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Research Article

PHYSICOCHEMICAL, PHYTOCHEMICAL ANALYSIS AND HPTLC FINGER PRINTING OF *EUGENIA JAMBOLANA*

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ABSTRACT

Considerable literature on medicinal uses of *Eugenia jambolana* (Jamun) is available in the traditional as well conventional system of medicine. Jamun has been used in many traditional particularly Unani system of medicine since long. The present study aimed to standardize the crude drug "*Eugenia jambolana*" by doing qualitative and quantitative analysis of different extracts. Physic-chemical analysis (determination of loss on drying, determination of the pH value, swelling index, ash values, different extractive values and fluorescent analysis) was done on powdered of the seeds of the drug. The moisture content and the ash value were found within the recommended normal range. The value of different hot extracts (Petroleum ether, Methanol, chloroform and Aqueous) is more the cold extracts. Phyto-chemical screening revealed presence of protein, carbohydrate and phenols in aqueous extract while alkaloids and phenols are ruled out in methanol extract. HPTLC finger prints of methanolic extract shows different peaks confirming the presence of various constituents. Rf values indicates the presence of different chemical constituents like glycosides, tannins, proteins, triterpenes, saponins, steroids, and amino acids in the drug.

Keywords: *Eugenia jambolana*, HPTLC, Phyto-chemical analysis, Rf values.

INTRODUCTION

Jamun is a large, evergreen widely dispersed forest mostly found in India, Sri Lanka, Malaysia and Australia which is also cultivated for its edible fruits. The genus comprises around 1100 species and has a native range that extends from Africa and Madagascar through southern Asia. It is widely distributed all over India, Ceylon-Malaya and Australia.[1-3]

Jamun is a large evergreen tree of around 30 m height. The young bark is pale brown in colour, while the mature are darkish brown and scaly. The leaves are leathery, obovate-elliptic with 6 to 12 cm long with broad tip and less acuminate (may vary in shape, smooth and glowing with numerous nerves which unite in the margin), the tip being broad and less acuminate. The trees flowers once in a year and in the Indian subcontinent it is mostly during the month of June–July. Flowers are scented, greenish- white 7.5-13 mm across in branched clusters at stem tips, calyx cuplike; 4 petals, fused into a cap; many stamens. Fruits are found in the form of berries which are variable in size up to 1.5 to 3.5 cm long, ellipsoid or oblong, black with pink juicy pulp. These fruits are of dark-purple or nearly black, luscious, fleshy, and edible with a single large seed. Taste of fruits is a combination of sweet, mildly sour and astringent and tends to colour the tongue purple.[1]

Different parts of *Eugenia jambolana* Lam. (Jamun) used for medicinal purpose are fruits, leaves, dried seeds and bark.[2,4,5] Various pharmacological studies have been conducted on *Eugenia jambolana* and proven its antibacterial activity,[6-8] antifungal activity[9] free radical scavenging activity[10], anti-diabetic activity [11-14] hepato-protective effect[15] gastro-protective effect[16] hypolipidemic effect[8,17] anti-neoplastic effect.[18]

The therapeutic efficacy of the drug depends greatly on the use of proper and genuine raw material. Due to that reason, the assurance of safety, quality and subsequent efficacy of the drug have now become a major key issue, so the standardization of plant materials is needed. Standardization is imperative to get reproducible pharmacological activities and best quality product. Physicochemical properties and phytochemical analysis of drug helps in positive identification of the same. Keeping in view the medicinal properties of *Eugenia jambolana*, the present study aimed to analyze powder of the seed and extracts for physic chemical, phyto chemical properties and HPTLC fingerprinting of the drug.

MATERIALS AND METHODS

Collection and authentication of drug

The seeds of Jamun *(Eugenia jambolana)* were purchased from Khari Baoli, Old Delhi and taxonomically identified by taxonomist at Botany Department of Aligarh Muslim University, Aligarh and certificate is taken from the department regarding identification.

