Antimicrobial Activity of *Anogeissus Leiocarpus* and *Lannea Microcarpa* on Some Microbes Isolated From Vegetables in Sokoto

Garba, H. Danladi, Sanusi Muhammad, Adamu A. Aliero, and Habiba M. Muhammad

***Abstract*—**The antimicrobial activity of aqueous leaf and bark extracts of *Anogeissus leiocarpus* and *L. microcarpa* were tested *In-vitro* against two fungi (*Aspergillus nigeri* and *Fusarium oxysporium*) and bacteria, *Pseudomonas syringae* isolated from some vegetables sold in Sokoto markets. The facilities of Usmanu Danfodiyo University, Sokoto were used. 90mm petri dishes were used for agar impregnation method for anti-fungal activity, while agar well diffusion was employed in control of bacteria. 2mm of fungi were inoculated on the impregnated plates, while streak was carried out for bacteria. 5mg/l and 80mg/l of fulcin tablets were used as control. The activity of leaves was found to be more than the bark extracts. The lower concentrations of 10mg/ml and 20mg/l had no effect on all pathogens. The higher concentrations of 40mg/ml and 80mg/ml had varying effects, with the highest being by *A. leiocarpus* leaves on *P. syringae* (50%). Significant inhibition ($p \leq 0.05$) was also recorded by *L. microcarpa* leaves on *F. oxysporium* (46.7%), *P. syringae* (42.2%) and 27.8% on *A. niger*. 80mg/ml of *L. microcarpa* leaves and barks had significant effects on *F. oxysporium* (33.3) and (30%) respectively. It also recorded 22.2% on *A. niger*. The most inhibited organism was *F. oxysporium* while the least inhibited was A. niger. Inhibition by *A. leiocarpus* leaves showed the most effects, very close to fulcin, while bark extracts were generally less effective**.**

Keywords— *Anogeissus leiocarpus, Lannea microcarpa,* fungi, Bacteria and inhibition

I. Introduction

THE use of plants as sources of cure for ailments has a place in history. In recent times, efforts are being made to see that plants are further exploited in order to provide for alternative sources of cure for either plant or animal diseases. The diseases are usually caused by different pathogens belonging majorly to fungi, bacteria or virus groups. *Aspergillus niger* is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found, they are ubiquitous in nature, widely distributed geographically, with a wide range of habitats due to their ability to colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte, but is also associated with many plant diseases [10].

F. oxysporum produces three types of asexual spores: microconidia, macroconidia, and chlamydospores [1]. In general, the aerial mycelium first appears white, and then may change to a variety of colors - ranging from violet to dark purple - according to the strain (or special form) of *F. oxysporum.* If sporodochia are abundant, the culture may appear cream or orange in color [[31]

*P. syringa*e is a Gram negative, plant-pathogenic bacterium, strains of which are noted for their diverse and host-specific interactions with different plant species. Specific strains are assigned to one of the over 50 known pathovars based on their ability to infect different plant species (pseudomonas syringae.org). Microorganisms especially fungi are known to be the major cause of market and field losses of crops [25]. Many plants especially spices have been used severally in the preservation of plants and animal products and in the treatment of various plant and animal diseases,[9], [25],[17],[18].

The development of nontoxic, safe and effective biodegradable alternative to synthetic fungicides has in recent years, led to global at screening various plant for bioactivity against plant pathogenic organisms [25], [33]. However, it is estimated that about 10% of the over 250,000 different plant species in the world today have been examined chemically for antimicrobial activity [11]. The *Lippia* leaf extract was found to exhibit fungicidal action through inhibition of growth of some fungi [22], [16]. Also, ethanolic leaf extract of *Lippa. multiflora*, *B. perotitiana* and *Azadirachta indica* have been shown to exhibit varying levels of inhibition on mycelia growth of *A. niger* and *Fusarium verticilloides* [19]. It has been revealed that both fungicides and extracts of plant origin caused inhibition in mycelial growth and spore germination of *Fusarium oxysporum*, [35]. Certain protective fungicides although hazardous to environment are still used for the control of fungal diseases [32]. Botanicals though being researched as alternatives to chemicals, have not yet taken over the antimicrobial use in any large scale. Use of pesticides of plant origin have also been suggested by some workers as alternative to synthetic chemicals in order to counter the potential hazardous effect on the environment associated with the use of synthetic chemicals [4],[12],[30],[3]. *A. sativum* was shown to have anti-fungal activity [28]. Similar results were found by Bowers and Locke [7], using *Allium sativum*

Garba H. Danladi is with the Kebbi state University of Science and Technology, Aliero, Nigeria: +2348033463043; (danladihani@gmail.com)

Sanusi Muhammad, is with Usmanu Danfodio University, Sokoto, Nigeria.(smsamdiri@gmail.com).

Adamu A. Aliero, is with the Usmanu Danfodio University, Sokoto, Nigeria. (+2348034635145: aaaliero@gmail.com).

