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PHYTOCHEMICAL SCREENING AND HPTLC FINGERPRINTING PROFILE OF STEM BARK EXTRACTS OF *MAYTENUS EMARGINATA*

Umesh Prabhakar Joshi * and Rajendra Dayaram Wagh

Department of Pharmacognosy, DCS's A. R. A. College of Pharmacy, Nagaon, Dhule - 424006, Maharashtra, India.

Keywords:

Phytochemical screening, Physical constants, HPTLC, Petroleum ether extract, Chloroform extract, Methanolic extract, *Maytenus emarginata*

Correspondence to Author:
Umesh Prabhakar Joshi

Assistant Professor,
Department of Pharmacognosy,
DCS's A. R. A. College of Pharmacy,
Nagaon, Dhule - 424006,
Maharashtra, India.

E-mail: joshiup2008@gmail.com

ABSTRACT: Objective: To establish the preliminary phytochemical screening and fingerprint profile of stem bark Extracts of *Maytenus emarginata by* using high-performance thin layer chromatography (HPTLC) technique. **Methods:** Preliminary phytochemical screening was done, physical constants were evaluated, and HPTLC studies were carried out. CAMAG make HPTLC system equipped with Linomat 5 applicator, TLC scanner 3, server vision CATS-server PH, version 2.5.18072.1 software were used. **Results:** Preliminary phytochemical screening of the extract showed the presence of flavonoids, glycosides, tannins, alkaloids, terpenoids, phenolic compounds, proteins, reducing sugars, fats, and oils. The fingerprint analysis of petroleum ether extract showed four peaks; chloroform extract showed four peaks and methanolic extract showed six peaks in 10 µl of the sample analyzed. **Conclusions:** It can be concluded that HPTLC fingerprint analysis of Petroleum ether, chloroform and methanolic extract of stem bark of *Maytenus emarginata* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations.

INTRODUCTION: Natural products continue to form a significant proportion of drugs in current use and those of under investigation. It is estimated that 56% of the lead compounds for medicines in the British National Formulary are natural products [1]. There are many plants in Indian Medicinal Plants, which are used for formulating therapeutic preparation according to Ayurveda and another traditional system of medicine [2]. The phytochemical analysis which is carried out during the 1970s and 1980s have discovered some alkaloids and other pharmacologically active substances that are currently being studied, and that can serve as models for new synthetic compounds[3].

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Standardization of plant materials is the need of the day. Several pharmacopeias containing mono-graphs of the plant materials describe only the physicochemical parameters. Hence, the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [4, 5]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [6].

Plants are a source of a large number of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials, *etc*. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide.

It is now believed that nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against some diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers [7].

Medicinal plants have been in use from time immemorial, and their utilization has been increasing day by day in the present time. Naturally obtained compounds are used as safer and easily biodegradable than synthetic compounds, and the problem of drug resistance observed in synthetic drugs is also reduced. Plants represent a source of leads for many pharmaceutical compounds and the phytochemical compounds, and secondary metabolites present in plants have been used in treating some human ailments [8].

Herbal medicines widely used in health-care in both developed and developing countries are complex chemical substances prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally. According to the estimation of the World Health Organization (WHO), about 80% of the world population uses herbs and other traditional medicines for their primary health care needs. Herbal drugs are finished labeling products that contain active chemical constituents such as aerial or underground parts of the plant or other plant material or a combination thereof, whether in the crude state or as herbal preparations [9]. Phytochemical substances are chemicals derived from plants, and the term is often used to describe a large number of secondary metabolic compounds found in plants. Preliminary phytochemical screening assay is a simple, quick and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemical substances in a mixture and an important tool in bioactive compound analysis [10]. HPTLC technique is a more efficient, faster with more reliable and reproducible results. HPTLC in combination with digital scanning profile, also provides accurate and precise retention factor (R_f) values and quantitative analysis of sample by *in-situ* scanning densitometry aided by formation of easily detected derivatives by post-chromatographic chemical reactions as needed, as well as a record of the separation in the form of a chromatogram with fractions represented as peaks with defined parameters including absorbance, R_f value, height and area [11]. HPTLC fingerprinting analysis could be used in proper identification of medicinal plants, as a valuable analytical tool in the routine quality control and standardization of herbal drugs [12] and as a chemotaxonomical tool in the plant systematic [13], for determination of medicinally bioactive components of the herbal medicine [14].

