Journal of Ecobiotechnology 2011, 3(3): 05-10
ISSN: 2077-0464
www.scholarjournals.org

Journal of Ecobiotechnology

www.journal-ecobiotechnology.com

JEBT-Microbial Biotechnology

Pharmacognostic Evaluation of *Hyptis Suaveolens* (L.Poit) Lamiaceae

G.L. Pachkore[1] and D.A. Dhale[2*]

[1]*Department of Botany, P.V.P. College, Patoda. Dist Beed (MS) India*
[2]*P.G. Department of Botany, SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule-424005 (India)*

Article Info

Article History

Received : 04-02-2011
Revised : 25-03-2011
Accepted : 29-03-2011

*Corresponding Author

Tel : +91 9422658646
Fax : 02562 272562

Email:
datta.dhale@yahoo.com

Abstract

The present communication deals with the pharmacognostic investigations on the *Hyptis Suaveolens* (L. Poit) Lamiaceae. The plant is stimulant, carminative, antispasmodic antirheumatic, antisuporitic bath. It is also used for parasitical cutaneous diseases, infection of uterus, and as sudoritic in catarrhal condition, headache, stomach, snuff to stop bleeding of the nose. Macroscopic and microscopic examinations of the organs and differential chemical tests were carried out. The morphology of the entire plant, anatomy of the leaves, stem and root and the physico-chemical standards of the powder in the present study can be used to identify the crude drug. Preliminary phytochemical screening shows the presence of volatile oil, Alkaloid, phenolics, tannin, saponin, protein, carbohydrates etc. Extractive values as well as quantitative estimation of various phytochemicals have been studied for different plant organs.

Key Words: Phytochemical, Pharmacognosy, *Hyptis Suaveolens*

Introduction

Ethanobotanical studies carried out by various workers have revealed that the tribal communities. In India used about 7,500 species of plants for a variety of medicinal purposes (Anonymous, 1995). The scientific evaluation of ethno-medicinal important plants has become much more common, particular as a numbers of drug discovery programmes have begun the regular screening of traditional herbal remedies. In recently chemical analysis and biological assays have lead to the discovery of novel bioactive Phyto-chemicals. An analysis of the data on the plants products exhibiting biological activity (wanger and wolf, 1977) indicate that alkaloids, triterpenoids and phenolics are the three major which shows curatives properties, only limited numbers of medicinal plants of India, has been scientifically and experimentally screened.

Although a vast indigenous knowledge about the medicinal plants is available in India. A large number of plants posse's curative properties. The ancient system of medicine such as Ayurvedic, Unani and Sidha recommanded plants or their extracts to prevent or combat every type of diseases. The present study, pharmagostical studies have been carried out on the leaves, stem and root of the medicinal important plant *Hyptis suaveolens* (L. poit) which is used by various tribal communities of Maharashtra, Marathwada region to cure various diseases like, parasitical cutanous, diseases, infection of utrus, and as a sudorofic in cutarrhal condition. The plant is stimulant carminative, antiplasmodic, antirhenmatic, antisuportic bath. It is also used for headache, stomaches, and snuff to stop bleeding of the nose (Nadkarni 1976).

In view of its diverse medicinal applications and in order to ensure the quality of the drug, in view of adulteration and substitution, the present pharmacognostic investigation on the leaves in *H. Suaveolens* has undertaken.

Materials and Methods

The fresh plant organs (Leaves, Stems, Roots, etc) of *H. Suaveolens* were collected from the Botanical garden of Government Institute of Science, Caves Road, Aurangabad. The voucher specimen is preserved in the Department of Botany Government institute of science, Aurangabad. (M.S., India). The collected plants were washed repeatedly with tap and finally with distilled water. Then sliced in root, stem and leaves. They were dried and powdered with help of grindings and filtered through sieves and stored for chemical analysis. (Daniel M. 1965, Sadashivam and Manikramal 1992, Khandelwal 1985, Kokate *et al* 2005). Some plant material was also preserved in 70% alcohol. Leaf epidermal studies were carried out on fresh specimens. Peels were removed mechanically using some chemicals. They were stained in 1% safranin mounted in glycerine and made semi-permanent by ringing with DPX solution. Stomatal index (SI) was calculated as defined by Salisbury (1927, 1932) viz.,

