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Meera Devi Sri Paramashivam
Consultant (Microbiology), Captain Srinivasa Murti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-600106, Tamil Nadu, India

Ramasamy Duraisamy
Assistant Professor, PG & Research Department of Chemistry, Govt. Arts College, Ariyalur-621713, Tamil Nadu, India

Arul Vasu C
Assistant Professor, Department of Zoology, University of Madras, Guindy Campus, Chennai-600025, Tamil Nadu, India

Ilavarasan R
Assistant Director In-Charge, Captain Srinivasa Murti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-600106, Tamil Nadu, India

Correspondence:
Meera Devi Sri Paramashivam
Consultant (Microbiology), Captain Srinivasa Murti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-600106, Tamil Nadu, India

A Comparative study on pytoconstitutents of *Punica granatum* flowers of Normal and Ornamental variety using TLC/HPTLC methods

Meera Devi Sri Paramashivam, Ramasamy Duraisamy, Arul Vasu C, Ilavarasan R*

Abstract

Punica granatum is a shrub that has a bushy appearance, have a good number of varieties, cultivated either as commercial crop for the production of pomegranates or as an ornamental crop for its beautiful flowers. Among the ornamental crop of *Punica granatum*, the "Double flower" producing plant gains much significant value as it is used in Unani drug under the name of Gulnar for treating many ailments. The present study was aimed to investigate and compare the phytoconstitutents present in both the flowers using TLC and HPTLC methods. The normal and double flowers of *P. granatum* were extracted with chloroform and alcohol separately using soxhlet apparatus. The TLC and HPTLC finger print profile of the extracts with marker compounds were studied using HPTLC Instrument, CAMAG (Switzerland). The TLC and HPTLC profile of chloroform and alcohol extract of normal and double flowers of *P. granatum* were exploited. The compound gallic acid and ethyl gallate, chromatographed together served as marker compound. The results revealed the similar and different phytoconstituents of both the flowers. The TLC and HPTLC finger print chromatograms not only demonstrates the similarities and differences between the flowers but also shows the presence of tested marker compounds gallic acid and ethyl gallate in alcohol extracts of both the flower types apart from acting as a quality control tool.

Keywords: *Punica granatum* -Normal flower, Ornamental flower, TLC, HPTLC finger prints, phytoconstituents.

INTRODUCTION

Punica granatum is a shrub that tends to develop multiple trunks, has a bushy appearance and grows up to a height of 12 to 16 feet [1]. In different pomegranate growing areas of the world, a good number of varieties have been identified and cultivated either as commercial crop for the production of pomegranates or as an ornamental crop for its beautiful flowers. Usually the flowers of commercial crop are large, red, white or variegated and have a tubular calyx that becomes the fruit, whereas the ornamental *P. granatum* cultivars produces different types of flowers. Among them, the "Double flower" producing *P. granatum* gains much significant value. As the name implies, theses plants produces double flowers, wherein numerous stamens are modified into petals and look like large attractive rose flowers [2,3]. Majority of these ornamental flowers are abortive and does not set fruits however they yield fruits when manually pollinated. These ornamental *P. granatum* cultivars are found in India, Russia, China and Turkmenistan [4,5]. As per the literature survey the commercial samples of the Unani drug used under the name Gulnar have been identified as double flowers of horticulture form of *P. granatum* which does not produce fruits [6,7]. Considering the medicinal activities of both the flowers types (commercial and ornamental), the present study was aimed to investigate and compare the phytoconstitutents present in both the flowers using TLC and HPTLC methods.

MATERIAL AND METHODS

Flower samples

The normal and double flowers of *P. granatum* (Fig. 1) were collected from the herbal garden of Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu during the month of

April to June 2012 and were authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, India. A voucher specimen of normal flowers *P. granatum* (No. 00443) and double flowers of *P. granatum* (No.00517) were deposited in the Herbarium of Department of Botany, Captain Srinivasa Murti Research Institute for Ayurveda and Siddha Drug Development, Chennai, India.

