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Chemical Composition Variability of Ethiopian Rosemary (*Salvia rosmarinus* Schleid) Accessions

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Abstract: Essential oil of forty-five Ethiopian rosemary accessions was analyzed using gas chromatography-mass spectrometry (GC-MS) to investigate the variability of essential oil composition.A total of 42 compounds, representing 95.85-98.89% of the total oil composition were detected. The oils were dominated by α-pinene (5.08-40.62%), 1, 8-cineole (8.13-38.48%), camphor (2.15-23%), verbenone (1.83-20.25%), β-caryophyllene (2.12-9.39%), endo-borneol (1.79-12.56%), camphene (1.69-7.86%,), bornyl acetate (1.55-9.65%), limonene (1.65-6.07%), α-terpineol (1.66-6.37%), β-pinene (1.55-6.45%) and linalool (1.58-3.91%). Among these, α-pinene, 1, 8-cineole, camphor and verbenone were the most ubiquitous constituents and found to present in all accessions, while the rest varied among the accessions. Correlation analysis showed that α-pinene, 1, 8-cineole and verbenone were correlated negatively with the majority of the major compounds, while the association of camphor with the entire main constituent was not significant, except with α-pinene ($r = -0.46^{***}$) and linalool ($r = -303^{*}$). Based on the relative concentration of the main constituents of the essential oils, six distinct chemotypes were identified for Ethiopian rosemary accessions. The chemotypes were: α-pinene/1, 8-cineole/camphor; α-pinene/1, 8-cineole/verbenone; α-pinene/1, 8-cineole/endo-borneol; 1, 8-cineole/camphor/α-pinene; verbenone/α-pinene/camphor and camphor/1, 8-cineole/verbenone. The defined chemotypes demonstrated the presence of high chemical variability among individual plants that makes it difficult to describe a single chemotype based on geographic origin. Interestingly, more contribution of genotype for the chemical variability than environmental factor was noticed in the present study, indicating the inherent nature of the essential oil constituents. Overall, the observed high essential oil constituent variability among the tested accessions reflected the enormous potential of Ethiopian rosemary germplasm for wider applications in different destinations that are predominated by rosemary products.

Key words: Chemical Composition · Chemotypes · Essential Oil · *Salvia rosmarinus* · Variability

INTRODUCTION

Plant secondary metabolites, such as essential oils are recognized for their various biological effects like antimicrobial, antifungal, antibacterial, insecticidal and antioxidant activities [1-6] and applied in the fields of cosmetics, sanitary, pharmacology and food preservation [7-11]. Of essential oil-bearing plants, *Salvia rosmarinus* Schleid., commonly known as rosemary is ancient plant that has been widely assessed for the quality of its essential oils and traded all over the world [12-13]. The essential oil of rosemary owns significant antimicrobial, anti-cancerous, anti-lipid peroxidation and several other medicinal activities [14-18]. It also possesses high antioxidant activity and widely used as natural preservatives in the food and cosmetic industry [19-23]

Notably, rosemary essential oil is characterized by the presence of various chemical compounds which are primarily responsible for its different biological activities and anti-oxidant properties [18, 24, 25]. Studies carried out elsewhere showed that chemical compositions such as α-pinene, bornyl acetate, camphor and 1, 8-cineole are among the main compounds responsible for the anti-microbial activities of rosemary essential oil [26-27].

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The biological activities of various rosemary oil chemical compositions have been also described in different literatures [14, 28-30].

Due to consumers' concern about the negative side effects of synthetic products and the growing demand for natural agents [31-33], rosemary extracts have received significant attention because of their strong natural antioxidant and antimicrobial properties [34-35]. Thus, characterizing the chemical composition of rosemary essential oils and understanding the extent of chemical variability is crucial to exploit the available germplasm for the desired compositions.

