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Research Article

HPTLC Fingerprint Analysis of Bastard Oleaster (*Elaeagnus latifolia* Linn): An Edible Plant Used by the Tribal Community of Northeast India

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ABSTRACT

The present study is aimed to investigate the phytochemistry along with TLC and develop High Performance Thin Layer Chromatography (HPTLC) fingerprint profile for the first time of the three different extracts viz. acetone, methanol and aqueous extracts of the flower of *Elaeagnus latifolia*, the wild edible plant used by the tribal people of Meghalaya, India. Camag HPTLC system equipped with TLC Linomat V applicator, Camag TLC scanner III and winCATS software were used. Acetone, methanol and aqueous extracts of the flower of *Elaeagnus latifolia* were developed in suitable mobile phase using standard procedures and scanned under UV at 254nm and 366nm. Phytochemical screening showed presence of phytosterols, glycoside and saponins. The HPTLC fingerprint scanned at 366nm revealed 7 peaks with R_f value in the range of 0.14 to 0.79 for acetone extract, 6 peaks with R_f value 0.13 to 0.80 for methanol extract and 10 peaks with R_f value 0.16 to 0.80 for aqueous extract with mobile system I, similarly 8 peaks with R_f value in the range of 0.27 to 0.91 for acetone extract, 9 peaks with R_f value 0.02 to 0.89 for methanol extract and 4 peaks with R_f value 0.07 to 0.41 for aqueous extract with mobile system II. Phytochemical screening, TLC and HPTLC analysis of the flower of *Elaeagnus latifolia* can provide standard reference for the proper identification, authentication and quality control of the drug and will be helpful in differentiating the species.

Keywords: *Elaeagnus latifolia*, Elaeagnaceae, HPTLC fingerprinting, Standardization.

INTRODUCTION

The Northeastern region of India is rich in diversity of wild edible plant species, particularly in Meghalaya due to its diverse culture and home of large number of tribal people[1]. Meghalaya is one of the biodiversity rich states in India in terms of flora and fauna, which is due to varied altitude, climate, topography and status of soil[2]. Tribal knowledge on wild edible plants of Meghalaya, are used as edibles either in raw or in cooked forms[3]. This knowledge is passed on from generation to generation which is based on their, observation, trial and error and long experience[1]. Modern medicine has evolved from traditional system only after thorough chemical, pharmaceutical screening of plants. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern techniques and applying suitable standards[4]. Several pharmacopoeias contain monographs of the medicinal plant materials which describe only the physicochemical parameters, hence the modern methods of identification and quantification of active constituents in the plant material may be useful for proper standardization of medicinal plants and its formulations[5,6]. HPTLC fingerprinting is an important tool for quantification of active ingredients, authentication and quality control of medicinal plants. It is a modern, simple, rapid and accurate tool for detecting the marker compounds in the plant sample[7]. *Elaeagnus latifolia* Linn, belongs to the family Elaeagnaceae. It is commonly known as Bastard Oleaster. It is distributed throughout the hilly parts of India, Sri Lanka and China. In Northeastern states of India, it is common in Assam, Nagaland, Khasi and Jaintia hills of Meghalaya at an altitude of 1500m[8]. Flowers are many in a cluster, fruit 2.5cm long, seeds are ellipsoid with 8 strong blunt ribs and are two centimeters long[9]. The fruit is edible when ripe. It is pleasantly acidic and refreshing, used in the preparation of tarts and jellies. The flowers are considered cardiac and astringent. Bark is light yellow in color and are used as fuel[10]. The literature survey revealed that flowers of *Elaeagnus latifolia* has no scientific claims for HPTLC fingerprint profile.

MATERIALS AND METHODS

Plant Materials

The fresh flowers of *Elaeagnus latifolia* Linn. (Elaeagnaceae) were collected in bulk from the horticulture farm of ICAR Research complex, NEH Region, Shillong, Meghalaya, India, in the month of November 2014. The collected specimen were identified and authenticated by the Botanist Dr. A. A. Mao, Scientist – F, BSI, Eastern Regional Centre, Shillong, India and the

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Figure 1: Flower of *Elaeagnus latifolia*.

herbarium was deposited in S.K Patel College of Pharmaceutical Education and Research, Department of Pharmacognosy, Ganpat University, India.

Preliminary phytochemical screening

The powdered drug was subjected to systematic phytochemical screening by successively extracting them in different solvents and testing for the presence of various chemical constituents such as phytosterols, glycoside and saponins in different extracts by using standard procedures[4].

