Food and Nutrition Sciences, 2015, 6, 1381-1394
Published Online November 2015 in SciRes. http://www.scirp.org/journal/fns
http://dx.doi.org/10.4236/fns.2015.615144

Isolation of Six Microorganisms from Rotten *Dioscorea alata* (Water Yam), and Antimicrobial Sensitivity Test with Nine Plant Extracts

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Received 3 October 2015; accepted 17 November 2015; published 20 November 2015

Abstract

Six microorganisms: four fungi—*Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifera*, *Penicillium marneffei*, two bacteria—*Erwinia carotovora* and *Pseudomonas aeruginosa* were isolated and identified from three rotten *Dioscorea alata* (water yam) varieties from two sites each in two local government areas of Benue State, Nigeria, in West Africa, between the months of May 2014 and May 2015. Pathogenicity test carried out using the microorganisms confirmed them to be the pathological agents of the rot. Antimicrobial activity test with aqueous extracts of nine plants: *Terminalia catapa*, *Passiflora edulis*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, and *Mangifera indica* of fresh and dry material showed that three pathogens, *Rhizopus stolonifera* (fungi), *Erwinia carotovora* and *Pseudomonas aeruginosa* (bacteria) isolated were completely inhibited each by a plant. The result obtained shows that *Passiflora edulis* had the best antimicrobial activity for both fungi and bacteria; indeed it inhibited completely *Rhizopus stolonifera* which was stubborn with most of the other plants. *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, and *Mangifera indica* were also able to inhibit most of the fungi but not completely. *Terminalia catapa* and *Jatropha tanjorensis* were most effective against the bacteria. *Erwinia carotovora* was completely inhibited by *Terminalia catapa* and *Pseudomonas aeruginosa* was completely inhibited by *Jatropha tanjorensis*. *Daniella oliveri* and *Ceiba pentandra* had the least inhibition against the isolates. Generally, the fresh plant extract shows more activity as compared to the dry plant extract.

Keywords

Bacteria, Fungi, Inhibition, Plant Extracts, Yam Rot

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How to cite this paper: Onuh, J.O., Shiriki, D., Ubwa, S.T. and Shambe, T. (2015) Isolation of Six Microorganisms from Rotten *Dioscorea alata* (Water Yam), and Antimicrobial Sensitivity Test with Nine Plant Extracts. *Food and Nutrition Sciences*, **6**, 1381-1394. http://dx.doi.org/10.4236/fns.2015.615144

1. Introduction

Dioscorea alata (*D. alata*, water yam) belongs to the genus *Dioscorea*, family Dioscoreaceae [1] [2] and it ranks next to *Dioscorea rotundata* (*D. rotundata*, white yam) [2]-[5]. In some countries where there is no white yam, its production is the highest amongst yams. The water yam production is limited to once a year (perennial vines) [2] [6], usually planted by yam tuber cut or yam sets on heaps in the months of December to April and is harvested only once between November and January of the following year; it is known as ten months yam because it takes nine to ten months to mature unlike white yam which takes four to six months to mature [5] [7]. Its requirement for soil rich in manure is certainly less than that of *D. rotundata*, where fertilizer supplementation is required. The white yam and water yam are often intercropped with white yam covering three quarters of the farm land and water yam only one quarter. Harvested crops often show that the yield of the white yam is two thirds and water yam one third clearly showing that the yield of water yam is higher even though less fertilizer was applied. This fact has been verified by discussion with the local farmers and by personal farming experience. White yam has about 78% carbohydrates and has the highest calories per hectare when compared with other starchy tubers like cassava and potatoes [5] [8]. *D. alata* contains about 75.65% carbohydrate and has several good nutrients [4] [5]. The advantage of *D. alata* over *D. rotundata* is that, it is often recommended for diabetic patients and those slimming due to its low glycemic index [4] [5] [9] [10]. The market value of *D. alata* is lower than that of *D. rotundata* but with the increasing number of diabetic patients, its economic value is coming up [6]. *D. rotundata* is sweeter than *D. alata* and has better pounding characteristics. Taking ten months to mature is an added disadvantage affecting its cropping [5]. It is to be noted that some species of the family Dioscoreacea which *D. alata* belongs to have high phytochemicals which may be of great disease treatment value [11]; the phytochemicals in these species, like phytate, lectins, phenolic compounds, amylase inhibitors and saponins, are reported to reduce blood glucose, plasma cholesterol, triglycerides level and control cancer risks [11] [12]; others have a reasonable quantity of saponins and are used for medicinal purposes as a source of natural antibiotic used by the body to fight off infections and microbial invasion [13].

Water yam tubers have better storage properties than the white yam tubers; all the same it is subject to post harvest losses as the white yams. In storage, heat or high temperature causes rot in the stored tubers [14]-[17]. Water yam tubers respire continuously and need adequate aeration; this is often achieved by providing adequate ventilation or putting on fans to enhance air circulation in the store house or storage area [14] [18] [19]. Rodents attack the yam tubers causing wounds [20] [21]; in storage, this can be prevented by screening the store house or storage area with wire mesh. Nematodes attack the yam tubers on the farm causing wounds which may serve as entry points for rot causing microorganisms [20]-[22]; this can be prevented by the use of chemicals [23].

