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Facultad de Ciencias Agropecuarias

Universidad Nacional de Trujillo

RESEARCH ARTICLE

Bursera graveolens essential oil: Physiochemical characterization and antimicrobial activity in pathogenic microorganisms found in *Kajikia audax*

Kevin Carlos Noel-Martinez[1]; Gerardo Juan Francisco Cruz[2]; Rosa Liliana Solis-Castro[3,*]

[1] Ingeniería Agroindustrial, Universidad Nacional de Tumbes, Av. Universitaria s/n, Tumbes, Tumbes. Peru.
[2] Laboratorio Análisis Ambiental, Universidad Nacional de Tumbes, Av. Universitaria s/n, Tumbes, Tumbes. Peru.
[3] Laboratorio Biología Molecular, Facultad de Ciencias de la Salud, Universidad Nacional de Tumbes, Av. Universitaria s/n, Tumbes, Tumbes. Peru.

* Corresponding author: rsolisc@untumbes.edu.pe (R. L. Solis-Castro).

Received: 11 January 2021. Accepted: 17 June 2021. Published: 19 July 2021.

Abstract
Essential oils are products from aromatic plants, which, due to their biological, allelopathic, antioxidant and antimicrobial effects, are important for food preservation. *Kajikia audax* is a fish of great commercial importance, however, it is highly perishable, requiring strategies to extend its shelf life. *In vitro* antimicrobial activity of *Bursera graveolens* essential oil was determined against microorganisms isolated from *K. audax*. Essential oil was extracted by steam distillation, obtaining a yield of 1.25%, a density of 0.83 g/ml and a refractive index of 1.473°, in addition, it was determined by GC-MS that *D*-limonene (77.6%) is the majority compound. Antimicrobial tests showed that the minimum inhibitory concentration (MIC) for *Aeromonas salmonicida* and *Pichia kudriavzevii* was 1.62 mg/ml and 6.48 mg/ml respectively, and the minimum bactericidal concentration (MBC) was 25.92 mg/ml for both microorganisms. *Pseudomonas aeruginosa* showed total resistance against the concentrations used. *B. graveolens* essential oil turned out to be a potential product to control the growth of microorganisms isolated from *K. audax*, however, it should be tested against species of the genera *Vibrio*, *Flavobacterium*, *Shewanella*, *Lactococcus* and *Streptococcus* that cause spoiling of hydrobiological products.

Keywords: spoiling microorganisms; *Bursera graveolens*; essential oil; minimum inhibitory concentration; minimum bactericidal concentration; marine food.

DOI: https://dx.doi.org/10.17268/sci.agropecu.2021.033

Cite this article:
Noel-Martinez, K. C., Cruz. G. J. F., & Solis-Castro, R. L (2021). *Bursera graveolens* essential oil: Physiochemical characterization and antimicrobial activity in pathogenic microorganisms found in *Kajikia audax*. *Scientia Agropecuaria*, *12*(3), 303-309.

1. Introduction
Pathogenic microorganisms are the main cause of hydrobiological food contamination (Walczak et al., 2017). In fish, these pathogens alter their quality and reduce fish shelf-life, making them not able for human consumption (Peña et al., 2019). Due to these pathogens, foodborne diseases also occur, which constitute a serious health problem worldwide (Soto et al., 2016).
Pathogens can cause alterations in the nutritional and organoleptic properties of foods (Rani et al., 2016); therefore, their detection is very important (Law et al., 2015). To solve this problem, synthetic antimicrobial agents are used although it is currently replaced by compounds from natural origin (Mostafa et al., 2018; Pastrana-Puche et al., 2017). There has been an increase in the search for efficient alternative for food preservation (Savoldi et al., 2020; Bachir & Benali, 2012); and different "in vitro" studies demonstrate the efficacy of spices to inhibit pathogenic microorganisms (Huong et al., 2021; El Jery et al., 2020).
There are many possible useful bioactive substances that are derived from plants (Bajpai et al., 2012). It is the case of essential oils which have antimicrobial properties. They are composed of a variety of active components such as: terpenes, terpenoids, carotenoids, coumarins and curcumin. (Calo et al., 2015; Cevallos & Londoño, 2017; Pandey et al., 2017). Due to the inhibitory activity that reduces the growth of bacteria, yeasts and fungi, essential oils are a promising alternative for food preservation (Mancuso et al., 2019).
Most essential oils are obtained by steam distillation and hydro-distillation; these techniques are widely used because of its high performance; the purity of the obtained essential oil and it does not require sophisticated

technology (Patiño & Martínez, 2014). However, there are others extraction methods such as hydrodistillation assisted by microwave, solvents extraction, among others. To determine the antimicrobial activity "*in vitro*" against different pathogenic microorganisms, different methods are used such as: macrodilution or tube dilution method, microdilution or plate dilution, disk diffusion or Kirby-Bauer method, among others which evaluate minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Laith, 2021; Parvekar et al., 2020; Wijesinghe et al., 2021).

