International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

VALIDATED HPTLC METHOD FOR SIMULTANEOUS DETERMINATION OF QUERCETIN AND KAEMPFEROL IN METHANOLIC EXTRACT OF *TRIDAX PROCUMBENS*

Saraswathy Nagendran[1]*,
Vahbiz Patel[1], Nidhi Sharma[1], Jay Savai[1], Meena Chintamaneni[1]

[1]Shobhaben Pratabhai Patel School of Pharmacy & Technology Management,NMIMS, Mumbai, India.

ABSTRACT

A rapid and sensitive High-performance thin layer chromatography (HPTLC) method was developed and validated for the simultaneous determination of Quercetin and Kaempferol in the methanolic extract of Tridax procumbens plant. Tridax procumbens plant was collected from Dhule District, Shirpur, India and comparative study was performed for presence of standards - Quercetin and Kaempferol using HPTLC technique. HPTLC of flavonoids was performed on F_{254} TLC plates with Toluene: Ethyl acetate: Methanol: Formic acid (6:4:0.5:0.2) as mobile phase. Densitometric determination of flavonoids was performed at λ = 254 nm in reflectance/absorbance mode. The linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range of 100-1000ng with r^2= 0.997 for Quercetin and r^2= 0.906 for Kaempferol with respect to peak area. The recovery for Quercetin was 98.60% and for Kaempferol was 90.08% indicating accuracy of method. HPTLC method analyzed; represented an excellent technique for simultaneous determination of Quercetin and Kaempferol in the methanolic extract of Tridax procumbens plant, with good sensitivity, selectivity, precision and accuracy.

KEYWORDS : *Tridax Procumbens, Quercetin, Kaempferol, Flavonoids, HPTLC, Accuracy, Recovery, Sensitivity.*

Correspondence to Author

SARASWATHY NAGENDRAN

Shobhaben Pratabhai Patel School of Pharmacy & Technology Management,NMIMS, Mumbai, India.

Email: saraswathy76@yahoo.co.in

INTRODUCTION

Tridax procumbens is a species of flowering plant in the daisy family (Asteraceae) [1]; common names include coat buttons, ghamra and Kambarmodi (Figure 1).

Figure 1 - Image of *Tridax procumbens* plant

It is best known as a widespread weed and pest plant[1]. The plant bears daisy like yellow -centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. This weed can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climates[1,2,3].

Tridax procumbens is known for several potential therapeutic activities like antiviral, antimicrobial, wound healing activity[18,19], insecticidal[20] and anti-inflammatory activity [2].

Reports from tribal areas in India, states that the leaf juice can be used to cure fresh wounds, to stop bleeding and as a hair tonic. Despite these known benefits, it is still listed in the United States as a Noxious Weed and regulated under the Federal Noxious Weed Act. [8,9,10]

Quercetin and Kaempferol are constituents present in the extract of *Tridax procumbens* [1,2] and can be used as markers. So far, no report on simultaneous determination and Validation of Quercetin and Kaempferol in *Tridax procumbens by* HPTLC method is found in literature. In this study, HPTLC was used for the simultaneous determination of Quercetin and Kaempferol. The results indicate that this method is fast, sensitive and suitable for quantitative assessment of both Quercetin and Kaempferol simultaneously.

Quercetin is a plant-derived flavonoid found in fruits, vegetables, leaves and grains. It may also be used as an ingredient in supplements, beverages or foods. Quercetin has been shown to be used for treatment of cancer[12,13,14,15,16,17], fibromyalgia, as an antiviral and as an anti-inflammatory [2,4].

Kaempferol a type of natural flavonoid, that has been isolated from tea[11] broccoli, *Delphinium*, Witch-hazel, grapefruit, cabbage, kale, beans, endive, leek, tomato, strawberries, grapes, brussels sprouts, apples and other plant sources. [1]

Quercetin and Kaempferol are two constituents which are present in *Tridax procumbens* plant. There is so far no report present on simultaneous determination of Quercetin and Kaempferol in *Tridax procumbens* using HPTLC. Hence the objective of this study is to determine presence of Quercetin and Kaempferol using HPTLC.

MATERIALS AND METHODS

Equipments

The chromatographic system is HPTLC- DESAGA Applicator AS 30, 230 V, with HPTLC Densitometer CD 60, 230V, with Windows® software ProQuant®.

Reagents and materials

Reagents and chemicals

Methanol, toluene, ethyl acetate and formic acid were of LR grade, purchased from Thermo fisher Scientific India Pvt. Ltd.

Quercetin and Kaempferol standards were purchased from Sigma Aldrich (USA).

Plant material

Tridax procumbens were obtained from Dhule District, Shirpur and identified and

authenticated by the Department of Botany, St.Xavier's College, Mumbai.