D. alata and *D. rotundata* have a dormancy period of two months after harvesting [24], thereafter sprouting commence. Sprouting during storage uses up the reserve carbohydrates meant for humans. Sprouting can be arrested by removal with hand, by spraying with plant extract [25]-[28] and use of gamma irradiation [23] [29]-[32]. Spraying with plant extract and gamma irradiation gives better result when sprayed in their period of dormancy [23] [24] [32].

Microbial attacks on water yam and white yam result into dry rot, soft rot or wet rot [8] [17] [33]. In the dry rot, the infected tissues become hard and dry with varying coloration depending on the microorganism involved. It is caused by the *Fusarium species*, *Aspergillus niger*, *Aspergillus flavus*, and *Pseudomonas aeruginosa* [17] [34]-[36]. Soft rot causes the infected tissues to become soft ramified by the fungal mycelium and turn brown and sometimes wet due to a rapid collapse of the cell walls inducing pinkish with yellowish border on the affected tissues. Fungi associated with soft rot include *Armillariella mellea*, *Mucor circinelloides*, *Rhizoctonia solani*, *Rhizopus* spp., and *Penicillium* spp. [34] [37] [38]. The wet or watery rot of yam tuber is characterized by the oozing of whitish fluid from the tissues when pressed. It is usually associated with *Erwinia carotovora*, a bacterium [17] [34] [38].

D. alata like *D. rotundata* tubers have outer cover, whose microorganisms cannot easily penetrate, but it is easily wounded by rodents, nematodes and man during weeding, harvesting and postharvest handling. Such wounds facilitate the penetration and development of rot microorganisms [17] [20] [22] [36] [38]. Pathogenic fungi of both *D. alata* and *D. rotundata* tubers include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillustamari*, *Botryodiplodia theobromae*, *Cladosporium herbarum*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, *Penicillium chrysogenom*, *Penicillium oxalicum*, *Penicillium cyclopium*, *Penicillium sclerotigenum*, *Rhizopus stolonifera*, *Rhizopus nigrican*, *Rhizopus nodosus*, *Rhizopus oryzae*, *Rhizoctonia* spp., *Mucor mucedus*,

Mucor circineloides, *Saccharomyces cerevisiae*, *Colletotrichum gloesporioides*, *Cylindrocapus radicola*, *Gleiocladium roseum*, *Geotricum candidum*, *Roselina* spp. and *Trichoderma viride* [17] [37] [39]-[41]; while the bacteria pathogens include *Serretia marcescens*, *Erwinia carotovora*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa* [36].

Control of microbial rot of food using microorganisms in crops as well as soil has earlier been reported [42] [43], but it requires special skills that not many farmers may have. Aqueous extracts of the five plants earlier studied [36] showed that some rot causing microorganisms were completely inhibited in *D. rotundata*; and that *Azadirachta indica* and *Moringa oleifera* extracts inhibit activity of microorganisms that are plant pathogens [25] [44]. In the case of Dioscoreaceae, inhibition of microorganisms is best achieved and prolonged during the dormancy period [23]. Pathogenicity test carried out on yams shows that different sections of yam react differently to microorganisms [17]; it is opined that, this is possibly due to variation of free sugar concentration along the length, which is carbon source to the microorganisms.

Plant extracts have been used for treatment of about 80% of humans for all forms of ailments particularly in rural areas of developing countries over the ages [45]-[53]. The use of plant extract for treatment of ailment is now preferred or should be preferred in fighting microbial infections instead of synthetic antimicrobial drugs that most microorganisms pathogenic to humans and perhaps even those pathogenic to plants are presently becoming resistant to [23] [46] [54]-[61]. More attention has been paid in the use of plant extracts for treatment of humans and animals with little attention to the treatment of plants and indeed food crops. It is also observed that antimicrobial activity of plants varies with their place of growth [62]. It is thus important that even though these plants might have been worked on, it is necessary to study those grown in other environments. This work is intended to be easily accessed by peasant farmers to reduce postharvest losses. For this reason only aqueous extract was used instead of methanol, ethanol or other solvents often used to obtain maximum extraction.

In the light of the above, the microorganisms causing *D. alata* rot were identified and treated with nine aqueous plant extracts for their activities.

2. Materials and Methods

2.1. Source of Materials

Thirty six (36) samples, twelve (12) pieces of each variety (Azawele wele, Kor and Banada) of rotten and twenty seven pieces of unrotten *D. alata* were collected from the Local Governments, Tarka and Vandeikya in Benue State, in May 2014 for this study and treated as earlier reported [36] and the whole work lasted up to May of, 2015.

The water yam samples were collected, properly labelled and packaged in cellophane bags and taken to the microbiology laboratory of the Benue State University for analyses.

