December 2007 Volume 3 Issue 3

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 3(3), 2007 [159-165]

Lignan and other compounds from the indian medicinal plant, *Hyptis suaveolens*

Amruta Indane*, Alka Chaturvedi

P.G.T.D. Botany, R.T.M.Nagpur University, Nagpur, M.S., (INDIA)

Tell : 9223502879

E-mail: aaj_23580@rediffmail.com

Received: 11th September, 2007 ; Accepted: 16th September, 2007

ABSTRACT

Four extract prepared by using different solvents in their increasing order of polarity. All extracts were screened for the presence of a number of medicinally active compounds. Petroleum ether and n-hexane extracts showed presence of lignan while 70% methanol and water extracts dissolves moderately polar glycosidic components of plants identified as antheracene derivatives, coumarins, flavonoids,phenolic acids, different terpenoids, etc. The results obtained in present investigation revealed great potential for production of lignan, there remains a need for identification and confirmation of specific lignan.

KEYWORDS

Hyptis suaveolens;
Lignans;
Phenolic compounds;
Terpenoids;
n-hexane;
TLC,
HPTLC methods.

INTRODUCTION

Lignans were known as natural products, are distributed widely in plant kingdom. The breadth of the biological activities of these compounds has come to be appreciated relatively recently. Much interest has been focused on their effectiveness as antineophlostic agent and research in this area has revealed several modes of action by which they can regulate the growth of mammalian cells. Additionally, these lignans have various biological activities like they display an antitumour activity that is particularly true of the podophyllotoxin group of lignans, constituents of the medicinal resin extracted from *Podophyllum* species[1].

Lignans possess antimitotic activity, antiviral activity, inhibits enzyme activcity, catharctic activity, cardiovascular activity, allergenicity, pesticidal activity, antimicrobial activity, fungistatic activity. They may influence nucleic acid metabolism; show activity on central nervous system both as depressant and antidepressants; protection activity against hepatotoxins; stress reducing activity[1]. Availability of the compounds from natural resources is an important issue for pharmaceutical companies that manufacture podophyllotoxin drug[2].

H.suaveolens is an aromatic herb found in Deccan Pennisula, North East India, Andaman and Nicobar Island. The plant is said to be useful as a stimulant, carminative, sudorfic and galactogogue. In the form of infusion the plant is used in catarrhal conditions, infections of the uterus and parasitic skin disease. The plant is said to have antiseptic properties. In Philippines, the leaves and tops are considered to be antispasmodic and used in antirheumatic and antisuporific baths[3]. *H.suaveolens* also possesses antiiflammatory and free radical scavenging activity[4]. Water extract of the aerial parts of *Hyptis* suaveolens were found as potent in-

Full Paper

hibitors of HIV-RT[5,6].

One new and six known lignans, as well as sideritoflavone and rosmarinic acid were isolated from *Hyptis verticillata*. The known lignans were identified as dehydropodophyllotoxin, dehydrodesoxypodophyl lotoxin, 4-demethyldeoxy podophyllotoxin, podophyllotoxin, podorhizol and epipodorhizol[7]. Investigation on the aerial parts of *Phlomis integrifolia* (Lamiaceae) yielded in the isolation of iridoids, phenylethanoid glycosides, lignans, neolignans, flavonoids, monoterpene glucosides and diterpenoids[8]. Potential production of podophyllotoxin based on yields and abundance was evaluated in some genera including *Hyptis, Nepeta* and *Thymus*[9]. Therefore, present investigation was undertaken to screen the availability of lignan and other compounds in *H.suaveolens*.

EXPERIMENTAL

The plant selected for the present study i.e. *Hyptis suaveolens* was collected from its wild habitats in the Nagpur city Aug-Nov, 2002 and 2003. A voucher specimen for plant was deposited at the Herbarium, Department of Botany, Nagpur University, Nagpur (Figure 1). The healthy plants were collected. They were washed free of the dirt and other impurities and dried immediately. A slow drying preferably shade-drying or drying in an oven at 40-60^{0}C was done. The dried plant material were powdered with the help of mixer grinder and used for extraction.

The classical procedure of hot extraction was used, where the plant material is boiled in Soxhlet's extractor.