Thin Layer Chromatography (TLC)

Thin layer chromatography studies of the different extracts were carried out in various solvents using Precoated silica gel 60 F_{254} as an adsorbent which were procured from (E. Merck Ltd, Germany)[12,13]. The plates were developed and observed under UV at both 254nm and 366nm and showed prominent band separation with chloroform: toluene: methanol (4:4:2) and later sprayed with 5% sulphuric acid. The R_f values were calculated for different bands.

Preparation of sample

The flowers were shade dried at room temperature for 10 days and coarsely powdered. The powdered crude drug was macerated with acetone, methanol and water respectively. The extracts were filtered, and the filtrate were evaporated and dried under reduced pressure to yield the acetone, methanol and aqueous extracts. All the extracts were dissolved in respective solvents and were centrifuged at 3000rpm for 5mins, then filtered. The filtrate was used as the sample solution. The samples were inoculated on the pre-coated Silica gel 60 F_{254} aluminum sheets.

Chromatographic condition

Chromatogram was developed on 10 × 20cm aluminum Thin Layer Chromatography (TLC) plate pre-coated with 0.2mm layer of silica gel 60 F_{254} (E. Merck, Germany) stored in a desiccator. The application was done by Camag Linomat syringe (100µL sec^{-1}), mounted on a Linomat V applicator. Application of bands of each extract with different concentration were carried out at a distance of 8mm with the help of Linomat V applicator attached to Camag HPTLC system, which was programmed through winCATS software (Version 1.3.0) at λ_{max} 254 and 366nm using deuterium light source, the slit dimensions were 8 × 0.4mm and at λ_{max} 410nm using tungsten light source. The chromatograms were recorded[11-13].

Developing solvent system

The samples were applied in the form of bands at a distance of 8mm (distance to the lower edge was 10mm) was performed at 25ºC with dichloromethane: methanol: ammonia (90:9:1) as mobile system I and chloroform: toluene: methanol (4:4:2) as mobile system II in a Camag chamber previously saturated with solvent vapor for 20mins. The concentration of the sample 8,16,24,36µl for acetone and methanol and 5,10,15,20µl for aqueous extract were applied on the track at a distance of 8mm. After development, the plate was dried at 60°C in an oven for 5mins. Scanning was then performed with a Camag TLC Scanner III equipped with the winCATS Software.

Detection of spots

The chromatograms were scanned by the densitometer at 254 and 366nm. The R_f values and fingerprint data were recorded and the plate was kept in photo documentation chamber and captured the images.

RESULTS

The preliminary phytochemical screening of petroleum ether, chloroform, methanol, acetone and aqueous extracts of the flower of *Elaeagnus latifolia* showed the presence of phytosterols, glycoside and saponins (Table 1). The different solvent systems with different polarities were prepared for developing the TLC system for identification of constituents in aqueous extracts and the one showing better resolution was selected as the solvent system for the study. The plates were developed and observed under UV at both 254 nm and 366nm. The R_f values were calculated for different bands (Table 2). The HPTLC fingerprint scanned at 366nm revealed 10 peaks with R_f value 0.16, 0.22, 0.46, 0.48, 0.50, 0.51, 0.57, 0.66, 0.72 and 0.80 for aqueous extract (Table 3), 6 peaks with R_f value 0.13, 0.22, 0.26, 0.52, 0.63 and 0.80 for methanol extract (Table 4) and 7 peaks with R_f value 0.14, 0.31, 0.39, 0.53, 0.56, 0.58 and 0.79 for acetone extract (Table 5) when developed in mobile system dichloromethane: methanol: ammonia (90:9:1). Similarly, HPTLC fingerprint when developed in chloroform: toluene: methanol (4:4:2), when scanned at 366nm revealed 4 peaks with R_f value 0.07, 0.32, 0.35 and 0.41 for aqueous extract (Table 6), 9 peaks with R_f value 0.02, 0.04, 0.13, 0.33, 0.51, 0.63, 0.66, 0.81 and 0.89 for methanol extract (Table 7) and 8 peaks with R_f value 0.27, 0.36, 0.40, 0.46, 0.50, 0.56, 0.76 and 0.91 for acetone extract (Table 8).

