Colegio de Postgraduados

Antibacterial activity of the *Calendula officinalis* L. essential oil on *Escherichia coli*

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ABSTRACT
In developing countries, the risk of getting sick from eating food contaminated with *Escherichia coli* is very high. As a consequence of the multidrug resistance of this bacterium, a therapeutic alternative has been sought in the plant kingdom. The **Objective** of this research was to evaluate the antibacterial effect of pot marigold (*Calendula officinalis*) essential oil (EO) on the growth of *E. coli*. **Design/Methodology/Approach**: The antibacterial activity was determined using a Kirby-Bauer disk diffusion susceptibility test. A 90 to 60% dilution of EO generated 24 to 22 mm halos. The EO was subjected to a GC/MS analysis. The **results** showed that cadinene (53.8%) was the main constituent, followed by germacrene (22.5%). The minimum inhibitory concentration was 7 μg mL^{-1}. **Findings/Conclusions**: *C. officinalis* EO can be considered as an option in the treatment against this enterobacteria.

Keywords: *Calendula officinalis*, pot marigold, antimicrobial resistance, essential oil (EO), antibacterial compound.

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INTRODUCTION

Infectious diseases are a public health problem whose frequency and lethality are increasing by leaps and bounds and is therefore considered as one of the greatest threats to world health, food security, and development (WHO, 2020). The pathogenic microorganisms' antibiotic resistance is a consequence of the indiscriminate or wrong use of drugs and the presence of natural processes that produce mutations in bacteria and fungi (Aslam *et al.*, 2018). Antibiotic resistance also leads to a great increase in the costs of the health sector, because the number of patients admitted to hospitals and, therefore, the number of medicines supplied have increased (Dadgostar, 2019).

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Antibiotics only kill sensitive bacteria. Resistant bacteria survive, reproduce, and thrive by natural selection (Aslam *et al*., 2018), creating multidrug-resistant microorganisms. *Escherichia coli* is part of the common gut flora found in the intestine of humans and animals. Nonetheless, it is one of the main agents of infections transmitted by water, food, and direct contact with infected people. Approximately 30% of the bacteremia cases in hospitals are caused by this bacterium —for example, a 71% increase in the bloodstream infections reported by the European Antimicrobial Resistance Surveillance Network. This figure is responsible for approximately 280 million cases of diarrhea and bloodstream infections and 400,000 deaths every year, most of them among young children from all over the world (Salame-Khouri *et al*., 2018; Steffen *et al*., 2015). In addition, the antibiotic resistance of some *E. coli* strains has been detected in all parts of the world and is one of the major public health challenges. In Mexico City, multidrug-resistant strains of *E. coli* have been reported: 80% are resistant to rifaximin, 30% to ampicillin, and 50 to 90% to fosfomycin, trimethoprim-sulfamethoxazole, neomycin, furazolidone, chloramphenicol, and ciprofloxacin (Novoa-Farias *et al*., 2016).

This situation, as well as the side effects of certain antibiotics, have encouraged researchers to find new antimicrobial agents in phytotherapy, especially in plants which have been traditionally used in Mexico. *Calendula officinalis* L. is an herbaceous plant of the Asteraceae family that contains phytochemicals with antibacterial activity, including flavonoids, saponins, carotenoids, triterpenoids, and tannins. Therefore, the purpose of this work was to research the effect of pot marigold EO on a multidrug-resistant strain of *E. coli*.

MATERIALS AND METHODS

Inflorescence production

Except for the gas chromatography analysis, all phases of this work were carried out at the Colegio de Postgraduados, Campus Montecillo. Pot marigold was sown in a peat moss:agrolite:soil (2:2:1 v/v) substrate, inside 5-cm diameter black polyethylene bags, at 20 to 30 °C, under greenhouse conditions. After 120 days, the seedlings were transplanted into the open field. The rows were established 70 cm apart from each other and there were 30 cm between plants. The agronomic management included irrigation, weed removal, and control of pests and diseases. The flower heads were harvested with a 0.5-cm peduncle cut. The harvested inflorescences were shade dried at room temperature (18 °C) for 7 days. An average yield of 420 kg ha^{-1} of dry heads was obtained.

