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 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 alkaloids, saponins, tannins, flavonoids, steroids and phenolic. 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 locally and 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-globe 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% anacacic 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 is 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 amenorrheae 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 Aframomum danielli, Aframomum melegueta and chemical disinfectants like (parazone), sodium chloride and sodium benzoate at mild form has been suggested to reduce the losses due to storage mounds [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...
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* *Phyllantus amarus* and *Acalypha fimbriata* free from insect and pathogen attack were collected from different areas within Abraka community. Abraka (Ethiopia 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:

\[
\text{Number of times a fungus was encountered} \times 100 \div \text{Total fungal isolations}
\]

**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 laboratory for processing. The 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 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

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

| Fungal isolate | No of times isolated | Percentage frequency (%) | Pathogenicity of isolates |
|----------------|----------------------|--------------------------|--------------------------|
| *Aspergillus niger* | 80                   | 69.6                     | +                        |
| *Fusarium solani* | 35                   | 30.4                     | +                        |

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

| Phytochemicals | *P. amarus* | *E. hirta* | *E. heterophylla* | *A. fimbriata* |
|----------------|------------|------------|------------------|---------------|
| Saponins       | +          | +          | +                | +             |
| Alkaloids      | -          | +          | +                | +             |
| Tannins        | -          | +          | +                | +             |
| Flavonoids     | +          | +          | -                | -             |
| Steroids       | +          | +          | +                | +             |
| Glycosides     | -          | +          | -                | -             |
| Terpenes       | -          | -          | +                | +             |
| Phenols        | +          | +          | +                | +             |

* + = Presence  
  - = Absence

Data analysis
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-dimethyllethyl) 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*,
Table 3: Major identified constituents of the plant extracts.

| Plant Extracts | Peak No | Retention time (min) | % peak | Compound formula | Name of compound |
|----------------|---------|----------------------|--------|------------------|------------------|
| *P. amarus*    | 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}O2    | Hexadecanoic acid, methyl ester |
|                | 3       | 30.888               | 5.51   | C_{16}H_{36}O2    | Hexadecanoic acid, ethyl ester |
|                | 4       | 31.068               | 9.17   | C_{16}H_{36}O2    | 11,14-octadecadienoic acid, methyl ester |
|                | 5       | 31.745               | 22.92  | C_{19}H_{34}O2    | 10-octadecanoic acid, methyl ester |
|                | 6       | 31.802               | 8.89   | C_{19}H_{38}O2    | Octadecanoic acid, methyl ester |
|                | 7       | 32.223               | 19.33  | C_{19}H_{38}O2    | Octadecanoic acid, ethyl ester |
|                | 8       | 32.405               | 3.85   | C_{20}H_{34}O2    | 9,12,15-octadecatrienoic acid, ethyl ester (Z,Z,Z) |
|                | 9       | 33.205               | 1.99   | C_{21}H_{42}O2    | Eicosanoic acid, methyl ester |
|                | 10      | 34.017               | 2.31   | C_{34}H_{22}O7    | Carissanol dimethyl ether |
| *E. hirta*     | 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}O2    | Hexadecanoic acid, methyl ester |
|                | 3       | 30.867               | 8.85   | C_{16}H_{32}O2    | n-Hexadecanoic acid, methyl ester |
|                | 4       | 31.061               | 14.71  | C_{18}H_{36}O2    | Hexadecanoic acid, ethyl ester |
|                | 5       | 31.798               | 9.22   | C_{19}H_{36}O2    | Hexadecanoic acid, ethyl ester |
|                | 6       | 32.093               | 22.22  | C_{22}H_{42}O2    | Erucic acid |
|                | 7       | 32.401               | 9.33   | C_{19}H_{38}O2    | Octadecanoic acid, methyl ester |
| *E. heterophylla* | 1 | 3.070                | 2.42   | C_{14}H_{22}O     | 1,4-Benzenedimethanol, alpha, alpha, dimethyl |
|                | 2       | 5.43                 | 10.52  | C_{14}H_{10}O     | Xylene/benzene1,2-dimethyl |
|                | 3       | 25.372               | 1.70   | C_{17}H_{34}O2    | Phenol3,5-bis(1,1-dimethylethyl) |
|                | 4       | 30.537               | 5.83   | C_{16}H_{32}O2    | Hexadecanoic acid |
|                | 5       | 30.867               | 17.42  | C_{16}H_{32}O2    | n-Hexadecanoic acid |
|                | 6       | 31.057               | 7.95   | C_{16}H_{32}O2    | Hexadecanoic acid, ethyl ester |
|                | 7       | 31.797               | 18.47  | C_{16}H_{32}O2    | 10-Octadecanoic acid, methyl ester |
|                | 8       | 32.093               | 28.88  | C_{16}H_{32}O2    | Erucic acid |
|                | 9       | 32.401               | 3.25   | C_{16}H_{32}O2    | Octadecanoic acid, ethyl ester |
| *A. fimbriata* | 1       | 3.070                | 2.42   | C_{14}H_{22}O     | 1,4-Benzenedimethanol, alpha, alpha, dimethyl |
|                | 2       | 5.43                 | 10.52  | C_{14}H_{10}O     | Xylene/benzene1,2-dimethyl |
|                | 3       | 25.372               | 1.70   | C_{17}H_{34}O2    | Phenol3,5-bis(1,1-dimethylethyl) |
|                | 4       | 30.537               | 5.83   | C_{16}H_{32}O2    | Hexadecanoic acid |
|                | 5       | 30.867               | 17.42  | C_{16}H_{32}O2    | n-Hexadecanoic acid |
|                | 6       | 31.057               | 7.95   | C_{16}H_{32}O2    | Hexadecanoic acid, ethyl ester |
|                | 7       | 31.797               | 18.47  | C_{16}H_{32}O2    | 10-Octadecanoic acid, methyl ester |
|                | 8       | 32.093               | 28.88  | C_{16}H_{32}O2    | Erucic acid |
|                | 9       | 32.401               | 3.25   | C_{16}H_{32}O2    | Octadecanoic acid, ethyl ester |
|                | 10      | 34.015               | 3.56   | C_{16}H_{32}O2    | Cis-9-Hexadecanen |

