April 2008 Volume 4 Issue 1

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 4(1), 2008 [26-41]

Medicinally important secondary metabolites detected in weed plants of lamiaceae

Amruta Jain-Indane*, Alka Chaturvedi

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

Tel : + 91- 9223502879

E-mail : aaj_23580@rediffmail.com

Received: 15th March, 2008 ; Accepted: 30th March, 2008

ABSTRACT

Four weed plants were screened for medicinally important secondary metabolites. 70% methanol extract and water decoction dissolves maximum components i.e. moderately polar glycosidic components. Presence of coumarins, Flavonoids, Lignan, Phenolic acids, Essential oils, triterpenes was observed almost in all samples. But there are variations in the amount and number of bands in each sample. For their preliminary detection and optimization of separation conditions, TLC method was used and further confirmation was done using HPTLC techniques.

KEYWORDS

70% methanol extract;
Secondary metabolites;
TLC;
HPTLC methods;
Water decoction;
Weed plants.

INTRODUCTION

In the entire plant kingdom, family Lamiceae is having a place of significance due to its aromatic and medicinal properties. Since from long time members of Lamiaceae are well known for their medicinal uses as discussed earlier. It still possesses various genera, which are not worked out, especially weeds, plants of wasteland, which may be used as alternative and economically cheap source for some commercially as well as medicinally important phytochemical constituents. Considering the medicinal and commercial importance of different members of Lamiaceae and its dominance in the Vidarbha region, the four plants of this family were selected depending on their availability and medicinal uses mentioned in literature[1,2] such as:-

- *Anisomeles indica* (Linn.) Ktze., plant used as carminative, astringent, tonic. The oil obtained from it is used in uterine affections. On the other hand HIV- inhibitory diterpenoids were isolated from *Anisomeles indica*[3].
- *Lavandula bipinnata* O.Ktze., said to be used in medicine and as antidote to snake poison. The powdered leaves are given for inhalation to the person who has been bitten by a serpent in order to prevent him from falling into sleep. In the book "Dravyaguna Vijnāna" mentioned various uses of *Lavandula stoechas* Linn., synonym of *Lavandula bipinnata*[4].
- *Leucas cephalotes* Spreng., used as stimulant, diaphoretic, insecticide; fresh juice externally applied in scabies, flowers in the form of a syrup used as remedy for cough and colds. The leaves, in combination with other drugs, are prescribed for scorpion-sting (Vagbhata); but are not antidote to scorpion venom.
- *Leucas cephalotes* mentioned as synonym of *Leucas aspera* Spreng., its therapeutic uses as antipyretic and insecticide. It is a reputed home remedy for worms, fever and intestinal catarrh in children. It is antipyretic, antiseptic, carminative, febrifuge, and wormifuge, antihistaminic. It is used

in anorexia, cough, dyspepsia, fever, jaundice, psoriasis, scabies and chronic skin eruptions. An alcoholic extract of leaves showed antibacterial activity against *Micrococcus pyogenes* and *Escherichia coli*[5].

Also, in "Dravyaguna Vijnāna" mentioned various uses of *Leucas cephalotes*[4].

- *Nepeta hindostana* (Roth) Haines, largely used in fevers and as cardiac tonic, internally taken in gonorrhoea. Decoction used as gargle in sore throat.

Hence, considering the today's increasing demands for biologically active constituents or natural products, the present investigation is proposed.

EXPERIMENTAL

All the plants/herbs selected for the present study was collected from their wild habitats viz; *Anisomeles indica*, and *Leucas cephalotes* were collected from different location in the Nagpur, *Lavandula bipinnata* from Mukttagiri (M.P.) and *Nepeta hindostana* from Chikhaldhara (M.S.) during Aug.- Nov.2002 and 2003. A voucher specimen for each plant was deposited at the Herbarium, Department of Botany, Nagpur University, Nagpur (Plate- 1 to 4).

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. 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 % MeOH, 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.

Plate 1: *Anisomeles indica* (a) inflorescence and (b) voucher specimen of herbarium sheet

Plate 2: *Lavandula bipinnata* (a) habit and (b) voucher specimen of herbarium sheet

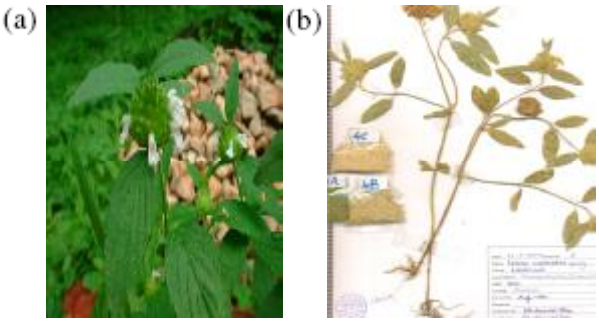

Plate 3 : *Leucas cephalotes* (a) inflorescence and (b) voucher specimen of herbarium sheet

Plate 4 : *Nepeta hindostana* (a) habit and (b) voucher specimen of herbarium sheet

Water extracts of the plants were prepared following the methods used by the herbal healers (as per rec-

Full Paper

ommendations 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 and filtered. The extract was reduced by evaporation.

Special extraction procedure (mainly for)

a. Alkaloids- Powdered plant material extracted with 10% AcOH in EtOH, leaving to stand for at least 4h. 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[6].
b. Cardiac glycosides-2g powdered drug extracted by heating for 15min under reflux with 30ml 50% EtOH, with the addition of 10ml 10% lead- (II)-acetate solution. After cooling and filtration, the solution is extracted by shaking three times with 15 ml quantities of dichloromethane/isopropanol (3:2) and used for chromatography[7].
c. Essential Oils- The assay of essential oils in crude drugs, spices and oleoresins involve water distillation of the comminuted material and measurement of the volume of the separated oil, for this an apparatus devised by Clevenger and adopted by the Pharmacopoeia of United States (USP) was used. Procedure described earlier was used for collection of essential oil in the glass vial[8].

Percentage of the essential was calculated by using following formula- Percentage of the essential Oil = v/w × 100 in the sample

where, v is the volume of separated oil, w is the weight of the plant material used for determination.

Sapogenins and phytosterols

Powdered plant material hydrolyzed with 2 M HCl for 2-6 h., 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.

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 etc[6,7,9,].

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

All the four plants showed poor alkaloidal content. A single orange band after Dragendorff's spraying was observed in the leaf extracts of four plants along with stem extract of *L.cephalotes* at the Rf 0.74 (TABLE 1) as compared with the extract of *Rauvolfia* sp. The leaf extract of *A.indica*, *L.bipinnata* and *N.hindostana* indicated presence of alkaloid in chloroform extract as single orange band. The stem and root extracts of rest of the plants reacted negatively with Dragendorff reagent (TABLE 1).

Anthracene derivatives (anthraquinone and anthranol) were detected by their orange red (anthraquinone) and bright yellow (anthranols) band after 10% KOH spray. For separation of anthracene derivatives, the different mobile phases were tried, and anthracene derivatives were well separated with reproducibility of results in mobile phase consisting of EtOAc:MeOH:H_2O (100:13.5:10) (TABLE 1).

They were found to be present in the methanolic and water extracts of leaf samples of four plants (TABLE 1). Further their presence was detected in the extracts of stem samples of *A.indica* and *L.cephalotes* only. They were found to be absent in extracts of stem samples of *L.bipinnata* and *N.hndostana* as well as in root samples of all the plants. All leaf samples show separation of 2-4 bands (Rf 0.23-0.97) of anthracene derivatives (TABLE 1). In the stem samples of *A.indica,* and *L.cephalotes* 2-3 bands (Rf 0.21-0.66) were separated. The four major red-orange bands (anthraquinone)