.Habiba M. Muhammad, is with Ibrahim Badamasi Babangida University, lapai, Nigeria (+2348065647965; habibamaliyumaliyu@gmail.com).

against eighteen different fungi including *Fusarium* spp.[5],[6]. The suppressive effect of some phytochemical compounds on nematode population has been well documented in several pathological systems [8]. Significant reduction was observed in the multiplication of plant-parasitic nematodes *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, and *Helicotylenchus indicus* and in the frequency of parasitic fungi such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Phyllosticta phaseolina*, and *Sclerotium rolfsii* by the application of botanicals to soil, However, the frequency of saprophytic fungi *Aspergillus niger*, *Trichoderma viride*, and *Penicillium digitatum* was significantly increased [34]. [27] observed the Nematicidal potential of oil-seed cakes in amended soil and found a reduced number of root galls caused by *M. incognita* on tomato.

Fungi produce mycotoxins which present health hazards to consumers of contaminated produce. The Turkey X disease outbreak in England was traced to contaminated peanuts from Brazil and this led to the discovery of aflatoxin produced primarily by *Aspergillus niger* and *A. flavus*. Equine leukoncephalomalacia is doubtless caused by toxins from *Fusarium moniliforme* and alimentary toxic aleukia was thought to be caused by the trichothecenes mycotoxins. Sithese mycotoxins producing fungi grow staple foods of both humans and animals, and affect their populations. Also, products such as eggs, milk, dairy products, and meat can be contaminated through the ingestion of feed containing mycotoxins by the producing animals [21]. The use of *Khaya senegalenisi* bark to protect maize against insects increased the risk of aflatoxin development [15].

The isolation of the Test pathogens from vegetables in Sokoto markets [10] means that consumers are relatively exposed to the dangers of not only the fungal diseases that may result from consumption of infected vegetables, but also from the mycotoxins assocated with them. It is imperative therefore, to continue the struggle for a risk-free antibiotic solution to the problems posed by these pathogenic microbes, especially the use of botanicals.

II. Materials And Method

*Media Preparation***:** Potato dextrose agar (PDA) and nutrient agar (NA) were used for this research. PDA was prepared according to manufacturer's (Micro master Thane, Maharanshta India) instructions. Thirty nine grams fresh agar was weighed using METTLER 166AA balance and mixed with small quantity of steril distilled water, shaken and then made up to 1000 mls in 1ltr conical flask. It was shaken vigorously to obtain an even mixture. Similarly, twenty eight grams (28g) of nutrient agar was placed into 1ltrconical flask and mixed with 1000 mls distilled water, according to manufacturer's (Antec diagnostic products, United Kingdom) instructions. The media were sterilized by autoclaving at 121^{o} C for 15 minutes. The agar were then allowed to sufficiently cool to 47^{o} C before being poured into sterile 90 mm Petri dishes in the incubation room. They were allowed to stay for 24 hrs to properly solidify before inoculation.

*Collection of plant materiaks***:** Two (2) kg of each plant (bark and leaves) of *Anogeissus leiocarpus* and *Lannea microcarpa* were collected using sterile knives and placed in sterile polythene bags, tied and labelled appropriately. They were taken to the laboratory and dried in hot air oven (Gallenkamp 1 H150) until constant weight was obtained. The dried parts were ground with mortar and pestle into fine powder.

*Aqueous Extract Formation***:** Four hundred (400) g of the fine powdered plant part was soaked in 400 ml distilled water and allowed to stand for 24 hrs. the suspension was then sieved with muslin cloth, followed by heating the filtrate of each sample in a water bath at 70^{o} C, until the water dried out leaving behind, dissolved plant particles in powdered form.

*Agar incorporation***:** The sensitivity of the fungi to the extract was evaluated *in vitro*. Saboraud Dextrose Agar (SDA) was incorporated with the extracts for mycocidal effect. Varying concentrations of the extract (5, 10, 20, 40, and 80 mg/ml) were prepared by mixing 0.9, 1.8, 3.6, 7.2, and 14.4 g of the powdered extract in 180 ml of water in respective conical flasks. Five (5) ml of each extract concentration was aseptically mixed in 15 ml SDA and poured into a Petri dish and allowed to solidify. Two (2) mm of the isolates were then inoculated into the incorporated Petri dishes (in 3 replicates) in the pre-sterilized incubation room and the growth thereafter was observed. Three lines of measurement were taken daily for each mycelial growth until the organisms filled the control plates.

Paper disc: was used for the bacteria pathogen; 5 mm paper discs were obtained by punching Whatman No1 filter paper using sterile cork borer and placed in Petri dishes. They were sterilized by autoclaving at 121^{o} C for 15 minutes, later, 5 mls of the different extracts were poured into test tubes. Ten discs were placed into each test tube. They were allowed to soak for 24 hrs, before aseptically removing them and drying in hot air oven at 40^{o} C. Bacterial isolates were then streaked into already poured media containing Petri dish, and one disc, representing different concentrations was placed at equidistant place and adequately labelled. The bacterial growth around the discs was observed and the percentage inhibition calculated.

