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Antimicrobial Activities of Indigenous Botanicals in the Control of Bacterial Soft Rot of Sweet Potato in Umuahia, Abia State, Nigeria

***[1]Ibeh, M. C., [1]Uzoma, P.G. and [2]Agu, J.C.**

[1]Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, PMB 7267 Umuahia Abia State, Nigeria
[2]National Root Crops Research Institute, Umudike
*Corresponding Author's email: maryjoyibeh@gmail.com

Abstract
The antibacterial activities of aqueous and ethanol extracts of selected botanicals in the control of bacterial soft rot disease of sweet potato in Umuahia, Abia State, Nigeria were studied. Diseased sweet potato roots sourced from markets in Umuahia were taken to the laboratory to isolate and identify the causal organisms. Extracts of *Vernonia amygdalina* (Bitterleaf) and *Bidens pilosa* (Black jack) leaves and *Syzygium aromaticum* (Clove) seeds were tested in vitro against the bacterial isolates by Disk Diffusion Method in a Completely Randomized Design with three replications. The pathogenicity test revealed *Erwinia* spp (46.51%) and Bacillus (26.45%) to be pathogenic. Inhibition patterns differed with the plant extract, extraction solvent, and the organism tested. *Vernonia amygdalina* ethanolic extracts were the most active, against *Erwinia* spp (16.67mm), while *B. pilosa* ethanolic extracts highly inhibited *Bacillus* spp.(15.67mm). However, both were significantly different ($P \leq 0.05$) from aqueous *Syzygium aromaticum* extracts as it recorded the lowest inhibition of 8.67mm for Bacillus spp. and 9.67mm for *Erwinia* spp. All the botanicals were compared favourably with the standard antibiotic which had 21.67mm and 22.67mm against *Erwinia* and *Bacillus* pathogens respectively. Bitter leaf and Black Jack ethanol has shown significant potential in the pharmacognosy for the control of the isolated bacteria pathogens of sweet potato and is recommended for their control.

Keywords: Antibacterial activities, Plant extracts, Sweet potato, Bacterial pathogens

Introduction

Sweet potato (*Ipomoea batatas* L.) belongs to the *Convolvulaceae* family and order *Solanales* (Agu *et al*., 2015) with approximately 500 - 600 species; making up the largest number of species within the *Convolvulaceae* family. The family is made up of about 45 genera and 1,000 species. It is a large, sweet-tasting; starchy tuberous roots that have heart-shaped leaves and funnel-shaped flowers (Austin *et al*., 2006). The origin and domestication of sweet potatoes occurred in Central America (Huang and Sun, 2000).

Nigeria is one of the largest producers of sweet potatoes in sub-Saharan Africa with annual production estimated at 3.46 million tons per year (Udemeze, 2019). A brief analysis of potato production in various countries of Africa reveals that Egypt is Africa's number one potato producer, followed by Malawi while Nigeria is known as the fourth biggest producer in Africa (Ugonna *et al*., 2013). Sweet potato ranks among the five most important food crops in over 50 developing countries (Abdelhameed, 2008). China tops the list of the world's largest producers of sweet potatoes with about 100 million metric tons, accounting for 84.4% of total world output (Gao, et al., 2000). It has high protein content and carbohydrate content, providing a good source of dietary energy and essential micronutrients to consumers. The protein content of Sweet potato is very high (FAO, 2008), compared to other roots and tubers. It is a good source of vitamin C and pro-vitamin A, and can be substituted for maize in livestock production (Okeke *et al.*, 2020). They are moisture and dietary fibre rich, also rich in ascorbic acid, lysine and minerals. They also provide as much as 359 KJ of energy and have low total lipid content (USDA, 2014).

Sweet potato tubers suffer from post-harvest losses as a result of some physical, pathological and physiological factors or a combination of all three factors. The crop is cheap and easily cultivated, yet it is facing a 1ot of production and post-harvest challenges. Attack by fungi, bacteria and viruses are probably the most serious causes of post-harvest losses of between 25% and 60% of the initial weight of sweet potatoes depending on variety when stored for six and half months in a semi-