The present study was aimed to investigate HPTLC fingerprinting profile of petroleum ether, chloroform and methanolic extract of stembark of *Maytenus emarginata* for analyzing marker chemical constituents. *Maytenus emarginata* (Willd) D. Hou. belonging to the family Celastraceae is an evergreen tree that generally grows as small trees, bushes or lianas and has resinous stems and leaves. They tolerate various types of stresses of the desert, locally known as vickado, “Kankero” in Hindi, “Thorny staff tree” in English. Various parts of this plant contain immense medicinal properties such as shoots of the plant help for mouth ulcer [15]. The bark is ground to a paste and applied with mustard oil to kill lice in the hair. Pulverized leaves are given in milk to children as a vermifuge [16]. A decoction of the leaf twigs is used as a mouthwash to relieve a toothache. Ash of leaves is used to heal up sores, and wound gives a cooling effect. The leaves are burnt and mixed with ghee to form an ointment used to heal sores [17]. The tender leaves are chewed raw in the treatment of jaundice. The fruit is used in medicines to purify blood [18].

MATERIALS AND METHODS:

Plant Collection: The fresh stem barks of plant *Maytenus emarginata* were collected from Haripura and Manudevi region of Taluka Yawal, District Jalgaon, India. The selected plant was authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS’s, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra with authentication no. Bot/011/2016-17. Stem barks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of a grinding machine.

Preparation of Plant Extract: The extraction process was carried out using a continuous Soxhlet extraction method. About 300 gm of dry powdered plant material or bark was extracted in Soxhlet apparatus with 500 ml of petroleum ether for 16 h and successively with chloroform and methanol as solvent. After extraction, the solvent was removed using rotary vacuum evaporator to give a concentrated extract at 60 ºC in a water bath. It was then dried aseptically with the help of drier and subjected to preliminary phytochemical screening and HPTLC is fingerprinting [19, 20].

Phytochemical Screening: The different qualitative phytochemical tests were used for identification of the phytoconstituents present in the petroleum ether, chloroform and methanolic extracts of *Maytenus emarginata* stem barks for the identification of the various active chemical constituents like as alkaloids, phenols, flavonoids, tannins, terpenoids, glycosides, reducing sugars, fats and oils [21, 22]. The positive tests were noted as present (+) and absent (-).

Evaluation of Physical Constants: [23] Foreign matter, moisture content, total ash value, water soluble ash value, acid insoluble ash value, alcohol soluble extractive value, water-soluble extractive value was carried out [20]. The results are presented in **Table 2**.

HPTLC Fingerprinting Equipments: [24, 25, 26, 27]

Sample Preparation: The plant extracts residue was redissolved in 1ml of chromatographic grade methanol, which was used for sample application on Merck HPTLC plates pre-coated silica gel 60F 254 aluminum sheets.

Developing Solvent System: A number of solvent systems were tried for petroleum ether, chloroform, and methanolic extract, but the satisfactory resolution was obtained in the solvent system Toluene: Methanol (9:1) for petroleum ether extract, Toluene: Ethyl alcohol (7:3) for chloroform extract and Toluene: Ethyl acetate: Methanol: formic acid (5:4:1:1) for methanolic extract.

Sample Application: Application of bands of each extract was carried out using a spray technique. Sample was applied in duplicate on pre-coated silica gel 60F 254 aluminum sheets (20 × 10 cm) with the help of Linomat 5 applicator attached to CAMAG make HPTLC system, which was programmed through Vision CATS software (2.5.18072.1). The samples (10 µl) were spotted in the form of bands of width 8 mm with a 100 microlitre sample using a Hamilton syringe.

Development of Chromatogram: After the application of sample, the chromatogram was developed in Twin trough glass chamber 20 × 10 cm saturated with the solvent system Toluene: Methanol (9:1) for petroleum ether extract, Toluene: Ethyl alcohol (7:3) for chloroform extract and Toluene: Ethyl acetate: Methanol: formic acid (5:4:1:1) for methanolic extract for 20 min.

Detection of Spots: The air-dried plates were viewed in ultraviolet radiation to midday light. The chromatograms were scanned by densitometer at 540 nm 254 nm & 366 nm in Densitometry TLC Scanner 4. The R_f values and fingerprint data were recorded by Vision CATS software.

