ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EX TRACT OF COCONUT ROOT

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CERTIFICATION

This is to certify that this project work was carried out and submitted by AR UNA BALIKIS with matriculation number: HND/23/SLT/FT/0680 to the Departme nt of Science Laboratory Technology, Biochemistry Unit, Kwara State Polytechnic, Ilorin, and has been read and approved in accordance to the partial fulfillment of t he requirement for the Award of Higher National Diploma (HND) in SLT.

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This project is dedicated to Almighty Allah, the Lord of the universe for his goodness, mercy, guidance, protection and his exceptional intervention in ensurin g my success in this programme.

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My sincere appreciation goes to my parents Mr. and Mrs. Aruna, and to my wonderful sis Mrs. Suliat Garba and husband Mr. Taiwo Garba. May Almighty All ah bless you all.

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ABSTRACT

Cocos nucifera (Arecaceae) is commonly called the "coconut tree" and is the most naturally widely consumed in different forms by indigene of many countries. It is used in preparation of some drinks aid oil. Cocos Nucifera is believed to have ma ny potential, nutritional, medicinal and pharmaceutical uses. The study is on eval uation of phytochemical and antimicrobial analyzed using AOAC and AAS metho d respectively show appreciable of phenol, tannin, steroids and alkaloid present a nd flavoid is absent.the constituent of c.nucifera have some biological effects, su ch as anti-helminthic, anti-inflammatory, anti-nociceptive, antioxidant, anti-fungal antimicrobial, and antitumor activities.

CHAPTER ONE

1.0 Introduction

1.1.1 Coconut

Cocos nucifera (L.) is an important member of the family Arecaceae (palm family) popularly known as coconut, coco, coco-da-bahia, or coconut-of-the-beach (Tejano, 1984). The plant is originally from Southeast Asia (Mal aysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans. From that region, the fruit of the coconut palm is believed t o have been brought to India and then to East Africa. After the discovery of t he Cape of Good Hope, this plant was introduced into West Africa and, from there, dispersed to the American continent and to other tropical regions of t he globe (Abdulhameed, 2011).

The plant is an arborescent monocotyledonous tree of around 25m in height (giant coconut) with a dense canopy. The root of the coconut system is fasciculated. The stem is an unbranched type, and at its apex, a tuft of lea ves protects a single apical bud. The pinnate leaves are feather-shaped, havi ng a petiole, rachis and leaflets. Under favorable environmental conditions, t he giant adult coconut emits 12-14 inflorescence spikes per year, while the adult dwarf coconut can emit 18 spikes in the same period. The auxiliary infl orescence has globular clusters of female flowers. The plant is monoecious (male and female reproductive organs on the same plant) (Popenoe, 1969).

The coconut fruit comprises an outer epicarp, a mesocarp, and an inn er endocarp. The epicarp, which is the outer skin of the fruit, and the mesoc arp, which is heavy, fibrous, and tanned when dry, have many industrial uses.

The endocarp is the hard dark core. Inside is a solid white albumen of varied thickness, depending on the age of the fruit, and with an oily pulp consisten cy and a liquid albumen called coconut water that is thick, sweet, and slightl y acidic (Popenoe, 1969).

The term "coconut" (or the archaic "cocoanut") (Jeron, 2012) can refer to the whole coconut palm, the seed , or the fruit, which botanically is a drup e , not a nut . The term is derived from the 16th-century Portuguese and Spa nish word Coco, meaning "head" or "skull" after the three indentations on the coconut shell that resemble facial features (Jeron, 2012). Coconuts are kno wn for their versatility of uses, ranging from food to cosmetics (Khon et al.; 2010). The inner flesh of the mature seed, as well as the coconut milk extra cted from it, forms a regular part of the diets of many people in the tropics and subtropics. Coconuts are distinct from other fruits because their endos perm contains a large quantity of clear liquid, called "coconut water "or "coc onut juice" (Khon et al.; 2010).

Mature, ripe coconuts can be used as edible seeds, or processed for oil and plant milk from the flesh, charcoal from the hard shell, and coir from the fibrous husk. Dried coconut flesh is called copra, and the oil and milk de rived from it are commonly used in cooking – frying in particular – as well a s in soaps and cosmetics. The hard shells, fibrous husks and long pinnate le aves can be used as material to make a variety of products for furnishing an d decorating. The coconut also has cultural and religious significance in cert ain societies, particularly in India, where it is used in Hindu rituals (Kaunitz, 1 986).

