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PSORALEA PINNTA ACTIVE COMPONENTS SEPARATED, IDENTIFIED AND QUANTIFIED USING HPLC-UV & HPLC- MS

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ABSTRACT:
Medical Science advancements have made it possible to develop a large number of drugs. A qualitative analysis identifies the presence or absence of certain components in a sample in order to provide us with information about the sample's nature. The potential pharmacological properties of many plants are constantly being investigated (such as inflammatory, hypotensive, hypoglycaemic, amoebicidals, antifertility, cytotoxic and antibiotics*). Psoralea corylifolia* belongs to the family Fabaceae which is commonly known as blue pea. There are 130 species in the Fabaceae family's Psoralea genus, most of which are found in South Africa, North and South America, and Australia. Phytochemical evaluation is used to determine the nature of Phytoconstituents present in the plant by using suitable chemical tests as per standard procedure. Solvent mixture was selected on the basis of the phytoconstituents present in each extract. The separation of constituents in the extract of *Psoralea corylifolia* was performed by HPLC-UV and MS in negative ion mode. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract. Powdered drugs are generally identified by their physiochemical parameters. Physiochemical property characterization has gained strength in the pharmaceutical industry and has become a standard procedure. Loss on drying, total ash, soluble and insoluble ash, extractive values are reliable aids for detecting adulteration. The HPTLC chromatogram for *Psoralea corylifolia* (HAEPC) showed significant separation of three phytoconstituents with Rf values ranging from 0. 43 to 0.83. HPTLC studies indicate the plant extract contains three phytoconstituents. We studied macroscopy and physicochemical parameters to identify adulterants. Carbohydrates, Proteins, Steroids, Terpenoids, and Flavonoids were detected in the crude drug and formulated lozenges. This will help in preventing variation in the quality of the drug because the physicochemical constants were determined. P. corylifolia contains bavachin, bakuchiol, and psoralen, all of which are commercially available.

Keywords: *Psoralea pinnata*, HPLC-UV, HPLC-MS, Primary Active constituents, Herbal medicine

INTRODUCTION

"Health is wealth," the saying goes. All humans have a fundamental desire to have a healthy physique. The first requirement for enjoying life and everything else that humanity has to offer is good health. People are more interested in their health today. Using accurate diagnostics and efficient treatment, the medical model of health seeks to eliminate disease. Medical science advancements have made it possible to develop a large number of drugs [1]. Every year, more and more medications are introduced to the global market. There are either brand-new or minimally modified substances in these medications. Quantification is used from the very beginning of the discovery of a medicine to determine its quality and efficacy. Analytical methods or observing the effect of drug on various animal models are used to evaluate quality and efficacy [2]. To make sure that products are efficient, quality standards are applied using the analytical approach. There has been tremendous advancement in the field of pharmaceutical analysis as a result of the pharmaceutical industry's recent rapid growth, which has led to the introduction of several pharmaceutical formulations into the healthcare system [3]. With the introduction of analytical methods, physical properties are measured to determine a substance's chemical composition, resulting in a drastic change in how substances are classified. Analytical instruments are devices (or sets of devices) that provide information about a sample's chemical composition (or) its physical properties (or both) [4]. Pharmaceutical agents derived from plants account for at least 122 different chemical compounds. About 25% of drugs in developed countries are derived from plants or are modified versions of plants first isolated from plants. A variety of purposes may require this information, such as testing materials, maintaining standards, verifying physical phenomena, monitoring the process stream, and controlling product quality and safety management.

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Such measuring devices are developed through analysis instrumentation, a science of technology [5]. As the demand for our products has increased and the possibility of substitution has increased, the importance of quality control is of almost importance. Today, a large number of drugs are available in monographs that include descriptions, tests for identity and purity, assays for active constituents [6].

A qualitative analysis identifies the presence or absence of certain components in a sample in order to provide us with information about the sample's nature. Using pure compounds, including synthetic drugs, has its limitations, and herbal medicine and homoeopathy, which rely heavily on plant sources, have seen a resurgence of interest in recent years. The potential pharmacological properties of many plants are constantly being investigated (such as inflammatory, hypotensive, hypoglycaemic, amoebicidals, antifertility, cytotoxic and antibiotics). Due to historical and cultural reasons, herbal medicines have often maintained their popularity alongside modern medicine [7]. In contrast, herbal products were typically marketed and regulated as dietary supplements in the USA. These criteria are not required for preapproval of a product in this category.

