

**DETERMINATION OF ACTIVE PHYTOCOMPOUNDS FROM
FORMULATED SUNSCREEN CREAM CONTAINING *PONGAMIA
PINNATA* LEAVES AND *PUNICA GRANATUM* PEEL EXTRACT BY
HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY**

Sneha Patil^{*}, Bharti Fegade, Urvashi Zamindar and V.H. Bhaskar

Gahlot Institute of Pharmacy, Koparkhairane, Navi Mumbai.

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***Correspondence for**

Author

Sneha Patil

Gahlot Institute of
Pharmacy, Koparkhairane,
Navi Mumbai.

ABSTRACT

Objective: The present investigation has been focused on the determination of photo protective activity of the 10% formulated sunscreen cream containing *Pongamia pinnata* and *Punica granatum* extract (3:2) in combination. And the quantitative estimation of phytoconstituents from both the extracts and in the formulated Sunscreen cream by High Performance Thin Layer Chromatography (HPTLC) method. **Methods:** Formulated Sunscreen cream was evaluated for Sunscreen activity. It is reported that Ellagic acid (polyphenol) and Karanjin (flavonoid) is responsible for the Sunscreen activity of *Punica granatum* and *Pongamia pinnata* respectively.

Therefore Ellagic acid was used as a standard marker for *Punica granatum* and Karanjin was a Standard marker for *Pongamia pinnata*. Ellagic acid and karanjin were chromatographed on TLC aluminium sheets silica gel plates using Chloroform: methanol: formic acid (9:1:2 v/v/v) and Toluene: ethyl acetate (7:3 v/v) as Mobile phase respectively. Detection and Quantification of both the extracts as well as formulated sunscreen cream were performed at Lambda 273 nm. **Results:** The formulated Sunscreen cream has shown good Photoprotective activity. The RF values of standards were 0.23 for Ellagic acid and 0.6 for Karanjin. The total peak areas of the standards and the corresponding peak areas of extracts were composed and the statistical analysis was carried out. **Conclusion:** From the present findings, it was concluded that there is a presence of Ellagic acid and Karanjin in *Punica granatum* and *Pongamia pinnata* respectively. As well as both the standards are present in 10% formulated Sunscreen cream in good quantity indicates Photo-protective effect of the formulation.

KEYWORDS: HPTLC, Ellagic acid, Karanjin, Sunscreen activity.

INTRODUCTION

Skin is the outermost and largest organ of the body. When the mammalian skin is exposed long term to U.V radiation; it induces the oxidative stress by generating the reactive oxygen species which trigger the development of sunburn, erythema, skin cancer etc.^[1] Synthetic sunscreen formulations available in market pose variety of adverse effects. Some of the active ingredients (oxybenzone, retinoic acid, benzophenone etc.) of synthetic sunscreen formulation are potentially carcinogenic. Therefore, the researchers have turned their attention towards developing herbal sunscreen agents which are effective with less or no side effects.^[2] Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades (HPTLC) has emerged as an important tool for the qualitative, semi-qualitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes developing TLC fingerprint profiles and estimation of chemical markers. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase.^[3]

Punica granatum (Family: Punicaceae) is commonly known as Pomegranate. From the ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years. The rind of the fruit and the bark of the pomegranate tree are used as a traditional remedy against diarrhea, dysentery and intestinal parasites.^[4] In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, the majority of research has focused on pulp and juice of fruit. But new scientists from the institute of Hygiene and environmental medicine have reported that the peel offers high yields of phenolics, flavonoids and proanthocyanidins than the pulp.^[5] It is stated that Ellagic acid, a polyphenol antioxidant found in large quantities in pomegranates, helps in healing sunburns and is known to reverse sun damage. Scientists have found that the incredible antioxidant activity in pomegranates may reduce the harmful effects of ultra violet radiations and inhibit the growth of skin tumors.^[6]

Pongamia pinnata (Family: Febaceae) is commonly called as Karanj. It is used in Ayurveda and Siddha traditional medicinal systems to treat various skin conditions from eczema and psoriasis to leprosy. It is believed to enhance the UV absorbing properties of conventional sunscreen.^[7] The methanolic extract of *Pongamia pinnata* is having 88.5% of U.V absorbing

property. It is rich in phenolics compounds & bioflavonoid like Karanjin and Pongamol that have excellent antioxidant property. The antioxidant property of the components of extract in conjugation with the U.V absorbing property renders its usage as a highly effective sunscreen, thus preventing wrinkles, premature skin aging, skin cancer etc.^[8]