Bursera graveolens it is a deciduous tree that grows in the lower part of the western slopes of northern Peru, and it is called "palo santo" in these zones (Puescas, 2010). It is an aromatic plant that is used for traditional medicine. However, the production of the essential oil of this species is attractive because of its applications (Preedy, 2015). Fon-Fay et al. (2017) analyzed "in vitro" antimicrobial activity of B. *graveolens* against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enteritidis*, *Aspergillus niger* and *Penicillium citrinum*; finding a MIC of 0.005 µl/ml for *Staphylococcus aureus*. Celina Luján-Hidalgo et al. (2012) studied the antimicrobial activity of the essential oil from *B. graveolens* leaves against *S. aureus* and *B. subtilis* (MIC of 7.45 mg/ml and 7.29 mg/ml, respectively). Additionally, the authors, found MIC of 1.71 mg/ml to 11.99 mg/ml for yeast. The antimicrobial activity of the essential oil of *B. graveolens* branches has also been investigated by Sotelo-Méndez et al. (2017). It was found that that the essential oil has significant antimicrobial properties against *S. aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *E. coli*, *Salmonella choleraesuis*, and *Candida albicans*

Although the antimicrobial capacity of *B. graveolens* essential oil has been demonstrated in the previous studies, the origin (type of food) of the microbial strains used is not specified. These results might not be applicable for the most of kind of food. To stablish effective strategies for specific kind of food, the antimicrobial test must be done against microorganisms isolated from the same food. In the present study, the "*in vitro*" antimicrobial activity of *Bursera graveolens* essential oil was determined against microorganisms isolated and molecular identified from *Kajikia audax*.

2. Materials and methods

2.1 Essential oil extraction from *B. graveolens*

The extraction process was carried out by steam distillation. The raw material was obtained in the district of Rica Playa- Quebrada La Sesina (3-48'42"S 80-31'54"W), belonging to the national park Cerros de Amotape Tumbes – Peru which consisted of 3 pieces of the trunk of *B. graveolens* with weights of: 2.61 kg, 3.32 kg and 2.41 kg, of which with the help of an electric carpentry planer a total of 6.98 kg was obtained. The swarf was put in an autoclave machine and without coming in contact with the water, was subjected to a heat source for 1 h 45 minutes, the steam produced by the autoclave was circulated as a water coolant by a distiller. Once becoming an aqueous solution, with the help of a separatory funnel, the oil was separated from the water by difference of weights, thus obtaining 105 ml *B. graveolens* essential oil that was then stored in an amber bottle in refrigeration.

2.2 Yield determination

The yield of *B. graveolens* essential oil, was obtained in terms of percentage by weight (%) based on the mass of the dry material used (Kg), using the following formula (Cruz et al., 2013): $Y\% = [(Mass.EO)/(Mass.MV)]\times 100$; where *Y%*: yield in %; *Mass.EO*: mass of essential oil; *Mass.MV*: mass of the material placed in extractor "g".

2.3 Chemical characterization of the essential oil

Chemical characterization was performed by Agilent Technologies 7890A gas chromatograph with Agilent Technologies 5975C mass spectrophotometer detector, with the following chromatographic conditions: column J&W 122-1545.67659 DB-5ms, 325 °C: 60 m x 250 m x 0.25 mm. Thermal profiling: starting at 40 °C it rises 5 °C/min up to 180 °C; then 2.5 °C/min up to 200 °C where it stays for 5 min then finally rises 10 °C/min up to 300 °C for 3 min. The sample injected was 1 µl which was obtained from the mixture of 20 µl of essential oil in 1 ml of dichloromethane. The run time was 54 minutes, and the carrier gas was helium (1 ml/min).

2.4 Determination of density, refractive index and maximum absorbance

The density was obtained using a standardized pycnometer, which was weighed beforehand on an analytical balance for an accurate measure, then the weight of the essential oil sample was scaled. For the measurement of refractive index, a type of refractometer (ABBE Schmidt Haensh, Germany) was used. The method based on ISO 4735 was used to measure the maximum absorbance in the ultraviolet radiation range, which is based on the determination of the absorption spectrum of the alcoholic dissolution of the essential oil (250 mg in 100 ml ethyl alcohol), using pure ethanol, in the wavelength range between 260 and 340 nm, a spectrophotometer was used for these measurements (LAMBDA 365 double-beam UV/Vis Spectrophotometer by PerkinElmer, USA) (Cruz et al., 2013).

2.5 Isolation and identification of microorganisms

In a flask, a sample of 25 g of *Kajikia audax* was weighed, then at room temperature 225 ml of diluent (1% peptone water) was added. Successive dilutions were performed up to 10^{-3}, and in petri dishes 0.1 ml of this dilution was inoculated with agar: Eosin methylene blue (EMB) agar or Endo agar for isolation of *Escherichia coli* and enterobacteria; Baird-Parker agar for staphylococci; Thiosulfate-citrate-bile salts-sucrose (TCBS) agar for vibrio; *Salmonella Shigella* (SS) agar for *Salmonella* and Sabouraud agar for fungi and yeast (DIGESA, 2008). To demonstrate cell morphology Gram staining was used and for the identification of each strain the PCR (*Polymerase chain reaction*) of the 16S rRNA gene was used for bacteria (Wang et al., 2011) and ITS (*Internal transcribed spacer*) for fungi (White et al., 1990) with prior sequencing by the company Macrogen Inc (Korea).