The photo documentation of aqueous, methanol and acetone extracts observed at 254 and 366nm is given (Figure 2A, B) when developed in the mobile system I and (Figure 3A, B) when developed in the mobile system II. Photo documentation of HPTLC Chromatogram of track 4, 8 and 12 are given (Figure 4A, B, C) with mobile system I and similarly (Figure 5A, B, C) with mobile system II. Photo documentation of HPTLC fingerprint profile of all the tracks at 254nm is also given (Figure 6). These separated bands had different R_f values and the percentage areas of these were given in the Table 3,4,5 for solvent system I and Table 6,7,8 for solvent system II respectively. The HPTLC images shown in Figure 2A, B and Figure 3A, B indicated that all sample constituents were clearly separated without any tailing and diffuseness. The

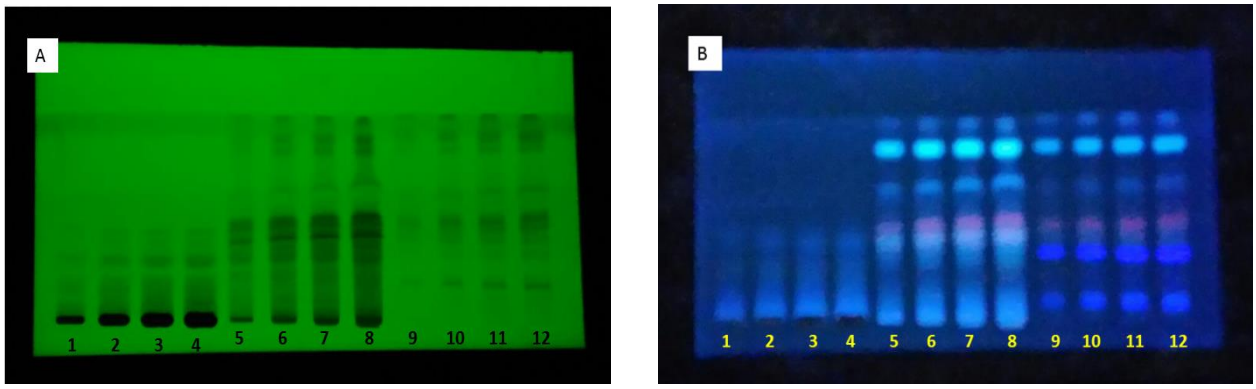

Figure 2: (A) TLC profile at 254nm, (B) TLC profile at 366nm with mobile system I - dichloromethane: methanol: ammonia (90:9:1).
Track 1,2,3,4 - 5,10,15,20µl respectively (Aqueous extract)
Track 5, 6,7,8 – 8,16,24,32µl respectively (Methanol extract)
Track 9,10,11,12 – 8,16,24,32µl respectively (Acetone extract)

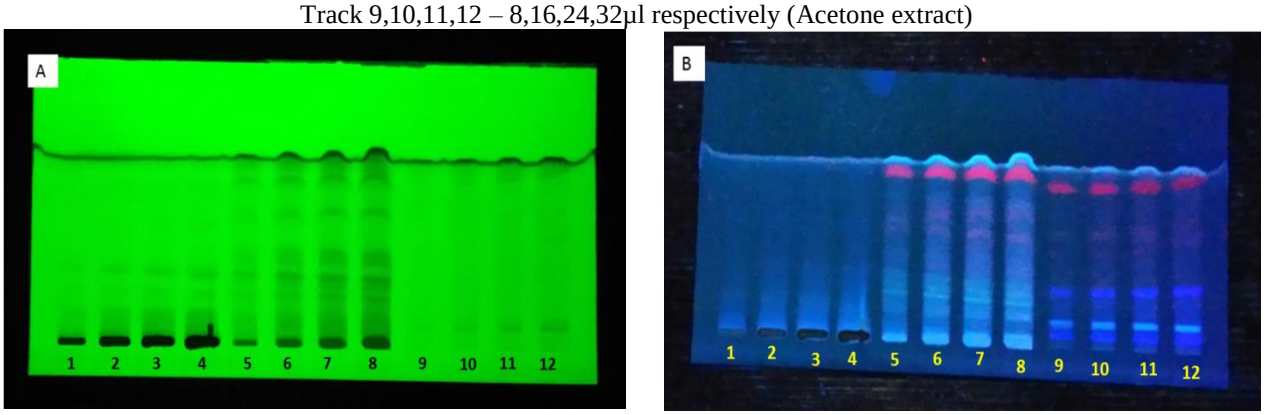

Figure 3: (A) TLC profile at 254nm, (B) TLC profile at 366nm with mobile system II - chloroform: toluene: methanol (4:4:2).
Track 1,2,3,4 - 5,10,15,20µl respectively (Aqueous extract)
Track 5, 6,7,8 – 8,16,24,32µl respectively (Methanol extract)
Track 9,10,11,12 – 8,16,24,32µl respectively (Acetone extract)

Table 1: Phytochemical constituents of the flower of *Elaeagnus latifolia*.

