Evaluation Of Antibacterial Activity In *Pelargonium Graveolens* Extracts

Paula Sofía Rojas Pedraza[1], Oscar Eduardo Rodriguez Aguirre [2,3], Janeth del Carmen Arias Palacios[1].

[1]*Department of Microbiology, Faculty of Science Biotechnology Ambiental and Industrial Microbiology Group. Pontificia Universidad Javeriana, Bogotá, Colombia.*

[2]*Environmental Engineering Program, Faculty of Engineering, Universidad El Bosque, Research Group CHOC- IZONE, Bogotá, Colombia,*

[3]*School of Medicine, Fundación Universitaria Juan N. Corpas, Bogotá, Colombia*

Email:corresponding author: rodriguezoscare@unbosque.edu.co

Abstracts: The antibacterial activity of the total ethanolic extract and fractions of *Pelargonium graveolens* against *Candida albicans*, *Escherichia coli*, *Pseudomonas spp*, *Staphylococcus aureus* and *Bacillus spp*. Methods: To measure the relative antimicrobial activity, the percentage of inhibition was calculated by the plate and well method. Results: It was established that the highest antimicrobial activity was presented by the oily extract without preference for Gram positive or Gram negative, the acetone fraction with a value of 58.53% inhibition. The hexane fraction has activity greater than 100% only against *S. aureus*. Conclusions: The extracts of *P. graveolens* present antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts, especially the oily extract, which presented the highest percentage of inhibition recorded against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, respectively; while for *Pseudomonas aeruginosa* and *Bacillus subtilis*, the extract that presented the highest percentage of inhibition was ethyl acetate.

Keywords: Antibacterial Activity, *Pelargonium Graveolens, Inhibition Percentage, S. Aureus, E. Coli, P. Aeruginosa, C. Albicans, B. Subtillis.*

1. INTRODUCTION

Since ancient times, people have sought to cure their own diseases using nature, just as the use of animals was initially instinctive, such instinctive use was also applied to plants. In fact, it is estimated that the plants have been cultivated as drugs for approximately 60,000 years. Manuals on medicinal plants date back nearly 5,000 years in India, China, and Egypt, and at least 2,500 years in Greece and Central Asia.

In fact, according to the WHO, more than 80% of the world's population depends more frequently on traditional medicines, mainly plants, which serve as the main source of health care, in addition, it is estimated that currently more than 50% of Available medicines are derived in some way from medicinal plants [1].

Secondary metabolites of medicinal plants are the material basis for their clinically curative effects and, in fact, it is these metabolites that form the basis of many commercial drugs, as well as herbal remedies derived from medicinal plants. The different chemical components of medicinal plants have biological activities that can improve human health through production processes in the pharmaceutical and food industries, but also represent an important value in the perfumery, agrochemical and cosmetic industries. There are three main groups of these metabolites in plants based on their biosynthetic pathway, including nitrogen-containing compounds (cyanogenic glycosides, alkaloids, and glucosinolates), phenolic compounds (flavonoids and phenylpropanoids), and terpenes (isoprenoids) [2].

The genus *Pelargonium* (Geraniaceae) are aromatic plants cultivated for their essential oil, derived from *Pelargonium graveolens*, *P. capitatum, P. radens and P. odoratissimum*, among other species, this is known as one of the 20 best essential oils in the world. This genus is known for its pharmacological properties in the treatment of

bronchitis, fever, diarrhea, cough, gastroenteritis and other ailments related to the respiratory tract. In addition, *Pelargonium essential* oils have been well known for their antifungal, antimicrobial, anti-inflammatory, and spasmolytic properties, other findings suggest that the pharmacological activity of Pelargonium species is attributed in part to their richness in bioactive phytochemicals including oxygenated coumarins, derivatives gallic acid, flavonoids, phenolic and hydroxycinnamic acid derivatives [3].

The natural antioxidants obtained from this genus are of greater benefit compared to the synthetic ones, therefore, the development and use of antioxidants of natural origin obtained from more effective botanical sources are desirable in preventive medicine and in the food industry for his interest in the protection of the organism against oxidative stress and for the management of various diseases [3], these are complementary to the antimicrobial activity; For this reason, the objective of this work is to evaluate and demonstrate the antimicrobial capacity of extracts and fractions of *Pelargonium graveolens* using the plate and well method, in order to obtain more information about its potential for future Phytotherapeutic products.

Pelargonium graveolens native to South Africa, belonging to the Geraniaceae family, where the Xhosa and Zulu tribes of South Africa use these species to treat coughs, diarrhea and tuberculosis. The active medicinal ingredients are found in the bitter-tasting roots of the plants. A commonly used medicine produced in Germany called “Umckaloabo” originates from the roots of *P. sidoides* and *P. reniform*. This herbal medicine is widely used in Germany for bronchitis, antibacterial and antifungal infections [9, 10].

Pelargonium graveolens is a high-value, multi-crop aromatic plant (Figure 1), mostly cultivated for its essential oils, widely used in the cosmetics industry and as a food flavoring, where its use has been approved by the FDA [11].

2. MATERIALS AND METHODS

2.1. Soxhlet extraction. It is the extraction method that was used in this work, it is defined as the action of separating a specific fraction of a sample with a liquid, leaving the rest as complete as possible. The solid-liquid extraction is the most frequent and is carried out in a Soxhlet equipment (Figure 2), in which the plant of interest is initially taken and the extraction process is carried out, based on the following stages [15]:

1) placement of the solvent in a balloon.

2) boiling of the solvent which is evaporated to a reflux condenser.

3) the condensate falls on a container containing a porous cartridge with the sample inside.