2.6 Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) by tube dilution method

A stock solution was prepared by adding 2 ml of *B. graveolens* essential oil, 2ml of DMSO (Dimethyl sulfoxide) and 12 ml of Mueller Hinton Broth (MHB) to a flask (Hili et al., 1997). The inoculum was prepared with microorganisms developed in Muller Hinton Broth for 18 to 24 hours, with a bacterial suspension adjusted to the McFarland standard of 0.5 and a 1/10 dilution getting a final concentration of 10^7 CFU/ml. From the stock solution, serial dilutions were made, preparing 10 test tubes with the following concentrations of essential oil: 207.33 mg/ml; 103.67 mg/ml; 58.3 mg/ml, 25.91 mg/ml; 12.96 mg/ml; 6.48 mg/ml; 3.24 mg/ml; 1.62 mg/ml; 0.81 mg/ml; 0.41 mg/ml. To each tube, 10 µl of the inoculum was added. Two test tubes were kept without being inoculated: a broth tube as a negative growth control and a tube with essential oil as a positive growth control. The concentration of essential oil that does not show growth is the minimum inhibitory concentration (MIC). With the help of an inoculation loop a sample from the tubes where no turbidity was observed was transferred to a plate with Mueller-Hinton agar. Thus, the content of the positive control tube should develop growth in the dish, and the concentration where no growth appears in the plate will correspond to the minimum bactericidal concentration (MBC) (Servicios Antimicrobianos, 2012).

3. Results and discussion

3.1 Yield and characterization of *B. graveolens* essential oil

Table 1 presents the organoleptic and physicochemical properties of the essential oil. The density and refractive index were close to those reported before in the essential oil from the leaves and fruits of *B. graveolens* (Cruz et al., 2013; Luján-Hidalgo et al., 2012; Rey-Valeirón et al., 2017). As for the yield, it was much higher than that found by Cruz et al. (2013) with a value of 0.09% and that found by Luján-Hidalgo et al. (2012) with a value of 0.10%. Consequently, it should be considered that certain circumstantial factors may have influenced the characteristics of the oil such the location from which the raw material was extracted (Leal-Torres et al., 2013), as well as the distillation time and the condition of the material (Meyer-Torres et al., 2018).

Table 1
Organoleptic and physicochemical properties of *Bursera graveolens* essential oil

Parameter	Observation
Color	Clear light yellow
Odor	Intense and pungent
Taste	Spicy
Density	0.83 g/ml
State	Liquid
Yield	1.25%
Refractive Index	1.473°

Furthermore, the maximum absorption of the essential oil analyzed in Figure 1 paralleled with a wavelength (nm) close to 272.5 nm in the UV-VIS wavelength range, which was found below that reported by Cruz et al. (2013) at a value of 293 nm.

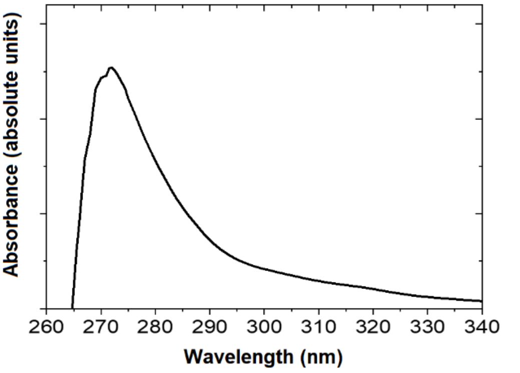

Figure 1. Absorption of *Bursera graveolens* essential oil obtained by Double-Beam UV-Vis Spectrophotometer.

The gas chromatography analysis of the extracted essential oil allowed the identification of 28 chemical compounds making up 82.35% of the chemical composition of the essential oil, with 6 others being unknown. Table 2 shows the most important compounds, such as terpenes, monoterpenes, diterpenes, sesquiterpenes, and some oxygenated compounds found in this work in comparison with previous and recent works. The main constituents were menthofuran (5.16%), α-terpineol (4.01%), germacrene D (2.33%), carvone (0.83%). The largest compound was monoterpene D-limonene with 77.06%.

Studies related to the chemical composition of *B. graveolens* essential oil using the same method of analysis or a variation of it, mention monoterpenes as main constituents, namely limonene and menthofuran (Carmona et al., 2009; Monzote et al., 2012; Jaramillo-Colorado et al., 2019; Rey-Valeirón et al, 2017) or sesquiterpenes as cadinene, farnesene and germacrene-D (Luján-Hidalgo et al., 2012; Sotelo-Méndez et al., 2017). However, Sotelo-Méndez et al. (2017), Luján-Hidalgo et al. (2012), Rey-Valeirón et al. (2017) and Carmona et al. (2009) found other constituents of *B. graveolens* essential oil, which were not found with gas chromatography in the present study.

We may explain this difference by the source of where the oil was extracted, and some authors obtained the oil of the leaves (Luján-Hidalgo et al., 2012; Monzote et al., 2012) or fruits (Rey-Valeirón et al., 2017) or a mixture of stems and leaves (Jaramillo-Colorado et al., 2019) of *B. graveolens*, while in this research it was attained specifically from the part of the stem. Additionally, it may be due to some ecological conditions under which the plants persisted which may alter the qualitative and quantitative characteristics of the essential oil, such as those caused by the specific season the samples were gained (Sotelo-Méndez et al., 2017). In the chemical composition of the essential oil different terpenic compounds were found, such as monoterpenes and sesquiterpenes, that are aromatic compounds which contribute to the menthol aroma of the dry trunk of *B. graveolens* (Yukawa et al., 2006).

3.2 Isolation of microorganisms

From the sample of *Kajikia audax*, 6 strains were isolated out of which 2 were not viable. The identified microorganisms were: *Aeromonas salmonicida* (MK1), *Pseudomonas aeruginosa* (PS1), *P. aeruginosa* (PS2) and *Pichia kudriavzevii* (lev). The molecular identification is shown in Table 3, such as percent of similarity and the accession number of the organism type.

The microscopic characteristics of bacteria closely resemble Gram-negative bacteria and that of fungi to yeast (Figure 2).

