August 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.2 • 329-335

ASSESSMENT OF THE ANTIBACTERIAL CAPACITY OF EXTRACTS OF *Sinapis alba* L. BY THE METHOD OF PLATES AND WELLS

Camacho Carrillo M.A.[1], Arias-Palacios J.[1], Rodríguez Aguirre O.E.[2,3]

[1]Departamento de Microbiología, Grupo Biotecnología Ambiental e Industrial, Pontificia Universidad Javeriana, Bogotá, Colombia

[2]Facultad de Ingeniería, Universidad El Bosque, Bogotá Colombia

[3]Departamento de Química, Pontificia Universidad Javeriana, Bogotá, Colombia

Email address: jdcarias@javeriana.edu.co

Abstract

The antibacterial effect of the essential oils of *Sinapis alba* L is known, since these contain mainly allylisothiocyanate, with an inhibitory effect on a great variety of bacteria. This study was carried out to known the antibacterial effects of *Sinapis alba* L. extracts to three different bacteria strains. The plates and wells methodology was used.The results allowed to determine that all the vegetal organs of *Sinapis alba* had an inhibitory effect to *S. aureus*, however, no evidence inhibitory effect against *E. coli* or *Pseudomonas*. And the highest percentage of inhibition was with petroleum ether solvent.

Keywords: *Sinapis alba, Antibacterial Capacity*, *S. aureus*.

Introduction

Sinapis alba L is a plant belonging to the family Cruciferaceae or Brassicaceae. Its Latin genus "sinapis" which means mustard, and the Latin species "alba" which means white, refers to the light color of its seeds [1,2]. It is widely recognized as the producer of mustard, however, in addition to its culinary use has medicinal advantages. It's seeds and oil are consumed as palliative treatments for cancer, bronchitis, pneumonia, among other diseases. In addition, it is believed that the plant has emollient and sedative properties, even narcotic [3]. The antibacterial effect of the essential oils of *Sinapis alba* L is also known, since these contain mainly allylisothiocyanate, which contains an inhibitory effect on a great variety of bacteria such as *E. coli, P. fluorescens, S. aureus, Bacillus subtilis, L. brevis, K. pneumoniae, P. aeruginosa* [4,5].

The method used for the extraction of *Sinapis alba* L (Root, stem, leaves and flowers) was Soxhlet, in which by boiling and an organic solvent, the oils contained in the parts of the plant are extracted [6].

The microorganisms tested during this experiment, both Gram positive and Gram negative, are known to produce diseases such as gastroenteritis, meningitis, pneumonia, among others; that at present are controlled with medicines elaborated from chemical synthesis; however, it is of great interest to test the effect produced by *Sinapis alba* L as a different alternative for the treatment of these. In the present study it is proposed to demonstrate that the extracts of the *Sinapis alba* L plant have a bactericidal effect against the aforementioned microorganisms.

Materials and methods

Obtaining the strains

The microorganisms used were obtained from the bacterial strain collection of the Pontifica Universidad Javeriana.

Preparation of culture medium

The assembly of the experiment was carried out facing each part of the plant with each of the strains, so 52 g / L of BHI agar was prepared.

Preparation of the inoculum

A suspension of each microorganism was made in BHI broth from each strain, this inoculum had a concentration of 1x108 CFU / mL with reference to pattern 1 of Mc Farland. Finally, for the control of the inoculum, confirmation of the initial concentration was made by plate count on BHI agar.

Extraction

The extraction process of the plant was carried out in the same way for the four parts of the plant: stem, root, leaves and flowers. A sample was taken which was introduced in a thimble, then the Soxhlet extraction process was started using first as petroleum ether solvent, after finishing the process and collecting the extract, the sample was allowed to dry to repeat the Soxhlet extraction with ethanol and finally with dichloromethane, the extracts were finally obtained with a pasty consistency.

