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HPTLC Fingerprinting and Qualitative Identification of Phenolic Compounds in the Methanolic Extract of Padina tetrastromatica (Hauck)

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Abstract

Padina tetrastromatica (Hauck), a brown marine macroalga belonging to the order Dictyotales, is known to contain a variety of biologically active secondary metabolites with potential pharmaceutical significance. The present study was undertaken to characterize the phytochemical profile of the methanolic extract of *P. tetrastromatica* using High-Performance Thin-Layer Chromatography (HPTLC). Fresh seaweed samples were collected from the coastal region of Mandapam, Ramanathapuram District, Tamil Nadu, India, and authenticated by a qualified marine botanist. The collected material was shade-dried, powdered, and extracted with methanol by maceration for three days. HPTLC analysis was performed on silica gel 60 F254 precoated aluminium plates using toluene acetate acid (3:5:1.6:0.4, v/v/v/v) as the mobile phase. Densitometric scanning was carried out at 254 nm using a CAMAG TLC Scanner III. The chromatographic profile of the extract was evaluated against authentic reference standards, including quercetin, rutin, gallic acid, caffeic acid, and ellagic acid. The methanolic extract produced several well-resolved chromatographic bands, indicating the presence of diverse phytoconstituents. Comparison with the reference standards showed chromatographic similarity with quercetin (Rf ≈ 0.83), rutin (Rf ≈ 0.18), and gallic acid (Rf ≈ 0.76), whereas additional bands suggested the presence of other phenolic and flavonoid compounds. Overall, the HPTLC fingerprint established in this study provides a reproducible qualitative profile of *P. tetrastromatica* extract and may serve as a useful tool for its authentication and quality assessment. The detection of phenolic constituents also highlights the potential of this brown seaweed as a source of bioactive compounds for further pharmacological investigation.

INTRODUCTION

Marine macroalgae are considered as valuable sources of structurally diverse bioactive compounds with significant applications in the pharmaceutical, nutraceutical and cosmetic industries. Brown algae (Phaeophyceae) are particularly rich in phenolic compounds, flavonoids, terpenoids, carotenoids, sterols, sulfated polysaccharides, and other secondary metabolites possessing antioxidant, anti-inflammatory, antimicrobial, anticancer and wound-healing activities.

Padina tetrastromatica (Hauck), a marine brown alga belonging to the order Dictyotales is widely distributed in the tropical coastal areas of India. The therapeutic efficacy of herbal and marine algal extracts is mainly dependent on their phytochemical composition. Standardization of extracts is therefore a necessary condition for quality, reproducibility and efficacy. High-performance thin layer chromatography (HPTLC) is a simple, rapid, sensitive and reproducible chromatographic technique widely used for the qualitative fingerprinting and authentication of herbal drugs. This technique helps to detect multiple phytoconstituents simultaneously and compare with authentic reference standards.

In present investigation, methanolic extract of Padina tetrastromatica prepared by maceration was subjected to HPTLC fingerprint analysis using reference compounds like quercetin, rutin, gallic acid, caffeic acid and ellagic acid. The study was conducted to develop a characteristic chromatographic fingerprint to be used as quality control marker for future phytochemical and pharmacological investigations. (Manivannan et al. 2008; Dong et al. 2025).

MATERIALS AND METHODS

Collection and Authentication of Seaweed

The brown seaweed Padina tetrastromatica was collected from R.K. Algae Project Centre, 12/172 Meenavar Colony, Mandapam, Ramanathapuram District, Tamil Nadu, India (GPS coordinates: 09°12.370' N, 079°08.411' E). It was identified by the marine botanist Mr. M. Rajendra Kumar. The seaweed was collected and washed thoroughly with sea water and then distilled water to remove adhering impurities and epiphytes. It was then dried in the shade, powdered with the help of a mechanical grinder and stored in an airtight container until further analysis (Fig. 1).

