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HPTLC Fingerprint Profile of Leaf Extracts of *Pongamia pinnata* (L.) Pierre

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Abstract
Thin layer chromatography which involves separation of different components, HPTLC is help in better resolution of compounds with lower limits of detection and quantifies separated components with the use of an integrated software platform .*Pongamia pinnata (L.) Pierre* is a Fabaceae tree from family Papilionaceae found in this region, all plant parts such as root leaves and stem flower and seeds used in traditional medicine. Leaves are also having medicinal properties. Attempts have been made to study complete profile of leaf extract by using High performance thin layer chromatography. The densitometric analysis shows fingerprinting, RF value, peaks of densitogram and chemical variation, this technique is useful for drug identification, adulteration, and also acts as biomarker in plant industry.

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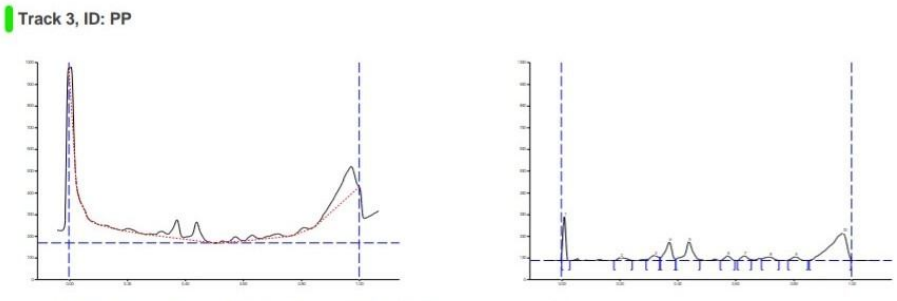
INTRODUCTION

Phytomedicines are standardized herbal preparation consisting of complex mixture of one or more plants which are used in different countries for the management of various diseases India recognizes more than 2500 plant species which have medicinal values. However, large flora is waiting for investigation for their medicinal properties (Joy et. al, 1998). Raigad district of Konkan region is very well known for its huge Biodiversity of flora and fauna. The Raigad district in Maharashtra state lies between 17^0 53'l and 19^0 08'N. Latitude and 72^0 51'and 73^o 43 E. Longitude and covers an area of 719,889 Sq.kms the district is bounded on the west by Arabians sea and the major sahyadri scrap adjoining Ratrangeri district on the North by Thane district and on the south the savitri river which flows along the boundary over a stretch of 30 Kms on the South east the Satara district and on the east the Pune district,

The use of medicinal plants as a source of medicine and human substances has been in vogue since antiquity India has rich heritage of use of plants as medicines and near about 805 medicines obtained from plants. Raigad district of Kankan region is very well known for its huge Biodiversity of flora and fauna. The main range of sahyadri, spurs and valleys form important botanical pockets of high biodiversity. The north–east and east stretches of sahyadri supports luxuriant growth of vegetation in Maharashtra state. The area has forest situated on its surrounding mountains. Sahyadri hills has huge reservoir of enormous natural resources including vegetation wealth and traditional knowledge of medicinal plants.

MORPHOLOGY OF PLANT

Pongamia pinnata (Linn.) Pierre. is a small or middle sized tree, branches drooping leaves pinnate, leaflets opposite (Harborne,1988), broadly ovate, Flowers in axillary racemes, shorter than leaves, corolla pinkish-white, pods woody, seed solitary, reniform, brown. The roots of *Pongamia pinnata (L.)* Pierre is good for ulcers, cleaning teeth, strengthening gums and gonorrhoea (Tanaka, 2002). Bark is sweet and mucilaginous to taste. Leaves are good for digestive, laxative and diarrhoea, seeds are carminative anthelmintic (Sajid, 2012) inflammation, chronic fever anaemia and haemorrhoids. Seed oil is anthelmintic, leprosy ulcer and also used as a biodiesel. WHO, (1989) and Pawar et. al., (2011) reported that 80% populations rely mainly on traditional therapies, involving the use of plant extracts or their active constituents. Tambekar (2007) emphasized the need to ensure the quality of medicinal plant products using modern technique (Chaudhay, et. al., 2008). HPTLC is the best technique for better resolution and estimation of constituents with reasonable accuracy within a short period (Deepanjan et. al., 2004). Objective behind the present study was to carry out phytochemical analysis of leaf extract of *Pongamia pinnata (L.)* Pierre.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	29.2	0.01	201.7	32.66	0.03	0.0	1655.1	11.89	unknown *
2	0.18	0.2	0.20	13.7	2.21	0.24	1.5	347.1	2.49	unknown *
3	0.29	5.1	0.32	23.9	3.87	0.34	15.2	535.7	3.85	unknown *
4	0.34	14.6	0.37	85.6	13.86	0.39	6.8	1669.1	11.99	unknown *
5	0.39	7.3	0.44	87.1	14.11	0.48	3.6	1879.1	13.50	unknown *
6	0.55	0.4	0.57	21.7	3.51	0.60	1.3	396.8	2.85	unknown *
7	0.61	0.1	0.63	22.5	3.65	0.65	3.9	421.0	3.02	unknown *
8	0.69	8.8	0.72	16.5	2.68	0.75	0.9	462.5	3.32	unknown *
9	0.78	1.9	0.81	17.8	2.89	0.85	0.1	370.1	2.66	unknown *
10	0.86	0.1	0.97	127.0	20.56	1.00	3.9	6183.6	44.42	unknown *

