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Original Research Article

Antimicrobial susceptibility study of three medicinal plants on selected food and cash crops pathogens

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Abstract

The use of chemicals for the control of pathogens in agriculture is of great concern especially because of their detrimental effect in the environment. Studies have shown that plants contain bioactive antimicrobial compounds but few studies address the effect of these compounds on plant pathogens. Here, we evaluate the antimicrobial activities of methanolic extracts of plants on plant pathogens. The phytochemicals present in the plants studied include alkaloids, saponins, tannins, flavonoids and cardiac glycoside. Also, the plants contain nutrients in varying composition with highest percentage in carbohydrate content and lowest percentage in moisture content. The mineral present also include Na, K, Ca, Mg, Zn, Fe, Cu, Mn and P. The radial mycelial growth and dry mycelial assay were used to test for activities against common phytopathogens at varying concentrations. The comparative study showed that *Ficus exasperata* exhibited the strongest antimicrobial activities against all the test organisms. The results of this study showed that the plants studied contains bioactive compounds which may serve as alternative to chemical based biocides for the control of food and cash crops pathogens.

Keywords: Bioactive compounds, environment, food crops, cash crops, phytopathogens

INTRODUCTION

Plants have been a rich source of bioactive compounds reported to be used in health care settings in developing countries (Fagbohun et al., 2012a; Opara and Nwankwo, 2015). Plants of medical importance are getting attention than ever because of the potential benefits to society especially in the line of medicine and pharmacology (Onifade, 2006). Although, there are several reports on the successes of plants extracts in the treatment of human related infections but there has been neglects on the treatment of plant infections using bioactive compounds from plants (Opara and Nwankwo, 2015).

Food and cash crops have been a great blessing for human race because of their nutritional and economic values however, their yield have been affected over the years as a result of microbial attack both on the growing plants and their produce (FAO, 2007). The disease of food crops and cash crops is currently controlled through the massive use of chemical biocides (Kanah and AlNajar, 2008). In past decades the use of chemicals has increased the productivity and quality of crops and has also helped in controlling disease of food and cash crops but in recent times, the inappropriate and non-discriminatory use has led to some identifiable problems (Opara and Nwankwo, 2015) such as persistence of chemical residues in farm produce, phytotoxicity, environmental pollution, development of resistance in target organisms, high cost of disease control, at times non-availability and hazards to man and his environments renders them unattractive to adopt by farmers hence the need for an alternative (Al-Rahmah et al., 2013; Abd

Table 1. The ethnobotanical data of the plant parts employed

Plant codes	Plant species	Family	Common name	Plant part used
A	*Aspilia africana*	Asteraceae	Haemorrhage plant	Leaf
B	*Ficus exasperata*	Moraceae	Forest sand paper	Leaf
C	*Icacina trichantha*	Icacinaceae	Sweet Bush mango	Leaf

El-Ghany et al., 2015).

The biological inhibitions of food and cash crop pathogens by different bioactive compounds and crude extracts of plants of different extracting solvents have been investigated. Jasso de Rodriguez et al. (2005) evaluated antifungal activity of *Aloe vera* pulp on mycelial growth of *Rhizoctonia solani* and *Fusarium oxysporum* isolated from food and cash crops and reported a reduced colony growth rate at 105µl^{-1} concentration of the extract. Moreover, *Eucalyptus* leaf extracts were reported to inhibit mycelial growth of *Rhizoctonia solani*, *Phytophthora*, *F. oxysporum* and many other fungi with concentrations above 20% (Al-Rahmah *et al*., 2013).

However, this work aimed to study antimicrobial activities of methanol crude extracts of three medicinal plants on phytopathogens isolated from food and cash crops.

METHODOLOGY

Plant materials

Plant materials (leaves) of three plant species belonging to three (3) botanical families included in this study (Table 1) were collected from Ado Ekiti metropolis, Ekiti State, Nigeria. The collected plants were carried in polyethylene bags to the laboratory, washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite and dried indoor with low moisture for 28 days. The dried material of each plant species was grounded into a powdered material using a waring blender to pass 100 mm sieve and the mince was sealed and stored in air-tight container until extraction.

