



GC-MS analysis of bioactive compounds from *Lessertia montana* leaf extract

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Received September 10, 2021; Revised September 24, 2021; Accepted September 24, 2021, Published October 31, 2021

DOI: 10.6026/97320630017846

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Abstract:

Lessertia montana is an indigenous medicinal plant with wide spectrum of useful bioactive compounds against numerous diseases in the Basotho traditional medicine. Therefore, it is of interest to identify phyto-chemical compounds from the leaf extract of the plant using GC-MS analysis. The results show that the extract possessed 30 bioactive compounds including 1-ethyl-3,5-dimethylbenzene and Prehnitene with % peak areas of 13.57 and 11.88, respectively for further consideration in drug discovery.

Keywords: *L. montana*, Extraction, GC-MS analysis, ADME properties

Background:

Medicinal plants are used in traditional medicine to cure a variety of diseases [1]. Modern medicine has evolved from folk medicines that use plant as a source of drugs [2]. Currently 80% of the world population depends on plant-derived drugs to provide beneficial effects to humans because of its availability, affordability, fewer or no side effects and numerous endowed compounds with significant pharmacological potential, many of which may serve as lead compounds in the development of new drugs [3]. Plants are rich source of a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids etc., which have several biological properties [4]. Consequently screenings of active compounds from natural sources have become relatively simpler and played a major part in the development of new drugs from medicinal plants with efficient protection and treatment roles against various diseases including cancer and Alzheimer's diseases [5]. Hence the need for

development of effective methods to discover bioactive compounds from natural sources is deemed necessary. Interestingly, there are several medicinal plants available in the nature, which has not been investigated for their medicinal potential [6]. Moreover, the development of compounds is not only encouraging for therapeutic prospective, but also plays an active role towards invention of novel semi-synthetic and synthetic compounds [7, 8]. Thus, the screening of plant extracts is a novel strategy to find therapeutically active compounds in many plant species achieved through Gas Chromatography-Mass Spectroscopy (GC/MS) combined analytical techniques. *Lessertia Montana* is a medicinal plant and the member of the Fabaceae family indigenous to India. It is a soft-wooded shrub of 0.5-1.0 m height endowed with beautiful silvery green foliage, large red flowers and attractive bladderly pods [9]. Traditionally, the plant is used for the treatment of numerous diseases such as diabetes, cardiac ailment as well as in sedative use. Previous studies on

this plant from our laboratory established the antioxidative and antidiabetic effects of the leaf extract [10]. Therefore, it is of interest to identify phytochemical compounds from the leaf extract of the plant using GC-MS analysis.

Materials and Methods:

Plant collection and extract preparation:

Fresh *L. montana* Leaves were collected in the area of Nilgiris, Tamil Nadu, India and it rinsed with distilled water in order to remove dust particles and were spread on a brown cardboard paper for exposure to air at room temperature. Following days of drying, they were separately ground into fine powder materials using a laboratory blender. Exactly 10 g each of the powdered samples were exhaustively extracted with 50 mL of ethanol (100%) for three days. The extracting flask was placed on a horizontal platform shaker at 110 rpm to allow for proper agitation. The extract was initially filtered using Whatman No. 1 filter paper. A dry powdered extract was obtained rotary evaporator under reduced pressure at 40 °C. The leftover extract was kept in air-tight contained and refrigerated (4 °C) for future analysis.

GC-MS analysis:

GC-MS analysis of ethanolic extract was performed using a Thermo GC -Trace ultra Ver: 5.0 Thermo MS DSQ II systems and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with DB 5 - MS capillary standard non-polar column (30mmX0.25mm 1D X 1 J.Mdf). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow

rate 1mL/min and an injection volume of 1µL was employed (split ratio of 10:1); Injector temperature 80°C; Ion-source temperature 250°C. The oven temperature was programmed from 70°C (isothermal for 2 min), with an increase of 6°C/min, to 260°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 50 to 650 Da. Total GC running time was 25 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass [11].

Identification of bioactive compounds:

The identification of components was based on Willey and NIST libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Willey) attached to the GC-MS instrument and the results were obtained. The name, molecular weight and the structure of the components of the test materials were ascertained while the relative percentage composition of each component was calculated by comparing its average peak area to the total area [12].

