PHYTOCHEMICAL INVETSIGATION AND THIN LAYER CHROMATOGRAPHY OF METHANOL EXTRACT OF *Psoralea Corylifolia* AND *Emblica Officinalis* LEAVES

ABSTRACT

The current study focuses on phytochemical screening of *Psoralea corylifolia* and *Emblica officinalis* leaf extracts in methanol solvent. The antibacterial compounds found in both leaf extracts of *Psoralea corylifolia* and *Emblica officinalis* plants were investigated using phytochemical studies. The extracts contained flavonoids, terpenoids, tannins, alkaloids, saponins, and phenolic chemicals, according to preliminary phytochemical screening. The solvent systems of toluene: ethyl acetate: methanol (24:5:2) and chloroform: ethyl acetate: acetic acid (50:50:1) yielded the most phytochemicals from methanolic extracts of *Psoralea corylifolia* and *Emblica officinalis* plants, respectively. On TLC plates, these chemicals were separated, resulting in the discovery of different spots in both leaf extracts. The Rf values of methanol extract of *P. corylifolia* run under toluene: ethyl acetate: methanol (24:5:2) solvent system was 0.12, 0.19, 0.30, 0.41, 0.53, 0.65, 0.77, 0.84, 0.89, and 0.92, respectively, while Rf values of methanol leaf extract of *E. officinalis* run under chloroform: ethyl acetate: acetic acid (50:50:1)solvent system was 0.23, 0.31, 0.41, 0.64, 0.76 and 0.88 respectively. The results of the investigation will be used to confirm the proper identification of antibacterial fractions from *P. corylifolia* and *E. officinalis* crude plant extracts. The optimum solvent for extracting antibacterial components from *P. corylifolia* and *E. officinalis* leaves was methanol.

Key words: *Psoralea corylifolia*, *Emblica officinalis*, Phytochemical analysis, Extraction, TLC, compounds, chloroform, methanol extract.

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INTRODUCTION

The major source for a wide range of newer herbal antibacterial compounds is to be from different medicinal plants. These plants have provided civilization with useful, and occasionally lifesaving, pharmaceuticals for many generations. When a connection between structure of chemical and biological activity was discovered in modern agriculture, empirical research gave way to rational use of antimicrobial plants. Because of the conceptual cooperation of chemistry and biology, this novel technique to find and develop possible new fungicides is largely effective. As a result, such plants should be studied further to learn more about their qualities, safety, and efficacy. Fungicides derived from plants have been used in agriculture for a long period.

The *Psoralea corylifolia* is a weed commonly known as babji, bakuchi and bavanchi. Is belongs to Fabaceae family. Bakuchi grows throughout India, *Psoralea corylifolia* Linn, has multiple purpose uses as it is an important component of Ayurveda. In the present investigation it was found that phytochemicals such as phenols, alkaloids, tannins, flavonoids and saponin were present in the seeds of *Psoralea corylifolia* plant. TLC and HPLC also confirmed these results (Pandey et al., 2013).

Emblica officinalis is a deciduous tree, commonly known as Indian gooseberry or amla and 'Nelli' in Tamil. It belongs to the family Phyllanthaceae. It is widely grown in all over India. The antimicrobial activity of plant extracts against some gram positive and gram-negative pathogenic microorganisms have been assessed the chemical constituents in the plant extracts (Kanthimathi and Soranam., 2013).

For the management of plant diseases, many chemical compounds have been produced. However, as people become more aware of the harmful side effects of these chemicals, greater emphasis is being placed on the use of biocontrol agents. In the study of plant pathology, there is currently a huge difficulty in introducing some environmentally acceptable and safe alternative control tactics for agriculture, which has encouraged researchers to focus on plants and microorganisms as a biocontrol agent. Bavchi and Anola both can produce large number of antimicrobial compounds, which ultimately lead people to move towards use of plant extracts as a source of biocontrol agents against major pant diseases caused by bacterial pathogens.

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Furthermore, pharmacological investigations have confirmed the importance of medicinal plants as a source of bioactive phytochemicals. These bioactive compounds are generally present in all plant cells as secondary metabolites, although their concentration varies depending on the plant part, season, climate, and growth phase. Scientists have now developed strategies to separate natural elements with desired biological activity and use them against phytopathogenic bacteria. In India, many plant-based treatments are used to cure various plant diseases, but little research has been done on the scientific validation of these plants importance in the agrochemical industry. As a result, there is need for systematic and scientific research to support the use of medicinal plants against phytopathogenic bacterial pathogens.

