Effect of harvesting age and drying methods on essential oil yield of rosemary (*Rosmarinus officinalis* L.) leaves in Wondo Genet, Ethiopia

A.A. Melese[1,*], G. Abebe[2], A. Befa[3], G. Moges[2] and B. Degu[3]

[1] Southern Agricultural Research Institute, Jinka Agricultural Research Center, Jinka 4420, Ethiopia
[2] Wondo Genet College of Forestry and Natural Resources, Hawassa University, Hawassa 0000, Ethiopia
[3] Ethiopian Institute of Agricultural Research, Wondo Genet Agricultural Research Center, Addis Abeba 1165, Ethiopia
* Corresponding author: aytonasnake7@gmail.com
Submission: 21 April 2023 Revised: 9 August 2023 Accepted: 9 August 2023

ABSTRACT

Background and Objectives: *Rosmarinus officinalis* L. is extensively used in Ethiopian cuisine for flavoring dishes like roasted meats and spice blends. This study aimed to analyze how harvesting age and drying methods affect the essential oil content of rosemary leaves.

Methodology: Employing a 3 × 4 factorial design with 3 replications, this research explored the effects of harvesting age and drying methods on extracted essential oil yields from rosemary leaves. Data underwent a two-way ANOVA analysis using SAS software, with statistical significance set at $P < 0.05$.

Main Results: Harvesting age, drying methods, and their interactions significantly impacted rosemary leaf essential oil content ($P < 0.05$). Leaves harvested at 6 months after transplanting (MAT) exhibited the lowest essential oil content volume per weight (EOCV/W) for both fresh (0.55 ± 0.21%) and dry (1.34 ± 0.52%) leaves, as well as essential oil content weight per weight (EOCW/W) for fresh (0.50 ± 0.17%) and dry (1.22 ± 0.43%) leaves. Leaves collected at 12 MAT displayed the highest EOCV/W (fresh: 0.96 ± 0.27%; dry: 2.29 ± 0.69%) and EOCW/W (fresh: 0.84 ± 0.24%; dry: 2.01 ± 0.60%) values. Intermediate values emerged for leaves harvested at 18 MAT, with EOCV/W (fresh: 0.84 ± 0.16%; dry: 2.14 ± 0.43%) and EOCW/W (fresh: 0.74 ± 0.14%; dry: 1.88 ± 0.37%). During oven, sun, and shade drying processes, the mean EOCV/W losses for fresh leaves were 26.45, 27.72, and 25.19%, respectively, while EOCW/W losses were 27.12, 31.94, and 30.22%. For dry leaves, EOCV/W losses were 5.85, 7.32, and 8.62%, while EOCW/W losses were 10.41% and 29.31%, respectively, compared to the essential oil content of fresh leaves.

Conclusions: Optimal rosemary leaf harvesting occurred at 12 MAT, utilizing fresh- and shade-dried methods for essential oil extraction via hydro distillation. Harvesting at one year post-transplanting, along with oil extraction from fresh- and shade-dried leaves, proved most efficient. These findings hold significance for enhancing rosemary essential oil production in both pharmaceutical and culinary sectors.

Keywords: Rosemary, essential oil content, harvesting age, drying methods

Thai J. Agric. Sci. (2023) Vol. 56(2): 102−114

INTRODUCTION

Rosemary *(Rosmarinus officinalis* L.) is a Lamiaceae family medicinal aromatic plant commonly known as Yesga metibesha in Ethiopia. It is an attractive, evergreen, and highly branched plant (Johnson, 1982). Its origin is in the Mediterranean region, but it has now spread all over the world (González-Minero *et al.,* 2020). The Lamiaceae family is well known for its essential oil that is used in the pharmaceutical, food, and cosmetics industries (Naghibi *et al*., 2005). Rosemary is a perennial crop that can live for 5 years or more, depending on the crops grown area (Johnson, 1982). The plant prefers light, dry soil with a pH range of neutral to alkaline, preferably in areas with rocky soil (Hailemariam, 2016). It can grow to a height of one to two meters and has thick, hard, and attractive green fragrant needle-like leaves with beautiful small clusters of flowers. Its oil consists of 40.9% 1, 8-cineole, 5.2% bornyl acetate, 13.9% α-pinene, and 7.1% β-pinene has money applications and continues to play an important role in modern drugs for the treatment of human and animal diseases (Batanouny *et al*., 1999; Mukherjee *et al*., 2008). It is also the most important and widely grown crop in every Ethiopian home garden (Zigene *et al*., 2022). In Ethiopia, rosemary grows in vast agricultural lands in agricultural extension systems on commercial farms in Wolaita, Hadiya, Gurage, Sidama, Arssi, North Shewa, Gondar, Harari, and other parts of the country for flavoring agents and essential oil extractions (Zigene *et al*., 2022). Ethiopians used rosemary leaves for various applications including roasting meats, fish, vegetables, spice tea, food ingredients for pea mash, and pepper powders. Further women also believe that rosemary essential oil could encourage their skin hair follicle growth and keep their hair healthy by removing dandruff (Habtemariam, 2016). Its essential oil has a strong effect on the brain clears the mind, aids concentration, and fresh odor (Aronson *et al*., 2007; Gottschalck and Breslawec, 2012; Polat and Satil, 2012; Polat *et al*., 2015; Fiume *et al*., 2018). Rosemary's leaves essential oil is a natural plant-derived volatile substance used worldwide for numerous applications (Zorpeykar *et al*., 2022). The essential oil content of rosemary leaves is determined by the metabolic state of the leaf tissue and is closely related to the physiology of the plant's entire leaf maturity (Guenther, 1972; Khorshidi *et al*., 2009).