Physico-chemical analysis

Physico-chemical standardisation of the test drug was done on the parameters like loss on drying, determination of the pH value, swelling index, ash values, different extractive values and fluorescent analysis as per the recommended guideline of the WHO.[19, 20]

Preliminary phytochemical screening

Test for protein- It was done by Millon's test and Ninhydrin test.[21]
Test for carbohydrates-Fehling's solution test, Molisch's test and Iodine test was done to trace out carbohydrate in test drug.[21]
Test for phenols and tannins- Two millilitre of 2% solution of $FeCl_3$ was mixed with extract and observed for formation of black or blue green colour.[21]
Test for flavonoids- Shinoda test was performed to rule out the flavonoids in the drug.[21]
Test for glycosides- Glycosides were ruled out by performing Liebermann's test[21]
Test for alkaloids-Mayer's test, Dragendorff's reagent and Hager's test was performed to rule out alkaloids in the extract of the drug.[22]

Determination of extractive values

Cold extraction, hot extraction and successive extraction of the drug was done as per the procedure by WHO guideline.[19,23]

Thin layer chromatography (TLC)

Thin layer chromatography (TLC) assay was conducted using aluminium sheets of silica gel (TLC silica gel 60 $F_{254,}$ Merck). Extract of plant material was dissolved in a volatile (easily evaporated) solvent to produce a very dilute solution. A small amount of extract solution was applied on plate or stationary phase. The plate was developed in the developing chamber containing shallow pool of solvent just below the level of at which the sample was applied. The solvent was drawn up through the particles on the plate through the capillary action and as the solvent moves over mixture each compound dissolved in the solvent and moved up the plate. When the solvent front was moved to top end of adsorbent (within about 1 cm), the plate was removed from the developing chamber. Solvent was allowed to evaporate and the solvent front was marked. The plate was placed in iodine chamber and then visualized under UV light in UV chamber. Measurement of R_f was determined and value was expressed as decimal fraction and was calculated by dividing the distance the compound travelled from the original position by the distance the solvent travelled from the original position (solvent front).[24]

High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis of Jamun (*Eugenia jambolana)* extract was carried out for their qualitative analysis. Hence, simultaneous finger printing analysis of extracts was carried out using newly developed HPTLC method following the ICH guidelines.[25]

HPTLC sample preparation and chromatographic conditions

The methanolic extract of Jamun (*Eugenia jambolana)* was reconstituted using methanol (HPLC grade) and prepared 10 mg/mL concentration of the extracts. The samples were spotted in the form of bands (4.0 mm width), with a CAMAG microlitre syringe on pre-coated HPTLC silica gel aluminium plates ($60F_{254}$; 20 × 10 cm, Merck KGaA, Germany) using a CAMAG Linomat V (Muttenz, Switzerland) and were controlled by Win CATS software (CAMAG). A constant application rate of 10 µL/s was employed and the space between two bands was 6.0 mm. The slit dimension was maintained at 5.0 × 0.3 mm, and 20 mm/s scanning speed was employed. The solvent system of the Jamun (*Eugenia jambolana)* seed extracts consisted of Chloroform: Methanol: Formic acid 10%, (8:2:1.5, v/v/v). Linear ascending development was carried out in a 20 × 10 cm twin trough glass chamber, saturated with the solvent system. The optimized chamber saturation time for the solvent system was 15 min at room temperature. The length of chromatogram run was 80 mm. Subsequent to development, HPTLC plates were dried in an oven at 60°C for five min. Densitometric scanning was performed on a CAMAG TLC scanner IV (absorbance mode 530 nm) with Win CATS software after spraying the developed plate with anisaldehyde-sulphuric acid reagent and heating it on a hot air oven at 110°C for five min.

