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Variation of Yield and Chemical Composition of Essential Oil from *Cupressus lusitanica* Growing in Different Agro-ecological Zones of Rwanda

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PN, JC, JM and TM designed the study. Author PN collected samples and performed laboratory analyses. Author TM provided analytical support and analyzed the collected data. Author PN performed literature search and wrote the first draft of the manuscript. All authors revised and approved the final manuscript.

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ABSTRACT

Chemical composition and essential oil contents among essential oil-bearing plants are mostly influenced by different factors including ecological features of habitat. In this study, variation in yield and chemical composition of essential oils (EOs) from the leaves of *Cupressus lusitanica* Mill. (Cupressaceae) in different regions of Rwanda was investigated. Extraction of essential oils from fresh leaves of *C. lusitanica* collected in March 2021 and April, 2021 from three different ecological zones of Rwanda, Buberuka highland zone (Burera), Central plateau zone (Huye) and Eastern

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savannah zone (Kayonza) was realized through steam distillation. The chemical compositions of distilled EOs were analyzed using both Fourier transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS), while their yields were determined by simple calculus. The average yields of the EOs were 0.27 ± 0.02, 0.34 ± 0.02 and 0.39 ± 0.01% (v/w) for Burera, Huye and Kayonza, respectively. Results of FT-IR analysis were confirmed by those of GC-MS analysis, and indicated the presence of different groups of compounds including aliphatic alkanes, carboxylic acids, alkenes, aldehydes, aromatics and ketones in the EOs. GC-MS results revealed that sabinene (20.84%), myrcene (19.63%), α-pinene (10.23%) and δ-3-carene (10.13%) were the dominant chemical constituents for EOs of *C. lusitanica* from Burera. Umbellulone (24.21%), δ-3-carene (16.76%), sabinene (10.54%) and α-pinene (8.21%) were the main constituents for EOs of *C. lusitanica* from Huye, while γ-terpinene (18.77%), umbellulone (18.16%), isobornyl acetate (9.972%), and myrcene (7.20%) were the major components of EOs of *C. lusitanica* from Kayonza. The current results demonstrated an intraspecific variation in content and chemical profile of *C. lusitanica* EOs from one geographical region to another. The observed variations are mostly due to the interactions of *C. lusitanica* species with climatic and environmental conditions of ecological habitat. However, it could also be the effects of various biotic factors, as well as maturity of plant and stage of plant growth. Further studies are needed to establish the influence of different geo-climatic and environmental factors on each single major component of *C. lusitanica* EOs.

Keywords: Steam distillation; Cupressus lusitanica; essential oils; yield variation; chemical composition; ecological zone.

1. INTRODUCTION

Global diversity of vegetation is noticeably influenced by different factors including climatic and ecological conditions of habitat through alteration of the life cycle, phytochemical composition and distribution of plant species, as well as development of new physical traits [1,2]. Such changes are often seen as forcible adaptations of plant ecosystem against biotic and abiotic factors in natural habitat [2,3]. As abiotic factors influence, type of soil and topography play a significant role in the variability of environments, leading to the physiognomic distinction of plant species [4,5].

Medicinal plants are continuously showing benefits and potential applications through a large variety of their secondary metabolites; among them, essential oils [6,7]. On the other hand, majority of inhabitants in Sub-Saharan Africa depend on plants as sources of their primary health care on account of their properties of inhibiting growth of microbes, socio-cultural acceptability and curative effects against different diseases [6,8].

Essential oils (EOs) are widely distributed in the plant kingdom, but only aromatic plants contain extractable amounts, and they are accumulated in all parts of the plant with most amounts being less than 1% and rarely reach 15% of yield [9]. Like other plant secondary metabolites, EOs have multiple ecological functions including mediation of plant competition via allelopathic effects on other plant species, and signaling processes such as attraction of beneficial insects for pollination and seed dispersal [1,10]. Some plants produce very concentrated EOs of unpleasant odours to repel plant enemies like parasites, herbivores and pests [11]. Thus, they play a great role for plant self-defense against biotic and abiotic stresses [1,6,12].

The synthesis and accumulation of EOs among essential oil-bearing plants are often influenced by different abiotic factors such as light, altitude, temperature, soil properties, precipitation (water availability) and biotic factors like plant parasites and pests, genetics, maturity and stage of growth [13]. Some factors alter or inhibit the metabolic pathways for production of some phytochemicals, which lead to biosynthesis of different compounds [14]; this may confirm that there is a relationship between the phytochemistry of the plant species and their ecological environment [2].

The higher sensitivity of some essential oil constituents to climatic variations and environmental stresses is related to qualitative variation of EOs among aromatic plants [13, 15, 16]. Previous studies have revealed that several plants of arid regions increase their phenolic contents and decrease their protein and carbon metabolites as an adaptive strategy to droughts

[17]. In other words, the plants synthesize a wide range of active metabolites that are involved in their adaptation to certain habitats [18].