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 in 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...
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, glycossides, 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. Dembling 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 Alcyphra fimbristii, Phyllanthus amarus and Euphorbia hirta. While ethanolic extracts of Alcyphra fimbristii was the most effective in inhibiting the growth of Fusarium solani followed by Ephyphora heterophylla, Euphorbia hirta and Phyllanthus amarus. Previous studies have shown that ethanolic leaf extracts of E. hirta, E. heterophylla, A. fimbristii, 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.

**References**

1. Ehiagbonare JE, Onyibe HI, Okoegwale EE (2008) Studies on the isolation and Chemical Research 29: 320-325. 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.

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

| Conc. (%) | P. amarus | E. hirta | E. heterophylla | F. fimbristii |
|----------|-----------|----------|----------------|-------------|
| 0        | 0"        | 0"       | 0"             | 0"          |
| 20       | 27.91"    | 51.86"   | 45.81"         | 55.51"      |
| 40       | 48.14"    | 54.65"   | 55.43"         | 59.77"      |
| 60       | 56.98"    | 73.72"   | 62.09"         | 80.37"      |
| 80       | 70.47"    | 78.61"   | 76.21"         | 85.58"      |
| 100      | 78.61"    | 84.07"   | 80.93"         | 90.31"      |

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.

| Extract conc. (mg/ml) | P. amarus | E. hirta | E. heterophylla | F. fimbristii |
|-----------------------|-----------|----------|----------------|-------------|
| 0                     | 0%        | 0%       | 0%             | 0%          |
| 20                    | 78.61%    | 78.47%   | 76.05%         | 90.31%      |
| 40                    | 70.47%    | 78.61%   | 76.21%         | 85.58%      |
| 60                    | 56.98%    | 73.72%   | 62.09%         | 80.37%      |
| 80                    | 27.91%    | 51.86%   | 45.81%         | 55.51%      |
| 100                   | 0%        | 0%       | 0%             | 0%          |

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