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BIOACTIVE VOLATILE COMPOUNDS OF THREE MEDICINAL PLANTS FROM NEPAL

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ABSTRACT
The volatile organic compounds of *Asparagus racemosus*, *Bergenia ciliate* and *Terminalia chebula* were isolated by simultaneous distillation–extraction (SDE) technique and then analyzed by gas chromatography–mass spectrometry (GC-MS). A total of 55, 48 and 56 compounds were identified from and compounds camphor, borneol, capric acid, furfural, myrtanal, α-pinene, α-terpeniol, perillaldehyde, 2-carene, butyrophenones, furfural, β-caryophyllene, 2-nitropropane were detected as a bioactive compounds with various proportions among the studied plants.

Key Words: Nepalese medicinal plants, volatile organic compounds, GC-MS, camphor, bioactivity

INTRODUCTION
The widespread use of herbal remedies and healthcare preparations has been traced to the occurrence of natural products with medicinal properties [1]. Increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [2]. The percentage composition of the volatile components and characteristics of Volatile Organic Compounds (VOCs) provide an important parameter for the characterization of the plant [3]. Careful identification of VOCs for fragrance and pharmacologically active ingredients will show the presence of numerous useful compounds. They are gaining increasing interest because of their relatively safe status and their exploitation for potential multi-purpose functional use. Most of the constituents are terpenoids, generally monoterpenes and sesquiterpenes, as well as sometime diterpenes and aromatic compounds derivatives. The VOCs present in essential oils has been reported for their anti-spasmodic, restraining, diuretic, anti-biotical, antimicrobial, antifungal, insectisidal, and anthelmintic efficiency [4].

Due to species climatic and geographical conditions, temperate and alpine plants of the Himalaya offer greater possibilities of having novel molecules and even largest quantities of the active compounds [5]. Therefore the number of people and institutions seeking information on Himalayan medicinal plant is increasing very rapidly. In our previous studies, randomly searched plants from Nepal were found to be good source of essential oils and bioactive volatile compounds [6-9]. In continuation of our efforts to evaluate the efficacy of traditional medicine, recently we have reported the bioactivities of several medicinal plants from various geographical locations of Nepal based on the ethnopharmacological information [10-12]. We are randomly searching the bioactive phytochemicals on Nepalese plants. For

this study we have taken *Asparagus racemosus* Willd, *Bergenia ciliata* (Haw.) sternb, and *Terminalia chebula* Retz, plants. Advances in our basic biological understanding of will contribute a benefit in Nepalese traditional medicine.

MATERIALS AND METHODS

Plant Materials

Plants *A. racemosus*, *B. ciliata*, *T. chebula* were collected from Kavre, Sindhuplanchawok and Kathmandu, Nepal. Samples were taxonomically identified and voucher specimens are deposited in Central Department of Botany, Tribhuvan University, Nepal. They were dried at room temperature, packed on vacuum free condition by removing air from the package and stored at -18 ^{0}C before the experiment.

Reagents

The regents used in experiments were purchased from Sigma Co. (USA) and Fisher Scientific (USA). HPLC grade organic solvents (*n*-pentane & diethylether), used for extraction and chromatography, were redistilled using a spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and Milli-Q water that was generated with a water purification system (Millipore Corporation, Bedford, USA).

Extraction of volatile organic compounds

Fifty grams of sample were homogenized in a blender (MR 350CA, Braun, Spain) and mixed with 1L of distilled water. After adjusting the pH at 6.5 with 1% NaOH, 1 μl *n*-butylbenzene was added as an internal standard. The resultant slurry was used for extraction of VOCs with 200 ml redistilled *n*-pentane:diethylether (1:1, v/v). The extraction of volatile organic compound was carried out for 2 h, using simultaneous distillation-extraction (SDE) apparatus of Nikerson and Likens (1966) type [12]. The solvent, containing compound extract, was dehydrated for 12 h using 10 g anhydrous Na_2SO_4 and then concentrated to approximately 1.5 ml using the vigreux column. This extract was further concentrated to 0.5 ml under gentle stream of N_2 gas and used for gas chromatography-mass spectrometry (GC-MS) analysis.