Figure 1: Chromatogram of *Pongamia pinnata (L.)* Pierreleaf extract

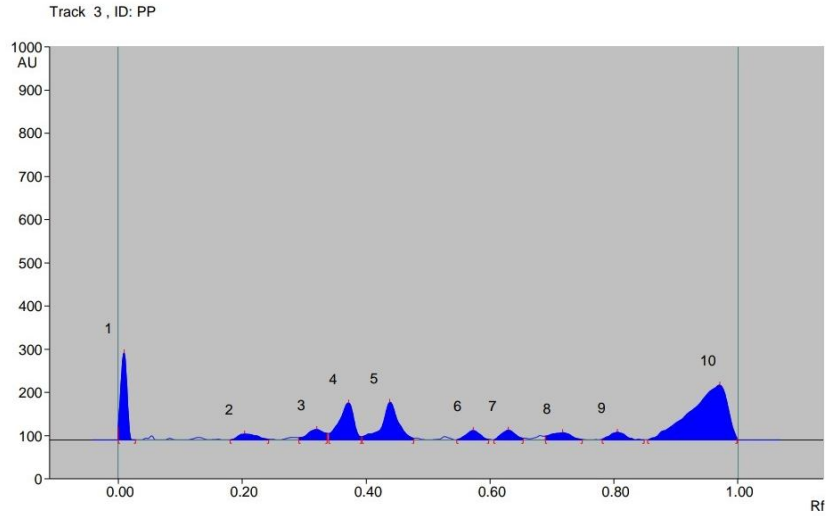


Figure 2: HPTLC Chromatogram of *Pongamia pinnata (L.)* Pierreleaf extract.

Table 1: RF Value of leaf extract of *Pongamia pinnata (L.)* Pierre Leaf at UV254nm

Peak	Start Position	Start Height	Max position	Max height	Max %	End Position	End Height	Area	Area %
1	0.00	29.2AU	0.01Rf	201.7AU	32.66%	0.03Rf	0.0AU	1655.1AU	11.89%
2	0.18	0.2AU	0.20Rf	13.7AU	2.21%	0.24Rf	1.5AU	347.1AU	2.49%
3	0.29	5.1AU	0.32Rf	23.9AU	3.87%	0.34Rf	5.2AU	535.7AU	3.85%
4	0.34	14.6AU	0.37Rf	85.6AU	13.86%	0.39Rf	6.8AU	1669.1AU	11.99%
5	0.39	7.3AU	0.44Rf	87.1AU	14.11%	0.48Rf	3.6AU	1879.1AU	13.50%
6	0.55	0.4AU	0.57Rf	21.7AU	3.51%	0.60Rf	1.3AU	396.8AU	2.85%
7	0.61	0.1AU	0.63Rf	22.5AU	3.65%	0.65Rf	3.9AU	421.0AU	3.02%
8	0.69	8.8AU	0.72Rf	16.5AU	2.68%	0.75Rf	0.9AU	462.5AU	3.32%
9	0.78	1.9AU	0.81Rf	17.8AU	2.89%	0.85Rf	0.1AU	370.1AU	2.66%
10	0.86	0.1AU	0.97Rf	127.0AU	20.56%	1.00Rf	3.9AU	6183.6AU	44.42

Figure 3: HPTLC plate Scanned at 366 nm.

