Quantification of total phenolic, flavonoid content and HPTLC fingerprinting of *Senecio laetus*

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Abstract
Senecio laetus was chosen for this study because it lacks data, making it necessary to carry out its phytochemical screening, total phenolic and flavonoid content analysis, TLC and HPTLC fingerprinting, and establish the standard pharmacognostical parameters for this plant. The quality and purity of the plant species can be determined with the use of these factors. To find several phytoconstituents, preliminary qualitative phytochemical screening was conducted. Additionally, the contents of phenolic and flavonoid compounds were quantified. Also, methanolic extract HPTLC fingerprinting was carried out. Different fractions contained carbohydrates, alkaloids, glycosides, saponins, flavonoids, tannins, phenols, etc., According to preliminary phytochemical analysis. With 785.52 mg/g of GAE and 202.33 mg/g of RE, respectively, ethyl-acetate has the highest phenolic and flavonoid content among all the fractions. Alcoholic extract's HPTLC fingerprinting revealed seven polyvalent phytoconstituents.

Keywords: *Senecio laetus*; total phenolic content; total flavonoid content, HPTLC fingerprint.

1. INTRODUCTION

Senecio is a genus of daisy family (*Asteraceae*). It belongs to the tribe *Senecioneae* and is one of the largest genera [1] of flowering plants comprising about 1500 species and is distributed worldwide with remarkable morphological variations[2]. The word "Senecio" is derived from the Latin word "senex," which means "old" (an old man), in reference to the grey pappus on the cypsela, which is made up of modified calyces[3].Senecio species are most frequently used in traditional medicine as anti-inflammatories andantiemetics[4]. The fatalities of countless grazing animals have been associated with some species' deadly pyrrolizidine alkaloids and furanoeremophilanes[5].

A native medicinal plant called *Senecio laetus,* also referred to as *Senecio chrysanthemoides*, can be found between 2400 and 3600 meters above sea level in the northwest Himalayas. Traditionally, mouth and throat irritation has been treated using *S. laetus*[6].

In this study, the plant under investigation is evaluated for its qualitative phytochemical screening, total phenolic and flavonoid content, TLC analysis and HPTLC fingerprinting. All these parameters serve as an important guide for authentication, identification, purification, and quality control of the plant.

2. MATERIALS AND METHODS

2.1 Plant Identification, Collection and Authentication

Whole aerial parts *i.e.,* stem, leaf and flowers of *Senecio laetus*were collected from Yanihama, district Ganderbal, Kashmir, India. at an altitude of 1700 m in the month of July.
Prof. Akhtar H. Malik Curator, Department of Botany, Centre for Biodiversity and Taxonomy (CBT), University of Kashmir, identified and authenticated the plant under voucher specimen no. 2823- (KASH). For future reference, the plant specimen was also submitted to the University of Kashmir's herbarium.

2.2 Extract preparation

The plants aerial parts were cleaned and dried in shadow for around 15 days. The dried plant was then powdered using a grinding mill. The milled plant material was weighed and then subjected to cold maceration method of extraction with ethanol and water (8:2). The plant extract was filtered and then completely dried using rotary evaporator at 40 ºC. Fractionation of the extract by liquid-liquid extraction method using hexane, dichloromethane (DCM), ethyl-acetate and n-butanol was carried out sequentially. All the fractions were dried using rotary evaporator. The percentage yield was calculated separately for each fraction and was stored in air-tight containers at 4℃.

2.3 Qualitative phytochemical screening

Several fractions of *S. laetus* were evaluated for qualitative phytochemical screening, carried out according to protocol [7-12].

2.4 Total phenolic content

The content of total phenolics in various fractions was determined in 96-well plate using *Folin–Ciocâlteu reagent* (FCR) as per the method defined by Karou [13] with minor variations. 100 µL of various plant fractions (1 mg/mL) with 400 µL of distilled water was treated with 150 µL of *Folin–Ciocâlteu reagent* (10% v/v). The mixture was incubated at room temperature for 3-5 min after which 500 µL of sodium carbonate (20% w/v) was added. It was then shaken and then incubated for 1-2 hr in dark and the absorbance was read at 765 nm with a microplate reader (Bio-Rad, Belgium) against a blank (the reagent was replaced by water). Gallic acid (10- 90 µg mL^{-1}) was used as standard and the phenolic content was expressed as mg of Gallic Acid Equivalents (GAE) per gram of extract (mg GAE/g of extract)[14].

