HPTLC Fingerprint Profile Of Some *Cinnamomum* Species

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Abstract

In the present communication, finger print of four medicinally and economically important leaves of *Cinnamomum* species has been developed. Hexane extract of these plants were developed in the mobile phase of toluene : ethyl acetate (8:1, v/v) and scanned under UV at 254 nm and after dipping in vanillin-sulphuric acid reagent followed by heating at 105°. The four species showed differentiating fingerprints on densitomentric scanning at different wavelengths. These finger prints would be helpful in the authentication of these species.

Keywords: *C. malabatrum, C. sulphuratum, C. tamala,* Densitometry, Lauraceae, Petiole.

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INTRODUCTION

About 270 species of *Cinnamomum* Schaeffer (Family: Lauraceae) are distributed in Asia, America and Australia out of which 20 specie occur in South India.[1–2] Several Cinnamomum species are medicinally and economically important. The genus plays an important role in the search for new source of aroma chemicals and it is a prime area where perfume industries are interested.[3–4] Investigation of *Cinnamomum* species lead to the discovery of aroma chemicals having unique flavor due to aroma chemicals like eugenol, linalool, safrole and benzyl benzoate.[5–7] The aroma principles eugenol and linalool possess different biological properties.[9–15] Four economically important species viz., *C. malabatrum, C. sulphuratum, C. tamala* and *C. zeylanicum* were taken up for the HPTLC finger print studies using eugenol and linalool as appropriate chemical markers.

MATERIALS

Leaves of *Cinnamomum* viz. *C. malabatrum, C. sulphuratum, C. tamala* and *C. zeylanicum* growing in the evergreen forests of Coorg district of Karnataka were collected in the month of January and were authenticated with the help of Regional floras.[17] The leaves were shade dried and the petioles were separated from the lamina portion and used for the study. Analytical reagents *viz., n*-hexane, toluene, ethyl acetate were purchased from SRL chemicals, Mumbai, India. TLC plates were obtained from Merck, Bombay, India. Eugenol and linalool were obtained from M/s. Sigma-Aldrich chemicals, Bangalore, India.

METHODS

1 g of finely crushed petiole of the selected plants were separately extracted with *n*-hexane using Soxhlet apparatus. The extracts were freeze dried. The residues were dissolved in 10 ml of *n*-hexane. 10 mg of each of eugenol and linalool were dissolved in 10 ml of *n*-hexane.

TLC aluminium plates precoated with silica gel 60 F_{254} (Merck) of 0.2 mm thickness was used as stationary phase. 10 µl of *C. malabatrum, C. sulphuratum, C. tamala*, 20 µl of *C. zeylanicum* and 4 µl of eugenol and linalool were applied as 6 mm bands at 6 mm distance on the TLC plates using CAMAG Linomat IV sample applicator. The speed of the application of extracts was maintained at 5 sec/µl. Then the plate was developed using the mobile phase of Toluene : Ethyl acetate (8:1, v/v) in a CAMAG twin trough chamber up to a height of 8 cm. The developed plate was air dried and scanned at a wavelength of 254 nm using deuterium lamp in the CAMAG scanner 030618 equipped with CATS V 4.06 software. The chromatograms were recorded. Then the plate was dipped in vanillin-sulphuric

acid reagent. The plate was heated in an air circulated oven at 105°C till the development of colour of the spots. Then the plate was scanned immediately in the visible region at a wavelength of 620 nm using tungsten lamp. UV spectrum of marker compounds and their corresponding spots in the extracts were also recorded.

RESULTS

From the HPTLC finger prints, the peak of eugenol was found at R_f 0.69 and linalool was found at R_f 0.57. The chromatograms obtained were shown in Fig. 1–11. The R_f of peaks under UV 254 nm were listed in Table 1. The R_f of peaks under visible light at 620 nm were listed in Table 2. Peaks with R_f value less than 0.05 and higher than 0.96 were not included in the table as the former represents the point of application and latter represents the movement of the constituents to the solvent front.

