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

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**ABSTRACT**

*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 anti-diabetic 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 IC50 value for the *in vitro* DPPH radical scavenging assay was 0.50 μg/ml and in vitro anti-diabetic assay displayed IC50 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.

**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 Kumaon at altitudes of 8,000-11,000 ft. In its natural habitat, it grows on dry banks, among rocks and ruines 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. Medicinally, it is used as an expectorant, carminative, anti-inflammatory, anti-catarhral, 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.

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. Several studies have reported the composition of essential oil isolated from *H. officinalis*, the major constituents were found to be pino-carvone, iso-pino-camphone, β-pinene, 1, 8-cineole and pino-carvone. 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. 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.

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.\textsuperscript{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 petrol 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\textsubscript{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 caffeeic 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\textsuperscript{-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.\textsuperscript{14}

Antioxidant activity

The antioxidant potential of methanolic extract was evaluated by three methods viz. DPPH radical scavenging assay,\textsuperscript{15} reducing power assay\textsuperscript{16} and total anti-oxidant capacity\textsuperscript{17} to estimate the hydroxyl radical scavenging activity of \textit{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.\textsuperscript{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 \textit{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.

High performance thin layer chromatography

The method for quantification of phenolic was previously developed. HPTLC profile of methanolic extract of \textit{H. officinalis} was done by CAMAG HPTLC System with winCATS-3 software. The marker
Hyssopus officinalis extract was determined by 50% inhibition of HIV-1, IL-12 and IL-10 production. Inhibitory activity of the extract was measured in vitro by means of biochemical activity of the alpha amylase enzyme. Starch iodine color assay, respectively (Figure 5). 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.

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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.

**REFERENCES**

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.