The name coconut derives from seafarers during the 16th and 17th century for its resemblance to a head. (Dicitionary.com, 2017) [Not in citation given] 'Coco' and 'coconut' apparently came from 1521 encounters by Portuguese and Spanish explorers with Pacific islanders , with the coconut shell reminding them of a ghost or witch in Portuguese folklore called coco(Kaunitz, 1986).

The specific name nucifera is Latin for "nut-bearing".

The coconut palm starts fruiting 6 to 10 years after the seed germinates and reaches full production at 15 to 20 years of age. It continues to fruit until it is about 80 years old with an annual production of 50 to 200 fruits per tree. The fruits require about a year to develop and are generally produced regularly throughout the year (Chan, 2006).

The palm tree has a smooth, columnar, light grayish brown trunk, and topped with a terminal crown of leaves. Tall selections may attain a height of 80 to 100 feet while dwarf selections are shorter in stature. The trunk is slender and slightly swollen at the base. It is usually erect but may be leaning or curved.

The leaves are pinnate with feather-shaped, up to 18 feet long and 6 feet wide. The leaf stalks are 3 to 5 feet in length and thornless. Flowers are small, light yellow, and in clusters which emerge from canoe-shaped sheaths among the leaves.

The seed is roughly ovoid, up to 15 inches long and 12 inches wide, composed of a thick, fibrous husk surrounding a somewhat spherical nut with a hard, brittle hairy shell. The nut is 6 to 8 inches in diameter and 10 to 12 i

nches long. Three sunken holes of softer tissue called "eyes" are at one end of the nut. Inside the shell is a thin, white, fleshy layer, about ½ inches thick a t maturity, known as the "meat". The interior of the nut is hollow but partially filled with a watery liquid called "coconut milk". The meat is soft and jelly-lik e when immature but it becomes firm at maturity. The coconut milk is abun dant in unripe fruits but it is gradually absorbed as ripening proceeds. The fr uits are green at first turning brownish as they mature. Yellow varieties go fr om yellow to brown (Onyeike and Acheru, 2002).

The coconut is the most extensively grown and used nut in the world and the most important palm. It is an important commercial crop in many tr opical countries, contributing significantly to their economies.

1.1.2 Husk and Shells

The husk and shells can be used for fuel and are a source of charcoal. Activated carbon manufactured from coconut shell is considered superior t o those obtained from other sources, mainly because of small macropores structure which renders it more effective for the absorption of gas and vapo r and for the removal of color, oxidants, impurities and odor of compounds (Jackson, et al.; 2004).

A dried half coconut shell with husk can be used to buff floors. It is kn own as a *bunot* in the Philippines and simply a "coconut brush" in Jamaica. T he fresh husk of a brown coconut may serve as a dish sponge or body spon ge. *Tempurung* as the shell is called in the Malay language can be used as a soup bowl and if fixed with a handle a ladle. In India, coconut shells are also used as bowls and in the manufacture of various handicrafts, including butt

ons carved from dried shell. Coconut buttons are often used for Hawaiian al oha shirts. In Thailand, the coconut husk is used as a potting medium to pro duce healthy forest tree saplings. The process of husk extraction from the c oir bypasses the retting process, using a custom-built coconut husk extract or designed by ASEAN-Canada Forest Tree Seed Centre (ACFTSC) in 1986. Fresh husks contain more *tannin* than old husks.

Tannin produces negative effects on sapling growth (Jackson, et al.; 2004). In parts of South India, the shell and husk are burned for smoke to repel mo squitoes.

1.1.3 Coconut Water

Coconut water serves as a suspension for the endosperm of the coc onut during its nuclear phase of development. Later, the endosperm mature s and deposits onto the coconut rind during the cellular phase. Coconut wat er contains sugar, dietary fiber, proteins, antioxidants, vitamins and minerals, and provides an isotonic electrolyte balance. It is consumed as a refreshing drink throughout the humid tropics, and is gaining popularity as a sport drin k. Mature fruits have significantly less liquid than young immature coconuts, barring spoilage. Coconut water can be fermented to produce coconut vine gar (Vestlund, 2010). The health benefits of the coconut water include: Boo sting of the immune system, Detoxification and fighting of viruses and also help cleanse the digestive tract.

1.1.4 Coconut Milk

Coconut milk, not to be confused with coconut water, is obtained pri marily by extracting juice by pressing the grated coconut's white kernel or b

y passing hot water or milk through grated coconut, which extracts the oil a nd aromatic compounds. It has a fat content around 17%. When refrigerated and left to set, coconut cream will rise to the top and separate from the mil k. The milk can be used to produce virgin coconut oil by controlled heating a nd removal of the oil fraction (Vestlund, 2010).