Preparation of Standard solutions

Standard stock solutions of Quercetin and Kaempferol were prepared in Methanol, at concentration of 100µg/ml.

Preparation of Sample solution

The fresh plant of *Tridax procumbens* was powdered and extraction was carried out by cold maceration and continuous hot percolation using methanol as a solvent (Figure 2). Methanolic extract was prepared and stored in vacuum desiccator.

30mg of dried methanolic extract was dissolved in 1ml methanol (30mg/ml) and filtered through 0.45µ Membrane filter (Millipore) and used for all the validation parameters[5].

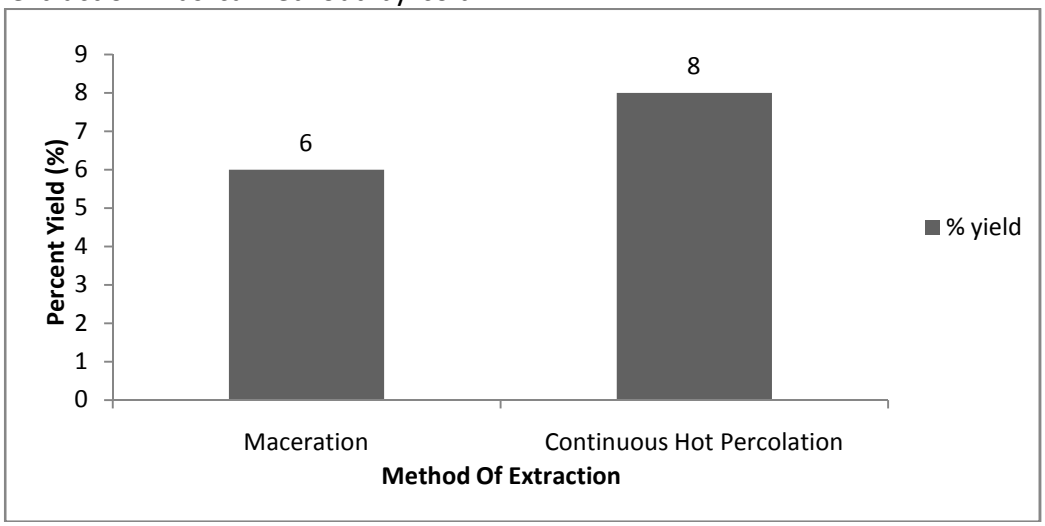

Figure 2 - Graph showing the percentage of the methanolic extract of *Tridax procumbens* plant

Chromatographic conditions

Chromatographic analysis was carried out by separating the Quercetin and Kaempferol using HPTLC technique. The mobile phase used was Toluene - Ethyl acetate - Methanol - Formic acid (6:4:0.5:0.2).

Quercetin and Kaempferol were quantified by densitometric determination, performed at λ= 254 nm in reflectance/absorbance mode. All chromatographic operations were carried out at ambient temperature.

RESULTS AND DISCUSSION

TLC analysis was carried out by spotting two standards viz Quercetin and Kaempferol as well as the methanolic extract of *Tridax procumbens.* The methanolic extract of *Tridax procumbens* showed presence of Quercetin (R_f=0.47) and Kaempferol (R_f=0.56) (Figure 3).

Figure 3 - TLC plate derivitized using Anisaldehyde reagent. Band 1: Methanolic extract of *Tridax procumbens*; Band 2: Rutin Standard ; Band 3: Quercetin Standard; Band 4: Kaempferol Standard

The standard spectra of Quercetin and Kaempferol were compared to the spectra of Quercetin and Kaempferol in the methanolic extract of *Tridax procumbens* plant. The method was found to be specific for Quercetin and Kaempferol. (Figure 4-7).

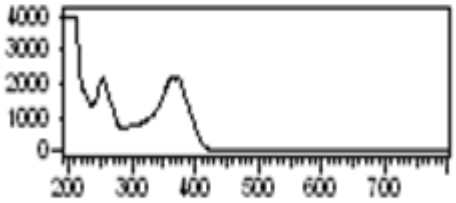

Figure 4 - Spectra Of Quercetin Standard

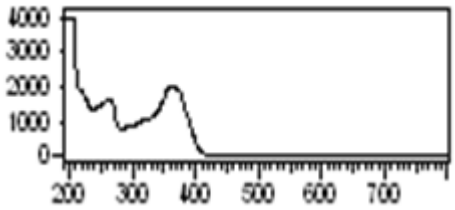

Figure 5 - Spectra of Kaempferol Standard

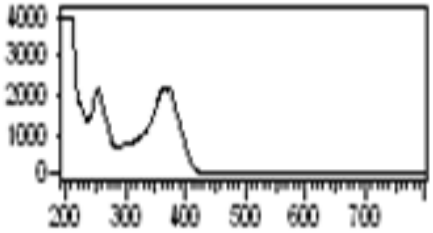

Figure 6 - Spectra of Quercetin in *Tridax procumbens* extract.

