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Research Article

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Simultaneous determination and validation of gallic acid and quercetin in *Anisomeles malabarica* R. Br. Ex Sims using high performance thin layer chromatography

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ABSTRACT

To analyse the two bioactive components Gallic acid and Quercetin from the methanolic extract of aerial part of Anisomeles malabarica R.Br. Ex Sims. Chromatography Separation was carried out on aluminium plate precoated with silica gel 60F_{254} with toluene: ethyl acetate: formic acid (5.0:2.5:0.5, v/v/v) as solvent system. Densitometry scanning was performed under reflectance- absorbance mode at 366 nm to quantify the spots. The R_f values of Gallic acid and Quercetin were found to be 0.24 and 0.40 respectively. Linearity of r^2 = 0.999 and 0.998, LOD 0.6 and 0.7 µg/band, LOQ 0.7 and 0.8 µg/band and recovery of 97.62% and 97.55% respectively were satisfactory for Gallic acid and Quercetin. The HPTLC method was validated as per the ICH guideline. The developed simple, precise, specific, sensitive and accurate method can be used for routine quality control of raw material of Anisomeles malabarica R.Br. Ex Sims as well as formulation containing any of these bioactive compounds.

Keywords: HPTLC**,** *Anisomeles malabarica*, Gallic acid and Quercetin.

INTRODUCTION

Anisomeles malabarica R.Br. Ex Sims Plants belong to family Lamiaceae. It is commonly found in Western Ghats from Maharashtra to Karnataka, Andhra Pradesh, Kerala and Tamil Nadu [1]. *Anisomeles malabarica* is useful in halitosis, epilepsy, hysteria, amentia, anorexia, dyspepsia, colic, flatulence, intestinal worms, fever arising from teething children, intermittent fever, gout, swelling and diarrhea [2].

Phytochemical examination revealed the presence of essential oils with citral as the chief chemical constituent. The petroleum ether extract of plant contains a triterpenic acid known as betulinic acid [3] while the hexane extract consists of β-sitosterol and diterpenoids (ovatodiolide and anisomelic acid) [4]**.** The aerial parts contain anisomelolide, malabaric acid, 2-acetoxymalabaric acid, anisomelyl acetate, anisomelol and anisomelin [5]**.** The seeds are mainly comprised of proteins and amino acids, viz., aspartic acid, threonine, serine, glutamine, proline, glycine, alanine, valine, metheonine, isoleucine, leucine, tryptophan, phenylalanine, histidine, lysine and arginine [6]**.** TLC methods for detection of quercetin, β-sitosterol, stigmasterol, catechin and ovatodiolide have been conducted [7] and estimation of Quercetin from the *Anisomeles malabarica* plant was also conducted [8].

Nowadays, HPTLC has become a routine analytical technique due to its reliability quantitation of analytes at nano level estimation and cost effectiveness [9]**.** HPTLC method can be used for fingerprinting of the bioactive phytoconstituents present in the plant extracts. However, HPTLC method for simultaneous quantification of phytoconstituents Gallic acid and Quercetin from *Anisomeles malabarica* has not been reported in literature. Because of HPTLC ample application in routine quality control a need was felt to develop a method for simultaneous analysis of Gallic acid and Quercetin present in the *Anisomeles malabarica* R.Br. Ex Sims plant and this developed method thus can be used for standardization of the formulation containing these two bioactive compounds.

EXPERIMENTAL SECTION

2.1 Collection and Authentication:
The whole plant of *Anisomeles malabarica* R.Br. was collected from Dindigul district region of Tamil Nadu, India. The plant was botanically authenticated. A voucher specimen (MVS-1) of the plant has been deposited at the herbarium of the Botanical Survey of India, Pune.

2.2 Chemicals and Reagents:
Gallic acid and Quercetin was procured from [Sigma Aldrich, Germany]. All reagents of analytical grades; toluene, ethyl acetate, formic acid, and methanol and silica gel $60F_{254}$ precoated TLC aluminium plates purchased from [E-Merck].

2.3 Preparation of standard solutions and Plant samples:
A stock solution of Gallic acid and Quercetin was prepared by dissolving 10 mg of accurately weighed Gallic acid and Quercetin in methanol and making up the volume to 10mL with methanol to get the final concentration of 1mg/mL.

1.0g of plant powder was weighed and 10mL of methanol added and sonicated for 15 minutes. The final concentration of plant extract was made to 10mg/mL.

2.4 Chromatography:
Chromatography was performed, as described previously on 20 cm × 10 cm aluminium plate precoated with silica gel $60F_{254.}$ Samples were applied 6 mm wide and 10 mm apart by means of Camag (Switzerland) Linomat V sample applicator equipped with 100µL syringe. The development of plate was performed using toluene: ethyl acetate: formic acid (5.0:2.5:0.5, v/v/v) as solvent system in 20 cm × 10 cm twin-trough glass chamber previously saturated with mobile phase for 15 minutes.

