

The Influence of Harvesting Season, Wilting Period and Leaf Position on Essential Oil Content of *Eucalyptus camaldulensis* Leaves

¹Fikremariam Haile, ¹Degnechew Genene, ³Yohannes Fekadu,
²Zewdineh Damtew and ³Solomon Abate

¹Ethiopian Environment and Forest Research Institute (EEFRI),
Wood Technology Research Center, Bioenergy and Biochemical Research Division;
P.O. Box: 2322, Addis Ababa, Ethiopia

²Wondogenet Agricultural Research Center, EARI; P.O. Box: 198, Shashemene, Ethiopia

³Ethiopian Agricultural Research Institute; P.O. Box: 30708, Addis Ababa, Ethiopia

Abstract: The aim of this research is to determine the essential oil content variation at harvesting day (EOC₀) and essential oil content at wilting day (EOC_w) of *Eucalyptus camaldulensis* was examined the influence of harvesting season (Long Rain Season and Dry Season), the leaf type (top, middle and bottom) and wilting period from 0 h (W₀) to 96 h (W₄) for obtaining the highest quantity of the essential oil from *Eucalyptus camaldulensis* leaves. A completely randomized design (CRD) with three replications was used in the experiment. The oil was obtained by hydrodistillation of *E. camaldulensis* leaves using Clevenger apparatus for 3 h. The distillate oil was measured and the essential oil content (EOC) was expressed in percent based on calculations of harvesting day and wilting day. EOC₀ and EOC_w of *E. camaldulensis* were highly significantly ($p < 0.001$) affected by harvesting season (HS), leaf position (LP) and wilting period (WP). The interaction effect of harvesting season, leaf position and wilting period on EOC₀ and EOC_w of *E. camaldulensis* is significant at 0.001 probability level. The maximum yield for EOC₀ was found at wilting period of 48 h (W₂) with long rain season in top leaf part with value 0.69%. The maximum yield for EOC_w were found at wilting period of 96 h (W₄) with both harvesting seasons (i.e. long-rain and dry seasons) with values of 1.13% and 1.11%, respectively; while, statistically similar maximum yield of EOC_w were obtained at wilting period of 72 h (W₃) and 48 h (W₂) with respective values of 1.06% and 1.08% for the same leaf position. In view of these results, it can be concluded that the top leaf part and day four wilting period are the most opportune to obtain maximum yield of essential oil extracted when the *E. camaldulensis* leaves were distilled.

Key words: *Eucalyptus camaldulensis* • Hydro-distillation • Leaf type • Leaf position • Wilting day

INTRODUCTION

Eucalyptus camaldulensis (Dehnh.) with common name river red gum is a tree of the genus *Eucalyptus*. It is one of approximately eight hundred (800) genus in the family of *Myrtaceae*. It is a plant species in a number of parts of the world, however it is indigenous to Australia, where it is widely distributed and cultivated in tropical and sub-tropical areas of the world [1], particularly alongside inland water courses. In Ethiopia, *E. camaldulensis* is known as a 'Key-Bahir-Zaf' (in Amharic) or a "red eucalypt" and one of the 55 types

of *eucalypts* introduced in the country and among the most widely adopted and used in plantations for farmers [2, 3]. Furthermore, *Eucalyptus camaldulensis* is one of the most-extensively cultivated and planted pulpwood species. It has also been planted predominately for its leaves that are plentiful in essential oil content (EOC) and are exploited commercially for their use in chemicals and allied industries, as ingredient in pharmaceuticals, cosmetics and perfumery and as an additives in food processing industries [4-6]. *E. camaldulensis* leaves and the essential oil extract (i.e. the natural extract) obtained from the leaves has a number of applications in daily

human life because of its biological activities, like anti-carcinogenic, anti-inflammatory, anti-viral, carminative, anti-pyretic, spasmolytic, anti-septic, hepatoprotective and anti-microbial attributes [7-11].

