The essential oil of Mentha viridis L, chemical characterization and the relationship with its biological activities

Walter Francisco Quezada-Morenoa*, Walter David Quezada-Torres[b], Moraima Cristina Mera-Aguas[c], Reina Medina-Litardo[a]; Marcia Proaño-Molina[d]

[a]Facultad de Ciencias Agrarias- Grupo Agrotecnología sostenible-Universidad de Guayaquil

[b]Facultad de Estudios de Postgrado e Investigación-Universidad Tecnológica Empresarial de Guayaquil

[c]Escuela de Ciencias Agrícolas y Ambientales-Pontificia Universidad Católica Ecuador-Sede Ibarra

[d]Universidad de las Américas (UDLA), Facultad de Posgrados. Ecuador.

El aceite esencial de Mentha viridis L,
caracterización química y relación con sus actividades biológicas

L'oli essencial de Mentha viridis L,
caracterització química i relació amb les seves activitats biològiques

RECEIVED: 25 JUNE 2021; REVISED: 27 SEPTEMBER 2021; ACCEPTED: 30 SEPTEMBER 2021

ABSTRACT

The objective of the study was to determine the yield and quality of the essential oil of *Mentha viridis L.*, obtained by water-vapor distillation using cohobation. The oil extraction process required an experimental design DCA of 3^3 with the application of factors such as sample moisture, water volume and extraction time in three levels (high, medium and low). These factors, evaluated by the oil yield response variable, were analyzed using the statistical software statgraphics plus. For the oil components, a gas chromatograph coupled to a mass detector (GC-MS) was used. Extraction results show significance to the oil yield applied to dehydrated leaves, extraction times and amount of water at medium and high levels. The volatile chemical composition of the sample essential oil revealed the presence of 34 components, highlighting DL-carvone with 36.5%, anethole with 15.3%, germacrene D with 8.8% and limonene with 7.2%; Consequently, it is concluded that these components provide aromatic and antioxidant characteristics for functional fragrances to be used in pharmaceutical, cosmetic and food industries. Due to its biological activity, the essential oil is also recommended for the agricultural sector as a bactericidal and insecticidal inhibitor, with prospects for research and immediate application.

Keywords: Peppermint, Oil extraction, Yield, Chemical composition

RESUMEN:

El objetivo del estudio fue determinar el rendimiento y la calidad del aceite esencial de Mentha viridis L., obtenido por destilación con vapor de agua mediante cohobación. El proceso de extracción de aceite requirió un diseño experimental DCA de 33 con la aplicación de factores como humedad de muestra, volumen de agua y tiempo de extracción en tres niveles (alto, medio y bajo). Estos factores, evaluados por la variable respuesta rendimiento de aceite, fueron analizados mediante el software estadístico statgraphics plus. Para los componentes del aceite se utilizó un cromatógrafo de gases acoplado a un detector de masas (GC-MS). Los resultados de la extracción muestran importancia en el rendimiento de aceite aplicado a las hojas deshidratadas, los tiempos de extracción y la cantidad de agua a niveles medios y altos. La composición química volátil del aceite esencial de muestra reveló la presencia de 34 componentes, destacándose DL-carvona con 36,5%, anetol con 15,3%, germacreno D con 8,8% y limoneno con 7,2%; En consecuencia, se concluye que estos componentes aportan características aromáticas y antioxidantes a fragancias funcionales para ser utilizadas en la industria farmacéutica, cosmética y alimentaria. Por su actividad biológica, el aceite esencial también es recomendado para el sector agrícola como inhibidor bactericida e insecticida, con perspectivas de investigación y aplicación inmediata.

Palabras clave: Menta, extracción de aceite, rendimiento, composición química

***Corresponding author: mfrancisco473@gmail.com**

RESUM:

L'objectiu de l'estudi va ser determinar el rendiment i la qualitat de l'oli essencial de Mentha viridis L., obtingut per destil·lació al vapor d'aigua mitjançant cohobació. El procés d'extracció d'oli va requerir un disseny experimental DCA de 33 amb l'aplicació de factors com la humitat de la mostra, el volum d'aigua i el temps d'extracció en tres nivells (alt, mitjà i baix). Aquests factors, avaluats per la variable de resposta del rendiment del petroli, es van analitzar mitjançant el programari estadístic statgraphics plus. Per als components del petroli, es va utilitzar un cromatògraf de gasos acoblat a un detector de masses (GC-MS). Els resultats de l'extracció mostren la importància del rendiment d'oli aplicat a les fulles deshidratades, els temps d'extracció i la quantitat d'aigua a nivells mitjans i alts. La composició química volàtil de l'oli essencial de la mostra va revelar la presència de 34 components, destacant la DL-carvona amb un 36,5%, l'anetol amb un 15,3%, el germacre D amb un 8,8% i el limonè amb un 7,2%; En conseqüència, es conclou que aquests components proporcionen característiques aromàtiques i antioxidants per a fragàncies funcionals per ser utilitzades en la indústria farmacèutica, cosmètica i alimentària. Per la seva activitat biològica, l'oli essencial també es recomana per al sector agrícola com a inhibidor bactericida i insecticida, amb perspectives d'investigació i aplicació immediata.

Paraules clau: Menta, extracció d'oli, rendiment, composició química

1. INTRODUCTION

Essential oils are mixtures of components whose structure includes terpenes (C_5H_8), various oxygenated organic compounds and phenolic compounds. They are fluid or thick in appearance, volatile, and have a characteristic smell and color, depending on the plant species. Essential oils are aromatic liquids obtained from different parts of the plant and widely used in the food, cosmetic and pharmaceutical industries[1]. They are products that result from the secondary metabolism of plants that are made up of a number of volatile chemical components, mainly terpenoids, as well as aromatic and aliphatic compounds that provide characteristics such as smell, taste and biological activity[2].

