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HPTLC FINGERPRINT PROFILE FOR QUANTITATIVE DETERMINATION OF VARIOUS PHYTOCONSTITUENTS IN *ANISOMELES* SPECIES

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ABSTRACT

In the present communication, finger print of two ethano-botanically important *Anisomeles* species has been developed. A sensitive and reliable densitometric High Performance Thin Layer Chromatography (HPTLC) method has been developed for the quantification of quercetin, β-sitosterol, stigmasterol, catechin and ovatodiolide present in *Anisomeles indica* and *Anisomeles malabarica*. Chromatographic analysis was performed using methanol, chloroform, acetone and ethanol extract of these plants were developed in the different solvents such as toluene, chloroform, ethyl acetate, methanol at various proportions. Detection and quantification of all phytoconstituents was done by densitometric scanning at different wavelengths. These finger prints would be helpful in the standardization of these species.

Key words: HPTLC, *Anisomeles indica*, *Anisomeles malabarica*, phytoconstituents, standardization

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INTRODUCTION

Anisomeles Linn. R. Br. one of the largest genera of the Lamiaceae, is a genus of herbs or under-shrubs, distributed in tropical Asia and Australia. Over all the world 29 species of genus *Anisomeles* are found with huge diversity. Three species found in India yet, *Anisomeles indica, Anisomeles malabarica* and *Anisomeles heyneana*.[1] Out of these *A. indica* and *A. malabarica* were investigated for their biological activities from last 2 decades in India (Anonymous, 1959). *Anisomeles indica* are used in folk medicine all over the India. It is popularly known as 'Jirnya' in northeastern part of India, where it receives widespread used as folk medicine, predominantly in the treatment of intestinal disorders and intermittent fever. *Anisomeles indica* have anti-microbial, astringent, carminative, ethanolic extract (50%) of the herb showed hypothermic activity and when burnt acts as a mosquito repellant. The essential oil present in the herb is useful in uterine affections, [2,3] recently the valued plant investigated for its herbaceous activity.[4] Whereas, *Anisomeles malabarica* useful in halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, intestinal worms, fever arising from teething children, intermittent fever, gout, swelling and diarrhea.[5]

TLC methods for detection of quercetin, β-sitosterol, stigmasterol, catechin and ovatodiolide have been conducted[6]. However, HPTLC method for quantification of phytoconstituents from *Anisomeles* species has not been reported in literature. Densitometric HPTLC, a widely used method for the phytochemical evaluation of herbal drugs, has been developed in the present work for the quantification of phytoconstituents from various extracts of *Anisomeles* species.

MATERIAL AND METHODS

Plant Collection and Identification

Plant samples of the 2 species were collected from their type localities in October 2009. The identity of the plant material was verified by Dr. (Prof.) D.A. Patil botanist, SSVP'S Science College, Dept. of Botany, Dhule (MS), and Dr. (Prof.) H.B Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India. Voucher specimen of the plant material has been deposited at Institute level (HNSIPER/Herb-05 & 06). The collecting localities of the species, Toranmal (MS) and Dindigul, (TN); India.

Preparation of plant extracts

The dried powdered of aerial parts (Stem, leaves, flowers and fruits) of the *A. indica* (AIA) & *A.* malabarica (AMA) were extracted separately with ethanol & acetone using Soxhlet apparatus for 6 hrs. It gives 10.38% ethanolic extract (AIAEE), 34.26 % acetone extract (AIAAcE) of *A.*

indica and, 11.50 % ethanolic extract (AMAEE) and 14.82% acetone extract (AIAAcE) of *A.* malabarica. The methanolic extract of *A. indica* (AIAME -11.50%) & *A.* malabarica (AMAME-11.07%) prepared by refluxing the plant material, separately. The crude extracts are stored in desiccators for future use. For HPTLC fingerprinting, 10 mg of all extracts were dissolved in 10 ml of respective solvent, separately to give concentration 1 µgµL^{-1}.

Preparation of Standard (Marker)

2 mg standard was dissolved in 2 ml of respective solvent, separately to give concentration 1 µgµL^{-1}

Reagents and standard

Toluene, acetone chloroform, ethanol, ethyl acetate and formic acid used were of analytical grade. Standard quercetin and catechin was procured from Yucca Enterprises, Mumbai (India). β-sitosterol and stigmasterol were purchased from Natural Remedies, Mumbai (India).

