Research Article

TLC Based Chemotaxonomic Approach of some Laurels Present in Sub-Himalayan Terai & Duars Region of West Bengal, India

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ABSTRACT

Lauraceae, an aromatic plant family has many medicinal uses which include antioxidant, antimicrobial and anti-inflammatory activities. The study was planned to investigate the phytochemical components present in the bark extracts of eight economically important plants of Terai and Duars region of North Bengal, India by thin layer chromatography. The results of the work revealed the occurrence of phytochemical constituents such as essential oil, anthrax quinones, bitter principle, flavonoids and phenolics in the bark of these plants. Further, the thin layer chromatographic profiles may also provide the fingerprint characteristics as well as the relation between them by cladogram which is prepared by XLSTAT 2009 software. It could be concluded that as the plants contained rich variety of phytochemicals and the study of phytochemicals itself through TLC-based separation profile and fingerprint thus appears to be much helpful in taxonomic study of the genus *Cinnamomum* and *Litsea.*

Keywords: TLC, Phytochemicals, Lauraceae, Terai and Duars.

INTRODUCTION

Aromatic plants and medicinal constitute are the major segments of the flora and they provide raw materials in pharmaceuticals, cosmetics and drug industries.[1]The separation and purification of plant components are generally carried out through chromatographic techniques based on their size, shape, or charge.[2] Thin layer chromatography (TLC) is generally considered as reproducible and authentic method for analysis of different drugs. It is extensively adopted for the rapid analysis of drugs and drug preparations. This technique provides a chromatographic drug fingerprint in very short time. It is therefore suitable for observing the identity and purity of drugs, and for detecting adulterations as well as substitutions.[3] Not only that, this method can help in chemotaxonomic clustering for solving phylogenic problems. Through chemotaxonomy, many authors compared different category of phytochemicals, present in genera and classify different groups by preparing cladogram on the basis of TLC spots appeared after processing.[4,5]

Cinnamomum and *Litsea* (specially the oil yielding plants) have some similar morphological characters. Blooming season is very restricted for this Lauraceae family. In view of the diversity of different phytochemicals and essential oil known for this family, a detailed chemotaxonomic study of these secondary metabolites of different economically important medicinal and aromatic plants of these two genera may contribute valuable information towards a comparison of the genera. Since no recorded information was previously available on the essential oil and phytochemicals of these plants grown in Terai and Duars region of West Bengal, in this paper, the study was carried out to evaluate qualitative phytochemical constituents of various secondary metabolites present in the bark of eight Laurels by using TLC and compared them through Agglomerative Hierarchical Clustering (AHC) by Ward's method.[6]

MATERIALS AND METHODS

Collection and authentication of plant material

For present study eight species representing two genera of Lauraceae were chosen. Plants were collected from different region of Terai-Duars region of West Bengal. The collected materials were: *Cinnamomum bejolghota* (Buchanon–Hamilton) Sweet, *Cinnamomum camphora* (Linnaeus) J. Presl, *Cinnamomum tamala* ((Buchanon–Hamilton) Nees&Ebermaier, *Cinnamomum verum* J. Presl, *Litsea assamica* Hooker *f.*, *Litsea glutinosa* (Loureiro) Robinson, *Litsea laeta* (Nees) Hooker *f.* and *Litsea monopetala* (Roxburgh) Persoon. Plant specimens were identified taking help of relevant taxonomic literature including[7-11] and by matching with the previously identified specimens at NBU and CAL. The material has been deposited in the 'NBU Herbarium' and recorded against the voucher herbarium specimen number 9679, 9680, 9678, 9677, 9639, 9640, 9641, 9642 dated 11-06-11.

Extraction of the plant samples

For extraction of different secondary metabolites, barks of eight Laurels were used. Bark specimens were surgically separated from plants, washed thoroughly and one gm of each bark material was weighed separately and extracted with 5ml of methanol for 10 min. Methanolic filtrate was concentrated through vacuum rotary evaporator for application on TLC plates.

Extraction of essential oil

Essential oil was extracted by using the method proposed by Lee and Shibamoto.[12] Bark of eight Laurels (200 g) was placed in a 3 litre round-bottom flask with 1 litre of distilled water. The solution was steam distilled at 55°C for 8 hrs. Then, 900 ml distillate was fractionated with 100 ml of dichloromethane for 6 h. After the dichloromethane extract was dried over anhydrous sodium sulphate, the solvent wasremoved until the volume was reduced to 2 ml.