Preparation of plant extracts

For investigations, methanolic plant extracts were prepared by soaking 250 g of dry powder plant material from each plant species in 1000ml of methanol with stirring for 96 h and then filtered through double layers of muslin, centrifuged at 9000 rpm/min for 10 min and finally filtered again through Whatman filter paper no. 41 to remove leaf debris and obtain a clear filtrate. The filtrates were evaporated and dried under reduced pressure at temperature below 40°C with Rotary evaporator. The solvent extract was stored in the refrigerator at 4^{o}C until when required.

Phytochemical screening

Quantitative phytochemical screenings to determine the presence of alkaloids, tannins, saponins steroids, phlobatannin, terpenoids, flavonoid and cardiac glycosides using standard methods as described by Sofowora (2008) and Fagbohun et al., (2012a) were carried out.

Proximate analysis

The proximate analyses of the sample for moisture, ash, fibre and fat were done by the method of AOAC, (2005). The nitrogen was determined by micro-Kjeldahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying with 6.25. Carbohydrate was determined by difference. All determinations were performed in duplicates. All the proximate values were reported as percentage.

Mineral analysis

The mineral was analysed by using a flame photometer (Model 405 Corning, UK), using NaCl and KCl to prepare the standards. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were done in duplicates. All chemicals used were analytical grade (BDH, London). Earlier, the detection limit of the metals was determined according to Techtron (1975). The optimum analytical range was 0.1 – 0.5 absorbance unit with a coefficient of variation of 0.87 - 2.20%. The minerals were reported as milligram/100 grams (Fagbohun et al., 2012a).

Fungal cultures

Standard strains of *Aspergillus niger, Aspergillus flavus*, *Aspergillus glaucus*, *Fusarium oxysporum* and

Table 2. Phytochemical analysis of three medicinal plants

Plant samples	Alkaloids	Saponins	Tannins	Flavonoids	Terpenoids	Phlobatannin	Cardiac glycoside
A	+	+	+	+	+	-	+
B	+	+	+	+	-	+	+
C	+	+	+	+	+	+	+

Legend: A: *Aspilia africana*, B: *Ficus exasperata*, C: *Icacina trichantha*

Table 3. Quantitative analysis of phytochemical constituents (anti- nutrients) of medicinal plants

Plant samples	Tannin (%)	Alkaloids (%)	Saponin (%)	Phytate (mg/g)	Phytin phosphorus (mg/g)	Flavonoid (%)	Cyanide (mg/kg)	Oxalate (mg/g)
A	3.49	2.56	5.40	17.28	4.85	2.34	1.27	3.68
B	4.82	3.03	4.40	19.76	5.56	1.64	0.51	4.13
C	2.83	4.32	7.22	15.63	4.42	4.15	1.17	3.17

*Legend: A: *Aspilia africana*, B: *Ficus exasperata*, C: *Icacina trichantha*

Rhizoctonia solani were obtained from Plant pathology Unit, Department of Microbiology, Ekiti State University while pure cultures of *Botryodiplodia theobromae* and *Phytophthora palmivora* were obtained from Cocoa Research Institute of Nigeria, Idi-Ayunre, Ibadan, Oyo State, Nigeria. The fungal isolates were maintained on sterilized potato dextrose agar plates.

Microbiological assay

The microbiological assay was done according to the method described by Fagbohun *et al*., (2012b).

Radial mycelial growth

The following concentrations (50.0, 100.0, 150.0, 200.0 and 250.0 mg/ml) of the extract were aseptically dispensed into different sterile petri-dishes. About 15 ml of sterilized molten potato dextrose agar was added to the plates and were swirled gently to mix content evenly. The mycelial discs measuring 6 mm in diameter were taken with a sterile cork-borer from the advancing edges of 3-5 days old cultures of the test fungi were placed centrally on the cooled seeded plates and incubated at 28^{o}C for 7days. The control experiments were carried out as described above, but only 1ml of the extracting solvent was added to each of the plates. The radial mycelial growth of both test and control plates were measured using an electronic caliper. The net growth was obtained by subtracting the diameter of the inoculum plugs from the test.