Fish and shellfish have a diverse microbiota, which on some occasions belong to altering microorganisms and on others, to microorganisms that cause food-borne illnesses (Karthiga Rani et al., 2016). Among the pathogenic and spoilage bacteria in fish are the gram-negative rods of the families Enterobacteriaceae and Vibrionaceae, the *Flavobacterium* genus, the *Pseudomonas* genus and the *Aeromonas* genus. However, the species of these bacterial groups can be isolated from fresh fish, without causing appreciable disease (Walczak et al., 2017).

Within the *Aeromonas* genus, *A. salmonicida* is the etiological agent of furunculosis in salmonids, however, it is also isolated in non-salmonid fish (Sudheesh et al., 2012) such as *Kajikia audax*. Although its pathogenicity is referred to fish, its presence in food is associated to food spoiling and outbreak food contamination in humans (Pękala-Safińska et al., 2021).

Pseudomonas species are usually isolated in a significant percentage (63%) in fish samples, and it is considered as a microbiological quality indicator microorganism in food (Karthiga Rani et al., 2016). The presence of the genus *Pseudomonas* and the *Shewanella* genus has been observed both in the initial and final microbiota of the shelf-life fish, measured by 16 days (Parlapani et al., 2015). Therefore, its presence in isolates from fresh fish samples is frequent, as in the case of this study.

The presence of *Pichia kudriavzevii* (syn. *Candida krusei* syn. *Issatchenkia orientalis*) is associated to the microbiota of plants and soil. However, it has been suggested that the ecology of pathogenic yeasts is diverse, so it is possible that this yeast could be found in additional environments (Opulente et al., 2019), as in the case of fish species.

Table 2
Chemical composition of *Bursera graveolens* essential oil analyzed by gas chromatography

Constituents (NIST08.L)	% in the sample (relative peak areas)						
	Water steam distillation		Hydro-distillation				
	This work	Luján-Hidalgo et al., 2012	Carmona et al., 2009	Monzote et al., 2012	Sotelo-Méndez et al., 2017	Rey-Valeirón et al., 2017	Jaramillo-Colorado et al., 2019
β-Myrcene	0.49	1.59	0.9	0.7	0.02	1.2	--
α-Phellandrene	0.29	--	0.2	0.1	--	37.64	--
m-Cymene	0.67	--	--	--	--	--	--
D-Limonene	77.06	14.29	30.7	26.5	0.19	49.89	42.0
β- Phellandrene	0.25	12.97	--	--	0.48	--	--
Eucalyptol	0.26	--	--	--	--	--	--
trans-p-Mentha-2,8-dienol	0.34	--	0.2	0.1	--	--	--
cis-p-Menth-2,8-dienol	0.37	--	0.2	Traces	--	--	1.6
Menthofuran	5.16	21.17	3.5	5.1	--	6.08	--
α-Terpineol	4.01	--	0.6	0.3	--	Traces	1.0
Dihydrocarvone	0.10	--	0.1	0.1	--	--	--
cis-2-metil-5-(1-metiletenil)-2-Ciclohexen-1-ol	0.64	--	--	--	--	--	--
trans-2-metil-5-(1-metiletenil)-2-Ciclohexen-1-ol	0.27	--	--	--	--	--	--
Pulegone	0.34	24.19	1.7	1.7	--	0.11	20.9
Carvone	0.83	24.29	0.2	0.1	--	--	7.5
δ-Elemene	0.18	--	--	--	--	--	--
Ylangene	0.08	--	--	0.2	--	--	--
β-Elemeno	0.43	31.33	11.3	14.1	--	--	1.9
α-Gurjunene	0.52	32.35	0.1	0.2	--	--	--
γ-Muurolene	0.25	--	--	--	--	--	--
α-Amorphene	0.07	--	--	--	--	--	--
Germacrene D	2.33	36.67	0.6	0.4	0.92	1.33	--
α-Farnesene	0.16	37.57	--	--	--	--	--
α-Muurolene	0.08	--	--	--	--	--	--
γ-Cadinene	0.15	--	0.2	0.4	--	--	--
δ-Cadinene	0.13	38.72	0.5	1.2	--	0.13	--
β-Cadinene	0.15	--	--	--	--	--	--

Table 3
Molecular bacterial identification of the strains isolated from *Kajikia audax* (Nucleotide BLAST)

Strain	Sequence size	Closer identification	% Similarity	Access number
MK1	1418	*Aeromonas salmonicida*	99.86%	CP048223.1
PS1	1375	*Pseudomonas aeruginosa*	100.0%	CP053028.1
PS2	1388	*Pseudomonas aeruginosa*	100.0%	CP053028.1
Lev	505	*Pichia kudriavzevii*	98.58%	LC389009

Although the microbiota of fish is diverse, also considering the species of microorganisms that arrive by handling the product, it should be considered that the different temperatures and atmospheres that are applied can affect the composition and growth of the microorganisms considered as altering microbiota (Parlapani et al., 2015).

3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The concentrations used were ranging from 0.41 mg/ml to 207.33 mg/ml. Table 4 shows the results of "*in vitro*" antimicrobial activity of *B. graveolens* essential oil as the MIC and MBC for each microorganism. Similarly, MBC plates can be observed on Figure 3.

Table 4
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the essential oil against the microorganisms isolated from *Kajikia audax*

Microorganism	MIC (mg/ml)	MBC (mg/ml)
Aeromonas salmonicida	1.62	25.92
Pseudomonas aeruginosa	>207.33*	>207.33*
Pseudomonas aeruginosa	>207.33*	>207.33*
Pichia kudriavzevii	6.48	25.92

*Inhibition above the concentration of 207.33 mg/ml was not tested.

