Ethnopharmaceutical importance of under-explored plant species *Hyptissuaveolens* (L.)

Rajani Srivastava[1]*, Shreya Pandey[1] and Ambrish Singh[2]

[1]*Environmental Science (Environmental Technology) Institute of Environment & Sustainable Development, RGSC, BHU*

[2]*Pharmacy ayurveda, Faculty of Ayurveda, IMS, RGSC, BHU*

Abstract

Plants store a variety of important secondary metabolites with pharmacognostic and pharmacological implications, some of which have the potential to become super medicines in the future. In-vivo generation of these metabolites is influenced bya number of biotic and abiotic factors resulting in a constant accumulation of various phytochemicals and their derivatives that could be relevant in future medication research and development. There are over 70,000 plant species are employed ethnomedicinally in various ancient medical systems such as Ayurveda, Siddha, and Unani, as well as in Allopathy. The goal of this study is to look into the therapeutic potential of secondary metabolites as well as the probable pharmacological and pharmacognostic significance of the under-explored/underutilized plant *Hyptissuaveolens* (L.) Poit.Almost all parts of this plant are being employed in conventional drug to treat various diseases. It has been reported that it shows protection against peptic ulcer diseases and has anti-cancerous properties. The leaves of *Hyptissuaveolens*secreted essential oil by hydrodistillation have been linked to the genus Hyptis' broad range of biological activity. It contains phytochemicals like alkaloids, tannins, saponins, flavonoids, terpenoids, minerals (like calcium, magnesium, sodium) and metals (like zinc and iron). The ursolic acid found in *H. suaveolens* can be used as a COVID-19 virus treatment agent. In addition, the ethnobotanical study claims that the beneficial plant has neuroprotective, antioxidant, antibacterial, antidiarrhoeal, anthelmintic, anti-inflammatory, wound healing, insecticidal, antimitotic, anti-proliferative, antisecretory, hepatoprotective, and acaricidal properties. The phytochemicals and extracts obtained from the plant have a great deal of therapeutic promise. As a result, we can use this plant for a variety of purposes.

Keywords: *Hyptissuaveolens*, Lamiaceae, Peptic ulcer diseases (PUD), Hydro distillation, Ethnobotanical

*Corresponding author Email: rajani.env@bhu.ac.in

1. Introduction

Plants store a variety of important secondary metabolites and are widely used in Ayurveda and other traditional therapeutic systems. They are source of many chemical components that can medicate a broad spectrum of diseases [1]. Traditional medicines are used over 70% of the world's population, according to a World Health Organization (WHO) report [2]. It is critical to investigate the phytochemical composition of plants found in various climatic settings, as climatic stress causes changes in the plant's phytosociological behaviour.Secondary metabolite variations can contribute to distinct pharmacological activities, making plant species a potential source of novel drugs[3].The Lamiaceae (mint family) is one of the major families, having 236 genera and over 7000 species. It's a large family with a lot of diversity and variety [4]. The presence of essential oils is a characteristicfeature of this family, which makes several members medicinal species with widespread applications in the pharmaceutical, cosmetic, and perfume industries [5]. *Hyptissuaveolens* (L.)Poit.is a wild medicinal plant native to tropical America that has received little attention [6]. The majority of this plant's bioactive compounds are used in medicinal preparations for aextensivevariety of ailments, which include respiratory, and digestive tract infections, indigestion, stomachpain, fever, burns, injuries, cramps, and various skin complaints, as well as an antirheumatic and antisuporific bath [7].

1.1 Medicinal relevance of *Hyptissuaveolens*

*Hyptissuaveolens*is anfragranttraditional herb. It has a lot of polyphenolic and flavonoid components in its leaves. The hepato- and neuroprotectivefeatures of polyphenolics have been discovered [8]. The majority of plant species in the Lamiaceae family is able to counteract free radicals which reveal its antioxidativeproperties [9,10]. Cancer treatment is available in modern medicine, but they have major negative impacts. The essential oil of *H. suaveolens* contains terpenoids such as sabinene,trans-caryophyllene, E-spatulenol, Beta-elemene, Rimuene, Eucaliptol, 1-8-cineole and other ingredients as reported by [11].