Full Paper

TABLE 1:Compounds found to be present in different parts of the selected plants

S. no.	Comp.	Mobile phase used	Rf Values							
			A. indica				*L. bipinnata*			
			Lvs.	St.	Lvs.	St.	Lvs.	St.	Lvs.	St.
1.	Alkaloid	MeOH:Con.NH_4OH (200:3)	0.78	-	-	×	0.74	-	-	×
2	Anthracene derivatives	EtOAc:MeOH:H_2O (100:13.5:10)	0.23,0.5, 0.73	0.51	-	0.5, 0.73	0.66	-	-	0.66
3	Cardiac glycosides	EtOAc:MeOH:H_2O (81:11:8)	0.95 (kedde's)	-	-	-	0.59, 0.82 ($SbCl_3$) 0.65 (kedde's)	-	-	0.65 (kedd)
4	Coumarins	EtOAc:Formic acid:G-AcOH:H_2O (100:11:11:26)	0.1, 0.25, 0.62	0.25, 0.6	-	0.1, 0.25, 0.62	0.25, 0.61, 0.72	0.25, 0.61	0.25, 0.61	0.61, 0.72
5	Flavonoids	EtOAc:Formic acid:G-AcOH:H_2O (100:11:11:26)	0.33, 0.4, 0.79, 0.86, 1	0.71	0.58, 0.83, 0.96, 1	-	0.65, 0.87, 1	0.96	-	
6	Lignan	$CHCl_3$:MeOH (97:3)	0.38, 0.67, 0.74, 0.8, 0.86	-	-	×	0.82	-	-	×
7	Phenolic acids	EtoAC:Formic acid: G-AcOH:H_2O (100:11:11:26)	0.9, 0.95	0.72, 0.89, 0.9	0.75, 0.92	0.4, 0.72, 0.89,0.95	0.57, 0.83, 0.9	0.48, 0.92	0.9, 0.95	0.9, 0.98
8	Essential oils	PhMe:EtOAc (93:7)	0.45, 0.5, 0.53, 0.58, 0.64, 0.71, 0.77, 0.98	-	-	0.45,0.53, 0.64,0.71, 0.77, 0.98	0.4, 0.5, 0.58, 0.6, 0.71, 0.9	-	-	0.4, 0.5, 0.6, 0.71, 0.9
9	Phytosterols	PhMe:EtOAc (93:7)	0.45	-	-	×	0.5	-	-	×
10	Saponin and Sapogenin	$CHCl_3$:G-AcOH: MeOH:H_2O(60:32:12:8)	0.66	-	-	×	0.24, 0.4, 0.58, 0.76	-	-	×
S. no.	Comp.	Mobile phase used	*L. cephalotes*				*N. hindostana*			
			Lvs.	St.	Lvs.	St.	Lvs.	St.	Lvs.	St.
1.	Alkaloid	MeOH:Con.NH_4OH (200:3)	0.74	0.74	-	×	-	-	-	×
2	Anthracene derivatives	EtOAc:MeOH:H_2O (100:13.5:10)	0.39, 0.66, 0.91, 0.97	0.52	-	0.39, 0.66, 0.91	0.58, 0.66	-	-	0.58, 0.66
3	Cardiac glycosides	EtOAc:MeOH:H_2O (81:11:8)	0.14, 0.8 ($SbCl_3$)	-	-	×	-	-	-	×
4	Coumarins	EtOAc:Formic acid:G-AcOH:H_2O (100:11:11:26)	0.1, 0.24, 0.61, 0.73, 0.79	-	-	0.24, 0.6, 0.73, 0.79	0.3, 0.45	-	-	0.45
5	Flavonoids	EtOAc:Formic acid:G-AcOH:H_2O (100:11:11:26)	0.21,0.33,0.42, 0.5, 0.83, 0.89, 0.96	0.72	-		0.38, 0.72	0.72	-	
6	Lignan	$CHCl_3$:MeOH (97:3)	0.6, 0.73	-	-	×	-	-	-	×
7	Phenolic acids	EtoAC:Formic acid: G-AcOH:H_2O (100:11:11:26)	0.72, 0.79, 0.93	0.5, 0.72, 0.89	0.89, 0.95	0.9, 0.98	0.89, 0.92	0.6, 0.9	0.4,0.62, 0.95	0.6, 0.98
8	Essential oils	PhMe:EtOAc (93:7)	0.28, 0.42,0.48, 0.61, 0.66,0.72, 0.98	-	-	0.42,0.48, 0.61, 0.72, 0.98	0.4, 0.48, 0.6	-	-	0.4, 0.48, 0.6
9	Phytosterols	PhMe:EtOAc (93:7)	0.45	0.4	-	×	0.5	-	-	×
10	Saponin and Sapogenin	$CHCl_3$:G-AcOH: MeOH:H_2O(60:32:12:8)	0.24, 0.41, 0.48, 0.6	-	-	×	0.48, 0.5	-	-	×

note- 'Lvs'- leaves extract, 'St'- stem extract, 'Rt'- root extract, 'Arl'- aerial parts extract, '-'- absent , '×'- not tried, SbCl3 and kedde's- spray reagents

Full Paper

seen in leaf samples of *Leucas cephalotes* after derivatization with 10%KOH, indicates that major quantity was present in this sample. Presence of anthraquinone was identified and observed at Rf 0.66 on the basis of colour reaction and Rf value of standard (emodin) mentioned in literature as well as compared with *Aloe* extract.

Coumarins generally give intense blue or blue green, yellow colours in UV 365nm after KOH treatment. Bright blue bands were observed in the extracts of leaf, stem and root samples indicating the presence of coumarins in comparison with the extract of *Melilotus officinalis*. Their absence was observed in roots of *A.*indica, *L.cephalotes*, *N.hindostana* and stem extracts of *L.cephalotes* and *N.hindostana* (TABLE 1).

The amount varies from part to part but its major amount was found in leaf samples. Two to five bands were observed with blue fluorescence in UV 365nm in leaf samples. For separating coumarins two mobile phases viz., toluene:ethyl acetate (93:7) and ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26) were used. Both the systems exhibited good separation. The coumarin aglycones were separated in non-polar PhMe:EtOAc (93:7) whereas glycosides resolved in polar phase consisting of ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26). The numbers of bands separated in each sample (Rf 0.1-0.79) are tabulated in TABLE 1.

Flavonoid is a large group consisting of flavanone, flavone and flavanol. All of them give very bright fluorescing bands of yellow or orange colour after derivatisation with NP/PEG reagent as compared with extract of *Arnica* sp. It was observed as major compound in all the four plants. Presence of flavonoids was observed in methanolic and water extracts of all parts viz., leaf, stem and root extracts of all the plants, except in the root extracts of *L.bipinnata*, *L.cephalotes* and *N.hindostana*. For separation of these different flavanoid components eight mobile phases were tried (TABLE 1). Among all these, mobile phase containing EtOAc:Formic acid:Glacial acetic acid:H_2O (100:11:11:26) gave good separation as well as resolution for all samples.

There are variations in the amount and number of bands in each sample. But methanolic and water extracts of leaf samples from *A. indica* and *L.cephalotes* showed five and seven bands respectively (Rf- 0.21-1.0) (TABLE 1). The root samples of *L.bipinnata*, *L.cephalotes* and *N. hindostana* reacted negatively. Leaf samples of *Anisomeles indica* showed prominent band and in leaf sample of *Leucas cephalotes* two major bands observed in visible range of light and seven bands in UV- 365 nm which are identified on the basis of fluorescence after derivatization with NP/PEG.

In the mobile phase containing $CHCl_3$:MeOH (97:3) the petroleum ether and n-hexane extract of leaf samples of *A.indica*, *L.bipinnata* and *L.cephalotes* show presence of lignan with separation of two-five bands of pink colour (Rf 0.22-0.86) 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:Formic acid:Glacial acetic acid:H_2O (100:11:11:26) good separation of phenolic acid 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 Rf0.4-0.98 (TABLE 1).

Cardiac glycosides are water-soluble compounds. In the mobile phase consisting of EtOAc:MeOH:H_2O (81:11:8) better separation was obtained for it. The plates are derivatised using two reagents viz., $SbCl_3$ in $CHCl_3$ and Kedde spray reagent, presence of pink bands in visible range of spectrum indicates presence of cardiac glycosides in comparison with extract of *Digitalis* sp.

Using $SbCl_3$ in $CHCl_3$ reagent for detection, their presence was found in leaf samples of *L.bipinnata* and *L.cephalotes* (TABLE 1). In, this samples one band gave colour reaction and Rf value corresponding to the bufadienolid aglycone scillirosidin (at Rf~0.8) when compared with the extract of *Scillae* bulbus. Whereas, using Kedde reagent for detection indicating presence of cardenolides, only two leaf samples of *Anisomeles indica* and *Lavandula bipinnata* shows single band at Rf-0.95 and 0.65 respectively. All the extracts of stem and roots showed its absence (TABLE 1).

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. All the four members showed presence

Full Paper

of essential oil with particular aroma. *L.bipinnata* was found to be rich in essential oils as compared to others (TABLE 1). All leaf samples showed its presence. Whereas stem samples of *L.bipinnata* and *N.hindostana* shows poor essential oil content was poor and in rest of the samples it was found to be absent. In the mobile phase consisting of PhMe:EtOAc (93:7) the oil from leaf samples gave good separation with 3-8 prominent bands between the range Rf- 0.28-0.98 (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. For detection of steroids, hydrolysed chloroform extract was used and developed in mobile phase consisting of PhMe:EtOAc (93:7). Single band was seen in leaf extract of each plant at Rf 0.45 (TABLE 1). It was found to be present also in extracts of stem samples of *L.cephalotes*. However it was not observed in remaining stem samples and all the root samples (TABLE 1).