(a) (b)

Figure 1: *Hyptis suaveolens* (a) habit and (b) voucher specimen of herbarium sheet

All chemicals used for extraction and preparing mobile phase were of AR grade. The Petroleum ether(60-80^{0}C) was used for extracting all lipidic compounds specially carotenoids, chlorophylls, etc. Next to this the residue was dried and again extracted with 70% methanol, as it is more effective because this solution combines the properties both of methanol and water. The extraction process was continued till de-coloration of plant material in the extractor. After complete extraction, the extract was distilled to remove the excess solvent and the extract was reduced to comfortable volume (approximately 5-8ml), by evaporation.

Water extracts of the plants were prepared following the methods used by the herbal healers (as per recommendations of the herbal healers). The dried powder of the aerial parts of each plant (approximately 5g) was boiled at 100^0 C in 100ml of water for 15-30min, cooled to room temperature for 15min, and filtered. The extract was reduced by evaporation. Special Extraction Procedure used for some compounds explained below-

Alkaloids

Powdered plant material extracted with 10% acetic acid in ethanol, leaving to stand for at least 4hr. concentrate the extract to one-quarter of the original volume and precipitate the alkaloid by drop wise addition of NH_4OH. Collected by centrifugation, washed with 1% NH_4OH. The residue dissolved in a few drops of Chloroform[10].

Cardiac glycosides

2g powdered drug extracted by heating for 15min under reflux with 30ml 50% ethanol, with the addition of 10ml 10% lead-(II)-acetate solution. After cooling and filtration, the solution is extracted by shaking three times with 15ml quantities of dichloromethane/isopropanol(3:2) and used for chromatography[11].

Sapogenins and phytosterols

Powdered plant material hydrolyzed with 2M HCl for 2-6hr., neutralized with NH_4OH and the solid matter dried and extracted with petroleum ether (60-80^{0}C). The extract was taken to dryness and the residue dissolved in chloroform. The solution was then concentrated and subjected to TLC[10].

During the present studies initially TLC plates were

used for the separation, after preliminary analysis HPTLC technique and plates were employed for confirmation. Sample was applied on plate in the form of spots of liquid extract.

Ascending development was used during analysis. The saturation of the chamber was done using suitable solvent for 5-10min as per requirement, while for some compounds better separation achieved without chamber saturation also. For the TLC screening, the selection of mobile phases was done using available literature of similar studies on other members of Lamiaceae and by pioneer workers[10,11,12]. In addition to the absorption of visible light(coloured substances), UV light (i.e.254nm and 365nm) was primarily used for detection. After preliminary inspection in UV-254nm and UV-365nm light, each chromatogram was analyzed for the presence of drug constituents by spraying with reagent for particular group.

RESULTS

Hyptis suaveolens(L.) Poit. in Ann.Mus.Par. 7:472, 1806; Hook .f. Fl. Brit. India 4:630, 1885; Naik, Fl. Osmanabad 270,1979. *Ballota suaveolens* L. Syst. ed. 10:1100. 1790. Whole plant used in medicine.

Common name

English-Wild spikenard, Chan; French-Gros baume, Hyptis a Odeur; Hindi- Wilayati tulasi; Marathi-Ran tulas; Other languages-Beng.-Bilayati tulasi; Oriya-Ganga tulasi, Purodo; in Bihari-Bhunsri, Dimbubuha, Ara Gusumpuru, Mumutun (Guam).

Morphological description

Erect strongly aromatic, pubescent herbs or shrubby annuals, 100-170 cm tall. Leaves ovate, rounded or subcordate at base, crenate-dentate, pubescent on both the surfaces; the upper ones rather smaller. Flowers in lower leaf axills, 2-4 together in pedunculate cymes; bracts ovate, minute; pedicel short. Calyx tubular, 5-8 mm long, toothed with 10 ribs, glandular pubescent and with a ring of hairs in the throat; teeth subulate, mucronate. Corolla tubular, 5-6mm long, bluish; upper lip shortly two lobed; lower one 3-lobed. Nutlets two, quadrate, compressed with a median rib, rugulose, brown. Fls. and Frts. -October-March.

Out of many secondary metabolites, which are used in medicine and other commercial purpose, major ten groups of secondary metabolites were screened in the present study, which are

- Nitrogenous compounds -1. Alkaloids;
- Phenolic compounds-2. Anthracene derivatives, 3. Coumarins ,4. Flavonoids, 5. Lignan, 6. Phenolic acids;
- Terpenoids-7. Cardiac glycosides, 8.Essential oils (di-, mono- and sesqui- terpenes), 9. Phytosterols, 10. Saponins and Sapogenins.

