RESEARCH ARTICLE

Isolation of Ethyl Acetate Fraction of Methanolic Extract of *Cinnamomum malabatrum* Leaves by Column Chromatography

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ABSTRACT

Cinnamomum malabatrum (Lauraceae) is available in the Western Ghats and south India, the leaves were collected shade dried and made into fine powder form, and the powder was subjected to extraction by different solvents like petroleum ether, methanol, and water. Further, these extracts were subjected to thin layer chromatography.

C. malabatrum ethyl acetate fraction of methanolic extract was given strong evidence for active components, based on evidence ethyl acetate fraction was subjected for further chromatographic separation; column chromatography was used for isolation of component of *C. malabatrum* ethyl acetate fraction of methanolic extract was subjected to column chromatography by using different ratios of mobile phases (in Hexane and Methanol). Flavonoids and phenols found present.

Keywords: *Cinnamomum malabatrum*, Flavonoids, Phenols, Column chromatography.

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Conflict of interest: None

INTRODUCTION

The purpose of this investigation was to detail the procedure for isolating the active ingredients using column chromatography.[1] Separating molecules in complicated mixtures is a frequent application of column chromatography.[2] *Cinnamomum malabatrum* ethyl acetate fraction of methanolic extract was gave strong evidence for active components, based on this evidence ethyl acetate fraction was subjected for further chromatographic separation.[3,4]

MATERIALS AND METHODS

All the materials and equipment were purchased from UV Scientifics, Hyderabad.

Protocol

The size of the column-chromatography stand's long cylindrical glass column should be determined by the number of samples to be taken. The ethyl acetate fraction was dried completely and then powdered with silica gel for use in a pre-packed silica gel column. Carefully cover the sample powder with a layer of cotton and set it in the column. The extract was fractionated by passing solvents through a column at a constant rate while using gravity. Thin layer chromatography (TLC) was used to further analyze the fractions, which were each collected in their own test tube and numbered in order. TLC is beneficial for verifying the purity of fractions. A capillary tube is used to apply each fraction to activated TLC plates half an inch separately from the lesser side of the plates; are then placed in a developing chamber equipped with a solvent appropriate for the application. After removing the TLC plate; it is allowed to dry. Bands or spots on a TLC chromatoplate can be utilized as indicators of the presence of specific substances through pictorial findings with UV light at 254 nm in an iodine chamber and application of spray chemical. Marking the spots where components can be seen on the chromatoplate to determine Rf value for each spot makes up. Additional spectrum analysis can be performed based on the chemicals' composition.[5,6]

Method

Fractionation of ethyl acetate extract by Column chromatography

It was used to separate phytoconstituents from ethyl acetate extract (EAE). A vertical column made of borosilicate glass (40 mm x 60 cm) was used for separation (Figure 1). Before being packed, the column was given a thorough acetone rinsing and dried thoroughly. A piece of glass wool was inserted into column's base using the rod. Added sea sand with a particle size between 50 and 70 was applied to the top of the glass wool. A centimeter up was a layer of sea sand with 50–70 micron particles. The sand grains were cleaned with the solvent. By turning off the flow of Hexane, the column was filled to within

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three-quarters of its capacity. A 200 grams of 60–120 mesh silica gel were utilized as the packing material. To aid in the packing of the column, a hexane-silica slurry was made and poured from the top, filling about two-thirds of the column while the solvent was drained at the same time. Silica slurry was topped off with 1-cm of sea sandand sand particles were washed clean with solvent. From top of column, 20 gm of EAE was combined with the smallest possible amount of hexane, and the mixture was poured down the sides and rinsed off. One centimeter of sea sand was piled on top of the extract. To keep column from drying out, the amount of solvent was kept at a constant 6 cm above the extract. By altering the ratio of low-polarity (i.e. hexane) to high-polarity (i.e. methanol) solvents, we were able to isolate distinct fractions of EAE (Table 1). The volume of solvent collected per fraction averaged 40 mL, and the flow rate (5 mL/min).[7]

TLC of fractions

TLC (Silica gel 60 F254) analysis was performed on each fraction separately to discover phytoconstituents. Spraying TLC plates with a vanillin-con. H_2SO_4 solution (15 g vanillin in 250 mL of ethanol + 2.5 mL of con.H_2SO_4) and then drying them at 100 C in a hot air oven for 20–30 minutes. An Rf value was calculated for every spot. The combination was concentrated using a rotary evaporator after being blended with other substances having similar Rf values. Dry weights of the fractions were determined. Then, the EAE and concentrated fractions were tested for phenolic antioxidants.[8]

HPLC analyses of fractions and EAE

HPLC outlines of EAE and separated portions of *C. malabatrum* were obtained using two distinct procedures, each employing a different mobile phase, and utilising variablestages of solvent schemes in prescribed retention times and elute findings. Binary gradient mode, Luna 5µ C18 reverse-phase analytical column (250 × 4.6 mm), SPD-M10A VP (PDA), 1-mL/min total flow rate, 25°C column oven temperature, and 280 nm detection wavelength were used to analyse all samples on a Shimadzu LC-10 AT VP. Five pieces of EAE, each weighing 5 mg, were liquefied in 3 mL of methanol for analysis. Mobile phases were filtered via a millipore membrane and degassed before being used. Quercetins, ascorbic, gallic, benzoic acids and resorcinol were all used as standards in the analysis of phenolic composites.

