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Formulation of Psoralea Ethosomes and their Evaluation

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ABSTRACT:

Psoralea corylifolia known as 'babchi' is a medicinal plant for the treatment large scale level in pharmaceutical industries to cure various skin diseases. In India, indigenous herbal remedies such as Ayurveda and other Indian traditional medicines have since ancient times used plants in treatment of various disorders. In our present investigation, preliminary phytochemical analysis of Psoralea corylifolia has been evaluated for the presence of bioactive constituents using various polarity solvents including hexane, n-butanol, ethanol and water. The present phytochemical screening of the seeds extracts revealed the presence of maximum compounds including carbohydrates, terpenoids, alkaloids, phenols, tannins, amino acid, cardiac glycosides and proteins. The results suggest that the ethanolic extracts of Psoralea corylifolia has promising therapeutic potential and can be used as a base for the development of novel potent drugs in phytomedicines.

Introduction:

The skin is the largest and most easily accessible organ of the body. It serves as a potential route of drug administration for systemic effects. However, the outer layer of the skin, the stratum corneum, represents the most resistible barrier to drug penetration across the skin, which limits the

transdermal bioavailability of drugs. Therefore, special carriers are required to combat the natural skin barrier to deliver drug molecules with different physicochemical properties to systemic circulation.

Transdermal drug-delivery systems offer many advantages, such as avoidance of first-pass

metabolism by the liver, controlled delivery of drugs, reduced dosing frequency, and improved patient compliance, as they are non-invasive and can be self-administered.¹

Bakuchi (*Psoralea corylifolia* L) is a vulnerable and medicinally important plant that grows throughout India. It has multiple uses as it is an important component of Ayurvedic as well as Allopathic systems of medicines. Bakuchi is one of the main herbs in traditional Indian and Chinese herbal medicine. It has been used in the treatment of skin disorders, eczema, hair loss, leucoders, leprosy, inflammation, dental caries disease of the skin and diarrhea (leaves). It possesses important activities like antibacterial, anti-inflammatory, antiplatelet, antitumor, immune-modulatory.

P. corylifolia L. seed has been reported to contain several phytoconstituents including coumarins and flavone components, such as psoralen, isopsoralen, psoralidin, neo-bavaisoflavone, bavachin, corylin, bavachalcone and possess antibacterial, anti-inflammatory, antifungal, antioxidant, antiparasitic, estrogenic, antitumour, and immuno-modulatory activity.

The medicinal values of plants lie in their component phytochemicals such as alkaloids, flavonoids, tannins and other phenolic compounds, which produce a definite physiological effect on the human body. Systematic searches on bioactive compounds of useful medicinal plants are now considered to be a rational approach in nutraceutical and drug research. Therefore, the present work has been

designed to evaluate the phytochemical constituents of *P. corylifolia*.²

Material and Methods

Collection of plant materials

Dried fruits of *Psoralea corylifolia* were purchased from a local store and their authenticity was confirmed by comparing them with the herbarium specimen at the NISCAIR/RHMD/consult/2015/2935-128, National Institute Of Science Communication And Information Resources, New Delhi.

The seeds were washed thoroughly 1-2 times with tap water and once with sterile distilled water, air dried at room temperature on a sterile bottle. After complete drying seeds were powdered well using a mixer. Then the powdered material was weighed and kept in air tight container and stored in refrigeration for further use.³

Extraction of plant materials

Hot extraction- About 200gm of powdered plant materials was taken and subjected to successive solvent extraction. The extraction was carried out for 26 h with ethanol.

Cold extraction- About 100gm of the powdered plant materials was taken and subjected to an iodine flask then macerated. The extraction was carried out for 24 h with ethanol.⁴

Preliminary Phytochemical Studies of

Psoralea corylifolia Seeds^{5,6}

Extract i.e, ethanol was subjected to qualitative chemical investigation through different chemical

tests in order to detect the presence of various phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins and free amino acids, saponins etc. the different qualitative tests were performed for establishing a profile of a given extract for its chemical composition.