In TLC screening, alkaloid was detected in the form of light orange coloured bands by spraying the plate with Dragendorff's solution. In all six mobile phases were tried to separate alkaloids. The mobile phase consisting of MeOH: NH_4OH (200:3) exhibited good resolution of alkaloidal band. The plant showed poor alkaloidal content. A single orange band after Dragendorff's spraying was observed in the leaf extracts at the Rf 0.74 (TABLE 1) as compared with the extract of *Rauvolfia* sp. The stem and root extracts reacted negatively with dragendorff reagent.

Anthracene derivatives(anthraquinone and anthranol) were detected by their orange red (anthraquinone) and bright yellow(anthranols) band after 10% KOH spray. It is well separated with reproducibility of results in mobile phase consisting of EtoAC: MeOH: H_2O(100:13.5:10). They were found to be present in the methanolic extract of leaf as 2-4 bands (Rf 0.23-0.97) and stem samples 2-3 bands(Rf 0.21-0.66)(TABLE 1) as well as water extract. No bands were observed in the root extract. Coumarins generally give intense blue or blue green, yellow colours in UV 365nm after KOH treatment in comparison with the extract of *Melilotus officinalis*. For separating coumarins ethyl acetate: formic acid: glacial acetic acid: water(100:11:11:26) was used. Two to five bands were observed with blue fluorescence in UV 365nm in leaf samples(Rf 0.1-0.79). It was found to be present in stem, root and water extract also(TABLE 1).

Flavonoid is a major group found to be of common occurance in plants. It gives very bright fluorescing bands of yellow or orange colour after derivatisation with NP/PEG reagent as compared with extract of *Arnica* sp. Presence of flavonoids was observed in methanolic and water extracts of all parts viz., leaf, stem and root ex-

Full Paper

TABLE 1 : Detection of secondary metabolites in different parts of *H.suaveolens*

S. No.	Compound	Mobile phase used	Rf values in different parts			
			Lvs.	St.	Rt.	Ar.
1.	Alkaloid	MeOH: Con.NH_4OH (200:3)	0.74	-	-	-
2	Anthracene derivatives	EtOAc: MeOH: H_2O (100:13.5:10)	0.27,0.58	0.21, 0.61,0.66	-	0.27, 0.58
3	Cardiac glycosides	EtOAc: MeOH:H_2O (81:1:8)	0.81($SbCl_3$)	-	-	0.81 ($SbCl_3$)
4	Coumarins	EtOAc: Formic acid: G-AcOH: H_2O (100:11:11:26)	0.1, 0.25, 0.61	0.25, 0.6	0.6	0.1, 0.25, 0.6
5	Flavonoids	EtOAc: Formic Acid: G-AcOH:H_2O (100:11:11:26)	0.37, 0.92, 1	0.72, 0.98	0.96, 1	0.37, 0.72, 0.95, 1
6	Lignan	$CHCl_3$: MeOH (97: 3)	0.22, 0.37	-	-	0.22, 0.37
7	Phenolic acids	EtOAc: Formic Acid: G-AcOH: H_2O (100:11:11:26)	0.9, 0.92	0.5, 0.62, 0.93	0.95	0.9, 0.95
8	Essential oils	PhMe: EtOAc (93:7)	0.43, 0.48, 0.73, 0.99	-	-	0.43, 0.48, 0.73, 0.99
9	Phytosterols	PhMe: EtOAc (93:7)	0.45	0.4	-	×

Note-'Lvs.'- leaves extract, 'St.'- stem extract, 'Rt'- root extract, 'A.'- aerial parts extract

tracts. Mobile phase containing EtoAC: FA: GA: H_2O (100:11:11:26) gave good separation as well as resolution of flavanoids. There are variations in the amount and number of bands in each sample. The leaf and stem samples of *H.suaveolens* showed major bands of flavonone with intense yellow green fluorescence after reacting with NP/PEG reagent.

For separation of lignans four mobile phases were tried(TABLE 2). In the mobile phase containing $CHCl_3$: MeOH(97: 3) the petroleum ether and n-hexane extract of leaf sample show it's presence with separation of two bands of pink colour(Rf. 0.22-0.37) in visible range of spectrum after reacting with 50% H_2SO_4 at 110^0C(TABLE 1) as compared with extract of *Podophyllum peltatum*.

