Histological and phytochemical studies on aromatic plant, *Hyptis suaveolens (l.)*of family Lamiaceaea (MS) India

Ulhe S K[1] and S D Narkhede

[1]Department of Botany, Institute of Science Nagpur (M.S.)
Government Science College,Gadchiroli, (M. S.)
sushama_narkhede@yahoo.com

ABSTRACT

Hyptis suaveolens plant collected from Gorewada Lake forest, Nagpur district (Maharashtra state).This plant belongs to family -Lamiaceae and is commonly known as "Ran tulsi" which is evergreen aromatic, perennial, erect herb and measuring about 120-170cm tall. The roots are typically tap root and weak herbaceous type .The stem is quadrangular. The leaves are covered by hairs and inflorescence is of verticillasters type. In the present study anatomical characters and phytochemical analysis of the leaves were reported. Anatomical characters: Both outer and lower epidermis layers of the leaves were covered by multi-cellular hairs. Pallisade was poorly developed while mesophyll is filled with compact parenchyma. Midrib and Vascular bundles were well developed having secondary growth with broad vessels .Phytochemical Analysis: Fresh leaves were shaded dried and 50gm powder was used. The aqueous extract was prepared in six solvents viz.Petroleum ether, benzene, chloroform, acetone, ethyl alcohol and water to screen the active chemical constituents. The qualitative analysis confirmed the presence of alkaloids, tannin and saponin in leaves. Alkaloids showed high scores while tannins showed moderate scores but saponins indicated low scores. Aromatic oil is found in 6% in 3gm of dry weight of powder of leaves of *H. suaveolens*. The present investigations concluded that the leaves of *Hyptis suaveolens* contains alkaloids>tannins> saponins in this order, and contains double percent amount of aromatic oil. These chemicals are widely used in Ayurvedic traditional medicinal system.

Key words: *Hyptis suaveolens,* Lameaceae*,* leaves, Phytochemical, anatomical, analysis.

INTRODUCTION

The plant is being used by the local peoples and tribal of Maharashtra as ethno medicineon various ailments. It is fever associated with cold.Pounded fresh material applied as poultice may be used for treatment of snake bites. Used as an external wash for dermatitis Eczema.Leaf paste is applied on sores and fungal skin infections.Juice of leaves used for athlete's foot, applied daily to interdigital.Aromatics plants and species have great importance for food, cosmetics and pharmaceutical industries. Plant has an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). The final extract of each solvent was use to analyze for the presence of different phytochemical constituents (Harborne, 1973). Lamiaceae family species are important for its medicinal properties among plants. This family is represented by 45 genera and 574 species (Erik and Tarikahya, 2004). Chemical constituents of plants can be classified in different ways (*kurin et al.,* 2007) and hence phytochemistry would refer to the study of chemicals derived from plants.

Naturally growing Lamiaceae members have been used as tea, spice and for various medicinal purposes. Also used to treat fever, cough, headaches, wound healing heart diseases and stomachaches. (Nurdan and Aysel, 2007).SS In the present study, Authors have concentrated on the anatomical features of the leaves and preliminary, screening and qualitative separation of secondary metabolites from leaves of plant, *Hyptis suaveolens*.

MATERIALS AND METHODS

The anatomical study of the species Double Staining Method was used with some modification, (Microsc 1953) preliminary Phytochemical screening of plant and oil percentage in leaves of from family Lamiaceae was carried out.

Phytochemical Test:

In this context it is significant to undertake a preliminary Phytochemical screening of plants for detection of various plants constituent. Plant cells are highly sophisticated chemical factories, where large varieties of chemical compounds are synthesized through definite pathways. Separation of active substance from crude drug is called as extraction and it involved the use of different solvents. The choice of plant material for extraction depends on its nature and the compounds required being isolated. The dry powder of the plant material is commonly used for extraction. The expanding knowledge of the phytochemical screening has revolted that existence of close relationship between chemical constituent of plants and their taxonomical status. The alkaloids, tannins and saponins are more important chemical constituents. The alkaloids are poisonous in nature but when used in small quantities exert useful physiological and hence they have secured significant place in medicines.

