

Extraction Methods and Inhibition Studies of Ten Plant Extracts on Nine Yam Rot Pathogenic Microorganisms

Dooshima Shiriki^{1*}, Simon Terver Ubwa², Mohammed Ikagu Yusufu³, Tsea Shambe²

¹Department of Biological Sciences, Benue State University, Makurdi, Nigeria

²Department of Chemistry, Benue State University, Makurdi, Nigeria

³Department of Food Science and Technology, University of Agriculture, Makurdi, Nigeria

Email: *dshiriki@bsum.edu.ng

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Abstract

Nine microorganisms, comprising of four bacteria, *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella oxytoca* and five fungi, *Rhizopus stolonifera*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium marneffeii*, isolated from rotten yam tubers of 2016 harvest year, were treated with ten plants extracts (*Passiflora edulis*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, *Mangifera indica*, *Terminalia catapa* and *Senna alata*), singly and synergistically by incorporation of extract in media for inhibition test. Two plant extracts singly and completely inhibited the growth of three organisms: *Terminalia catapa* at 100% and 10⁻¹ showed complete inhibition (a) of *Erwinia carotovora*. *Passiflora edulis* at undiluted (100%) concentration completely inhibited *Rhizopus stolonifer* and *Penicillium marneffeii*, respectively. Synergistic plant extract recorded complete inhibition (a) of all the four bacteria isolates at 2 mL extract incorporation; ten (10) mL extract incorporation in media recorded complete inhibition (a) of three out of the five fungi isolates: *Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffeii*, respectively; the other two fungi recorded high inhibition (b) of *Aspergillus niger* and *Aspergillus flavus*, respectively. Hot aqueous synergistic plants extract recorded poor inhibition of the isolates as compared to the cold. Soxhlet solvent extracted synergistic plants extract, however, recorded lower inhibition as compared to hot aqueous synergistic plants extract and cold aqueous synergistic plants extracts. Room temperature solvent extracted synergistic plants extracts recorded inhibition that was same as that obtained with cold aqueous synergistic plants extract. This research indicates that heat employed extractions recorded less inhibition activity.

Keywords

Microorganisms, Rotten Yam, Plant Extracts, Inhibition, Synergistic

1. Introduction

The use of plant extract for treatment of ailment is now preferred or recommended 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 [1]-[10]. There are about 250,000 to 500,000 species of plants on Earth [8] [9]. However, it is only a small percentage (1% to 10%) of these plants that are used as foods by both humans and other animal species [9] [10]. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s [9] [11]. The antimicrobial properties of plants have been investigated by researchers globally. In Latin America, a research tested 122 known plant species used for therapeutic treatments in Argentina [9] [12]. It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococcus aureus*, ten inhibited *Escherichia coli*, and four inhibited *Aspergillus niger*; also was reported that the most potent compound was one extracted from *Tabebuia impetiginosa*. The antimicrobial properties of compounds obtained from *Parthenum argentatum* against *Candida albicans*, *Torulopsis*, *Hansemula*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were detected [9] [13] [14]. Research work conducted revealed that the substances extracted from nine known plants in Uruguai did not show any activity against *C. albicans* and *Saccharomyces cerevisiae*, but inhibited the growth of *Bacillus subtilis*, *E. coli* and *P. aeruginosa* [9] [14] [15] [16]. In many developing countries, traditional medicine is one of the primary healthcare systems [17] [18] [19]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [20]. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they had been very successful, especially in the areas of infectious disease and cancer [21]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, amongst others, which have been found *in vitro* to have antimicrobial properties [22] [23].

Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine [24]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [25].

Most investigations in the use of plant extracts as alternative to synthetic drugs are bias for treatment of humans and animals with little attention to the

treatment of plants and indeed food crops. Researchers who have worked on the use of some single plant extracts to inhibit yam rot microorganisms reported findings that yielded varying degrees of inhibition but no complete inhibition of all the rot causing microorganisms that exist in associative growth have been recorded [7] [26] [27] [28]. Hence, the use of some plants extracts reported to have antimicrobial activity for single and synergistic inhibition studies on the yam pathogenic microbes that were previously isolated from rotten yam tubers and confirmed as etiology of the rot in this study; also to probe further on for possible complete inhibition of the microorganisms that are present in associative growth; positive findings shall be recommended for adoption by farmers for fresh yam tuber preservation for long duration storage (shelf life elongation).

2. Materials and Methods

2.1. Microorganisms

Previously isolated and maintained yam rot pathogenic microorganisms: Nine microorganisms, comprising of four bacteria, *Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella oxytoca* and five fungi, *Rhizopus stolonifera*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium marneffeii*, of 2016 harvest year [29] were obtained from the laboratory, department of Biological Sciences, Benue State University, Makurdi where they were preserved and used for the antimicrobial inhibition test.

2.2. Plant Extract Preparation

2.2.1. Single Plant Extract Preparation

The method of [30] was used. Freshly harvested plant leaves of *Passiflora edulis*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorensis*, *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, *Mangifera indica*, *Terminalia catapa* and *Senna alata* were collected along km 4 Gboko road Makurdi and were authenticated by a plant taxonomist in the Department of Biological Sciences, Benue State University, Makurdi and used. The leaves were washed in clean water and put in a clean plastic basket to drain. They were separately cut to fine shredding with a clean stainless steel knife and 10.0 g of each was weighed and wet milled in a warring blender with 50 mL sterile distilled water, the blends were individually filtered with No. 1 Whatman filter paper into labelled sterile 100 mL volumetric flask, the residue was washed with 30 mL of the sterile water, it was made up to mark using the sterile distilled water 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 was made up to mark with sterile distilled water. The extracts were stored up in the refrigerator and used for inhibition (antimicrobial activity) test.