Development and determination of the solvent system

A highly sensitive and accurate HPTLC method was developed and used for *Anisomeles* species. Chromatographic separation was carried on 10 cm × 10 cm aluminum plates pre-coated with silica gel 60F254 (Merck) as the stationary phase for different extracts prepared from *Anisomeles* species. 10 µl of the sample was applied and different solvent systems were selected for different extracts as per Table 1. The scan was performed at a wavelength of 366 nm and also at visible range. A saturation time of 25 minutes was allowed before chromatographic run.

The sample was spotted on the TLC plate in triplicate with the help of automatic TLC applicator system of the Linomat5_171103" S/N 171103 (1.00.12) on the Merck precoated aluminum sheets of silica gel 60F254. After trying with various solvent systems with variable volume ratio, the suitable solvent system selected is as stated in the Table 1 in proportional ratio and developed in the twin through chamber of TLC to the maximum height of the plate so that it can be able to separate all the components on the polar phase of silica gel and that of mobile phase of solvent system. The components get separated by the principle of adsorption, having differential migration rates of individual component towards the phases.[7]

Development of HPTLC technique

After the development, TLC plate is then removed, dried completely and detected with the suitable detection system as 5% $FeCl_2$ or UV cabinet system for detection of spots. Further it was scanned with the Densitometer (CAMAG, Switzerland) under the UV range of 366 nm. A corresponding densitogram was then obtained in which peaks are appeared for the corresponding

spots being detected in the densitometer while scanning, and the peak area under the curve corresponds to the concentration of the component in the sample for the concentration applied on the TLC plate is given in the Table 2 for different extracts.

Table 1: TLC profile of different extracts of *Anisomeles* species and standards along with R_f values

Solvent System	Detection	Name of Extract	R_f Values
Toluene: Ethyl acetate: Formic acid (5:4:1)	Under UV light at 365nm[9]	AIAME	0.41, 0.55, 0.58, **0.62**
		AMAME	0.41, 0.52, 0.58, **0.62**, 0.78, 0.90, 0.99
		Quercetin	**0.61**
Chloroform: Methanol[10] (8:0.6)	H_2SO_4 (10% in ethanol) Heated in oven at 110^0C for 5-10 min	AIACE	0.31, 0.35, **0.39**, 0.88, 0.94
		AMACE	0.29, 0.30, 0.37, **0.51**, 0.87, 0.96
		β-sitosterol	**0.39**
		Stigmasterol	**0.50**
Toluene: Ethyl acetate: Methanol (4:3:3)	5% $FeCl_3$ solution	AIAAcE	0.11, **0.23**, 0.48
		AMAAcE	0.13, **0.21**
		Catechin	**0.23**
Toluene: Ethyl acetate: Formic acid[11] (7:3:1)	Under UV light at 365nm	AIAEE	0.03, 0.07, **0.58**, 0.72, 0.86, 0.94
		AMAEE	0.04, 0.20, **0.57**, 0.71, 0.91
		Ovatodiolide	**0.58**

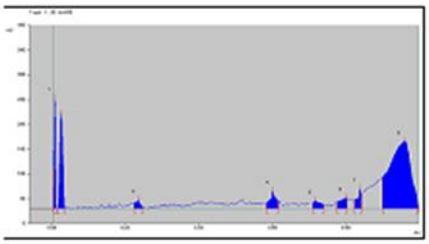
Retention factor R_f and area under curve (AUC) for sample and standard were determined using integration software WinCATS. The quantity of quercetin, β-sitosterol, catechin and ovatodiolide in various extracts were calculated by using linear equation and against the known standard (marker) concentration.[8]

RESULTS AND DISCUSSION

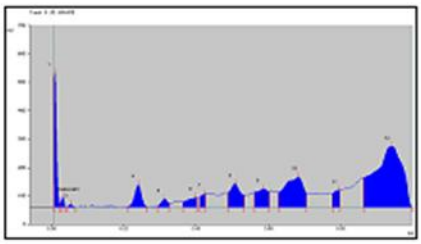
From the HPTLC finger prints, the peak of quercetin was found at R_f 0.60 (AUC=3756.9 for 20µL), β-sitosterol found at R_f 0.39 (AUC=3503.5 for 10µL), catechin found at R_f 0.20 (AUC=1710.2 for 20µL) and ovatodiolide was found at R_f 0.69 (AUC=9686.4 for 20µL). The various extract matches the R_f values with standard, there AUC and quantitative finding given in Table 2. The chromatograms obtained were shown in Figure 1–4.