Chemical tests for detection of organic constituents

(A) Test for carbohydrates

- **Molisch's test:** The test solution was treated with a few drops of alcoholic α -naphthol solution. The solution was shaken and concentrated H_2SO_4 was added slowly through the sides of test tube. A purple-to-violet color ring may form at the junction of the two liquids.

(B) Test for reducing sugars

- **Fehling's test:** Equal volumes of Fehling's A and Fehling's B reagents were mixed and few drops of sample were added and boiled in water bath for 5-10 min. the formation of a brick-red precipitate of cuprous oxide may occur to correspond to presence of reducing sugars.
- **Benedict's test:** Equal volumes of test solution and Benedict's reagent were mixed and heated in a water bath. The appearance of green, yellow or red color may correspond to the presence of reducing sugars.

(C) Test for monosaccharides

- **Barfoed's test:** The test tube containing 1ml reagent and 1 ml of a solution of the compound was heated in a beaker of boiling water; if red courous oxide formed within two minutes may reveal the presence of monosaccharides.

(D) Test for pentose sugars

- **Bial's orcinol test:** To boil Bial's agent few drops of test solution were added. Green or purple coloration may appear.

(E) Test for hexose sugar

- **Selwinoff's test:** 1ml test solution was added to about 3ml of reagent and boiled for 1-2 min. the formation of red color may ensure the presence of fructose.
- **Tollen's phloroglucinol test for galactose:** To 100mg of compounds, 2ml of Tollen's reagent was added gently. A silver mirror obtained inside the wall of the test tube may indicate the presence of aldose sugar.

(F) Test for non-reducing polysaccharides

- **Iodine test:** 3ml test solution was mixed with a few drops of dilute iodine solution. The blue color thus obtained may disappear on boiling and reappear on cooling.
- **Tannic acid test:** With 20% tannic acid, the test solution may produce off white

precipitate which may correspond to the presence of polysaccharides.

(G) Test for Tannic and phenolic compounds

To 2-3 ml of aqueous or ethanolic extract was added with a few drops of-

- **Gelatin solution:** The test solution may give a white precipitate.
- **5% Ferric chloride solution:** The test solution may show deep blue-black color to confirm the presence of free tannins. A brownish-green precipitate may appear if condensed tannins were present.
- **Lead acetate solution:** The test solution may give a white precipitate.
- **Bromine water:** The test solution may show discoloration of bromine water.
- **Acetic acid solution:** The test solution may give the red color solution.
- **Potassium dichromate:** Red precipitate may appear.
- **Dilute HNO₃:** The test solution may show transient red color.
- **Dilute NH₄OH and potassium ferricyanide:** The test solution may give a red color solution.
- **Dilute potassium permanganate solution:** The test solution may show discoloration.

(H) Test for alkaloids

- **Mayer's test:** 2-3 ml test solution may give a cream color precipitate with

Mayer's reagent to ensure the presence of alkaloids.

- **Dragendorff's test:** Reddish brown colored precipitate may appear when 2-3 ml test solution was added to Dragendorff's reagent to reveal the presence of alkaloids.
- **Wagner's test:** 2-3ml test solution may give a reddish brown precipitate with Wagner's reagent which may correspond to the presence of alkaloids.
- **Haber's test:** Yellow-coloured precipitate may appear when 2-3 ml of test solution was mixed with Hager's reagent.

(I) Test for glycosides

- **Baljet test:** A thick section may show a yellow to orange color with sodium picrate.
- **Legal test:** to the solutions of aqueous or alcoholic extract 1ml pyridine and 1ml alkaline sodium nitroprusside solution were added. Pink to red color may appear.
- **Keller killani test:** 2ml extract of the drug was added to glacial acetic acid containing a trace amount of 0.5% ferric chloride. It was transferred to a small test tube; 0.5ml of concentrated sulphuric acid was carefully added by the side of the test tube. Reddish brown color if appeared at the junction of the two liquids and the upper layer appeared bluish-green, may correspond to the presence of glycosides.

