

Growth Inhibition Potentials of Leaf Extracts from Four Selected Euphorbiaceae against Fruit Rot Fungi of African Star Apple (*Chrysophyllum albidum* G. Don)

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Abstract

The efficacy of ethanolic leaf extracts from *Phyllanthus amarus*, *Euphorbia hirta*, *Euphorbia heterophylla* and *Acalypha fimbriata* in inhibiting the growth of post-harvest fruit rot fungi of *Chrysophyllum albidum* was investigated at the concentrations of 100, 80, 60, 40 and 20 mg/ml *in-vitro*. The fungi isolated from rotted fruits and their frequency of occurrence includes *Aspergillus niger* (69.6%) and *Fusarium solani* (30.4%). These fungal isolates were cultured on different leaf extracts agar and their radial mycelia growth was observed. The antifungal activities increased with increase in concentrations of the plant extracts with *E. heterophylla* extract most effective in inhibiting the growth of *A. niger* while *A. fimbriata* extract was more effective in the inhibition of *F. solani* than other extracts. Phytochemical screening of the plant extracts revealed the presence of saponins, alkaloids, glycosides, terpenes, steroids, flavonoids, tannins and phenols. Gas Chromatography Mass Spectrometry (GC-MS) analysis revealed the presence a complex mixture of constituents ranging from 7 compounds in *E. hirta*, 10 compounds in *A. fimbriata*, 11 compounds in *E. heterophylla* and 14 compounds in *P. amarus*. The result of this study is an indication that these Euphorbiaceae could be a potential source of antifungal agents.

Keywords: Growth inhibition; Leaf extracts; Euphorbiaceae; Rot fungi; *Chrysophyllum albidum*

Introduction

Chrysophyllum albidum G. Don commonly called African star apple and locally called udara (Igbo), agbalumo (Yoruba) belongs to the family Sapotaceae [1]. It features prominently in the compound agro forestry system for fruit, food, cash income and other auxiliary uses including environmental purposes. It is also a tree that is common throughout the Tropical Central, East and West Africa regions for its sweet edible fruit and various ethnomedical uses [2].

C. albidum fruits are widely eaten in Southern Nigeria. The fruit is seasonal (December-March), when ripe. It is flattened seeds or sometimes fewer by abortion. The fruit is ovoid to sub-globose pointed at the apex and up to 6 cm long and 5 cm in diameter. The skin or peel is grey when immature turning orange red, pinkish or light yellow within the pulp having three to five seeds arranged as a star [3].

The fruit has been found to have the highest content of ascorbic acid with 1000 to 3330 µg of ascorbic acid per 100 gm of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew. It is also an excellent source of vitamins B and D as well as iron [4]. Umoh [5] and Ureigho [6] reported on the proximate composition, minerals and vitamins content of *Chrysophyllum albidum*.

The fruit has immense economic potential, especially following the report that jams that could compete with rasp berry jams and jellies could be made from it and it is eaten especially as snack by both young and old [2]. The fruits contain 90% anacardic acid, which is used industrially in protecting wood and as a source of resin. The fruits can be used in the preparation of wine, soft drink, jams and jellies [3,6].

The seed are used for local games; it is also a source of oil, which used for diverse purposes [7]. The seeds along with those of other Sapotaceae are used as anklets in dancing. It was also discovered in the removal of Ni²⁺ ions from synthetic wastewater [8]. The cotyledons are

useful in the preparation of medicine for the treatment of infertility problems in both male and female; infertility due to the presence of abnormalities within the uterus and female tubes, abdominal pains in dysmenorrheal, secondary ammenorrhoea in women (loss or absence of menstrual cycle). The seed cotyledon has been reported to possess anti-hyperglycemic and hypolipidemic effects [9].