Thin layer chromatography (TLC)

Methanolic bark extracts of each Laurals were subjected to qualitative Phytochemical detection as well as DPPH based antioxidant fingerprint through TLC.[3] It was performed by using silica gel-60 F_{254} chromatographic plates of 8cmx2cm with 3mm thickness to confirm the presence of secondary metabolites. For the separation of phytochemical compounds, the methanolic bark extracts were spotted manually using micro-pipette. The spotted plates were put in a solvent chamber which contained various solvent systems to detect the suitable mobile phase. After this paration of phytochemicals, various spray reagents specific for detection of special class of secondary metabolites were used to identify the compounds (Table 1).[13] The colour ofthe spots was noted and hR_f values were calculated by using the following formula:

$$\text{Retention factor } (hR_{f)} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled the solvent}} \times 100$$

Statistical Analysis

All experiments were conducted in triplicate and statistical analysis was done. hR_fvalues were done through MS-Excel and Dendrogram wasprepared by XLSTAT 2009 software.[14]

RESULT AND DISCUSSIONS

There have always been debates regarding the taxonomic complexity of the species associated with the family Lauraceae. The species related to this family have shown variations and similarities in different morphological aspects.[15,16] There are many morphological limits of the species of Lauraceae regarding identification due to the short flowering and fruiting time. For this reason, this family has been re-classified many times but no satisfactory revision of this whole section has yet been worked out. The boundaries between many of the species of this family are still ill-defined, with several of the 'separately' described 'new' taxa have established themselves with morphological variants. Many authors suggested that the presence or absence of certain compounds in plantssupplied several taxonomic markers with valuable information regarding the systematic position of this species within a family.[4, 5, 17, and 18]

The qualitative analysis of essential oil, anthra quinones, bitter principle, flavonoids and phenolics of economically important eight laurels was done by Thin Layer Chromatography. In TLC, the qualitative analysis of these components was done on aluminium based silica gel plates using specific solvent systems for each secondary metabolite's group (Figure 1a-f).

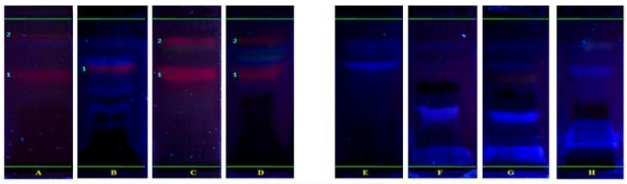

Figure 1(a): Detection of anthraquinones in bark of eight Laurels

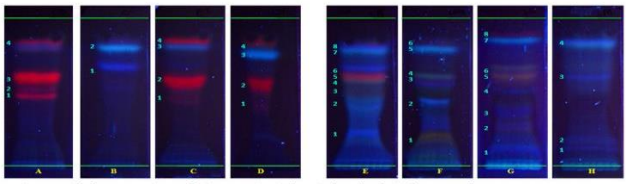

Figure 1(b): Detection of bitter principles in bark of eight Laurels

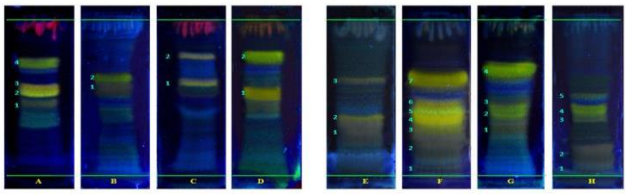

Figure 1(c): Detection of flavonoids in bark of eight Laurels

[A] *Cinnamomum bejolghota,* [B] *C. camphora,* [C] *C. tamala,* [D] *C. verum,*
[E] *L. assamica,* [F] *L. glutinosa,* [G] *L. laeta,* [H] *L. monopetala*
1,2,3,4..... are the bands of different compounds

When the methanolic extracts of these eight species were subjected to the solvent system ethyl acetate: methanol: water (100:13.5:10), which is specific for anthraquinones,[19,20] *Cinnamomum*spp showed many red coloured bands on preparative silica gel plates under UV-365nm indicating the presence of various anthraquinone derivatives (Figure 1a).

