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Phytochemical screening and GC MS analysis of *Medinilla beddomei* C B Clarke Leaf

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Abstract
Medinilla beddomei C B Clarke is an epiphytic shrub belongs to the family melastomataceae. The present study was carried to determine the phytochemical components present in the petroleum ether, chloroform, acetone and methanol leaf extracts of *M. beddomei.* The study revealed that leaf extracts of *M. beddomei* consist of different phytochemical compounds like alkaloids, phenols, flavonoids, tannins, terpenoids, saponins and steroids. Acetone leaf extracts of the plant had the highest amount of phenol and flavonoid contents (26.81±0.42 mg gallic acid equivalent/g and 3.84±0.20 mg quercetin equivalent/g respectively). GC MS analysis of acetone leaf extract showed 11 phytocompounds. Presence of these compounds makes the plant more valuable for treating various ailments.

Keywords: *Medinilla beddomei*, phytochemicals, GC MS analysis, acetone extract, fever

Introduction
Medinilla Gaudich. ex. DC, is an old Afro-asiatic genus consist of 430 species. Among these four species are from Penisular India. *M. malabarica, M. beddomei, M. anamalaiana and M. sahyadrica* are endemic to the Southern Western Ghats [1]. The forests of Western Ghats of Kerala are a rich house of traditional medicinal and commercially beneficial plants. Phytochemical investigation suggests that members of the genus Medinilla possesses valuable phytochemical compounds like phenol, flavonoids and tannin and also used in traditional medicine [2-4]. *Medinilla beddomei* C B Clarke is an epiphytic climbing shrub native to Southern Western Ghats [5]. Leaves of this epiphytic plant are eaten freshly by Muthuva and Irular, tribal groups in Idukki for reducing the body heat due to fever [6, 7] and also it is used as a refresher while going into the forest. [8]. The present study deals with the phytochemical screening of the petroleum ether, chloroform, acetone and methanol leaf extracts of *M. beddomei* and GC MS analysis of the acetone leaf extract of *M. beddomei* to assay the phytochemical compounds.

Fig 1: *Medinilla beddomei* Habit

Materials and Methods
Collection of the plant sample
Medinilla beddomei was collected from Sholayar reserve forest, Thrissur during the month of June 2021 and taxonomically identified based on the flora of presidency of madras. The voucher specimen No. 18372 was deposited at KFRI, Thrissur for further reference.

Preparation of the Plant extracts
The plant sample was collected from the natural habitats and thoroughly washed to remove the dirt and debris. The leaves were air dried and powdered with mechanical grinder. Forty gram of the powder was successively extracted with 200 ml of petroleum ether, chloroform, acetone and methanol using Soxhlet extractor for 8-12 h at a temperature of the boiling point of the solvents. The residues of the plant samples were obtained and stored in a refrigerator at 4 °C for further studies.

Qualitative and Quantitative analysis of the plant extracts
Phytochemical analysis of the *M. beddomei* leaf in different extracts were carried out according to the standard methods [9, 10]. Quantitative estimation of total phenol was conducted using modified protocol [11] and the total phenolic content in the samples were expressed as milligrams of gallic acid equivalent per gram of dry weight of the sample and the total flavonoid was quantified based on the method of Chang *et al*., 2002 [12]. Quercetin was used as standard for the study.

Identification of the components
Gas chromatography is a widely used method to detect the phytochemical compounds present in the plant extracts. Here volatile components of acetone extract of *M. beddomei* leaf were identified and quantified by using GC MS analysis. The components were identified by comparing the MS fragmentation pattern of unknown compounds with the pattern of known compounds stored in National Institute of

Standards and Technology (NIST) and Wiley mass spectra libraries.