Saponins are water-soluble secondary metabolites, which are simply detected by froth formation during extraction. Their presence was further confirmed by developing the plate in $CHCl_3$:Glacial acetic acid:MeOH:H_2O (60:32:12:8) followed by spraying it with AS reagent. Distinct blue or gray bands of saponins were observed in all leaf samples (Rf- 0.24-0.76) same as those in extract of Ginseng root, except poor content in *N.hindostana*. They were absent in stem and root samples of all the plants (TABLE 1). The heavy and consistent foam formation during extraction was observed in leaf samples of *L.cephalotes*.

Sapogenin is the aglycone part of saponin, which can be observed in non-polar systems. Four different mobile phases were tried for separation of sapogenins. Good separation for hydrolysed extracts of all leaf samples was obtained in mobile phase containing hexane:acetone (4:1). The detection is in concurrence with the earlier saponin detection. In stem and root samples of all plants its absence was observed. One-four bands of sapogenins were separated.

Hptlc screening

From TLC screening the major compounds were selected as listed below for further confirmation using standard marker compounds for each group and HPTLC (fingerprint) methods.

Anthracene	⇒ Emodin
Coumarin	⇒ Scopoletin
Flavanols(fl.gly)	⇒ Quercetin-rutin
Phenol carboxylic acids (PCA)	⇒ Chlorogenic acid and Ferulic acid

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.

1. HPTLC analysis of samples for RCF

Mobile phase : Ethyl acetate:formic acid:glacial acetic acid:water (10:1:1:2.6).

Reagent : Natural products-polythene glycol reagent (NP/PEG)

Results : Each extract showed a characteristic fingerprint of varying colours corresponding to the yellow-orange and/or yellow-green flavonoid glycosides and blue fluorescent phenol carboxylic acids markers (Plate 5). Standard compounds viz., rutin, chlorogenic acid and ferulic acid were found as bright yellow-orange zone (Rf-0.45) of rutin and blue fluorescent zones of chlorogenic acid (Rf- 0.5) and ferulic acid (Rf-0.95).

In methanolic extract of leaf sample of *A.indica* (1) total nine peaks were detected at 365nm. One orange zone (Rf-0.68) and one yellowish green zone kaemferol derivative (Rf-0.62) were found. While three-four blue fluorescent zones of PCA were observed (Rf-0.2-0.97) (Figure 1).

In the methanolic extract of stem four orange fluorescent zone of quercetin derivatives (Rf- 0.41-0.72)

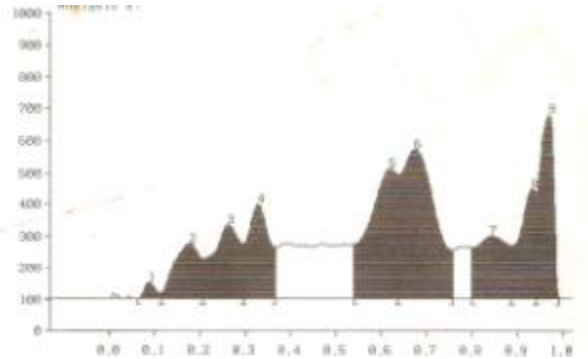

Figure 1: Chromatogram of methanolic extract of leaf of *Anisomeles indica (for Rutin, chorogeic acid and ferulic acid)*

Full Paper

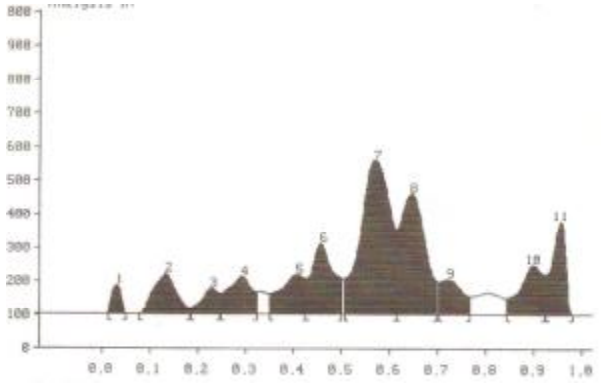

Figure 2: Chromatogram of methanolic extract of stem of *Anisomeles indica (for Rutin, chorogeic acid and ferulic acid)*

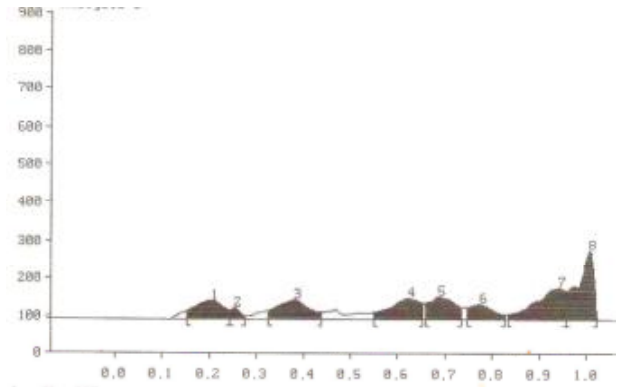

Figure 3: Chromatogram of methanolic extract of root of *Anisomeles indica (for Rutin, chorogeic acid and ferulic acid)*

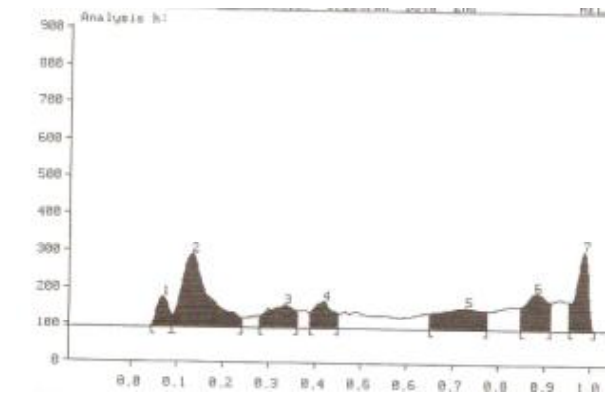

Figure 3: Chromatogram of methanolic extract of aerial parts of *Anisomeles indica (for Rutin, chorogeic acid and ferulic acid)*

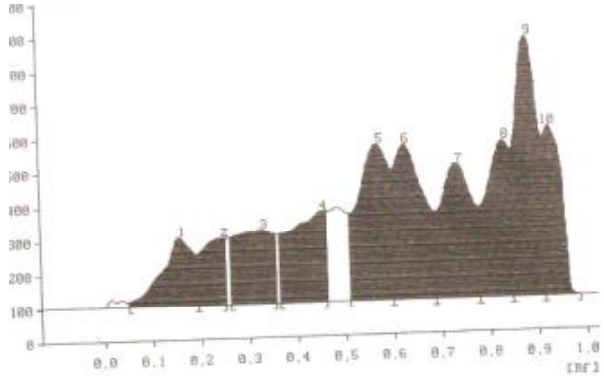

Figure 5 : Chromatogram of methanolic extract of leaf of *L.bipinnata (for Rutin, chorogeic acid and ferulic acid)*

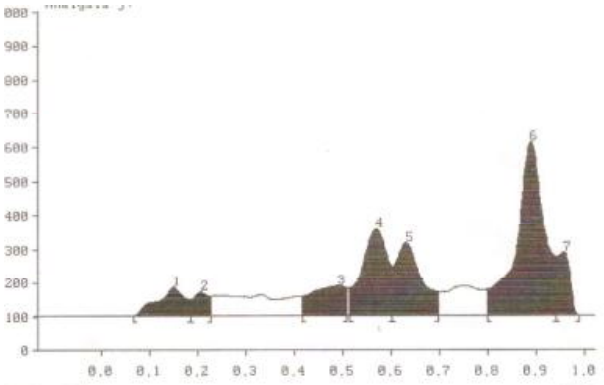

Figure 6 : Chromatogram of methanolic extract of stem of *L.bipinnata (for Rutin, chorogeic acid and ferulic acid)*

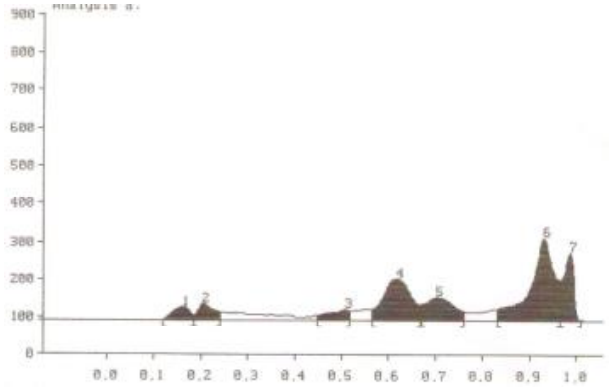

Figure 7: Chromatogram of methanolic extract of root of *L.bipinnata (for Rutin, chorogeic acid and ferulic acid)*

were found, among these rutin (Rf-0.46) in low concentration and one major zone of flv. gly.(Rf-0.57) was observed (Figure 2). The root sample showed blue fluorescent zone (Rf-0.94, 1.0) indicating presence of phenol carboxylic acid, which may be of caffeic acid or ferulic acid (Plate 6; Figure 3). While in aqueous extract of aerial parts, orange fly. gly. zone (Rf 0.6) was found. Blue fluorescent zone (Rf- 0.99) was found similar to that of ferulic acid (Plate 6; Figure 4).