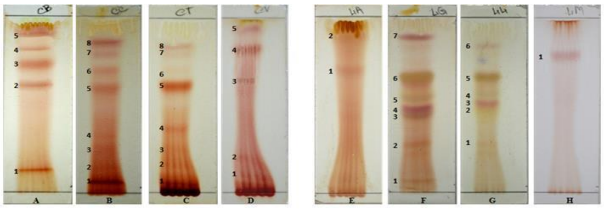

Figure 1(d): Detection of phenolics in bark of eight Laurels

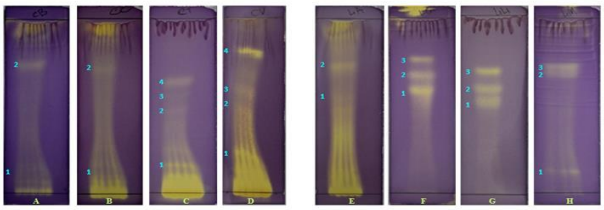

Figure 1(e): Detection of DPPH free radical scavenging potency in bark of eight Laurels

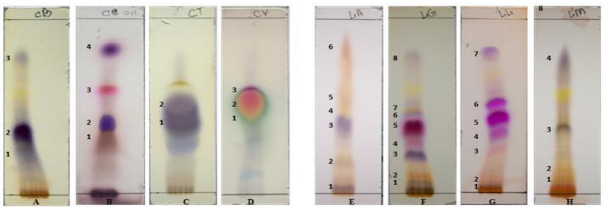

Figure 1(f): Detection of essential oils in bark of eight Laurels

[A] *Cinnamomum bejolghota,* [B] *C. camphora,* [C] *C. tamala,* [D] *C. verum,*
[E] *L. assamica,* [F] *L. glutinosa,* [G] *L. laeta,* [H] *L. monopetala*
1,2,3,4..... are the bands of different compounds

Table 1: Profile of different secondary metabolites of different species of Laurels identified through Thin Layer Chromatography (TLC)

Compound Class	Spraying Reagent	Detection	Appearance of band colour	Expected compound diversity	Number of TLC Bands among different Laurels							
					Cb	*Cc*	*Ct*	*Cv*	*La*	*Lg*	*Ll*	*Lm*
Anthraquinones	10% Ethanolic KOH reagent	UV-365 nm	Red	Frangulin A & B; Glucofrangulin A & B; Emodin and Rhein	2	1	2	2	0	0	0	0
Bitter principles	Vanillin and Sulphuric acid reagent	UV-365 nm	Red violet, Blue and Brown- red	Neohesperidin; Naringin; Harpagoside; Foliamenthin; Menthiafolin; Quassin; Marrubiin; Absinthin; Cnicin; Gentiopicroside and Swertiamarin	5	3	5	5	8	6	8	4
Flavonoids	Natural products-Polyethylene glycol	UV-365 nm	Orange, Yellow-green and Orange-yellow	Leutedin; Quercetin; Myricetin; Kaempferol; Isohamnetin and Apigenin	4	2	2	3	3	7	4	5
Phenolics	Fast blue reagent	Visible	Pinkish orange	Phenolics	5	8	8	5	2	7	6	1
DPPH scavenging capacity	0.2% DPPH Solution	Visible	Yellow	Antioxidants	2	2	4	4	2	3	3	3
Essential oil	Anisaldehyde and Sulphuric acid	Visible	Strong blue, Brown, Red and Green	Essential oils	3	4	2	3	5	8	7	4

Cb- Cinnamomumbejolghota, Cc- C. camphora, Ct- C. tamala, Cv- C. verum, La- Litseaassamica, Lg- L. glutinosa, Ll- L. laeta, Lm-L. monopetala

