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Phytochemical Screening and Fingerprinting of Basella alba L. leaf extract

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Abstract
There are various medicinal plants present in this world. India is one of the most important countries in means of medicinal plants, as traditional medicines were one of the chief practices started by Indians. Plants show medicinal property due to numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc [9,10,15]. In present study, the attempt of screening phytochemicals from the leaves of the Basella alba and also it's fingerprinting by using chromatography technique were carried out. The phytochemical screening of B.alba revealed the presence of alkaloids, phenols, saponin glycosides and tannins. The methanolic leaves extract was subjected to HPTLC, the extract showed five bands. The band with Rf value 0.13 is lutein, 0.44 is rutin and 0.25 is quercetin which was accordance with the standard, which have been observed in both 254nm and 366 nm.

Keywords: Basella alba, HPTLC fingerprinting, Lutein, Phytochemicals, Quercetin, Rutin.

Introduction

Basella alba belongs to the family Basellaceae, commonly called as Malabar spinach, Malabara palak, Mayalu or poi. Basella alba is a wildly cultivated, cool season vegetable with twining herbaceous vine growth habit. The stem of the plant is green, succulent, branched, smooth and elongated. The lamina of the leaves are fleshy, cordate, stalked. Spikes are long, axillary and solitary; the fruits of the plant are fleshy, sessile, ovoid or spherical, 7 mm long. Intially green in color, turns purple at maturity. Mainly the leaves and stems have ethnobotanical importance due to presence of vitamins, carbohydrates and proteins. The plant is also used for medicinal purposes [10]. (Figure 1)

Figure 1: Basella alba

Figure 2: Basella Leaf Extract

Materials and Method

Basella plant was bought from the plant nursery and propagated in laboratory for a month. Plant took 2 months to acclimatize and fully grow in new pot. (Figure1)

Preparation of Plant powder

The fresh leaves were collected from new grown plant, weighed and kept for drying in an oven for 48hrs.The dry weight was recorded and then the dried leaves were finely powdered using mortar & pestle. The plant powder was stored in air tight container and used for further analysis.

Preparation of Extract: 10gms of plant powder was soaked in 100ml of methanol for 24 hours. After 24 hours, the extract was filtered and evaporated to dryness on water bath. After evaporation the extract was reconstituted in 100ml of methanol. Thus the methanolic extract was used further for all analysis. (Figure 2)

Qualitative analysis of phytochemicals of Basella leaf extract [13]

The extract was tested for presence of alkaloids, tannins, phenols, glycosides and saponin (Table 1).

Table 1: Qualitative analysis of phytochemicals of Basella extract

Metabolites	Test	Observation
Alkaloid	Mayer's test: 1ml extract + 6 drops of Mayers reagent	Cream yellow ppt
	Drangendroff's test: 1ml extract + 6 drops of Drangendroff's reagent	Orange yellow ppt
Tannin & Phenols	1 ml extract + 5% FeCl3	Blue – Black ppt
Glycosides	Keller-Kiliani test : 1 ml extract + 1 ml GAA + 0.8 ml FeCl3 + 0.5 conc H2SO4	Reddish brown ring at junction and upper layer is blue color
Saponin	1ml extract + 20 ml d/w, shake for 15 mins	Formation of foam layer

Detection of phytochemicals of Basella leaf extract by Thin Layer Chromatography technique.

TLC studies were performed for the development of characteristic finger print profile for methanolic Basella leaf extract. The stationary phase used was commercially available pre-coated plates with standardized adsorption layers, i.e., Silica gel 60 F254, (Merck, Germany). The extract was used to get the TLC fingerprints by using solvent system Petroleum ether: Diethyl ether: Acetic acid (80:20:1). The

chromatograms were developed in glass chambers on 10 × 10 cm plates till the mobile phase travelled up to a distance of 8 cm from starting point. After developing, the plate was dried under room temperature for 5-10 minutes and observed under UV-254 nm. Photographs were taken and the R_f value was recorded.

HPTLC fingerprinting of Basella leaf extract

HPTLC fingerprint development of Basella alba sample was performed with 5 µL and 10 µL. Methanolic leaf extract of each concentration was filtered with nylon filter of pore size 0.45 microns. These extracted samples were applied on TLC Silica Gel 60 F254 (Merck, HX16775754) for the development. The entire experiment was carried by maintaining the temperature at 25^0 C and humidity at 40%. HPTLC instrument used was of Linomat 5. Mobile Phase used for fingerprint development was Petroleum ether: Diethyl ethyl ether: Glacial acetic acid (8:2:0.1 - v/v/v). Double run was performed in the same mobile phase using twin trough chamber. The chamber was saturated by Whatmann filter paper no 1. The development distance of 70mm from the application point was maintained. To obtain a successful chromatogram and photo documentation at 254nm and 366nm Scanner 3 of Camag was used.

RESULT and DISCUSSION

Qualitative analysis of phytochemicals of Basella leaf extract

The preliminary qualitative analysis is one of the useful methods which detect various bioactive compounds which are responsible for several pharmacological activities like antioxidant value which depends upon the presence of flavonoid and phenolic compounds, further contributed to cure cancer and boost immunity etc.