Essential oils are compounds that contain terpenes, which together or not with other components, generate the smell of the plant[3], they are insoluble in water and soluble in fats, nonpolar organic solvents (they lack positive and negative pole in its molecules) and alcohols. Volatile oils, which are responsible for the aroma, are distillable by steam entrainment.

Essential oils are not pure substances, but a mixture of volatile organic components found in aromatic plants. The essential oil producing plants mainly belong to the families Apiaceae, Lauraceae, Myristicaceae, Lamiaceae, Asteraceae, Myrtaceae, Rosaceae, Piperaceae, Verbenaceae and Rutaceae[4].

The genus Mentha comprises about 25 species and several hybrids from Europe, Asia, North America, and Africa[5]. From these species only one is officially registered in Venezuela (Mentha spicata L.) hierba buena[6], Hierbabuena / yerbabuena *Menta viridis L.* [7], known as green mint. The genus *Mentha* includes in this study 5 foreign species with medicinal applications in Colombia, among the most mentioned is yerbabuena (*Mentha piperita*)[8]. Some *Mentha* species are used in Ecuador in traditional medicine, in infusions for their stimulating and digestive properties. Hierbabuena, menta romana, spearmint (engl.), menthe verte (engl.), hortelã-comum (port.), hortelã-preta (port.), hortelã-verde (engl.) *Mentha spicata* L. (*Lamiaceae*), *Mentha viridis* (L.)[9], are all aromatic plants of industrial importance for the extraction of essential oils, extracts and biocides.

Given the importance of the genus *Mentha* in the production of essential oils (monoterpenes) and especially components such as menthol, menthone and carvone, they constitute an important sustainable economic resource to be used in industries for the preparation of insecticides, antiseptics, medicines, sweets, jams, liqueurs and / or perfumes[5]. Natural essential oils are found in products used in daily life such as: drinks, perfumes, toiletries, cosmetics, food, paints, varnishes, plastics, pharmaceuticals[10], as stimulants, digestives and antispasmodics in traditional medicine. Essential oils have different biological activities and several of these secondary plant metabolites exhibit marked antimicrobial effects that have made their use as antiseptic and / or preservative in food, well known since ancient times[11]. Essential oils are bioactive compounds with antifungal, antibacterial and antioxidant properties[12].

The extraction of essential oil by steam entrainment consists of applying the steam stream directly on the material to be distilled, at a suitable pressure, a temperature close to 100 ° C. The plant sample, generally fresh, is enclosed in an inert chamber and is subjected to a stream of superheated water vapor[13]. Then the steam drags the volatile fractions to condense and separate them in a container known as a "florentine glass," considering that organic components (essential oils) are insoluble in water and form two phases (soluble and insoluble), thus facilitating separation.

Steam extraction is carried out when steam is injected into the volatile component and other non-volatile components and this yields its latent heat to the components of the mixture to cause their vaporization. The extraction of essential oils by steam entrainment follows Dalton's law or partial pressure law.

According to Dalton's law of partial pressures, the total pressure by a mixture of gases is equal to the sum of the partial pressures of each of the constituent gases. This law establishes that the pressure of a mixture of gases that do not react chemically is equal to the sum of the partial pressures that each of them would exert if only one of them occupied the entire volume of the mixture, without changing the temperature[14]. At the moment of steam entrainment, the two pressures of both oil (Pa) and water (PH_2O) are added, which is equal to the total pressure (Pt) generated in the extractor ($Pt = Pa + PH_2O$)[15]. Consequently, in a process of extraction of essential oil by steam entrainment, Dalton's Law explains the behavior of the components in the aqueous phase where pressure, temperature, mass and quantity of water vapor are specially considered variables of study. For this reason, the principle of this Law becomes an alternative for optimizing traditional processes to achieve significant yields in the extraction of essential oils in rural sectors for sustainability purposes.

When distilling a mixture of two immiscible liquids, its boiling point will be the temperature at which the sum of the vapor pressures equals atmospheric pressure, where the temperature will be lower than the boiling point of the most volatile component. This causes the compound to decompose at or near its boiling temperature. In general, this technique is used when the compounds meet the conditions of being volatile, immiscible in water, provided that they operate at low vapor pressure and high boiling point.

The presence of the liquids in two phases throughout the extraction (organic and aqueous), makes each liquid behave as if the other were not present. That is, each liquid generates its own vapor pressure that will correspond to that of a pure liquid at a reference temperature. As the vapor is rich in the component more volatile than the original liquid, it is possible to drag the two components by vaporization for their condensation and separation, considering that the volatile component is insoluble in water.

The distillation as a technique allows the separation of liquid mixtures with different boiling points and its effectiveness depends on the difference between their boiling points (the greater the difference in the boiling point, the greater the efficiency in distillation), a fast and economical application method. especially in rural areas, which is vital for the development of the essential oils industry.

From the prior analysis, the scientific question posed in the research proposal was answered: what factors affect the yield in the extraction of essential oil using the vapor entrainment method? and what is, according to the scientific literature, the chemical component present in the highest percentage that allows to evaluate its biological potential?

Consequently, the study was aimed at determining the yield and quality of the essential oil of *Mentha Viridis L.*, obtained by water-steam distillation by cohobation and the assessment of its biological activity was supported by the scientific literature in respect to its use and applications.

It is important to indicate that at the country level the essential oil industry is incipient, the use of aromatic plants in beverages such as tea and in traditional medicine is widespread. In reference to its relationship with biological activity, there is no evidence of significant results from the use and application of essential oils; therefore, the need for studies in production, industrialization and application activities in different fields arises. They could constitute a new source of antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties. However, more research is required on the isolation of the main compounds[16]. In addition, an in vivo assay is necessary to confirm the antimicrobial activity of M. viridis[17].

