutin, Gallic acid and Caffeic acid in Saffron.

Track number	Name/ amount of sample in µl	Rf values of compounds in extract/ standard	Rf value of the marker in extracts	Name of marker in extracts	Area of standard marker in sample	Amount of marker present in µl/ 10µl of extract / 5µl of standards	% of marker in extracts
T -1	(MEKMS-1) Saffron purchased from Kulumanali /10µl	0.21,0.25,0.34,0.39 ,0.51,0.60,0.66,0.73, **0.86**	0.86	Caffeic acid	10202.4	1.1742	0.1174%
T -2	(MEGSSC-2) Saffron purchased from Grocery shop /10µl	0.66,0.73,0.79,**0.83**,0.88	0.83	Quercetin	1395.9	1.1922	0.1192%
T -3	(MEMBSK-3) Market brand Kesari tatva /10µl	0.05,0.12,**0.19**,0.25, 0.32,0.42,0.50,0.57, 0.64,**0.77**,**0.84**,0.90	0.84	Quercetin	6073.9	5.1879	0.5187%
			0.19	Rutin	338.6	0.2288	0.0228%
			0.77	Gallic acid	1015	1.2264	0.1226%
T -4	Quercetin /5µl	0.19,0.57,0.64,0.76,**0.84**	0.84	Quercetin	11707.7	5	100%
T -5	Rutin /5µl	**0.18**,0.25,0.40,0.46,0.47,0.82	0.18	Rutin	14797.7	5	100%
T -6	Gallic acid/5µl	0.12,0.26,0.46,**0.76**,0.84	0.76	Gallic acid	8275.7	5	100%
T -7	Caffeic acid/5µl	0.06,0.11,0.33,0.53,**0.85**,0.89	0.85	Caffeic acid	8689.8	5	100%

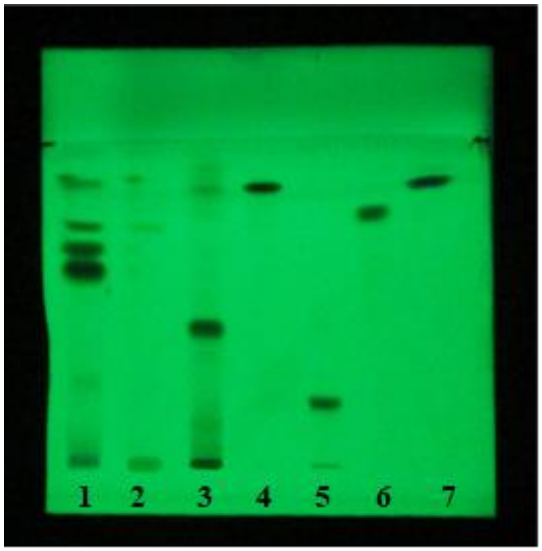

Fig 1: TLC Profile of Saffron extract and four Standards after development in Mobile phase.1. MEKMS-1, 2. MEGSSC-2, 3. MEMBSK-3, 4. Standard- Quercetin, 5. Standard- Rutin 6. Standard- Gallic acid, 7. Standard- Caffeic acid.

