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RESEARCH ARTICLE

Physicochemical and Phytochemical Evaluation of *Psoralea corylifolia* (Seed) and its Extract

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Abstract

Background: Herbal phytochemicals play an important role in the treatment of various dreadful diseases. *Psoralea corylifolia* is an annual herbaceous plant widely grown in India and other countries Secondary metabolites of *P. corylifolia* play a crucial role in various skin disorders like psoriasis, eczema and vitiligo as per literary and current reported studies. Various studies have indicated that the seeds of *P. corylifolia* possess antimicrobial and anti-inflammatory properties. **Aim:** To evaluate the physicochemical and phytochemical analysis of *P. corylifolia.* **Methods:** Psoralen, which is the main active chief constituent present in *P. corylifolia*, has great therapeutic potential for managing psoriasis. The utilisation of such constituents in Novel Drug Delivery System (NDDS) like micro-emulsions, nano-emulsions, phytosomes, etc. leads to enhanced therapeutic potential. But before proceeding, the formulation development quality control standardisation of every drug is a must for the effective performance of the drug. The standardisation of herbal drugs plays a crucial role in their enhanced therapeutic value. All the standardisation parameters of herbal drugs are widely available in various pharmacopoeias as well as in the Ayurvedic formulary. This article is an attempt to revalidate the physicochemical evaluations of *P. corylifolia* seeds along with the High-Performance Thin Layer Chromatography (HPTLC) profiling by using potent phytochemical markers. **Results:** The phytochemical investigation of the extract of *P. corylifolia* shows the presence of alkaloids, tannins, triterpenoids, glycosides, flavonoids etc. **Conclusion:** Based on findings, it is concluded that prepared extract can be utilised for further activities like formulation development where the extract can play an important role as compared to simple powder.

Keywords: Pharmacognostic Evaluation, Phytochemical Analysis, Psoriasis

1. Introduction

Psoralea corylifolia is an annual erect herb that is widely used in traditional systems of medicine throughout the world. *Psoraleos* genus is derived from a Greek word that means affected by itching or leprosy. In India, it is also known by its various synonyms which are available in the database of classical Ayurvedic works of literature i.e., *Babchi, Bakuchi, Vakuchi, Bawachi*, etc[1-4]. "*P. corylifolia*" also has some other biological names, such as *Cullen corylifolium*, *Cullen corylifolia*, *Psoralea patersoniae* and *Trifolium unifolium*[5]. *P. corylifolia* plant is widely distributed in tropical and subtropical regions of the world, especially China[6,7]. The height of this plant is approx. 25–170cm and it grows in warm areas. Sandy soil is best for the growth of *P. corylifolia* plant. March and April are the best months for the cultivation of this plant which matures in November. The plant grows within 6-7 years from the day of its cultivation. *P. corylifolia* fruit is perennial and bears a small reddish

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flower. Its fruit has no odour and has an unpleasant taste[8].

This plant is widely used in traditional as well as Chinese systems of medicine, mainly for skin diseases and anthelminthic activity[9-11]. *P. corylifolia* seeds have great medicinal potency in traditional systems of medicine and act as laxatives, anthelmintics and antimicrobial agents due to their major phytoconstituents. The major chemical constituents present in *P. corylifolia* and their therapeutic efficacy is mentioned in Table 1. External application of *P. corylifolia* seed either in the form of novel drug delivery or in the form of a cream or ointment has great therapeutic efficacy in various dreadful diseases like leprosy, psoriasis and inflammatory diseases of the skin such as eczema[12,13].

Table 1. List of chief constituents with their therapeutic efficacy[14-28]

S. No.	Chief Constituents	Plant Part	Therapeutic Efficacy
1	Bakuchiol	Seed	Antibacterial, Antifungal
2	Bavachinin	Seed	Antibacterial
3	Bakuchicin	Seed	Topoisomerase inhibitor
4	Bavachinone A	Seed	Antibacterial
5	Bavachinone B	Seed	Antibacterial
6	Corylifolin	Seed	Antioxidant
7	Psoralen	Seed	Antipsoriatic
8	Neobavaisoflavanone	Seed	Antbacterial
9	Isopsoralen	Seed	Antiprotozoal
10	Bakuisoflavone	Seed	Antibacterial
11	Corylifols	Seed	Antibacterial
12	Coryaurone A	Fruit	Anti-bacterial
13	Hydroxy bakuchiol	Seed	Lymphangiogenesis
14	Angelicin	Seed	Antibacterial
15	Bavacoumestan C	Seed	Antibacterial