The genus *Cupressus* is a part of cupressaceae family, which comprises about 30 genera and 130 species of evergreen coniferous trees. *Cupressus lusitanica* is a coniferous tree in Spermatophya phylum, native to Mexico and Central America [19, 20].The morphological characteristics of *C. lusitanica* are more distinguishable; it has rough sub-cylindrical branchlets aligned along a single plane, and their transverse section is retrogonal. The leaves are green while cones are sub-ovulate with six to ten scales [21]. The general ecological features favorable for *C. lusitanica* species include moist climate and altitude variation of 1,000 to 4,000 m above sea level, with average annual rainfall range from 800 to 4,000 mm and mean annual temperature of 12 to 30 ^{o}C [21].

The wildly grown *C. lusitanica* is prone to fluctuation of environmental factors, but it is very adaptive to deep, drained and moist soil with neutral to little acidic property, and it can also tolerate the short dry season and drought. Though, it cannot withstand waterlogging [21, 22].

Cupressus lusitanica has become the major plantation in Eastern Africa and it has economic importance like timber and fire wood production, and wind breaking [23]. *C. lusitanica* leaves are traditionally used to fend off insect pests from stored products, and as flies repellent in rural areas [24– 27], while its EOs are good for treatment of cough and rheumatism, and it is also important in making fresheners and various cosmetics like deodorants, perfumes and soaps [23,28]. Moreover, the EOs from different Cupressaceae species were previously reported to demonstrate moderate toxicity against mosquitoes [29].

The temperate climate of Rwanda is very favorable for growth of *C. lusitanica* where it is commonly seen on the hedges of many homes, and it is also a part of major plantations in the country [21,30].

The geographic location and connected environmental and climatic features affect the total content and chemical profile of plant's essential oil [13,31]. Therefore, *C. lusitanica* species in different ecological habitats could normally produce unequal amount of EOs of different chemical profiles and bioactivities, which may affect their crucial uses. So, the determination of individual or population's chemical features and oil contents is very helpful to select the population with distinct bioactive constituents and to fully utilize the therapeutic, pharmacological and other properties owned by this species.

The main goal of this study was to investigate the intraspecific variations of the yield and chemical constituents of EOs from fresh leaves of *C. lusitanica* growing in different agroecological zones of Rwanda. To the best of our knowledge, no similar scientific work was reported in open literature on this species growing in different agro-ecological zones of Rwanda.

2. MATERIALS AND METHODS

2.1 Brief Description of the Study Area

Rwanda is a hilly and mountainous country, geographically located in central Africa (Fig. 1) between 1° 04' and 2°51' latitude South, 28° 45' and 31° 15' longitude East with 26,338 km^2 of surface area and altitude variation of 900 to 4,507 m above sea level [32–35]. The six major agro-ecological zones of Rwanda (Fig. 1) are grouped into three altitudinal regions [36] ; The North-West of Rwanda is a part of highlands region occupied by Congo-Nile crest, Buberuka highland and volcanic highland zones, with more than 2,000 m above sea level, while the East is part of lowlands region, which is relatively flat with altitudes below 1,500 m and it consists of Eastern Savannah and Eastern plateau zones. The central plateau is part of midlands that consist of wavy hills with altitude of 1,500 to 1,900 m above sea level [37]. Therefore, such topographic pattern is responsible for the moderate and cool climate of the country, with the annual average temperature and precipitation of 20 ^{o}C and 1,250 mm, respectively [36– 41].

The current study was based on three sampling sites within each of the three agro-ecological zones (Fig. 1). Table1. is showing the geo-climatic features and geographical coordinates of the study-based regions.

2.2 Sample Collection

Fresh leaves of mature *C. lusitanica* plants (5 kg each) were manually collected from three selected agro-ecological zones of Rwanda

Table 1. Climatic and geographical information of study-based habitats of *C. lusitanica* populations

Region	AEZ	Altitude (m)	Temperature (^{o}C)	Rainfall (mm/year)	Soil type	Sampling sites	Latitude (S)	Longitude (E)
Highlands	Buberuka highland	1900-2300	< 18	1000-1500	Laterite soil	Burera / Gahunga	1°29'13.8" 1°27'10.2" 1°29'19.9"	29°40'44.7" 29°41'53.7" 29°39'44.3"
Midlands	Central plateau	1500-1900	18-20	1200-1300	Humiferous, loamy soil from granite and gneissic	Huye/ Ngoma	2°35'30.3" 2°35'30.3" 2°37'11.3"	29°43'53.6" 29°43'59.3" 29°44'30.4"
Lowlands	Eastern savannah	1200-1400	> 21	800-1000	Sandy, weathered soil	Kayonza/ Gahini	1°51'29.3" 1°51'26.2" 1°51'26.6"	30°29'23.7" 30°29'22.8" 30°29'18.8"

AEZ, agro-ecological zone: Secondary data adapted from Verdoodt & Ranst [36], Iiyama et al.[39], Ocimati et al.[41], Uwizeyimana et al.[42].