Dry Mycelial Growth Assay

Five mycelial discs each of the test fungi obtained as described were introduced into 120 ml Erlenmeyer flasks containing 25 ml of sterile potato dextrose broth. Different concentrations (50.0, 100.0, 150.0, 200.0 and 250.0 mg/ml) of the induced extract were introduced into each of the Erlenmeyer flask containing inoculum plugs of the test fungi. The flasks were incubated at 28^{o}C for 7 days. For control, only 1 ml of the extracting solvent was added to each of the flask and treated as above. At the end of the 7th day of incubation, the content of each flask was decanted into a funnel containing pre-dried and pre-weighed Whatman filter paper. The content of the filter paper was dried at 50-60^{o}C in an oven until constant weight was obtained for each test fungi. The mycelial weight was determined by subtracting the weight of the filter paper from the total weight of the dry mycelial mass.

Statistical analysis

All experiments were conducted in triplicates for each treatment and the data were reported as mean ± SE (standard error). The data were also analysed statistically using One-way analysis of variance (ANOVA) and differences among the means were determined for significance at P = 0.05 (by SPSS, 17.0 Chicago, USA).

RESULTS

Phytochemical analysis

The results of the phytochemical analysis of the three

Table 4. Proximate composition of the three medicinal plants in percentage

Plants samples	Ash	Moisture content	Crude Protein	Fat	Fibre	Carbohydrate
A	11.06	2.84	18.45	7.55	7.08	53.03
B	9.14	2.68	18.92	8.79	11.53	49.05
C	11.59	2.75	21.40	5.95	7.84	50.48

*Legend: A: *Aspilia africana*, B: *Ficus exasperata*, C: *Icacina trichantha*

Table 5. Mineral contents of the medicinal plant samples (mg/100g)

Plants	Na	K	Ca	Mg	Zn	Fe	Cu	Pb	Mn	P
A	72.89±0.01^{c}	48.38±0.01^{c}	75.66±0.01^{c}	80.80±0.01^{c}	58.68±0.01^{a}	6.73±0.01^{c}	0.93±0.01^{c}	ND	2.56±0.01^{c}	68.31±0.01^{b}
B	64.56±0.01^{b}	33.69±0.01^{a}	61.54±0.01^{b}	71.42±0.01^{a}	60.55±0.01^{b}	5.38±0.01^{b}	0.37±0.01^{b}	ND	1.67±0.01^{b}	75.82±0.01^{c}
C	62.38±0.01^{a}	42.54±0.01^{b}	53.68±0.01^{a}	73.62±0.01^{b}	63.48±0.01^{c}	4.56±0.01^{a}	0.32±0.01^{a}	ND	0.88±0.01^{a}	61.84±0.01^{a}

*Legend: A: *Aspilia africana*, B: *Ficus exasperata*, C: *Icacina trichantha*
**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

plants (*Aspilia africana, Ficus exasperata* and *Icacina trichantha*) are shown in Tables 2 and 3. All the plants were found to contain alkaloids, saponins, tannins, flavonoids and cardiac glycoside. Phlobatannin was present in both *F. exasperata* and *I. trichantha* but absent in *A. africana* while terpenoids was present in both *A. africana* and *I. trichantha* but absent in *F. exasperata.* The quantitative analysis of the phytochemicals in percentage (%) showed that *F. exasperata* had the highest value for tannin with 4.82% while *I. trichantha* had the lowest with 2.83%. *I. trichantha* had the highest value of alkaloids, saponin and flavonoids of 4.32%, 7.22% and 4.15% respectively.

Proximate analysis

The results of the proximate analysis of the methanolic extracts of the three plants used for this study showing their nutrient composition are shown on Table 4. The results obtained showed that among the extracts, *I. trichantha* had the highest composition of Ash (11.59%) while *F. exasperata* had the lowest (9.14%). The data obtained for their moisture content showed that *A. africana* had the highest composition which is (2.84%) while *F. africana* had the lowest (2.68%). *F. exasperata* was found to contain the highest percentage fat (8.79%) and fibre content (11.53%). *I. trichantha* had the highest crude protein of 21.40% with *A. africana* having the lowest (18.45%). Carbohydrate content was found to be highest in *A. africana* (53.03%) while *F. exasperata* had the lowest (49.05%). This result showed that the plant extracts showed that the plant extracts have nutrients in varying composition with highest percentage in their carbohydrate content and lowest percentage in moisture content.

