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Research Article

Phytochemical Analysis and Evaluation of Lupeol and β-Sitosterol in Genus *Heliotropium*

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Abstract

In the present study, four species of genus *Heliotropium* are phytochemical analyzed. Species like *Heliotropium bacciferum* Forssk., *H. curassavicum* L., *H. ovalifolium* Forssk. and *H. supinum* L. were collected from different regions of Rajasthan, Indian hot desert. These species have been selected to estimate the concentration of lupeol and β-sitosterol through HPTLC. The genus is of vital importance in the folk medicine and is also believed to be useful in treating malaria, abdominal pain, fever, dermatitis, venereal diseases, diarrhoea, insect bites, menstrual disorder, urticaria, sore throat, etc. All the four species are phytochemically rich, of which *H. supinum* L. has been found to be rich in both lupeol and β-sitosterol.

Keywords: *Heliotropium;* HPTLC; Lupeol; β-sitosterol

Introduction

Plants are known to be used for medicinal purpose since ages in India, the evidence of which may be found in the Indian Vedas. Medicinal plants are considered as rich sources of compounds which can be used in drug development. Now a day, people are more interested in exploring the medicinal plants for different biologically active phyto-constituents which exhibit important therapeutic effects. Although the search for new potent drugs from the medicinal plants is undergoing but there are plenty of plants which still remain unexplored and need attention. India is one of the richest countries in plant wealth and medicinal plants. History of medicinal plants roughly begins with Vedic period (3500-800BC). Rigveda, Ayurveda, Manu Samhita, Brihat Samhita etc. are Vedic texts which deal with uses of plant for community and their health regards. Charak Samhita and Sushruta Samhita are two monumental books for India which deal with 1200 plants for drug preparation and specific therapeutic applications. The oldest written evidence of medicinal plants usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 plants. The study and description of medicinal plants in India was carried further in 19th and 20th century. Other workers include Watt [1] who in his book "A Dictionary of Economic Products of India" has described indigenous medicinal plants, their use and cultivation for yield of drugs, while Dymock., *et al*. [2] mentioned medicinal plants from India in his book '*Pharmacographia Indica*'.

Rajasthan symbolizes royalty, rich cultural heritage, safaris and sand-dunes. The state is the largest in North-Western part of India; geographically it lies between 23° 3′ to 30° 12′N longitude and 69° 30′ to 78° 17′S latitude with an area of 3, 42, 269 Km2 out of which about 1, 98,100 Km^2 is arid and the rest is semi-arid. The elevation of land surface varies from 214 to 1727 m.

Materials and Methods

In the present study, different parts of the collected plants were subjected to phytochemical screening of pharmacologically important compounds. The plants were air dried, powered in grinder and were stored at room temperature.

Preparation of plant extract

The powdered plant parts of each sample were dissolved in 20 ml of aqueous methanol for overnight. The extract was concentrated and dried using rotary evaporator under reduced pressure. 2 mg of each dried extract was again dissolved in 2ml of methanol to obtain 1mg/1ml concentration and stored at 4°C till further analysis.

HPTLC instrumentation and conditions

Concentration range from 200-10,000 mg of standard solutions were spotted on silica gel 60 F254 HPTLC plate (Merck, India) using CAMAG Linomat V automatic spotter (Dosage speed: 150nL/s, Syringe size: 100µL, Band length: 6.0 mm). Plates were developed in a twin-through chamber (20×10 cm) to a distance up to 8 cm.

The data regarding the bioactive markers used, composition of the solvent system, derivatizing reagent used and wavelength of the entire marker compounds. After development, the plates were first air dried and then oven dried at 105°C for 3-4 min. Further, the plates were derivatized with p-anisaldehyde sulphuric acid. In some cases, the plates were air dried and scanned densitometric using CAMAG TLC Scanner IV. p-anisaldehyde sulphuric acid was prepared by dissolving 1 ml of p-anisaldehyde solution in 2 ml of concentrated sulphuric acid and 100 ml of acetic acid. Afterwards, measurements were made by winCATS software. Peak areas were recorded and calibration curve of standards was obtained. The plates were scanned at specific wavelength (525nm) and peak area, peak height, absorption spectra were recorded.

