

Effects of Ethanolic Extracts of *Enantia chlorantha* (Oliv) Bark Against Fungal Spoilage of *Dacryodes edulis* (G. Don, H. J. Lam)

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Abstract: This research investigated the effects of ethanolic extracts of *Enantia chlorantha* compared to standard antifungal drug (ketoconazole) against pathogenic fungi; *Phoma glomerata* (Corda) Wollenw. & Hochapfel and *Rhizopus stolonifer* (Vuillemin) using potato dextrose agar (PDA) medium. The experiment was laid out in Forest Pathology Unit, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt in a completely randomized design (CRD) with five replications. Data collected were subjected to analysis of variance (ANOVA) and the mean separation was done using New Duncan Multiple Range Test (NDMRT) at the probability of 5%. The quantitative and qualitative phytochemical analysis of *E. chlorantha* (Oliv)bark was carried out in the Department of Plant Science and Biotechnology Laboratory, University of Port Harcourt Choba, using chemical methods to screen the phytochemical constituent present in the *E. chlorantha* bark. Results of the screening showed that the plant had the highest amount of phenol (2.48%), saponin (5.82%), flavonoid (4.13%), tannin (2.59%), alkaloid (2.32%) and the least was cynogenic glycoside (2.00% mg/kg). The results on the effects of ethanolic extract of *E. chlorantha* bark significantly ($P \leq 0.05$) reduced the mycelial growth of *P. glomerata* and *R. stolonifer*. The results indicates that at varying level of concentrations of the extracts; 2 to 8mls extracts significantly ($P \leq 0.05$) inhibited the mycelial growth of the test fungi. However, 8ml of the *E. Chlorantha* competed favorably with the standard antifungal drug (ketoconazole) ($0.00\text{cm} \pm 0.01 - 0.00\text{cm} \pm 0.01$). It is therefore recommended that the use of ethanolic *E. chlorantha* bark extracts reduced the growth of some fruit-borne fungi of African pear and the plant (*E. chlorantha*) is eco-friendly and readily available for use by farmers and alike to improving the yield of three fruit.

Keywords: African pear, ethanolic extract, *Enantia chlorantha*, Ketoconazole Phytochemical, constituent.

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I. Introduction

African pear (*Dacryodes edulis*) is an important fruit in the tropical Africa. The tree is common in the region and belongs to the family Burseraceae which are mainly shrubs and trees with resinous aromatic gum on their bark. The fruit has a central core with fleshy edible layers as epicarp and mesocarp. It is found in the rain forest zone of Africa ranging from Serria Leone, Ghana, Nigeria, Equatorial Guinea and Cameroon (Ejiofor and Okafor, 1997, Onuorah, *et al.*, 2001). It is called by different names among the Nigerian tribes, Ube (Igbo), Eben (Efik) and Elemi (Yoruba) (Chukunda, 2014).

The African pear, African plum or Safou, locally called 'Ube' among the Igbos in South eastern part of Nigeria belongs to the family of Burseraceae and botanically known as *D. edulis*. It is an indigenous fruit tree grown in the humid low lands and Plateau regions of West, Central African and Gulf of Guinea countries. In South-eastern Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October. It is an annual fruit of about 3cm in diameter and contains a leathery shelled stone surrounded by a pulpy pericarp about 5mm thick. The pericarp is butyraceous, i.e. having the qualities of butter. It is this portion of the pear which is eaten, either raw or cooked that forms a sort of 'butter'. Besides, the pulp contains 48% oil and a plantation can produce 7-8 tonnes of oil per hectare. This makes it useful as feedstock for biodiesel production. (Awono *et al.*, 2002).

D. edulis fruit has high dietary contributions as it is softened in various ways, (hot water, hot-ash, or grilled) and eaten with maize and yam boiled, roasted or fried (Okigbo, 1977; Nwanekezi, 2005). It has also been reported as a source of oil for both domestic and industrial uses (Okorie *et al.*, 2000; Osagie and Odotuga, 1980). Though African pear has high nutritional values and said to be rich in proteins, fats, carbohydrates, high energy value, its keeping qualities are quite low due to microbial deterioration (Leakey, 1991, Yumbi *et al.*, 1989). This means that much of the products are lost following microbial attacks and spoilage.

