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Factors influencing the yield of essential oil content from *Eucalyptus globulus* leaves grown in Southern Ethiopia

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Abstract
This study aims to investigate the effect of three experimental factors such as harvesting seasons (dry season, long rain and short rain seasons), leave types (immature twin leaves, matured but lignified leaves and matured leaves) and leaves position (top, medium and bottom) on moisture content (MC), essential oil content (EOC) and essential oil yield (EOY) from *Eucalyptus globulus* leaves. This experiment was conducted to determine MC, EOC and EOY, which are expressed by percent on moisture content and essential oil content, while gm/tree on essential oil yield on the basis of dry leaf weight. The oil was obtained by hydro distillation of *E. globulus* leaves using Clevenger apparatus for 3 hrs. Except, EOC, EOY and optical rotation (O.R), the main effect of harvesting season (HS) significantly (p<0.05) influenced MC, refractive index (RI) and specific gravity (SG) of E. *globulus* leaf oil. The effect of leaf position (LP) and leaf type (LT) on all parameters considered in the experiment, vis. MC, EOC and EOY were also significant except EOY in LP. When the interaction effect between (HS*LP), (HS*LT) and (LP*LT) were observed, it has not significantly influenced all parameter measured except EOY at the interaction effect between (HS*LT) and (LP*LT). In the experiment, the main effects of all factors, higher MC was recorded for HS 53.96% on dry season (DS), for LP 54.48% on top position and for LT 58.5% on immature twin leaves and least moisture content was recorded for matured and lignified leaves (ML) (50.14%) on LT. The main effect of leaf position showed significantly (p<0.01) higher EOC (23.67%) at top leaf position than at bottom leaf position and 10.25% at top leaf position than medium leaf position. The main effect of leaf type showed significantly (p<0.01) higher EOC (13.28%) at immature twin leaves than at matured and lignified leaves and 8.86% at immature twin leaves than at matured leaf type. The interaction effect between harvesting season and leaf type showed a significantly higher EOY for matured but lignified leaves (28.74%) at long rain session, which was followed by matured leaves (21.5%) at short rain season. Comparing the interaction effect between leaf position and leaf type, the maximum essential oil yield was recorded with parallel statistical significance for matured but lignified leaves (24.83%), matured leaves (23.10%), matured but lignified leaves (23.48%) and matured leaves (19.97%) at all harvesting period. The Physico-chemical characteristics of the oil on the main effect of HS has significantly (p<0.05) influenced refractive index (R.I) and has a highly significant (p<0.01) influence on the specific gravity (S.G) of the oils.

Keywords: Essential oil, *Eucalyptus globulus*, immature leaf, matured leaf, lignified leaf

Introduction
The genus *Eucalyptus*, family *Myrtaceac*, is a large genus comprising more than 700 species [1]. The genus is native to Australia [2] and widely grown in many parts of the world, of which at least 500 species produce some type of essential oils [3]. Currently, growing Eucalyptus trees at a farm level have become very popular practice among farmers [4]. The planting rate is increasing due to the high demand for the trees, especially for fuel, poles, construction materials and other domestic consumptions. Some of the species produce quality timber and others possess paper-pulping quality. Among various aromatic plants, the genus *Eucalyptus* consists of tall, magnificent, and evergreen trees with aromatic foliage rich in oil glands and is an excellent source of commercially important oil [5].
Eucalyptus globulus has the most important *Eucalyptus* oil sources [6]. *Eucalyptus* oils are found in the leaves, fruits, buds and bark of the red. However, the most important commercial oil is isolated from the leaves by steam distillation [6]. *Eucalyptus globulus* is rich in leaf oils which contain high proportions of 1, 8-cineole (70-95%, however, some studies have shown as little as 4% in its oil [7, 8].