Method A

Table 1: Column chromatography yield of *C. malabatrum N – Haxane: Methanol 20 : 80, 10 : 90 and 0 : 100*

Number of elutes	*Solvent phase*	*Fraction*	*Yield of fraction*
1–25	H : M (100 : 0 and 90:10)	Fraction 1	2.23
26–74	H : M (80 : 20 and 70: 30)	Fraction 2	3.34
75–89	H : M (60: 40 and 50: 50)	Fraction 3	3.97
90–118	H: M (40 : 60 and 30: 70)	Fraction 4	4.32
119–132	H : M (20 : 80 and 10 : 90 and 0 : 100)	Fraction 5	5.45

In approach A, HPLC analysis was used to determine the concentrations of ascorbic, gallic, benzoic acids and resorcinol. Solvent A (Acetonitrile) and solvent B (Methanol) were utilized in a gradient elution to determine concentrations.We started our gradient elution programme with 92% solvent B and maintained that concentration for the first 0 to 35 minutes. After that, for the next 35 to 45 minutes, solution B consisted of 78%. The whole thing took 45 minutes.

Method B

Method B HPLC analysis was carried out. The chemicals were measured using a gradient elution technique with two solvents, solvent A (acetonitrile), and solvent B (methanol). The gradient elution protocol started with solvent B at 100% concentration and was maintained there for zero to four minutes. After then, the concentration of solvent B was increased to 50% for 4 to 10 minutes, dropped to 20% for 10 to 20 minutes, and then returned to 50% for the remaining 30 minutes. A total of 60 minutes was the full length.[9,10]

RESULTS

The CM EAE silica gel column chromatography fractions were sprayed with vanillin-con. H_2SO_4 spray and dried at 100ºC in a hot air oven for 20 to 30 minutes before being verified for finding of numerous phytoconstituents by TLC.[11] Similar Rf values among phytocompounds were grouped together.[12] After combining, following was the sum of active fractions: Fraction A was made up of aliquots of 40 mL each that had been diluted in medium schemes H:M (100:0 and 90:10); Fraction B was made up of aliquots of 40 mL each that had been diluted H:M (80:20, 70:30, and 60:40); Fraction C was made up of aliquots of 40 mL each that had been diluted H:M (50:50). Tables 2 to 7 display yields of obtained fractions. Five chemicals, including ascorbic acid, resorcinol, gallic acid, benzoic acid and quercetin, were analysed using HPLC profiles for both EAE and *C. malabatrum* fractions. A 2.875 minute ascorbic acid time, a 6.097-minute gallic acid time and a 12.850 minute resorcinol time. Quercetin (13.694) and benzoic acid (39.809 minutes). Fraction –1: Ascorbic acid (2.909 minutes), benzoic acid (40.074 minutes) and quercetin (14.004 minutes) were present and gallic acid and resorcinol were not clear. Fraction –2 : Ascorbic acid (2.879 minutes), gallic acid (5.977 minutes), resorcinol (12.629 minutes), benzoic acid (40.710 minutes), quercetin (13.859 minutes). Fraction – 3: Ascorbic acid (2.888 minutes), gallic acid (5.795 minutes), resorcinol (12.746 minutes), quercetin (13.714 minutes). Fraction –4: Ascorbic acid (2.862 minutes), Resorcinol (12.469 minutes), quercetin

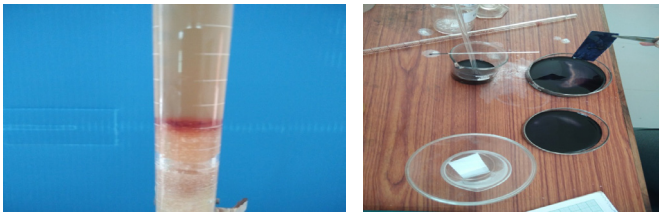

Figure 1: Column separation of components Development of TLC Plates

Table 2: Retention time's of compounds present in EAE of *C. malabatrum*

Phenolic compounds	*Retention time*	*Area*	*Height*	*Conc.*
Ascorbic acid	2.875	49,673	17,083	74.211
Gallic acid	6.097	23,216	242	0.4121
Resorcinol	12.850	17,439	148	0.5234
Benzoic acid	39.809	517,234	20,457	248.00
Quercetin	13.694	59,139	10,164	4.219