Fungi have been reported to be associated with post harvest deterioration of agricultural products in Nigeria. However, *F. solani*, *L. theobromae*, *Rhizopus spp* and *A. flavus* have been reported to be associated with *C. albidum* [10]. Since most microbial spores are small in size and light, they could settle on the surface of African Star Apple fruits resulting in the range of microbial group isolated from them.

Preserving the freshness of these fruits for many days or months is therefore the problem, which most farmers and the traders seek to solve. Control of fruit rot by employing the use of local preservatives (plant extracts) like *Afromomum danielli*, *Afromomum melegueta* and chemical disinfectants like (parazone), sodium chloride and sodium benzoate at mild form has been suggested to reduce the losses due to storage moulds [10].

The objective of this study is therefore to isolate and identify fungi associated with *C. albidum* fruits rot in storage as well as to

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determine the effects of various concentrations of ethanolic extracts of *Phyllanthus amarus*, *Euphorbia hirta*, *Euphorbia heterophylla* and *Acalypha fimbriata* on the identified fungi.

Materials and Methods

Collection of plant materials for the study

Mature healthy and rotted *C. albidum* fruits were purchased at Abraka Main Market, Delta State. Fresh and healthy leaves of *Euphorbia hirta*, *Euphorbia heterophylla*, *Phyllanthus amarus* and *Acalypha fimbriata* free from insect and pathogen attack were collected from different areas within Abraka community. Abraka (Ethiope East Local Government Area of Delta State lies within latitude 05° 47'N and longitude 06° 06'E of the Equator with an annual rainfall of 3,097.8 mm, annual relative humidity of 83% and annual mean temperature of 30.6°C [11]. The plants were identified using Akobundu and Agyakwa [12].

Isolation and identification of fungi

Isolation and identification of fungi from diseased *C. albidum* fruits was carried out using the method adopted from Ilondu [13]. Sections, 4 mm long, excised from the margins of diseased spot with sterile razor blade were surface-sterilized for 2 min in 2% aqueous solution of commercial bleach (sodium hypochlorite solution), rinsed in two changes of sterile distilled water. The disinfected tissue pieces were blotted between sterile Whatman No. 1 filter paper and aseptically plated on potato dextrose agar (PDA) plates (3 pieces per plate). The plates were then incubated at room temperature (32 ± 2°C) for five days. Any observed mycelial growth was repeatedly transferred to fresh PDA plates until pure cultures of isolates were obtained.

The frequency of isolations of the different types of fungi associated with *C. albidum* fruit rot diseases was determined. The number of times each fungus was encountered was recorded. The percentage frequency of occurrence was calculated with the formula below:

$$\frac{\text{Number of times a fungus was encountered}}{\text{Total fungal isolations}} \times \frac{100}{1}$$

Plant sample preparation and extraction procedures

The plants were collected into polyethylene bags and taken to the laboratory for processing. The leaves were separately plucked and rinsed in flowing tap water, shade dried on the bench in a ventilated section of the Department of Botany herbarium at ambient temperature (30°C ± 2) for two weeks [14]. Dried leaves were separately ground into powder using an electric blender before extraction. For extraction procedures, one hundred gram of each pulverized sample was put into Soxhlet extractor and three hundred milliliter of absolute ethanol (HPLC grade) was added and extracted for 8hrs for each batch of sample. The extracts were evaporated on a rotary evaporator at 40°C to remove excess alcohol. The solvent free extracts were stored at 4°C till needed.

Phytochemical tests

One gram of powdered sample was subjected to phytochemical test for alkaloid (Myers reagent), Flavonoids were determined by magnesium rebbon test, Sapoin by chloroform and H₂SO₄ tests, Tannins, by Ferric salt test, Sterol by Chloroform-acetic anhydride, Terpenes and phenols by following the procedures of Oyewale and Audu [15].