The chemical composition of rosemary essential oil has been widely studied; and its high variability in relation to genetic factors, geographic origin and isolation methods were reported [1, 36-39]. High variability in rosemary essential oil was evident by the presence of at least 13 different rosemary oil chemotypes elsewhere, based on their relative percentages of α-pinene, 1, 8-cineole, camphor, borneol, verbenone and bornyl acetate [40]. Different findings indicated that the chemical variability of rosemary essential oil is mainly dependent on the genetic factors rather than on environmental conditions and geographic locations [1, 21], suggesting the possibilities of genetic manipulations to improve the crop for desired compounds.

An effective improvement program, in turn, largely depends on the prior characterization of the germplasm for the extent of genetic variability in the required characters. Although several studies were carried-out to investigate the chemical composition of rosemary essential oil in different countries, there is no effort made to examine the chemical composition of rosemary essential oil in Ethiopia except a study conducted by Bekri *et al.* [41] on chemotypic characterization of only three Ethiopia rosemary varieties.The current study was, therefore, designed to assess the variability exists in essential oil compositionof rosemary germplasms grown in Ethiopia.

MATERIALS AND METHODS

Experimental Site and Plant Materials: A total of 45 rosemary accessions were used for the study. Detail information of the accessions and experimental site is given in chapter 2 (sections 2.2.1 and 2.2.2). The accessions were multiplied via stem cutting to get sufficient planting materials for the experiment. After multiplication of the accessions, soft stem cuttings were taken and seedlings were raised in the nursery before being transplanted to the experimental field. After being raised in the nursery, healthy, equal-size and well performed seedlings were transplanted in the main experimental field with inter and intra row spacing of 60 cm. Prior to chemical composition analyses, all accessions were cultivated for more than 2 years (from seedling multiplication to field experimentation) under homogeneous field management conditions to minimize the influence of environmental factor on chemical composition of rosemary essential oil as suggested by Li *et al.* [21].

Sample Preparation and Essential Oil Extraction: For essential oil extraction, fresh leaves of composite samples (300 g) was taken from each accession and subjected to hydro-distillation for 4 hrs using a Clevenger-type apparatus. Then essential oil was collected after drying with an anhydrous Na_2SO_4 and stored in a refrigerator until GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: GC-MS analysis was carried out in the Natural Products Laboratory at Wondo Genet Agricultural Research Center. The identification of the essential oil composition was performed using a GC-MS (Agilent model 7820A) equipped with auto sampler (Agilent model G4513A), MS detector (5975) and HP-5ms capillary column (0.25 mm i.d. × 30 m × 0.25 µm film thickness). A 0.2% (w/v) of the essential oil solution was prepared by diluting in n-hexane and the instrument was conditioned with a splitless injector mode. Oven temperature of GC was programmed as: initiation at 60°C for 1 min, increase to 80°C at 5°C/min intervals, which were kept for 3 min and increased to 180°C at 4°C/ min interval and held for 3 min and finally increased to 300°C at 25°C/min interval with a holding time of 6min. The injection part temperature was set at 250°C. Helium was used as carrier gas and controlled in constant flow mode at a linear velocity of 36.6 cm/sec. The mass spectrometer interface temperature was set at 260°C and operated on scan mode in 40-500 m/z range, with ion source and transfer line temperatures of 230°C and 260°C, respectively. Injection of the sample was operated on a split ratio of 1:5 with an injection volume of 1µL. The GC-MS total run time was 46 min with solvent delay time of 3.4 min. The identification of volatile compounds was primarily based by comparing their mass spectra, performed with MSDChem software (Agilent), with NIST/WILEY libraries and retention times.