Figure 1: Normal and double flowers of *P. granatum*

Extraction of flowers for TLC / HPTLC finger prints

The powder of normal and double flowers (each 5 gm) were extracted with chloroform and alcohol separately using soxhlet apparatus and made upto 10 ml in a standard flask.

Preparation of markers

Each 1 mg of gallic acid and ethyl gallate were dissolved in ethanol and made up to 2 ml in a volumetric flask separately.

TLC/HPTLC studies and instrument conditions

The chloroform and alcohol extracts of both flowers were chromatographed using toluene: ethyl acetate (8.5: 1.5), toluene: ethyl acetate : formic acid (1: 0.5 : 0.1) respectively. The marker compounds (gallic acid and ethyl gallate) were also chromatographed along with alcohol extracts of both the flowers as per standard methods [9-10].

Instrument: CAMAG (Switzerland), sample applicator : ATS4 applicator with N_2 gas flow, photo documentation system : Digi store - 2 documentation system with win cats & video scan software, scanner : Camag HPTLC scanner - 3 (030618), win cats-IV, development chamber : Camag HPTLC 10 x 10, 10 x 20 twin trough linear development chamber, quantity applied : 10 µl for extracts and 4 µl for standards, stationary phase : Aluminium plate precoated with silica gel 60 F_{254} (E. Merck), plate thickness : 0.2 mm, scanning wavelength : 254 nm, laboratory condition : 20 ± 5°C and 53 % relative humidity.

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 nm and 366 nm. The HPTLC finger print profiles were also recorded at 254 nm. Finally the plates were derivatized using vanillin sulphuric acid reagent heated at 105°C till colour spots appeared.

RESULTS

The flowers of *P. granatum* normal and double flowers were studies and results were discussed as follows.

TLC profile of chloroform extracts of *P. granatum* flowers

The TLC profile of chloroform extract of *P. granatum* flowers is shown in Fig. 2. The corresponding R_f values of various spots for chloroform extract is given in Table 1. At 254 nm 6 and 4 spots were observed for normal and double flowers respectively. Normal flower showed 7 spots and double flower showed 3 spots at 366 nm. The plate when derivatized with vanillin sulphuric acid, showed 5 spots each for both extracts.

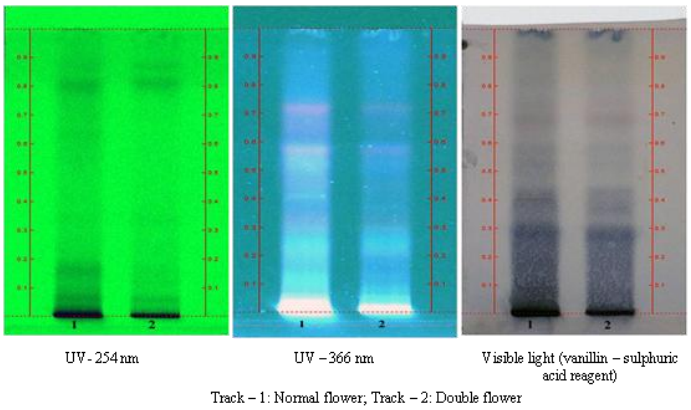

Figure 2: TLC profile of chloroform extracts of *P. granatum* flowers

Table 1: R_f Values of chloroform extract

R_f Values (UV-254 nm)	
Track–1(Normal flowers)	**Track – 2 (Double flowers)**
0.87 Green	0.87 Green
0.80 Green	0.81 Green
0.62 Green	----
0.34 Green	0.34 Green
0.17 Green	0.16 Green
0.10 Green	----
R_f Values (UV-366 nm)	
0.72 Pink	0.72 Pink
0.57 Pink	0.57 Pink
0.52 Blue	----
0.41 Blue	----
0.32 Pink	----
0.25 Blue	0.24 Blue
0.13 Blue	----
R_f Values after derivatised with vanillin – sulphuric acid reagent	
0.82 Grey	0.68 Pink
0.67 Pink	0.58 Grey
0.58 Violet	0.41 Violet
0.40 Violet	0.38 Violet
0.29 Violet	0.28 Violet