It has anti-cancer action in the MCF-7 cell line or a human breast cancer cell line. The ethanol extract of *H. suaveolens* induces apoptosis by inhibiting the production of the anti-apoptotic protein Bcl2 [12]. By targeting carcinogenic enzymes via proteasome degradation, ursolic acid and other known triterpenoids cause malignant cell lines to enter a cell cycle arrest [13]. *H. suaveolens* essential oil contains antibacterial flavonoids and phenolic compounds that are effective against pathogenic bacteria [14, 15]. Its essential oil shows antifungal activity against *Aspergillus* spp. (*A. flavus, A. parasiticus, A. niger, A. ochraceus, A. fumigatus*), *Saccharomyces cerevisiae, Mucor* sp., and *Fusariummoniliforme*. In streptozotocin-induced diabetic rats, a methanolic extract from the leaves of *H. suaveolens* has antihyperglycemic activity [17]. Malaria is commonly treated with this plant. 13alpha-epi-dioxiabiet-8(14)- en-18-ol, a diterpenoid isolated from *H. suaveolens* leaves in a petroleum ether extract, also has antiplasmodial activity [18]. *H. suaveolens* alcohol, chloroform, and petroleum ether extract increased hydroxyproline concentration, collagen deposition, dry weight of granulation tissue, and wound healing activity in granuloma cells by enhancing free radical foraging and increasing antioxidant enzymes [19]. The larvae of the yellow fever mosquito Aedesegypti were suppressed by an ethanolic extract of H. suaveolens. Sharma et al. [20] discovered that *H. suaveolens* hydro-distillate leaves had an acaricidal effect in ruminants. H. suaveolens contains ursolic acid, which can be used as an antiviral drug against fatal viral infections including HIV and influenza [21].Jee et al. [22] confirmed that ursolic acid can inhibit the transcriptase-replicase enzyme which can be effective against life-threatening RNA viruses. It affectstheduplication and assemblage of viral protein by acting on the protease (M^{pro}) and chymotrypsin like ($3CL^{pro}$) of SARS-CoV2[23,24]. It can be used as an effective therapeutic agent against metabolic and infectious human illnesses in the future, based on its pharmacological expansion.

Thus, because of its multipurpose benefits, this research focused on structural characterization and analysis of pharmacognosy quality and the phytochemical profilelayingof species *Hyptissuaveolens*.

2. Material and Methods

2.1 Study Area

The research was conducted at four locations in natural habitats around Rajiv Gandhi South Campus, Banaras Hindu University, and Mirzapur (Figure 1). Three replicate samples of *Hyptissuaveolens*were gathered for examination at each site. *Hyptissuaveolens* plant was confirmed and authenticated (voucher

specimen no. 2022-0.7) by Faculty of Ayurveda, Banaras Hindu University. The harvested plants were dried in the shade at room temperature, ground into powder, and stored in sealedvessels at normal room temperature for further extract preparation, powder study, and phytochemical screening at the Ayurveda Faculty, IMS, BHU. Petroleum ether, chloroform, ferric chloride, and methanol were all analytical grade reagents.

