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Phytochemical Screening, Isolation and Characterization of Bioactive Compounds from Ethanol Extract of *Memecylon lushingtonii* Gamble

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The primary goal of this study was to isolate and characterize the bioactive compounds present in the *M. lushingtonii* Gamble leaves using ethanol as solvent for the extraction using Soxhlet apparatus for 8 h. Based on the chemical analysis, column chromatography using different solvents depending on polarity further isolated the components. Isolation of the extract was carried out using toluene and ethyl acetate solvents with varying concentrations and showed two single spots by TLC at the rentation time (Rt) of 0.66 and 0.72 for the solvent's toluene and ethyl acetate (8:12 and 2:18), respectively. Various spectroscopy techniques (UV, NMR, IR, and MASS) were employed for the characterisation of the compounds and elucidation of their structures. The results confirmed the presence of alkaloids, phenolics, flavonoids, tannins, sterols and terpenoids. The spectroscopic analysis revealed the compounds were quercetin (R_f: 0.66) and luteolin (R_f: 0.72) which were both flavonoid group and contains five and four –OH groups, respectively in their basic structure (C6-C3-C6). The results demonstrated that the leaves of *M. lushingtonii* Gamble exhibited a higher concentration of polyphenolic chemicals, which contribute to the diverse therapeutic properties.

Keywords: *M. lushingtonii* Gamble leaves, Bioactive compounds, Phenolics, Flavonoids.

INTRODUCTION

Plant chemistry contributes for the discovery of thousands of novel compounds every year. The chemical modification, derivatization and various pharmacological screening of these natural compounds are important methods for the newer drug discovery [1]. Moreover, plant secondary metabolites have intriguing pharmacological and chemical characteristics for the human health. Interestingly, plant secondary metabolites contain various chemicals and versatile therapeutic applications for the human health benefit [2,3]. Working with plant bioactive compounds is the greatest challenge since it requires using the predicted theoretical approach to extract the expected chemical and then analyzing the spectroscopic data to understand and complete elucidation of the structure. Nowadays, chemicals from the alkaloids, glycosides, flavonoids, tannins, phyto sterols, terpenoids and saponins are used as medications or to prevent a variety of chronic diseases including chronic cancer, neuronal disorders, cardiovascular diseases, *etc.* [2,4]. Among these compounds, flavonoids are the largest group belongs to the polyphenolic compounds procured from the various parts of the plants and utilized for versatile applications [5-7]. These activities are mainly due to structural variation in the flavonoids compounds and their sub-classes *viz.* flavanols, flavanones, flavones, isoflavones, chalcones, flavonols, anthocyanins, *etc.* [8,9]. Primarily, their oxidative qualities contribute to the prevention and treatment of a wide range of diseases, including diabetes, inflammation, microbial infections, oxidative stress, free radical scavenging activity, various hypersensitivity reactions, cardiovascular diseases, malignancies, *etc.* [10-12].

In context of conventional healing practices, *Memecylon lushingtonii* Gamble, belongs to the Melastomataceae family, stands out as a particularly precious resource among the Memecylon species. Genus Memecylon contains around 300-400 species which are distributed throughout Asia, Africa, America [13]. This pale-blue flowered dicot tree is widely spread across India, particularly in the states of southern India [14] and the pale-blue flowered dicot tree can reach a height of 7 meters,

and its globular berries contain a single seed [15]. Some key pharmacological properties including effective post coital contraceptive [16], antibacterial [17], antioxidant properties [18], *etc* are also reported in the literature. These efficacies are due to presence of many bioactive compounds *viz.* amyrin, memecylaene, sitosterol, tartaric acid, malic acid, ursolic acid, triterpenes, flavonoids, *etc*. [19,20]. *M. lushingtonii* Gamble tree became endemic due to over exploitation and deforestation, but it has high economic demand since it contains antioxidant activity [18], but no important compounds have been reported or isolated. This study established a foundation for future research by isolating and characterizing several bioactive compounds for the first time.

EXPERIMENTAL

Plant material: The *M. lushingtonii* Gamble leaves were collected from Horsley Hills station of Tirupati city (3.66ºN 78.40ºE) and authenticated by Prof. Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati (Voucher no: 0893). Further, the leaves sample was stored in the Department of Pharmacognosy, SRM College of Pharmacy, as herbarium (Her. no: SRM-PCOG/2023-24/ML-leaves-0123) for future reference.