Table 2: Qualitative analysis of phytochemicals of Basella leaf extract

Metabolites	Result
Alkaloid	++
Tannin & Phenols	++
Glycosides	++
Saponin	++

(Table 2) reveals the presence of alkaloids, phenols, saponin, glycosides and tannins in the leaf extract of Basella alba. The presence of saponins shows anit-inflammatory, vasodilator actions, antioxidant properties [6] and anticancer property [10]. The plants also contain flavonoids, ascorbic acid, phenolic compounds and possess antioxidant activity[6].

T.L.C of Basella leaf extract

As the phytochemical screening confirmed the presence of secondary metabolites further the sample was tested for few of the bioactive compounds such as pigments, flavonoids and phenols.

The Basella sample was loaded and only one band was observed by the TLC method. The Rf value was found to be 0.13. To confirm and quantitate the visible band the samples were subjected further for HPTLC analysis.

HPTLC fingerprint analysis of Basella leaf extract

The methanolic leaves extract of 5 µL and 10 µL were subjected to HPTLC, the extract showed five bands in both 254 and 366 nm.

Figure 3: HPTLC fingerprint at 254nm & 366nm

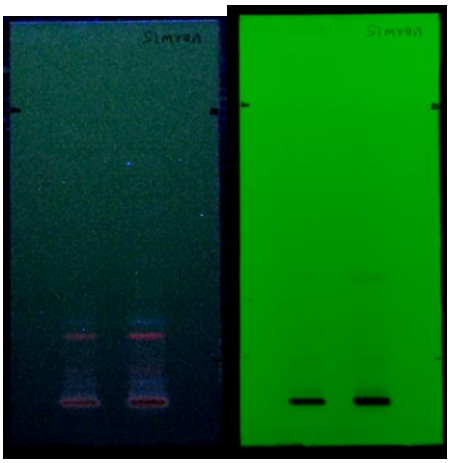


Figure 4: HPTLC profile of Basella alba at 5.0 µL

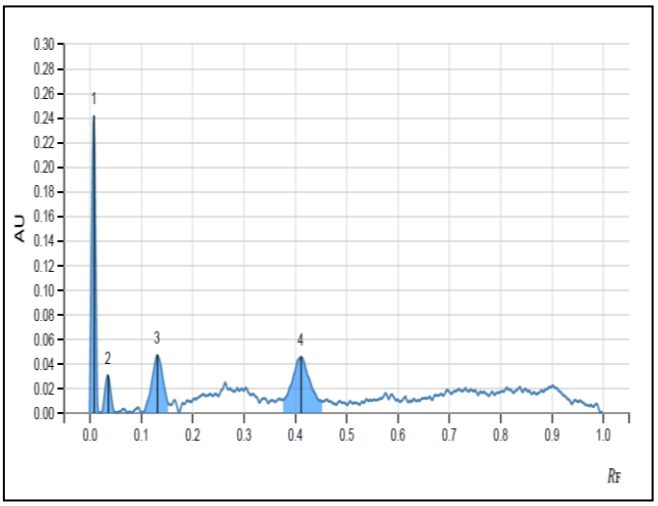


Figure 5: HPTLC profile of Basella alba at 10.0 µL

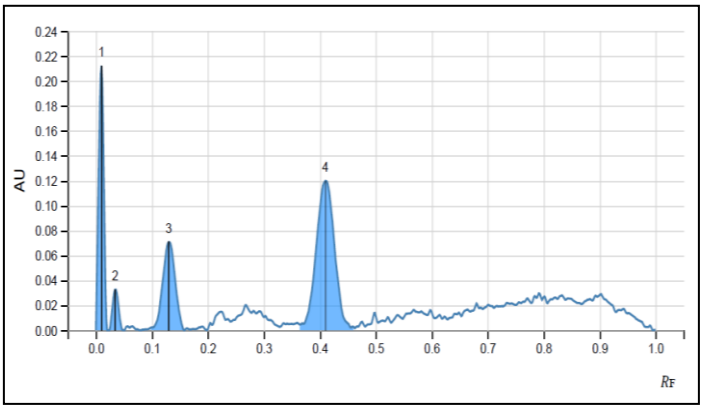


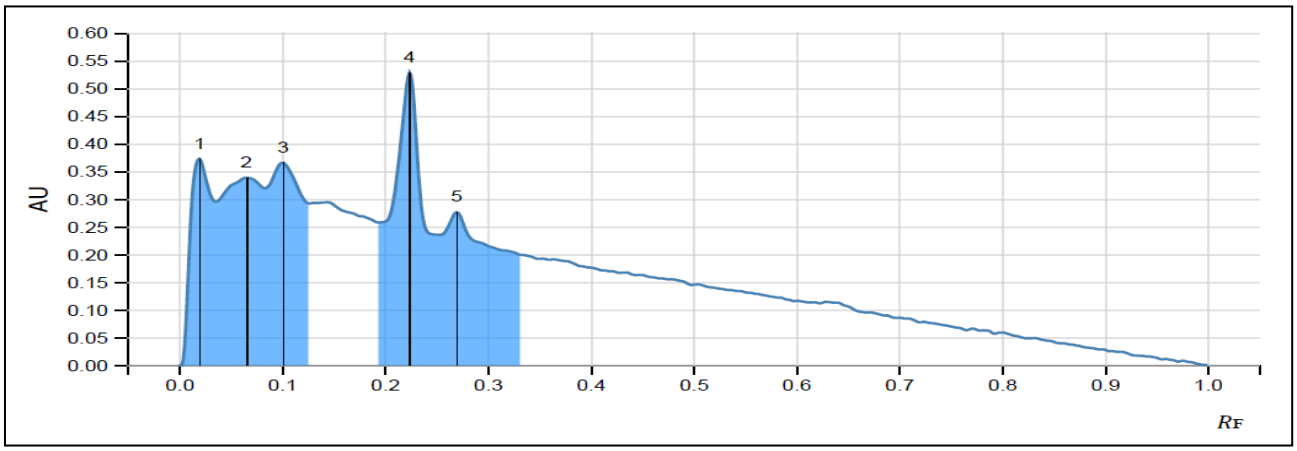
Table 3: HPTLC profile of Basella alba at 5.0 µL.

Peak	Start		Max			END		Area		Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.000	0.00	0.009	0.2414	66.44	0.017	0.00	0.00213	37.89	
2	0.023	0.00	0.036	0.0302	8.30	0.050	0.0000	0.00039	6.84	
3	0.104	0.0002	**0.131**	0.0465	12.80	**0.157**	0.0062	0.00119	**21.22**	Lutein
4	0.377	0.0104	**0.411**	0.0453	12.46	**0.453**	0.0090	0.00192	**34.05**	Rutin

Table 4: HPTLC profile of Basella alba at 10.0 µL

Peak	Start		Max			End		Area		Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.000	0.000	0.010	0.2122	48.75	0.020	0.000	0.00226	24.90	
2	0.024	0.00000	0.034	0.0326	7.48	0.049	0.000	0.00004	4.39	
3	0.094	0.0010	**0.130**	0.0707	16.24	**0.159**	0.000	0.00183	**20.15**	Lutein
4	0.364	0.0045	**0.410**	0.1198	27.52	**0.461**	0.0020	0.00458	**50.56**	Rutin

Figure 6: HPTLC profile of Basella alba at 5& 10.0 µL



Peak	Start		Max			End		Area		Substance Name
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.003	0.0000	0.020	0.3722	19.80	0.036	0.295	0.008	11.79	
2	0.036	0.2951	0.066	0.3384	18.01	0.084	0.319	0.015	20.74	
