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Evaluation of antibacterial activity of essential oil Origanum vulgare (oregano)

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Abstract

The aim of the research is to evaluate the antibacterial activity of the essential oil of Origanum vulgare (oregano) against pathogenic bacteria to man. Methodology: The essential oil is obtained by maceration of leaves and stems, then purified by filtration assisted distillation separated by rotavapor, the chemical characterization was determined by HPLC and FTIR spectroscopy. The antibacterial evaluation was made by the diffusion method on agar plates against Gram positive (Staphylococcus aureus) and Gram negative bacteria (Escherichia coli, Proteus mirabilis and Klepsiella pneumoniae) using dilutions of 1:10, 1: 100 and 100% of essential oil of oregano. Results: The chromatogram confirming the presence of thymol, carvacrol, ferulic acid and caffeic acid latter two greater intensity responsible for the antibacterial and antioxidant activities. FTIR spectroscopy study by the presence of group's functions as phenols, alcohols and carboxylic acids were identified. The bacteria tested showed sensitivity in dilutions of 1:10, in determining the minimum bactericidal concentration (MBC) showed greater inhibition in microdilutions 1: 100 Essential oil to positive gram-negative bacteria. Conclusion: The essential oil has bacteriostatic and bactericidal activity against bacteria tested showing different degrees of sensitivity.

Antimicrobial, Evaluation, metabolite.

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Introduction

Today has increased the search for natural ingredients to replace synthetic substances which mostly can be harmful to health. Essential oils of plants are mixtures of several chemicals that can be used in industries perfumes, soaps, disinfectants and similar products.

Oregano is a wild aromatic plant since ancient times is used as a seasoning in various dishes; Arcila Garcia as 2003 and 2013 have shown that according to their chemical composition can be used in the pharmaceutical industry, in Mexico more than 40 species of oregano are known, however it is grown mostly for export purposes.

The essential oil of Origanum vulgare (oregano) on its chemical composition has thymol and carvacrol (Garcia 2013), which according to studies are responsible for antibacterial activity. (Davidson and Naidu 2000).

General oregano

Origanum vulgare its botanical name, which derives from Greek, "splendor of the mountain." It is a native plant to Central Europe, South and Central Asia. The Spanish introduced the country oregano around 1970 in the central area, where later was grown extensively (Ávila-Sosa
2008).

In the international market Turkey, Greece and Mexico are the main suppliers of dried oregano manufactured and not manufactured in the world, however, Turkey and Mexico provide 65 to 31% of production respectively the rest of the production bring other countries Mediterranean.

Essential oil

Essential oils are used as therapeutic agents, because of its smell and flavor, many others were used as key ingredients in the industries of perfumes, soaps, disinfectants and similar products. In the food industry they are extensively used as flavoring in a wide variety of food and drink (Holley and Patel, 2005).

They have shown that the essential oil of Origanum vulgare (oregano) has activity against the gam-negative bacteria: Escherichia coli, Salmonella typhimurium, Vibrio cholerae, Klebsiella pneumoniae, Proteus mirabilis and gram-positive bacteria: Staphylococcus with different degrees of sensitivity (Albado, 2001).

Chemical composition

There are several studies on the chemistry of oregano, using aqueous extracts and oils essential. Flavonoids have been identified as apigenin and luteolin, aliphatic alcohols, terpenes and compounds derived phenylpropane (Dardioi, 1997).

In Origanum vulgare they found coumerico acid, ferulic, caffeic, r - hydroxybenzoic and vanillin. The essential oils of Lippia species containing limonene, - cariofilene b, r -cymene, camphor, linalool, a-pinene and thymol, which can vary according to chemotype.

Thymol and carvacrol are natural phenolic compounds considered as potential antioxidants, antifungal and antibacterial agents, present in significant amounts in essential oils and Lippia Origanum genus, species widely used as spices and herbal teas (Burdock, 2005).

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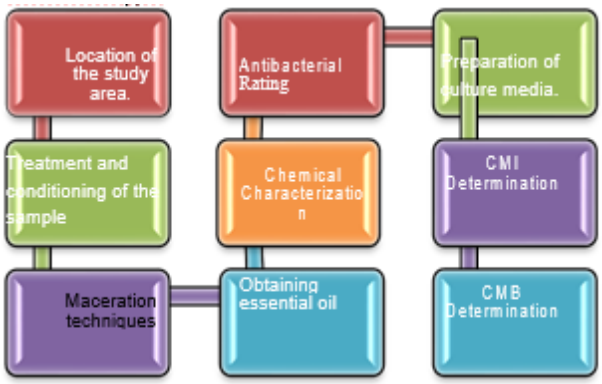
Uses and properties

One of the most common uses for oregano is as relish typical dishes of each country (Aranda Ruiz et al., 2009). In Mexico, it is used as a spice for flavoring and as a preservative in various fresh and processed foods of national and international dishes numbers (Huerta, 1997).

