Performance, chemical composition and antibacterial activity of the essential oil of Ruta chalepensis and Origanum vulgare

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Abstract. The antibacterial effectiveness of Origanum vulgare and Ruta chalepensis essential oils cultivated in three municipalities of Norte de Santander, Colombia, on gram positive and gram-negative bacteria was determined in vitro. The oil extraction process was carried out at zoey perfumery company by the steam dragging method from 5 kg of vegetable material, the oil yield obtained compared with the vegetable material collected was calculated using mathematical formulas and its chemical composition was determined by mass spectrometry since is an analytical technique with great potential that allows to elucidate the structure and chemical properties of molecules. For the determination of the minimum inhibitory concentration and the minimum bactericidal concentration of the extracted oils, the mother solution of 2.5 g/mL was prepared and from this solution, the different dilutions at concentrations from 1000 mg/mL up to 15.62 mg/mL were made. A suspension equal to tube 0.5 of the McFarland scale of each of the microorganisms (Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) was then prepared with sterile saline solution and inoculated in microplates with volumes of diluted solution, soy tripticasa broth and dimethyl sulfoxide, was then incubated at 37 °C for 18 hours and proceeded to inoculate in Müeller-Hinton agar to verify its minimum bactericidal concentration. The results show that the yield of Origanum vulgare essential oil was 0.8% and for Ruta chalepensis 0.1%. The chemical analysis of the oils revealed the major components of Origanum vulgare such as β-mircene 1.6%, α-terpinene 15.7%, 1.8-cineol 3.8%, γ-terpineno 2.6%, terpine-4-ol 1.1%, timol methyl ether 17.4%, timol 30.6%, carvacrol 8.1%, trans-β-caryophyllene 6.3%, α-humulene 1%, cariophylene oxide 3.1% and Ruta chalepensis as nonanone 37.1%, undecanone 39.4%, nonanyl acetate 2.2%, decanone 2.8%. The results obtained show that essential oil of Ruta chalepensis at concentration of 500 mg/mL stop growth of Escherichia coli and Pseudomonas aeruginosa and at a concentration of 1000 mg/mL stop growth of S. aureus while the essential oil of Origanum vulgare was the most effective for the inhibition of all the microorganisms evaluated, requiring a concentration of 15.62 mg/mL for Escherichia coli and Staphylococcus aureus and for Pseudomonas aeruginosa, a concentration of 125 mg/mL was necessary. It is concluded that according to the chemical composition, materials of vegetable origin such as Ruta chalepensis and Origanum vulgare essential oils can be taken for the elaboration of products with potential in artisanal cosmetics and even in pharmaceutical products.
1. Introduction

Essential oils are defined as mixtures of volatile components, products of the secondary metabolism of plants, composed mostly of hydrocarbons of the polymethylene series of the group of terpenes corresponding to the formula (C6H8)n, together with other compounds almost always oxygenated that transmit to the essential oils the aroma that characterizes them [1] and that in turn can be obtained from different parts of the plant (root, stem, leaves, flowers, fruit, seed) and its chemical composition may vary depending on the type of plant, the area where it grows, the climate, and the parts of the plant [2]. Aromatic plants are a group of plants that produce all or part of their active ingredients in the form of essential oils [2]. Using different types of extraction for the species, in this study we use the method called steam-driven distillation. In steam-driven distillation, the plant sample is subjected to a stream of water vapor, thus the essence is dragged, condensed and separated from the aqueous fraction. It is widely used at industrial level due to its high yield, the purity of the oil obtained and because it does not require sophisticated technology, this method allows us to evaluate the yield, which in most plants is obtained from 0.01% to 10% essential oil content. The average amount found in most aromatic plants is around 0.1% to 2% [3]. In addition, essential oils have been studied to show antimicrobial activity in pathogenic bacteria [3].