The contribution of fruits and vegetables in human nutrition cannot be neglected but is limited due to the presence of fungal attack which make some essential nutrients unavailable for human nutrition. There is a geometric increase in demand for fruits/vegetables in Nigeria (Ikhuoria *et al.*, 2007; Oyolu, 1980).

The production and commercialization of African pear has been on the increase in the past few years and transactions are now known to cut across some national and international boundaries (Awono *et al.*, 2002). The fruit is rich in lipids infact investigations into the nutritional composition of the fruit has focused on the oil content (Omote *et al.*, 1987; Kinkela *et al.*, 1993). Kapseu *et al.*, (1996). Studies on the lipid and fatty acid content of the fruit. Gadet *et al.*, (2005) and Lam *et al.*, (1987) reported that the oil composition of the fruits depend on the fruit origin and ripening conditions as well as the variety. However, *D. edulis* is consumed in Nigeria as raw, roasted or boiled in hot water and is eaten alone or used in the garnishing of fresh maize (Arisa *et al.*, 2008; Lam,1985). Despite the increased consumption of *D. edulis* fruits in Nigeria, particularly in the Port Harcourt Metropolis, the fruits have faced huge waste due to lack of proper post-harvest preservation. Microorganisms penetrate the intact cuticle of the fruits through natural openings or wound during harvest (Nwufo *et al.*, 1989). Microbial deterioration of the fruits have paved a way for the investigation of nutritional composition of *Dacryodes edulis*.

Enantia chlorantha according to the Thesaurus of Agricultural Organizations (1990) is otherwise known as 'African whitewood'. It is an ornamental tree found in the rainforests of Nigeria, Liberia, and Cote d' Ivoire, *Enantia chlorantha* is a fair sized ornamental forest tree that can grow to heights of 30 m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, Moambe Jaune and Annikia chlorantha. The tree grows to about 30m high with dense foliage and spreading crown with fluted stem which produces a sulfurous yellow dye as reported by Iwu (1993). It is used locally across Africa to make unpainted furniture and veneers. It is referred to locally by the Nigerian Yoruba tribe as "Dokita Igbo" which literally means "Doctor of the forest" due to medicinal use in rural West Africa to treat several ailments. In Nigeria, traditionally it is used in the treatment of malaria as reported by Gbadamosi and Oni (2005).

Gill and Akinwunmi (1986) also reported the use of the infusion of *E. chlorantha* stem bark for the treatment of pulmonary tuberculosis and infected wounds. The stem bark is made up of an inner bark which is bright yellow and an outer cork which is dark brownish.

For these reasons, the need to detect the fungi responsible for the spoilage of *Dacryodes edulis* fruits in Port Harcourt Metropolis and therefore became imperative, as this research would provide information on the food safety of *Dacryodes edulis* for human and animal consumption and possibly the use of plant based extracts as an alternative to chemicals in controlling fungi pathogen in an infected fruits.

Specific objectives of the research are to;

1. determine the percentage occurrence of fungi found in fruits of *Dacryodes edulis* sold in Port Harcourt Metropolis.
2. identify the fungi species isolated from the infected fruits.
3. determine the phytochemical Constituents of *Enathia chlorantha* bark
4. evaluate the effect of *ethanolic* extract of *Enantia chlorantha* bark to control isolated pathogens.

II. Materials And Methods

Study Area

This study was carried out at the Laboratory of Forestry and Environment (Forest Pathology Unit) Rivers State University, Nkpolu-Oroworukwo, Port Harcourt and Department of Plant Science and Biotechnology Laboratory, University of Port Harcourt Choba, Nigeria.

Sources and Collection of plant Materials

African pear (*Dacryodes edulis*) fruits was collected from three (3) markets in Port Harcourt Metropolis, namely, Choba, Oil Mill and Mile Three Markets.