Phytochemical constituents	Flower extract
Alkaloids	-
Glycosides	+
Saponins	+
Phytosterols	+
Phenolics and tannins	-
Carbohydrates	-
Gums and mucilage	-

"+" Present, "-" Absent

Table 2: Qualitative chemical examination of the aqueous extract of flower.

Bands	R_f Values
1	0.10
2	0.22
3	0.37
4	0.46
5	0.56
6	0.66
7	0.71
8	0.93

Table 3: R_f value of the chromatogram of track 4 Aqueous extract of the flower of *Elaeagnus latifolia* when developed in the solvent system dichloromethane: methanol: ammonia (90:9:1).

Track	Peak	Max R_f	Area %
4	1	0.16	6.57
4	2	0.22	4.49
4	3	0.46	6.86
4	4	0.48	7.89
4	5	0.50	7.37
4	6	0.51	7.59
4	7	0.57	33.91
4	8	0.66	16.33
4	9	0.72	5.43
4	10	0.80	3.55

difference in number of peaks and R_f values indicates qualitative variations of the components in the extracts. The appearance of the peaks, R_f values and their areas provide corresponding fingerprint profiles for the flower of *Elaeagnus latifolia*. The chromatographic fingerprints obtained can be stored without any errors and changes for further investigation.

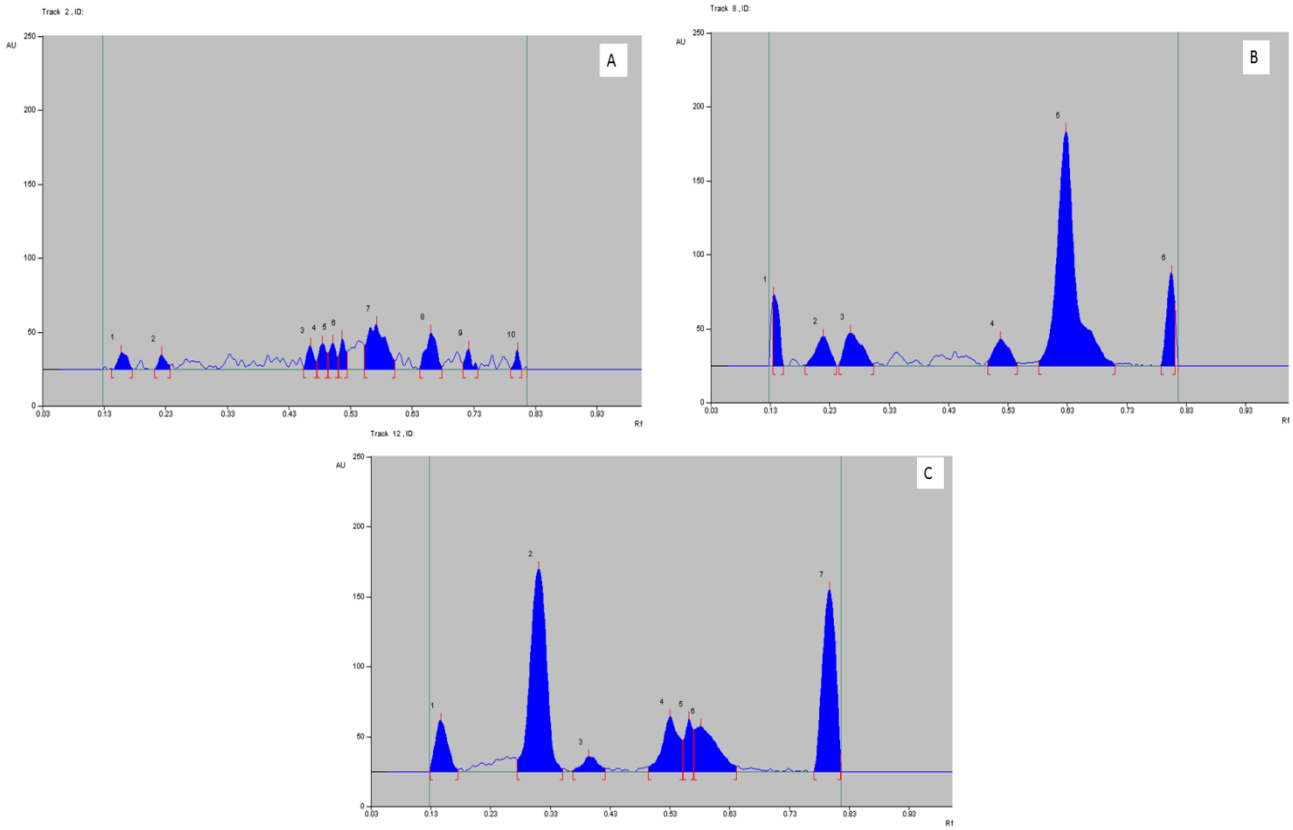

Figure 4: A- HPTLC Chromatogram of track 4(Aqueous extract) at 366nm, B- HPTLC Chromatogram of track 8(Methanol extract) at 366nm, C- HPTLC Chromatogram of track 12(Acetone extract) at 366nm with mobile system dichloromethane: methanol: ammonia (90:9:1).

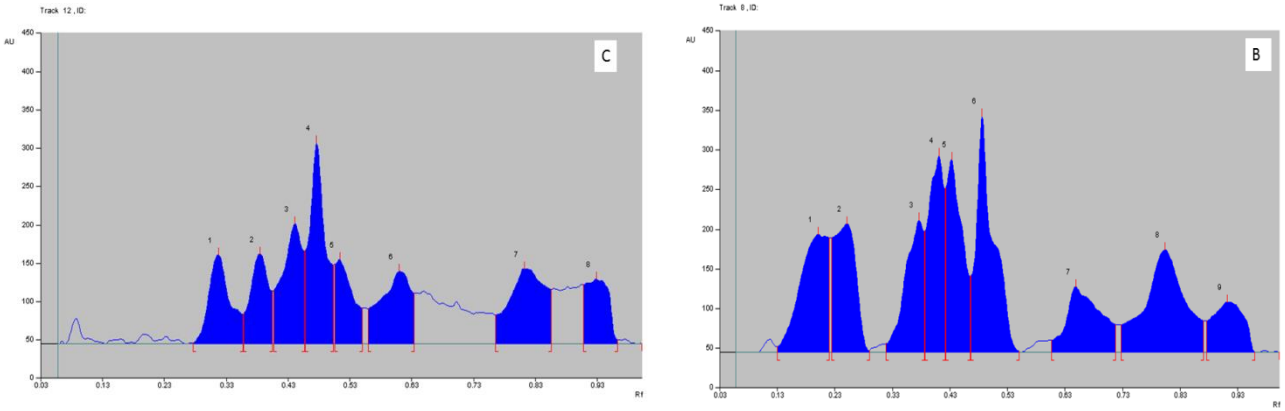