The study made it possible to observe the effect exerted by Origanum vulgare and Ruta chalepensis on highly pathogenic bacteria such as Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), and Pseudomonas aeruginosa (P. aeruginosa). For this reason, it is said that the contribution to the strengthening of science, engineering and technological development of the scientific community is in the contribution of new knowledge through the research of natural antimicrobials extracted from plant material of great value for the cosmetic and pharmaceutical industry at the national level. Given the above, it is important to assess the performance of essential oils allowing to identify the cost-benefit generated through the use of oils in study allowing the entry of the departmental industry in new markets with the generation of new products with bactericidal properties.

2. Materials and methods

2.1. Collection of plant material

Samples of plant material were collected from the municipalities of Cácoa, Pamplona and Ragonvalia, Norte de Santander, Colombia, gathering about 5 kg of this approximately, which was dried in the shade, shredded and stored in amber bottles until the time of extraction.

2.2. Extraction of essential oil by steam distillation

Vegetable material from Origanum vulgare and Ruta chalepensis was used. Each of these samples was subjected to a stream of water vapor (100 °C -105 °C) for 2 hours, so the essence was washed away, condensed and separated from the aqueous fraction. The separated essential oil was measured in a burette and stored in an amber bottle at 4 °C [4]. The yield of the extraction of essential oil from each of the samples (leaves, bark and wood) was obtained by the Equation (1) [5].

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\text{%yield} = \frac{\text{Essential oil mL} \times 100}{\text{Vegetal material weight g}} \times 100\%
\]  

(1)

2.3. Determination of the minimum inhibitory concentration by micro-dilution in plate

2.3.1. Preparation of stock solution

An analytical balance weighed 2.5 g of the oil under study and added 1 mL of dimethyl sulfoxide and then mixed by vortex.

2.3.2. Preparation of oil dilutions

Tubes were marked with the corresponding dilutions and concentrations from 1000 mg/mL to 15.62 mg/mL as shown in Table 1.
2.3.3. Preparation of the bacterial suspension
A bacterial suspension of approximately $10^8$ microorganisms per milliliter equivalent to the Mc Farland 0.5 standard was prepared for each one of the microorganisms to be tested (E. coli ATCC 25922, S. aureus ATCC 25923 and P. aeruginosa) and 96 wells were assembled on microplates distributing in triplicate the volumes of diluted solution, soy tripticasa broth, bacterial suspension and dimethyl sulfoxide (Table 2). It incubated at 37 °C for 18 hours.

**Table 1. Preparation of oil dilutions.**

| Concentration (mg/ml) | Dilution | Mother solution volume (µl) | Soy tripticasa broth volume (µl) |
|-----------------------|----------|-----------------------------|----------------------------------|
| 1000.0                | 1/2      | 400.0                       | 600                              |
| 500.0                 | 1/4      | 200.0                       | 800                              |
| 250.0                 | 1/8      | 100.0                       | 900                              |
| 125.0                 | 1/16     | 50.0                        | 950                              |
| 62.5                  | 1/32     | 25.0                        | 975                              |
| 31.3                  | 1/64     | 12.5                        | 988                              |
| 15.6                  | 1/128    | 6.3                         | 994                              |

**Table 2. Volumes used for mounting the plates.**

| Dilutions               | Diluted solution (ul) | Soy tripticasa broth (ul) | Bacterial suspension (ul) | Dimethyl sulfoxide (ul) |
|-------------------------|-----------------------|---------------------------|---------------------------|-------------------------|
| Negative control        | 100                   | 200                       | 100                       |                         |
| Positive control        | 100                   | 100                       | 100                       |                         |
| CDS control             | 100                   | 100                       | 100                       | 10                      |

2.3.4. Determination of the minimum bactericidal concentration
The inoculation was carried out of 10 µl of the well in Müller Hinton agar to verify its minimum bactericidal concentration and incubated for 24 hours at 37 °C. The absence of growth in the sowing indicated its minimum bactericidal concentration.

2.4. Chemical analysis
The analysis of the samples was developed according to the method accredited by ISO 7609-1985(E) [6]; using as reference standard the certified hydrocarbon mixture C₆-C₅ (Accu Standard, New Haven, CT). Sample preparation was carried out by dilution and direct injection of the essential oils into the chromatographic equipment. The chromatographic analysis was performed on an AT 6890 Series Plus gas chromatograph (Agilent Technologies, Palo Alto, California, USA) [7].