RESULTS

Table 1: Findings of Physio-chemical Standardisation

S. No.	Parameters	Mean ± SEM
1.	**Loss on drying**	7.86 + 0.46
2.	**Swelling Index**	1.1 ± 0.10%
3.	**Ash values**	
	Total ash	5.53 + 0.29
	Acid insoluble ash	2.46 ± 0.13
	Water soluble ash	1.0 ± 0.11
4.	**pH**	
	pH 1% solution	5.29 + 0.04
	pH 10% solution	4.82 ± 0.01
5.	**Hot extraction**	
	Petroleum ether	6.84 + 0.29
	Chloroform	12.8 + 0.79
	Methanol	16.86 + 1.04
	Aqueous	20.23 + 0.52
6.	**Cold Extraction**	
	Petroleum ether	1.23 ± 0.08
	Chloroform	1.66 ± 0.27%
	Methanol	7.73 + 0.87%
	Aqueous	16.1 + 0.47 %
7.	**Successive Extract Value**	
	Petroleum ether	6.8 + 0.15
	Chloroform	4.76 + 0.84
	Methanol	7.8 + 0.30
	Aqueous	**9.56 ± 0.32**

Table 2: Phytochemical Screening of Individual Extracts

Constituents	Test	Extracts			
		Petroleum ether	Chloroform	Methanolic	Aqueous
Alkaloids	Mayer's test	-	-	+	_
	Dragendroff's test	-	+	+	+
	Hager's test	-	-	+	_
	Wagner's test	-	+	+	-
Proteins	Millon's test	-	-	-	+
	Ninhydrin test	-	-	-	+
Carbohydrate	Fehling's solution test	-	-	-	+
	Benedict's reagent test:	-	-	-	+
	Molisch's test	-	-	-	+
Phenols	Shinoda test	-	+	+	+

HPTLC finger print analysis

Optimization of the solvent system for HPTLC and various chromatographic conditions:

The composition of the solvent system was optimized by testing different solvent compositions of varying polarities. Different tested compositions of solvent system, the desired resolution of the compounds, together with symmetrical and reproducible peaks, was achieved using the Chloroform: Methanol: Formic acid 10%, (8:2:1.5, v/v/v) solvent system. Well-separated and compact bands were visualized using anisaldehyde-sulphuric acid spraying reagent. HPTLC fingerprinting of extract has been shown in below Tables and Figures showing the qualitative analysis of extract.

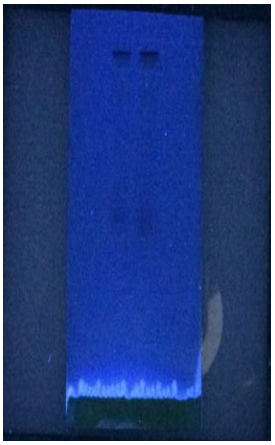

Figure 1: HPTLC finger print of Methanolic extract of EJ at 254 nm

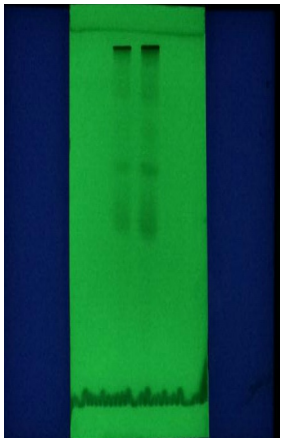

Figure 2: HPTLC finger print of Methanolic extract of EJ at 366 nm

Table 3: Rf values for Methanolic extract of EJ at 254 nm (Track 1 and Track II)

	Track I				Track II			
Peak	**Rf value**	**Max height**	**Max%age**	**Area %age**	**Rf value**	**Max height**	**Max%age**	**Area %age**
1	0.17 Rf	93.4 AU	6.53%	4.04%	0.16 Rf	48.2 AU	3.97%	2.59%
2	0.23 Rf	79.2 AU	5.54%	3.86%	0.21 Rf	48.8 AU	4.02%	2.32%
3	0.28 Rf	88.3AU	6.17%	5.80%	0.25 Rf	57.1 AU	4.70%	6.63%
4	0.40 Rf	46.5AU	3.25%	6.75%	0.39 Rf	31.9 AU	2.62%	5.60%
5	0.63 Rf	126.2AU	8.82%	9.86%	0.61 Rf	88.8 AU	7.31%	10.01%
6	0.80 Rf	120.5 AU	8.43%	22.83%	0.80 Rf	93.7 AU	7.71%	24.01%

