Academic Journal of Life Sciences
ISSN(e): 2415-2137, ISSN(p): 2415-5217
Vol. 3, No. 8, pp: 47-51, 2017
URL: http://arpgweb.com/?ic=journal&journal=18&info=aims

Isolation of the Pathogenic Organism of Bacterial Blight of *Psidium guajava* and the Antibacterial Effects of Aqueous Ethanolic Leaf Extracts of *Ocimum gratissimum, Cymbopogon citratus, Vernonia amygdalina and Annona muricata* on the Pathogen

Eneh Frank Uchenna* Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Okeke Chidi Benjamin Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Igbokwe Gabriel Ejike Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Nwachukwu Godslove Pephel Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Okpala Chukwuemeka Obumneme Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Abstract: The research work was aimed at isolation, characterization and identification of pathogenic organism responsible for the infection of guavas (*Psidiumguajava*) referred to as bacterial blight of guava and also the antibacterial potency of ethanolic leaf extracts of *Vernoniaamygdalina, Ocimumgratissimum, Cymbopogoncitratus* and *Annonamuricata* were screened against bacterial blight isolate *(Erwiniapsidii)* using agar disk diffusion method. The leaf extracts were screened for antibacterial activities at 200mg/ml concentration. Antibacterial efficacy of extracts against the bacterial isolate was indicated by the appearance of clear zones of inhibition around the disks. The extracts of *Vernoniaamygdalina* and *Ocimumgratissimum* showed inhibitory activities against the bacteria isolate with zones of inhibition of 13mm and 9mm respectively while extracts of *C.citratus* and *A.muricata* showed no zone of inhibition. Result of the antagonistic activity of the extracts compared favourably with the activity of standard fumigant. The minimum inhibitory concentration of *V.amygdalina* and *O.gratissimum* were 25mg/ml and 50mg/ml respectively and not determined in *C.citratus* and *A.muricata.*

Keywords: Psidium guajava; Zone of inhibition; Antibacterial acitivity; Gram staining; Biochemical tests.

1. Introduction

Antibacterial agents otherwise serves as drugs that counter the growth of bacteria, and according to World Health Organization, WHO reported by Santos, *et al.* [1] medicinal plants would be the best source to obtain a variety of drugs, and that certain herbs and plants have therapeutic potency for the treatment of some diseases. A variety of local plants exist which have been proven to possess antibacterial properties. Amongst such local plants are Bitter leaf (*Vernoniaamygdalina*), Soursop (*Anonamuricata L.*), African Basil (*Ocimumgratissimum*) and Lemon grass (*Cymbopogoncitratus*).

According to Kedari and Ayesha [2], plants extracts of A. muricata exhibit diverse categories of pharmacological activities such as Antihyperglycemic, Anti-Herpes Simplex Virus, Anticancer/Acetoginins, Antiltyperlipidemia, Anti-depression, Antimicrobial, Cytotoxicity, Chemopreventive/Skin papillomagenesis, Antioxidant, Antibacterial, Anti-Proliferative.

In a research work carried out by Mbajiuka, *et al.* [3], it was reported that the inhibitory activities of ethanolic extracts of *Ocimumgratissimum* against bacteria were found to be little greater than aqueous (cold) crude extracts which according to Sofowora [4] the active principles of the plant herb may be more soluble in ethanol as employed in traditional medicine.

*Corresponding Author

According to Ogundare [5], the extracts *Vernoniaamygdalina*, *Ocimumgratissimum*, *Corchorousolitorius*and*Manihot palmate*)showed varying antimicrobial activities among the plant extracts, with *V. amygdalina*, and *O. gratissimum* appearing to exhibit the highest antibacterial activity.

The guava fruit is in fact a berry whose shape can vary from round to pear-shaped. The skin is yellow with some patches of pink and the interior of the fruit consists of a juicy central pulp dark-pink coloured with several yellowish seeds. [6, 7].

The economic and nutritional benefits of Guava are compromised when it is attacked by plant pathogens. It is with this background that plants with anti microbial properties against the pathogens of Guava are investigated in order to control the huge nutritional and economic losses associated with the attack of pathogens. It is also noteworthy that plant extracts or phyto-chemicals are safer to the human systems and the environment.