Determination of the inhibitory effect of each of the extracts on agar plates

From the previously prepared inocula, each was mixed separately with the BHI broth. In each 100x15mm petri dish approximately 27mL of inoculated medium was served, then allowed to solidify under refrigeration. Subsequently, to each agar plate two central perforations were made with an inverted pipette of 10mL. Finally, 100 uL of each extract was added in triplicate. This procedure was repeated in the same way for the 3 microorganisms under study [7] (Table 1).

Incubation

All microorganisms were incubated at 37 ° C for 3 days.

Controls

Growth control of each bacterium in the culture medium without perforation was carried out. To perform the blank control, 100uL of dimethylsulfoxide (DMSO) was used in duplicate, and a commercial antibacterial was used as a positive control (5.25% sodium hypochlorite) [7].

Measurement of relative antimicrobial activity

After the incubation, the measurement of the halos of each assembly was carried out and the relative antimicrobial activity with respect to the

commercial antibacterial and DMSO was calculated using the following formula [8].

$$\% \, inhibition = \frac{\text{diameter halo extract} - \text{white halo diameter}}{\text{halo diameter positive control} - \text{white halo diameter}}$$

Diameter of the white halo (mm): DMSO

Diameter of the control (mm): 5.25% sodium hypochlorite.

Results

Antibacterial activity of Sinapis alba L extracts on E. coli

From the procedure carried out in the practical stage of this work of degree, the results for E. coli were obtained, in the Table 2 it can be shown that they did not present inhibition halos with respect to the total extracts of root, stems, leaves and flowers with none of the extraction solvents.

In relation to the controls, haloes of inhibition of 5 mm with sodium hypochlorite and 2 mm with DMSO were evidenced.

Antibacterial activity of extracts of Sinapis alba L on P. aeruginosa.

In Table 2, the results of the inhibition of the plant on P. aeruginosa are presented, where it is shown that the microorganism was resistant to the effect of the plant. Additionally, *P. aeruginosa*, where there was growth inhibition only in the control with sodium hypochlorite.

Antibacterial activity of the extracts of Sinapis alba L on S. aureus

From the results obtained, it was observed that Sinapis alba L has an inhibitory effect on the microorganism, this can be seen in Table 2.

For *S. aureus*, the percentages of inhibition were obtained from equation 1, where the extract that had the highest percentage of inhibition was the stem with petroleum ether in 5.3%, followed by root with petroleum ether with 3.9%, leaves with petroleum ether and flowers with dichloromethane with 3.8%; in the cases of root with petroleum ether, root with ethanol and leaves with dichloromethane there was no inhibition.

In turn, halos of inhibition were presented against the control with sodium hypochlorite, this being 5 mm and for the control with DMSO of 2 mm.

Analysis of diameters

As can be seen in Table 3a and Figure 1a and 2a, the stem extract shows that, on average, the diameter of the highest haloes is due to the stem extract. The above shows that the extract of the stem on average presents better antibacterial activity.

As for the solvent, the Petroleum ether shows a greater average growth in the diameter of the haloes as can be seen in Table 3b and Figure 1b and 2b. However, it is observed that, on average, the petroleum ether solvent shows greater activity, since its average is better bought with the extract.

In figure 1, we can see that the extracts with the highest percentages of inhibition were stems and flowers, where values of 33% and 31% were obtained, respectively, compared to the root extract that only has 15% inhibition. These results can also be observed in table 2, where *S. aureus* had a greater inhibitory effect with these extracts.

Discussion

The *Sinapis alba* L plant belonging to the *Brassicaceae* family contains different types of glucosinolates and / or degradation by-products that have been known for a long time for their fungicidal, bactericidal, nematicidal and allelopathic properties [9]; the nature of these depends on the hydrolysis conditions and the particular glucosinolate [10]. It has been reported that these products of hydrolysis are responsible for the inhibition of Gram positive and negative bacteria such as *E. coli, S. aureus, P. aeruginosa*, among other bacteria, however, their action on microorganisms is unknown.

In a study conducted by Aliakbarlu (2013), where Sinapis alba extracted from distilled water was used, no type of inhibition against E. coli was obtained, this is related to the results of this study, where this microorganism was found to be resistant to all treatments (Table 2), which could serve as evidence to emphasize that the plant with or without solvents is not efficient in the inhibition of the microorganism.

