**ANTIMICROBIAL EFFECT OF *PERSEA AMERICANA* AVOCADO PEAR PEEL**

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**ENUGU STATE**

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**TITLE PAGE**

**ANTIMICROBIAL EFFECT OF *PERSEA AMERICANA* AVOCADO PEAR PEEL**

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**PROJECT**

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**JULY, 2016**

**APPROVAL PAGE**

This research work has been presented and accepted by Godfrey Okoye University Enugu in partial fulfillment of the requirement for the award of B.Sc Microbiology degree in Microbiology in the Department of Biological Sciences.

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**CERTIFICATION**

This is to certify that this research work by Nze Hope Chinaza has been examined and approved as meeting the requirements for award of B. Sc. Microbiology degree Microbiology in the Department of Biological Sciences, Faculty of Natural and Applied Sciences Godfrey Okoye University, Enugu.

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**DEDICATION**

This work is dedicated to GOD ALMIGHTY for his love, mercy, and sustenance in doing this work and to my lovely parents and siblings for their support and prayers in handling this work.

**ACKNOWLEDGEMENT**

My profound gratitude goes to Almighty God, my distinguished H.O.D and my Lectures for their immense efforts in impacting knowledge which was a great help in writing this work. I also wish to appreciate the support and encouragement of my beloved parents in making this work a success. I am also grateful to my wonderful sisters, in-laws, friends, and my colleagues for their care, encouragement, and support.

**ABSTRACT**

In the for anti microbial effect the back peel of *Persea americana* (AVACADO) was investigated for activities . the study was to evaluate the antimicrobial efficacy of the crude ethanolic and aqueous extract of the peel of *Persea americana* against selected clinical isolates. The agar well diffusion method was used in study also phytochemical screening was conducted on the plant which revealed the presences of saponin, carbohydrates, tannins the present invention relates to extracts from *Persea americana* enriched in bioactive compounds which can be used as antimicrobial, antibacterial or spore germinating inhibiting agents the process for obtaining the extract is to isolate molecules and method for using the extracts enriched in bioactive compounds for providing antimicrobial inhibiting effect*.*

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**CHAPTER ONE**

**INTRODUCTION**

**1.1 BACKGROUND TO THE STUDY**

Medicinal plants are plants which contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Medicinal plant have continued to attract attention in the global search for effective methods of using plants’ parts(e.g. seeds, stems, leaves, roots, peel, bark etc) for the treatment of many diseases affecting humans. Many important drugs used in medicine today are directly or indirectly derived from plants due to its bioactive constituents such as; alkaloids, steroids, tannins e.t.c. (Hill, 1952).

In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents. Thus is anticipated that photochemical with adequate antimicrobial efficacy will be use for treatment of bacterial infections.

The avocado (*Persea Americana*) belongs to the *Lauraceae* family of tropical and Mediterranean trees and shrubs; other members of this family include: laurel, cinnamon, safaris and green-heart (a timber of the Guiana’s). The avocado is thought to have originated from Mexico and Central South America; for thousands of years and till today, it has been a popular food in those places. In the mid 17th Century, they were introduced to West Africa, Mauritius and India.

The world is facing explosive increase in resistant strains of microorganisms. It poses a serious challenge to primary health care in un developing countries, with negative consequences on the economy. Antibiotics such as penicillin and erythromycin which used to have a high efficacy against bacteria species and strains, have become less effective, due to the increased resistance of many bacteria.

Previous and recent studies have shown that the leaf, back and seed extract of avocado pear (*P. americana*) are rich sources of photochemical such as saponins, steroids, alkaloids, as such a source of (Idris*et al* 2010. and Ilozue*et al*, 2014). However information on antimicrobial effect of avocado pear peel extract in Enugu is scanty. Therefore this works aims to determine and evaluate the antimicrobial activity of avocado pear peel extract in Enugu.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1** **AVOCADO**

The avocado is a tropical tree which grows well and abundantly in Samoa. It is one of the many agricultural produce which is highly under-utilized in the country. The unpopularity of the fruit amongst consumers results in a lot of fruits going to waste every time it is in season. This makes the need for value added products using such local agricultural produce very important not only to reduce food waste but also for the economic benefit of the country.

