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Original Article

Pharmacognostic and Pharmacological Evaluation of *Hyssopus officinalis* L. (Lamiaceae) Collected from Kashmir Himalayas, India

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ABSTRACT

Introduction: *Hyssopus officinalis* L. is a well-known herb for its culinary and medicinal significance. The purpose of this study was to perform the pharmacognostic evaluation. **Methods:** Physicochemical and phytochemical analysis, HPTLC quantification and *in vitro* antioxidant and antidiabetic activity were done. **Results:** Preliminary screening revealed the presence of phytomolecules such as alkaloid (0.99%), tannin (1.75%), sugar (1.96%) and starch (0.68%). Total phenolic and flavonoid content were found to be 2.32% and 1.16% respectively. HPTLC quantification data showed that the content of ferulic acid (0.034%) was higher than caffeic acid (0.0064%) on dry weight basis The IC_{50} value for the *in vitro* DPPH radical scavenging assay was 0.50 µg/ml and *in vitro* anti diabetic assay displayed IC_{50} value of 0.8366 mg/ml. **Conclusion:** The study suggests presence of considerable amount of phenolic acids and antioxidant activity in the plant which supports its use in the traditional systems of medicine.
Key words: Antioxidant, DPPH, *Hyssopus officinalis,* HPTLC, Phenolic acids.

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INTRODUCTION

Hyssop (*Hyssopus officinalis* L., family: Lamiaceae) is a native of South European countries. In India, it is found in the Western Himalayan region from Kashmir to Kumaun at altitudes of 8,000-11,000 ft.[1] In its natural habitat it grows on dry banks, among rocks and ruins with a height ranging from 50 to 120 cm. The genus *Hyssopus* comprises of aromatic perennial herbs or sub-shrubs known for their culinary as well as medicinal properties for hundreds of years and the leaves are mainly used as an aromatic condiment. Hyssop oil from aerial part finds its greatest use in flavoring preparations for alcoholic beverages, meat products and seasonings.[2] Medicinally, it is used as an expectorant, carminative, anti-inflammatory, anti catarrhal, antispasmodic and as traditional medicine in many parts of the world. The herb was used to alleviate digestive disorders, cure laryngitis and to accelerate wound healing in Turkish folk medicine, relaxation of peripheral blood vessels and to promote sweating.[3,4,5] It is used in tea blends for cough relief, antispasmodic effects and relieving catarrh. Apart from this, *H. officinalis* exhibit various other pharmacological activities i.e. anti-bacterial, anti-fungal, anti-oxidant, sedative, spasmolytic, anti-viral, cytotoxic and anti platelet activities of Hyssop extract have been also reported.[6,7] Several studies have reported the composition of essential oil isolated from *H. officinalis*, the major constituents were found to be pino-camphone, iso-pinocamphone, β-pinene, 1, 8-cineole and pino-carvone.[8] Antibacterial, antifungal and antioxidant properties of hyssop have been attributed to the presence of pinocamphone, iso-pinocamphone and β-pinene. Antiviral activity has probably been attributed to the presence of caffeic acid and tannins.[9,10] As evident from available literature that majority of work was focused on chemical characterization of *H. officinalis* oil. Hence, this study aimed for physicochemical standardization of *H. officinalis* aerial part. In addition, the phenolics were quantified through HPTLC and their bioactivity was analyzed by different models. This aids in quality regulation of raw material for more pronounced culinary use as well as this also promotes the cultivation of species in its natural location.

MATERIALS AND METHODS

Plant material

The aerial part of *H. officinalis* used in the present study was collected from Kashmir-Leh highway (India) in the month of October 2016 at an altitude of 11,562 ft. The plant was found in flowering condition. The collected germplasm was authenticated by Dr. Sharad Srivastava, principal scientist, CSIR-NBRI, Lucknow. A voucher number was assigned (LWG No 262569) and the herbarium specimen was deposited in the national repository of CSIR-National Botanical Research Institute, Lucknow.