Statistical Analysis: Compounds that were not detected in the study were given a value of zero. Twelve compounds at levels more than 1.5% were used for analysis and discussion. The compounds were: α-pinene, 1, 8-cineole, camphor, verbenone, β-caryophyllene, endo-borneol, camphene, bornyl acetate, limonene, α-terpineol, β-pinene and linalool. Descriptive analysis using MINITAB version 17 software was conducted and percentage of compounds across accessions were presented as mean ± SE. Correlations analysis among the major compounds was performed using the same software

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oils: GC-MS analysis of the essential oil extracted from the 45 rosemary accessions allowed identification of forty-two compounds, accounting for 95.85-98.89% of the total oil composition. The main constituents identified in most of the accessions were α-pinene (5.08 - 40.62%), 1, 8-cineole (8.13 - 38.48%), camphor (2.15 - 23%), verbenone (1.83 - 20.25%), β-caryophyllene (2.12 - 9.39%), endo-borneol (1.79 - 12.56%), camphene (1.69 - 7.86%,), bornyl acetate (1.55 - 9.65%), limonene (1.65 - 6.07%), α-terpineol (1.66 - 6.37%), β-pinene (1.55 - 6.45%) and linalool (1.58 - 3.91%) (Table, 1). The level of identified compounds revealed the presence of high chemical variability among rosemary accessions grown in Ethiopia.

It was interesting to note that α-pinene, 1, 8-cineole, camphor and verbenone dominated the essential oil composition and were found to present in all accessions, while the rest varied among the accessions. Previous studies explained that the presence of α-pinene, 1, 8-cineole, camphor and verbenone as the major characteristic components of this species [13, 42-45], which is in consistent to our data. In fact, several biological activities of rosemary oils were also attributed to the presence of these major compounds [27, 46].

Among the major compounds, α-pinene was the most ubiquitous constituent varied from 5.08 (Ros41) to 40.62% (Ros38) across accession with an average value of 24.57% (Table 6.1). Congruent to this result, several studies reported an abundant distribution of α-pinene in rosemary genotypes elsewhere [12, 13, 21, 40]. 1, 8-cineole was the second most abundant constituent found in the range of 8.13% (Ros23) to 38.48% (Ros33) between accessions with an overall mean value of 18.88% (Table 1), an outcome similar to that described by different authors [40, 47-48]. The relative quantities of camphor and verbenone ranged between 2.15% (for Ros34) and 23% (for Ros29); and 1.83% (for Ros21) and 20.25% (for Ros24), respectively. In agreement with this finding, wide variation in camphor (7.27–13.02%) and verbenone (0.15–6.61%) contents of different rosemary varieties essential oil were reported [18, 39]. It is also important to mention the existence of some accessions with an essential oil rich in endo-borneol as much as: 10.58% (Ros05), 11.97% (Ros17), 12.56% (Ros18) and 10.11% (Ros34). The result obtained here is in accordance with an earlier study by Bekri *et al*. [41], who defined an abundant presence of α-pinene, 1, 8-cineole, camphor, verbenone and endo-borneol in the essential oil of Ethiopian rosemary verities.

Rosemary essential oils are known to have different biological activities which depend on their chemical composition [29, 47, 49] and the different compositions are likely to present different biological activities [1, 50]. Concerning this, different researchers reported antimicrobial and antioxidant activity of rosemary essential oil rich in 1, 8-cineole, camphor and borneol [1, 17, 28, 30]. (Similarly, strong antimicrobial activity of rosemary oil rich in α-pinene, bornyl acetate, camphor and 1, 8-cineolewas described at different times [18, 26, 32]. Several biological activities of 1, 8-cineol, α-pinene and camphor from rosemary essential oil was also stated by different authors elsewhere [27, 51, 52]. Thus, the major compounds detected in the essential oil of the studied accessions indicated the richness of the oil for various biological activities.

Besides, rosemary essential oil has wider application in cosmetics and food industries [14, 53, 54]. Hence the higher essential oil composition variability observed in the tested accessions lies with the great potential of Ethiopian rosemary accessions for broader application either by direct selection or through improvement activities.