Preparation of Methanolic Extract

The cleaned samples of seaweed were shade dried at room temperature to a constant weight. The dried material was powdered in a mechanical grinder to a coarse powder. The extraction was performed in methanol for three days (72 hours) with occasional stirring to facilitate the efficient extraction of phytoconstituents by the maceration method for about 100 g of the powdered seaweed. The extract was filtered through Whatman No. 1 filter paper and the solvent was concentrated under reduced pressure by rotary evaporator. The concentrated extract was stored in sealed containers at 4°C until further analysis.

HPTLC Analysis

HPTLC fingerprinting was performed on a CAMAG HPTLC system (Muttenz, Switzerland) equipped with a Linomat 5 sample applicator and TLC Scanner III controlled by winCATS software.

Methanolic extract and reference standards (quercetin, rutin, gallic acid, caffeic acid, ellagic acid) were prepared in methanol. Samples were applied as 6 mm bands on pre-coated silica gel 60 F254 aluminium TLC plates using a CAMAG Linomat 5 applicator. The different volumes of application of seaweed extract (2, 5 and 10 µL) were analyzed along with the standards.

Chromatographic development was performed in CAMAG horizontal developing chamber. The mobile phase was toluene: ethyl acetate: formic acid: methanol (3:5:1.6:0.4, v/v/v/v). The solvent front was allowed to migrate up to 80 mm and the plates were dried in an oven at 60°C for 5 min. Densitometric scanning was performed at 254nm with CAMAG TLC Scanner III, chromatograms were evaluated with the winCATS software. The chromatographic profile of the extract was compared with authentic standards based on their Rf values and peak characteristics to identify the major phytochemical constituents. (Vijayan and Mathew 2017; Yende et al. 2020) (Garde et al. 2026).

RESULTS AND DISCUSSION

HPTLC fingerprint analysis of methanolic extract of Padina tetrastromatica showed characteristic chromatographic profile with several well resolved peaks which confirmed the presence of complex mixture

of phytochemicals. The constituents could be efficiently separated by the optimized mobile phase toluene: ethyl acetate: formic acid: methanol (3:5:1.6:0.4).

The extract showed several chromatographic bands at different volumes of application (2, 5 and 10 µL) suggesting good reproducibility of the fingerprint profile. (Fig. 2) The methanolic extract showed the presence of a major phytoconstituent as evidenced by a consistent prominent peak at Rf 0.84–0.85. Apart from this dominant peak several low-intensity peaks were detected all over the chromatogram, indicating the chemical diversity typical for brown marine algae.

Figure 3 Three-dimensional HPTLC densitogram of the methanolic extract of *Padina tetrastromatica* and reference standards scanned at 254 nm showing the chromatographic fingerprint and distribution of phytochemical constituents.

Comparison with authentic reference standards revealed chromatographic correspondence with rutin (Rf ≈ 0.18) and gallic acid (Rf ≈ 0.76), thus indicating the probable presence of these phenolic compounds in the extract (Table 1, 2, 3,4). An unidentified major peak (Fig. 4) with Rf 0.84, which was close to the Rf of the quercetin reference standard (Rf 0.83) was observed. The standards of caffeic acid and ellagic acid were successfully resolved, but their presence in the extract could not be conclusively proved under the present analytical conditions.

Quercetin is one of the most powerful naturally occurring flavonoids, showing impressive antioxidant, anti-inflammatory, antifibrotic and wound healing properties. Rutin is a flavonoid with potent antioxidant, anti-inflammatory, collagen modulating and wound healing properties and gallic acid exhibits potent free radical scavenging, antimicrobial and anti-fibrotic activities. These compounds are possibly responsible for the previously reported pharmacological properties of Padina tetrastromatica especially its antioxidant and tissue regenerative potential.

The chromatogram shows several unidentified peaks indicating the presence of other phytochemicals such as flavonoids, phlorotannins, phenolic acids, pigments and other secondary metabolites characteristic of Dictyotales seaweeds. The broad spectrum of biological activities shown by the species can be attributed to the synergic action of these compounds.