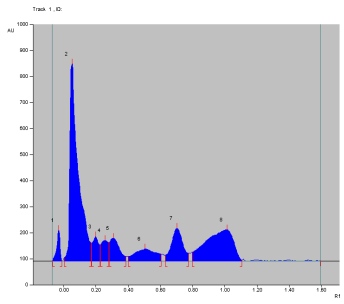

Figure 3: Chromatogram of extract at 254 nm (Track 1)

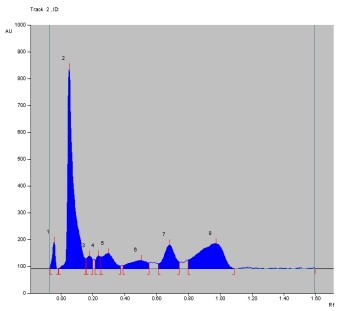

Figure 4: Chromatogram of extract at 254 nm (Track 2)

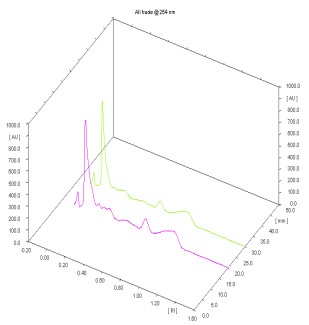

Figure 5: Overlay of extract at 240 nm

Table 4: Rf values for Methanolic extract at 366 nm (Track 1 and Track II)

Track I					Track II			
Peak	**Rf value**	**Max height**	**Max %age**	**Area %age**	**Rf value**	**Max height**	**Max %age**	**Area %age**
1	0.16 Rf	48.2 AU	3.97%	2.59%	0.09Rf	246.2 AU	13.56%	13.18%
2	0.21 Rf	48.8 AU	4.02%	2.32%	0.15 Rf	221.1 AU	12.18%	18.03%
3	0.25 Rf	57.1 AU	4.70%	6.63%	0.24 Rf	113.3 AU	6.24%	7.36%
4	0.39 Rf	31.9 AU	2.82%	5.60%	0.32 Rf	22.4 AU	1.24%	0.57%
5	0.61 Rf	88.8 AU	7.31%	10.01%	0.36 Rf	19.0 AU	1.05%	0.38%
6	0.80 Rf	93.7 AU	7.71%	24.01%	60.40 Rf	70.7 AU	3.89%	2.20%
7					0.46 Rf	18.7 AU	1.03%	0.59%
8					0.59 Rf	16.6 AU	0.91%	0.64%
9					0.65 Rf	43.6 AU	2.40%	0.82%
10					0.68 Rf	37.7 AU	2.08%	1.99%
11					0.73 Rf	35.9 AU	1.98%	1.21%
12					0.79 Rf	28.7 AU	1.58%	1.35%
13					0.86 Rf	30.9 AU	1.70%	2.19%
14					0.94 Rf	37.9 AU	2.09%	2.10%
15					0.98 Rf	44.1 AU	2.43%	5.10%
16					1.16 Rf	21.2 AU	1.17%	0.85%
17					1.30 Rf	17.2 AU	0.94%	1.39%
18					1.39 Rf	27.2 AU	1.50%	0.46%
19					1.42 Rf	19.9 AU	1.09%	0.47%
20					1.52 Rf	178.4 AU	9.62%	6.04%

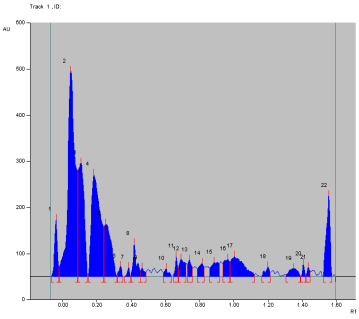

Figure 6: Chromatogram of extract at 366 nm (Track 1)

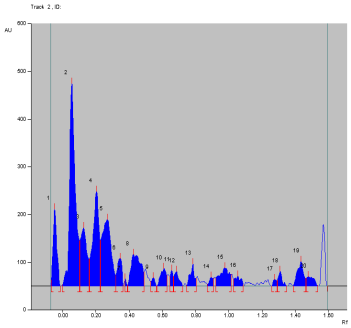

Figure 7: Chromatogram of extract at 366 nm (Track 2)