MATERIALS AND METHODS

The study began with the analysis of the scientific information of the plant species *Mentha viridis L* and with the extraction of essential oils. The collection of the plant was carried out in climatic floors between 1600 to 2000 meters above sea level, difference altitude of 400 meters above sea level that does not affect the chemical composition, but does affect the concentration; however, the type of soil and other climatic conditions are significant. In the extraction by steam entrainment, the selective vaporization of the components was carried out, using a water-vapor distillation equipment with a capacity of 4 kilograms of stainless steel with "return of condensed liquid from the water (cohobation) to the container once the oil is released"[15,18,19], as shown in figure 1.

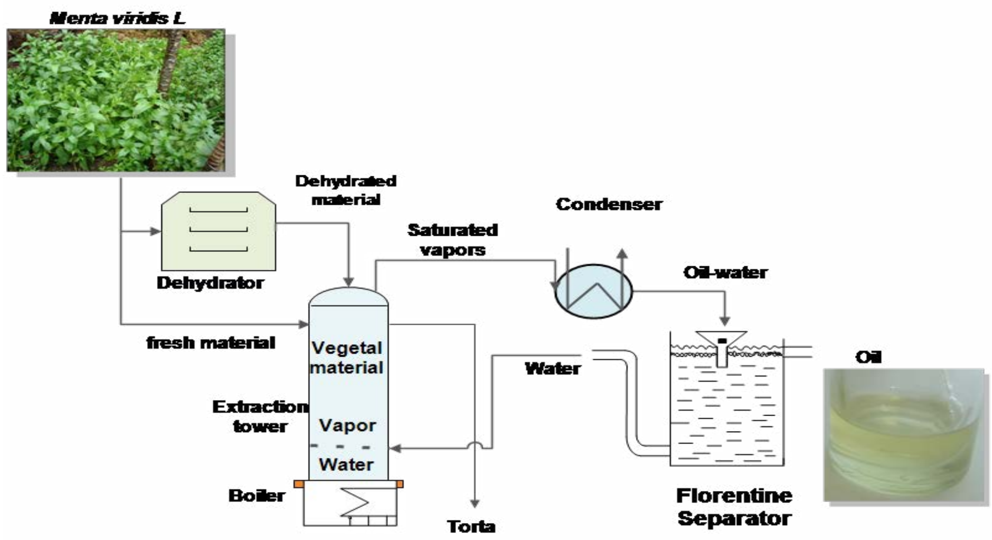

Figure 1. *Extraction process of essential oil from Mentha viridis L.*

A sample of *Mentha* plants was collected, with an initial fresh moisture of 80.56% and the material was dehydrated at a temperature of 50 ° C, using a tray dehydrator until moisture of 60 and 40% (± 2) in the material vegetable and for the control an infrared analyzer (Mettler-Toledo) with moisture sensor was used. The amount of water used was 3, 5 and 7 liters and the extraction time of 1.5, 2.5 and 3.5 hours; volume and time that were adjusted to operations in essential oil extraction processes by "steam entrainment that are between 1 to 3.5 hours"[15] and that are decisive in the yield of oil obtained.

For the extraction of essential oil, 2000 grams of plant material was used adjusted to the capacity of the equipment and to an experimental design 3^3 with two repetitions, according to three factors (moisture, water volume and extraction time) at three levels (high, medium and high) and a response variable, as shown in table 1.

The evaluation of the results of the extraction process was carried out using the statistical software Statgraphics plus 5.1; and, the quality analysis of the volatile fraction (highest yielding sample) was was carried out with a gas chromatograph coupled to a mass detector (GC-MS). The relationship of the essential oil components and their biological activity was supported by the scientific information analyzed regarding the benefits and applications.

Table 1. *Factors, levels and response variable in the oil extraction process*

Factors	Units	Levels			Response Variable
		Low	Medium	High	
Material moisture (X_1)	Percentage	**40**	**60**	**80,56**	Oil Volume (ml) Yield (%)
Water Volumen (X_2)	Liter	**3**	**5**	7	
Extraction time(X_3)	Hour	**1,5**	**2,5**	**3,5**	

DISCUSSION OF RESULTS

The analysis of the experiment with the statistical software Statgraphics plus 5.1 allowed to obtain the recommended regression in the extraction yield of the essential oil of the *Mentha* with three factors (moisture, volume and time) that were evaluated in three levels and two repetitions applying a multilevel factorial design. which consisted of 27 runs with completely randomized experiments. These results are shown in Table 2.

Extraction results showed oil yields averaging 0.22% and density 0.908 g / cm3. The moisture of the sample and extraction time influenced the amount of oil obtained. Comparable results of the yield with *Mentha spicata* of fresh leaves was 0.128%[20], 0.142% of leaves and 0.209% of leaves and stems[21] values and for Mint viridis of 0.8%[17].

Table 2. *Factors, levels and response variable of essential oil extraction process of Mentha*