The CAMAG software reported that quantitative estimation could not be performed because the standards were analyzed using single-level calibration, resulting in calibration errors. Therefore, the present investigation serves as a qualitative HPTLC fingerprint rather than a quantitative assay. Nevertheless, the developed chromatographic profile can be used as a reliable reference for authentication, quality control, and standardization of *P. tetrastromatica* extracts.

CONCLUSION

The present study successfully established an HPTLC fingerprint profile of the methanolic extract of *Padina tetrastromatica*, a brown marine alga belonging to the order Dictyotales collected from the Mandapam coastal region of Ramanathapuram District, Tamil Nadu. The chromatographic analysis revealed a chemically diverse phytochemical composition with probable identification of quercetin, rutin and gallic acid

based on their corresponding Rf values. The presence of these phenolic constituents supports the antioxidant and therapeutic potential of the seaweed. Although quantitative estimation was not possible because of single-level calibration, the HPTLC fingerprint generated in this study provides a reproducible qualitative marker for authentication, quality control, and standardization of *P. tetrastromatica*. Further studies involving validated quantitative HPTLC methods, LC–MS/MS characterization, and bioactivity-guided isolation are recommended to identify the major active constituents responsible for its pharmacological properties.

Declarations

I declare that the manuscript titled " HPTLC Fingerprinting and Qualitative Identification of Phenolic Compounds in the Methanolic Extract of *Padina tetrastromatica (Hauck)*" is an original work by the author. All data, results, and interpretations in this study are original and come from scientific investigation. We have appropriately acknowledged all sources of information and cited all references. The manuscript does not include any plagiarized material, made-up data, or falsified results. The author(s) take full responsibility for the accuracy, integrity, and authenticity of the work presented.

Ethics approval-

Not Applicable

Competing interests-

The authors declare no competing interests

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Availability of data and material-

All data created or examined during this study are included in this published article and its supplementary information files.

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Tables

Table 1: HPTLC sample application details of *Padina tetrastromatica* extract and reference standards

S.NO	Sample ID	Application volume	Sample
1	T1	2.0 µL	Seaweed extract
2	T2	5.0 µL	Seaweed extract
3	T3	10.0 µL	Seaweed extract
4	T4	5.0 µL	Quercetin standard
5	T5	5.0 µL	Rutin standard
6	T6	5.0 µL	Gallic acid standard
7	T7	2.0 µL	Caffeic acid standard
8	T8	5.0 µL	Ellagic acid standard

Table 2: HPTLC peak profile of T1 -2 µL methanolic extract of *Padina tetrastromatica*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	1.5	-0.06	13.9	5.17	-0.05	0.3	88.1	1.26	Unknown*
2	-0.04	0.3	-0.01	74.8	27.70	0.03	27.0	1834.3	26.32	Unknown*
3	0.05	22.8	0.06	29.7	11.00	0.10	21.7	791.2	11.35	Unknown*
4	0.13	17.55	0.15	21.4	7.92	0.19	3.9	592.9	8.51	Unknown*
5	0.22	1.2	0.25	24.0	8.87	0.27	13.7	390.9	5.61	Unknown*
6	0.79	1.2	0.85	106.2	39.35	0.89	21.0	3271.6	46.94	Unknown*

Table 3: HPTLC peak profile of T2 -5 µL methanolic extract of *Padina tetrastromatica*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	13.6	-0.06	13.8	2.57	-0.05	0.1	72.6	0.33	Unknown*
2	-0.04	0.6	-0.02	42.4	7.89	-0.01	17.5	553.8	2.55	Unknown*
3	0.00	20.8	0.03	81.5	15.15	0.08	40.1	2987.4	13.75	Unknown*
4	0.08	40.1	0.09	40.9	7.60	0.17	16.5	2117.1	9.75	Unknown*
5	**0.17**	**16.7**	**0.18**	**29.0**	**5.39**	**0.20**	**4.6**	**320.1**	**1.47**	**Rutin**
6	0.23	10.9	0.26	61.2	11.38	0.34	14.2	2078.6	9.57	Unknown*
7	0.51	1.9	0.54	10.7	1.99	0.57	1.4	204.0	0.94	Unknown*
8	0.71	2.2	0.85	258.3	48.03	0.90	4.4	13388.5	61.64	Unknown*

