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| cetlogo ***CHEMICAL ENGINEERING TRANSACTIONS***  ***VOL. 92, 2022*** | A publication of  aidiclogo_grande |
| The Italian Association  of Chemical Engineering  Online at www.cetjournal.it |
| Guest Editors: Rubens Maciel Filho, Eliseo Ranzi, Leonardo Tognotti  Copyright © 2022, AIDIC Servizi S.r.l. **ISBN** 978-88-95608-90-7; **ISSN** 2283-9216 | |

Development of a Biopesticide as an Eco-Safe Candidate for the Management of Agricultural Crops in the Department of Bolivar, Cartagena – Colombia

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The increased use of synthetic pesticides, and the risks to human health and the environment that they represent, have led to the recent promulgation of the development of sustainable agriculture. In the department of Bolivar, Colombia, agriculture represents one of the most important activities in the economic sector; however, the incidence of different types of pests and diseases in the main production systems is considered the main challenge to maintain the productivity of different agricultural crops. To conduct pest control through methodologies that contribute to environmental sustainability, the use of botanical products has been considered as an alternative to chemical control with conventional synthetic pesticides. Considering the above, this research sought to develop a biopesticide from avocado seeds with anthelmintic and bactericidal properties. The soxhlet method for extracting compounds from the pesticide seed with two solvents was used, then the extract was diluted in water to perform the tests with biological models such as Caenorhabditis elegans for anthelmintic experiments with endpoints such as mortality and change in gene expression, and for Gram-positive bacteria: Staphylococcus aureus (ATCC 35696), Staphylococcus epidermidis (ATCC12228), Klebsiella pneumonia (ATCC 10031), Escherichia coli (ATCC 25922) and Pseudomonas aeruginosa (ATCC 27853) using the broth dilution method, for which the minimum inhibitory concentration was taken into account. Preliminary results show a high percentage of lethality in nematodes and bacteria, demonstrating that residual biomass such as avocado seed is a strategy for the preservation of human health and the environment in general in its application as an organic pesticide in agricultural crops.

* 1. Introduction

Population growth and high demand for food has made it necessary to use pesticides to make most of agricultural products. However, the use of this substance causes the accumulation of toxic waste in the environment, depositing it in bodies of water, soil and air (Tudi et al., 2021). It can also have adverse human health effects, such as thyroid cancer and neurological problems in children (Lerro et al., 2021; Roberts et al., 2018).

This situation makes it necessary to search for alternatives friendly to the environment and human health. One of these alternatives are biopesticides, pesticides from living organisms, such as plants, microorganisms or animals, whose main compounds are organic, not harmful to human health and have little or no impact on the environment (Tudi et al., 2021).

*Persea americana* is the tree that has the avocado as its fruit, this variety presents better adaptation to the climatic conditions of Colombia from 18-26 °C, suggesting that it probably originated in South America and more exactly on the North coast of Colombia. For its part, one of the main characteristics of this variety is the large size of its fruits, which can be from 250 to 2,500 grams in weight, oval, round or pear-shaped; They have a shiny, smooth or leathery, flexible, thin, non-granular bark that is green, yellowish green, bright green, reddish yellow, red, purple or black, and with pulp very low in fat (5 to 15%) and high in sugar (5%). Among the variety *Persea* *americana* are the commercial varieties: Butler, Fuchs, Hulumanu, Lorena, Peterson, Pinelli, Simmonds and others. It´s seed has been sowed to have bioactive with biological properties that benefit human health. This seed has shown activity against bacteria, fungi and other microorganisms of environmental concern. Therefore, avocado seed extracts can be considered biopesticide candidates for the maintenance of agricultural crops (Flores et al., 2019).

In this article, some studies performed by different authors that show the antibacterial activity of avocado seed are analysed, as well as the results of preliminary tests of anthelmintic activity of avocado seed extracts against Caenorhabditis elegans as evidence of biocide activity presented by these extracts on microorganisms of ecological relevance.