Repetition	Material moisture	Water volume	Extraction time	Oil volume	Yield
	%	Liters	Hours	ml	%
1	1	-1	1	4,8	0,24
1	-1	-1	-1	2,4	0,12
1	0	0	-1	6,5	0,32
1	0	1	0	6,8	0,34
1	1	1	0	4,7	0,23
1	0	-1	0	5,6	0,28
1	0	0	1	6,8	0,34
1	-1	1	1	2,2	0,11
1	1	1	1	4,5	0,22
1	0	0	0	6,6	0,33
1	0	-1	-1	6,5	0,32
1	0	-1	1	6,8	0,34
1	1	0	-1	3,9	0,19
1	1	0	1	4,5	0,22
1	-1	0	-1	2,3	0,11
1	-1	1	0	2,6	0,13
1	-1	-1	1	2,4	0,12
1	1	-1	-1	3,8	0,19
1	0	1	-1	6,2	0,31
1	1	-1	0	4,8	0,24
1	1	1	-1	4,2	0,21
1	-1	-1	0	2,2	0,11
1	-1	0	0	2,2	0,11
1	-1	1	-1	2,5	0,12
1	-1	0	1	2,7	0,13
1	0	1	1	6,5	0,32
1	1	0	0	4	0,2
2	1	-1	1	4	0,2
2	-1	-1	-1	2,3	0,11
2	0	0	-1	5,5	0,27
2	0	1	0	6,9	0,34
2	1	1	0	4,5	0,22
2	0	-1	0	6,5	0,32
2	0	0	1	6,8	0,34
2	-1	1	1	2,4	0,12
2	1	1	1	4,5	0,22
2	0	0	0	6,7	0,33
2	0	-1	-1	6,1	0,3
2	0	-1	1	6,7	0,33
2	1	0	-1	3,6	0,18
2	1	0	1	4,1	0,2
2	-1	0	-1	2,3	0,11
2	-1	1	0	2,6	0,13
2	-1	-1	1	2,5	0,12
2	1	-1	-1	4,1	0,2
2	0	1	-1	5,8	0,29
2	1	-1	0	4,5	0,22
2	1	1	-1	4,2	0,21
2	-1	-1	0	2,2	0,11
2	-1	0	0	2,2	0,11
2	-1	1	-1	2	0,1
2	-1	0	1	2,6	0,13
2	0	1	1	7,1	0,35
2	1	0	0	4,7	0,23

The Analysis of Variance allowed determining the significant effect (P <0.05) for the yield at different levels of three separate factors, which indicated that there is statistical significance in each effect compared with an estimate of the experimental error. The p value less than 0.05 evidenced in 5 effects, indicates that they are significantly different from zero with a confidence level of 95%.

Consequently the null hypothesis is rejected; all this adjusted to an R-squared of 98.23% of the variability in Yield and the R-squared (adjusted for gl) = 97.4701%, which according to the Durbin-Watson statistic = 1.40045 (p = 0,0298) and shows that there is a positive correlational dependence between the dependent variable and the independent variables, since the value is less than 2.

The equation of the adjusted model according to the essential oil yield data (Y) is the dependent variable expressed as a predicted response and the factors X1, X2 and X3 as independent variables. The model shows a value of bo = 0.323 and that when optimizing the yield response, the value improves to 0.35, therefore the best conditions for conducting the process are given by the variable X1 (moisture) at medium and high levels for the variable X3 (Temperature), the same happens for the variable X2 (volume of water) but with little significance. The permanence of water in sufficient quantity in the process is ensured by the return of the condensed liquid applied by cohobation (Equation 1).

$$Y=0,323+0,049X_1 + 0,0114X_2 + 0,022X_3 - 0,156X_1^2 + 0,0X_1{}^*X_2 + 0,0025X_1{}^*X_3 + 0,004X_2^2 - 0,00042X_2{}^*X_3 - 0,007X_3^2 + 0,0X_1^{2*}X_2 + 0,0X_1^{2*}X_3 + 0,007X_1{}^*X_2^2 - 0,021X_1{}^*X_2{}^*X_3 - 0,008X_1{}^*X_3^2 + 0,0\,X_2^{2*}X_3 - 0,0096\,X_2{}^*X_3^2.$$

The Pareto diagram (figure 2) shows the value of each independent variable in decreasing order from left to right, where the moisture of the plant material and the extraction time obtained the greatest proportionally significant effect and the volume of water showed little and progressive significance as the level was increased to medium and high. The importance of conditioning the material (Factor A) by reducing the humidity to a medium level, Factor A stands out significantly in the yield of extracted oil.

In the graph of main effects for oil Yield (figure 3), plant moisture has a greater positive effect at medium level and decreases at high level. High dehydration of the plant material should be avoided since it affects the yield due to the volatility of the oils. It was shown that in times of extraction at increasing levels, greater extraction of the essential oil is achieved; while, the volume of water exerts a low significant effect with respect to both the variable and the interactions where the significance is < 0,5.

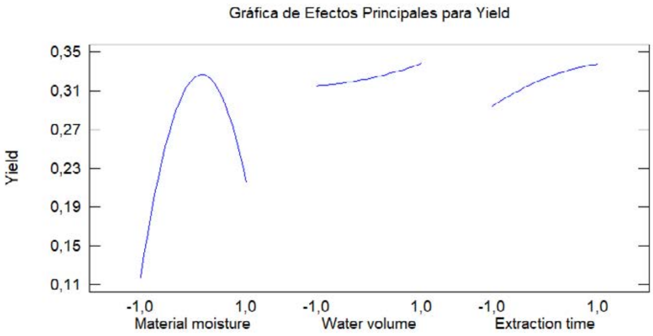

Figure 3. *Graph of Main Effects for Oil Yield*

For the interactions (figure 4), the effect is positive and visible at intermediate levels for the variables moisture and water volume, and the same effect is evidenced for the variables moisture and extraction time. The water volume and extraction time interactions are important at positive values, not at negative values or low levels.