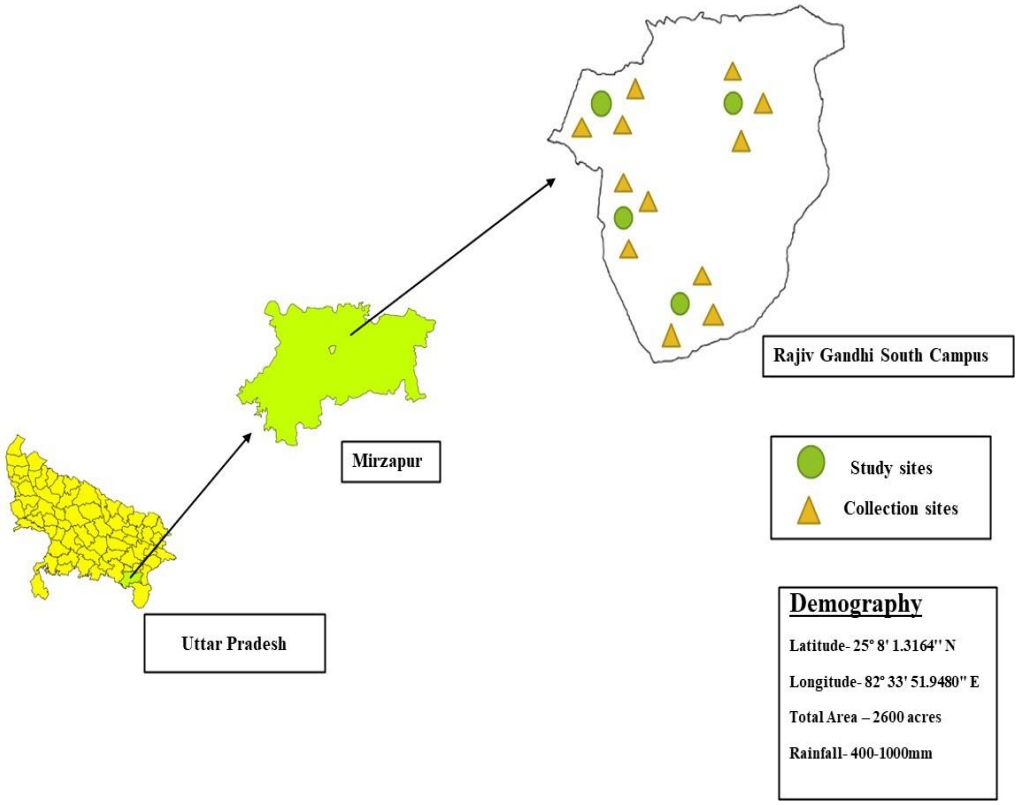

Figure 1 Study site

2.2 Macroscopic and Organoleptic Analysis

The macroscopic inspection of a medicinal plant aids in the rapid identification of plant material as well as the standardisation of crude drugs. Different plant parts like roots, stems, and leaves were used in the macroscopical and organoleptic testing. For the study of organoleptic characteristics, the plant's size, form, odour, colour, taste, and other significant characteristics were taken into account. The macro-morphological features of the root, leaf, and stem were studied under a magnifying lens (10x).

2.3 Microscopic and Powder Analysis

For microscopic and powder identification, dried herbs were crushed to a granular powder and kept in a suitable container.Transverse sections of the root, stem, and leaves were used in the anatomical study. Diagnostic characters were examined after staining the sections with Safranin. Powder microscopy aids in the investigation of the structure and kind of tissues found in a crude drug powder. Fine dried powder of root, stem and leaf analysis was performed by mixing the fine powder with safranine and examining it under a microscope.The transverse section (stem, root, and leaf) as well as the powder section were photographed. With 10x microscope objective lenses, the powdered section was examined. The powder section is made by using chloral hydrate or diluted glycerine as cleaning agents. Chloral hydrate and diluted glycerine were prepared as per procedures discussed in general rules in the protocol for testing of Ayurvedic, Siddha &Unani Medicine (Government of India, Department of Ayush, Ministry of Health and Family Welfare Pharmacopeial laboratory for Indian Medicine Ghaziabad).

2.4 Fluorescence Analysis

A little amount of residue was taken on a clean microscopic slide, along with 1 to 2 drops of newly made reagent solution, and the slide was gently tilted for a minutes to mix everything together. After enabling the powder to react with various chemical reagents, a Fluorescence study onthe drug *Hyptissuaveolens*was conducted, using short UV (254 nm) and long UV (366 nm) according toChase and Pratt [26] and Kokoski et al [25] and then the fluorescence pattern was perceived [27].