HPTLC finger print profile of chloroform extract of *P. granatum* flowers

HPTLC finger print profile of chloroform extract of normal flowers showed 8 peaks of which 2 were major peaks at R_f 0.18 and 0.93 whereas others were moderately smaller peaks (Fig. 3). The chloroform extract of double flowers showed 6 peaks of which one peak at R_f 0.93 was major whereas others were moderately smaller peaks (Fig. 4).The densitometric chromatogram of both the flowers of *P.granatum* was also recorded at 254 nm (Fig. 5).

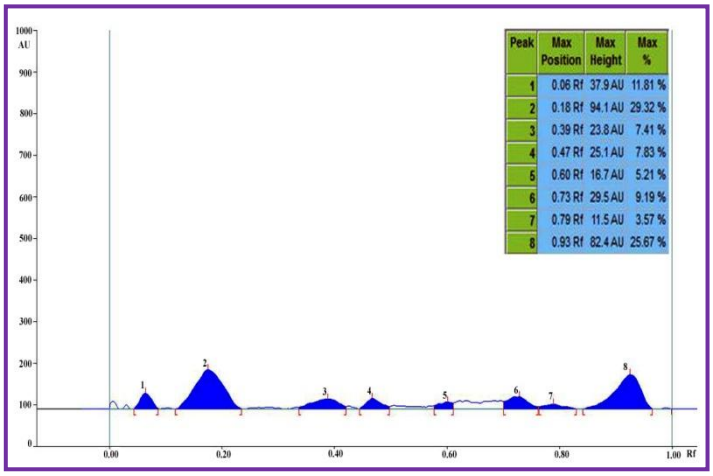

Figure 3: HPTLC profile of *P. granatum* normal flowers at 254 nm chloroform extract

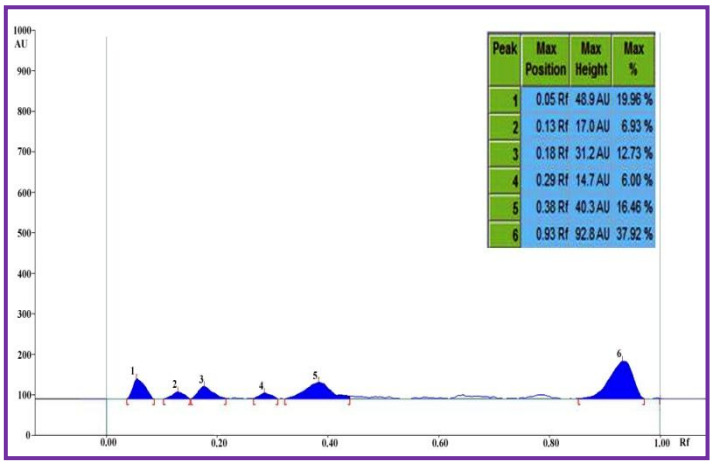

Figure 4: HPTLC profile of *P. granatum* double flowers at 254 nm chloroform extract

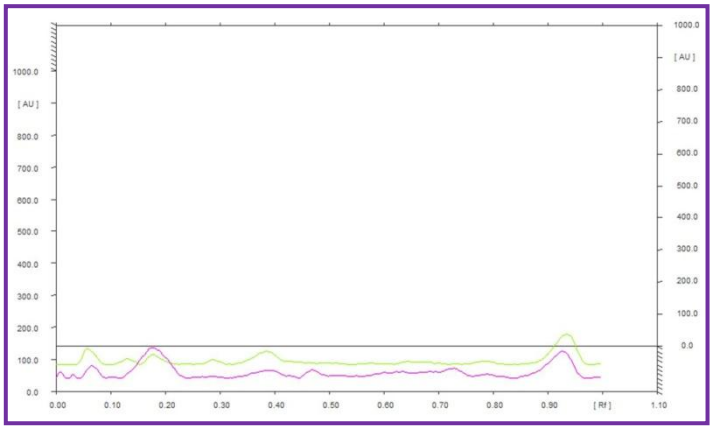

Figure 5: Densitometric chromatogram of *P. granatum* flowers at 254nm chloroform extract