In methanolic leaf extract of *L.bipinnata* (3) the flv. gly. found as orange-yellowish green zones (Rf-0.63-0.88) and blue fluorescent zone of ferulic acid (Rf-0.93)

Full Paper

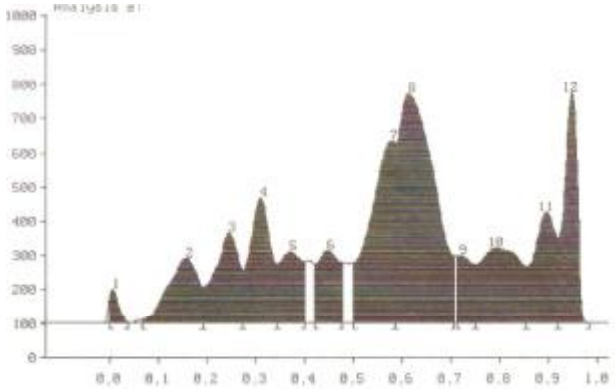

Figure 8: Chromatogram of methanolic extract of leaf of *L.cephalotes (for Rutin, chorogeic acid and ferulic acid)*

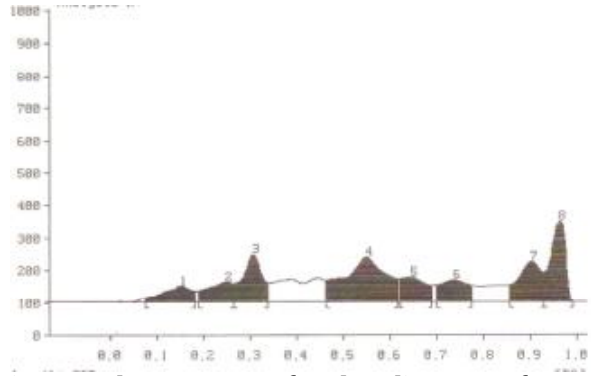

Figure 9: Chromatogram of methanolic extract of stem of *L.cephalotes (for Rutin, chorogeic acid and ferulic acid)*

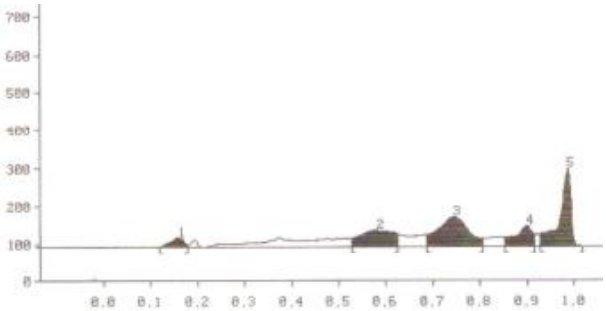

Figure 10: Chromatogram of methanolic extract of root of *L.cephalotes (for Rutin, chorogeic acid and ferulic acid)*

(Figure 5). The stem sample of *L.bipinnata* showed presence of yellowish green to orange colour flv. gly. zone (Rf 0.57-0.89) as quercetin and kaemferol derivatives (Plate 5; Figure 6).

The blue fluorescent PCA zones were found in root samples of *Lavandula bipinnata*. Blue fluorescent chlorogenic acid zone (Rf- 0.5) and whitish blue fluorescent zones (Rf- 0.93 and 0.99) were identified as that of caffeic acid and ferulic acid respectively. One

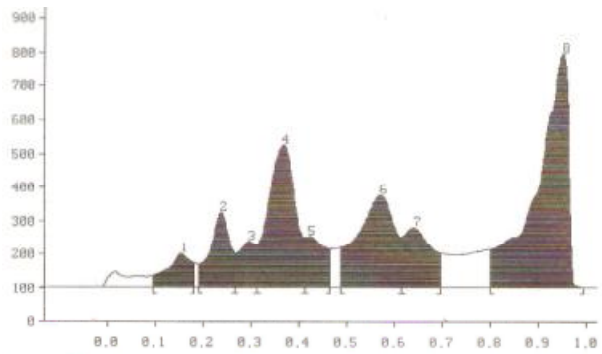

Figure 11: Chromatogram of methanolic extract of leaf of *Nepeta hindostana (for Rutin, chorogeic acid and ferulic acid)*

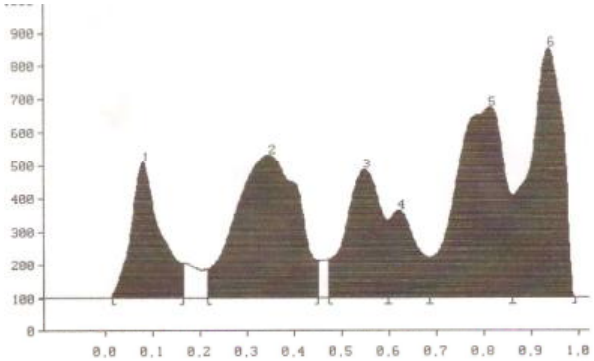

Figure 12 : Chromatogram of methanolic extract of stem of *Nepeta hindostana (for Rutin, chorogeic acid and ferulic acid)*

orange flv. gly. zone (Rf 0.56) was also found as depicted in the spectra of figure 7. In the aqueous extract very faint blue fluorescent zone was seen at the front, might be of PCA (Plate 6).

Leaf extract of *L.cephalotes* (4) showed presence of flv. gly. as orange-yellowish green zones (Rf-0.62-0.9) (Plate 5) as well as blue fluorescent ferulic acid zone (Rf-0.95) (Figure 8).

In the stem extracts presence of yellowish green fly. gly. zone (Rf-0.74) was found (Figure 9). Faint blue carboxylic acid zones (Rf 0.5-0.99) were observed in root extract (Figure 10). Blue fluorescent zone (Rf 0.3-0.36) accompanied by whitish blue fluorescent chlorogenic acid zone (Rf- 0.52) (Plate 6).

The fly. gly. as yellow green-orange zones (Rf-0.37-0.64) were found in methanolic leaf extract of *Nepeata hindostana* (5) along with blue fluorescent ferulic acid zone (Rf-0.95) (Figure 11). The stem extract showed presence of three yellowish green-orange flv. gly. zones(Rf- 0.08-0.55) (Figure 12). In root extract total

Full Paper

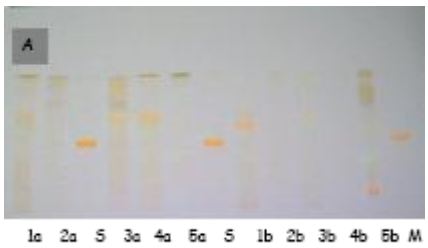

Plate 5 : HPTLC Fingerprint for RCF of leaf and stem extracts :- 1a-*A. indica* leaf (MeOH); 1b- *A.indica* stem (MeOH); 3a- *L.bipinnata* leaf (MeOH) ; 3b-*L.bipinnata* stem (MeOH); 4a-*L.cephalotes* leaf (MeOH); 4b-*L.cephalotes* stem (MeOH); 5a-*N.hindostana* leaf (MeOH); 5b-*N.hindostana* stem (MeOH);S-Standard marker compound (RCF)