2.5 Phytochemical Analysis

For phytochemical analysis, dried plant materials were ground to a granular powder and kept in a suitable container. The 70% methanol, petroleum ether, aqueous solution and chloroform were used extraction of powder. The bioactive compounds like alkaloid, carbohydrate, steroid, saponins, amino acid, flavonoid, cardiac glycoside and tannin were screened ascertain by analyzing for theircoloration reactions which usually be added chemical reagents such as alkaloid (Dragendorff Reagent Test, Mayer Test, Hager Test, Wagner Test), carbohydrate (Benedict, Fehling and Molisch test), Steroid (Amylum Test),Steroid (Salkowski Test), Saponin (Forth Formation), Amino acid (Ninhydrin Test), Flavonoid (Zinc hydrochloride Test), Cardiac glycoside Test (Raymond Test) and Tannin(Ferric Chloride Test)[27].

2.6 Pharmaceutical Standardization

For numerous formulations, physical parameters such as bulk density, tap density, angle of repose, and Hausner ratio were computed.The number of particles or granules packed together is mentioned as bulk density.

The formula for calculating bulk density (Db) is Db = M/Vb, where M denotes particle mass and Vb denotes the entire volume of packing.

To calculate the volume of packing, a graded cylinder mounted on a mechanical tapping device (jolting volumeter) with a specifically cut spindle can be utilised. The formulation powder was carefully transferred into the cylinder using a funnel. The sample was tapped until no more volume reduction was detected, then the beginning volume was recorded. The original volume and bulk density values were used to calculate the bulk density value. The angle of repose is estimated by fixed funnel and free-standing cone methods both use equipment with its tip set at a specific height (H) above the paper on a level horizontal surface.The powder or grains were carefully poured into the funnel until the peak of the conical pile just touched the funnel's tip.

Thus, $\tan\alpha$ = H/R or α = arc tan H/R, where α is the angle of repose, with R being the radius of the conical pile.

The Hausner ratio is calculated using the equation D_f / D_o, where D_f is the tapped density and D_o is the bulk density.

The capacity of a powder to be compressed is known as compressibility. The percentcompressibility of the powder can be calculated using the following formula based on the apparent bulk density and tapped density.

% Compressibility = [(tapped density – bulk density) / tapped density] × 100 [29] (Bahuguna Y et al, 2014)

2.7 Thin Layer Chromatography

The approach of semi-quantitative examination could be used to determine TLC. It confirms the extract's presence of several components. TLC was done on a glass plate covered with a thin layer of absorbent material and a stationary stage of silica gel "G." The chromatogram was establishedby a mobile phase made up of a mixture of various solvents of varied polarity. Some phytoconstituentslike alkaloids, flavonoids, glycosides, and phenolics were detected using a variety of imaging reagents [28].

2.8 Heavy MetalAnalysis

Atomic absorption spectroscopy was used to examine the medicinal plant's methanolic extract. Analytical grade nitric acid (HNO_3) was used for wet digestion of the materials. Three different concentrations of standard solution were made for all the heavy metals to obtain a calibration curve by diluting a stock standard solution of concentration 1000ppm. The radiation source was hollow cathode lamps for Cd, Pb, and Zn, with air acetylene as the fuel.

3. Results and Discussion

3.1 Macroscopic Character

The macroscopical or morphological studies aid in the identification of plant species and are the first stage in determining the crude drug description. The microscopic and macroscopic identification is necessary as the substitutes or adulterants may appear to be quite similar to the actual substance.

