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Chemotypic Characterization and Antioxidant Activities of *Rosemarinus officinalis* Essential Oil from Ethiopian Cultivars

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Abstract

In Ethiopia, *R. officinalis* is locally known as "Yetibs Qitel" used for flavor of various cuisine. Three varieties of *R. officinalis* were registered at Wendo Genet Agricultural Research Center and aimed to define the Physico-chemical properties and antioxidant activities of their essential oils. Hydro distillation of fresh *R. officinalis* leaves was acquired an essential oil yield (v/w) of 1.02% (Rosemary-01), 0.84% (Rosemary-02) and 0.65% (Rosemary-03). GC-MS analysis of the essential oils also revealed that major abundance of Eucalyptol (22.25%), *α*-Pinene (20.39%) and camphor (8.04%) for Rosemary-01; Eucalyptol (21.46%), *α*-Pinene (17.27%) and Verbenone (13.65%) for Rosemary-02 and Eucalyptol (17.33%), *α*-Pinene (15.75%) and endo-Borneol (9.09%) for Rosemary-03 variety. *R. officinalis* essential oils show a free radical scavenging activity against DPPH with an IC_{50} value of the range 457.01-589.68 µL/mL. The synergy of genotype and environmental conditions makes variability on yield, chemical composition and biological activities of *R. officinalis* essential oils to be established in Ethiopia.

Keywords: Antioxidant; Essential oil; Ethiopia; Cultivar; Chemotype; *Rosemarinus officinalis*

Introduction

Aromatic plants have been used since ancient times for various purposes such as preservative and flavors in food, perfumery in fragrance and aromatherapy. One of the products obtained from those plants are essential oils, Concrete, Absolutes and Resinoids [1]. *Rosemarinus officinalis* L. (Labiatae) is among the essential oil-bearing plants which is widely used in fragrance, cosmetics, food and pharmaceutical industry. Besides, it has a potential of antimicrobial and antioxidant activity which are claimed to extend the shelf life of food products [2-4].

In Ethiopia, *R. officinalis* L. locally known as " Yetibs Qitel" and its leaf used as a flavor of varieties of dishes and spices ingredients on preparation of Capsicum annum food colorant powder. The *R. officinalis* essential oil has also used for aromatherapy, cosmetics and flavoring, and preservation of food products. Enemor and Aner Wereda from Gurage Zone and Sebeta from Oromia region are the known commercialized area and source of *R. officinalis* leaves for central market.

One of the research thematic plants conducted at Wendo Genet Agricultural Research Center is *R. officinalis* and has been released three known varieties namely, Rosemary-01, Rosemary-02 and Rosemary-03. Chemotypic characterizations of these three varieties are crucial to provide a specification-based application. The important concepts related to quality of essential oils are the chemical composition and its biological activities. Those physico chemical property and biological activities of the plant's product can vary accordingly to climate, soil composition, plant organ, age and vegetative cycle stage [5,6]. As of the above concept, this work was done to address physicochemical variability and antioxidant activities among three varieties of *R. officinalis* essential oil in Ethiopia (Figure 1).

Figure 1: General View of Rosemary Plants.

Materials and Methods

Sample preparation

The plants before flowering (10 months old) were harvested on mid of January 2017 from Wendo Genet Agricultural Research Center experimental field (1800 m a.s.l., N 39° 1' 44" E 8° 25' 59"). The leaves were striped from the stem and subjected to extract.

Sample extraction

The fresh leaves were distilled through hydro distillation by using Clevenger type apparatus for 3 hours and collected the pale-yellow essential oil after drying with anhydrous Na_2SO_4. The essential oils were subjected to determine the physical property, antioxidant activity and chemical composition profile.

Physical property of essential oil

The yields of the essential oil were calculated based on the formula described below.

Oil yield (v/w (%))=(Amount of distiled oil (mL))/(Amount of fresh distiled leaf (g)) × 100.

The specific gravity of the essential oil was measured by using Picno meter. The refractive indexes of the essential oils were measured by Refractometer (Reichert, AR200) and the optical rotations of the essential oils were measured by a Polari meter (Bio base, Automatic) (Table 1).