So in the present study sunscreen cream was made by the combination of the two extracts in the 3:2 ratios. And high performance thin layer chromatography was performed to detect and quantify the presence of Ellagic acid in *Punica granatum* and karanjin in *Pongamia pinnata*. And presences of this active compound in the formulated cream were also determined by HPTLC.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh mature *Punica granatum* (pomegranates) fruits were procured from agriculture farm of the Aurangabad region. The fresh leaves of *Pongamia pinnata* (Karanj) were procured from botanical garden of our college (Gahlot institute of Pharmacy, Koparkhairane). The both plant species were authenticated by Dr. H.M Pandit of Botany Department, Guru Nanak Khalsa College, matunga, Mumbai and both species were deposited at herbarium. The peel were separated from whole fruits of *Punica granatum* (Pomegranate) and dried in direct sunlight. The leaves of *Pongamia pinnata* (Karanj) were dried in shade. The dried fruit peels of Pomegranate and leaves of Karanj were crushed in to coarse powder by using grinder. The both powders were stored in a vessel for future use.

Preparation of plant extracts

Preparation of ethanolic extract of *Punica granatum* (Pomegranate)

70 gm of coarsely powdered dried *Punica granatum* (Pomegranate) fruit peel material was evenly packed in the column of soxhelt assembly and about 400ml of absolute ethanol was taken in a round bottomed flask. The apparatus was set up in the mantle and extraction was carried out for 24 hrs at 45°C.

Preparation of methanolic extract of *Pongamia pinnata* (Karanj)

70 gm of coarsely powdered dried leaves of *Pongamia pinnata* (Karanj) was evenly packed in Soxhelt apparatus and then extraction was done with absolute methanol for 24 hrs at 45°C. After that both the extracts were filter through muslin cloth, and concentrated on water bath at 60°C.

Chemicals and Materials

All the materials used in formulation and evaluation of formulation were of analytical grade.

Formulation of Sunscreen Cream

Sunscreen cream was formulated by using simple o/w borax cream base formula. 10% Sunscreen cream was formulated by incorporating *Pongamia pinnata* leaves and *Punica granatum* peel extract (3:2) into the cream base and were evaluated for various physical and physicochemical parameters.^[9]

Determination of Sun Protection Factor by using Mansur equation ^[10]

The efficacy of a sunscreen is expressed by the Sun Protection Factor (SPF). An in vitro method of determining SPF of the sunscreens is by using Mansur equation

$$\text{SPF (spectrophotometric)} = \text{CF} \times \sum \text{EF} \times \text{I} \times \text{A}$$

Where, CF- correction factor (=10); EE-erythral effect spectrum; I -solar intensity spectrum;

A- Absorbance of sunscreen product.

This was used for determining the SPF of the formulated sunscreen cream. For this, 1 gm of finished Preparation was diluted with 50 ml of ethanol in volumetric flask (1000µg/ml). 1ml and 5 ml of stock solution was diluted to 50 ml with ethanol to prepare 50µg/ml and 100µg/ml dilutions respectively. Also 5 ml of stock solution was diluted to 25ml with ethanol (200µg/ml). The absorption of each aliquot prepared were determined from 290-320 nm, taking ethanol as a blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 2 determinations were made at each point, followed by the application of Mansur equation.

Estimation of phytochemicals by using HPTLC method

Instrumentation

Analysis was performed on a Camag HPTLC (High Performance Thin Layer Chromatography) system equipped with a sample applicator Linomat v, twin trough development chamber (10×10) size, TLC Scanner III, Wincats integration software was used.

Reagents and Chemicals

Analytical grade Toluene, Methanol, Ethyl acetate, Chloroform, Formic acid was used for analysis. Pure Karanjin and Ellagic acid were obtained from Yucca Enterprise Pvt Ltd, Mumbai. Pre coated TLC aluminium sheets silica gel F₂₅₄ (20×20cm, 0.2mm thick) plates were used.

Preparation of standards – Karanjin and Ellagic acid solution

1 mg of Ellagic acid and Karanjin were accurately weighted and dissolved into 10 ml volumetric flask, by means of ultrasonication for 15 minutes. The solution was diluted up to 10 ml with methanol (100µg/ml).

Preparation of sample (methanolic extract)

Dried *Punica granatum* (Pomegranate) extract was accurately weighed (200mg) in to 10 ml volumetric flask, dissolved in 6 ml methanol and then it was getting filtered through Whatmann filter paper (33.3µg/µl). Dried methanolic extract of *Pongamia pinnata* (Karanj) was accurately weighed (187mg) in to 10 ml volumetric flask, dissolved in 6 ml methanol and then it was getting filtered through Whatmann filter paper (32µg/µl).

