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ANTIMICROBIAL EFFECT OF COCCOLOBA UVIFERA ON ESCHERICHIA COLI STRAINS ISOLATED FROM SHRIMP OBTAINED IN THE MARKET OF SAN FRANCISCO DE CAMPECHE, IN THE STATE OF CAMPECHE

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Abstract: For a long time the human being used plants to cover some of their nutritional needs, thanks to this for years some medicinal plants have been taken into account for the cure of diseases as an alternative to antibiotics Currently it is known that through food different types of foodborne diseases (ETA's) are obtained, these constitute an important public health problem in the regions, with social, economic, political and cultural implications.The microbiological analysis of the collected samples was carried out according to the standards established in the methodology of the Official Mexican Standard NOM-113-SSA1-1994, Method for counting total coliform microorganisms and the Official Mexican Standard NOM-031-SSA1-1993. The resistance profiles were carried out following the Kirby Bauer methodology. For theextract preparation and antimicrobial activity: the methodology described by Tirado torres et al., 2019.(67%), Cobaltin and Cefotaxime (50%) was followed. Low microbial activity was observed using the sea grape aqueous extract with inhibition halos of 8mm and 9mm.

INTRODUCTION

For a long time the human being uses plants to cover some of their nutritional needs, these mostly, apart from having a great nutritional contribution, help strengthen the immune system and therefore the prevention of certain diseases, thanks to this for years Some medicinal plants have been taken into account for the cure of diseases as an alternative to antibiotics, since with their use they have been able to treat the most common conditions in humans, being able to take into account some or all of their parts (leaves, stems, roots, seeds, fruits, etc.), in addition to the fact that the most vulnerable populations often do not have the necessary antibiotics for certain types of diseases. This being the case.

It is currently known that different types of foodborne diseases (ETA's) are obtained through food, these constitute an important public health problem in the regions, with social, economic, political and cultural implications. special interest within the period 1997-2000, both by public health authorities and consumers and producers(PAHO, 2002).

Most cases of food poisoning are due to common bacteria such as E. Coli, Salmonella, Staph, etc.(Garcia AR, 2014).

Faced with the increasingly common presence of these diseases and by providing antibiotics for their dissipation, bacteria have formed mechanisms of resistance to antibiotics, as a form of evolution or survival(Forbes, 2009).

That is why natural alternatives are sought for the inhibition or elimination of these microorganisms, in addition to the fact that, as previously mentioned, the main factor for the contagion of this type of microorganism is through food.

Shrimp was investigated as one of the most consumed foods in the San Francisco de Campeche region, which is eaten fresh and often raw (cocktail and ceviche) and in order to identify one of the main pathogenic microorganisms (E. coli), using standardized and appropriate methods to obtain updated and objective information. The objective of this research work was to investigate a natural alternative with antimicrobial capacity of Sea Grape (Coccoloba Uvifera).

METHODOLOGY

The microbiological analysis of the collected samples was carried out according to the standards established in the methodology of the Official Mexican Standard NOM-113-SSA1-1994, Method for counting total coliform microorganisms and the Official Mexican Standard NOM-031-SSA1-1993. Fresh-chilled and frozen bivalve molluscs.78 shrimp samples were collected in batches of 8 and 10 samples per week for 8 weeks at different distribution points within the San Francisco de Campeche market from September to November 2018, which were transferred to the laboratory of the Faculty of Chemical Sciences. -Biological from the Autonomous University of Campeche (UAC) to be analyzed.

Sample preparation: 25g of shrimp sample were weighed under sterile conditions and transferred to a sterile bag. The 25g of sample was placed in 225ml of peptone water. Dilutions: Volumes of 1 ml of the pre-enriched sample were transferred to the tubes with saline for the -2 dilution, from which 1 ml was transferred to another tube with saline for the -3 sample.

Inoculation: 1ml of the pre-enriched sample (dilution at -1) was transferred to the tubes with 9ml of lactose broth (with Durham hood) for the dilution at -2 and from this 1ml was taken for another tube with lactose broth for the dilution at -3.

Tubes were incubated with lactose broth for 24 hours at 35°C. From the tubes that showed turbidity and gas production, a hoe was taken and seeded in an equal number of tubes with 2% brilliant green bile broth, incubated in a water bath at 45 °C for 24 hours.