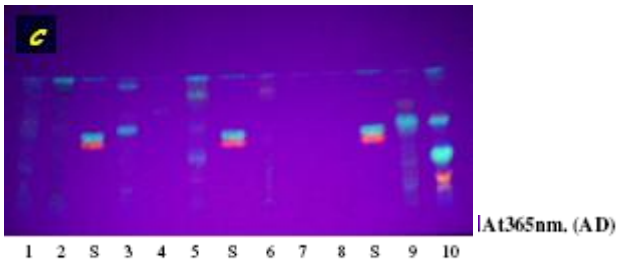

Plate 6 : HPTLC Fingerprint for RCF of root and aerial parts extracts: 1-*A.indica* root (MeOH) ; 3- *L.bipinnata* root (MeOH); 4-*L.cephalotes* root (MeOH) ; 5-*N.hindostana* root (MeOH); S Spike(RCF); 6- *A.indica* aerial parts (H_2O); 8-*L. bipinnata* aerial parts (H_2O); 9-*L. cephalotes* aerial parts (H_2O) 10- *N.hindostana* aerial parts (H_2O)

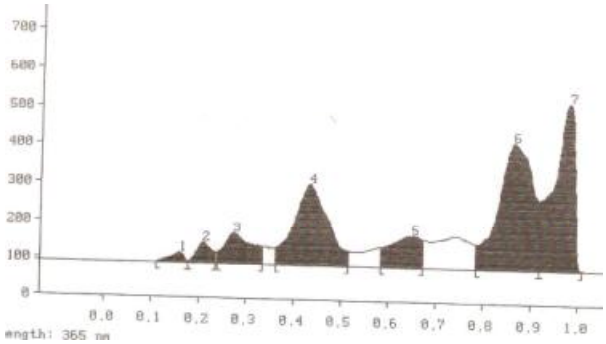

Figure 13 : Chromatogram of methanolic extract of root of *Nepeta hindostana (for Rutin, chorogeic acid and ferulic acid)*

five fluorescent zones were found. Among them the orange fluorescent zones of quercetin derivatives (Rf~0.28) accompanied by two blue fluorescent zones (0.4, 0.65) of *Nepeta hindostana*. Above it there is a yellow-green fluorescent zone indicating presence of kaemferol glucoside or apigenin (Rf~0.86). Blue fluorescent ferulic acid zone (Rf~0.98) was found (Plate 6; Figure 13).

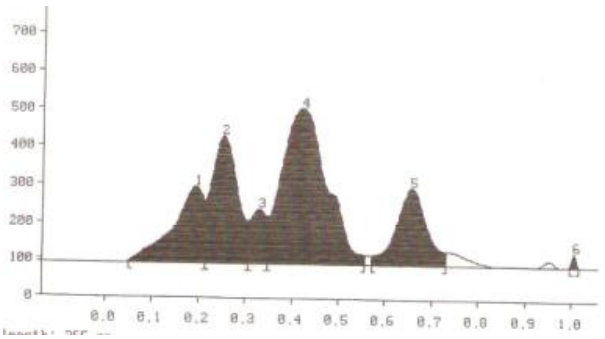

Figure 14 : Chromatogram of aqueous extract of aerial parts of *Nepeta hindostana (for Rutin, chorogeic acid and ferulic acid)*

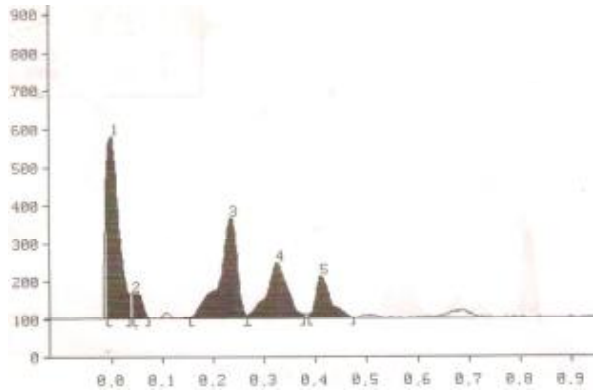

Figure 15 : Chromatogram of methanolic extract of leaf of *Anisomeles indica (for Emodin and Scopoletin)*

Three prominent orange flv. gly. zones (Rf~0.1-0.66) accompanied by two major blue fluorescent zones (Rf~0.33 and 0.42) were found in aqueous extract of *N.hindostana*. Slightly fluorescent zone of PCA was seen at the front (Plate 6; Figure 14).

2. HPTLC Analysis of samples for ES

Mobile phase : Toluene:ethyl acetate:methanol:formic acid:glacial acetic acid (10:1.5:1:0.2:0.1).

Reagent : Potassium hydroxide reagent (KOH)

Results : In *A.indica* (1), methanolic extracts of leaf contain scopoletin (Rf~0.32), which is seen as blue fluorescent zone accompanied with blue fluorescent zone of umbelliferone or substituted coumarin (Rf~0.41) (Figure 15). Four peaks were detected in methanolic extract of stem with very low conentration of coumarin (i.e. scopoletin) (Rf~0.29) (Plate 7; figure 16). In methanolic extract of root as well as aqueous extract very low concentration of all constituents was detected. The scopoletin (Rf~0.31) was detected in low concentration in methanolic leaf extract of *L.bipinnata* (3)

Full Paper

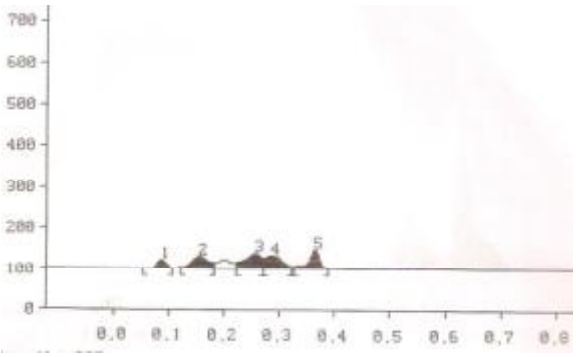

Figure 16 : Chromatogram of methanolic extract of stem of *Anisomeles indica (for Emodin and Scopoletin)*

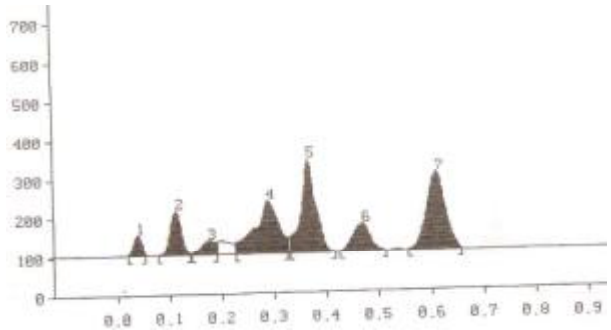

Figure 19 : Chromatogram of methanolic extract of leaf of *Nepeta hindostana (for Emodin and Scopoletin)*

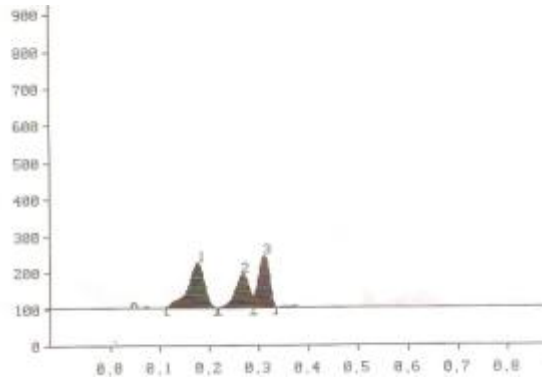

Figure 17 : Chromatogram of methanolic extract of leaf of *Lavandula bipinnata (for Emodin and Scopoletin)*

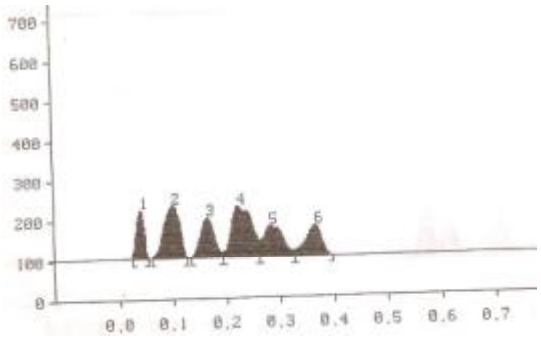

Figure 20 : Chromatogram of methanolic extract of stem of *Nepeta hindostana (for Emodin and Scopoletin)*

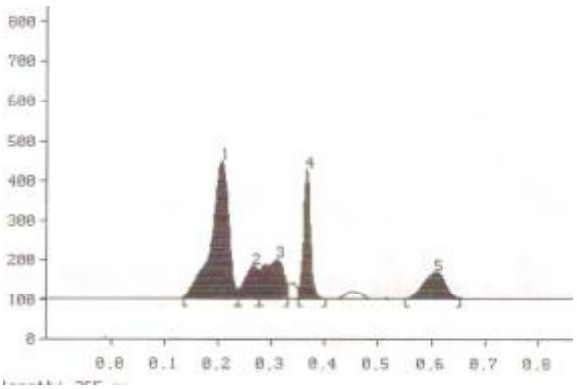

Figure 18 : Chromatogram of methanolic extract of leaf of *Leucas sephalotes (for Emodin and Scopoletin)*