2.2.2. Cold Aqueous Synergistic Plants Extract Preparation

The method of [31] was used with little modifications; plant leaves were blended in a ratio of 1:1. Fresh leaves of ten plants (*Ceiba pentandra*, *Jatropha tanjoren-*

sis, *Azadirachta indica*, *Moringa Oleifera*, *Carica papaya*, *Mangifera indica*, *Daniella oliveri*, *Terminalia catapa*, *Passiflora edulis* and *Senna alata*) were collected and washed in clean sterile water, it was put to drain in a clean plastic basket for 10 minutes. The leaves were separately cut to fine shredding with a stainless steel kitchen knife and weighed (20.0 g each) individually using an electronic digital weighing balance. They were mix and blended using a warring blender into fine paste. The mix (50.0 g) was weighed into a sterilized 500 mL conical flask, 200 mL of sterile water was added and stirred for 10 minutes, it was plugged with cotton wool and kept to extract for 12 hours at room temperature. The extract was filtered with Whatman No 1 filter paper and the residue was washed with 50 mL of water to a concentration of 0.2 g/mL; the combined volume was decanted into a sterile bottle, properly corked; stored in the refrigerator and used for antimicrobial inhibition test.

2.2.3. Hot Aqueous Synergistic Ten Plants Extraction

The method of natives (Tiv, unpublished work), was used with little scientific modification (measurements and aseptic techniques). The blended mix (50.0 g) was weighed into a sterilized 500 mL beaker, 250 ml of clean water was added, stirred and heated to boiling; it was allowed to boil on for 15 minutes for proper extraction. It was to cooled and filtered with Whatman No 1 filter paper into a 250 mL volumetric flask and made up to the mark with sterile water washings of the boiled plant mix. It was corked and stored in the refrigerator for antimicrobial inhibition test.

2.2.4. Ethanol and Methanol Solvents Soxhlet Synergistic Ten Plants Extraction

The method of [32] was used with little modification. The blended mix (100 g) were packed into timbles and placed inside the soxhlet extractor. The solvents (ethanol and methanol) were taken in round bottom flask of the extractor, respectively; heat was applied to the bottom of the flask from heating mantles and extraction was carried out for 10 hours. The solvents were recovered and the extracts were respectively turned into pre-weighed and labeled beakers, it was evaporated in evaporation bath for 24 hours. The extract was reconstituted with sterile water to a concentration of 0.2 g/ml and used for the inhibition test.

2.2.5. Room Temperature Ethanol and Methanol Solvents Synergistic Ten Plants Extraction

The method of [33] was used with some modification. The blended mix (50.0 g) each was weighed into two sterilized 500 mL beaker respectively, 250 mL of solvents ethanol and methanol were added separately to individual beaker contents, stirred and left to extract on a shaker bath at room temperature for 12 hours. The extract was filtered through Whatman No. 1 filter paper into a sterilized conical flask, poured into pre weighed sterilized glass dishes respectively and exposed in laminar air flow chamber for the solvents evaporation. The yield was turned into a 250 mL volumetric flask and reconstituted with sterile water to a

concentration of 0.2 g/mL and stored away from light under room temperature to be used for the inhibition test.

2.3. Plant Extract Incorporation

2.3.1. Single Plant Extract Incorporation

The method of [27] was used. The plant extracts solution of 10 g/50 mL of solvent (stock solution), and its serial dilutions of 10^{-1} and 10^{-2} were prepared by adding 1.0 mL each of the plant extract stock solutions in 10.0 mL and 100 mL standard volumetric flask then making it up to the mark with sterile distilled water respectively. A 1.0 mL of the undiluted stock and 1.0 mL each of the two dilutions of the single plant extracts were added into labelled sterile petri dishes respectively and molten nutrient Agar and potatoe dextrose agar held at 45°C was poured onto it, respectively; it was mixed properly by rotating the plates on the flat surface, the plates were allowed to set for 30 minutes, they were incubated for sterility check and only sterile plates were used for inhibition test.

2.3.2. Synergistic Plant Extract Incorporation

The method of [34] was used. The extract, 1.0 mL - 10.0 mL, each were pipette into sterile petri dishes and 20 mL or 15 mL (agar quantity reduced with increasing extract quantity) of molten nutrient agar or potato dextrose agar held at 45°C was poured onto it respectively and mixed thoroughly. The plates were allowed to set, packed and incubated overnight for sterility test and sterile plates only after incubation were used for the inhibition test.

2.3.3. Inhibition Test

The methods of [27] was used with little modifications; the bacteria and fungi isolates were seeded individually on the plants extract incorporated nutrient agar and potato dextrose agar respectively, controls were set up by seeding on media without plant extract addition; plates were incubated at 37°C for 48 hours for the bacteria and 7 days for the fungi. The plates were observed for microbial activity. Growth diameter where present was measured and it was compared with the control to device a percentage growth width using a transparent plastic ruler.

3. Results and Discussion

3.1. Results

3.1.1. Single Plant Inhibition Test

Table 1 present results of single plant extract inhibition (antimicrobial sensitivity) test of the ten plants (*Passiflora edulis*, *Daniella oliveri*, *Ceiba pentandra*, *Jatropha tanjorencis*, *Azadirachta indica*, *Carica papaya*, *Moringa oleifera*, *Mangifera indica*, *Terminalia catapa* and *Senna alta*) on the nine Isolates. *Passiflora edulis* at undiluted (100%) concentration completely inhibited *Rhizopus stolonifer* and *Penicillium marneffeï*; high inhibition was obtained in *Rhizopus stolonifer* and *Penicillium marneffeï* at 10^{-1} dilution along with *Aspergillus flavus* and *Fusarium oxysporum* at undiluted concentrations. Good inhibition was recorded

Table 1. Single plant extract inhibition test on the isolates.