Parameters	Rosemary-01	Rosemary-02	Rosemary-03
Essential oil yield (%v/w) fresh based	1.02 ± 0.02	0.84 ± 0.05	0.65 ± 0.06
Specific gravity (g/mL)	0.89	0.87	0.87
Refractive index (25°C)	1.46758	1.46878	1.47068
Optical rotation (0)	17.54	32.819	11.211
FRSA, IC50 (µL/mL)	18.72	457.01	589.68

Table 1: Physical property and FRSA activity of *R. officinalis* varieties essential oil. FRSA: free radical scavenging activity.

Free radicals scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The DPPH free radical scavenging activities of the essential oils were determined by the method described by Williams et al. [7]. Five concentrations (100, 50, 25, 12.5 and 6.25 µL/mL) of 50 µL samples were mixed with 5 mL of 0.004% methanol solution of DPPH. The mixture was incubated for 30 minutes at 37°C. After incubation, the absorbance of the mixture was read at 515 nm using UV-Vis spectrophotometer. Ascorbic acid was used as a positive control. Tests were carried out in triplicate and average values were taken. Inhibition of DPPH free radical was calculated by using the equation:

I(%)=(Ao-As)/Ao × 100,

Where Ao is the absorbance of DPPH solution (containing all reagents except the test sample), and as is the absorbance of the tested sample. The IC_{50} value which represented the concentration of the samples that caused 50% inhibition was determined based on the linear equation of concentrations of the *R. officinalis* oil versus inhibition for all tested samples.

GC-MS analysis

GC-MS (Agilent model 7820 A) was used to determine the chemical composition profile of the essential oil. Solutions of essential oils (2%) were prepared by dissolving with N- Hexane. The instrument was conditioned with a split/ split less injector mode, MS detector (5975), and HP-5 SM capillary column (0.25 mm i.d. × 30 m × 0.25 µm film thickness). Injector was operated on a split ratio of 1:5 with an injection volume were 1 µL and injector temperature was set at 250°C. The MSD interface temperature was set 260°C. Helium was used as carrier gas and controlled in constant flow mode at a linear velocity of 36.6 cm/sec. The oven temperature was programmed to started at 60°C, which is held for 1 minute and ramped at 5°C/min to 80°C with 3 minutes holding; next to the temperature was ramped at 4°C/min to 180°C, which was held for 3 minutes, finally raised at 25°C/min to 300°C with 6 minutes held. The MSD was operated on scan mode in 40-500 m/z range, with ion source and transfer line temperatures held at 230°C and 260°C, respectively. The solvent delay time was 3.4 minutes and taken 43.8 minutes for total run.

Results and Discussion

The oil yield obtained from Rosemary-01 variety is superior (1.02%) and followed Rosemary-02 (0.84%). Rosemary-03 yielded less essential oil (0.65%). Comparable results were reported irrespective of variety from different origins of *R. officinalis* such as Pakistan (0.35%) [8]; Nepal (0.5%), South Africa (0.8%) and Australia (0.9%) [9].

Different physical properties were observed by the varieties of *R. officinalis* essential oils. A higher Optical rotation value was scored by Rosemary-02 variety (32.82o) and next to Rosemary-01 (17.54o). The variability of specific rotation value is occurred due to presence of different enantiomeric distribution and concentration among varieties. Principally the abundance of Verbenone in the *R. officinalis* essential oil enhances positive value of optical rotation due to favors 100% (R) enantiomer in nature and contrarily the abundance of Borneol in the *R. officinalis* essential oil may reduce the optical rotation value as favor of >90% (S) enantiomer [9,10].

From the DPPH assay result, the IC_{50} value of the *R. officinalis* varieties essential oils were varied with 457.01 µL/mL (Rosemary-02) to 589.68 µL/mL (Rosemary-03) and the control Ascorbic acid had an IC_{50} value of 577.04 µg/mL. The variability of FRSA among varieties came due to having different concentration of chemical constituents in their essential oils (Figures 2 and 3).