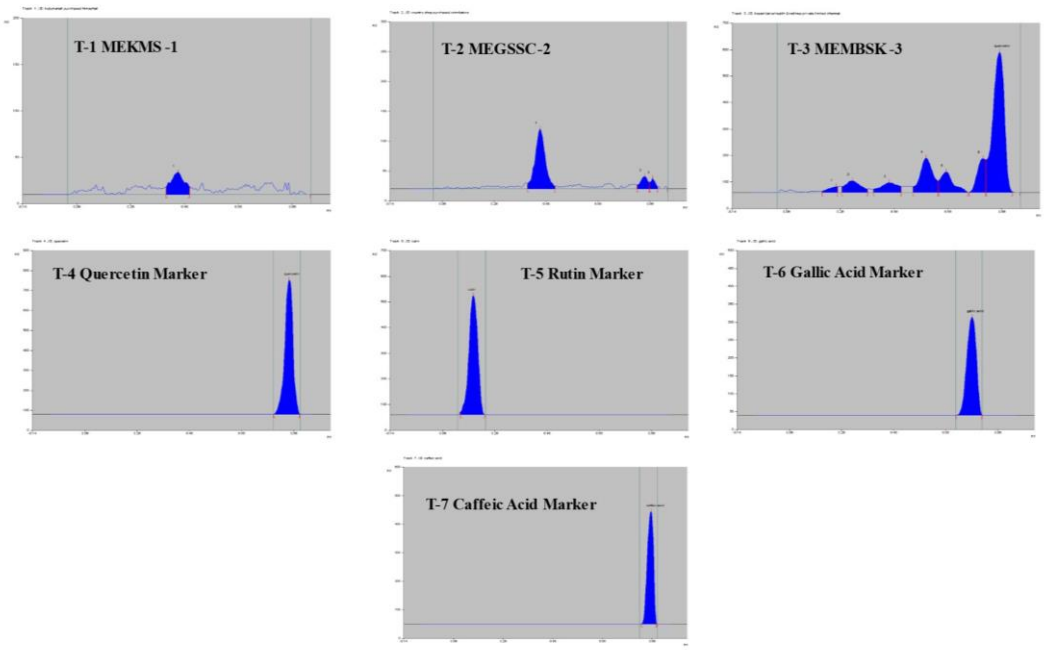

Fig 2: Chromatogram of Saffron extract and four Standards after development in Mobile phase.

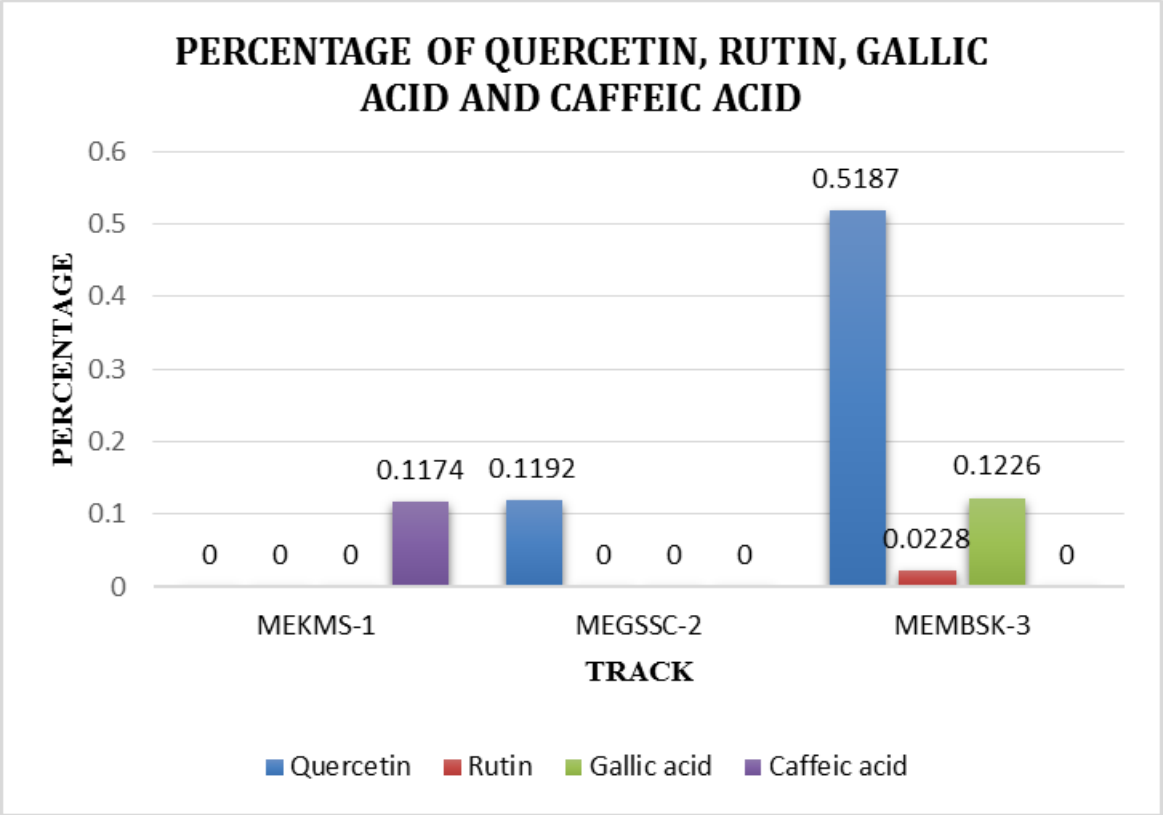

Fig 3: Percentage of free radical scavengers Quercetin, Rutin, Gallic acid and Caffeic acid in Marketed formulations of Saffron.