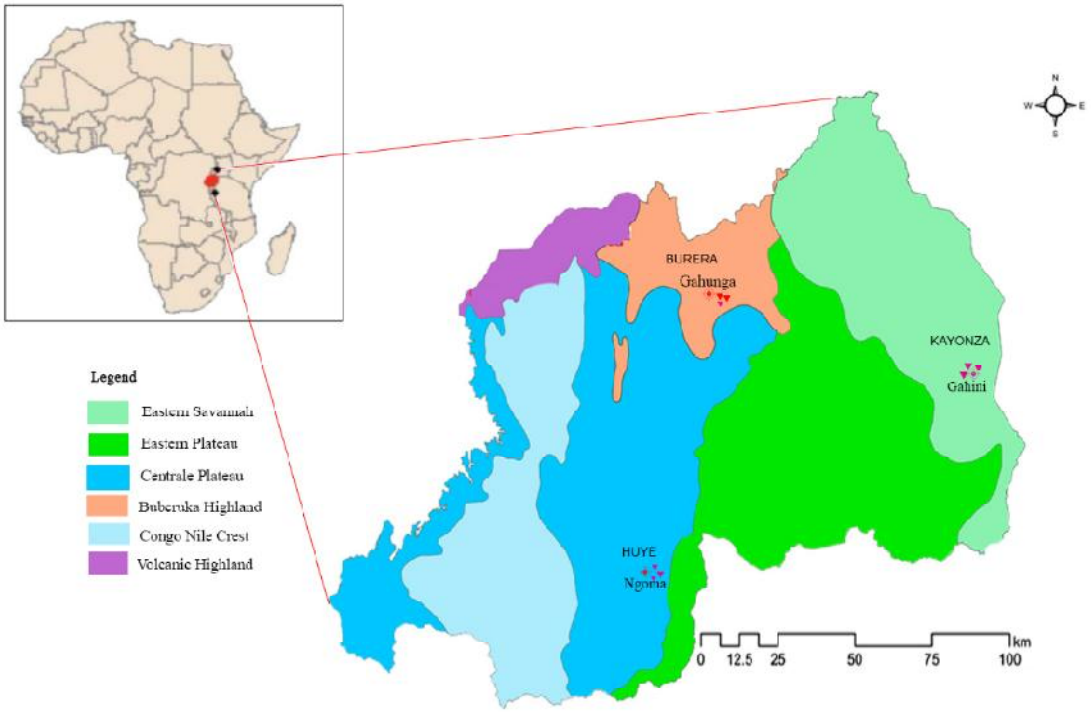


Fig. 1. Map showing six major Agro-Ecological Zones of Rwanda with study sites (adapted from Mukuralinda et al.[40]

between March 2021 and April 2021. Three locations from each zone were sampled and their geographic coordinates are indicated in Table 1. Botanical identification of the plant species was carried out by a botanist and the voucher specimens (No. 14427/001, 14427/002, 14427/003) were deposited at the National herbarium of Rwanda, Huye district, Rwanda. The laboratory samples were packaged in polyethylene bags and then transported to the Chemistry Laboratory, College of Science and Technology, University of Rwanda, Kigali (Rwanda) where they were stored in refrigerator at 4°C until extraction on the next day.

2.3 Extraction of Essential Oils

From each zone, a total mass of 2.40 kg of fresh leaves of *C. lusitanica* was separately subjected to steam distillation in four replicates for 3 hours. Following the procedural steps described by Campolo et al.[11] and Ahmet [43]: The weighed 600 g (composite sample made of leaves from sampled locations in each zone) of fresh leaves of *C. lusitanica* were packed into biomass flask (2,000 mL) connected to boiling flask (2,000 mL) contained around 1,750 mL of boiling water and allow the steam to pass through the plant samples for 3 hours. The distilled essential oils were dried over anhydrous sodium sulfate and the total EO amount of 9.30, 8.10 and 6.50 mL for Kayonza, Huye and Burera, respectively were kept in tightly closed amber glass vials at 4°C for analysis. The percentage yields of EOs were then calculated using equation (Eq.1).

$$Yield = \frac{m_1}{m_0} \times 100 \qquad \text{(Eq.1)}$$

with m_0 and m_1, the mass of fresh leaves packed in biomass flask (g) and volume of extracted essential oil (mL), respectively.

2.4 Fourier Transform Infrared Spectroscopy of the Essential Oils

The FT-IR analysis of EOs was done using a FT-IR spectrometer (Bruker Alpha II, 111311, Germany) equipped with a Diamond Crystal ATR (Attenuated Total Internal Reflectance) accessory. The FT-IR spectra of essential oil were recorded in the spectral range of 4000 to 400 cm^{-1} with the scanning resolution set to 2.0 cm^{-1} for 24 scans on each essential oil sample. The analysis was repeated twice for confirmation of spectra.

The liquid sample (2 drops ~ 0.1 mL) of essential oil was put on diamond crystal plate and allowed the infrared beams to pass through the essential oil sample. Then, FT-IR spectra (Fig. 3) were generated. The functional groups of compounds present in the essential oil were determined by comparing the wavenumbers of essential oil spectra with those on an IR correlation chart and the previous studies [44–46].

2.5 Identification of Chemical Components by Gas chromatogram-phy/mass spectroscopy

Gas chromatography/mass spectroscopy (GC-MS) analysis of *C. lusitanica* EO was performed using a Hewett-Packard GC (Agilent 8890A) with Agilent 5977 mass selective detector equipped with a HP-5 MS ultra-inert column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) and a mass system with ionization energy of 70 ev. Helium was the carrier gas at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 250 □C and 280 □C, respectively. The oven temperature was programmed from 110 □C with an increase of 10 □C/min to 200 □C, and finally to 280°C at 5°C/min. Diluted samples (1:100 v/v in hexane) of 1.0 µl were injected manually in the split-less mode. The components were identified by comparing their relative retention times and mass spectra with those of standards library (NIST 11) and installed Mass Hunter Software, as well as the data reported in literature. Results were further confirmed by comparing the elution order of the compounds with their relative retention indices on non-polar phases.