subterranean (Suleiman and Falaiye, 2013). Ameinyo and Ataga (2006) reported *Rhizopus oryzae* and *Aspergillus niger* as being responsible for sweet potato rot. The principal factor responsible for losses during storage of *I. batatas* has been reported to be infection by microorganisms resulting in tuber decay Bacteria causing rots in potatoes have been reported to produce a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases, and proteases. These enzymes are also responsible for tissue maceration and cell death (Olivieri *et al*., 2004). *Pectobacterium* spp. initially known as *Erwinia* spp. (George *et al*., 2004) induce soft rot on these crops both during the vegetation cycle and in storage and is therefore considered the most threatening bacterium on potatoes and other tubers worldwide (Hadas *et al*., 2001). The production of sweet potato, especially vegetable potato, is seriously affected by rots. The use of protective chemicals on ware tubers is not advisable; it can render roots and tubers unfit for consumption as they undergo little processes before consumption. The adoption and use of indigenous plant materials that are environment-friendly, cost-effective and available make up a vital component of sustainable agriculture. The aim of this work, therefore, was to isolate, characterize and identify the bacteria pathogens associated with post-harvest loss of sweet potato and to determine the antimicrobial properties of selected botanicals against these isolated pathogens.

Materials and Methods

Sample collections and source of materials

The test botanicals, leaves of (*Vernonia amygdalina* and *Bidens pilosa*) were collected from homestead farms within the National Roots Crop Research Institute (NRCRI), Umudike while clove (*Syzygium aromaticum)* was purchased from Orie-Ugba Market, Ibeku, Umuahia; Abia State. The test sweet potato tubers (healthy and diseases) were sourced from the markets.

Media Preparation

The media used for the isolation of the microorganisms from the test tubers and sensitivity (susceptibility) test of extracts of the botanicals were Nutrient Agar and Muller Hinton Agar respectively. Each medium was prepared according to the manufacturer's directives.

Isolation of Test Pathogens

The method described by the International Commission on Microbiological Specification of Food (ICMSF, 1998) was used. The diseased roots were first surface disinfected by swabbing with cotton wool soaked in 70% ethanol solution. Thereafter, the tuber was cut open with the aid of a flamed sterilized kitchen knife, to reveal the inside. Freaky samples were cut out at the boundary area between the diseased part and the healthy path (to capture the pathogens responsible for the disease progression). The collected sub-samples were aseptically crushed to homogeneity in a surface-sterilized porcelain laboratory motor with a pestle. The homogenized sample was diluted serially in ten (10) folds. The inoculated plates were allowed to cool and solidify before being sealed, labelled approximately and incubated. The plates were examined daily for growth. On the establishment of growth, the distinct organisms were purified using the streaking method, characterized and identified.

Purification of Microorganisms isolated

Purification of the bacteria was done by sub-culture. The bacteria inoculation in the sub-culture plates was done by streaking method.

Identification and Characterization of microbial isolates

Bacteria isolates were identified and characterized by a study of their respective colonial, structural and biochemical characteristics, with existing taxa in standard manuals. Bergey's Manual of systematic bacteriology and the manual for the identification of medical bacteria were used (Buchaman and Gibbons, 2004). Bacteria isolates were closely examined visually while in culture plates and their respective colony features were recorded including the extent of growth, colony colour, elevation, margin form, consistency and light passage among others were recorded. Observations were recorded against each isolate. Furthermore, each bacterial isolate was mounted on a slide, gram stained and examined under an oil immersion objectives lens (x100). The cell shape and arrangement as well as the reaction to the principal dye (gram reaction) were recorded. Some further tests were done to determine their reactions to specific dyes as indications of the presence of specific features like flagella, capsule, spore etc. and state of motility). Observable features were recorded accordingly. The bacteria isolates were then tested on some biochemical reactions indicating their ability to produce enzymes such as catalase, urease, oxidase, and coagulase, to reduce nitrate, utilize citrate and ability to use specific sugars with the production of acid, gas or both. The observed activities were also recorded accordingly.

Occurrence of bacteria isolates in spoilt sweet potato

The occurrence of bacteria isolates from diseased sweet potato tubers was determined by the methods of Sampo *et al*. (1997), which is:

$$\% \text{ occurrence} = \frac{\text{Number of plates in which the species appeared}}{\text{otal number of plates incubated}} \times 100 \ldots\ldots 1$$

Pathogenicity Test

The tests were conducted to determine the ability of each isolate to cause diseases (bacterial soft rot) on healthy sweet potato tubers. In this regard, the method described by (Cheesbrough, 2006) was employed. The surface of healthy roots was washed in distilled water, dried and surface sterilized by swapping the entire surface with cotton wool moistened with 70% ethanol. The method of Okigbo and Ikediugwu (2000) was employed. Carefully, with the aid of a flamed cork borer (5mm diameter), a hole was bored on the root and 0.5 ml inoculum of the test organism, from a relatively fresh culture, was carefully introduced into the hole and the