The methods [40] [48] [59] [63]-[67] were used to culture, isolate, identify and evaluate the pathogenicity and antimicrobial sensitivity of the rot causing microorganisms using aqueous extracts of nine plants, fresh and dry plant material.

Culture media potatoe Dextrose Agar, Nutrient Agar, Nutrient broth, MacConkey Agar, Triple Sugar Iron Agar (TSIA), Simon Citerate Agar were procured from Titan Biotech TM, Guangdong Huakai Microbial Sci. & Tech. Co. Ltd., Scharlau-Spain, Piotec Laboratories Ltd., UK respectively. Grams staining reagents (crystal violet, lugol's iodine, absolute alcohol, safranin); catalase reagent(hydrogen peroxide); Covacs reagents; oxidase reagent; Sodium hypochlorite, were procured from BDH Chemicals England; M & B, Laboratory Reagent, England; Reckitt Benckiser, Nigeria respectively and used.

Terminalia catapa (fruit plant-leaves), *Passiflora edulis* (passion fruit-fruit peels), *Daniella oliveri* ([Chiha-Tiv]-leaves), *Ceiba pentandra* ([Vambe-Tiv]-leaves) and *Jatropha tanjorensis* (Catholic plant-leaves) were collected at Tse-Abu, Makurdi, Benue; while *Azadirachta indica* (neem [dogonyaro-Hausa]-leaves), *Carica papaya* (Paw paw [Mbue-Tiv]-leaves), *Moringa oleifera* (Drumstick [Jegeregede-Tiv]-leaves) and *Mangifera indica* (mango [Mongur-Tiv]-leaves) were collected within Benue State University and used for the antimicrobial sensitivity test.

2.2. Microbial Isolation

2.2.1. Sample Preparation

The methods of [36] [40] were used in the preparation of the sample without any modifications.

2.2.2. Media Preparation
The culture media Potatoe dextrose agar + chloramphenicol, Nutrient Agar and MacZonkey Agar Nutreint broth, Simon citrate agar, and TSIA were prepared according to manufacturer directions. The prepared media plates were subjected to sterility testing by incubating them at 37˚C for 24 hours and observing for absence of microbial growth. The sterile media plates were then used for the inoculation. The media were poured and used as reported by [36] [63].

2.2.3. Inoculation
Inoculation was carried out using the methods of [36] [40] without any modifications.

2.2.4. Identification
The methods of [36] [63] were used for the various tests and examinations. Identification of bacteria and fungi isolates was carried out using the methods of [64] [65] [67] respectively.

2.2.5. Optimal Growth Temperature
The methods of [36] [63] were used without any modifications. The various isolates were each inoculated and incubated at temperatures ranging from 28˚C to 42˚C and their radial growth diameter were observed, measured and recorded.

2.2.6. Pathogenicity Test
The test was carried out using the methods of [36] [40] without any modifications, using the healthy yam tubers.

2.2.7. Plant Extract Preparation
The methods of [36] [48] were used. The fresh and dry plant leaves of *Terminalia catapa*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadrichta indica*, *Carica papaya*, *Moringa oleifera*, *Mangifera indica* and fruit peels of *Passiflora edulis* were used. They were washed in clean water. Fresh material extraction was carried out by first, fine shreading 10.0 g of the plant parts then grinding it in a warring blender with sterile distilled water, the blends were filtered with No 1 Whatman filter paper into a sterile 100 mL volumetric flask, made up to mark and labeled. Serial dilutions of 10^{-1} and 10^{-2} concentrations of the extracts were carried out by pipetting 1.0 mL of each filtrate into a 10 mL and 100 mL volumetric flask respectively and the volume made up to mark. Samples were also dried and treated as above. The extracts were all kept in the refrigerator for antimicrobial activity test.

2.2.8. Plant Extract Incorporation
The methods of [36] [66] were employed using the plant extracts prepared above. The samples (1.0 mL) were pipetted into labelled sterile petri dishes respectively and molten nutrient agar or potatoe dextrose agar held at 45˚C were poured onto it and mixed properly by rotating the plates on the flat surface, the plates were allowed to set for 30 minutes; subjected to sterility test and used for antimicrobial sensitivity test.

2.2.9. Antimicrobial Sensitivity Test
The methods of [36] [66] were used. The isolates were cultured on the fresh and dry plant extract incorporated media plates and incubated for 24 hours (bacteria) and for up to 7 days (fungi); control for each of the isolates were carried out by seeding on media plates poured on 1 mL sterile distilled water, instead of the extract. The effect of the extracts on the microbial growth and survival was observed and recorded.

3. Results and Discussion

3.1. Results

Four fungi; *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifera* and *Penicillium marneffei and* two bacteria; *Erwinia carotovora* and *Pseudomonas aeruginosa* were isolated from the water yam samples (**Table 1**).

The incidence of occurrence of these organisms at the sampled sites of the two local government areas reveals that within the same local government area, organism present in same water yam variety from one site may be absent at the second site. Also observed was similarity or variance of their occurrence across the two local

Table 1. Microorganisms isolated from rotten water yam and their incidence ratio.