- **Borntrager's test:** 3ml test solution was added to dilute sulphuric acid in a test tube which was boiled for 5 min and filtered while hot, to cold filtrate equal volumes of benzene or chloroform were added and shaken well. Dilute ammonia was added to the separated organic layer. A rose pink to red color may appear in the ammoniacal layer to ensure the presence of glycosides.
- **Modified Borntrager's test:** 5ml extract was added to a mixture of 5ml 5% FeCl_3 and 5 ml dilute HCL which was then after heated for 5 min in a boiling water bath. The mixture was cooled and benzene was added. The solution was shaken well. The organic layer was separated and an equal volume of dilute ammonia was added. A rose pink to red color may appear in the ammoniacal layer.

(J) Test for flavonoids

- To a small quantity of residue, add lead acetate solution. The yellow-colored precipitate is formed.
- To a small amount of residue, the addition of an increasing amount of sodium hydroxide to the residue shows yellow coloration. Which decolorizes after the addition of acid.

(K) Test for saponins glycosides

- **Foam test:** Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

Chemical Tests for Detection of Inorganic Constituents

Ash of drug material was prepared was prepared. 50% v/v HCL or 50% v/v HNO_3 was added to the ash, kept for 1hr or longer and filtered. With filtrate, the following tests were performed.

(A) Test for Iron

- 5 ml test solution was added to a few drops of 2% potassium ferricyanide. Dark blue coloration may appear.
- 5 ml test solution was added to a few drops of 5% potassium thiocyanate. The solution may turn blood red.

(B) Test for sulphate

- 5 ml test solution was added to a few drops of 5% BaCl_2 solution. The white crystalline BaSO_4 (precipitate) thus appeared may be insoluble in HCL.
- With lead acetate reagent. It may give a white precipitate soluble in NaOH.

(C) Test for chloride

- 3 ml test solution prepared in HNO_3 was added to few drops of 10% AgNO_3 solution. A white precipitate of AgCl may appear which was soluble in a dilute ammonia solution.
- To about 5-7 ml filtrate was added 3-5 ml lead acetate solution. White precipitate soluble in hot water may appear to correspond to the presence of chloride.

Determination of Extractive Values

100 g of air-dried drug gives – 80x g of the

Determination of alcohol, ethanol, chloroform and water-soluble extractives

alcohol (90%) soluble residue

- Weigh about 5 g of the powdered drug in a weighing bottle and transfer it to a dry 250ml. conical flask.
- Fill a 100 ml graduated flask to the delivery mark with the solvent (90% ethanol, 90% petroleum ether, 90% chloroform and 100 ml water). Wash out the weighing bottle and pour the washings. Together with the remainder of the solvent into the conical flask.
- Cork the flask and set it aside for 24 hr, shaking frequently. (Maceration)
- Filter into a 50 ml cylinder. When sufficient filtrate has been collected, transfer 25 ml, of the filtrate to a weighed, thin porcelain dish, as used for the ash values determination.
- Evaporate to dryness on a water-bath and complete the drying in an oven at 100°C.
- Cool in a desiccator and weigh.
- Calculate the percentage w/w of extractive with reference to the air-dried drug.

Calculation:

25 ml of alcoholic extract
gives = x g of residue

100 ml of alcoholic extract
gives = 4x g of residue

5 g of air dried drug gives – 4x g of
alcoholic (90%) soluble residue

Alcohol (90%) soluble extractive values
of the sample = 80%

Determination of Moisture (Loss on Drying)

- Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish.
- Dry in the oven at 100°C.
- Cool in the desiccator and watch. The loss in weight is usually recorded as moisture.

Determination of Swelling Factor

- Take 1 g of the seeds in a 25 ml stoppered cylinder.
- Add water up to 25 ml marking.
- Shake occasionally during 23 hr.
- Keep aside for 1 hr.
- Measure the volume occupied by the swollen seeds.

TLC Profile of *Psoralea Corylifolia* Seed Ethanolic Extract**Procedure**

TLC was performed by dissolving seed extract in methanol. The extract was spotted on pre-coated TLC plates made up of silica gel GF₂₅₄ (Sigma Aldrich). Mobile phase (Toluene: Ethyl Acetate; 7.5:2.5) was prepared, poured in TLC chambers and kept aside for saturation. TLC plates with the spots of extract were placed in saturated TLC chambers and allowed to run to 3/4th height of the plates. After the development of TLC plates, the plates were air dried and spots were detected by various means like exposure to iodine vapours, spray with spraying reagents like 10% methanolic KOH, & anisaldehyde-sulphuric acid and visualized under visible light and UV356.