In traditional medicine, the infusion of the leaves is used for the treatment of skin infections, anti-fungal, respiratory diseases, cough, bronchitis, fever and amenorrhea problems to regulate menstruation and hepatic disorders such as diuretic, antihypertensive and antimicrobial, as repellent, abortion, and local anesthetic perspiratory (Arcila Lozano et al., 2007).

The essential oil extracted from oregano leaf is used in the soft drink industry, liquor, pharmaceutical and cosmetology (FAO 2000), as well as in the preparation of soaps and aromatherapy products (Sanchez et al., 2007) and for the production of oil for aeronautics and automotive parts cleaning, and making candles (SEMARNAT, 2007).

Methodology



Location of the study area

The Origanum vulgare serves as an object of study is collected in the community of Karistay Papantla municipality located in the north-central state of Veracruz part.

Treatment and conditioning of the sample

The collected Origanum vulgare plants are washed with water to remove dirt that may contain, after the washing process a selection of leaves and stems that are in good condition subsequently smaller parts is reduced by a grinding process is performed mechanic.

Maceration

The sample C is placed to sun exposure for drying the same for a period of about two weeks at a temperature of 35 °. After drying the sample, placed in a container with cane alcohol 25 ° marinate for three weeks to extract the secondary metabolites.

Oil extraction assisted by distillation method (Broken steam). 400 mL of the mash at 80 rpm solution are placed 55C, obtaining 5% of essential oil of oregano, with a time of two hours.

Once the essential oil is obtained is necessary to filter to purify it, then deposit it in a glass bottle amber and place it in a cool environment to avoid degradation by the effects of light, moisture absorption, evaporation of volatile constituents, oxidation by air and changes due to the action of microorganisms.

HPLC chromatography

A sample of 5 mL of Origanum vulgare essential oil is taken and brought to the facilities of Nanoscience and Nanotechnology Micro and IPN for analysis by HPLC. procedure:

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1. They should be substances with a HPLC purity.

2. They should be filtered with filters of pore diameters about 20 .mu.m and 45μm.

3. You have to wash the tubes that contain the sample with HPLC grade deionized water.

4. Purge to homogenize the column and no a mobile phase residues from an above experiment to ensure that the separated compounds are solely those we add to the machine.

Transform infrared spectrophotometry

FTIR Fourier

A sample of 5 mL of essential oil of oregano is taken and brought to the facilities of Nanoscience and Nanotechnology Micro and IPN for analysis by infrared spectrometry with Fourier Transform (FTIR). Procedure:

1. Preparation, offline sample in aqueous medium.

2. Introduction of the sample system.

3. Removing -in line- of Origanum vulgare essential oil to the organic phase and separating the organic and aqueous phases.

4. Acquisition FTIR spectrum.

5. Analysis of the spectra.

Antibacterial Evaluation

Sterilization of laboratory equipment used.

The pressure steam sterilization is performed in a autoclave.

This device uses saturated steam at 15 pounds pressure allowing the chamber reaches 121 ° C. The sterilization time is usually 15 minutes.

Preparation of culture media.

Petri dishes of 9 cm in diameter are used for 1 cm, which is added 20 mL of nutrient agar solid basis. 5 species of bacteria obtained from the microbiology laboratory of the IMSS regional hospital in Poza Rica de Hidalgo, View assessed. Which are Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis.

Planting strain

With the previously sterile loop, taking a hoe a sample and plot grooves closest to each other inside the box with the culture medium used subsequently incubated in bacteriological stove, in a period of 24 to 48 hours at a constant temperature of 36 ° C.

Preparation of oil dilution.

Dilutions of essential oil and ethyl alcohol according to the following concentrations 1:10, 1 are used: 100 and 100% for each evaluated using sensidiscs bacteria cellulose, leaving to incubate for 48 hours at a temperature of 37 ° C, analyzing results every 24 hours.

Bacteriostatic and bactericidal evaluation.

Once the seed and placed in the sensidiscs bacteria incubated and take two readings of the know (24 and 48 hours) to determine the presence of inhibition zones (lysis) and thus show the antimicrobial activity of oil as shown in the Table 1.

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Determination of minimum inhibitory concentration (CMI)

This technique incorporates the essential oil of Origanum vulgare (antimicrobial) to sensidiscs filter paper. Introduction allows determination of the inhibition of the bacterial strains.