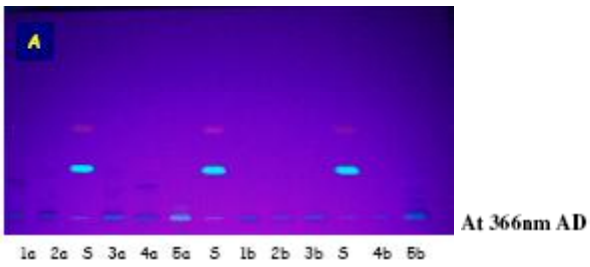

Plate 7 : HPTLC Fingerprint for ES of Leaf and Stem extracts:- 1a- *A.indica* leaf (MeOH) ; 1b *A.indica* stem (MeOH); 3a- *L.bipinnata* leaf (MeOH); 3b-*L.bipinnata* stem (MeOH); 4a-*L.cephalotes* leaf (MeOH); 4b-*L.cephalotes* stem (MeOH); 5a-*N.hindostana* leaf (MeOH); 5b-*N.hindostana* stem (MeOH); S-Spike (Emodin + Scopoletin)

(Figure17). Its absence was found in methanolic extract of stem and root as well as aqueous extracts of aerial parts.

Methanolic extract of *L.cephalotes* (4), leaf shows orange-red fluorescent zone (Rf ~0.27-0.61) of emodin accompanied with blue fluorescent scopoletin (Rf ~0.31) zone (Figure 18). It found to be present in low concentration in methanolic extracts of stem (Plate 7). It was absent in methanolic extract of root and aqueous extract of its aerial parts.

In leaf extract of *N.hindostana* (5), two orange-red fluorescent zones found (Rf ~0.29-0.61) as orange-red fluorescent emodin (Rf ~0.61) zone exactly similar to that of standard were found (Plate 7; Figure 19). Methanolic extract of stem contains the substituted coumarin, which are seen as blue fluorescent zone (Plate 7; Figure 20). In methanolic extract of root and aqueous extract of *N. hindostana* show poor content of constituent.

Full Paper

DISCUSSION

Alkaloids, Anthracene derivatives, coumarins, cardiac glycosides, essential oil, flavonoids, lignans, phenolic acids, steroids, saponins and sapogenins were observed during these studies in the selected plant species whose presence may be attributed to the medicinal properties of these plants[10, 11]. It is believed in the folklore practice that the plant preparation as a whole is therapeutically effective and the concept of a single active principle is generally not acceptable. The activity of plant material does not depend on a single substance but believed to be influenced by a large number of other components in the herbal medicine[12]. As the traditional health care systems like ayurveda or ethnomedicines suggest, most of the drugs used are in the form of paste, powder (churna), ash of single or mixture of different plants (bhasma) or in the form of water decoction (kadha). Therefore, keeping this classical approach in mind methanolic and water extracts of different parts of selected plants were studied.

Alkaloids are reported to have dramatic physiological activities. They act mainly on central nervous system. Many drugs used as hallucinogens, mental stimulants and mental depressant contain alkaloids[13]. In present investigation leaf extract of all the plants showed its presence as single orange coloured band after reacting with Dragendorff reagent (TABLE 1). However all the genera showed very poor alkaloidal content. Its presence was reported in other genera of family Lamiaceae viz., *Scutellaria* sp., *Rosmarinus* sp., *Leonurus* sp., *Lallemantia* sp., *Leonurus* sp., *Lamium* sp., *Salvia* sp., etc[14]. A note on the fatty acid profile of seed-oil and isolation of an antimalarial alkaloid from *Leonotis nepetaefolia* was presented[15]. However no report is available on the plants of the present study. Also rare occurrence of alkaloids in the family Lamiaceae was reported[16].

Anthracene derivatives present in the methanolic as well as water extracts of all leaf samples resulted in separation of two-four bands (Rf 0.23-0.97). The four major red-orange bands seen in leaf samples of *L.cephalotes*, indicates that major quantity was present in this sample. Presence of anthraquinone was identified and observed at Rf 0.66 on the basis of colour reaction and Rf value of standard mentioned in literature as well as compared with *Aloe* extract. Anthraquinones known to have antimicrobial activity[17,18]. Emodins are used as cathartics[13]. In HPTLC screening, all the samples were compared with emodin. In *L.cephalotes* (4), methanolic extract of leaf show orange-red fluorescent zone (Rf ~0.27-0.61) of which one at 0.61 corresponded to that of emodin. Also in *Nepeta hindostana* (5), two orange-red fluorescent zones were found (Rf ~0.29-0.61) which were exactly similar to that of standard emodin (Plate 7). As per the literature studied, it was found that there are no reports on the presence of anthracene derivatives in these members of Lamiaceae. Therefore further studies are required by preparatory column chromatography and spectrometry.

Coumarins reputed to have anticoagulation, estrogenic, vasodialation, antibacterial and anti-helminthic properties[19]. During the TLC screening, coumarins were found to be present in almost all the samples. In HPTLC screening, *L.cephalotes* (4) (Plate 7), methanolic extract of leaf showed orange-red fluorescent zone (Rf ~0.27-0.61) accompanied with blue fluorescent scopoletin (Rf ~0.31) zone. The scopoletin was detected in low concentration in methanolic extract of leaf of *L.bipinnata*. In *A.indica* (1), methanolic extracts of leaf contain scopoletin (Rf ~0.32), which is seen as blue fluorescent zone accompanied with blue fluorescent zone of umbelliferone or substituted coumarin (Rf ~0.41). Four peaks were detected in methanolic extract of stem with very low conentration of coumarin (i.e. scopoletin) (Rf~0.29) (Plate 7). 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*[20].

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[21]. In the present investigation all the plants were found to be rich in different components of flavonoids. Among the flavonoids major content of quercetin derivative was observed in maximum samples, which was further confirmed by using HPTLC tech-

nique (Plate 5) and rutin as standard marker compound.

Two new flavonoid glycosides, together with known flavonoid aglycones were isolated from the bark of *Colebrookea oppostifolia*[22]. Nine species belonging to three subgenera of *Ocimum* surveyed for flavonoid glycosides[23]. Many species in subgenus *Ocimum* also produced the more unusual compound, quercetin 3-O-(6''-Osmalonyl) glucoside and small amounts of flavone O-glycosides. Flavonoid glycosides isolated during the phytochemical analysis of the whole plant of *Salvia moorcroftiana* Wall[24].

In the methanolic root extract of *Nepeta hindostana* five fluorescent zones were found. One orange fluorescent zone of quercetin derivatives (Rf 0.28) accompanied by two blue fluorescent zones (0.4, 0.65). Above it there is a yellow-green fluorescent zone indicating presence of kaemferol glucoside or apigenin (Rf~0.86).

Phenolic acid (rosmarinic acid) was observed in *Mentha spicata, Nepeta apulaei, Nepeta sp., Thymus maroccanus* and *Thymus saturoides*, all belonging to subfamily Nepetoideae of Lamiaceae[25]. Researchers concentrate on ascorbic acid, tocopherols and carotenoids as well as on plant extracts containing various individual antioxidants such as flavonoids (quercetin, kaempherol, myricetin), catechins or phenols (carnosol, rosmanol, rosmaridiphenol) and phenolic acids (carnosic acid, rosmarinic acid)[26]. Hence, there is lot of scope to commercialize these plants as antioxidants.

Studies on anti-inflammatory constituents of *Leucas mollissima* WALL. Var. chinensis Benth. were done and isolated eight known compounds-oleanolic acid, apigenin, apigenin glucoside, cirsimaritin, mixture of beta-sitosterol and stigmasterol. Among these, compound apigenin exhibited potent anti-inflammatory activity[27]. In the present investigation also a yellow-green fluorescent zone indicating presence of kamferol glucosides or apigenin (Rf 0.86) was found in the methanolic root extract of *Nepeta hindostana*. Earlier findings suggest that flavonoids of apigenin and luteolin are potentially useful for the development of therapeutic treatment of cancer and are characterized as selectively inhibitors of the growth of leiomyomal smooth muscle cells[28].

The presence of iridoid glycosides and phenolic compounds such as flavonoid and phenyl propanoid glycosides was reported in different species of *Phlomis* during preliminary phytochemical analysis by thin layer chromatography using specific reagents and observed antinociceptive effects[29]. 14 different flavones found during HPLC survey of the leaf surface flavonoids of 38 species of *Nepeta* and four species of the related genera[30]. The most frequently encountered flavones in *Nepeta* were cirsimaritin; 8-hydroxycirsimaritin and genkwanin. In addition, two flavonols were found.