DISCUSSION

In the chromatograms derived under UV 254 nm (Fig. 1-5), the peaks at R_f 0.73 in all four plant extracts corresponds to eugenol and linalool was not visible in UV light. The peak area of eugenol in *C. zeylanicum* was comparatively higher than other species. Peaks at R_f 0.25, 0.33, 0.63, 0.73, 0.87 & 0.95 were common to *C. sulphuratum* and *C. tamala*; but peaks at R_f 0.25 was minor in *C. sulphuratum* whereas major in *C. tamala*. Peak at R_f 0.43 of *C. sulphuratum* was specific to this specie and it was not seen in other species. Similarly, a major peak at R_f 0.37 of *C. tamala* was found to be specific to this plant.

Table 1. RF VALUES OF PEAKS OBSERVED UNDER UV 254 NM

Sl.No.	Cm	Cs	Ct	Cz	Marker
1	-	0.06 (Minor)	-	-	
2	-	0.10(Minor)	0.09(Minor)	-	
3	-	-	0.14(Minor)	-	
4	-	0.25(Minor)	0.25(Major)	-	
5	-	0.33(Minor)	0.33(Minor)	-	
6	0.39(Minor)	-	0.37(Major)	-	
7	-	0.43(Major)	0.46(Minor)	-	
8	0.59(Minor)	-	-	-	
9	-	0.63(Major)	0.63(Major)	-	
10	0.73(Minor)	0.73(Major)	0.73(Major)	0.73(Major)	0.73(Eugenol)
11	-	0.87(Major)	0.87(Major)	0.86(Minor)	
12	0.95(Major)	0.95(Major)	0.94(Major)	0.94(Minor)	

Table 2. RF VALUES OF PEAKS OBSERVED UNDER UV 620 NM

Sl. No	Cm	Cs	Ct	Cz	Marker
1	0.05	-	-	0.07	
2	0.13	0.13(Major)	0.13(Minor)	0.13	
3	-	0.18(Major)	0.18(Minor)	-	
4	0.25	0.25(Major)	0.25(Major)	0.27	
5	-	0.29(Minor)	0.32(Minor)	0.31	
6	0.31(Major)	-	0.39(Major)	0.40(Major)	
7	0.40(Major)	0.41(Major)	-	-	
8	-	0.51(Major)	-	-	
9	0.57(Major)	0.57(Major)	0.57(Major)	0.57(Major)	0.57(Linalool)
10	-	0.64(Major)	0.66(Minor)	-	
11	0.73(Major)	0.73(Major)	0.73(Major)	0.73(Major)	0.73(Eugenol)
12	-	0.78(Major)	0.78(Major)	0.78(Major)	
13	-	-	-	0.93(Major)	

Cm- Cinnamomum malabatrum; Cs- Cinnamomum sulphuratum; Ct-Cinnamomum tamala; Cz- Cinnamomum zeylanicum