Phytochemical Analysis:

Successive solvent extraction about 50 gm of the dry powder was successively extracted with the following solvents in a "Soxhlet Extractor". Petroleum Ether ,Chloroform ,Acetone ,Ethyl Alcohol ,Water The liquid extract so obtained in each solvent was concentrated by distilling of the solvent and then evaporated to dryness the water bath at 50^0C. The solidified extract with each solvent was weighted and calculated the percentage in terms the dry weight of plant materials. Each time before extracting a residual part with the next solvent it was dried in even at 40^0C to 50^0C.

Detection of Alkaloids:

Alkaloids were tested (Smolenski *et al*, 1972). The solidified extracts were taken with a few drops of dilute hydrochloric acid and filtered it. Filtrate were tested carefully with variety of reagent such as Mayer's reagent (cream ppt), Dragendoff's reagent (orange brown ppt), Hanger's reagent (yellow ppt) and Wagner's reagent (reddish brown ppt) for alkaloid test. If all the test are positive, then only presence of alkaloids was reported.

Mayer's test, Wagner's test, Hager test is carried out.

Dragendoff's Reagent:

5.2 gm Bismuth carbonate and 4 gm sodium iodide were boiled for few minutes with 50 ml glacial acetic acid. After 12 hour the precipitate of sodium acetate crystals were filtered using glass funnel. 40 ml of clear red brown filtrate was mixed. 160 ml ethyl acetate and 1 ml of water and stored in Amber colored bottle.

Detection of Tannins:

Tannin were tested in the ethanol and water extract by gelatin salt block test (Farnworth, 1966) Small quantity of Petroleum ether, chloroform acetone, ethyl alcohol and water extract were taken and addition of dilute ferric solution (5%) black green color indicates tannin. Ferric Chloride Test, Gelatin Test, Lead Acetate Test are carried out.

Alkaline Reagent Test:

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution.Yellow fluorescence indicates the presence of flavonoids.

Detection of Saponins:

Presence of Saponin was tested (Cambie *et al*., 1961) in water extract. The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins. 1 gm of petroleum ether, chloroform, Acetone, Ethanol and water extract were taken followed by adding distilled water to it. It was shakes thoroughly again with the addition of the water till volume become 20 ml and further shake in graduated cylinder thoroughly for 15 minutes. Appearances of one cm. layer of f indicate the presence of saponin.

Detection of Oils

Reagent: Petroleum ether or hexane

Oil content in sample (% dry wt. basis) = [(b – a) x 100] / wt of sample (gm)

Observations and Results:

Hyptis suaveolens (L) poit, Ann, Mus. Matl. Hist Nat 7:472 t Syst Nat. ed. 10, 1100, 1759

Distribution – It is native to tropical American weed naturalized in the area on waste land barren fields. Locality – It is common plant collected from Gorewada Lake forest, Nagpur. Family – Lamiaceae

Botanical Name – *Hyptis Suaveolens*

Vernacular name – Rantulsi

Fls & Frts – October, March

Morphological Description:

Evergreen, perennial, erect, herb being approximately 2 meters (120-180 cm) high.

Roots are typically tap root, aerial weak herbaceous.

Stem, woody erect, light green in color, branching racemose as well as cymose angular young branches cylindrical. Leaves covered by hairs, simple estipulate, opposite decussate, sub sessile unicostate with crenate margin with dimension 8.7-10 x 6.2-7 cm, acute apex triangular petiole, color green. Flower is sessile, hypogynous, actinomorphic, and blue in color, tetracyclic. Inflorescence a terminal, branched, raceme of verticillaster. Calyx consists of five sepals gamosepalous. Corolla consist of five petals gamopetalous two upper teeth become united forming an almost regular, tetramerous 3-lobed with middle one bent downwards. Stamens 4, didynamous, epipetalous and alternate with corolla lobes. Gynoecium bicarperally, syncarpous ovary bilocular style gynobasic ovary superior. Fruits nutlets.The placentation: axil.

Fig:1: *Hyptis suaveolens* (L)

Fig. 2: T.S.Leaf of *Hyptis Suaveolens*

Anatomical character of Leaf:-

Both outer and lower epidermis covered by multi-cellular hairs. Palisade poorly developed. Mesophyll with compact parenchyma. Midrib and Vascular bundles are well developed having secondary growth. Vessels are broad.