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Physicochemical and phytochemical characterization

Physicochemical and phytochemical studies viz. extractive values, total ash, acid insoluble ash, total sugar, starch, tannin and phenols were estimated from shade-dried and powdered plant material were also carried out.[11,12,13]

HPTLC quantification

Preparation of plant extract

Accurately weighed 5 g of powdered sample was cold macerated with absolute methanol (25 ml), kept on shaker for 08 hrs and allowed to stay for 16 hrs at room temperature (25 ± 2˚C). Extraction was repeated thrice, filtered (Whatman No. 4) and pooled filtrate was dried in rotatory evaporator (Buchi, USA) under standard conditions of temperature (45± 2˚C) and pressure (40 mbar) and finally lyophilized (Labconco, USA) to solid residue. Before extraction, the sample was defatted using petroleum ether to remove the fatty materials/impurity. Extractive yield (%) was calculated on dry weight basis.

Instrumentation and chromatographic conditions

HPTLC, quantification of phenolics were carried out on silica gel G60 F_{254} precoated aluminum plate with 0.2 mm thickness (Merck, Germany) as stationary phase. Prior to HPTLC profiling, the stock solution of marker compounds and plant sample was freshly diluted with methanol and filtered through a 0.45 µm Millipore membrane filter (pall, USA) to prepare working solution of 0.1 mg/ml and 10 mg/ml respectively. Working solutions of plant sample (15µl) and marker compounds viz. ferulic acid and caffeic acid (2µl) were applied on plate as 6 mm wide bands positioned 10 mm above the bottom and 15 mm from the side of the plate, using CAMAG Linomat V automated TLC applicator (the nitrogen flow providing a delivery speed of 150 nl $s^{-1)}$ from the application syringe. The chromatogram was developed with mobile phase of Toluene: Ethyl acetate: Formic acid (6:3:1, v/v/v) in CAMAG twin through chamber. After development, plates were dried for 30 min and scanning was performed using CAMAG TLC Scanner 3 at wavelength of 300 nm, operated by win CATS Software (version 3.2.1). The slit dimensions were 4×0.45 mm and the scanning speed was 100 mm/s. Quantification (% dry weight basis) was done based on regression analysis of area versus concentration of marker compounds dilutions.[14]

Antioxidant activity

The antioxidant potential of methanolic extract was evaluated by three methods viz. DPPH radical scavenging assay,[15] reducing power assay[16] and total anti-oxidant capacity[17] to estimate the hydroxyl radical scavenging activity of *H. officinalis*.

Antidiabetic activity

The antidiabetic assay was performed based on the alpha amylase inhibition assay with slight modification based on the starch-iodine test.[18]

RESULTS

Physicochemical standardization

Physicochemical standardization parameters were carried out as per standard protocols of Ayurvedic Pharmacopoeia of India (1989) to furnish data that can be used as quality regulation for herbal product development. The ash values viz. acid insoluble ash, water insoluble ash was found to be 12.40%, and 10.98% respectively. Alcohol extractive (%) was 1% and hexane extractive was 2.65%. However, the water extractive was found to be significantly high i.e. 15% in the plant (Figure 1).

Quantitative estimation of secondary metabolites

The phytochemical screening (qualitative) of *H. officinalis* leaf extract shows presence of various bioactive compounds like alkaloid, carbohydrate, tannin, flavonoid, sterols and terpenoids. Sugar and starch content were found to be 1.96% and 0.68%. Total phenolic content was found to be higher than flavonoid contents in the methanolic extract. Tannin content was recorded as 1.75% and alkaloid content was 0.99% (Figure 2). The results indicate that the plant is rich in various phytomolecules.