ULTRAVIOLET SPECTROSCOPY

Compounds with multiple bonds normally absorb a portion of electromagnetic radiation in the ultraviolet and visible regions when it passes through them. A compound's structure and the wavelength of the radiation determine the amount of absorption. When electromagnetic radiation in the visible and ultraviolet regions of the spectrum is absorbed, ions and molecules undergo changes in their electronic structures [8].

DRUGS ANALYSIS USING HPLC METHODS

A variety of industries have used high performance liquid chromatography [HPLC] for sample analysis and purification, including the pharmaceutical, biotechnological, environmental polymer and food industries. The HPLC instrument consists of eight basic components: the mobile phase reservoir, solvent delivery system, sample introduction device, column, detector, waste reservoir, and connective tubing.

There are several steps involved in the chromatographic method of a separation;

- The act of adsorbing or retaining a substance on a stationary phase
- The mobile phase separates the adsorbed substance.
- By flowing the mobile phase continuously, the separated substance can be recovered. It is called elution.
- The eluted substance was analyzed quantitatively and qualitatively

Parameters for Method Validation

The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature

- Specificity study
- Linearity and range study
- Limit of detection and Limit of quantitation study
- Precision study
- Accuracy study
- Robustness study
- Solution stability study
- System suitability

Plant introduction

Psoralea corylifolia belongs to the family Fabaceae which is commonly known as blue pea. There are 130 species in the Fabaceae family's Psoralea genus, most of which are found in South Africa, North and South America, and Australia. Several of them have been used extensively as herbal medicines in China, India, and other nations. The species in the complex may have inflorescences that are pseudo-spicate, pseudo-racemose, or pseudo-capitate. *Psoralea* was also characterized by the presence of a unique, cup-shaped structure (cupulum) present at different positions on each flower pedicel. Total number of identified secondary metabolites from the genus Psoralea amounts to 129, including flavonoids, coumarins, phenols, benzofurans, benzopyrans, quinines, sesquiterpenoids, triterpenoids, steroids, and some other components upto 2015. In addition, a key component called bakuchiol and its related many bioactivities of chemicals, including the suppression of caspase-3, monoamine transporters, and immunosuppressive effect hepato protective effect, antibacterial activity, and apoptosis DNA polymerase inhibition, an anti-inflammatory impact, and cytotoxic effects, topoisomerase II, including anti-diabetic effects [9].

Methodology

Procurement of Plant materials

The leaf powder of *Psoralea corylifolia* was purchased from Registered Herbal store, Telengana and Various organoleptic characters like colour, odour, taste and nature observed.

Physiochemical Evaluation

The powdered material of *Psoralea corylifolia* was used for the analysis of various physiochemical parameters which is useful in the determination of quality and purity of crude drug powder. Ash values, extractive values, loss on drying were determined as per the standard WHO guidelines which is very much useful in the determination of quality and purity of the crude drugs.

Preparation of Hydroalcholic Extract of *P. corylifolia* (HAEPC)

The powder plant material was defatted with petroleum ether by maceration method. Defatted marc was extracted with 70% ethanol and 30% water as a solvent by continuous hot percolation method for a period of 72 hours. Then the extract was filtered and evaporated under reduced pressure using Buchi rotary evaporator and the extract thus obtained is used for further experimental studies.

Phytochemical Investigations

Phytochemical evaluation is used to determine the nature of Phytoconstituents present in the plant by using suitable chemical tests as per standard procedure.

Analytical Studies

Thin Layer Chromatography [10]

TLC Plate Preparation

The plates were prepared using Stahl TLC spreader. 40g of silica gel G was mixed with 85mL of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in desiccators.

Selection of mobile phase

Solvent mixture was selected on the basis of the phytoconstituents present in each extract. Factors such as nature of components, stationary phase, polarity, influence the rate of separation of constituents was considered. From the vast analysis, best solvents were selected which showed good separation with maximum number of components.

HPTLC (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY) [11]

HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract.

Chromatographic condition:

The estimation has been done using the following chromatographic conditions. HPTLC analysis was performed on 20 cm× 10 cm aluminium backed HPTLC plates coated with a 0.2 mm layer of silica gel G 60 F254. The plates were washed with methanol and activated in an oven at 110°C for 15 min prior to the analysis. The reference standards and samples were applied onto the plates in the form of 6 mm long bands, 8 mm from bottom edge of the plate and 14 mm from side the edges, by means of a CAMAG automatic TLC applicator CAMAG Linomat V. The plates were scanned immediately at 254 nm in absorbance–reflectance mode, by means of TLC Scanner IV (CAMAG) with vision CATS software, version 2.5.18262.1, using a deuterium lamp.