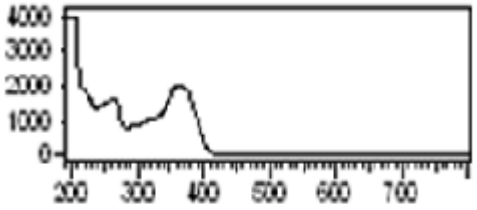

Figure 7 - Spectra of Kaempferol in *Tridax procumbens* extract.

A series of standard mixture solutions of Quercetin and Kaempferol were used to determine the linear relationship between the standard mixture concentration and peak areas (Figure 8).

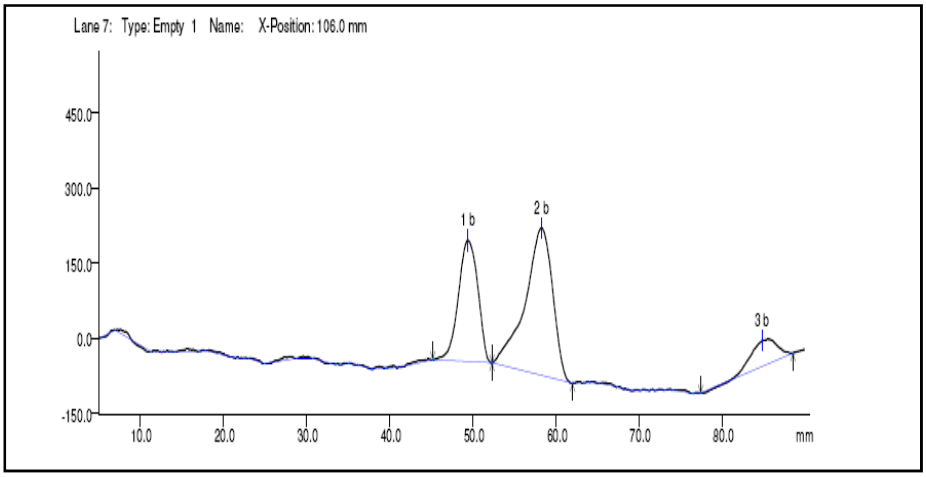

Figure 8 - Chromatogram representing Quercetin Peak- 1b, Kaempferol peak- 2b.

The linear regression analysis data for the calibration plots showed a good linear relationship in the concentration range of 100 - 1000ng with r^2= 0.997 for Quercetin and r^2= 0.906 for Kaempferol with respect to peak area and the detection limit (Limit of detection – Limit of Quantitation) for Quercetin was observed to be 70ng to 212ng and for Kaempferol was 120ng to 364ng. (Table 1).

TABLE 1: VALIDATION DATA FROM CALIBRATION CURVES OF THE QUERCETIN AND KAEMPFEROL IN THE EXTRACT OF *TRIDAX PROCUMBENS*

Compound	**Regression equation**	**Correlation coefficient (R^2)**	**Linear Range (ng)**	**Detection Limit (ng)**
Quercetin	Y = 13.62x – 6.544	0.997	100 - 1000	70 to 212
Kaempferol	Y= 12.87x + 296.1	0.906	100 – 1000	120 to 364
X concentration (ng), Y peak area.				

The intra-day and inter-day precisions expressed as the relative standard deviation (R.S.D.) for peak area was determined for both standards Quercetin and Kaempferol by repeated analysis (*n* = 6) (Figure 9).

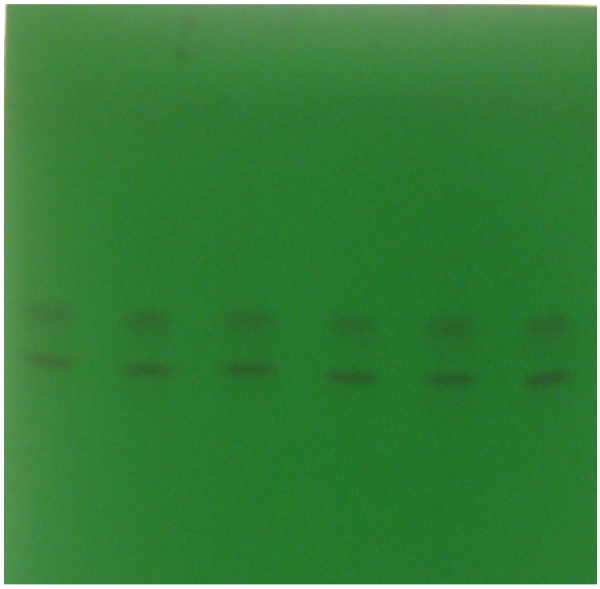

Figure 9 - Precision plate developed by HPTLC observed at 254nm. Bands (1-6): Quercetin and Kaempferol Standard

Intra-day relative standard deviation of Quercetin was between 3.02 and 10% and of Kaempferol was between 2.46 and 15.21% while Inter-day relative standard deviation of Quercetin was between 1.80 and 11.88% and of Kaempferol was between 4.70 and 9.34% (Table 2).

