World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

HPTLC finger printing profile of flower extract of *Punica granatum*

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Received: 21-05-2017 / Revised Accepted: 21-07-2017 / Published: 01-08-2017

ABSTRACT

The main objective of the study is to investigate the fingerprint profile of *Punic granatum* using high performance thin layer chromatography (HPTLC) technique. Camag HPTLC system equipped with TLC Linomat V applicator, Camag TLC scanner III and winCATS software were used. Hydroalcoholic extracts of the flower of *Punic granatum* was developed in suitable mobile phase using standard procedures and the plates were kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV light at 254 nm and 366 nm. The fluorescent band (under 254 nm and 366 nm) at R_f 0.56 in mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1) was found and the marker compound Quercetin was quantified.

Keywords: *Punica granatum*, HPTLC Fingerprint, Phytoconstituent, Quercetin

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How to Cite this Article: R. Nalini, R. Anuradha and S. Kamalaveni. HPTLC finger printing profile of flower extract of *Punica granatum.* World J Pharm Sci 2017; 5(8): 136-142.

INTRODUCTION

The world Health Organization (WHO) defined health as "a complete state of physical, mental and social wellbeing and not merely the absence of disease of infirmity. Medicinal plants play an important role in the human health care and are one of the oldest forms of health care known to mankind. Isolation of biologically active compounds from plant has always been of great interest for scientists, as these drugs have come from botanical sources [1]. Modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening; plants remain a major source of medicinal compounds. Synthetic drugs causes side effects as a result people are more favorable to use natural compounds obtained from plants [2].

HPTLC is a chromatography technique used to separates mixtures. Modern High Performance TLC (HPTLC) is an efficient instrumental analysis and optimized quantitative HPTLC using a densitometry evaluation can produce results analogous to those obtained with gas chromatography (GC) and HPLC (Wagner *et al* ., 2001) [3]. Thus, HPTLC 'finger print analysis' may be a powerful tool for the quality control of raw plant material and may be an alternative technique, particularly in the analysis of crude plant extracts. An important over conventional TLC, HPTLC is an instrumental technique where by special plants and instrumental resources for sampling are used and the quantitative evaluation of separation is aided by densitometry (Nile *et al*., 2004) [4].

Several pharmacopoeia containing monographs of the plant materials describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards (Peterson *et al*., 2004 [5] and Lanfranco *et al.*, 1999) [6]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time (WHO, 2001) [7]. HPTLC remains one of the most flexible, reliable and cost –efficient separation technique ideally suited for the analysis of botanical and herbal drugs. Used with standardized procedures, it guarantees reproducible results, a vital element in the routine identification of complex finger printing of plant extracts and pharmaceutical products. Several advantages of using HPTLC for the analysis of compounds as compared to other techniques , like HPLC, Spectrophotometry, Trimetry, etc (Renger *et al*., 2001) [8].

Plants which have one or more of its organ containing substances that can be used for the therapeutic purpose are called medicinal plants.[9] The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of photochemical constituents.[10] Phytochemical are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemical are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds. [11] Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins.

Punica granatum L. (Punicaceae), known as pomegranate, is a deciduous small tree, up to 8 m in height with attractive reddish scarlet edible fruits. The species originated in Iran, Afghanistan and Baluchistan, found wild in the warm valleys of the Himalayas and is cultivated throughout India. [12] The dried flowers, known as Gulnar, are efficacious to treat haematuria, haemoptysis, diarrhoea, dysentery, nasal hemorrhage [13] and in Unani literature as a remedy for diabetes.[14, 15] Flower juice is recommended as a gargle for sore throat, in leucorrhoea, hemorrhages and ulcers of the uterus and rectum. The root bark and stem bark of the plant are astringent and used as anthelmintic especially against tapeworms. Fruit rind is valued as an astringent in diarrhea and dysentery. The powdered flower buds are useful in bronchitis. The seeds are reputed as stomachic and the pulp as cardiac and stomachic. The green leaf paste is applied to relieve conjunctivitis.[16] The aqueous-ethanol (50%, v/v) extract of the flowers leads to significant blood glucose lowering effect in normal, glucose-fed hyperglycemic and alloxan-induced diabetic rats.[17] In Chinese medicine these flower are also used for the treatment of injuries from falls and grey hair of young man.[18] In addition *Punica granatum* is considered as "a pharmacy unto itself" in ayurvedic medicine and is used as an antiparasitic agent, a blood tonic, and to ulcers.[19]