Phytochemical analysis of Leaf

Alkaloids: Fresh weight of leaf – 58.6 gm

Dry weight of leaf – 22.1 gm

High concentration (+++) of alkaloid was observed in Benzene, whereas moderate concentration (++) of alkaloid was found in petroleum ether, chloroform, Acetone and ethyl alcohol while water extract exhibited (-ve) test for alkaloids.

Tannin: High concentration (+++) of tannin was observed in Acetone extract, while moderate concentration (++) of tannin was found in petroleum ether (chloroform, ethyl alcohol) whereas low (+) concentration was found in water.

Saponin: Chloroform extract shows (+++) high concentration for saponin, while petroleum ether and benzene extract shows moderate concentration (++) whereas Acetone and Ethyl Alcohol and water extract shows low (+) concentration for Saponin.

The important properties of medicinal plants are perhaps due to presence of various secondary metabolites such as alkaloids, flavonids, glycosides, phenol, saponins etc. The above results indicate that the leaves of plant investigated that it is rich in alkaloids. Anatomical study of leaf was carried out to observe the anatomical details as well as to points out site of chemical constituents. Leaves were dorsiventral, palisade to only upper sides spongy parenchyma well developed with prominent inclusion of chemical constituents. Phytochemical analysis of this plant high concentration observed in benzene, modrate contration in petroleum ether and low in ethyl alcohol. Alkaloids analysis in *H.suaveolens* (L.) Poit, carried out Maxwell *et al.,* (1995) and Edeoga, *et al.,* (2004),Maxwell *et al., (*1995)Singh and Sawhney,(1988) and they have reported Alkaloids as important medicinal constituent in family Lamiaceae. In *H.suaveolens*(L.) Poit, high concentration was observed in Benzene extract, moderate in ethyl alcohol, Acetone and Chloroform is found to negative for this test. Tannins are consider as food product is plant vegetable. Tannins like isoflavones were detected by Harborne, (1998)for member of Lamiaceae and saponin was reported in low concentration.

The presence of these chemical constituents in the investigated plant accounts for their usefulness as medicinal plants. Alkaloids are known to play some metabolic role and control development in living system. They are used in medicine especially steroidal alkaloids. The Phytochemical analysis of leaves of this plant has high concentration observed in benzene, modrate contration in petroleum ether and low in ethyl alcohol. Singh and Sawhney,(1988),Maxwell *et al.,* (1995) and Edeoga *et al.,*(2004) have reported alkaloids as important medicinal constituent in family Lamiaceae. In the present study also reported that alkaloids are the major constituent in the leaf of H.suaveolens. Tannin is considered as food product in plant vegetable. Tannins like isoflavones were detected by Harborne,(1998)for member of Lamiaceae. In the leaves of *H. suaveolens* tannin is found at considerable level and thus supports Harbone's view .The presence of tannin in above plant may be the reasons why most of the animal does not graze this plant.

The present investigations concluded that the leaves of *Hyptis suaveolens* contains alkaloids>tannins> saponins in this order, and contains double percent amount of aromatic oil. These chemicals are widely used in Ayurvedic traditional medicinal system**.** The presence of tannin in above plant may be the reasons why most of the animal does not graze this plant.

Table No.1 .Qualitative chemical analysis of various extract obtained by solvent extraction of leaves of *Hyptis suaveolens*

Test	Reagent Used	Petroleum Ether	Benzene Extract	Chloroform Extract	Ethyl alcohol Extract	water
Colour	Colour of extract	Pale green	Dark brown	Greenish black	Bottle green	Dark brown
Alkaloid	Mayer's Reagents	-	+	+	+	-
	Dragendroffs Reagents	++	+	++	-	-
	Wagner's Reagents	-	+++	-	++	-
	Hager's Reagents	+	++	-	-	-
Tannin	Ferric Chloride Test	++	+	-	-	-
	Gelatin Test	-	-	++	-	+
	Lead Acetate Test	+	++	+	-	+
	Alkaline Test	-	-		-	-
Saponin	Foam Test	+	++	+++	+	+

Low +, Moderate ++, High+++.Absent

Table 2: Analysis of oil percentage in leaf

S No.	Empty flask weight	Flask weight with oil	Oil in percentage
1	124.770gm	124.950gm	6%

Bhoganokar PY and Devarkar VD, 1999. *Addition to the flora of Melghat, Technical Buletin* Mo VIII, Directorate project Tiger, Melghat Amravati. pp 1-67.