Phenolic acids were found to be present in almost all samples In mobile phase consisting of EtOAc: Ferulic acid: G-AcOH: H_2O(100:11:11:26) good separation as well as resolution was obtained for both methanolic as well as water extracts. One-three bands were separated in each sample as bright blue-bluish white bands between Rf 0.5-0.95(TABLE 1). For separation of cardiac glycosides mobile phase consisting of EtOAc: MeOH: H_2O(81:11:8) was used. All the extracts show its absence.

Essential oil is a general term given to the aromatic mono-(C_{10}), sesqui-(C_{15}) di-(C_{20}) terpenes and all of them can be detected by their aroma and blue, gray, green, pink, red or brown coloured bands after AS derivatisation. *H.suaveolens* leaf sample was found to be rich in essential oil, in rest of the samples it was found to be absent. In the mobile phase consisting of toluene: ethyl acetate (93:7) the oil from leaf sample gave good separation with 4 prominent bands between the range Rf- 0.43-0.99(TABLE 1). Phytosterols or plant steroids are tetracyclic triterpenes and give peculiar light pink bands after spraying the plates with LB reagent followed by heating. The plates were developed in mobile phase consisting of toluene: ethyl acetate(93:7). Single band was seen in leaf and stem extract of at Rf 0.4, 0.45(TABLE 1). Saponin and sapogenins were found to be present in negligible amounts.

From TLC screening the major compounds were selected and their presence was confirmed by using standard marker compounds for each group. For HPTLC screening two separate mobile phases were developed for two groups one consists of rutin, chlorogenic acid and ferulic acid (RCF); second consists of emodin and scopoletin (ES), which are explained below.

- Ethyl acetate: formic acid: glacial acetic acid: water (10:1:1:2.6) for RCF.
- Toluene: ethyl acetate: methanol: formic acid: glacial acetic acid (10:1.5:1:0.2:0.1) for ES.

In *H.suaveolens*, the methanolic extract of leaf shows presence of flavonoid glycosides (flv. gly.) in low concentrations as very faint orange and yellow-green zones (Rf-0.26 and 0.90) were observed (Figure 2). In methanolic extract of stem of *H.suaveolens*, flv. gly. were found in low concentration as orange fluorescent zone (Rf 0.64-0.74) (Figure 3). Blue fluorescent zone of ferulic acid (Rf-0.95) was found in root extract (Figure 4). The aqueous extract of aerial parts shows very faint blue fluorescent PCA zone(Rf-0.91-0.99) but in very low concentrations (Figure 5). For RCF detection

Full Paper

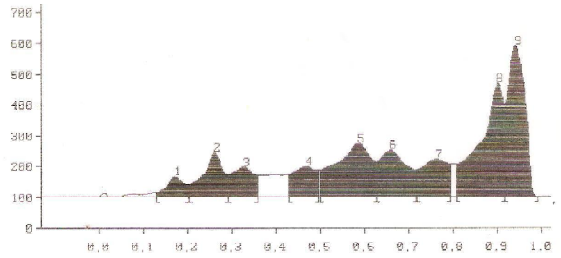

Figure 2 : Chromotogram of methanolic extract of leaf of *Hyptis suaveolens*(for rutin, chlorogenic acid and ferulic acid)

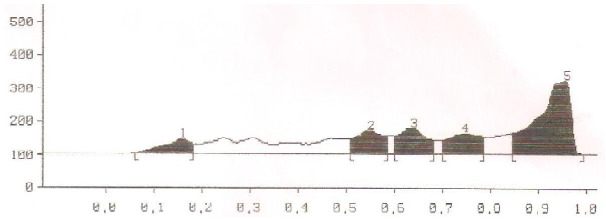

Figure 3 : Chromotogram of methanolic extract of stem of *Hyptis suaveolens*(for rutin, chlorogenic acid and ferulic acid)

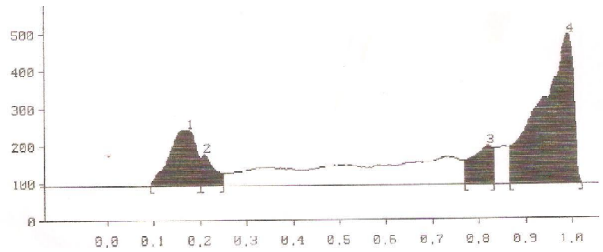

Figure 4 : Chromotogram of methanolic extract of root of *Hyptis suaveolens*(for rutin, chlorogenic acid and ferulic acid)