Plate 1: Photograph of African Pear

The bark of *Enantia chlorantha* plant was collected from Cross Rivers State National Park Akamkpa. The sample was subjected to surface stabilization using 5% alcohol and then air dried and further grounded into powder using blender (Monlinex 530, 240) and parked in polythene bags for further analysis (Chukunda *et al.*, 2019).

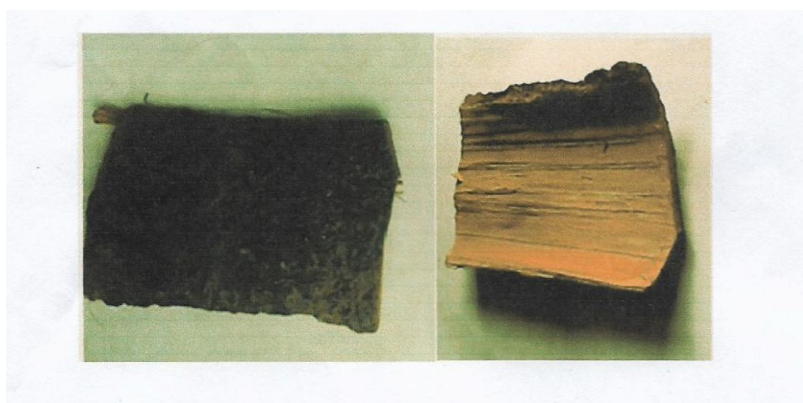


Plate 2: Photograph of *Enantia chlorantha* Bark

Preparation of Extracts

Twenty grams (20g) each of the *Enantia chlorantha* bark powder was extracted with 90% ethanol in 250ml beakers which 72 hrs. The extracts was collected in separate container and concentrated to dryness in a flash evaporator (Buch type) under reduced pressure to obtained the enthanolic extracts (Uma, 2009; Chukunda *et al.*, 2019).

Isolation of Pathogen

The infected fruit parts showing typical symptoms of infection was cut and placed on a Petri dishes for fungi sporulation (Chukunda *et el* 2019) The fungal culture was maintained in Potato Dextrose Agar (PDA) medium stored at 40°C to be used in determining the antifungal activity of *Enantia chlorantha* plant (Uma, 2009; Chukunda, *et al.*, 2019).



Plate 3: Picture Showing Pure Culture of *Rhizopus stolonifer* and *Phoma glomerata*

Effect of Ethanolic Extracts of *Enantia chlorantha* Bark against Fungi Mycelia Growth

The antifungal activity assay was carried out using potato dextrose agar methods (Chukunda *et al*, 2019) Ethanol extracts of *Enantia chlorantha* was made up of 2, 4, 6 and 8mls concentration respectively each percentage concentration of the extracts sample was mixed with potato dextrose agar (PDA) and allowed to solidify before introducing a 5mm disc of the fungus and incubated at a room temperature of ($27 \pm 2^\circ\text{C}$). The linear mycelia growth of the fungus on the extracts was measured along transect in two directions at right angles to each other after 14 days of incubation and a mean diameter value of fungus growth inhibition was measured and recorded (Chukunda *et al*, 2019, Elenwo, 2009).

However, 200mg Ketoconazole was added in each separate Petri dishes which served as standard control and the Petri dishes were incubated at room temperature of ($27 \pm 2^\circ\text{C}$). Later the linear fungus growth inhibition was measured and recorded to compare with the extracts using a transparent meter rule (Chukunda, 2014; NCCLS, 1998).

Phytochemical Analysis of *Enantia chlorantha* Bark

Phytochemical screening of the extract of *Enantia chlorantha* bark was carried out according to the methods described by Trease and Evans (1989) and Mann *et al.*, (2008). Ten grams (10g) of the ground bark samples was separately soaked in 200ml of ethanol and allowed to stand for 72 hours for extraction. After the 72 hours, it was filtered using No. 1 Whatmman filter paper. The filtered samples was sterilized and later evaporated to dryness for the detection of active components like tannins, alkaloid, saponin, cynogenic, glycoside, flavonoid and phenol.