Figure 5: A- HPTLC Chromatogram of track 4(Aqueous extract) at 366nm, B- HPTLC Chromatogram of track 8(Methanol extract) at 366nm, C- HPTLC Chromatogram of track 12(Acetone extract) at 366nm with mobile system chloroform: toluene: methanol (4:4:2).

DISCUSSION

Phytochemical screening of the flower showed the presence of phytosterols, glycoside and saponins. An important observation from phytochemistry point is the absence of alkaloids. The TLC profile of aqueous extract indicated presence of eight compounds with the solvent system chloroform: toluene: methanol (4:4:2). HPTLC fingerprinting is a rational option to meet the need for more effective and powerful quality assessment of the traditional system of medicine throughout the world. It is a modern, rapid, accurate and simple tool for detecting the marker compounds in the plant sample[6]. The separation and resolution are better, the results are much more reliable and reproducible as compared to the TLC technique. HPTLC was performed in various solvents, dichloromethane: methanol: ammonia (90:9:1) and Chloroform: Toluene: Methanol (4:4:2) were found to be the most suitable solvents for proper elution of compounds with good separations. The present study gives information regarding various phytoconstituents present in acetone, methanol and aqueous extracts when scanned at 254 and 366nm. The separated spots had different R_f values and the percentage areas. The HPTLC images indicate that all sample constituents were clearly separated without any tailing and