2.5 Total flavonoid content

The content of total flavonoids in various fractions was also determined using 96-well plate as per the method defined by Herald [15] with few variations. 300 µL of various plant fractions (1 mg/mL) were mixed with 3 mL methanol (30 %). This was followed by adding 150 µL of $NaNO_2$ (0.5 M) and 150 µL of $AlCl_3$ (0.3 M), mixed properly and incubated for 5 min at room temperature. To this was added 1mL of NaOH (1M). The 96-well plate was shaken for few seconds and the absorbance was read at 510 nm with a microplate reader (Bio-Rad, Belgium) against a blank for 30 s in the plate reader prior to absorbance measurement at 510 nm. Rutin (50-250 µg mL^{-1}) was used as a standard to generate a calibration curve the flavonoid content was expressed as mg of Rutin Equivalents (RE) per gram of extract (mg RE/g of extract)[16].

2.6 Thin layer chromatography analysis

Two grams of the powdered plant material was extracted with 20 ml of alcohol and refluxed on water bath for half an hour. The solution was filtered and the filtrate was concentrated under vacuum to 5 ml. Applied 3 µl of the alcoholic extract on TLC plate and developed using Toluene: Ethyl acetate: Formic acid (7: 2: 0.01) as mobile phase. The plate was then dried in air after development and examined under UV (254 nm, 366 nm). Then dipped the plate in vanillin-sulphuric acid reagent followed by heating at 110 ºC for 5 min and observed under visible light.

2.7 High performance thin layer chromatography profile

HPTLC studies were performed using the method of Harborne [17] and Wagner [18]. To achieve HPTLC fingerprinting, a Swiss CAMAG-HPTLC machine with a Linomat 5 sample applicator was employed. Chromatographic separation was performed using precoated silica gel HPTLC aluminum plates 60F-254 (20 cm 20 cm, 0.2 mm thicknesses, 5–6 m particle size, E-Merck, Germany). TLC plate was developed using Toluene: Ethyl acetate: Formic acid (7: 3: 0.01) as mobile phase subsequently experimenting with several solvent system combinations to achieve great separation and distinct peaks for analysis. After developing them, the plates were allowed to dry in air, recorded the finger print and densitometric chromatogram of the sample at 254 and 366 nm.

3. RESULTS:

3.1 Qualitative phytochemical screening

The qualitative phytochemical screening showed the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids, tannins, phenols, etc. as shown in Table 1.

Table 1: Phytochemical analysis of various fractions of *Senecio laetus*

S. No.	Phytochemical Test	Fraction				
		Hexane	Dichloro methane	Ethyl acetate	Butanol	Residual
1.	Alkaloids	+	+	+	-	-
2.	Carbohydrates	-	-	+	+	+
3.	Proteins	-	-	+	+	+
4.	Amino Acids	-	-	+	+	+
5.	Saponins	-	-	-	+	+
6.	Tannins	-	-	-	+	+
7.	Glycosides	+	+	-	-	-
8.	Steroids	+	+	+	+	-
9.	Flavonoids	-	+	+	+	+
10.	Gum & mucilage	-	-	-	-	-
11.	Phenolics	+	+	+	+	+
12.	Fixed oils and fat	+	+	-	-	-
13.	Terpenoids	+	+	-	-	-
14.	Diterpenes	+	+	+	+	+

3.2 Total phenolic content (TPC) and total flavonoid content (TFC)

Since they are robust antioxidants and have a higher therapeutic rate in the management of many diseases brought on by oxidative stress, phenol and flavonoids have drawn a lot of attention. The results of TPC and TFC of various fractions are depicted in the Table 2. The phenolics present in

various fractions are expressed as mg Gallic acid equivalents per gram of fraction (mg GAE/g) evaluated using regression equation: y = 0.0116x + 0.0371 and R^2 =0.9978 [x = absorbance; y = GAE]. Similarly, the flavonoids in various fractions are expressed as mg Rutin equivalents per gram of fraction (mg RE/g) evaluated using regression equation y = 0.0015x + 0.0305 and R^2 = 0.9961 [x = absorbance; y = RE].

TPC of various fractions showed variation from 12.18 to 785.52 mg GAE/g dry weight of fraction. ethyl-acetate contains highest phenolic content followed by butanol, residual and dichloromethane, with least TPC in hexane fraction as shown in Figure 1. TFC of various fractions varied from 101.67 to 202.33mg RE/g dry weight of fraction. Ethyl-acetate contains highest flavonoid content followed by butanol, residual and dichloromethane fraction as shown in Figure 1.

Table 2 Total Phenolic and Flavonoid contents of various fractions of *Senecio laetus*.