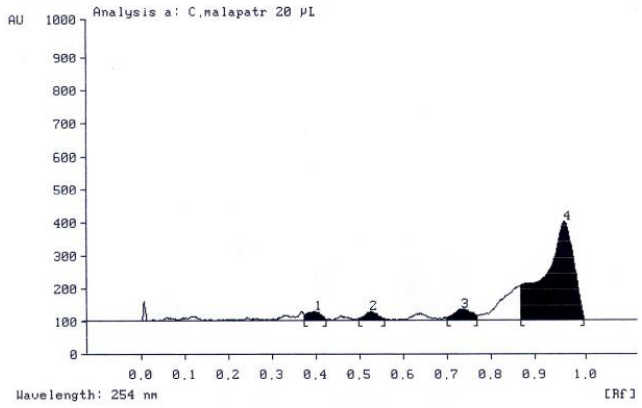

FIGURE 1. *FINGER PRINT OF C. MALABATRUM AT • 254 NM*

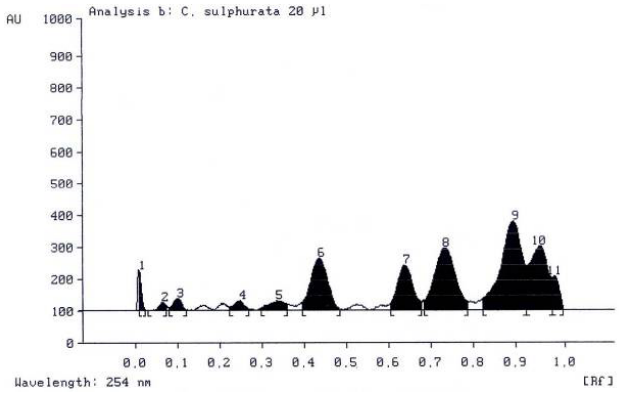

FIGURE 2. *FINGER PRINT OF C. SULPHURATUM AT • 254 NM*

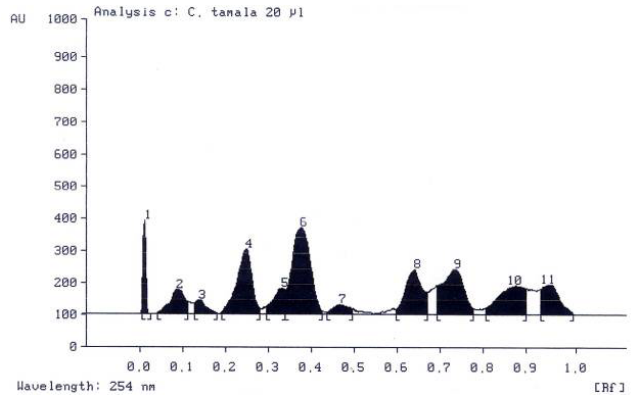

FIGURE 3. *FINGER PRINT OF OF C. TAMALA AT • 254 NM*

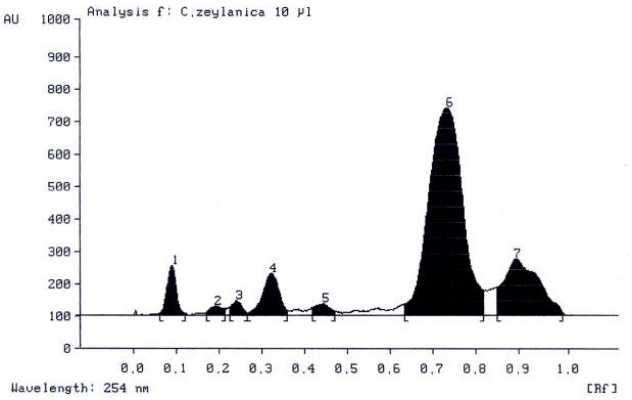

FIGURE 4. *FINGER PRINT OF OF C. ZEYLANICUM AT • 254 NM*

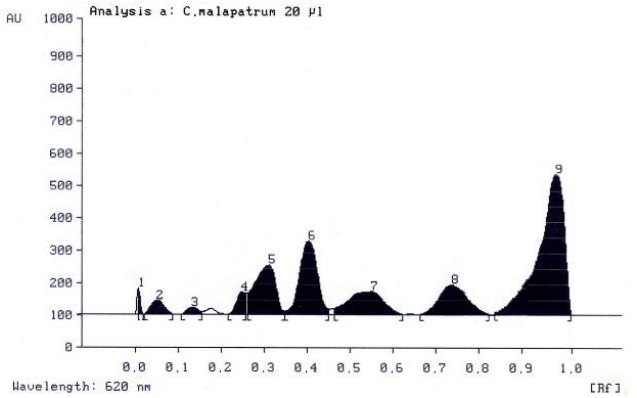

FIGURE 5. *FINGER PRINT OF OF C. MALABATRUM AT • 620 NM*

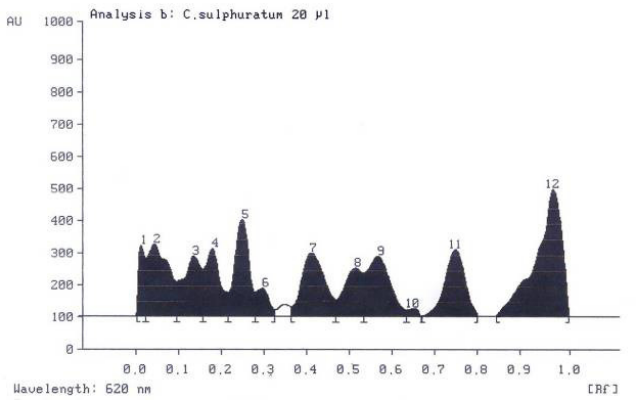

FIGURE 6. *FINGER PRINT OF C. SULPHURATUM AT • 620 NM*