Extract analysis

GC-MS analysis was done at National Research Institute for

Chemical Technology (NARICT) Zaria, Kaduna state, Nigeria. A SHIMADZU GCMS-QP 2010 Plus system was used. The GC-MS was operated under the following conditions: Column oven temperature: 70°C; Injection temperature: 250°C; Injection mode: split; Pressure: 104.1 kPa; Total flow: 6.2 ml/min; Column flow: 1.59 ml/min; Linear velocity: 46.3 cm/sec; Purge flow: 3.0 mL/min; and Split ratio: 1.0. The generated chromatogram was recorded. The identification of the components was carried out using the peak enrichment technique of reference compounds and computer matching with those of NIST.05 library mass spectrum [14,16].

Effect of extracts on fungal growth

Different concentrations (100, 80, 60 40 and 20 mg/ml) were prepared from each of the extracts. One millilitre of each level of concentration was aseptically incorporated into 20 ml of cool molten PDA in sterile test tube. Each medium was homogenized by gentle agitation before dispensing into sterile 9 cm Petri dishes. The control was set up using extract free PDA plates. The plates were allowed to set for 3 hr. The effect of the extracts on fungal growth was determined using the method of Chohan et al. [17]. This was done by inoculating at the Centre of 90 cm Petri plates with a mycelia disc (4 mm) obtained from the colony edge of 7-day old culture of the test fungi. Three replicates of both the control and PDA-extract plates per isolate were incubated at room temperature (28 ± 2°C) and radial growth was measured with a metric ruler daily for seven days. Colony diameter was taken as the means along two directions on two perpendicular lines drawn on the reverse of the plates. The percentage inhibition was calculated by the method of Ayodele et al. [18].

Data analysis

Data obtained were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 17.0 and means were separated according to Duncan's Multiple Range Test (DMRT) at 5% probability level.

Results

The fungi isolated from the diseased *Chrysophyllum albidum* fruits were *Aspergillus niger* and *Fusarium solani*. *A. niger* occurred more frequently with 69.6% followed by *F. solani* with 30.4% (Table 1). The classes of natural products present in the plant investigated are shown in Table 2. Tannins, saponins, steroids and phenols were present in all

Fungal isolate	No of times isolated	Percentage frequency (%)	Pathogenicity of isolates
<i>Aspergillus niger</i>	80	69.6	+
<i>Fusarium solani</i>	35	30.4	+

Table 1: Percentage occurrence of fungi associated with *Chrysophyllum albidum*.