Plant	Organism/Extract concentration					
	Fungi			Bacteria		
	<i>Rhizopus stolonifer</i>			<i>Erwinia carotovora</i>		
	100%	10 ⁻¹	10 ⁻²	100%	10 ⁻¹	10 ⁻²
<i>Passiflora edulis</i>	+++++ ^a	++++ ^b	+++ ^c	+++ ^c	++ ^d	+ ^e
<i>Daniella olivera</i>	- ^f	- ^f	- ^f	- ^f	- ^f	- ^f
<i>Ceiba pentandra</i>	- ^f	- ^f	- ^f	+ ^e	+ ^e	+ ^e
<i>Jathropha tanjorensis</i>	+++ ^c	+ ^e	- ^f	+++ ^c	++ ^d	++ ^d
<i>Azadirachta indica</i>	+ ^e	- ^f	- ^f	+++ ^c	+ ^e	- ^f
<i>Carica papaya</i>	+ ^e	- ^f	- ^f	++++ ^b	+ ^e	- ^f
<i>Moringa oleifera</i>	- ^f	- ^f	- ^f	+++ ^c	+ ^e	- ^f
<i>Mangifera indica</i>	- ^f	- ^f	- ^f	+++ ^c	+ ^e	- ^f
<i>Terminalia catapa</i>	++ ^d	+ ^e	+ ^e	+++++ ^a	+++++ ^a	++++ ^b
<i>Senna alata</i>	++++ ^b	+++ ^c	++ ^d	+++ ^c	+ ^e	+ ^e
Summary	a = 1 b = 2 c = 3	d = 2 e = 5 f = 17		a = 2 b = 2 c = 6	d = 3 e = 10 f = 7	
				<i>Aspergillus niger</i>		<i>Pseudomonas aeruginosa</i>
<i>Passiflora edulis</i>	+++ ^c	+++ ^c	+ ^e	+ ^e	+ ^e	+ ^e
<i>Daniella olivera</i>	+ ^e	- ^f	- ^f	++ ^d	++ ^d	+ ^e
<i>Ceiba pentandra</i>	- ^f	+ ^e	+ ^e	+++ ^c	+++ ^c	++ ^d
<i>Jathropha tanjorensis</i>	- ^f	- ^f	- ^f	++++ ^b	++++ ^b	++++ ^b
<i>Azadirachta indica</i>	+++ ^c	++ ^d	- ^f	++ ^d	+ ^e	- ^f
<i>Carica papaya</i>	+++ ^c	++ ^d	- ^f	+++ ^c	++ ^d	+ ^e
<i>Moringa oleifera</i>	+++ ^c	+ ^e	- ^f	++ ^d	+ ^e	+ ^e
<i>Mangifera indica</i>	++ ^d	+ ^e	- ^f	++ ^d	+ ^e	- ^f
<i>Terminalia catapa</i>	+ ^e	- ^f	- ^f	+++ ^c	++ ^d	+ ^e
<i>Senna alata</i>	++++ ^b	++ ^d	+ ^e	++ ^d	+ ^e	+ ^e
Summary	a = 0 b = 1 c = 5	d = 4 e = 8 f = 12		a = 0 b = 3 c = 4	d = 9 e = 12 f = 2	
				<i>Aspergillus flavus</i>		<i>Serratia marcescens</i>
<i>Passiflora edulis</i>	++++ ^b	+++ ^c	+ ^e	- ^f	- ^f	- ^f
<i>Daniella olivera</i>	+ ^e	- ^f	- ^f	+ ^e	- ^f	- ^f
<i>Ceiba pentandra</i>	+ ^e	+ ^e	+ ^e	++ ^d	+ ^e	- ^f
<i>Jathropha tanjorensis</i>	++ ^d	+ ^e	+ ^e	+ ^e	- ^f	- ^f
<i>Azadirachta indica</i>	+++ ^c	++ ^d	- ^f	+ ^e	+ ^e	- ^f
<i>Carica papaya</i>	+++ ^c	++ ^d	- ^f	++ ^d	+ ^e	- ^f
<i>Moringa oleifera</i>	+++ ^c	++ ^d	- ^f	+ ^e	+ ^e	- ^f
<i>Mangifera indica</i>	++ ^d	+ ^e	- ^f	+ ^e	+ ^e	+ ^e
<i>Terminalia catapa</i>	++ ^d	+ ^e	- ^f	- ^f	- ^f	- ^f
<i>Senna alata</i>	+++ ^c	++ ^d	+ ^e	++ ^d	+ ^e	+ ^e
Summary	a = 0 b = 1 c = 5	d = 7 e = 10 f = 7		a = 0 b = 0 c = 0	d = 3 e = 13 f = 14	

Continued

	<i>Fusarium oxysporum</i>			<i>Klebsiella oxytoca</i>		
<i>Passiflora edulis</i>	++++ ^b	++ ^d	++ ^d	+ ^e	+ ^e	+ ^e
<i>Daniella olivera</i>	+ ^e	+ ^e	++ ^d	+ ^e	+ ^e	+ ^e
<i>Ceiba pentandra</i>	+++ ^c	+++ ^c	+ ^e	++ ^d	+ ^e	+ ^e
<i>Jathropha tanjorensis</i>	+++ ^c	++ ^d	+ ^e	++++ ^b	++ ^d	++ ^d
<i>Azadarichta indica</i>	++ ^d	+ ^e	+ ^e	++ ^d	+ ^e	- ^f
<i>Carica papaya</i>	++ ^d	++ ^d	+ ^e	+++ ^c	++ ^d	+ ^e
<i>Moringa oleifera</i>	+ ^e	+ ^e	- ^f	+ ^e	+ ^e	- ^f
<i>Mangifera indica</i>	+ ^e	+ ^e	- ^f	+ ^e	+ ^e	+ ^e
<i>Terminalia catapa</i>	+ ^e	+ ^e	+ ^e	+ ^e	+ ^e	+ ^e
<i>Senna alata</i>	+++ ^c	++ ^d	+ ^e	+ ^e	+ ^e	+ ^e
Summary	a = 0 b = 1 c = 4	d = 8 e = 15 f = 2		a = 0 b = 1 c = 1	d = 5 e = 21 f = 2	
	<i>Penicillium marneffeï</i>					
<i>Passiflora edulis</i>	+++++ ^a	++++ ^b	+++ ^c			
<i>Daniella olivera</i>	- ^f	- ^f	- ^f			
<i>Ceiba pentandra</i>	- ^f	- ^f	- ^f			
<i>Jathropha tanjorensis</i>	++++ ^b	+++ ^c	+ ^e			
<i>Azadarichta indica</i>	+ ^e	+ ^e	- ^f			
<i>Carica papaya</i>	+ ^e	- ^f	- ^f			
<i>Moringa oleifera</i>	- ^f	- ^f	- ^f			
<i>Mangifera indica</i>	- ^f	- ^f	- ^f			
<i>Terminalia catapa</i>	+++ ^c	++ ^d	+ ^e			
<i>Senna alata</i>	+ ^e	- ^f	- ^f			
Summary	a = 1 b = 2 c = 3	d = 1 e = 6 f = 17				

Summary of overall inhibition: a = 4, b = 13, c = 31, d = 41, e = 100, f = 60; Key: = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition, b = high inhibition, c = good inhibition, d = moderate inhibition; e = mild inhibition, f = no inhibition.

by *Rhizopus stolonifer* at 10^{-2} dilution, *Aspergillus niger* at undiluted and 10^{-1} concentrations along with *Aspergillus flavus*, and *Penicillium marneffeï* and the bacteria *Erwinia carotovora* at 10^{-2} and undiluted concentrations.