Several newly studied species and further accessions of the Lamiaceae have been analyzed for their exudates flavonoid profiles[31]. Hypolaetin 8-O-glucuronide and related flavonoids from *Lavandula coronopifolia* and *Lavandula pubescens* are reported[32]. In the present investigation also flavonoids were found to be present *L.bipinnata*.

Three diterpenes, a mixture of two related diterpenes, in addition to the acylated flavone apigenin were isolated and identified from the aerial parts of *Leucas neufliseana*[33]. Leaf flavonoids were reported as systematic characters in the genera *Lavandula* and *Sabaudia*[34].

Lignans were known as natural products. 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[35]. In the present investigation also lignans were found to be present in leaf samples after two phase development in two different mobile phases containing Chloroform:methanol (9:1) and toluene :acetone (6.5 :3.5). But reproducibility in results was found to be less.

One new and six known lignans, as well as sideritoflavone and rosmarinic acid were isolated from *Hyptis verticillata* (lamiaceae)[36]. Investigation on the aerial parts of *Phlomis integrifolia* (Lamiaceae) yielded in the isolation of iridoids, phenylethanoid glycosides, lignans, neolignans, flavonoids, monoterpene glucosides and diterpenoids[37]. Potential production of podophyllotoxin was evaluated on the basis of yields and abundance in some genera including *Hyptis, Nepeta*

and *Thymus*[38].

During present studies also the major components observed in all the four plants were flavonoids and the phenol carboxylic acids other than essential oils. Chemotaxonomic significance of two bioactive caffeic acid esters, nepetoidins A and B was revealed in subfamily Nepetoideae of Lamiaceae[39]. Nepetoidin B show greater antioxidant activity than gallic, rosmarinic and caffeic acids and both compounds were antifungal. It is observed that a ternary mixture of methanol, water and methyl acetate, 1+1+8 (v/v), extracted more caffeic acid derivatives than when the solvents were used individually[40].

It was reported that the main components of *Orthosiphon stamineus* are the pharmacologically active polyphenols:the polymethoxylated flavonoids and the caffeic acid derivatives in leaves extracts[41]. The compounds occurring in most of the samples of *Stachys* taxa found to be chlorogenic acid, isoquercetin, luteolin 7-O-glucoside, rutin and quercetin[42]. In the present investigation, the blue fluorescent PCA zones were found in root samples of *L. bipinnata*. Blue fluorescent chlorogenic acid zone (Rf- 0.5) and whitish blue fluorescent zones (Rf- 0.93 and 0.99) were identified as that of caffeic acid and ferulic acid respectively. In the root extract of *L.cephalotes* Blue fluorescent zone (Rf 0.3-0.36) accompanied by whitish blue fluorescent chlorogenic acid zone (Rf- 0.52).

Two new phenolic glycosides were isolated from the aqueous extract of *Salvia prionitis* together with seven known phenolic acids[43]. The presence of polar phenolic analytes in aqueous extracts from selected Lamiaceae species was confirmed and screened for antioxidant properties[44]. During the present studies presence of anthracene derivatives, coumarins, flavonoids and phenolic acids were found as major compounds in all the selected plants.

Preliminary investigation showed the presence of cardiac glycosides in *A.indica*, *L.bipinnata* and *L.cephalotes*. Kedde reagent, which is specific for cardiac glycosides, reacted positively with leaf extract of *A.indica* and *L.bipinnata* confirming presence of cardiac glycosides. The bands were compared with the extract of *Digitalis*, which is rich source of cardiac glycosides. However till today no cardiac glycosides have been reported in members of Lamiaceae. Hence, further investigations are required to confirm its presence in these plants.

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 oils were found to be present in the leaves of all the four members. 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 of the selected plant is extracted and using TLC method preliminary analysis was done. It revealed presence of 6 bands in *A.indica*, 7 different bands in *L.bipinnata*, 6 bands in *L.cephalotes* and only 3 bands in *N.hindostana*. *L.bipinnata* was found to be rich in essential oil content. In case of *Lavandula stoechas* , inflorescence contained more fenchone, myrtenyl acetate and alpha-pinene, while leaves contained more 1,8-cineole and camphor[45].

Composition of essential oil of *Nepeta persica* Boiss and forty-one components were characterized. The major components were 1,4-hexadiene-2, 3,4,5 tetramethyl and 4 abeta, 7 alpha, 7 alpha- nepetalactone [46]. In composition of the volatile oil of *Nepeta macrosiphon* Boiss., the main components of the oil were spathulenol, alpha-caddinol, bicyclogermacrene, beta-caryophyllene and linalool[47]. While in present studies *Nepeta hindostana* was found to be poor in oil content as only two components were characterized. Essential oil obtained from aerial parts of *Anisochilus carnosus* (Linn. fil.) Benth. and oil content was 0.13 % (v/w), on a fresh weight basis. Carvacrol (27.9%), camphor (14.1%) and alpha-cis-bergamotene were the most abundant components[48].

Steroids and triterpenoids are known to possess anti-inflammatory, lipolytic and anticholesterenic activities[49]. Single pink coloured band was seen in leaf extract of each plant at Rf 0.45 corresponding with that of stigmasterol. A chlorinated monoterpene ketone, acylated beta-sitosterol glycosides and a flavanone glycoside were reported from *Mentha longifolia*[50]. Presence of earlier known compounds sitosterol and ∝-amyrin was confirmed[51]. In the present investigation also presence of phytosterol i.e. stigmasterol was observed almost in all the plant samples (TABLE 1). In

chemical group test of the tincture of *Leucas lavandulaefolia* Rees., with 60 % (v/v) alcohol, the presence of steroids, tannins and reducing sugars were confirmed[52].

Saponins are well known for their expectorant, spasmolytic and anti-tussive activities[53,54,55]. The heavy and consistent foam formation during extraction was observed in leaf samples of *L. cephalotes* indicates the presence of saponin. Distinct blue or gray bands of saponins (Rf- 0.24-0.76) same as those in extract of Ginseng root were observed in all samples except poor content in *Nepeta*.

The detection of sapogenins, which is aglycone part of saponin, is in concurrence with the earlier saponin detection. Two-six bands were separated during TLC screening in the mobile phase consist of hexane:acetone (40:10) with Rf 0.11-0.76. There are no reports found in the studied literature on the presence of saponin and sapogenins compounds.

Hptlc studies

As HPTLC is modern version of TLC, both are closely related. Hence almost similar the chromatographic condition were applied during HPTLC screening as those used for TLC analysis.

For the selection of the optimal combination of systems, two approaches are possible the first one is to determine and compare an amount of information and discriminating power for all possible combinations of TLC systems. The second approach is to classify the TLC system into groups with similar separation properties and to select the best system from each group[56]. Both of these approaches were followed during the phytochemical studies in the present investigation. Hence as per the second approach mentioned above for the HPTLC screening such TLC system was tried which separates the groups with similar separation properties.

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*[56]. It has been shown that the most favourable TLC systems for the separation of investigated compounds are ethyl acetate-formic acid-acetic acid-water (100:11:11:27 v/v) and ethyl acetate- formic acid-water (8:1:1 v/v). It was observed that flavonoids resolved in mobile phase containing ethyl acetate-formic acid-acetic acid-water(100:11:11:27v/v)[7]. 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 (100:11:11:27 v/v). Vundac et al.[42] also used 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[42].

The success of this investigation will result in method development for separation of group of compound that may responsible for their medicinal activities. The HPTLC screening results in confirmation of the presence of some secondary metabolites viz., rutin, chlorogenic acid and ferulic acid, scopoletin and emodin in selected plants.

Abbreviations

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

ACKNOWLEDGEMENT

We are thankfull to Dept. of Pharmaceutical Chemistry, University Campus, R.T.M. Nagpur University for facilitating HPTLC analysis.

REFERENCES

[1] K.R.Kirthikar, B.D.Basu; 'Indian Medicinal Plants', Lalit Mohan Basu Publishers, Allahabad **(1984)**.
[2] R.N.Chopra, S.L.Nayar, I.C.Chopra; 'Glassary of Indian Medicinal Plants', CSIR Publication, New Delhi, **(1992)**.
[3] M.Shahidul, M.A.Quader, M.A.Rashid; Fitoterapia, **71(5)**, 574-576 **(2000)**.
[4] P.V.Sharma; 'Dravyaguna Vijñana, Chaukhambha Bharati Academy, Varanasi, India, **2**, 17-18, 707-708 **(1983)**.
[5] S.G.Joshi; 'Medicinal Plants', Oxford and IBH publishing Co.Pvt.Ltd., New Delhi, India, 222-223 **(2000)**.
[6] J.B.Harborne; 'Phytochemical methods:A guide to modern technique of plant analysis'.Champman and Hall, London, **(1998)**.