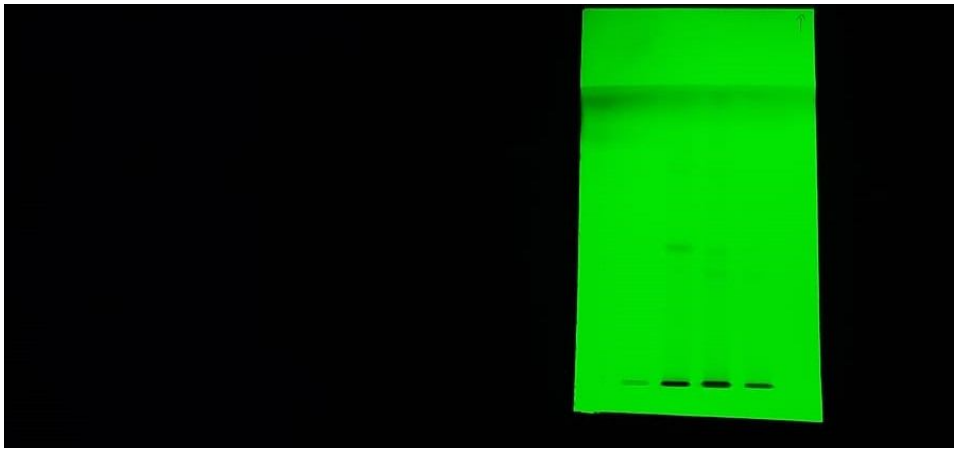


Figure 4: HPTLC profile of *Pongamia pinnata (L.)* Pierre plant ethanol extract at 254 nm.

MATERIAL AND METHODS

Preliminary phytochemical analysis of leaf extracts of *Pongamia pinnata (L.)* Pierreis done by as per method described by Wagner et. al, (1998), Harborne, (1988) and Eike et. al (2006). HPTLC profiling was done by using CAMAG HPTLC System with WIN CATS software.

Collection of Plant material: The leaves of *Pongamia pinnata (L.)* Pierre was collected from Poladpur district. Raigad and brought to laboratory for further analysis and washed gently with running tap water to remove surface dust and pollutants. The leaves were dried under the shade. The dried plant material was made powder using mixture grinder.

Extraction of Plant Material: About 10 gm. powder of *Pongamia pinnata (L.)* Pierrewas extracted separately using 70% ethanol in a Soxhlet Extractor (Borosil) for about six hours. After extraction the extracts were evaporated to dryness. The dried extracts were dissolved in 5 ml ethanol and filtered using Whatmann filter. The filtered extracts were later used for further phytochemical and HPTLC analysis (Khandelwal, 2005). Win CATS Planar Chromatography Manager. The sample of leaf extract of leaf extracts of *Pongamia pinnata (L.)* Pierrewere filtered through the whatman filter paper No.1 and injected analysis the Following peaks were obtained in fig No. 2. The leaf extract of *Pongamia pinnata (L.)* Pierre was shows peaks which may be Karanja, karanjin (Maurya et. al., 2004), Glabrachalcone, karangin, isopongachromene, pongamol, pongagalabrone, pongapin, pinnatin, kanjone (Kirtikar et. al., 1995).