Mineral analysis

The results of the mineral contents of the three plants used for this study are shown on Table 5. The results obtained showed that that contains sodium, potassium, calcium, magnesium, zinc, iron, copper, manganese and phosphorus in varying concentrations. Sodium content ranged from 62.38 mg/100g in *I. trichantha* to 72.89 mg/100g in *A. africana*. Potassium ranged from 33.69 mg/100g in *F. exasperata* to 48.38 mg/100g in *A. africana.* Calcium ranged from 53.68 mg/100g in *I. trichantha* to 75.66 mg/100g in *A. africana.* Magnesium ranged from 71.42 mg/100g in *F. exasperata* to 80.80 mg/100g in *A. africana.* Zinc ranged from 58.68 mg/100g in *A. africana* to 63.48 mg/100g in *I. trichantha*. Iron ranged from 4.56 mg/100g in *I. trichantha* to 6.73 mg/100g in *A. africana.* Copper ranged from 0.32 mg/100g in *I. trichantha* to 0.93 mg/100g in *A. africana*. Manganese content of the plants ranged from

Table 6. Effect of methanolic extract of *Aspilia africana* on the radial mycelial growth of the test fungi measured in mm

Test fungi	Control	Concentration of crude extract (mg/ml)									
		50		100		150		200		250	
		A	B	A	B	A	B	A	B	A	B
A. flavus	20.0±0.6^{a}	17.0±0.6^{a}	15.0	12.0±0.6^{b}	40.0	8.0±0.7^{b}	60.0	4.0±0.3^{bc}	80.0	0.0±0.0^{a}	100.0
A. glaucus	38.0±0.6^{e}	27.0±0.6^{d}	29.0	18.0±0.6^{d}	53.0	15.0±0.6^{e}	61.0	7.0±0.6^{d}	82.0	3.0±0.6^{b}	92.0
A. niger	30.0±0.6^{c}	23.0±0.6^{c}	23.0	18.0±0.6^{d}	40.0	10.0±0.6^{c}	67.0	5.0±0.6^{c}	83.0	0.0±0.0^{a}	100.0
B. theobromae	33.0±0.6^{d}	20.0±0.6^{b}	39.0	15.0±0.6^{c}	55.0	12.0±0.6^{d}	64.0	10.0±0.6^{d}	70.0	4.0±0.6^{c}	88.0
P. palmivora	25.0±0.6^{b}	18.0±0.6^{a}	28.0	10.0±0.9^{a}	60.0	0.0±0.0^{a}	100	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
Rhizoctonia solani	43.0±0.6^{f}	33.0±0.6^{f}	23.0	21.0±0.8^{e}	51.0	11.0±0.4^{cd}	74.0	4.0±0.6^{bc}	91.0	0.0±0.0^{a}	100.0
Fuarium oxysporum	39.0±0.6^{e}	31.0±0.6^{e}	21.0	20.0±0.6^{e}	49.0	8.0±0.7^{b}	80.0	3.0±0.6^{b}	92.0	0.0±0.0^{a}	100.0

*Legend: A: Mean radial mycelial growth in mm, B: % inhibition of the radial mycelial growth

**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

Table 7. Effect of methanolic extract of *Ficus exasperata* on the radial mycelial growth of the test fungi measured in mm

Test fungi	Control	Concentration of crude extract (mg/ml)									
		50		100		150		200		250	
		A	B	A	B	A	B	A	B	A	B
A. flavus	30.0±0.6^{b}	15.0±0.6^{b}	50.0	8.0±0.6^{b}	73.0	4.0±0.6^{a}	87.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
A. glaucus	40.0±0.6^{e}	30.0±0.6^{e}	9.0	25.0±0.6^{f}	38.0	20.0±0.6^{d}	50.0	12.0±0.6^{e}	70.0	3.0±0.6^{b}	93.0
A. niger	25.0±0.6^{a}	10.0±0.6^{a}	60.0	5.0±0.6^{a}	80.0	3.0±0.6^{a}	88.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
B. theobromae	35.0±0.6^{d}	30.0±0.6e	14.0	20.0±0.6^{e}	43.0	10.0±0.6^{b}	71.0	9.0±0.6^{d}	77.0	5.0±0.6^{c}	86.0
P. palmivora	30.0±0.6^{b}	15.0±0.6^{b}	50.0	7.0±0.6^{b}	77.0	3.0±0.6a	90.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
Rhizoctonia solani	32.0±0.6^{c}	19.0±0.6^{c}	40.6	17.0±0.6^{d}	46.9	12.0±0.6^{c}	62.5	7.0±0.6^{c}	78.1	2.0±0.6^{b}	93.8
Fuarium oxysporum	30.0±0.6^{b}	22.0±0.6^{d}	26.7	16.0±0.6^{c}	46.7	9.0±0.6^{b}	70.0	3.0±0.6^{b}	90.0	0.0±0.0^{a}	100.0