Full Paper

[7] H.Wagner, Bladt; 'Plant Drug Analysis:A Thin Layer Chromatography Atlas', 2nd Eds., Springer- Verlag Publication, Berlin, Germany, **(1996)**.

[8] L.G.Chatten; 'Pharmaceutical Chemistry, Theory and Application, Marcel Dekkar, Inc., New York, **1**, **(1966)**.

[9] E.Stahl (Eds.); 'Thin layer Chromatogrphy:A laboratory Hand Book', Springer-Verlag Publication, Berlin, **(1969)**.

[10] L.D.Kapoor, A.Singh, S.L.Kapoor, S.N.Srivastava; Loydia, **32 (3)**, 297-304 **(1989)**.

[11] L.S.Gill, H.G.K.Nyawame, E.J.Eseobor, I.S.Osagie; Ethnobotany, **5(1,2)**, 129-142 **(1993)**.

[12] R.T.Sane; Indian Drugs, **39(3)**, 184-189 **(2002)**.

[13] S.C.Chhabra, F.C.Usio, E.N.Mshiu; Indian Journal of Ethnopharmacology, **11**, 157-179 **(1984)**.

[14] R.F.Raffaut; 'A Handbook of Alkaloids and Alkaloid containing plants', Wiley-Interscience, New York, **(1970)**.

[15] U.P.Reddy, K.Vaidyanath; Advances in Plant Sciences, **14(1)**, 169-172 **(2001)**.

[16] C.K.Kokate, A.P.Purohit, S.B.Gokhale, 'Pharmacognosy', Nirali Publication, Pune, India, **(2002)**.

[17] J.L.Rios, M.C.Recio, A.Villar; Journal of Ethnopharmacology, **21**, 134-152 **(1987)**.

[18] R.Diaz, J.Que Vedo-Sarmeinto, A.Ramas-Carmeuzava, P.Cabo, J.Cabo; Fitoterapia, **59(4)**, 329-333 **(1988)**.

[19] M.H.Reddy; Journal Economic and Taxonomic Botany, Additional Series, **12**, 37-39 **(1996)**.

[20] P.B.Andrade, R.M.Seabra, P.Valentao, F.Areia; Journal of Liquid Chromatography and Related Technologies, **21(8)**, 2813-2820 **(1998)**.

[21] V.D.Tripathi, R.P.Rastogi; J.Sci.Ind.Res., **40**, 116-124 **(1981)**.

[22] F.Yang, X.C.Li, H.Q.Wang, C.R.Yang; Phytochemistry, **42 (3)**, 867-869 **(1996)**.

[23] R.J.Grayer, G.C.Kite, S.P.Veitch, A.J.Paton; Biochemical Systematics, Ecology, **30(4)**, 327-342 **(2002)**.

[24] M.Zahid, O.Ishrud, Y.Pan, M.Asim, M.Riaz, V.U. Ahmad; Carbohydrate Research, **337(5)**, 403-407 **(2002)**.

[25] F.Mouhajir, J.A.Pedersen, M.Rejdali, G.H.N.Towers; Pharmaceutical Biology, **39(5)**, 391-398 **(2001)**.

[26] N.Yanishlieva, E.M.Marinova; E.J.Lip.Sci.Tech., **103(11)**, 752-767 **(2001)**.

[27] C.T.Ku, S.C.Chen, J.P.Wang, J.B.Wu, S.C.Kuo; Chinese Pharmaceutical Journal Taipei, **52 (5)**, 261-273 **(2000)**.

[28] H.D.Kim, T.K.Lee, L.S.Lim, H.Kim, Y.C.Lee, C.H. Kim; Applied Pharmacology, **205(3)**, 213-224 **(2005)**.

[29] P.Sarkhil, M.Abdollahi, A.Shafiee; Pharmacological Research, **48(3)**, 263-266 **(2003)**.

[30] Z.Jamzad, R.J.Grayer, G.C.Kite, M.S.J.Simmonds, M.Ingrouille, A.Jalili; Biochemical Systematic and Ecology, **31(6)**, 587-600 **(2003)**.

[31] V.K.M.Valant, J.N.Roitman, E.Wollenweber; Biochemical Systematics and Ecology, **31(11)**, 1279-1289 **(2003)**.

[32] El Garf Ibrahim, R.J.Grayer, G.C.Kite, N.C.Veitch; Biochemical Systematics and Ecology, **27 (8)**, 843-846 **(1999)**.

[33] T.Khalil, S.R.Gedara, M.F.Lahloub, A.F.Halim; Phytochemistry, **41(6)**, 1569-1571**(1996)**.

[34] T.M.Upson, R.J.Grayer, J.R.Greenham, C.A. Williams, F.Al-Ghamdi, F.H.Chen; Biochemical Systematic and Ecology, **28(10)**, 991-100 **(2000)**.

[35] W.D.MacRae, G.H.Neil Towers; Phytochemistry, **23(6)**, 1207-1220 **(1984)**.

[36] M.Kuhnt, H.Rimpler, M.Henrich; Phytochemistry, **36(2)**, 485-489**(1994)**.

[37] I.Saracoglu, M.Varel, Í.Çalis; Turk J Chem., **27**, 739-747 **(2003)**.

[38] E.Bedir, I.Khan, R.M.Moraes; 'Bioprospecting for Podophyllotoxins'.reprinted from:J Janick, A Whipkey (eds.); Trends in new crops and new uses, ASHS Press, Alexandria, VA, **(2002)**.

[39] 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)**.

[40] K.Olah, D.Hanganu, R.Oprean, C.Mogosan, N.Dubei, S.Gocan; Journal of Planar Chromatography, Modern-TLC, **17(1)**, 18-21 **(2004)**.

[41] K.Olah, L.Radu, C.Mogosa, D.Hanganu, S.Gocan; Journal of Pharmaceutical and Biomedical Analysis, **33(1)**, 117-123 **(2003)**.

[42] V.B.Vundac, M.Zeljan, P.Misko, G.Petra, C.C. Biserka; J.Planar Chromatography.Modern TLC, **18(104)**, 269-273 **(2005)**.

[43] L.M.Zhao, X.T.Liang, L.N.Li; Phytochemistry, **42(3)**, 899-901 **(1996)**.

[44] H.J.D.Dorman, O.Bachmayer, M.Kosar, R. Hiltunen; Journal of Agricultural and Food Chemistry, **52(4)**, 762-770 **(2004)**.

[45] S.Melpomeni, A.Chedly, K Eugene; Biochemical Systematics and Ecology, **24(3)**, 255-260 **(1996)**.

[46] K.Javidnia, R.Miri, F.Safavi, A.Azarpira, A.Shafiee; Flavour and Fragrance Journal, **17(1)**, 20-22

(2002).

[47] K.Javidnia, R.Miri, A.Jafari, H.Rezai; Flavour and Fragrance Journal, **19(2)**, 156-158 **(2004)**.

[48] F.Senatore, F.Lentini, F.Venza, M.Bruno, F. Napolitano; Flavour and Fragrance Journal, **18(3)**, 202-204 **(2003)**.

[49] S.Chawla, S.S.Handa, A.K.Sharma, B.S.Kaith; J Scientific and Industrial Research, **46**, 214-223 **(1987)**.

[50] M.S.Ali, M.Saleem, W.Ahmad, M.Parvez, R. Yamdagni; Phytochemistry, **59(8)**, 889-895 **(2002)**.

[51] Ulubelen, U.Sönmez, G.Topcu, C.B.Johansson; Phytochemistry, **42(1)**, 145-147 **(1996)**.

[52] S.Mukherjee, B.Ghosh, S.Jha; Plant Cell Reports, **15(9)**, 691-694 **(1996)**.

[53] R.Benerji, D.Prakash, G.K.Patnaik, S.K.Nigam; Indian Drugs, **20(2)**, 51-54 **(1982)**.

[54] R.Sood, A.Bajpai, M.Dixit; Indian J.Pharmacol., **17**, 187-189 **(1985)**.

[55] S.K.Nigam, G.Misra; Phytochemistry:'basic principles in relation to ethnobotany', In S.K.Jain; 'Methods and approaches in Ethnobotany', Society of Ethnobotanists, Lucknow, India, 125-140 **(1989)**.

[56] S.M.Madic and Z.Males; Pharmazie, **54(5)**, 362-364 **(1999)**.