4) rise in the level of the solvent covering the cartridge to a point where reflux occurs that returns the solvent with the material extracted to the balloon.

5) This process is repeated until the sample is exhausted. What is extracted is concentrated in the solvent balloon [15].

2.2. Microorganisms. The planet earth has approximately one trillion species of microorganisms, where only 1% have been identified [16]. Among these, there are about 1,400 known species of human pathogens (including viruses, bacteria, fungi, protozoa, and helminths) [17]. Among them are: *Candida albicans*, a polymorphic fungus that is the cause of superficial mucosal infections, such as oral and vaginal candidiasis, is also capable of causing life-threatening diseases (such as immunodeficiency and blood infections), accounting for significant mortality rates, (40 %) in immunosuppressed patients and in immunosuppressive treatment [18]. *Escherichia coli* is a Gram-negative bacillus that is known to be part of the normal intestinal flora, but can also be the cause of intestinal and extra-intestinal diseases in humans. When found outside the intestinal tract, it can cause urinary tract infections

(UTIs), pneumonia, bacteremia, and peritonitis, among others [19]. *Staphylococcus aureus* is a Gram-positive coccus that has caused a wide variety of clinical illnesses. *S. aureus* infections are common in hospitals and treatment remains a challenge due to the emergence of multidrug-resistant strains such as MRSA (methicillin-resistant *Staphylococcus aureus*) [20].

Pseudomonas sp is a genus of Gram-negative bacilli, which usually reside in sewage, soil, or animal and human feces. They can mainly cause nosocomial infections; however, in immunosuppressed individuals with infected wounds, they are fearsome and dangerous pathogens. They rapidly induce antimicrobial resistance and contribute to the formation of biofilms that are highly resistant to therapeutic interventions. *P. aeruginosa* stands out as one of the most dangerous opportunistic pathogens in hospitals, in addition, it is resistant to high concentrations of salts and dyes, weak antiseptics and many commonly used antibiotics [21, 22]. Finally, there is *Bacillus sp*, Gram-positive, spore-forming, strictly aerobic or facultative anaerobic bacilli. Only a few Bacillus species are known to cause disease in animals and humans [23]. *Bacillus subtilis* is a microorganism that is considered non-pathogenic for humans, however, the eye has been the most commonly infected organ, mainly by direct inoculation, and reports of infection of the central nervous system by *B. subtilis* have previously been published, all in the form of purulent meningitis [24].

This is a small approach and perspective to what infections or diseases caused by microorganisms and antimicrobial resistance generally imply, which in bacterial pathogens is a worldwide challenge associated with high morbidity and mortality. Patterns of multidrug resistance in Gram-positive and negative bacteria have led to infections that are difficult to treat or even untreatable with conventional antimicrobials.

2.3. Obtaining extracts and strains. Extracts of *Pelargonium graveolens* were obtained, which are: total ethanolic extract, hexane fraction, dichloromethane fraction, ethyl acetate fraction, aqueous fraction and oil, while the microorganisms were obtained from the collection of strains of the Pontifical Javeriana University.

Preparation of culture media. 52 grams of BHI agar were suspended per liter of distilled water, mixed well and dissolved by heating with frequent stirring, then autoclaved at 121°C for 15 minutes [26, 27].

2.4. Inoculum preparation. A suspension of each microorganism was made in saline solution, this inoculum should have an approximate concentration of 6.0E+8 CFU/mL with reference to Mc Farland pattern 2. Finally, for the inoculum control, the initial concentration was confirmed by plate count on BHI agar [27, 28].

2.5. Preparing plates and wells. Of the previously prepared inocula, each was mixed separately with the BHI broth. Approximately 27 ml of inoculated medium were poured into each 100 x 15 mm Petri dish and then allowed to solidify under refrigeration at approximately 4°C. Subsequently, each agar plate was perforated six times with a 10 mL inverted pipette. Finally, 10 ppm, 20 ppm, 30 ppm and 50 ppm of each extract/fraction and the positive (5.25% NaClO) and negative (99.5% DMSO) controls were added, performing each extract in triplicate. This procedure was repeated in the same way for the 5 microorganisms under study [27].

2.6. Measurement of relative antimicrobial activity. After incubation, the measurement of the halos of each assembly was performed and the relative antimicrobial activity with respect to hypochlorite and DMSO should be calculated using the following formula [27]

$$\%\ \boldsymbol{inhibition} = \frac{Extract\ halo\ diameter - White\ halo\ diameter}{Positive\ control\ halo\ diameter - White\ halo\ diameter}\ x\ 100$$

White halo diameter (mm): 99.5% DMSO

Control diameter (mm): 5.25% sodium hypochlorite.

3. RESULTS

The total ethanolic extract, hexane fraction, dichloromethane fraction, ethyl acetate fraction, aqueous fraction and *Pelargonium gravveolens* oil, at concentrations of 10 ppm, 20 ppm, 30 ppm and 50 ppm, together with the positive (NaClO) and negative controls (DMSO) against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*, from which the following results were obtained:

Table 1. Measurement of inhibition halos in millimeters (mm) and percentage of inhibition of the total ethanolic extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans.*