TLC profile of alcohol extract *of P. granatum* flowers

The TLC profile of alcohol extract of *P. granatum* flowers is shown in Fig. 6. The corresponding R_f values of various spots in the TLC profile is given in Table. 2. At 254 nm 8 spots each were seen in normal and double flowers. At 366 nm, 12 and 13 spots were observed for the extracts of normal and double flowers respectively. The plate showed 11 and 10 spots for normal and double flowers when derivatized with vanillin sulphuric acid. The spots at R_f 0.34 and 0.47 corresponded to gallic acid and ethyl gallate respectively and confirms its presence in the alcohol extract.

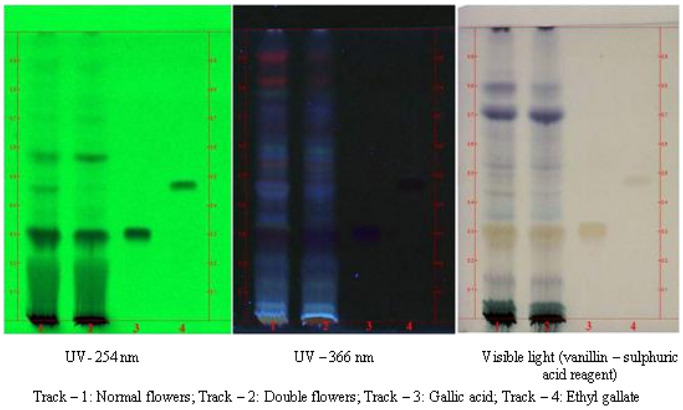

Figure 6: TLC profile of chloroform extracts of *P. granatum* flowers with markers

Table 2: R_f Values of alcohol extracts with marker compounds

***Rf*Values (UV-254 nm)**	
Track–1(Normal flowers)	**Track – 2 (Double flowers)**
0.88 Green	0.88 Green
0.81 Green	0.76 Green
0.70 Green	0.70 Green
0.57 Dark green	0.57 DarkGreen
0.45 Dark green (**Ethyl gallate**)	0.45 Green (**Ethyl gallate**)
0.37 Green	0.37 Dark green
0.30 Dark green (**Gallic acid**)	0.30 Dark green (**Gallic acid**)
0.18 Dark green	0.18 Dark green
***Rf* Values (UV-366 nm)**	
0.90 Pink	0.91 Pink
0.82 Pink	0.82 Pink
0.78 Blue	0.78 Blue
0.72 Violet	0.73 Violet
0.67 Violet	0.69 Blue
----	0.61 Blue
0.59 Yellowish green	0.59 Yellowish green
0.54 Pink	0.54 Pink
0.47 Blue (**Ethyl Gallate**)	0.47 Blue (**Ethyl Gallate**)
0.37 Blue	0.37 Blue
0.34 Violet (**Gallic acid**)	0.34 Violet (**Gallic acid**)
0.21 Blue	0.20 Blue
0.14 Blue	0.14 Blue
***Rf* Values after derivatised with vanillin – sulphuric acid reagent**	
0.80 Violet	0.80 Violet
0.71 Violet	0.71 Violet
0.63 Violet	0.63 Violet
0.53 Violet	0.53 Violet
0.50 Violet	0.50 Violet
0.47 Brown (**Ethyl gallate**)	0.47 Brown (**Ethyl gallate**)
0.43 Violet	0.43 Violet
0.37 Violet	0.37 Violet
0.34 Brown (**Gallic acid**)	0.34 Brown (**Gallic acid**)
0.26 Brown	0.26 Brown
0.14 Violet	----