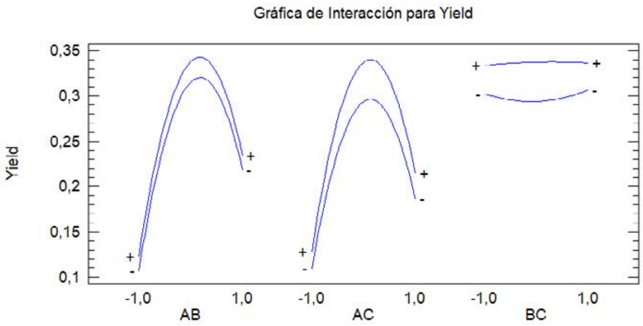

Figure 4. *Graph of interactions for Yield*

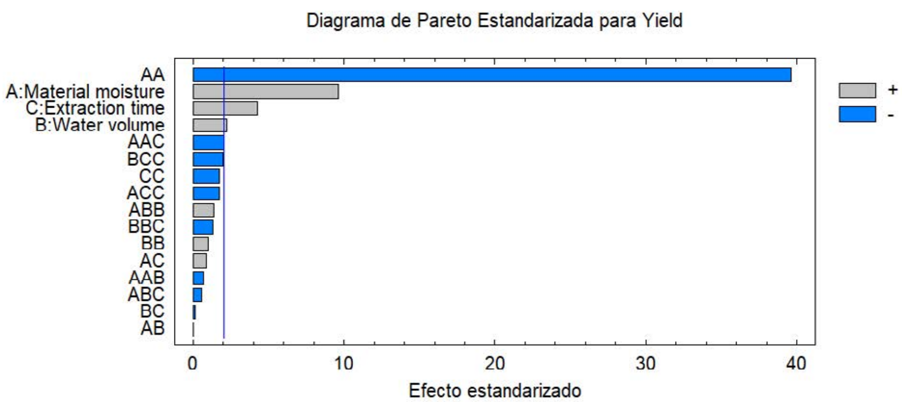

Figure 2. *Standardized Pareto diagram for Yield*

Figure 5 of response surface shows the trend towards the right, optimization of the extraction process (oil yield) at intermediate levels, whose behavior is reflected by moisture and extraction time especially.

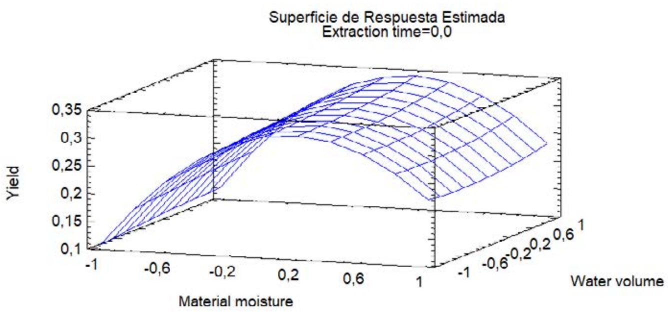

Figure 5. *Graph of factor response surface and response variable*

Experimentally it is shown that high yields are achieved in the extraction of essential oil according to values studied in the experiment (table 1) and that correspond to the best extraction yields obtained for treatments 4, 7 and 12 (table 2). For factors (Material moisture, Water volume and Extraction time) at levels (medium, high and medium), treatment 4 corresponds, for treatment 7 (medium, medium and high) and for treatment 12 at levels (medium-low and high), consequently the performance depends in its order of importance on the humidity of the material, extraction time and volume of water the latter because the process was carried out with water return (cohobation).

In the figures (2,3,4 and 5) it is evident that depend on the moisture of the plant at a medium level especially (60%) at medium and high extraction times; therefore, they must be adjusted to optimize the process for the sustainability of the activity.

The chemical characterization of the volatile fraction of *M. viridis L.* analyzed using the gas chromatograph coupled with a mass detector (GC-MS), yielded 34 components that are visible in table 3 and the chromatographic peaks reached are shown in figure 6.

In our study, the results showed that the aromatic compound with the highest presence is carvone with 36.53%, followed by anethole with 15.35%, germacrene with 8.81%, limonene with 7.2% and others in a lower proportion. Carvone with "its molecular ion is 150 amu and the molecular formula $C_{10}H_{14}O$, is a monoterpene-ketone"[22]. Carvone (p-menta-6,8-dien-2-one) is a flavoring substance widely used in pharmaceutical products[23] with an "antibacterial and / or antimicrobial effect and as an insecticide"[24], antifungal activity against A. fumigatus and C. Krusei[25], antiherpetic activity that is used in industries of flavors and products for oral hygiene[26] and sweet mint smell, such as mint leaves. Carvone and limonene can exhibit prophylactic and curative properties against skin, liver, lung, pancreas and breast cancer[25].

Table 3. *Composition of the essential oil of M. viridis L*

POLAR COLUMN				
Nº pico	**Tr**	**%**	**IK (cal)**	**Asignación**
1	6,24	1,38	1167	Beta-mirceno
2	7,29	7,25	1201	Limoneno
3	7,50	2,54	1206	1,8-cineol
4	8,67	1,60	1236	Cis-ocimeno
5	9,29	0,42	1252	Trans-ocimeno
6	13,78	0,20	1358	Desconocido
7	19,33	0,23	1477	Citronela
8	19,48	0,43	1481	Alpha-copaeno
9	20,71	2,26	1507	Beta-bourboneno
10	21,19	0,57	1517	Alpha-gurjuneno
11	22,78	0,40	1551	Linalool L
12	23,18	0,53	1560	Beta-cubebene
13	24,20	3,15	1582	Trans-cariofileno
14	24,77	0,59	1594	Cis-dihidrocarvone
15	26,19	1,66	1625	Gamma-muuroleno
16	27,40	0,31	1652	alpha-humuleno
17	27,56	2,29	1656	(+)-epi-biciclosesquife-landreno
18	27,92	4,99	1664	Estragol
19	28,17	0,29	1669	Trans-beta-farneseno
20	28,32	0,27	1673	Peral
21	29,31	8,81	1695	Germacreno D
22	30,50	36,53	1722	DL-carvona
23	31,46	1,97	1744	Dihidrocarveol
24	32,27	1,40	1763	Desconocido
25	32,87	0,34	1777	Alfa-cadineno
26	34,66	15,35	1819	Anetol
27	35,09	0,38	1830	(E)-carveol
28	36,33	0,47	1860	(Z)-carveol
29	36,68	0,24	1868	Carvacril acetato
30	43,31	0,36	2036	Germacreno D-4-ol
31	43,51	0,84	2041	Desconocido
32	44,43	0,50	2066	Viridiflorol
33	52,99	0,37	2304	Desconocido
34	63,33	0,64	2565	fitol

Where:

Tr	Retention time
%	Percentage of the compound in the sample
Ik (cal)	Calculated Kóvats Index
*	Percentage of compounds identified in the polar column

Aromatic substances such as carvone and anethole help to expel gases from the digestive tract [27,28]. Anethole is an organic compound with the molecular formula $C_{10}H_{22}O$, derived from phenylpropene, with a characteristic smell of anise oil and a sweet taste found in some essential oils [29]. Anethole is used for flavoring in the food and alcoholic beverage industries; Furthermore, they are used in perfumes, soaps and detergents and as precursors in organic synthesis for pharmaceutical formulations. These compounds present various biological activities such as insecticide, bactericide, anti-inflammatory [28] and larvicidal activity [31]. Anethole supports the digestive system, helps maintain healthy cell function, supports healthy blood flow, and calms the nerves[22].