Correlation among the Major Constituents: In order to investigate the relationship among chemical variables, correlation analysis was performed (Table 2). Examining the association among the major compounds, α-pinene correlated negatively with camphor (r = -0.462***), endo-borneol (r = -0.632***), α-terpineol (r=0.328*), verbenone (r=-0.405**), bornyl acetate (-0.51***) and β-caryophyllene (-0.41**) and positively with camphene (r = 0.434**). Similarly, the association of 1, 8-cineole with linalool (r=-0.321*), endo-borneol (r=-0.434**), α-terpineo (r = -0.528***), verbenone (r = -0.357*), bornyl acetate (r = -0.52***) and β-caryophyllene (r = -0.54***) was negative. Verbenone correlated negatively with camphene

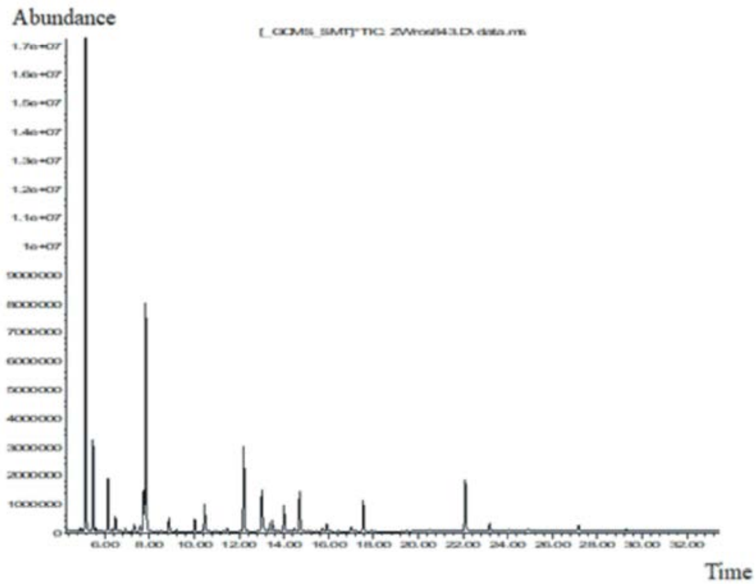

Fig. 1: GC-MS chromatogram of Ethiopian rosemary essential oil

Table 1: Retention times, range and mean of the 12 main essential oil constituents across 45 rosemary accessions

			Range		
No.	RT(min)	Compounds	Minimum (%)	Maximum (%)	Mean ±SE
1	5.14	α-Pinene	5.08	40.62	24.57± 1.39
2	5.48	Camphene	1.69	7.86	4.59±0.23
3	6.16	β-Pinene	1.55	6.45	3.15±0.14
4	7.74	Limonene	1.65	6.07	3.59±0.13
5	7.84	1, 8-cineole	8.13	38.48	18.88±0.94
6	10.47	Linalool	1.58	3.91	2.37±0.10
7	12.22	Camphor	2.15	23.00	9.40±0.70
8	13.03	Endo-borneol	1.79	12.56	4.95±0.38
9	14.01	α-Terpineol	1.66	6.37	3.20 ±0.19
10	14.72	Verbenone	1.83	20.25	7.04±0.74
11	17.56	Bornyl acetate	1.55	9.65	3.85 ±0.32
12	22.11	β-Caryophyllene	2.12	9.39	4.97±0.29

Table 2: Pearson's correlation coefficient matrix for 12 main volatile constituents of the essential oil of 45 rosemary accessions