Thus, the aim of this paper is to study the phytochemicals and partial characterization of a bioactive antibacterial compounds from methanol extract of *Psoralea corylifolia* and *Emblica officinalis* plants against citrus canker causing *Xanthomonas axonopodis* pv. *citri* bacteria.

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Material and methods

Collection and preparation of plant leaves-

The leaves of *Psoralea corylifolia* and *Emblica officinalis* plants used in this study were collected from the campus of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola city of Maharashtra.

Collected leaves (*Psoralea corylifolia* and *Emblica officinalis*) were thoroughly washed under tap water to remove impurities. The leaves were dried separately under shade with alternate shifting for about 3 to 4 weeks. The dried leaves were powdered with grinder and stored in airtight container until further use (Dhawan and Gupta, 2017).

Extraction of leaves using different solvents: -

For anola and bavchi leaf extracts, methanol, acetone, chloroform, dichloromethane, and petroleum ether solvents were utilized for extraction of leaves. The extraction was done using Soxhlet's apparatus. The 250 ml solvent was added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on heating mantle. The powder (50g) of dried leaves was loaded into the thimble, which is placed inside the Soxhlet extractor. The solvent was heated using the heating mantle and began to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. This process is run for a total of 16 hours.

The supernatant from flask was filtered separately through Whatman No. 1 filter paper and evaporated at room temperature. Air dried extracts were weighed separately and transferred into small vials and kept in refrigerator at 5˚C until further use. The percentage of extraction yield was calculated by using following formula (Khan *et* al. 2010).

$$\text{Extraction yield \%} = \frac{\text{Weight of extract}}{\text{Weight of ground floral material}} \times 100$$

The resultant crude extracts were used for phytochemical analysis, growth inhibition assay against *Xanthomonas axonopodis* pv. *citri* and for chromatographic analysis.

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Preliminary phytochemical screening of plant extract

Preliminary phytochemical analysis of anola and bavchi crude leaf extract were performed for analysis of different phytochemicals like cardio glycosides, saponins, fixed oils and fats, alkaloids, steroids, flavonoids, tannins, and phenolic compounds by following method given by Prashanth and Krishnaiah (2014).

i) Test for cardiac glycosides:

Take 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5 % ferric chloride were added in test tube. Carefully 0.5 ml of concentrated sulphuric acid was added by the sides of test tube. Formation of brown ring in acetic acid layer indicates the presence of cardiac glycosides.

ii) Test for saponins:

Take 2 ml extract and dilute with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

iii) Test for alkaloids:

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.

iv) Test for steroids and triterpenoids:

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken, and allowed to stand. If the lower layer turns red, sterol is present. Formation of reddish-brown color indicates the presence of terpenoids.

v) Test for flavonoids:

The extract was treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow colour, which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoids.

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vi) Test for tannins and phenolic compounds:

A small amount of extract was dissolved in distilled water. To this solution of 2 ml of 5 % ferric chloride solution was added. Formation of blue, green, or violet colour indicates presence of phenolic compounds.

Thin layer chromatography and Bioautography: -

Based on in vitro growth inhibition assay methanol extract of anola and bavchi were selected for chromatographic analysis. This method was used to study the preliminary screening of phytochemicals. Thin layer chromatography for separation of different antimicrobial compounds presents in crude methanol extract and bioautography for isolation of active bioactive compounds.

Thin layer chromatography (TLC)

Thin layer chromatography was carried out to know the chemical profile of anola and bavchi crude leaf extract.

Preparation of TLC plates

The TLC plates were prepared as described by Harborne (1998). Briefly, 25 g of silica gel-G (Hi media, Manufactured, India) was mixed with 50 ml of distilled water and the slurry formed was uniformly spread over TLC plates with a thickness of 0.25 mm using the spreader. The plates were allowed to dry at room temperature and heated in an oven at 110˚ C for 1 hr.