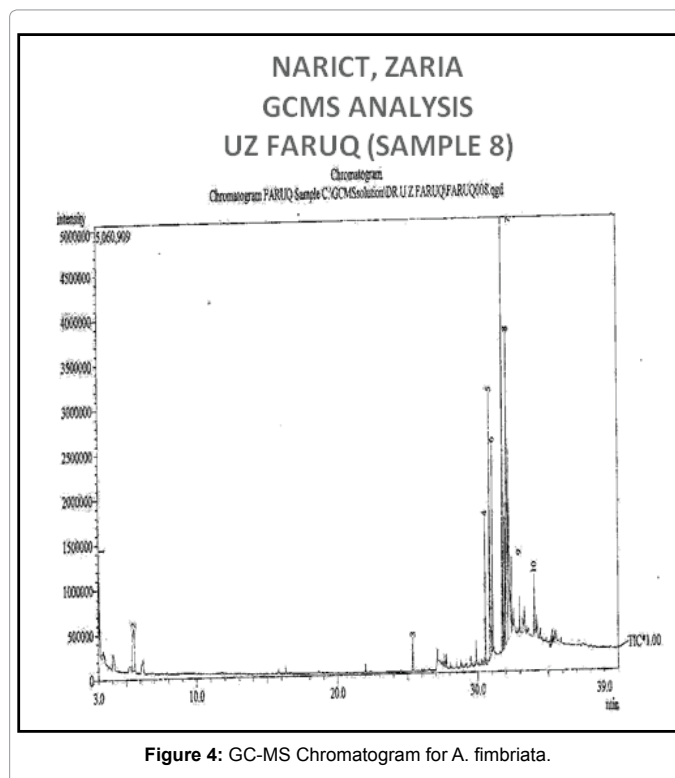
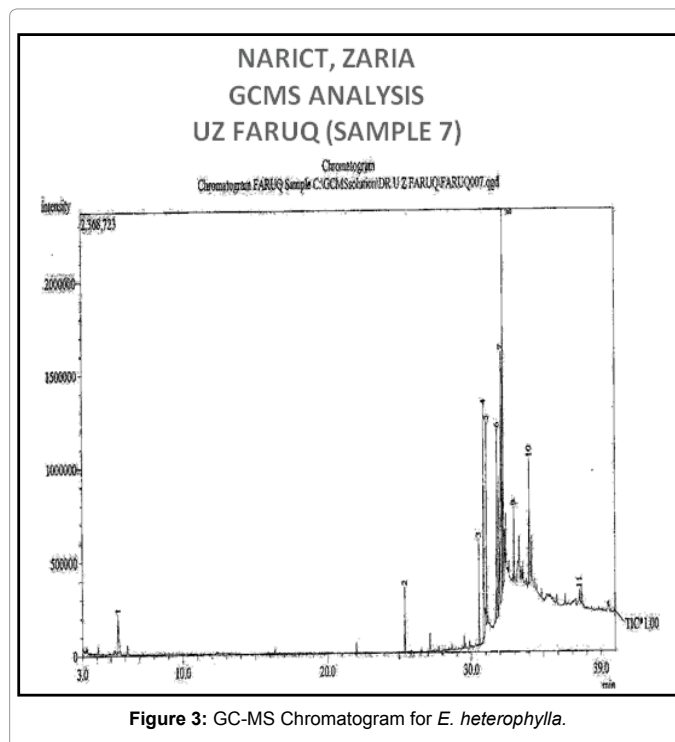
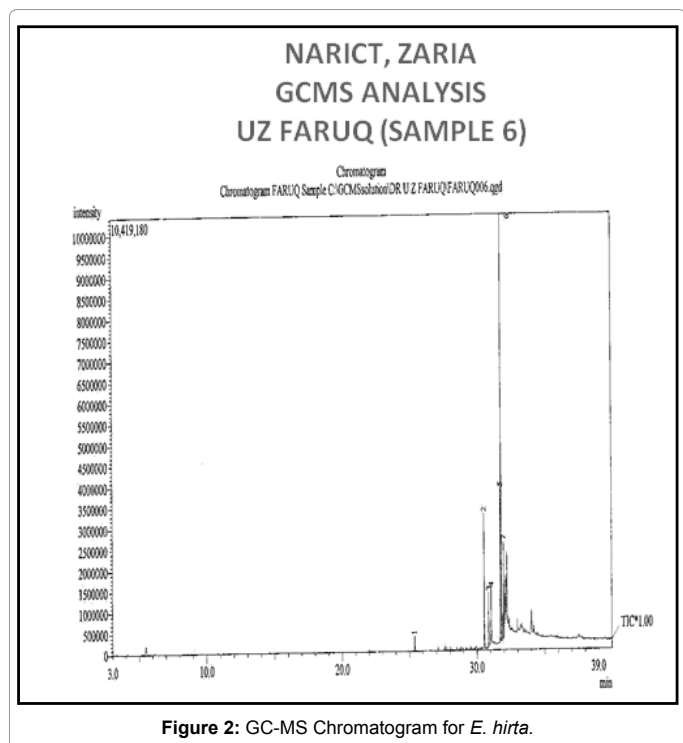
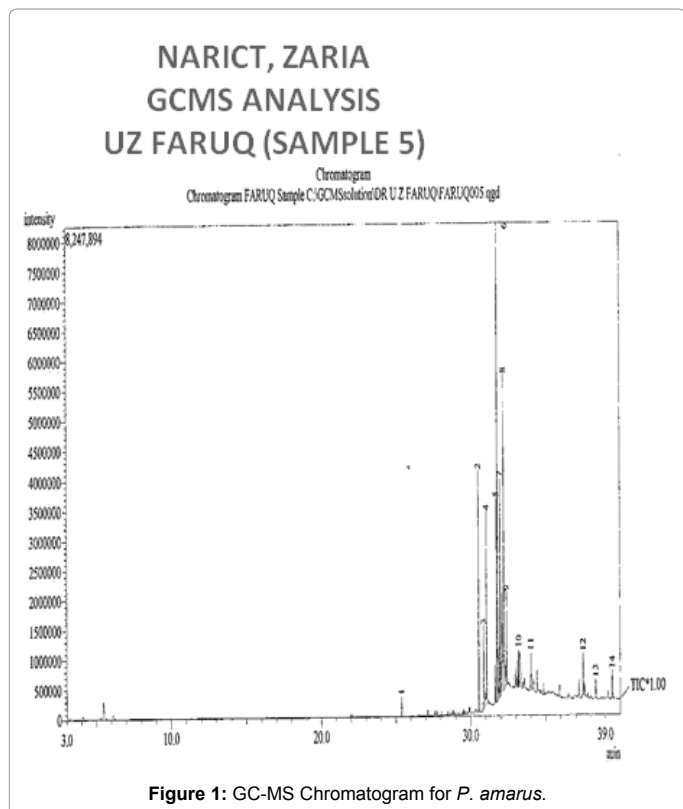
Phytochemicals	<i>P. amarus</i>	<i>E. hirta</i>	<i>E. heterophylla</i>	<i>A. fimbriata</i>
Saponins	+	+	+	+
Alkaloids	-	+	+	+
Tannins	+	+	+	+
Flavonoids	-	+	-	-
Steroids	+	+	+	+
Glycosides	-	+	-	+
Terpenes	-	+	-	+
Phenols	+	+	+	+