1.1.5 Coconut Oil

Another by-product of the coconut that is rapidly growing in popularit y is coconut oil. It contains fatty acids, Lauric, Caprylic, and Capric. Aside fro m its many non-culinary uses, such as a hair conditioner or health suppleme nt, it is frequently used as a cooking ingredient. It can be used in the same a pplications for which most other oils are used - pan frying and deep frying - but it can also be added directly to food, similar to the way one would add o live oil to bread or a salad. It is extracted from copra. It can promote weight loss by improving digestion. The Coconut Oil is notable for its anti-microbial properties that fight of bacteria, viruses, and fungi, and has been used for m edical purposes in tropical countries for centuries. Recent studies have even shown that the use of Coconut Oil helps the body and skin to heal and repair faster protecting it from deadly disease (Vestlund, 2010).

CHAPTER TWO

2.0 Phytochemistry

Phytochemistry study of the coconut fiber (mesocay) ethanolic extra ct revealed that the phenols, tannis, leucoanthocyanidins, flavornolds, triterp enes, sterolds and alkaloids, butanol extract recovered triterpenes saponis and condense tannins. Notably comp, flavornolds having antixiodant action are widely distributed in edible vegetable, fruit and condensed tannins are re ported to possessed antihelminthic activity by binding to prevent in the cuti

cle oral activity the esophagus and cloaca of nematodex thus intensing the and chemical damage in helminth.

The lyophilized of extract and fraction as well as ethylacetate extract, from the calcifera rich in polyphenols, compound such as catechins, epicate chnics, tannis and flavolloids.

The constituents of the liquid albumen were identified as vitamin B, ni cotinic acid (B3, 0.6ug/ml), panthothenic acid, (B5, 0.52ug/ml), biotin (0.02u g/ml), riboflavin (B2, <0.01ug/ml), folic acids, L-arginine, plant hormones (au xin, 1-3-diphenylurea, cytokinin) enzymes (and phosphor catalase, dehydrog enase, diastase, peroxidase, RNA polymerases) and growth promoting (Onif ade, et al.; 2003).

Root phenolic compounds were identified as flavonoids and saponin s. Compound identified in leaf epicuticular wax were lupeol methyl ether, ski mminalli (3b-25- ethyl-9-19, cyclolanost-24(241)-ene), and isoskimmiwallin (3b-methoxy -24- ethyl-9,19-25(251)-ene) (Onifade, et al.; 2003).

2.1 Pharmacological Activities of Extracts, Fractions and Isolated Cons tituents

Several studies have been conducted to identify the active molecules in coconut and their pharmacological and biological activities. Various extra cts, fractions and isolated compound of different parts of the coconut fruits were tested showing different activities including antihypertensive, analgesi a, vasodilatation; protection of kidney, heart and liver functions; protection a

gainst anti-inflammatory, anti-oxidant, anti-osteoporosis, anti- diabetes, anti-neoplastic, bactericidal, anti- helminthic, anti-malarial, leishmanicidal, antifungal anti-viral activities (these are described below and also listed in supplementary Table) (Jackson, et al.; 2004).

2.1.1 Analgesic Activity

Crude root-fiber extract and two aqueous extract fractions of molecular weight less than (F1) greater than (F2) 1KDa were studied for their analgesic activity by acetic acid- induced and witting, tail-flick, and hot plate tests in mice (Z. Villareal, et al, 2005). All three extracts induced peripheral and antinociceptive activity. Oral administration of the crude extract (50, 100 or 150mg/kg) sign inhibited writhing by 24%, 34%, and 52.4% respectively, when compared with a control gram fraction F1 and F2 reduced total writhing at 10 and 50mg/kg. In the tail-flick test, oral process with crude extract (100 and 150mg/kg), F1 (10 and 50mg/kg) or F2 (10 and 50mg/kg) produce effects better or similar to morphine (5mg/kg) until 80min. (Jackson, et al.; 2004).

However, with the exception of (10mg/kg, 60min after administration), neither crude extract (150mg/kg) nor F2 (50mg/kg) increased the latency of mice response to thermal stimulation in the hot-plate test.

In another study, an ethanol extract of the root fiber (40, 60 or 80mg/kg) show significance properties as indicated by a reduction in the number of writhes and stretches induce in acetic acid (Jackson, et al.; 2004).

The results were similar to those in animals that received aspirin (60 mg / kg), or morphine sulfate (1.15mg/kg).

Furthermore, administration of extract along with morphine or pethidine not only produced analgesia in mice but also potential analgesic effect of these two drugs. These studies were performed using coconut root fiber extracts, suggesting that this part of a highly potent analgesic. Cocos nucifera , may enable the production of new low cost media, several ailments and may provide a very expensive source of new analgesic drugs. Further investigations were warranted.