Peaks with R_f value in respect to 20 µL included in the table as the former represents the point of application and latter represents the movement of the constituents to the solvent front. In the chromatograms derived under UV 365 nm(Figure 1), the peaks at R_f 0.68 in AIAME & AMAMe

Chromatogram 1a: Peak display of AIAME



Chromatogram 1b: Peak display of AMAME



Chromatogram 1c: Peak display of standard Quercetin

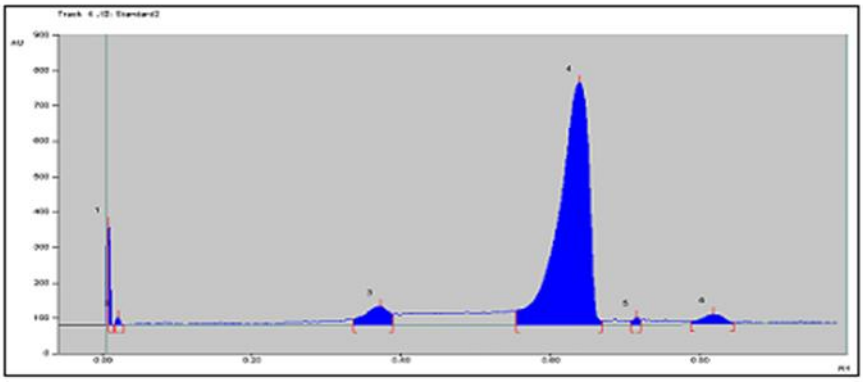
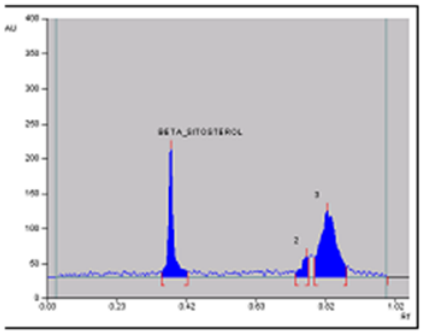
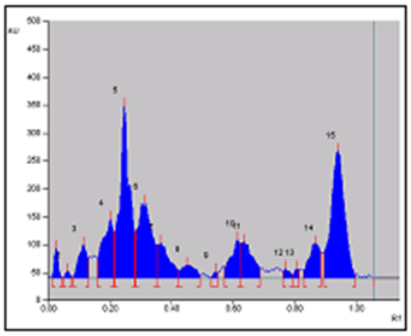


Figure 1: Fingerprint of AIAME, AMAME & Quercetin

Chromatogram 2a: Peak display of AIACE



Chromatogram 2b: Peak display of AMACE



Chromatogram 2c: Peak display of standard β-sitosterol

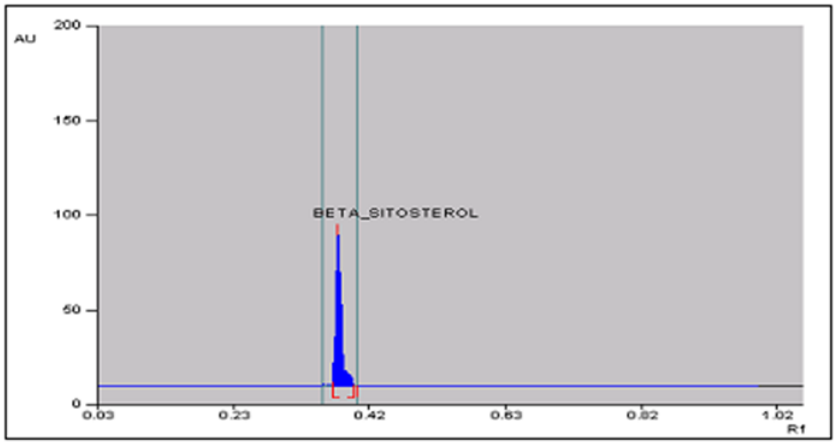
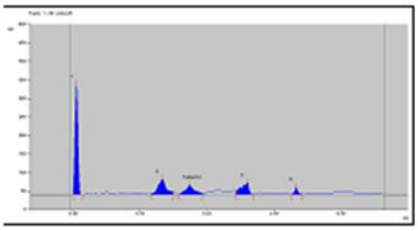
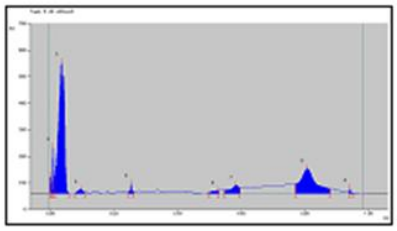