Finally, chromatography is not only useful for studying the composition of an essential oil, but it is also useful for assessing quality. In figure 6, you can see a typical chromatogram where each separated substance produces a chromatographic peak, whose area is proportional to its amount in the injected mixture, where the major components in the essential oil of *Mentha Viridis L.* are evidenced: carvone as the main compound reaching the highest peak, followed by anethole, germacrene and limonene.

CONCLUSIONS

The application of technologies for extraction processes of essential oils of *Mentha viridis L,* by steam entrainment according to the water-vapor distillation method in a single body by cohobation, is a sustainable alternative for rural sectors, where the control of variables is important to achieve efficiency in the processes.

According to factors (material moisture, water volume and extraction time) at three study levels (low, medium and high) and assessed with the response variable (essential oil yield) the best extraction results were obtained (3,4 %). They were reached at only medium moisture levels, water volume (high, medium and low) due to the effect of water recirculation and extraction time (medium, high and low) for three treatments in their order respectively. Therefore, the lack of knowledge of those variables in the extraction processes will affect the yield of essential oil, consequently the sustainability of the activity.

In the chemical characterization of the volatile fraction of *Mentha viridis L*, 34 components were identified, the ones with the highest presence in their order from highest to lowest: carvone, anethole, germacrene and limonene. Due to the biological activity of these components, this oil can be used for antibacterial and / or antimicrobial and insecticidal, antifungal, antherpetic and is widely used for the industry of flavors and products for oral hygiene. These are reasons for giving some convenient executive steps in favor of immediate application in the industry and production.

REFERENCES

1. Ochoa, K.; Paredes, L.; Bejarano, D.; Silva, R. Extracción, caracterización y evaluación de la actividad antibacteriana del aceite esencial de *Senecio graveolens* Wedd (Wiskataya). Scientia Agropecuaria. 2012, 3, 291–302. Available online: https://dialnet.unirioja.es/descarga/articulo/4104068.pdf (accessed on 3 March 2021).
2. Ordaz, G.; Armas, H.; Yáñez, D.; Moreno, S. Composición química de los aceites esenciales de las hojas de *Helicteres guazumifolia* (Sterculiaceae), *Piper tuberculatum* (Piperaceae), *Scoparia dulcis* (Arecaceae) y *Solanum subinerme* (Solanaceae), recolectadas en Sucre. ISSN-0034-7744. 2011,

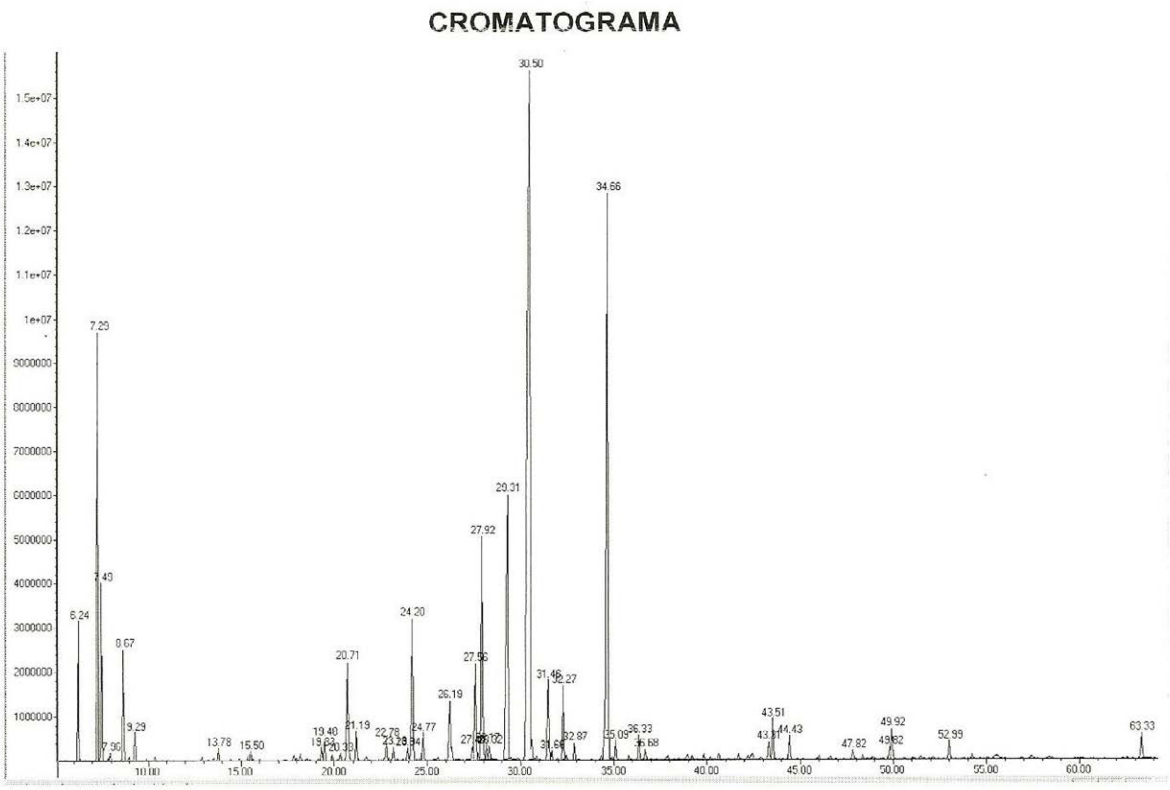

Figure 6. *Chromatographic profiles of the components of the essential oil of Mentha viridis L*

