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Antimicrobial Activity and Composition of *Rindera lanata* (LAM.) Bunge var. *canescens* (A.D.C.) Kosn. Essential oil Obtained by Hydrodistillation and Microwave Assisted Distillation

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Abstract: The composition of essential oils of *Rindera lanata* (LAM). Bunge var. *canescens* (A.D.C) Kosn. obtained by hydro-distillation (HD) and microwave assisted distillation (MW) by GC, GC/MS. Thirty three and thirty nine compounds were identified in the oils representing 81.20% and 78.57% of the oils obtained by HD and MW respectively. Aldehydes were shown to be the main group of constituents of the MW 40.11% and 15.23%, respectively. However, the major group in the HD was found to be 25.35% alcohols and 23.78% hydrocarbons. 6-Methyl heptan-2-ol (15.97%) was the main compound of the HD. Furthermore, in the MW assisted essential oil, the major compound present was decane (10.50%). Terpenoid class compounds were found in essential oils and oxygenated monoterpenes were determined as major group (13.34% and 7.19%). Antimicrobial activity of the isolated essential oils of the plant was also investigated and they showed moderate antimicrobial activity against the tested microorganisms.

Keywords: Boraginaceae; *Rindera lanata*; hydro-distillation; microwave; essential oil.

1. Plant Source

The genus *Rindera pallas.* (Boraginaceae) comprises 25 species, mainly in central and western Asia [1]. All species of this genus are widespread in Anatolia, Iran, Iran-Azerbaijan, Iraq and Transcaucasus [2-4]. Rindera genus is represented by 4 species comprising 5 taxa including two endemics in Turkey [5]. One of these is *Rindera dumanii* diagnosed by H. Duman, which only grows in Beyşehir, Ankara, in the village of Akseki [5,6]. *Rindera caespitosa* is also found in Erzurum, Turkey [7,8]. Furthermore, *Rindera gracea* is an endemic plant growing South-East Europe, the Mediterranean Basin and founds a natural habitatoin rocky terrain of Greece [1,9].

Plant material and isolation of the essential oil *Rindera lanata* (Lam.) Bunge var. *canescens* (A.D.C) Kosn. (Boraginaceae) was collected in June 2011 from grassland and meadow areas in

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Aydıntepe-Bayburt, Turkey (at altitudes of ~1540 m) in the northeastern part of Turkey. The plant was authenticated by Salih Terzioglu and voucher specimens were deposited at KTU, Herbarium of the Faculty of Forestry, KATO (KATO 8764), Karadeniz Technical University, Turkey.

R. lanata var. *canescens* is a perennial gramineous plant, growing in meadows, on grassland and volcanic slopes (~ca.1300-3500m) [7]. In Turkey, when in flower, *R. lanata* (Lam.) var. *canescens* has a white fuzz and purple-pink fruit, the leaves of which are covered with a medium down [10].

2. Previous Studies

Rindera pallas. (Boraginaceae) genus is generally known to be a rich source of pyrrolizidine alkaloids (PAs) group of secondary metabolites [11,13]. In previous work, it has been established that PAs have a neurotoxic, mutagenic, carcinogenic and antitumor activities [14,15]. The Boraginaceae family, which includes 455 genus and 9551 species, has abundant fatty acids [16,18]. Fatty acids, the most important fatty acids of which are Omega 3 and Omega 6, are major sources of energy and they are the initiators of essential substances in the body (structural and functional) [18].

The aim of this study is that whether *R. lanata* var. *canescens* has biological activity or not because of the biological activity results from the PAs in the *Rindera* genus. In this study, essential oils obtained with two different ways (HD and MW) are compared, in order to find the best methods for identifying much more compound from *R. lanata* var. *canescens*. The palynological contribution to the system of *Rindera* genus was performed in previous work [1]. There is a study about FAs values and lipids, lipophilic components in the essential oils of *R. pallas* genus [19]. In previous studies about *Rindera oblongifolia* was worked the acids of triglycerides of the seed oil and lipids from fruit [20,21]. No study has been found concerning the essential oil composition obtained from HD and MW assisted distillation and antimicrobial activity of *R. lanata* var. *canescens.*

3. Present Study

The fresh aerial parts (170g and 165g) of *R. lanata* var. *canescens* were subjected to hydro-distillation in a modified Clevenger type apparatus and microwave assisted distillation.

In the hydro-distillation Clevenger type apparatus was used for 4 h to produce oil and in the microwave distillation was carried out at atmospheric pressure using Milestone DryDIST microwave apparatus with a fixed power of 600 W at 110 ºC (40 minute). Previously an infrared (IR) sensor had been used to control the temperature [22,24].