RESULTS AND DISCUSSION: The preliminary qualitative phytochemical screening of the crude powder of *Maytenus emarginata* stem barks was done to assess the presence of bioactive components. The presence of various phyto-constituents like alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid, and phenolic compounds was determined **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING ON PETROLEUM ETHER, CHLOROFORM AND METHANOLIC STEM BARKS EXTRACTS OF *MAYTENUS EMARGINATA*

S. no.	Phytochemical constituents	Petroleum ether extract	Chloroform extract	Methanolic extract
1	**Alkaloids**			
	a) Dragendorff's test	++	+	++
	b) Mayer's test	+	++	++
	c) Hagers's test	-	-	-
	d) Wagner's test	+	+	+
2	**Carbohydrates**			
	a) Molisch's test	-	+	+
	b) Fehlings test	-	+	+

	c) Benedict test	-	+	+
	d) Barfoed's test	-	+	+
3	**Saponins**			
	a) Foam Test	-	-	+
	b) Heamolytic test	-	-	+
4	**Steroids and Triterpenoids**			
	a) Salkowski test	++	++	-
	b) Liebermann - Burchard Reaction	++	++	-
	c) Lieberman's Reaction	++	+	-
5	**Phenolic compounds & Tannins**			
	a) Ferric chloride test	-	++	+++
	b)Lead acetate test	-	++	+++
	c) Potassium Dichromate test	-	+	+++
	d) Dilute HNO3	-	+	+++
6	**Proteins & Amino acids**			
	a) Biuret test	-	+	+
	b) Millions test	-	+	+
	c) Xanthoprotein test	-	-	-
	d) Ninhydrin test	-	+	+
7	**Flavone & Flavonoids**			
	a) Lead acetate test	-	++	+++
	c) Ferric chloride test	-	+	+++
	c) Sodium Hydroxide test	-	++	++
	d) Shinoda test	-	+	++

+++ = maximum; ++ = moderate; + = minimum; - = absent

TABLE 2: PHYSICAL CONSTANTS OF STEM BARK OF *MAYTENUS EMARGINATA*

S. no.	Evaluation Parameter	Value (%)
1	Foreign matter	0.25
2	Moisture content	8.25
3	Total Ash value	8.75
4	Acid-insoluble Ash Value	1.58
5	Water Soluble Ash Value	3.34
6	Alcohol soluble extractive value	16.24
7	Water-soluble extractive value	8.61

The HPTLC chromatogram, peaks, R_f values and area obtained for solvent extracts after scanning at 366 nm and UV R White are depicted in respective **Fig. 1** and **2** and **Table 3, 4** and **5**. The chromatograms of *Maytenus emarginata* at UV R White and 366 nm revealed that all sample constituents were separated without any tailing and diffuseness. It is evident from **Table 2** that in petroleum ether extract of *Maytenus emarginata* stem barks, eleven peaks are indicating the occurrence of at least eleven different components in petroleum ether extract. The components with R_f values were found to have % area ranging between 2.83 and 16.57 as shown in **Fig. 1**. The chloroform extract exhibited four spots indicating the occurrence of at least three different components. In this case, the components with R_f values were found to be more predominant as the % area was ranging from 10.97 to 63.66.

TABLE 3: R_f VALUES OF PEAK FORMED OF *MAYTENUS EMARGINATA* PETROLEUM ETHER EXTRACT

S. no.	Peak	R_f	Height	Area
1	1	0.029	0.0820	3.81
2	2	0.087	0.2106	9.79
3	3	0.121	0.2965	13.78
4	4	0.149	0.1561	7.26
5	5	0.227	0.1919	8.92
6	6	0.265	0.2915	13.55
7	7	0.368	0.2052	9.54
8	8	0.479	0.1387	6.45
9	9	0.623	0.0609	2.83
10	10	0.854	0.1617	7.52
11	11	0.945	0.3564	16.57