*Legend: A: Mean radial mycelial growth in mm, B: % inhibition of the radial mycelial growth

**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

0.88 mg/100g in *I. trichantha* to 2.56 mg/100g in *A. africana.* Phosphorus ranged from 61.84 mg/100g in *I. trichantha* to 75.82 mg/100g in *F. exasperata.*

Antimicrobial activities of the extracts

The result of the antimicrobial activities of the crude methanolic extract of the three plants studied on the radial mycelial growth of selected plant pathogens are shown in Tables 6 – 8. It was observed that the net radial mycelial growth of the phytopathogenic fungi selected decreased with high concentration of the extracts for all the three plants extracts. For *A. africana, Aspergillus flavus* was more susceptible to the extract at 50 mg/ml with percentage inhibition of 15.0% while at 150 mg/ml, *Phytophthora palmivora* was more susceptible with zone of inhibition of 100% with others showing a percentage inhibition of 60% and above. It was observed that five (*Aspergillus flavus, A. niger, Phytophthora palmivora, Rhizoctonia solani* and *Fusarium oxysporum*) of the seven selected plant pathogens were completely inhibited at the highest concentration of 250 mg/ml.

Table 8. Effect of methanolic extract of *Icacina trichantha* on the radial mycelial growth of the test fungi

Test fungi	Control	Concentration of crude extract (mg/ml)									
		50		100		150		200		250	
		A	B	A	B	A	B	A	B	A	B
A. flavus	23.0±0.6^{a}	20.0±0.6^{a}	13.0	17.0±0.6^{a}	26.0	11.0±0.6^{b}	52.0	6.0±0.6^{b}	74.0	2.0±0.6^{b}	91.0
A. glaucus	35.0±0.6^{e}	30.0±0.6^{e}	14.0	25.0±0.6e	29.0	20.0±0.6^{f}	49.0	12.0±0.6^{e}	66.0	7.0±0.6^{c}	80.0
A. niger	32.0±0.6^{d}	23.0±0.6^{bc}	28.0	15.0±0.6^{a}	53.0	13.0±0.6^{cd}	59.0	10.0±0.6^{d}	69.0	6.0±0.6^{c}	81.0
B. theobromae	25.0±0.6^{b}	22.0±0.6^{b}	12.0	18.0±0.6^{b}	28.0	12.0±0.6^{bc}	52.0	9.0±0.6^{cd}	64.0	3.0±0.6^{b}	88.0
P. palmivora	30.0±0.6^{c}	20.0±0.6^{a}	33.0	18.0±0.6^{b}	40.0	7.0±0.6^{a}	77.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
Rhizoctonia solani	30.0±0.6^{c}	24.0±0.6^{cd}	20.0	20.0±0.6^{c}	33.3	14.0±0.6^{d}	53.3	8.0±0.6^{c}	73.3	3.0±0.6^{b}	90.0
Fuarium oxysporum	29.0±0.6^{c}	25.0±0.6^{d}	13.8	22.0±0.6^{d}	24.1	16.0±0.6^{e}	44.8	12.0±0.6^{e}	58.6	6.0±0.6^{c}	79.3

*Legend: A: Mean radial mycelial growth in mm, B: % inhibition of the radial mycelial growth

**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

Table 9. Effect of methanolic extract of *Aspilia africana* on the dry mycelial growth weight of the test fungi measured in mm