Table 3: Retention time of FRACTION – 1 Solvent System H: M (100: 0 and 90:10)

Phenolic compounds	*Retention time*	*Area*	*Height*	*Conc.*
Ascorbic acid	2.909	30.891	12,114	40,281
Gallic acid	-	-		-
Resorcinol	-	-	-	-
Benzoic acid	40.74	314,210	69,49	210,011
Quercetin	14.040	66,372	14,781	60,201

Table 4: Retention time of FRACTION – 2 Solvent System H: M (80: 20 and 70: 30)

Phenolic compounds	*Retention time*	*Area*	*Height*	*Conc.*
Ascorbic acid	2.879	40,621	11367	71.50
Gallic acid	5.977	6592	621	1.149
Resorcinol	12.629	3145	189	1.078
Benzoic acid	40.710	49,127	3648	40.008
Quercetin	13.859	72,124	11219	5.981

Table 5: Retention time of FRACTION – 3 Solvent System H: M (60: 40 and 50: 50)

Phenolic compounds	*Retention time*	*Area*	*Height*	*Conc.*
Ascorbic acid	2.880	29,254	10,481	51.210
Gallic acid	5.795	1458	149	0.327
Resorcinol	12.746	2497	185	2.241
Benzoic acid	-	-	-	-
Quercetin	13.714	521,258	91,458	44.128

Table 6: Retention time of FRACTION – 4 Solvent System H: M (40: 60 and 30: 70)

Phenolic compounds	*Retention time*	*Area*	*Height*	*Conc.*
Ascorbic acid	2.862	28418	10614	51.12
Gallic acid	-	-	-	-
Resorcinol	12.469	43258	3214	18.320
Benzoic acid	39.918	7841	729	5.239
Quercetin	13.958	10,458	10541	7.251

Table 7: Retention time of FRACTION - 5 Solvent System H: M (20: 80 and 10: 90 and 0: 100)

Phenolic compounds	*Retention time*	*Area*	*Height*	*Conc.*
Ascorbic acid	2.868	58215	19210	114.21
Gallic acid	5.994	16587	1098	2.215
Resorcinol	12.585	5982	621	3.148
Benzoic acid	40.552	12754	2181	6.421
Quercetin	13.562	10714	21851	9.218