Alkaloid Determination

1ml of 1% hydrochloric acids was added to 3ml of extracts of bark of – *Enantia chlorantha* in a test tube. The respective mixture was heated for 20 minutes, cooled and filtered. Two drops of Mayer’s reagent was added to 1ml of the extract. A creamy precipitate was an indication of the presence of alkaloids (Mann *et al*, 2008; Abalaka *et al.*, 2010; Chukunda *et al.*, 2019).

Cynogenic Glycosides Determination

10ml of 50% sulphuric acid (H_2SO_4) was added to 1ml of the extracts of *Enantia chlorantha* bark and the mixture respectively was heated in boiling water for about 15 minutes. 10ml of fehling’s solution was then added and the mixture boiled for 5 minutes, a brick red precipitate was formed confirming for the presence of glycosides (Abalaka *et al.*, 2010; Chukunda *et al.*, 2019).

Tannins Determination

1ml of freshly prepared 10% potassium hydroxide (KOH) was added to 1ml of the extracts *Enantiachlorantha* bark. A dirty white precipitate showed the presence of tannins (Hagerman, 2002).

Saponins Determination

Two (2ml) of the extracts of *Enantia chlorantha* bark was vigorously shaken in the test tube for 2 minutes and no fothing was observed, 5 drops of olive oil was added to 3ml of the extract in the test tube and vigorously shaken, absence of stable emulsion formed showed absence of saponins (Akharaiyi, 2011).

Flavonoid Determination

One millimeter (1 ml) of 10% sodium hydroxide (NaOH) was added to 3ml of the extracts of *Enantia chlorantha* bark, there was yellow coloration which is indicative of the presence of flavonoids (Chukunda, *et al.*, 2019).

Experimental Design and Statistical Analysis

The experimental was laid in a completely randomized design (CRD), the treatment was replicated three times. Data collected was analyzed using analysis of variance (ANOVA) using SPSS Genstat software as described by steel and Torrie (1980). Duncan Multiple Range test at a probability of 5% (DMRT) to separate the means.

III. Results And Discussion

The results on the quantitative and qualitative phytochemical constituent of *Enantia chlorantha* bark are presented in Tables 1a and b. Results in the quantitative phytochemical analysis showed that phenol has the highest percentage value of (8.77%) followed by the presence of saponin (2.82%), flavonoid (4.13 %) tannin (2.59 %), alkaloid (4.32%), and the least cynogenic glycoside (2.00% mg/kg). In the qualitative values, the presence of alkaloid, flavonoid and phenol were moderately found to be higher compared to saponin and cynogenic glycoside were the least found in the plant tissues.

Consequently, this suggests that in quantitative analysis phenol has the highest percentage followed by saponin, flavoniod, alkanoid & cynogenic glygoside while in qualitative, the presence of alkaloid, flavanioid and phenol were moderately found in the plant tissue.

In this study *E. chlorantha* was attributed to contain saponin, which are known to have deleterious haemolysing effect on circulating erythrocytes (Sofowora, 1993) while tannin are often determine by reducing power and scavenging activities. This is in consonance with the findings of Igwe and Onabanjo (1989).

The results on the effect of ethanolic extract of *Enantia chlorantha* bark against the mycelial growth of *P. glomerata* and *R. stolonifer* are presented in Table 2. The results indicate that at varying concentrations of the extracts from 2ml to 8mls of the plant extract significantly ($P < 0.05$) inhibited the growth of the test fungi. However, 8ml of the *E. chlorantha* completed favorably with the standard antifungal drugs (Ketoconazole) both treatments inhibited the growth of fungal pathogen significantly.

Many studies had revealed that most modern drugs could no longer solve the problem of these infection of pathogens have been identified in laboratory and field studies and these development of some modern drugs could not be possible in the absence of the phytochemical constituent of plant and *Enantia chlorantha* is not an exception (Akharai, 2011). The use of folklore medicine due to its analgesic and antiasthmatic activities (Oni, *et al.*, 2007). Similarly, Chukunda *et al.*, (2019) reported on the ethanolic effect of *Datura metel* in the mycelial inhibition of *Lasiodiplodia theobroma* that infected *Khaya grandifolia*.