3. Results and discussion
Table 3 shows the yield of essential oils obtained from dried leaves at room temperature for 72 hours using steam distillation for extraction.

The results show that the plant with the highest yield in oil extraction was *Origanum vulgare* (1.2%), it should be noted that the *Origanum vulgare* used in this research was wild. These data differ from those obtained by Reyes and Ortega [8], whose oil yield was 2.55% in natural populations, 2.14% under irrigation conditions and Flores, et al. [9] with 3.2%.

On the other hand, the hydrodistillation of the *Ruta chalepensis* leaves produced an oil of intense and penetrating odor. The yield was 0.12%, lower than those obtained for this species by other authors (5.51% [9], 0.8% [10], and 0.3% [11]), which is why it can be said that the total essential oil content in the plants can be affected by the genotype, climate, growing location, rainfall and harvesting regime [12]. Yield variability can also be associated with the existence of subspecies [11], differences in the drying of plant material [10], variations in distillation parameters [9,13,14], among other factors.
Different yields according to the time and mode of process from its collection to its extraction. In the essential oil, it has been recognized that the economic value of essential oils and their industrial applicability are directly related to their chemical composition and biological activity. Hence, O. vulgare oil may have greater industrial applicability in the pharmaceutical and food areas as it shows a rich content in phenolic monoterpenes such as thymol (30.6%), terpinene (15.7%) and carvacrol (8.1%), which have been recognized as inhibitory activity against bacteria, fungi and protozoa. However, its mechanism of action is not yet fully understood. In this sense, the results show that the essential oil of Ruta chalepensis (Cáucota) showed a minimum inhibitory concentration of 125 mg/ml against P. aeruginosa, while for S. aureus and E. coli it reached values of 15.62 mg/ml. On the other hand, the essential oil of Origanum vulgare (Pamplona) and Ragonvalia, Colombia, requires a lower concentration (15.62 mg/ml to 62.5 mg/ml) to exert some effect against E. coli, P. aeruginosa, and S. aureus in comparison with Ruta chalepensis oil. These results coincide with Albado E., et al. [15], who affirm that the essential oil of Origanum vulgare is highly active in the inhibitory process of some microorganisms thanks to the fact that it contains up to 56 compounds, of which quantitatively significant differences have been found in two isomeric phenols, carvacrol (0.1%-56.6%) or non-crystallizable phenol and thymol (7.9%-53.6%) or crystallizable phenol. Similarly, the effect of this oil on P. aeruginosa at a concentration of 62.5 mg/ml can be seen, which contradicts Arcila C., et al. [16], who argue that carvacrol and thymol phenols have the highest levels of activity against gram negative microorganisms, except for P. aeruginosa, which is the most active thymol. On the other hand, the essential oil of Ruta chalepensis (Cáucota) showed a minimum inhibitory concentration of 125 mg/ml against P. aeruginosa, while for S. aureus and E. coli it reached values of 15.62 mg/ml, this activity can be due to the presence of oxygenated compounds, with a contribution of more than 75% of relative abundance. Among them, 2-undecanone, the most abundant ketone in this oil, inhibited the bacteria Escherichia coli and Bacillus subtilis in a previous study [15], which strengthens the possible linkage of this compound with the effect observed in the present research.

Table 3. Yield obtained from the oils under study.

| Oil               | Study material | Extraction time (h) | Performance (%) |
|-------------------|----------------|---------------------|-----------------|
| Origanum vulgare  | Dry leaf       | 2                   | 1.2             |
| Ruta chalepensis  | Dry leaf       | 2                   | 0.12            |

Table 4. Antimicrobial activity of essential oils against E. coli, S. aureus, P. aeruginosa.