Table 4: HPTLC peak profile o fT3 -10 µL methanolic extract of *Padina tetrastromatica*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.06	5.4	-0.04	97.3	8.47	-0.03	71.5	1150.3	3.00	Unknown*
2	-0.03	72.3	-0.01	124.5	10.84	-0.00	64.7	1731.6	4.51	Unknown*
3	-0.00	69.3	0.00	85.1	7.41	0.01	71.4	696.6	1.82	Unknown*
4	0.01	72.4	0.03	134.0	11.67	0.08	58.0	4541.9	11.84	Unknown*
5	0.14	47.7	0.17	76.5	6.66	0.19	42.8	2109.3	5.50	Unknown*
6	0.22	47.4	0.25	100.5	8.75	0.28	49.5	3021.7	7.87	Unknown*
7	0.30	46.8	0.31	48.6	4.23	0.39	14.3	2286.9	5.96	Unknown*
8	0.41	10.0	0.43	12.2	1.06	0.47	1.7	268.8	0.70	Unknown*
9	0.49	5.1	0.52	33.7	2.93	0.56	0.3	954.6	2.49	Unknown*
10	**0.66**	**2.2**	**0.76**	**120.8**	**10.52**	**0.77**	**118.6**	**3452.1**	**9.00**	**Gallic acid**
11	**0.77**	**118.7**	**0.84**	**315.1**	**27.44**	**0.90**	**5.8**	**18161.2**	**47.33**	**Quercetin***

Figures

Figure 1

Brown seaweed *Padina tetrastromatica*

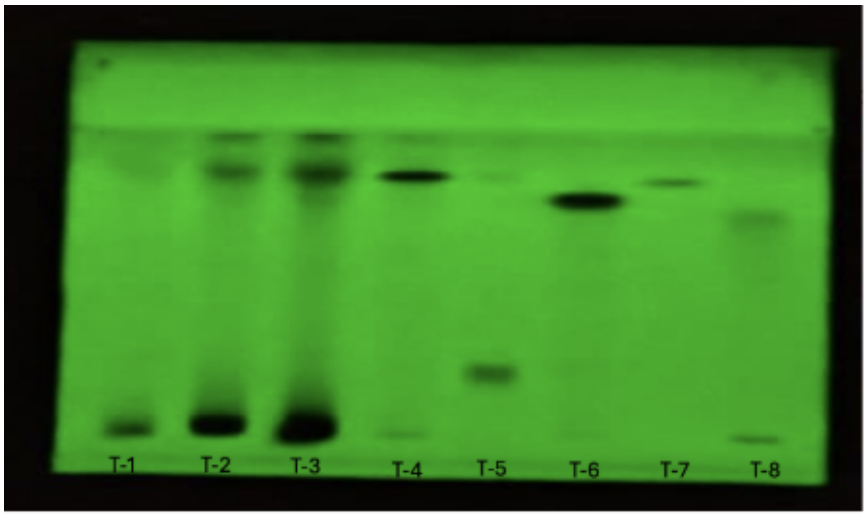

Figure 2

HPTLC plate image.