The result of the antifungal activity of ethanolic extract of *Enantia chlorantha* showed that test fungi were highly sensitive to the extract and the standard antifungal (Ketoconazole).

The result of the present finding agrees with the report of Huda *et al.*, (2015) who reported that ethanolic extract of *Enantia chlorantha* completed favourably with the standard antibiotic such as ketoconazole, kanamycin, cefotloxine, streptomycin and Refampin to fight against microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis* and *Kiebsiella pneumonia*.

Alharaiyi (2011) had earlier reported that *E. chlorantha* is a natural source of antioxidant and having antimicrobial activities. Reddy (2009) similarly, observed that the extract of *E. chlorantha* showed significantly antimicrobial activity against *staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas acryginosa*, *Escherichia coli*, *Aspergillus niger* and *Fusurium* species. This report agreed with the present finding whereby the plant extracts significantly inhibited the mycelial growth of *R. stolonifer* and *Phoma glomerata*. Medicinal plant such as *Enantia chlorantha* has been used for certain ailment caused by several microbial diseases such as malaria due to their vulnerable effects in the health care (Amjad *et al.*, 2005). The plant is used in alternative medicine for providing remedy against many diseases (Akroum *et al.*, 2009).

Fasola *et al.*, (2011) who reported on the potentials of *E. chlorantha* extracts control yellow fever virus. Similarly, Agbaya and Onabanjo (1994, 1998) expressed the analgesic anti-pyretic, and anti-malarial actions to *E. chlorantha* against some laboratory pathogens.

IV. Conclusion And Recommendations

Conclusion

The antifungal activities of *E. chlorantha* can be comparable to the standard antifungi (Ketoconazole). Therefore, this research offers scientific information on the use of *Enantia chlorantha* plant extract for the treatment of infection that could be caused by microorganism against plant and human. Result also established that 2 to 8ml extract of *E. chlorantha* showed fungicidal and fungistatic potency for the treatment of any pathogenic fungi.

In conclusion, the plants chemical found in *Enantia chlorantha* are thought to have the potentiality of useful drugs if properly harness since it is ecofriendly

Recommendations

- (1) The results observe considered the plant with high phytochemical quality for antifungal effectiveness.
- (2) The plant extract of *Enantia chlorantha* should be subjected to further analysis to screen for its toxicity and side effect for perfect use.
- (3) It is also recommended that 2 to 8ml of ethanolic extract of *E. chlorantha* inhibited growth of the test fungi isolated from *Dacryodes edulis* fruit.

Table 1a: Quantitative Phytochemical Constituents of *E. chlorantha*

Sample	Alkaloid (%)	Flavonoid (%)	Saponin (%)	Phenol (%)	Tannin (%)	Cynogenic glycoside (mg/kg)
	4.32 ± 09.02	4.13 ± 0.03	2.02 ± 0.04	8.77 ± 0.02	2.59 ± 0.03	2.00 ± 0.03

Table 1b: Qualitative Phytochemical Constituent of *Enantia chlorantha*

Phytochemical constituents	Description	Symbols of Conc
Saponin	Moderately present	++
Alkaloid	Highly present	+++
Flavonoid	Highly present	+++
Phenol	Highly present	+++
Cynogenic glycoside	Moderately present	++

Table 2 Ethanolic Extract of *Enantia chlorantha* on the mycelial growth of *P. glomerata* and *R. stolonifer*

Ethanolic Extract of <i>Enantia chlorantha</i> (ml)	<i>R. stolonifer</i>	<i>P. glomerata</i>	Anti-fungal Ketoconazole
2	0.6 ± 0.01 ^d	0.4 ± 0.02 ^d	
4	0.4 ± 0.01 ^c	0.3 ± 0.01 ^c	
6	0.2 ± 0.02 ^b	0.2 ± 0.02 ^b	0.00 ± 001
8	0.0 ± 0.01 ^a	0.0 ± 0.001 ^a	

Mean value with the same superscript (a,b,c) in the same column are not significantly ($p \leq 0.05$) different by DMRT ($p \leq 0.05$).

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