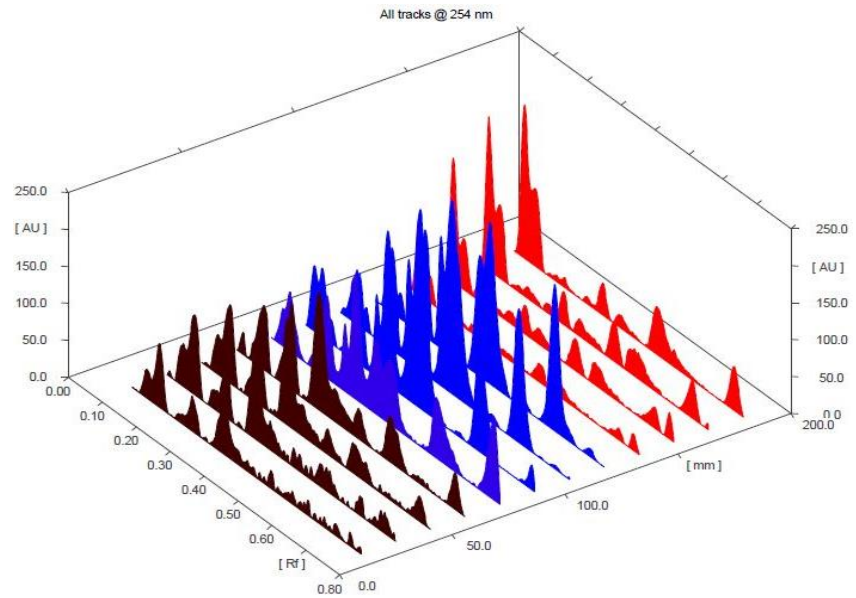

Figure 6: HPTLC fingerprint profile of all the tracks at 254nm of the flower of *Elaeagnus latifolia* with mobile system dichloromethane: methanol: ammonia (90:9:1).

Table 4: R_f value of the chromatogram of track 8 Methanol extract of the flower of *Elaeagnus latifolia* when developed in the solvent system dichloromethane: methanol: ammonia (90:9:1).

Track	Peak	Max R_f	Area %
8	1	0.13	6.67
8	2	0.22	6.00
8	3	0.26	8.33
8	4	0.52	6.10
8	5	0.63	62.81
8	6	0.80	10.08

Table 5: R_f value of the chromatogram of track 12 Acetone extract of the flower of *Elaeagnus latifolia* when developed in the solvent system dichloromethane: methanol: ammonia (90:9:1).

Track	Peak	Max R_f	Area %
12	1	0.14	7.25
12	2	0.31	36.95
12	3	0.39	2.68
12	4	0.53	10.02
12	5	0.56	4.59
12	6	0.58	11.53
12	7	0.79	26.98

Table 6: R_f value of the chromatogram of track 4 Aqueous extract of the flower of *Elaeagnus latifolia* when developed in the solvent system chloroform: toluene: methanol (4:4:2).

Track	Peak	Max R_f	Area %
4	1	0.07	17.05
4	2	0.32	34.90
4	3	0.35	31.96
4	4	0.41	16.09

diffuseness. The number of peaks and R_f values indicates qualitative variations of the components in the extracts.

The appearance of the peaks, R_f values and their areas provide corresponding fingerprint profiles for the flower of *Elaeagnus latifolia*. The chromatographic fingerprints obtained can be stored as an electronic image without any errors and change for further investigation.

CONCLUSION

Phytochemical screening of the flower showed the presence of phytosterols, glycoside and saponins. The TLC profile of aqueous extract indicated presence of eight compounds. The results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of the flower of *Elaeagnus latifolia* can provide standard fingerprints with selected solvent system and can be used as a reference for the proper identification, authentication and quality control of the drug and will be helpful in differentiating the species. Further, isolation and characterization of the active constituents from the plant is to be evaluated and reported in near future.

Table 7: R_f value of the chromatogram of track 8 Methanol extract of the flower of *Elaeagnus latifolia* when developed in the solvent system chloroform: toluene: methanol (4:4:2).

Track	Peak	Max R_f	Area %
8	1	0.02	8.84
8	2	0.04	21.84
8	3	0.13	22.67
8	4	0.33	4.34
8	5	0.51	4.02
8	6	0.63	5.41
8	7	0.66	2.55
8	8	0.81	15.33
8	9	0.89	15.00

Table 8: R_f value of the chromatogram of track 12 Acetone extract of the flower of *Elaeagnus latifolia* when developed in the solvent system chloroform: toluene: methanol (4:4:2).

Track	Peak	Max R_f	Area %
12	1	0.27	11.08
12	2	0.36	9.69
12	3	0.40	14.15
12	4	0.46	18.60
12	5	0.50	8.79
12	6	0.56	12.85
12	7	0.76	15.97
12	8	0.91	8.85

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