	Inhibition in mm of corresponding concentrations									
	Réplica	**Cntrl (+)**	**10 ppm**	**% inhibición**	**20 ppm**	**% inhibición**	**30 ppm**	**% inhibición**	**50 ppm**	**% inhibición**
Escherichia coli	1	10	10	100	12	120	13	130	16	160
	2	9	10	111,1	12	133,3	12	133,3	15	166,7
	3	9	9	100	11	122,2	13	144,4	15	166,7
Staphylococcus aureus	1	11	9	81,8	12	109,1	12	109,1	14	127,3
	2	14	9	64,3	11	78,6	13	92,8	14	100
	3	13	8	61,5	11	84,6	13	100	15	115,4
Pseudomonas aeruginosa	1	6	9	150	10	166,7	14	233,3	14	233,3
	2	8	8	100	9	112,5	11	137,5	13	162,5
	3	8	11	137,5	11	137,5	14	175	18	225
Bacillus subtilis	1	13	9	69,2	10	76,9	11	84,6	14	138,5
	2	12	7	58,3	10	83,3	12	100	16	133,3
	3	11	8	72,7	9	81,8	11	100	14	127,3
Candida albicans	1	20	9	45	13	65	15	75	15	75
	2	23	10	43,5	11	47,8	13	56,5	15	65,21
	3	24	10	41,7	13	54,2	14	58,3	15	62,5

Figure 5 shows the averages of the percentages of inhibition corresponding to each concentration in each microorganism, where in all concentrations, the percentage for *Pseudomonas aeruginosa* was higher, obtaining a percentage of 129.2 at 10 ppm, a percentage of 138.9 at 20 ppm., at 30 ppm a percentage of 181.9, and at 50 ppm

a percentage of 206.9. The concentration with the highest percentage of inhibition in all cases was 50 ppm, where the highest was *P. aeruginosa*, the next was *E. coli* with 164.5%, then B. subtilis with 133%, *S. aureus* with 114.2 % and finally *C. albicans* with 67.6%, the latter obtained percentages less than 100% in all cases.

Additionally, it can be inferred that this extract has a greater inhibitory activity on Gram negative bacteria, then Gram positive bacteria and finally yeasts.

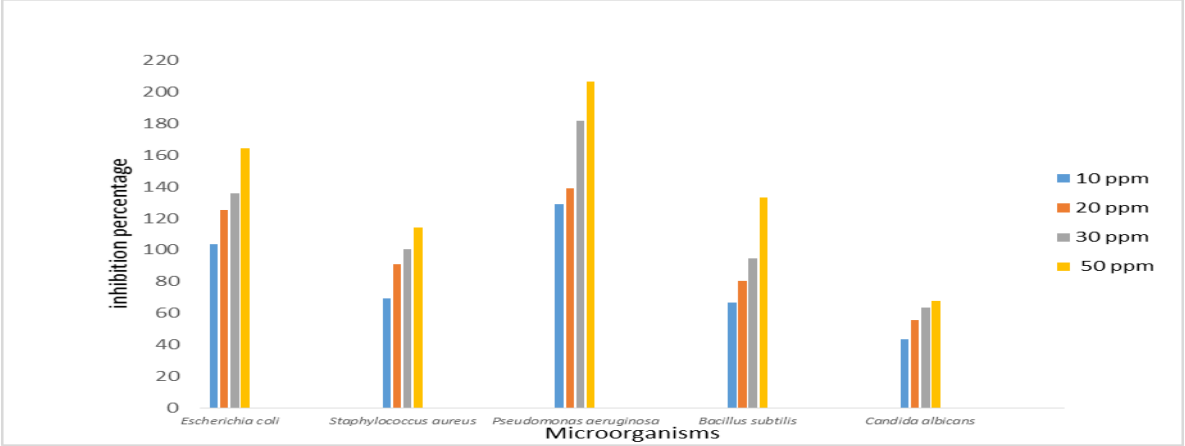

Figure 5. Average by concentration of percentages of inhibition of total ethanolic extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

Table 2. Measurement of inhibition halos in millimeters (mm) and percentage of inhibition of the hexane fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans.*

		Inhibition in mm of corresponding concentrations								
	Réplica	**Cntrl (+)**	**10 ppm**	**% inhibición**	**20 ppm**	**% inhibición**	**30 ppm**	**% inhibición**	**50 ppm**	**% inhibición**
Escherichia coli	1	10	0	0	0	0	0	0	0	0
	2	11	0	0	0	0	0	0	0	0
	3	10	0	0	0	0	0	0	0	0
Staphylococcus aureus	1	10	0	0	7	70	9	90	11	110
	2	13	0	0	7	53,8	7	53,8	10	76,9
	3	11	0	0	7	63,6	8	72,7	9	81,8
Pseudomonas aeruginosa	1	8	0	0	0	0	0	0	0	0
	2	6	0	0	0	0	0	0	0	0
	3	7	0	0	0	0	0	0	0	0
Bacillus subtilis	1	11	0	0	0	0	0	0	0	0

	2	9	0	0	0	0	0	0	0	0
	3	12	0	0	0	0	0	0	0	0
Candida albicans	1	24	0	0	0	0	0	0	0	0
	2	23	0	0	0	0	0	0	0	0

Figure 7 shows the averages of the percentages of inhibition corresponding to each concentration in each microorganism, where results are only observed for *S. aureus* at concentrations of 20 ppm, 30 ppm and 50 ppm, obtaining 62.5%, 72.2% and 89.6 %, respectively. Additionally, it can be seen that this fraction has activity only against *S. aureus* among the microorganisms evaluated and this exceeds 100% in percentage of inhibition only on one occasion in 50 ppm in one of the replicas as observed in table 2.