HPLC-UV METHOD [12]

Chemical and materials

The reference standards of Bavachin, isobavachalcone, bavachinin and bakuchiol which having purity ≥ 99% were obtained from sigma Aldrich, ANJ Biomedicals and Natural Remedies Pvt. Ltd. Bangalore, India. The solvent which is used in this study having HPLC grade were purchased from local commercial market sources.

Stock Solution

The standard stock solution of bavachin, isobavachalcone, bavachinin and bakuchiol were prepared by dissolving 25 mg of each in 7.5 mL methanol to yield a concentration of 4.00 mg/mL and kept at 4^{o}C. The bavachin, isobavachalcone, bavachinin and bakuchiol stock solutions were diluted with methanol to obtain calibration solutions ranging from 10-1000, 20-2000, to 40-4000 µg/ml.

Instrumentation

HPLC-UV: performed by 1100 HPLC instrument (Agilent Technologies, California, USA). Detector used: UV detector; Column used- DL- Cl8 column (5.0 mm, 250 mmˇ4.6 mm, Japan) at 30^{o}C; Flow rate- 0.5ml/min; Mobile Phase- Acetonitrile (A) and 0.01M formic acid (B); Elution type- Gradient elution; Injection volume- 10µL; Detection Wavelength- 246nm

LC-MS- Agilent 1100 HPLC system was coupled on-line to an LC/MSD Trap SL Plus spectrometer (Agilent Corp, Waldbronn, Germany); Mode: Negative ion mode; Drying gas: Nitrogen gas; Gas Temp^{o}- 350^{o}C; Mass range- 50 to 1000 m/z; Detection of bavachin, isobavachalcone, bavachinin and bakuchiol was performed in selected ion monitoring (SIM) mode with (m/z) 323, 323, 337 and 255, respectively.

RESULTS

Determination of Foreign Matter

The powder material was found to be free from contamination by moulds, insects or other animal contamination.

Macroscopic Evaluation

The Macroscopical features of the *Psoralea corylifolia Linn* are tabulated in Table No: 1

Physiochemical Evaluation

The physiochemical standards of *Psoralea corylifolia* were listed below.

Phytochemical Investigations

The various chemical tests were performed for HAEPC extract for the identification of phytoconstituent. The results were displayed in table 5

HPLC-UV and LC-MS study on hydroalcoholic extract of *Psoralea corylifolia* (HAEPC)

The separation of constituents in the extract of *Psoralea corylifolia* was performed by HPLC-UV and MS in negative ion mode. A typical total ion chromatogram (TIC) of the identified compounds with MS detection is displayed in Fig. 7. The typical chromatograms with UV detection are shown in Fig 8

Table1: Characteristics of leaf powder of Psoralea corylifolia.

S.NO	Characteristics	Observation
1	Colour	Greyish green powder
2	Odour	Characteristic
3	Taste	Bitter taste

Table 2:

S.No	Ash value				Extractive value			Moisture content (Loss on drying)
	Total ash	Acid insoluble ash	Water soluble ash	Sulphated ash	Water soluble extractive	Alcohol soluble extractive	Ether soluble extractive	
1	7.28±0.01	4.21±0.1	5.18±0.15	6.25±0.01	49%	19.2%	10%	1.3%

*****Values are expressed as Mean ± SD, n=3**

Table 3: Preliminary phytochemical screening of HAEPC

S. NO	TESTS	RESULTS
1	Carbohydrates	+
2	Monosaccharide	+
3	Starch	+
4	Protein	+
5	Amino acid (tyrosine, cysteine)	+
6	Steroid	-
7	Flavonoid	+
8	Alkaloids	-
9	Terpenoids	+
10	Mucilage	-
11	Tannins	+
12	Volatile oil	+

Thin Layer Chromatography

Table 4: Thin Layer Chromatography of Hydroalcoholic extract of Psoralea corylifolia

S. No	Extract	Solvent System	No. of spots	Rf value
1	Hydro alcohol	Toluene: Ethyl acetate: Glacial acetic acid	4	0.41 0.49 0.74 0.81

Table 5: HPTLC profile of HAEPC extract and standards

Mobile Phase	Detector	Name of the sample	Rf value	Results
Toluene: ether: glacial acetic acid	UV Detector-254nm	**Standard**		**Indicates the presence of bavachin, bakuchiol and psoralen**
		Spot A	**0.43**	
		Spot B	**0.83**	
		Spot C	**0.77**	
		Test		
		Spot A	**0.41**	
		Spot B	**0.85**	
		Spot C	**0.74**	