Therefore, the yield of essential oil of rosemary may be influenced by harvesting age, either collecting too young or too mature leaves or drying methods. Hence, essential oil content in Lamiaceae and Asteraceae families may depend on harvesting age, cutting height, harvesting season, time, drying method, and distillation time (Mallavarapu *et al.,* 1999; May *et al*., 2010; Zigene *et al.,* 2012). Therefore, scientific knowledge about harvesting age and drying methods is essential for extracting the maximum amount of essential oil content from rosemary leaves (Rocha *et al*., 2014). The harvesting age influences the essential oil content, total biomass, and active constituents of rosemary leaves (Rocha *et al*., 2014; Tsasi *et al.,* 2017; Banjaw and Wolde, 2019; Bekele *et al*., 2019). Similarly, when leaves are exposed to higher temperatures, drying methods may cause essential oil loss by converting it into other types of compounds, and chemical reactions such as oxidation, isomerization, cyclization, or dehydrogenation may occur during the drying process (Turek and Stintzing, 2013; Hailemariam, 2016; Hazrati *et al*., 2021). Therefore, the optimal harvest age and drying methods for rosemary leaves for more essential oil extractions should be known. Essential oils are natural plant-derived volatile substances that are used worldwide for numerous applications (Zorpeykar *et al*., 2022). The different studies illustrate that the essential oil yield from dried herbs is still lower than that of fresh herbs in Wondo Genet (Zigene, 2010). However, there is insufficient scientific evidence to determine how much the effects of different drying methods and harvesting ages affect the essential content of *R. officinalis* leaves in the Wondo Genet. Therefore, determining the appropriate plant harvesting age and drying methods for essential oil extractions from rosemary leaves is important. Thus, the objective of this research was to determine the effects of harvesting age, drying methods and their interactions on the essential oil content of *R. officinalis* leaves. This research will provide a new perspective on the effect of harvesting and drying methods on the essential oil content of rosemary leaves across a study area for farmers, agricultural extension, governments, non-governmental organizations, and other small businesses in Ethiopia to engage in full-scale production of rosemary essential extractions.

MATERIALS AND METHODS

Experimental Site

This study was conducted at the Ethiopian Institute of Agricultural Research, Wondo Genet Agricultural Research Center, Ethiopia from 2018 to 2020. The geographical location experimental site ranges from 38° 37’13’’ to 38° 38’20’’ E and 7° 5’23’’ to 7° 5’52’’ N with an altitude of 1,780 m above sea level. Southeast of Shashemene about 14 and 267 km South of Addis Abeba (Figure 1). The site receives a mean annual rainfall of 1,128 mm, with minimum and maximum temperatures of 11.47 and 26.51 °C, respectively. The soil textural class of the experimental site is sandy loam with a pH of 7.2 (Abayneh *et al*., 2006).

The Experimented Design and Treatments

The study was carried out using 3 × 4 factorials in a completely randomized design with 3 replications testing the effect of 3 levels of harvesting ages (6, 12, and 18) months after transplanting (MAT) and 4 levels of drying methods (fresh, shade, sun, and oven), as shown in Table 1.

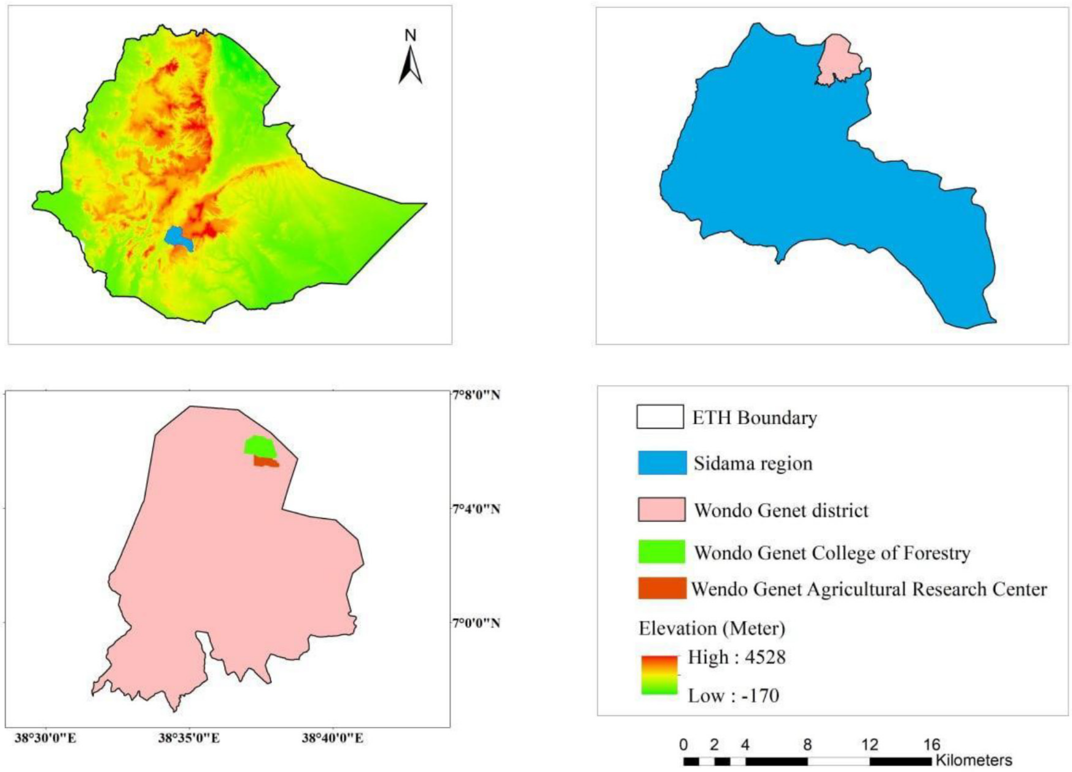

Figure 1 The study area at the Ethiopian Institute of Agricultural Research, Wondo Genet Agricultural Research Center, Ethiopia

Table 1 Treatment combinations of 3 × 4 factorial arrangement

Harvesting age	Drying method			
	D_1 (fresh leave)	D_2 (oven drying)	D_3 (sun drying)	D_4 (shade drying)
A_1 (6 MAT)	$A_1 \times D_1$	$A_1 \times D_2$	$A_1 \times D_3$	$A_1 \times D_4$
A_2 (12 MAT)	$A_2 \times D_1$	$A_2 \times D_2$	$A_2 \times D_3$	$A_2 \times D_4$
A_3 (18 MAT)	$A_3 \times D_1$	$A_3 \times D_2$	$A_3 \times D_3$	$A_3 \times D_4$

Note: A = harvesting age (A_1, A_2, and A_3 = levels of harvesting age), D = drying methods (D_1, D_2, D_3, and D_4 = levels of drying methods), MAT = months after transplanting.