Daniella olivera recorded mild to moderate inhibition of the organisms with the exception of *Rhizopus stolonifer*, *Erwinia carotovora* and *Penicillium marneffeï* that recorded no inhibition respectively.

Ceiba pentandra showed good inhibition (c) of *Pseudomonas aeruginosa* and *Fusarium oxysporum* at 100% and 10^{-1} dilutions, respectively. Other organisms recorded mild (e) to moderate inhibition (d), respectively; with the exception of *Penicillium marneffeï* that recorded no inhibition (f).

Jatropha tanjorensis at undiluted concentration (100%) showed high inhibition (b) of *Pseudomonas aeruginosa* (both at 100% and the dilutions), along with *Klebsiella oxytoca* and *Penicillium marneffeï*. It also recorded good inhibition (c) at 100% concentration on *Rhizopus stolonifer*, *Erwinia carotovora*, *Fusarium oxysporum* and *Penicillium marneffeï* at 10^{-1} dilution, respectively.

Azadirachta indica in the undiluted concentration only, showed good inhibition (c) of *Erwinia carotovora*, *Aspergillus niger* and *Aspergillus flavus*, respectively.

Carica papaya at undiluted concentration recorded high inhibition (b) of *Erwinia carotovora*, and good inhibition (c) of *Aspergillus niger*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca*, respectively.

Moringa oleifera at undiluted concentration only recorded good inhibition (c) of *Erwinia carotovora* and *Aspergillus niger*. Other organisms recorded moderate to mild inhibition with the exceptions of *Rhizopus stolonifer* and *Penicillium marneffeï*, that showed no inhibition (resistant).

Mangifera indica at undiluted concentration recorded good inhibition (c) of *Erwinia carotovora*, moderate to mild inhibition of the other organisms with the exception of *Penicillium marneffeï* that showed no inhibition.

Terminalia catapa at 100% and 10^{-1} showed complete inhibition (a) of *Erwinia carotovora*, and recorded high inhibition (b) at 10^{-2} dilution. It recorded good inhibition (c) of *Pseudomonas aeruginosa* and *Penicillium marneffeï*, respectively. It also recorded mild to moderate inhibition of the other organisms with the exception of *Serratia marcescens* which recorded no inhibition (resistant).

Senna alata at undiluted concentration recorded high inhibition (b) of *Rhizopus stolonifer* and *Aspergillus niger*. It showed good inhibition (c) of *Rhizopus stolonifer* at 10^{-1} dilution and *Erwinia carotovora*, *Aspergillus flavus* and *Fusarium oxysporum* at 100% concentrations, respectively. It recorded mild to moderate inhibition of other organisms.

3.1.2. Cold Aqueous Synergistic Ten Plants Inhibition Test on Isolates

Table 2 present results of cold aqueous synergistic ten plants extract inhibition test on the nine isolated microorganism.

Synergistic effect of the ten plants extract on the isolates reveals that 1.0 mL addition of extracts in the culture media recorded moderate inhibition (d) of *Erwinia carotovora* and *Klebsiella oxytoca*, and mild inhibition (e) of *Pseudomonas aeruginosa* and *Serratia marcescens*, respectively; the fungi isolates however showed no inhibition (f). 2.0 mL incorporation of the extract recorded complete inhibition (a) of all the four bacteria isolates (*Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella oxytoca*), the fungi isolates showed no inhibition (f). Addition of 3.0 mL of extract showed mild inhibition (e) of *Aspergillus niger* and *Penicillium marneffeï* without affecting the other three fungi; 4.0 mL incorporation of the extract showed moderate inhibition (d) of *Aspergillus niger*, *Aspergillus flavus* and *Penicillium marneffeï* and mild inhibition (e) of *Rhizopus stolonifer* and *Fusarium oxytoca*, respectively.

Table 2. Cold aqueous synergistic ten plants extract inhibition test on isolates.

Microorganism	Extract volume applied									
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	7 mL	8 mL	9 mL	10 mL
<i>Rhizopus stolonifer</i>	-f	-f	-f	+e	+++c	+++c	+++c	+++c	++++b	++++a
<i>Aspergillus niger</i>	-f	-f	+e	+e	+++c	+++c	++++b	++++b	++++b	++++b
<i>Aspergillus flavus</i>	-f	-f	-f	+e	+++c	++++b	++++b	++++b	++++b	++++b
<i>Fusarium oxysporum</i>	-f	-f	-f	+e	+++c	+++c	++++b	++++b	++++a	++++a
<i>Penicillium marneffeii</i>	-f	-f	+e	++d	++++b	++++b	++++b	++++b	++++a	++++a
<i>Erwinia carotovora</i>	+d	++++a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	-f	++++a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Serratia marcescens</i>	-f	++++a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Klebsiella oxytoca</i>	+d	++++a	NA	NA	NA	NA	NA	NA	NA	NA

Summary of inhibition: a = 9, b = 15, c = 9, d = 5, e = 6, f = 13; Key: = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition, b = high inhibition, c = good inhibition, d = moderate inhibition; e = mild inhibition, f = no inhibition.

There was marked increase in the inhibition with the addition of 5.0 mL of the extract, showing high inhibition (b) of *Penicillium marneffeii* and good inhibition (c) of *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*, respectively. Incorporation of 6 mL of the extract showed high inhibition (b) of *Aspergillus flavus* and *Penicillium marneffeii*, it recorded good inhibition (c) of *Rhizopus stolonifer*, *Aspergillus niger* and *Fusarium oxysporum*, respectively. Addition of 7 mL and 8 mL of extract to test media showed high inhibition (b) of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium marneffeii*, it recorded good inhibition (c) of *Rhizopus stolonifer*, respectively.