2. Material and Methods

2.1. Sources of Plant Samples

The infected Guavas were collected from Abia community in Udi Local Government Area of Enugu state and the fresh samples of *Ocimumgratissimum*, and *Annonamuricata* leaves were collected from Umuaguwhile *Cymbopogoncitratus*, and *Vernoniaamygdalina* leaves were collected fromUmuegbu all in Nnobi community, Idemili South Local Government Area in Anambra State between the hours of 6-8 am at a prevailing temperature of about 28±2^{o}C. All the collections were done in the month of May.

The plant leaves and the infected Guava fruits were identified and authenticated by Mr. Nwatu, the taxonomist at Botany Department of Faculty of Biosciences, NnamdiAzikiwe University, Awka, Anambra State.

2.2. Extraction of Samples

The plant leaves were collected in bulk, washed under running tap water to remove dirt and rinsed with distilled water. The harvested leaves were air dried for fourteen days in the Applied Biochemistry laboratory and the leaves pulverized in a mechanical grinder.

The powdered leaves (50g) were extracted with 250 ml of 70% ethanol which were done by soaking the powdered leaves in 70% ethanol for 32hours, and then filtered by first , using a clean muslin cloth and then, No. 1 Whatman filter paper. The filtrates were concentrated to dryness.

2.3. Isolation of Micro-Organism from the Infected Sweet Orange

2.3.1. Procedures

The infected part was cleaned with 70% ethanol to disinfect the infected part. The infected part was excised using an ethanol-cleaned knife and cut into tiny bits with a spatula. The fragmented sample (0.1g) was placed in a test tube containing 10ml of distilled water and serially diluted using ten-fold dilution for 10,000 times (serially diluted for four times).

A quantity (1ml) of 10,000 times diluted test organism was transferred to the various prepared agar medium and spread evenly using spread plate method described by Madigan, *et al.* [8].

The petri-dishes were incubated at 37^{o} C for 16 – 24hours for growth.

2.4. Morphological Characterization

2.4.1. Gram staining Technique

Procedures: The test organism was heat-fixed (a suspension of the test organism was passed over a flame from a Bunsen burner) on a slide and the slide flooded with 1% aqueous solution of crystal violet and allowed to stand for a minute. The crystal violet stain was poured off, washed with water and the slide flooded with gram Iodine solution and allowed a contact time of a minute. The slide was gently rinsed with water from a squirt bottle and blotted by air-drying to remove excess water without allowing smear to become dry.

The slide was discoloured with ethanol, washed and air dried and counterstained using 2% solution of safranin (dye), which was then washed briefly with water and blotted (air dried) and viewed with a light microscope. Oil immersion lens was used (x100).

2.5. Biochemical Tests

2.5.1. Slide Coagulase Test

Principle: Coagulase test is used to differentiate Staphylococcus aureus (positive) from coagulase negative Staphylococcus (CONS). Coagulase is an enzyme produced by S.aureus that converts (soluble) fibrinogen in plasma to (insoluble) fibrin.

Procedures: The slide was divided into two sections with grease pencil, one labeled as “test” and other as “control”. A small drop of distilled water was placed on each area. One or two colonies of the test organism were emulsified on each drop (placed on the water) to make a smooth suspension. The test suspension was treated with a drop of plasma (from human blood) and mixed well with a loop and observed for clumping. The control suspension serves to rule out false positivity due to agglutination.

2.6. Catalase Test (Slide Method)

Principle: Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them by mediating the breakdown of hydrogen peroxide into oxygen and water.

Procedures: Some colony of the test organism was gently transferred unto a slide using a sterile wire loop and 4 – 5 drops (200µl) of a 3% H_2O_2 added to it. The slide was covered against a dark background and observed for effervescence.

2.7. Plasma Preparation

Procedures: Human blood (4ml) was added in a centrifuge tube containing 0.8ml of Ethylene diaminetetraacetate (EDTA). It was centrifuged for 15minutes at1000 – 2000xg using an electric centrifuge. The resulting supernatant (plasma) was transferred into a clean tube using a Pasteur pipette.

2.8. Determination of Antibacterial Activities of Plant Extracts Using Disk Diffusion Method

Procedures: Disk of 6mm in diameter which was cut out from What man No.1 filter paper, was impregnated in various concentrations of each plant extract, dried in an oven (at 50^{o}C) under an aseptic condition.