Fraction	Total Phenolic Content in GAE (mg/g)	Total Flavonoid Content in RE (mg/g)
Hexane	12.18 ± 0.54	ND
Dichloromethane	361.12 ± 5.71	197.67 ± 4.06
Ethyl-acetate	785.52 ± 11.96	202.33 ± 5.53
Butanol	289.5 ± 6.08	191.06 ± 5.07
Residual	280.08 ± 5.98	145.29 ± 3.85

The values are represented as mean ± SD (n=3)

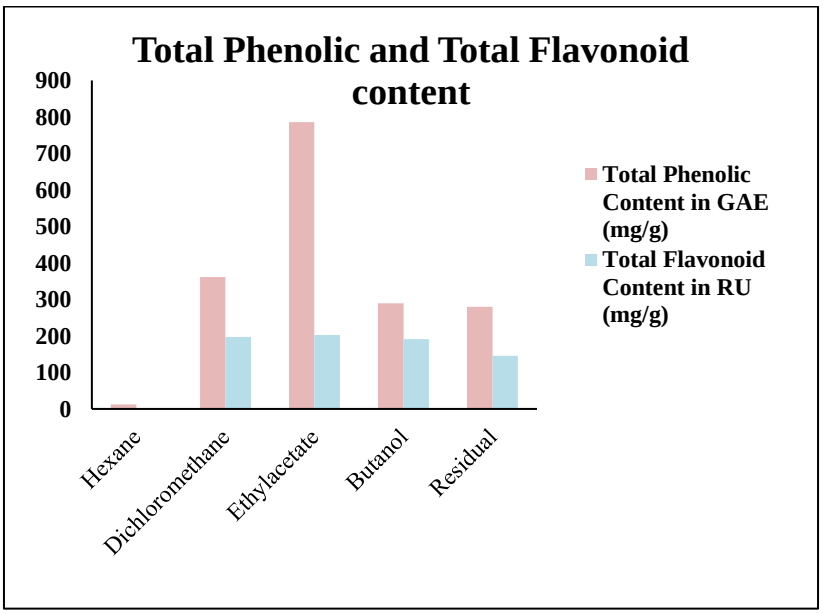
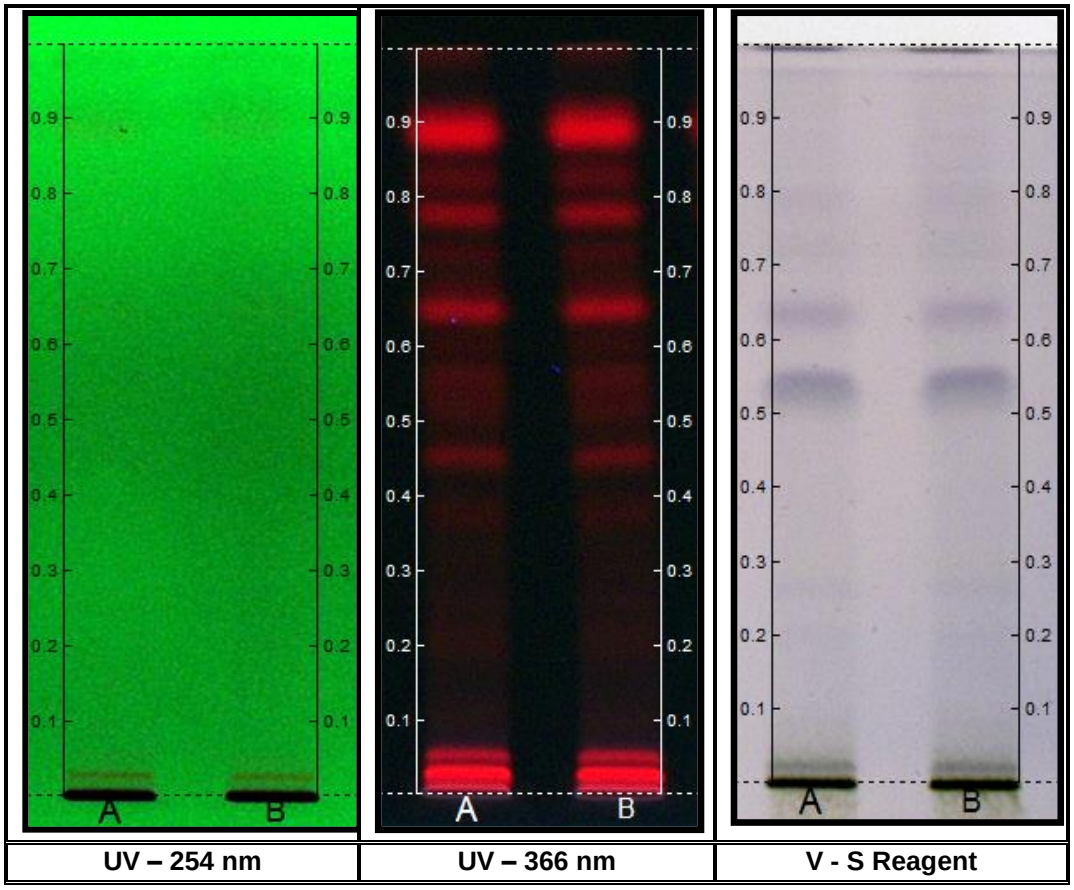


Figure 1 Total Phenolic and Flavonoid contents of various fractions of *Senecio laetus*.