Figure 7. Average by concentration of percentages of inhibition of the hexane fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

Table 3. Measurement of inhibition halos in millimeters (mm) and inhibition percentage of the dichloromethane fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans.*

		Inhibition in mm of corresponding concentrations								
	Réplica	**Cntrl (+)**	**10 ppm**	**% inhibición**	**20 ppm**	**% inhibición**	**30 ppm**	**% inhibición**	**50 ppm**	**% inhibición**
Escherichia coli	1	14	7	50	9	64,3	11	78,6	13	92,9
	2	13	8	61,5	10	76,9	11	84,6	12	92,3
	3	12	7	58,3	9	75	10	83,3	11	91,7
Staphylococcus aureus	1	15	7	46,7	8	53,3	10	66,6	11	73,3
	2	19	6	31,6	7	36,8	8	42,1	10	52,6
	3	18	7	38,9	8	44,4	9	50	10	55,6

Pseudomonas aeruginosa	1	11	6	54,5	7	63,6	7	63,6	11	100
	2	9	5	55,5	7	77,8	8	88,8	10	111,1
	3	8	6	75	7	87,5	8	100	9	112,5
Bacillus subtilis	1	20	10	50	11	55	13	65	15	75
	2	15	7	46,7	9	60	11	73,3	13	86,7
	3	17	6	35,3	8	47	9	52,9	11	64,7
Candida albicans	1	17	6	35,3	6	35,3	8	47	10	58,8
	2	22	6	27,3	7	31,8	8	36,4	10	45,4

Figure 9 shows the averages of the percentages of inhibition corresponding to each concentration in each microorganism, where in all concentrations, the percentage for *Pseudomonas aeruginosa* was higher, obtaining a percentage of 61.7 at 10 ppm, a percentage of 76.3 at 20 ppm. , at 30 ppm a percentage of 84.1 and at 50 ppm a percentage of 107.9, this being the only value in the entire analysis to exceed 100% in percentage of inhibition.

The concentration with the highest percentage of inhibition in all cases was 50 ppm, where the highest was *P. aeruginosa*, the next was *E. coli* with 92.3%, then *B. subtilis* with 75.5%, *S. aureus* with 60.5 % and finally *C. albicans* with 52.1.

It can be inferred that the only microorganism against which this fraction would be effective is *P. aeruginosa*, however, it must be at a concentration of 50 ppm or greater, so that it exceeds the 100% inhibition percentage.

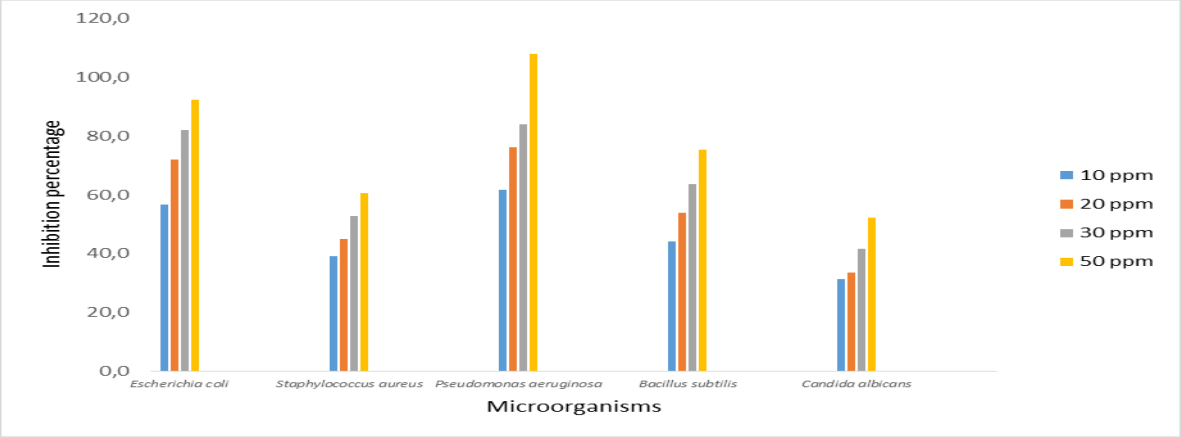

Figure 9. Average by concentration of percentages of inhibition of the dichloromethane fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

Table 4. Measurement of inhibition halos in millimeters (mm) and inhibition percentage of the ethyl acetate fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

		Inhibition in mm of corresponding concentrations								
	Réplica	**Cntrl (+)**	**10 ppm**	**% inhibición**	**20 ppm**	**% inhibición**	**30 ppm**	**% inhibición**	**50 ppm**	**% inhibición**
Escherichia coli	1	12	9	75	11	91,6	11	91,6	14	116,7
	2	9	10	111,1	10	111,1	11	122,2	15	166,6
	3	11	11	100	12	109,1	13	118,2	17	154,5
Staphylococcus aureus	1	17	11	64,7	13	76,5	15	88,2	18	105,9
	2	15	10	66,7	12	80	15	100	17	113,3
	3	17	9	52,9	11	64,7	14	82,4	19	111,8
Pseudomonas aeruginosa	1	6	8	133,3	10	166,7	13	216,7	15	250
	2	6	7	116,7	9	150	11	183,3	12	200
	3	6	8	133,3	9	150	11	183,3	13	216,7
Bacillus subtilis	1	9	10	111,1	11	122,2	12	133,3	15	166,7
	2	12	9	75	10	83,3	12	100	15	125
	3	15	9	60	11	73,3	12	80	16	106,7
Candida albicans	1	25	12	48	13	52	15	60	17	68
	2	27	11	40,7	14	51,8	15	55,6	17	63
	3	28	11	39,3	12	42,9	14	50	16	57,1

Figure 11 shows the averages of the percentages of inhibition corresponding to each concentration in each microorganism, where in all concentrations, the percentage for *Pseudomonas aeruginosa* was higher, obtaining a percentage of 127.8 at 10 ppm, a percentage of 155.6 at 20 ppm., at 30 ppm a percentage of 194.4, and at 50 ppm a percentage of 222.2. The concentration with the highest percentage of inhibition in all cases was 50 ppm, where the highest was *P. aeruginosa*, the next was *E. coli* with 145.9%, then *B. subtilis* with 132.8%, *S. aureus* with 110.3 % and finally *C. albicans* with 62.7%, the latter obtained percentages less than 100% in all cases.