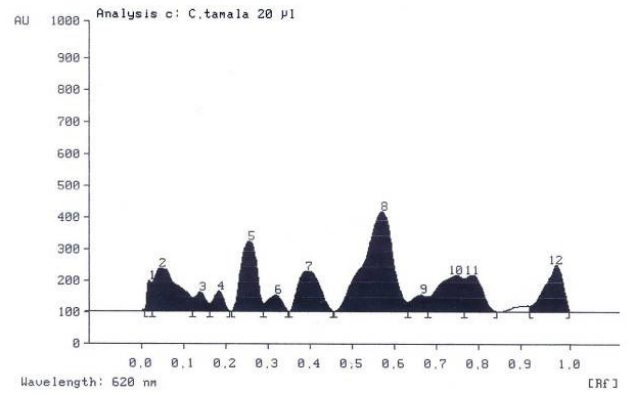

FIGURE 7. *FINGER PRINT OF C. TAMALA AT • 620 NM*

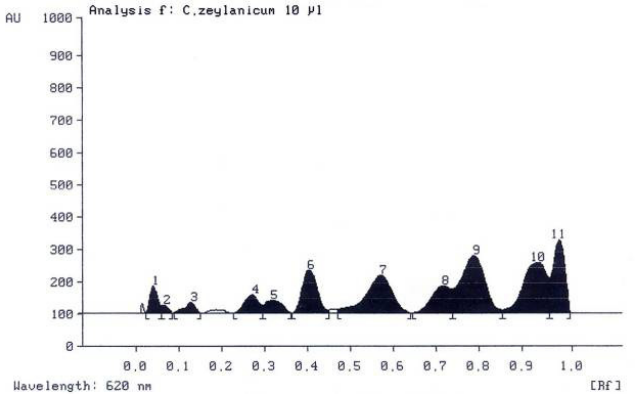

FIGURE 8. *FINGER PRINT OF C. ZEYLANICUM AT • 620 NM*

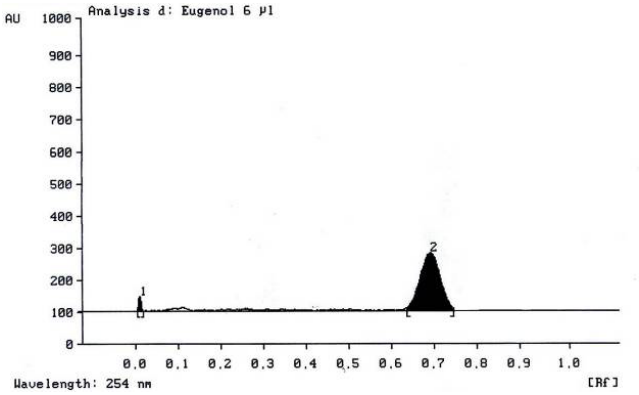

FIGURE 9. *FINGER PRINT OF C. EUGENOL AT • 254 NM*

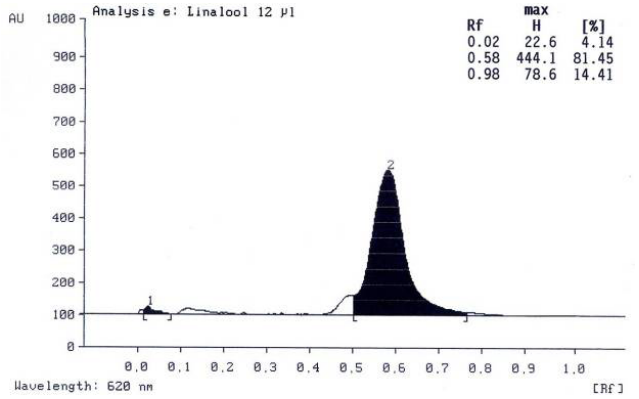

FIGURE 10. *FINGER PRINT OF LINALOOL AT • 620*

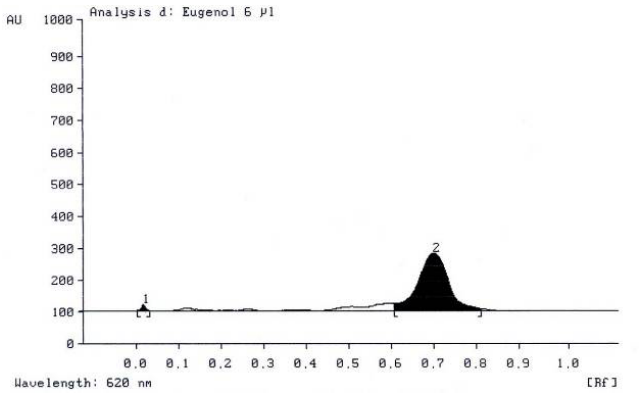

FIGURE 11. *FINGER PRINT OF EUGENOL AT • 620 NM*

In the chromatograms derived under visible light at 620 nm (Fig.6-11), peaks at R_f 0.57 and 0.73 corresponds to linalool and eugenol respectively and their UV super imposable spectra were shown in Fig.12 & 13. *C. malabatrum* showed 7 spots and other species showed 9 peaks each. Peak at R_f 0.13 was common in all species; but major in *C. sulphuratum* and minor in others. Similarly, peak at R_f 0.25 was common in all species but major in *C. sulphuraturm* and *C. tamala*. Peak at R_f 0.31 was major in *C. malabatrum* whereas it is minor in other species. Minor peak at R_f 0.64 of *C. sulphuratum* and 0.66 of *C. tamala* were comparable. Peak at R_f 0.93 of *C. zeylanicum* is specific to it.

