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Effects of Aqueous Extract of *Enantia chlorantha* (Oliv) Bark Against Fungal Spoilage of Avocado Pear *(Persea gratissima:* CF. Gaertn*)*

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Abstract: *The aim of this research was to investigate the antifungal efficacy of aqueous extract of Enantia chlorantha with standard antifungal drug 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, Port Harcourt in a completely randomized design (CRD), with three replicates. Data collected were subjected to analysis of variance (ANOVA) and the mean separation was done using Duncan Multiple Range Test (DMRT) at the probability of 5%. The quantitative and qualitative phytochemical analysis of E. chlorantha (Oliv) bark were 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 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 aqueous extract of E.chlorantha bark significantly reduced the mycelial growth of P. glomerate and R. stolonifer. The result indicates that at varying level of concentrations of the extracts of2 to 8mlsof the plant extracts significantly ($p \leq 0.05$) inhibited the growth of the test fungi. However, 8ml of the E. chlorantha competed favorably with the standard antifungal drug ketoconazole (0.20±0.25 – 2.00±0.12). It is therefore recommended that the use of aqueous E. chlorantha bark extracts reduced the growth of some fruit-borne fungi of avocado pea and the plant is eco-friendly, cheap and readily available.*

Keywords: *Antifungal, Aqueous extract, Enantia chlorantha, Ketoconazole Phytochemical*

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I. Introduction

Avocado *(Persea gratissima*. C.F. Gaertn*)* commonly called Avocado pear is a member of the family Lauraceae, which are mainly shrubs and trees that yield resinous aromatic gum from their cut bark. It is among the well-known indigenous fruit trees in the tropical and subtropical rain forest zone of the Southern regions of West Africa (extending eastward from Sierra Leone to Nigeria and Western regions of Central Africa, which includes Cameroun, Equatorial Guinea, Gabon, Democratic Republic of Congo, Congo Brazzaville and Angola).

The fruit is a pome, characterized by a central core surrounded by edible fleshy layers (Barry, 2001). The Avocado fruit has a pulpy mesocarp of 3 to 9 mm^2 thickness, 7cm-20cm long, weighs 100g – 1000g and has a large central seed, 5cm – 6.4cm long. The skin texture is finely pebbled and dull green when ripe (Barry, 2001). Avocado fruit is a major and cheap source of nutrients containing protein (2g), moisture (72.23g), fiber (6.7g), fat (14.66g) and carbohydrate (8.53g) and high energy value of 160 kcal per 100g. They are also rich in fatty acids, amino acids, potassium, B-vitamins, vitamins K and E. Avocado fruit is much cherished by many people and it makes a significant dietary contribution, as it improves the food problems in developing countries. Besides, it is available at most seasons including strategic periods of the year when conventional staples that are difficult to store are scarce (Wogu, 2014).

The oils from the pulps and seeds can be used in foods, pharmaceuticals and cosmetics manufacturing as well as numerous industrial uses. They are rich in monounsaturated fatty acids and are comparable to other currently used vegetable oils (Dennis C. , 2013).

Avocado fruit used in commerce are picked hard and green and kept in coolers at 3.3^0 to 5.6^0C, until they reach their final destination. Once picked, avocados ripen in a few days at room temperature. The fruit has a very short shelf-life. It can averagely be stored 3-6 days before spoilage.

The poor shelf life of the fruit has led to its high perishability, huge losses and market glut during harvest as noticed by large heaps of unsold rotten fruits in the refuse dumps of village and urban markets. These

characteristics of Avocado fruits are serious setback for export market as well as industrial uses, as it does not offer flexibility throughout the market channels. The avocado fruit is vulnerable to bacterial, viral, and fungal diseases which lead to its spoilage. Diseases and microorganisms can affect the fruit causing spotting, rotting, cankers, pitting and discoloration (Samson and Van Reenen-Hoekstra, 1988). Numerous species of microorganisms easily attack the fruit. The high spoilage rate of Avocado fruit coupled with its high nutritional contents pre-supposes that an array of microorganisms may be involved in its spoilage of avocado fruits.