Formulated sunscreen cream was approximately weighted and oily phase of cream was separated from aqueous phase by using partition coefficient method by using hexane: methanol .after that methanolic phase was separated from the oily phase which will be use for the analysis.

Development of HPTLC technique

The Sample of methanolic extracts of *pongamia pinnata* leaves and *Punica granatum* Peel, standards karanjin ,Ellagic acid and aqueous phase of formulated sunscreen cream were spotted in the form of bands by using automatic TLC applicator Linmat 5,10 mm from the bottom on a Precoated TLC aluminium sheets silica gel F₂₅₄ (10×20cm,0.2mm thickness) plates. Chloroform: methanol: formic acid (9:1:2, v/v/v) was used as a mobile phase for *Pongamia pinnata* leaves extract, karanjin and formulated cream while Toluene: ethyl acetate (7:3, v/v) was used as a mobile phase for *Punica granatum* peel extracts, Ellagic acid and formulated cream. Following saturation of the solvent systems, the plates were kept in Camag twin trough chamber for 15 mins. After development the plates were dried by hair dryer at 60°C for 5 mins and scanned by using CAMAG TLC Scanner III absorbance at 273 nm and operated by Win CATS software 4.03 versions.

Summary of Estimation of Ellagic acid in *Punica granatum* peel extract and formulated sunscreen cream

Stationary phase: silica gel plates

Mobile phase: Chloroform: Methanol: Formic acid (9:1:2, v/v/v)

Sample 1: *Punica granatum* peel extract (33.3 μ g/ μ l) - 4 μ l

Standard 1: Ellagic acid (0.1mg/ml) -2 μ l

Sample 2: formulated sunscreen cream extract -5 μ l

Migration distance: 80mm

Scanning Wavelength: 273nm

Mode of scanning: Absorption.

Lamp used: D2

Measurement type: Remission

Summary of Estimation of karanjin in *Pongamia pinnata* leaves extract and formulated sunscreen cream-

Stationary phase – silica gel plates

Mobile phase – Toluene: ethyl acetate (7:3, v/v)

Sample 1: *Pongamia pinnata* leaves extract (32 μ g/ μ l) - 5 μ l

Standard 1: Karanjin (0.1mg/ml) -3 μ l

Sample 2: formulated sunscreen cream extract -15 μ l

Migration distance: 80mm

Scanning Wavelength: 273nm

Mode of scanning: Absorption.

Lamp used: D2

Measurement type: Remission.

Quantification

Sample solution and standard solution were spotted on HPTLC plate. The percentage of Ellagic acid and Karanjin in methanolic extract of *Punica granatum* peel and *Pongamia pinnata* leaves respectively was calculated by comparison of the areas measured for standard solution also the quantities of both the standards in the 10% formulated cream were calculated.

Linearity

The linearity of Ellagic acid, Karanjin and both plant extract was determined by applying standard solution of different concentrations ranging from 0.5 μ l to 3 μ l spot on 20 \times 20cm HPTLC plates, Precoated with silica gel G F₂₅₄ in the form of sharp 6 mm bands, the distance between two adjacent bands was 9.5mm. The plate was developed in a solvent systems up to the distance of 80mm, at room temperature. The plate was dried by hair dryer. The detector response for was measured for each band at wavelength of 273 nm using Camag TLC scanner and win CAT software. The peak area of above mentioned phytoconstituents was obtained by plotting a graph of peak vs. applied concentration (in μ g)

RESULTS AND DISCUSSION

Medicinal plants are important for pharmacological research and drug development, not only when plants constituents are used directly as therapeutic agents, but also as starting materials for synthesis of drugs or as models for pharmacologically active compounds. Identification and quality evaluation of crude drugs is a fundamental requirement of industry and other organizations dealing with natural health products (NPH). The fact must be taken in to consideration that the plant material to be examined has a complex and inconsistent compositions based on its contents of secondary metabolites.^[9]

Determination of Sun Protection Factor by using Mansur equation

Measurement of SPF is ultimate way to determine effectiveness of sunscreen formulation. The higher the SPF, the more protection a sunscreen offers against UV-light. Sunscreens are used to aid the body's natural defense mechanism to protect against harmful UV radiation from the sun. SPF of 10% formulated sunscreen cream containing *Punica granatum* peel and *Pongamia pinnata* leaves extract was checked by absorption spectroscopy using mansur equation method. Absorbances obtained were considered for SPF calculations and depicted in Table no.1.