| Oil               | Bacteria       | Pamplona | Ragonvalia | Cáucota |
|-------------------|----------------|----------|------------|---------|
|                   | MIC (mg/ml)    | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) | MIC (mg/ml) | MBC (mg/ml) |
| Ruta chalepensis  | E. coli        | 500      | 1000.0     | 1000      | 1000      | 15.62       | --          |
|                   | S. aureus      | 1000     | 62.5       | 1000      | 1000      | 15.62       | --          |
|                   | P. aeruginosa  | 500      | 500.0      | 1000      | 1000      | 125.00      | --          |
| Origanum vulgare  | E. coli        | 15.62    | --         | 15.62     | 15.62     | 500         | 15.2        |
|                   | S. aureus      | 15.62    | --         | 15.62     | 15.62     | 1000        | 62.5        |
|                   | P. aeruginosa  | 125.00   | --         | 62.50     | 125.00    | 500         | 125.0       |

Table 4 shows the inhibitory and bactericidal activity of the oils analyzed against the three test microorganisms. In this sense, the results show that the essential oil of Origanum vulgare from the municipalities of Pamplona and Ragonvalia, Colombia, require a lower concentration (15.62 mg/ml to 62.5 mg/ml) to exert some effect against E. coli, P. aeruginosa, and S. aureus in comparison with Ruta chalepensis oil. These results coincide with Albado E., et al. [15], who affirm that the essential oil of Origanum vulgare is highly active in the inhibitory process of some microorganisms thanks to the fact that it contains up to 56 compounds, of which quantitatively significant differences have been found in two isomeric phenols, carvacrol (0.1%-56.6%) or non-crystallizable phenol and thymol (7.9%-53.6%) or crystallizable phenol. Similarly, the effect of this oil on P. aeruginosa at a concentration of 62.5 mg/ml can be seen, which contradicts Arcila C., et al. [16], who argue that carvacrol and thymol phenols have the highest levels of activity against gram negative microorganisms, except for P. aeruginosa, which is the most active thymol. On the other hand, the essential oil of Ruta chalepensis (Cáucota) showed a minimum inhibitory concentration of 125 mg/ml against P. aeruginosa, while for S. aureus and E. coli it reached values of 15.62 mg/ml, this activity can be due to the presence of oxygenated compounds, with a contribution of more than 75% of relative abundance. Among them, 2-undecanone, the most abundant ketone in this oil, inhibited the bacteria Escherichia coli and Bacillus subtilis in a previous study [15], which strengthens the possible linkage of this compound with the effect observed in the present research.

Table 5 shows the chemical composition of the essential oils of Origanum vulgare and Ruta chalepensis. According to Stashenko, et al. [7], the economic value of essential oils and their industrial applicability are directly related to their chemical composition and biological activity. Hence, O. vulgare oil may have greater industrial applicability in the pharmaceutical and food areas as it shows a rich content in phenolic monoterpenes such as thymol (30.6%), terpinene (15.7%) and carvacrol (8.1%), which have been recognized as inhibitory activity against bacteria, fungi and protozoa. However, its mechanism of action is not yet fully understood. In addition, other studies concerning timol have shown that this terpenoid can interact with phospholipid membranes, altering the permeability of the membrane by lipophilic compounds [16]. In addition, other studies concerning timol have shown that this terpenoid can interact with phospholipid membranes, altering the permeability of the membrane by lipophilic compounds [16]. Therefore, not only do the structure, functional groups and composition of essential oils play an important role in determining their antimicrobial activity [4,18,19] but also quantitative differences can be attributed to the methods of production, date and geo-botanical conditions of the crop as well as to the time elapsed between collection and the process of production of the essential oil.

Different studies on the chemical composition of the essential oil of Origanum vulgare, report different yields according to the time and mode of process from its collection to its extraction. In the present investigation, thymol, terpenoids, phenols and compounds metabolically related to carvacrol
were obtained, being products of interest for their application in the Food Pharmaceutical Industry and in agriculture [18,19]. The monoterpenoids are responsible for the fragrances and odour sensations of many plants. Structurally and biologically they are very different, being classified up to 35 groups. On the other hand, thymol and carvacrol are natural phenolic compounds, considered as possible antioxidants, antifungal and antibacterial agents, acaricides, analgesics, antiacne, antispasmodics, deodorants, dermatogens, expectorants, insecticides, larvicides, pesticides and vermicide present in significant quantities in the essential oils of the genus Thymus, Origanum vulgare, Satureja, Thymbra and Lippia [20].