The R_f value of various spots was calculated using formula:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{distance travelled by solvent}}$$

Preparation of Ethosomal Vesicles⁷

Psoralea corylifolia ethosomal formulations were prepared by 'Hot Method'. The drug concentration was fixed as 0.1 g w/w. The soya lecithin (phospholipid) and ethanol concentration was in the range of 1- 3% w/w and 20-30% w/w respectively. An accurately weighed quantity of the drug was dissolved in ethanol and propylene glycol was added to it. The soya lecithin was dissolved in water at 40°C in another beaker. The drug solution was then added slowly to soya lecithin dispersion of water at 40°C with 1,700 rpm in a closed vessel and was stirred for 30 minutes. The final preparation was subjected to ultra-sonication for an hour with a cycle of 15 minutes. The evenly dispersed ethosomal vesicle was formed.

Table 1: Composition of *Psoralea corylifolia* ethosomal vesicles⁸

Formulation code	Drug extract (gm)	Soya lecithin (ml)	Ethanol (ml)	Propylene glycol (ml)	Water
F ₁	0.1	1	20	20	q.s
F ₂	0.1	1	30	20	q.s
F ₃	0.1	2	20	20	q.s
F ₄	0.1	2	30	20	q.s
F ₅	0.1	3	20	20	q.s
F ₆	0.1	3	30	20	q.s

Characterization of Ethosomes⁹

Vesicle morphology

(A) Optical microscopy

A drop of ethosomal suspension was spread on a slide and covered with a cover slip and observed by optical microscopy and photomicrographs were taken.

(B) Scanning electron microscopy (SEM)

One drop of ethosomal system was mounted on a stub covered with clean glass homogeneously. A polaron E5100 sputter coated the samples with gold and the samples were examined under a scanning electron microscope at an accelerating voltage of 20 KV.

(C) Determination of entrapment efficiency¹⁰

The total volume of the ethosomal suspension was measured and 1 ml of this formulation was transferred to 1.5 ml centrifuge tube. The suspension was diluted with ethanol. Ethosomes were separated by ultracentrifugation at 45,000 rpm for 30 min. Supernatant and sediment were recovered and their volume was measured. The untrapped and entrapped drug contents were analyzed by estimating drug in supernatant by UV-spectroscopic method.

$$\text{Percentage entrapment efficiency} = \frac{D}{Dt} \times 100$$

Where =

D = amount of drug in sediment

Dt = total amount of drug is supernatant and sediment

Results:

Detection of organic compounds

Table 2: Phytochemical tests for the detection of organic constituents

Extract		Ethano lic hot extract	Ethano lic cold extract
Chemical class	Tests		
Carbohydrates	Molish's test	+	+
	Fehling test	+	+
	Barford test	+	+
	Benedict test	+	+
	Bial test	-	+
	Selwinoff reagent	+	+
	Tallen test	+	+
	Iodine test	-	-
	Tannin test	+	+
Tannis & phenolic compounds	5%-FeCl ₃ solution	+	+
	Lead acetate	+	+
	Gelatin solution	+	+
	Bromine water	+	+

	Acetic acid solution	+	+
	Potassiou m dichromate	+	+
	Dil. HNO ₃	+	+
	Dil NH ₄ OH + potassium ferricyanide solution	+	+
	Dil NH ₄ OH + potassium ferricyanide solution + KMnO ₄	+	+
Alkaloids	Dragender off reagent	+	+
	Mayer reagent	+	+
	Hager reagent	+	+
	Wagner reagent	+	+
Glycosides	Legal test	+	+
	De-oxysugar	+	+
	Borntrager test	+	+

	Modified borotrager	+	+
Flavonoids	Lead acetate test	+	+
	NaOH test	+	+

	Hot extracts	Cold extracts
Moisture content (loss on drying)	0.2	0.1

Detection of inorganic constituents:

Table 3: Detection of inorganic elements

Tests	Filtrate	
	Hydrochloric acid	Nitric acid
Tests for calcium	-	-
Tests for potassium	-	-
Tests for magnesium	-	-
Test for iron	+	+
Test for sulphate	+	+
Tests for phosphate	-	-
Test for chloride	+	+

Detection of extractive values

Table 4: Quantitative determination of extractive values

Extracts	% Yields (w/w)	Consistency
Ethanol	125.9	Sticky
Petroleum ether	110.0	Sticky
Chloroform	109.3	Sticky
Water	122.7	Sticky

Determination of moisture content

Table 5: Quantitative determination of moisture content

Parameter	% Moisture content
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Determination of swelling index

Table 6: Quantitative determination of swelling index

Parameter	Swelling index (cm)
Swelling index	1

The mobile phase consisting of Toluene: Ethyl Acetate (7.5 : 2.5) with spray reagents, 10% methanolic KOH and anisaldehyde- sulphuric acid showed 10 spots each (Figure 1 & 2) while with iodine vapours (figure 3) gave 9 spots. Rf values of all the detected spots are given in table 1. TLC plate sprayed with anisaldehyde-sulphuric acid reagent showed violet coloured bands at Rf 0.31 (Hot Extract) and Rf 0.34 (Cold extract), indicating the presence of psoralene among other phytoconstituents.

Table 7: TLC profile of *Psoralea corylifolia* seed ethanolic extract

S. No.	Ex tr ac t	Solv ent syst em	Vis uali zing age nt (Spr ay rea gen t)	Visible light			UV 356		
				N o f o f S p o t s	R f v a l u e s	C o l o r	N o f o f S p o t s	R f v a l u e s	Fluo resce nce Colo ur
1	Co ld ext rac t	Tolu ene: ethy l acet ate (7.5: 2.5)	10 % met han olic KOH solu tion	7	0.31	yellow	10	0.34	Greenish yellow

					0.66777	brownish yellow	0.520550.610.6660.7770.87	Brownish Orange Yellow Yellow
	Hot extract	Toluene: ethyl acetate (7.5:2.5)	10% methanolic KOH solution	7	0.310.480.50.520.590.650.74	yellow yellow yellow yellow yellowish orange Brownish Blue flour essence Brownish Orange Yellow Yellow	10.270.310.350.390.440.480.530.580.630.680.730.780.830.880.930.98	Greenish yellow Yellow Greenish yellow Yellow Yellowish orange Brownish Blue flour essence Brownish Orange Yellow Yellow

								0.740.84	nc brownish orange yellow
2	Cold extract	Toluene: ethyl acetate (7.5:2.5)	Anisaldehyde spray reagent		0.320.40.490.580.670.81	yellow yellow Greenish yellow Yellow Dark yellow Dark yellow Bluish green Pink Yellow Yellow flour essence	10.280.320.360.400.440.480.520.560.600.640.680.720.760.800.840.880.920.96	Sky blue Yellow Light yellow Greenish yellow Dark yellow Dark yellow Bluish green Pink Yellow Yellow flour essence	
3	Hot extract	Toluene: ethyl acetate	Anisaldehyde spray	6	0.320.40.490.580.67	yellow yellow yellow yellow yellowish orange Brownish Blue flour essence Brownish Orange Yellow Yellow	10.270.310.350.390.440.480.530.580.630.680.730.780.830.880.930.98	Sky blue Yellow	

		(7.5: 2.5)	reagent		0.48	greenish yellow	320	Light yellow
					0.59	yellow	40	Dark yellow
					0.68	dark yellow	0	Dark yellow
					0.83	dark yellow	48	Bluish green
						yellow	59	Pink
						yellow	60	Yellow
						yellow	66	Yellow
						dark yellow	88	flour
						yellow	77	esence
						yellow	77	
						yellow	83	
						yellow	80	
						yellow	87	
						yellow	7	
4	Cold extract	Toluene: ethyl acetate (7.5: 2.5)	Iodine	9	0.13	-	-	-
					0.21	-	-	-
					0.22	-	-	-
					0.31	-	-	-
					0.32	-	-	-
					0.33	-	-	-
					0.36	-	-	-
					0.56	-	-	-
					0.66	-	-	-
					0.63	-	-	-
					0.68	-	-	-
					0.80	-	-	-
					0.88	-	-	-
					0.87	-	-	-
5	Hot extract	Toluene: ethyl acetate	Iodine	9	0.14	-	-	-
					0.22	-	-	-
					0.21	-	-	-