On the other hand, according to a study conducted by Das (2012) using an extract of Moringa oleifera, a plant of the same order of *Sinapis alba*, showed that the extract with petroleum ether had no antibacterial activity against Gram negative bacteria including *P. aeruginosa* , additionally Saadabi and Abu (2011) reported in their study that the petroleum ether extract showed no activity against *E. coli.* This has a direct relationship with this study, since one of the solvents used was petroleum ether, which for these two Gram negatives had no effect on its growth.

It is known that Gram negative bacteria, such as *E. coli* and *P. aeruginosa*, have an intermembrane space, as well as an outer membrane that protects them against harmful agents [11]. This would indicate that Gram-negative microorganisms may be less susceptible to the action of antibacterials, thanks to the outer membrane that surrounds the cell wall that restricts the diffusion of hydrophobic compounds through its lipopolysaccharide coating [12]. This can be related to the results obtained for *P. aeruginosa* and *E. coli* (Table 2), which showed that they were resistant to the inhibitory effect of *Sinapis alba*. In turn, *S. aureus* was the most sensitive to being inhibited by the four evaluated extracts, this may be due to the fact that Gram-positive bacteria have a less complex outer cover than Gram-negative bacteria and that they only have one layer of peptidoglycan in mesh shape that makes them more permeable to extracts [13].

In relation to *S. aureus*, according to a study conducted by Peng (2014), they analyzed the compositions of mustard seed essential oil to determine its antimicrobial activity, focusing on allyl isothiocyanate (AITC) where they reported that it represented 71.06 % *Sinapis alba* essential oil. In this study, they performed the extraction procedure with DMSO as a solvent and, using the disc diffusion technique, they determined that *S. aureus* had a greater inhibition diameter, this being 15.57 mm; This is consistent with the data obtained in this trial, where *S. aureus* showed an inhibitory effect on the plant, showing close values, since the extract with the highest inhibition for this microorganism was 18 mm (Table 2); with this, it could be said that the AITC are possibly related to the inhibition of the microorganism since, in addition, they used a solvent different from those used in this study, which would show that the inhibitory effect of *Sinapis alba* on *S. aureus* is not influenced by the type of solvent if not by plant action, however, its structure-function relationship, its comparative spectra of activity or the mechanism of action of glucosinolates or their byproducts are not experimentally identified and does not allow deductions on the actual efficacy of these chemical compounds [14].

It is known that Gram negative bacteria, such as *E. coli* and *P. aeruginosa*, have an intermembrane space, as well as an outer membrane that protects them against harmful agents [11]. This would indicate that Gram-negative microorganisms may be less susceptible to the action of antibacterials, thanks to the outer membrane that surrounds the cell wall that restricts the diffusion of hydrophobic compounds through its lipopolysaccharide coating [12]. This can be related to the results obtained for *P. aeruginosa* and *E. coli*, which showed that they were resistant to the inhibitory effect of *Sinapis alba*. In turn, *S. aureus* was the most sensitive to being inhibited by the four evaluated extracts, this may be due to the fact that Gram-positive bacteria have a less complex outer cover than Gram-negative bacteria and that they only have one layer of peptidoglycan in mesh shape that makes them more permeable to extracts [13].

Conclusion

Regarding the extracts used, it was determined by the percentage of inhibition that the extract with the highest antibacterial spectrum on *S. aureus*, was the stem extract with petroleum ether, followed by the root extract with petroleum ether, leaves with ether of oil and flowers with dichloromethane.

With the test, it was also possible to show that based on the extract, stem and root had a higher percentage of inhibition in all cases and that, in turn, dichloromethane also had better results in comparison with the other solvents.

Acknowledgments

The authors are please to acknowledge Pontificia Universidad Javeriana and universidad El Bosque for support this project.