$$SI = \frac{S}{E + S} \times 100$$

Where 'S' = number of stomata per unit area and 'E' = number of epidermal cells in the same area. Stomatal frequency and stomatal index have been calculated out of an average of 10 readings. Palisade ratios (PR) was calculated as the average of palisade cells (P) beneath each epidermal cell (E). Vein islet number is defined as the number of vein islets per mm^2 of the leaf surface midway between the midrib and the margins. The line and cellular sketches of the figures were drawn using a Camera Lucida. Transections of leaf, petiole, stem and root were taken by free hand. Fresh and preserved

materials were used. Sections were stained in safranin (1 %), light green (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of leaf, petiole, stem and root sections were taken by using Jenaval and Mirax Laborec Cameras affixed to microscope. Histochemical tests were performed on fresh plant materials according to the methods of Johansen (1940) and Guerin (1971). For the observation of leaf architecture leaves were first cleaned by in 10 to 20% aqueous sodium hydroxide solution followed by trichloroacetic acid and phenol solution (2:1 by weight) and then stained with kores stamp pad purple ink.

The moisture content was determined by heating the drug at 105^{o}C to constant weight and calculated the loss of weight. The extracts were prepared using various solvents and total ash, acid insoluble ash, and acid soluble ash value were obtained (Anonymous, 1985).

Nitrogen (N) content in dry plant material was estimated by micro Kjeldal's method (Bailey, 1967) and crude protein was expressed as N x 6.25. Calcium (Ca) content was determined A.O.A.C (1975) method. Phosphorus (P) content was estimated by the colorimetric method (Oser, 1979). Potassium (K) content was determined on a flame photometer (model Mediflame- 127) as suggested by (Jackson, 1973). The amount of phenol, amino acids, and reducing sugar were estimated following Sadasivam and Manickam (1992).

TLC plates 60F 254 were obtained from Merck. Solvents and other chemicals used were of laboratory grade.

Results and Discussion

Macroscopic characters

A tall coarse, branched, very sweet smelling herb, varying in stature but attaining 1.8 to 2 m in congenial situation, with obtusely 4 angled stem often 1.3 cm diameter, lower 11.5 X 9 cm, Slightly cordate, hairy, upper smallest not cordate. Flowers small blue when young often capitates, 2-4 together on an axillary penduncle in (globosely heads) or in bracteates axiliary recemiform cymes or in luxuriant specimens, collected into thyrriform almost leafless panicles 30 cm or more long. Filament hairy, calyx compressed sub – 2 lipped some what deflexed, tube 6 mm. Corolla tubular, 5-6 mm long, bluish; upper lip shortly 2 lobed; lower 1 -3 lobed or long in fruit lo-nerved meeting in a marginal nerve and with an in flexed ring of hairs in the mouth, teeth subulate aristae strong. Nutlets 2, quadrate, 2-3 mm long.

Compressed with a median rib, rugulose brown. Tropical American weed introduced long ago naturalized along road sides, hedges on hill – slopes (Naik, 1996).

Flowers and Fruits : October to March

Parts used : Whole plants.

Microscopic characters

Epidermal features: The epidermal cells of leaf are polygonal, isodimetric and elongated, on the both surfaces. The cell walls may be wavy or sinuous on both the surfaces. The cell walls of adaxial epidermal cells are large with thick walls. The epidermal cells on veins are elongated.

Cuticular ornamentation: The upper epidermal cells are bigger than the lower epidermal cells. A well developed cuticle is always present on both the surfaces of the leaf. The cuticle on the epidermis of leaf may be smooth or may show papilose structure on a pattern of striatious.