Incorporation of 9 mL recorded complete inhibition (a) of *Fusarium oxysporum* and *Penicillium marneffeii* and high inhibition (b) of *Rhizopus stolonifer*, *Aspergillus niger* and *Aspergillus flavus*, respectively. Incorporation of 10 mL of extract recorded complete inhibition (a) of *Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffeii* and high inhibition (b) of *Aspergillus niger* and *Aspergillus flavus*, respectively.

3.1.3. Hot Aqueous Synergistic Ten Plants Extract Test

Table 3 shows results of hot aqueous synergistic ten plant extract inhibition test on the isolates. There was no inhibition of any of the isolated organism by 1 mL incorporation; addition of 2 mL of extract showed mild inhibition (e) of the four bacteria isolates (*Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella oxytoca*), respectively, there was no inhibition of the fungi. Addition of 3.0 mL of extract showed complete inhibition (a) of the four bacteria isolates, the fungi isolates recorded no inhibition. Incorporation of 4 mL recorded mild inhibition (e) of *Penicillium marneffeii*, the other four fungi showed no inhibition. Addition of 5 mL extract recorded moderate inhibition of

Table 3. Hot aqueous synergistic Ten-Plant extract inhibition test.

Microorganism	Extract volume applied									
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	7 mL	8 mL	9 mL	10 mL
<i>Rhizopus stolonifer</i>	- ^f	- ^f	- ^f	- ^f	+ ^e	+++ ^c	+++ ^c	++++ ^b	++++ ^b	++++ ^b
<i>Aspergillus niger</i>	- ^f	- ^f	- ^f	- ^f	+ ^e	+++ ^c	+++ ^c	+++ ^c	++++ ^b	++++ ^b
<i>Aspergillus flavus</i>	- ^f	- ^f	- ^f	- ^f	+ ^e	+++ ^c	+++ ^c	++++ ^b	++++ ^b	++++ ^b
<i>Fusarium oxysporum</i>	- ^f	- ^f	- ^f	- ^f	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^b	++++ ^a
<i>Penicillium marneffeii</i>	- ^f	- ^f	- ^f	+ ^e	++ ^d	+++ ^c	++++ ^b	++++ ^b	++++ ^b	++++ ^a
<i>Erwinia carotovora</i>	- ^f	+ ^e	++++ ^a	NA	NA	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	- ^f	+ ^e	++++ ^a	NA	NA	NA	NA	NA	NA	NA
<i>Serratia marcescens</i>	- ^f	+ ^e	++++ ^a	NA	NA	NA	NA	NA	NA	NA
<i>Klebsiella oxytoca</i>	- ^f	+ ^e	++++ ^a	NA	NA	NA	NA	NA	NA	NA

Summary of inhibition: a = 6, b = 13, c = 10, d = 2, e = 8, f = 23; Key: = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition, b = high inhibition, c = good inhibition, d = moderate inhibition; e = mild inhibition, f = no inhibition.

Fusarium oxysporum and *Penicillium marneffeii*, with mild inhibition (e) of *Fusarium oxytoca*, *Aspergillus niger* and *Aspergillus flavus*, respectively. Incorporation of 6 mL and 7 mL recorded good inhibition (c) of the fungi isolates with the exception of *Penicillium marneffeii* which recorded high inhibition (b) at 7 mL extract incorporation.

Incorporation of 8 mL and 9 mL extract, recorded high inhibition (b) of the fungi isolates with the exception of *Aspergillus niger* which recorded good inhibition (c) with 8 mL extract addition. Addition of 10 mL of extract recorded complete inhibition of *Fusarium oxysporum* and *Penicillium marneffeii*; it also recorded high inhibition (b) of *Rhizopus stolonifer*, *Aspergillus niger* and *Aspergillus flavus*, respectively.

3.1.4. Ethanol and Methanol Solvents Soxhlet Extracted Synergistic Ten-Plants Inhibition Test on the Isolates

Table 4 and **Table 5** present results of ethanol and methanol solvents - soxhlet extracted synergistic ten plants inhibition test on the isolates; results obtained from both solvents were the same. Incorporation of 1 mL extract showed no inhibition for all isolates. Addition of 2 mL extract recorded mild inhibition (e) of *Erwinia carotovora* and *Klebsiella oxytoca* only. Incorporation of 3 mL recorded good inhibition (c) of *Klebsiella oxytoca*; moderate inhibition (d) of *Erwinia carotovora* and mild inhibition (e) of *Pseudomonas aeruginosa*, *Serratia marcescens* the fungi recorded no inhibition. Incorporation of 4 mL of extract recorded high inhibition (b) of *Erwinia carotovora* and *Klebsiella oxytoca*, it showed good inhibition (c) of *Pseudomonas aeruginosa* and *Serratia marcescens*, and mild inhibition (e) of the fungi *Penicillium marneffeii*, respectively. Addition of 5 mL extract completely inhibited the four bacteria isolates; it recorded moderate inhibition of *Penicillium marneffeii* and mild inhibition of *Rhizopus stolonifer*,

Table 4. Ethanol soxhlet extracted synergistic Ten-Plants inhibition test on the isolates.

Microorganism	Extract volume applied									
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	7 mL	8 mL	9 mL	10 mL
<i>Rhizopus stolonifer</i>	-f	-f	-f	-f	+e	+++ ^c	+++ ^c	+++ ^c	++++ ^b	++++ ^b
<i>Aspergillus niger</i>	-f	-f	-f	-f	+e	+e	++ ^d	++++ ^b	++++ ^b	++++ ^b
<i>Aspergillus flavus</i>	-f	-f	-f	-f	+e	+e	++ ^d	+++ ^c	++++ ^b	++++ ^b
<i>Fusarium oxysporum</i>	-f	-f	-f	-f	+e	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^a
<i>Penicillium marneffeii</i>	-f	-f	-f	+e	++ ^d	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^b
<i>Erwinia carotovora</i>	-f	+e	++ ^d	++++ ^b	++++ ^a	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	-f	-f	+e	+++ ^c	++++ ^a	NA	NA	NA	NA	NA
<i>Serratia marcescens</i>	-f	-f	+e	+++ ^c	++++ ^a	NA	NA	NA	NA	NA
<i>Klebsiella oxytoca</i>	-f	+e	+++ ^c	++++ ^b	++++ ^a	NA	NA	NA	NA	NA

Summary of inhibition: a = 5, b = 12, c = 11, d = 4, e = 11, f = 25; Key: = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition; b = high inhibition; c = good inhibition; d = moderate inhibition; e = mild inhibition; f = no inhibition.