	α-Pinene	Camphene	β-Pinene	Limonene	1, 8 cineole	Linalool	Camphor	endo-Borneol	α-Terpineol	Verbenone	Bornyl acetate
α-Pinene											
Camphene	0.43^{**}										
β-Pinene	0.1^{ns}	0.22^{ns}									
Limonene	0.03^{ns}	-0.15^{ns}	-0.31^{*}								
1, 8 cineole	0.14^{ns}	-0.26^{ns}	0.09^{ns}	0.004^{ns}							
Linalool	-0.16^{ns}	-0.21^{ns}	0.23^{ns}	0.21^{ns}	-0.32^{*}						
Camphor	-0.46^{***}	-0.15^{ns}	-0.11^{ns}	-0.27^{ns}	0.19^{ns}	-0.30^{*}					
endo-Borneol	-0.63^{***}	-0.18^{ns}	0.06^{ns}	0.06^{ns}	-0.43^{**}	0.26^{ns}	-0.07^{ns}				
α-Terpineol	-0.33^{*}	-0.04^{ns}	-0.27^{ns}	0.15^{ns}	-0.53^{***}	-0.02^{ns}	-0.08^{ns}	0.46^{***}			
Verbenone	-0.41^{***}	-0.31^{*}	-0.47^{***}	0.10^{ns}	-0.36^{*}	0.08^{ns}	-0.02^{ns}	0.18^{ns}	0.31^{*}		
Bornyl acetate	-0.51^{***}	-0.24^{ns}	0.03^{ns}	-0.14^{ns}	-0.52^{***}	0.26^{ns}	-0.27^{ns}	0.77^{***}	0.34^{*}	0.33^{*}	
β-caryophyllene	-0.41^{**}	-0.1^{ns}	-0.08^{ns}	0.07^{ns}	-0.54^{***}	0.28^{ns}	0.19^{ns}	0.31^{*}	0.56^{***}	0.04^{ns}	0.32^{*}

(r =-0.308*) and β–pinene (r = -0.467***) and positively with α-terpineol (r = 0.310*) and bornyl acetate (r =0.33*). The association of camphor with the entire major constituent was non-significant except its negative association with α-pinene (r = -0.46^{***}) and linalool (r = -303*). 1, 8-cineole and α-pinene doesn't correlated to each other (r = 0.143^{ns}), but each of them strongly correlated to the other constituents, creating variable chemical groups.

Understanding the relationship among the main chemical constituents would give insight to the breeders on how to manipulate and improve the crop for the desired chemical constituents. In this study, it is observed that α-pinene and camphene correlate positively to each other, showing an increase in one of them might result in an increase of the other. On the other hand, a significant negative correlation of α-pinene, 1, 8-cineole and verbenone with the majority of the main constituents indicates that any one of these constituents would increase as the other decreases. So this relation should be considered during selection activities aimed at the improvement of these constituents. Moreover, the positive associations of endo-borneol, α -terpineol, bornyl acetate and β-caryophyllene to each other indicate the possibility of simultaneous improvement of these chemical traits.

Chemotypes of Rosemary Accessions: Based on the percentage of the investigated major constituents, the 45 rosemary accessions were grouped into six different chemotypes as follows:(1) α-pinene/1, 8-cineole/camphor, (2) α-pinene/1, 8-cineole/verbenone, (3) α-pinene/1, 8-cineole/endo-borneol, (4)1, 8-cineole/camphor/α-pinene, (5) verbenone/α-pinene/camphor and (6) camphor/1, 8-cineole/verbenone (Table .3). The result was consistent with Satyal *et al.* [40], who classified rosemary population from different countries into a mixed chemotypes of (i) α-pinene/1, 8-cineole, (ii) verbenone/α-pinene/camphor/1, 8-cineole, (iii) myrcene/1, 8-cineole/camphor, (iv) 1, 8-cineole/camphor/α-pinene and (v) α-pinene/β-pinene/camphene. Our finding was also in agreement with Jordan *et al.* [14], who identified six mixed chemotypes for essential oils of rosemary populations from Murcia.

Chemotype 1 consisted of one released variety (Ros08) and 25 accessions (57.78%) from all collection regions except commercial farm. Chemotype 2 has four accessions (8.89%) including two accessions from Hadiya, one from Arssi and one released variety (Ros01). The third chemotype was made up of four accessions (8.89%) all from Harari except one released variety (Ros05). These three chemotypes were dominated by α-pinene but they differed from each other in their third major constituents. The result was comparable with previous findings that reported α-pinene as leading constituents in rosemary populations from France [39, 55], Spain [42], Iran [45] and Romania [56].