Additionally, it should be noted that these results in Table 4 are similar to those in Table 1, that is, the total ethanolic extract and ethyl acetate fraction have a similar behavior. On the other hand, it can be inferred that this extract has a greater inhibitory activity on Gram negative bacteria, then Gram positive bacteria and finally yeasts.

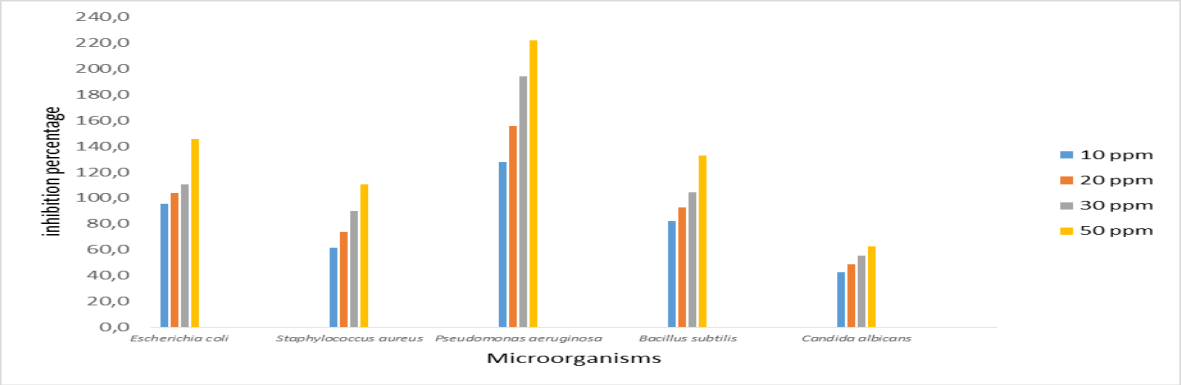

Figure 11. Average by concentration of percentages of inhibition of the ethyl acetate fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans.*

Table 5. Measurement of inhibition halos in millimeters (mm) and percentage of inhibition of the aqueous fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

		Inhibition in mm of corresponding concentrations								
	Réplica	**Cntrl (+)**	**10 ppm**	**% inhibición**	**20 ppm**	**% inhibición**	**30 ppm**	**% inhibición**	**50 ppm**	**% inhibición**
Escherichia coli	1	10	9	90	11	110	13	130	14	140
	2	11	8	72,7	12	109,1	12	109,1	14	127,3
	3	9	9	100	11	122,2	13	144,4	12	133,3
Staphylococcus aureus	1	15	6	40	7	46,7	10	66,7	12	80
	2	17	6	35,3	6	35,3	10	58,8	13	76,5
	3	15	6	40	6	40	11	73,3	14	93,3
Pseudomonas aeruginosa	1	9	6	66,7	7	77,8	7	77,7	8	88,9
	2	6	6	100	7	116,7	8	133,3	9	150
	3	7	6	85,7	7	100	7	100	10	142,8
Bacillus subtilis	1	19	6	31,6	8	42,1	9	47,4	10	52,6
	2	16	9	56,2	10	62,5	11	68,8	11	68,8
	3	12	8	66,7	10	83,3	11	91,7	12	100
Candida	1	29	10	34,5	14	48,3	15	51,7	16	55,2

albicans	2	32	12	37,5	15	46,9	15	46,9	16	50
	3	27	11	40,7	14	51,8	15	55,6	17	63

Figure 13 shows the averages of the percentages of inhibition corresponding to each concentration in each microorganism, where in all concentrations, the percentage for *Escherichia coli* was higher, obtaining a percentage of 87.6 at 10 ppm, a percentage of 113.8 at 20 ppm. , at 30 ppm a percentage of 127.8, and at 50 ppm a percentage of 133.5.

The concentration with the highest percentage of inhibition in all cases was 50 ppm, where the highest was E. coli, the next was *P. aeruginosa* with 127.2%, then *S. aureus* with 83.3%, *B. subtilis* with 73.8%. % and finally *C. albicans* with 56.1%.

On the other hand, it can be inferred that this extract has a greater inhibitory activity on Gram negative bacteria, since *E. coli* together with *P. aeruginosa* were the only microorganisms to have values that exceeded 100% in percentage of inhibition compared to the positive control (NaClO).

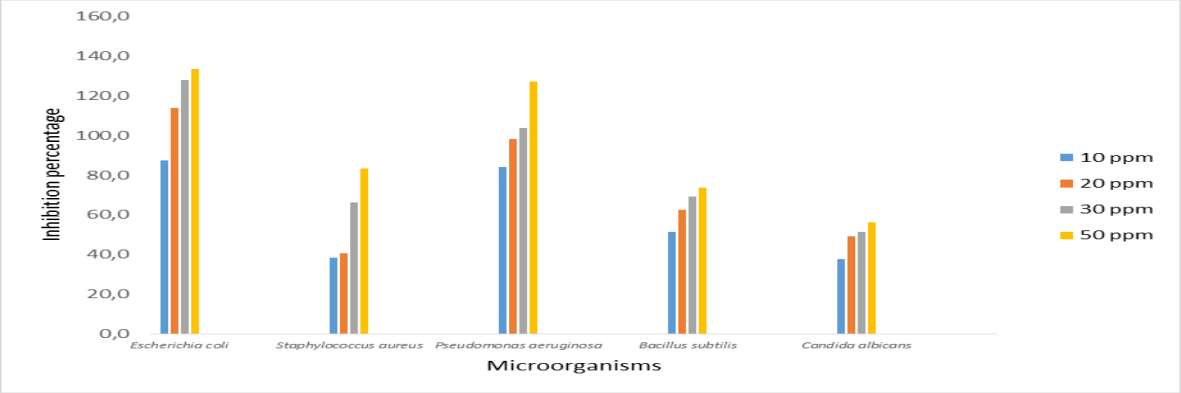

Figure 13. Average by concentration of percentages of inhibition in the aqueous fraction against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