Further bio assay guided fractionation and isolation of specific are highly recommended so that the chemical moiety responsible for the activity and can be its mechanism of action established.

2.1.2 Anti- Inflammatory

Aqueous crude extracts of root fiber of c. nucifera are used to treat arthritis other inflammatory ills in Northeastern Brazils traditional medicine (George, 1993).

A study using animal models of inflammation (formalin test and subcutaneous air pouch method) showed that aqueous crude extracts of c. nucifera var. Typica (50,100mg/kg) significant ($p < 0.05$) the time that animals spent licking their formalin infected paws and reduced inflammatory induced by subcutaneous carrageenan injection by reducing cell ,migration, extra valuation of and TNF- production (George, 1993).

Root fiber extracts were also tested on rat paw edema induced by carrageenan, histamine and serotonin (Jackson, et al.; 2004). Animals were pre- tested by oral administration of crude extract (50, 100mg/kg), F1 or F2 (1, 10, or 50mg/kg), promethazine (30mg/kg) or methysergide (10mg/kg). Cru

de extract significantly ($p<0.05$) reduced histamine at (150mg/kg) and sero tonin induced edema at (100 and 150mg/kg). Even when mice were treated with 1mg/kg of F1, q significance inhibitory effect was observed in histamin e and serotonin- induced edema. However F2 did the edema induced by any pro-inflammatory agent.

Animal tests revealed significant activity supporting the use of the ro ot fiber extracts in the medicine (George, 1993).

2.2 Antibacterial, Antifungal and Antiviral Activities

Brushing the teeth with fibrous coconut husks is a common oral hygi ene practice among rural people of South India (Jackson, et al.; 2004). In thi s context, the antimicrobial properties of alcoholic extracts of the husk agai nst common oral pathogens were analyzed by the agar well diffusion metho d (Jose *et al., 2014*). There was significant concentration-dependent antimi crobial activity, expressed as a zone of inhibition with respect to all tested o rganisms except Actinomyces species. However, the effect of the C. nucifer a extract was less than that of chlorhexidine.

Ethanolic (cold and hot percolation), dry-distilled, and aqueous extrac ts of coconut endocarp were compared with gentamicin and ciprofloxacin f or their antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA), *methicillin-sensitive S. aureus, Pseudomonas aeruginosa, Es cherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Citrobacter fr eundii, Enterococcus, Streptococcus pyrogens, Bacillus subtilis, and Microc occus luteus* using the Kirby-Bauer disc diffusion method. The endocarp ext racts showed strong antimicrobial activity against *B. subtilis, P. aeruginosa,*

S. aureus, and M. luteus but had no effect on *E. coli* (Jackson, et al.; 2004). The dry-distilled extract (1 mg/mL and 200 mg/mL) could inhibit the growth of *B. subtilis and Aspergillus spp.* but was inactive against *R. oligosporus* at all concentrations (Singla *et al.,* 2011). The crude aqueous extract of husk fi ber and five fractions obtained by thin layer chromatography (TLC) were als o tested (10, 50, and 100 mg/kg) against *E. coli, S. aureus,* and MRSA via ag ar diffusion; they were active only against *S. aureus* and MRSA, with a minim um inhibitory concentration (MIC) of 1024 mg/mL for both (Jackson, et al.; 2004).

In another study, the antimicrobial activity of mesocarp powder extra cted with six common organic solvents was evaluated by the disk diffusion method (Verma *et al.,* 2012). The pathogens *E. coli* and *S. typhi* were used. The antimicrobial activity against E. coli was higher with the benzene solven t, while bioactivity toward *S. typhi* was more effective with the diethyl ether extract. Potential bio-components responsible for the antimicrobial activity were identified as tocopherol, alcohol palmitoleyl, cycloartenol, and b-sitost erol.

The in vitro anti listerial activities and time kill regimes of crude aqueo us and n-hexane extracts of the husk fiber of C. nucifera were tested (Akinye le *et al.,* 2011). The aqueous extracts were active against 29 of 37 Listeria is olates examined, while the n-hexane extracts were active against 30 (both at 25 mg/mL). The diameters of the zones of inhibition were 12-17 mm and 1 2-24 mm, respectively, while those of the control antibiotics were 20-50 mm for ampicillin and 22-46 mm for tetracycline. The MICs of the susceptible b

acteria were 0.6-2.5 mg/mL for the aqueous fraction and 0.6-5.0 mg/mL for the n-hexane extract. The mean reduction in viable cell count in the time kill assay with the aqueous extract ranged from 0.32 to 3.2 log10 CFU/mL after 4 h of interaction and from 2.6 to 4.8 log10 CFU/mL after 8 h at 1_ and 2_ MIC. With the n-hexane extract, the values were 2.8-4.8 log10 CFU/mL after 4 h of interaction and 3.5-6.2 log10 CFU/ mL after 8 h in 1_ and 2_ MIC. For the aqueous extract, bactericidal activity was observed against three of the tested Listeria strains at a concentration of 2_ MIC after 8 h exposure, while the n-hexane fraction was bactericidal against all five test bacteria at both MICs after 8 h.