		(7.5: 2.5)			0.22			
					26			
					0.33			
					50			
					0.55			
					44			
					0.66			
					20			
					0.69			
					88			
					00			
					0.99			
					1			

Prepare anisaldehyde-sulfuric acid spray reagent:

For detection of phenols, sugars, steroids, and terpenes

Spray with a solution of freshly prepared 0.5ml p-anisaldehyde in 50ml glacial acetic acid and 1ml 97% sulfuric acid. and heat to 105°C until maximum visualization of spots. The background might be brightened by water vapor. Results: Lichen constituents, phenols, terpenes, sugars, and steroids turn violet, blue, red, grey or green.

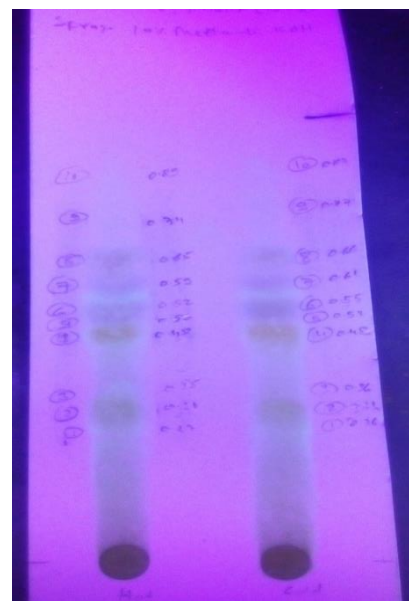


Figure 1a:
Figure 1b:

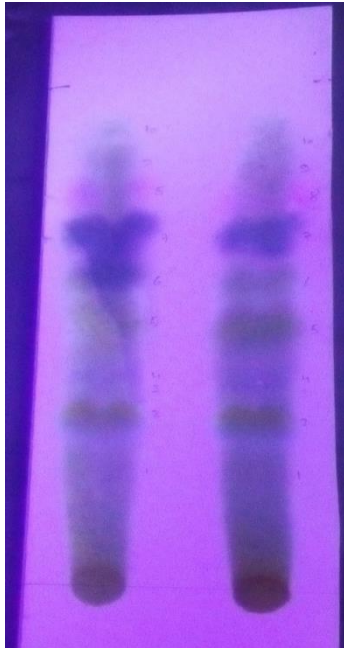


Figure 2a:

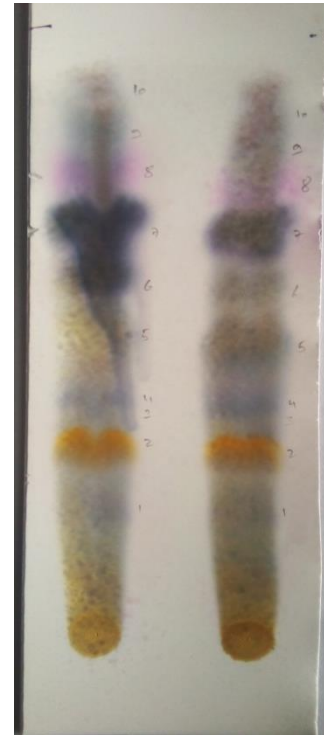


Figure 2c:

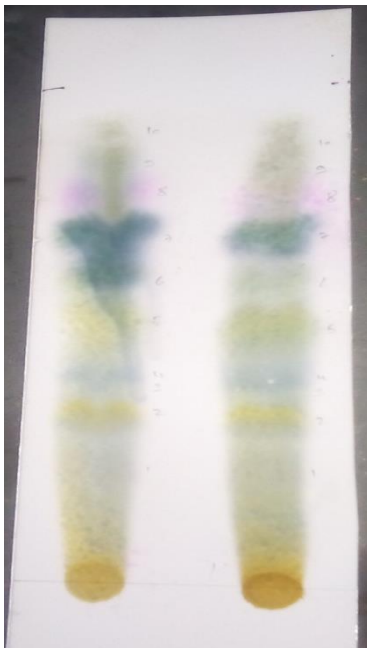


Figure 2b

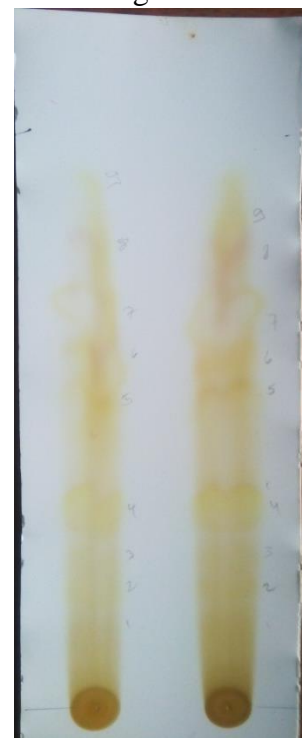


Figure 3a

Optical microscopy

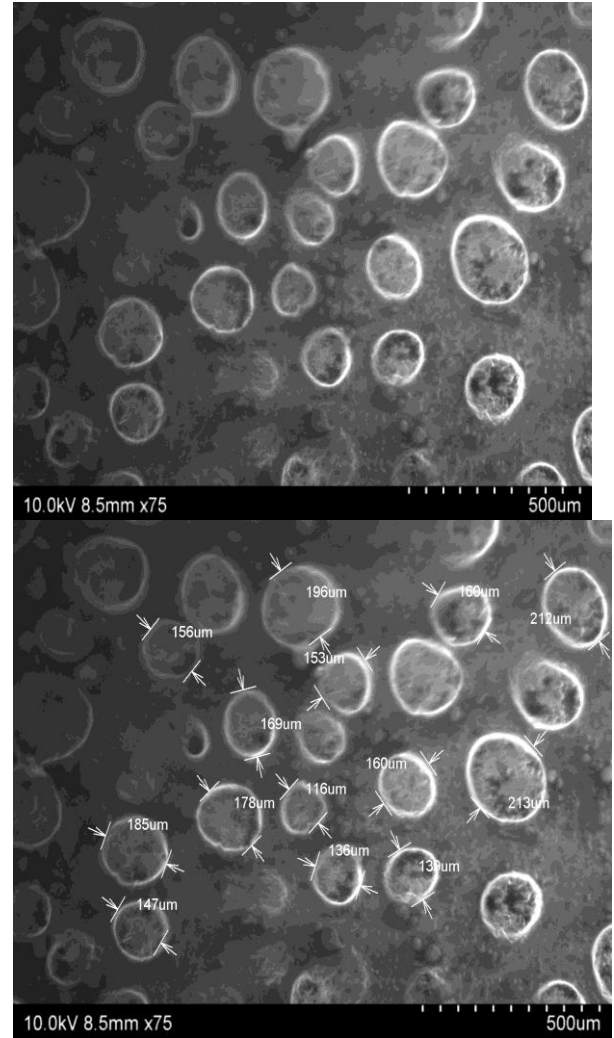
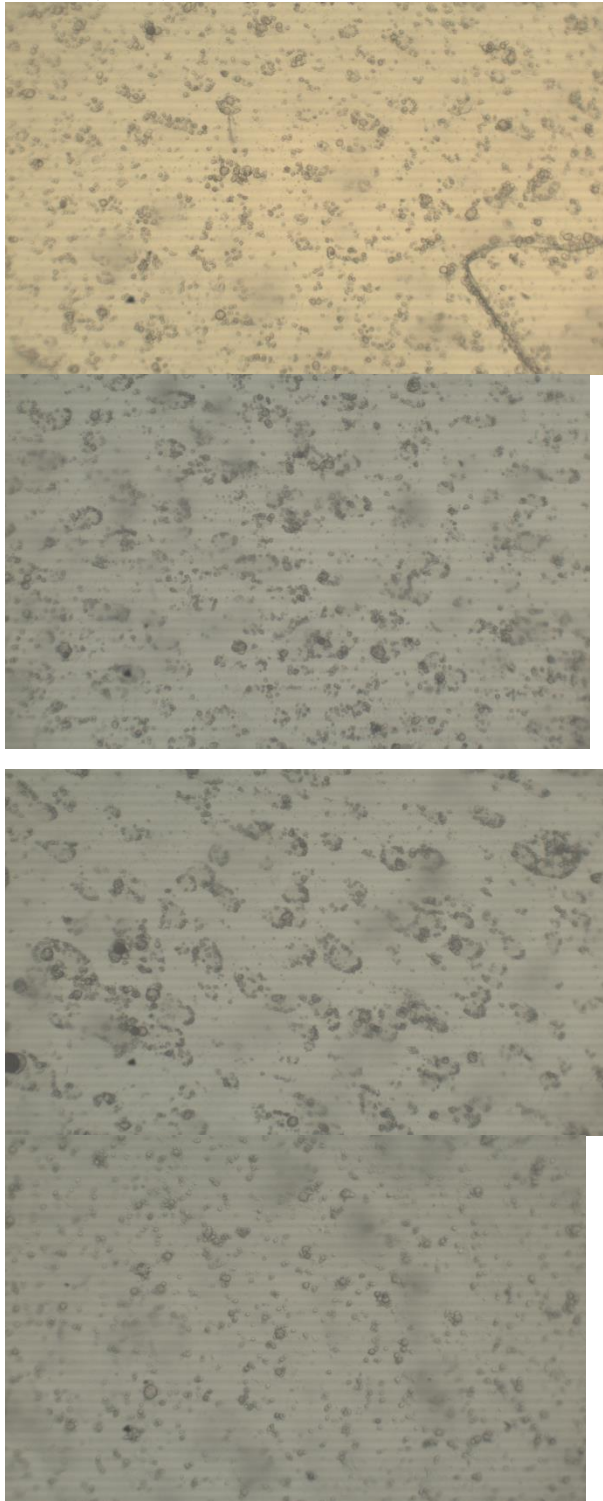