The plates were dried at room temperature in air and then in oven at 105^0C for 5 minutes. After development and drying the plates were scanned densitometrically at 366 nm for Gallic acid and Quercetin. The peak areas were recorded.

2.5 Method Validation:
ICH guidelines were (CPMP/ICH/381/95; CPMP/ICH/281/95) followed for the validation of the analytical procedure. The method was validated for precision, repeatability and accuracy.

The repeatability of the method was checked by repeated scanning of the same spot of Gallic acid and Quercetin, seven times and was expressed as coefficient of variance (% CV).

Variability of the method was studied by analyzing aliquots of the standard solution of Gallic acid and Quercetin on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % CV.

Table 1: Validation parameters for Gallic acid and Quercetin

Parameters	Gallic acid	Quercetin
Linear working range(µg/band)	0.6-1.3	0.7-1.4
Correlation coefficient(r)	0.999	0.998
Limit of Detection(LOD)(µg/band)	0.6	0.7
Limit of Quantification(LOQ)(µg/band)	0.7	0.8
Repeatability (% R.S.D, n =6) Aerial powder of *Anisomeles malabarica* R.Br	0.05	0.13
Intermediate precision (% R.S.D, n= 18) Aerial powder of *Anisomeles malabarica* R.Br	0.040	0.049
Stability of standard solution	Stable for 24 hours	Stable for 24 hours
System suitability	1.31	1.84
R_f(% R.S.D. , n =6) Peak area (% R.S.D., n=6)	0.013	0.465
Assay (mg/g) Aerial powder of *Anisomeles malabarica* R.Br	0.360	0.459
Percent recovery Aerial powder of *Anisomeles malabarica* R.Br	97.62%	97.55%

Accuracy of the method was tested by performing recovery studies at three levels (50%, 100% and 150% addition). The percent recovery as well as average percent recovery was calculated. For the determination of limit of detection and limit of quantification, different dilutions of the standard solutions of Gallic acid and Quercetin were applied along with methanol as well as the blank and determined on the basis of signal to noise ratio.

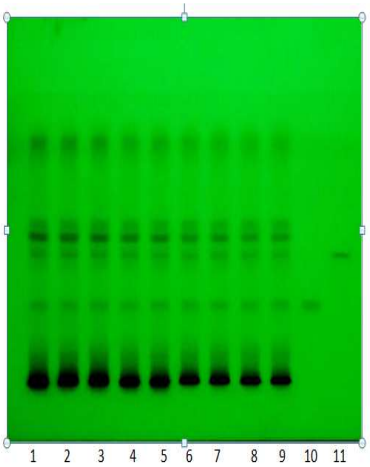

Figure 1: Photograph of developed TLC plate

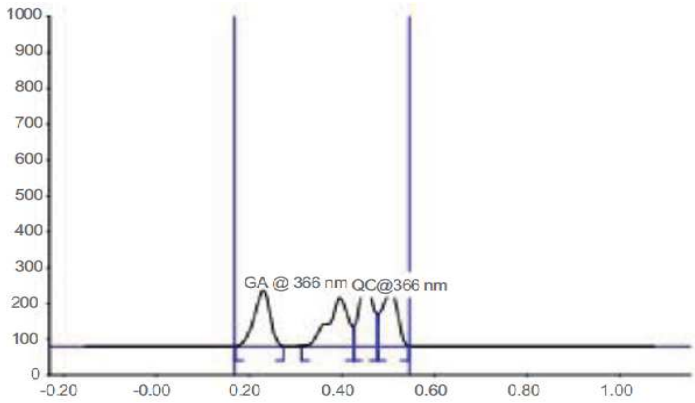

Figure 2: HPTLC chromatogram of methanolic extract of aerial powder of Anisomeles malabarica

RESULTS AND DISCUSSION

Different concentration of standard solution of Gallic acid and Quercetin were applied in triplicate on HPTLC plates and methanolic extract of plant extract was applied on HPTLC plate for estimation of Gallic acid and Quercetin. The HPTLC plates were developed in a solvent system toluene: ethyl acetate: formic acid (5.0: 2.5: 0.5, v/v/v) and dried in air and scanned densitometrically at 366 nm for Gallic acid and Quercetin.

The peak areas were recorded. The method was validated in terms of precision, repeatability and accuracy (Table 1). The relationship between the concentration and peak response was linear within the range of 0.6 to 1.3µg/band for Gallic acid and 0.7 to 1.4µg/band for Quercetin with correlation coefficient of 0.999 and 0.998 respectively.

The % recovery of Gallic acid and Quercetin were found to be 97.62% and 97.55%, 99.28% respectively.

CONCLUSION

The HPTLC method was developed for simultaneous quantification of Gallic acid and Quercetin in presence of other plant constituents. The proposed method was found to be precise, simple, specific and sensitive.

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