The avocado fruit is rich in nutrients, high in proteins, antioxidants and dietary fiber is perhaps the most poorly conceived and misunderstood fruit of all times. This is mainly attributed to its high fat and calorie content and so most nutritionists and dieticians either advise against it or to use it “sparingly” (Bergh, 2012).

**2.2 HISTORICAL ORIGIN OF THE PLANT**

The avocado (alligator pear or aguacate as commonly known) is scientifically known as *Persea Americana* of the family Lauraceae and is a native plant of Southern Mexico and Central America . The word avocado comes from the Aztec word *ahuacatl* which is translated by the Spaniards as aquacade meaning “testicle” due to its shape. Historical records of the usage of the plant exist from 7000 B.C. of its cultivation from 6000 B.C. and continuous use in all the well known archeological sites of Mexico. (Doughari, 2010)

**2.3 VARIETIES OF AVOCADO**

There are three races of avocado:

Guatemalan

Mexican

West India

While each has distinctive features, cross-pollination permits the development of unlimited varieties.

Mexican is the hardest of the group and the most tolerant of cold conditions. Mature trees can tolerate temperatures to 5oC without damages, however flowers are frost prone. Zutano, Bacon, Shephard and the rootstock Duke are all Mexican types.

The Guatemalan race is from the tropical highlands. It requires a cool tropical climate without extremes of humidity and temperature. Trees can withstand light frost to 2oC. Gwen and Reed are varieties from this race.

The West Indian race originated in the humid low lands of tropical Central America. This race is the most tolerant to saline soil and water. They are the most susceptible to cold weather. (Murakoshi, 2010)

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**Figure 2.1: Avocado pear**

**Image from google (Assessed on 20th june 2016)**

**2.4 SCIENTIFIC CLASSIFICATION OF AVOCADO PEAR**

**Taxonomy**

Kingdom *Plantae*

Phylum *Angiosperm*

Super class *Mannolianae*

Super order *Laurales*

Family *Lauraceae*

Genus *Persia*

Specie *P. Americana*

Authourity Miller

**Common names**

English: alligator pear, avocado, avocado –pear, butter fruit

Amharic*: avocado*

Burmese: *htaw bat, kyese*

Creole: *zaboka*

Filipino: *avocado*

French*: avocet, avocatier, zabelbok, zaboka*

German*:Allgatorbine, Avocadobirne*

Indonesian: *adpukat, avocado*

Khmer*: avokaa*

Malay: *apukado, avocado*

Mandika: *avocado*

Pidgin English: *bata*

Spanish agucate*: pagua*

Swahili: *mparachichi*

Thai*: awokado*

In some Nigerian Languages: Igbo Ube-oyibo, Yoruba pia Efik : eben, Benin: orumwu. (Wigg*et al*, 2013)

**2.5 CULTIVATION**

The tree grows to 20 m (66 ft), with alternately arranged leaves 12–25 cm (4.7–9.8 in) long. The flowers are inconspicuous, greenish-yellow, 5–10 mm (0.2–0.4 in) wide. The pear-shaped fruit is 7–20 cm (2.8–7.9 in) long, weighs between 100 and 1,000 g (3.5 and 35.3 oz), and has a large central seed, 5–6.4 cm (2.0–2.5 in) long (Dowling and Morton, 2011). The subtropical species needs a climate without frost and with little wind. High winds reduce the humidity, dehydrate the flowers, and affect pollination. The trees also need well-aerated soils, ideally more than 1 m deep. Yield is reduced when the irrigation water is highly saline. These soil and climate conditions are available in southern Nigeria especially in Enugu State.