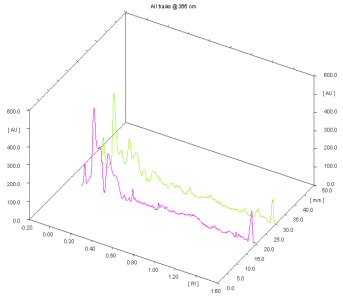

Figure 8: Overlay of extract at 366 nm

Table 5: Rf values for Methanolic extract at 540 nm (Track 1 and Track II)

Track I					Track II			
Peak	**Rf value**	**Max height**	**Max %age**	**Area %age**	**Rf value**	**Max height**	**Max %age**	**Area %age**
1	0.10 Rf	123.0 AU	7.98%	8.47%	0.08 Rf	188.4 AU	2.25%	17.87%
2	0.16 Rf	197.9 AU	12.84%	13.16%	0.15 Rf	181.3 AU	14.68%	24.79%
3	0.23 Rf	139.5 AU	9.05%	14.09%	0.31 Rf	20.2 AU	1.63%	0.83%
4	0.32 Rf	57..6 AU	3.74%	2.90%	0.35 Rf	18.4 AU	1.49%	0.80%
5	0.37 Rf	15.3 AU	0.99%	0.23%	0.40 Rf	54.3 AU	4.40%	2.80%
6	0.39 Rf	66.0 AU	4.28%	7.29%	0.59 Rf	11.4 AU	0.93%	0.59%
7	0.53 Rf	17.1 AU	1.11%	0.61%	0.64 Rf	34.7 AU	2.61%	1.15%
8	0.57 Rf	37.1 AU	2.41%	2.26%	0.68 Rf	36.0 AU	2.91%	3.35%
9	0.65 Rf	32.8 AU	2.13%	0.95%	0.73 Rf	35.5 AU	2.87%	2.73%
10	0.67 Rf	30.0 AU	1.95%	1.54%	0.79 Rf	21.7 AU	1.76%	2.07%
11	0.75 Rf	45.9 AU	2.98%	2.08%	0.91 Rf	11.4 AU	0.92%	0.85%
12	0.87 Rf	18.2 AU	1.18%	0.73%	0.1.16 Rf	25.4 AU	2.06%	1.55%
13	0.92 Rf	38.2 AU	2.48%	4.09%	1.30 Rf	22.0 AU	1.78%	2.85%
14	1.03 Rf	20.7 AU	1.35%	1.41%	1.40 Rf	19.5 AU	1.58%	0.46%
15	1.26 Rf	13.9 AU	0.90%	0.51%	1.52 Rf	156.9 AU	12.70%	8.40%
16	1.29 Rf	30.6 AU	1.98%	1.27%				
17	1.39 Rf	51.9 AU	3.37%	3.45%				
18	1.47 Rf	19.1 AU	1.24%	1.62%				

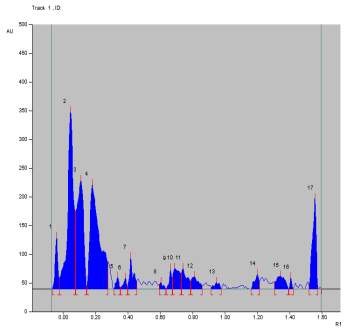

Figure 9: Chromatogram of extract at 540 nm (Track 1)

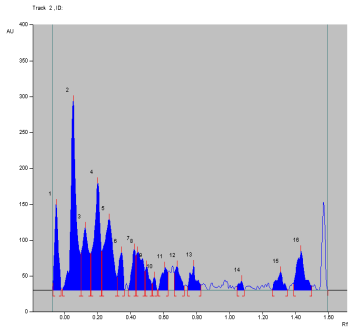

Figure 10: Chromatogram of extract at 540 nm (Track 2)

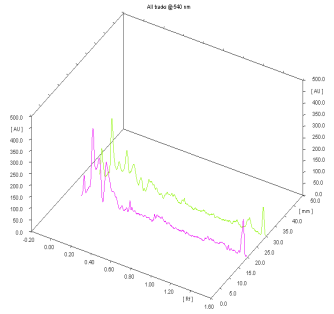

Figure 11: Overlay of extract at 540 nm

Table 6: Rf values for Methanolic extract at 640 nm (Track 1 and Track II)