Hyptissuaveolens is a branching, erect, glandular perennial herb with a green hairy quadrate and a woody stem that develops to be 0.7–2 m tall (Figure 2).The root is taproot type. Leaves are opposite, simple with a rich fragrant scent, roughly ovate with finely serrated margins and acute apex and the bottom is thick and hairy with a long slender and hairy petiole. In 2–3 months, the plant begins to flower and produces 2-4 blue flowers in tiny cymes along branches with decreased leaves.Theflower is complete, zygomorphic, pedicellate and bracteolate. It has a bilabiate calyx with lobes that are subequal and subulate. Long, tubular, bilabiate, and pubescent bluish corolla with lower lip saccate and rounded top lip is present. Androecium has four stamens that are linked to a didynamous that is inserted below the neck. The stigma is bifid, and the fashion is gynobasic. With a single basal ovule in each lobe, the ovary is deeply lobed. The nutlike or seedlike fruit is dry, indehiscent, and uniseminated. The flowers are fertilised by a large number of pollinators, resulting in a large amount of seed production. The plant has a characteristic minty scent when crushed. On the surface of leaves, hair glands can be observed. Essential oil and secondary metabolites are produced by the glandular trichomes or hair glands present inaromatic plants [31]. Seeds can germinate at temperatures ranging from 10 to 40 degrees Celsius, but growth appears to be best at 25 to 30 degrees Celsius[32]. When seeds come in contact with water, they develop mucilage[33]. It blooms abundantly from July to November, and its typical habitats are rail lines, crossroads or slopes of open woods, woodland clearings, and wastelands[34](Figure2, A-J).

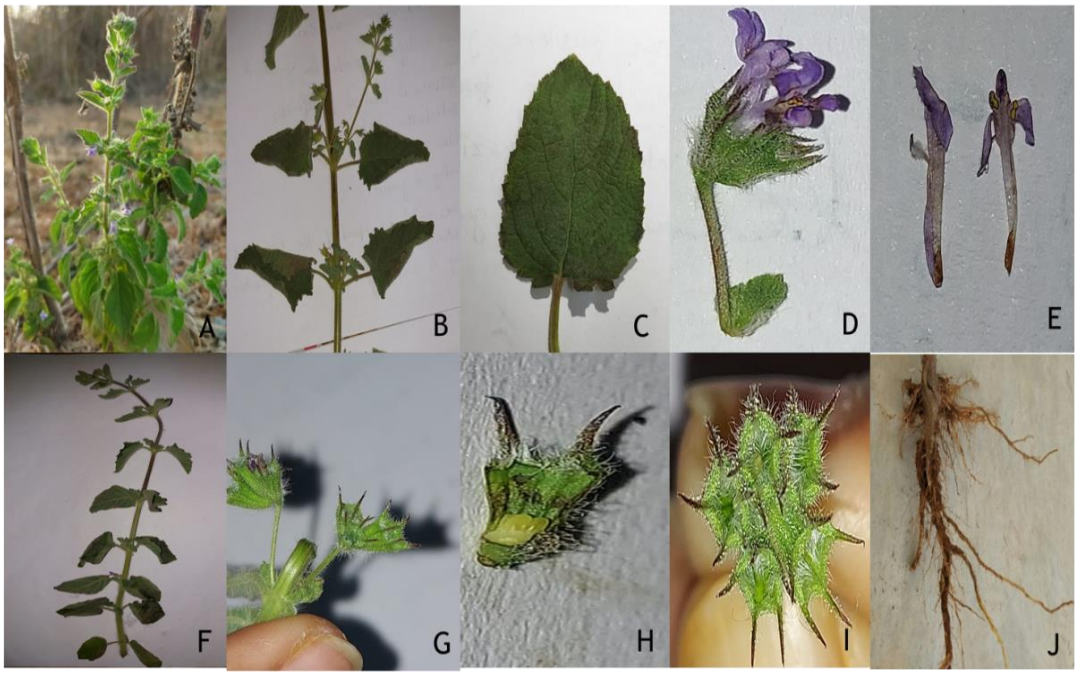

Figure 2: A,Habit and habitat of *Hyptissuaveolens*; B, Stem; C, Leaf; D, Flower; E, L.S of Flower; F, Plant Sample;G, Cluster of immature fruit; H, Immature fruit; I, Axillary cyme inflorescence J, Tap Root

3.2 Organoleptic study

Organoleptic qualities contribute in the detection of contaminated medications. Our sensory organ is used to perform the analysis, which is based on shape, colour, and odour. Siddiqui et al. [30] outlined the strategies for analysing the characteristics. Table 1 shows the organoleptic structures of *Hyptissuaveolens* root, stem and leaf.