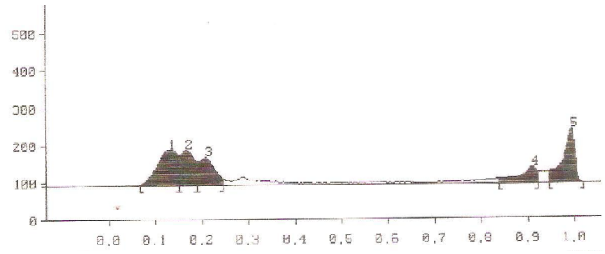

Figure 5 : Chromotogram of aqueous extract of aerial parts of *Hyptis suaveolens*(for rutin, chlorogenic acid and ferulic acid)

Natural products-polythene glycol reagent(NP/PEG) was used.

Leaf sample shows presence of scopoletin(Rf ~0.32) in low concentration as faint blue fluorescent

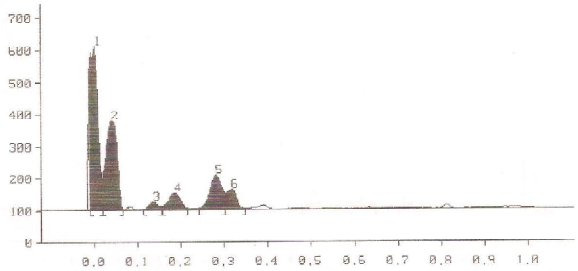

Figure 6 : Chromotogram of methanolic extract of leaf of *Hyptis suaveolens*(for emodin and scopoletin)

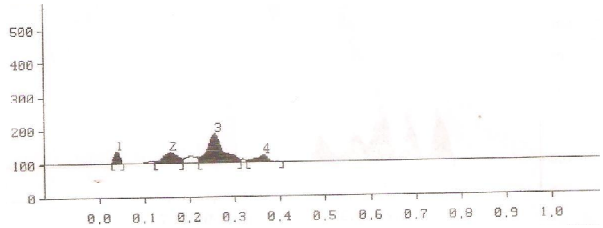

Figure 7 : Chromotogram of methanolic extract of stem of *Hyptis suaveolens*(for emodin and scopoletin)

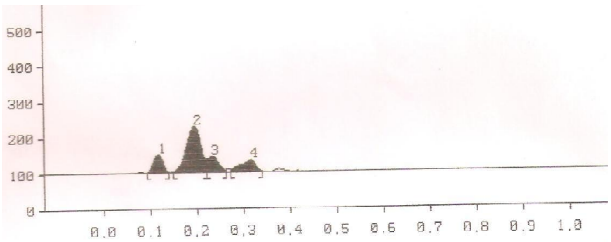

Figure 8 : Chromotogram of methanolic extract of root of *Hyptis suaveolens*(for emodin and scopoletin)

zone(Figure 6). Methanolic extract of stem and root also show its presence in low concentration(Figure7 and 8). No separation/constituents obtained in aqueous extract. Potassium hydroxide reagent(KOH) was used for detection of ES.

DISCUSSION

Herbal medicines have been used for thousands of years in many parts of the world. Still there are plenty of opportunities for future developments including specific morphological plant products.

The genus showed very poor alkaloidal content. Anthraquinones known to have antimicrobial activity[13,14]. Emodins are used as cathartics[15]. They were found to be present in the methanolic extract of leaf as 2-4 bands (Rf 0.23-0.58) and stem samples 2-3 bands

Full Paper

(Rf 0.21-0.66)(TABLE 1) as well as water extract. However, there are no reports found on the presence of anthracene derivatives in this genus. Coumarins reputed to have anticoagulation, estrogenic, vasodialation, antibacterial and anti-helminthic properties[16]. The scopoletin was detected in low concentration in methanolic extract of leaf. A simple and accurate reversed phase HPLC procedure proposed for the determination of 19 phenolic compounds including flavonoids, phenolic acids and coumarins in seven medicinal species including *Lavandula officinalis*, *Mentha piperita* and *Salvia officinalis*[17].

Flavonoids have antiviral, anti-inflammatory and cytotoxic activities and used in the treatment of capillary fragility, retinal haemorrhage, hypertension, diabetic retinopathy, rheumatic fever, and arthritis and as antioxidants[18]. In methanolic extract of stem flv. gly. were found in low concentration as orange fluorescent zone (Rf 0.64-0.74).