Vol. 59 (2): 585-595. Available online: https://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77442011000200005 (accessed on 3 April 2021).
3. Usano, J., Pála, J.; Díaz, S. Aceites esenciales: conceptos básicos y actividad antibacteriana. Reduca (Biología). Revista RE. Serie Botánica. 7 2014, (2): 60-70. Available online: http://revistareduca.es/index.php/biologia/article/view/1553/1747 (accessed on 10 May 2021).
4. Linde, G.A., Colauto, N.B., Albertó, E.; Gazim, Z.C. Quimiotipos, Extracción, Composición y Aplicaciones del Aceite Esencial de Lippia alba. Rev. Bras. Pl. Med., Campinas, 2016, v.18, n.1, 191-200. Available online: https://www.scielo.br/j/rbpm/a/k8wnWR3SQY5Q9BYV6cBd5Hh/?lang=es (accessed on 12 March 2021).
5. Bonzani, N., Costaguta, M.; Barboza, G. Estudios anatómicos en especies de *Mentha* (Fam. Lamiaceae) de Argentina. ISSN: 1815-8242. 2007, 14 (1): 77-96. Available online: file:///C:/Users/Walter/Downloads/CONICET_Digital_Nro.49a63224-06f6-42c4-b29c-e8af01a3079f_A%20(3).pdf (accessed on 24 May 2021).
6. Orsini, G. Morfoanatomía del género Mentha (Mentheae, Nepetoideae, Lamiaceae) en Venezuela. Universidad Central de Venezuela. 2015, Research. Abril, 25. [CrossRef]
7. Fonnegra, R.; Jiménez S. Plantas medicinales aprobadas en Colombia. 2ª edición. Ed. Universidad de Antioquia. Medellín, Colombia. 2007, 368. Available online: https://books.google.com.pe/books?id=K8eI-7ZeFpsC&printsec=frontcover&hl=es&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false (accessed on 25 May 2021).
8. Yesid, H.; García, H.; Quevedo, G. Pautas para el conocimiento, conservación y uso sostenible de las plantas medicinales nativas en Colombia. Estrategia Nacional para la Conservación de Plantas. Ministerio de Ambiente, Vivienda y Desarrollo Territorial 2011, 61. Available online: http://repository.humboldt.org.co/handle/20.500.11761/31427 (accessed on 25 May 2021).
9. Domínguez, G.; Castro, A. Usos medicinales de la familia Labiatae en Chiapas, México. Etnobiología 2002. 2: 19-31. Available online: https://dialnet.unirioja.es/servlet/articulo?codigo=5294447 (accessed on 26 May 2021).
10. Pérez, J. Caracterización química de los aceites esenciales de plantas aromáticas, de uso medicinal y de importancia económica de Guatemala. Proyecto FINDECYT/FODECYT 017-2019. https://fondo.senacyt.gob.gt/portal/images/yootheme/17-2017.pdf (accessed on 09 June 2021)
11. Mancianti, F.; Ebani, G. Biological Activity of Essential Oils. Molecules 2020, 25, 678. [CrossRef]
12. Arango, O.; Hurtado, A.; Castillo, P.; Santacruz, M. Estudio de las condiciones de extracción por arrastre con vapor del aceite esencial de laurel de cera (*Morella pubescens*) study of extraction conditions of "laurel de cera" *(Morella pubescens)* essential oil by means of steam distillation. 2009, Vol 7. No. 2. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1692-35612009000200006 (accessed on 29 May 2021).
13. Moreno, J.; López, G.; Siche, R. Modelación y optimización del proceso de extracción de aceite esencial de eucalipto (*Eucalyptus globulus*). Modeling and optimization of extraction process of eucalyptus essential oil (*Eucalyptus globulus*)**.** Scientia Agropecuaria 2010, 1, 147–154. Available online: https://revistas.unitru.edu.pe/index.php/scientiaagrop/article/view/27 (accessed on 02 June 2021).
14. Moore; Stanitski; Wood, Kotz. El mundo de la Química. Conceptos y aplicaciones. Segunda edición. Ediciones Pearson. México. 2000.
15. Bandoni, A. Los recursos vegetales aromáticos en Latinoamérica. Su aprovechamiento industrial para la producción de aromas y sabores. Ed. Ciencia y Tecnología para el Desarrollo (Cyted), Argentina. 2003, 143-148-149. Available online: https://www.academia.edu/15574491/Bandoni_LOS_RECURSOS_VEGETALES_AROM%C3%81TICOS_EN_LATINOAM%C3%89RICA_ (accessed on 03 June 2021).
16. Abdelhakim Bouyahya, Fatima Lagrouh, Nasreddine El Omari, Ilhame Bourais, Meryem El Jemli, Ilias Marmouzi, Najoua Salhi, My El Abbes Faouzi, Omar Belmehdi, Nadia Dakka, Youssef Bakri. 2020. Los aceites esenciales de compuestos fenólicos ricos en Mentha viridis muestran importantes propiedades antioxidantes, antidiabéticas, dermatoprotectoras, antidermatofitas y antibacterianas. Revsita Biocatálisis y biotecnología agrícola. Volume 23, January 2020. **https://www.sciencedirect.com/science/article/abs/pii/S1878818119315324?via%3Dihub** (accessed on 9 september 2021).
17. Mounira Mkaddem , Jalloul Bouajila , Monia Ennajar , Ahmed Lebrihi , Florence Mathieu , Mehrez Romdhane 2009. Composición química y actividades antimicrobianas y antioxidantes de los aceites esenciales de *mentha* **(** *longifolia* **L. y** *viridis* **).** JOURNAL OF FOOD SCIENCE—Vol. 74, Nr. 7. JFS M: Food Microbiology and Safety. **https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1750-3841.2009.01272.x** (accessed on 08 september 2021).
18. Quezada, W.; Quezada, WD.; Gallardo, I.; Cevallos, E.; Arias, G.; Travez, A.; Zambrano, Z.; Rojas, O. Extraction and chemical characterization of the essential oil of *Tagetes pusilla*, in fresh and stored samples. AFINIDAD LXXVI, 2019, 588. Available online: file:///C:/Users/Walter/Downloads/361885-Other-522187-1-10-20191230%20(1).pdf (accessed on 03 June 2021).
19. Quezada, W.; Quezada, WD.; Travez, A.; Arias, G.; Cevallos, E.; Zambrano, Z.; Brito, H.; Salazar, K. Essential oil of Minthostachys mollis: extraction and chemical composition of fresh and stored samples. Arabian Journal of Medicinal and Aromatic Plants. 2019, 5 (1), 59-71. Available online:

https://revues.imist.ma/index.php/AJMAP/article/viewFile/15684/8726 (accessed on 03 June 2021)
20. Mamani, B. Actividad antibacteriana de aceite esencial de *Mentha Spicata* L. sobre flora mixta salival. Cirujano Dentista. Tesis Universidad Nacional Mayor de San Marcos. Perú, 2013. Available online: https://cybertesis.unmsm.edu.pe/bitstream/handle/20.500.12672/3424/Mamani_cb.pdf?sequence=1&isAllowed=y (accessed on 04 June 2021)
21. Orellana, A. Caracterización del aceite esencial de hierbabuena *(Mentha Spicata* L.) obtenido por el método de arrastre con vapor. Tesis. Universidad Nacional de Callao. Perú, 2017. Available online: http://repositorio.unac.edu.pe/handle/UNAC/3612 (accessed on 04 June 2021)
22. Hill, D. Manual de Química de aceites esenciales dōTERRA. Chapter 1: An Introduction to Essential Oils, 2018. Available online: https://media.doterra.com/us/es/brochures/essential-oil-chemistry-handbook.pdf (accessed on 04 June 2021)
23. Barrera, R.; Alarcón, E.; González, L.; Villa, A.; Montes de Correa. Síntesis de carveol, carvona, verbenol y verbenona. Ingeniería y Competitividad, Universidad del Valle. Cali, Colombia. 2008, vol. 10, núm. 1, 43-63. Available online: https://revistaingenieria.univalle.edu.co/index.php/ingenieria_y_competitividad/%20article%20/view%20/2480 (accessed on 04 June 2021)
24. López, M. Toxicidad volátil de monoterpenoides y mecanismos bioquímicos en insectos plaga del arroz almacenado. PH.D.Tesis. Universidad de Murcia. España. 2008. Available online: https://tdx.cat/handle/10803/11037#page=1 (accessed on 04 June 2021)
25. Benavides, O.; Villota, J.; Tovar, D. Caracterización del aceite esencial presente en las hojas de *Phyla nodiflora* (L.) Greene (OROZUL). Revista Centro de Estudios en Salud. 2010, Año 10 Vol. 1 Nº 12, 57- 64. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0124-71072010000100008 (accessed on 04 June 2021)
26. Agudelo, L.; Gómez, G.; Durán, D.; Stashenko, E.; Betancur, L. Composición química y evaluación de la actividad antiherpética *in vitro* de aceites esenciales de *Lippia alba* (Mill) N.E. Brown y sus componentes mayoritarios. Revista Salud UIS. Universidad Industrial Santander. Colombia. Salud UIS 2010; 42, 230-239. Available online: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0121-08072010000300006 (accessed on 04 June 2021)
27. Medi ambient. Plantas aromáticas para huertos urbanos. Cultivos y Propiedades. DIPUTASIO DE VALENCIA, 2014. Available online: https://www.dival.es/sites/default/files/medio-ambiente/Estudio3.pdf (accessed on 06 June 2021)
28. Iñiguez, R.; Esqueda, H.; Escoto, T.; Ochoa, H.; Rodríguez, A.; contreras, H. Caracterización química de aceites esenciales y extractos de madera de dos especies de pinos del bosque La Primavera. Revista Mexicana de Ciencias Forestales. 2014, Vol.6 (28): 42-57. Available online: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-11322015000200004 (accessed on 06 June 2021)
29. Bolívar, G. Anetol: estructura, propiedades, usos, toxicidad. Lifeder. 2019. Recuperado de https://www.lifeder.com/anetol/ Available online: https://www.lifeder.com/anetol/#:~:text=El%20anetol%20es%20un%20compuesto,an%C3%ADs%20y%20un%20sabor%20dulce.&text=Este%20compuesto%20se%20utiliza%20como%20diur%C3%A9tico%2C%20carminativo%20y%20expectorante. (accessed on 08 May 2021)
30. Muñoz, A.; Bottia, E.; Cárdenas, C; Patiño, J.; Díaz, O.; Martínez, J.; Kouznetsov, V.; Stashenko, E. Estudio comparativo sobre la capacidad de atrapamiento del catión radical ABTS+. por los aceites esenciales de especies aromáticas con alto contenido de *trans*-anetol y estragol. Revista Scientia et Technica, 2007. Año XIII, No 33. Available online: https://revistas.utp.edu.co/index.php/revistaciencia/article/view/6137. (accessed on 08 May 2021)
31. Andrade, S.; Sánchez, LE.; Nevárez, GV.; Camacho, A.; Nogueda, B. Aceites esenciales y sus componentes como una alternativa en el control de mosquitos vectores de enfermedades. Biomédica 2017;37(Supl.2):224-43. [CrossRef]