Plant Material Collection

R. officinalis seedlings were obtained from the Ethiopian Institute of Agricultural Research, Wondo Genet Agricultural Center, from the crop research and multiplication directorate experimental site. The seedlings were propagated vegetatively by cutting the branches in the middle portion of the branches. Small pieces and/or cuttings were transplanted into plastic tubes or pots (30 cm in diameter and 50 cm in height) at the nursery level, where seedling management is well practiced. After 3 months, the seedlings were transplanted to an experimental field labeled the planted date (Figure 2H). Plants were collected at random from the central rows of each plot, excluding the borders (Figure 2C). Harvesting was carried out in the second half of April 2019 from disease-free plants by cutting these individuals with their respective age classes 20–30 cm above the soil surface, depending on the plant's wood height. The collected plants were taken to the laboratory as soon as they were harvested, and the leaves were separated from the stems and divided into 4 batches; one for fresh, and the other 3 batches were dried using oven drying, sun drying, and shade drying methods (Figures 2E, 2I, and 2J). Thus, for this study, only leaves were used for essential oil extraction. After harvesting, the leaves from 3 different ages of plants were separated and dried at different drying temperatures in 4 separate categories: fresh, sun, oven, and shade-dried methods, until they reached equilibrium moisture content. The total experiment consisted of 12 leaf samples collected from separate plants of each of the 3 age classes and 4 drying methods, yielding a total of 32 samples prepared for essential oil extraction by hydrodistillation (Table 1). The effects of other environmental factors such as ambient temperature, cultivation methods, irrigation, and other management activities were not tested because all of these factors were held constant across experimental types.

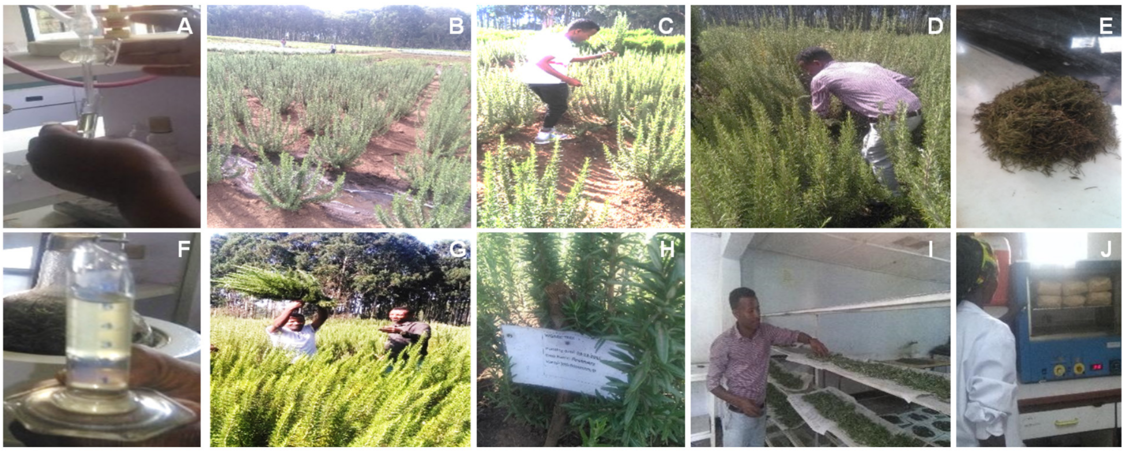

Figure 2 Sample collection, preparations and essential oil extraction using hydrodistillation: (A and F) harvesting of essential oil, (B) plant management, (C, D, and G) harvesting of rosemary leaves, (H) experimental field with planted date label, (E, I, and J) drying leaves.

Drying Procedure

The collected rosemary leaves were dried in the sun, oven, and shade. The weight loss in all drying treatments was measured using an analytical balance until the equilibrium moisture content was attained. Because the 3 drying methods used different heat sources during the drying process, drying times varied: shade drying took more time than sun drying and sun drying used a longer duration than oven drying methods at 40 °C. The rosemary leaves were dried for 7, 5, and 1 day under shade at an average relative humidity of 23–39%, in the sun at an average temperature of 25 °C at the same relative humidity, and in an oven at 40 °C, respectively. The plants were dried separately until the moisture content reached equilibrium. Therefore, the temperature differences varied because one was exposed to full sunlight, an oven, shade, and fresh leaves. The drying methods were shade drying was done in a well-ventilated, wire-constructed room where leaf samples were spread on a hard, dry, wire-constructed surface allowing free circulation of air for 7 days, while the sun-dried leaves were directly exposed to sunlight for 5 days. However, oven-dried leaves were exposed to oven drying at 40 °C for 24 h (Tambunan *et al.*, 2001).

The Procedure of Essential Oil Extractions

The essential oil of rosemary leaves was extracted at the Wondo Genet Agricultural Research Center Laboratory using a Clevenger apparatus by using hydrodistillation following procedures of Clevenger (1928). A total of 300 g of fresh rosemary leaves and 650 mL of H_2O were used in the distillation which lasted for 3 h after the mixture reached the boiling point. After drying, the leaf sample was measured, and the percentage of mass loss from each dried leaf was calculated based on the original fresh leaf weight. The prepared sample was subjected/soaked to hydrodistillation at atmospheric pressure using a Clevenger apparatus extraction device in a round-bottomed flask that contained distilled water for the extraction of essential oils under optimal operating conditions. The leaves were completely immersed in water and boiled for 3 h on a heating mantle. During the distillation process, vapor containing both essential oils and water vapor is passed to the condenser, which converts the steam into liquid form and drops it into harvesting material with a lower density than water which is then placed on top of the water. After 3 h of monitoring the distillation, droplet collection decreased thoroughly, and 2 phases were observed: an aqueous phase (aromatic water) and a less dense organic phase (essential oil). Thus, the less dense material is easily removed by opening the steam coil until nearly all of the water has been lost, then closing the steam coil and harvesting essential oil with its closing mechanism. The essential oil was then separated from the water by decanting or skimming it off the top with a pressure-paced pipet, the most important piece of equipment used to take essential oil from top to bottom and carefully record its amount in mL and g on the datasheet. The extraction of essential oils was done in triplicate. The amount of total essential oil extracted was calculated based on the percentage volume per weight (EOCV/W, % v/w) and weight per weight (EOCW/W, % w/w) for fresh and dry based as follows:

$$\text{Moisture content \%} = \frac{W1 - W2}{W1} \times 100 \quad \text{------- (1)}$$

where W1 = original weight of the sample before drying and W2 = weight of the sample after drying

$$\text{EOCV/W fresh based} = \frac{\text{Volume of oil (mL)}}{\text{Weight of fresh distillation sample (g)}} \times 100 \quad \text{------- (2)}$$

$$\text{EOCV/W dry based} = \frac{\text{Volume of oil (mL)}}{\text{Weight of dry distillation sample (g)}} \times 100 \quad \text{------- (3)}$$

$$\text{EOCW/W fresh based} = \frac{\text{Weight of oil (g)}}{\text{Weight of distillation of fresh sample (g)}} \times 100 \quad \text{------- (4)}$$

$$\text{EOCW/W dry based} = \frac{\text{Weight of oil (g)}}{\text{Weight of distillation dry sample (g)}} \times 100 \quad \text{------- (5)}$$

$$\text{Weight of distilling sample dry (g)} = \frac{\text{Weight of distilled fresh sample (g) – (100 – Moisture content)}}{100} \quad \text{------- (6)}$$

$$\text{Percentage change of essential oil content} = \frac{EOC_2 - EOC_1}{EOC_1} \times 100 \quad \text{------- (7)}$$

where EOC_1 = essential oil gets from fresh leaves and EOC_2 = essential oil gets from dried leaves

Statistical Analysis

All data collected was subjected to an ANOVA factorial analysis using SAS software to determine the effect of harvesting age and drying method and interactions on the extracted essential oil yield of rosemary leaves. A least significant difference (LSD; P < 0.05), was used to determine the significance of treatment means for essential oil yields for harvesting age, drying methods, and their instructions. The analysis data is presented as mean values ± standard deviation.

RESULTS AND DISCUSSION

Effects of Harvesting Age and Drying Methods on the Moisture Content of *Rosmarinus officinalis* L. Leaves

From the analysis data, fresh and shade-dried leaves had the highest moisture content (58.71%; 59.61%), (59.83%; 57.60%), and (61.15%; 61.52%), while the smallest moisture content occurred in sun- and oven-dried leaves (58.86%; 58.41%), (57.58%; 57.27%), and (60.60%, 58.90%), respectively, during the leaves harvested 6, 12, and 18 MAT (Figure 3). Based on the results, the moisture content of rosemary leaves was higher for fresh and shade-dried leaves, while oven- and sun-dried leaves had a lower percentage of moisture content. Similarly, Argyropoulos *et al*. (2012) found that *Melissa officinalis* had higher moisture content in fresh leaves and air/shade-dried leaves, but sun and oven drying had a significant effect on the percentage of moisture content. In this study, the water loss of rosemary leaves during drying was measured to determine the drying effects on essential oil content through evaporation, and the mean weight loss recorded was 150, 143, and 102 g during the sun, oven, and shade drying process respectively. The most weight loss occurred in large amounts of leaves harvested at a younger age under sun- and oven-dried leaves. According to the data analysis, the essential oil content was decreased during the drying process. Therefore, the study's findings demonstrated that essential oil content is directly proportional to the moisture content during drying leaves (Figure 3 and Tables 2–3). Thus, in this study, the amount of essential oil content and the amount of moisture content lost during the drying of *R. officinalis* leaves were found to have been significantly correlated. It may be that the amount of water removed during the drying process is related to a loss of the chemical constituents of the leaves, resulting in a decrease in the essential oil content from leaves (Figiel and Michalska, 2016).

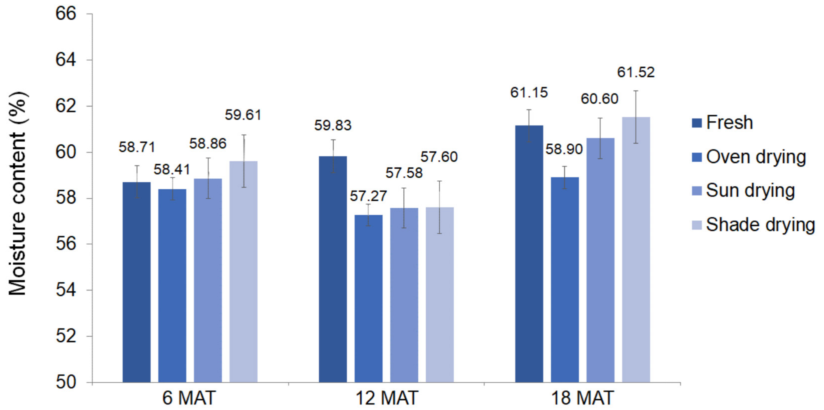

Figure 3 Influence of harvesting age and drying methods on rosemary leaves' moisture content (%). MAT = months after transplanting.

Effect of Harvesting Age on the Essential Oil Content of *Rosmarinus officinalis* L. Leaves

The analysis of variance showed that harvesting age had a significant ($P < 0.05$) effect on essential oil content. The EOCV/W (0.55 ± 0.21 and 1.34 ± 0.52% respectively) and the EOCW/W (0.50 ± 0.17 and 1.22 ± 0.43% respectively) for fresh and dry leaves were lowest at 6 MAT. The highest EOCV/W (0.96 ± 0.27 and 2.29 ± 0.69% respectively) and EOCW/W for fresh and dry leaves (0.84 ± 0.24 and 2.01 ± 0.60% respectively) occurred at 12 MAT (Table 2). This is in agreement with the findings of Zorpeykar *et al*. (2022), who identified that the yield of *Peppermint* essential oil content increased with plant age. Similarly, at 18 MAT, the EOCV/W for fresh and dry was 0.84 ± 0.16 and 2.14 ± 0.43% and the EOCW/W for fresh and dry was 0.74 ± 0.14 and 1.88 ± 0.37% respectively. Therefore, based on the analysis, the leaves harvested from 12 MAT had the highest EOCV/W and EOCW/W followed by leaves harvested from 18 MAT, when compared to 6 MAT (Table 2). This result is in agreement with the findings of those who observed that leaf maturation is the physiological component most responsible for increasing the percentage of essential oil content (Zigene, 2010). Therefore, this study provides evidence that when the harvesting date was extended from 6 months to one year, the essential oil content increased. This is also supported by the results obtained from most medicinal aromatic plants (Ismail *et al*., 2021).