3.3 Thin layer chromatography

The TLC chromatogram after development, under UV (254 nm) showed major spots at R_f 0.90 (Green), 0.002 (light yellow). Under UV (366 nm), it showed major spots at R_f 0.90, 0.85, 0.78, 0.72, 0.65, 0.60, 0.58, 0.52, 0.45. 0.37, 0.05 and 0.02 (Red) (Figure 2).



Solvent System: Toluene : Ethyl acetate : Formic acid (7 : 3 : 0.01)

Figure 2 TLC profile of alcoholic extract of aerial parts of *Senecio laetus*.

3.4 High performance thin layer chromatography profile

The results from HPTLC finger print scanned at wavelength 254 nm for alcoholic extract showed there are seven polyvalent phytoconstituents with their Rf value starting from 0.02 to 0.97. All the constituents were present in concentration > 4 (Table 3). The highest concentration of the phytoconstituent was found to 26.52 % and its corresponding Rf value was found to be 0.02. The HPTLC finger print and the densitometric chromatogram of the alcoholic extract at 254 nm are shown in Figure 3 and Figure 4 respectively.

The results from HPTLC finger print scanned at 366 nm (Absorbance mode) for alcoholic extract showed there are nine polyvalent phytoconstituents with their Rf value starting from 0.02 to 0.86. All the constituents were present in concentration > 2 (Table 4). The highest concentration of the phytoconstituent was found to 42.62 % and its corresponding Rf value was found to be 0.02. The HPTLC finger print and the densitometric chromatogram of the alcoholic extract at 366 nm (Absorbance mode) are shown in Figure 5 and Figure 6 respectively.

The results from HPTLC finger print scanned at 366 nm (Fluorescence mode) for alcoholic extract showed there are eight polyvalent phytoconstituents with their Rf value starting from 0.00 to 0.86. All the constituents were present in concentration ≥ 3 (Table 5). The highest concentration of the phytoconstituent was found to 27.80 % and its corresponding Rf value was found to be 0.00. The HPTLC finger print and the densitometric chromatogram of the alcoholic extract at 366 nm (Fluorescence mode) are shown in Figure 7 and Figure 8 respectively.

Table 3: Rf value at 254 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	4.3 AU	0.03 Rf	70.6 AU	26.52 %	0.04 Rf	1.1 AU	489.8 AU	6.48 %
2	0.16 Rf	0.2 AU	0.22 Rf	11.7 AU	4.40 %	0.24 Rf	6.0 AU	351.1 AU	4.64 %
3	0.24 Rf	6.1 AU	0.27 Rf	24.0 AU	9.03 %	0.29 Rf	2.4 AU	528.1 AU	6.99 %
4	0.43 Rf	13.3 AU	0.46 Rf	29.1 AU	10.92 %	0.49 Rf	23.7 AU	939.2 AU	12.42 %
5	0.63 Rf	32.3 AU	0.66 Rf	43.8 AU	16.45 %	0.76 Rf	0.1 AU	2709.0 AU	35.84 %
6	0.87 Rf	0.4 AU	0.92 Rf	61.3 AU	23.02 %	0.97 Rf	7.1 AU	2201.2 AU	29.12 %
7	0.97 Rf	6.7 AU	0.99 Rf	25.7 AU	9.65 %	0.99 Rf	19.1 AU	341.2 AU	4.51 %