The Nutrient agar medium was prepared and kept to gel. Nutrient broth (5ml) was placed in a test tube and labeled. Suspension of test organism was made in the nutrient broth with reference to 0.5M McFarland turbidity. A swab was dipped in the nutrient broth containing the test organism and streaked uniformly on the prepared solid agar. Disks containing various fixed concentration (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml) were placed on the center of the plate with the growing organism and incubated at 37^{o}C for 16 – 24 hours. Diameters of zones of inhibition were measured in millimeters.

2.9. Determination of Minimium Inhibitory Concentration (MIC)

This was carried out using disk diffusion method as described by Olutiola, *et al.* [9].

Procedures: Different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml) of the extracts were used. Plates were incubated for growth at 37^{o}C for 24hours, after which they were observed for clear zones around the disk, indicating inhibition. The concentration above that in which there is no inhibition was noted as minimum inhibitory concentration (MIC).

2.10. Standard Fumigant Sensitivity Assay

Procedures: An 18 – 24hrs old culture of the test organism on nutrient agar was uniformly smeared on a solid-fixed 20ml nutrient agar.

This was carried out using disk diffusion method. Disk impregnated in 200mg/ml of standard fumigant, 2,3-dichlorovinyl diphosphate (DDVP was placed on a nutrient agar containing the test organism and allowed to stand on the bench for 1hr for proper diffusion of the standard fumigant and thereafter, incubated at 37^{o}C for 24hours.

The resulting diameter of inhibition zones were measured in (mm).

2.11. Fumigant Preparation Using Vernoniaamygdalinaethanolic Leaf Extracts

The powdered *V. amygdalina* (240g) was soaked in 1 litre of 70% ethanol for 32hours. The extracts were first filtered using a clean muslin cloth, and then, filtered using a No.1 Whatman filter paper. The filtrate was concentrated to dryness in a waterbath (at 80^{o}C).

The dry extract (16g) were dissolved in 200ml of propan-2-ol, giving a concentration of 80mg/ml which was packaged in a fumigant bottle, ready for spraying.

2.12. Test for Toxicity of the Local Fumigant on Plant Leaves

The prepared fumigant was sprayed on leaves (fronds) of Coconut and Orange. After spraying, the fumigant stuck on their leaves. Their leaves were observed for seven days for effects such as leaf folding and change of colour.

3. Results and Discussion

The study was aimed at isolation, identification and characterization ot the organism in an infected guava fruit (*P. guavaja*and determination of the sensitivity of the organism to aqueous ethanolic leaf extracts of V. amygdalina and O. gratissimum as well as a standard fumigant, 2,3- dichlorovinyldiphosphate.

Isolation, identification and characterization of the organism from an infected Guava (Table 1, showed that a rod-like gram-negative organism which was positive to catalase test (it can breakdown hydrogen peroxide into oxygen and water), negative to slide coagulase test (it cannot convert fibrinogen in plasma into fibrin) is responsible for the disease of the guava fruit. The organism was identified as *ErwiniaPsidii.*

Table-1. Result showing biochemical tests of microorganism isolated from infected guava fruit.

S/N	TEST	RESULTS	INFERENCE
1	Catalase	+ ve	Can break down hydrogen peroxide into water and oxygen.
2	Slide coagulase	- ve	Cannot convert fibrinogen in plasma into fibrin.

Table-2. Results of Diameter of zones of inhibition (in mm) at different concentrations.

Concentrations Of extracts. (mm/ml)	*V. amygdalina*	*C. citratus*	*O.gratissimum*	*A.muricata*
300	13	-	9	-
100	11	-	8	-
50	10	-	6	-
25	9	-	7.5	-

Key (-): No zone of inhibition

3.1. Result for Antimicrobial Sensitivity Test

Table-3. Diameter of Zone of inhibition (mm) at Concentration of 200mg/ml

Sources of lant extracts/inhibitor	*V.amygadlina*	*C.citratus*	*O.gratissimum*	*A.muricata*	2,3- Dichlorovinyldihoshate (DDV
Zones of inhibition	13 mm	-	9 mm	-	14 mm

Key: - = No zone of inhibition.

Leaf extracts used in this study showed that only *V.amygdalina* and *O.gratissimum* inhibited the organism (Table 2). The ethanolic extract of *V. amygdalina* inhibited the organism with zone of inhibition of 13mm. However, *O. gratissimum* extracts recorded a zone of inhibition of 9mm.