Extraction of leaves: Ethanol solvent was used for the extraction of *M. lushingtonii* Gamble leaves using temperature controlled Soxhlet apparatus (Stericox India Pvt. Ltd., New Delhi, power supply: 220 Volts 50 Hz). Leaves (500 g) were cleaned with running water followed by 28 days shade dried and grounded coarsely by mixer grinder. Soxhlet extraction was carried out for 8 h maintained at the oven temperature of 40 ºC. Extracted liquid was dried using rotary flash evaporator for 45 min at 40 ºC. Viscous liquid was collected and cthen the extract was collected and preserved in small glass bottle at 4 ºC.

Screening of phytochemicals: The chemical examinations were used to analyze the preserved *M. lushingtonii* Gamble leaf extract for the presence of several phytoconstituents [21, 22]. Following the identification and separation of the compounds using TLC, further isolation was also done.

Isolation of compounds: Column chromatography was used for the isolation of the compounds from the ethanol extract of *M. lushingtonii* Gamble. Wet packing was done using activated column silica gel (60/120 mesh size) for column bed preparation. Various solvents in combination were used depending on polarity and ran for TLC analysis to obtain a single spot.

Characterization: Isolated compounds were characterized by UV, IR, NMR and Mass spectroscopic method. The ethanol extract containing the bioactive compounds were analyzed by Shimadzu UV-2450 model of UV-VIS spectrophotometer (scanned at 500 to 190 nm) in methanol. The IR FTIR) spectra were measured with $CHCl_3$ measured on Perkin Elmer FTIR spectrophotometer. The NMR (^1H and ^{13}C) spectra were recorded $CDCl_3$ on Bruker 300 MHz and Avance Neo 500 MHz spectrophotometer, respectively and MASS spectra was obained on Thermo-Finnigann TSQ Quarter Ultra (Triple Quad) instrument. Silica gel 60 and 70-230 mesh ASTM (Merck 7734) were used for column chromatography. TLC Aluminum sheets and PTLC (215 cm × 15 cm silica gel 60 F_{254}) were employed in the TLC analysis. The TLC spots were visualized under UV light (254 nm).

RESULTS AND DISCUSSION

In present study, ethanol was used for the extraction of *M. lushingtonii* Gamble leaves and the yield was found to be 25.82 g. The ethanol solvent was chosen due to its ability to dissolve a substantial quantity of phytoconstituents, attributed to its high dielectric constant, non-toxic nature and easy availability [23,24]. The presence of phytochemicals were performed with various chemical test and revealed presence of alkaloids, terpenoids, phenolics, flavonoids, glycosides in ethanol extract of *M. lushingtonii* Gamble (Table-1).

TABLE-1
PHYTOCHEMICAL ANALYSIS RESULTS OF THE ETHANOL EXTRACT OF *Memecylon lushingtonii* Gamble LEAVES

Constituents	Results
Alkaloids	++
Glycosides	++
Tannins	+
Saponins	–
Flavonoids	++
Phenolics	++
Sterols	+
Terpenoids	++
Resins	–
Proteins	–
Lipids	–

(++) = Present prominently; (+) = Present; (–) = Absent
(++) showed the compounds are strongly present whereas (+) showed the compounds are lightly present and (–) indicated some specific compounds were absent.

Based on the presence of bioactive constituents, further TLC was performed for identification and separation of flavonoids compounds present in the ethanol extract of *M. lushingtonii* Gamble. A solvent system including toluene, ethyl acetate, and formic acid at a ratio of 5:4:1 was utilized, resulting in the separation of different flavonoids under UV light. A total 10 different flavonoids were identified at different rention time (Rt) of 0.23, 0.26, 0.37, 0.39, 0.66, 0.68, 0.72, 0.83, 0.86 and 0.88, respectively.

Isolation of constituents was further carried out with various solvents at different concentrations. The eluted bands were separated and collected into a test tube. Each time TLC was performed with the varying ratio of different solvents and checked the purity to get single spots. Every time, TLC was run and various bands were separated out. Finally, in the solvent system of toluene-ethyl acetate fraction (12:8), compound-1 was eluted out (yield: 132.8 mg). The TLC was performed using toluene:ethyl acetate:formic acid with the ratio of 5:4:1, a single component on TLC plate was confirmed at R_f of 0.66. Further, with toluene-ethyl acetate (18:2) fraction, compound-2 was eluted out (yield: 98.2 mg) and confirmed with same solvent system with the R_f of 0.72 (Table-2).