An essential oil is a volatile oil, which available in some plants and tree species. It can be found in the fruits, trunks, leaves, blossoms, stems and/or roots of the considered trees. Even though it is known as oil, it is quite distinct from common vegetable oils, since it is very volatile, light, non-greasy, absorbed quickly into the skin [12]. Several essential oils are required and utilized by chemicals and allied industries as a source of particular compounds for succeeding transformation to a variety of synthetic products like pesticides, vitamins, aroma chemicals. They are also valuable in the production of essential medicated ointments, shampoos, balms, soaps, perfumes and other cosmetics. Moreover, *E. camaldulensis* essential oils are used in medicine to treat certain skin diseases and respiratory infections [13, 14]. According to previous study, Pino *et al.* [15], *E. camaldulensis* oils, are rich in 1,8-cineole, are predominantly used in the pharmaceuticals processing industries. A number of research findings have been carried out on an essential oils of *E. camaldulensis* and shown that it has significant roles such as, anti-fungics, analgesics, anti-bacterial activities, insecticide and anti-inflammatory properties of these essential oils [16-19]. The bio-chemical activity of this essential oil is based on the oil compositions and dosages. Several factors like harvesting seasons (i.e. stage of the plants), origin, species variety, storage of biomass, wilting period and leaf position can inherently influence the physico-chemical compositions and nature of the essential oil [20]. Abiotic stresses such as drought and nutrient deficiency and biotic stresses such as disease or herbivory can also affect mainly the quality and yield of essential oil and its corresponding compositions [21, 22]. The rationale of this study is to investigate the influence of harvesting season, wilting period and leaf position on essential oil content (EOC) of *E. camaldulensis* leaves.

MATERIALS AND METHODS

Site Description: *Eucalyptus camaldulensis* tree leaves were taken from Wondo Genet Agricultural Research Center experimental site. Geographically, Wondo Genet is located at 07° 03' 19.1" to 07° 04' 00.2" North latitude and from 38° 30' 08.4" to 38° 31' 01.8" East longitude. It covers a wide altitudinal range of 1600 - 2580 m.a.s.l. [23]. 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 [24]. 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 [25].

Sample collection and preparation: *Eucalyptus camaldulensis* trees were randomly selected from Wondo Genet Agricultural Research Center. The experimental site and samples of leaves were harvested during the long rain season from August, 2015 to October, 2015 and the dry season from March, 2016 to May, 2016. A total of 30 treatment combinations comprising 5 levels of wilting periods (0, 24, 48, 72 and 96 h after harvest), 3 levels of leaf positions (Top, Medium and Bottom) and 2 levels of harvesting season (Long Rain Season and Dry Season) were used for the experiment. The design of the experiment was completely randomized design (CRD) with three replications. For each treatment constant weight of leaf samples were taken and subjected to open air for wilting purposes under shade having an average temperature of 25 °C for five days.

Essential oil Extraction: Wilted leaves of *E. camaldulensis* for every treatment with three replications was subjected to hydro-distillation for 3 h using Clevenger-type apparatus, according to the method recommended by European Pharmacopoeia (1983) [26-28]. The distillate oil of each sample was measured and the percentage of essential oil content was calculated by using the following mathematical methods:

- The weight of distilled oil was divided by fresh leaf weight and multiplied by hundred, it was given essential oil content (EOC) at harvested day (EO C₀) [29].
- The weight of distilled oil was divided by wilted leaf weight and multiplied by hundred, it was given essential oil content at wilting day (EOC_w) [29-30].

Data Analysis: The statistical analysis was done with SAS software version 9.0 and SAS Studio (Which is free university license and very good for assumption checking). The classical general linear model with two-way ANOVA fits the data very well as shown in the results. Mean separation was carried out using LSD at ($P < 0.001$).

RESULTS AND DISCUSSION

Variation in Essential Oil Content (EOC) of *Eucalyptus camaldulensis*:

The EOC₀ and EOC_w of *E. camaldulensis* were highly significantly ($P < 0.001$) affected by harvesting season, leaf position and wilting period (Table 1). These results have shown similar tendency with previous study by Fikremariam *et al.* [31], which has shown that the effect of harvesting season, wilting period and leaf position on the yield of the resulting essential oil extracted from the *E. globules* leaves and indicated that the post-harvest wilting period before distillation of the *E. globules* leaves up to 96 h (W₄) gave significantly high essential oil contents than early distillations in each leaf type during the harvesting season. An average of essential oil content on wilting period for both harvesting season, increases variably between top and middle (0.11%) and top and bottom (0.53%) for EOC_w. In addition, the present study is comparable with other research findings made by Solomon *et al.* [32], showed that the effect of wilting period on the yield of the resulting essential oil produced from the grass species (i.e. Lemongrass, Palmarosa and Citronella) wilted up to day three gave significantly higher amount of EOC_w than that of early distillations and the amount of the corresponding EOC_w increases within the range 0.21-0.85%, 0.27-0.82% and 0.83-1.53% for Lemongrass, Palmarosa and Citronella, respectively. Interaction effect of harvesting season, leaf position and the wilting period was also highly significant on EOC₀ and EOC_w of *E. camaldulensis* (Table 1).