**2.6 AVOCADO PROPAGATION**

Growing avocado directly from the seed is not favorable because it bears fruits only after 4 – 6 years of growth and it rarely resembles the parent cultivar. The avocado has hypogeal germination, meaning that the shoot grows directly from the epicotyls in the soil. Commercial orchards are thus usually propagated by either grafted trees or rootstocks. Rootstocks are propagated by seeds (seedling rootstocks) and layering (clonal rootstocks). One common method is the etiolation technique used for propagating the desired clonal rootstock specific for disease and soil conditions. Lateral and terminal grafting is normally used and carried out for the young plants after one year of growth in green houses(Berg, 2012).

**2.7 PEST AND DISEASES AFFECTING AVOCADO**

A soil borne fungus known *as phytophthoracinnamomi* is a very sever disease which causes root rot of the trees. The disease is easily transported by equipment, tools and shoes from infected soil, so farmers are highly encouraged to use disease free and certified plants or rootstocks. Once a tree is infected it is difficult to treat except to cut back on water supply.

*DothiorellaBotryosphaeria* ribs canker is another fungus which infects the trunk and results in dead patches which spreads to maturing fruits causing rancid smelling, darkened spots on the flesh. This disease which starts upon harvest cannot be detected on the outside and has no means of control. A viral disease known as Sun blotch causes shrinking of new leaves, yellowed streaking of young stems, cracking of the trunk and occasional fruit deformation. It is spread by the use of contaminated tools and scion and so using virus-free propagating wood is a must (Platt-Aloia and Thomson, 2010).

Pests include rats, leaf caterpillars, avocado brown mite, six spotted mite and also snails.

**2.8 HARVEST AND POST HARVEST OF AVOCADO**

Avocado fruits are strange in that they only start to ripen and turn soft when they are picked. They remain hard and continue to grow when mature on the tree until they fall off. The fruits can be left on the tree up to (4 – 6 months) after being fully developed and will ripen very quickly once picked. The taste of the fruits at the time of harvest depends on their oil content which in turn is dependent on their stage of maturity. Avocados can ripen quickly when stored together with other fruits like banana and apples due to the production of ethylene gas. The fruits must be handled with care when harvested to minimize physical damage and bruising which results in undesirable discoloration and softening of the pulp. The fruit ripening process is slowed considerably by low temperature. (Platt-Aloia and Thomson, 2010) reported that it is high activities of wall hydrolytic enzymes during ripening that result in ultra structural changes in the cell walls of ripened avocado fruits. Extended cold storage results in chilling injury which is marked by improper softening of flavor development and discoluoration of the mesocarp. The major storage component of the avocado fruit is the oil contained in its mesocarp. Avocados are usually sold in the market or the road sides in Enugu where one can buy them ripped or unripe (Platt-Aloia and Thomson, 2010).

**2.9 HEALTH BENEFIT OF AVOCADO**

Bergh, 2011, described the avocado fruit to be nutrition-rich while others in the industry call it a functional food due to its additional health benefits from certain phytochemicals. It contains high amounts of vitamin A, B, C, E and other nutrients like folacin, niacin, iron (Fe), magnesium (Mg), folate, Pantothenic acid and contains 60% more potassium than bananas.

Vitamin E, C and beta carotene (vitamin A precursor) are natural antioxidants which protect against dangerous “free radicals” which are by-product of life processes due to oxygen (Bergh, 2011). These free radicals results in cataracts from eye tenses, cancer due to cell mutation, arthritis, advanced aging process and heart disease due to cholesterol buildup. These antioxidants are specifically effective in reducing the oxidation of the low density lipoprotein (LDL) which leads to plaque deposits in arteries. The role of vitamin E involves in slowing down the aging process makes avocado very important in cosmetic industry.

 Avocado protein has also been proven to contain all the essential amino acids for human nutrition attributes not provided by any other plant source (Bergh, 2011). Its fiber content was also noted to be high in both the soluble and insoluble forms and this is considered very advantageous due to fiber’s lowering effects on cardiovascular disease, hypertension, diabetes and obesity. Pectin in particular a water-soluble fiber is known to be most effective in maintaining heart health.