Yam Variety/Sample Area	Fungi Isolated				Bacteria Isolated	
	Aspergillus niger	*Aspergillus flavus*	*Rhizopus stolonifera*	*Penicillium marneffei*	*Erwinia carotovora*	*Pseudomonas aeruginosa*
			Azawele-wele			
1	+	+	+	−	−	+
2	−	−	−	+	−	+
3	+	+	+	−	+	−
4	+	−	+	+	+	−
Incidence Ratio	**3/4**	**2/4**	**¾**	**2/4**	**2/4**	**2/4**
			Kor			
1	+	+	+	−	−	+
2	+	+	+	−	−	+
3	+	−	+	+	−	+
4	+	+	+	−	−	+
Incidence Ratio	**4/4**	**¾**	**4/4**	**1/4**	**0/4**	**4/4**
			Banada			
1	+	+	−	+	−	+
2	+	+	+	−	+	−
3	+	−	+	+	−	+
4	+	+	+	−	−	+
Incidence Ratio	**4/4**	**¾**	**¾**	**2/4**	**1/4**	**¾**

Key: First Site: 1 & 2 in Tarka Local Government Area Second Site: 3 & 4 in Vandeikya Local Government Area. + = presence of the microorganism; − = absence of the microorganism.

government areas. *Aspergillus niger* shows high occurrence incidence in all the water yam varieties in all the sampled areas, followed by *Rhizopus stolonifera*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Penicillium marneffei* and *Erwinia carotovora. Erwinia carotovora* was observed to be completely absent in Kor variety (**Table 1**).

Biochemical tests conducted on the isolated bacteria confirmed them to be *Erwinia carotovora* and *Pseudomonas aeruginosa* (**Table 2**).

The optimal growth temperature recorded for both the bacteria and fungi species was 38˚C. At 28˚C to 30˚C, the growth of the bacteria *Pseudomonas aeruginosa* was very well supported, the fungi *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifera*, *Penicillium marneffei* andbacteria *Erwinia carotovora*, were only fairly supported; at 32˚C to 38˚C all isolates grew very well. The growth of all the fungi isolates and the bacteria *Erwinia carotovora* were highly retarded at temperatures above 40˚C while the growth of *Rhizopus stoloniferaw*as only slightly retarded (**Table 3**).

Pathogenicity test carried out with the isolates shows varying degrees of rot with different organisms. Soft rot was recorded with the fungi *Penicillium marneffei* and the bacteria *Erwinia carotovora* either as singly or in associative growth with the other organisms, but it was also recorded where the fungi *Rhizopus* and the bacteria *Pseudomonas aeruginosa* were found in associative growth. Wet rot was recorded with the bacteria *Erwinia carotovora* and in the associative growth of all bacteria, all bacteria and all fungi. Also, associative growth of all fungi and all bacteria recorded only soft rot. The water yam varieties that did not show presence of certain isolates, shows rot initiation on introduction of the microorganisms. The results shows that the organisms isolated were responsible for the rot (**Table 4**).

Table 2. Biochemical test for identification of *Erwinia carotovora* and *Pseudomonas aeruginosa.*

Test(s)	Microorganisms	
	Erwinia carotovora	*Pseudomonas aeruginosa*
Gram staining reaction	−	−
Catalase	+	+
Oxidase	−	+
Citrate utilisation	−	+
Gas	−	−
H_2S (hydrogen sulphide)	−	−
Motility	+	+
Pigment	−	Blue
pH (in nutrient broth medium)	7.47	7.43
Acid	−	+

Key: + = Positive Reaction; − = Negative Reaction.

Table 3. Optimal growth temperature determination.

Temperature (˚C)	Fungi				Bacteria	
	Aspergillus niger	*Aspergillus flavus*	*Rhizopus stolonifera*	*Penicillium marneffei*	*Erwinia carotovora*	*Pseudomonas aeruginosa*
28	5.4	4.1	4.8	4.1	2.1	3.1
30	5.6	4.3	5.6	4.2	2.1	3.2
32	5.9	4.5	6.1	4.3	2.5	2.8
34	6.3	4.9	7.5	4.6	2.6	2.8
36	6.6	5.2	7.9	4.8	2.6	2.8
38	6.6	5.4	7.9	5.0	2.6	2.7
40	4.9	4.0	6.1	4.5	1.2	1.8
42	4.0	3.8	5.8	4.0	−	1.3

Key: − = No Growth.

Table 4. Pathogencity test-rot measurement.

