SHORT REPORT

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Chemical Characterization and Acetylcholinesterase Inhibition Potential of Volatile Components of Aerial Parts of *Pluchea lanceolata* (DC.) Oliv. & Hiern

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Abstract: *Pluchea lanceolata* (DC.) Oliv. & Hiern (Rasana) is an important medicinal plant due to its usage in number of *Ayurvedic* formulations. First time, chemical composition of essential oil from the aerial part of *P. lanceolata* was analyzed by gas chromatography-mass spectrometry (GC-MS) and NMR spectroscopy. *Ex-vivo* cholinesterase inhibitory activity of the essential oil was also evaluated using mouse brain homogenate. The major components were linalool (32.2%), β-caryophyllene (8.5%), α-terpineol (8.0%), spathulenol (7.4%), linalylacetate (5.6%), naphthalene, 1,6-dimethyl-4-(1-methylethyl)- (4.3%), α-copaene (3.6%), epi-cubebol (3.6%) and trans-α-bergamontene (3.1%). The experimental results showed that hydrodistilate of *P. lanceolata* significantly inhibited acetylcholinesterase activity (IC_{50} value 2.54 ± 0.03 µg/mL).

Keywords: *Pluchea lanceolata;* volatile organic contents (VOCs); acetylcholinesterase inhibition; GC-MS; NMR.

1. Plant Source

The aerial parts of plant *Pluchea lanceolata* (DC.) Oliv. & Hiern were collected at the experimental field of our Institute in June, 2011. Voucher specimen No 12555 was deposited at Herbarium Botany Department of Central Institute of Medicinal and Aromatic plants Lucknow, U.P. India.

2. Previous Studies

Flowering plant genus *Pluchea* belongs to Asteraceae family, comprising camphor weeds, or sour-bushes. These resinous and bushy shrubs are found in sandy or saline soils and distributed throughout the greater part of India, and neighboring Asian countries viz. Pakistan, Bangladesh, and Afghanistan [1]. In *Ayurvedic* texts, *Pluchea lanceolata* (DC.) Oliv. & Hiern is categorized as '*Vata- samanam'* (diseases involving nervous system) *'Rasayana'* (herb for rejuvenation). Multi-herbal and/or herbo-mineral formulations based on this plant are advised for the management and cure of anxiety, dementia, depression malaria and epilepsy by the traditional healers [1-3]. Alzheimer's disease (AD) is the most common cause

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of progressive cognitive dysfunction that results from a deficiency in cholinergic activity in brain [4]. In this regard acetylcholinesterase (AChE), plays an important role in release of acetylcholine (ACh). The Inhibition of AChE, serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia, ataxia, myasthenia gravis and Parkinson's disease [5].

In a recent review, plant extracts and various phytochemical moieties viz. alkaloids (139), monoterpenes (27), coumarins (18), triterpenes (17), flavonoids (14), benzenoids (13), diterpenes (8), oxygen heterocycles (5), sesquiterpenes (5), stilbenes (3), lignans (2), sulfur compounds (2), proteids (2), polycyclic (1), quinoid (1), benzoxazinone (1), carotenoid (1) and alycyclic (1) were reported for acetylcholinesterase inhibition [6-7]. Several AChE inhibitors such as tacrine, donepezil, rivastigmine and galanthamine [8] have also been approved by US Food and Drug Administration (US-FDA). Adverse effects of these drugs such as gastrointestinal disturbances and challenges of bioavailability have forced to search better AChE inhibitors from natural resources [9]. Therefore, the aim of this work was to characterize the composition and to evaluate the acetylcholinesterase inhibitory potential of hydro-distillate of fresh plant material of *P. lanceolata* aerial parts collected at flowering phase.

3. Present Studies

3.1. Chemical characterization of VOCs of P. lanceolata

The GC and GC/MS analyses of the *Pluchea lanceolata* essential oil facilitate the identification of 46 compounds, accounting for 97% of the total. The identification of the compounds was achieved on the basis of retention time, Kovats Indexes, literature reported retention index and mass spectra [10-11]. The essential oils showed to be complex mixtures of several components, predominating oxygenated monoterpenes and sesquiterpenes (Table 1). It must be emphasized that the crossing data achieved from the RI, MS and NMR, allowed the identification of almost all individual compounds detected, even those that presented very similar mass spectra but different NMR spectra could be distinguished. The essential oils from *P. lanceolata* were characterized by high percentages of oxygenated monoterpene (53.4%) for which the four major constituents were linalool (32.2%), α-terpineol (8.0%) and linalylacetate (5.6%), sesquiterpene hydrocarbon (20.9%) consisted mainly of α-copaene (3.6%), trans-caryophyllene (8.5%) and oxygenated sesquiterpene mainly comprising of epi-cubebol (3.6%), trans-α-bergamotene (3.1%), spathulenol (7.4%) and aromatic hydrocarbon comprising of naphthalene, 1,6-dimethyl-4-(1-methylethyl) (4.3%) (Figure 3S). α-Pinene- a typical chemical marker in other *Pluchea* species of South America was not detected in plant under study i.e. *P. lanceolata*. Monoterpene hydrocarbons and oxygenated diterpenes were also found but in very less amounts i.e. 1.1% and 1.7%, respectively. The chemical characterization of essential oil of *P. lanceolata* is reported first time. Efforts were also made to achieve a limited metabolomic fingerprinting of *P. lanceolata.*