2.6 Statistical Analysis

The yields of EOs were expressed as mean values ± standard error of replicates using one-way analysis of variance (ANOVA). Significant differences between mean values were established through Tukey's honest significant difference (HSD) test. All analyses were performed at 95% confidence interval using Minitab statistical software (Release 17, Minitab Inc., USA).

3. RESULTS AND DISCUSSION

3.1 Percentage yield of essential oils from *C. lusitanica* leaves

The fresh leaves of *C. lusitanica* collected from Kayonza (lowlands) had the highest average yield of EOs (0.39 ± 0.01%, v/w), followed by leaves collected from Huye (midlands) and Burera (highlands) which yielded 0.34 ± 0.02 and 0.27 ± 0.02 % (v/w), respectively (Fig. 2).

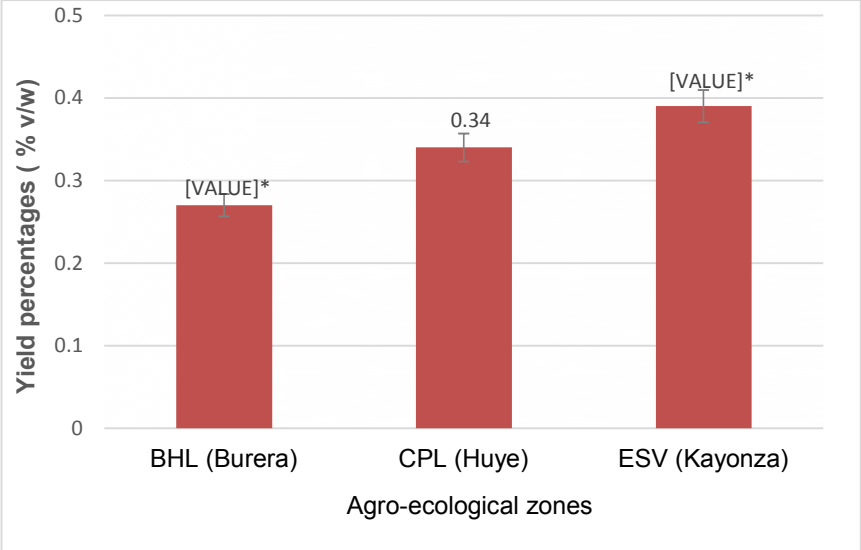


Fig. 2. Comparison of yield percentages (%) of *Cupressus lusitanica* essential oil from agro-ecological zones of Rwanda

BHL: Buberuka Highland, CPL: Central Plateau, ESV: Eastern Savannah. Mean values ± standard error of four replicates; mean values followed by (*) are significantly different (Tukey HSD test at 95% CI, Minitab 17).

The average yield of EOs of *C. lusitanica* leaves from Burera was significantly lower than that from Kayonza (*P* = .02). However, it showed no statistical difference to the yield of EOs from Huye (*P* = .13). Similarly, the average yield of EOs from Huye was also lower than that from Kayonza but the two were not significantly different (*P* = .27).

The yield percentages of *C. lusitanica* EOs in the current study were a little higher than the yields of 0.35 and 0.125% obtained for hydrodistilled EOs from fresh leaves of *C. lusitanica* in Kenya as reported by Bett et al.[47], and Kimutai & Mainya [23]. Hassanzadeh et al.[26] also reported a yield ranging from 0.28 to 0.58 % in Costa Rica, while by hydro-distillation, Kuiate et al.[20] reported a yield of 0.33% for *C. lusitanica* fresh leaves from Cameroon. The higher yields of EOs obtained in the current study could therefore be attributed to the extraction method used (no data were previously reported on use of steam distillation method for EOs extraction from *C. lusitanica species*) or the use of fresh leaves rather than dried ones [25,47]. In addition, the synthesis and accumulation of EOs are also affected by other factors, which are linked to the physiology of the plant species itself or variability in characteristics and components of plant habitat [12,48].

The production of EOs in numerous aromatic plants was demonstrated to increase under water-stress conditions [49]. Different authors reported the increase of monoterpene amounts as a response to drought, water stress and hot climate, that enable many aromatic plants under stressed conditions to lower their photosynthetic activities and significantly reduce the emission of their terpene compounds, which results in their accumulation within secretory glands [50, 51]. The statement was also supported by findings of El Hamrouni-Aschi et al. [52] who reported the higher yields of EOs from *cupressus simpervirens* in semi-arid regions of Tunisia. In contrast, some authors reported different statement, regarding reduction of essential oil production due to the water stress for different plant like *S. officinalis* [53]*, Mexican oregano* [12], and *Achillea eriophora* [54].

3.2 FT-IR analysis results of *C. lusitanica* essential oil

The results of FT-IR analysis of the EOs showed almost similar spectra for all EOs samples with significant peaks at around 2923-2933 cm^{-1}, 1710-1723 cm^{-1}, around 1450 cm^{-1}, 1370-1373 cm^{-1} and 875-878 cm^{-1} (Fig. 3).