### Table 5. Chemical composition of the essential oils of *Origanum vulgare* and *Ruta chalepensis.*

| Compound               | *Origanum vulgare* Concentration (%) | *Ruta chalepensis* L. Concentration (%) |
|------------------------|--------------------------------------|----------------------------------------|
| Timol                  | 30.6                                 | --                                     |
| Timol methyl ether     | 17.4                                 | --                                     |
| α-Terpinene            | 15.7                                 | --                                     |
| Terpinene              | 2.6                                  | --                                     |
| Terpineno-4-ol         | 1.1                                  | --                                     |
| 1,8-Cineol             | 3.6                                  | --                                     |
| Carvacrol              | 8.1                                  | --                                     |
| α-Humulene             | 1.0                                  | --                                     |
| β-Myrcene              | 1.6                                  | --                                     |
| Trans-β-caryophyllene  | 6.3                                  | --                                     |
| Caryophyllene Oxide    | 3.1                                  | --                                     |
| 2-Undecanone           | --                                   | 39.4                                   |
| Nonanone               | --                                   | 37.1                                   |
| Decanone               | --                                   | 2.8                                    |
| Nonanoyl acetate       | --                                   | 2.2                                    |

As for *R. chalepensis* oil, the results show a high concentration in undecanone (39.4%) and nonanone (37.1%), which places it as a useful oil in perfumery; in the food industry as a flavoring; besides possessing insecticidal activity [12], repellent [12] and antifungal [21]. Its chemical composition is characterized by an abundance of ketones [12,21]. However, the results of this study show that *R. chalepensis* oil from Pamplona and Ragonvalia, Colombia, has a low antimicrobial activity against *E. coli*, *S. aureus* and *P. aeruginosa* compared to oil extracted from Cárcota which showed a high inhibitory action against *E. coli* and *S. aureus* (15.62 mg/ml) and low against *P. aeruginosa* (125 mg/ml), reaffirming the theory of Conti, *et al.* [18]. Those who ensure that the concentration of essential oil components depends on the geobotanical conditions of the crop and the part of the plant from which it was obtained [11].

The other side, the 2-undecanone, due to its presence as a major component in the essential oils of several Ruta species, is considered a marker for this genus [4]. The prevalence of this compound in the oil studied allows it to be included in the 2-undecanone chemotype, in correspondence with its relative abundance value, which is in the range of 20.40% to 82.74% previously reported [11]. The content of this ketone found in the corresponding research was 39.4% higher than that found in the study chemical composition and antibacterial activity of the essential oil of *Ruta chalepensis* L.

### 4. Conclusions

The essential oil of *Origanum vulgare* was the most active for the inhibition of all the microorganisms evaluated, however, there was a greater activity in the inhibition of the growth of *E. coli* and *S. aureus* than for the bacterium *P. aeruginosa*, which allows inferring that a complementary treatment based on *Origanum vulgare*, could generate positive results before an infection caused by *E. coli* and *S. aureus*, since an average concentration of 39.06 mg/ml was necessary for the inhibition of these bacteria.
The antibacterial activity reported by oregano essential oil evidences the opportunity to develop a product for the pharmaceutical industry with inhibitory activity for bacteria such as S. aureus, E. coli, and P. aeruginosa.

The yield of the essential oils under study confirms the importance of assessing the geo-botanical conditions of the crop that allow the producer to standardize the handling of the crop with a view to offering a homogeneous product without variation in quality.