Obtaining the EO

The dried plant material was subjected to steam distillation for 2 h in a 60-L distiller. Afterwards, the water-oil emulsion was recovered and three applications of 50 mL of methylene chloride (CH_2Cl_2) were added to separate the oil. Anhydrous sodium sulfate (Na_2SO_4) was then added to completely remove the water. Subsequently, the result was concentrated in a Buchi B-480 Water Bath at 40 °C (baine-marie). The EO was transferred with a Pasteur pipette to a 1.5-mL vial and stored at 4 °C.

Gas chromatography analysis of the EO

The samples were analyzed at the Unidad de Servicios de Apoyo a la Investigación y a la Industria, Facultad de Química, UNAM, in order to determine the chemical composition of pot marigold EO, using a Pegasus 4D gas chromatography/mass spectrometry (GC/MS) (LECO), with a Time of Flight analyzer. The GC/MS system used a stationary phase HP-5MS (DB5) / 10m $\times$ 0.18mm $\times$ 0.18mm thick column and Ultra High Purity Grade 5.0 helium (Praxair) was used as carrier gas at a flow of 1 mL min^{-1}. The following standards were used: naphthalene, 1, 2, 3, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-,1S-cis) and 1,6-Cyclodecadiene, 1-methyl- 5-methylene-8-(1-methylethyl)-,[s-(E,E)].

Bioassays

The Instituto de Ciencias Básicas e Ingeniería de la Universidad Autónoma del Estado de Hidalgo provided a strain of the multidrug-resistant enterohemorrhagic *Escherichia coli* (EHEC), which can resist the following antibiotics: amoxicillin-clavulanic acid, colistin, erythromycin, gentamicin, and neomycin (Gómez -Aldapa *et al.*, 2016). It was cultivated in a Petri-dish with an Eosin-Methylene Blue (EMB) Agar (BD®) solid culture medium. Antibacterial activity was assessed using the Kirby-Bauer disk diffusion susceptibility test. The bacterial inoculum was 100 μL at 1×10^8 cells mL^{-1} and had a radiant streaking method. We evaluated 13 treatments: pure EO, 10-90% dilutions of EO, and controls (water and amikacin). A 5-mm diameter Whatman® No. 1 filter paper disc was placed in the center of each Petri-dish, with 10 μL per treatment and four repetitions. Petri-dishes were incubated at 37 °C for 24 h.

Inhibition diameter

After incubation, all Petri dishes were photographed and scanned with an HP Deskjet multifunctional. The GIMP (GNU Image Manipulation Program) free software was used to edit the threshold of the files, converting them into black and white images. Inhibition zone diameters were measured using the IMAGEJ digital image processing program. An analysis of variance and a Tukey's mean comparison (α, 0.05) were performed using the SAS® v. 9 statistical package.

Determination of minimum inhibitory concentration (MIC)

MIC values were determined considering the lowest concentration of the crude extract than can inhibit the visible growth of *E. coli* after the incubation period.

RESULTS AND DISCUSSION

Phytochemical analysis

Some climate components, crop management, and the phenological stage of the plant at the time of harvest affect its content of secondary metabolites (Russo *et al.*, 2015).

The analysis showed that the EO is composed of 95.85% sesquiterpenes and 4.15% other hydrocarbons. The most abundant components (germacrene and cadinene) are non-oxygenated sesquiterpenes that account for 76.4% of the total oil. These results match the findings of Salome (2015), who determined that cadinene was the most abundant compound in all the floral morphotypes of pot marigold that they studied.

As seen in Figure 1, there are several important peaks with retention times of 606.816 s (cadinene) and 604.066 s (germacrene).

Antibacterial activity

The essential oil has great potential for antibacterial activity against *E. coli*, according to both the interpretation of the inhibition zones (in mm) in the Kirby-Bauer disk diffusion susceptibility tests and the significant differences in the various concentrations of the essential oil.

Figure 2 shows the susceptibility groups found with the statistical analysis. The bacterium turned out to be susceptible to treatments with a 100 to 70% dilution of EO, with average diameters of 30, 28, 27, and 26 mm. However, 60-40% concentrations had an intermediate effect (23, 20, and 16 mm). Finally, levels of 30-10% allowed the bacteria to grow relatively undisturbed. All dilution levels showed a visible effect and had a metallic green sheen resulting from the rapid lactose fermentation and strong acid production (Figure 3).