Microorganism	Water Yam Varieties					
	Azawele wele		Kor		Banada	
	Width	Depth	Width	Depth	Width	Depth
Aspergillus niger	1.9	3.6	1.4	2.3	1.4	2.5
Aspergillus flavus	2.4	3.6	2.0	2.4	1.8	2.2
Rhizopus stolonifera	2.2	4.1	2.3	3.1	1.6	2.5
Penicillium marneffei	1.7	2.3	1.2	2.0	1.2	1.9
Erwinia carotovora	1.5	2.9	1.4	2.7	1.6	2.5
Pseudomonas aeruginosa	1.6	2.8	1.4	2.7	2.0	2.5
All bacteria	1.8	3.4	1.7	3.0	1.5	2.9
All fungi	3.5	3.6	2.6	3.3	2.2	3.0
All bacteria and fungi	4.3	4.0	3.2	3.9	2.8	3.4

Antimicrobial sensitivity tests reveal antifungal and antibacterial activity of both the fresh and dry plant material extracts, the fresh samples were more effective than the dry samples. *Passiflora edulis* shows high inhibition of *Aspergillus niger* and *Aspergillus flavus* at undiluted concentration and 10^{-1} dilution in the fresh extract followed by *Azadirachta indica*, *Carica papaya*, *Moringa oleifera* and *Mangifera indica* all having good inhibition at undiluted concentration. In the dry extracts similar trend was observed, *Passiflora edulis* shows good inhibition at the undiluted concentrations while the others show moderate to mild inhibition. The fresh extracts all recorded inhibition at least in the undiluted form (**Table 5(a)** & **Table 5(b)**).

Passiflora edulis fresh extract at undiluted concentration and 10^{-1} dilution completely inhibited the growth of *Rhizopus stolonifera* and shows high inhibition at 10^{-2}, while *Jatropha tanjorensis* shows high inhibition at undiluted concentration and good inhibition at 10^{-1} dilution along with *Terminalia catapa* at undiluted concentration. In the dry extracts *Passiflora edulis* shows high inhibition at undiluted concentration and 10^{-1} dilution, good inhibition at 10^{-2} dilution along with *Jatropha tanjorensis* at undiluted concentration. *Rhizopus stolonifera* was not sensitive to most of the other extracts (**Table 5(c)**).

Passiflora edulis fresh extract shows high inhibition of *Penicillium marneffei* in all the concentrations along with *Azadirachta indica*, *Carica papaya*, *Moringa oleifera* and *Mangifera indica* at undiluted concentrations. Their dilutions shows moderate to mild inhibition. In the dry extract *Passiflora edulis* shows good inhibition in all the concentrations while the others show moderate to mild inhibition (**Table 5(d)**).

Terminalia catapa fresh extract shows complete inhibition of *Erwinia carotovora* in all the concentrations followed by *Passiflora edulis*, *Jatropha tanjorensis* and *Carica papaya* which shows high inhibition at undiluted concentrations; *Jatropha tanjorensis* at 10^{-1} dilution, *Azadirachta indica*, *Moringa oleifera* and *Mangifera indica* at undiluted concentrations shows good inhibition. In the dry extract *Terminalia catapa* still shows complete inhibition at undiluted concentration and 10^{-1} dilution; high inhibition at 10^{-2} dilution along with *Carica papaya*. *Passiflora edulis* and *Jatropha tanjorensis* shows good inhibition (**Table 5(e)**).

Jatropha tanjorensis fresh extract at undiluted concentration and 10^{-1} dilution shows complete inhibition of *Pseudomonas aeruginosa*, high inhibition at 10^{-2} dilution; *Terminalia catapa*, *Ceiba pentandra*, and *Carica papaya* at undiluted concentrations also shows high inhibition. *Daniella oliveri*, *Azadirachta indica* and *Moringa oleifera* at undiluted concentrations shows good inhibition along with 10^{-1} dilutions of *Terminalia catapa*, *Daniella oliveri* and *Ceiba pentandra*. In the dry extracts, *Jatropha tanjorensis* shows high inhibition in all the concentrations; *Ceiba pentandra* shows good inhibition in undiluted concentration and 10^{-1} dilution along with *Terminalia catapa* in undiluted concentration (**Table 5(f)**).

3.2. Discussion

This study reveals the types of fungi and bacteria species associated with rot of water yam tubers in some parts of Benue State, Nigeria. The microorganisms isolated were four fungal species; *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifera* and *Penicillium marneffei* and two bacteria species; *Erwinia carotovora* and *Pseudomonas aeruginosa*. The results obtained from fungal rot organisms isolated are in agreement with earlier results [2] [6] [7] [15] [25] [26]. Brown hard rot was recorded with the fungi; *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifera* in all the water yam varieties, and the bacteria *Pseudomonas aeruginosa.* This is similar to earlier findings on white yam rot organisms [17] [36]. The fungi; *Aspergillus niger*, *Aspergillus flavus* and the bacteria *Pseudomonas aeruginosa* isolated are known to be human pathogenic or opportunistic human pathogenic organisms [44] [68]. They may even secrete substances that are harmful to humans [43] [69].