Table 1Organoleptic character of *Hyptissuaveolens*

Characteristic	Leave	Stem	Root
Shape	Roughly ovate	Quadrangular	Cylindrical
Colour	Green	Brown	Brown
Order	Minty	Characteristic	Characteristic

3.3 Microscopic character

3.3.1 Stem anatomy

The stem's T.S. is quadrangular in shape, with glandular and non-glandular trichomes in ridges and furrows. The epidermis is a single-layered layer of tiny rectangular cells. 2-9 layered hypodermisis present under this and in the furrowschlorenchyma present. In ridges, discrete patches of collenchyma are present. The pericycle is made up of sclerenchymatous bands that are distinct. The pith is thick and parenchymatous (Figure 3A).

3.3.2 Leaf Anatomy

The leaf has a dorsiventral orientation. The upper epidermis cells are bigger, round to oval in form, and bordered with cuticles and many trichomes. The lower epidermis cells are smaller and have thinner cuticles. In the midrib area, there is a non-glandular trichome. On both sides, stomata can be found. There are no papillae. Unilayered palisade and four-layered spongy tissue are two types of mesophyll. Palisade is one layered and has significant intercellular gaps, whereas spongy tissue is four to six layers thick. Palisade and spongy both have uneven shapes. There are both glandular and non-glandular types of trichomes present. On both sides, there are major lateral veins (Figure 3B). A huge collateral vascular bundle makes up the bowl-shaped midrib. The xylem pieces are arranged in a straight line and face upwards. The sieve tubes, companion cells, and phloem parenchyma make up the phloem, while the vessels, tracheid, and xylem parenchyma make up the xylem. The epidermis is followed by uni-layered collenchyma on the abaxial surface, and bi- and tri-layers of collenchymatous cells on the adaxial surface. The parenchymatous cortex, which has a vascular bundle, follows the collenchymatous hypodermis. (Figure 3B).

3.3.3 Root Anatomy

The root cross-section is circular in outline. Small cells make up the epidermis. The development of the periderm is noticed. Cork is a multi-layered material with rectangular cells on one side and a cortex on the other. The secondary xylem is the most visible part of the segment when compared to phloem. Two or three rows of cells make up the medullary rays. Pith is not present. More xylem vessels and phloem fibres were found in secondary root growth, as well as reduced primary xylem, 4-5 medullary rays, periderm, and cork cells (Figure 3C).