Table 5. Methanol soxhlet extracted synergistic Ten-Plant inhibition test on the isolates.

Microorganism	Extract volume applied									
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	7 mL	8 mL	9 mL	10 mL
<i>Rhizopus stolonifer</i>	-f	-f	-f	-f	+e	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^b
<i>Aspergillus niger</i>	-f	-f	-f	-f	+e	+e	++ ^d	++++ ^b	++++ ^b	++++ ^b
<i>Aspergillus flavus</i>	-f	-f	-f	-f	+e	+e	++ ^d	+++ ^c	++++ ^b	++++ ^b
<i>Fusarium oxysporum</i>	-f	-f	-f	-f	+e	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^a
<i>Penicillium marneffeii</i>	-f	-f	-f	+e	++ ^d	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^b
<i>Erwinia carotovora</i>	-f	+e	++ ^d	++++ ^b	++++ ^a	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	-f	-f	+e	+++ ^c	++++ ^a	NA	NA	NA	NA	NA
<i>Serratia marcescens</i>	-f	-f	+e	+++ ^c	++++ ^a	NA	NA	NA	NA	NA
<i>Klebsiella oxytoca</i>	-f	+e	+++ ^c	++++ ^b	++++ ^a	NA	NA	NA	NA	NA

Summary of inhibition: a = 5, b = 12, c = 10, d = 7, e = 10, f = 25 Key: = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition; b = high inhibition; c = good inhibition; d = moderate inhibition; e = mild inhibition; f = no inhibition.

Aspergillus niger, *Aspergillus flavus* and *Fusarium oxysporum*, respectively. Incorporation of 6 mL of extract recorded good inhibition (c) of *Rhizopus stolonifer*, it showed moderate inhibition (d) of *Fusarium oxysporum* and *Penicillium marneffeii*, and mild inhibition (e) of *Aspergillus niger* and *Aspergillus flavus*, respectively. Addition of 7 mL recorded good inhibition of *Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffeii*, it showed moderate inhibition of *Aspergillus niger* and *Aspergillus flavus*, respectively. Incorporation of 8 mL of extract recorded high inhibition (b) of *Aspergillus niger* and good inhibition (c) of *Rhizopus stolonifer*, *Aspergillus flavus* *Fusarium oxysporum* and *Penicillium*

marneffeï, respectively. Addition of 9 mL of extract recorded high inhibition (b) of the five fungi isolates, respectively. Incorporation of 10 mL extract recorded complete inhibition (a) of *Fusarium oxysporum* and high inhibition (b) of *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium marneffeï*, respectively.

3.1.5. Ethanol and Methanol solvents, Bench Extracted Synergistic Ten-Plants Inhibition Test on Isolates

Table 6 and **Table 7** present results of ethanol and methanol bench extracted synergistic ten plants inhibition test on isolates, the results are same with those obtained from cold aqueous synergistic extract. Addition of 1.0 mL of extracts in the culture media was able to inhibit *Pseudomonas aeruginosa* and *Serratia marcescens* mildly (e); *Erwinia carotovora* and *Klebsiella oxytoca* were inhibited moderately (d), respectively; the fungi isolates however showed no inhibition. Incorporation of 2.0 mL of the extract recorded complete inhibition (a) of all the four bacteria isolates (*Erwinia carotovora*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Klebsiella oxytoca*); the fungi isolates were still not inhibited. Addition of 3.0 mL of extract showed mild inhibition (e) of *Aspergillus niger* and *Penicillium marneffeï* without affecting the other three fungi; 4.0 mL incorporation of the extract showed mild inhibition (e) of *Rhizopus stolonifer* and *Fusarium oxysporum*; moderate inhibition (d) of *Aspergillus niger*, *Aspergillus flavus* and *Penicillium marneffeï*. There was marked increase in the inhibition with the addition of 5.0 mL of the extract, showing good inhibition (c) for *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*; and high inhibition (b) for *Penicillium marneffeï*. Incorporation of 6 mL of the extract showed good inhibition of *Rhizopus stolonifer*, *Aspergillus niger* and *Fusarium oxysporum*; and high inhibition of *Aspergillus flavus* and *Penicillium marneffeï*, respectively. Addition of 7 mL and 8 mL of extract to test media showed good inhibition (c) of *Rhizopus stolonifer* and high inhibition (b) of *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Penicillium marneffeï*, respectively.

Incorporation of 9 mL recorded complete inhibition (a) of *Fusarium oxysporum* and *Penicillium marneffeï* and high inhibition (b) of *Rhizopus stolonifer*, *Aspergillus niger* and *Aspergillus flavus*, respectively. Incorporation of 10 mL of extract recorded complete inhibition (a) of *Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffeï* and high inhibition (b) of *Aspergillus niger* and *Aspergillus flavus*, respectively.

3.1.6. Cold Aqueous Synergistic Ten-Plant Extract Inhibition Test on Weak Spores

Table 8 shows result of cold aqueous synergistic ten plants extract inhibition test on weak spores; the two fungi, *Aspergillus niger* and *Aspergillus flavus* were not completely inhibited in the first treatment with the extract dose (10 mL) that completely inhibited the other three fungi (*Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffeï*). The second and third treatments showed

Table 6. Ethanol bench extracted synergistic ten plants inhibition test on isolates.

