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HPTLC PROFILING AND PHYTO-PHARMACEUTICAL STUDY OF FLOWER OF GMELINA ARBOREA ROXB.

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INTRODUCTION

India has one of the most diverse cultural traditions associated with the use of medicinal plants and this knowledge is accessible from thousands of medical texts and manuscripts. The substances having medicinal value have been used extensively for treating numerous disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Primary metabolites like amino acids, carbohydrates and proteins are vital for the maintenance of life processes while others such as alkaloids, tannins, phenols, glycosides etc. known as secondary metabolites are having pharmacological, ecological and toxicological importance [1]. Numerous medicinal plants, traditionally used for thousands of years are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some need to be scouted by means of various experimental methods [2].

As like all *Gmelina arborea* Roxb. is also one of the commonly used plant in the Indian traditional systems of medicine. It is widely distributed in tropical and subtropical regions worldwide (Greaves, 1981). It has been used traditionally in Ayurvedic treatment as one of the ingredient of "*Dashamoola*". Other parts including roots are also having the medicinal value. Flowers are the one of the important part of the plant for reproduction as well as for many pharmacological actions. The flowers of *gambhari* are said to be *vatahara, grahi, pittahara, vrushya, balya and raktapradarahara* in *Nighantus* [3,4] and having *madhura-rasa, sheeta-guna, tikta, vatakara, kashaya* and *madhura-vipaka*. Flowers and bark is also used in the preparation of *Chandanadi taila.* (*Charak* Samhita). Flowers are acclaimed for leprosy, skin and blood diseases [5]. Major chemical constituents like beta-sitosterol, ceryl alcohol, gmelinol, butyric and tartaric acid, arborone, isoarboreol, gmelanore, octacasanol etc. are obtained from the plant. Verbascoside isolated from the flowers and roots of *G. aborea* has been reported to possess both the activities cytotoxicity on liver and hepatoprotective activity [6]. The objective of this study is to generate the HPTLC profile of flower (methanolic extract) and also assess the phytochemical present in the sample.

MATERIALS AND METHODS

Collection of plant material

The flowers of *Gmelina arborea* Roxb. were collected from appropriate sources in Jamnagar, Gujarat, India and sample was authenticated at Pharmacognosy Lab. under the authentication no. IPGT & RA PHM. 6262/18-19 in IPGT & RA Gujarat Ayurved University, Jamnagar.

Habitat and Morphology of the plant *Gmelina arborea* Roxb.

Fig-1. Natural habitat

Fig-2. Herbarium

Fig-3. Flowering Twig

Fig-4. Flowers Powder

Preparation of plant extract

Fresh flowers were shade dried at room temperature for 10 days and powdered coarsely using electric blender. The powder (5gm) was taken and mixed with Methanol (100ml). The mixture was stirred frequently for 6hrs. and then kept undisturbed for 18 hrs. Then the extract was filtered with a muslin cloth [7] and used for phytochemical screening. The remaining extract was dried out and used for further study.

Preliminary phytochemical screening

Phytochemical analysis of the extract was conducted as per the standard procedure [8]. By this procedure, the presence of several phytochemicals like alkaloids, glycosides, flavonoids, tannins, saponins, sugars and glycosides were tested.

High Performance Thin Layer Chromatography (HPTLC) Analysis

High Performance Thin Layer Chromatography (HPTLC)[7,9] is an invaluable quality assessment tool for the evaluation of herbal materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses. Performing thin-layer chromatographic separation on HPTLC layers has several advantages over those on conventional layers:

- Higher resolution of zones due to higher number of theoretical plates.
- Shorter developing times.
- Less solvent consumption.
- Less background noise due to narrow size distribution of particles.

However, suitable instruments are required to get the best results. In most cases instrumental thin-layer chromatography utilizes pre-coated layers. Not only are they more convenient, their quality is far superior to that of layers prepared from the adsorbents available for self-coating. The pre-coated layers have a smoother and more durable surface.

STEPS INVOLVED IN HPTLC:

1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning and documentation

SAMPLE FOR HPTLC:

For the HPTLC study sample of methanolic extract of *Gmelina arborea* Roxb. of flowers was prepared. Mobile Phase: Toluene: Ethyl acetate: Acetic acid (7: 2: 1 v/v/v) is used as mobile phase for the extracted matter.

CHROMATOGRAPHIC CONDITONS:

⇨ Application mode	:	Camag Linomat V
⇨ Development Chamber	:	Camag Twin trough Chamber.
⇨ Plates	:	Precoated Silica Gel GF254 Plates.
⇨ Chamber Saturation	:	30 min.
⇨ Development Time	:	30 min.
⇨ Development distance	:	8 cm.
⇨ Scanner	:	Camag Scanner III.
⇨ Detection	:	Deuterium lamp, Tungstan Lamp
⇨ Data System	:	Win cats software.