Standardisation is parameters that determine the quality, identity and quantity of active ingredients present in a drug and ensure that the drug is genuine[29]. Standardisation is a pre-requisite parameter before making any formulation[30]. For treating diseases, herbal phytochemicals play an important role and standardization helps in the quantification of these phytochemicals[31]. The identification, as well as authentication of herbal drugs, are essential for pharmaceutical companies as well as for the public healthcare system[32]. Figure 1 represents the herbal drug standardisation.

Physical standardisation includes analysis of loss on drying, ash value extractive values etc. Biological standardization includes microbial contamination, pharmacological evaluation, biological assays and toxicological analysis. Chemical standardisation includes chromatographic analysis and heavy metal and pesticide residue analysis. Botanical standardiation includes analysis of colour, odour, taste and microscopic analysis[33,34].

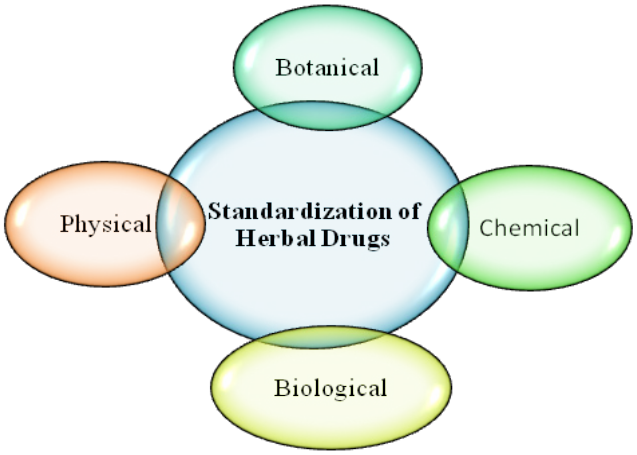

Figure 1. Schematic representation of herbal drug standardization.

2. Materials and Methods

2.1 Procurement of *P. corylifolia* Seeds

Psoralea corylifolia seeds were obtained as a gift sample from Shree Dhanwantri Herbals Amritsar Punjab.

2.2 Authentication of *P. corylifolia* Seeds

The authentication of *P. corylifolia* seeds was done at Shree Guru Nanak Dev University, Amritsar, Punjab, by submitting the sample in the herbarium file with the reference Boucher No. 0374.

2.3 Morphology of *P. corylifolia* Seeds[35-37]

Psoralea corylifolia, is an erect annual herbaceous plant with a size range from 0.5 to 1.1 meters. The plant has simple, hairy and wide oval leaves. The petioles have glands and are hairy. The blue flowers have a racemes-like look and the pungent and oblong seeds are deep brown.

2.4 Powder Microscopy of *P. corylifolia*[38]

Organoleptic powder microscopy shows unicellular trichomes, volatile oil, pollen grains, simple fibres, prismatic crystals and stone cells.

2.5 Physicochemical Analysis of *P. corylifolia* Seeds[39]

The seed of the *P. corylifolia* was coarsely powdered with the help of a grinder and then the mixture was passed through a 60 # sieve. The obtained coarse powder was subjected to evaluation parameters to check its identity, purity and strength. Seeds of *P. corylifolia* were examined morphologically and microscopically, followed by foreign matter evaluation, loss on drying, total ash, acid-insoluble ash and extractive values. i.e., water-soluble and alcohol-soluble extractive values as per the Ayurvedic Pharmacopoeia of India.

2.6 Preparation of *P. corylifolia* Extract[40]

5g of *P. corylifolia* seeds were ground to a coarse powder. Then take this coarse powder and make a thimble of (What man No. 1) filter paper fitted with a 250ml round bottom flask containing 150ml of extracting solvent. The extraction process was carried out at the boiling point of each solvent for up to 5 hours in the case of petroleum ether and chloroform, respectively. In the case of aqueous extraction, the period of extraction was longer, i.e., 7-8 cycles by the recycling process. After the completion of the first extraction, the material remaining in the thimble was re-extracted again with a suitable amount of solvent.