MATERIALS AND METHODS

Collection and authentication of plant material: The flowers of *Punica granatum* were collected from in and around the Mannargudi, Thiruvarur District, Tamilnadu, India. They were identified and authenticated by Dr. S. John Britto, Department of Botany, Rabient Herbarium and

Center for Modular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India.

Preparation and Extraction of Plant Material: Collected plant material were thoroughly washed with distilled water and then dried under shade at room temperature for few days. The dried plant samples were ground well into a fine powder using blender. The powdered samples were then stored in airtight containers for further use at room temperature.

Extraction of Plant Material: The hydroalcoholic extract was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). 65g of course powder were weighed and the material was subjected to soxhelt extraction separately and successively with 300 ml of Ethanol (70%) as solvent. These extract was concentrated under reduced pressure in a rotary evaporator until solvent are completely evaporated from the extract. This obtained hydroalcoholic extracts were put in air tight container stored in a refrigerator.

HPTLC FINGER PRINTING ANALYSIS

Standard Preparation: 25 mg of standard Quercetin was dissolved in 25 ml of methanol.

Sample preparation: The plant extracts residue about 105.3mg was redissolved in 10ml of 7:3 Ethanol and water, which was used for sample application on 20×10cm HPTLC plates pre-coated silica gel 60 F_{254} Aluminium sheets.

Developing solvent system: A number of solvent systems were tried for extract, but the satisfactory resolution was obtained in the solvent Toluene: Ethyl acetate: Formic acid (5:4:1).

Sample application: Applied 2µl of standard and 5µl, 10µl of test solutions were spotted on a precoated silica gel 60 F_{254} HPTLC plate (E.Merck) of uniform thickness 0.2mm using Linomat5 sample applicator attached to CAMAG make HPTLC system, which was programmed through winCATS software .

Development of chromatogram: After the application of sample, the chromatogram was developed in Toluene: Ethyl acetate: Formic acid (5:4:1) solvent system to a distance of 8 cm. The plates were dried at room temperature in air.

Detection of spots:
The plate was scanned densitometrically at 254 nm using TLC scanner 3 and the plate was observed under UV light at 254 nm and 366 nm using CAMAG REPROSTAR 3. The R_f values and colour of the resolved bands were noted.

RESULTS AND DISCUSSION

HPTLC studies have shown that it is more resourceful than ordinary TLC methods, as the spots are well resolved. It is an invaluable quality assessment tool for the assessment of botanical materials, and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. It is helpful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The exclusive characteristic of the picture like image of HPTLC coupled with digital scanning profile is progressively attractive to herbal analysis to construct the herbal chromatographic fingerprint. In the last few decades (HPTLC) has become known as an important tool for the qualitative, semi- qualitative and quantitative phytochemical analysis of herbal drugs and formulation. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. The major advantage of HPTLC is several samples can be analyzed simultaneously using a small quantity of marker compound and mobile phase with very less time (Kashirsagar *et al*., 2008) [20]. The chemical constituents and their amounts in herb can be different, due to growing conditions, such as climate, soil fertility, the harvest season, age of the leaves, the drying process, etc. In the present investigation the flavanoids compounds were analyzed by HPTLC. A densitometry HPTLC analysis was performed for the progress of characteristic fingerprint sketch which may be used as markers for quality evaluation and standardization of the herbal drug.