Enantia chlorantha according to the Thesaurus of Agricultural Organisms (1990) is otherwise known as 'African whitewood'(Adesokan, 2015). 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 sulphurous yellow dye as reported by (Eldeen and Van-Staden, 2007). 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).

Nyong, Odeniyi & Moody (2015) 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.

The use of fungicides is the primary method of control of postharvest fungal decay of fruits. This has raised several reasons of public concern for human health conditions and environmental pollution associated with pesticide usage in orchards, persistence of residues on treated fruits, development of resistance of fungal pathogens to fungicides and high costs of new chemicals have motivated the search for alternative approaches such as Biological control. Hence the use of the plant extract of *Enantia cloranthia* in this research.

Specific objectives of the research are to;

1. isolate the fungi associated with the spoilage of avocado pears stored at room temperature.
2. identify the fungi isolated using macro-morphological features of the fungi.
3. determine the phytochemical constituents *Enantia chlorantha* bark.
4. evaluate the effect of aqueous extract of *Enantia chlorantha* bark against the fungi growth as compared with antifungal ketonaczonal drugs.

II. Materials And Methods

Study Area

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

Sources and Collection of Plant Materials

Avocado fruit was collected from two markets in Port Harcourt Metropolis; Fruit Market at D-line, Nkpolu-Oroworukwo market Port Harcourt.

The bark of *Enantia chlorantha* was collected from Cross River State National Park Akamkpa. The sample was subjected to surface stabilization using 50% alcohol and then air dried and further ground into powder using blender (Monlinex 530, 240) and parked in polythene bags for further analysis.

Plate 2 Photograph of of Avocado Pear (*Perseagratissima)*

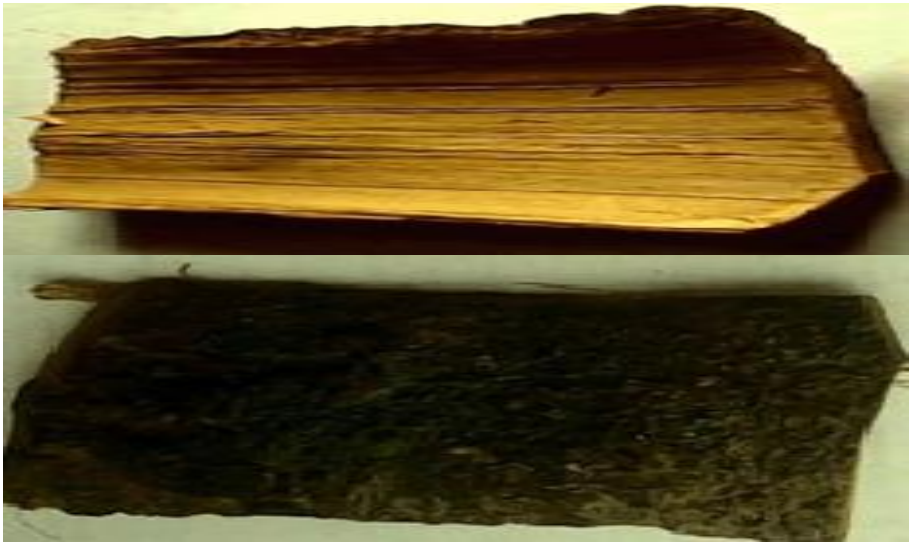

Plate 2 Photograph of *Enantia chlorantha* bark

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 1 Whatmman filter paper. The filtered samples was sterilized and later evaporated to dryness for the detection of active components like tannins, alkaloids, saponins, glycosides and flavonoids.

Alkaloid Determination

1ml of 1% hydrochloric acids was added to 3ml of *Enantia chlorantha* bark extracts in a test tube. The respective mixture was heated for 20 minutes, cooled and filtered. About 2 drops of Mayer's reagent 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).

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 solution was added and the mixture boiled, a brick red precipitate was confirmatory 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 *Enantia chlorantha* bark. A dirty white precipitate will show the presence of tannins (Hagerman, 2002; Chukunda *et al.,* 2019).