Table 2 The effect of harvesting age on essential oil content of *Rosmarinus officinalis* L. leaves

Harvesting age	EOCV/W fresh based (%)	EOCV/W dry based (%)	EOCW/W fresh based (%)	EOCW/W dry based (%)
6 MAT	0.55 ± 0.21[c]	1.34 ± 0.52[c]	0.50 ± 0.17[c]	1.22 ± 0.43[b]
12 MAT	0.96 ± 0.27[a]	2.29 ± 0.69[a]	0.84 ± 0.24[a]	2.01 ± 0.60[a]
18 MAT	0.84 ± 0.16[b]	2.14 ± 0.43[b]	0.74 ± 0.14[b]	1.88 ± 0.37[a]
$LSD_{0.05}$	0	0.14	0.05	0.12
CV (%)	8.23	8.51	8.45	8.67

Note: Means in each column followed by the same letter are not significantly different ($P < 0.05$). EOCV/W = essential oil content calculated based on the percentage volume per weight, EOCW/W = essential oil content calculated based on the percentage weight by weight, MAT = months after transplanting, LSD = least significant difference, CV = coefficient of variation.

Nevertheless, in this study, the essential oil content of rosemary leaves increased from 6 to 12 MAT; however, the essential oil content decreased after 12 months (Table 2). This is in agreement with the findings of Zorpeykar *et al*. (2022), who found no significant differences in essential oil content in *Peppermint* after one year of age. It is also supported by Tajidin *et al*. (2012) and Maione *et al.* (2013), who observed that as lemongrass (*Cymbopogon citratus*) matured, its essential oil content became decreased. Therefore, variance analysis from this study indicated that harvesting age had a significant effect on the percentage of EOCV/W and EOCW/W from fresh and dry-based essential oil extractions (Table 2). This result is in agreement with Getachew and Aynalem (2017), who found that harvesting age had a highly significant effect on the yield of *C. citratus* essential oil. Similarly, rosemary leaves harvested 18 MAT had a yellow color, making their appearance less attractive, and had sharked leaves. Therefore, rosemary leaves should ideally be harvested one year after transplanting, resulting in a higher amount of essential oil content when extracted either in fresh or shade-dried leaves with hydrodistillation.

Effect of Drying Methods on the Essential Oil Content of *Rosmarinus officinalis* Leaves

The variance analyses indicated that drying methods had a significant ($P < 0.05$) effect on the essential oil content of *R. officinalis* leaves (Table 3). Fresh rosemary leaves had the highest EOCV/W (0.90 ± 0.20 and 2.25 ± 0.52%) and EOCW/W (0.81 ± 0.18 and 2.02 ± 20.48%) for fresh and dry, followed by shade drying leaves with EOCV/W of 0.85 ± 0.19 and 2.09 ± 0.47% and EOCW/W of 0.74 ± 0.16 and 1.82 ± 0.41% for fresh and dry, respectively. The smallest amount of EOCV/W and EOCW/W for fresh and dry based were obtained from the sun- and oven-dried rosemary leaves (Table 3). This is in agreement with the results of Fadil *et al*. (2014), who observed that drying *R. officinalis* leaves in the shade had no significant effects on essential oil content. Therefore, the results showed that drying methods caused significant losses of essential oil content when compared to the fresh state. This is in agreement with the results of Rohloff *et al*. (2005), who found that sun- and oven-drying rosemary leaves at 50–65 °C cause an unpredictably significant decrease in the essential oil content.

Generally, from this investigation variances of analysis shows that the highest essential oil content of rosemary leaves was obtained from fresh leaves, followed by shade-dried leaves, whereas the lowest essential oil content was obtained from sun- and oven-dried leaves (Table 3). This result is in agreement with the previous studies on *Mentha longifolia* (Asekun *et al*., 2007). Therefore, from this study, the effects of drying methods on the percentage change in essential oil content of leaves during drying was investigated by comparing them with fresh leaves. Therefore, the mean of EOCV/W (26.45 and 27.72%), EOCW/W (27.12 and 1.94%), EOCV/W (25.19 and 30.22%), EOCW/W (26.87 and 29.31%), EOCV/W (5.85 and 7.32%), and EOCW/W (8.62 and 10.41%) were lost during the oven, sun and shade drying process for fresh and dry based respectively when compared essential oil content fresh leaves. This result is in agreement with the results of Ibanez *et al*. (1999), who found that drying rosemary leaves at 30 and 45 °C resulted in 16–23% essential oil losses, whereas drying at 60 °C resulted in 65% essential oil losses. Therefore, the essential oil content of rosemary dried in shade was similar to that of fresh rosemary leaves. Similarly, another finding also confirmed that fresh and shade drying methods are excellent methods for extracting essential oils from *Lippia berlandieri* Schauer (Yousif *et al*., 2000). Therefore, this study investigated how drying methods affect the percentage of essential oil content in rosemary leaves, and fresh and shade-dried leaves are recommended for essential oil extractions from rosemary leaves by hydrodistillation.

Table 3 The effect of drying methods on the essential oil content of *Rosemarynus officinalis* L. leaves

Drying methods	EOCV/W fresh based (%)	EOCV/W dry based (%)	EOCW/W fresh based (%)	EOCW/W dry based (%)
Fresh	0.90 ± 0.20[a]	2.25 ± 0.52[a]	0.81 ± 0.18[a]	2.02 ± 0.48[a]
Oven drying	0.69 ± 0.26[b]	1.70 ± 0.63[c]	0.61 ± 0.22[c]	1.46 ± 0.53[c]
Sun drying	0.70 ± 0.25[b]	1.65 ± 0.62[c]	0.62 ± 0.20[c]	1.50 ± 0.51[c]
Shade drying	0.85 ± 0.19[a]	2.09 ± 0.47[b]	0.74 ± 0.16[b]	1.82 ± 0.41[b]
$LSD_{0.05}$	0.05	0.06	0.12	0.14
CV (%)	14.98	15.08	14.58	14.43

Note: Means in each column followed by the same letter are not significantly different (P < 0.05). EOCV/W = essential oil content calculated based on the percentage volume per weight, EOCW/W = essential oil content calculated based on the percentage weight by weight, LSD = least significant difference, CV = coefficient of variation.