Luján-Hidalgo et al. (2012) evaluated the "in vitro" antimicrobial activity of *B. graveolens* over *E. coli* and *Citrobacter freundii* and found that the essential oil did not show antimicrobial activity on these enterobacteria. This may prove that essential oils do not show the same antibacterial effect with strains of the same or different microbial family or class since *A. salmonicida*, of the Aeromonadaceae family, we determined an MIC of 1.6197 mg/ml and an MBC of 25.9156 mg/ml and as for the representatives of the Pseudomonadaceae family they had shown resistance. It should also be noted that the Enterobacteriaceae, Pseudomonadaceae, as well as the Aeromonadaceae families belong to the Gammaproteobacteria class of the Proteobacteria phylum.

Moreover, Luján-Hidalgo et al. (2012) found that for gram-positive bacteria such as S. aureus, the MBC was 7.45 mg/ml and 7.29 mg/ml for *Bacillus subtilis*. In this case the results show a more effective antimicrobial effect for other Gammaproteobacteria. On the other hand, Sotelo-Méndez et al. (2017) found that when using essential oil obtained from dry logs of *B. graveolens*, it demonstrated a 100% inhibition against gram-positive bacteria, gram-negative bacteria and yeasts, due to the purity of the oil. Furthermore, the bacteria were sensitive to chloramphenicol, oxytetracycline and fluconazole.

In the present work, bacteria were also proved to be sensitive to amikacin, *A. salmonicida* with inhibition halo of 30 mm, *P. aeruginosa* (PS1) 25 mm and *P. aeruginosa* (PS2) 18 mm. *B. graveolens* had shown a strong "in vitro" antimicrobial activity over *E. coli*, *S. aureus*, *B. subtilis*, *Salmonella enteritidis*, *Aspertillus niger and Penicillium citrinum* which made it necessary to set a range from 0.005 to 5 µl/ml for MIC for such bacteria (Fon-Fay et al., 2017). Similarly, Canales-Martínez et al. (2017) found antimicrobial activity of *Bursera morelensis* essential oil against *Streptococcus pneumoniae*, *Vibrio cholerae* and E. coli with a MIC of 0.125 mg/ml and an MBC of 0.25 mg/ml. The oil had also inhibited the filamentous fungi. In the case of yeasts, the present study had found higher values of MIC and MBC to that mentioned previously (6.4789 mg/ml and 25.9156 mg/ml, respectively). The caused inhibition in enterobacteria and yeast may be due to the high percentage of the monoterpene, D-limonene; and, to a lesser extent, the sesquiterpenes and diterpenes found in the chemical composition (Sotelo-Méndez et al., 2017; Luján-Hidalgo et al., 2012; Fernández-Ruiz et al., 2018). According to Loor & Coello (2019), D-limonene is an antioxidant metabolite of pharmaceutical interest, with anti-inflammatory, antibacterial and antifungal activity.

Researchers note that the antimicrobial activity of essential oils against the different pathogenic microorganisms present in food is due to its photochemical composition.

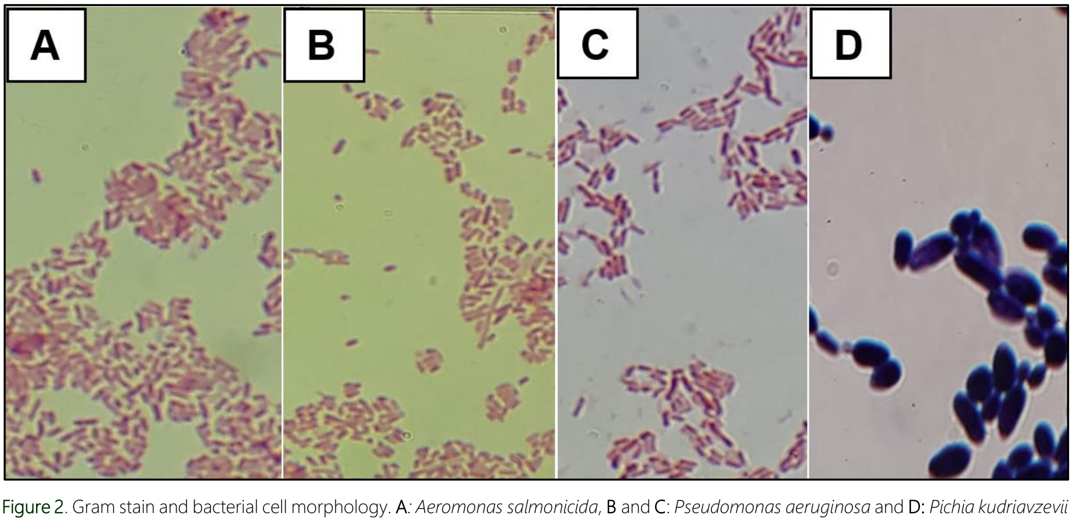

Figure 2. Gram stain and bacterial cell morphology. A: *Aeromonas salmonicida*, B and C: *Pseudomonas aeruginosa* and D: *Pichia kudriavzevii* (Direct microscopy, 1000x).