	Track I				Track II		
Peak	**Rf value**	**Max height**	**Max %age**	**Area %age**	**Max height**	**Max %age**	**Area %age**
1	0.10 Rf	87.7 AU	7.80%	8.94%	87.7 AU	7.80%	8.94%
2	0.16 Rf	149.4 AU	13.29%	13.96%	149.4 AU	13.29%	13.96%
3	0.23 Rf	98.6 AU	8.77%	13.64%	98.6 AU	8.77%	13.64%
4	0.32 Rf	52.7 AU	4.69%	3.55%	52.7 AU	4.69%	3.55%
5	0.39 Rf	57.1 AU	5.07%	3.52%	57.1 AU	5.07%	3.52%
6	0.44 Rf	53.1 AU	4.72%	5.50%	53.1 AU	4.72%	5.50%
7	0.49 Rf	33.7 AU	2.99%	2.04%	33.7 AU	2.99%	2.04%
8	0.53 Rf	18.1 AU	1.60%	0.80%	18.1 AU	1.60%	0.80%
9	0.57 Rf	31.7 AU	2.82%	3.01%	31.7 AU	2.82%	3.01%
10	0.67 Rf	33.1 AU	2.95%	2.96%	33.1 AU	2.95%	2.96%
11	0.75 Rf	34.0 AU	3.02%	3.17%	34.0 AU	3.02%	3.17%
12	1.05 Rf	12.7 AU	1.13%	0.84%	12.7 AU	1.13%	0.84%
13	1.26 Rf	25.2 AU	2.24%	2.64%	25.2 AU	2.24%	2.64%
14	1.39	54.4	4.83	6.72	54.4	4.83	6.72

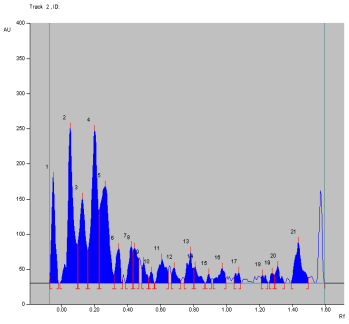

Figure 12: Chromatogram of extract at 640 nm (Track 1)

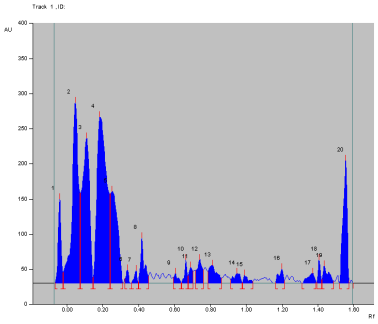

Figure 13: Chromatogram of extract at 640 nm (Track 2)

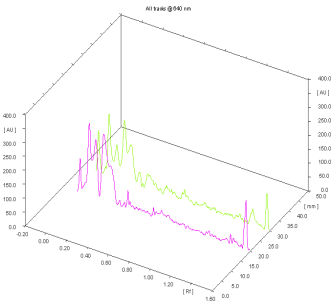

Figure 14: Overlay of extract at 640 nm

DISCUSSION

Standardization is the quality control tool establishing standards or innate characteristics, definitive quantitative and qualitative values that gives assurance of quality, efficacy and safety of the drug[26]

The procured test drug was authenticated by taxonomist at department of Botany, Faculty of Science, Aligarh Muslim University, Aligarh and was standardized based on ash value, loss on drying, determination of pH, swelling index[19] and fluorescence analysis.[20] The loss on drying of powdered drug and swelling index was found 7.86% and 1.1% respectively. Loss on drying indicates the amount of moisture content of the drug. The ash value was 5.53% of the dry weight. The water soluble and acid insoluble ash was found to be 1.0% and 2.46% respectively. Ash values indicate the presence of high inorganic content. However, the ash content is possibly due to the Na^+ and Ca^{2+} salts which are not harmful. pH value of drug is determined 5.29 at 1% solution and 4.82 at 10% solution.