The reading of positive tubes in gas and turbidity was made and the reading of the most probable number was made according to theNOM-112-SSA1-1994. They were inoculated with a loop in Petri dishes with EMB agar and incubated at 35 °C for 24 hours. The growth of typical colonies in EMB agar was identified and three colonies were selected and transferred to different media: peptone water, Simmons citrate and MR-VP medium. They were incubated at 35°C for 24 hours. MR-VP medium was incubated for 48 hours.

Reading of biochemical tests

Peptone water: After the incubation time, 2-3 drops of Kovac's reagent were added and the appearance of a red ring on the surface was observed, indicating a positive test.

Simmons citrate: It was observed whether or not there was a change in the color of the medium, discarding those that turned to a bluish color.

MR-VP medium: After the incubation time, 2-3 drops of methyl red reagent were added, and a change in the color of the tube to a red tone was observed, which would indicate a positive test.

All positive samples were identified and transferred to the standard method.

Preparation of the extract: the methodology described by Tirado torres et al., 2019 was followed.

Inoculum preparation and resistance profiles

E. coli strains isolated from shrimp samples were inoculated into tubes with 3ml of trypticasein soy broth and incubated at 35 ± 2 °C for 18 hours. Cultures were centrifuged for 15 min at 3500 rpm. Subsequently, the tubes were decanted and two washes were carried out, adding 5 ml of SSI and centrifuging at 3500 rpm for 20 min in each wash. A third and fourth wash was performed using 5 ml of sterile peptone water and centrifuging at 3500 rpm for 20 min. The last wash was decanted and 5 ml of sterile peptone water was added and vortexed to take 100 µl of the suspension and add it to Mueller Hinton agar plates using the plate extension technique, sensidiscs were placed and incubated at 35 ± 2 °C for 24 hours.

Resistance tests with sea grape extract.

For this test, the strains that showed resistance to at least six antibiotics of the sensidiscs used in the previous stage were used.

Plates with Mueller Hilton agar were used, to which 100µl of the previously made suspension of strains and sterile peptone water were placed, again using the plate extension technique. Discs made with previously sterilized filter paper were placed using sterile tweezers, after which 10µl of extract was added to the discs, once the extracts were absorbed by the agar, the plates were incubated for 24 hours at 35 ± 2 °C.

At the end of the incubation time, the diameter of the inhibition halos was measured with a vernier caliper and averaged for each extract.

RESULTS AND DISCUSSION

Of the 78 samples of E. coli, 47 strains were positive in the confirmatory test, for the reading of the most probable number according to theNOM-112-SSA1-1994 thus reporting E. coli figures between 4 and 11 Log CFU/g. In turn, a prevalence of E. coli of 31% (24 samples) was obtained. The strains isolated from the shrimp samples were subjected to resistance tests with antibiograms and a prevalence of 25% of multi-resistant strains to antibiotics (6 strains) was obtained. Table 1 shows the resistance profiles obtained in which it is observed 100% resistance to Ampicillin, carbenzillin Ynetilmicin; followed by sulfamethoxazole (83%), chloramphenicol and Nitrofurantoin (67%), Cephalotin and Cefotaxime (50%).

In counterpart amikacin, ciprofloxacin, gentamicin and norfloxacin show effective inhibition with 0% microbial resistance.

Antimicrobial activity of sea grape.

In this study, low microbial activity was observed using the aqueous extract of sea grape with inhibition halos of 8mm and 9mm. Although a low inhibition of bacterial growth is reflected, there are no references that compare with the data obtained.

ANTIBIOTIC	CONCENTRATION	# OF RESISTANT STRAINS	REPRESENTATIVE %
AMPICILLIN (AM)	10µg	6	100
CARBENCYLINE (CB)	100µg	6	100
NETILMICIN (NET)	30µg	6	100
SULFAMETOXASOLE + TRIMETROPRIM (STX)	25µg	5	83
CHLORAMPHENICOL (CL)	30µg	4	67
NITROFURANTOIN (NF)	300µg	4	67
CEPHALOTIN (CF)	30µg	3	fifty
CEFOTAXIME (CFX)	5µg	3	fifty
AMIKACIN (AM)	30µg	0	0
CIPROFLOXACIN (CFX)	5µg	0	0
GENTAMICIN (GE)	10µg	0	0
NORFLOXACIN (NOF)	10µg	0	0

Table 1 Resistance profile of E. coli strains isolated from shrimp.

CONCLUSION

The present study gives a clear perspective on the presence of E. coli strains in shrimp samples showing a prevalence of 31%. It is also concluded that shrimp may be participating in the dissemination of resistant strains among the consuming population, therefore it is necessary to implement adequate hygiene practices during the capture, washing, storage and transport of this food, as well as the cooking of this crustacean.