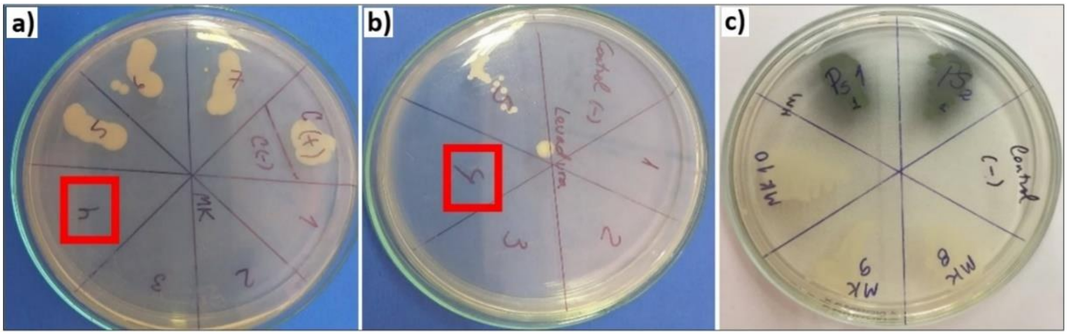

Figure 3. Minimum Bactericidal Concentration (red) of *B. graveolens* essencial oil over a) *Aeromonas salmonicida* (MK), b) *Pichia kudriavzevii* (Levadura), c) *Pseudomonas aeruginosa* (PS1 and PS2). Both *P. aeruginosa* strains presented MBC > 207.33 mg/ml, while the other strains presented MBC = 25.92 mg/ml.

Components such as terpenes, monoterpenes, sesquiterpenes, oxygenated and phenolic compounds are found to be responsible for a variety of biological activities (Pinto, 2014). To evaluate their antimicrobial activity, researchers had studied exclusively the specific chemical compounds present in the chemical composition of *B. graveolens* essential oil. Such is the case of the compound (+)-α–pinene which was dissolved in 1% Tween® 80 (otherwise known as Polysorbate 80) and in 5% DMSO (dimethyl sulfoxide) - that inhibited *E. coli* (ATCC® 25922™) - with a concentration of 160 µl/ml with an arithmetic mean of 12 mm of inhibition halo diameter (Farias et al., 2017).
Another compound find in essential oils is carvone, it has also been assessed "*in vitro*" against the bacterium, *L. monocytogenes*, attaining a MIC and MBC of 2.5 µL/ml, led to the conclusion that the bacteria are sensitive to this chemical component (Shahbazi, 2015). Lastly, the compound, α-terpineol has shown antifungal activity against *Geotrichum citri-aurantii* with a 2.00 µl/ml MIC and a minimum fungicidal concentration (MFC) of 4.00 µl/ml, causing an impairment in the integrity of the membrane of the cell and leakage of intracellular components (Zhou et al., 2014).

4. Conclusions

Chemical characterization of *B. graveolens* essential oil - attained by steam distillation- presented the following compounds as primary constituents identified by gas chromatography–mass spectrometry (GC-MS): D-limonene (77.06%), menthofuran (5.16%), α-terpineol (4.01%), germacrene D (2.33%) and carvone (0.83%). Furthermore, during the isolation of microorganisms of *K. audax*, gram-negative bacilli (*A. salmonicida* and *P. aeruginosa*) and a yeast (*P. kudriavzevii*) were distinguished. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) found for sensitive microorganisms showed a correlation to the antimicrobial properties present in the chemical composition of *B. graveolens* essential oil of, such as monoterpenes diterpenes, sesquiterpenes, oxygenated and phenolic compounds. This sensitivity makes it possible that *B. graveolens* essential oil can be utilized to control the growth of microorganisms in hydrobiological products. Nevertheless, several environmental, chemical factors influencing toxicity as well as the susceptibility of other microorganisms in relation to the essential oil or its purified components must still be examined.

Acknowledgements
The authors thank to Universidad Nacional de Tumbes, Universidad Nacional de Ingeniería and Fondo Nacional de Desarrollo Científico, Tecnológico y de Innovación Tecnológica (Contrato N° 02-2018-FONDECYT-BM-IADT-MU).

Conflict of interest statement
No potential conflict of interest was reported by the authors.

ORCID
K. C. Noel-Martinez https://orcid.org/0000-0001-5707-2054
G. J. F. Cruz https://orcid.org/0000-0001-6096-0183
R. L. Solis-Castro https://orcid.org/0000-0002-1813-8644

References

Bachir, R. G., & Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*, *2*(9), 739–742.

Bajpai, V. K., Baek, K. H., & Kang, S. C. (2012). Control of *Salmonella* in foods by using essential oils: A review. *Food Research International*, *45*(2), 722–734.

Calo, J. R., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2015). Essential oils as antimicrobials in food systems - A review. *Food Control*, *54*, 111-119.

Canales-Martínez, M., Rivera-Yáñez, C., Salas-Oropeza, J., López, H., Jiménez-Estrada, M., et al. (2017). Antimicrobial Activity of *Bursera morelensis* Ramírez Essential Oil. *African Journal of Traditional, Complementary and Alternative Medicines*, *14*(3), 74-82.

Carmona, R., Quijano-Celís, C. E., & Pino, J. A. (2009). Leaf oil composition of *Bursera graveolens* (Kunth) Triana et planch. *Journal of Essential Oil Research*, *21*(5), 387–389.

Cevallos, V., & Londoño, L. (2017). Aceites esenciales en la conservación de alimentos. *Microciencia*, *6*, 38–50.

Cruz, G., Feijoo, C., Carrión, L., Pirila, M., Keiski, R., & Cruz, J. (2013). Rendimiento y calidad del aceite esencial de hojas de *Bursera graveolens* y *Myroxylon peruiferum* procedentes del Área de Conservación Regional Angostura-Faical, Tumbes, Perú. *Manglar*, *1*, 10.