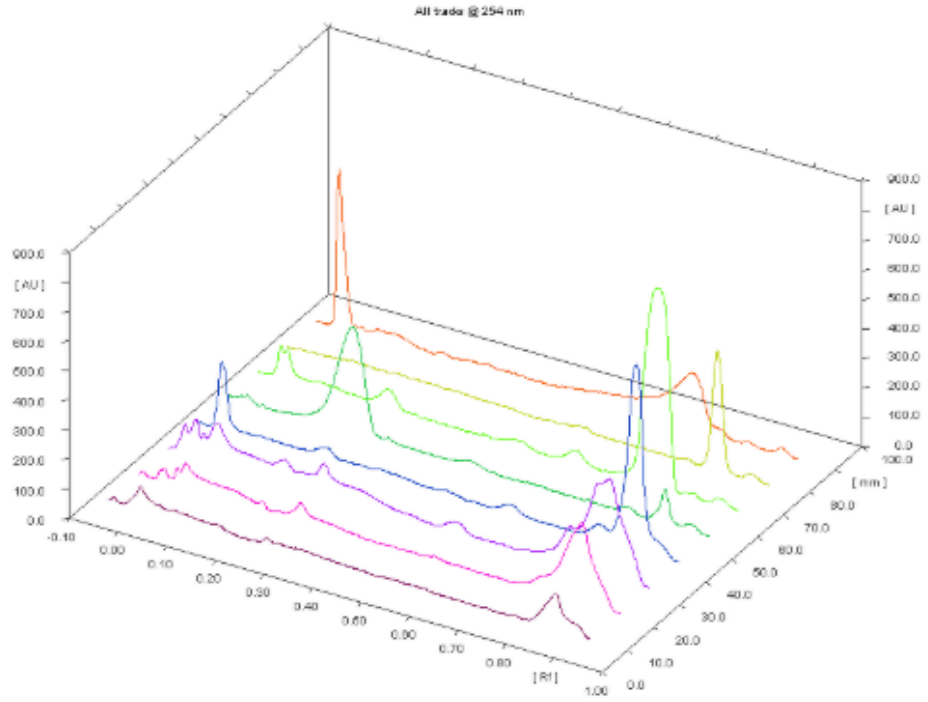

Figure 3

Three-dimensional HPTLC densitogram of the methanolic extract of *Padina tetrastromatica* and reference standards scanned at 254 nm showing the chromatographic fingerprint and distribution of phytochemical constituents.