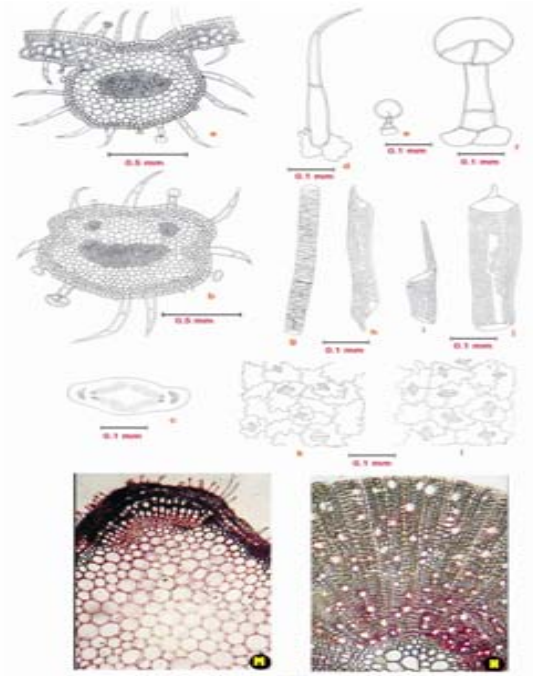

Fig.1: *Hyptis suaveolens* (L) Poit.

a: T. S. Leaf
b:T. S. Petiole
c:T. S. Node
d-f: Trichome
g-j: Vessels
k-l:Stomata
M: T. S. Stem
N: T. S. Root

Stomata: The leaves are amphistomatic. The number of stomata per unit are is always higher on the lower surface than on the upper surface (Table 2) The shape of stomata is oval and elliptical in outline. The stomata are mostly diacytic (Fig. 1 k, l).

Trichomes: Trichomes are observed on both the surfaces of the leaves. They are also present on the inter-costal region and on the veins. The trichomes are more on upper epidermis as compaired to lower epidermis. Non-glandular trichomes is of uniseriate malticellular type with two basal cells and upper body consisting, of three cells with curved, terminal end. (Fig. 1 d) Two types of Glandular trichomes are observed i.e. one with three celled head two-celled stalk with two basal cells (Fig. 1 f) and other with one celled head, two celled stalk with one basal cells (Fig. 1 e).

T. S. of Leaf: The leaf is dorsiventral and hypostomatic. The cells of upper epidermis are larger. The cells of lower epidermis are smaller with thin cuticle. Stomata are present on both surfaces. Papillae are absent. The mesophyll is differentiated into palisade and spongy tissue. Palisade is one layered and spongy tissue is of four to six layered. Glandular and Non-Glandular trichomers are presents.

The vasculature consists of one median vascular bundle. Xylem elements are in linear rows and facing upwards. Papillae are absent on the epidermis. Phloem consists of sieve tubes, companion cells and phloem parenchyma along with xylem consists of vessels, tracheids and xylem parenchyma. The epidermis in midrib region is followed by one layered collenchyma on abaxial surface and two to three layers of collenchyma on adaxial surface. The collenchymatus hypodermis is followed by parenchymatous cortex in which vascular bundle is present (Fig 1 a).

Leaf microscopic characters are summarized in Table 2.

T. S. of Petiole: The shape of petiole is more or less circular in outline. Epidermis is made up of small sized cells with cuticle are present Glandular and Non-Glandular trichomers are presents. Below the epidermis on both the lateral sides of petiole, chlorenchymatous patches are presents. Epidermis is followed by two to three layered collenchymatous hypodermis. Hypodermis is followed by five to six layered parenchymatous cortex.The petiole vasculature consists of one median and two lateral vascular bundle. Xylem elements are in linear rows and facing upwards. Papillae are absent on the epidermis (Fig1 b).

T. S. of Stem: The stem is quadrangular in out line. The epidermis is made up of small rectangular cells which is one layered non-glandular and glandular trichomes are presents. At four corners distinct patches of collenchyma are presents in additions to 2-9 layered hypodermis. Pericycle is in the form of discrete sclerenchymatous bands. Vasculture is in the form of a continuous cylinder, pith is large parenchymatous. (Fig. 1 M).

T. S. of Node: The node shows unilacunar one traced condition. (Fig. 1 c).

Vessel elements: Vessel elements are long, cylindrical, appearing, singly or in groups or two. The length width performation plate, end walls, and lateral walls thickenings were noted in Table 1 (Fig. 1 g, h, i, j).

T. S. of root: In cross section of the root is circular in out line. Epidermis is made of small cells. Periderm formation is observed. Cork is many layered, made up of rectangular cells followed by cortex. Secondary xylem is the prominent part of the section as compared to phloem. Medullary rays are composed of 2-3 rows of cells. Pith is absents (Fig. 1 N).

Histology:

Histological results indicates presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides (Table 5).