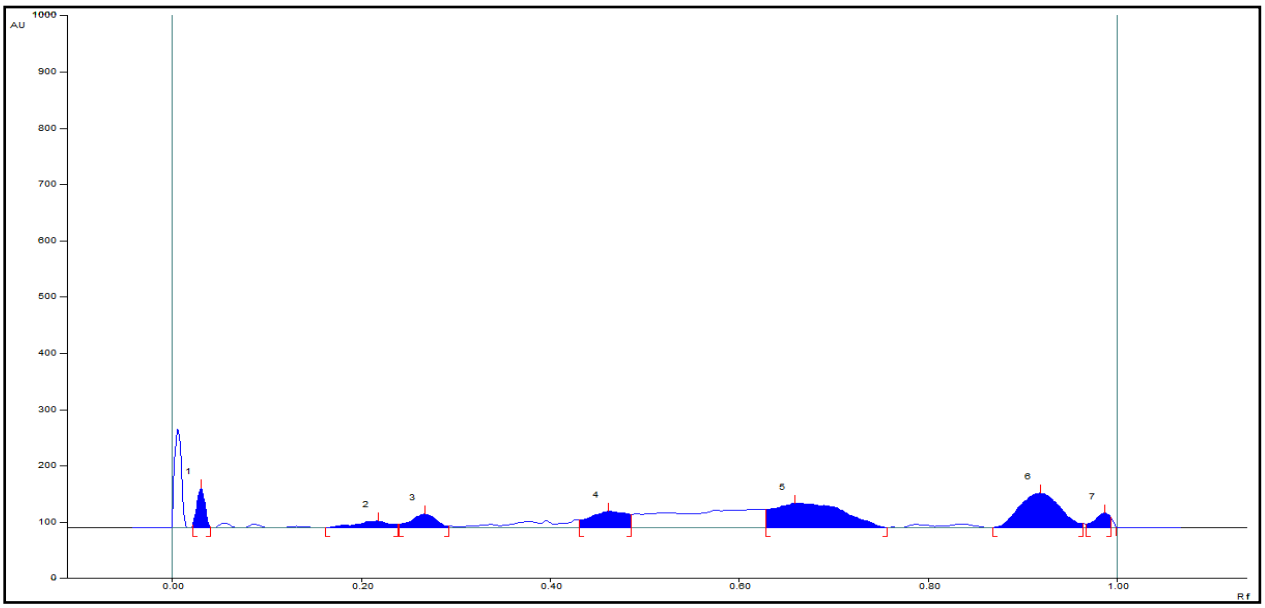


Figure 3 HPTLC finger print at 254 nm (Absorbance mode)

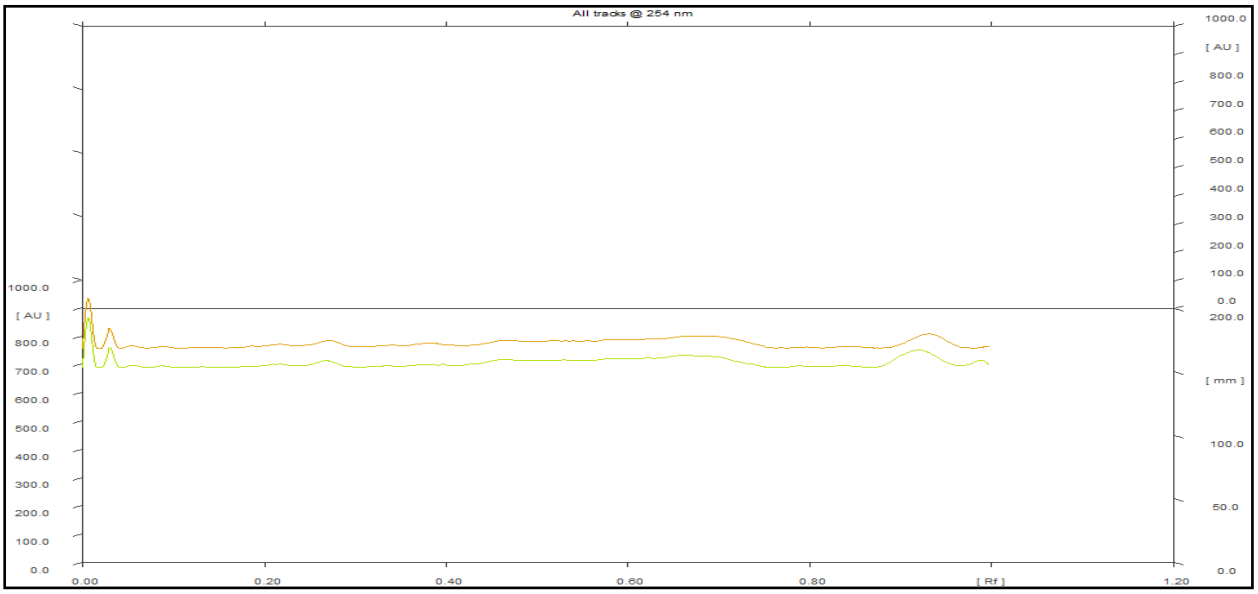


Figure 4 Densitometric chromatogram at 254 nm (Absorbance mode)

Table 4 R_f values at 366 nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	6.5 AU	0.03 Rf	254.2 AU	42.62 %	0.04 Rf	5.0 AU	2090.2 AU	20.31 %
2	0.05 Rf	2.5 AU	0.06 Rf	52.0 AU	8.71 %	0.07 Rf	0.9 AU	466.9 AU	4.54 %
3	0.24 Rf	0.8 AU	0.27 Rf	18.6 AU	3.12 %	0.31 Rf	0.5 AU	432.0 AU	4.20 %
4	0.42 Rf	0.5 AU	0.46 Rf	30.8 AU	5.16 %	0.49 Rf	9.5 AU	807.1 AU	7.84 %
5	0.50 Rf	9.3 AU	0.54 Rf	23.1 AU	3.87 %	0.60 Rf	5.2 AU	1043.1 AU	10.14 %
6	0.63 Rf	9.0 AU	0.66 Rf	51.9 AU	8.70 %	0.71 Rf	4.8 AU	1316.4 AU	12.79 %
7	0.76 Rf	1.1 AU	0.79 Rf	38.9 AU	6.52 %	0.82 Rf	5.2 AU	797.1 AU	7.75 %
8	0.82 Rf	5.3 AU	0.83 Rf	14.0 AU	2.34 %	0.86 Rf	3.2 AU	310.6 AU	3.02 %
9	0.86 Rf	3.6 AU	0.90 Rf	113.1 AU	18.96 %	0.95 Rf	9.9 AU	3027.9 AU	29.42 %