Microorganism	Extract volume applied									
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	7 mL	8 mL	9 mL	10 mL
<i>Rhizopus stolonifer</i>	- ^f	- ^f	- ^f	+ ^e	+++ ^c	+++ ^c	+++ ^c	++++ ^b	++++ ^b	+++++ ^a
<i>Aspergillus niger</i>	- ^f	- ^f	+ ^e	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^b	++++ ^b	++++ ^b
<i>Aspergillus flavus</i>	- ^f	- ^f	- ^f		+++ ^c	++++ ^b	++++ ^b	++++ ^b	++++ ^b	++++ ^b
<i>Fusarium oxysporum</i>	- ^f	- ^f	- ^f		+++ ^c	+++ ^c	++++ ^b	++++ ^b	+++++ ^a	+++++ ^a
<i>Penicillium marneffeii</i>	- ^f	- ^f	+ ^e	++ ^d	++++ ^b	++++ ^b	++++ ^b	++++ ^b	+++++ ^a	+++++ ^a
<i>Erwinia carotovora</i>	++ ^d	+++++ ^a	NA	+ ^e	NA	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Serratia marcescens</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Klebsiella oxytoca</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA

Summary of inhibition: a = 9, b = 15, c = 9, d = 5, e = 6, f = 13. Key: - = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition; b = high inhibition; c = good inhibition; d = moderate inhibition; e = mild inhibition; f = no inhibition.

Table 7. Methanol bench extracted synergistic ten plants inhibition test on isolates.

Microorganism	Extract volume applied									
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	7 mL	8 mL	9 mL	10 mL
<i>Rhizopus stolonifer</i>	- ^f	- ^f	- ^f	+ ^e	+++ ^c	+++ ^c	+++ ^c	++++ ^b	++++ ^b	+++++ ^a
<i>Aspergillus niger</i>	- ^f	- ^f	+ ^e	++ ^d	+++ ^c	+++ ^c	++++ ^b	++++ ^b	++++ ^b	++++ ^b
<i>Aspergillus flavus</i>	- ^f	- ^f	- ^f	++ ^d	+++ ^c	++++ ^b	++++ ^b	++++ ^b	++++ ^b	++++ ^b
<i>Fusarium oxysporum</i>	- ^f	- ^f	- ^f	+ ^e	+++ ^c	+++ ^c	++++ ^b	++++ ^b	+++++ ^a	+++++ ^a
<i>Penicillium marneffeii</i>	- ^f	- ^f	+ ^e	++ ^d	++++ ^b	++++ ^b	++++ ^b	++++ ^b	+++++ ^a	+++++ ^a
<i>Erwinia carotovora</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Pseudomonas aeruginosa</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Serratia marcescens</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA
<i>Klebsiella oxytoca</i>	++ ^d	+++++ ^a	NA	NA	NA	NA	NA	NA	NA	NA

Summary of inhibition: a = 9, b = 15, c = 9, d = 5, e = 6, f = 13. Key: - = no inhibition; + = mild inhibition (0.5% - 34% inhibition); ++ = moderate inhibition (35% - 54% inhibition); +++ = good inhibition (55% - 74% inhibition); ++++ = high inhibition (75% - 99% inhibition); +++++ = complete inhibition; NA = not applicable; a = complete inhibition; b = high inhibition; c = good inhibition; d = moderate inhibition; e = mild inhibition; f = no inhibition.

Table 8. Cold aqueous synergistic ten plants extract inhibition test on weak spores.

Microorganism	Application times			
	1 st	2 nd	3 rd	4 th
<i>Aspergillus niger</i>	+	+	+	-
<i>Aspergillus flavus</i>	+	+	+	-

Key: - = absence of growth; + = presence of growth.

markedly reduced growth and retarded sporangium formation; seeding of the weak spores from third treatment plate onto a fresh and fourth treatment plate yielded complete inhibition (no growth). This implies that to successfully keep out the fungi, treatment has to be repeated.

3.2. Discussion

Single plant extraction inhibition test (antimicrobial sensitivity test) results (Table 1) showed antifungal and antibacterial activity of the fresh plant leaves extracts in varying degrees. Single plant extract inhibited completely the growth of three organisms by two plants: *Terminalia catapa* at 100% and 10⁻¹ showed complete inhibition (a) of *Erwinia carotovora* and *Passiflora edulis* at undiluted (100%) concentration completely inhibited *Rhizopus stolonifer* and *Penicillium marneffei*. The result is in agreement with reports of several researchers who observed that, of the several methods adopted for controlling losses due to post harvest disease of yam; biological control method has been preferred in some cases because it is selective with no side effect and cheap. Resistance to biological control is rare and biological control agents are self-propagations and self-perpetuating [6] [35]; the advantage of plant extract products includes its local availability, presence of little or no toxicity to humans and simple preparation procedures [8] [28].

Cold aqueous synergistic ten plants extract inhibition Test on Isolates as shown in Table 2 recorded better inhibition. There was complete inhibition (a) of all the four bacteria isolates at 2 mL extract incorporation; 10 mL extract incorporation in media recorded complete inhibition (a) of three out of the five fungi isolates (*Rhizopus stolonifer*, *Fusarium oxysporum* and *Penicillium marneffei* and high inhibition (b) of the other two (*Aspergillus niger* and *Aspergillus flavus*), respectively. Further extract quantity addition was no longer necessary because at 10 mL incorporation the media was too soft, again in treatment, there is room for repeated dosage. The adoption of synergistic plant extract has been reported by other researchers as more effective, [36] [37].

Hot Aqueous Synergistic Ten Plants Extract Inhibition Test as shown in Table 3, recorded reduced activity when compared to that of cold aqueous synergistic extract. This is most probably that some of the active ingredients are denatured by heat. This result is in agreement with the findings from other researchers, who observed that Zones of inhibition were also diminished by heating the extract, although its antimicrobial activity was not totally lost when heated [38] [39] [40].