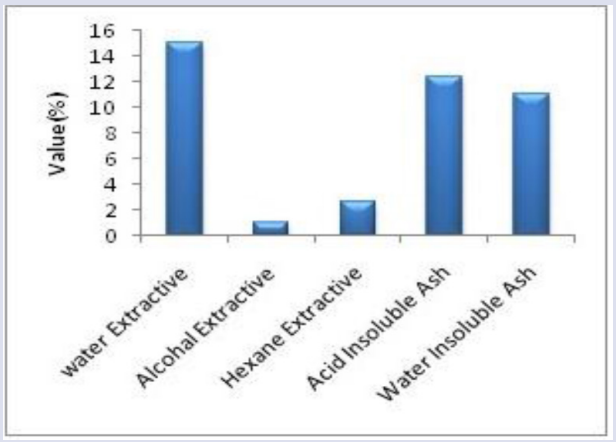

Figure 1: Physicochemical values of *H. officinalis.*

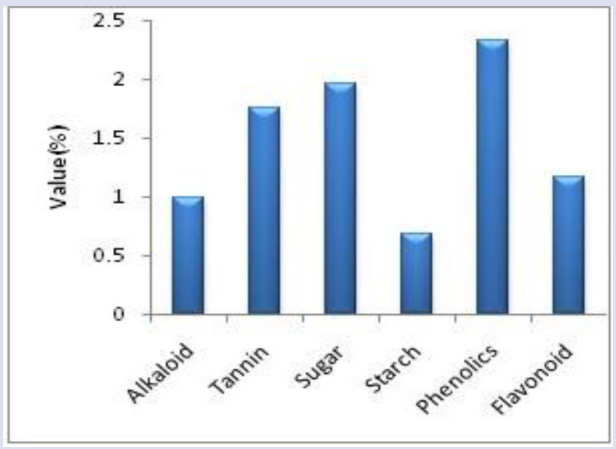

Figure 2: Phytochemical evaluation of *H. officinalis.*

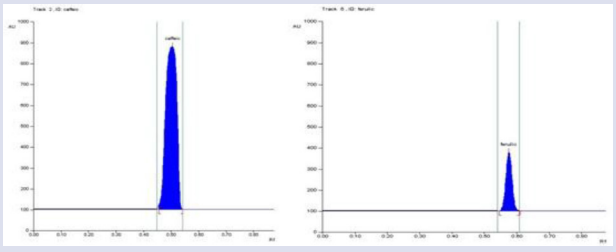

Figure 3: HPTLC denistometric scan profile of ferulic and caffeic acid showing distinct R_f values.

High performance thin layer chromatography

The method for quantification of phenolic was previously developed. HPTLC profile of methanolic extract of *H. officinalis* was done by CAMAG HPTLC System with winCATS-3 software. The marker

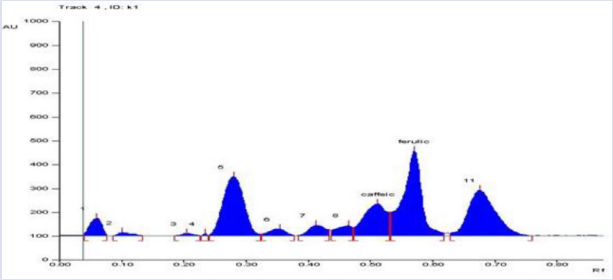

Figure 4: HPTLC densitometric scan profile of *H.officinalis* L. extract showing presence of ferulic and caffeic acid.

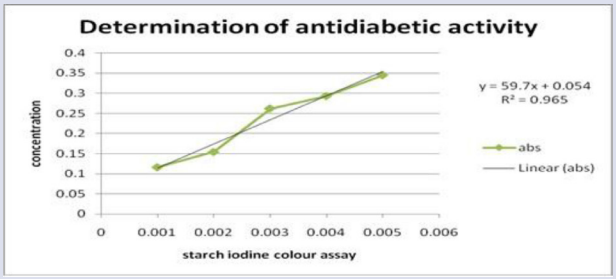

Figure 5: *In vitro* antidiabetic activity of *H. officinalis* extract by α-amylase inhibition methods

compounds were identified by matching the UV spectra and R_f of bands in the plant sample with marker compounds (Figure 3). The densiometric scanning reveals the presence of caffeic acid and ferulic acid in the crude extract at R_f of 0.50 and 0.57 respectively (Figure 4). The content of ferulic acid (0.034 %) was higher than caffeic acid (0.0064 %) on dry weight basis in crude drug.