According to the IR guide of Bruker optics (Germany), the significant peak on FT-IR spectra of *C. lusitanica* essential oil around absorption band of 3000-2850 cm^{-1}, is attributed to the presence of asymmetrical and symmetrical C-H stretches in CH_3, and CH_2 for alkanes, like aliphatic group of terpenes, whereas the peaks located around 1725-1700 cm^{-1} indicated the presence of carbonyl group (C=O) for carboxylic acids, 1720-1705 cm^{-1} (C=O) for saturated ketones, and 1720-1740 cm^{-1} (C=O) for saturated aldehydes.

Other significant peaks were located at 1450 cm^{-1} for C-OH stretch for tertiary alcohol, 1375-1370 cm^{-1} for –C-O-CH_3 (alkyl substituted ether). The vibrational frequency at ~1190 cm^{-1} confirmed the presence of -CH_2- stretch (methylene-cyclohexane ring vibration) [55]. The peaks around 900-800 cm^{-1} are attributed to the vibrations of out-of-plane bending patterns of aromatic rings and alkenes such as monocyclic and bicyclic terpenes, whereas the absorption bands at 1166 and 1111 cm^{-1} suggested the presence of terpenes with tertiary and secondary alcoholic functions [45, 56].

3.3 GC-MS results of essential oil from *C. lusitanica* leaves

The GC-MS analysis led to the identification and quantification of 37, 36 and 30 major compounds corresponding to 97.47%, 96.65% and 97.44% of the EOs of *C. lusitanica* leaves from Burera, Huye and Kayonza, respectively. Table 2 represents the major compounds of EOs and their relative abundances represented by chromatogram peaks (Fig. 5).

The EOs of *C. lusitanica* leaves from Burera was dominated by hydrocarbons and oxygenated monoterpenes at 80.06% and 16.16%, respectively. The major monoterpene hydrocarbons found were Sabinene (20.84%), Myrcene (19.63%), α-Pinene (10.23%) and δ-3-Carene (10.13%), while the oxygenated monoterpenes were mainly Linalool (6.83%), Umbellulone (3.23%), and Camphene hydrate (1.38%). On the other hand, the total compositions of EOs of *C. lusitanica* leaves from Huye was dominated by monoterpene hydrocarbons (51.26%), with dominance of δ-3-Carene (16.76%), Sabinene (10.54%), α-Pinene (8.21%), and α-Terpinene (5.84%). However, the

major compound was found to be Umbellulone, an oxygenated monoterpene which constituted a total of 24.21%. Other major oxygen-containing compounds were Camphene hydrate (3.47%), α-Terpineol (3.18%), 1,8-Cineole (2.36%) and Linalool (2.16%). Contrastingly, γ-Terpinene (18.77%), Myrcene (7.20%), Limonene (5.53%), α-Pinene (5.24%) and δ-3-carene were dominant among monoterpene hydrocarbons that occupied about 51% of chemical compositions of EOs from *C. lusitanica* growing in Kayonza, while about 46% portion was occupied by oxygenated monoterpenes, with major compounds; Umbellulone (18.16%), Isobornyl acetate (9.72%), Linalool (8.71%) and Camphene hydrate (2.30%).

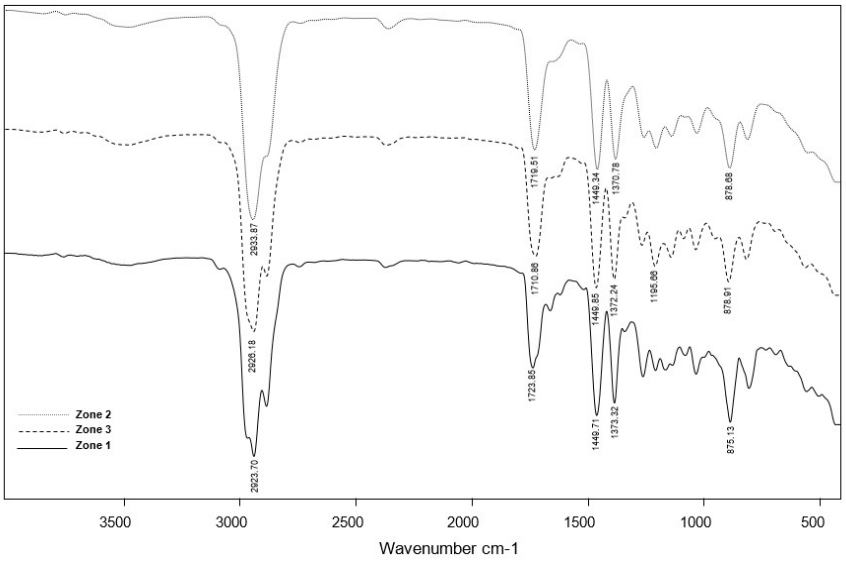


Fig. 3. FT-IR spectra of *C. lusitanica* EOs from studied ecological zones of Rwanda. *Zone 1, Burera (highlands); Zone 2, Huye (midlands); Zone 3, Kayonza (lowlands)*