Each plant was placed in the bottom of the Clevenger-type apparatus in a cooling bath (-15 ºC) and MW resistant flask of Clevenger type apparatus (yields v/w: 0.04 and 0.09%) with 50 mL water, respectively. The oils were extracted with HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulfate and stored in sealed vials at 4-6 ºC before analysis by GC/FID and GC/MS.

Gas chromotography (GC) and Gas chromotgraphy-mass spectrometry (GC/MS); The capillary GC/FID and GC/MS analyses were performed using Agilent-5973 Network System, equipped with a FID (supplied with air and hydrogen of high purity) and a split inlet. HP-5 capillary column (30m×0.32 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas, at a flow rate of 1mL/min. The injections were performed in splitless mode at 230 °C. Two µicrolitres of essential oils solutions in hexane (HPLC grade) was injected and analysed with

Table 1. Identified components and the chemical class distribution in the essential oils of *R. lanata* var. *canescens.*

	Compounds	A	B	A	B	Lit. RI	Lit.Ref.
		% Area[a]	% Area[a]	Ex. RI[b]	Ex. RI[b]		
1	Nonane	-	2.92	-	901	900	[26]
2	6-Methyl heptan-2-ol	15,97	-	958	-	965	[26]
3	2-Pentyl furan	-	2.01	-	989	991	[25]
4	Octanal	2.10	3.52	992	996	998	[26]
5	Decane	1.59	-	1004	-	1000	[25]
6	(2*E*,4*E*)-Heptadienal	-	0.80	-	1004	1000	[30]
7	2-Phenylacetaldehyde	1.74	3.26	1046	1043	1042	[26]
8	Octanol	2.03	-	1074	-	1068	[25]
9	Linalool	5.40	2.54	1101	1104	1097	[26]
10	Nonanal	3.15	7.20	1105	1103	1101	[26,27]
11	(2*E*,6*Z*)-Nonadienal	-	2.80	-	1156	1155	[26]
12	Nonanol	4.28	1.31	1162	1173	1169	[26]
13	α-Terpineol	2.67	-	1187	-	1189	[26,30]
14	Dodecane	-	0.39	-	1196	1200	[25]
15	Decanal	4.46	10.50	1205	1205	1202	[28]
16	(2*E*,4*E*)-Nonadienal	1.93	-	1210	-	1212	[29]
17	β-Cyclocitral	-	0.46	-	1216	1221	[26]
18	Geraniol	1.81	-	1256	-	1253	[26]
19	(2*E*)-Decanal	-	0.89	-	1263	1264	[28]
20	(2*E*,4*Z*)-Decadienal	-	0.52	-	1295	1293	[26,29]
21	Undecanal	1.13	2.04	1303	1309	1307	[26,29]
22	(2*E*,4*E*)-Decadienal	-	1.34	-	1318	1317	[26,29]
23	(*E*)-β-Damascenone	0.66	-	1380	-	1385	[26]
24	(*Z*)-Jasmone	-	0.37	-	1393	1393	[26]
25	Dodecanal	-	2.30	-	1405	1409	[26]
26	Geranyl acetone	0.37	0.48	1458	1447	1455	26,27
27	(*E*)-β-Ionone	1.25	2.05	1482	1485	1489	[26]
28	Pentadecane	-	0.41	-	1495	1500	[26]
29	Tridecanal	0.39	2.64	1506	1506	1510	[26]
30	Tetradecanal	0.34	1.01	1618	1618	1613	[26]
31	Heptadecane	-	0.49	-	1704	1700	[26]
32	(2*E*)-Tetradecen-1-ol	2.84	-	1721	-	1715	[25]
33	Octadecane	-	0.45	-	1798	1800	[26]
34	Hexadecanal	-	1.23	-	1815	1814	[27]
35	Hexahydrofarnesyl acetone	0.72	0.58	1855	1847	1848	[25]
36	Nonadecane	0.98	1.57	1895	1897	1900	[26]
37	Farnesyl acetone	0.46	0.71	1910	1911	1915	[27]
38	Methyl hexadecanoate	0.78	1.07	1930	1944	1938	[26]
39	Eicosane	1.30	0.56	2002	2002	2000	[26]
40	Ethyl hexadecanoate	0.30	0.34	2008	2000	1993	[26]
41	Methyl linoleate	0.44	4.24	2100	2095	2095	[26,30]
42	Heneicosane	0.39	0.71	2106	2101	2100	[26]
43	γ-Linolenic acid methyl ester	2.25	-	2109	-	2101	[30]
44	Ethyl linoleate	0.66	1.42	2170	2170	2172	[30]
45	Docosane	10.62	3.72	2201	2196	2200	[26, 31]
46	Tricosane	1.44	3.20	2293	2305	2300	[26, 31]
47	Tetracosane	6.22	2.38	2397	2400	2400	[26, 31]
48	Pentacosane	1.25	3.98	2502	2502	2500	[26, 31]
	Terpenoids	13.34	7.19				
	Aldehydes	15.24	40.11				
	Hydrocarbons	23.78	22.79				
	Ester	5.12	7.92				
	Alcohols	25.35	1.31				
	Total	81.20%	78.57%				

A: Hydro-distillation B: Microwave distillation.