Standardisation of solvent system

Each sample of the crude extract of anola and bavchi were diluted in methanol solvent. The prepared TLC plates were marked 1 cm from bottom and 10 µl each sample was applied on TLC plates at equal distance with the help of capillary tubes. For separation of maximum bands on TLC plates, different solvent systems were used according to polarity and from that toluene: ethyl acetate: methanol (24:5:2) for bavchi and chloroform: ethyl acetate: acetic acid (50:50:1) for anola were selected as standard solvent system.

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List 1 solvent system used during experiment for *Psoralea corylifolia* methanol extract:

Sr. No.	**Solvent system**	**Proportion**
1	Ethyl acetate: methanol	3:7
2	Ethyl acetate: acetone	4:6
3	Toluene: ethyl acetate: methanol	24:5:1.5
4	Methanol	100%
5	Ethyl Acetate: acetic acid: petroleum ether	19:1:5
6	Ethyl Acetate: acetic acid: petroleum ether	15:6:4
7	Ethyl Acetate: acetic acid: petroleum ether	20:6:4
8	Ethyl acetate: methanol: petroleum ether: water	19:3:3
9	Ethyl acetate: methanol: benzene	20:6:3
10	Ethyl acetate: methanol: butanol	19:1:6
11	Petroleum ether: ethyl acetate	02:01

List 2 solvent systems used during experiment for *Emblica officinalis* methanol extract.

Sr. No.	**Solvent systems used**	**Proportion**
1	Chloroform: hexane: ethyl acetate	50:50:1
2	Toluene: ethyl acetate: methanol	7:2:1
3	Chloroform: ethyl acetate: acetic acid	50:50:1
4	Acetone: methanol	1:1
5	Toluene: ethyl acetate: acetic acid: formic acid	20:45:20:5
6	Methanol: chloroform	20:80

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7	Toluene: ethyl acetate	9:2
8	Hexane: Ethyl acetate	3:1
9	Ethyl acetate: methanol: water	81:11:8

TLC plate was kept in chromatography chamber, containing Toluene: ethyl acetate: methanol (25:4:2) for bavchi and Chloroform: ethyl acetate: acetic acid (50:50:1) for anola as solvent system and allowed to run until it reaches as 3/4th position. The developed chromatogram on TLC plates was allowed to air dry and observed under visible, UV light (both at 360 nm and 254 nm). The bands were noted and the Rf value (Relative front) of separated bands were calculated by measuring the distance travelled by solute and the solvent. It is given by formula,

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Isolation of antibacterial compounds by Bioautography

The TLC plate was developed in Toluene: ethyl acetate: methanol (25:4:2) solvent system for *Psoralea corylifolia* and Chloroform: ethyl acetate: acetic acid (50:50:1) for *Emblica officinalis.* All the prepared chromatogram was dried for complete removal of solvent. The bioautography agar overlay method was used to analyze antibacterial component present in *Psoralea corylifolia* (bavchi) and *Emblica officinalis* (anola) crude extract. The bacteria *Xanthomonas axonopodis* pv. *citri.* was grown in the nutrient broth. This broth was distributed over a prepared TLC plate. After solidification of the suspension, the TLC-bioautography plate was incubated at 37°C for 24 h. The bioautogram that developed was sprayed with a 1% aqueous solution of 2,3,5-tri phenyl tetrazolium chloride (TTC) and incubated at 37°C for 4 h. Inhibition zones indicated as the white color zone against pink background as the presence of active compounds. Growth inhibition areas were compared with the Rf of the related spots on the reference TLC plates.

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The bioactive compounds resulted from bioautography was scraped from TLC plates using preparative TLC. Purified samples were collected in separate eppendorf tube and dissolved in respective solvents. Then it was centrifuged at 10,000 rpm for 10 minutes in centrifuge machine. The supernatant was collected in small 2ml eppendorf tubes and were evaporated using vacuum evaporator for complete evaporation of solvent.

RESULTS AND DISCUSSION

Extraction yield of plant leaf extracts

Extraction yield of plant leaves in each solvent were determined as described in "Materials and Methods". Extraction yields of each plant leaves in different solvents are presented in Table 1.

Extraction yield of *Psoralea corylifolia*

Methanol exhibited (14.50%) maximum extraction from *Psoralea corylifolia* leaves whereas minimum extraction yield was observed in dichloromethane (9.75%).

Extraction yield of *Emblica officinalis*

Maximum extraction yield (15.75%) of *Emblica officinalis* leaves was reported in distilled water solvent and minimum in petroleum ether (0.65%).