The Interaction Effect of Harvesting Season, Wilting Period and Leaf Position on Essential Oil Content at Harvest (EOC₀) and Essential Oil Content at Wilting Day (EOC_w) of *Eucalyptus camaldulensis*: As shown in Table 1, the interaction effect of harvesting season,

wilting period and leaf position on EOC₀ and EOC_w of *E. camaldulensis* is significant at $P = 0.001$. The maximum yield for EOC₀ was found at wilting period of 48 h (W₂) for long-rain season in the top leaf part with value of 0.69%. The maximum yield for EOC_w were found at wilting period of 96 h (W₄) with both harvesting seasons (i.e. long-rain and dry seasons) with values of 1.13% and 1.11%, respectively; while, statistically similar maximum yield of EOC_w were obtained at wilting period of 72 h (W₃) and 48 h (W₂) with respective values of 1.06% and 1.08% for the same leaf position (Table 2). These results are in compliance with the previous studies on the essential oil obtained from the wilted leaves of *E. camaldulensis* plants collected from five localities of the Montenegro coastline, which was reported in the range of 0.63-1.59 % [33]. In comparison with other literature, which was reported for *Eucalyptus* genus, where the essential oil content was found in the range of 0.2 - 0.7% (w/w) based on the dried/wilted leaves in Côte d'Ivoire at near Abidjan [34]. The smallest values of EOC₀ (0.23%) and EOC_w (0.231%) were obtained for samples harvested during dry season from bottom part and distilled without wilting (W₀) and after wilting, respectively (Table 2). These results have shown the same tendency with the research work corporate by Fikremariam *et al.* [31]. In general, from the experimental data it has been observed that the percentage of EOC₀ and EOC_w come across wilting period from 0 h (W₀) to 96 h (W₄) in all positions of leaves (i.e. top, middle and bottom) of *E. camaldulensis* for both harvesting seasons, the sign of irregular increment was noted; nonetheless there were slightly statistical uniformity. While computing along with leaf type maximum yield of EOC₀ (0.63%, 0.50%) and EOC_w (0.86%, 0.89%) (Figure 1) were observed on average in leaf tip part than medium and bottom parts at long rain season and dry season, respectively.

Table 1: Analysis of Variance for Essential oil content of *Eucalyptus camaldulensis*.

Source of variation	DF	Mean Square	
		EOC ₀	EOC _w
Harvesting season	1	0.243***	0.009*
Leaf position	2	0.394***	1.152***
Wilting period	4	0.014***	0.560***
Interaction effect	2	0.007***	0.020***
Error	60	0.06	0.001
CV (%)		7.24	5.58
R-Square		0.95	0.98

***= Significant at $P < 0.001$; **= Significant at $P < 0.01$; *= Significant at $P < 0.05$; ns= Non-significant at $P < 0.05$, EOC₀ = Essential oil content at harvest, EOC_w = Essential oil content at wilting day

Table 2: Interaction effects of harvesting time, leaf position and wilting period on essential oil content at harvest (EOC₀ %) and essential oil content on the wilting day (EOC_w %) of *Eucalyptus camaldulensis*

		Means			
		Harvesting Season			
		Dry season		Long rain season	
Leaf Position	Wilting Period	EOC ₀ (%)	EOC _w (%)	EOC ₀ (%)	EOC _w (%)
Top	W ₀ (0 h)	0.49 ^{ef}	0.49 ^{hi}	0.65 ^{ab}	0.65 ^{efg}
	W ₁ (24 h)	0.47 ^{efgh}	0.71 ^{cde}	0.64 ^b	0.74 ^c
	W ₂ (48 h)	0.56 ^d	1.08 ^a	0.69 ^a	0.91 ^b
	W ₃ (72 h)	0.51 ^e	1.06 ^a	0.57 ^{cd}	0.89 ^b
	W ₄ (96 h)	0.47 ^{efgh}	1.13 ^a	0.61 ^{bc}	1.11 ^a
Middle	W ₀ (0 h)	0.45 ^{fgh}	0.45 ^{ij}	0.33 ^{kl}	0.33 ^k
	W ₁ (24 h)	0.37 ^{jk}	0.49 ^{hi}	0.48 ^{efg}	0.54 ^b
	W ₂ (48 h)	0.4 ⁱ	0.70 ^{cde}	0.51 ^e	0.68 ^{cdef}
	W ₃ (72 h)	0.33 ^{kl}	0.64 ^{ef}	0.43 ^{hi}	0.68 ^{cdef}
	W ₄ (96 h)	0.33 ^{kl}	0.69 ^{cdef}	0.44 ^{ghi}	0.71 ^{cde}
Bottom	W ₀ (0 h)	0.23 ⁿ	0.23 ^l	0.27 ^{mn}	0.27 ^l
	W ₁ (24 h)	0.28 ^m	0.36 ^k	0.37 ^{jk}	0.42 ^j
	W ₂ (48 h)	0.28 ^m	0.45 ^{ij}	0.45 ^{fgh}	0.61 ^g
	W ₃ (72 h)	0.31 ^m	0.55 ^h	0.48 ^{efg}	0.72 ^{cd}
	W ₄ (96 h)	0.33 ^{kl}	0.66 ^{efg}	0.44 ^{ghi}	0.72 ^{cd}