The outcome of this study has shown that leaf extracts of *V. amygdalina* and *O. gratissimum* possess inhibitory potentials against the organism with the leaf extracts of V. amygdalina showing the highest potency. The leaf extracts of *C. citratus* and *A. muricata* exhibited no antagonism against the test organism (Table 2).

Table-4. Result of minimum (mic) inhibitory concentrations of plant extracts.

Organism	*V.amygdalina*	*C.citratus*	*O. Plant Extracts A.muricata*
Minimum Inhibitory Concentration			
Organism	25mg/ml	ND	50mg/ml ND

Key: ND = Not Determined.

Antimicrobial activities in plants have been reported to be as a result of bioactive components present in the plants, such as alkaloids, saponins, tannins, flavonoids, steroids etc [10].

The extracts of *V. amygdalina* compared favourably with commercial fumigant, 2,3-dichlorovinyl diphosphate (DDVP), in its bacterial inhibitory potentials. This was seen as recorded in Table 3. This result is a pointer to the fact that, if the crude extracts were subjected to purification, the active components could record same (if not higher) zone of inhibition than the commercial fumigant. It is possible that combinations of different extracts could produce synergistic effects that could produce higher inhibitory effects than the commercial fumigant, DDVP. On the other hand, the presence of impurities could have lowered the potency of the crude extracts, which when removed could exhibit higher potency [11].

The minimum inhibitory concentration values of 25mg/ml and 50mg/ml were obtained from *V. amygdalina* and *O. gratissimum* respectively (Table 4), indicating a higher sensitivity of *Erwinapsidii*to *V. amygdalina*than*O. gratissimum*, and a greater potency of *V. amygdalina*to the organism than *O. gratissimum*.

3.2. Results for Toxicity Test

The sprayed coconut leaves (fronds), guava leaves,and Orange leaves incurred no harm as no leaf color change or leaf folding was seen. This shows that the prepared fumigant is not toxic to the plants.

4. Conclusion

V. amygdalina in this project work has proven to have more antibacterial (inhibitory) activity against the gram-negative bacteria, *Erwiniapsidii* responsible for guava bacterial blight disease. However, it is suggested that more plant extracts be tested for synergism in order to produce an antibacterial compound or preparation with an overwhelming potency, without any hazard to the human systems and the environment.

References

[1] Santos, P. R. V., Oliveira, A. C. X., and Tomassini, T. C. B., 1995. "Controle microbiogico de productos fitoterapicos." *Rev. Farm. Bioquim.*, vol. 31, pp. 35-38.

[2] Kedari, T. S. and Ayesha, A. K., 2014. *American Journal of Research Communication*, vol. 2,

[3] Mbajiuka, C. S., Obeagu, E. I., Ochei, K. C., and Nnadi, D. C., 2014. *International Journal of Microbiological Research*, vol. 5, pp. 124-129.

[4] Sofowora, E. A., 1983. *An address proceedings on the 5th international symposition on medical plants*. Published at Unniversity of Ile-Ife Nigeria, p. 7.

[5] Ogundare, A. O., 2011. *Journal of Microbiology and Antimicrobials*, vol. 3, pp. 77-86.

[6] Samson, J. A., 1986. *Tropical fruits*. 2nd ed. New York: Longman Scientific & Technical. p. 335.

[7] Sancho, E. and Marcia, B., 1999. *Frutasdeltropico; Guiafotografica; fruits of the tropics: photographic guide*. E.Sancho B. Sanjose, p. 64.

[8] Madigan, M. T., Martinko, M. J., and Parker, J., 2002. *Biology of Microorganisms*. 9th ed. Upper Saddle River New Jersey: Prentice Hall, Inc. pp. 983-986.

[9] Olutiola, P. O., Famurewa, O., and Sonntag, H. G., 1991. *An introduction to general microbiology: A practical approach*. 1st ed. Germany: HygieneinstitutderUniversitat Heidelberg. p. 155.

[10] Odugbemi, T., 2006. *Outline of medicinal plants in Nigeria*. 1st ed. Nigeria: University of Lagos Press. p. 77.

[11] Slayer , A. A. and Whiff, D. D., 1994. *Bacteria pathogens*. Ist ed. Washington D.C: AMS Press. pp. 120 – 240.