Table 8: Determination of drug entrapment efficiency

Formulation code	Drug extract (g m)	Soya lecithin (ml)	Ethanol (ml)	Propylene glycol (ml)	Water	% Entrapment efficiency
F ₁	0.1	1	20	20	q.s	100.3
F ₂	0.1	1	30	20	q.s	102.01
F ₃	0.1	2	20	20	q.s	100.22
F ₄	0.1	2	30	20	q.s	97.09
F ₅	0.1	3	20	20	q.s	107.1

Scanning electron microscopy:

F ₆	0.1	3	30	20	q.s	100.1 8
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Conclusion:

Psoralea Corylifolia ethosomes were prepared and evaluated for various parameters. 6 batches were prepared for the optimization process. Phytochemical tests for the detection of organic constituents were performed in hot and cold extract separately and it was found that it contains carbohydrates, tannins and phenolic compounds, alkaloids, glycosides and flavonoids. Phytochemical tests for the detection of inorganic constituents were performed in HCl and HNO₃ filtrate of seed extract and it was found that it contains iron, chloride, sulphate. In the quantitative determination of extractive values ethanol extract % yield was found to be 125.9% w/w and have sticky consistency, petroleum ether % yield was found to be 110.0% w/w and have sticky consistency, chloroform % yield was found to be 109.3% w/w and have sticky consistency, water extract % yield was found to be 122.7% w/w and have sticky consistency. Moisture content in hot extract was found to be 0.2% and in cold extract 0.1%. Lichen constituents, phenols, terpenes, sugars, and steroids turn violet, blue, red, grey or green while TLC evaluation. Swelling index of ethosomes was found to be 1 cm. Drug entrapment efficiency was found to be highest in batch F₅ i.e., 107.1%.

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Declared none.

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