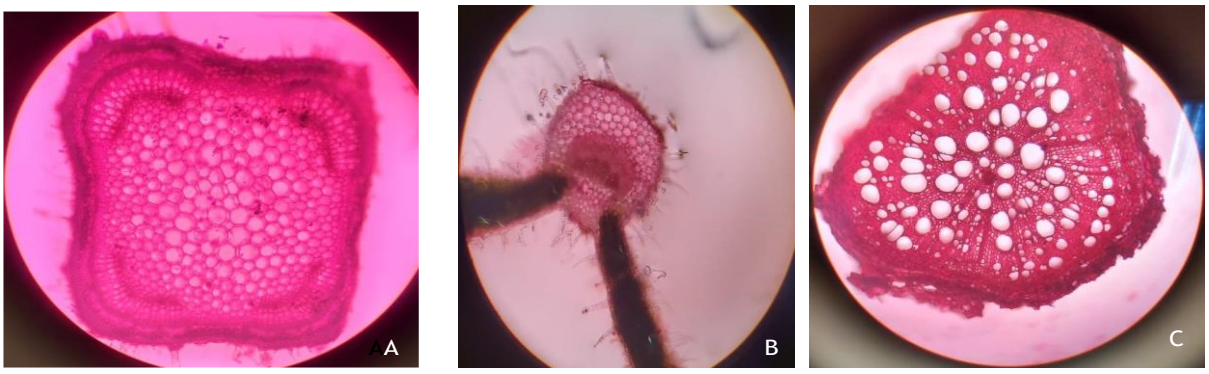

Figure 3 A, T.S of Stem; B, T.S of Leaf; C, T.S of Root

3.4 Powder study

This study is conducted to determine the tissue and cellular inclusion in the powder and the powder was dark green. The observation under a microscope shows different anatomical characteristic features like periderm tissue, tracheid, annular vessel, spiral thickening and phloem fibres (Figure 4).

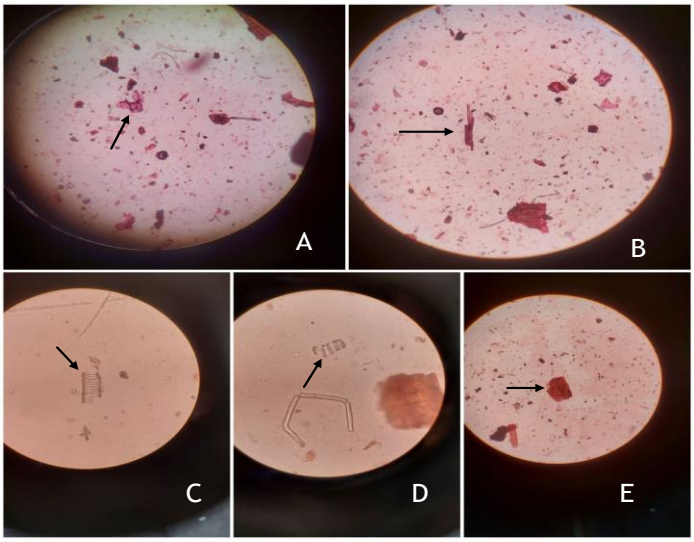

Figure 4 A, Periderm Tissue; B, Tracheid; C, Annular Vessel; D, Spiral thickening; E,Phloem fibres

3.5 FluorescenceAnalysis

The behaviour of crude drug powder of *H. suaveolens* following treatment with different reagents displayed variable colours under visible, short, and long UV light. UV fluorescence is used to show the fluorescence of numerous natural goods that is not apparent in natural sunshine. If the substance itself isn't fluorescent, it can be transformed into fluorescent derivatives or breakdown products with the use of various reagents. As a result, this method can be used to assess the quality of crude drugsand is an essential pharmacognostic technique [35]. When the crude drug powder was treated with different solvents and chemical reagents, distinct colors were obtained. Treating powdered drugsseparately with the reagents such as 5%NaOH, Iodine solution, dilute HCl, 0.1 N KOH, 1N HCl and dilute ammonia showed identical colors(Table 2).

Table 2 Fluorescence analysis of *Hyptissuaveolens*

Solvent	Long UV (254 nm)	Short UV (366nm)	Visible Light
I_2 Solution	Ivory black	Purple	Vandyke Brown
Dilute HCl	Silver Grey	_	Brown
5% NaOH	Dark Black	Black	Brown Ochre

0.1 N KOH	Yellow Green	_	Olive
1N HCl	Dark Green	Black	Gold
Dilute Ammonia	Moss Green	_	Olive Green

3.6 Phytochemical Analysis

Table 3 demonstrations the occurrence of various compounds in the phytochemical extract. Flavonoids, alkaloids, carbohydrates, steroids, saponin, ammino, flavonoid, and tannin were found in abundance in methanolic extract. All of the extracts were determined to be devoid of cardiac glycoside and starch. Using conventional processes outlined in the Ayurvedic Pharmacopoeia of India, these phytoconstituents were identified by distinctive colour changes.This perception highlighted that the powder of *Hyptissuaveolens* phytochemicals may be effectively extracted by the use of polar solvents (Table 3).