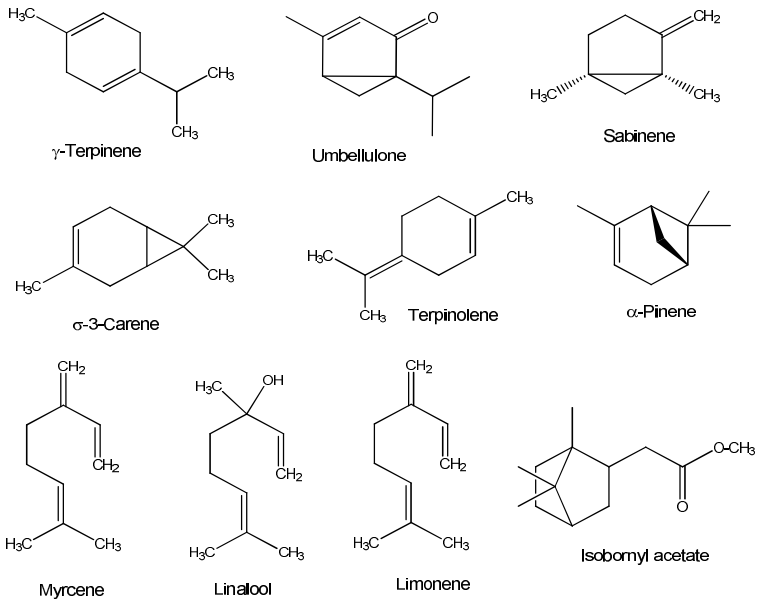


Fig. 4. Some of the major compounds identified in essential oils from leaves of *C. lusitanica* growing in Rwanda

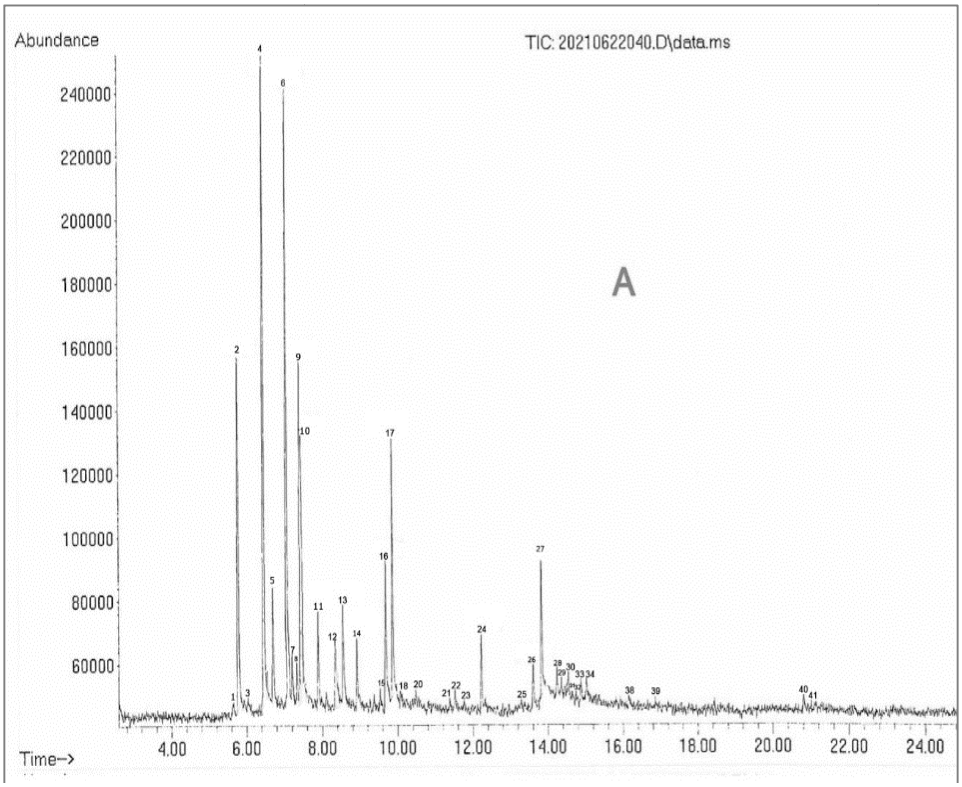
Table 2. Major chemical constituents of essential oils from leaves of *C. lusitanica* from different ecological zones of Rwanda

