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Antibacterial Activity of *Vernonia Amygdalina* on Post Harvest Organisms Associated with Cocoyam (*Colocasia Esculentus L*) Corms Rot

Ijato James*

Department of Plant Science and Biotechnology, Faculty of Science, Ekiti State University, Ado-Ekiti, P.M.B 5363, Ekiti State, Nigeria

***Corresponding author:** Xuemei John, Department of Biomedical Data Science, Geisel School of Medicine, Hanover, USA, Tel: 08146144519; E-mail: considerureternity@gmail.com

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Abstract

Cocoyam is a root crop that is produced in regions of the tropical or sub-tropical developing countries. In certain countries like Ghana, cocoyam production is surplus but post harvest losses are high due to mechanical damage of corms during harvest which predisposes the corms to microbial attack in storage. Antibacterial activities of the test plant were determined using agar diffusion method. Five bacteria strains were isolated from rotten cocoyam corm viz: *Bacillus cereus, Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa* and *Proteus mirabilis*. Cold aqueous extract of 20% of the test plant mostly inhibited *Staphylococcus aureus* (0.66) followed by Bacillus cereus (0.65). The list inhibited organisms being *Pseudomonas aeruginosa* (0.36) and Proteus mirabilis (0.38). Hot water extract (20%) of the test plant mostly inhibited Bacillus cereus (0.52) followed by *Staphylococcus aureus* (0.49). The results showed that higher concentrations of V. *Amygdalina* extracts inhibited the growth of organisms more than lower concentrations. The use of plant based biocide against bacterial isolates proves efficacious as they are effective, affordable and are less harmful to man the environment.

Keywords: Cocoyam; Antibacterial; V. *Amygdalina*

Introduction

Cocoyam is an herbaceous plant belonging to the family *Aracaceae.* They are grown primarily for their edible root, although all the plant parts are edible. Cultivated cocoyam as food crops belong to either the genus *Colocasia* or *Xanthosoma* [1]. Cocoyam thrives best when planted in full sunlight or partial shade. The plant can survive for short period of 100C but will be damaged or killed by lower temperature [2]. Cocoyam leaves are locally used for wrapping kolanut and bitter cola [3,4]. Reported dietary fibre of cocoyam as being aider of digestive system and easy passage of excreta. Cocoyam is prone to pest and disease attack which can account for 60% of corm loss [5]. Microbial deterioration can be mitigated through the use of disease free planting material [6]. Weeding and treatment with cooper based copper pesticide. High moisture has been found to promote bacterial rot of cocoyam corms.

Materials and Methods

Preparation and Sterilization of laboratory wares

All glass wares used for this finding were washed using detergent in running water; these were air dried before sterilization in the autoclave. The inoculating chamber and other working surfaces were sterilized by swabbing with 70% alcohol.

Sample collection and identification

Fresh leaf samples of V. *amygdalina* were collected from a vegetation site in Ado Ekiti. The identity of the collected plant was authenticated in the herbarium unit of Ekiti State University, Ado Ekiti. The leaf samples were air dried at room temperature for two weeks before grinding into fine powder. The powdered samples were stored in a clean air tight container in the laboratory before use.

Preparation of media

Twenty eight (28 g) of nutrient agar was weighed on Melter weighing balance and poured into 100ml beaker. The medium was dissolved by boiling in a water bath in order to give room for homogenization. This was later removed and allowed to cool down at room temperature before dispensing into sterile Maccartney bottles before it was autoclaved at 1210C for 15min.

Preparation of plant extracts

Hundred grams (100 g) of test plant powder was weighed into 200ml of distilled water and this was allowed to stand overnight at room temperature. This was later filtered usung muslin cloth and the filtrate (served as the extract) was stored in the sterile bottle at 40C.

Isolation of bacteria

After extraction, 1 ml of infected cocoyam corm broth was taken using syringe and dispensed into 9 ml of sterile water. This

process was serially diluted and the final diluent was stored in the test tube and corked using cotton wool to avoid contamination.

Pathogenicity Tests

Pathogenicity tests were carried out using established protocols and techniques in bacteriology. Healthy cocoyam corms were washed in sterile distilled water and surface sterilized with 0.1% ethanolic solution. A sterilized corn borer was used to cut (creating core) the corms and then cultures of the bacterial isolates were introduced into the openings and the cores were placed back. Petroleum jelly was smeared to completely seal the hole to guide against contamination. These were incubated for five (5) days. Upon observation of lesions, inoculums from the infected corms were taken and cultured. The symptoms were identical to those of naturally infected cocoyam.