* + 1. Avocado seed as a biopesticide

Currently, avocado seed represents an underutilized resource and waste problem for avocado processors. There is ethno-pharmacological information on the use of seeds for treating health-related conditions, mainly in South American regions, where this fruit is endemic. The phenolic compounds of the seed would be responsible for the benefits on human health. Additionally, other compounds such as terpenes and alkaloids to which antimicrobial activity is attributed (Dabas et al., 2013).

The development of analytical chemistry and tools such as liquid and gas chromatography has made it possible to clarify the presence of metabolites in the avocado seed to which biocidal properties can be attributed. Most of these compounds are apolar or moderately apolar, some of them identified as sesquiterpenoids, esters of unsaturated fatty acids and polyunsaturated fatty acids (Soledad et al., 2021). Likewise, there are morphological variations in the avocado fruit, which in turn generates changes in the metabolites of the variations, therefore, some variations will present a greater biocidal activity than others (Alves et al., 2019). This information is confirmed by the different studies reviewed below.

* + 1. Antibacterial activity of avocado seed extracts

Numerous studies have been based on obtaining bioactives from the pulp, peel and seed of avocado, which allow the discovery of therapeutic alternatives for different diseases (Flores et al., 2019). Although the seed represents a considerable percentage of the total fruit, scientific research on the phytochemistry and biological effects of avocado seeds is in the early stages. Currently, the seed represents an underutilized resource and waste problem for avocado processors. There is ethno-pharmacological information on the use of seeds to improve hypercholesterolemia, and to be useful in the treatment of hypertension, inflammatory conditions and diabetes (Dabas et al., 2013). There are many studies focused on evaluating the antibacterial activity of avocado seed extracts, obtaining various results. However, most of them point to a possible bacteriostatic activity by bioactive present in this seed. Table 1 shows the main results of investigations carried out to determine the antibacterial activity of avocado seed extracts by means of micro-dilution methods in broth and determination of minimum inhibitory concentration (MIC).

Table 1: Research evaluating the antibacterial activity of avocado seed extracts

|  |  |  |  |
| --- | --- | --- | --- |
| Bacterial strains | Avocado variety/ Extract | Result | Reference |
| *Staphylococcus aureus* (ATCC 14458)  *Bacillus cereus* (ATCC 11778)  *Salmonella typhi* (ATCC 06539)  *Escherichia coli* (ATCC 10536) | Hass/Ethanolic | *S. aureus* MIC was 5 mg/mL, for the rest of the strains it was 20 mg/mL. | (Alves et al., 2019) |
| Quintal/ Ethanolic | *S. aureus* MIC was 2.5 mg/mL, for the rest of the strains it was 10 mg/mL. |
| Fortuna/ Ethanolic | *S. aureus* MIC was 1.25 mg/mL, for the rest of the strains it was 10 mg/mL. |
| Margarida/ Ethanolic | *S. aureus* MIC was 2.5 mg/mL, for the rest of the strains it was 20 mg/mL. |
| *Enterococcus faecium* (Aislado clínico)  *Enterococcus faecalis* (ATCC51299) | -/Acetone | *E. faecium* MIC was 0.0009 mg/mL and *E. faecalis* was 0.0083 mg/mL | (Nogbou et al., 2021) |
| -/ Ethanolic | *E. faecium* MIC was 0.0014 mg/mL y *E. faecalis* was 0.0026 mg/mL |
| -/Methanolic | *E. faecium* MIC was 0.0022 mg/mL y *E. faecalis* was 0.0015 mg/mL |
| *Salmonella enterica* subsp. enterica serovar *Typhimurium* (ATCC 14028) and *Staphylococcus aureus* (ATCC 2913). | Criollo/ Ethanolic | Inhibition of both strains at a concentration of 2000 mg/L | (Soledad et al., 2021) |
| Criollo/ Acetone | Maximum antimicrobial activity at 1000 mg/L |
| *Listeria monocytogenes* (ATCC 7644),  *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus*  *aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212),  *Escherichia coli* (ATCC 25922), *Salmonella Enteritidis*  *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella*  *Typhimurium* (ATCC 13311), and *Enterobacter aerogenes* (ATCC 13048). | Shepard/ Ethanolic | MIC for *S. epidermidis* and *E. coli* was greater than 500 μg/m. For the rest of the strains, it was between 166–416.7 μg/mL | (Raymond-Chia & Dykes, 2010) |
| Hass/ Ethanolic | MIC for *S. enteritis*, *C. freundii*, *P. aeruginosa*, *E. aerogenes* was between 125 and 250 μg/mL. For the rest of the strains, it was higher than 500 μg/mL |
| Fuerte/ Ethanolic | *S. epidermidis*, *E. coli*, *S. typhimurium* and *E. aerogenes* MIC was higher than 500 μg/mL, while for the rest of the strains it was between 125–416.7 μg/mL |
| *S. aureus* ATCC 25923, *E. coli* ATCC 8739 y P. aeruginosa PA01 y clinical isolates of *S. aureus* y *E.coli* | -/ Methanolic | MIC for all the bacteria evaluated ranged from 64 to 128 μg/m | (Ekom et al., 2022) |