Drug-like properties prediction:

The active compounds from *L. Montana* was checked for their ADME properties using QikProp (QikProp, Schrödinger, LLC, New York, NY, 2017). This helps to analyze the pharmacokinetics and pharmacodynamics of the ligands by accessing the drug-like properties. The significant ADME properties such as molecular weight (MW), H-Bond donor, H-Bond acceptor and log P (O/W) were predicted [13].

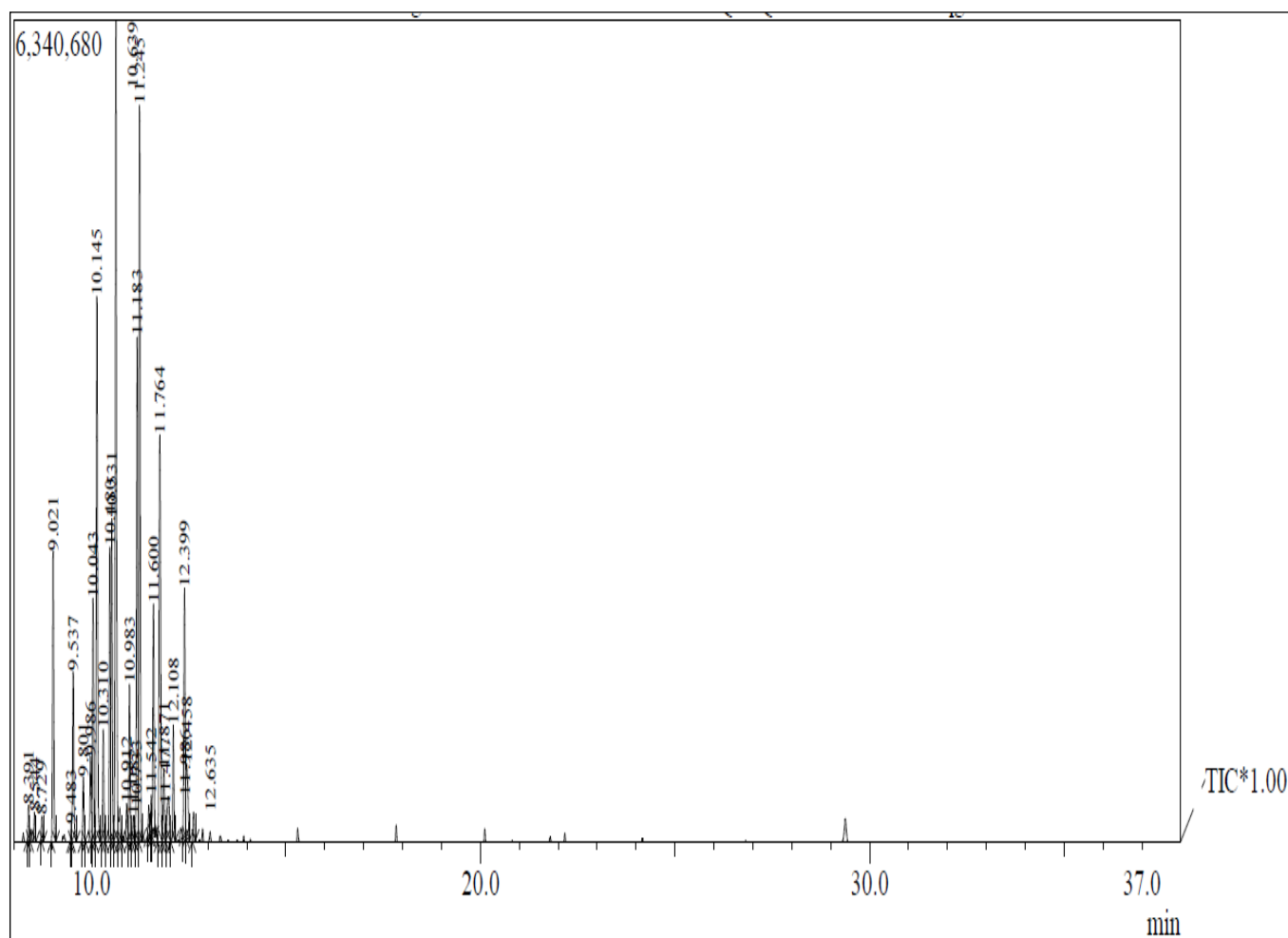


Figure 1: GC-MS chromatograph of ethanolic extract of *Lessertia montana* leaf

Table 1: Identified bioactive compounds from the GC-MS chromatograph of *Lessertia montana* leaf showing the retention time and percentage composition