Figure 2: Fingerprint of AIACE, AMACE & β-sitosterol

Chromatogram 3a: Peak display of AIAAcE



Chromatogram 3b: Peak display of AMAAcE



Chromatogram 3c: Peak display of standard Catechin

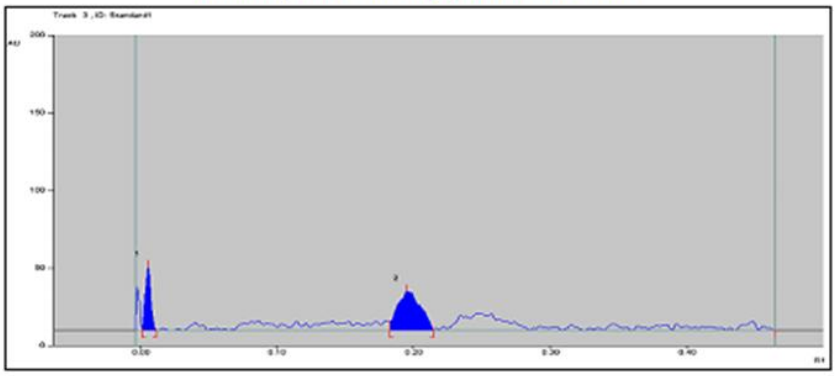
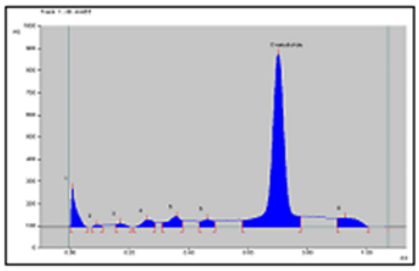
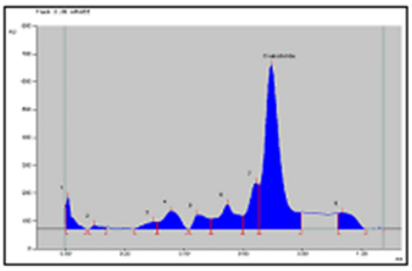