The avocado fruit contains more calories per gram than most other foods and thus people tend to avoid it because of the well known adverse effects of cholesterol on humans. The fat content of avocado which is the cause for much misconception however is another valuable aspect of the fruit. More than 70% of its fat is monounsaturated fat with low levels of polyunsaturated and saturated fat with slight variations according to cultivars and fruit maturity stage (Arpaia *et al,* 2011). Monounsaturated fat in particular has been noted by (Bergh, 2011) in two of his papers to be highly beneficial is that it not only lowers the level of harmful cholesterol (LDL) but also maintains the levels of the beneficial high density lipoprotein (HDL) or good cholesterol which protects the heart (Eyres *et al,* 2012).

**2.10 CHEMICAL COMPOSITION OF AVOCADO**

**2.10.1 FATTY ACID COMPOSITION**

Typical avocado oil is comprised mostly of monounsaturated fatty acids (74%), 11% polyunsaturated fatty acid and about 13% saturated (Arpaia*et al,* 2011). These percentages vary slightly with cultivars and other influential factors but the oil is very similar to olive oil. It is this high level of monounsaturated fat which gives the desirable effect of being “anticholesterol” as it prevents the formation of clots, the major cause of coronary heart disease.

**2.10.2 PHYTOCHEMICALS COMPOSITION**

The naturally occurring phenolic compounds found in vegetables and fruits have been proven to have equal or greater cholesterol lowering properties than unsaturated fatty acids (Nicolosi and Orthoeter, 2010). Beta-Sitosterol (a phytosterol) is one of the healthy plant compounds found to be most abundant in avocado. It is widely proven to be responsible for the non-absorption of the bad cholesterol (LDL) and maintaining the good HDL cholesterol in the intestine which then lowers total plasma cholesterol (Arpaia *et at,* el 2011).

The phytosterol content has the same skin penetrating abilities of lanolin and for this reason avocado oil is highly valuable in the cosmetic industry. Lutein or carotene is also highly abundant in avocado oil. This phytochemical is effective in providing protection against prostate cancer, eye diseases and macular degeneration.

**2.10.3 PHARMACOLOGICAL ACTIVITIES OF AVOCADO**

**Analgesic and anti inflammatory activity**

The aqueous extract of *P. americana* leaves caused a significant and dose-dependent inhibition of the control writhes. The inhibition by 1600 mg/kg extract was similar to that produced by 100mg/kg of acetylsalicylic acid (57.2% and 58.0%, respectively). The inhibition (87.2%) shown by 800 mg/kg of the extract was same as morphine

(2mg/kg, 87.0%). There was a significant and dose-dependent inhibition of both phases, by the extract. A greater inhibition (77.1%) was produced by the extract (800 mg/kg) compared with acetylsalicylic acid (68%) in phase II of the test. The aqueous leaf extract of *P. americana* (800 mg/kg) produced a significant inhibition of the swelling caused by carrageenan at 3h. This effect was similar to that produced by indomethacin in the same duration.(Adeyemi*et al*., 2012)

**Antiviral activity**

the ethanol extract did not show any activity under the experimental conditions employed (de Almeida *et al*., 2008).

**Effect on body weight**

It is evident from Brail *et al* study that the administration of aqueous and methanolic back peel extracts of *P.Americana* caused a reduction in body weight compared with the hyperlipidemic controls. It could be that *P. americana* back peel extracts increase the catabolism of lipids accumulated in the adipose tissue, resulting in a decrease in the mean body weight (Brai *et al*., 2008 Pliego and Litz, 2008).

**2.11 ALLERGIES**

Some people have allergic reactions to avocado. There are two main forms of allergy: those with a tree-pollen allergy develop local symptoms in the mouth and throat shortly after eating avocado; the second, known as latex-fruit syndrome, is related to latex allergy and symptoms include generalized urticaria, abdominal pain, and vomiting and can sometimes be life- threatening (Brehler *et al*., 2014).