Saponins Determination

Two (2ml) of the extracts of *Enantia chlorantha* bark was vigorously shaken in the test tube for 2 minutes and no forthing 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 will show absence of saponins (Akharaiyi, 2011; Chukunda *et al.,* 2019).

Flavonoid Determination

One millimeter (1ml) of 10% sodium hydroxide (NaOH) was added to 3ml of the extracts of *Enantia chlorantha* bark, there was no yellow coloration which is indicative of the absence of flavonoids.

Preparation of Extracts

Twenty grams (20g) each of the *Enantia chlorantha* bark Powder was extracted with 500ml beakers of distilled water. The extracts was collected in separate container and concentrated to dryness in a flash evaporator (Buch type) under reduced pressure to obtained the Aqueous extracts (Uma, 2009; Chukunda *et al.,* 2019)

Micro-Organism Used

Stock culture of *Rhizopus stolonifer* (Veillemin) and *Phoma glomerate*,(Wollenw and Hochapfel) collected from spoilt avocado pears. The fungal culture was maintained in Potato Dextrose Agar (PDA) medium and was stored at 40^0C then used in determining the antifungal activity of *Enantia chlorantha* plant (Uma, 2009; Chukunda *et al.,* 2019).

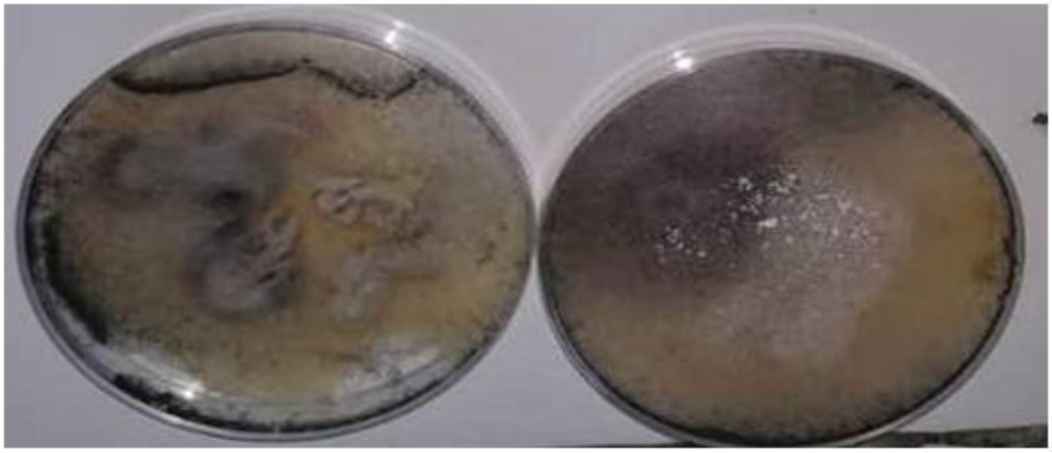

Plate 1: Picture showing stock pure culture of *Rhizopus stolonifer*

Plate 2: Picture showing stock pure culture of *Phoma glomerata*

Effect of Aqueous Extracts of *Enantia chlorantha* Bark Agaiot Fungal Mycelia Growth

The antifungal activity assay was carried out using potato dextrose agar methods (Chukunda *et al*, 2019). Aqueous extracts of *Enantia chlorantha* was serially diluted to 2ml, 4ml, 6ml and 8ml concentrations respectively each milligram (ml) concentration of the extracts of 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 ± 2^0C). The linear extension of the fungus on the aqueous extracts of the samples concentrated will be measured along transect in two directions at right angles to each other after 7 to 14 days of incubation and a mean diameter value of fungus growth inhibition was recorded (Chukunda, Baraka, & Umoren, 2019).

However, ketoconazole (Antifungal drug) was added in each separate Petri dish which served as standard control and the petri dishes were incubated at room temperature of ($27\pm2^{\circ}$). Later the linear fungus growth inhibition was measured and recorded using a transparent meter rule (Chukunda, Baraka, & Umoren, 2019).