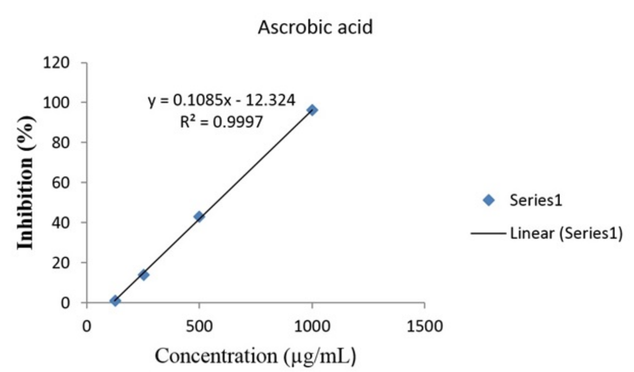

Figure 2: Plots of inhibition of free radical scavenging activities of Ascorbic acid.

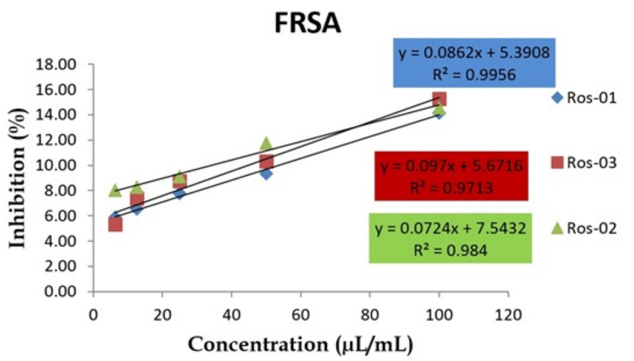

Figure 3: Plots of inhibition of free radical scavenging activities of *R. officinalis* varieties essential oils.

GC-MS analysis reports of *R. officinalis* varieties essential oils revealed that a total of 31 compounds were identified. Eucalyptol and α-Pinene were the dominantly found compounds of the three varieties essential oils with 22.25% and 20.39% (Rosemary-01), 21.46% and 17.27% (Rosemary-02) and 17.33% and 15.75% (Rosemary-03) respectively. Rosemary-01 had higher amount of Camphor (8.04%) and Rosemary-02 had higher amount of Verbenone (13.65%) which is the natural R isomer that made higher value of optical rotation. The third variety (Rosemary-03) was superior in endo Borneol content (9.09%) which is the bitter taste that made to be unpleasant taste among three varieties. This results are comply with the composition of the *R. officinalis* oils reported by Satyal et al. [9-12], from USA, Nepal, South Africa, Kenya, Yemen and Australia origins which are an abundance of α - Pinene (13.5%-38.1%), 1,8-cineole (16.1%-29.4%), Verbenone (0.8%-18.6%), Borneol (2.1%-7%), camphor (0.7%-7.0%), and limonene (1.6%-4.4%) (Figures 4 and 5; Table 2).

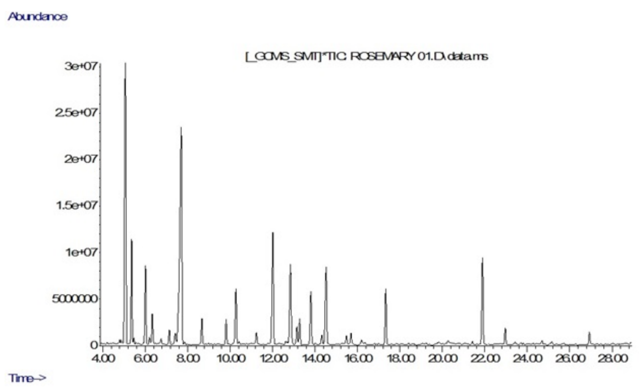

Figure 4: GC chromatogram of *R. officinalis* essential oil.