2.7 Phytochemical Screening of *P. corylifolia* Seed Extract[41-44]

Phytochemical screening plays an important role in the qualitative analysis of herbal drugs. The seed extract was subjected to the evaluation of various primary and secondary metabolites by following the standard procedures, which include alkaloids, glycosides, terpenoids etc.

2.8 HPTLC Analysis of *P. corylifolia* with Marker Gallic Acid[42,43]

HPTLC analysis is an important parameter for the detection of chief active constituents present in a drug. HPTLC was performed on 20cm x 10cm Thin Layer Chromatography (TLC) aluminium plates having a thickness of "Silica gel 60 F^{254}". Using 100 microliter sample syringes and an applicator, the sample was applied in a bandwidth of 6mm. A fixed application rate of 150L s^{-1} was used. The mobile phase containing solvent i.e. toluene, ethyl acetate and acetic acid in a ratio of 15:12:3 was carried out in a twin trough glass chamber saturated with vapours of the mobile phase. Following an 80mm development distance, plates were air-dried. Camag TLC scanner was used for the scanning. All the observations were recorded with a comparison of standard as well as test compounds at various concentrations applied and observed under 366 and 254nm.

2.8.1 Standard and Sample Preparation

Standard preparation: 27.3mg standard was taken in 25ml of methanol. Sample Preparation: 1.333g sample was taken in 25ml of methanol to which 1 drop of 0.1M hydrochloric acid was added and sonicated for 10 minutes. After that, it was filtered with 0.42-micron filter paper evaporated to dryness and reconstituted with 10ml methanol and aspirated 2µl, 4µl and 6µlin TLC plate. Comparison within the R_f value of standard i.e. gallic acid and sample i.e. *P. corylifolia* extract was done.

2.8.2 Calibration

Gallic acid stock solution (27.3 micrograms/ml) was prepared in methanol and dilution was carried out for further analysis. To complete 10-100 mg/spot of gallic acid, different volumes of the solution (2, 4, 6, 8, 10, 12, 14 **µl**) were applied in duplicate to a plate. Peak area data and the corresponding amounts were treated by linear least square regression analysis.

3. Results

3.1 Result for Morphology of *P. corylifolia*

The morphological characters of *P. corylifolia* seeds mentioned in Table 2, Figures 2, 3 and 4 show the powder characters of seeds of *P. corylifolia.*

3.2 Results for Powder Microscopical Characters of *P. corylifolia* Seed

The powder microscopy of *P. corylifolia* seed represented in Figure 5, shows the presence of a Parenchymatous

Table 2. Organoleptic characters of *P. corylifolia* seed

Sr. No.	Organoleptic Characters	Observations
1	Colour	Dark brownish
2	Odour	Oily
3	Taste	Characteristic

Figure 2. Shows *P. corylifolia* seeds.

Figure 3. Shows authenticated seeds of *P. corylifolia*.

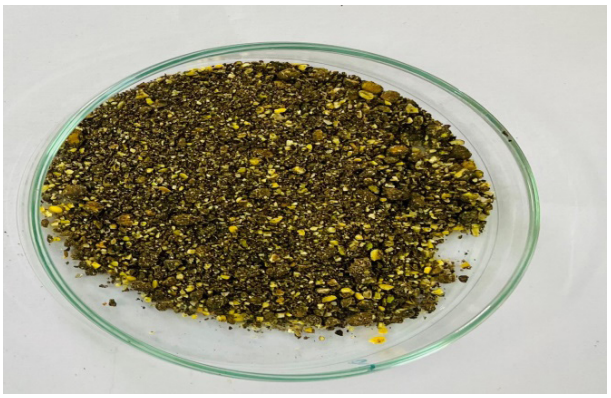

Figure 4. Powder of *P. corylifolia* seed.

cell, simple fibre, pitted vessel, covering trichome and stomata cell.