Table 6. Measurement of inhibition halos in millimeters (mm) and inhibition percentage of the oil extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

		Inhibition in mm of corresponding concentrations								
	Réplica	**Cntrl (+)**	**10 ppm**	**% inhibición**	**20 ppm**	**% inhibición**	**30 ppm**	**% inhibición**	**50 ppm**	**% inhibición**
Escherichia coli	1	12	13	108,3	13	108,3	20	166,7	21	175
	2	9	11	122,2	15	166,7	18	200	19	211,1
	3	11	12	109,1	16	145,4	20	181,8	24	218,2
Staphylococcus aureus		9	29	322,2	32	355,6	33	366,7	39	433,3

Pseudomonas aeruginosa	1	6	0	0	0	0	0	0	0	0
	2	10	0	0	0	0	0	0	0	0
	3	6	0	0	0	0	0	0	0	0
Bacillus subtilis		14	11	78,6	16	114,3	18	128,6	10	71,4
Candida albicans		23	30	130,4	32	139,1	36	156,5	37	160,9

Figure 15 shows the averages of the percentages of inhibition corresponding to each concentration in each microorganism, where in all concentrations, the percentage for *Staphylococcus aureus* was higher, obtaining a percentage of 322.2 at 10 ppm, a percentage of 20 ppm of 355.6, at 30 ppm a percentage of 366.7, and at 50 ppm a percentage of 433.3, almost doubling the highest value obtained with the ethyl acetate extract. The concentration with the highest percentage of inhibition in almost all cases was 50 ppm (except for *B. subtilis* that obtained a higher percentage of 30 ppm of 128.6) where the highest was *S. aureus*, the next was *E. coli* with 201.4% and finally *C. albicans* with 160.9%, compared to the positive control (NaClO), while *P. aeruginosa* did not show growth.

Additionally, although this extract has the highest antimicrobial activity, it does not have a preference for Gram positive or negative.

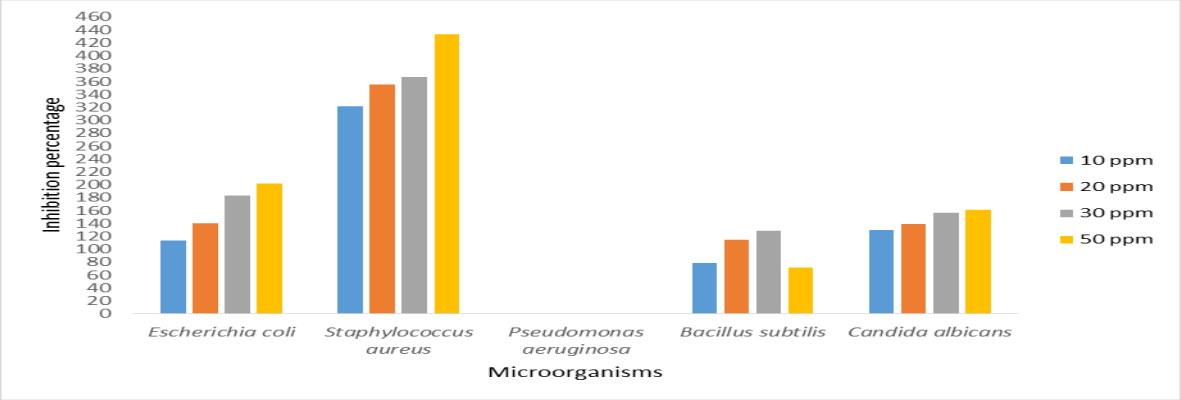

Figure 15. Average by concentration of percentages of inhibition in oil extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans*.

4. DISCUSSION

Aanachi et al., 2020, evaluated a methanol extract of *P. graveolens* that showed the highest antibacterial activity against *P. aeruginosa* [12], which is consistent with this work, since the extract in this research with the closest polarity, the total ethanolic extract [29], also presents the highest antibacterial activity against *P. aeruginosa* as observed in table 1. In that same study, antimicrobial activity is observed in the hexane extract, where greater antibacterial activity was obtained against *P. aeruginosa. P. aeruginosa* [12], however, this does not agree with the results observed in table 2, since although this extract does not show activity against *P. aeruginosa*, it does show antimicrobial activity against *S. aureus*. The last extract studied by Aanachi in the article was dichloromethane, which showed greater antimicrobial activity against *P. aeruginosa* [12], which agrees with what is shown in table 3, where it is observed that the microorganism with the highest percentage of inhibition it was *P. aeruginosa*.

The literature reports that, by using ethyl acetate as a solvent to make plant extracts, in many cases it has a better potential to inhibit bacterial pathogens and *C. albicans*, as seen in extracts of *Curculigo orchioides* and *Piper retrofractum* [30, 31]. , which can be verified in this work, since the second extract with the best and highest percentages of inhibition was that of ethyl acetate, as can be seen in figure 11.

Hussain et al. reported that Gram-positive bacteria are more sensitive to plant essential oils than Gram-negative bacteria, especially E. coli [32], however, fixed oil was used in this work and the results of this project as shown. seen in Figure 15, it is observed that there is greater activity in *S. aureus*, a Gram positive bacterium and the second highest result is against *E. coli*, a Gram negative bacterium, the third was against *C. albicans*, a yeast; finally, as the fourth and last microorganism against which antimicrobial activity was evidenced with this extract, *B. subtilis*, the other Gram-positive bacteria studied.