Chromatographic analysis

Chromatographic analysis was carried out using a Shimadzu GC-MS (Model QP-5000, Shimadzu Co., Kyoto, Japan) in EI (electron impact) mode. The ionization voltage was 70eV and temperatures of ion source and injector were 230^{o}C and 250^{o}C respectively. The capillary column used was a DB-WAX (60m × 0.2mm, i.d., and 0.25 μm, film thickness; J & W, USA). The oven temperature programmed at 40^{o}C (Isothermal for 3 minutes) was ramped to 150^{o}C at 2^{o}C/min and to 220^{o}C at 4^{o}C/min (Isothermal for 20 minutes) followed by 230^{o}C at 5^{o}C/min. Helium was used as the carrier gas at a flow rate of 1ml/min, with injector volume of 1μl using 1:20 split ratio. The standard value of retention index (RI) was determined by two different mixture of *n*-alkane, mixture I (C_7 ~ C_{17}) and mixture II (C_{13} ~ C_{23}) considering as standard (Fig. 1) . 1 µL mixture of alkane was analyzed to find out the retention time (RT) of standards by GC-FID. RI of each peak was established by a basic program that substituted the RT of each peak of *n*-alkane confirmed at GC chromatogram.

Identification of volatile organic compounds

Qualitative analysis of volatile compounds was carried out by identification of compounds from mass spectra with the aid of mass spectral data book [13]. The spectrum of each

analyzed volatile compound agreed with that present in the mass spectrum library of WILLY 139, NIST 12 and NIST 62. The content of the volatile flavor compounds was calculated on a dry weight basis by comparing with peak area percent of the internal standard. The mass spectrometer scanned was ranged from 41 to 450 *m/z*. The following formula was used for quantitative analysis of volatile compounds.

$$\text{Compounds Content (mg/kg)} = \frac{C \times 1000}{A \times B}$$

A : Peak area of internal standard
B : Amount of sample (g)
C : Peak area of each compounds in sample

RESULTS AND DISCUSSION

Volatile organic compounds

The essential oil of *A. racemosus* was extracted by solvent extraction method for 2 h using SDE apparatus and analyzed by GC/MS. Investigation confirmed that Nepalese originated *A. racemosus* contained small amount (59.61 mg/kg) of essential oil. GC/MS chromatogram is presented in fig. 1 (A) and the differences among the components are also evident from table 1. Total 49 volatile compounds, belonging to chemical classes of acid (5), alcohol (15), aldehyde (12), ester (1), hydrocarbon (8), ketone (5), N-containing compounds (1), and miscellaneous (1) were tentatively identified. Alcohol was the dominant phytochemical family with the highest proportion accounting by 49.82% of total content. Five alcohols, out of 15, were monoterpene alcohols. The major alcohol compounds were borneol (26.40 %), myrtanol (13.72 %), pinocarveol (2.37%) and 2-ethylhexanol (1.76 %). Aldehyde was characterized as second largest chemical group containing 16.70%. Perillaldehyde (8.97%) was abundant aldehyde compound and 4-[1-hydroxyethyl]benzaldehyde (1.55%), hexanal (1.34%) and furfural (1.17%) were also detected in considerable amount. Acid and ketone containing 8.97% and 6.98% respectively were also characterized as major chemical groups present in essential oil of *A. racemosus*. Decanoic (4.19%) and undecanoic (2.72%) acids were important components while camphor (3.33%) and 6,10,14-trimethyl pentadecanone (1.71%) were characterized as important ketone components. The percentage of total hydrocarbons was 5.27%. All the hydrocarbons except [*E*]-4-hexadecen-6-yne were monoterpenes. Remaining chemical classes i.e. ester, S-containing compound and N-containing compounds were detected at levels lower than 3%. Only three compounds; borneol, myrtanaol and paraldehyde could occupy 45.09% of the whole content. The analysis of terepenoids in this result shows that the oil dominated by terpenes (mainly monoterpene and its derivatives) accounting more than fifty percent of the oil. This result indicated the presence of a high percentage of oxygenated monoterpenes (49.73%) in essential oil of *A. racemosus*. Conclusively the prime volatile composition of *A. racemosus* was borneol and some major compounds ranged in content order as follows: myrtanol, perillaldehyde, decanoic acid, camphor, α-pinene oxide and pinocarveol.