The extraction yields of the various solvents employed in this study are given in Table 1. The polarity and capacity of a solvent to extract additional chemical compounds from the *Psoralea corylifolia* plant determine the extractability of that solvent. *Psoralea corylifolia* was discovered to yield more bioactive compounds when extracted with distilled water compared with other solvents. Like this, Kumar et al. (2015) demonstrated that *Psoralea corylifolia* seeds had the best extraction yield in methanol. Also, it was found for increasing extraction yield and was best to extract more compounds or plants used to extract more substances had more substances that ideally dissolve in methanol (Wavare et al., 2017).

Preliminary phytochemical analysis

Preliminary phytochemicals present in methanol extract of *Psoralea corylifolia* and *Emblica officinalis* were analysed by following standard procedure as explained under

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"Materials and Methods". Observations on presence or absence of phytochemicals namely, cardio glycosides, saponins, fixed oils and fats, alkaloids, steroids, flavonoids, tannins, and phenolic compounds were noted as + sign for presence and - sign for absence and are presented in Table 2.

Alkaloids, saponins, tannins and phenolics, fixed oils and fats, cardio glycosides and flavonoids compounds were observed in methanolic extract of *Psoralea corylifolia*. Similarly, alkaloids, saponins, flavonoids, tannins and phenolic compounds were observed in methanolic extract of leaves of *Emblica officinalis*.

Present findings agree with previous investigation of Patil et al. 2013 who reported the presence of alkaloids, saponin, flavonoids and tannins in the *Emblica officinalis* methanolic leaves extract. The methanolic leaf extract was found to be negative for the presence of oil and fats, glycosides, and sterols. These findings correlate with the result obtained by previous authors (Badoni et al. 2016, Alegar et al. 2014). The presence of glycosides, phenolics, tannins and flavonoids in methanolic extract of *Psoralea corylifolia* was detected by Suman et al. (2013).

Chromatography

Based on *in vitro* results, methanol extracts of *Psoralea corylifolia* and *Emblica officinalis* were selected for further partial purification by chromatographic analysis. All these methanol extract of *Psoralea corylifolia and Emblica officinalis* were screened for preliminary phytochemical analysis and thin layer chromatography. Observations were recorded for presence or absence of phytochemicals, number and Rf values of bands (compounds) present in extract.

Thin Layer Chromatography (TLC)

Thin layer chromatography was used for separation of different chemical constituents present in methanol extract of *Psoralea corylifolia and Emblica officinalis* respectively, as described under 'Materials and Methods'.

Standardization of solvent system

Various solvent systems were screened for efficient separation of bands according to polarity. Total 19 solvent systems were used in present investigation to know most suitable solvent system for separation of compounds in methanol *Psoralea corylifolia* and *Emblica*

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officinalis. The Rf values and colour of separated bands in different solvent systems under UV transilluminator are summarised in Table 3a and 3b.

It is observed from data presented in Table 3a and 3b, different solvent systems showed differences in number of bands and their Rf values in methanol *Psoralea corylifolia* and *Emblica officinalis*. Among all the tested solvent systems most promising solvent systems produced good results on TLC plates were toluene: ethyl acetate: methanol (25:5:2) and chloroform: ethyl acetate: acetic acid (50:50:1) for methanol extracts of *Psoralea corylifolia* and *Emblica officinalis.*

The Rf values of methanol extract of *Emblica officinalis* run under chloroform: ethyl acetate: acetic acid (50:50:1) solvent system was 0.23, 0.31, 0.41, 0.64, 0.76 and 0.88 (Table 3a). The Rf values of methanol extract of *Psoralea corylifolia* run under toluene: ethyl acetate: methanol (25:5:2) solvent system was 0.12, 0.19, 0.30, 0.41, 0.53, 0.65, 0.77, 0.84, 0.89 and 0.92. (Table 3b).

Our findings agree with previous investigation of Alam et al. (2012) who reported six Rf value from ethanol extract of *Emblica officinalis* using solvent system Toluene: ethyl acetate: acetic acid: formic acid 20:45:20:05 showing Rf values 0.02, 0.13,0.37,0.70,0.84 and 0.91. The ethanol extracts produced three fractions having Rf 0.76, 0.69 and 0.39 on TLC under ethyl acetate: methanol (3:7) solvent system using *Psoralea corylifolia* (Pandey et al., 2013). More et al. (2016) also showed ten fractions under Petroleum ether: Ethyl acetate (02:01) solvent system of Rf value 0.04, 0.07, 0.14, 0.18, 0.25, 0.35, 0.45, 0.61, 0.70 and 0.84.