TABLE 2**:** INTRADAY AND INTERDAY PRECISION

Compound	Concentration (ng)	Intra- day R.S.D. For peak area (%)	Inter- day R.S.D. For peak area (%)
Quercetin	400	10.00	11.88
	600	6.88	6.65
	800	3.02	1.80
Kaempferol	400	15.21	9.34
	600	7.08	6.20
	800	2.46	4.70

The results showed that intra-day and inter-day relative standard deviations for peak area are quite low and precise. The recovery experiments of the Quercetin and Kaempferol were performed by spiking methanol extract to the standards and the procedure was repeated for three times. The recoveries for the two flavonoids were between 90.08 and 98.60% (Table 3).

TABLE 3: RECOVERIES OF QUERCETIN AND KAEMPFEROL IN THE METAHNOLIC EXTRACT OF PLANT OF TRIDAX PROCUMBENS

Compound	Amount added (ng)	Recovery (%)
Quercetin	1000	98.60
Kaempferol	1000	90.08

The HPTLC method analyzed here represented an excellent technique for simultaneous determination of Quercetin and Kaempferol in the extract of *Tridax procumbens plant*, with good sensitivity, precision and accuracy. Furthermore, this method can be used as quality control of

Quercetin and Kaempferol in the extract of *Tridax procumbens plant.*

REFERENCES

1. Russell GE, Welman WG, Reitief E, Immelman KL, Germishuizen G, Pienaar BJ, Wyk MV and Nicholas A, List of species of southern African plants, Mem. Bot. Surv. S. Africa. 1987, (2), 1–152.
2. Funk VA, Berry PE, Alexander S, Hollowell TH, Kelloff CL, Checklist of the Plants of the Guiana Shield (Venezuela: Amazonas, Bolivar, Delta Amacuro; Guyana, Surinam, French Guiana), Contr. U.S. Natl. Herb, 2007, 55: 1–584.
3. Galinato MI, Moody K and Piggin CM, Upland Rice weeds of South and Southeast Asia, International rice research institute. 1999, 25.
4. Edited by Tandon N and Sharma M, Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research. 2010, 8(13).
5. Handbook of African medicinal plants, Iwu MM. Boca Raton, FL, CRC Press, 1993.
6. Standardization of Botanicals: Testing and Extraction Methods of Medicinal Herbs, Volume 1, 2011.
7. Suseela L, Sarsvathy A, Brindha P, Pharmacognostic studies on Tridax procumbens L.(Asteraceae), Journal of Phytological Research, 2002, 15: 141–147.
8. Bessey CE, Principles and Practices of Plant Taxnomy, 1915, 127-130.
9. Graves WJ, Weed Initiated Pest Risk Assessment for Tridax procumbens L. 2000.
10. Park JS, Rho HS, Kim DH, Chang IS, Enzymatic Preparation of Kaempferol from Green Tea Seed and Its Antioxidant Activity, 2006; 54 (8): 2951–2956.
11. Verschoyle RD, Steward WP, Gescher AJ, Putative cancer chemopreventive agents of dietary origin-how safe are they, Nutrition and Cancer 59 (2), 2007, 152–62.
12. Rietjens IM, Boersma MG, Woude H, Jeurissen SM, Schutte ME and Alink GM, Flavonoids and alkenylbenzenes: mechanisms of mutagenic action and carcinogenic risk, PubMed. 2005, 574 (1): 124–38.
13. Neuhouser ML, Dietary flavonoids and cancer risk: evidence from human population studies 2004, 1–7.
14. Murakami A, Ashida H, Terao J, Multitargeted cancer prevention by quercetin, 2008, 315–25.
15. Nöthlings U et al, Flavonols and pancreatic cancer risk, American Journal of Epidemiology 2007, 166 (8):924–931.
16. Paliwal S, Sundaram J, Mitragotri S, Induction of cancer-specific cytotoxicity towards human prostate and skin cells using quercetin and ultrasound, British Journal of Cancer 2005, 92(3):499–502.
17. Udupa AL, Kulkarni DR, and Udupa SL, Effect of Tridax Procumbens Extracts on Wound Healing, Pharmaceutical Biology 1995, 37-40.
18. Udupa SL, Udupa AL and Kulkarni DR, Influence of Tridax procumbens on Lysyl Oxidase Activity and Wound Healing, Planta Med. 1991,57(4):325-7.
19. Pathak AK, Dixit VK, Insecticidal and insect repellent activity of essential oils of Tridax procumbens and Cyathocline lyrata, Fitoterapia,1988, 211-214 .

*****