Test fungi	Control	Concentration of crude extract (mg/ml)									
		50		100		150		200		250	
		A	B	A	B	A	B	A	B	A	B
A. flavus	141.0±1.0^{b}	119.0±1.0^{c}	16.0	104.0±1.0^{d}	26.0	90.0±1.0^{d}	36.0	75.0±1.0^{e}	47.0	40.0±1.0^{e}	72.0
A. glaucus	180.0±1.0^{e}	154.0±1.0^{e}	14.0	136.0±1.0^{e}	24.0	124.0±1.0^{f}	31.0	71.0±1.0^{d}	61.0	23.0±1.0^{c}	87.0
A. niger	178.0±1.0^{e}	167.0±1.0^{f}	6.0	165.0±1.0^{f}	7.0	98.0±1.0^{e}	45.0	43.0±1.0^{c}	76.0	12.0±1.0^{ab}	93.0
B. theobromae	125.0±1.0^{a}	105.0±1.0^{a}	18.0	90.0±1.0^{c}	28.0	81.0±1.0^{c}	35.0	38.0±1.0^{b}	70.0	9.0±1.0^{a}	93.0
P. palmivora	144.0±1.0^{bc}	122.0±1.0^{c}	15.0	89.0±1.0^{c}	38.0	49.0±1.0^{b}	66.0	43.0±1.0^{c}	70.0	32.0±1.0^{d}	78.0
Rhizoctonia solani	147.0±1.0^{c}	111.0±1.0^{b}	25.0	77.0±1.0^{b}	48.0	47.0±1.0^{b}	68.0	31.0±1.0^{a}	84.0	17.0±1.0^{b}	94.0
Fuarium oxysporum	166.0±1.0^{d}	127.0±1.0^{d}	24.0	67.0±1.0^{a}	60.0	41.0±1.0^{a}	75.0	29.0±1.0^{a}	83.0	13.0±1.0^{b}	92.0

*Legend: A: Mean measurement of the dry mycelial growth weight in mm, B: % inhibition of the dry mycelial weight

**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

This similar trend of susceptibility was also observed for *F. exasperata.* At 50 mg/ml, *A. glaucus* showed the least percentage inhibition of 9.0% while *A. niger* showed the highest percentage inhibition of 60.0%. At 100 mg/ml, 80.0% of the radial mycelial growth *A. niger* had been inhibited while only 38.0% of *A. glaucus* was inhibited. Similarly at 200 mg/ml, *A. flavus, A. niger* and *P. palmivora* were completely inhibited while at 250 mg/ml, *F. oxysporum* was also completely inhibited.

The susceptibility of the pathogens to the extracts of *I. trichantha* showed that at 50 mg/ml, all except *P. palmivora* had a percentage inhibition of less than 30% while at 100 mg/ml, *A. niger* had the highest percentage inhibition of 53.0%. On the other hand, at 150 mg/ml, *P. palmivora* had the highest percentage inhibition. At 200 and 250 mg/ml concentration, *P. palmivora* other pathogens showed a percentage inhibition that ranged from 64.0% in *B. theobromae* at 200 mg/ml to 91.0% at 250 mg/ml concentration of the extract.

The result of the antimicrobial activities of the crude methanolic extract of the three plants studied on the dry mycelial weight of the selected plant pathogens are shown in Tables 9 – 11. It was observed that the dry was completely inhibited by the extract while mycelial weight of the phytopathogenic fungi selected decreased with high concentration of the extracts for all the

Table 10. Effect of methanolic extract of *Ficus exasperata* on the dry mycelial growth weight of the test fungi measured in mm