+ = Presence
- = Absence

Table 2: Phytochemical Screening of Plants used in the study.

the plants. Alkaloids were present in *E. hirta*, *E. heterophylla* and *A. fimbriata* except *P. amarus*. Flavonoids was only present in *E. hirta*. Glycosides and terpenes were present only in *E. hirta* and *A. fimbriata*.

The gas chromatography profiles of the plants extracts used in the study were shown in Figures 1-4. The analysis of the extract revealed



complex mixture of constituents ranging from 7-14 compounds in the samples (Table 3).

Phenol 3,5-bis (1,1-dimethylethyl) were recorded in all plant. Hexadecanoic acid, methyl ester were recorded in all the plant except *A. fimbriata*. 10-Otadecenoic acid, methyl ester was recorded to be the most abundant of all the (14) compounds identified in *P. amarus*,

Plant Extracts	Peak No	Retention time (min)	% peak	Compound formula	Name of compound
<i>P. amarus</i>	1	25.381	1.09	C ₁₄ H ₂₂ O	Phenol3,5-bis(1,1-dimethylethyl)
	2	30.543	9.45	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester
	3	30.868	5.51	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid
	4	31.066	9.17	C ₁₈ H ₃₆ O ₂	Hexadecanoic acid, ethyl ester
	5	31.745	9.50	C ₁₉ H ₃₄ O ₂	11,14-octadecadienoc acid, methyl ester
	6	31.802	22.92	C ₁₉ H ₃₄ O ₂	10-octadecenoic acid, methyl ester
	7	31.978	8.69	C ₁₉ H ₃₈ O ₂	Octadecanoic acid, methyl ester
	8	32.223	19.33	C ₂₀ H ₃₄ O ₂	9,12,15-octadecadienoic acid, ethyl ester (z,z,z)
	9	32.405	3.85	C ₂₀ H ₄₀ O ₂	Octadecanoic acid, ethyl ester
	10	33.205	1.99	C ₂₁ H ₄₂ O ₂	Eicosanoic acid, methyl ester
	11	34.017	2.31	C ₁₈ H ₃₄ Cl ₁₀	9,12-octadecadienoyl chloride (z,z)
	12	37.501	2.71	C ₂₂ H ₂₈ O ₇	Carissanol dimethyl ether
	13	38.330	1.20	C ₃₄ H ₂₂	Dibenz(a,h) anthracene, 12-diphenyl
	14	39.463	2.29	C ₁₀ H ₁₄ O ₂	Benzene, 4-ethyl-2-dimethoxy-
<i>E. hirta</i>	1	25.383	1.94	C ₁₄ H ₂₂ O	Phenol, 3,5-bis(1,1-dimethylethyl)
	2	30.542	12.68	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester
	3	30.869	8.85	C ₁₆ H ₃₂ O ₂	n-hexadecanoic acid
	4	31.067	6.33	C ₁₈ H ₃₆ O ₂	Hexadecanoic acid, ethyl ester
	5	31.747	16.27	C ₁₉ H ₃₄ O ₂	9,12-octadecadienoic acid methyl ester
	6	31.806	44.9	C ₁₉ H ₃₆ O ₂	9-octadecenoic acid (z)-, methyl ester
	7	31.98	9.33	C ₁₉ H ₃₆ O ₂	Octadecanoic acid, methyl ester
<i>E. heterophylla</i>	1	5.496	4.78	C ₈ H ₁₀	O-xylene
	2	25.386	3.47	C ₁₄ H ₂₂ O	Phenol3,5-bis(1,1-dimethylethyl)
	3	30.542	3.94	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester
	4	30.868	14.71	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid, methyl ester
	5	31.061	7.84	C ₁₈ H ₃₆ O ₂	Hexadecanoic acid, ethyl ester
	6	31.798	9.22	C ₁₉ H ₃₆ O ₂	10-Octadecenoic acid, methyl ester
	7	32.093	22.22	C ₂₂ H ₄₂ O ₂	Erucic acid
	8	32.224	21.39	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid (z,z)-
	9	32.944	2.97	C ₃₇ H ₇₄ NO ₆ P	Hexadecanoic acid
	10	34.020	8.33	C ₁₈ H ₃₄ O	13-Octadecenal
	11	37.644	1.13	C ₁₀ H ₁₄ O ₂	1,4-Benzenedimethanol, alpha, alpha, dimethyl
<i>A. fimbriata</i>	1	3.070	2.42	C ₅ H ₁₂ O	1-Butanol,3-methyl-/isopentyl alcohol
	2	5.43	10.52	C ₈ H ₁₀	Xylene/benzene1,2-dimethyl
	3	25.372	1.70	C ₁₄ H ₂₂ O	Phenol3,5-bis(1,1-dimethyl ethyl)
	4	30.537	5.83	C ₁₇ H ₃₄ O ₂	Pentadecanoic acid
	5	30.867	17.42	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid
	6	31.057	7.95	C ₁₈ H ₃₆ O ₂	Hexadecanoic acid ethyl ester
	7	31.797	18.47	C ₁₉ H ₃₆ O ₂	10-Octadecenoic acid, methyl ester
	8	32.093	28.88	C ₂₂ H ₄₂ O ₂	Erucic acid
	9	32.401	3.25	C ₂₀ H ₄₀ O ₂	Octadecanoic acid, ethyl ester
	10	34.015	3.56	C ₁₆ H ₃₀ O	Cis-9-Hexadecenal

Table 3: Major identified constituents of the plant extracts.

9-Octadecenoic acid (Z)-methyl ester was most abundant among the (7) compounds in *E. hirta*, Erucic acid was most abundant in *E. heterophylla* and *A. fimbriata*.