3	0.084	0.3193	**0.101**	0.3652	19.43	0.127	0.291	0.014	**18.83**	Lutein
4	0.194	0.2575	**0.224**	0.5278	28.08	**0.251**	0.235	0.019	**25.07**	Quercitin
5	0.254	0.2354	**0.270**	0.2759	14.68	**0.333**	0.198	0.017	**23.57**	Quercitin

Table 5: HPTLC profile of Basella alba at 5.0 & 10.0 µL

The band with Rf value 0.13 is lutein as compared with standard [2].The percentage AUC of lutein for 5µL is 21.22 %(Figure 3 & 4 , Table 3) and 20.15% for 10 µL (Figure 3 & 5 , Table 4). When both were run together the lutein percentage was 18.83%. (Figure 6 & Table 5).

Lutein is also found in vegetables, such as kale, spinach, and broccoli. Now a days on commercial scale lutein is isolated at very high rate from marigold flowers [4] stated its physiological role in eye protection from harmful UV light. Lutein also useful to quench singlet oxygen, a highly reactive free radical that can damage DNA reported by [2,7] During inflammation, lutein in immune tissues is depleted and the level of depletion is dependent on dietary lutein intake. There are many applications of lutein which include pigmentation of poultry products, drugs and cosmetics, coloration of food and prevention of age related macular degeneration.[3,8,11]

Basella alba leaf extract also showed band with Rf value 0.23 which can be a band of quercetin [14].The AUC percentage of quercetin is 23.57% (Figure 6 & Table 5). The presence of quercetin was also found in Nymphaea stellata with Rf value 0.25[14]. Quercitin is a aglycone derived from plants which acts as a powerful antioxidant which scavenges free radicals that destroys erythrocytes during smoking. It

improves heart health and kills cancer cells. It is one of an important bioflavanoid which treats various diseases [1].

HPTLC analysis of Basella alba leaf extract also revealed one more band with Rf value 0.41 at 5µL and 10µL, which can be of rutin [5] (Table 3 & 4, Figure 4 & 5). The presence of rutin has also been reported in Alchemilla sp with Rf value 0.44 [5].Rutin has anticonvulsant and neuroprotective effects. Rutin has various beneficial effects on human health such as a protective effect against hepatotoxicity, inflammation, and spatial memory impairment [12].

Conclusion

Basella alba leaf extract can be a good source of flavonoids (quercetin & rutin) and pigment (lutein) shows potent content of secondary metabolites. Through TLC and HPTLC lutein, quercetin & rutin were screened and quantified from Basella alba leaf extract. More purification can be analysed through FTIR.

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