Interaction Effect of Harvesting Age and Drying Methods on the Essential Oil Content of *Rosmarinus officinalis* Leaves

The variance analyses indicated that the interaction of harvesting age and drying methods had a significant ($P < 0.05$) effect on the essential oil content of *R. officinalis* leaves (Table 4). The highest level of essential oil content was obtained from fresh and shade-dried leaves, while the lowest level of essential oil content was observed in oven- and sun-dried leaves at 6, 12, and 18 MAT in all four studied traits (Table 4). However, the essential oil content rises with plant age and provides more oil when extracted from fresh or shade-dried leaves than from younger leaves. This result is in agreement with Turek and Stintzing (2013), who stated that the essential oil content of aromatic herbs may be lost during the drying process due to various chemical reactions depending on the nature and age of the leaves. Thus, the combined effect of more matured leaves containing higher oil content than younger leaves from all drying methods. Similarly, Baritaux *et al*. (1992) found that drying has a significant impact on the surface morphology of the leaves depending on their maturity, structure, and position, resulting in essential oil content reductions in *Ocimum basilicum*. Therefore, this study demonstrates that when drying matured rosemary leaves do not easily rapture their oil glands as the younger leaves do. This result is also in agreement with Ascrizzi *et al*. (2018) findings that changes in essential oil content during the drying process are influenced by biological factors. Thus, from this study, the mean comparison of the interaction of harvesting age and drying methods revealed that rosemary leaves dried at different temperatures at an early age had significantly lower essential oil content than rosemary leaves harvested at a later age across all studied traits (Table 4). Even though the essential oil content increased by harvesting age (from 6 to 12 MAT), the essential oil content began to decline after 12 to 18 MAT, indicating that the essential oil storage potential of the leaves has been attained. This is in agreement with the findings of Zorpeykar *et al*. (2022), who found no significant differences in essential oil content in *Peppermint* after one year of age. In this study, rosemary leaves harvested from 6 MAT had juvenile structures, indicating that they contained a smaller amount of natural products than matured leaves and that high drying temperatures easily destroyed the oil glands, resulting in rapid evaporation of oil and essential oil losses during drying. This is in agreement with the results obtained by Ascrizzi *et al.* (2018). Based on the results of the combined analysis, it is necessary to decide that the optimum harvesting age of rosemary leaves is 12 MAT and that fresh and shade-dried methods for essential oil extractions under hydrodistillation are more desirable.

Table 4 Interaction effect of harvesting age and drying methods on the essential oil content of *Rosemarinus officinalis* leaves

Harvesting age	Drying methods	EOCV/W fresh based (%)	EOCV/W dry based (%)	EOCW/W fresh based (%)	EOCW/W dry based (%)
6 MAT	Fresh	0.62 ± 0.25^{e}	1.49 ± 0.66^{cd}	0.59 ± 0.26^{ef}	1.43 ± 0.62^{cd}
	Oven	0.55 ± 0.21^{e}	1.32 ± 0.59^{d}	0.50 ± 0.17^{efg}	1.19 ± 0.50^{def}
	Sun	0.52 ± 0.28^{e}	1.25 ± 0.76^{d}	0.47 ± 0.15^{gh}	1.15 ± 0.44^{ef}
	Shade	0.52 ± 0.23^{e}	1.30 ± 0.54^{d}	0.45 ± 0.19^{h}	1.09 ± 0.44^{f}
12 MAT	Fresh	1.02 ± 0.19^{b}	2.54 ± 0.41^{a}	0.90 ± 0.16^{b}	1.06 ± 0.35^{ab}
	Oven	0.91 ± 0.16^{c}	2.12 ± 0.39^{b}	0.79 ± 0.14^{c}	0.61 ± 0.35^{e}
	Sun	0.75 ± 0.16^{d}	1.75 ± 0.36^{c}	0.66 ± 0.13^{de}	0.83 ± 0.29^{cd}
	Shade	1.16 ± 0.14^{a}	2.74 ± 0.36^{a}	1.01 ± 0.13^{a}	0.86 ± 0.33^{c}
18 MAT	Fresh	1.06 ± 0.20^{ab}	2.72 ± 0.55^{a}	0.93 ± 0.19^{ab}	2.40 ± 0.50^{a}
	Oven	0.61 ± 0.33^{e}	2.23 ± 0.74^{b}	0.55 ± 0.28^{fg}	1.35 ± 0.63^{cde}
	Sun	0.83 ± 0.38^{cd}	2.10 ± 0.86^{b}	0.74 ± 0.33^{cd}	1.87 ± 0.75^{b}
	Shade	0.86 ± 0.26^{c}	2.50 ± 0.59^{a}	0.74 ± 0.22^{cd}	1.92 ± 0.51^{b}
$LSD_{0.05}$		0.21	0.52	0.18	0.44
CV (%)		15.82	16.05	15.38	15.26

Note: Means in each column followed by the same letter are not significantly different (P < 0.05). EOCV/W = essential oil content calculated based on the percentage volume per weight, EOCW/W = essential oil content calculated based on the percentage weight by weight, MAT = months after transplanting, LSD = least significant difference, CV = coefficient of variation.

CONCLUSION

The results showed that the essential oil content of *R. officinalis* was significantly (P < 0.05) affected by harvesting age, drying methods, and their interactions. The highest essential oil content was found in leaves harvested 12 MAT using fresh and shade-dried leaves, while the lowest essential oil content was obtained from leaves harvested 6 MAT from oven- and sun-dried leaves for all studied traits. Even though increasing the essential oil content from 6 to 12 MAT, the essential oil content began to decline after 12 to 18 MAT. Therefore, it is necessary to conclude that harvesting rosemary leaves at 12 MAT and using fresh and shade-dried leaves for essential oil extractions under hydrodistillation is preferable. As a result, rosemary leaves should ideally be harvested after one year of transplanting and should use fresh and shade-dried leaves for essential oil extraction methods under hydrodistillation. Further research is needed to determine the rate of evaporation and ingredients that evaporate quickly when *R. officinalis* leaves are dried and to identify the primary and secondary metabolites that arise during the crop's age.