Similarly, the study on *B. ciliata* enabled the identification of the 43 volatile constituents of *B. ciliata* oil. Identified compounds belonged to chemical classes of acid (7), alcohol (13), aldehyde (5), ester (4), hydrocarbon (3), ketone (8), N-containing compounds (2) and miscellaneous (1). Acid group compounds were present in *B. ciliata* with the highest proportion accounting for 34.06% of the total content. The major acid compounds were capric (decanoic) (24.27 %), caproic (hexanoic) (2.48%), and pelargonic (nonanoic) (2.31%)

acids. Fatty acids such as valeric (pentanoic) acid, enanthoic (heptanoic) acid and caprylic (octanoic) acid, were also detected. Ketone group of chemical class (33.01%) was characterized as a second major chemical group containing 5,6-dihydro-2-pyranone (29.74%) as a dominant compound. Some of the N-containing compounds such as hexanenitril (1.49%) and 2-nitropropane (0.03%) were also detected. Alcohols and hydrocarbons containing 13.77 % and 5.16% respectively were also detected at high levels. Aliphatic alcohols were dominant among this group while linalool (7.51%) contributed the major portion of alcohol. Remaining alcohol compounds were detected lower than 2%. Hydrocarbon group was also detected in this oil including some aliphatic and aromatic constituents. Compounds limonene (1.89%), β-phellandrene (0.34%) and *β*-caryophyllene (2.71%) were the major hydrocarbons related to terpene group. Only six terpenoids occupied 11.8% of total constituent. In conclusion, the prime volatile compound of *B. ciliata* was 5,6-dihydro-2-pyranone and major compounds can be ranged in content the following order: decanoic acid, linalool, nonanoic acid, *β*-caryophyllene and hexanal.

Fifty three compounds of the essential oil from *T. chebula* so far belonging to chemical classes of acid (7), alcohol (16), aldehyde (11), ester (5), furan (2), hydrocarbon (2), ketone (6), N-containing compounds (3), miscellaneous (1) were tentatively identified. Identified compounds represent above 80% of the total peak area. Aldehyde group contained the highest proportion (29.36 %) of the total volatile content. Furfural (12.59%), α-tolualdehyde (7.64 %) and 5-methylfurfural (2.98 %) were detected as major aldehyde compounds. Alcohol (25.61 %) was characterized as second major chemical group. Aliphatic compounds were the main compounds among the alcohols. Acid and aldehyde containing 16.73% and 10.53 % respectively were characterized as major chemical groups. The prime composition was furfural (12.59%) and other ranged in content order as follows: *α*-tolualdehyde (7.64%), camphor (6.13%), 2-heptadecanone (5.77%), nonanoic acid (4.38%) and 5-methyl furfural (2.98%). The analysis shows that fatty acids; butyric acid, valeric acid, caproic acid, enanthoic acid, caprylic acid and pelargonic acid contained 8.65% of total oil. The analysis of terpenoid showed terpenes achieved 10.27% of the oil.

Comparison of bioactive compounds

The present study shows that *A. racemosus* oil largely composed of terpenes, mainly oxygenated monoterpenes dominated by two compounds, borneol and myrtanol. Such essential oils, containing monoterpene as their major constituents are known highly effective for pharmacological activities [14,15]. Compounds myrtanal, α-pinene, perillaldehyde, 2-carene and butyrophenones are well known for their biological activities but their concentrations were relatively lower proportions. Oxygen-containing monoterpes have apparent antispasmodic, sedative and tranquilizing action and beneficial to various systems and metabolic processes in human organism [16]. Compounds borneol, myrtanol and camphor were detected by high amounts in *A. racemosus* . Borneol, a major constituent of *A. racemosus*, is an important ingredient in many Japanese incense formulas, used for analgesia and anesthesia in traditional Chinese and Japanese medicine as well as known for antimicrobial activities [17-19]. Myrtanol, a second major constituent of this oil, exhibits activity as an insect repellent for lice [20]. Camphor, a major constituent of this oil is well-known chemical with its pronounced antimicrobial potentials [21, 22]. Some of the hydrocarbon compounds such as structural isomers of pinene and 2-carene, detected in this sample are very important bioactive compounds as mentioned in litereatures [2324].