Investigating the pathogenic bacterium it is inoculated into one or more agar plates and on the surface thereof impregnated sensidiscs of different dilutions made above are introduced.

The plates were incubated for 24 and 48 hours and growth is analyzed therein. The diameter of the inhibition zone formed around each disc is evaluated.

In this way you know if the pathogenic bacteria is sensitive, intermediate or resistant to each of the solutions, for this study the symbols shown in the table was used.

Sensitive	Intermediate	Resistant
+	++	-

Table 1 Bacterial inhibition

Determination of minimum bactericidal concentration (MBC)

Its objective is to determine the lowest concentration of an antimicrobial that is able to kill a bacterial strain, in order to compare it with that reached in a given location.

Procedure:

1. Perform microdilutions 1:10 and 1: 100 for essential oil of Origanum vulgare, alcohol as solvent.

2. Enter within the sensidiscs microdilutions filter paper.

3. Perform planting by massive groove of each bacteria in Mueller-Hinton agar and place 4 sensidiscs according to the clock hands.

4. Incubate in bacteriological stove for 24 hours at 36 ° C

5. Interpretation of results.

Determine if the microorganism is sensitive (+), Intermediate (++) or resistant (-) to each solution used during the study.

Results

Location of the study area

The raw material (Origanum vulgare) was collected in the community of Karistay town of Papantla, ., With coordinates Latitude 20 ° 38'26.15 "N, longitude 97 ° 18'29.54 "W; - Tuxpan (Microbiology Lab) collected material to the facilities of the University of Veracruz Poza Rica was carried Campus.

Obtaining essential oil

Method was used for maceration for extraction of secondary metabolites and for separation of the essential oil assisted distillation (broken steam) was used, was obtained with 400 mL of the mash solution (leaves and stems of oregano) 20 mL oil oregano essential having a yield of 5%.

Chemical characterization

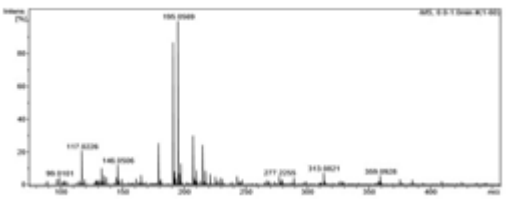

Figure 1 Interpretation of HPLC Origanum vulgare essential oil.

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Figure 1 shows the components found in the essential oil of oregano molecular weights considering the presence of ferulic acid is inferred with an approximate molecular weight 05 to 195 gmol; Carvacrol and thymol with a molecular weight of 146.05 g / mol.

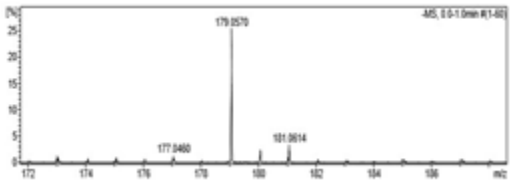

Figure 2 Identification of caffeic acid.

Figure 2 highlights the presence of caffeic acid with a molecular weight of 179.05 and Dihidroactinidiolida with 181.06 g / mol, molecular weights approaching those found in the spectrum, so the presence of secondary metabolites oregano is confirmed.

FTIR interpretation Origanum vulgare essential oil

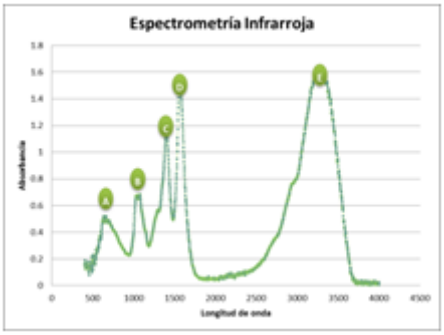

Figure 3 Identification of secondary metabolites by ETIR

absorbance	Functional group
A 624.89 cm^{-1}	cycloalkanes
B 1024.76 cm^{-1}	Fluoroalkanes / aliphatic amines
C 1393.00 cm^{-1}	carboxylic acids
D 1560.38 cm^{-1}	Carboxylic acids / Sales
E 323.06 cm^{-1}	carboxylate / Amines

Table 2 Oregano functional groups by FTIR antibacterial evaluation

The bacteria were obtained through donation Clinical Laboratory Analysis Mexican Social Security Institute in the city of Poza Rica; which they were: Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae. Seeded each bacteria mentioned above in standard culture medium, subsequently reseeding was performed to purify.