Antioxidant activity

The antioxidant potential of the plant extract was determined by the total antioxidant capacity, reducing power assay, and DPPH radical scavenging assay. The total antioxidant capacity of *H. officinalis* extract was measured by phospho-molybdenum method based on regression analysis of ascorbic acid, against regression equation (y = 196.4x + 0.179) and coefficient; r^2 = 0.990 at a different concentration (0.001 to 0.005 µg/ml). Reducing power of the plant extract increases linearly against regression equation (y = 0.112x + 0.121) and coefficient; r^2 = 0.985 at a concentration (2 to 10 µg/ml), like the standard. Estimation of free radical scavenging activity through DPPH assay is most widely used for screening of medicinal plants having anti oxidant activity. The mechanism involves the decolorization of solution through electron donated by anti oxidant moiety (standard/plant extract) and thus stabilizing the DPPH radical. The IC_{50} value for the *in vitro* DPPH assay was at 0.50 µg/ml against regression equation (y = 028252x + 35.65) and coefficient; r^2 = 0.905. The results clearly indicate the significant antioxidant activity in the plant which is in positive coorelation with the presence of phenolic acids and flavonoids which are known to impart antioxidant potential to the plant.

Antidiabetic activity

The antidiabetic activity of *H. officinalis* extract was determined by inhibition of biochemical activity of the alpha amylase enzyme. Starch iodine assay model systems involved. Inhibitory activity of the extract on α-amylase was observed in the range 0.1-0.5 mg/ml. The IC_{50} of the methnolic extract was found to be 0.8366 mg/ml. However, the IC_{50} of the reference compound, acarbose, was found to be less than 0.025 mg/ml in the starch iodine color assay, respectively (Figure 5).

DISCUSSION

The results obtained suggest that caffeic acid being present in significant amount in the plant can act as a marker compound for the chemical identification of the plant and can be used to monitor the batch to batch consistency of the herbal product using this plant. *H. officinalis* has considerable antioxidant and antidiabetic activity. It can be used as a major cure for diseases resulting from damage caused by free radicals. Thus, the need for identifying and exploring the therapeutic potential of such useful medicinal plants is of very much significance to conserve our indigenous traditional knowledge and commercial formulation for market acceptability and competency.

CONCLUSION

In the present study physicochemical standardization parameters and phytochemical constituents identified will be helpful in the identification, standardization and quality control of this herbal drug.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

There are no conflicts of interest.

ABBREVIATIONS

HPTLC: High-performance thin-layer chromatography; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IC:** Inhibitory concentration; **API:** Ayurvedic Pharmacopoeia of India.

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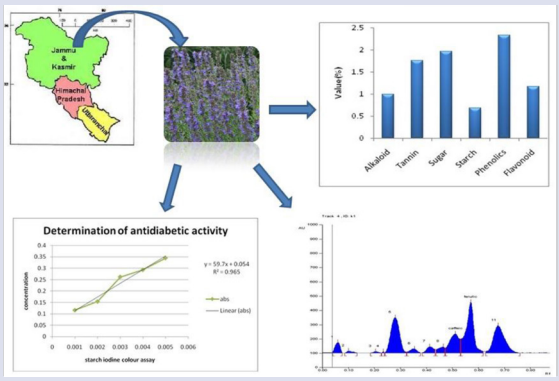
GRAPHICAL ABSTRACT



SUMMARY

- Physicochemical and phytochemical study was done to evaluate the quality standard of raw drug.
- The content of ferulic acid was found to be higher than caffeic acid.
- *In vitro* antidiabetic and antioxidant activity exhibit potential activity in targeted species.
- Evaluated pharmacognostical standards are useful for identification and authentication purposes.

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