SPF of 10% formulated sunscreen cream was found to be for 50 μ g/ml, 100 μ g/ml, and 200 μ g/ml. A continuous increase in the SPF values with an increase in the concentration of the formulation under study signifies that the SPF of the formulation is depends on the concentration of both the extracts .so by varying the concentration of both extract added to the formulation, it may serve different purpose.

Table no.1 Determination of sun protection factor values of 10% Formulated sunscreen cream

Conc. µg/ml	Wavelength	290	295	300	305	310	315	320	SPF
	EF×I	0.015	0.081	0.287	0.327	0.1864	0.083	0.018	
50	A	0.47	0.42	0.38	0.34	0.33	0.32	0.31	3.6
	EF×I×A	0.007215	0.0348	0.1092	0.1140	0.06151	0.02693	0.0057	
100	A	0.82	0.72	0.64	0.53	0.55	0.54	0.53	6.06
	EF×I×A	0.0123	0.058	0.1856	0.1917	0.1025	0.04530	0.0095	
200	A	1.55	1.41	1.17	1.06	1.03	1.00	0.99	11.1
	EF×I×A	0.0233	0.113	0.318	0.330	0.193	0.0843	0.0178	

Estimation of phytochemicals by using HPTLC method

In HPTLC analysis, the R_f values of standards such as Ellagic acid is found to be 0.23 (Figure-1), Karanjin is found to be 0.6 (Figure-2) and the peak areas covered are represented in graph, Ellagic acid (2µl)-3212.7(Figure-3), Karanjin (5µl)-6763.7(Figure-4). Our samples Punica granatum and Pongamia pinnata extract has shown the amount of Ellagic acid and Karanjin was found to be 0.3183% and 0.16 %, karanjin respectively, shown in Table no.2

Table no. 2 Recovery of phytochemicals from both the extracts

	R _f value	Amount of Sample loaded	Area	Recovery	Yield% in extracts (w/w)	Quantity of standards in cream (g)
1.	Ellagic acid	2µl	3212.74	212 ng	0.31%	0.0012g
2.	Karanjin	5µl	6763.70	265 ng	0.16%	0.009g

The formulated 10% sunscreen cream has shown 0.0012g of Ellagic acid and 0.009g of karanjin.

Many alkaloid, Tannic and phenolics and flavonoids had been reported in the Pongamia pinnata and Punica granatum. The method of sample preparation and the development of a suitable mobile phase are two important steps in devising an analytical procedure. With respect to phytomedicines, these steps are more significant because of the complexity of the chemical composition and the affinities of the components towards various solvents. by analyzing different mobile phases for the effective separation of both the extract and formulated sunscreen cream and its active phytoconstituents by HPTLC, the desired resolution of Ellagic acid and Karanjin with symmetrical and reproducible peaks were

achieved by using Chloroform: methanol: formic acid (9:1:2 v/v/v) and Toluene: Ethyl acetate (7:3 v/v) respectively as suitable mobile phase which was supported by many researchers. Thus, HPTLC method is more rapid, very precise, more sensitive, and simple, which can be utilized for quantitative monitoring of phytoconstituents of *Pongamia pinnata* and *Punica granatum* by using markers.

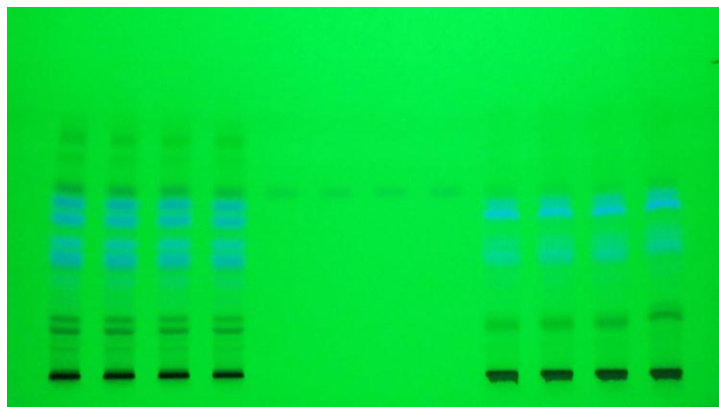


Fig 1: Shows the presence of Ellagic acid at 270nm

Picture shows that, lane 1 to 4 denotes Pomegranates peel extracts (4 μ l), 5 to 8 denotes Ellagic acid (2 μ l), and 9 to 12 denotes 10% Formulated sunscreen cream (5 μ l)