Organisms Identification

Pure isolates obtained from the diseased cocoyam fruits were identified subjecting for identification purposes. Each isolate was subjected to macroscopic and microscopic examinations during which their structural features were observed. Identification of bacteria was based on the growth patterns, colour of culture and microscopic examinations of bacteria.

Determination o f antibacterial activity of the test plant

Determination of antimicrobial effects of the test plant was by pour plate method. Molten nutrient agar was poured into sterile Petri dishes, allowed to stand, cool down to 450 C and the bacterial inoculum was streaked on the medium.

Wells were punched into the agar gel using 4 mm corn borer and the wells were illed with 1 ml of the test plant extracts. The plates were incubated at 370 C for 24 hr.

The antibacterial activity of the test plant was determined by measuring the diameter of the zone of inhibition using metre rule.

Results

Table 1 showed the effects of different concentrations of aqueous extracts of V. *amygdalina* against post harvest bacteria isolated from rotten cocoyam corm as contaminants viz: *Bacillus cereus, Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and Proteus mirabilis.* All the concentrations (5to20%) of the test plant mostly inhibited *Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and Proteus mirabilis.* Cold aqueous extract of 20% of the test plant mostly inhibited *Staphylococcus aureus* (0.66) followed by Bacillus cereus (0.65).

The list inhibited organisms being *Pseudomonas aeruginosa* (0.36) and *Proteus mirabilis* (0.38).

Table 1: Effects of different concentrations of cold aqueous extracts of V. amygdalina against post harvest bacteria of cocoyam corm.

Concentration of extract%	**Bacterial isolates**				
	S.aureus	*A. hydrophila*	*P. aeruginosa*	*P. mirabilis*	*B. cereus*
Control	6.41c	8.60b	6.91c	5.81c	9.57a
5	0.44c	0.56a	0.36c	0.38b	0.51b
10	0.50c	0.61a	1.41a	1.45c	0.52b
15	0.55c	0.65c	0.43c	0.49d	0.58b
20	0.61b	1.66c	0.46c	0.52c	0.65c
LSD	1.45	0.08	0.07	0.08	0.8

Values followed by the same letter are n ot significantly different at ($p<0.05$ at Fischer's LSD)

Table 2 showed that all the concentrations (5to20%) of the test plant mostly inhibited *Staphylococcus aureus, Aeromonas hydrophila, Pseudomonas aeruginosa and Proteus mirabilis.* Cold aqueous extract of 20% of the test plant mostly inhibited Bacillus cereus (0.52) followed by *Staphylococcus aureus* (0.49) while the list inhibited organism was *A. hydrophila* (0.40).

Table 2: Effects of different concentrations of hot aqueous extracts of V. Amygdalina against post harvest bacteria of cocoyam corm.

Concentration of extracts %	Bacterial isolates				
	S.aureus	*A.hydrophila*	*P.aeruginosa*	*P.mirabilis*	*B.cereus*
Control	6.41c	8.60b	6.91c	5.81c	9.57a
5	0.31c	0.42a	0.32c	0.34b	0.42a
10	0.34d	0.44a	1.36c	1.43b	0.44a
15	0.37c	0.46b	0,40b	0.46b	0.48a
20	0.40c	1.49b	0.42c	0.48b	0.52a
LSD	1.45	0.05	0.05	0.04	0.91

Values followed by the same letter are not significantly different at ($p<0.05$ at Fischer's LSD)

Discussion and Conclusion

Plants produce antimicrobial agents as secondary metabolites for self-defense against pathogenic invasion [7]. The use of plant extracts remains one of the major sources of natural products for a new therapy mainly in the developing countries as plant extracts cost less. It is also effective against a broad range of antibiotic resistant microbes. Additional benefit of using plant based biocide is that, natural products have less adverse consequence on man and the environment [8]. Chemical application creates numerous environmental and attendant resistant by microbes [9]. Reported high antibacterial effects of *V. amygdalina* against yam rot pathogens. Plant extracts have effectively controlled various phytopathogens [10]. Successfully controlled rice blast pathogens both *in vitro* and in vivo using *A. indica*. In Nigeria, plant extracts have been used by various researchers to mitigate plant diseases such as cowpea [11]. Banana [12]. Yam [13]. Cocoyam (Nwachukwu and Osuji 2008 and sweet potato [14]. Both cold and hot aqueous extracts of *V. amygdalina*
proved efficacious against isolated bacteria from rotten cocoyam corms. Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) obtained in this study could be attributed to low concentration of active compounds and vice versa as this has been reported by who asserted that variations in the values of MIC and MBC could be attributed to low amount of active compound contained in the extracts [15-16]. Reported that plant extracts are antagonistic against bacterial pathogens, as plant extracts play important role in crop production, this will have prominent role in the development of future commercial pesticide for crop production strategy in the management of plant diseases.

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