Likewise, the results showed that, in the control treatments with methylene chloride (CH_2Cl_2), bacterial growth was 100%, ruling out any toxic effect of the solvent. The inhibition of bacterial growth reached MIC values of 7 μg/mL.

Radioza *et al.* (2007) found that pot marigold EO inhibited the in vitro growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Its

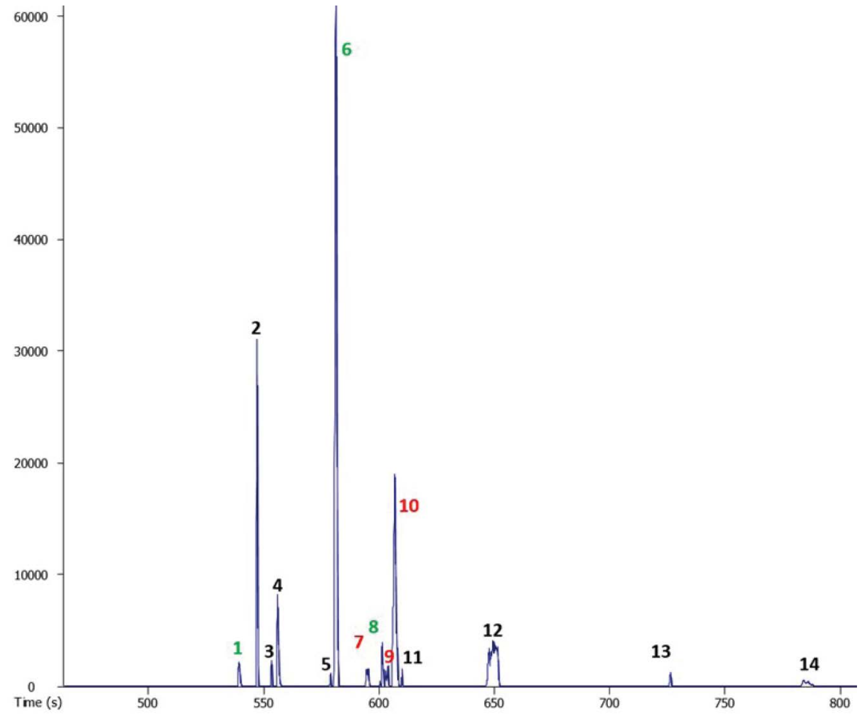

Figure 1. Phytochemical profile of pot marigold EO obtained with a gas chromatography/mass spectrometry: 1, 6, and 8 Germacrene; 2 Cubebene; 3 Copaene; 4 -caryophyllene; 5 Isoledene, 7, 9 and 10 Cadinene; 11 Seychellene; 12 Muurolene; 13 Eicosane; 14 Heptadecane.

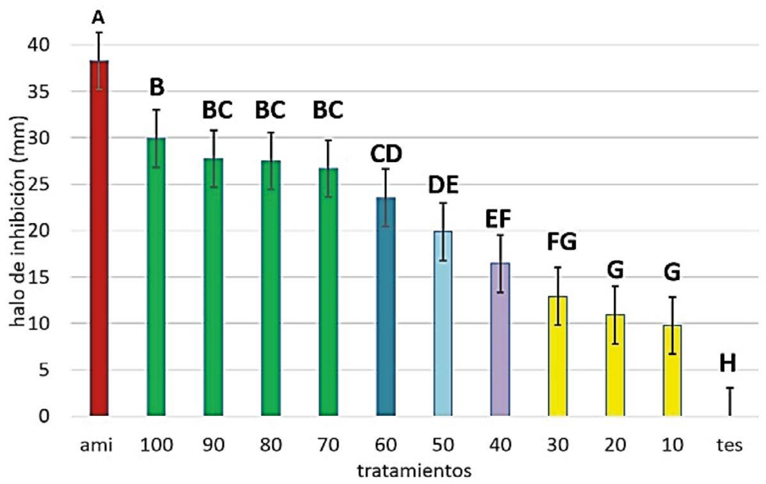

Figure 2. Inhibition halos of the *in vitro* growth of *E. coli* produced by different concentrations (10-100%) of *Calendula officinalis* EO, with amikacin (ami) and water (tes) as positive and negative controls. Each value is the mean of 4 replicates ± SE. Different letters are treatments with statistical significance ($p>0.0001$, $\alpha=0.05$, Tukey).