Results and Discussions

Every plant or plant part has a specific phytochemical profile which can be used for the specific purpose of selection. HPTLC is a unique technique which allows us to simultaneously identify different compounds in the same plant or the specific compounds in different plants in a single attempt. HPTLC produces visible chromatograms complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously so that reference and test samples can be compared for identification. Similarities and differences are immediately apparent and with the help of the image comparison. Several chromatograms can be compared directly, even from different plates. In addition to the visible chromatograms, analog peak data are also available from the chromatogram [3]. Chromatographic fingerprint analysis has shown to be a sound and realistic approach for the quality assessment and species authentication of traditional medicines [4,5]. It uses chromatographic techniques to develop specific patterns of identification for herbal drugs. The developed fingerprint pattern of components can then be utilized to decide not only for the absence or presence of markers of interest, but also the proportion of all detectable analytes. The specific fingerprints of species are developed through HPTLC and can be used to check adulteration at any stage. HPTLC is an important tool to measure phytochemical constituents from herbal drug formulations, both qualitatively and quantitatively. Using this technique, various workers have worked out different compounds in different plants and this technique has come out to be best suitable for identification, visualization and quantification of biologically active compounds. The linear regression equation with the correlation coefficient values for the compounds is given in Table 1. The content of compound in plant samples has been measured by using calibration curve of the standard compounds.

Sr. No.	Marker compounds	Linearity range (mg/spot)	Linear equation (y)	R_f	Correlation coefficient ($R^{2)}$
1.	Lupeol	2-10	y=908.31x	0.61	0.9953
2.	β-sitosterol	2-10	y=1641.6x	0.54	0.9952

Table 1: Table showing Linearity range, Linear equation, R_f value and Correlation coefficient.

Lupeol exhibits anti-cancerous and anti-inflammatory properties [6]. Mixture of Toluene: Ethyl acetate: Glacial Acetic Acid (14.5: 4.5: 1.0 v/v/v) was used as mobile phase to separate lupeol. The compound is detected at 525 nm (Figure 1-3). The R_f value of lupeol has been found to be 0.61 (Table 1). HPTLC densitometric chromatogram of standard tracks and sample tracks of four species of *Heliotropium* are visible in figure 1 and 2. In the present study, Lupeol has been detected in all the four species of *Heliotropium* however, maximum amount was observed in whole plant sample of H_4 (13.39 ± 0.22 mg/g of DW), followed by leaf sample of H_1, stem sample of H_1, leaf sample of H_2, leaf sample of H_3, stem sample of H_2 and the stem sample of H_3 (2.21 ± 0.28 mg/g of DW). Among the stem and leaf samples of H_1, H_2, and H_3, leaf samples contain more amount of lupeol than the stem samples. Among H_1, H_2, H_3 and H_4, both H_1 and H_4 have been found to be rich in lupeol

Figure 1: HPTLC profile of Lupeol and β-sitosterol in four Heltropium species at 525.

Track 1-5 =Standard Lupeol

Track 6,8,10 = H1, H2, H3 stem samples

Track 7,9,11 = H1, H2, H3 leaf samples

Track 12 = whole plant sample of H4

Track 13-17 = Standard β-sitosterol

Figure 2: 3-D chromatogram of tracks

Figure 3: absorption spectra of a) Lupeol b) β-sitosterol

Compounds Samples code		**Lupeol** (mg/g of DW)	β-sitosterol (mg/g of DW)
H_1 *H. supinum* L.	Stem	13.39 ± 0.22	28.07 ± 0.50
	Leaf	15.02 ± 0.15	9.67 ± 0.19
H_2 *H. ovalifolium* Forssk.	Stem	2.406 ± 0.29	3.62 ± 0.57
	Leaf	6.93 ± 0.24	15.48 ± 0.47
H_3 *H. curassavicum* L.	Stem	2.21 ± 0.28	7.92 ± 0.38
	Leaf	4.39 ± 0.20	6.51 ± 0.20
H_4 *H. bacciferum* Forssk.	Whole plant	21.51 ± 0.95	0.59 ± 1.75