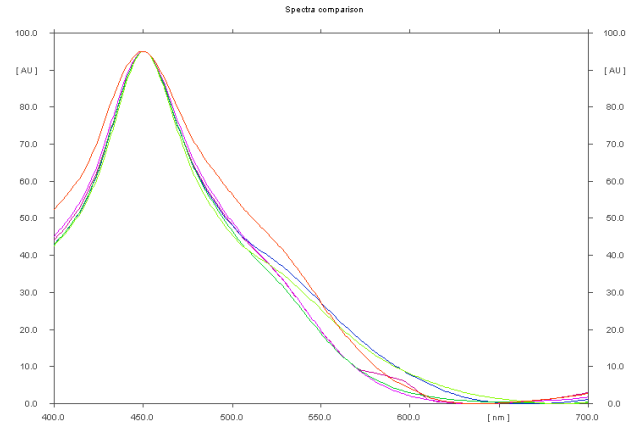

FIGURE 12. *SUPERIMPOSABLE UV SPECTRA OF EUGENOL*

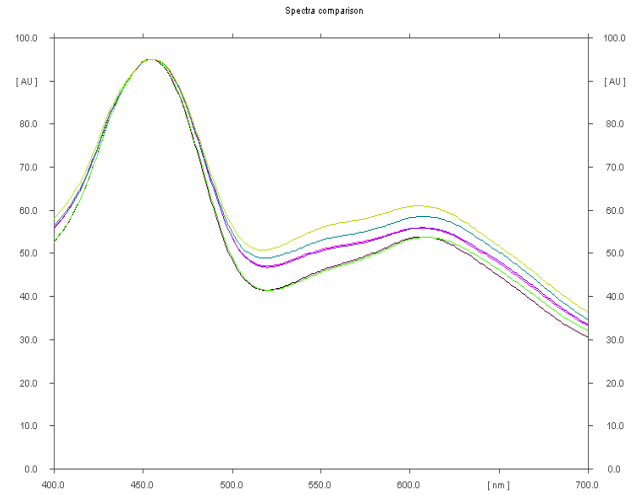

FIGURE 13. *SUPERIMPOSABLE UV SPECTRA OF LINALOOL*

CONCLUSION

C. sulphuratum and *C. tamala* were comparable to each other. The solvent system used in the study was able to differentiate the four species.

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REFERENCES

1. Kostermans AJGH. *The South Indian species of Cinnamomum Schaeffer (Lauraceae)*. Bull Bot. Surv. India. 1986; **25**: 90-133.

2. Cooke TCIE. *Flora of the presidency of Bombay*. London: Taylor & Francis. Vol.**1**; 1903; 282–283.
3. Senanayake VM, Wijesckera ROB. *The volatiles of the Cinnamomum species, Proceedings of the 11th International Congress of Essential Oils*. Frag and Flav. 1990; **4**: 103–120.
4. Nath SC, Baruah A, Hazarika AK. Essential oils of the leaves of *Cinnamomum* Schaefer members. *Indian Perfumer*. 1999; **43**: 473–478.
5. Hussain RA, Kim J, Hu TW, Soejarto DD, Kinghorn AD. Isolation of highly sweet constituent from *Cinnamomum osmophloem* leaves. *Planta Med*. 1986; **5**: 403–404.
6. Nath, S.C.; Baruah, K.S. Eugenol as the major component of the leaf oils of *Cinnamomum impressinervium* Meis. *J Essent. Oil Res*. 1994; **6**: 211–212.
7. Zhou R. *A new species of safrole containing plant Cinnamomum petrophilum N. Chao. In Proceedings of 9th International Congress of Essential Oils, Book 1*, Essential Oils Association of Singapore: Singapore; 1983; 102–103.
8. Peana AT, D'Aquila PS, Panin F, Serra G, Pippia P, Moretti MD. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine* 2002; **9**: 721–726.
9. Maria do Socorro S, Rosa Mendonca-Filho RR, Bizzo HR, Igor de Almeida R, Soares RMA, Souto-Padron T, Alviano CS, Lopes AHCS. Antileishmanial Activity of a Linalool-Rich Essential Oil from *Croton cajucar*. *Antimicrobial Agents and Chemotherapy*. 2003; **47**: 1895–1901.
10. Peana AT, Marzocco S, Popolo A, Pinto A. (-)-Linalool inhibits in vitro NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sci*. 2006; **78**: 719–23.
11. Perrucci S, Cioni PL, Cascella A, Macchioni F. Therapeutic efficacy of linalool for the topical treatment of parasitic otitis caused by *Psoroptes cuniculi* in the rabbit and in the goat. *Medical and Vet. Entomol*. 2008; **11**: 300–302.
12. Benencia F, Courrèges MC. *In vitro* and *in vivo* activity of eugenol on human herpesvirus. *Phytother. Res*. 2000; **14**: 495–500.
13. Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, Fabbri D, Dettori MA, Delogu G, Ponzoni M, Rozzo C. Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. *Molecular Cancer*. 2007; **6**: 8.
14. Ogata M, Hoshi M, Urano S, Endo T. Antioxidant Activity of Eugenol and Related Monomeric and Dimeric Compounds. *Chem. Pharm. Bull*. 2000; **48**: 1467–1469.
15. Guenette SA, Beaudry F, Marier JF, Vachon P. Pharmacokinetics and anesthetic activity of eugenol in male Sprague–Dawley rats. *J. Vet. Pharmacol. & Therapeut*. 2006; **29**: 265–270.