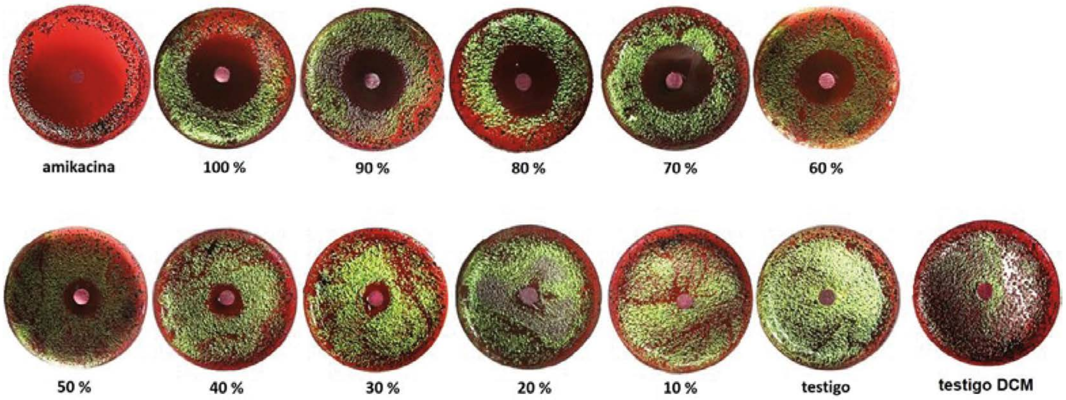

Figure 3. Inhibition halos caused by different dilutions (expressed as a percentage) of pot marigold EO on the growth of *E. coli in vitro*. The 100-10% dilutions are compared with a commercial antibiotic (amikacin), the control (distilled water), and the DCM control (methylene chloride).

abundant content of sesquiterpenes —which act as phytoalexins, antibiotic compounds that are capable of intercalating in the lipids of the bacterial cell membrane— distorted the structure of their membranes and make them more permeable (Devi *et al.*, 2010). Salazar-Gómez *et al.* (2020) have likewise reported that this group of compounds has anticancer, anti-inflammatory, and antimicrobial activity. Although the antibacterial effect of an essential oil depends on its mixture of metabolites, its activity can be attributed to its major components. Pot marigold EO is rich in compounds such as: cadinene (Salome-Abarca *et al.*, 2015), which has anti-inflammatory and antiseptic properties (Kaufman *et al.*, 1999); germacrene, which has insecticidal activity against mosquitoes, in addition to its well-known antimicrobial (De Lima *et al.*, 2010), cytotoxic (Palazzo *et al.*, 2009), and antibacterial effect against *Pseudomonas syringae*; caryophyllene, which has proven to be active against *S. aureus* (De Lima *et al*., 2010); and cubeben, which inhibits the growth of *S. aureus*, *B. subtilis*, *Klebsiella pneumoniae*, and *P. aeruginosa* (Sharifi-Rad, 2014).

Based on the evidence found in the scientific literature and the results of this work, sesquiterpenes could be the most important active principle in the antibacterial action of this plant, given both their pharmacological actions and their abundance in pot marigold. Nevertheless, the action of other active principles, such as flavonoids —to which antibacterial and antiallergic actions are attributed—, has not been ruled out. The antihemorrhagic, anti-inflammatory, antipyretic, antiseptic, and phlebotonic properties of this species, reported by Melgarejo *et al*. (2008), could also support the patient's full recovery.

CONCLUSION

Due to the abovementioned results, the EO of *C. officinalis* could be used to support the treatment of the disease caused by the antibiotics-resistant *E. coli*.

REFERENCES

Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z. 2018. Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist., 10*(11):1645-1658. doi: 10.2147/IDR.S173867. PMID: 30349322; PMCID: PMC6188119.

Bauer AW, Kirby WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol., 45*(4): 493-496. PMID: 5325707

Dadgostar P. 2019. Antimicrobial resistance: implications and costs. *Infect Drug Resist*., 20;12:3903-3910. doi: 10.2147/IDR.S234610. PMID: 31908502; PMCID: PMC6929930.

Devi KP, Nisha SA, Sakthivel R, Pandian SK. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of ethnopharmacolo gy.130*(1):107-15. doi: 10.1016/j.jep.2010.04.025.