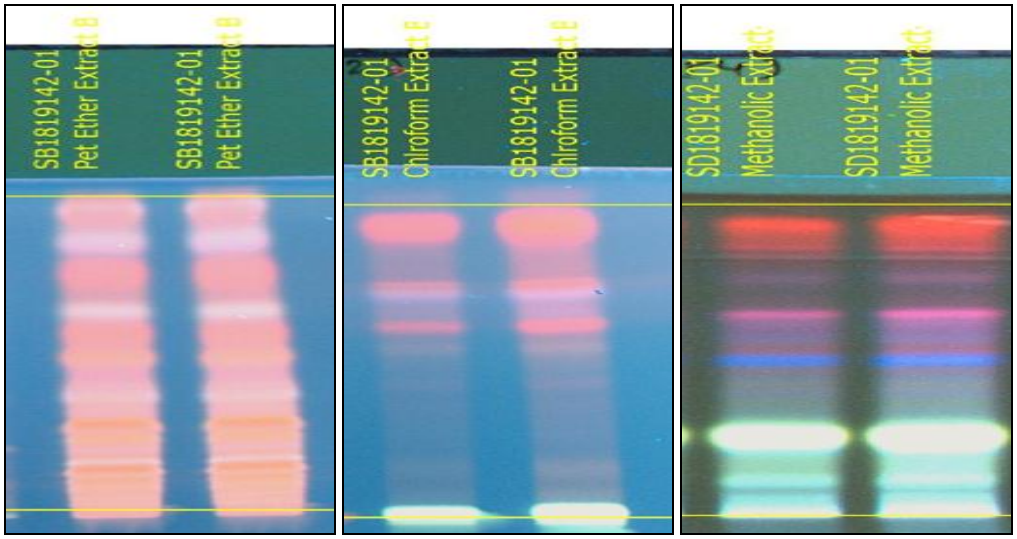
TABLE 4: R_f VALUES OF PEAK FORMED OF *MAYTENUS EMARGINATA* CHLOROFORM EXTRACT

S. no.	Peak	R_f	Height	Area
1	1	0.524	0.0415	10.97
2	2	0.708	0.2408	63.66
3	3	0.915	0.0959	25.37

TABLE 5: R_f VALUES OF PEAK FORMED OF *MAYTENUS EMARGINATA* METHANOLIC EXTRACT

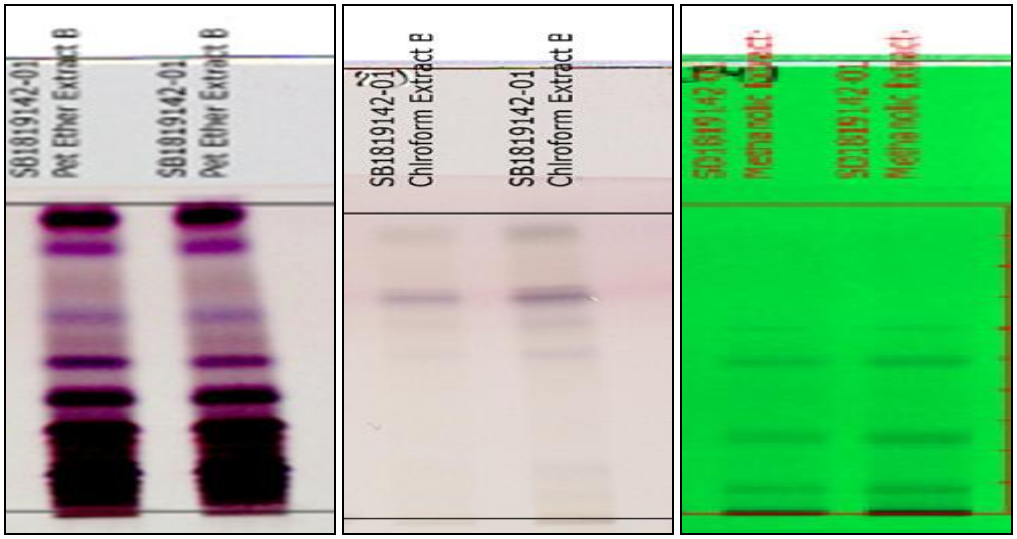
S. no.	Peak	R_f	Height	Area
1	1	0.009	0.3135	46.22
2	2	0.088	0.0813	11.98
3	3	0.256	0.1308	19.28
4	4	0.503	0.1060	15.63
5	5	0.607	0.0467	6.89

The methanolic extract exemplified five peaks in this case, the components with R_f values were found to be more predominant as the % area was ranging from 6.89 to 46.22. HPTLC fingerprinting is a valuable quality assessment tool for the evaluation of botanical materials, it allows for the analysis of a broad number of compounds both efficiently and cost-effectively. HPTLC 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 used for quality control analysis and quantitative determination of the plant material. The qualitative determination of HPTLC profiles from medicinal plants is a fast expanding field of research to identify the chemical profiles with curative properties [28]. 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 the image of HPTLC coupled with digital scanning profile is progressively attractive to herbal analysis to construct the herbal chromatographic fingerprint.