Physio-chemical characters

The physio-chemical characters summarized in Table 3.

Phytochemical evaluation

The studies revealed evaluation of various phytochemicals *viz.,* volatile oil (Properties of the oil are summarized in Table 6), total alkaloids, carbohydrates, lipids, proteins and phenols. The value obtained for various phytochemicals in drug sample are presented in Table 3.

Effects of Chemical on Powdered drug of *H. suaveolens* are summarized in Table 4.

The results of the have been given in the Table: 3 Four distinct spots were observed when the solvent system – 1 was used for phenolics compounds. All these spots were visible only after the treatment of the TLC plate in iodine vapour. When the solvent system II was used to test the steroidal compounds, seven different spots with various RF values were observed only three spots (0.13, 0.24, 0.32) were observed without the iodine vapour treatment while all the seven different spots were observed after treating the TLC plate with iodine vapour. The RF values obtained from TLC of Ethanol Extract of Powder of *H. suaveolens* are summerised in Table 7.

Table No. 1: Vessels Elements of *Hyptis Suaveolens*

Sr. No.	Organ	V.I.	A.V.L.	V.D.	A.V.D.
1.	In Stem (%)	200.0 to 680.0	344.4	16.0 to 32.0	22.7
2.	In Root (%)	160.0 to 420.0	258.0	32.0 to 68.0	42.2

Note: V.I.= Vein Index, AVI= Average Vein Index, V.D.=Vein Density. A.V.D= Average Vein Density

Table 2: Quantitative microscopy of *H. suaveolens*

Parameter	*Mean*
Stomatal frequency	
a) Adaxial Epidermis	44.8*
b)Abaxial Epidermis	127.2*
Stomatal index	
a) Adaxial Epidermis	12.33*
b)Abaxial Epidermis	23.14*
Vein islet number	35/mm^2
Vein termination	20 /mm^2
Palisade ratio	5.10 Palisade/Epidermal cell

* – Microscopic Field

Table 3: Physico-Chemical evaluation of *H. suaveolens*

Physical evaluation (%)				Chemical evaluation (%)			
	Leaf	Stem	Root		Leaf	Stem	Root
Moisture content	8.17	7.10	6.15				
Extractive values				Volatile Oil	0.4	0.2	0.1
a) Ethyl Acetate	34.14	15.20	10.12	Total Alkaloids	3.2	1.8	1.5
b) Alcohol	50.10	21.20	20.70	Carbohydrates	2.9	3.2	2.7
c) Water	51.20	49.05	27.25	Lipids	5.86	3.12	2.02
Ash values				Proteins	2.78	0.74	0.5
a) Total ash	4.7	2.18	2.46	Phenolics	0.3	0.3	0.04
b) A.S.A.	1.96	1.70	0.42				
c) A.I.A.	2.74	0.48	2.04				
d) W.S.A.	2.85	1.48	2.11				
e) W.I.A.	1.85	0.70	0.35				

Note : A.S.A.= Acid Soluble Ash ; A.I.A= Acid Insoluble Ash ; W.S.A=Water Soluble Ash ; W.I.A= Water Insoluble Ash

Table 4: Effects of Chemical on Powdered drug of *H. suaveolens*

Sr. No.	Reagent	Leaf	Stem	Root
1	Powder	Olive green	Light brown	Light brown
2	Powder+ Iodine	Light brown	Orange	Light brown
3	Pd + 5% Ferric Chloride	Pale yellow	Light brown	Light brown
4	Pd + 1.N.NaOH	Redish brown	Dark brown	Light brown
5	Pd + Acetic acid	Dark green	Yellowish brown	Faint brown
6	Extracts + Acetic acid + 50% H_2SO_4	Yellowish brown	Faint yellow	Faint brown
7	Pd + 50% H_2SO_4	Green	Faint yellow	Faint brown
8	Pd+ 50% Concentrate HCl	Green	Light green	Faint brown
9	Pd + Ammonia.	Dark green	Light green	Faint brown
10	Pd + Ammonia + Pot. Ferrocyanide.	Dark green	Light yellow	Faint brown
11	Extracts + 4%NaOH + 1% $CuSO_4$	Yellowish brown	Green	Dark green
12	Extracts + 40%NaOH + 1% Lead acetate	Faint redish brown	Lemon yellow	Light orange
13	Pd + 50%.Nitric acid + Picric acid.	Faint redish brown	Yellowish brown	Light orange
14	Pd + Saturated picric acid.	Brownish orange	Dark yellow	Light orange