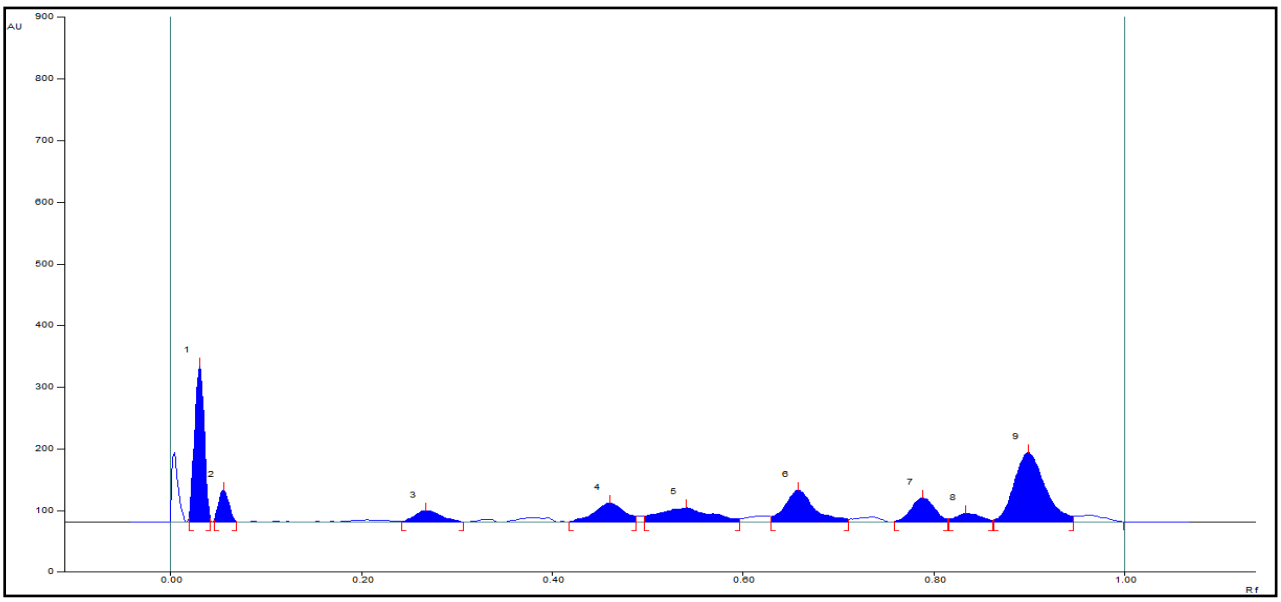


Figure 5 HPTLC finger print at 366 nm (Absorbance mode)

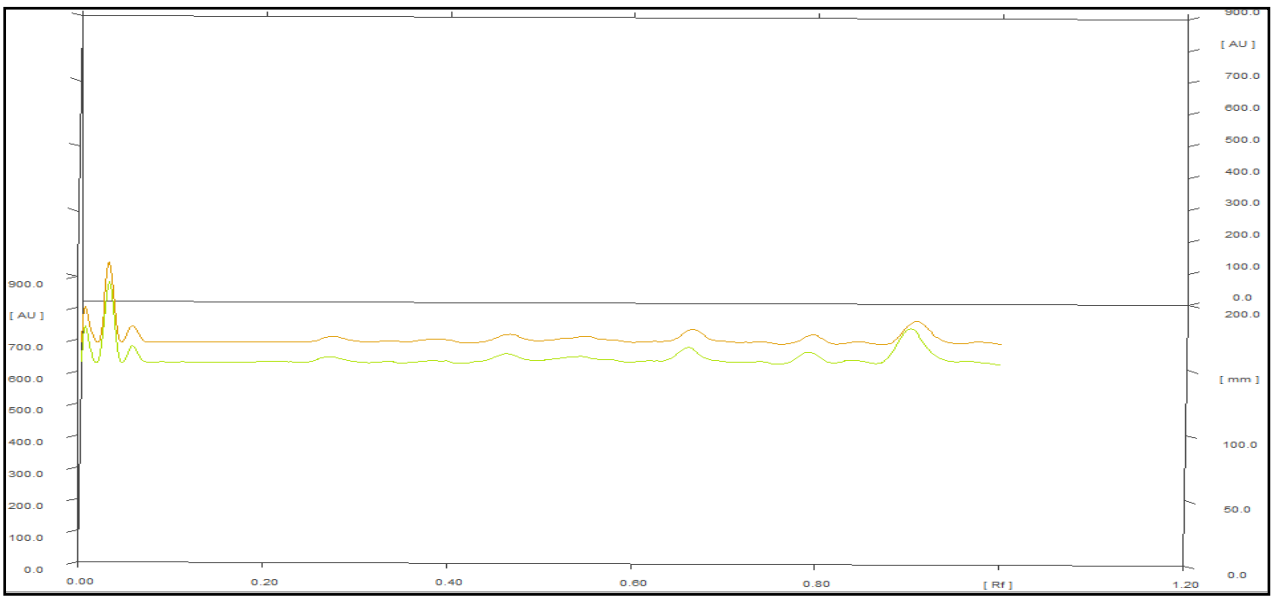


Figure 6 Densitometric chromatogram at 366 nm (Absorbance mode)