The two fungi were very sensitive to various concentrations of the plant extracts tested since the extracts significantly reduced the mycelia growth of the fungi at all concentrations (Table 4). However, the effectiveness of the plant extracts increased with increased concentration and this was significantly different (p<0.05) when compared to the control. Similarly, percentage growth inhibition generally increased with increase in concentration of the leaf extracts when compared to the control. Although, the plant extracts could not give complete inhibition at the highest concentration tested, their effectiveness increased with increase concentrations.

There was no significant difference in the inhibitory effect of *P. amarus* on *A. niger* at the concentrations of 20 and 40 mg/ml, 60

and 80 mg/ml concentrations with *E. heterophylla* as well as 80 and 100 mg/ml concentrations with *A. fimbriata*. Similarly, there was no significant difference in the inhibitory effect of *A. fimbriata* extract on *F. solani* from 60-100 mg/ml concentrations (Table 5). *A. niger* was most sensitive to *E. heterophylla* followed by *A. fimbriata*, *P. amarus* and *E. hirta* respectively. Similarly, *F. solani* was most sensitive to *A. fimbriata* followed by *E. heterophylla*, *E. hirta* and *P. amarus*.

Discussion

The present study showed that two fungi were associated with post harvest fruit rot disease of *Chrysophyllum albidum*, which include *Aspergillus niger* and *Fusarium solani*. These fungi have previously been reported as fruit rot pathogens [13,19,20].

Aspergillus niger has the highest percentage occurrence of 69.6% followed by *F. solani* which is 30.4%. This was enhanced by the light

Extract conc. (mg/ml)	<i>P. amarus</i>		<i>E. hirta</i>		<i>E. heterophylla</i>		<i>A. Fimbriata</i>	
	<i>A. niger</i>	<i>F. solani</i>	<i>A. niger</i>	<i>F. solani</i>	<i>A. niger</i>	<i>F. solani</i>	<i>A. niger</i>	<i>F. solani</i>
0	4.30 ^a	4.30 ^a	4.30 ^a	4.30 ^a	4.30 ^a	4.30 ^a	4.30 ^a	4.30 ^a
20	2.07 ^b	3.1 ^b	2.83 ^b	2.83 ^b	1.53 ^b	1.90 ^b	1.87 ^b	1.53 ^b
40	1.95 ^c	2.23 ^c	2.40 ^c	1.93 ^c	0.95 ^c	1.73 ^b	1.40 ^c	0.58 ^c
60	1.13 ^c	1.85 ^d	1.97 ^d	1.63 ^c	0.72 ^c	0.85 ^c	0.92 ^d	0.42 ^c
80	0.92 ^d	1.27 ^e	1.50 ^e	1.03 ^d	0.62 ^c	0.62 ^c	0.75 ^d	0.30 ^c
100	0.84 ^d	0.92 ^f	1.03 ^f	0.82 ^d	0.47 ^c	0.42 ^c	0.60 ^d	0.22 ^c

Values with the same superscript(s) in the same column are not significantly different at P>0.05 by DMRT.

Table 4: Radial mycelia growth (cm) of fungi isolated from *Chrysophyllum albidum* fruits when exposed to various concentrations of plant leaf extracts.

Conc. (%)	<i>P. amarus</i>		<i>E. hirta</i>		<i>E. heterophylla</i>		<i>A. fimbriata</i>	
	<i>F. Solani</i>	<i>A. niger</i>	<i>F. Solani</i>	<i>A. niger</i>	<i>F. Solani</i>	<i>A. niger</i>	<i>F. Solani</i>	<i>A. niger</i>
0	0 ^f	0 ^d	0 ^f	0 ^f	0 ^f	0 ^e	0 ^d	0 ^d
20	27.91 ^e	51.86 ^d	45.81 ^e	34.19 ^e	55.51 ^e	64.42 ^d	64.42 ^c	56.51 ^c
40	48.14 ^d	54.65 ^c	55.43 ^d	44.18 ^d	59.77 ^d	77.91 ^c	86.49 ^b	67.44 ^b
60	56.98 ^c	73.72 ^b	62.09 ^c	54.19 ^c	80.37 ^c	83.43 ^b	90.23 ^a	78.75 ^{ab}
80	70.47 ^b	78.61 ^a	76.21 ^b	65.12 ^b	85.58 ^b	85.58 ^b	93.02 ^a	82.68 ^a
100	78.61 ^a	80.47 ^a	80.93 ^a	76.05 ^a	90.31 ^a	89.07 ^a	94.89 ^a	86.05 ^a

Values with the same superscript(s) in the same column are not significantly different at P>0.05 by DMRT.