Table 5: Histochemical Test of *H. Suaveolens*

Sr.No.	Test	Leaf	Stem	Root
1.	Volatile oil	+	+	+
2.	Starch	+	+	+
3.	Protein	+	+	+
4.	Tannin	+	+	+
5.	Saponin	+	-	-
6.	Fat	+	+	+
7.	Alkaloids	+	+	+
8.	Glycoside	+	+	+

Table 6: Physical Properties of the Volatile oils of *H. Suaveolens*

Sr. No.	Physical Exam	Observation
1.	Colour	Yellow
2.	Odour	Aromatic
3.	Solubility	54 %
4.	Boiling Point	205^0 C.
5.	Optical Rotation	10^0 to 15^0
6.	Refractive Index	1.4362 to 1.4370
7.	Weight Per ml.	0.995 to 1.1015

Table No.7: RF values obtained from TLC of Ethanol Extract of Powder of *H. Suaveolens*

Sr. No.	Solvent System	R.F. Values	Colour of the spots without any treatment	After treating with Iodine Vapour.
1.	Toluene. Ethylacetate (97 : 3) for phenolic compounds	0.21	Colourless	Greenish Yellow
		0.35	Colourless	Greenish Yellow
		0.83	Colourless	Greenish Yellow
		0.52	Colourless	Golden Yellow
		0.10	Pale Green	Golden Yellow
2.	Chloroform : Benzene for Volatile Oil	0.21	Pale Green	Light Green
		0.32	Pale Green	Pink
		0.40	Green	Pink
		0.47	Colourless	Brown
		0.78	Colourless	Brown
		0.67	Colourless	Purple

Acknowledgement:

The authors wish to thank Head of Botany Department, Government Institute of Science, Aurangabad for providing the necessary laboratory facilities.

References

Annonymous (1985) "*Indian Pharmacopoeia*" vol. 2,3rd Ed., Govt. of India, Ministry of Health, Controller of Publications, New Delhi, India. pp. A74 - A75.

Anonymous (1995) "Indian pharmacopocia" Miaistry of Health and family welfare govt. of India, countroller, publication, New Delhi. Vol. II PPA – 74 A.124.

AOAC (1975) "*Official and tentative methods of analysis",* XI Edn., Association of official Analytical chemists, Washington, D. C.

Bailey, R. L. (1967). "*Techniques in Protein Chemistry", 11 Edn., Elsevier Publishing Co. Amsterdam.*

Daniel M. 1991, "Plant Economic botany and Methods of Phyto chemistry" Kalyani publishers ; Ludhiana and New Delhi.

Guerin H.P., Delaveau P.G. and Paris R.R. (1971) "*Bullein de La Societe Botanique de France* " 118: 29

Johansen D.A. (1940) "*Plant Microtechnique"*, Tata McGraw Hill Publishing Company Ltd., New Delhi.

Khandelwal K.K. 1985 "Practical Pharmacognosy" Nirali Prakashan Pune.

Kokate C.K., A.P. Purohit and S.B. Gokhale, 2005. 'Pharmaeognosy, Nirali Prakashan, Pune.

Nadkarni K.M. , 1976. Indian Materia Medica. Mumbai, India ; Popular Pakistan 976 ; p 671.

Naik V.N., 1996. "Flora of Marathwada" Amrut Prakashan, Aurangabad.

Oser B.L. (1979) "*Hawk's Physiological Chemistry*". 14th Edn. Tata Mc Graw Hill Publishing Co. Ltd. New Delhi, India.

Sadasivam S. And Manickam A., 1992. "*Biochemical methods for agricultural sciences"*. Wiley Eastern Ltd., New Delhi, India.

Salisbury E.J. (1927). "*Phil Trans".* Roy, Soc. London, 216: 1.

Salisbury E.J. (1932). "*Beih. Bot. Zeni-ralb"*. 49: 408.

Wagner, H. and P. Wolff, 1977. New Natural Products and plant Drugs with pharmacological, Biological and therapentical Activity Spriuger – Veriag London.