3.3 Results for the Physico-chemical Analysis of *P. corylifolia* Seed

The physicochemical analysis of *P. corylifolia* seed shows the results mentioned in Table 3 and its solubility rate is presented in Table 4.

3.4 Results for the Phytochemical Screening of *P. corylifolia* Seed

The results for the phytochemical screening of seeds of *P. corylifolia* are shown in Table 5.

3.5 Results for the HPTLC Analysis of *P. corylifolia* Seed

All the observations were recorded with the comparison of Standard as well as test compounds at various concentrations applied and observed under 366 and

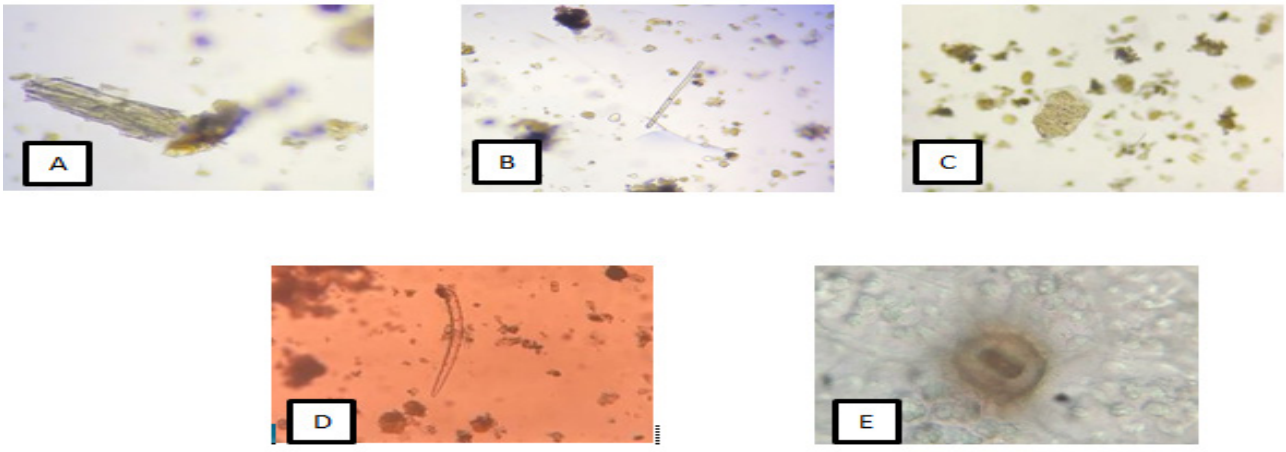

Figure 5. Powder microscopy of *P. corylifolia* seed; (A). Parenchymatous cell; (B). Simple fibre; (C). Pitted vessel; (D). Covering trichome; (E). Stomata cell.

Table 3. Shows the results of physicochemical and heavy metal analysis

Drug	Parameters	API Value %	Obtained Value %
P. corylifolia	Foreign Matter	NMT 1	Nil
	Total Ash	NMT 8	4.82
	Acid Insoluble Ash	NMT 2	0.71
	Alcohol Soluble Extractive Value	NLT 13	59.48
	Water Soluble Extractive Value	NLT 11	68.91
	Loss on drying	-	9.10
	Heavy Metal Analysis		
	Lead (as Pb) Cadmium (as Cd) Mercury (as Hg) Arsenic (as As)	NMT 10.0ppm NMT 0.3ppm NMT 1.0ppm NMT 3.0ppm	Not Detected Not Detected Not Detected Not Detected

Table 4. Solubility rate of *P. corylifolia* seed in different solvents

Name of Drug	Solvent			
	Petroleum Ether	Chloroform	Water	Mean
P. corylifolia	22.13±0.23	30.96±0.43	42.10±0.18	31.73±0.27

254nm. Table 6 and Figure 6 give a detailed analysis of the chromatogram, peaks and chromatographic images.