The incidence of occurrence of these organisms at the sampled sites of the two local government areas revealed that within the same local government area, an organism present in same water yam variety from one site may be absent at the second site. Also observed was similarity or variance of their occurrence across the two local government areas; this is most probably due to the weather condition at the time of harvest, if the day is dry, windy and the tubers are bruised, it dries easily reducing entry time of the microorganisms but under humid weather, the organisms stick fast and grow causing damages, this type of change can also influence variation in occurrence on a farm. *Aspergillus niger* showed high occurrence incidence in all the water yam varieties in all the sampled areas, this is most probably due to the fact that the time required for it to survive and cause rot is shorter than most microorganisms found on yam; it creates pre-harvest and post-harvest food rot all over the world for tubers as well as nuts [43] [54] [70]. This was followed by *Rhizopus stolonifera*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Penicillium marneffei* and *Erwinia carotovora*. *Erwinia carotovora* was observed to

Table 5. Antimicrobial sensitivity test. (a) Antimicrobial sensitivity test on the fungi *Aspergillus niger*; (b) Antimicrobial sensitivity test on the fungi *Aspergillus flavus*; (c) Antimicrobial sensitivity test on the fungi *Rhizopus stolonifera*; (d) Antimicrobial sensitivity test on the fungi *Penicillium marneffei*; (e) Antimicrobial sensitivity test on the bacteria *Erwinia carotovora*; (f) Antimicrobial sensitivity test on the bacteria *Pseudomonas aeruginosa*.

(a)

Plant	Fresh Extract Concentrations			Dry Extract Concentrations		
	100%	10^{-1}	10^{-2}	100%	10^{-1}	10^{-2}
Terminalia catapa	++^{d}	–^{f}	–^{f}	+^{e}	–^{f}	–^{f}
Passiflora edulis	++++^{b}	++++^{b}	++^{d}	+++^{c}	+++^{c}	+^{e}
Daniella oliveri	++^{d}	–^{f}	–^{f}	+^{e}	–^{f}	–^{f}
Ceiba pentandra	+^{e}	++^{d}	++^{d}	–^{f}	+^{e}	+^{e}
Jatropha tanjorensis	++^{d}	++^{d}	+^{e}	+^{e}	+^{e}	+^{e}
Azadirachta indica	+++^{c}	++^{d}	–^{f}	++^{d}	–^{f}	–^{f}
Carica papaya	+++^{c}	++^{d}	–^{f}	++^{d}	–^{f}	–^{f}
Moringa oleifera	+++^{c}	+^{e}	–^{f}	+^{e}	–^{f}	–^{f}
Mangifera indica	++^{d}	+^{e}	–^{f}	+^{e}	–^{f}	–^{f}
Summary:		*a* = 0; *d* = 9 *b* = 2; *e* = 4 *c* = 3; *f* = 8			*a* = 0; *d* = 2 *b* = 0; *e* = 10 *c* = 2; *f* = 12	

Key: 100% = undiluted plant extract; +++++ = complete inhibition ++++ = high inhibition; +++ = good inhibition; ++ = moderate inhibition; + = mild inhibition; − = no inhibition. Variance of plant extracts (fresh and dry) inhibition of various microorganisms: a = Complete inhibition, b = high inhibition, c = good inhibition, d = moderate inhibition, e = mild inhibition, f = no inhibition.

(b)

Plant	Fresh Extract Concentrations			Dry Extract Concentrations		
	100%	10^{-1}	10^{-2}	100%	10^{-1}	10^{-2}
Terminalia catapa	++^{d}	+^{e}	–^{f}	+^{e}	–^{f}	–^{f}
Passiflora edulis	++++^{b}	++++^{b}	++^{d}	+++^{c}	+++^{c}	+^{e}
Daniella oliveri	++^{d}	+^{e}	–^{f}	+^{e}	–^{f}	–^{f}
Ceiba pentandra	+^{e}	+^{e}	+^{e}	–^{f}	+^{e}	+^{e}
Jatropha tanjorensis	++^{d}	+^{e}	+^{e}	+^{e}	+^{e}	–^{f}
Azadirachta indica	+++^{c}	++^{d}	–^{f}	++^{d}	+^{e}	–^{f}
Carica papaya	+++^{c}	++^{d}	–^{f}	++^{d}	+^{e}	–^{f}
Moringa oleifera	+++^{c}	++^{d}	–^{f}	++^{d}	–^{f}	–^{f}
Mangifera indica	+++^{c}	++^{d}	–^{f}	+^{e}	–^{f}	–^{f}
Summary:		*a* = 0; *d* = 7 *b* = 2; *e* = 7 *c* = 4; *f* = 6			*a* = 0; *d* = 3 *b* = 0; *e* = 10 *c* = 2; *f* = 12	

(c)