[a]%Area obtained by FID peak-area normalization.

[b]RI calculated from retention times relative to t hat of *n*-alkanes (C_5-C_{32}) on the non-polar HP-5 column.

Lit.Ref.: Literatur References.

the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp. The identify of each compound was supported by comparing their indices (RI) with published values [25-31]. The percentage compositions of the oils were computed from GC peak areas without using correction factors. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column is same as GC/FID. Helium was used as carrier gas, at a flow rate of 1mL/min. The injections were performed in splitless mode at 230 °C. Two μicrolitres of essential oils solutions in hexane (HPLC grade) was injected and analysed with the column held initially at 60 °C for 2 min and then increased to 240 °C with a 3 °C/min heating ramp.

The constituents of the essential oils were carried out by a comparison of their retention indices (RI) of the using *n*-alkanes (C_5-C_{32}) as the standards and their mass spectra with those of mass spectral libraries (NIST and WILEY) and literature comparison [25-31]. The GC peak area values from the HP-5 column separation were evaluated to find the ratio of the components of each of essential oils.

Chemical compositions, their percentage and experiment retention indices (RI) and literature retention indices (RI) are presented in Table 1. The main components in the hydro-distillation are 6-methyl heptan-2-ol (15.97%); docosane (10.62%); tetracosane (6.22%); linalool (5.40%) and α-terpineol (2.67%), in the MW assisted distillation decanal (10.50%); nonanal (7.20%); methyl linoleate (4.24%); linalool (2.54%) and *(E)*-β-ionone (2.05%). The hydro-distillation oil identified the presence of 33 compounds, representing 81.20% of the total oil, while 39 components were shown in the MW assisted distillation, accounting 78.57% of the total oil.

The chemical class distributions of the essential components are listed in Table 1. All of compounds were classified into five classes, which are terpenoids, aldehydes, hydrocarbons, esters and alcohols. The major components were alcohols in HD and MW (25.35% and 1.31%), hydrocarbons (23.78% and 22.79%) and aldehydes (15.24% and 40.11%), respectively. Moreover, the value of determined terpenoids (oxygenated monoterpene, oxygenated sesquiterpene) was 13.34% and 7.19%, respectively.

Also, it is known rich in terms of fatty acids of *Rindera* genus, but in this study the ratio of esters is fairly low according to other components classes (5.12% and 7.92%). The major components of esters are γ-linoleic acid methyl ester (2.25% in HD), methyl linoleate (4.24%) and ethyl linoleate (1.42%) in the MW assisted distillation.

As can be seen from the previous data, there are differences in the results regarding esters in the *Rindera* genus. This result is normal because environmental factors affect the chemical composition of the essential composition. According to these data, in terms of the types of components, essential oils are different; however, there is not much difference between the two methods in terms of number of compounds (33 and 39).

The antimicrobial effects of the substances were tested quantitatively in broth media, using double microdilution and the minimal inhibition concentration (MIC) values (μg/100 mL) were determined, respectively [32]. The essential oils of *R. lanata var. canescens* were tested for antimicrobial activity using the agar-well diffusion method. The microorganism and antimicrobial activities of essential oils are shown in Table 2.

While Boraginaceae family contain PAs group have activities neurotoxic, mutagenic, carcinogenic and antitumor activities, *R. lanata var. canescens* don't showed significant biological activity. But it was established that in high concentrations they have a strong antimicotic effect only against *C. albicans* bacteria.

Hexane with a dilution of 1:10 was used as solvent control, by using ampicillin and fluconazole as standard antibacterial and antifungal agents [33]. All test microorganisms were

obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey). All the newly extracted compounds were weighed and dissolved in hexane to prepare an extract stock solution of between 489-107200 microgram/milliliter (µg/100mL).

Table 2. Screening for antimicrobial activity of essential oil in *R. lanata* (Lam.) Bunge var. canescens Kosn. (µg/ 100mL)

Sample	Stock sol. (µg/mL)	Microorganisms and Minimal Inhibition Concentration							
		Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca
R.lanata MW	4890	-	-	-	-	-	-	-	611.2
R.lanata HD	11690	-	-	-	-	-	-	-	584.5
Ampicilin	10	2	32	>128	2	2	<1		
Streptomisin	10							4	
Fluconazole	5								<8

Escherichia coli (*E. coli*) ATCC35218, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *Enterococcus faecalis* (*E. faecalis*) ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B.cereus)* 709 Roma, *Mycobacterium smegmatis* (*M. smegmatis*) ATCC607 and *Candida albicans* (*C. albicans*) ATCC60193.

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