GC MS analysis

GC/MS analysis was done with Shimadzu QP-2010 Plus with thermal desorption system TD 20, fitted with a 60 m × 0.25 mm × 0.25 m WCOT column coated with diethylene glycol (AB-Innowax 7031428, Japan). Helium was used as the carrier gas with a flow rate of 1.21 mL/min at a column pressure of 77.6 kPa. The injector and detector temperatures were set at 260 °C. Samples (8 µL) were injected into the column with a split ratio of 10:0. Component separation was achieved with a linear temperature program of 70 - 260 °C with a total run time of 65.32 min. The MS parameters were: electron ionization (EI) voltage 70 eV, peak width 2 s, mass range 40 - 700 m/z and detector voltage 1.5 V. Compounds were identified by their retention time (RT) and elucidation of mass spectra. It was compared with NIST and WILEY libraries.

Results

The phytochemical Analysis

The qualitative analysis of the phytochemicals of petroleum ether, chloroform, acetone and methanol leaf extracts of *M. beddomei* revealed the presence alkaloids, glycosides, phenols, flavonoids, tannins and saponins. Coumarins was absent in all the extracts. Results of the preliminary phytochemical analysis are depicted in table 1

Table 1: Preliminary phytochemical screening of *M. beddomei* leaf extracts

Sl. No.	Phytochemicals	Petroleum Ether	Chloroform	Acetone	Methanol
1	Alkaloid	+	+	+	+
2	Glycosides	+	+	-	+
3	Phenol	+	+	+	+
4	Flavonoids	-	+	+	+
6	Terpenoids	+	+	+	+
7	Tannins	+	+	+	+
8	Steroids	-	-	+	-
9	Saponins	-	+	-	+
10	Coumarins	-.	-	-	-

Quantitative estimation of total phenol and flavonoid content were estimated and represented in table 2. The average triplicate assays showed that phenol content of all the leaf extracts of *M. beddomei* varied from 14.43 mg gallic acid equivalent/g to 26.81 mg gallic acid equivalent/g. Also total flavonoid content showed variations from 0.28 mg quercetin equivalent/g to 3.84 mg quercetin equivalent/g. *M. beddomei* leaf extracts exhibited more phenol content than flavonoid (Figure1).

Table 2: Quantitative estimation of total phenol and flavonoid content of *M. beddomei* leaf extracts

Sl. No.	Total phenolics (mg GAE/g ± SD)	Total flavonoid (mg QE/g ± SD)
Petroleum Ether	$14.43^{\pm 0.13}$	$0.28^{\pm 0.03}$
Chloroform	$20.80^{\pm 0.10}$	$2.21^{\pm 0.03}$
Acetone	$26.81^{\pm 0.42}$	$3.84^{\pm 0.20}$
Methanol	$23.74^{\pm 0.11}$	$2.48^{\pm 0.08}$