Chemotype 4 is dominated by 1, 8-cineole which has five accessions (11.1%) those from commercial farm, Wolaita and Gurage and was in agreement with different authors [1, 18, 57, 58] who described 1, 8-cineole as a leading constituent for rosemary population from various countries. Similar findings were also stated by different writers [26, 36, 59]. Chemotype 5 is dominated by verbenone and is comprised of four accessions all from North Shewa. Corresponding to this finding [60] reported verbenone as a leading constituent for rosemary populations from Sicily.

The six chemotype was represented by only two samples from Arssi (4.44%). The essential oil of the accessions in this chemotype was dominated by camphor and has a lower amount of α-pinene than all the rest accessions. Consistent with this finding, [43, 48] found the prominent distribution of camphor for India and Spain rosemary essential oils, respectively. The overall finding of the current study was also in line with the result of other researchers who found α-pinene, 1, 8-cineole and camphor chemotypes for rosemary essential oils in different places [21, 61, 62].

This study demonstrated the presence of high chemical variability among rosemary accessions, which is mainly dependent on genetic factor than geographic location. This could be explained by the fact that the studied accessions were cultivated in the same location for more than two years prior to chemical characterization and yet presented variability in their chemical profile. This can further supported by the observed chemical similarity between accessions collected from geographically distant locations; and chemical dissimilarity between accessions from geographically closer areas. The study also pointed out the existence of diverse accessions within collection regions, which makes it difficult to define a single chemotype based on the area of collection. Our finding at morphological and molecular level diversity analysis in the previous chapters also corroborated this and showed the existence of high within collection region diversity than between collection region. This could be resulted from presence of gene flow due to planting material exchange among growing communities.

Table 3: Percentage of the dominant compounds and chemotypes of rosemary essential oils across the 45 accessions

			Dominant compounds				
Accessions	Origin	α-pinene	1, 8-cineole	camphor	Verbenone	Endo-borneol	Chemotypes
Ros01	Hadiya	33.13	20.58	-	16.25	-	AP-E-V
Ros02	Wolaita	27.71	26.09	11.2	-	-	AP -E-C
Ros03	Wolaita	31.06	19.54	7.85	-	-	AP -E-C
Ros04	Hadiya	20.34	20.11	-	16.01	-	AP-E-V
Ros05	Wolaita	15.57	11.2	-	-	10.58	AP -E-EB
Ros06	Gonder	31.41	18.71	7.65	-	-	AP -E-C
Ros07	Gonder	31.71	18.92	7.44	-	-	AP -E-C
Ros08	Gurage	32	20.79	9.38	-	-	AP -E-C
Ros09	Harari	35.41	16.73	7.51	-	-	AP -E-C
Ros10	Harari	34.76	18.65	8.02	-	-	AP -E-C
Ros11	Harari	24.63	18.57	9.92	-	-	AP -E-C
Ros12	Arssi	35.71	15.81	7.7	-	-	AP -E-C
Ros13	Sidama	32.84	19.26	7.57	-	-	AP -E-C
Ros14	Wolaita	33.21	18.85	7.27	-	-	AP -E-C
Ros15	Hadiya	31.84	17.31	7.7	-	-	AP -E-C
Ros16	Hadiya	21.71	20.22	8.68	-	-	AP -E-C
Ros17	Harari	17.51	14.98	-	-	11.97	AP -E-EB
Ros18	Harari	16.01	14.89	-	-	12.56	AP -E-EB
Ros19	Harari	33.27	17.79	7.52	-	-	AP -E-C
Ros20	N. Shewa	31.21	17.53	8.3	-	-	AP -E-C
Ros21	N. Shewa	30.16	17.49	7.78	-	-	AP -E-C
Ros22	N. Shewa	14.34	-	13.41	14.55	-	V= AP -C
Ros23	N. Shewa	14.58	-	13.1	15.3	-	V= AP -C
Ros24	N. Shewa	11.18	-	10.24	20.25	-	V= AP -C
Ros25	N. Shewa	17.15	-	12.8	17.26	-	V= AP -C
Ros26	Arssi	25.64	17.63	3.83	16.48	-	AP -E-V
Ros27	Arssi	22.91	19.69	7.75	-	-	AP -E-C
Ros28	C.Farm	16.52	22.5	21.1	-	-	E-C- AP
Ros29	C.Farm	21.36	30.12	23	-	-	E-C- AP
Ros30	Gurage	39.19	18.95	7.3	-	-	Al-E-C
Ros31	Gurage	16	38	17.06	-	-	E-C- AP
Ros32	Gurage	28.01	17.23	7.95	-	-	AP -E-C
Ros33	Gurage	7.01	38.48	10.97	-	-	E-C- AP
Ros34	Harari	21.35	18.36	-	-	10.11	AP -E- EB
Ros35	Wolaita	33.93	19.38	6.74	-	-	AP -E-C
Ros36	Wolaita	11.5	19.97	12.15	-	-	E-C- AP
Ros37	Hadiya	23.59	18.47	-	16.88	-	AP -E-V
Ros38	Gurage	40.62	28.02	11.44	-	-	AP -E-C
Ros39	Gurage	38.22	28.22	5.82	-	-	AP -E-C
Ros40	Arssi	-	15.85	17.88	11.32	-	C-E-V
Ros41	Arssi	-	13.98	20.63	11.15	-	C-E-V
Ros42	Sidama	24.17	17.62	10.21	-	-	AP -E-C
Ros43	Sidama	20	17.97	10.1	-	-	AP -E-C
Ros44	Sidama	29.31	16.31	9.69	-	-	AP -E-C
Ros45	Sidama	15.76	15.2	12.31	-	-	AP -E-C