Plant	Fresh Extract Concentrations			Dry Extract Concentrations		
	100%	10^{-1}	10^{-2}	100%	10^{-1}	10^{-2}
Terminalia catapa	$+++^{c}$	$++^{d}$	$+^{e}$	$++^{d}$	$+^{e}$	$+^{e}$
Passiflora edulis	$+++++^{a}$	$+++++^{a}$	$++++^{b}$	$++++^{b}$	$++++^{b}$	$+++^{c}$
Daniella oliveri	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Ceiba pentandra	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Jatropha tanjorensis	$++++^{b}$	$+++^{c}$	$+^{e}$	$+++^{c}$	$+^{e}$	$-^{f}$
Azadirachta indica	$+^{e}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Carica papaya	$+^{e}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Moringa oleifera	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Mangifera indica	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Summary:		$a = 2$; $d = 1$ $b = 2$; $e = 4$ $c = 2$; $f = 16$			$a = 0$; $d = 1$ $b = 2$; $e = 3$ $c = 2$; $f = 19$	

(d)

Plant	Dry Extract Concentrations			Dry Extract Concentrations		
	100%	10^{-1}	10^{-2}	100%	10^{-1}	10^{-2}
Terminalia catapa	$++^{d}$	$-^{f}$	$-^{f}$	$+^{e}$	$-^{f}$	$-^{f}$
Passiflora edulis	$++++^{b}$	$++++^{b}$	$++++^{b}$	$+++^{c}$	$+++^{c}$	$+++^{c}$
Daniella oliveri	$++^{d}$	$+^{e}$	$-^{f}$	$+^{e}$	$-^{f}$	$-^{f}$
Ceiba pentandra	$+^{e}$	$+^{e}$	$-^{f}$	$+^{e}$	$+^{e}$	$-^{f}$
Jatropha tanjorensis	$++^{d}$	$-^{f}$	$-^{f}$	$+^{e}$	$-^{f}$	$-^{f}$
Azadirachta indica	$++++^{b}$	$++^{d}$	$+^{e}$	$++^{d}$	$+^{e}$	$+^{e}$
Carica papaya	$++++^{b}$	$++^{d}$	$+^{e}$	$++^{d}$	$+^{e}$	$+^{e}$
Moringa oleifera	$++++^{b}$	$++^{d}$	$+^{e}$	$++^{d}$	$+^{e}$	$+^{e}$
Mangifera indica	$++++^{b}$	$++^{d}$	$+^{e}$	$++^{d}$	$+^{e}$	$+^{e}$
Summay:		$a = 0$; $d = 5$ $b = 7$; $e = 7$ $c = 0$; $f = 6$			$a = 0$; $d = 4$ $b = 0$; $e = 13$ $c = 3$; $f = 7$	

(e)

Plant	Fresh Extract Concentrations			Dry Extract Concentrations		
	100%	10^{-1}	10^{-2}	100%	10^{-1}	10^{-2}
Terminalia catapa	$+++++^{a}$	$+++++^{a}$	$+++++^{a}$	$+++++^{a}$	$+++++^{a}$	$++++^{b}$
Passiflora edulis	$++++^{b}$	$+++^{c}$	$++^{d}$	$+++^{c}$	$++^{d}$	$+^{e}$
Daniella oliveri	$+^{e}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$	$-^{f}$
Ceiba pentandra	$++^{d}$	$+^{e}$	$+^{e}$	$+^{e}$	$+^{e}$	$+^{e}$
Jatropha tanjorensis	$++++^{b}$	$+++^{c}$	$++^{d}$	$+++^{c}$	$++^{d}$	$++^{d}$
Azadirachta indica	$+++^{c}$	$+^{e}$	$-^{f}$	$++^{d}$	$+e$	$-^{f}$
Carica papaya	$++++^{b}$	$+^{e}$	$-^{f}$	$++++^{b}$	$+^{e}$	$-^{f}$
Moringa oleifera	$+++^{c}$	$+^{e}$	$-^{f}$	$++^{d}$	$+^{e}$	$-f$
Mangifera indica	$+++^{c}$	$+^{e}$	$-^{f}$	$++^{d}$	$+^{e}$	$-^{f}$
Summary:		$a = 3$; $d = 3$ $b = 3$; $e = 7$ $c = 5$; $f = 6$			$a = 2$; $d = 6$ $b = 2$; $e = 8$ $c = 2$; $f = 7$	

(f)

Plant	Fresh Extract Concentrations			Dry Extract Concentrations		
	100%	10^{-1}	10^{-2}	100%	10^{-1}	10^{-2}
Terminalia catapa	++++^{b}	+++^{c}	+^{e}	+++^{c}	++^{d}	+^{e}
Passiflora edulis	++^{d}	++^{d}	+^{e}	+^{e}	+^{e}	+^{e}
Daniella oliveri	+++^{c}	+++^{c}	++^{d}	++^{d}	++^{d}	+^{e}
Ceiba pentandra	++++^{b}	+++^{c}	++^{d}	+++^{c}	+++^{c}	++^{d}
Jatropha tanjorensis	+++++^{a}	+++++^{a}	++++^{b}	++++^{b}	++++^{b}	++++^{b}
Azadirachta indica	+++^{c}	+^{e}	–^{f}	++^{d}	+^{e}	–^{f}
Carica papaya	++++^{b}	++^{d}	+^{e}	++^{d}	+^{e}	+^{e}
Moringa oleifera	+++^{c}	++^{d}	+^{e}	++^{d}	+^{e}	–^{f}
Mangifera indica	++^{d}	+^{e}	–^{f}	++^{d}	–^{f}	–^{f}
Summary:		a = 2; d = 7 b = 4; e = 6 c = 6; f = 2			a = 0; d = 8 b = 3; e = 9 c = 3; f = 4	

be completely absent in Kor variety, this is most probably due to the fact that the substrate in Kor as well as the weather at harvesting time were unfavorable for it to cause yam rot. The result on the water yam rot is similar to those earlier obtained. The higher prevalence of yam rots with more microorganisms in the rain forest areas support the above observations [40] [71]-[73].