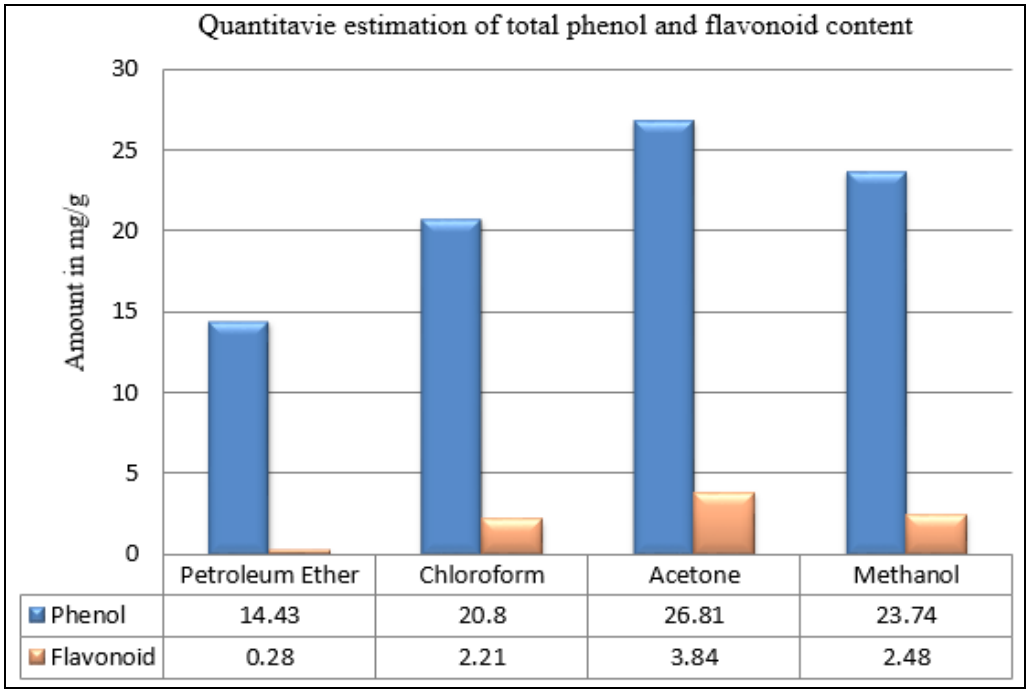

Fig 2: Graphical analysis of Total phenol and flavonoid content in *M. beddomei* leaf extracts

GC MS analysis

Gas chromatography is a widely using tool for the detection of volatile and semi volatile components. Even trace amount of volatile components can be detected through this technique. Acetone extract of *M. beddomei* leaf revealed the presence of 11 compounds. The phytochemical compounds with their Retention Time (RT), molecular formula, molecular weight and peak area in percentage are presented in table 3.