				Composition (%)		
Peak No	**Retention Time (min)**	**Retention Index**	**Compound**	**Burera (highland)**	**Huye (midlands)**	**Kayonza (lowlands)**
1	5.66	938	Thujene	0.12	tr	0.10
2	5.78	943	α-Pinene	**10.23**	**8.21**	**5.24**
3	5.93	949	Tricyclene	0.11	0.11	tr
4	6.46	969	Sabinene	**20.84**	**10.54**	**4.05**
5	6.71	978	β-Pinene	2.58	2.26	1.52
6	7.07	992	Myrcene	**19.63**	1.31	**7.20**
7	7.21	997	α-Phellandrene	1.06	0.73	0.66
8	7.33	1001	β-Phellandrene	1.04	0.39	0.41
9	7.42	1004	δ-3-Carene	**10.13**	**16.76**	3.13
10	7.45	1005	α-Terpinene	**6.72**	**5.84**	2.88
11	7.89	1017	*p*-Cymene	2.11	1.73	1.32
13	8.55	1036	(Z-), β-Ocimene	2.08	0.65	0.42
14	8.92	1047	Limonene	1.27	2.08	**5.53**
16	9.69	1069	γ-Terpinene	*3.14*	0.65	**18.77**
18	10.08	1080	Terpinolene	tr	-	tr
32	14.76	1198	δ-2-Carene	tr	-	tr
		Monoterpene hydrocarbons		*81.06*	*51.26*	*51.23*
12	8.38	1031	1,8 -Cineole	1.22	2.36	0.71
15	9.23	1056	Sabinene hydrate	0.14	0.26	-
17	9.87	1074	Linalool	**6.83**	2.10	**8.71**
19	10.23	1084	Linalool oxide	-	0.27	-
20	10.63	1090	2-Nonanone	0.11	-	-
21	11.38	1115	Camphor	tr	1.21	-
22	11.53	1118	α-Thujone	tr	0.39	-
23	11.83	1121	Borneol	tr	tr	tr
24	12.23	1135	Camphene hydrate	1.38	3.47	2.33
25	13.23	1160	*p*-Cymen-8-ol	tr	0.15	-
26	13.61	1169	Benzyl alcohol	1.07	2.33	2.13
27	13.84	1175	Umbellulone	3.23	**24.21**	**18.16**
28	14.24	1185	Terpinen-4-ol	0.53	2.08	1.50
29	14.35	1188	p-menth-2-en-1-ol	0.47	tr	-
30	14.50	1191	α-Terpineol	0.32	3.18	0.38
31	14.55	1192	*cis*-Carveol	tr	tr	-
33	14.93	1202	γ-Terpinen-7-al	0.43	0.52	0.50
34	15.02	1204	Verbenone	0.43	1.71	0.54
35	15.24	1209	Peperitol	-	0.12	-
36	15.69	1220	Eucarvone	-	-	0.81
37	15.97	1226	Isobornyl acetate	tr	-	9.72
38	16.16	1231	Peperitone	tr	0.92	0.57
41	21.02	1344	α-Terpinyl acetate	tr	-	-
		Oxygenated Monoterpenes		*16.16*	*45.28*	*46.06*
39	16.83	1247	α-Cubebene	0.12	-	-
40	20.80	1341	β-Cedrene	0.13	tr	0.15
42	21.89	1367	β- Elemene	-	0.11	-
		Sesquiterpene hydrocarbons		*0.25*	*0.11*	*0.15*
		Total identified compounds		*97.47%*	*96.65%*	*97.44%*

tr, trace < 0.10%; (-) not detected; retention index calculated from retention times in relation to the series n-alkanes on a HP-5 MSUI capillary column. Compounds are listed in elution order, and the % composition in bold represents Major compounds.

Different reports have often pointed out umbellulone, α-pinene, germacrene-D, limonene and terpinen-4-ol as the major compounds in the EOs of *C. lusitanica* growing in different regions of the world [20,26,27,57,58]. However, the amount concentrations of components vary from one region to another due to the influence of many factors, including harvest season, climate, soil type, age of the plants and the extraction method [35,59,60]. For example, Bett et al.[58] reported the dominance of oxygenated monoterpenes in the leaf EOs of *C. lusitanica* growing in Kenya with umbellulone (18.38%), α-pinene (9.97%), sabinene (8.16%) and limonene (7.91%) as major compounds. Almost similar results were reported by Kuiate et al. [20] for EOs from *C. lusitanica* leaves in Cameroon with dominance of umbellulone (18.30%), germacrene-D (8.20%), α-pinene (7.40%), *epi*-zonarene (5.0%), limonene (3.5%) and terpinen-4-ol (2.6%). However, the oil was dominated by sesquiterpenes (34.70%) followed by oxygenated monoterpenes (28.0%). Different findings were however reported in Cameroon with the dominance of sesquiterpenes like germacrene-D (18.5%), *epi*-zonarene (8.2%), *cis*-calamenene (8.2%), and oxygenated monoterpenes like terpinen-4-ol (6.30%), linalool (6.0%) and umbellulone with 6.0% [27]. In contrast to the foregoing findings from Cameroon [20, 27], Kenya [58] and Costa Rica [26], the EOs of *C. lusitanica* growing in Brazil was reported to contain β-pinene, and β-(Z)-ocimene as major monoterpenes and oxygenated monoterpenes like *endo*-fenchol, whereas the main sesquiterpenes were α-acoradiene, α-amorphene, thujopsan-2α-ol and 7α-*epi*-selinene [57]. A strong justification for this variation could not be only related to different climatic and edaphic conditions across different regions, which directly influence the metabolism of the plants, but also due to exposure to different biotic components and age of plants from which the leaves were harvested [57,61].

The current chemical compositions study of EOs of *C. lusitanica* from Rwanda indicated similar results with those from previous reports. However, in spite of their previous reported presence [20, 26, 27, 47], Germacrene-D and some sesquiterpenes and their oxidative compounds including *epi*-zonarene, *cis*-calamenene, amorphene, *endo*-fenchol and thujopsan-2α-ol, were not detected in all EOs from *C. lusitanica* leaves from the studied regions.