CMI determination of essential oil of oregano

bacteria	inhibition Sensible (+), Intermediate (++), Resistant (-)		
Gram positive	1:100	1:10	100%
Staphylococcus aureus	-	+	-
Gram negative			
Proteus mirabilis	+	+	+
Escherichia coli	-	+	++
Klebsiella pneumoniae	+	+	-

Table 3 Determination of oil CMI

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According to the assessment antibacterial essential oil of oregano has antibacterial activity to Staphylococcus aureus (gram positive) and Proteus mirabilis, Escherichia coli, Klebsilella pneumoniae (Gram negative) concentration 1:10. Table 4.

CMB determination of essential oil of oregano

bacteria	microdilutions	
Gram positive	1:100	1:10
Staphylococcus aureus	++	+
Gram negative		
Proteus mirabilis	++	+
Escherichia coli	+	-
Klebsiella pneumoniae	++	+

Table 4 Determination of OMB According to the evaluation of the minimum bactericidal concentration (MBC)

Table 4 is presented in Table oregano essential oil has antibacterial activity, using microdilutions to Staphylococcus aureus (gram positive) and Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae negative). The concentration of 1: 100 exhibits greater inhibition against all strains tested.

Conclusions

Based on the results obtained it is concluded that the method of extraction by maceration of the essential oil of oregano (Origanum vulgare) was ineffective showing a 5% yield.

The characterization study using HPLC and FTIR spectroscopy confirmed the presence of thymol and carvacrol, responsible for the antimicrobial properties of oregano.

Compared to the 2013 study by García components are found in greater intensity ferulic acid and caffeic acid.

In the analysis of the Minimum Inhibitory

Concentration

(CMI) has inhibitory results dilutions 1:10 Staphylococcus aureus and gram-negative bacteria Escherichia coli, Proteus mirabilis and Klepsiella pneumoniae.

In the analysis of the minimum bactericidal concentration (MBC) with the use of microdilutions 1: 100 Essential oil increased bacterial inhibition with gram positive bacteria Staphylococcus aureus and gram-negative bacteria Escherichia coli, Proteus mirabilis and Klepsiella pneumoniae was presented.
The essential oil of oregano finally concluded that the microbiological evaluation gives bacteriostatic and bactericidal activity with greater intensity as small dilutions shown inhibitory effects.

Referencies

Albado E, Sáez G, Gabriel S. 2001. Composición química y actividad antibacteriana del aceite esencial del Origanum vulgare (orégano). Rev Med Hered 12 (1), 2001 (Rev Med Hered 2001; 12: 16-19).

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods - a review. International Journal of Food Microbiology. 94. 223– 253.

Davidson PM, Naidu AS. 2000. Phyto-Phenols. In: Naidu, A.S. (Ed.), Natural Food Antimicrobial Systems. CRC Press, Boca Ratón, FL, pp. 265-294.

ISSN-On line: 2410-4191

Cela, R., R. A. Lorenzo, et al. (2002). Técnicas de separación en química analítica. Madrid, Editorial Síntesis.

Rzedowki, J. y G. Calderon. 2002. FAMILIA VERBENACEAE. Fascículo 100. Flora del bajío y de regiones adyacentes. Instituto de Ecología A.C. Centro Regional del Bajío. Conacyt y CONABIO, México.

Andrews MJ. Determination of Minimum Inhibitory Concentration. J Antimicrob Chemother 2001

Sanchez, O., Medellin, A. Aldama, B. Goettsch, J. Soberon 2007. Capítulo 8 "Evaluación del riesgo de extinción de Lippia graveolens de acuerdo al numeral 5.7 de la NOM-058-SEMARNAT-2001".

Flores, J. E., Percepción remota de contaminantes atmosféricos mediante dos métodos espectroscópicos en la zona centro de la Ciudad de México: FTIR y DOAS, tesis Maestría (Maestría en Ciencias de la Tierra)-UNAM, Centro de Ciencias de la Atmosfera, 2003.

Callejas T., Pablo A. Obtención de extractos de plantas en medios ácidos y/o alcohólicos para aplicaciones medicinales y alimenticias. Tesis. Universidad Nacional de Colombia. Manizales. 2002

González, V. Ángela A. Obtención de aceites esenciales y extractos etanólicos de plantas del Amazonas. Tesis. Universidad Nacional de Colombia. Manizales. 2004.

Madigan, M., Parker, J. 2002. Brock. Biología de los microorganismos. Décima Edición. Ed. Pearson.

Romero C, Raúl. Microbiología y parasitología humana: bases etiológicas de las enfermedades infecciosas y parasitarias. Tercera Edición. Editorial Médica Panamericana, 2007.

Villavicencio G., E., O. U. Martínez B. Y A. Cano P. 2005. OREGANO Recurso con alto potencial. Rev. Ciencia y Desarrollo.

ISSN-On line: 2410-4191