**CHAPTER THREE**

**3.0 MATERIALS AND METHOD**

**3.1 MATERIALS**

**3.1.1Chemicals and Reagents**

Ethanol

MULLER HINTON Agar

Distilled water

**3.2 Equipments and Glass wares**

Electronic incubator

Electronic digital weighing balance

Weighing balance

Burnsen burner

Petri dishes

Wire loop

Matches

Marker

Muslin cloth`

Black nylon

Hand glove

1000ml measuring beaker

1000ml measuring cylinder

500ml measuring cylinder

50ml conical flask

**3.3 SAMPLE COLLECTION**

The fruits of *Persea Americana* (Avocado pear) were purchased from New market in Enugu North L.G.A of Enugu state, Nigeria in June 2016. It was then conveyed to Biology department, Godfrey Okoye university Enugu state where it was identified and authenticated. It was afterwards taken to the Biology laboratory where it was allowed to ripen before it was cut open and its butter removed while the peel was washed with distilled water and air dried.

**Preparation of growth media**

**Muller Hinton Agar**

With the help of a weighing balance 6.84g of Muller Hinton Agar was weighed into 1000ml conical flask to which 500ml of distilled water was added and autoclaved for 15mins at 121oC and allowed to cool to 40 – 50oC, and then 20ml was aseptically dispensed into each Petri dishes.

**Preparation of Paper Discs**

Discs were prepared using Whatmann filter paper which were sterilized by autoclaving at 180oC for 1hour. (Ilozue*et al*, 2014)

**3.4 Plants Extract preparation**

Using an electronic blinder, the dried plant material was homogenized to fine powder. The plant material (15g) was weighed using a weighing balance in a plastic bottle to which 100ml of ethanol (%) was added using 100ml measuring cylinder, soaked and covered with viva black nylon and kept at room temperature. After 3days, the soaked mixture was filtered using a filter paper. The filtrates were exposed to 78oC in water bath for 24hours for ethanol to evaporate.

**3.5 Test Organism used for the analysis**

Three clinical isolates of bacteria species, Gram-positive Staphylococcus aureus, Gram-negative Escherichia coli, *penicillium* and *Aspergellus* of which Staphylococcus aureus was isolated from throat swab using swab stick, and Escherichia coli was isolated from urine using inoculating loop to inoculate and streak on the blood agar, which was allowed to grow for some days. These microbes were all obtained from Goldlife medical laboratory, Enugu state Nigeria

**3.6 Antimicrobial Susceptibility test**

Crude extract with a concentration of 20mg/ml was tested for antimicrobial activity by using agar well diffusion method (Bedi*et al*, 2010). 19ml of sterile Muller Hinton Agar and 1ml of sterile blood agar were dispensed in four different sterile Petri plates and autoclaved for 15mins at 37oC for 1hour. Using electronic weighing balance, 20mg of avocado peel extract was weighed into two different 50ml conical flask respectively with the addition of ethanol and distilled water Both were thoroughly shaken and allowed to stand for 20mins in order to determine the solubility of both solvents towards the sample. Result shows that ethanol dissolved more than water. The selected microbes were inoculated and streaked on the surface of the Muller Hinton Agar using a wire loop. The flamed cork borer was used to create a well in the Petri plates of Muller Hinton agar and was specified with numbers and horizontal line drawn on it.

Sterile micropipette was used to introduce about 0.1ml of each of the 20mg/ml of aqueous and ethanol extract solution of *Persea americana* peels into each of the wells. Finally, the Petri plates were incubated at 37oC for 24hours. The effect was compared with that of the control (water and ethanol), the zones showing complete inhibition were measured and the diameters of the zones were measured to the nearest millimeter. By the antibiotic zone scale the area of inhibition was measured for each extracts.