removed flesh was replaced carefully while the outside was sealed with sterile paraffin gel. This was done for each test organism on separate roots and in each case, a control was set up in which the hole was dug and the flesh replaced without the addition of any organisms. The inoculated roots were labelled properly and kept on the shelf at room temperature and examined over a period of two (2) weeks. The onset of disease was determined by symptoms which included softening, discolouration, offensive odour and viable growth of microorganisms. The test tubers with any of such symptoms were carefully cut open to observe the inside alongside that of the control. Bacteria isolates which caused clear visible rots were considered pathogenic when compared with the control roots. The extent of pathogenicity was calculated with reference to the control. A general formula as shown below was used:

$$\frac{D-RL}{D} \times 100 = \text{Severity of diseases} \quad \ldots\ldots 2$$

$$\text{Effective pathogenicity} = ST - SC\,(\%)$$

D= Diameter of test Tuber, RL=Rot length, ST=Severity in Test Pathogen, SC= Severity in Control Experiment

Production of Extracts

Production of the plant extracts was done in line with the cold solvent extraction method described by Akinbosun and Itedjere (2013). The leaves of *Vernonia amygdalina* (Bitterleaf) and *Bidens pilosa* (Black jack) and *Syzygium aromaticum* (Clove) seeds were prepared by washing thoroughly in distilled water, drained and air dried at 30^0C room temperature. Thereafter, they were ground with a sterile manual grinder (Amadioha, 2004). The pulverized flour samples were weighed out separately in 10g portions each and in duplicates. Each 10g of the three (3) samples was soaked in 100ml ethanol in the ration (1:20 w/v), while the other 10g of each sample was soaked in 100ml distilled water. The soaked samples in their respective air-tight container were allowed to stand for 48 hours being shaken for 15 minutes every 12 hours. Therefore, they were separately filtered through Whatman filter papers to obtain the crude extract solution which were evaporated to dryness leaving the crude extract. The filtrates were collected in weighed beakers and after evaporation, the beakers and their contents were labelled and their respective extract yields were calculated as follows:

$$\%\ \text{yield} = 100x\frac{W1-W2}{W} \ldots\ldots 3$$

Where: W=Weight of sample extracted, W_1=Weight of emptying extraction container, W_2=Weight of container with dry extract

Antimicrobial Activity Test

These tests were conducted to test the ability of the botanical extracts to inhibit the growth of the isolated pathogens in-vitro. The diffusion technique method (Akinbosun and Itedjere, 2013) was employed. Accordingly, the obtained extracts from each plant were separately reconstituted in the aqueous and ethanol solvents respectively in minimum volume ≤ 1ml and used for the test. The medium used for the antibacterial activity test was Mullar Hunton Agar (Cheesbrough, 2006). A solid sterile Muller Hunton Agar plate was inoculated with each test bacterial in a spread plate inoculation. With the aid of a flamed wire lop, a loopful (about 0.1ml) of the test organism was inoculated onto the sterile plate and spread evenly over the entire plate lawn using a flamed bent glass rod (glass hockey). Then carefully and working under aseptic practice, a flamed cork borer (5mm diameter) was used to make a shallow well (about 3mm deep) on the inoculated agar at about equidistant position and also creating sufficient space from the edge of plate. Carefully, about 0.1ml of each reconstituted plant extract was introduced into each of the three (3) agar wells on the inoculated plate. This was done for each pathogen isolate versus each plate extract. A standard was set up containing a standard antibiotic (streptomycin) in place of the extract and treated as described. All inoculated treated plates were well labelled, sealed and incubated for 24 hours at 37^oC. They were observed for the presence of inhibition zone (clear areas) around any of the treatment spot as a mark of positive activity. The extent of inhibition was determined by the diameter of the inhibition zone as measured with a transparent ruler.

Data Analysis

Data was subjected to analysis of variance (ANOVA) and the mean separation was done using Fishers' Least Square Difference (LSD). The statistical package used was R and GenStat Edition 4.

Results and Discussion

Percentage Extract Yield of the Test Botanicals

Table 1 shows the extract yield of the test botanicals, bitter leaf, clove and blackjack. There are variations in the extract yields of the different plants and extraction solvents. From the results of the yield extracts, there was a higher yield from ethanol extract than from the water extracts. There were also variations in the extract yield from the different plants. Comparatively, the bitter leaf had the highest yield of 23.83% and 38.44% for water and ethanol extracts respectively while *Bidens pilosa* had the lowest extract of 17.58% and 29.13% for water and ethanol extracts respectively. The variations in the extracted content could be attributed to genetic and agronomic factors. This could be due to the fact that some plants are endowed with peculiar metabolites such as bitter principles in bitterleaf and *utazi.* What plants synthesize is determined by what they are able to absorb from the soils and there is a wide heterogeneity in soils.