Similarly aldehyde compounds such as myrtenal and perillaldehyde were also identified in this study. Myrtenal is terpene-derived aldehydes considered to be produced by tropospheric oxidation of α-pinene [25]. Perillaldehyde inhibits the vasoconstriction as well as therapeutic agents against infections caused by fungus [26, 27]. Beside a flavouring use of furfural, it has a wide variety of uses such as a weed killer, fungicide, affects yeast survival and also affects biochemical enzyme activities [28]. Butyrophenones are widely used drugs for treatment of psychoses and are frequently encountered in forensic chemistry and clinical toxicology [29]. But it is remarkable that compounds *β*-pinene, myrtenal, 2-carene, butyrophenone were detected in very small amounts i.e. below 1% of this oil. Although they usually occur as complex mixtures, their activity can generally be accounted for in terms of their major components.

The characteristic of some VOCs in *B. ciliate* is also discussed here after the identification of compounds. Linalool is important substance used in foodstuffs as a food additive [30,31] and bioactive compound [32, 33]. Litereatures confirm that limonene is an antiseptic chemotherapeutic agent beside its fragrance value [34,35]. *α*-Terpineol has myorelaxant and antispasmodic effects [36]. *β*-Caryophyllene has been commonly used as a fragrance chemical since the 1930s and recent litereature described as woody and spicy odour [37]. Camphor is well-known chemical with its pronounced antimicrobial potentials [21, 22]. But, 2-nitropropane has been found to cause hepatotoxicity in occupationally exposed humans [38]. Compounds *α*-terpineol, camphor and 2-nitropropane contained by less than 1% concentration while compounds *β*-caryophyllene contained by 2.71% concentration of this oil.

The result obtained on *T. chebula* demonstrates only a few VOCs of pharmacological applications. Furfural has a wide variety of uses including weed killer and fungicide, affects yeast survival and also affect biochemical enzyme activities as described previously [39]. Tolualdehyde is used as an additive in non-alcoholic beverages, ice cream and pharmaceuticals. Camphor is well-known chemical with its pronounced antimicrobial potentials, antiseptic, stimulant and antispasmodic properties [21, 22]. On the basis of the above investigation, it may be concluded that the *T. chebula* can yield small quantity of essential oil with a few important VOCs. But due to the low concentrations of VOCs, it is not feasible to commercial production of such oil in large volume.

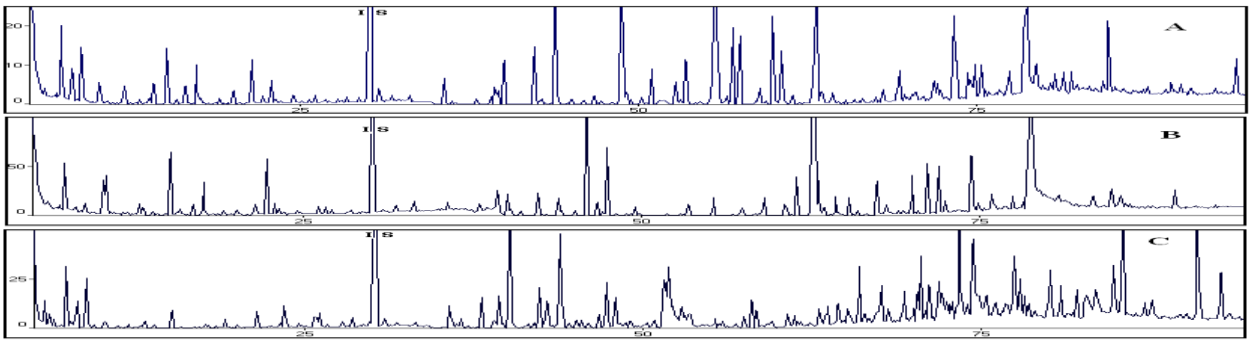

Figure 1. GC/MS chromatogram of volatile organic compounds obtained from *A. racemosus* (A), *B. ciliate* (B) *T. chebula* (C).