The major chemical constituents of *P. lanceolata* current study along with other *Pluchea* species of Southern America origin are summarized in Table 1S. Selin-11-en-4α-ol in *P. carolinensis* and sesquilavandulyl acetate in *P. quitoc* were the major chemicals while entirely different composition of *P. sagittalis* [12] with predominance of monoterpenoids viz. α-Pinene, α-Humulene and camphene could also be observed (Table S1). A high degree of variability characterizes *P. lanceolata* essential oils, mainly due to several biochemical pathways, high polyploidy and environmental conditions.

Table 1. Results of qualitative and quantitative of the volatile components from aerial part of *P. lanceolata*.

S. No	Compound name*	RI	LRI-(EQ-5)	% Content in Aerial part	Mode of identification
1	*n*-Hexanol	863	858	t	RI,^{13}C
2	6-Methylhept-5-ene-2-one	989	987	0.25	RI,
3	β-Myrcene	992	991	t	RI, ^{13}C
4	α-Phellandrene	1011	1007	0.83	RI, ^{13}C
5	*p*-Cymene	1024	1025	0.27	RI, ^{13}C
6	(*Z*)-β-Cimene	1040	1043	t	RI, ^{13}C

7	*trans*-Cinnamaldehyde	1045	1047	t	RI
8	Acetophenone	1069	1070	0.25	RI
9	Terpinolene	1087	1090	0.25	RI
10	Linalool	1100	1098	32.15	RI, MS, ^{13}C
11	*cis*-Thujone	1103	1112	0.38	RI
12	*p*-Cymene-8-ol	1188	1188	0.74	RI, ^{13}C
13	α-Terpineol	1192	1195	7.99	RI, MS, ^{13}C
14	Nerol	1227	1229	2.10	RI, MS, ^{13}C
15	Thymol methyl ether	1232	1235	0.11	RI
16	Neral	1241	1241	0.53	MS,RI
17	Linalyl acetate	1253	1256	5.62	RI, MS, ^{13}C
18	(*E*)-2,6-Octadienal,3,7-dimethyl	1270	1270	0.79	MS
19	Unidentified	1272		0.50	
20	Cycloisosativene	1371	1374	0.66	RI, MS, ^{13}C
21	α-Copaene	1379	1376	3.62	RI, MS, ^{13}C
22	Geranyl acetate	1385	1384	0.27	RI
23	(*Z*)-β-Damascenone	1405	1397	1.37	RI, MS
24	Cyperene	1409	1401	0.22	MS
25	Unidentified	1411		t	
26	β-Caryophyllene	1424	1421	8.49	RI, MS, ^{13}C
27	α-Humulene	1458	1454	0.85	RI, MS, ^{13}C
28	(*E*)-β-Farnesene	1465	1465	t	RI, ^{13}C
29	Germacrene D	1479	1480	0.58	RI, MS, ^{13}C
30	Unidentified	1487		0.26	
31	epi-Cubebol	1491	1496	3.60	RI, MS, ^{13}C
32	*trans*- β-Guaiene	1502	1508	0.87	RI, ^{13}C
33	Unidentified	1517		0.28	
34	Unidentified	1526		0.30	
35	Elemol	1547	1550	0.12	RI
36	δ-Cadinene	1557	1559	1.01	RI, MS, ^{13}C
37	Unidentified	1564		0.23	
38	Germacene D-4-ol	1579	1578	0.40	RI, ^{13}C
39	Unidentified	1581	-	0.28	RI
40	Carryophyllene oxide	1583	1584	t	RI
41	*trans*-α -Bergamotene	1586	1582	3.06	RI, MS, ^{13}C
42	Spathulenol	1589	1589	7.37	RI, MS, ^{13}C
43	Guaiol	1592	1594	0.20	RI
44	α -Bisabolol	1610	1685	0.78	RI, MS
45	Unidentified	1615		0.66	
46	β-Acorenol	1633	1634	0.19	RI
47	Unidentified	1639	-	0.39	
48	epi-α-Cadinol	1642	1645	1.19	RI, MS, ^{13}C
49	Unidentified	1645	-	0.15	
50	Geranyl tiglate	1647	1649	0.26	RI
51	Selin-11-ene-4-α-ol	1650	1651	tr	RI, ^{13}C