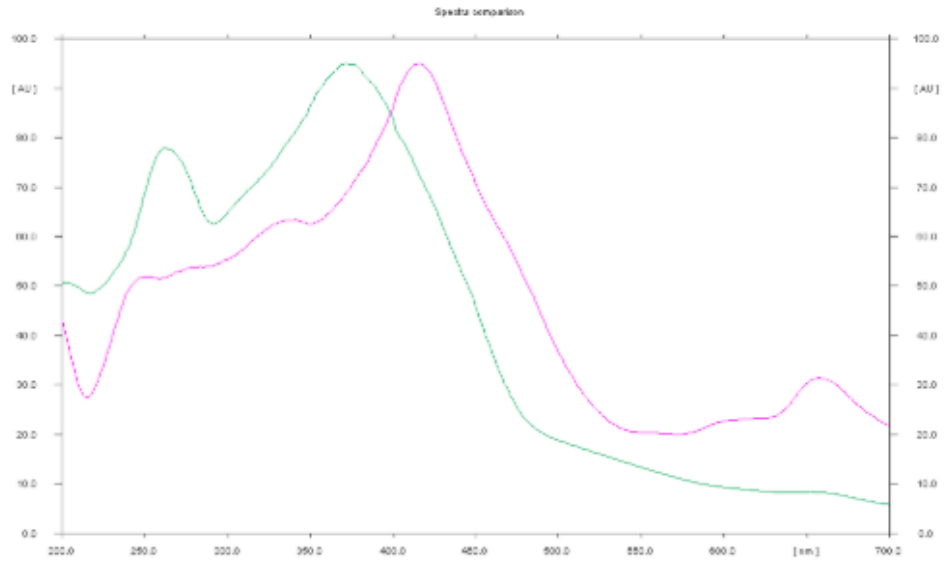

Figure 4

Overlay spectrum of quercetin

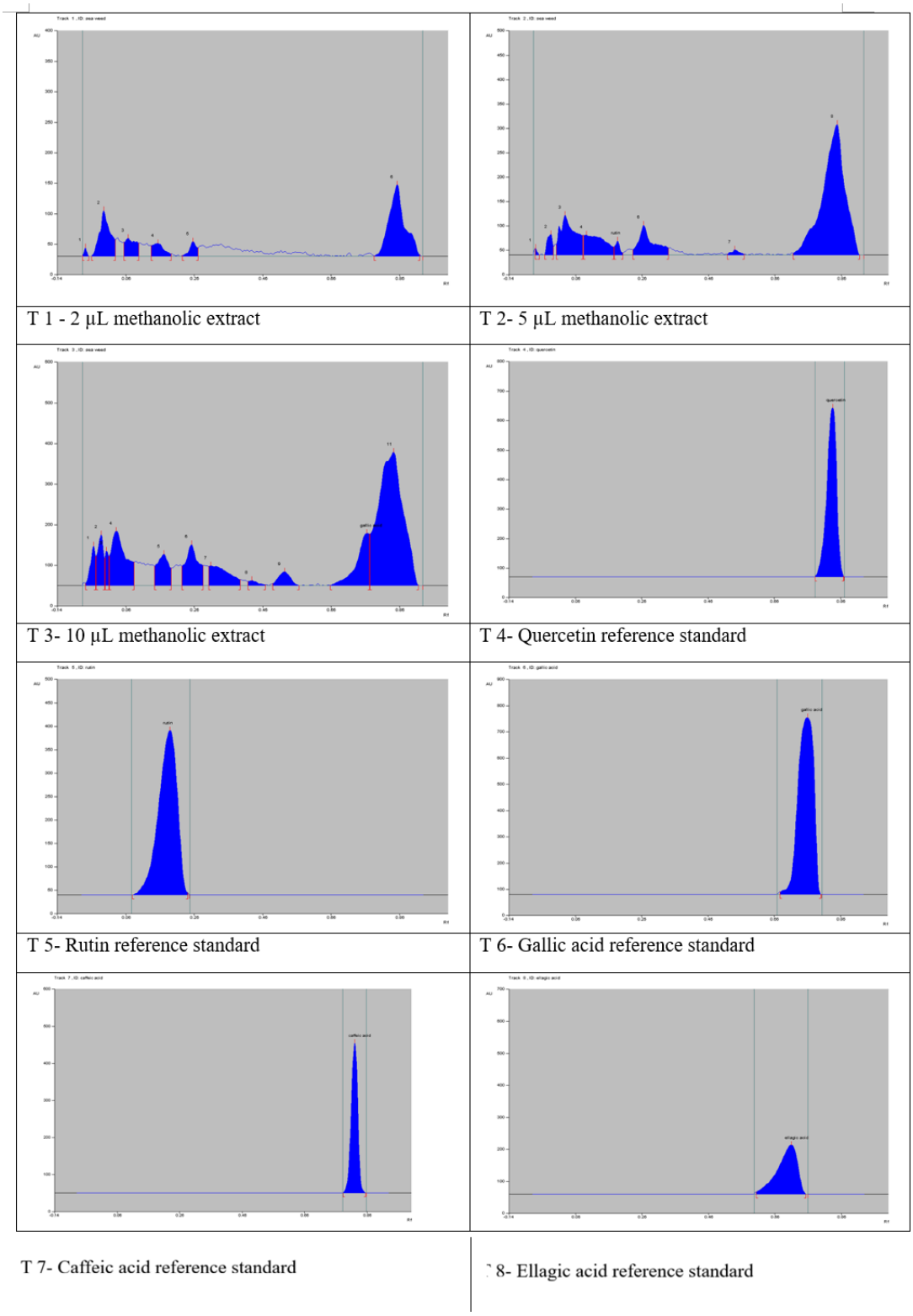

Figure 5

HPTLC densitometric chromatogram of T1, T2, T3, T4, T5, T6, T7, T8 scanned at 254 nm.