Table 5: Percentage growth inhibition of fungal isolates from *Chrysophyllum albidum* fruits after exposure to varying concentrations of leaf extract of various plants.

spores, which are easily dispersed by wind. Similarly *Aspergillus species* are capable of utilizing an enormous variety of substrates as the result of large number of enzymes they produce [21].

Phytochemical screening of the plants revealed the presence of saponin, alkaloid, tannin, steroids, Phenols, terpenes, glycosides, and flavonoids. The presence of these secondary metabolites could be responsible for their antifungal activity. Egwin et al. have earlier demonstrated the presence of tannins in *Euphorbia hirta* and opined that it may account for its antimicrobial activity. Tannins have been reported to be toxic to bacteria, filamentous fungal and yeast [22]. Ogbo and Oyibo [19] reported that the presence of alkaloids, saponins and terpenoids in the extract of *Ocimum gratissimum* may have accounted for the broad spectrum of activities on the fungal isolate tested.

The analysis of the plant extract of the leaves in this study showed a complex mixture of constituents. The total number of compounds identified varied from 7-14 in all the plant samples. It is possible that these compounds identified in the plant extracts were responsible for the observed fungi-toxic effects in the study. Sunderham [23] reported that the toxic action of the plant extract of *E. heterophylla* is due to the combined action of its constituents this is similar to the observations of Ilondu [14,16].

Erucic acid was the highest constituent found in *E. heterophylla* (22.22%) and *A. fambriata* (28.88%) extracts. Antimicrobial activity of *Eruca sativa* seed oil has been reported to be due to higher concentration of erucic acid present in the oil [24,25]. Varied concentrations of fatty acid including their ethyl and methyl esters were found abundant in all the plant extracts. Several researchers have reported the antifungal activity of fatty acid and their ethyl and methyl esters against pathogenic fungi [14,16,26].

The percentage inhibition of the mycelia growth of the tested fungi was found to increase as concentration of the plant extracts increased. This may be as a result of the presence of the biologically active antimicrobial compounds of the extracts in higher quantity at lower dilutions, this findings is in consonance with the work of Fernadex et al. [27] who suggested that with increasing concentrations the antagonistic property of the extract increased.

The above result clearly confirms that the test fungi varied widely in the degree of their susceptibility to the extracts. The extract of *Euphorbia heterophylla* was the most effective of all the extracts in inhibiting the growth of *Aspergillus niger* followed by *Acalypha fimbriata*, *Phyllanthus amarus* and *Euphorbia hirta*. While ethanolic extracts of *Acalypha fimbriata* was the most effective in inhibiting the growth of *Fusarium solani* followed by *Euphorbia heterophylla*, *Euphorbia hirta* and *Phyllanthus amarus*. Previous studies have shown that ethanolic leaf extracts of *E. hirta*, *E. heterophylla*, *A. fimbriata*, *P. amarus* and other species of these genera were capable of inhibiting the growth of bacteria, and fungi [13,23,28-32].

Conclusion

The result of this study is an indication that these Euphorbiaceae could be a potential source of antifungal agents. Knowledge of chemical constituents of non-economic plants is desirable because such information could be valuable in discovering new source of economic materials, which may be precursors for the synthesis of complex chemical substances. Such screening of various natural organic compounds and identification of active agents is the need of the century for the formulation of plant biofungicide and improvement of food security for the timing world population.

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