III. Results And Discussions

A. niger showed no sensitivity to any botanical at low concentrations but exhibited low sensitivity to high concentration of leaf extracts of *A. leiocarpus* and *L. microcarpa* (Table 1), where significant inhibition at $p \leq 0.05$ was recorded. *F. oxysporium* showed varying degrees of sensitivity to all the extracts. There was significant inhibition $p \leq 0.05$ at high concentrations (Table 2), with the highest inhibition by *A. leiocarpus* leaves with 46.7%. *P. syringae* displayed significant inhibition by *A. leiocarpus* leaf extracts at 40 and 80 mg/ml (Table 3). *A. leiocarpus* leaf extract had 50%. The bark extracts did not show any significant effect $p \leq 0.05$ on this pathogen.

From the study, *A. leiocarpus* and *L. microcarpa* leaf extracts showed good potentials as control agents for *F. oxysoprium*, *E. carotovora* and *P. syringae* than the bark extracts. This could be attributed to the risin and other active ingredients present in leaves, as reported by [13]. [14] reported that leaf extracts of *Nerium oleander* and *Pithecobium dulce*

achieved great inhibition of growth of *Bipolaris oryzae*. The production of better and more significant activity by high concentrations of the botanicals is related to the findings of [2], that high concentrations of *Nicotinia tabacum* significantly controlled *Colletotrichium destructivum.*

The inhibition of *P. syringae* by *A. leiocarpus* leaf extract came very close to that provided by Fulcin tablet at same 80 mg/ml concentration. It also showed good inhibition of *F. oxysoprium.* This can be explained by the report that glucosides described in this plant showed antimicrobial activities [29]. Also, fungitoxic activity of plant extracts was reported by [23] to be more than benomyl chemical fungicide.

TABLE I
INHIBITORY EFFECTS OF PLANT EXTRACTS ON *A. NIGER*

Plant	Plant part	Conc. (mg/ml)	Mn grth (mm)	inhibition %
Control	Water	20	90	0.0
	Fulcin tab	5	54	40.0
	"	80	32	64.4
A. leiocarpus				
	Leaves	5	90	0.0
		10	90	0.0
		20	90	0.0
		40	83	7.7
		80	65	27.8
	Bark	5	90	0.0
		10	90	0.0
		20	90	0.0
		40	90	0.0
L. microcarpa		80	90	0.0
	Leaves	5	90	0.0
		10	90	0.0
		20	90	0.0
		40	86	4.4
		80	70	22.2
	Bark	5	90	0.0
		10	90	0.0
		20	90	0.0
		40	90	0.0
		80	90	0.0

Mnrth = mean growth

TABLE II
INHIBITORY EFFECTS OF PLANT EXTRACTS ON F OXYSPORIUM

Plant	Plant part	Conc. (mg/ml)	Mngrth (mm)	inhibition %
Control	Water	20	90	0.0
	Fulcin tab	5	17.3	80.8
	"	80	9..5	89.4
A. leiocarpus				
	Leaves	5	90	0.0
		10	90	0.0
		20	75	20.0
		40	60	33.3
		80	48	46.7
	Bark	5	90	0.0
		10	90	0.0
		20	80	11.1
		40	68	24.4
L. microcarpa		80	52	42.2
	Leaves	5	90	0.0
		10	90	0.0
		20	80	11.1
		40	71	21.1
		80	60	33.3
	Bark	5	90	0.0
		10	90	0.0
		20	79	12.2
		40	70	22.2
		80	63	30.0

Mngrth = mean growth

Fig.1 5% trated *Fusarium oxysporium*

TABLE III
INHIBITORY EFFECTS OF PLANT EXTRACTS ON P. SYRINGAE

Plant	Plant part	Conc. (mg/ml)	Mngrth (mm)	inhibition %
Control	Water	20	18	0.0
	Streptoycin	5	12	33.0
	"	80	6	66.6
A. leiocarpus				
	Leaves	5	18	0.0
		10	18	0.0
		20	16	11.1
		40	1	33.3
		80	9	50.0
		5	18	0.0
	Bark	10	18	0.0
		20	18	0.0
		40	18	0.0
		80	16	11.1
L. microcarpa				
		5	18	0.0
	Leaves	10	18	0.0
		20	17	5.5
		40	15	16.7
		80	15	16.7
		5	1	0.0
	Bark	10	18	0.0
		20	18	0.0
		40	18	0.0
		80	18	0.0

Mngrth = Mean rowth

Traditionally, leaf decoctions of *L. microcarpa* are used to treat swelling and as dressing for wounds in Nigeria, and leaves barks roots and fruits are applied to treat mouth blisters, rheumatism, sore throat and dysentery [20]), attesting to the microbial activity of the plant.

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