TLC Bioautography

TLC plates run in Toluene: ethyl acetate: methanol (24:5:2) and Chloroform: ethyl acetate: acetic acid (50:50:1) system was used for bioautography technique to determine antibacterial activity of separated compounds against tested bacterium. The TLC plate after spraying with 2,3,5-tri phenyl tetrazolium chloride, showed a white colored inhibition against pink background around band which contain active principle responsible for antibacterial activity. The one compound from *Psoralea corylifolia* methanol extract showed well resolved inhibition of *Xanthomonas axonopodis* pv. *citri* at Rf- 0.77 showing pink color under UV transilluminator (Plate 2).

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Emblica officinalis methanol extract exhibited strong antibacterial activity on TLC plate with zone of inhibition at band with Rf 0.23 value against *Xanthomonas axonopodis* pv. c*itri* showing blue color under UV transilluminator. The compound from methanol extract of *Psoralea corylifolia* and *Emblica officinalis* showing inhibition band was denoted as compound: 1. and compound: 2.

In present study, bakuchiol compound of Rf 0.77 from *Psoralea corylifolia* seed showed prominent antibacterial activity using TLC bioautography against *Xanthomonas axonopodis* pv. *citri*. However, results of Purkayastha and Dahiya (2012) were in contrast with above findings, showing antibacterial activity of tannin compound of Rf 0.70-0.83 from *Psoralea corylifolia* oil against Enterococcus sp. Mehrotra et al. (2011) conducted TLC separation by contact-bioautography of ethanol extract of *Emblica officinalis* against *Helicobacter pylori* and noticed one spot of Rf value 0.16 in toluene: chloroform: acetone (40:25:35) and Rf value 0.46 in methanol: formic acid (1:1).

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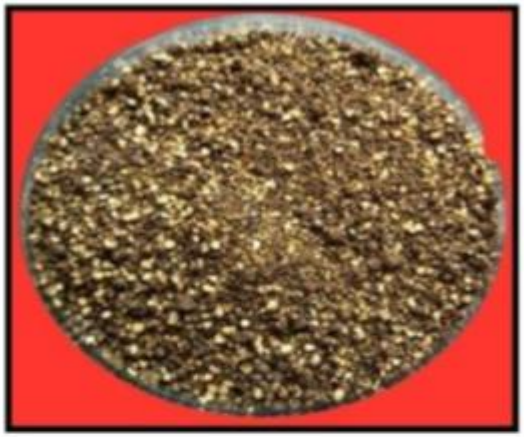


A) Dry seed powder of *Psoralea corylifolia*

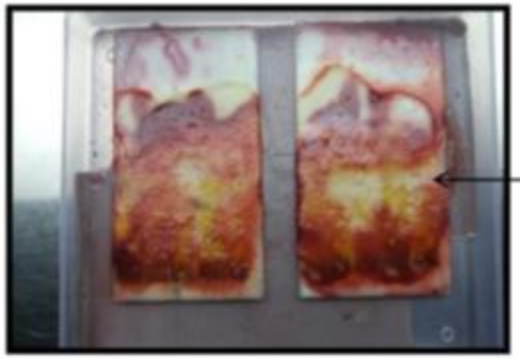
B) Dry leaves powder of *Emblica officinalis*

C) Crude extract

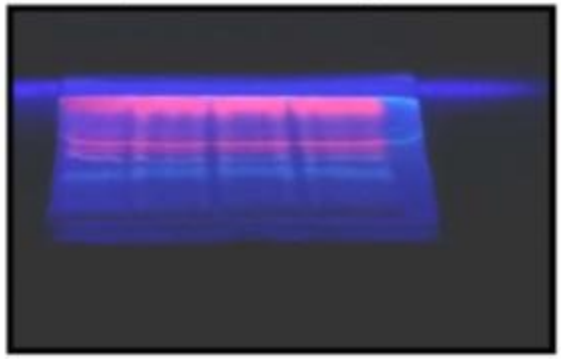
D) Conservation of extract

Plate 4 : Powder form and extraction of *Psoralea corylifolia* and *Emblica officinalis* .