HPTLC finger print profile of alcohol extract *P. granatum* flowers with gallic acid and ethyl gallate

HPTLC finger print profile of alcohol extracts of normal flowers showed 10 peaks of which 3 were major peaks at R_f 0.34, 0.54 and 0.66 whereas others peaks were moderately smaller peaks. The double flower

showed 9 peaks of which 2 were major at R_f 0.34 and 0.66 (Fig. 7, 8). The peaks at R_f 0.34 and 0.47 corresponded to gallic acid and ethyl gallate respectively (Fig. 9 & 10). The densitometric chromatogram of both the flowers of *P. granatum* together with gallic acid and ethyl gallate was also recorded at 254 nm (Fig. 11).

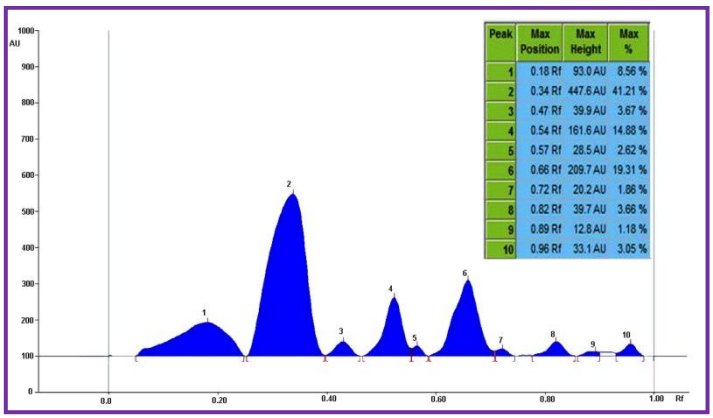

Figure 7: HPTLC profile of *P. granatum* normal flowers at 254 nm alcohol extract

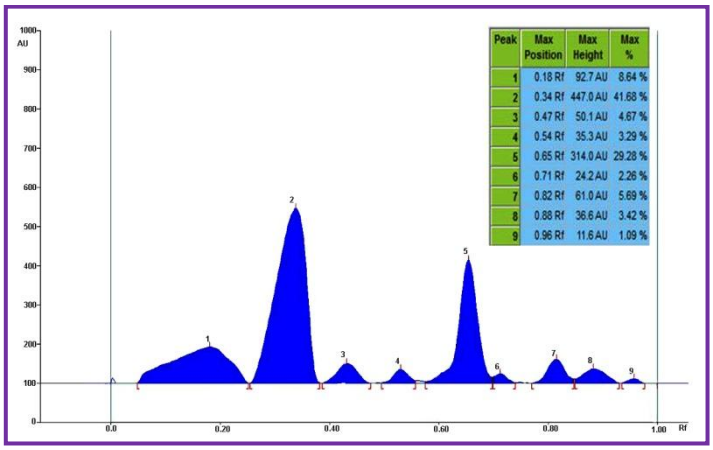

Figure 8: HPTLC profile of *P. granatum* double flowers at 254 nm alcohol extract