S. No	RT	Compounds	Molecular formula	Molecular Weight	Peak Area (%)
1	8.392	1-Methyl-4-ethylbenzene	C ₉ H ₁₂	120	0.53
2	8.542	1,2,3 Trimethylbenzene	C ₉ H ₁₂	120	0.60
3	8.725	2-Ethyltoluene	C ₉ H ₁₂	120	0.34
4	9.025	Hemimellitene	C ₉ H ₁₂	120	4.87
5	9.483	Phenyl ethyl ketone	C ₉ H ₁₀ O	134	0.26
6	9.533	Mesitylene	C ₉ H ₁₂	120	2.95
7	9.800	Indane	C ₉ H ₁₀	118	1.06
8	9.983	p-Diethylbenzene	C ₁₀ H ₁₄	134	1.26
9	10.042	m-Propyltoluene	C ₁₀ H ₁₄	134	3.84
10	10.142	1,4-Diethylbenzene	C ₁₀ H ₁₄	134	9.88
11	10.308	2-Phenylbutane	C ₁₀ H ₁₄	134	1.70
12	10.483	2-Ethyl-p-xylene	C ₁₀ H ₁₄	134	5.12
13	10.533	Prehnitene	C ₁₀ H ₁₄	134	4.66
14	10.642	1-Ethyl-3,5-dimethylbenzene	C ₁₀ H ₁₄	134	13.57
15	10.733	p-Cymol	C ₁₀ H ₁₄	134	0.64
16	10.908	5,9,9-Trimethylspiro[3.6]deca-5,7-dien-1-one	C ₁₃ H ₁₈	190	0.70
17	10.983	Prehnitene	C ₁₀ H ₁₄	134	2.42
18	11.083	Cumene, m-ethyl-	C ₁₁ H ₁₆	148	0.46
19	11.183	Durol	C ₁₀ H ₁₄	134	8.66
20	11.242	Prehnitene	C ₁₀ H ₁₄	134	11.88
21	11.475	p-Methylpropiophenone	C ₁₀ H ₁₂ O	148	0.46
22	11.542	3,5-Diethyltoluene	C ₁₁ H ₁₆	148	0.50
23	11.600	o-Allyltoluene	C ₁₀ H ₁₂	132	3.83
24	11.767	1,2,3,4-Tetramethyl-5-methylene-1,3-cyclopentadiene	C ₁₀ H ₁₄	134	8.76
25	11.875	trans-1-methyl-2-indanol	C ₁₀ H ₁₂ O	148	1.34
26	11.983	1,3-Diethyl-4-methylbenzene	C ₁₁ H ₁₆	148	1.19
27	12.108	Peroxide, bis(1-methyl-1-phenylethyl)	C ₁₈ H ₂₂ O ₂	270	1.73
28	12.400	Albocarbon	C ₁₀ H ₈	128	4.62
29	12.458	Isopropyl-p-xylene	C ₁₁ H ₁₆	148	1.78
30	12.633	1,1,2,2-Tetramethoxyethylene	C ₆ H ₁₂ O ₄	148	0.43

RT: retention time

Table 2: ADME properties of the active compounds from ethanolic extract of *Lessertia montana* leaf

S. No	Compounds	Molecular Weight (g/mol)	H-Bond donor	H-Bond acceptor	LogP (O/W)
1	1-Methyl-4-ethylbenzene	120.195	0	0	3.0
2	1,2,3 Trimethylbenzene	120.195	0	0	2.9
3	2-Ethyltoluene	120.195	0	0	3.6
4	Hemimellitene	120.195	0	0	3.8
5	Phenyl ethyl ketone	134.178	0	1	2.2
6	Mesitylene	120.195	0	0	3.4
7	Indane	118.179	0	0	3.2
8	p-Diethylbenzene	134.222	0	0	3.9
9	m-Propyltoluene	134.222	0	0	3.6
10	1,4-Diethylbenzene	134.222	0	0	3.4
11	2-Phenylbutane	134.222	0	0	3.7
12	2-Ethyl-p-xylene	134.222	0	0	3.9
13	Prehnitene	134.222	0	0	3.4
14	1-Ethyl-3,5-dimethylbenzene	134.222	0	0	3.2
15	p-Cymol	134.222	0	0	3.5
16	5,9,9-Trimethylspiro[3.6]deca-5,7-dien-1-one	190.286	0	1	2.7
17	Prehnitene	134.222	0	0	3.5
18	Cumene, m-ethyl-	148.249	0	0	4.5
19	Durol	134.222	0	0	3.4
20	Prehnitene	134.222	0	0	2.9
21	p-Methylpropiophenone	148.205	0	1	2.6
22	3,5-Diethyltoluene	148.249	0	0	3.9
23	o-Allyltoluene	132.206	0	0	3.8
24	1,2,3,4-Tetramethyl-5-methylene-1,3-cyclopentadiene	134.222	0	0	1.6
25	trans-1-methyl-2-indanol	148.205	0	1	2.0
26	1,3-Diethyl-4-methylbenzene	148.249	0	0	3.9
27	Peroxide, bis(1-methyl-1-phenylethyl)	270.372	1	2	1.9
28	Albocarbon	134.128	0	0	3.3
29	Isopropyl-p-xylene	148.249	0	0	3.8
30	1,1,2,2-Tetramethoxyethylene	148.158	0	4	0.8