References
[1] Isman M 2006 Botanical insecticides deterrents and repellents in modern agriculture and an increasingly regulated world Ann Rev Entomol 51 45
[2] Guenther E 2006 Essential oil 1st edition (Indonesia: UI-Press Jakarta)
[3] Gennari V 1996 Aromatherapy: A lifetime guide to healing with essential oils (USA: Prentice Hall Inc.)
[4] Bassolé I and Juliani H 2012 Essential oils in combination and their antimicrobial properties Molecules 17 3989
[5] Granados C, Yañez X, and Acevedo D 2014 Evaluation of Antioxidant Activity of the Essential Oil of Myrcianthes leucoxyla from Norte de Santander (Colombia) Información tecnológica 25 11
[6] International Organization for Standardization (ISO) 1985 Essential oils — Analysis by gas chromatography on capillary columns — General method, ISO 7609-1985 (Switzerland: International Organization for Standardization)
[7] Stashenko E, Martínez J, Ruiz C, Arias G, Duran C, Salgar W, and Cala M 2010 Lippia origanoides chemotype differentiation based on essential oil GC-MS and principal component analysis J Sep Sci 33 93
[8] Reyes C, and Ortega R 2002 Aprovechamiento, manejo y cultivo de orégano en la región lagunera folleto para productores No 6 (Matamoros: SAGARPA-INIFAP-CIRNOC-CELALA)
[9] Flores A, Hernández J, López J, Valenzuela L, Martínez M, and Madina veitia H 2011 Producción y extracción de aceite de orégano (Lippia graveolens Kunth) bajo cultivo en la comarca lagunera Rev Mex Cien For 2 113
[10] Mejrì J, Abderrabba M and Mejri M 2010 Chemical composition of the essential oil of Ruta chalepensis L: Influence of drying hydro-distillation duration and plant parts Industrial Crops and Products 32 671
[11] Boudiar T, Labed I, Safaei-Ghomi J, Kabouche A and Kabouche Z 2011 Analysis of the essential oil of ruta chalepensis subsp. angustifolia from Algeria Journal of Essential Oil Bearing Plants 14(6) 792
[12] Pino O, Sánchez Y, Rojas M, Abreu Y, Correa T, Martínez D and Montes de Oca R 2014 Chemical composition and antibacterial activity of the essential oil of Ruta chalepensis L Plant Protection Magazine 29(3) 220
[13] Haddouchi F, Chaouche T, Zaouali Y, Ksouri R, Attou A and Benmansour A 2013 Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria Food Chemistry 141 253
[14] Jamel J, Bouajila J, Aydi A, Barth D, Abderrabbaa M, and Mejri M 2012 Supercritical CO2 extract and essential oil of Ruta chalepensis L Growing in Tunisia: A natural source of Undecan-2-one Analytical Chemistry Letters 2(5) 290
[15] Albado E, Saez G, and Grabiel S 2001 Chemical composition and antibacterial activity of the essential oil of Origanum vulgare Herederal Medical Review 12(1) 16
[16] Arcila C, Loarca G, Lecona S, and Gonzalez E 2004 Origanum vulgare: properties composition and biological activity of its components Latin American Nutrition Archives 54(1) 21
[17] Gibka J, Kunicka-Styczynskab A and Gliński M 2009 Antimicrobial activity of Undecan-2-one, Undecan-2-ol and their derivatives Journal of Essential Oil Bearing Plants 12(5) 605
[18] Conti B, Leonardi M, Pestelli L, Prefeti R, Ouerghemmi I and Benelli G. 2013 Larvicidal and repellent activity of essential oils from wild and cultivated Ruta chalepensis L. (Rutaceae) against Aedes albopictus Skuse (Diptera: Culicidae), an arbovirus vector Parasitol Res 112 991
[19] Yang R and Shetty K 1998 Stimulation of Rosmarinaxis acid in shoot culture of orégano (Origanum vulgare) clonal line in response to proline analog and proline precursors J Agric Food Chem 46 2888
[20] Henao J, Muñoz L, Padilla L and Ríos E 2010 Extraction and characterization of the essential oil of H.B.K “orégano de monte” cultivated at Quindío and evaluation of antimicrobial activity Revista de Investigaciones de la Universidad del Quindío 21 82
[21] Burt S 2004 Essential oils: their antibacterial properties and their possible applications in food-a review Int J Food Microbiol 94 223