Experimental Design and Statistical Analysis

The experiment was laid out in a completely randomized design (CRD). The treatment was replicated three times. Data collected was analysed 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

Quantitative And Qualitative Phytochemical Constituents of *Enantia chlorantha*

The results on the quantitative and qualitative phytochemical constituents of *Enantia chloranta* bark are presented in Table 1a and 1b. results in the quantitative phytochemical analysis showed that phenol has the highest percentage value of (8.77%) followed by the presence of saponin (5.82%), flavonoid (4.13%) tannin (2.59%), alkaloid (2.32) and the least Cynogenic glycoside (2.00% Mg/Kg).in the qualitative phytochemical analysis of the *Enanthia chloranta* bark, the presence of Alkaloids, Flavonoid and phenol were found to be high compared to saponin and cynogenic glycoside which were moderately found in the plant tissues.

Antifungal efficacy of aqueous extract of *Enantia chlorantha* against mycelia growth of test fungi

The results on the antifungal efficacy of aqueous extracts of *Enantia chlorantha* bark against mycelia growth of *Rhizopus stolonifer* and *Phoma glomerata* are presented in Table 2 and Plates 3a and b. The results of the aqueous extract of *Enantia chlorantha* bark extract showed that at 8ml concentration there was significant ($P \leq 0.05$) difference in inhibition in the fungal mycelia growth of *Rhizopus stolonifer* (2.20±0.21) and *Phoma glomerata* (3.40±0.18). Generally the extracts of *Enantia chlorantha* bark competed favourably with the standard antibiotic (Ketoconazole) against the mycelial growth of *Rhizopus stolonifer* and *Phoma glomerata*.

Numerous antagonists of post-harvest pathogens have been identified in laboratory and field studies. The development of agricultural products based on beneficial microorganisms is relevant to reducing chemical contaminants in the food supply chain. (Olamide, 2013).

Microorganisms are ubiquitous and they have been found to colonize avocado pear fruits due to its high nutritional content that can support their growth and cause spoilage this is evident in there rapid proliferation within days of storage, as reported by Wogu (2014) who stated that the availability of nutrients is crucial to the increase or decrease of microbial number in fruits during spoilage. The result from this research identified two fungal isolates namely: *Rhizophus stolonifer and Phoma glomerata.*

These fungal species isolated from spoilt avocado pear in this study were also identified in a similar investigation carried out by (Efiuvwevwere, 2000), who additionally reported that the fungi is responsible for the soft rot in avocado pears.

Enantia chlorantha bark is highly effective against different types of diseases such as anti-diabetic and antiviral. *Enantia chlorantha* is a widely growing plant and well-known to have great pharmacological potential with great utility in folklore medicine. It contains saponin, flavonoid, glycoside, alkaloid, tannin, carbohydrate and protein (Amjad *et al*., 2005)**.**

Medicinal plants such as *Enantia chlorantha* bark extract has been used for certain ailment caused by several microbial diseases due to their vulnerable effects in the health care (Amjad *et al*., 2005). The plant is used in alternative medicine for providing remedy against man diseases (Akroum *et al*., 2009).

The problem of antibiotic resistance to most micro-organisms which has led to the resurgence of interest in herbal medicinal plant products as a source of suppressing or eradicating the ever-increasing problems of resistant micro-organisms against antibiotics (Akharaiyi, 2011).

The results of the antifungal activity of aqueous extract of *Enantia chlorantha* bark, showed that the test fungal were highly sensitive to the extracts and the standard antibiotic (ketoconazole).

The results of the present finding agreed with the report of (Huda *et al*., 2015) who reported that aqueous extract of *Enantia chlorantha* competed favourably with standard antibiotic such as Penicillin, Kanamycin, Streptomycin and Refampin to fight against micro-organisms such as *Escherichia coli*, *Pseudomonas eurogensa, Staphylococcus aureus., Proteus mirabilis* and *Klebsiella pneumonia.*

Akharaiyi (2011) had earlier reported that *Enantia chlorantha* extracts is a natural source of antioxidants and phytochemical constituent having antimicrobial activities.(Reddy, Tiwari, Elanchezhian, & Maheswari, 2009)similarly observed that the extracts of *Enantia chlorantha* showed significant antimicrobial activity against *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escheria coli, Aspergillus niger* and *Fusarium* species. This report agreed with the current findings whereby the *Enantia chlorantha* bark extracts significantly inhibited the mycelia growth of *Rhizopus stolonifer and Phoma glomerata*. This is consonance with the present result findings.