Table 1. Volatile organic compounds identified in Nepalese medicinal plants

No.	RI [b]	Compound name	*A. racemosus*		*B. ciliata*		*T. chebula*	
			mg/kg[c]	%[d]	mg/kg[c]	%[d]	mg/kg[c]	%[d]
1	804	Ethyl formate	-	-	-	-	0.24	0.54
2	862	Ethyl acetate	1.35	2.30	1.24	1.84	1.20	2.72
3	923	Ethanol	-	-	0.19	0.29		
4	891	2-Methyl butanal	0.18	0.30	-	-	0.20	0.45
5	896	3-Methyl butanal	0.55	0.94	-	-	0.49	1.11
6	921	2-Propanol	0.91	1.55	-	-	1.07	2.41
7	966	2-Pentanone	0.33	0.56	-	-	-	-
8	969	2-Pentanone	-	-	1.04	1.55	-	-
9	970	Pentanal	0.19	0.32			-	-
10	976	Unknown	-	-	0.76	1.14	-	-
11	1018	Thujene	0.24	0.40			-	-
12	1041	2,4-Dimethyl-3-pentanone	-	-	0.17	0.26	-	-
13	1060	Camphene	0.22	0.37	-	-	-	-
14	1078	Hexanal	0.79	1.34	1.48	2.21	0.32	0.71
15	1090	2-Methyl-1-propanol	0.02	0.03	0.07	0.10	-	-
16	1101	β-Pinene	0.22	0.38	-	-	-	-
17	1108	3-Pentanol	-	-	0.21	0.30	-	-
18	1116	Sabinene	0.53	0.90			-	-
19	1122	2-Pentanol	-	-	0.87	1.30	-	-
20	1145	Butanol	0.07	0.13	-	-	0.17	0.38
21	1161	α-Phellandrene	0.14	0.22	-	-	-	-
22	1181	Pyridine	0.71	1.22	-	-	0.37	0.83
23	1182	Heptanal	-	-	0.13	0.19	-	-
24	1193	Limonene	-	-	1.42	2.11	-	-
25	1201	β-Phellandrene	0.31	0.46	0.23	0.34	-	-
26	1207	3-Methyl butanol	-	-	0.09	0.14	-	-
27	1211	2-Pentynal	-	-	-	-	0.51	1.16
28	1214	[*E*]-2-Hexenal	-	-	-	-	0.08	0.17
29	1247	Unknown	-	-	-	-	0.20	0.45
30	1249	α-Ocimene	-	-	-	-	0.16	0.38
31	1252	2-Pyridyl nitrile	-	-	-	-	0.26	0.57
32	1252	Pentanol	-	-	0.25	0.37	-	-
33	1260	3-Methyl-4-hexen-2-one	-	-	0.07	0.11	-	-
34	1284	2-Nitropropane	-	-	0.02	0.03	-	-
35	1286	Octanal	-	-			0.14	0.31
I.S.	*1310*	*Butylbenzene*	-	-	-	-	-	-
36	1320	3-Methyl-1-pentanol	0.06	0.10	-	-	-	-
37	1335	[*E*]-4-Hepten-2-one			0.19	0.24	-	-
38	1355	Hexanol	0.03	0.05	0.19	0.29	-	-
39	1377	Dipropyl disulfide	0.02	0.02	-	-	-	-
40	1390	Nonanal	0.36	0.61	-	-	0.44	0.99
41	1390	Unknown	-	-	0.20	0.30		
42	1392	2,3-Dihydro-3-methyl furan	-	-	-	-	0.27	0.62
43	1403	3-Methyl-4-heptanone	-	-	-	-	0.09	0.21
44	1427	3-Methyl-3-penten-2-one	-	-	-	-	0.73	1.66
45	1448	Acetic acid	0.34	0.58	0.88	1.31	1.03	2.34
46	1452	2,2-Dimethyl hexanal	0.21	0.37	-	-		
47	1458	Furfural	0.68	1.17	-	-	5.56	12.58
48	1459	Heptanol	-	-	0.67	0.99	-	-
49	1463	2,4-Hexadienal	-	-	0.16	0.24	-	-
50	1491	2-Ethyl hexanol	1.03	1.76	0.88	1.31	1.16	2.63