**CHAPTER FOUR**

**4.0 RESULTS**

The antimicrobial activities of the ethanol and water extract of avocado pear peel extract against *Escherichia coli, Staphylococcus* *aureus,* *penicillium* and *Aspergellus* measured in terms of their average diameter of zone of inhibition are reported in the tale below:

**TABLE 2: RESULTS ON THE ANTIMICROBIAL EFFECT OF AVOCADO (*Perseaamericana* ) PEAR PEEL EXTRACT.**

|  |  |
| --- | --- |
|  | **RESULTS** |
| MICROORGANISM  | **Aqueous extract** | Ethanol extract | Water | Ethanol |
|  | **TEST CONTROL** |
| *Escherichia coli*  | 0.00mm | 0.00mm | 0.00mm | 0.00mm |
| *Staphylococcus aureus* | 0.00mm | 0.00mm | 0.00mm | 0.00mm |
| *Penicillium* | Positive (Clearance 6mm) | 0.00mm | 0.00mm | 0.00mm |
| *Aspergerllus* | Positive (Clearance 4mm) | 0.00mm | 0.00mm | 0.00mm |

**CHAPTER FIVE**

**5.1 DISCUSSION**

Avocado pear peel was used for the present study because of its medicinal properties shown in previous studies. The plant is known for its anti-inflammatory, anti-ageing, antiviral and antimicrobial properties.

The aqueous extract and ethanolic extract was tested against selected species of bacteria and yeast in terms of their diameter of inhibition zones as reported in Table 2, chapter four.

The water extract (2mg/ml) of air dried avocado peel extract demonstrated antimicrobial effect against the growth of Penicillium (4.00mm in terms of average zone of inhibition) and *Aspergellus* (6.00mm in terms of average zones of inhibition). The growths of other strains were not resisted by the water extract. This may be due to the resistance imposed by many microorganisms as a result of their enzymes that inhibit the antibiotics. This was why the water extract had no effect against the growth of *Escherichia coli* and *Staphylococcus aureus*.

The ethanol extract (2mg/ml) of air dried avocado peel demonstrated no noticeable antimicrobial activity against the four strains of microorganisms. Nevertheless there were blurred zones of inhibition around the growth of *Penicillium* and *Aspergerllus* whose measurements have almost the same diameter like that of water extract. The account for this blurriness might be because of the presence of the peel extract (2.0mg) present in the ethanol (10ml). However, if the concentration should be increased, the zone of inhibition may have a readable value. As such it is safe to assume that increased quantity would give a magnified effect. Ethanol extract also exhibited no inhibition against strains of *Escherichia coli* and *Staphylococcus aureus*. The controls, distilled water and standard ethanol demonstrated no activity against the four strains of microorganisms used.

Studies conducted on the antimicrobial activities of avocado pear so far were limited to the seeds and leaves and they all had promising results. (Idris *et al* 2010 and Ilozue *et al*, 2014). No known studies on the antimicrobial activity of the peel of avocado pear peel extract have been carried out or published.

This research, the antimicrobial activity of ethanol and water extract of the air dried avocado peel extracts against *Aspergerlluss*, Penicillium, *Staphylococcus aureus* and *Escherichia coli* was determined using Agar Well Diffusion method as used in other studies.

Generally, the antimicrobial activity exhibited by avocado pear peel extract was due to the presence of phenolic compounds, or *catechinprocyanidins* and hydroxycinnamic acids which are principle antimicrobial constituents found in the peels of avocado peels (Ilozue *et al*, 2014). This phytochemicals in the avocado peel extracts inhibited the growth of *Penicillium* and *Aspergerllius* and this was confirmed earlier by (Rodriguez *et al,* 2011) who tested the antimicrobial activity against strains of bacteria and fungi and was proven positive.

The Oleic found in the peels of avocado are found to have both antiseptic and antimicrobial activity and are of new interest in dermatology in treating acne and skin cancer.(Angioni, 2006, Ibtisam, 2011)

Thus, avocado peel is a chemotherapeutic agent against many pathogenic microbes such as *Candida albican* and *Proteus vulgaris* which may cause diseases.

**CONCLUSION**

This result has provided evidence to prove that Avocado pear peel contains considerable bioactive compounds hence a source of therapeutic agents.

**RECOMMENDATION**

 In view of the results obtained in this work, it is recommended that more research should be done to:

1. Isolate and identify the active compounds present in the ethanol and water extract of *parsea americana*
2. Screen more fruits in view of finding alternative treatments to microbial infection.

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