DIGESA. (2008). Dirección General de Salud Ambiental. Instructivo de preparación y dilución de muestras de alimentos para análisis microbiológico. Lima.

El Jery, A., Hasan, M., Rashid, M. M., Al Mesfer, M. K., Danish, M., & Ben Rebah, F. (2020). Phytochemical characterization, and antioxidant and antimicrobial activities of essential oil from leaves of the common sage *Salvia officinalis* L. from Abha, Saudi Arabia. *Asian Biomedicine*, *14*, 261-270.

Farias, T. C., Eduardo, L. S., Lima, Z. N., & Ferreira, S. B. (2017). Screening Antibacteriano do (+)-α– Pineno Frente a cepas bacterianas gram-negativas. In: *II Congresso Brasileiro de Ciência Da Saúde*, Campina Grande, Parabaíba, Brasil, 14-16 junho 2017.

Fernández-Ruiz, M., Yepes-Fuentes, L., Tirado-Ballestas, I., & Orozco, M. (2018). Repellent Activity of the essential oil of *Bursera graveolens* Jacq. ex L., against *Tribolium castaneum* Herbst, 1797 (Coleoptera: Tenebrionidae). *Anales de Biología*, *40*, 87–93.

Fon-Fay, F. M., Casariego, A., Falco A. S., & Pino J. A. (2017). Actividad Antimicrobiana de aceites esenciales de *Ocotea Quixos* (Lam.) Kosterm, *Bursera Graveolens* (Kunth) Triana y Planch, *Cymbopogon citratus* (DC) Stapf y *Curcuma longa* (L.) sobre microorganismos contaminantes de alimentos. *Ciencia y Tecnología de Alimentos*, *27*(3), 27-31.

Hili, P., Evans, C. S., & Veness, R. G. (1997). Antimicrobial action of essential oils: The effect of dimethylsulphoxide on the activity of cinnamon oil. *Letters in Applied Microbiology*, *24*(4), 269–275.

Huong, L. T., Viet, N. T., Sam, L. N., Giang, C. N., Hung, N. H., et al. (2021). Antimicrobial activity of the essential oils from the leaves and stems of *Amomum rubidum* Lamxay & NS Lý. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *20*(1), 81-89.

Jaramillo-Colorado, B. E., Suarez-López, S., & Marrugo-Santander, V. (2019). Volatile chemical composition of essential oil from *Bursera graveolens* (Kunth) Triana & Planch and their fumigant and repellent activities. *Acta Scientiarum - Biological Sciences*, *41*(1), 2020.

Karthiga Rani, M., Chelladurai, G., & Jayanthi, G. (2016). Isolation and identification of bacteria from marine market fish Scomberomorus guttatus (Bloch and Schneider, 1801) from Madurai district, Tamil Nadu, India. *Journal of parasitic diseases: official organ of the Indian Society for Parasitology*, *40*(3), 1062–1065.

Laith, A. A. (2021). Phytochemical analysis and antimicrobial activities of mangrove plant (*Rhizophora apiculata*) against selected fish pathogenic bacteria. In IOP Conference Series: *Earth and Environmental Science*, *718*(1), 012076.

Law, J. W. F., Mutalib, N. S. A., Chan, K. G., & Lee, L. H. (2015). Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. *Frontiers in Microbiology*, *5*, 1–20.

Leal-Torres, E., López-Malo-Vigil, A., & Sosa -Morales, M. E. (2013). Extracción, composición y caracterización de los aceites esenciales de hoja y semilla de cilantro (*Coriandrum sativum*). *Temas Selectos de Ingeniería de Alimentos*, *7*(1), 97–103.

Loor, L.; Coello, D. (2019). Estudio Comparativo de la Composición Química del Aceite Medicinal de Palo Santo de Illari vs. la Composición Química del Aceite Esencial de Palo Santo. *Ciencia*, *37*, 223-224.

Luján-Hidalgo, M. C., Gutiérrez-Miceli, F. A., Venturacanseco, L. M. C., Dendooven, L., Mendoza-López, M. R., Cruz-Sánchez, et al. (2012). Composición química y actividad antimicrobiana de los aceites esenciales de hojas de *Bursera graveolens* y *Taxodium mucronatum* de Chiapas, México. *Gayana - Botánica*, *69*(1), 7–14.

Mancuso, M., Catalfamo, M., Laganà, P., Rappazzo, A. C., Raymo, V., et al. (2019). Screening of antimicrobial activity of *Citrus* essential oils against pathogenic bacteria and Candida strains. *Flavour and Fragrance Journal*, *34*(3), 187-200.

Meyer-Torres, G., Sarmiento, O. I., Ramírez, R. I., & Guevara, O. (2018). Evaluación del rendimiento del aceite esencial de caléndula (*Calendula officinalis* L) obtenido por OAHD. *Revista ION*, *31*(1), 13–19.

Monzote, L., Hill, G. M., Cuellar, A., Scull, R., & Setzer, W. N. (2012). Chemical composition and anti-proliferative properties of *Bursera graveolens* essential oil. *Natural Product Communications*, *7*(11), 1531–1534.

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 361–366.

Opulente, D. A., Langdon, Q. K., Buh, K. V., Haase, M. A. B., Sylvester, K., et al. (2019). Pathogenic budding yeasts isolated outside of clinical settings. *FEMS Yeast Res.*, *19*(3), foz032.