51	1500	2-Acetylfuran	-	-	-	-	0.64	1.44
52	1515	Camphor	1.94	3.33	0.43	0.64	2.71	6.13
53	1518	Benzaldehyde	0.08	0.14	-	-	0.56	1.25
54	1541	2,3-Dimethyl-2-cyclopentenone	-	-	-	-	0.05	0.12
55	1549	Linalool	-	-	5.03	7.51		
56	1561	Octanol	-	-	0.29	0.43	0.20	0.45
57	1569	5-Methylfurfural	-	-	-	-	1.31	2.98
58	1572	*β*-Caryophyllene	-	-	1.82	2.71	-	-
59	1579	Cynopyrrolidine	-	-	-	-	0.90	2.04
60	1591	Perillaldehyde	5.24	8.97	-	-	-	-
61	1596	Unknown	0.11	0.16	-	-	-	-
62	1625	Butanoic acid	-	-	-	-	0.19	0.43
63	1627	Myrtenal	0.56	0.96	-	-	-	-
64	1637	*α*-Tolualdehyde	-	-	-	-	3.37	7.64
65	1642	Unknown	-	-	-	-	1.99	4.52
66	1656	Nonanol	0.42	0.72	-	-	-	-
67	1658	Furfuryl alcohol	-	-	-	-	0.17	0.38
68	1668	2-Methyl butanoic acid	-	-	0.41	0.62	0.35	0.80
69	1669	Isoboroneol	0.79	1.34	-	-	-	-
70	1697	*α*-Terpineol	-	-	0.47	0.70	-	-
71	1697	Unknown	0.13	0.19	-	-	-	-
72	1703	Borneol	15.42	26.40	-	-	-	-
73	1708	Unknown	0.86	1.30	-	-	-	-
74	1727	Pinocarveol	1.38	2.37	-	-	-	-
75	1737	Pentanoic acid	-	-	0.10	0.14	0.17	0.38
76	1737	[*E*]-4-Hexadecen-6-yne	1.31	2.25	-	-	-	-
77	1747	α-Farnesene	-	-	-	-	0.56	1.28
78	1753	Decanol	-	-	-	-	0.41	0.92
79	1764	2-Carene	0.19	0.29	-	-	-	-
80	1766	2,4-Nonadienal			0.37	0.56	-	-
81	1780	*α*-Pinene oxide	1.50	2.57	-	-	-	-
82	1792	*δ*-Hexalactone	-	-	0.12	0.18	-	-
83	1792	4-[1-Hydroxyethyl]benzaldehyde	0.91	1.55	-	-	-	-
84	1793	Butyrophenone	0.32	0.48	-	-	0.39	0.90
85	1795	Isobutyrophenone	-	-	0.19	0.29	-	-
86	1807	[*E,Z*]-2,4-Decadienal	0.02	0.03	-	-	-	-
87	1809	[*E,Z*]-2,4-Decadienal	-	-	0.91	1.36	-	-
88	1838	5,6-Dihydro-2-pyranone	-	-	19.94	29.74	-	-
89	1841	Unknown	0.24	0.37	-	-	-	-
90	1844	Hexanoic acid	-	-	1.67	2.48	0.36	0.83
91	1848	Myrtanol	8.02	13.72	-	-	-	-
92	1856	Unknown	-	-	0.15	0.19	-	-
93	1858	Guaiacol	0.11	0.19	-	-	0.52	1.16
94	1874	5-[2-Propenyl]-1,3-benzodioxole	-	-	0.39	0.58		
95	1874	Methyl-3-phenylpropenoate	-	-	-	-	0.34	0.76
96	1877	Benzylalcohol	-	-	-	-	0.51	1.16
97	1896	Dodecanol	-	-	-	-	0.10	0.24
98	1897	Unknown	-	-	0.28	0.35	-	-
99	1913	Benzeneethanol	-	-	-	-	1.30	2.96
100	1952	Heptanoic acid	-	-	1.02	1.52	0.31	0.71
101	1957	*p*-Creosol	-	-	-	-	0.87	1.96
102	1970	*α*-Methylbenzyl alcohol	0.15	0.22	-	-	-	-
103	1972	*α*-Phenylethyl alcohol	-	-	0.19	0.29	0.40	0.90
104	2002	Unknown	0.64	0.96	-	-	-	-