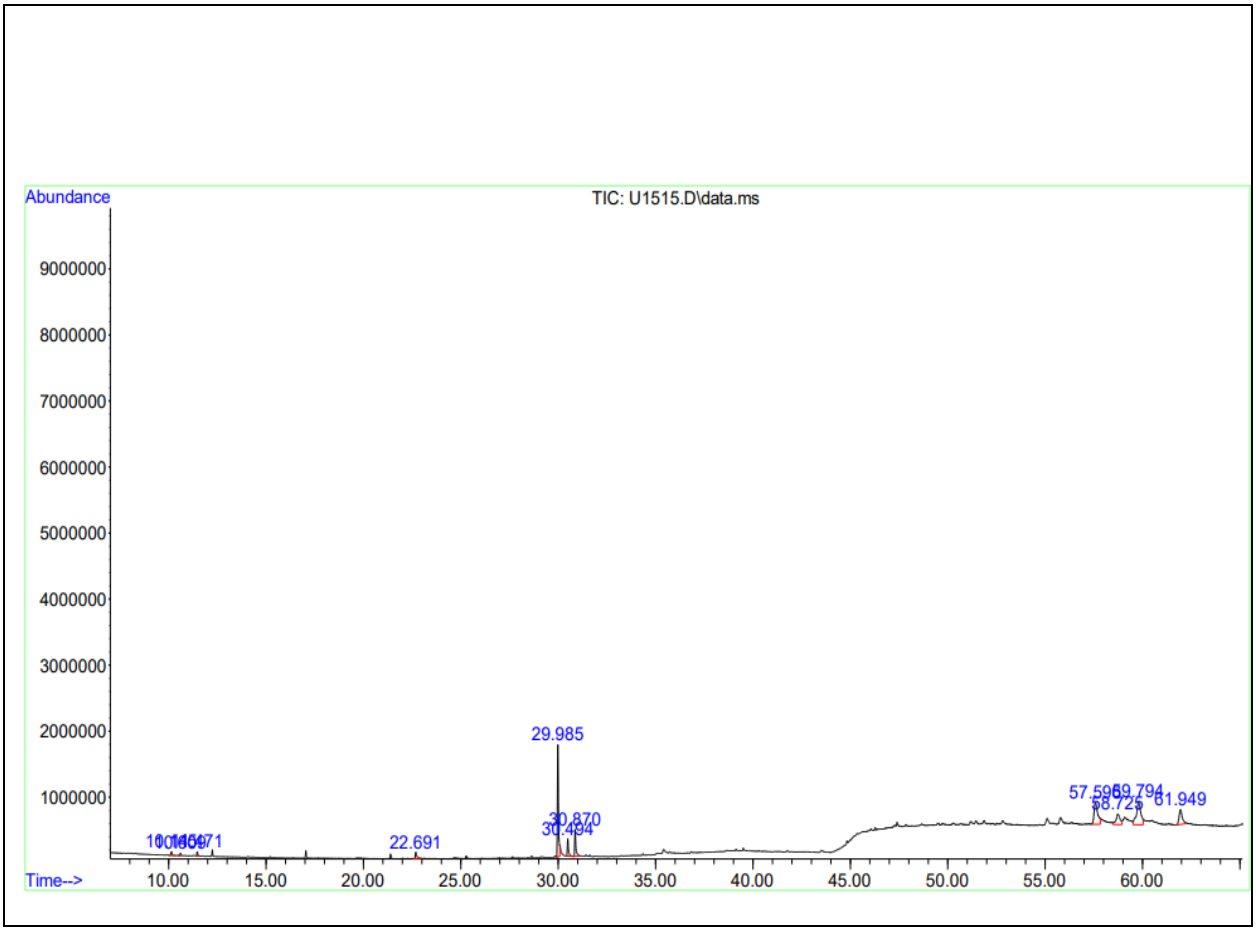

Fig 3: GC-MS chromatogram of the acetone extract of *M. beddomei* Leaf

Table 3: Phytochemical compounds detected in acetone leaf extract of *M. beddomei*.

Sl. No.	Retention Time	Area %	Compound Name	Molecular Formula	Molecular Weight
1	10.145	0.873	Cis decahydronaphthalene	$C_{10}H_{18}$	138
2	10.609	0.480	Hexachloromethane	C_2Cl_6	234
3	11.471	0.926	Spiro [4,5] decane	$C_{10}H_{18}$	138
4	22.691	2.026	2,4-Bis(1,1-dimethylethyl) phenol	$C_{14}H_{22}O$	206
5	29.985	24.498	Phytol	$C_{20}H_{40}O$	296
6	30.494	4.305	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
7	30.870	6.929	3-Eicosyne	$C_{20}H_{38}$	278
8	57.596	17.499	ɣ sitosterol	$C_{29}H_{50}O$	414
9	58.725	9.556	Olean-13 (18)-ene	$C_{30}H_{50}$	410
10	59.794	22.398	28-Norolean-17-en-3-one	$C_{29}H_{46}O$	410
11	61.949	10.510	Stigmast-4-en-3-one	$C_{29}H_{48}0$	412

Discussion and conclusion

The present study revealed that *M. beddomei* leaf extracts of petroleum ether, chloroform, acetone and methanol consist of valuable phytochemical compounds like alkaloids, flavonoids, phenol, glycosides, tannin and saponins. Also acetone leaf extract showed the increased amount of phenol and flavonoid content. Several studies showed that phenol and flavonoid possess many useful properties like antimicrobial, anti-allergic, antioxidant, antitumar and cytotoxic activities [13, 14]. GC MS analysis of acetone extract of *M. beddomei* revealed the presence of some active compounds like spiro [4, 5] decane, 2, 4-bis (1,1-dimethylethyl) phenol, phytol, ɣ sitosterol and stigmast-4-en-3-one. Phytol was present in the higher concentration in acetone leaf extract, which shows several pharmacological properties like antihyperalgesic, anti-inflammatory and antiarthritic effects [15, 16]. Also 2, 4-bis (1, 1-dimethylethyl) phenol is an another active component present in various plants known for antibacterial and anti-inflammatory activities [17]. Other compounds like ɣ sitosterol and stigmast-4-en-3-one showed antibacterial, anticancerous, antiviral and hypoglycaemic effects [18-21]. The present study revealed that *M. beddomei* is an important phyto pharmaceutical plant. It is traditionally used for the treatment of fever due to

the presence of various bioactive molecules. So further research is needed to identify their potential therapeutic activity.

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