Results and Discussion:

Gas chromatography separates the components of the mixture, and mass spectroscopy analyzes each of the components separately [14]. It is one of the best technique that can be employed to identify the bioactive constituents including long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc [15]. GC-MS is extensively applied in drug detection, environmental analysis, explosives investigation, medical, pharmaceutical, environmental and forensic applications as well as identification of unknown compounds of plants [16]. In recent times, investigation involving the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases continues to gain momentum [17]. Revelation evolving through

the GCMS analysis, a total of 30 natural compounds were identified from the ethanolic extract of *Lessertia montana*. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and % composition from the chromatogram of the ethanolic extract of the plant is presented in **Table 1** and **Figure 1**. The phytoconstituent prediction is based on NIST library revealing 30 compounds namely; 1-Ethyl-3,5-dimethylbenzene (13.57%), Prehnitene (11.88%), 1,4-Diethylbenzene (9.88%), 1,2,3,4-Tetramethyl-5-methylene-1,3-cyclopentadiene (8.76%), Durol (8.66%), 2-Ethyl-p-xylene (5.12%), Hemimellitene (4.87%), Prehnitene (4.66%), Albocarbon (4.62%), m-Propyltoluene (3.84%), o-Allyltoluene (3.83%), Mesitylene (2.95%), Prehnitene (2.42%), Isopropyl-p-xylene (1.78%), Peroxide, bis(1-methyl-1-phenylethyl) (1.73%), 2-Phenylbutane

(1.70%), trans-1-methyl-2-indanol (1.34%), p-Diethylbenzene (1.26%), 1,3-Diethyl-4-methylbenzene (1.19%), Indane (1.06%), 5,9,9-Trimethylspiro[3.6]deca-5,7-dien-1-one (0.70%), p-Cymol (0.64%), 1,2,3 Trimethylbenzene (0.60%), 1-Methyl-4-ethylbenzene (0.53%), 3,5-Diethyltoluene (0.50%), Cumene, methyl- (0.46%), p-Methylpropiophenone (0.46%), 1,1,2,2-Tetramethoxyethylene (0.43%), 2-Ethyltoluene (0.34%) and Phenyl ethyl ketone (0.26%). The ADME properties prediction of the screened compounds (**Table 2**) reflecting these parameters were under acceptable and permissible range which suggests that *L. montana* leaves could be further explored for bioprospecting of cheap, safe and affordable anticancer drug for the India and the world at large. Moreover, it must be noted that ADME properties of screened compounds do not predict any adverse effect that could be implicated in the failure of drugs [18]. Consequently, there is an increasing awareness in the early prediction of ADME properties reaching success rate of compounds development with the objectives [19]. The limitations of ADME properties are five depicted not more than 5 hydrogen-bond donors, not more than 10 hydrogen-bond acceptor, molecular mass should be less than 500 daltons, an octanol- water partition coefficient log P not greater than 5. Interestingly however, all these screened compounds were within the acceptable and permissible limits of ADME properties [20].

Conclusion:

We report the GC-MS analysis of bioactive compounds including 1-ethyl-3,5-dimethylbenzene and prehnitene from *Lessertia montana* leaf extract for further consideration in drug discovery.

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Edited by P Kanguane

Citation: Bioinformation 17(10): 846-850 (2021)

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