Finger printing analysis of sample was done through HPTLC method, and the selected solvent system Toluene: Ethyl acetate: Formic acid (5:4:1) was suitable for quantitative analysis. HPTLC fingerprinting of the plants under study were presented photo documentation under 254 nm and 366 nm along with Rf values (Fig.1, 2). Track 1 and Fig.3 showed (2 µl of standard), Track 2 and Fig.4 showed (5 µl of Sample) that total no. of spot was seven, respective Rf values: (0.13, 0.24, 0.37, 0.44, 0.52, 0.62, 0.97) and Track 3 and Fig.5 showed (10 µl of Sample) nine spots with the following Rf values (0.08, 0.13, 0.23, 0.24, 0.38, 0.44, 0.54, 0.63, 0.96). For quantitative analysis through HPTLC techniques, optimization of solvent system was done using combination of solvent system of varying polarity and the most suitable solvent system was found to be toluene: Ethyl acetate: Formic acid (5:4:1). Quantitative

analysis was performed through HPTLC techniques using Quercetin as standard marker compound in the *Punica granatum*. Standard quercetin Track 1 and Fig.3 showed single peak in HPTLC Chromatography. In the HPTLC fingerprinting, hydroalcoholic extract gave a band (Rf - 0.56) corresponding to Quercetin is visible in test solution track. HPTLC photograph of standard quercetin and hydroalcoholic extract of *Punica granatum* were presented in the Fig.3, and Fig.4 and 5. HPTLC method confirms the presence of high amount of flavonoid (Quercetin) in hydroalcoholic extract of *Punica granatum* (L). The chromatographic finger prints obtained can be stored as electronic images without any errors and change for further usage.

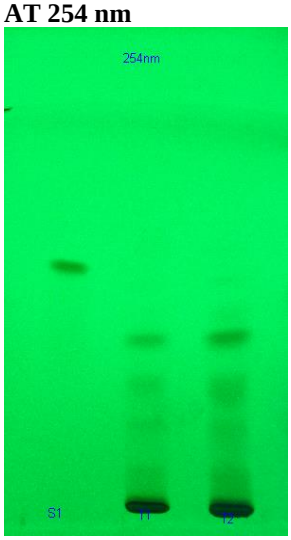
CONCLUSION

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants. HPTLC profile is a rapid, precise and powerful procedure to place the plant in taxonomic categories by analyzing the presence or absence of chemical constituent. The main goal of Pharmacognosy is to assess the value of raw materials and to ensure that the final product is of the required standard. Strict standardization procedures and pharmacognostical studies of medicinal plants would reduce drastically much of the accidents in wrong prescriptions of traditional herbal medicines [21]. The present HPTLC fingerprinting profile can be used as a diagnostic tool to identity and to determine the quality and purity of the *Punic granatum* in future studies. The HPTLC procedure provided excellent identification and quantification of flavonoids compounds presented in *Punica granatum* flower extract. Since these flavonoids have been of inserted of health benefits, the present analytical study could be a potential application to identify and quantify the flavonoid (Quercetin) compound present in the hydroalcoholic extract of the flower of *Punica granatum.* In future, the bioactive compounds will be isolated from this plant which may lead to the formation of new drugs against various diseases.

Conflict of interest: The authors declare that no conflict of interests existed in the organization, results, presentation and the finance of the research article.

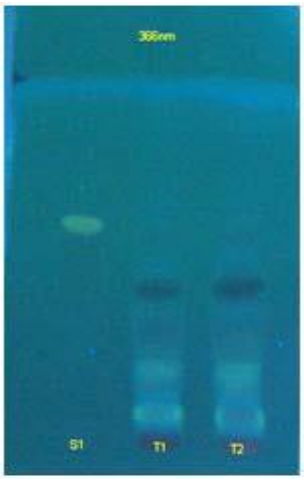
Figure 1: HPTLC FINGERPRINTING PROFILE OF *Punica granatum*
PHOTO DOCUMENTATION UNDER UV

AT 254 nm



S1 T1 T2

AT 366 nm



S1 T1 T2

Figure 2: 3D DISPLAY @ 254nm HPTLC Chromatogram of *Punica granatum* Flower Extract