52	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	1657	1650	0.89	RI, MS, ^{13}C
53	Unidentified	1659		0.46	
54	10-epi-α-Muurolol	1663	1658	0.70	MS, ^{13}C
55	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	1677	1675	4.34	RI, MS
56	Unidentified	1834	-	t	
57	Phytol	2111	2106	1.50	RI, MS, ^{13}C
58	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	2545	2541	0.15	RI, MS
	Group Identified				
	Monoterpene hydrocarbons (%)			1.12	
	Oxygenated monoterpenes (%)			53.37	
	Sesquiterpene hydrocarbons (%)			20.87	
	Oxygenated sesquiterpenes (%)			16.40	
	Oxygenated diterpenes (%)			1.68	
	Aromatic hydrocarbon (%)			4.40	
	Unidentified (%)			3.07	

RI, retention index on Equity-5 capillary column; LRI, retention index reported in the literature using same GC stationary phase. MS, mass spectra (tentatively identified by NIST, WILEY and Nbs computer library matching); t, trace (<0.1%);

3.2. Chemo-taxonomic fingerprint of P. lanceolata

Metabolomic fingerprinting of *P. lanceolata* essential oil, was defined with ^{1}H- and ^{13}C-NMR spectroscopic analyses) to confirm 26 compounds out of 58 detected by GC-FID/GC-MS. ^{1}H-NMR and ^{13}C-NMR of compounds confirmed by GC-MS were stored in the library. A representative NMR spectrum is presented in Figure 1S. Chemical shifts for major nine constituents were assigned as compiled in Figure 2S. In particular, linalool, β-caryophyllene, α-terpineol, spathulenol, linalylacetate, naphthalene, 1,6-dimethyl-4-(1-methylethyl), α-copaene, epi-cubebol and trans-α-bergamotene were confirmed identified by 1D spectroscopy (^{1}H and ^{13}C) exhibiting matching of highly diagnostic peaks in the stored NMR signals of *P. lanceolata* essential oil. ^{1}H-NMR spectrum revealed numerous specific signals for functional groups and arrangement of H-atoms in examined chemical moieties: multiplet at 2.0 ppm, double doublet at 5.89 ppm and multiplet at 5.23 ppm, 5.24 ppm, 5.20 ppm for linalool, multiplet at 2.0 ppm and triplet at 5.37 for α-terpineol, singlet at 2.01 ppm, doublets at 5.20 ppm, 5.23 ppm and 5.89 ppm for linalylacetate, triplet at 5.37 ppm and singlet at 1.71 ppm for α-copaene, triplet at 5.20 ppm and doublet at 4.63 ppm, 4.88 ppm for β-caryophyllene, broad peak at 2.0 ppm and singlet at 1.01 ppm, 1.06 ppm and 1.31 ppm for epi-cubebol, triplet at 5.37 ppm, 5.20 ppm and 2.62 ppm for trans-α-bergamotene, broader peak at 2.0 ppm and doublet at 4.63 ppm, 4.88 ppm for spathulenol and doublet at 7.68, 7.53, 7.14, 6.96, 6.93 for naphthalene, 1,6-dimethyl-4-(1-methylethyl). The overlapping of signals in ^{1}H was higher than ^{13}C due to broader spectral size. ^{1}H and ^{13}C NMR both further confirming the GC results, for identification and quality control. Moreover, these kinds of evidences reinforce the role of non-chromatographic approach as potential tool to discriminate chemotypes, cultivar, and hybrids [13-14].

3.3. Acetylcholinesterase inhibition

Number of clinically approved AChE inhibitors have undesirable effects including gastrointestinal disturbances and problems associated with bioavailability [6, 15]. The challenge forces researchers to search better AChE inhibitors from natural resources. The AChE inhibitory activity of the essential oils of *Pluchea* genus has never been explored before. Estimation of cholinesterase activity was performed on the using modified Ellman's method [16].

The inhibitory activity of different concentration of reference compound (physostigmine) and essential oil is summarized in Figure 4S. Under experimental condition, the EC_{50} value of physostigmine and essential oil of *P. lanceolata* was 1.03 µg/mL and 2.54 µg/mL respectively. The volatile organic

components such as linalool, linalyl acetate, spathulenol, α-terpineol, α-copaene, *trans*-caryophyllene have been reported to possess moderate to high AChE inhibition [17-18]. The terpene, ketones and hydrocarbon reported to have stronger AChE inhibition than the terpene alcohols [6]. The critical observation of major components of VOCs and their reported AChE values and some synergistic actions [6, 17-18]. Current study supports the use of *P. lanceolata* for the management of neurodegenerative ailments like dementia and Alzheimer's disease. Detailed pharmacological and toxicological studies are recommended to validate present finding.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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