S No	RT (min)	Name of compound	Molecular Formula	Molecular Weight	Percentage Composition		
					Rosemary-01	Rosemary-02	Rosemary-03
1	4.81	Tricyclene	$C_{10}H_{16}$	136	-	-	0.27
2	4.87	3-Thujene	$C_{10}H_{16}$	136	0.30	0.35	0.29
3	5.09	1R-α-Pinene	$C_{10}H_{16}$	136	20.39	17.27	15.75
4	5.37	Camphene	$C_{10}H_{16}$	136	5.17	3.79	4.72
5	5.47	2,4(10)-Thujadiene	$C_{10}H_{14}$	134	0.44	0.72	0.78
6	6.02	(-)-β-Pinene	$C_{10}H_{16}$	136	4.33	3.45	4.56
7	6.34	β-Myrcene	$C_{10}H_{16}$	136	1.40	1.93	1.48
8	6.75	α-Phellandrene	$C_{10}H_{16}$	136	-	-	0.35
9	7.14	α-Terpinene	$C_{10}H_{16}$	136	1.01	1.16	0.74
10	7.42	o-Cymene	$C_{10}H_{14}$	134	0.68	0.22	1.32
11	7.72	Eucalyptol	$C_{10}H_{18}O$	154	22.25	21.46	17.33
12	7.87	trans-β-Ocimene	$C_{10}H_{16}$	136	-	-	0.63
13	8.67	γ-Terpinene	$C_{10}H_{16}$	136	1.70	2.64	1.22
14	9.82	Terpinolene	$C_{10}H_{16}$	136	1.47	1.36	1.08
15	10.30	β-Linalool	$C_{10}H_{18}O$	154	3.43	3.76	4.38
16	11.25	Chrysanthenone	$C_{10}H_{14}O$	150	0.60	1.08	1.07
17	11.77	L-Pinocarveol	$C_{10}H_{16}O$	152	-	-	0.86
18	12.01	(-)-Camphor	$C_{10}H_{16}O$	152	8.04	4.48	3.46

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19	12.92	endo-Borneol	$C_{10}H_{18}O$	154	5.76	6.02	9.09
20	13.18	3-Pinanone, cis	$C_{10}H_{16}O$	152	1.08	1.22	2.56
21	13.30	(-)-4-Terpineol	$C_{10}H_{18}O$	154	1.62	1.71	1.66
22	13.82	α-Terpineol	$C_{10}H_{18}O$	154	3.52	2.00	2.80
23	14.37	(-)-Borneol	$C_{10}H_{18}O$	154	-	-	1.03
24	14.58	l-Verbenone	$C_{10}H_{14}O$	150	5.99	13.65	6.47
25	15.71	exo-2,7,7-trimethylbicyclo [2.2.1] heptan-2-ol	$C_{10}H_{18}O$	154	0.72	0.74	0.74
26	16.21	Geraniol	$C_{10}H_{18}O$	154	-	3.94	0.91
27	17.37	Bornyl acetate	$C_{12}H_{20}O_2$	196	3.35	2.25	7.22
28	21.42	Methyleugenol	$C_{11}H_{14}O_2$	178	-	0.35	0.97
29	21.90	Caryophyllene	$C_{15}H_{24}$	204	5.50	3.18	3.88
30	22.96	Humulene	$C_{15}H_{24}$	204	1.25	0.62	0.79
31	25.17	(-)-β-Cadinene	$C_{15}H_{24}$	204	-	-	1.20

Table 2: Chemical compositions of *R. officinalis* varieties essential oils.

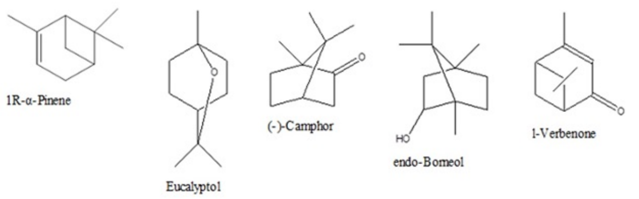

Figure 5: Structures of major abundant compounds of *Rosemarinus officinalis*.

Conclusion

Genotypic effect is making variability on the essential oil yield and physiology of the three varieties of *R. officinalis*. Unlike, the environment effect influenced to have similar major compounds which are dominated by Eucalyptol and α-Pinene. From relative concentration of the third majorly found compound of three *R. officinalis* varieties, it's proposed to be characterized as camphor (Rosemary-01), verbenone (Rosemary-02) and endo Borneol (Rosemary-03) chemo types. *R. officinalis* essential oils have a strong antioxidant activity against DPPH free radicals with comparable to the reference Ascorbic acid.

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