The essential oil from *E. globulus* is highly useful for production of perfumery and medicinal oils. The perfumery industries have showed great interest for *Eucalyptus* trees after the discovery of its essential oil in 1982. Its leaves normally contain essential oil (0.5-4%) although physiological rates with up to 7% oil are also reported [9].
The oil represents an important raw material for pharmaceutical, confectionery and cosmetic industries [10]. Several factors, such as types of species, genotypes, leaf position, leaf age and tree age could influence the yield of essential oil obtained from *E. globulus* [11]. Moisture content, wilting time, leave position and chopping size also affect essential oil production [12-14]. The aim of this study was to investigate the effect of seasonal variation, leaves position and leaves types on the yield of oils on the bases of leaves weight, essential oil contents and essential oil yield per tree production for giving methodical information on the production of essential oils from *E. globulus* in Ethiopia.

Material and Method

Site Descriptions

Wondo Genet is located at about 263 km South of Addis Ababa, and 17 km South East of *Shashemene* town, on the eastern escarpment of the Ethiopian Rift Valley in the South Nation Nationalities and Peoples Regional (SNNPR) State. Wondo Genet is surrounded by a green chain of mountains, and in the low-lying areas it contains an extensive flat marshland. Geographically, it is situated between 38°37′E - 38°42′E and 7°02′N - 7°07′N. It covers a wide altitudinal range of 1600 - 2580 m.a.s.l. [15]. The rainfall of Wondo Genet area is characterized by a bimodal distribution, with the main rainy season between July and October, which accounts for 50% of the total rainfall, and a short rainy season between March and May. The mean annual rainfall is 1247 mm, and the mean monthly temperature is 19.5 °C, with mean monthly maximum and minimum temperatures of 26.3 °C and 12.4 °C, respectively [16]. The main parent material of the soil of the study area is developed on volcanic deposits of ignimbrite, ash, lava and tuff, which have formed gentle and undulating terrain [17].

Sample Collection

Eucalyptus globulus leaves were taken from the garden of Wondo Genet Agricultural Research Center which was grown under experimental site. Three *Eucalyptus globulus* trees were randomly selected from the experimental garden and samples of *E.globulus* leaves were harvested at different seasonal period for distillation process. The collected leaves were categorized by leaves position (LP) (i.e. top, medium and bottom) and then sorted out by the leaf types (i.e. immature twin (IT), matured leaf (M) and matured and lignified leaf (ML) and the total weight of leaves per tree was recorded.

Laboratory Activities

Moisture Content Determination

7–10 gram of E. *globulus* fresh leaves were taken and cut into small pieces and placed on an empty aluminum tray. Then, the samples were placed into the oven drier for 2.5 - 3 hrs at 105 ± 1^0 C until the weight difference between two successive measurements got below 0.5 mg. The samples were taken from the oven drier and placed in desiccators until it got cool [6].

Extraction of Essential Oils

Per each extraction, an average of 250 gram sample of *Eucalyptus globulus'* leaves were taken into hydro-distillation and thereby oil distillation process has been carried out for 3 hrs. by using a Clevenger apparatus [18]. The distillated oil of each treatment was measured and EOC and EOY was calculated as follows:-

1. The weight of distilled oil was divided by moisture free leaf weight and multiplied by hundred [19].
2. The essential oil yield was also calculated by essential oil content in percent (DB) leaf weight (gram/tree)/100 and expressed as gram/tree [19, 20].

Physico-Chemical Studies of the Oil

The distilled essential oil was carried out by the instrument such as p20 Polarimeter, Refractometer and pycnometer for the analysis of physicochemical properties by applying the procedure used by the laboratory manual for plant products analysis volume I [6].

Laboratory Materials

Instruments used in the laboratory include sensitive balance to weigh distil sample; 2 liter capacity round bottom flask to keep samples during distillation; 300 mm length condenser to condense the volatile substance; 12 ml capacity Clevenger apparatus to separate oil from water; heating mantle as a source of heat energy for the distillation, p20 Polari-meter used to determine for specific optical rotation, Refractometer used for reading refractive index, pycnometer used to measured for density of the oil and amber glass vials to collect the separated essential oil.

Statistical Analysis

All statistical analyses were done using SAS software version 9. Two-way ANOVA was used to analyze the effect of seasonal variation, leaf position and leaf types on essential oil content and essential oil yields. The mean separation was carried out using Least Significant Difference (LSD) at ($P<0.001$) levels of significance.