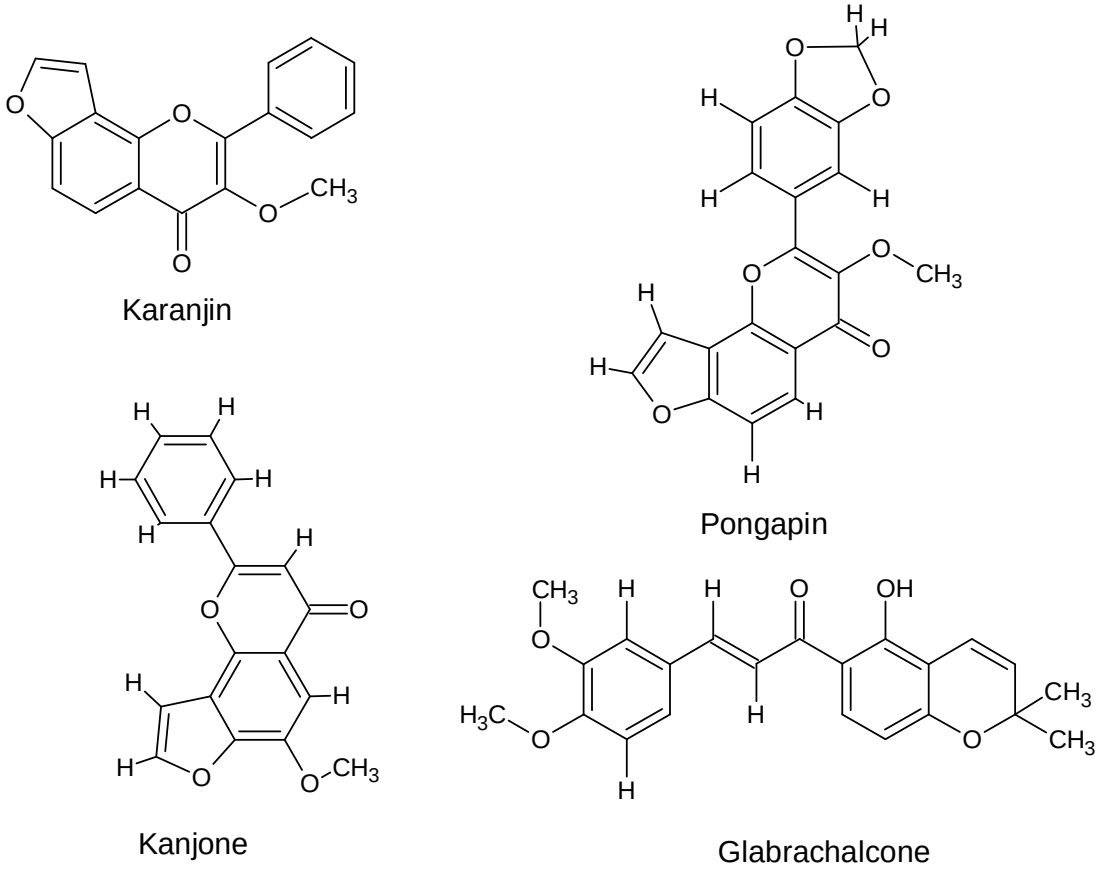


Figure 5: The Chemical structure of Kanjone, Glabrachalcone, Karanjin, Pongapin

RESULT AND DISCUSSION

The HPTLC analysis obtained high resolution and shows different peaks leaf extract of *Pongamia pinnata (L.)* Pierrewas runs along with the standard and perceived to validate the presence of phytochemical compounds from chromatogram after derivatization. The result from HPTLC finger print scanned at wavelength 254 nm for *Pongamia pinnata (L.)* Pierre shows polyvalent phytoconstituents and corresponding ascending order of Rf value are from 0.01 to 0.97 in which highest concentration of the phytoconstituents was found to be 32.66% and its corresponding RF value was found to be 0.01 respectively.

This is recorded in Table 1 ethanol is used as a solvent Rf value and different wavelength were obtained in picture plate at UV 254nm. The graphical representation shows different peaks of polyvalent phytoconstituents. The Rf value starts from 0.1 to 218.1 in which highest concentration of phytoconstituents were found and maximum percentage starts from 5.92 to 59.26% and maximum height from 0.00 to 2.8 control. The peak retention in ethanol extracts and is found with Rf start with 0.01, 0.33, 0.42, 0.86 and end with 0.03, 0.39, 0.48, 1.00 and maximum percentage is 59.26, 5.82, 6.3028.63 in Table 1 respectively.

The report of diabetic rat shows significant anti hyperglycaemic and anti-lipid peroxide effect with the leaf extract. It shows that the treatment of *Pongamia pinnata (L.)* Pierre extract could be used as a safe alternative anti-hyperglycaemic drug for diabetic patients (Punitha et. al., 2006), antiplasmodial activity, and also shows action on infectious diarrhoea (Tambekar et. al., 2007).

HPTLC finger printing is valuable quality assessment tool for the evaluation of botanical materials, it allows for the analysis of abroad number of compounds both efficiently and cost effectively. These studies have shown that it is more versatile than ordinary TLC methods as the spots are well resolved. The HPTLC method is simple, rapid, accurate, reproducible, selective and economic can be for quality and quantitative determination of plant material (Harborne et, al., 1988).

CONCLUSION

The study of HPTLC fingerprints profile of *Pongamia pinnata (L.)* Pierre useful to determine the quality of crude drug and, separation of secondary metabolites such as alkaloids, steroids, carbohydrates, tannin, flavonoids, terpenoids, coumarins, phenols quinone, and also useful for bioactive products which is use to understand biochemical, physiological mechanisms in plant.

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