AP, α-pinene; E, 1, 8-cineole; C, camphor; V, verbenone; EB, endo-borneol; C. Farm, Commercial Farm

Exchange of plant materials among communities will minimize genetic diversity among populations but increase variability among individuals. This could also be the reason for the observed high within growing region diversity than between regions diversity.

CONCLUSIONS

Essential oils of forty-five rosemary accessions were analyzed using GC-MS and a total of forty-two compounds, representing 95.85-98.89% of the total volatileswere identified. In general, the essential oil of the accessions were characterized by higher levels of α-pinene(5.08 - 40.62%), 1, 8-cineole (8.13 - 38.48%), camphor (2.15 - 23%) and verbenone (1.83 - 20.25%) and their presence in all accessions. There were also some accessions (Ros05, Ros17, Ros18 and Ros34) that are characterized by the presence of higher levels of endo-borneol constituents in their oil (10.11 -12.56%). Correlation analysis among the major compounds showed a strong negative association of α-pinene, 1, 8-cineole and verbenone with the majority of the major compounds, while the correlation of camphor with the entire main constituent was not significant, except with α-pinene ($r = -0.46^{***}$) and linalool (r = -303*), indicating the difficulty of improving these compounds simultaneously. A positive association of endo-borneol, α -terpineol, bornyl acetate and β-caryophyllene to each other was also noted, showing the possibility of concurrent improvement of these compositions.

Based on the percentage of the major constituents, six distinct chemotypes (α-pinene/1, 8-cineole/camphor; α-pinene/1, 8-cineole/verbenone; α-pinene/1, 8-cineole/endo-borneol; 1, 8-cineole/camphor/α-pinene; verbenone/α-pinene/camphor; and camphor/1, 8-cineole/verbenone) were identified for the studied accessions, demonstrating the presence of high levels of chemotypic variability among the accessions. Overall, the study indicated the presence of wide chemical variability among the tested accessions, which was mainly dependent on the genetic background rather than the area of growth. The observed variability was higher among individual plants within collection areas than between collection areas and was consistent with our findings at morphological and molecular level diversity analysis. The observed high chemical constituent variabilities revealed the potential of Ethiopian rosemary germplasm for wider application in different destinations that use rosemary products as raw materials.

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