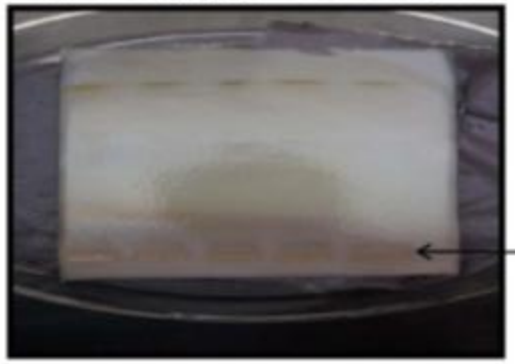
A) TLC of *Psoralea corylifolia*



B) Bioautography of *Psoralea corylifolia*



C) TLC of *Emblica officinalis*



D) Bioautography of *Emblica officinalis*

Plate 5: TLC and Bioautography of methanolic extract of of *Psoralea corylifolia* and *Emblica officinalis*.

Table 1. Effect of different solvents on per cent extraction yield from dry weight of leaves

Plant	Solvent	Yield in %
Psoralea corylifolia	Acetone	11.18
	Chloroform	10.77
	Dichloromethane	9.75
	Methanol	14.50
Emblica officinalis	Chloroform	2.5
	Acetone	7.12
	Methanol	15.75
	Petroleum ether	0.65

Table 2. Preliminary phytochemical analysis of methanol extract of *Emblica officinalis* and *Psoralea corylifolia.*

Test	*Emblica officinalis*	*Psoralea corylifolia*
Alkaloids	+	+
Terpenoids	_	+
Saponins	+	+
Tannins	+	+
Steroids	_	-
Phenolic compounds	+	+
Cardio glycosides	-	+
Flavonoids	+	+

+ presence, - absence.

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Table 3a. Standardization of solvent system for methanol extract of *Psoralea corylifolia*

Sr. No.	Solvent system	Proportion	Methanol extract of *Psoralea corylifolia*	
			Rf	**Color**
1	Ethyl acetate: acetone	4:6	0.70	Dark blue
			0.88	Dark black
2	Toluene: ethyl acetate: methanol	24:5:1.5	0.12	Brown
			0.19	Light brown
			0.30	Light blue
			0.41	Dark blue
			0.53	Dark black
			0.65	Light yellow
			0.77	Light blue
			0.84	Light black
			0.89	Light blue
			0.92	Red
4	Methanol	100%	-	Smear of compounds, no bands
5	Ethyl Acetate: acetic acid: petroleum ether	19:1:5	0.05	Blue
			0.62	Dark blue
			0.85	Black
6	Ethyl Acetate: acetic acid: petroleum ether	15:6:4		Smear of compounds, no

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				bands
7	Ethyl Acetate: acetic acid: petroleum ether	20:6:4		Smear of compounds, no bands
8	Ethyl acetate: methanol: petroleum ether: water	19:3:3		Smear of compounds, no bands
9	Ethyl acetate: methanol: benzene	20:6:3		Smear of compounds, no bands
10	Ethyl acetate: methanol: butanol	19:1:6		Smear of compounds, no bands
11	Petroleum ether: ethyl acetate	02:01		Smear of compounds, no bands

Table 3b. Standardization of solvent system for methanol extract *Emblica officinalis*

	Solvent systems used	**Proportion**	**Methanol extract *Emblica officinalis***	
			Rf	**Color**
1	Chloroform: hexane: acetic acid	50:50:1	0.17	Blue
			0.64	Red
2	Toluene: ethyl acetate: methanol	7:2:1	0.53	Yellow
			0.86	Red
3	Chloroform: ethyl acetate: acetic acid	50:50:1	0.23	Light black

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			0.31	Dark blue
			0.41	Light blue
			0.64	Light red
			0.76	Light blue
			0.88	Dark red
4	Acetone: methanol	1:1		Smear of compounds, no bands
5	Toluene: ethyl acetate: acetic acid: formic acid	20:45:20:5	0.51	Red
6	Methanol: chloroform	20:80	0.50	Black
			0.75	Red
7	Toluene: ethyl acetate	9:2		Smear of compounds, no bands
9	Ethyl acetate: methanol: water	81:11:8	0.40	Black
			0.78	Red

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