The research carried out by Alves et al. evaluated the antibacterial activity of ethanolic extracts from four varieties of avocado seed, obtaining minimum inhibitory concentrations ranging from 1.25 to 20 mg/mL, with the bacterial strain Staphylococcus aureus (ATCC 14458) being the which obtained the lowest inhibition level in all variations (Alves et al., 2019).

Nogbou et al. evaluated the antibacterial activity of three avocado seed extracts against two species of Enterococcus, showing very low minimum inhibitory concentrations in all cases; however, the most outstanding activity was obtained with the acetone extract (Nogbou et al., 2021).

Soledad et al. evaluated the antibacterial activity of ethanolic and acetone extracts from Creole avocado seeds, on the bacterial strains Salmonella enterica subsp. enterica serovar Typhimurium (ATCC 14028) and Staphylococcus aureus (ATCC 2913). In the results, it was observed that increasing the concentration of avocado seed extracts also increased microbial inhibition. The maximum concentration of extracts evaluated was 2000 mg/L, in which the growth of both strains was inhibited, however, the acetone extract showed maximum antimicrobial activity at 1000 mg/L (Soledad et al., 2021).

For their part, Raymond-Chia and Dykes evaluated the antibacterial activity of ethanolic extracts from three variations of avocado seed against reference strains of relevance in food safety, finding that the extracts showed antimicrobial activity (104.2 - 416.7 μg/mL) toward Gram- positive and Gram-negative (except Escherichia coli and Staphylococcus epidermidis) (Raymond-Chia & Dykes, 2010).

Ekom et al. evaluated the antibacterial activity of the ethanolic extract of P. americana on reference bacterial strains and clinical isolates, finding that MIC ranged between 64 and 128 μg/mL. The lowest MIC of 64 μg/mL were reported for S. aureus strains from clinical isolates, followed by S. aureus ATCC 25923, E. coli clinical isolates, E. coli ATCC 8739, and P. aeruginosa PA01(Ekom et al., 2022).

Other researchers have evaluated the antibacterial activity of avocado seed extracts using the disk diffusion method, such is the case of Pulp et al., who evaluated the antibacterial activity of acetone, methanol, and diethyl ether extracts from avocado seed. of “Maluma” variety, against the reference pathogens Shigella sonnei ATCC 9290, Escherichia coli ATCC 8739, Salmonella typhi ATCC 6539, Vibrio parahaemolyticus ATCC 17802, Proteus mirabilis ATCC 25933, Staphylococcus aureus ATCC 6538 and Bacillus cereus ATCC 11778. In this study, it was shown that the methanolic extract has greater potential to inhibit bacterial growth, the Gram-positive pathogens Bacillus cereus and Staphylococcus aureus (Pulp et al., 2021).