Table 3Phytochemical Analysis of plant species *Hyptissuaveolens*

Chemicals	Tests	Petroleum ether	Methanol	Aqueous methanol	Aqueous
Alkaloid					
	DragendorffReagent	+	+	++	+
	Mayer Reagent	-	-	++	+
	Hager Reagent	++	+	++	++
	Wagner Reagent	-	+++	++	+++
Carbohydrate					
	Benedick Test	+	+	-	+
	Fehling Test	-	-	-	-
	Molisch Test	+	+	+	+
Starch					
	Amylum Test	-	-	-	-
Steroid					
	Salkowski Test	+	+	-	+
Saponin					
	Forth Formation	-	-	+	+
Amino Acids					
	Ninhydrin Test	-	-	+	-
Flavonoid					
	Zinc Hydrochloride	-	+	-	+
Cardiac Glycoside					
	Raymond Test	-	-	-	-
Tannins					
	Ferric Chloride Test	-	+	-	-

3.7 Pharmaceutical Standardization

Standardization is necessary for determining the drug's quality based on the presence of active principles. The powder's packing capacity is determined by its density. The consolidation of powder is described by physical characteristics such as tapped density. The arch strength of a consolidated powder is likely to be higher. The Hausner's ratio of 1.13 suggests that the material is compressible. The lower the Hausner ratio, the better will be the powder's flow properties. As a result, the powder has a strong flow property. Because the angle of repose is related to interparticle cohesion, it can be used to estimate powder flowability indirectly (Table 4).

Table 4 Pharmaceutical Standardization of *Hyptissuaveolens*

Name	Value
Bulk Density	0.2 g/ml
Tapped Density	0.227 g/ml
Hausner's Ratio	1.13
Compressibility	11.89%
Angle of Repose	32.5º

3.8 Thin Layer Chromatography

On a silica gel "G" plate, the petroleum ether extract was identified and treated with the solvent system chloroform: Benzene (5:5). The produced chromatogram and Rf value will be particular to the solvent system chosen and will serve as a better tool for the standardization of the extract for the selected plant. It developed two colourless spots when observed in long UV but after treatment with iodine vapour then observed in long UV it developed two brown spots with Rfvalues 0.61 and 0.8 as present in Table 5.

Table 5Thin layer chromatography with petroleum ether extract

Solvent System	R.F value	Colour spot without treatment	After treatment with iodine,vapor observe in a long UV(254) Chamber
Chloroform:Benzene (For volatile oil)	0.61	colorless	Brown
	0.8	colorless	Brown

3.9 Heavy Metal Analysis by AAS

Heavy metal concentration in methanolic extract of *Hyptissuaveolens*was determined by using atomic absorption spectroscopy (AAS) and the result is tabulated in Table 6. It was found that the cadmium and lead concentration is below the prescribed limit by WHO which indicates that it can be further explored for pharmaceutical purposes.

Table 6 Heavy metal (AAS)

Metal	concentration	WHO permissible limits
Cd	0.1066 ppm	0.3 ppm
Pd	0.6269 ppm	10 ppm
Zn		

4. Conclusion

Pharmacognostic evaluation is necessary for the standardization of medicinal plants. Hyptis can be identified and authenticated by analyzing the macroscopic, organoleptic, microscopic, and powder properties of the plant. Natural bioactive substances present in plants are referred to asphytoconstituents. This study found that polar solvents could be used to extract therapeutically important phytochemicals for further research.

The herb was physiochemically standardized according to WHO criteria and the Indian herbal pharm acopeia.Standardization factors such as bulk density, tapped density, Hausner's ratio, compressibility, and angle of repose were employed in this study, indicating that it may be used as a reference for future research and demonstrating its potency. It exhibits a broad phytochemical profile, indicating that it could be beneficial

in the pharmaceutical industry. Further research is required so that Hyptis can be used to prepare a cost-effective therapeutic drug.

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