Similar solvent system was used for the experiment of bitter principles. After the spraying of vanillin-sulphuric acid reagent many coloured bands like red-violet, brown-red, blue-green and blue bands were appeared which were identified and shown in the Table 1 and Figure 1b. On the other hand, these plants were able to scavenge free radicals for the presence of different phytochemicals.[15] For that purpose, the methanolic extracts of all plants were applied to silica gel plates and developed in ethyl acetate: formic acid: acetic acid: water (100:11:11:27)for detection of flavonoids, phenolics and DPPH fingerprinting respectively.[20] The samples showed orange, orange-yellow and yellow-green bands on flavonoids' specific solvent system (Figure 1c),while pinkish orange bands on phenolics' specific solvent system indicating their existence in tested samples (Figure 1d). After separation on TLC plates, the antioxidant compounds of Laurels were determined *in situ* with DPPH reagent in which the TLC plate was observed through visible light (Figure 1e).As shown in Figure 1e, the separated components producing yellowish bands on the purple background were considered as antioxidants. Many authors examined that, the purple background colour was visualized with distinct yellow bands after spraying the plate with DPPH reagent when crude herbal drugs were separated through TLC.[21, 22] TLC was also used for identification of essential oils of these eight Laurels. After separation, the TLC plates were pulverized with anisaldehyde, warmed for spots colouring and studied in visible range (Figure 1f).

In 1972, Gottlieb[23]carried out extensive survey on most of the genera in Lauraceae, but insufficient records are available on the chemo-taxonomical aspects on this family. The results of the cluster analysis (Figure 2) provide useful chemotaxonomic correlations of two genera of this family. Dendrogram clustering analysis based on thin layer chromatographic data sorted out *C. camphora*as independent entities and it was found that *Litsea* genus is separated from the genus *Cinnamomum*. Close relationship was observed among *C. tamala, C. verum* and *C. bejolghota.* In case of *Litsea* firstly divided into two branches, one branch represented *L. laeta* and *L. glutinosa* and another branch stand for *L. monopetala* and *L. assamica*. A qualitative study of phytochemicals itself through TLC-based separation profile and fingerprint thus seems to be much useful in the taxonomic study of the genus *Cinnamomum* and *Litsea.*

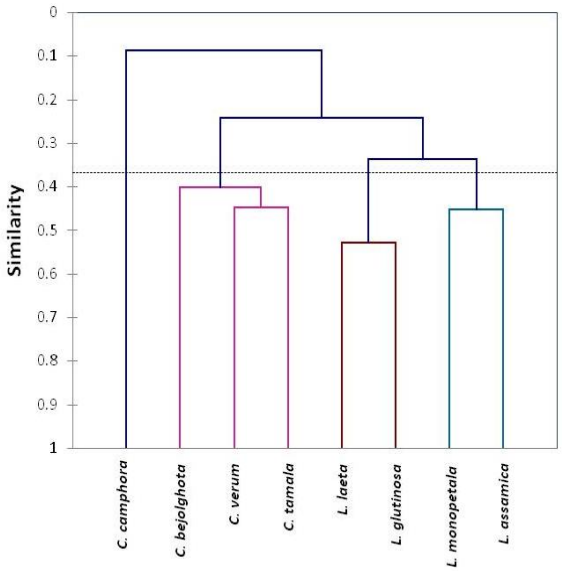

Figure 2. Dendrogram based on phytochemical constituents present in eight Laurels

CONCLUSION

Considerable variations were thus observed between different spices of Lauraceae in terms of antioxidant activity and Phytochemical attributes. Unsupervised pattern recognition techniques facilitated visualization of the complex dataset and clustered dendrogram was established on the basis of distribution of phytochemical attributes. The combination of chemical characters and multivariate data analysis reinforce easy interpretation of similarities and differences among eight laurels on their antioxidant activity and content of secondary metabolites.

Information from this study might be useful for choosing the specific type of spices for consumption in order to prevent lifestyle-related disorders among consumers. On the other hand, the work was also helpful in taxonomic study and cladogram analysis of the family Lauraceae.

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Dr. Palash Mandal was graduated and post-graduated from University of Kalyani, Kalyani, West Bengal, India. He was the recipients of gold medal at post graduation. During his Master's Course, he has taken specialization in Plant Physiology and Biochemistry. He is having fifteen years of teaching experience at North Bengal University, India. Currently he is working with the bioactivities of plant peptides along with *in vitro* antioxidant and anti-diabetic properties of medicinal and aromatic plants. He is also actively engaged in investigating the elicitor induced changes of nutraceutical and pharmacological activities of edible sprouts. Till now he has published 70 research articles in different reputed national and international journals with high impact factor along with four proceeding papers and two book chapters.