Optimal growth temperature studies show that all the fungi and the bacteria *Erwinia carotovora* isolated have 38˚C as optimum growth temperature; *Pseudomonas aeruginosa* recorded optimal growth temperature of 30˚C. The temperature of 28˚C was not favorable for all microorganisms as all of them showed retarded growth. On this basis, yam storage for temperature below 28˚C needs to be investigated further to reduce or stop post-harvest yam rot caused by microorganisms [17] [23].

Result of the Pathogenicity test reveals that the organisms isolated were responsible for the yam rot and it ranged from dry hard rot, soft rot and watery rot. Also collectively as all fungi, all bacteria or all fungi and bacteria combined produces higher degree of rot than when the organisms were introduced individually; this agrees with earlier findings in the study with *Dioscorea rotundata* [17] [36] [38] [74]. Microorganisms that were not isolated in the water yams from some local government areas were observed to cause rot when inoculated in them, showing that they are opportunistic pathogens.

Antimicrobial activity test with aqueous extracts of nine plants, *Terminalia catapa*, *Passiflora edulis*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*,and *Mangifera indica* of fresh and dry material showed that three pathogens, one fungi; *Rhizopus stolonifera* and the two bacteria isolates (*Erwinia carotovora* and *Pseudomonas aeruginosa)* were completely inhibited each by a plant; this agrees with earlier findings [58] [68] [75] [76]. The result obtained shows that *Passiflora edulis* had the best antimicrobial activity for both fungi and bacteria, indeed it inhibited completely *Rhizopus stolonifera* which was stubborn with most of the other plants; this is in agreement with earlier work [77]. The result reported with plant extracts on the microorganisms pathogenic in humans show that the extracts were not specific on human or plant pathogen but on the species of the microorganisms; thus the results obtained in this work are useful in the treatment of humans as well as control of post-harvest loses [55]. *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, and *Mangifera indica* were also able to inhibit most of the fungi but not completely. *Terminalia catapa* and *Jatropha tanjorensis* were most effective against the bacteria. *Erwinia carotovora* was completely inhibited by *Terminalia catapa* and *Pseudomonas aeruginosa* was completely inhibited by *Jatropha tanjorensis*. *Daniella oliveri* and *Ceiba Pentandra* had the least inhibition against the isolates; this agrees with earlier findings [55] [59] [68] [76] [78] [79]. The dried plant extracts show mild or low inhibition in most cases. This could be due to chemical changes brought about by enzyme hydrolysis which could bring about hydrolysis

of some compounds which may even induce intra or intermolecular reactions [43]. Some plant extracts could not inhibit other microorganisms but effectively inhibited others; this is probably due to the fact that, the active sites reacting with the various microorganisms were different or there were different substances in extracts inhibiting different microorganisms. This observation is similar to earlier reports [25] [68] [76] [78].

4. Conclusion

Seven plant extracts were found to be effective in inhibiting the six microorganisms isolated from water yam. Three out of the six microorganisms, one fungi, *Rhizopus stolonifera* and two bacteria, *Erwinia carotovora* and *Pseudomonas aeruginosa*, were completely inhibited by *Passiflora edulis*, *Terminalia catapa* and *Jatropha tanjorensis* respectively. The other four plants extracts *Azadirachta indica*, *Carica papaya*, *Moringa olifera* and *Mangifera indica* gave high to good inhibition on most of the isolates. *Daniella oliveri* and *Ceiba pentandra* were the least effective. Fresh leaves extracts effectively inhibited growth while dried leaves lost most of their ability to inhibit. There is a need to freeze dry fresh leaves and study the cause of loose of their ability to inhibit growth on drying gradually over a period. Comparative studies on the purified extracts from freeze dried samples and samples air dried gradually for at least a week will give us analysis of the product. Their ability to inhibit will then be compared and their differences will be noted.

Acknowledgements

We wish to thank the Centre for Food Technology and Research (CEFTER) of Benue State University, Makurdi for the facilities and finances made available to us to carry out this research. We also appreciate the farmers in the two local governments where the samples were obtained. Their useful discussions did not only encourage us but was helpful in carrying out the work. The cooperation of the staff of Biological Sciences and Chemistry Departments of Benue State University particularly Mr. Pius Utange is highly appreciated. Finally, this work is dedicated to the loving memory of T.S.'s dear wife, friend and companion; **Ankabu Mbatomon Shambe**.

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