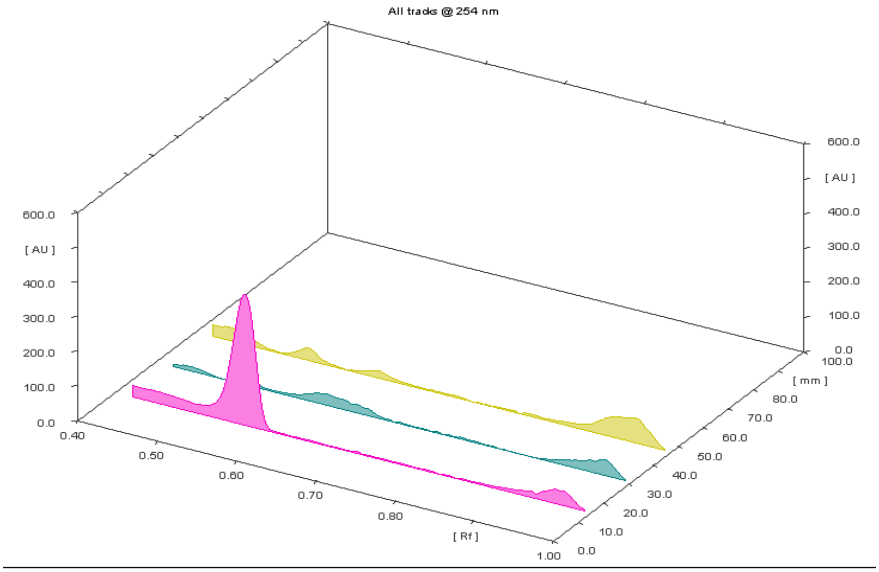
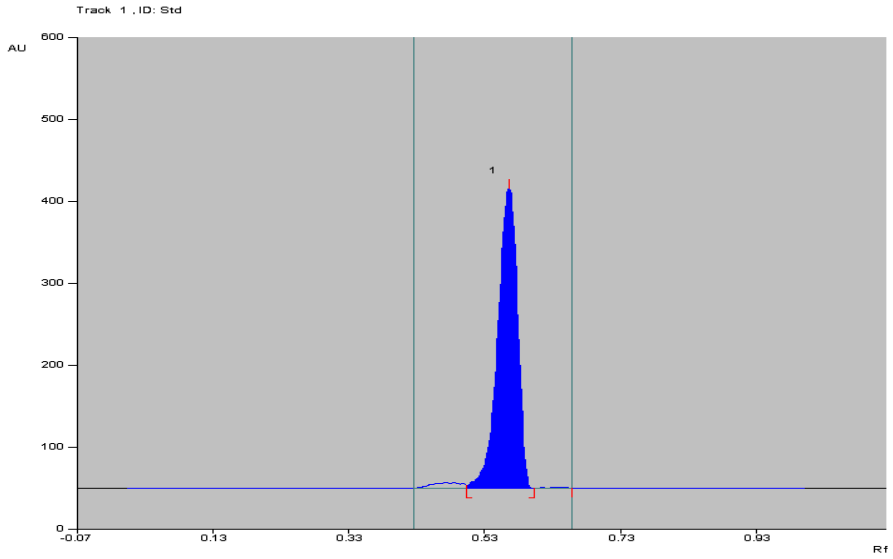
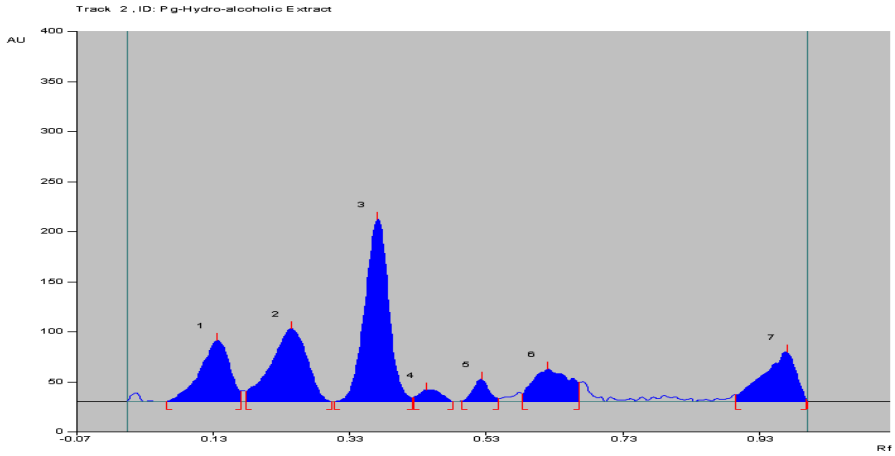