ACKNOWLEDGEMENT

Ethiopian Institute of Agricultural Research (EIAR), Wondo Genet Agricultural Research Center are thanked for providing plant material and laboratory assistance for this study. We would also like to express our gratitude to Wondo Genet College of Forestry and Natural Resources, Hawassa University for providing us with this opportunity.

REFERENCES

Abayneh, E., T. Demeke and A. Ashenafi. 2006. Soils of Wondo Genet Agricultural Research Center. National Soil Research Center, Addis Ababa, Ethiopia.

Argyropoulos, D., R. Alex, R. Kohler and J. Müller. 2012. Moisture sorption isotherms and isosteric heat of sorption of leaves and stems of lemon balm (*Melissa officinalis* L.) established by dynamic vapour sorption. LWT. 47(2): 324–331. https://doi.org/10.1016/j.lwt.2012.01.026.

Aronson, D.B., S. Bosch, D.A. Gray, P.H. Howard and P.D. Guiney. 2007. A comparative human health risk assessment of pdichlorobenzene-based toilet rimblock products versus fragrance/surfactant-based alternatives. J. Toxicol. Environ. Health B Crit. Rev. 10(7): 467–526. https://doi.org/10.1080/15287390600975103.

Ascrizzi, R., D. Fraternale and G. Flamini. 2018. Photochemical response of parsley (*Petroselinum crispum* (Mill.) Fuss) grown under red light: the effect on the essential oil composition and yield. J. Photochem. Photobiol. B. 185: 185–191. https://doi.org/10.1016/j.jphotobiol.2018.06.006.

Asekun, O.T., D.S. Grierson and A.J. Afolayan. 2007. Effects of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. subsp. *Capensis.* Food Chem. 101(3): 995–998. https://doi.org/10.1016/j.foodchem.2006.02.052.

Banjaw, D.T. and T.G. Wolde. 2019. Determination of appropriate harvesting age for *Aloe vera* yield and yield components at Wondo Genet, Southern Ethiopia. J. Nat. Sci. Res. 9(23): 23–25. https://doi.org/10.7176/JNSR/9-23-04.

Baritaux, O., H. Richard, J. Touche and M. Derbesy. 1992. Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum* L. Flavour Fragr. J. 7(5): 267–271. https://doi.org/10.1002/ffj.2730070507.

Batanouny, K.H., E. Aboutabl, M.C. Shabana and F. Soliman. 1999. Wild Medicinal Plants in Egypt: An Inventory to Support Conservation and Sustainable Use. Academy of Scientific Research & Technology, Cairo, Egypt.

Bekele, W., M. Tesema, H. Mohammed and K. Mammo. 2019. Herbage yield and bio-chemical traits as influenced by harvesting age of lemongrass (*Cymbopogon citratus* (DC) Stapf) varieties at Wondogenet, South Ethiopia. Int. J. Res. Agric. Sci. 6(3): 2348–3997.

Clevenger, J.F. 1928. Apparatus for the determination of volatile oil. J. Am. Pharm. Assoc. 17(4): 345–349. https://doi.org/10.1002/jps.3080170407.

Fadil, M., A. Farah, B. Ihssane, T. Haloui and S. Rachiq. 2014. The application of Plackett and Burman design in screening the parameters acting on the hydrodistillation process of Moroccan rosemary (*Rosmarinus officinalis* L.). Int. J. Innov. Appl. Stud. 8(1): 372–381.

Figiel, A. and A. Michalska. 2016. Overall quality of fruits and vegetables products affected by the drying processes with the assistance of vacuum-microwaves. Int. J. Mol. Sci. 18(1): 71. https://doi.org/10.3390/ijms18010071.

Fiume, M.M., W.F. Bergfeld, D.V. Belsito, R.A. Hill, C.D. Klaassen, D.C. Liebler, J.G. Marks Jr, R.C. Shank, T.J. Slaga, P.W. Snyder and L.J. Gill. 2018. Safety assessment of *Rosmarinus officinalis* (rosemary)-derived ingredients as used in cosmetics. Int. J. Toxicol. 37(suppl. 3): 12S–50S. https://doi.org/10.1177/1091581818800020.

Getachew, J. and G. Aynalem. 2017. Influence of harvesting age on yield and yield related traits of lemongrass (*Cymbopogon citrates* L.) varieties at Wondo Genet, Southern Ethiopia. Acad. Res. J. Agri. Sci. Res. 5(3): 210–215. https://doi.org/10.14662/ARJASR2017.018.

González-Minero, F.J., L. Bravo-Díaz and A. Ayala-Gómez. 2020. *Rosmarinus officinalis* L. (rosemary): an ancient plant with uses in personal healthcare and cosmetics. Cosmetics. 7(4): 77. https://doi.org/10.3390/cosmetics7040077.

Gottschalck, T.E. and H. Breslawec. 2012. International Cosmetic Ingredient Dictionary and Handbook. Personal Care Products Council, Washington, D.C., USA.

Guenther, E. 1972. The Essential Oils: History, Origin in Plants, Production, Analysis. Robert E. Krieger Publishing Co., Inc., Florida, USA.

Habtemariam, S. 2016. The therapeutic potential of rosemary (*Rosmarinus officinalis*) diterpenes for Alzheimer's disease. Evid. Based Complement. Alternat. Med. 2016: 2680409. https://doi.org/10.1155/2016/2680409.

Hailemariam, S. 2016. Extraction and Characterization of Essential Oil from Rosemary Leaves. Addis Ababa University, Addis Ababa, Ethiopia.

Hazrati, S., K. Lotfi, M. Govahi and M.T. Ebadi. 2021. A comparative study: influence of various drying methods on essential oil components and biological properties of *Stachys lavandulifolia*. Food Sci. Nutr. 9(5): 2612–2619. https://doi.org/10.1002/fsn3.2218.

Ibanez, E., A. Oca, G. de Murga, S. López-Sebastián, J. Tabera and G. Reglero. 1999. Supercritical fluid extraction and fractionation of different preprocessed rosemary plants. J. Agric. Food Chem. 47(4): 1400–1404. https://doi.org/10.1021/jf980982f.