Table 5 R_f values at 366 nm (Fluorescence mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.8 AU	0.01 Rf	178.4 AU	27.80 %	0.03 Rf	24.4 AU	2399.5 AU	19.18 %
2	0.03 Rf	125.7 AU	0.03 Rf	147.7 AU	23.00 %	0.05 Rf	67.8 AU	1476.3 AU	11.80 %
3	0.05 Rf	69.9 AU	0.05 Rf	81.8 AU	12.75 %	0.09 Rf	10.3 AU	1213.7 AU	9.70 %
4	0.42 Rf	3.9 AU	0.46 Rf	20.5 AU	3.19 %	0.49 Rf	7.4 AU	571.2 AU	4.57 %
5	0.49 Rf	7.4 AU	0.57 Rf	19.2 AU	3.00 %	0.63 Rf	6.1 AU	1211.2 AU	9.68 %
6	0.63 Rf	6.1 AU	0.66 Rf	53.1 AU	8.28 %	0.72 Rf	6.7 AU	1471.9 AU	11.76 %
7	0.76 Rf	5.7 AU	0.79 Rf	49.1 AU	7.65 %	0.82 Rf	7.6 AU	1160.3 AU	9.27 %
8	0.86 Rf	8.5 AU	0.91 Rf	92.0 AU	14.33 %	0.98 Rf	0.1 AU	3007.2 AU	24.04 %

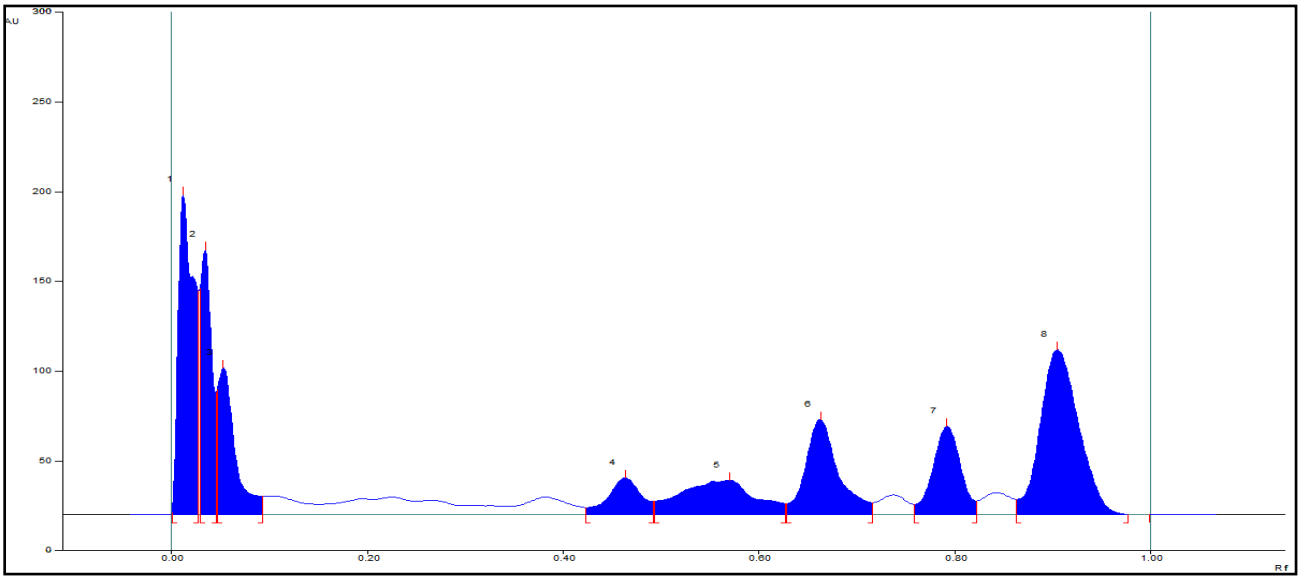


Figure 7 HPTLC finger print at 366 nm (Fluorescence mode)