Means followed by the same letters under the same column are statistically non-significant at 0.05 level of probability.



Fig. 1: Interaction between harvesting season, leaf position and wilting period on essential oil content of *E. camaldulensis*

LRS - Long Rain Season; DS - Dry Season; EOC₀ - Essential oil content at harvesting day; EOC_w - Essential oil content at wilting day

There is a significant variation between leaf positions (top, middle and bottom) in compliance with the corresponding nature of leaves (i.e. Immature Twin, Matured leaf, Matured and lignified leaf) and characters of leaves (i.e. leaf thickness, leaf area and leaf mass per area) [35]. Hence, specific oil cells or the glandular trichomes that are exist in parenchymal tissues of *E. camaldulensis* leaves [35-37] enables an extra growth in top leaf part than in (middle and bottom) leaf parts. In most cases, the percentage compositions of essential oil of the *E. camaldulensis* exhibits a significant increment with the loss of moisture for subsequent wilting which

was described by Solomon and Fikremariam [32]. These results are also in agreement with previous studies on the effect of dried/wilted under shade had shown significant effect on oil yield of *Eucalyptus sargentii* leaves [38].

CONCLUSION

This study has clearly shown the impact of harvesting season, leaf position and wilting period on EOC₀ and EOC_w of the *E. camaldulensis* leaves. Wilting after harvest before distillation of the *E. camaldulensis* leaves up to 96 h (W₄) gave significantly high essential oil

contents than early distillations in each leaf type, except tip and middle leaves type for EOC_w. In both harvesting seasons (i.e. long-rain and dry season), the amount of increase in variations between 0.19 (top and middle) and 0.34 (top and bottom) for EOC₀ and 0.28 (top and middle) and 0.38 (top and bottom) for EOC_w. Therefore, it can be concluded that this research finding has provided an excellent model for handling mechanisms of *E. camaldulensis* leaves before the leaf was distilled. The day four wilting period and top leaf part are the most appropriate to obtain maximum yield of essential oil recovered when the *E. camaldulensis* leaves was distilled and the experimental result has a significant contribution to save time, energy and minimize running cost or maximize production of essential oil from the considered leaves by distillation process at commercial scale.

ACKNOWLEDGMENT

Authors would like to gratefully thank Dr. Tesfaye Bekele, the director of the former Essential Oil Research Center, for his significant contributions, involvement and all rounded support during the study. Besides, the authors are also grateful and thankful to Mr. Birara Tilahun, for his valuable contributions in the collection of *E. camaldulensis* leaves from the field, as well as to Mr. Zerihun Jomba, for his active participation in the laboratory work and sample analysis during this study.