Table 2: Table showing amount of lupeol and β-sitosterol in four species of *Heliotropium.*

β-sitosterol one of the major Phytosterols was also determined. β-sitosterol is known for curing heart diseases and fighting high cholesterol levels in the body. Apart from this, it is also known to be helpful in prevention of different types of allergies, cancer, influenza, bronchitis, asthma, hair loss and migraine [7]. It is also known for its cholesterol lowering properties and reduces the risk of atherosclerosis and also possesses antioxidant and anti-diabetic properties [8]. The compound was separated using Toluene: Ethyl acetate: Glacial Acetic Acid (14.5: 4.5: 1.0 v/v/v) as mobile phase and the plate was scanned at 525 nm (Figure 1). The Rf value of the reference compound is found to be 0.54 (Table 1). β-sitosterol has been detected in all the four species of *Heliotropium*. The amount of β-sitosterol was found to be quite high in H_1, H_2, and H_3 where as in case of H_4 the amount was extremely low. Maximum amount of β-sitosterol was detected in H_1 stem sample (28.07 ± 0.50 mg/g of DW), followed by H_2 leaf sample, H_1 leaf sample, H_3 stem sample, H_3 leaf sample, H_2 stem sample and H_4 whole plant sample (0.59 ± 1.75 mg/g of DW). Among the leaf and stem samples, stem samples of H_1 and H_3, contain more amount of β-sitosterol than leaf samples. However, in case of H_2 reverse of it has been found where leaf sample has been found to contain more amount of β-sitosterol than stem sample. Among H_1, H_2, H_3 and H_4, H_4 has been found to be rich in β-sitosterol (Table 2).

Earlier, *Heliotropium* of family Boraginaceae have been widely studied by various workers, as the species of the genus have been used widely for centuries on warts and to treat inflammations and tumours and many more problems. Workers who have contributed to this work are Pal [9], Dattagupta and Datta [10] who evaluated pharmacognostic study of the leaf of *H. indicum* Linn., while Srinivas., *et al*. [11] have worked on anti-inflammatory activity of *H. indicum* Linn. in albino rats. Sharma and Alexander [12] carried out pharmacognostical and phytochemical investigations of roots of *H. indicum* Linn. Other workers including Dash and Abdullah [13], Roy [14] also have contributed to the study of *Heliotropium.* Tiwari and Masood [15] carried out analysis of chemical constituents of *H. ovalifolium* while Mohanraj., *et al*. [16] have given account on Heliofoline, a pyrrolizidine alkaloid from *H. ovalifolium*. Kulkarni., *et al*. [17] (Novel leads from *Heliotropium ovalifolium*, 4,7,8-trimethoxy-naphthalene-2-carboxylic acid and 6-hydroxy-5,7-dimethoxy-naphthalene-2-carbaldehyde show specific IL-6 inhibitory activity in THP-1 cells and primary human monocytes) and Sharma., *et al.* [18] also have worked on *Heliotropium* species. Earlier, Erosa-Rejón., *et al*. [19] isolated β-sitosterol along with other compounds from the organic extract of the leaves of *H. angiospermum*. Further, they carried out structural elucidation of the metabolites by analysis of their spectroscopic data and/or by comparison with those reported in the literature.

Conclusion

The employed statistical analysis ensures that the developed method is reproducible and selective. This method can be used as an important tool to ensure the therapeutic dose in herbal formulations, standardization and quality control of bulk drugs. Present study gives scope to use genus Heliotropium as an alternative source for the preparation of various pharmaceutical medicines.

Conflict of Interest Statement

We declare that we have no conflict of interest

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