In the TLC screening, one band observed in *Hyptis suaveolens* corresponded with that observed in *Podophyllum* extract and it was identified as of podophyl lotoxins on the basis of Rf value. Total synthesis of podophyllotoxin is an expensive process and availability of the compound from natural resources is an important issue for pharmaceutical companies that manufacture these drugs[2].

Podophyllotoxin is a natural lignan that is currently being used as a precursor to semi-synthetic anticancer drugs etoposide, teniposide and etopophos. These compounds have been used for the treatment of lung and testicular cancer as well as certain leukemias[19,20]. Podophyllotoxin preparations are also in the market for dermatological use to treat genital warts and recently immune-stimulatory activities of podophyllotoxin have been reported[21,22]. Recently, the presence of lignans particularly podophyllotoxin was reported in some members of lamiaceae[7,8,9].

Two bioactive caffeic acid esters, nepetoidins A and B in subfamily Nepetoideae of Lamiaceae found to be chemotaxonomically significant[23]. The main components of *Orthosiphon stamineus*(Lamiaceae) are the pharmacologically active polyphenols: the poly methoxylated flavonoids and the caffeic acid derivatives in leaves extracts[24]. During present studies also, the flavonoids and the phenol carboxylic acids were found to be present.

Secondary metabolites contribute to the aroma and flavour of many plants, which are highly valued by human. Hence extensive studies have been made on the chemistry of such plants. Essential oil was found to be present in the leaves. Members of Lamiaceae also have been studied by large number of workers and they reported numbers of components of essential oils with detailed account of GC-MS and NMR spectroscopy analysis. In the present investigation also essential oil is extracted and using TLC method preliminary analysis was done. It revealed presence 7 bands in *H. suaveolens*. The chemical polymorphism of essential oil of *H. suaveolens* from El Salvador was analyzed by GC-MS[25]. The principal component analysis of concentration data from **(32)** compounds showed differences of the chemical composition according to the geographic origin. In Southern area a fenchone-fenchol-chemotype was predominant, whereas in the Northern regions the plants mostly accumulated 1,8-cineole. *H.suaveolens* was studied for oil content during their project on new industrial crops in Northwestern Argentina and found 77-80% linoleic acid in the seeds[26].

Steroids and triterpenoids are known to possess anti-inflammatory, lipolytic and anticholesterenic activities[7]. Single pink coloured band was seen in leaf and stem extract at Rf 0.4 and 0.45 corresponding with that of stigmasterol. A chlorinated monoterpene ketone, acylated beta-sitosterol glycosides and a flavanone glycoside were reported from *Mentha longifolia*[28]. Presence of earlier known compounds sitosterol and ∝-amyrin was confirmed[29].

A strategy for a rapid selection of a set from eleven TLC systems for the separation of flavonoids and phenolic acids identified in the methanolic extract of *Lavandula flos* was discussed earlier[30]. It has been shown that the most favourable TLC systems for the separation of investigated compounds are ethyl acetate-formic acid-acetic acid-water(100:11:11:27v/v) and ethyl acetate- formic acid-water(8:1:1v/v). Flavonoids resolved in mobile phase containing ethyl acetate-formic acid-acetic acid-water(100:11:11:27v/v)[11]. Similar results were observed in the present investigation. Better separation for flavonoid glycosides and phenolic acid was obtained in ethyl acetate-formic acid-acetic acid-water(100:11:11:27v/v). Ethyl acetate-formic acid-

acetic acid-water(100:11:11:26) used as mobile for HPTLC determination of flavonoids and phenolic acids in seven croatian *Stachys* taxa[31].

Similarly another group consist of emodin and scopoletin shows good separation in mobile phase containing toluene: ethyl acetate: methanol: formic acid: glacial acetic acid(10:1.5:1:0.2:0.1). The results obtained in present investigation revealed great potential for production of medicinally important secondary metabolites. There remain a need for separation and identification of specific lignan particularly podophyllotoxin.

Abbreviations used *H.suaveolens*

Hyptis suaveolens, AR-Analyitical grade, NP/ PEG-Natural Product/ PolyEthylene Glycol, AS-Anisaldehyde sulphuric acid, LB-Libermann Burchard.