Test fungi	Control	Concentration of crude extract (mg/ml)									
		50		100		150		200		250	
		A	B	A	B	A	B	A	B	A	B
A. flavus	185.0±1.0^{c}	183.0±1.0^{d}	1.0	167.0±1.0^{f}	10.0	129.0±1.0^{g}	30.0	101.0±1.0^{f}	45.0	86.0±1.0^{e}	54.0
A. glaucus	220.0±1.0^{e}	196.0±1.0^{e}	11.0	153.0±1.0^{e}	30.0	95.0±1.0^{d}	57.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
A. niger	225.0±1.0^{f}	208.0±1.0^{f}	8.0	113.0±1.0^{d}	50.0	107.0±1.0^{f}	52.0	77.0±1.0^{e}	66.0	37.0±1.0^{c}	84.0
B. theobromae	174.0±1.0^{b}	126.0±1.0^{b}	28.0	111.0±1.0^{d}	36.0	103.0±1.0^{e}	41.0	67.0±1.0^{d}	61.0	43.0±1.0^{d}	75.0
P. palmivora	47.0±1.0^{a}	44.0±1.0^{a}	6.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0	0.0±0.0^{a}	100.0
Rhizoctonia solani	187.0±1.0^{c}	129.0±1.0^{b}	31.0	77.0±1.0^{b}	59.0	41.0±1.0^{b}	78.0	19.0±1.0^{b}	90.0	0.0±0.0^{a}	100.0
Fuarium oxysporum	199.0±1.0^{d}	143.0±1.0^{c}	28.0	97.0±1.0^{c}	51.0	68.0±1.0^{c}	66.0	44.0±1.0^{c}	78.0	19.0±1.0^{b}	90.0

*Legend: A: Mean measurement of the dry mycelial growth weight in mm, B: % inhibition of the mycelial weight

**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

Table 11. Effect of methanolic extract of *Icacina trichantha* on the dry mycelial growth weight of the test fungi measured in mm

Test fungi	Control	Concentration of crude extract (mg/ml)									
		50		100		150		200		250	
		A	B	A	B	A	B	A	B	A	B
A. flavus	135.0±0.5^{b}	133.0±0.8^{d}	1.0	98.0±1.0^{c}	27.0	86.0±1.0^{b}	36.0	55.0±1.0^{d}	59.0	10.0±1.0^{a}	93.0
A. glaucus	135.0±0.4^{b}	125.0±1.0^{c}	7.0	118.0±1.0^{e}	13.0	87.0±1.0^{b}	36.0	66.0±1.0^{e}	56.0	39.0±1.0^{e}	71.0
A. niger	285.0±0.5^{f}	251.0±1.0^{f}	12.0	198.0±1.0^{g}	31.0	178.0±1.0^{d}	38.0	51.0±1.0^{c}	82.0	28.0±1.0^{d}	90.0
B. theobromae	204.0±0.5^{d}	99.0±1.0^{b}	51.0	90.0±1.0^{b}	56.0	72.0±1.0^{a}	65.0	50.0±1.0^{c}	75.0	18.0±1.0^{b}	91.0
P. palmivora	112.0±0.7^{a}	89.0±1.0^{a}	21.0	75.0±1.0^{a}	33.0	73.0±1.0^{a}	35.0	32.0±1.0^{a}	71.0	10.0±1.0^{a}	91.0
Rhizoctonia solani	150.0±0.4^{c}	124.0±1.0^{c}	17.3	103.0±1.0^{d}	31.3	73.0±1.0^{a}	51.3	44.0±1.0^{b}	70.7	12.0±1.0^{a}	92.0
Fuarium oxysporum	220.0±0.1^{e}	180.0±1.0^{e}	18.2	159.0±1.0^{f}	27.7	123.0±1.0^{c}	44.1	70.0±1.0^{f}	68.2	22.0±1.0^{c}	90.0

*Legend: A: Mean measurement of the dry mycelial growth weight in mm, B: % inhibition of the mycelial weight

**Values represent mean of three replicates. Means with the same letter are not significantly different by Duncan's multiple tests*

three plants extracts. For *A. africana, R. solani* was more susceptible to the extract at all the concentrations of the extracts with percentage inhibition that ranged from 25.0% at 50 mg/ml to 94.0% at 250 mg/ml.

Similarly, the percentage inhibition of dry mycelial weight of the selected pathogens for *F. exasperata* showed that *R. solani* had the highest inhibition of 31.0% at 50 mg/ml while *P. palmivora* was completely inhibited at 100 mg/ml. At 150 mg/ml, *A. flavus* had the least percentage inhibition of 30.0%. The trend was also observed for 200 mg/ml but at 250 mg/ml, three (*A. glaucus, P. palmivora, R. solani*) out of seven selected pathogens were completely inhibited.

Similar trend was observed for *I. trichantha* only that none of the selected plant pathogen was completely inhibited at all the concentrations of the extracts.