105	2023	Hexanenitril	-	-	1.00	1.49	-	-
106	2031	Unknown	-	-	-	-	0.76	1.70
107	2039	Nerolidol	-	-	-	-	1.27	2.86
108	2055	Octanoic acid	-	-	1.36	2.03	0.84	1.92
109	2077	2,6-Dimethylphenol	-	-			1.11	2.51
110	2078	Methyl cinnamate	-	-	1.26	1.89	-	-
111	2083	Benzyl alcohol	0.28	0.48	-	-	-	-
112	2084	O-Cresol	-	-	-	-	0.66	1.49
113	2110	6,10,14-Trimethylpentadecanone	1.00	1.71	-	-	-	-
114	2103	2-Methoxy-4-propyl phenol	-	-	-	-	0.37	0.83
115	2111	2-Heptadecanone	-	-	-	-	2.54	5.77
116	2130	Octanoic acid	0.52	0.78	-	-	-	-
117	2134	Eugenol acetate	-	-	-	-	0.78	1.75
118	2136	3,4-Dimethylphenol	-	-	-	-	0.54	1.23
119	2139	*p*-Cymen-3-ol	0.50	0.75	-	-	-	-
120	2148	3-Methoxyacetophenone	0.59	0.90	-	-	-	-
121	2158	Methyl nonanoate	-	-	0.38	0.58	-	-
122	2161	2-Phenylisopropanol	-	-	0.12	0.18	-	-
123	2184	Nonanoic acid	0.41	0.70	1.55	2.31	1.93	4.38
124	2205	Decanoic acid	2.79	4.19	16.26	24.27	-	-
125	2211	Unknown	2.10	3.18	-	-	-	-
126	2373	Undecanoic acid	1.59	2.72	-	-	-	-
			59.61	**100.00**	**67.12**	**100.00**	**44.17**	**100.00**

[a]retention time, [b]retention index, [c]amount (mg/kg), [d]content (%)

Comparative profile of VOCs showed, aldehyde and alcohol groups were found among all the tested plants. Aldehyde group was dominant in *T. chebula* by accounting 29.36%. Similarly ketone and alcohols group were dominant in *B. ciliata* and *A. racemosus* respectively. A particular property of the aldehyde volatile oils is their insect repellent activity due to very strong scent. Similarly, ketone dominating species showed lipolytic, mucolytic, sedative, analgesic, anti-coagulant, anti-inflammatory, digestive, expectorant and stimulatant properties. This would be due to moderate electronegativity and strong polarity of ketones. Some ketones are known to be neurotoxic. Indeed, borneol and 5,6-dihydro-2-pyranone, were detected as major compounds in *A. racemosus*, *B. ciliata* respectively. Linalool, camphor and α-terpineol present in most of the oils show that all species have antinociceptive, antispasmodic, and antimicrobial activities. Comparatively *A. racemosus* possesses large number of bioactive compounds than other two plants.

CONCLUSIONS

Careful study of volatile organic compounds of three medicinal plants of Nepal showed that there are several bioactive volatile components present in the sample which could be isolated and used for the therapeutic purpose. Only six VOCs, ethyl acetate, hexanal, acetic acid, 2-ethyl hexanol, camphor and nonanoic acid were common among three plants. However, several bioactive compounds; camphor, borneol, capric acid, furfural, myrtanal, α-pinene, α-terpeniol, perillaldehyde, 2-carene, butyrophenones, furfural, β-caryophyllene, 2-nitropropane etc were detected with various proportions.

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