After the scanning was done in to the Camag Scanner III, the area under the curve of the methanol extract of flower of *Gmelina arborea* Roxb. was observed.

RESULT AND OBSERVATION

Phytochemical screening:

The qualitative analysis of bioactive compounds for the methanolic flower extract of *Gmelina arborea* Roxb. has been performed in this study and there is wide range of phytochemical compounds present in the extracts showing;

Table 1: Phytochemical screening of methanolic extract of *Gmelina arborea* Roxb.

S No	Test name		Extracts	
			Aqueous	Alcoholic
1	Alkaloids	Mayer's reagent	+	+
		Dragondorrf's	+	+
		Wagner's	+	+
2	Tannins and phenolic compounds	Gelatin solution	+	+
		5% $FeCl_3$	-	+
		Iodine	+	+
3	Glycosides	Baljet test	-	-
		Legal's test	+	+
		Test for Coumarin	+	+
4	Carbohydrates	Fehlings A and B	+	+
		Barfoerd' s test	+	+
		Ninhydrine	+	+
5	Saponin	Shaking in test-tube	-	-
6	Flavonoids	Lead Acetate	+	+
7.	Steroids	Salkowski reaction	-	+
9	Protein	Buiret test	+	+

+ indicates presence whereas – indicates absence

Table 2. Florescent analysis of flower of *Gmelina arborea* Roxb.:

Sr. no.	Sample	UV light		Visible light
		366nm	254nm	
1	H_2O	Light Dull green	Florescent greenish yellow	Transparent yellow
2	$FeCl_3$	Dark purple	Dark green	Brownish black Yellow florescence
3	HCl	Light purple	Dark Green light	Brown light
4	HCl 50%	Lightest Green	Lightest green	Light yellowish Brown
5	HNO_3	Lightest Green	Florescent yellow Green	Yellowish brown
6	HNO_3 50%	Yellowish green	Florescent yellow Green	Yellowish
7	H_2SO_4	Dark purple	Dark purple	Dark brown
8	H_2SO_4 50%	Dark Dull green	Brown	Brown
9	NH_3	Dark green	Greenish yellow	Brown
10	NaOH	Dark green	Light green	Brown

HPTLC:

High Performance Thin Layer Chromatography (HPTLC) is a valuable tool for reliable identity. The TLC procedure was optimized with a view to separate the compounds. The developing system consists of toluene: ethyl acetate: acetic acid (7:2:1 v/v/v) gave a sharp and well-defined band with Rf. The 3-D spectrum of track are scanned at 254nm and 366nm is shown as below:

HPTLC Fingerprinting profile and Densitogram of *G. arborea* flower extract

Fig-1. Visible light

Fig-2. UV Light 254nm

Fig-3. UV Light 366nm

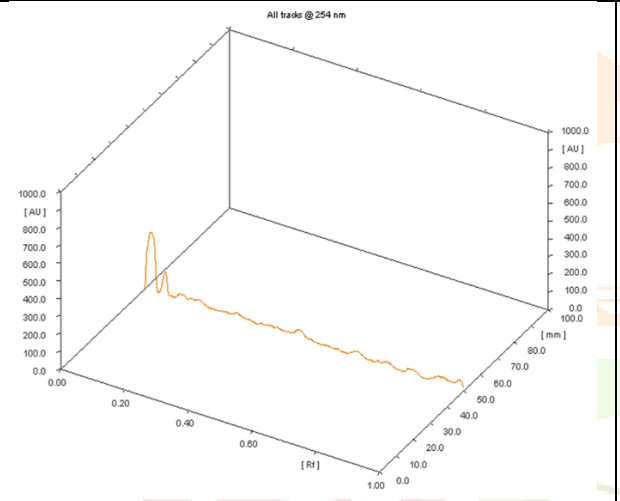

Fig-4. 3D display of *G. arborea* flower extract at 254nm

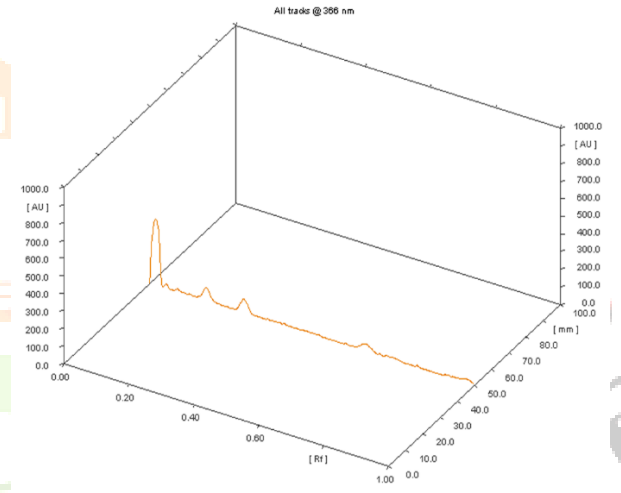

Fig-5. 3D display of *G. arborea* flower extract at 366nm

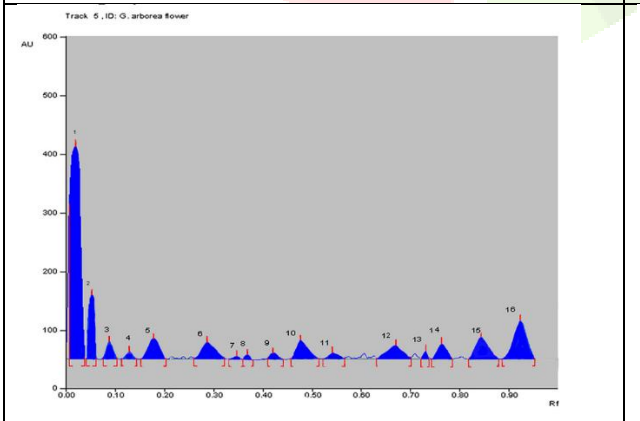

Fig-6. Densitogram of *G. arborea* flower extract at 254nm

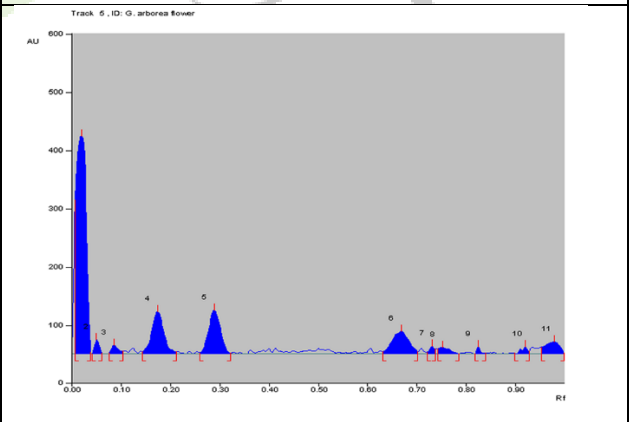

Fig-7. Densitogram of *G. arborea* flower extract at 366nm

Table- 3. Interpretation of Rf value at long and short wave length of UV Light:

Ultra-violet Light							
254nm UV Light				366nm UV Light			
Peak	**Stt Rf**	**Max Rf**	**End Rf**	**Peak**	**Stt Rf**	**Max Rf**	**End Rf**
1	0.01	0.02	0.04	1	0.01	0.02	0.04
2	0.04	0.06	0.07	2	0.04	0.05	0.06
3	0.09	0.12	0.13	3	0.08	0.09	0.10
4	0.13	0.15	0.16	4	0.14	0.17	0.21
5	0.16	0.17	0.20	5	0.26	0.29	0.32
6	0.27	0.29	0.31	6	0.63	0.67	0.70
7	0.32	0.33	0.35	7	0.72	0.73	0.74
8	0.35	0.36	0.37	8	0.74	0.75	0.78
9	0.41	0.41	0.43	9	0.82	0.82	0.84
10	0.45	0.48	0.51	10	0.90	0.92	0.93
11	0.52	0.52	0.55	11	0.95	0.98	1.00
12	0.63	0.67	0.70				
13	0.72	0.73	0.74				
14	0.74	0.77	0.79				
15	0.81	0.84	0.87				
16	0.88	0.93	0.95				

DISCUSSION

In HPTLC profile, each and every metabolite has played specific role and function in harmony with other metabolites within the organization framework of the cells in the defense mechanism of the plants. Chromatographic fingerprinting of phyto-constituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern. Here in this HPTLC study different peaks are observed at different Rf. Total 16 peaks are observed at 254nm, and 11 peaks are observed at 366nm of UV light out of which 5 peaks resembling each other at Rf 0.02, 0.17, 0.29, 0.67 and 0.73. The number of observed peaks shows the presence of numerous active constituents in the given sample of the flower.

According to WHO, it has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards.

CONCLUSION

The HPTLC fingerprinting analysis showed that various chemical constituents are present at different Rf values. Therefore, this study supports the use of flower extract of the *Gmelina arborea* Roxb. may be effective against several ailments. In future, it would be interesting to know about the chemical composition and better understanding of the mechanism of action of constituents present in the extract. It will help in the development of a new entity as drug for therapeutic application.

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