ACKNOWLEDGMENT

We are thankfull to our collogue Dr.K.Kogje for her help during the work and Dr.Tajne, Dept. of Pharmaceutical Chemistry, University Campus, R.T.M. Nagpur University for facilitating HPTLC analysis.

REFERENCES

[1] W.D.MacRae, G.H.Neil Towers; Phytochemistry, **23(6)**, 1207-1220 **(1984)**.
[2] C.Canel, R.M.Moraes, C.Burandt; Phytochemistry, **54**, 115-120 **(2000)**.
[3] K.R.Kirthikar, B.D.Basu; Indian Medicinal Plants, Lalit Mohan Basu Publishers, Allahabad **(1984)**.
[4] R.Shenoy, A.Shirwaikar; Indian Drugs, **39(11)**, 574-577 **(2002)**.
[5] L.V.Asolkar, K.K.Kakkar, O.J.Chakre; 'Second Supplement to Glossary of Indian Plants with Active Principles', Part I, Publication and Information Decorates(CSIR), New Delhi, India, **(1992)**.
[6] I.T.Matsuse, Y.A.Lim, M.Hattori, M.Correa, M.P. Gupta; Journal of Ethnopharmacology, **64(1)**, 15-22 **(1999)**.
[7] M.Kuhnt, H.Rimpler, M.Henrich; Phytochemistry, **36(2)**, 485-489 **(1994)**.
[8] I.Saracoglu, M.Varel, I.Calis; Turk J.Chem., **27**, 739-747 **(2003)**.
[9] E.Bedir, I.Khan, R.M.Moraes; 'Bioprospecting for Podophyllotoxins.Reprinted from: Trends in new crops and new uses', J.Janick, A.Whipkey (Eds.); ASHS Press, Alexandria, VA, **(2002)**.
[10] J.B.Harborne; Champman and Hall, London, **(1998)**.
[11] H.Wagner, Bladt; 'Plant Drug Analysis', A Thin Layer Chromatography Atlas, 2nd Eds.Springer-Verlag Publication, Berlin, Germany, **(1996)**.
[12] E.Stahl (Eds.); 'Thin layer Chromatogrphy: A laboratory Hand Book', Springer-Verlag Publication, Berlin, **(1969)**.
[13] J.L.Rios, M.C.Recio, A.Villar; Journal of Ethno pharmacology, **21**, 134-152 **(1987)**.
[14] R.Diaz, J.Que Vedo-Sarmeinto, A.Ramas-Carmeuzava, P.Cabo, J.Cabo; Fitoterapia, **59(4)**, 329-333 **(1988)**.
[15] S.C.Chhabra, F.C.Usio, E.N.Mshiu; Indian Journal of Ethnopharmacology, **11**, 157-179 **(1984)**.
[16] M.H.Reddy; Journal Economic and Taxonomic Botany, Additional Series, **12**, 37-39 **(1996)**.
[17] P.B.Andrade, R.M.Seabra, P.Valentao, F.Areias; Journal of Liquid Chromatography and Related Technologies, **21(8)**, 2813-2820 **(1998)**.
[18] V.D.Tripathi, R.P.Rastogi; J.Sci.Ind.Res., **40**, 116-124 **(1981)**.
[19] H.F.Stahelin, A.V.Wartburg; Cancer Res., **51**, 5-15 **(1991)**.
[20] F.Imbert; Biochimie., **80**, 207-222 **(1998)**.
[21] K.R.Beutner; 'Podophyllotoxins in the treatments of genital warts', In: E.P.Eischmann (Ed.); 'Sexually Transmitted Diseases: Advances and Treatment', **24**, 112-232, **(1996)**.
[22] N.Pugh, I.Khan, R.M.Moraes, D.Pasco; Immuno -pharmacology-Immuno-toxicology, **23**, 83-95 **(2001)**.
[23] R.J.Grayer, M.R.Eckert, N.C.Veitch, G.C.Kite, P.D. Marin, T.Kokubun, M.S.J.Simmonds, A.J.Paton; Phytochemistry, **64(2)**, 519-528 **(2003)**.
[24] N.K.Olah, L.Radu, C.Mogosa, D.Hanganu, S. Gocan; Journal of Pharmaceutical and Biomedical Analysis, **33(1)**, 117-123 **(2003)**.
[25] P.Grassi, M.J.Nunez, K.Var muza, C.Franz; Flavour and Fragrance Journal, **20(2)**, 131-135, **(2005)**.