REFERENCES

- Berhaut, J., 1979. Flore illustrée du Sénégal, TOME VI, Linacées à Lymphécées, pp: 530-531.
- Davidson, J., 1995. Eucalyptus tree improvement and breeding. Ministry of Natural Resources Development and Environmental Protection. Forestry Research Center, Addis Ababa, Ethiopia. H.
- FAO, 2001. Mean annual volume increment of selected industrial forest plantation species by Ugalde & Pérez. Forest Plantation Thematic Papers, Working Paper 1. Forest Resources Development Service, Forest Resources Division.
- Brooker, M.I.H. and D.A. Kleinin, 1992. A field Guide to Eucalyptus. South-Eastern Australia. Bloomings Melbourne, Australia, Vol. 1: 3rd edition.
- Khan, I.A. and E.A. Abourashed, 1996. Leung's Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics, 3rd edn. Wiley: New York, pp: 273-274.
- Marzoug, H.N.B., M. Romdhane, A. Lebrihi, F. Mathieu, F. Couderc, M. Abderraba, M.L. Khouja, J. Bouajila, 2011. Eucalyptus oleosa Essential Oils: Chemical Composition and Antimicrobial and Antioxidant Activities of the Oils from Different Plant Parts (Stems, Leaves, Flowers and Fruits). *Molecules*, 16: 1695-1709.
- Jeane, S.S., A. Worku, S.M. Sousa, V.G. Duarte, M.I.L. Machado and F.J.A. Matos, 2003. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *J Ethnopharmacol*, 89: 277-283.
- Kumar, A., V.D. Sarma, A.K. Sing and S. Kamla, 1988. Antimicrobial properties of different Eucalyptus oils. *Fitoterapia*, 59: 141-144.
- Ait-Ouazzou, A., L. Cherrat, L. Espina, S. Lorán, C. Rota and R. Pagán, 2011. The antimicrobial activity of hydrophobic essential oil constituents acting alone or in combined processes of food preservation. *Innovative Food Science and Emerging Technologies*, 12: 320-329.
- Wang, G., G., W. Tang and R.R. Bidigare, 2005. Terpenoids as therapeutic drugs and pharmaceutical agents, in *Natural Products: Drug Discovery and Therapeutic Medicine*. Eds. L. Zhang, A.L. Demain, Humana Press: Totowa, pp: 197-227.
- Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils - A review. *Food Chem. Toxicol.*, 46: 446-475.
- Guenther, E., 1972. *The Essential Oils*, Vol. I; Robert E. Kreiger Publishing Co., New York.
- Elaissi, A., Z. Rouis, S. Mabrouk, K.B.H. Salah, M. Aouni, M.L. Khouja, F. Farhat, R. Chemli and F. Harzallah-Shkhir, 2012. Correlation Between Chemical Composition and Antibacterial Activity of Essential Oils from Fifteen Eucalyptus Species Growing in the Korbous and Jbel Abderrahman Arboreta (North East Tunisia). *Molecules*, 17: 3044-3057.
- Sefidkon, F., M.H. Assareh, Z. Abravesh and M.M. Barazandeh, 2007. Chemical Composition of the Essential Oils of Four Cultivated Eucalyptus Species in Iran as Medicinal Plants (*E. microtheca*, *E. spathulata*, *E. largiflorens* and *E. torquata*). *Iranian Journal of Pharmaceutical Research*, 6(2): 135-140.
- Pino, J.A., R. Marbot, R. Quert and H. García, 2002. Study of essential oils of Eucalyptus resinifera Smith, E. tereticornis Smith and Corymbia maculata (Hook) K. D. Hill & L.A.S. Johnson, grown in Cuba. *Flavour Fragr. J.*, 17: 1-4.