TABLE-2
DIFFERENT FRACTIONS WITH R_f VALUES OF TLC BANDS

Toluene (mL)	Ethyl acetate (mL)	Bands appear	R_f
20	0	2	0.2, 0.21
18	2	1	0.72
16	4	3	0.38, 0.54, 0.57
14	6	0	–
12	8	2	0.37, 0.41
10	10	3	0.44, 0.47, 0.49
8	12	1	0.66
6	14	0	–
4	16	0	–
2	18	2	0.74, 0.83
0	20	0	–

The dried fractions were further characterized with UV, IR, ^{1}H NMR and MASS spectra. The UV analysis was performed from 500 nm to 190 nm and two peaks were observed for compound **1** at 375 and 250 nm (Fig. 1a) and 2 peaks were observed for compound **2** at 264 and 356 nm (Fig. 1b). Findings from the current study are in agreement with those from earlier research [25] that the isolated compound **1** must be quercetin, since it exhibited two distinct peaks at 375 and 250 nm, in comparison to standard quercetin. Similarly, for compound **2**, previous findings indicated that luteolin emitted two distinct peaks at 356 nm and 264 nm, which is consistent with the results conducted in the current study [26].

IR studies: In compound **1**, a broad peak at 3290.88 cm^{-1} (O-H *str.*) was observed due to the presence of hydroxyl groups and multiple -OH groups contributing to this peak. The IR band in the range of 2900.41-2355.62 cm^{-1} is attributed to the C-H stretching. Similarly, the other peaks at 1667.16 cm^{-1} (-C=C-), 1631.96 cm^{-1} (C=O str.), 1611.23 cm^{-1} (arom. -C=O), 1520.6 and 1514.89 cm^{-1} (arom. -C=C-), 1201.43 cm^{-1} (-C=O *str*.) and 1025.98 cm^{-1} (C-O *str.*) were also observed [27].

The IR Spectrum of compound **2** revealed the characteristic absorption bands at 3418.65 cm^{-1} attributed to the O-H stretching and also resulted several -OH groups attached with the parent compound. The other key peaks were observed at 1651.48 and 1598.63 cm^{-1} (*C=O str.*), 1655 and 1609 cm^{-1} (arom. -C=C), 1438.73 cm^{-1} (C-O-C bond), 1251, 1230 cm^{-1} (C-O-C), 1119.76 cm^{-1} (arom. C=C *str.*) and 1030.98 cm^{-1} (C-O *str.*) [28,29].

NMR studies: The ^{1}H NMR spectrum of compound **1** also reveals several key features for example, the signal at 12.506 ppm is the characteristic of hydroxyl group (-OH) attached to the aromatic ring of quercetin, particularly on the 5-hydroxy position of the A-ring. The region from δ 6.198 to 7.691 ppm contains signals typical of aromatic protons, while the peaks at δ 6.904 and 7.691 ppm correspond to the protons in the B and C-rings of quercetin (compound **1**). The peaks around δ 7.537 to 7.563 ppm indicate the presence of closely related protons in a conjugated system. The peaks between δ 9.323 to 9.607 ppm are indicative of additional hydroxyl groups (-OH) on the aromatic rings. Some small peaks are also appeared at δ 2.512 ppm, which might be due to solvent (DMSO) impurities. The C NMR data revealed that the aromatic carbons in the range 95-160 ppm and the carbonyl carbons (C=O) appear in the range 180-190 ppm (Table-3).

The ^{1}H NMR spectrum of compound **2** also reveals several key features, for example, the signal at δ 12.987 ppm is the characteristic of the hydroxyl group (-OH) attached to the aromatic ring. The region from 6.193 to 7.437 ppm contains signals typical of aromatic protons and the peaks around 7.401 to 7.437 ppm indicate the presence of closely related protons in the conjugated system. The peaks between 9.514 to 9.627 ppm are indicative of additional hydroxyl groups (-OH) on the aromatic rings. In ^{13}C NMR, the multiple peaks corresponding to the aromatic ring carbon atoms appeared at 95-160 ppm. The peaks at 180-190 ppm are due to the aromatic carbons, whereas the peaks in this region correspond to carbonyl groups present in the structure. The peaks in the range 40-90 ppm are associated with hydroxyl groups and other substituents in the structure (Table-3).

Mass spectral studies: For compound **1**, the molecular peak at *m/z* 303 may be represents the intact quercetin molecule with a mass of 303 Da. Whereas for compound **2**, the molecular