Result and Discussion

Except essential oil content (EOC), essential oil yield (EOY) and optical rotation (O.R), the main effect of harvesting season (HS) significantly ($p<0.05$) influenced moisture content (MC), refractive index (RI) and specific gravity (SG) of the *E. globulus* leaf oil. The effect of leaf position (LP) and leaf types (LT) on all parameters considered in the experiment, vis. Viz. MC, EOC and EOY was also significant except EOY in LP. When the interaction effect between (HS*LP), (HS*LT) and (LP*LT) were observed, it has not significantly influenced all parameter measured except EOY at the interaction effect between (HS*LT) and (LP*LT). EOY have highly significant ($P<0.001$) influence on (HS*LT) and (LP*LT) (Table 1).

Table 1: Analysis of variance for variability of *Eucalyptus globulus* leaves for different yield parameters.

		Mean Square					
Source of variation	**DF**	**MC**	**EOC**	**EOY**	**R.I**	**O.R**	**S.G**
HS	2	105.67**	0.52ns	302.01ns	5.59X106*	0.14ns	7.6*104***
LP	2	142.21**	12.57***	426.36ns			
HS*LP	4	26.43ns	0.38ns	105.76ns			
LT	2	2164.06***	3.68***	3420.80***			
HS*LT	4	41.03ns	0.09ns	1219.51***			
LP*LT	3	18.47ns	0.39ns	1808.32***			
HS*LP*LT	6	19.41ns	0.04ns	105.28ns			
Adj.R^2		0.45	0.34	0.32			
CV		8.35	19.27	70.45			

Moisture Content

Main effects of harvesting season have shown statistically uniform higher moisture content for dry season (DS) (53.96%) and long rain season (LRS) (53.87%). The least moisture content (52.49%) was observed for short rain season (SRS). In the experiment, the main effects of leaf position, observed at medium and bottom leaf positions, were statistically uniform and least moisture content was recorded 53.42% and 52.42%, respectively. The maximum moisture content (54.48%) was observed for top leaf position. The main effects of leaf types showed statistically highly significant difference (p<0.01) for moisture content. The maximum moisture content was recorded for immature twin leaves (IT) (58.5%) and least moisture content was recorded matured and lignified leaves (ML) (50.14%) (Table3). The least moisture content, which was 14.29% lower than the maximum, was observed on matured and lignified leaf types.

Essential Oil Content

Significantly (p<0.001) higher variation have shown on main effects LP and LT was observed, but in the case of interaction effect, there was no significant difference (Table 1). The main effect of leaf position showed significantly (p<0.01) higher essential oil content (23.67%) at top leaf position than at bottom leaf position and 10.25% at top leaf position than medium leaf position. The main effect of leaf types showed significantly (p<0.01) higher essential oil content (13.28%) for immature twin leaves than for matured and lignified leaves and 8.86% for immature twin leaves than for matured leaf types (Table 3). In this experiment, essential oil content for the main effect of harvesting season have shown insignificant different at different harvesting periods (Table 3). The finding of this study were compared to the previous study by Fikremariam 2018 [21] have been confirmed as having the same behavior. In both studies, the essential oil content in leaf tip part found maximum than at middle and bottom leaf parts.

Essential Oil Yield

The interaction effect between harvesting season and leaf types showed a significantly higher essential oil yield for matured but lignified leaves (28.74%) at long rain season, followed by matured leaves (21.5%) at short rain season (Table 2). The minimum essential oil yield showed statistically parallel significance (17.61%), (15.64%) for matured but lignified leaves 14.29% and 18.21% for matured leaves at harvested DS and LRS, respectively at different harvesting periods (Table 2 and Figure 1). Comparing the interaction effect between leaf positions and leaf types, the maximum essential oil yield was recorded with statistically parallel significance difference for matured but lignified leaves (24.83%), matured leaves (23.10%), matured but lignified leave (23.48%) and matured leaves (19.97%) at all harvesting period. The minimum essential oil yield was recorded with statistically parallel significance difference for matured leaves (10.93%), immature twin leaves (7.09%) and (10.38%) and matured but lignified leaves (13.67%) at all harvesting period (Table2 and Figure 2).