Pandey, A. K., Kumar, P., Singh, P., Tripathi, N. N., & Bajpai, V. K. (2017). Essential oils: Sources of antimicrobials and food preservatives. *Frontiers in Microbiology*, *7*, 1–14.

Parlapani, F. F., Kormas, K. A., Boziaris, I. S. (2015). Microbiological changes, shelf life and identification of initial and spoilage microbiota of sea bream fillets stored under various conditions using 16S rRNA gene analysis. *Journal of the Science of Food and Agriculture*, *95*(12), 2386-2394.

Parvekar, P., Palaskar, J., Metgud, S., Maria, R., & Dutta, S. (2020). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. *Biomaterial Investigations in Dentistry*, *7*(1), 105-109.

Pastrana-Puche, Y. I., De Paula, C. D., & Gallo-García, L. A. (2017). Evaluación de sustancias antimicrobianas naturales en la conservación de *Avena sinuana*. *Corpoica Ciencia y Tecnologia Agropecuaria*, *18*(2), 321–334.

Patiño, L., Saavedra, A., & Martínez, J. (2014). Extracción por arrastre de vapor de aceite esencial de romero. Handbook. *Ed. Ciencias Tecnológicas y Agrarias*. Sucre, Bolivia.

Pękala-Safińska, A., Tkachenko, H., Kurhaluk, N., Buyun, L., Osadowski, Z., et al. (2021). Studies on The Inhibitory Properties of Leaf Ethanolic Extracts Obtained from Ficus (Moraceae) Species Against *Aeromonas spp*. strains. *Journal of Veterinary Research*, *65*(1), 59-66.

Peña, Y. P., Castillo, V. L., López, N. A., Jardines, A. C., & Areas, R. T. (2019). Antimicrobial resistance in bacteria isolated in fish and shellfish. *Revista Habanera de Ciencias Médicas*, *18*(3), 500-512.

Pinto, R. 2014. Estudo da atividade antibacteriana da Carvona e seus derivados. Dissertacão de Mestrado. Universidade da Beira Interior, Covilha, Portugal.

Preedy, V. R. (Esed.). (2015). Essential oils in food preservation, flavor and safety. *Academic Press Elsevier*. United Kingdom.

Puescas, M. (2010). Estudio dendrológico de la especie *Bursera graveolens* - Palo Santo, Región Tumbes. In: http://planteetplanete.org/wp-content/uploads/2018/02/53.pdf

Rey-Valeirón, C., Guzmán, L., Saa, L. R., López-Vargas, J., & Valarezo, E. (2017). Acaricidal activity of essential oils of *Bursera graveolens* (Kunth) Triana & Planch and *Schinus molle* L. on unengorged larvae of cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari:Ixodidae). *Journal of Essential Oil Research*, *29*(4), 344–350.

Savoldi, T. L., Glamočlija, J., Soković, M., Gonçalves, J. E., Ruiz, S. P., Linde, G. A., et al. (2020). Antimicrobial activity of essential oil from *Psidium cattleianum* Afzel. ex Sabine leaves. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *19*(6), 614-627.

Servicios Antimicrobianos (2012). INEI – ANLIS "Dr. Carlos G. Malbrán"Malbrán C. Método de determinación de sensibilidad antimicrobiana por dilución, *MIC testing. M07-A9*,*32*(2).

Shahbazi, Y. (2015). Chemical Composition and in Vitro Antibacterial Activity of *Mentha spica*ta Essential Oil against Common Food-Borne Pathogenic Bacteria. *Journal of pathogens*, *2015*, 916305.

Sotelo-Méndez, A. H., Figueroa Cornejo, C. G., Césare Coral, M. F., & Alegría Arnedo, M. C. (2017). Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Bursera graveolens* (burseraceae) from Perú. *Indian Journal of Pharmaceutical Education and Research*, *51*(3), S429–S436.

Soto, Z., Pérez, L., & Estrada, D. (2016). Bacterias causantes de enfermedades transmitidas por alimentos: Una mirada en Colombia. *Salud Uninorte*, *32*(1), 105–122.

Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N., & Al-Habsi, S. (2012). Comparative pathogenomics of bacteria causing infectious diseases in fish. *International journal of evolutionary biology*, *2012*, 457264.

Walczak, N., Puk, K., & Guz, L. (2017). Bacterial flora associated with diseased freshwater ornamental fish. *Journal of veterinary research*, *61*(4), 445–449.

Wang, P., Liu, Y., Yin, Y., Jin, H., Wang, S., et al. (2011). Diversity of microorganisms isolated from the soil sample surround *Chroogomphus rutilus* in the Beijing region. *International Journal of Biological Sciences*, *7*(2), 209–220.

White, T.J., Bruns, T.D., Lee, S.B., et al. (1990). Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., Eds., PCR Protocols: A Guide to Methods and Applications, Academic Press, New York.

Wijesinghe, G. K., Feiria, S. B., Maia, F. C., Oliveira, T. R., Joia, F., et al. (2021). In-vitro Antibacterial and Antibiofilm Activity of *Cinnamomum verum* Leaf Oil against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Anais da Academia Brasileira de Ciências*, *93*(1), e20201507.

Yukawa, C., Imayoshi, Y., Iwabuchi, H., Komemushi, S., & Sawabe, A. (2006). Chemical composition of three extracts of Bursera graveolens. *Flavour and Fragrance Journal*, *21*(2), 234–238.

Zhou, H., Tao, N., & Jia, L. (2014). Antifungal activity of citral, octanal and α-terpineol against *Geotrichum citri-aurantii*. *Food Control*, *37*(1), 277–283.