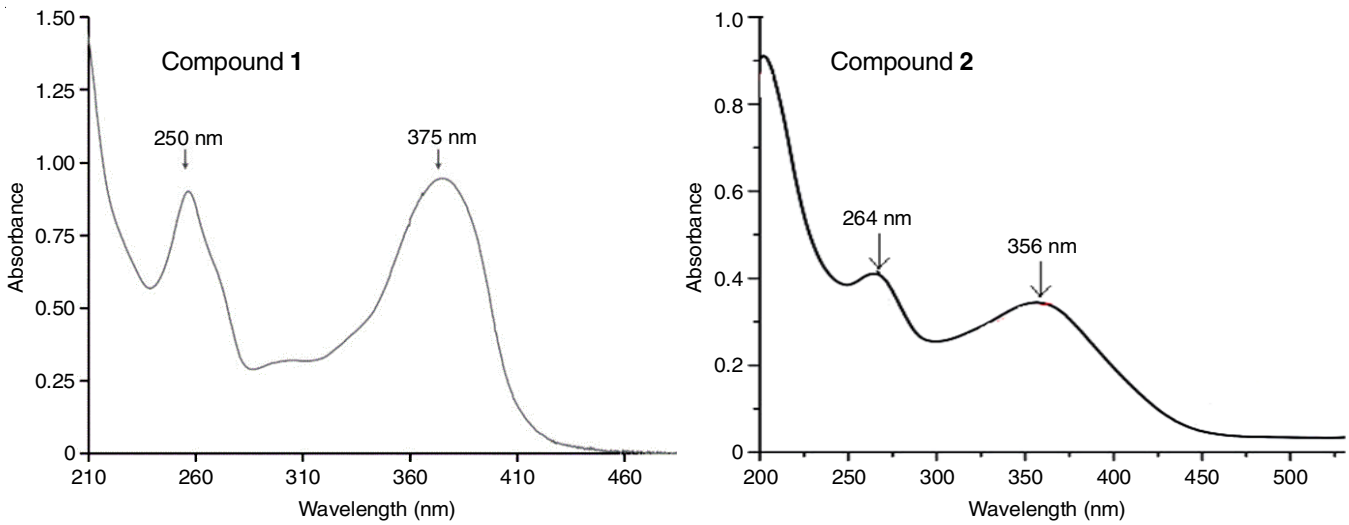

Fig. 1. UV spectra for isolated compounds **1** and **2**

TABLE-3
^{1}H NMR AND ^{13}C NMR CHEMICAL SHIFT OF ISOLATED COMPOUNDS FROM THE ETHANOL EXTRACT OF *Memecylon lushingtonii* Gamble LEAVES

Compound **1**			Compound **2**		
Position	^{1}H NMR	^{13}C NMR	Position	^{1}H NMR	^{13}C NMR
C-2	–	147.6	C-2	–	165.24
C-3	9.44 (1H, s)	136.7	C-3	6.77 (1H, s)	105.67
C-4	–	176.8	C-4	–	182.40
C-5	12.55 (1H, s)	161.9	C-5	12.90 (1H, s, Ar-OH)	161.42
C-6	6.22 (1H, d, *J* =2.05)	98.8	C-6	5.98 (1H, s)	99.11
C-7	10.84 (1H, s)	164.9	C-7	10.40 (1H, s, *J* =8.8)	166.96
C-8	6.44 (1H, d, *J* =2.03)	94.0	C-8	6.07 (1H, d, *J* = 2.1)	94.07
C-9		157.2	C-9	–	158.06
C-10		103.9	C-1'	–	124.46
C-1'		123.2	C-2'	6.61 (1H, d, *J* =2.1)	115.67
C-2'	7.71 (1H, d, *J* = 2.17)	115.7	C-3'	9.45 (1H, s, *J* =8.8)	145.15
C-3'	9.36 (1H, s)	146.1	C-4'	9.47 (1H, s)	147.78
C-4'	9.66 (1H, s)	148.6	C-5'	6.81 (1H, d *J* =8.8)	117.68
C-5'	6.93 (1H, d) *J* = 8.46)	116.2	C-6'	7.07 (1H, s, *J* = 8.8)	121.23
C-6'	7.56 (1H, dd, *J* = 2.24, 6.28)	120.7			

^{1}H NMR (δ_H in ppm, 400 MHz) and ^{13}C NMR (δ_C in ppm, 101 MHz)

ion peak (*m/z* 287) represents the intact luteolin molecule with a mass of 286 Da.

Based on the FTIR, NMR and MASS spectral data, the isolated compound **1** was quercetin whereas compound **2** was luteolin. Thereafter, TLC was run with the standard quercetin using mobile phase toluene, ethyl acetate and formic acid (5:4:0.5) and confirmed the isolated compound was quercetin (R_f: 0.66) and compound **2** was also run with standard luteolin with the same solvent system in TLC and confirmed that isolated compound was luteolin (R_f: 0.72).

Conclusion

The isolation and characterization of bioactive compounds from the ethanol extract of *Memecylon lushingtonii* Gamble leaves revealed the presence of various bioactive compounds especially flavonoids compounds. The separation process was conducted, resulting in the identification of two specific mobile phases that gave distinct spots. The spots were identified and characterized using IR, NMR and MASS spectroscopy and revealed the presence of quercetin and luteolin which further confirmed with TLC chromatogram by compared the spots with standard quercetin and luteolin, respectively.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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