DISCUSSION

The control of food and cash crops pathogens has been based on the application of chemical fungicides, crop rotation and the use of pathogen-resistant varieties over the years (Al-Rahmah et al., 2013). However, fungicide application has resulted in the accumulation of residual toxicity in soil crop juice (theobromine from cocoa), increase environmental pollution and alter the biological

balance in the soil by mounting selective pressure on non-target and beneficial microorganisms. Adverse effects of chemical based fungicides on the environment and human health are global environmental problem hence, a need to search for a new fungicides eco-friendly in nature.

In this study, three plants were analysed and assayed for their antimicrobial activities of selected food and cash crop pathogens. The result of the phytochemical analysis showed that all the plants were found to contain tannins, alkaloids, saponin and flavonoids. These compounds have been reported to be the active compounds that play a major role in the medicinal values and antimicrobial activities of plants against pathogens of both plants and animal origins (Iniaghe et al., 2009; Fagbohun et al., 2012a) while their abundance might correspond to their antimicrobial activities (Sofowora, 2008). Phytate and cyanide were found to be more abundant in *A. africana* while phytin phosphorus and oxalate were found to be abundant in *F. exasperata*. The values of the antinutrients reported in this study is in agreement with the findings of Fagbohun et al., (2012a) who analysed the phytochemicals extracted from the leaves of *Ocimum gratissimum* L., *Melanthera scandens* A. and *Leea guineensis*.

The proximate analysis of the plant extracts showed that the plant extracts have nutrients in varying composition. The analysis showed that all the extracts exhibited highest percentage in their carbohydrate content and lowest percentage in moisture content. This finding is in agreement with Opara and Nwankwo (2015) who studied the proximate composition of *Allium sativum, Zingiber officinale*, *Garcinia kola*, *Azadirachta indica* and *Jatropha curcas* and reported that the plant extracts have nutrients in varying composition with highest percentage in their carbohydrate content. However, the ratio of sodium to potassium is greater than 1 for all the three plants studied (1.5 for both *A. africana* and *I. trichantha,* 1.9 for *F. exasperata*) this implies that the plants studied may not be safe for direct consumption in human medicine as stated by FND (2002).

The antimicrobial susceptibility study of the three plant extracts showed that all the plant extract provided a significant inhibition of mycelial growth of the phytopathogenic fungi and their susceptibility to a given plant extract varied greatly.

A. africana showed complete inhibition of the radial mycelial growth of *A. flavus, A. niger, P. palmivora, R. solani* and *F. oxysporum* followed by the extract of *F. exasperata* which was found to completely inhibit the radial mycelial growth of *A. flavus, A. niger, P. palmivora* and *F. oxysporum*. The *F. exasperata* extract was found to be the most effective plant extract as it also showed a significant inhibitory effect on the test organisms by the complete inhibition of the dry mycelial weight of *A. glaucus, P. palmivora* and *R. solani.* These results are in agreement to the findings of Zaker and Mosallanejad, (2010), Fagbohun et al., (2012b), Al-Rahmah et al., (2013) and Ochola et al., (2015) who studied the antimicrobial activities methanolic extracts of different plants against test organisms and reported that all the extract showed a significant antimicrobial activities on the selected plant pathogens and concluded that the extract can serve as potential alternative to chemical based fungicides and contribute to the development of environmentally friendly plant based fungicides.

A variation in the antimicrobial activities of the extracts against the plant pathogens may be due to considerable variations in their constituents, origin of isolation and variation in the species of the plant pathogens (Alkhail, 2005; Fawzi et al., 2009). In addition, this study revealed that the three extracts of plants used can serve as potential source of antimicrobial compounds for the control of plant infections. The incorporation of the bioactive compounds of the plants into extract based formulation of biocides for the control of food and cash crops infection will help to reduce the negative environmental effect of chemical based biocides. Moreover, it will also reduce the concentration of chemical residues in the crops.

CONCLUSION

The detrimental effect of chemicals used for the control of phyto-pathogen in the environment is of public health concern and the best approach to minimise this is having an alternative. The plants studied contain bioactive compounds which may serve as alternative to chemical based biocides for the control of food and cash crops pathogens. Further study is needed to purify and carryout a field trial of these bioactive compounds.

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