Ismail, A.A., F. Pa'ee, M.N.F. Abd Rani and A.A. Mohd Noh. 2021. Effects of nitrogen rate and harvesting age on herbage and essential oil of *Cymbopogon citratus*. IOP Conf. Ser.: Earth Environ. Sci. 736: 012025. https://doi.org/10.1088/1755-1315/736/1/012025.

Johnson, A.F. 1982. Some demographic characteristics of the Florida rosemary *Ceratiola ericoides* Michx. Am. Midl. Nat. 108(1): 170–174. https://doi.org/10.2307/2425306.

Khorshidi, J., R. Mohammadi, M.T. Fakhr and H. Nourbakhsh. 2009. Influence of drying methods, extraction time, and organ type on essential oil content of rosemary (*Rosmarinus officinalis* L.). Nat. Sci. 7(11): 42–44.

Maione, F., C. Cicala, G. Musciacco, V. De Feo, A.G. Amat, A. Ialenti and N. Mascolo. 2013. Phenols, alkaloids and terpenes from medicinal plants with antihypertensive and vasorelaxant activities. A review of natural products as leads to potential therapeutic agents. Nat. Prod. Commun. 8(4): 539–544. https://doi.org/10.1177/1934578X1300800434.

Mallavarapu, G.R., R.N. Kulkarni, K. Baskaran, L. Rao and S. Ramesh. 1999. Influence of plant growth stage on the essential oil content and composition in Davana (*Artemisia pallens* Wall.). J. Agric. Food Chem. 47(1): 254–258. https://doi.org/10.1021/jf980624c.

May, A., E. Suguino, A.N. Martins, L.E.S. Barata and M.Q. Pinheiro. 2010. Biomass production and essential oil of rosemary (*Rosmarinus officinalis* L.) in function of the height and interval between the cuts. Rev. Bras. Plantas Med. 12(2): 195–200. https://doi.org/10.1590/S1516-05722010000200011.

Mukherjee, P.K., N. Satheesh Kumar and M. Heinrich. 2008. Plant made pharmaceuticals (PMPs)-development of natural health products from biodiversity. Indian J. Pharm. Educ. Res. 42(2): 113–121.

Naghibi, F., M. Mosaddegh, S.M. Motamed and A. Ghorbani. 2005. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iran. J. Pharm. Res. 4(2): 63–79. https://doi.org/10.22037/ijpr.2010.619.

Polat, R. and F. Satil. 2012. An ethnobotanical survey of medicinal plants in Edremit Gulf (Balikesir-Turkey). J. Ethnopharmacol. 139(2): 626–641. https://doi.org/10.1016/j.jep.2011.12.004.

Polat, R., U. Cakilcioglu, K. Kaltalioglu, M.D. Ulusan and Z. Turkmen. 2015. An ethnobotanical study on medicinal plants in Espiye and its surrounding (Giresun-Turkey). J. Ethnopharmacol. 163: 1–11. https:// doi.org/10.1016/j.jep.2015.01.008.

Rocha, R.P., E. de Castro Melo, L.C.A. Barbosa, R.H.S. dos Santos, P.R. Cecon, R. Dallacort and A. Santi. 2014. Influence of plant age on the content and composition of essential oil of *Cymbopogon citratus* (DC.) Stapf. J. Med. Plants Res. 8(37): 1121–1126. https://doi.org/10.5897/JMPR2013.5549.

Rohloff, J., S. Dragland, R. Mordal and T.H. Iversen. 2005. Effect of harvest time and drying method on biomass production, essential oil yield, and quality of peppermint (*Mentha* × *piperita* L.). J. Agric. Food Chem. 53(10): 4143–4148. https://doi.org/10.1021/jf047998s.

Tajidin, N.E., S.H. Ahmad, A.B. Rosenani, H. Azimah and M. Munirah. 2012. Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. Afr. J. Biotechnol. 11(11): 2685–2693. https://doi.org/10.5897/AJB11.2939.

Tambunan, A.H., Yudistira, Kisdiyani and Hernani. 2001. Freeze drying characteristics of medicinal herbs. Dry. Technol. 19(2): 325–331. https://doi.org/10.1081/DRT-100102907.

Tsasi, G., T. Mailis, A. Daskalaki, E. Sakadani, P. Razis, Y. Samaras and H. Skaltsa. 2017. The effect of harvesting on the composition of essential oils from five varieties of *Ocimum basilicum* L. cultivated in the Island of Kefalonia, Greece. Plants (Basel). 6(3): 41. https://doi.org/10.3390/plants6030041.

Turek, C. and F.C. Stintzing. 2013. Stability of essential oils: a review. Compr. Rev. Food Sci. Food Saf. 12(1): 40–53. https://doi.org/10.1111/1541-4337.12006.

Yousif, A.N., T.D. Durance, C.H. Scaman and B. Girard. 2000. Headspace volatiles and physical characteristics of vacuum-microwave, air, and freeze-dried oregano (*Lippia berlandieri* Schauer). J. Food Sci. 65(6): 926–930. https://doi.org/10.1111/j.1365-2621.2000.tb09394.x.

Zigene, Z.D. 2010. Cultivation of Artemisia (*Artemisia annua* L.) in Ethiopia: Cultural Practice and Chemistry. VDM Verlag, Riga, Latvia.

Zigene, Z.D., B.M. Kassahun and T.T. Ketaw. 2012. Effects of harvesting age and spacing on leaf yield and essential oil yield of rosemary (*Rosmarinus officinalis* L.). African J. Plant Sci. Biotech. 6(Special Issue 1): 9–12.

Zigene, Z.D., B.T. Asfaw and T.D. Bitima. 2022. Phenotypic diversity of rosemary (*Salvia rosmarinus* Schleid.) accessions for qualitative characters. Heliyon. 8(12): e11895. https://doi.org/10.1016/j.heliyon.2022.e11895.

Zorpeykar, S., E. Mirzaee-Ghaleh, H. Karami, Z. Ramedani and A.D. Wilson. 2022. Electronic nose analysis and statistical methods for investigating volatile organic compounds and yield of mint essential oils obtained by hydrodistillation. Chemosensors. 10(11): 486. https://doi.org/10.3390/chemosensors10110486.