Figure 3: HPTLC Chromatogram of Standard Quercetin (2 µl of Standard)



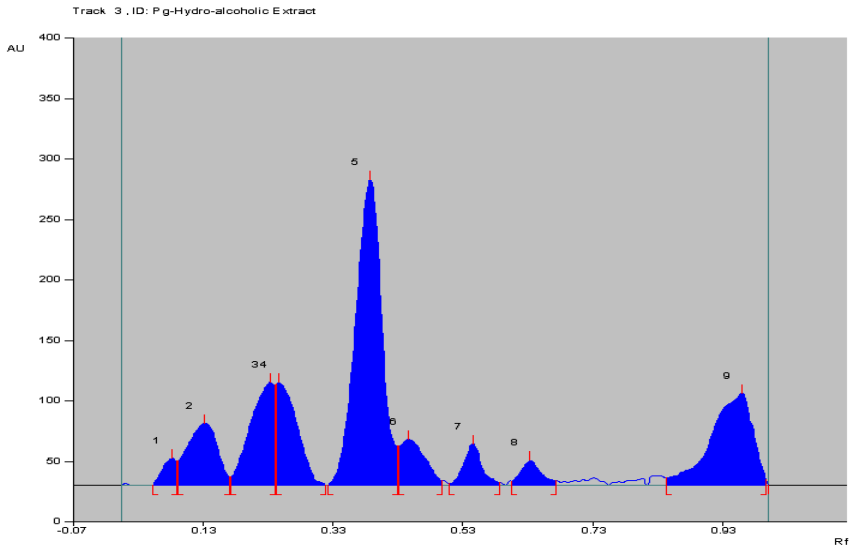
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.51	4.8	0.56	365.4	100.00	0.60	0.0	8119.1	100.00	unknown *

Figure 4: HPTLC Chromatogram of hydroalcoholic flower extract of *Punica granatum* (5 µl of Sample)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.1	0.13	61.0	14.07	0.17	10.5	2052.2	14.88	unknown *
2	0.17	10.3	0.24	73.1	16.88	0.30	0.2	2977.2	21.59	unknown *
3	0.30	0.0	0.37	182.5	42.13	0.42	4.1	4853.7	35.20	unknown *
4	0.42	4.3	0.44	11.9	2.76	0.48	0.1	305.2	2.21	unknown *
5	0.49	0.6	0.52	22.4	5.17	0.54	3.2	394.1	2.86	unknown *
6	0.58	7.9	0.62	32.4	7.48	0.66	19.0	1358.5	9.85	unknown *
7	0.89	7.0	0.97	49.9	11.51	1.00	2.5	1848.7	13.41	unknown *

Figure 5: HPTLC Chromatogram of hydroalcoholic flower extract of *Punica granatum* (10 µl of Sample)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	0.4	0.08	22.2	3.33	0.09	20.0	362.3	1.75	unknown *
2	0.09	20.0	0.13	51.5	7.73	0.17	6.9	1847.8	8.91	unknown *
3	0.17	7.2	0.23	85.2	12.79	0.24	82.6	2461.1	11.86	unknown *
4	0.24	83.5	0.24	85.3	12.80	0.31	0.2	2108.6	10.16	unknown *
5	0.32	0.2	0.38	252.9	37.97	0.43	32.6	7813.8	37.66	unknown *
6	0.43	32.6	0.44	38.0	5.71	0.49	3.7	1146.8	5.53	unknown *
7	0.51	1.5	0.54	34.5	5.18	0.58	2.1	736.6	3.55	unknown *
8	0.60	3.5	0.63	20.6	3.09	0.67	3.6	500.9	2.41	unknown *
9	0.84	5.9	0.96	75.9	11.40	0.99	4.8	3768.0	18.16	unknown *

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