The most effective antibiotics against E. coli were: amikacin, ciprofloxacin, gentamicin and norfloxacin. The indiscriminate use of antibiotics for prophylactic and therapeutic uses could be the reason why the resistance of this microorganism has increased. Future studies need to evaluate the antimicrobial effect of Moringa oleifera against other multiresistant microorganisms such as Shigella, L. monocytogenes, and clostridium.

REFERENCES

1. Ali Akbar and Anil Kumar Anal. Prevalence and antibiogram study of Salmonella and Staphylococcus aureus in poultry meat. Asian Pacific Journal of Tropical Biomedicine3 (2). 2013, Pp163-168

2. Noor Uddin GM., Larsen MH, Barco L, Minh Phu T, Dalsgaard A (2015) Clonal Occurrence of Salmonella Weltevreden in Cultured Shrimp in the Mekong Delta, Vietnam. PLoS ONE 10(7): e0134252. doi:10.1371/journal.pone.0134252

3. Cabrera-Diaz, Elisa; Barbosa-Cardenas, Claudia M; Perez-Montaño, Julia A; Gonzalez-Aguilar, Delia; Pacheco-Gallardo, Carlos; Barba, Jeannette. 2013. Occurrence, Serotype Diversity, and Antimicrobial Resistance of Salmonella in Ground Beef at Retail Stores in Jalisco State, Mexico. Journal of Food Protection 76 (12) 2004-10

4. Miranda J M ., Mondragón A C , Martinez B ., Guarddon M , Rodriguez J A . 2009. Prevalence and antimicrobial resistance patterns of Salmonella from different raw foods in Mexico. J Food Prot. 72(5):966-71. doi: 10.4315/0362-028x-72.5.966.

5. Robles-Reyes Rubén, Eusebio-Hernández M Guadalupe, Avilés-Ruiz David. Evaluation of the Reveal Quick Test for Salmonella detection in raw chicken meat. Rev Latinoam Microbiol 2001; 43 (2): 76-83

6. Charles-Hernández Glennda Lucía, Medina-Solís Carlo Eduardo, Hernández-Romano Jesús. Prevalencia de Salmonella sp en alimentos en el Estado de Tamaulipas durante el año 2005. Rev Invest Clin 2007; 59 (6): 437-443438

7. Heinitz ML, Ruble RD, Wagner DE, Tatini SR. Incidence of Salmonella in fish and seafood. J Food Prot. 2000; 63: 579–592. PMID: 10826714

8. Food and Agriculture Organization of the United Nations. FAO expert workshop on the application of biosecurity measures to control Salmonella contamination in sustainable aquaculture. 2010; FAO fisheries and aquaculture report no. 937.

9. Instituto Sinaloense de Acuacultura: Plan maestro de camarón de alta mar del estado de Sinaloa. 2009. Conapesca: Available at:https://cadenasproductivas.conapesca.gob.mx/pdf_documentos/comites/csp/Programa_Maestro_Estatal_CamaronAltamar_Sinaloa.pdf

10. Comisión Nacional de Acuacultura y Pesca. Available at: https://www.gob.mx/conapesca/articulos/produjo-mexico-47-mil-664-toneladas-de-camaron-en-la-temporada-de-captura-2019-2020-agricultura?idiom=es

11. SIAP 2020. Available at:http://www.campomexicano.gob.mx/raw_pesca_gobmx/seccionar_especie.php

12. Phillips I., Casewell M., Cox T., De Groot B., Friis C., Jones R. (2004): Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J Antimicrob Chemother 53(1):28–52.

13. Arora, D. S., Onsare, J. G. (2014): In vitro antimicrobial evaluation and phytoconstituents of moringa oleífera pod husks. – Ind Crops Prod 52(1): 125-135.

14. Brilhante, R. S. N., Sales, J. A., De Souza Sampaio, C. M., Barbosa, F. G., De Araújo, N. P. M., De Melo Guedes, G. M. (2015): Vibrio spp. from Macrobrachium amazonicum prawn farming are inhibited by Moringa oleifera Extracts. – Asian Pac J Trop Me 8(11): 919-922.

15. Peixoto, J. R., Silva, G. C., Costa, R. A., De Sousa, F. J. R., Vieira, G. H., Filho, A. A. (2011): In vitro antibacterial effect of aqueous and ethanolic Moringa leaf extracts. – Asian Pac J Trop Med 4(3): 201-204.