Ethanol and Methanol Solvents Soxhlet extracted synergistic ten plants Inhibition Test on the Isolates as shown in Table 4 and Table 5 recorded reduced activity when compared to the hot aqueous extract (boiled extract); Heat employed extractions generally recorded less inhibition (Plates 1-3). This finding is in agreement with [39] who observed that all the influential factors (temperature, solvents, agitation speed, among others) might have the ability to enhance

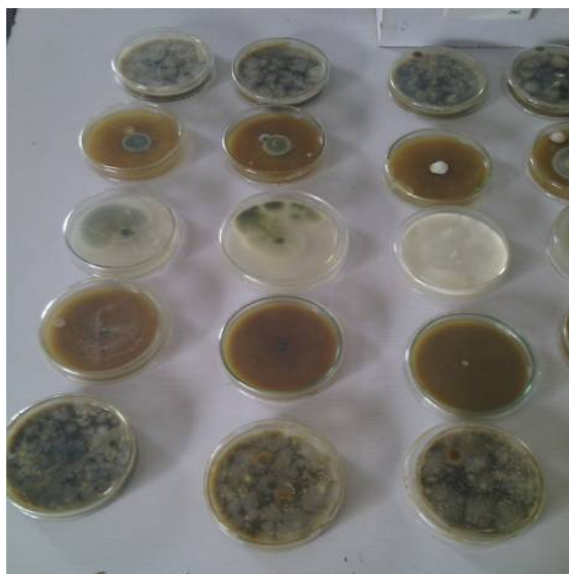


Plate 1. From Bottom to Top; Row 1: Non-inhibition by Soxhlet Methanol extracts on Fungi isolates (from right to left; *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium marneffeï*); Row 2: Inhibition by Cold Aqueous Synergistic Ten-Plant extracts on Fungi isolates (from right to left; *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium marneffeï*); Row 3: Fungi Control inoculations (from right to left; *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium marneffeï*); Row 4: Inhibition by Boiled Synergistic Ten-Plant extracts on Fungi isolates (from right to left); *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium marneffeï*. Row 5: Non-inhibition of Ethanol Soxhlet extracts on fungi isolates (from right to left; *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium marneffeï*).

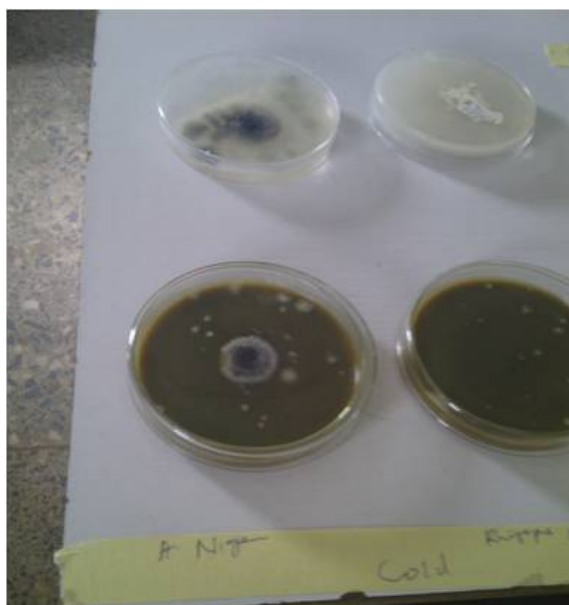


Plate 2. (from Left to right) Partial Inhibition of *A. niger* and Complete Inhibition of *R. stolonifer* by Synergistic Ten-Plants extract.

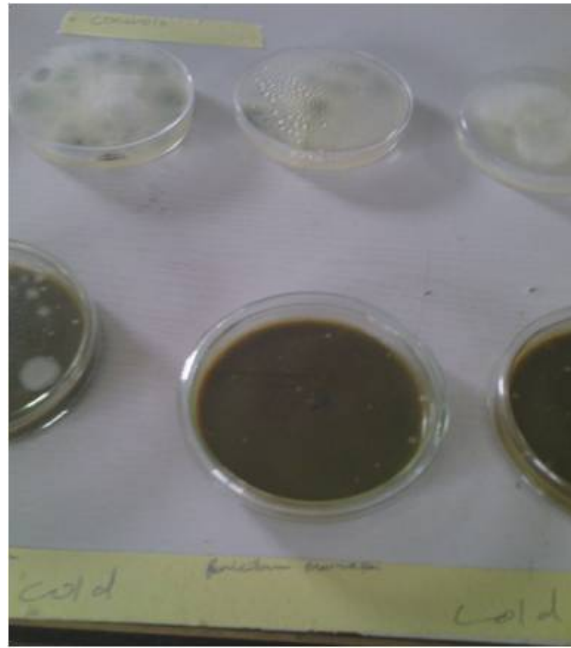


Plate 3. Complete inhibition of *P. marneffei* by Synergistic Ten-Plants extract.

extraction, but without proper judgment may cause degradation of compounds; also the Soxhlet extraction comes with disadvantage such as exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction. Again solvents used in the extraction system need to be of high-purity, that might add to the extraction cost [40]; also they might not be environmental friendly and may contribute to pollution problem [1].

Room temperature Ethanol and methanol solvents extracted synergistic ten plants inhibition test on isolates as shown in **Table 6** and **Table 7** recorded inhibition activity that was same with that obtained with cold aqueous synergistic ten plants extract. This finding is in agreement with, [39] [40], who observed and recommended that, the use of water as extraction solvent should be preferred over organic solvents when application on food for preservation is the target.

Cold aqueous synergistic ten plants extract inhibition test on weak spores and subsequently derived weak spores reduced their resistance until they were completely inhibited after the third treatment as shown in **Table 8**. This agrees with [41] who observed that, bacterial are more susceptible to plant extracts than fungi. Also it is common knowledge that, most often treatment of fungi infections require repeated dosage and application of antifungal agent.

4. Conclusion

Single plant extract recorded complete inhibition of bacteria *Erwinia carotovora* by *Terminalia catapa* and fungi *Penicillium marneffei* by *Passiflora edulis* only; *Pseudomonas aeruginosa* and *Klebsiella oxytoca* recorded good inhibition (b)

with *Jatropha tanjorensis* plant extract. Synergistic extract appears to enhance the complete inhibition of bacteria more than the fungi. The synergistic mixture comprising of all plants earlier used in single plant extractions gave better inhibition activity on the isolates. It was able to inhibit completely all the four bacteria isolates at 2 mL extract addition in culture media. All the fungi studied showed lower inhibition with aqueous synergistic ten plants extracts at lower concentrations when compared with aqueous single plant extracts and showed a gradual increase in inhibition with increase in concentration of the synergistic extract. Hot aqueous synergistic plants extract recorded poor inhibition of the isolates as compared to the cold. Soxhlet solvent extracted synergistic plants extract, however, recorded lower inhibition as compared to hot aqueous synergistic plants extract and cold aqueous synergistic ten plants extracts, respectively. Room temperature solvent extracted synergistic plants extracts recorded inhibition that was same as that obtained with cold aqueous synergistic plants extract.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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