Table 2: Interaction effect of harvesting time and leave type and leave position and leave type on essential oil yield of *Eucalyptus globulus.*

HS*LT		**EOY (gm/tree)**	**LP*LT**		**EOY (gm/tree)**
HS	LT		LP	LT	
DS	IT	Nos	BO	IT	Nos
	M	14.29^{cd}		M	10.93^{b}
	ML	28.74^{a}		ML	24.83^{a}
LRS	IT	Nos	ME	IT	7.09^{b}
	M	18.21^{bc}		M	23.10^{a}
	ML	15.64^{bc}		ML	23.48^{a}
SRS	IT	Nos	TOP	IT	10.38^{b}
	M	21.5^{b}		M	19.97^{a}
	ML	17.61^{bc}		ML	13.67^{b}

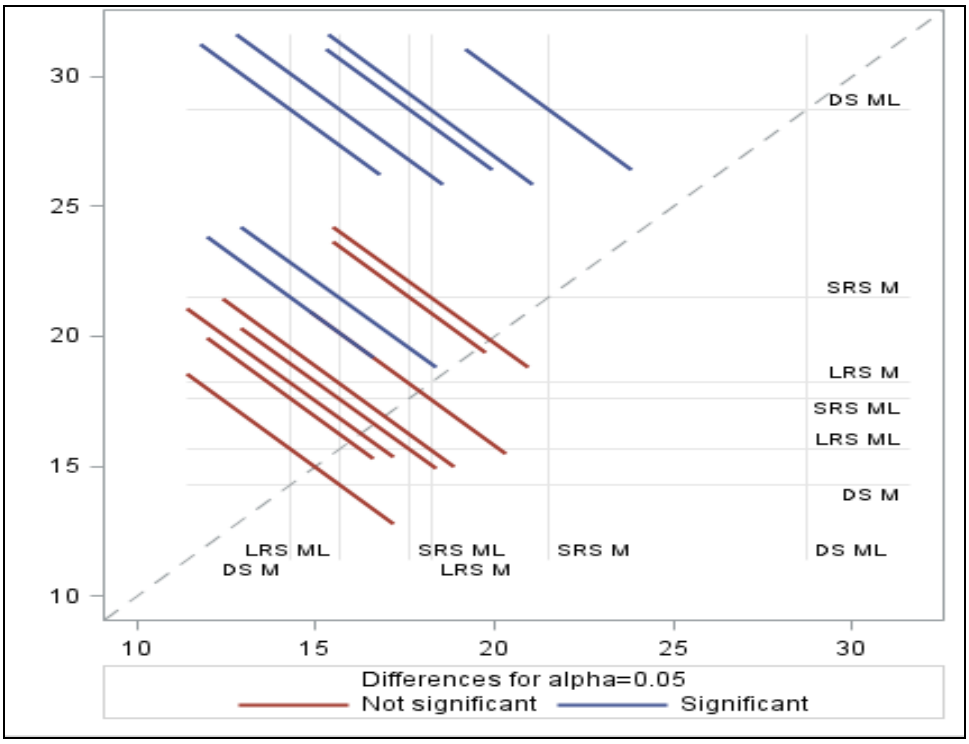

Fig 1: Eoy Comparisons for HS'LT

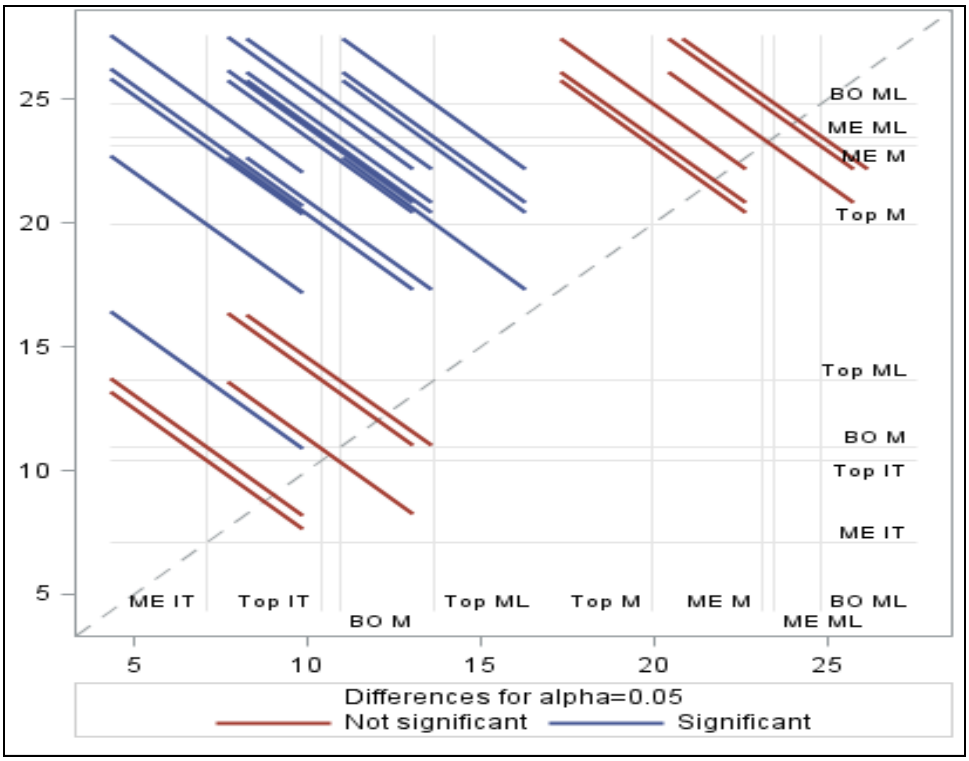

Fig 2: Eoy Comparisons for LP'LT

Table 3: Main effect of harvesting seasons, leaf positions and leaf types on moisture content and essential oil content of *Eucalyptus globules* oils

		MC	EOC
HS	DS	53.96[a]	Ns
	LRS	53.87[a]	Ns
	SRS	52.49[b]	Ns
LP	BO	52.42[bc]	2.16[c]
	ME	53.42[b]	2.54[b]
	TOP	54.48[a]	2.83[a]
LT	IT	58.5[a]	2.71[a]
	M	51.68[b]	2.47[b]
	ML	50.14[c]	2.35[c]

Physico-chemical Properties

The Physico-chemical properties analyzed in the experiment, except optical rotation, the main effect of HS significantly (p<0.05) influenced refractive index (R.I) and specific gravity (S.G) of the oils. The analysis of variance showed that, overall mean of S.G was found between DS, LRS and SRS with statistical difference and have S.G for LRS (0.9120) followed by DS (0.8948). The minimum overall average SG recorded was for SRS (0.8800) (Table 3). The least value of SG, which is 2.70% lower than the maximum, was observed on SRS, while compared by analysis of variance, overall mean of R.I was found between DS and LRS that have not

statistical difference and the least value on DS and LRS, which was recorded 1.4500 and 1.4497 respectively, on SRS have statistically difference than DS and LRS, which have maximum value (1.4522) was recorded (Table 4).