4. Discussion

The present study was focused towards the evaluation of *P. corylifolia* seed and its extract. Morphological, microscopical, physicochemical, phytochemical identification and HPTLC analysis were done for the evaluation of *P. corylifolia* seed. The physiochemical evaluation of the drug shows a positive result as compared to the standards mentioned in Ayurvedic Pharmacopoeia of India. The phytochemical investigation of the extract of *P. corylifolia* shows the presence of alkaloids, tannins, triterpenoids, glycosides, flavonoids etc. HPTLC was performed on 20cm x 10cm TLC aluminium plates having a thickness of "Silica gel 60 F 254". Using 100 microliter sample syringes and an applicator, the sample was applied in a bandwidth of 6mm. A fixed application rate of 150nL s^{-1} was used.

Table 6. Shows the band on the TLC plate along with their width area, Rf value against their volume and concentration applied

ID	Width	Band	Volume
1	22	1	19.6
2	24	1	25.14
3	28	1	31.38
4	22	1	0.32
5	23	1	1.45
6	24	1	1.67
ID	**Rf**	**Area**	**Volume**
2_1	0.326	792	25.14
5_1	0.318	598	1.45
Name of Sample	**Retardation factor**	**Volume**	**Conc. Applied**
P. corylifolia extract	0.318	1.45	213.296
Gallic Acid (Standard)	0.326	25.14	4.368

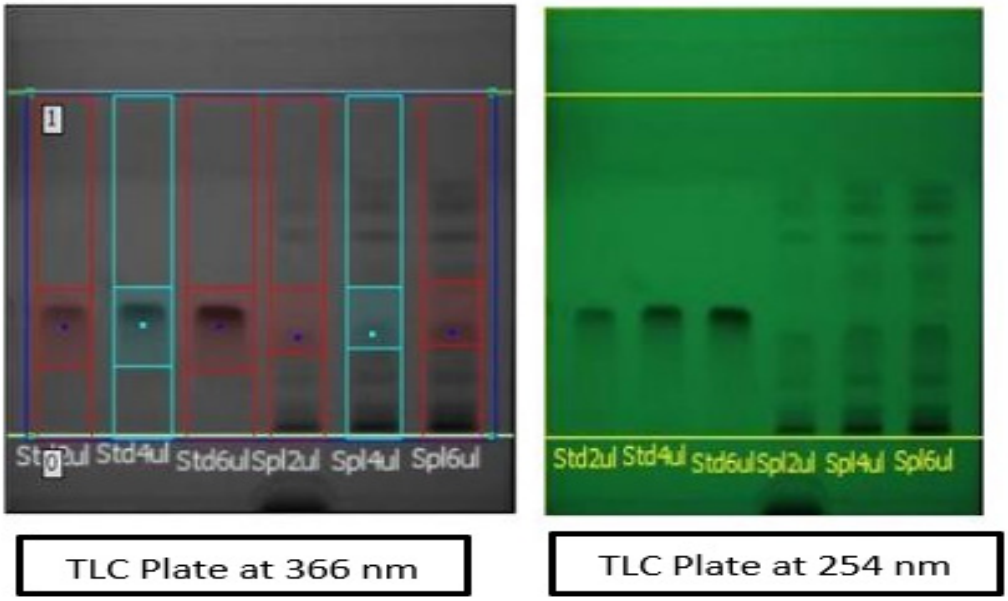

Figure 6. Shows HPTLC analysis of *P. corylifolia* seed extract.

The mobile phase containing solvent i.e. toluene, ethyl acetate and acetic acid in a ratio of 15:12:3 was carried out. The standard of gallic acid was used at a concentration of 27.3µg/ml. All observations were recorded and compared with the standard. The R_f was found to be 0.318 for the *P. corylifolia* extract and 0.326 for the standard (Gallic acid).

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7. Conclusion

After undergoing various physicochemical and chromatographic evaluations of *P. corylifolia* seed extracts, it is noteworthy that the drug showed the presence of important phyto-constituents in chromatographic analysis, like gallic acid. While conducting the physicochemical evaluations, the drug passed the limit of the official compendium. Hence, the drug was considered genuine. The prepared extract also shows the presence of important secondary metabolites as per the result of primary phytochemical evaluations. The present findings further established the analytical findings and evaluations of the extract of *P. corylifolia* seeds and confirmed the presence of noteworthy phytoconstituent extracts. Based on the findings, prepared extract can be utilised for further activities like formulation development where the extract can play an important role compared to simple powder.

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