De Lima SG, Citó MG, Lopes JAD, Neto JMM, Chaves MH, Silveira ER. 2010. Fixed and volatile constituents of genus croton plants: *C. adenocalyx* baill - Euphorbiaceae. *Revista latinoamericana de química, 38*(3), 133-144. Recuperado en 27 de enero de 2023, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0370-59432010000300001&lng=es&tlng=en. 33 – 144.

Gómez-Aldapa CA., Cerna-Cortes JF., Rangel-Vargas E., Torres-Vitela MR., Villarruel-López A., Gutiérrez-Alcántara EJ., Castro-Rosas J. 2016. Presence of Multidrug-Resistant Shiga Toxin-Producing *Escherichia coli*, Enteropathogenic *E. coli* and Enterotoxigenic *E. coli*, on Raw Nopalitos (*Opuntia ficus-indica* L.) and in Nopalitos Salads from Local Retail Markets in Mexico. *Foodborne Pathogens and Disease, 13*(5), 269-274. https://doi.org/10.1089/fpd.2015.2065

Kaufman PB, Cseke, LJ, Warber S, Duke JA, Brielmann HL. 1999. Natural Products from Plants. United States of América: CRC Press. 343 p.

Melgarejo López N, Álvarez Bustamente G, Alonso Abad A. 2008. Plantas medicinales. Guía para su uso en la atención primaria de salud. Argentina: Corpus. 109 p

Novoa-Farias O, Frati-Munari AC, Peredo MA, Flores-Juarez S, Novoa-Garcia O, Galicia-Tapia J, Romero-Carpio CE. 2016. Susceptibility of bacteria isolated from acute gastrointestinal infections to rifaximin and other antimicrobial agents in Mexico. *Rev Gastroenterol Mex 81*(1):3–10. doi: 10.1016/j.rgmx.2015.07.003.

Organización Mundial de la Salud. 2020. https://www.who.int/es/news-room/fact-sheets/detail/resistencia-a-los-antibi%C3%B3ticos

Palazzo MC, Agius BR, Wright BS, Haber WA, Moriarity DM, Setzer WN. 2009. Chemical compositions and cytotoxic activities of leaf essential oils of four Lauraceae tree species from Monteverde, Costa Rica. *Rec Nat Prod 3*(1): 32-37. EISSN:1307-6167

Radioza SA, Lurchak LD. 2007. Antimicrobial activity of *Calendula* L. plants. *Mikrobiol Z. 69*(5):21-5. Ukrainian. PMID: 18217350.

Russo DE, Rodríguez MM, Apóstolo NM. 2015. Flavonoides de Calendula officinalis L. bajo cultivo. Efecto de diferentes fechas de siembra y fertilización. *Dominguezia* - Vol. 31(1):23-29.

Salame-Khouri L, Contreras-Pichardo B, Arias-Rodríguez S. 2018. Epidemiología de las bacteriemias por Escherichia coli en dos hospitales de tercer nivel de la Ciudad de México. *An Med Asoc Med Hosp ABC.;63*(2):91-95.

Salazar-Gómez A, Ontiveros-Rodríguez J.C, Pablo-Pérez SS, Vargas-Día ME, Garduo-Siciliano L. 2020. The potential role of sesquiterpene lactones isolated from medicinal plants in the treatment of the metabolic syndrome - A review. *S. Afr. J. Bot*. 135:240–251. doi: 10.1016/j.sajb.2020.08.020.

Salomé-Abarca L, Soto-Hernández RM, Cruz-Huerta N, González-Hernández V A.

2015. Chemical composition of scented extracts obtained from *Calendula officinalis* by three extraction methods. *Botanical Sciences, 93*(3), 633-638. https://doi.org/10.17129/botsci.143

Sharifi-Rad J. Hoseini-Alfatemi SR, William NS. 2014. Chemical composition, antifungal and antibacterial activities of essential oil from *Lallemantia royleana* (Benth. In Wall.) *Journal of Food Safety*. 35: 19-25. https://doi.org/10.1111/jfs.12139

Steffen R, Hill DR, DuPont HL. 2015. Traveler's diarrhea: a clinical review. *JAMA*. 6:313(1):71-80. doi: 10.1001/jama.2014.17006. PMID: 25562268.