Table 4: Main effect of harvesting sessions on Physico-chemical properties of *Eucalyptus globules* oils

	HS		
	DS	**LRS**	**SRS**
Refractive Index at room temperature	1.4500[b]	1.4497[b]	1.4522[a]
Specific Gravity at room temperature	0.8948[b]	0.9120[a]	0.8880[c]

Conclusion

This research we have clearly shown the impact of leaf positions and leaf types on moisture and essential oil content of the *Eucalyptus glo*bulus leaves. The study showed that there has been higher essential oil content in the top leaf position and in immature twin leaf types. This value is in conformity with value observed by *List* [22]. The variation among the observed values might be permissible due to the regulation of oil accumulation by the specialized oil gland (i.e. glandular trichomes) and the oil gland capacity that was connected to leaf thickness and leaf area with leaf maturation may regulate oil content [23]. Moreover, that are present in parenchymal tissues of the top position and immature twin of *Eucalyptus glo*bulus leaves fragment are extra essential oil making during the secondary metabolic reaction other than the leaves type (M and ML) and leaves position (BO and ME), this is due to glandular trichome glands are full-fledged. Therefore, it can be concluded from the experiment that top leaf positions and all immature twin leaves are recommended to distillation so as to obtain high amount of essential oil. This result has significant importance as a guideline to produce essential oils from the *Eucalyptus globulus* for those commercially involved in the sector.

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