This latest study demonstrates that the bioactives present in the avocado seed cause the inhibition mainly of gram-positive bacteria, regardless of the sensitivity method used to evaluate the antibacterial activity. This can be attributed to the lipid bilayer present in gram-negative bacteria, which provides greater protection to the bacteria against antimicrobial agents (Soledad et al., 2021). Some researchers have combined seed extracts from different plant species to enhance the antibacterial activity. Such is the case of Diop et al., who mixed petroleum ether extract from avocado seed and methanolic extract from mango seed in equal proportions, which showed excellent results against S. aureus. The minimum inhibitory concentrations measured in Staphylococcus aureus (ATCC 6538) were 0.019 mg/mL for the mixture compared to 0.039 mg/mL for the avocado or mango extract alone (Diop et al., 2022).

Most of the investigations that evaluate the antibacterial activity of avocado seeds use bacterial strains of clinical and alimentary relevance, with a potential use of the evaluated extracts as antibiotics or antimicrobial additives, the antibacterial activity of these extracts on bacteria that cause it remains to be explored. of diseases in crops. However, due to poor wastewater management, bacteria of clinical and food relevance are increasingly common in agricultural environments in the department of Bolívar-Colombia (Conde et al., 2012). For this reason, the use of pesticides that prevent contamination of crops with this type of bacteria is essential.

* + 1. Anthelmintic activity of avocado seed

The nematode worm Caenorhabditis elegans has been used to evaluate the anthelmintic activity and mechanisms of action of molecules and plant extracts, drugs, since the life cycle of parasitic nematodes is usually complex, requiring a host for its propagation, for which makes it difficult to examine the impact of some substances or molecule on parasitic helminths. Therefore, C. elegans offers an excellent alternative for testing molecules and extracts, as it has a short life cycle and is capable of being easily maintained in the laboratory (Burns et al., 2015).

* 1. Materials and methods
     1. Avocado Seed Extracts

The avocado seed was washed with ultrapure water (UW) and crushed. The crushed seed was wrapped in filter paper and obtained the extracts with Soxhlet method. 500 mL of a 1:1 mixture of ethanol and water were used as solvents.

* + 1. Aqueous Extracts

The avocado seed extracts was mixed with ultrapure water (UW) in three dilutions, 50%, 75% and 100%.

* + 1. Strain

The wild type strain N2 was used for survival assay.

* + 1. Toxicity Assessment

Worms were subjected to age-synchronization via bleach solution (NaOH 0.5 M; HClO 0.8%). Strain was kept at 20 C in Petri dishes with K agar, prepared with KCl (0.03 M), NaCl (0.05 M), agar (17 g L-1), peptone (2.5 g L-1), cholesterol (25.8 µM), CaCl2 (1 mM) and MgSO4 (1 mM). Larvae were seeded with Escherichia coli OP50 Experiments were carried out using whole aqueous extracts.

2.4.1 Survival

Nematodes in larval age L4 were exposed for 24 h to whole extract and a 1:1 solution (53 mM NaCl, 32 mM KCl). 10 ± 1 nematodes for treatment were used. Four replicates for treatment were made and each experiment was done three. Subsequently, a visual inspection was conducted with an optical microscope to count the number of dead nematodes. Distilled water was used as a control. The results obtained were graphed in the GraphPad PRISMA 6 software (Liao, 2018).

* 1. Results

3.1 Survival

The results of lethality test to determine the anthelmintic activity of aqueous extracts of avocado seeds are show below (Figure 1).

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*Figure 1: Lethality test results*

* 1. Conclusions

The antibacterial activity of avocado seed has been documented recently using different bacterial strains, both reference and clinical isolates, showing a notable inhibition of the growth of gram-positive bacteria by methanolic extracts or acetone, mainly. However, the possible inhibitory effect of these extracts on Phytobacteria and their possible development as a biopesticide have yet to be explored. The aqueous extracts of avocado seed preliminarily showed high mortality in C. elegans, which possibly indicates bioactives with anthelmintic activity.

The biocidal properties of the extracts depend on the variety of the seed that is evaluated, since the metabolites vary from one variety of avocado to another. The anthelmintic and antibacterial activity referred to in this document qualify avocado seed extracts as potential biopesticides for the maintenance of agricultural crops.

Acknowledgments

This study was funded by the Biomedical, Toxicological and Environmental Sciences research group - BIOTOXAM and the Biomass Process Design and Utilization Research Group - IDAB of the University of Cartagena.

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