References

1. Singh S, Das S, Singh G, Perroti M, Schuff C, Catalán CA. Comparison of Chemical Composition, Antioxidant and Antimicrobial Potentials of Essential Oils and Oleoresins obtained from Seeds of Brassica Juncea and sinapis Alba. MOJ Food Process Technol. 2017;4(4):113–20.

2. Rosario S JM. Resistencia de Sinapis alba L.A herbicidas inhibidores de la ALS: bases agronómicas bioquímicas y moleculares. Córdoba; 2011.

3. Kozlowska H, Rotkiewicz DA, Zadernowski R, Sosulski FW. Phenolic acids in rapeseed and mustard. J Am Oil Chem Soc. 1983;60(6):1119–23.

4. Mejía-garibay B, Guerrero-beltrán JÁ, Palou E, López-malo A, Química DDI, Ambiental A, et al. Características físicas y antioxidantes de semillas y productos de mostaza negra (Brassica nigra) y amarilla (Brassica alba) . 2015;65:128–35. Available from: http://www.scielo.org.ve/pdf/alan/v65n2/art08.pdf

5. Boscaro V, Boffa L, Binello A, Amisano G, Fornasero S, Cravotto G, et al. Antiproliferative, proapoptotic, antioxidant and antimicrobial effects of sinapis nigra L. And sinapis alba L. Extracts. Molecules. 2018;23(11).

6. González ADD, Viatcheslav K, Alexander Guzmán M. Desarrollo de métodos de extracción de aceite en la cadena de producción de biodiesel a partir de microalgas. Prospect. 2009;7(2):53–60.

7. Durán Blanco M, Arias Palacios J, Rodríguez Aguirre O. Determination of the antifungal capacity of total extracts of sinapis alba L by the method of plates and wells. Asian J Sci Technol. 2017;8(12):7197–200.

8. Ramírez L, Díaz H. Actividad antibacteriana de extractos y fracciones del ruibarbo (Rumex conglomeratus). Scientia [Internet]. 2007;XIII(037):13–8. Available from: http://www.redalyc.org/html/849/84933113/%0Ahttp://redalyc.uaemex.mx/src/inicio/ArtPdfRed.jsp?iCve=84903790

9. J.w F, A.t Z, P T. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry. 2001;56.

10. Rincón V-, Sánchez-León •, Jiménez •. La Producción De Metabolitos Secundarios En La Familia Brassicaceae Secondary Metabolites Production By Brassicaceae Family. 2013;282–305.

11. Longbottom CJ, Carson CF, Hammer KA, Mee BJ, Riley T V. Tolerance of Pseudomonas aeruginosa to Melaleuca alternifolia (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. J Antimicrob Chemother. 2004;54(2):386–92.

12. Ratledge C, Wilkinson SG. An overview of microbial lipids. Microb lipids [Internet]. 1988 [cited 2019 Apr 7];1:3–22. Available from: https://scholar.google.com/scholar_lookup?title=An overview of microbial lipids&author=C. Ratledge&author=SG. Wilkinson&pages=3-22&publication_year=1988

13. Burt S. Essential oils: Their antibacterial properties and potential applications in foods - A review. Int J Food Microbiol. 2004;94(3):223–53.

14. Wilson AE, Bergaentzlé M, Bindler F, Marchioni E, Lintz A, Ennahar S. In vitro efficacies of various isothiocyanates from cruciferous vegetables as antimicrobial agents against foodborne pathogens and spoilage bacteria. Food Control [Internet]. 2013;30(1):318–24. Available from: http://dx.doi.org/10.1016/j.foodcont.2012.07.031

Table 1: Mounting model of each of the extracts

Microorganism	*Sinapis alba L.*			
	R1	**R2**	**R3**	**R4**
Escherichia coli	BHI	BHI	BHI	BHI
Staphylococcus aureus	BHI	BHI	BHI	BHI
Pseudomona aeruginosa	BHI	BHI	BHI	BHI

Table 2: Measurements of inhibition halos (mm) and percentage of inhibition of root extracts, stem, leaves and flowers against E. coli, S. aureus and P. aeruginosa