Figure 1: TLC Plate viewed in UV light at 254 nm

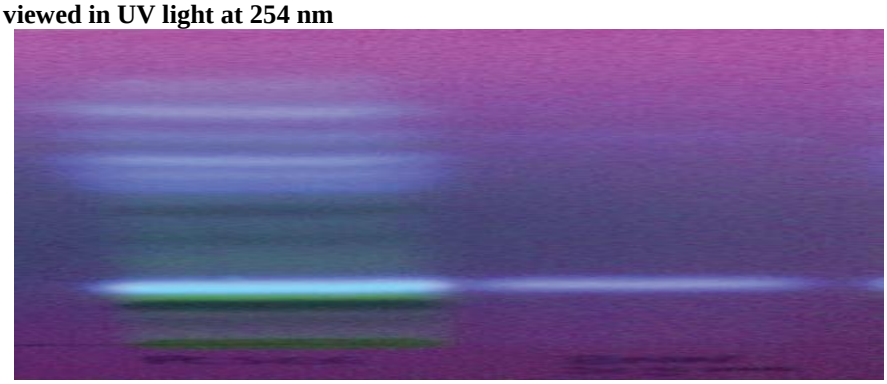


Figure 2: Densitograms of standards bavachin (A), bakuchiol (B), and psoralen (C) and test extract (D)

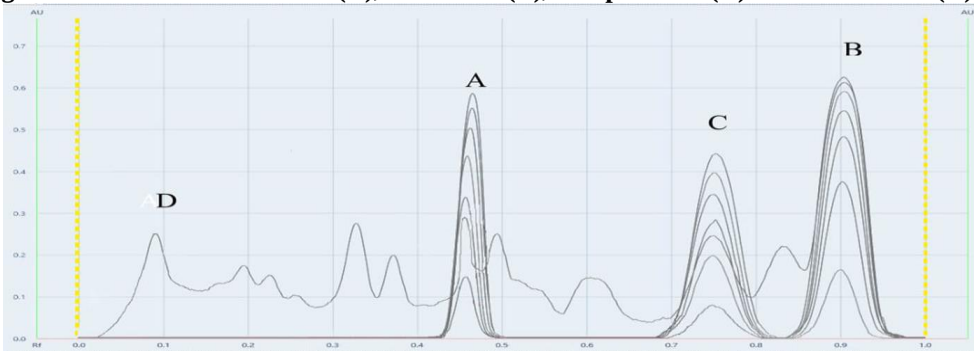


Figure 3: Total ion chromatogram (TIC) of the active compounds of extract of Psoralea corylifolia by LC-MS.

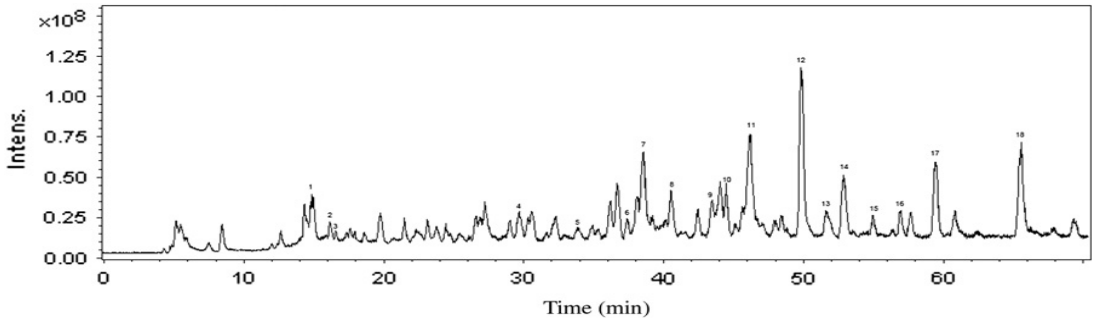
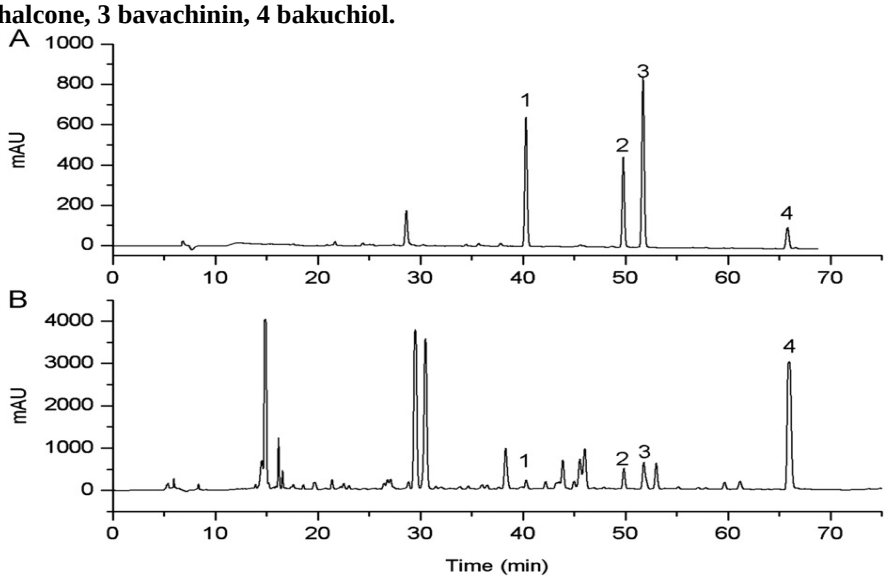