Occurrence of Bacteria Isolates in Spoilt Sweet Potato

The results from Table 2 show the occurrence of bacterial isolates; *Erwina*, and *Staphylococcus* were the most predominant as they were found in all the samples analysed (100%) while *Proteus* was the least predominant with 40% occurrence. Bacillus had an 80% occurrence. The predominance of Staphylococcus was attributed to the fact that the sweet potato tubers were in the open market where they were handled by prospective buyers and staphylococcus is a natural

occupant of the human body. This is in line with the work of Lowy, (1998). The equally very high presence of *Erwina* was perhaps due to the fact that it is a major pathogen of sweet potatoes. *Bacillus* have spores which can be airborne and reach many places within the environment. It is a common phenomenon that some microorganisms are transient and as such can be found in diseased plants even when they are not pathogenic to such plants. It is only tested on the pathogenicity of isolates that can show the role of their presence in the diseased plants.

Characterization and Identification

The isolates were identified by the colony morphology and physico-chemical properties listed in the Table 3.

Pathogenicity Test of Isolates

The result in Figure 1 shows that only two bacterial isolates, *Erwinia* and *Bacillus* demonstrated pathogenicity in healthy Sweet potato roots. *Erwinia* recorded 46.51% while *Bacillus* had 26.45% and both are significantly different ($P \leq 0.05$) from the control which recorded 3.47. With respect to this finding, *Erwinia* and Bacillus demonstrated pathogenicity within the 14 days test period. This is based on the population of the possible level of disease infection of 93.14% for *Erwinia* within one month and 52.8% for *Bacillus*. This result is in agreement with the report by Amadioha and Adisa (1993) which implicated *Erwinia* and *Bacillus* as pathogenic bacteria causing rot of sweet potato. Also in line with the findings of Nsofor (2020), *Erwinia* was found predominant and highly pathogenic in sweet potato causing rot. Moreover, the combined levels of pathogenicity of both pathogens in one sweet potato root could possibly result to synergy which effect can lead to total loss of the sweet potato roots during storage.

Antimicrobial Activity Test

Table 4 shows the antimicrobial activity of the test botanicals, extracts of bitterleaf (*Vernonia amygdalina)*, Clove seeds (*Syzigium aromaticum*) and Black jack (*Bidens pilosa*). There were variations in the activity of the different plant extracts against the pathogens isolated. The water extracts activity against *Erwnia spp* was in the range of 9.67mm (clove) to 12.33mm (bitter leaf) while the ethanol extract recorded inhibitions variations in the range of 15.00m (Clove) to 16.67mm (Bitterleaf). The corresponding activity against the *Bacillu*s spp isolates was between 8.67mm (clove) and 10.67mm (*B. pilosa* and *V. amydalina*) for the water extract while the ethanol extracts had inhibition diameter in the range of 13.67mm (clove) to 15.67mm (*B. pilosa*). The ethanol extracts were more potent than the water extracts. This may be due to the ability of ethanol to extract more active metabolites than water. This is in consonance with the work of Draz *et al*. (2019), who revealed the bioactivity of certain components of plant extracts with ethanol extracts showing more potency than water extracts even though both are effective in the control of plant diseases.

Conclusion

The extracts of *V. amygdalina, S. aromaticum* and *B. pilosa* have in this study, proven to possess significant potential in the pharmacognosy for the control of the bacterial pathogens isolated from sweet potato. Though the three botanicals compared favourably with the standard streptomycin, the use of *V. amygdalina* and *B. pilosa* should be adopted for the control of *Erwinia* spp and Bacillus-induced soft rot of sweet potato given their respective environmental friendliness and effectiveness.

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Table 1: Percentage extract yield of the test botanicals

Plant	W_1	W_2	W_2-W_1	Vol yield	Penicales
V. amygdalina (Ethanol)	41.331	45.175	3.844	38.44	10g sample was used
V. amygdalina (Water)	39.44	41.826	2.306	23.86	
S. aromaticum (Ethanol)	56.182	59.396	3.214	32.14	
S. aromaticum (Water)	44.118	46.084	1.966	19.66	
B. pilosa (Ethanol)	43.112	46.025	2.913	29.13	
B. pilosa (Water)	51.083	52.841	1.758	17.58	