Extractive values (cold and hot) of test drug were determined with different solvents including petroleum ether, methanol, water and chloroform. Cold extractive value of test drug (petroleum ether, chloroform, methanol, water,) was found to be 1.23%, 1.66%, 7.73% and 16.1% respectively. While as hot extractive values with same solvents were 6.84%, 12.8%, 16.86% and 20.23% respectively. Higher extractive values in hot extraction indicating the effect of elevated temperature on extraction. Successive extraction was done with different solvents by method of soxhletion and constant extractive values were recorded. Successive extractive values were 6.8%, 4.76%, 7.8%, 9.56% respectively. Extractive values denote the quantity of compounds in a drug.

Seeds of Jamun *(Eugenia jambolana)* were subjected to preliminary phytochemical screening involving successive

solvent extraction by different solvents in order of increasing polarity to obtain diverse polar and non-polar constituents possessing different solubility pattern followed by various chemical tests for qualitative detection of various chemical constituents. Preliminary phytochemical analysis of different seed extracts (chloroform, methanolic, aqueous) shows presence of phenols. Alkaloids were present highest in methanolic extract while in trace in the aqueous extract. Protein was only present in aqueous extract while alkaloids were found mostly in methanolic extract with traces in aqueous extract. Solvent polarities play a key role in increasing phenolic solubility and are good solvent systems for the extraction of polar antioxidant.[27]

TLC was conducted on the extracts (chloroform, methanol, pet ether) by running the plate in different solvent systems. Different solvent compositions of solvent system were tried to get desired resolution of compounds. Solvent system for performing HPTLC with desirable resolution of compounds together with symmetrical and reproducible peaks was achieved using Chloroform: Methanol: Formic acid 10%, (8:2:1.5, v/v/v). After spraying the plates with anisaldehyde- sulphuric acid reagent and heating it on hot air oven at 110^0C densitometric scanning was done by CAMAG TLC scanner IV at different wavelength of 254 nm, 366 nm, 540 nm and 640 nm. Rf values were determined by dividing the distance the solvent travelled from the original position and was expressed as decimal fraction. The results from HPTLC finger print scanned at wavelength 254 nm for methanolic extract of *Eugenia jambolana* (Jamun) revealed the presence of 06 polyvalent phytoconstituents. The Rf value ranged from 0.17 to 0.80. It is also clear from and chromatogram that out of 06 components, the component (6) with Rf value was found to be predominant as the %age area is 22.83% and highest concentration of the phytoconstituent was found to be 8.43% and its corresponding Rf value was found to be 0.80. Table 3, Figure 3-5.

HATS at 366 nm revealed again 06 components with a range of 0.16-0.80 with track 1 while 20 components with track 2 which lies in range of 0.09 to 1.52. In HATS, component 1 with Rf value of 0.09 having higher percentage of 13.56% and component 2 with Rf value of 0.15 showed maximum % sage area of 18.03%. Table 4, Figure 6-8.

At 540 nm HATS showed 18 no. of components with a range 0.10-1.47 having component 2 (Rf value 0.16) with higher %age of 12.84% and component 3 (Rf value 0.23) with maximum %age area of 14.09%. Table 5, Figure 9-11.

HPTLC scanning at 640 nm (Track 1 as well Track 2) revealed 14 components in HATS. Rf value ranged from 0.10 –1.39. The component with Rf value 0.16 has highest %age area of 13.96% and maximum % age of 13.29%. Table 6, Figure 12-14.

HPTLC finger print studies confirmed the results of phytochemical screening by the presence of various coloured bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytocompounds. HPTLC finger prints of methanolic extract shows different peaks confirming the presence of various constituents. Rf values indicates the presence of different chemical constituents like glycosides, tannins, proteins, triterpenes, saponins, steroids, and amino acids.[28]

CONCLUSION

In phytochemical analysis, protein, carbohydrate and phenols showed maximum amount. The present study also showed physico-chemical details of the seeds of the *Eugenia jambolana*. HPTLC fingerprinting of the drug confirms the presence of various constituent of the drug. The present work was taken up with a view to lay down standards, which could be useful to establish the authenticity of the drug in its medicinal use for maximum efficacy.

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