Figure 4: Typical chromatograms of four standard analytes (A) and sample (B) by HPLC-UV. Peak identification: 1 bavachin, 2 isobavachalcone, 3 bavachinin, 4 bakuchiol.



DISCUSSION

Powdered drugs are generally identified by their physiochemical parameters. Physiochemical property characterization has gained strength in the pharmaceutical industry and has become a standard procedure. Loss on drying, total ash, soluble and insoluble ash, extractive values are reliable aids for detecting adulteration. Adulteration can be prevented by using these simple and reliable standards. The preliminary phytochemical studies were performed and the results were revealed that the main focusing compound of flavonoid was presented in the extract of HAEPC. The HPTLC chromatogram for *Psoralea corylifolia* (HAEPC) showed significant separation of three phytoconstituents with Rf values ranging from 0. 43 to 0.83. HPTLC studies indicate the plant extract contains three phytoconstituents. HAEPC contains phytoconstituents with Rf values almost identical to standard bavachin, bakuchiol, and psoralen. In TIC chromatograms under negative-ion mode, the identified components accounted for 53.9% of the peak area. The extracts of *Psoralea corylifolia* contained many isomers, for example, three compounds (1–3) with identical [M–H] at 365, four compounds (5, 7, 10, 11) with identical [M–H] at 321, and two compounds (8, 9) with identical [M–H] at 323. The fragment ion m/z 285 for m/z 365 of psoralenoside and isopsoralenoside is identical ([M-Glu-H]) [13]. Deprotonated molecule ions ([M–H]) are 323, 323, 337, and 255, respectively, with retention times of

40.5, 49.8, 51.7, and 65.5 minutes. Bavachin, isobavachalcone, bavachinin, and bakuchiol are the compounds (6, 8, 9, 12). Molecular ions ([M–H]) of compounds (5, 7, 10, 11) are 321 m/z. Fragment ions 303 (M–H–H2O) of compounds (7 and 10) occur, while ion 285 of compound (7) is formed by losing H2O. Prorachromene is suggested by compound (15) and 18-prenyldaidzein by compound (7) [14]. There were 37.6% flavonoids, 3.3% coumarins, 4.0% benzofuran glycosides, and 4.4% meroterpenes in extracts of *Psoralea corylifolia*. It contains neobavaisoflavone, bavachin, corylin, 18-prenyldaidzein, psoralidin, isobavachalcone, bavachinin, corylifol A, prorachromene, and isobavachromene as its active components.

CONCLUSION

We studied macroscopy and physicochemical parameters to identify adulterants. Carbohydrates, Proteins, Steroids, Terpenoids, and Flavonoids were detected in the crude drug and formulated lozenges. This will help in preventing variation in the quality of the drug because the physicochemical constants were determined. *P. corylifolia* contains bavachin, bakuchiol, and psoralen, all of which are commercially available. Specificity was found in the HPTLC method. HPTLC was used to simultaneously measure bavachin, bakuchiol, and psoralen in *P. corylifolia* for the first time. *P. corylifolia* fingerprints were analyzed using HPLC. In order to evaluate its feasibility in quantitative analysis, we developed a suitable HPLC/MS method for identifying active compounds. *P. corylifolia* bavachin, isobavachalcone, bavachinin, and bakuchiol have been determined using HPLC-UV and HPLC-MS. 12 common peaks represent the characteristics of this herb's constituents in its fingerprint. *P. corylifolia's* quality can be evaluated comprehensively using this method. For the analysis of the four compounds in *P. corylifolia*, MS can provide high selectivity and sensitivity and UV can provide excellent repeatability.

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