Extract	Solvent	Inhibition halos (mm) *Escherichia coli*				% inhibition	Inhibition halos (mm) *Staphylococcus aureus*				*X*	% inhibition	Inhibition halos (mm) *Pseudomona aeruginosa*				% inhibition
		R1	R2	R3	R4		R1	R2	R3	R4			R1	R2	R3	R4	
Root	Dichloromethane	-	-	-	-	0	2	2	2	2	2,00	0,0	-	-	-	-	0
	Petroleum ether	-	-	-	-	0	15	15	15	10	13,75	3,9	-	-	-	-	0
	Ethanol	-	-	-	-	0	2	2	2	2	2,00	0,0	-	-	-	-	0
Stem	Dichloromethane	-	-	-	-	0	10	12	9	9	10,00	2,7	-	-	-	-	0
	Petroleum ether	-	-	-	-	0	15	17	20	20	18,00	5,3	-	-	-	-	0
	Ethanol	-	-	-	-	0	11	13	11	12	11,75	3,3	-	-	-	-	0
Leaves	Dichloromethane	-	-	-	-	0	-	-	-	-	0,00	0,0	-	-	-	-	0
	Petroleum ether	-	-	-	-	0	15	13	12	13	13,25	3,8	-	-	-	-	0
	Ethanol	-	-	-	-	0	12	11	11	13	11,75	3,3	-	-	-	-	0
Flowers	Dichloromethane	-	-	-	-	0	12	14	12	15	13,25	3,8	-	-	-	-	0
	Petroleum ether	-	-	-	-	0	10	11	11	9	10,25	2,8	-	-	-	-	0
	Ethanol	-	-	-	-	0	14	13	11	13	12,75	3,6	-	-	-	-	0
Positive control	Hypochlorite 5,25%	5	5	5	5		5	5	5	5	5,00		5	5	5	5	
Negative control	DMSO	2	2	2	2		2	2	2	2	2,00		-	-	-	-	

Table 3: Average diameter per extract (a), per solvent (b)

Extracto	Promedio halos
Root	5.92
Steam	13.25
Leaves	8.33
Flowers	12.08

Solvente	Diámetro Promedio
Dichloromethane	6.31
Petroleum ether	13.8
Ethanol	9.56

Figure 1: Halos of inhibition by extract. A. Average halos of inhibition by extract, B. Sum of diameter by extract.

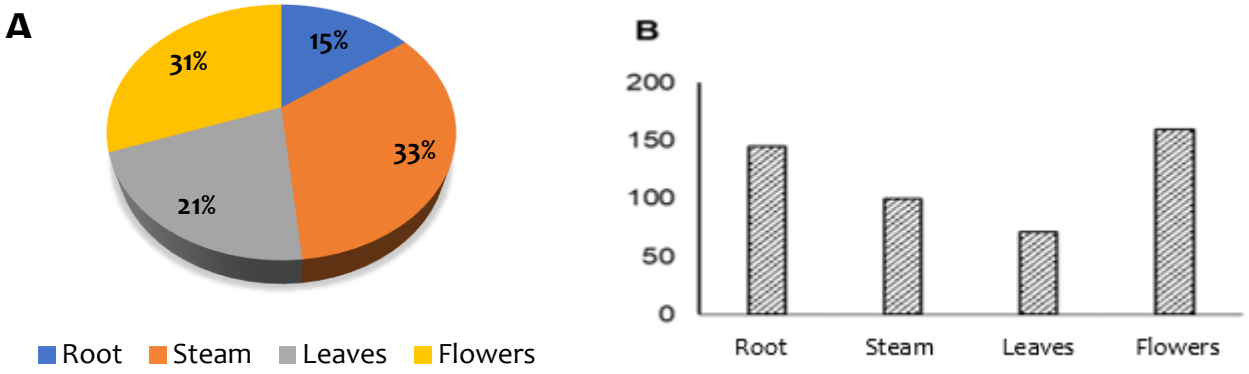

Figure 2: Halos of inhibition by extract. A. Average inhibition diameter per solvent, B. Total diameter per solvent