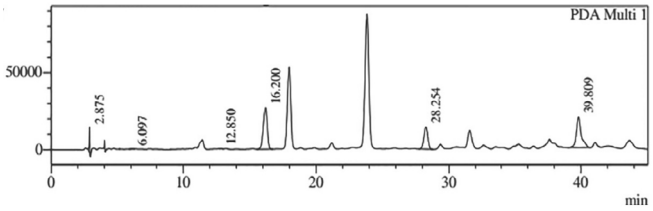

Figure 2: HPLC Data of Phenolics Present in CM Extract

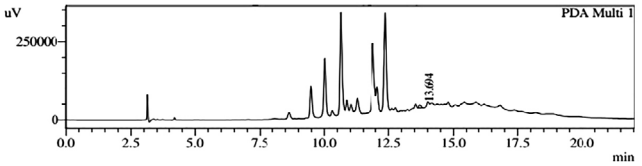

Figure 3: HPLC Data of Quercetin Existing in ethyl acetate fraction of CM

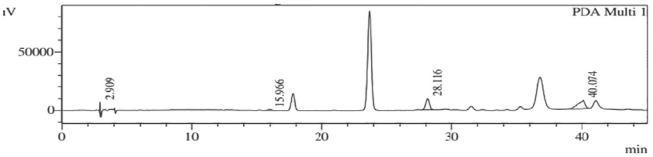

Figure 4: HPLC Data of Phenolics Existing in Fraction –1

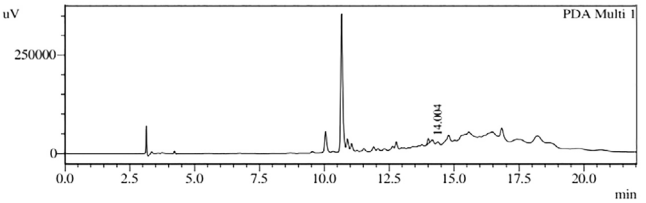

Figure 5: HPLC Data for Quercetin Existing in Fraction - 1

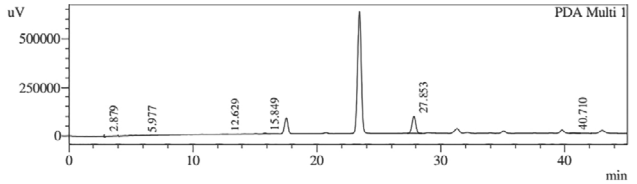

Figure 6: HPLC Data for Phenolics Existing in Fraction - 2

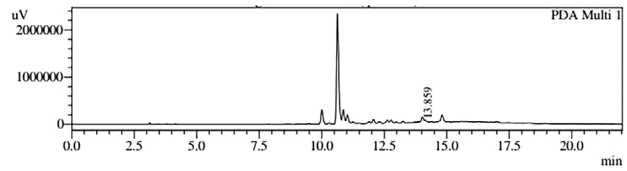

Figure 7: HPLC data for quercetin existing in Fraction - 2

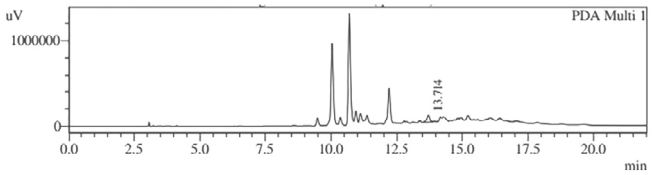

Figure 8: HPLC data for phenolics existing in Fraction - 3

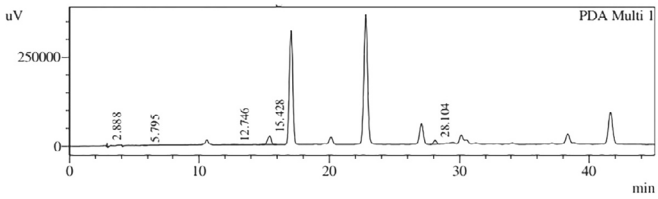

Figure 9: HPLC data for quercetin existing in Faction – 3

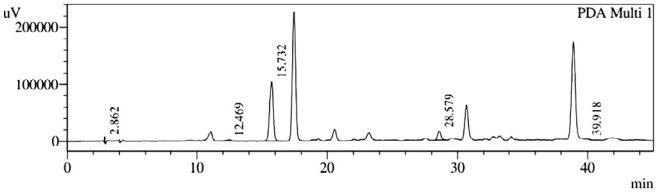

Figure 10: HPLC data for phenolics existing in Fraction - 4

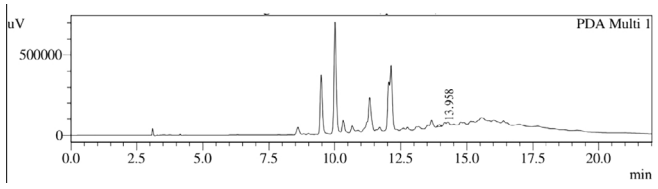

Figure 11: HPLC data for quercetin existing in Fraction – 4

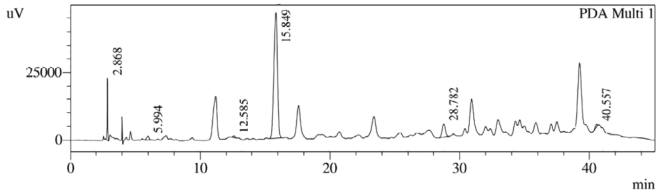

Figure 12: HPLC data for phenolics present in Fraction - 5

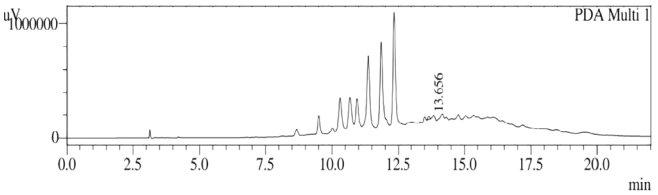

Figure 13: HPLC data for quercetin existing in Fraction – 5

(13.958 minutes) and benzoic acid (39.918 minutes). Fraction – 5: Resorcinol (12.585 minutes) benzoic acid (40.557 minutes), quercetin (13.656 minutes), ascorbic acid (2.868 minutes) and gallic acid (5.994 minutes) (Figure 2 to 13).

CONCLUSION

In column chromatography by using different ratios of mobile phases (n-Hexane and Methanol), as per bands formed in the column tube, mobile phase were collected distinctly and subjected to the TLC, and were developed using vanillin - con H_2SO_4, and also observed under UV light, then all fractions were subjected to HPLC study with PDA detector, here we have got deferent chromatograms with peaks and Rt. Based on the HPLC results, we reported the presence of flavonoids and phenols, As per literature evidence, which shows anticancer, anti-diabetic, anti-oxidant, and hepatoprotective activities and thus, further Pharmacological evaluation of selected fractions is recommended.

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