Bhattachrjee, SK and Jain Shashi, 2001. *Hand book of medicinal plant,* published by Pointer pubslisher vyas Building S.M.S. Highway Jaipur 302003 (Raj) India 220-222

Cambie RC Cain,BS Roche La, 1961. *A New Zealand Phytochemical survey (III) fern and fern allies*. New Zealand *Journal of Science* **4**:707-714.

Dhore MA, 1986. *Flora of Amravati District with Special Reference to the distribution of tree species,* Ph.D. Thesis Nagpur University, Nagpur.

Dipak koche Rupali shirsat Syed imran and Bandage DG, 2010. Phytochemical screeing of eight traditionally used Ehthnomedicinal plants from Akola district (ms)India. *Int. J. Pharma and BioScience,* **1**(4):2534-256.

Edeoga Ho, 2004. Nutrition Composition of three Nigeria Medicinal plants. *Food chemistry,* **57**(2):229-232.

Erdtman G, 1952. Pollen morphology and Plant Taxonomy Angiosperm Waltham Mass Chronica Botanica. Waltham, Mass

Erik S and Tarikahya B, 2004. Turkiye Florasi Uzerine. *kebikec*. **17**:139-163

Farnsworth NR, 1966. Biological and Phytochemical screening of plants. *Journal of pharmaceutical Sciences,* 225-276.

Geissman TA, 1963.Flavonoid compounds, tannins, lignins and related compound. In M. Florkin and E.H.Stotz(ed), Pyrrole pigment, so[renoid compounds an phenolic plant constituent, Elsevier, New York.**9**:265.

Harborne JB, 1973. Phytochemical methods, Chapman and Hall, London.

Harborne JB, 1998. Introduction to Ecological Biochemistry 3rd Edn.Acadamic Press. London, pp 10-15. Introduction to Ecological Biochemistry 3rd Edn.Acadamic Press.London,pp 10-15.

Microsc JR, 1953 A new simultaneous double-staining technique for histology of vascular plants. **73**(3):162-3.

Kurian A and Sankar MA, 2007. Medicinal plants 2 New India Pubishing Agency pp. 239-262.

Maxwell A, Seepers M, Pingal R, Mootoo DR, Rrynolds WF, 1995. 3- Belta-amino Sprisolane steroidal alkaloids from *Salanum triste. J. Matl Prod*, **58**:625-628.

Nurdan Sarac and Aysel Ugur, 2007. Antimicrobial activities and usage in folkloric Medicine of some Lamiaceae species growing in Mugla,turkey EurAsia *J. BioSci.,* **4**:28-37.

Singh R Sawhney SK, 1988. Advances in Prontier areas of plant Biochemistry Prentice Hall in India Private Ltd. New Delhi. Pp. **487.**

Sofowora A, 1993. Medicinal Plant and Traditional Medicine in Africa. 2nd ed. Spectrum Book Ltd. Ibadan, Nigeria. pp. 71-73,289.

Thimmaiah SR, 1999. Standard Methods of biochemical Analysis,Kalyanin Publishers, Ludiana-New Delhi-Noida .

Syed Imran SS Suradkar and Koche DK, 2012. Phytochemical analysis of *Leonotis Nepitifolia* (L)R BR,Awild medicinal plant of Lamiaceae. *Journal of Bioscience Discovery,* **3**(2):197-196.

Vyas SP, Sheela Bedi Tanja, 2008. A Handbook of aromatic and essential oil plant Jodhpur. *Agrobios,* **81:**7754-3545.

How to Cite this Article:

Ulhe SK and SD Narkhede, 2013. Histological and phytochemical studies on aromatic plant, Hyptis suaveolens (l.)of family Lamiaceaea (MS) India. *Sci. Res. Rept,* **3**(1):44-48.