(Gachande, 2013) reported that aqueous extracts of the *Enantia chlorantha* bark extracts showed good antimicrobial activities. This is consistent with the present research findings where the bark extracts of *Enantia chlorantha* inhibited the mycelia growth of *Rhizopus stolonifer* and *Phoma glomerata*.

IV. Conclusion And Recommendations

Conclusion

The antifungal activities of *Enantia chlorantha* bark extracts can be comparable to the standard antibiotic (ketoconazole). Therefore, this research offers a scientific basis for the use of the *Enantia chlorantha* bark extracts for the treatment of fungal spoilage of avocado fruits. Results also established that 8ml

concentration of the aqueous extract of *Enantia chlorantha* showed fungicidal and fungistatic potency for the treatment of *Rhizophus stolonifer and Phama glomarata*.

1) Based on the results, extracts of *Enantia chlorantha* bark should be used as fungistatic and fungicidal to control the fungal disease of avocado and other fruit tree diseases.

2) The results observed considered the plant with high phytochemical quality for antifungal effectiveness.

3) The plant extracts of *Enantia chlorantha* should be subjected to further analysis to screen for its toxicity and side effect for perfect therapeutic use on fruits.

Table 1a: Qualitative phytochemical constituent of *E.chlorantha* Bark

Sample	Alkaloid (%)	Flavonoid (%)	Saponin (%)	Tannin (%)	Cynogenic Glycosides mg/kg
	2.32	4.13	5.82	2.59	2.00

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

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

Table 2 Effects of aqueous extracts of *Enantia chlorantha* bark on mycelial growth of *Rhizopus stolonifer* and *Phoma glomerata* after one week (cm).

Conc. of the aqueous plant extracts of *Enantia chlorantha* (ml).	*Rhizopus stolonifer*	*Phoma glomerata*	*Ketoconazole*
8ml	2.20 ± 0.25^{a}	2.90 ± 0.23^{a}	
6ml	3.00 ± 0.22^{b}	3.00 ± 0.24^{b}	
4ml	3.20 ± 0.35b	3.80 ± 0.32^{c}	2.00±0.12
2ml	4.10 ± 0.40c	4.35 ± 0.43^{d}	
0ml	8.50 ± 0.00d	8.50 ± 0.00^{e}	

Mean values with the same superscripts (a,b,c..) in the same column are not significantly (P≤0.05) different by DMRT.

Plate 3a: Picture showing 100% Aqueous extract of *Enantia chlorantha* bark.

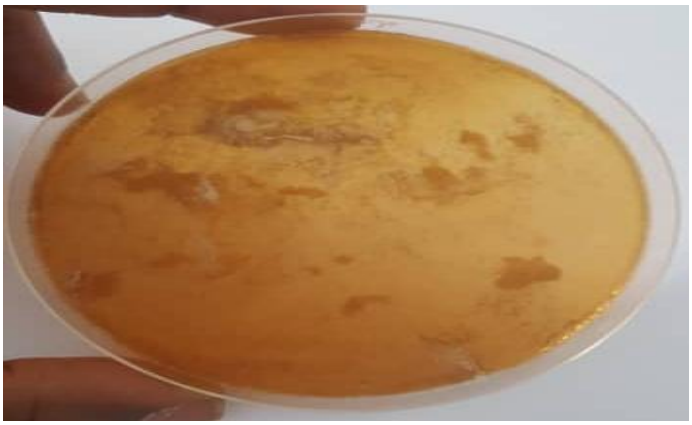

Plate 3b: Picture showing 100% antibiotic (Ketoconazole)

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