Footnote: W_1 is the weight of the container, W_2 is the weight of the extract

% ? ? = ? $\frac{? -}{?}$ ***= 10 (W1-W2)***

Where W_1 is the weight of the container, W_2 is the weight of the extract

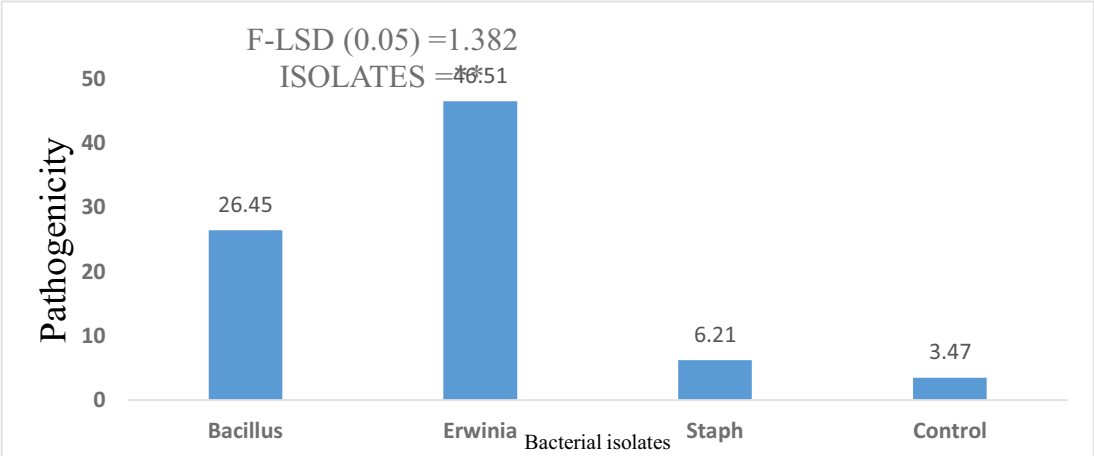
Table 2: Occurrence of Bacteria Isolates in Spoilt Sweet Potato

Cultivars	Staphylococcus	Bacillus	Erwina	Proteus
TIS 87/0087	+ve	+ve	+ve	+ve
UMUSPO1	+ve	+ve	+ve	-
UMUSPO2	+ve	-	+ve	+ve
UMUSPO3	+ve	+ve	+ve	-
UMUSPO4	+ve	+ve	+ve	-
Total % no of +ve	5	4	5	2
% occurrence	100%	80%	100%	40%

Table 3: Characterization and Identification of Isolates

Isolates	1st	2nd
Colony Morphology	Large colonies with irregular margin	Transparent, circular, slightly raised, shiny and creamy white. Produced Some pale yellow pigment
Microscopy	Gram reaction---------- (+ve) Spore ---------- (+ve) Flagella ---------- (+ve) Cell shape and arrangement ----------Rod shaped	Gram reaction----------(-ve) Spore ---------- (-ve) Flagella ---------- (+ve) Cell shape and arrangement ------- Rod with rounded ends
Biochemistry	Catalase ---------- (+ve) Oxidase ---------- (-ve) Coagulase ---------- (+ve) Indole ---------- (-ve) Nitrate ---------- (+ve)	Catalase ---------- (+ve) Oxidase ---------- (-ve) Coagulase ---------- (-ve) Indole ---------- (+ve) Nitrate ---------- (+ve)
Sugar Utilization	Glucose ---------- (+ve) Sucrose ---------- (+ve) Lactose ---------- (+ve) Maltose ---------- (+ve) Manitol ---------- (+ve) Xylose ---------- (+ve)	Glucose ---------- (+ve) Sucrose ----------(+ve) Lactose ---------- (+ve) Maltose ---------- (+ve) Manitol --------- (+ve) Xylose ---------- (+ve)
Suspected Isolates	*Bacillus* spp.	*Erwinia* spp.

+ve = positive; -ve= negative



Footnote: ** shows means are highly significantly different at 5% probability level
Figure 1: Pathogenicity Test of the Isolates

Table 4: Antimicrobial screening test of ethanolic and aqueous plant extracts against pathogenic bacterial isolates from the diseased Sweet potato.

Treatments	**Inhibition zones diameter (mm)**	
	***Bacillus* spp**	***Erwinia* spp**
Vernonia. amygdalina (Ethanol)	14.33	16.67
Vernonia. amygdalina (Water)	10.67	12.33
Bidens pilosa (Ethanol)	15.67	16.00
Bidens pilosa (Water)	10.67	11.67
Syzygium aromaticum (Ethanol)	13.67	15.00
Syzygium aromaticum (Water)	8.67	9.67
Control (Streptomycin)	22.67	21.67
LSD (P≤ 0.05)	1.09	1.94