16. Marzoug, H.N.B., M. Romdhane, A. Lebrihi, F. Mathieu, F. Couderc, M. Abderraba, M.L. Khouja and J. Bouajila, 2011. Eucalyptus oleosa Essential Oils: Chemical Composition and Antimicrobial and Antioxidant Activities of the Oils from Different Plant Parts (Stems, Leaves, Flowers and Fruits). *Molecules*, 16: 1695-1709.
17. Tolba, H., H. Moghrani, A. Benelmouffok, D. Kellou and R. Maachi, 2015. Essential oil of Algerian Eucalyptus citriodora: Chemical composition, antifungal activity. *Journal de Mycologie Médicale*, 25: 128-133.
18. Silva, J., W. Abebe, S.M. Sousa, V.G. Duarte, M.I.L. Machado and F.J.A. Matos, 2003. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *Journal of Ethnopharmacology*, 89: 277-283.
19. Ogendo, J.O., M. Kostyukovsky, U. Ravid, J.C. Matasyoh, A.L. Deng, E.O. Omolo, Kariuki, S.T. and E. Shaaya, 2008. Bioactivity of Ocimum gratissimum L. oil and two of its constituents against five insect pests attacking stored food products. *Journal of Stored Products Research*, 44: 328-334.
20. Raut, J.S. and S.M. Karuppaiyil, 2014. A status review on the medicinal properties of essential oils. *Industrial Crops and Products*, 62: 250-264.
21. Tang, C., W. Cai, K. Kohl and R.K. Nishimoto, 1995. Allelopathy: Organisms, processes and applications. In: Inderjit, Dakshini KMM, Einhellig FA, editors. Chapter 11, Plant stress and allelopathy. Washington, DC: ACS Symposium Series 582, American Chemical Society, pp: 348-362.
22. Leicach, S.R., A. Garau, A.B. Guarnaschelli, N. Sztarker and A. Dato, 2008. Changes in Eucalyptus camaldulensis essential oils with water stress. Conference presented at 5th World Congress on Allelopathy, 4. Allelopathy in Forest Systems. September 24, 2008. Saratoga Springs, NY, USA.
23. Hjelm, B., 2001. A management plan for the forest plantation of Wondo Genet College of Forestry 2001-2005, Wondo Genet College of Forestry.
24. Fikadu Getachew, 2006. Effect of land use type on soil physical and chemical properties in Wondo Genet, Ethiopia. M.Sc thesis Wondo Genet collage of forestry and natural resource.
25. Makin, M.J., T.J. King, A.E. Waddams, C.J. Birchall and T. Teferra, 1975. Development prospects in the southern Rift Valley, Ethiopia. Land Resource Study 21. LRD, Ministry of Overseas Development, Surbiton, xiv: 270
26. European Pharmacopoeia, 1983. Part 1. Maisonneuve SA, Sainte. Ruffine, pp: 4.58.
27. Khouzani, H.L., O.S. Fini and J. Safari, 2007. Essential oil composition of Rosa damascene Mill. Cultivated in Central Iran. *Scientia Iranica*, 14(4): 316-319.
28. Guenther, E., 1972. The essential oils: History-origins in plants production- analysis (voll), Robert E. Kriger publishing Co. Malabar, Florida, pp: 427.
29. Zheljzkov, V.D., C.L. Cantrell and T. Estates, 2010. Yield and composition of oil from Japanese cornmint fresh and dry material harvested successively. *Agronomy Journal*, 102(6): 1652-1656.
30. Bhuiyan, M.N.I., F. Akter, J.U. Chowdhury and J. Begum, 2010. Chemical constituents of essential oils from aerial parts of Adenosma capitatum and Limnophila aromatic. *Bangladesh Journal of Pharmacology*, 5: 13-16.
31. Fikremariam, H., D. Zedineh, F. Yohannes and A. Solomon, 2018. Influence of Harvesting Season, Leaf Position and Wilting Period on Essential Oil Content of Eucalyptus globules Leaves. *World Journal of Agricultural Sciences*, 7(4): 404-412, 2011. ISSN 1817-3047.
32. Solomon, A.M. and H. Fikremariam, 2010. Essential Oil Content Response of Lemongrass, Palmarosa and Citronella to Post Harvest Wilting and Chopin. *The African journal of plant science and biotechnology*.
33. Slavenko, G., O. Dejan, C. Maria, J. Emilija, B. Dušan, B. Kristina and M. Neda, 2010. Variation of essential oil composition of eucalyptus Camaldulensis (Myrtaceae) from the Montenegro coastline. *apteff*, 41: 1-203 (2010).
34. Kanko C., B. Soleymane Ramiarantsoa Tue, J. Chalchat, P. Chalard, G. Figueredo and C. Ahibo, 2012. Monoterpene hydrocarbons, major components of the dried leaves essential oils of five species of the genus Eucalyptus from Côte d'Ivoire. *Natural Science*, 4(2): 106-111.
35. Lewinsohn, E., N. Dudai, Y. Tador, I. Katzir, U. Ravid and E. Putievsky, 1998. Localization of citral accumulation in lemongrass leaves (*C. citratus* DC Stapf Poaceae). *Annals of Botany*, 81: 85-89.
36. Luthra, R., A.K. Srivastava and D. Gajewala, 2007. Histochemical localization of citral accumulating cite in lemongrass (*C. flexuosus* Nees ex Steud) Wats cultivar GRL-1. *Asian Journal of Plant Science*, 6: 419-422.
37. Sangwan, N.S., A.H.A. Farooqi, F. Shabih and R.S. Sangwan, 2001. Regulation of essential oil production in plants. *Plant Growth Regulation*, 34: 3-21.
38. Fathi, E. and F. Sefidkon, 2012. Influence of Drying and Extraction Methods on Yield and Chemical Composition of the Essential Oil Eucalyptus sargentii. *J. Agr. Sci. Tech.*, 14: 1035-1042.