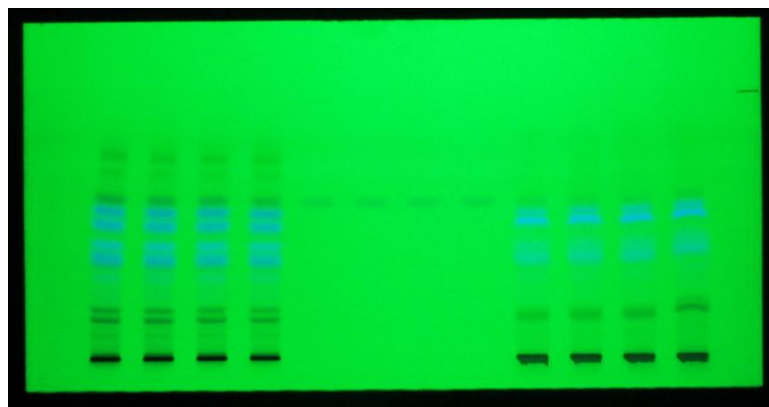


Fig 2: Shows the presence of Karanjin at 270nm

Picture shows that, lane 1 to 4 denotes *Pongamia pinnata* leaves extracts (5 μ l), 5 to 8 denotes Karanjin (3 μ l), and 9 to 12 denotes 10% Formulated sunscreen cream (15 μ l)

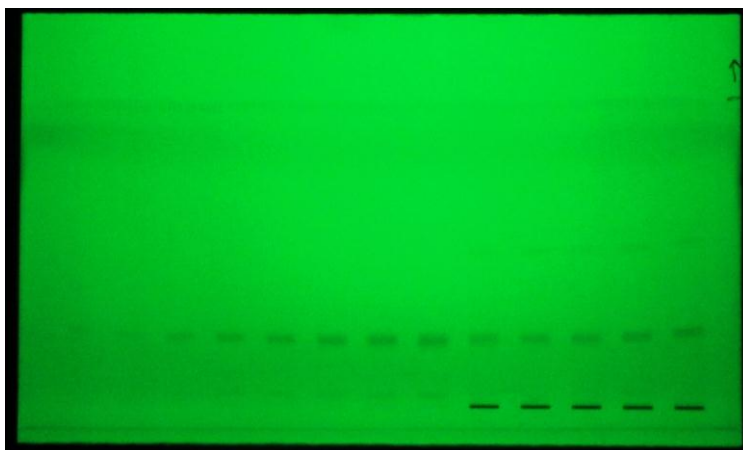


Fig 3: Quantification of Ellagic acid in pomegranate Extract at 270nm

Picture shows that, lane 1 to 8 denotes Ellagic acid (0.5-3.5 μ l), 9 to 13 denotes Pomegranate peel extract.

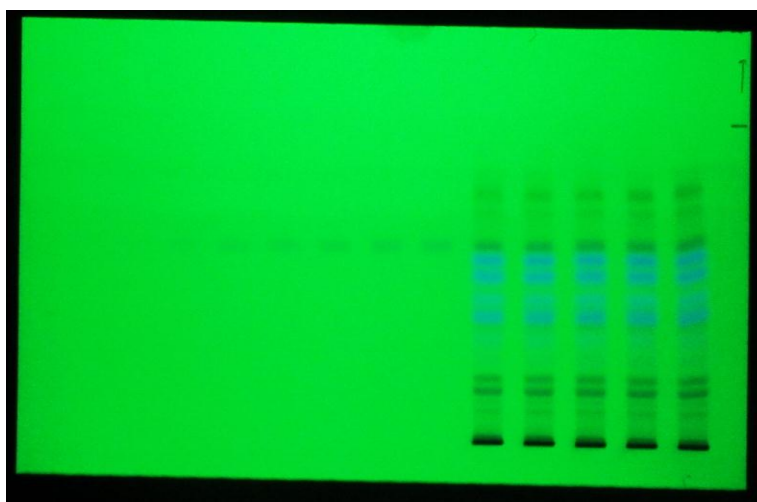


Fig 4: Quantification of Karanjin in *Pongamia Pinnata* Extract at 270nm

Picture shows that, lane 1 to 8 denotes Ellagic acid (0.5-3.5 μ l), 9 to 13 denotes *Pongamia pinnata* extract.

CONCLUSION

The developed HPTLC method was simple, accurate, precise and cost-effective and can be utilized for the routine analysis of quantitative determination of plant sample as well as for the developed formulation. The present study conclude that, *Punica granatum* and *Pongamia pinnata* contains, a number of markers of varying proportions, that are responsible for its sunscreen activity. The formulated sunscreen cream also shows the presence of Standard markers of both extract in good quantity which is responsible for its sunscreen activity. Hence the 10% formulated sunscreen cream has very good photoprotective activity. Increase

in the percentages of active phytochemicals in the formulation may give the greater sun protection against harmful UV rays.

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