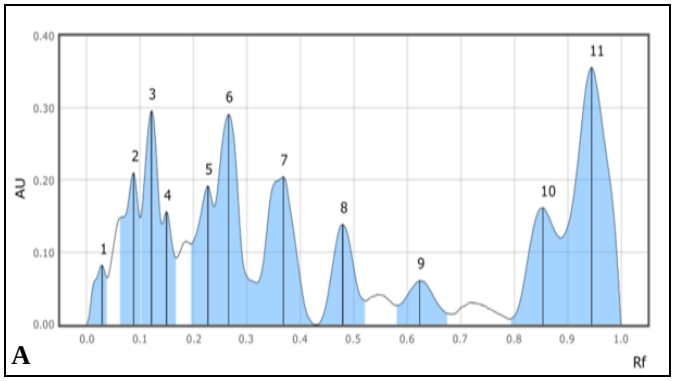
Petroleum ether extract **Chloroform extract** **Methanolic extract**

FIG. 1A: HPTLC FINGER PRINT PROFILE OF DIFFERENT SOLVENT EXTRACTS OF BARK OF *MAYTENUS EMARGINATA* AT UV 366 nm

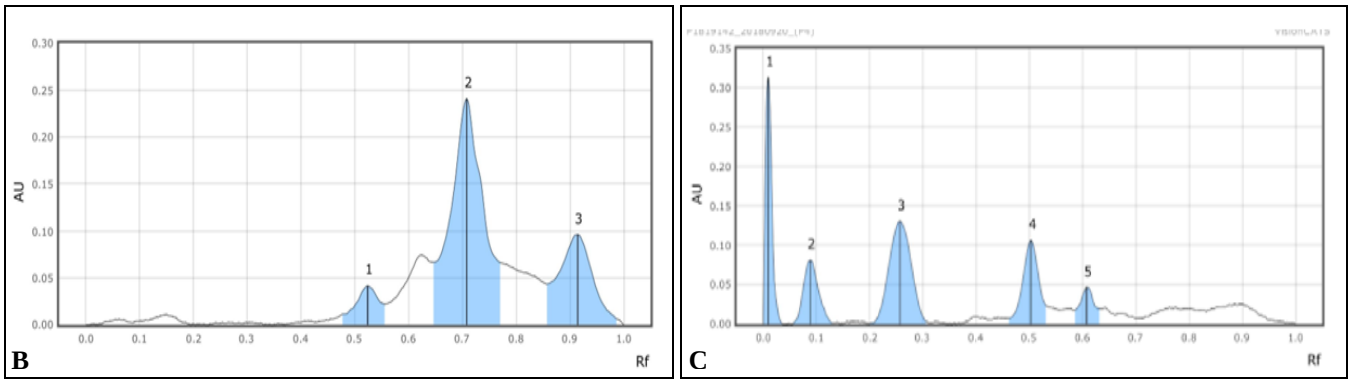


Petroleum ether extract **Chloroform extract** **Methanolic extract**

FIG. 1B: HPTLC FINGER PRINT PROFILE OF DIFFERENT SOLVENT EXTRACTS OF BARK OF *MAYTENUS EMARGINATA* AT R WHITE



PETROLEUM ETHER EXTRACT



CHLOROFORM EXTRACT **METHANOLIC EXTRACT**

FIG. 2: HPTLC CHROMATOGRAM OF DIFFERENT SOLVENT EXTRACTS BARK OF *MAYTENUS EMARGINATA* AT UV 366 nm

CONCLUSION: HPTLC fingerprint is nowadays best technique to check the genetic variability present in various medicinal plant species. HPTLC is a simple, renewable, less cost, defined and exact method in identifying various medicinal plant species and can also be used in standardization, characterization, and authentication of medicinally important plants. HPTLC finger printing helps in the identification and differentiation of the various adulterant, substitutes, and species. It can also serve as a biochemical marker for *Maytenus emarginata* in the plant studies and pharmaceutical companies.

Further research is necessary to carry out the characterization of the phytoconstituents and to execute quantitative estimation of phyto-constituents with the help of marker compounds is also important, but the HPTLC is fingerprinting data from the present study could be considered for setting up standards to *Maytenus emarginata*.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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