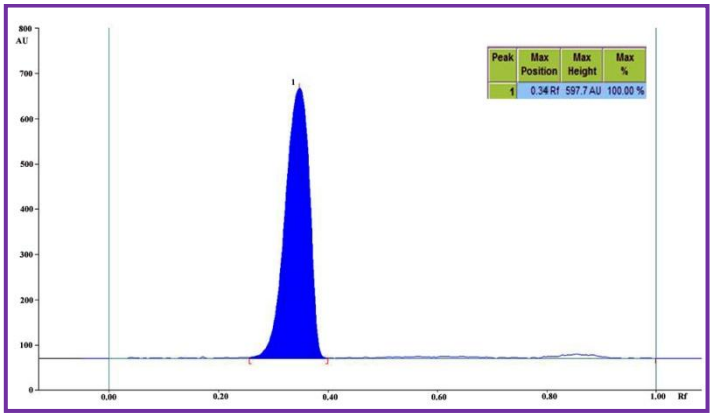

Figure 9: HPTLC profile of gallic acid marker at 254 nm alcohol extract

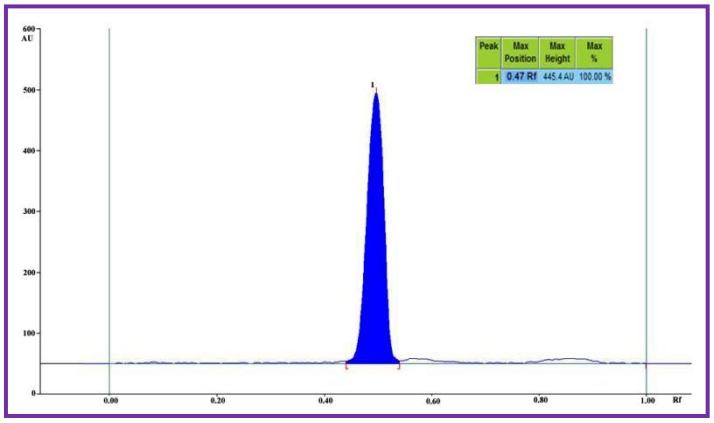

Figure 10: HPTLC profile of ethyl gallate marker at 254 nm alcohol extract

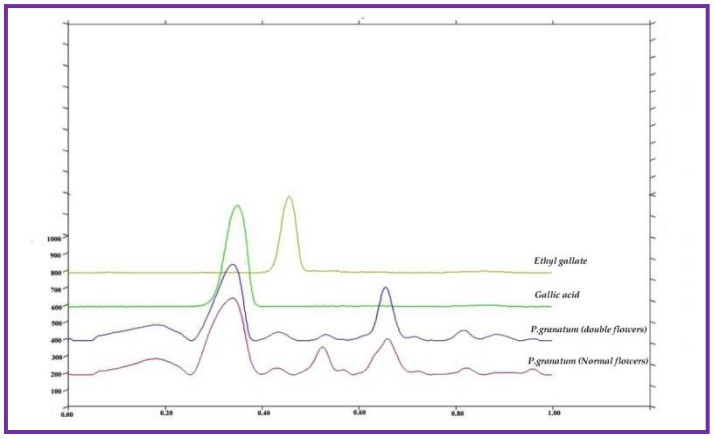

Figure 11: Densitometric chromatogram of *P. granatum* flower at 254 nm alcohol extract

DISCUSSION

High Performance Thin Layer Chromatography (HPTLC) is a modern method of TLC with improved versatility, separation efficiency and detection limits. HPTLC is useful in the identification of plants and their extracts, as each plant species produces a distinct chromatogram, with unique marker compounds. It is also used as quality control tool since comparison of chromatograms demonstrate the similarities and differences between the samples. In our present investigation, the suitable mobile phases with appropriate proportion have been determined for both chloroform and alcohol extract of *Punica granatum* flowers. Among the two solvents, the high polarity solvent alcohol, extracted higher quantity of secondary metabolites from both the flowers of *Punica granatum*. The flowers of *P. granatum* are reported to have higher amount of total phenol and flavonoids contents. According to one previous report, six cultivars of Iranian pomegranate flowers including ghojagh, rabbab, malas, shishegap, danesiah and golnar have been investigated. The total phenolic content of pomegranate flower extract expressed in terms of gallic acid equivalent, ranged from 25.94 % to 15.19 % mg gallic acid equivalents per gram of dry powder in ghojagh and golnar flower types respectively. Our reports also revealed the presence of gallic acid and its ester ethyl gallate in alcohol extract of both the flower types when used as marker compound.

The TLC and HPTLC fingerprints provided quantitative and semi quantitative information about the active constituents present in the normal and double flowers of *P. granatum* which aided us in designing the methods for isolation and characterization of bioactive compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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