CONCLUSION

It can be concluded that Quercetin, rutin, Gallic acid and caffeic acid were simultaneously detected in three marketed products of saffron. Presence of caffeic acid in Saffron purchased from Kulumanali, Quercetin in Saffron purchased from grocery shop and Quercetin, rutin, Gallic acid in Kesaritatva were detected. The marker compound selected for detection was well established for antioxidant activity. The above mentioned mechanism of antioxidant markers and validating antioxidant markers in the three methanolic extracts of formulations, we also conclude that according to the percentage of marker in formulations were confirmed for its therapeutic activity.

REFERENCES

1. Loriana Cardone, Saffron (*Crocus sativus* L.) the king of spices: An overview, Scientia Horticulturae, 15 October 2020; 272.
2. Azam Bolhassani, Bioactive Components of Saffron and Their Pharmacological Properties, Studies in Natural Products Chemistry, 2018; 58: 289-311.

3. Maryam Mashmoul, Saffron: A Natural Potent Antioxidant as a Promising Anti-Obesity Drug, Oct 29 2013; 2(4): 293–308.
4. Rayees Ahmad Bashi, Bioactive constituents of saffron plants: extraction, encapsulation and their food and pharmaceutical application, Applied Food Research, June 2022; 2.
5. Ahmad Reza Gohari, An overview on saffron, phytochemicals and medicinal properties, Pharmacognosy review, Jan-Jun 2013.
6. Marlene Suchareau, Improved quantification method of crocin in saffron extract using HPLC DAD after qualification by HPLC-DAD-MS, Food Chemistry, 15 Nov, 2021; 362.
7. Maryam Kabiri, Comparartive study of saffron constituents through HPLC and HPTLC methods, Oct 2017; 84.
8. Ibtissam Mzabri, Traditional and Modern Uses of Saffron (*Crocus Sativus*), *Cosmetics*, 2019; *6*(4): 63: 25 October 2019.
9. R N Kulkarni, R B Pandhare, V K Deshmukh, High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery, Journal Of Pharmaceutical and Biological Sciences, May 2021.
10. AboliGirme, Quantitative Determination and Characterization of Kashmir Saffron (*Crocus sativus* L.)-Based Botanical Supplement Using Single-Laboratory Validation Study by HPLC-PDA with LC–MS/MS and HPTLC Investigations, *CS Omega*, 2021; 6(36): 23460–23474.
11. A. Amirvaresi, Combining multivariate image analysis with high-performance thin-layer chromatography for development of a reliable tool for saffron authentication and adulteration detection, Journal of Chromatography A, 2020; 1628: 461461.
12. Samarghandian, Tahereh Farkhondeh, Tayebeh Zeinali, *Crocus sativus* L. (saffron) extract antioxidant potential and use in agingscience direct, 2020; 193-200.
13. Rajasekaran.A, Arivukkarasu.R. and Archana.D.HPTLC Method for estimation of gallic acid and Rutin in Haritaki –An Ayurvedic FormulationInternational Journal of Pharm Tech Research, 2011; 3(2): 986-999.
14. Arivukkarasu R., Bhuvanesh WDayanidhi, Deepaa V. C., Deepiga G., Dinesh D and Karunanithi M Detection And Estimation Of Rutin, Quercetin And Gallic Acid In Marketed Green Tea Formulations By Hptlc Technique World Journal of Pharmaceutical Research, 2023; 12(3): 690-697.
15. Maryam Mashmoul, Saffron: A Natural Potent Antioxidant as a Promising Anti-Obesity Drug, Antioxidants (Basel). 2013 Dec; 2(4): 293–308. Oct 29 2013.

16. Rachit Shukla, Prashant Kumar Singh, Savita Upadhyay, A Comprehensive Review of High-Performance Thin Layer Chromatography (HPTLC), International Journal Of Pharmacy And Pharmaceutical Research, April 2023; 27(1).
17. Pietta P. In Flavonoids in Health and Disease; Rice-Evans, CA; Packer, L., Eds.
18. Russell LH, Mazzio E, Badisa RB, et al. Autoxidation of gallic acid induces ROSdependent death in human prostate cancer LNCaP cells. Anticancer Res, 2012; 32(5): 1595-1602.
19. Cizmarova B, Hubkova B, et al. Caffeic acid: a brief overview of its presence, metabolism, and bioactivity, Bioactive Compounds in Health and Disease, 2020; 3(4): 74-81.