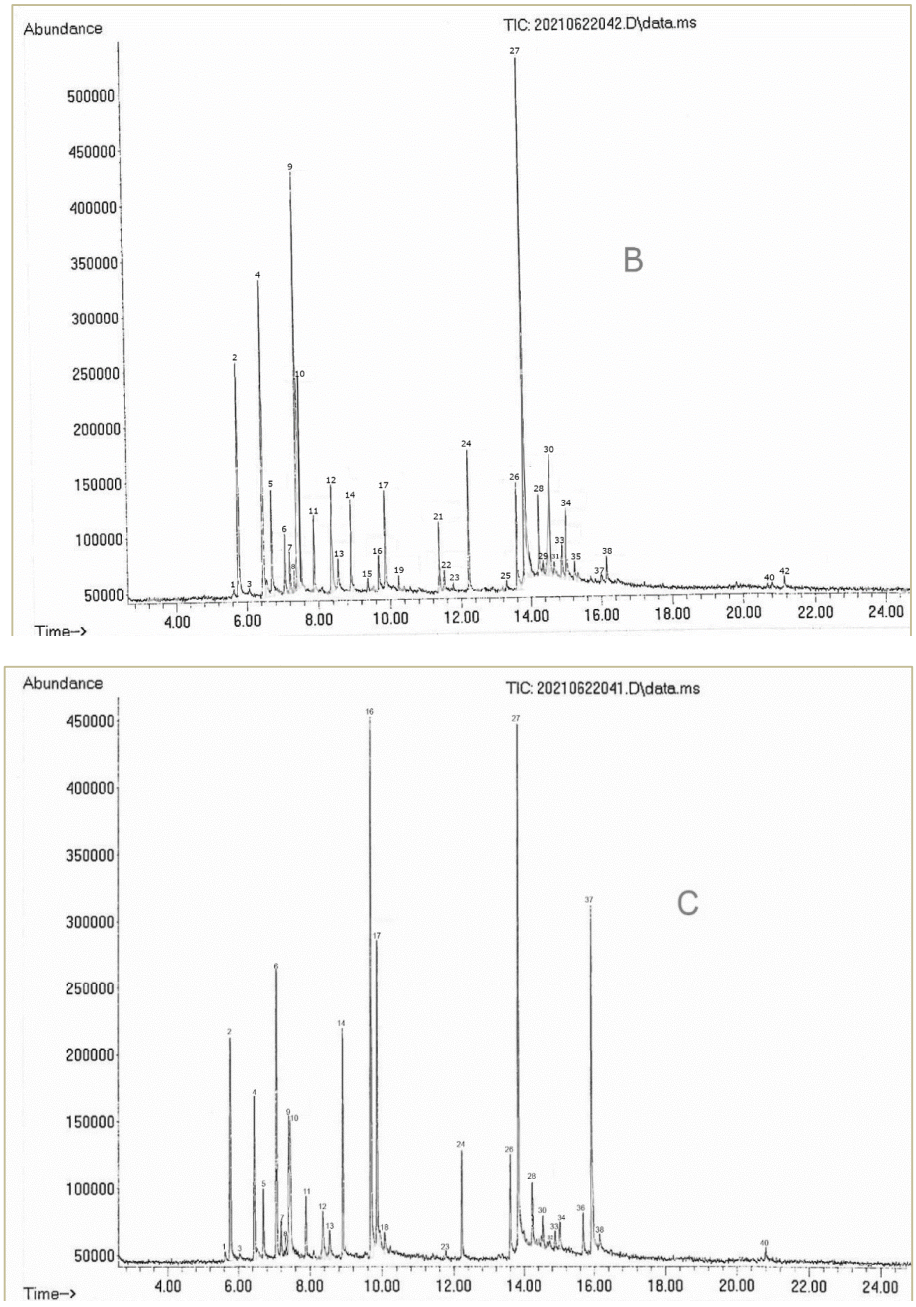


Fig. 5. GC-MS chromatogram of EO from *C. lusitanica* leaves collected from (A), Burera;(B), Huye and (C), Kayonza

4. CONCLUSION

Results of the current study showed that there is an intraspecific variation in the content and chemical profile of EOs from leaves of *C. lusitanica* growing in different geographical regions of Rwanda. A positive correlation between essential oil yield and temperature was proven by higher yield obtained from the lower altitude region of Kayonza in Eastern Savannah (semi-arid region) characterized by high annual temperature whereas the least yield was observed for leaves from Burera in the highlands region characterized by cooler climate and lower annual temperature.

The GC-MS results demonstrated that, the EOs of *C. lusitanica* leaves from Kayonza was very rich in γ-Terpinene, umbellulone, Isobornyl acetate and Linalool, whereas umbellulone, δ-3-Carene, sabinene and α-Pinene were dominant in essential oil of *C. lusitanica* from Huye. These compositions were different from that found in the oil of *C. lusitanica* from Burera, which was dominated by Sabinene, Myrcene, α-pinene and δ-3-Carene.

The observed variations in yields and chemical profile of *C. lusitanica* essential oil from different regions of Rwanda are mostly due to the interactions of this species with climatic and environmental conditions of ecological habitat. However, it could also be the effects of various biotic factors like competing plant species, parasites and fungi. Moreover, the maturity and stage of plant growth could also be the source of essential oil variation among plant species. Further studies are needed to determine the influence of different climatic and environmental factors on the essential oil synthesis and the effects of such factors on each single main component of *C. lusitanica* EOs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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