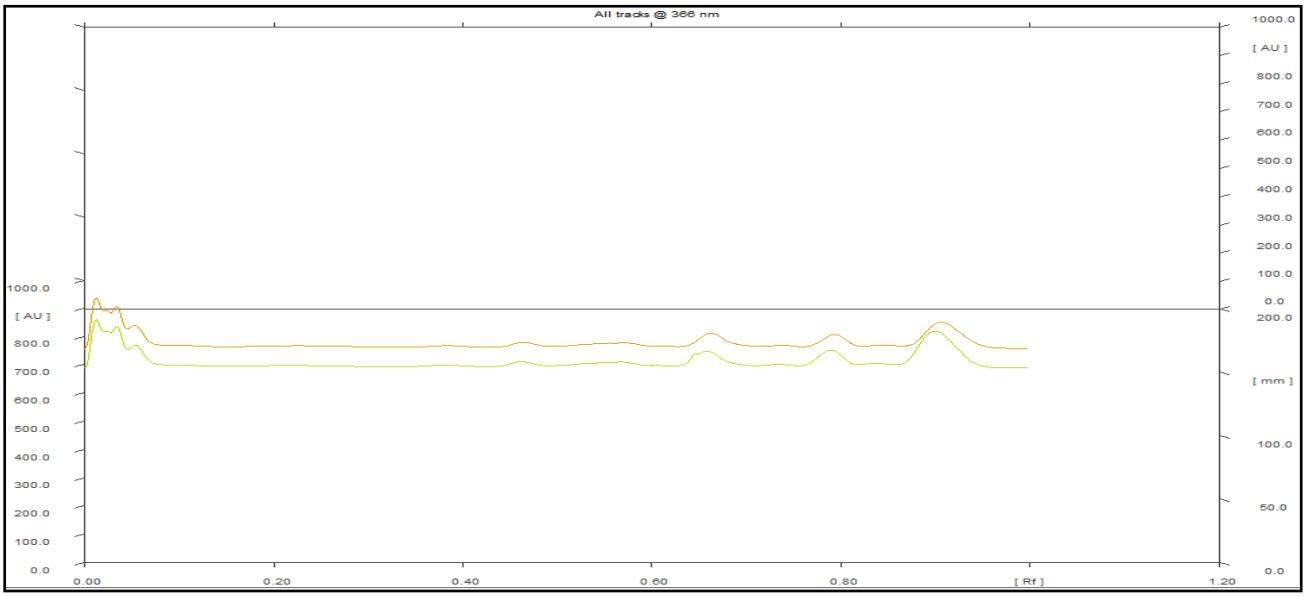


Figure 8 Densitometric chromatogram at 366 nm (Fluorescence mode)

4. DISCUSSION:

Typically, secondary metabolites are the substances accountable for therapeutic activity. Preliminary analysis of the phytochemicals in different *S. laetus* fractions revealed the presence of phenolics, saponins, lipids, steroids, flavonoids, tannins, proteins, alkaloids, carbohydrates, and amino acids. Therefore, the current preliminary phytochemicals screening could prove useful in the identification and further quantitative study of these key components for therapeutic use. In addition to being potent antioxidants, phenolic and flavonoid also have antibacterial, anti-allergic, anti-inflammatory, and anticancer properties [19]. The most prevalent phytochemicals are phenolic compounds, which are byproducts of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants [20]. These secondary metabolites are essential for growth and reproduction. Additionally, these substances offer defence against dangerous pathogenic microorganisms and predators [21]. In order to assess the quality of drugs, quantitative examination of such essential components is therefore crucial.

The total quantitative analysis of phenolic and flavonoid contents of various fractions of *S. laetus* was carried out by UV spectroscopic method.TPC of various fractions showed variation from 12.18 to 785.52 mg GAE/g dry weight of fraction. ethyl-acetate contains highest phenolic content followed by butanol, residual and dichloromethane, with least TPC in hexane fraction while as TFC of various fractions varied from 101.67 to 202.33mg RE/g dry weight of fraction. Ethyl-acetate contains highest flavonoid content followed by butanol, residual and dichloromethane fraction. These results showed that the ethyl acetate fractions of *S.laetus* contain significant quantity of phenolic and flavonoid compounds. For the detection, separation, estimation, and evaluation of several classes of natural compounds, HPLTC has become a significant and sophisticated analytical instrument. Chromatographic fingerprinting is thought to be a logical technique for stronger and more effective quality monitoring of herbal medications and their products [22].Results ofHPTLC fingerprinting of alcoholic extract showed there are seven polyvalent phytoconstituents with their Rf value starting from 0.02 to 0.97. All the constituents were present in concentration **>** 4**.** HPTLC finger print scanned at 366 nm (Absorbance mode) for alcoholic extract showed there are nine polyvalent phytoconstituents. HPTLC finger print scanned at 366 nm (Fluorescence mode) for alcoholic extract showed there are eight polyvalent phytoconstituents.

In conclusion, the *S. laetus* fingerprint images from this study's HPTLC analysis can be used as standard fingerprints as a guide for authentication, identification, purification, and quality control. As a result, the current study provides sufficient information about *S. laetus* identification and quality control.

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