Previous studies by Gâlea and Gabriel, 2014 [9], evaluated the antimicrobial activity of *Pelargonium roseum* oils on different microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, where they were compared against different antibacterial substances; Although the *P. roseum* oils had antimicrobial activity in *E. coli*, it was less than the inhibition of the antibacterials [9], unlike the fixed oil of *P. graveolens* with respect to the positive control (NaClO at 5.25%) , which was exceeded with a maximum of 201% inhibitory percentage, for this same microorganism (Figure 15). In the case of *S. aureus*, the antimicrobial activity of the oils in some cases was even better than that of the antibacterials [9], however, the fixed oil of *P. graveolens* had a maximum value of 433% over the positive control, for this same microorganism (Figure 15). For *P. aeruginosa*, the antimicrobial activity of the oils exceeded that of most antimicrobials [9], however, *P. graveolens* oils had no effect on this microorganism, therefore, there was no inhibition. Now, *C. albicans* was completely inhibited by *P. roseum* oils, while antibacterials had no effect on this yeast [9], instead, *P. graveolens* oils had the highest reported activity in *C. albicans* during the experiment, with a maximum inhibition percentage of 161% (Figure 15). Regarding the response of *B. subtilis*, it has been reported that *P. graveolens* oils have a powerful growth inhibition against this microorganism [33]. These findings are consistent with the results presented in Table 6, however, the highest percentage of inhibition presented with the oil, is lower than the maximum reported with the total ethanolic extract (Table 1) and ethyl acetate, (Table 4).

CONCLUSIONS

The extracts of *P. graveolens* present antimicrobial activity against Gram positive and Gram negative bacteria and yeasts, especially the oil extract, which presented the highest percentage of inhibition recorded against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*, with 201.4%, 433.3% and 160.9%, respectively; while for *Pseudomonas aeruginosa* and *Bacillus subtilis*, the extract that presented the highest percentage of inhibition was ethyl acetate , with 222.2% and 132.8%, respectively.

ACKNOWLEDGMENTS

The authors would like to thank Pontificia Universidad Javeriana Bogotá, School of Medicine, Fundación Universitaria Juan N. Corpas, Bogotá, Colombia, and Environmental Engineering Program, Faculty of Engineering, Universidad El Bosque, Research Group CHOC- IZONE, Bogotá, Colombia.

AUTHORS' CONTRIBUTIONS

All authors contribute to design the experiments; Janeth Del Carmen Arias Palacios, Paula Sofía Rojas Pedraza and Oscar Eduardo Rodriguez Aguirre, performed the experiments. All authors revised the article critically for important intellectual content.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

REFERENCES

[1] Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology [Internet]. 2018 Jan 1;7(1):1–7. Available from: http://www.herbmedpharmacol.com/Article/jhp-1198

[2] Li Y, Kong D, Fu Y, Sussman MR, Wu H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry. 2020 Mar;148:80–9.

[3] Ben ElHadj Ali I, Tajini F, Boulila A, Jebri M-A, Boussaid M, Messaoud C, et al. Bioactive compounds from Tunisian Pelargonium graveolens (L'Hér.) essential oils and extracts: α-amylase and acethylcholinesterase inhibitory and antioxidant, antibacterial and phytotoxic activities. Industrial Crops and Products [Internet]. 2020 Dec 15 [cited 2022 Apr 1];158:112951. Available from: https://www.sciencedirect.com/science/article/pii/S0926669020308682?casa_token=5AHxzvSI-v0AAAAA:wtkEsuoPZDpv575Nt6G5Zf4cIb9IpZ_VHRS7WjnwwkB_X44KmCeMdDCfaU41pgG_AnG7xspLZxY

[4] Streicher LM. Exploring the future of infectious disease treatment in a post-antibiotic era: Acomparative review of alternative therapeutics. J Glob Antimicrob Resist. 2021;24:285–95. Disponible en: https://doi.org/10.1016/j.jgar.2020.12.025

[5] Organización mundial de la salud (OMS). Informe para el secretario general de las naciones unidas. Organización mundial de salud (OMS) 2019.

[6] Kongkham B, Prabakaran D, Puttaswamy H. Opportunities and challenges in managing antibiotic resistance in bacteria using plant secondary metabolites. Fitoterapia. 2020;147(104762):104762. Disponible en: https://doi.org/10.1016/j.fitote.2020.104762

[7] Diaz-Sanchez S, D'Souza D, Biswas D, Hanning I. Botanical alternatives to antibiotics for use in organic poultry production. Poult Sci 2015;94:1419–30. Disponible en: https://doi.org/10.3382/ps/pev014

[8] Rakholiya KD, Kaneria MJ, Chanda SV. Medicinal plants as alternative sources of therapeutics against multidrug-resistant pathogenic microorganisms based on their antimicrobial potential and synergistic properties. In: Rai MK, Kon KV, editors. Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components. San Diego, CA: Elsevier; 2013. p. 165–79. Disponible en: https://doi.org/10.1016/B978-0-12-398539-2.00011-2

[9] Gâlea C, Gabriel H. Antimicrobial and Antifungal Activity of Pelargonium roseum Essential Oils. Adv Pharm Bull. 2014;4(Suppl 2):511–4. Disponible en: https://doi.org/10.5681/apb.2014.075

[10] Mativandlela SPN, Lall N, Meyer JJM. Antibacterial, antifungal and antitubercular activity of (the roots of) Pelargonium reniforme (CURT) and Pelargonium sidoides (DC) (Geraniaceae) root extracts. S Afr J Bot. 2006;72(2):232–7. Disponible en: https://doi.org/10.1016/j.sajb.2005.08.002