Figure 3: Fingerprint of AIAAcE, AMAAcE & Catechin

Chromatogram 4a: Peak display of AIAEE



Chromatogram 4b: Peak display of AMAEE



Chromatogram 4c: Peak display of standard Ovatodiolide

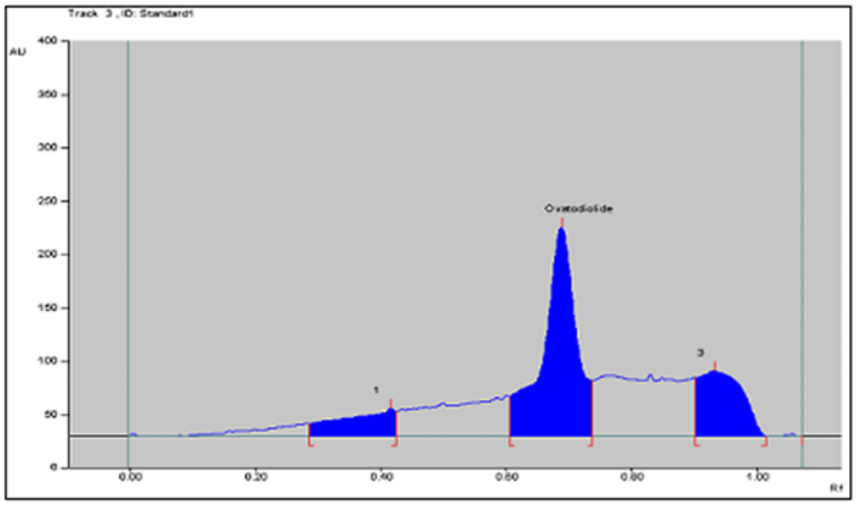


Figure 4: Fingerprint of AIAEE, AMAEE & Ovatodiolide

corresponds to quercetin was not visible in UV light. The peak area of quercetin in AMAME was comparatively higher than AIAME. In the chromatograms derived under visible light at 620 nm after spraying 10% H_2SO_4 (Figure 2), peaks at Rf 0.39 and 0.37 corresponds to β-sitosterol for AIACE and AMACE respectively. Figure 2a for AIACE showed 3 spots and 2b for AMACE showed 15 peaks. Similarly, the chromatograms derived under visible light after spraying 5% H_2SO_4 (Figure 2), peaks at Rf 0.21 and 0.18 corresponds to catechin for AIAAcE and AMAAcE respectively. At 365nm ovatodiolide in AIAEE & AMAEE gives correspond peak at R_f 0.70.

Table 2: AUC, Maximum R_f Values of Peaks Observed and Calculated Quantity for various extracts with respect to plant

Name of Extract	AUC	Maximum R_f Values	Quantityin Extract (µg)	Quantity in plant (%w/w)
AIAME	1032.7	0.68	16.60	0.19
AMAME	62146.9	0.64	60.45	0.669
AIACE	1018.6	0.39	2.90	0.54
AMACE	625.7	0.37	1.78	0.38
AIAAcE	154.2	0.21	1.80	3.08
AMAAcE	195.6	0.18	2.28	1.68
AIAEE	31415.5	0.70	1.855	0.97
AMAEE	28110.9	0.70	1.646	0.96

CONCLUSION

Anisomeles indica and *Anisomeles malabarica* were comparable to each other on basis of phytoconstituents quantities were find using HPTLC. It is also noted that, the proposed HPTLC method was found to be rapid, simple and accurate for quantitative estimation of quercetin, β-sitosterol, catechin and ovatodiolide in various *Anisomeles* species extract. The results of linearity range and correlation coefficient show that, within the concentration range indicated, there was a good correlation between peak area and corresponding concentration of all phytoconstituents.

This method of HPTLC for the different extracts of *Anisomeles* species was very much helpful in determining the quality of the crude drug and also helps to separate and isolate the components using other chromatographic techniques which can be used for further studies.

REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol-III. 4th ed., Dehradun: International Book Distributors; 2007: 2009-13, 2212-4.
2. Annonymous. A Dictionary of India Raw material and Industrial product. 'The wealth of India', Vol- VI. New Delhi: Council of Industrial and Scientific Research; 2003: 295-6.

3. Nadkarni KM. Indian Materia Medica, Vol- I. Mumbai: Popular Prakashan; 2000: 114-5.
4. Batish DR, *et al*: Phytotoxicity of a medicinal plant, *Anisomeles indica*, against *Phalaris minor* and its potential use as natural herbicide in wheat fields. Crop Protection 2007; 26: 948–952.
5. Asolkar, Kakkar and Chakre. Glossary of Indian Medicinal Plants with active principles. Part-I (A-K), New Delhi: National Institute of Science Communication and Information Resources; 1965-1981: 177-81.
6. Ushir YV, Chidrawar VR, Singh SK, Patel KN. Comparative Pharmacognostical standardization of Genus Anisomeles Linn. R. Br. (Lamiaceae). Int J Pharm Sci Res 2010; 1 (11): 65-73.
7. Stahl E. Thin Layer Chromatography: A Laboratory Handbook. 2nd ed., New York: Springer-Verlag Berlin Heidelberg; 2007: 60-102.
8. Wagner H and Bladt S. Plant Drug Analysis, a Thin Layer Chromatography. 2nd ed., New York: Springer-Verlag Berlin Heidelberg; 1996; 1-2: 149-51, 234.
9. Bruneton J. Pharmacognosy and Phytochemistry of medicinal plants. 2nd ed., New York: Lavoisier Publication; 1993: 384.
10. Harborne JB. Phytochemical methods: A guide to modern techniques of plant Analysis, 3rd ed., New Delhi: Springer (India) Pvt. Ltd.; 1998: 110-4, 129-33.
11. Gupta AK, Tandon N, Sharma M. Quality Standard of Indian Medicinal Plants, Vol- 6. New Delhi: Medicinal Plants Unit, Indian Council of Medicinal Research; 2008: 28-37.