[11] Boukhatem MN, Kameli A, Saidi F. Essential oil of Algerian rose-scented geranium (Pelargonium graveolens): Chemical composition and antimicrobial activity against food spoilage pathogens. Food Control. 2013;34(1):208–13. Disponible en: https://doi.org/10.1016/j.foodcont.2013.03.045

[12] El Aanachi S, Gali L, Nacer SN, Bensouici C, Dari K, Aassila H. Phenolic contents and in vitro investigation of the antioxidant, enzyme inhibitory, photoprotective, and antimicrobial effects of the organic extracts of Pelargonium graveolens growing in Morocco. Biocatal Agric Biotechnol. 2020;29(101819):101819. Disponible en: https://doi.org/10.1016/j.bcab.2020.101819

[13] Savoia D. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol. 2012;7(8):979–90. Disponible en: https://doi.org/10.2217/fmb.12.68

[14] Ringuelet J, Viña S. Productos naturales vegetales (1st ed., pp. 7-17). La Plata: Editorial de la Universidad Nacional de La Plata.

[15] Nuñez CE. Extracciones con equipo Soxhlet. Bioquímica y biología molecular 2008; 2 (3): 5.

[16] Researchers find that Earth may be home to 1 trillion species [Internet]. Nsf.gov. [cited 2021 Apr 21]. Disponible en: https://www.nsf.gov/news/news_summ.jsp?cntn_id=138446

[17] Microbiology by numbers. Nat Rev Microbiol. 2011;9(9):628. Disponible en: https://doi.org/10.1038/nrmicro2644

[18] Richardson JP, Moyes DL. Adaptive immune responses to Candida albicans infection. Virulence. 2015;6(4):327–37. Disponible en: https://doi.org/10.1080/21505594.2015.1004977

[19] Mueller M, Tainter CR. Escherichia Coli. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020. Disponible en: https://www.ncbi.nlm.nih.gov/books/NBK564298/

[20] Taylor TA, Unakal CG. Staphylococcus Aureus. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020. Disponible en: https://www.ncbi.nlm.nih.gov/books/NBK441868/

[21] Fejfarová V, Tibenská H, Niklová J, Bém R, Dubský M, Wosková V, et al. Benefits of acidifying agents in local therapy of diabetic foot ulcers infected by Pseudomonas sp: A pilot study. Int J Low Extrem Wounds. 2019;18(3):262–8. Disponible en: https://doi.org/10.1177/1534734619848573

[22] Pseudomonas aeruginosa [Internet]. Sciencedirect.com. [cited 2021 Apr 29]. Available from: https://www.sciencedirect.com/topics/medicine-and-dentistry/pseudomonas-aeruginosa

[23] Gu H-J, Sun Q-L, Luo J-C, Zhang J, Sun L. A first study of the virulence potential of a Bacillus subtilis isolate from deep-sea hydrothermal vent. Front Cell Infect Microbiol. 2019;9:183. Disponible en: https://doi.org/10.3389/fcimb.2019.00183

[24] Tsonis I, Karamani L, Xaplanteri P, Kolonitsiou F, Zampakis P, Gatzounis G, et al. Spontaneous cerebral abscess due to Bacillus subtilis in an immunocompetent male patient: A case report and review of literature. World Journal of Clinical Cases [Internet]. 2018 Dec 26 [cited 2022 Apr 2];6(16):1169–74. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6306642/

[25] Frieri M, Kumar K, Boutin A. Antibiotic resistance. J Infect Public Health. 2017;10(4):369–78. Disponible en: https://doi.org/10.1016/j.jiph.2016.08.007

[26] Sigmaaldrich.com. Disponible en: https://www.sigmaaldrich.com/catalog/product/sial/70138?lang=en&region=CO

[27] Camacho MA, Arias J, Rodríguez OE. Assessment of the antibacterial capacity of extracts of sinapis alba l. by the method of plates and wells. Pharmacology Online 2019; 2: 329-335.

[28] Giraldo PA, Andrade AC, Pombo L, Arias J, Rodríguez OE. DETERMINATION OF ANTIMICROBIAL ACTIVITY IN LEAVES AND FLOWERS OF CHROMOLAENA SCABRA (L. F.) R.M. KING AND H. ROB. Innovare 2020;

[29] Freed BK, Biesecker J, Middleton WJ. Spectral polarity index: a new method for determining the relative polarity of solvents [1]. J Fluor

Chem 1990;48:63–75. https://doi.org/10.1016/s0022-1139(00)82602-0.

[30] Panphut W, Budsabun T, Sangsuriya P. In vitro antimicrobial activity of Piper retrofractum fruit extracts against microbial pathogens causing infections in human and animals. Int J Microbiol 2020;2020:5638961. https://doi.org/10.1155/2020/5638961.

[31] Singh R, Gupta AK. Antimicrobial and antitumor activity of the fractionated extracts of Kalimusli (Curculigo orchioides). Int J Green Pharm 2008;2. https://doi.org/10.22377/ijgp.v2i1.393.

[32] Hussain AI, Anwar F, Shahid M, Ashraf M, Przybylski R. Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (Mentha spicataL.) from Pakistan. J Essent Oil Res 2010;22:78–84. https://doi.org/10.1080/10412905.2010.9700269.

[33] Ben Hsouna A, Hamdi N. Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from Pelargonium graveolens growing in Tunisia. Lipids Health Dis 2012;11:167. https://doi.org/10.1186/1476-511X-11-167.

DOI: https://doi.org/10.15379/ijmst.v10i2.3292