In studies with crude extract and five TLC fractions (I-V) of fiber meso carp of *C. nucifera* fruit, in vitro antimicrobial activity was seen in all trial stra ins of *S. aureus* tested with fractions II-V (Esquenazi *et al.,* 2002). Antifungal activity was demonstrated as growth inhibition of *Candida albicans, Cryptoc occus neoformans or Fonsecaea pedrosoi*. Antiviral action was only seen wi th the crude extract and fraction II. The antifungal, antimicrobial, and antivira l effects were attributed to condensed tannins and catechins present in the crude extract and fractions II-V, especially fraction II, which had a higher con centration of these compounds.

Studies with alcohol extract of ripe dried coconut shell have demonst rated action against *Microsporum canis, M. gypseum, M. audouinii, Trichop hyton mentagrophytes, T. rubrum, T. tonsurans, and T. violaceum* (Venkatara man *et al.,* 1980). This activity was attributed mainly to the high content of p henolic compounds. In another study, virgin oil from coconut pulp prevented

growth of *C. albicans* (Borate *et al., 2013*).

Coconut oil is very effective against a variety of viruses with lipid cap sules, such as visna virus, cytomegalovirus, and Epstein-Barr virus (Arora *et al.,* 2011). The medium chain saturated fatty acids from coconut oil destroy and break the membranes and interfere with viral maturation.

These reports indicate that various parts of *C. nucifera* should be furt her tested for antibacterial, antifungal, and antiviral activities in different ani mal models. Future studies should consider formulations and exact dose le vels suitable for use in humans to treat various strains of bacteria, viruses, a nd fungi.

2.3 Commercial Products

The primary coconut products traded internationally are derived from the fruit, copra and desiccated coconut, coconut cream and protein, whole mature nuts, coir and activated carbon from shells (Falck, et al.; 2000) Youn g drinking nuts, coconut water (fresh, canned or frozen), and palm sugar are important in local economics and have a ready market in developed countri es with large Asian populations. The other primary products in local econo mies include shell, mature nuts for cooking and food uses, brooms, ropes, a nd coconut shell products, some of which may find niche markets overseas. For many pacific island states, copra and its by-product, copra press cake, a re the only important exports (Ewansinal et al.; 2005).

2.4 Aims and Objective

The aims and objectives of this research work are to know the Anti-m icrobial and phytochemical analysis present in ethanolic extract of coconut r

oot.

CHAPTER THREE

3.0 Material and Methods

3.1 Materials

3.1.1 Mode of Collection of Sample

The coconut root use for this research work was bought in Ilorin, Kwara state.

3.1.2 Preparation of the Sample

Extraction

The type of solvent used for extraction was ethanol. Ethanol is a selective solvent, which dissolves alkaloids, volatile oils, glycosides, resins etc. The coarsely powdered root of Cocos nucifera Linn was used for the extraction procedure for the preparation of extracts. The shade dried and coarsely powdered root of Cocos nucifera Linn was extracted with 99.9% ethanol by cold maceration in a narrow mouthed bottle for seven days. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. The residue was then weighed and yield was recorded.

3.1.3 Reagent

- Ethanol
- Sodium hydroxide
- Dimethyl fruronamide (DMF)
- Ciprofloxacin hydrochloride
- Dragendorff's Reagent
- Mayer's Reagent

- HCL
- Wagner's Reagent
- Picric acid
- Agar

3.1.4 Apparatus

- Distillation flask
- Weighing balance
- Filter paper
- Mouthed bottle
- Test tube
- Autoclave
- Petri dish
- Uv lamp
- Incubator
- E. coli
- K. pneumoniac
- P. aeniginosa
- S. aureus.
- Sterile Disc

3.2 Method of Phytochemical

3.2.1 Phytochemical Screening

3.2.1.1 Determination for Alkaloids

A quantity (0.2g) of the sample was boiled with 5ml of 2% HCl on a st eam bath. The mixture was filtered and 1ml portion of the filtrate was meas

ured into four test tubes. Each of the 1ml filtrate was treated with 2 drops o f the following reagents.

i. **Dragendorff's Reagent:** A red precipitate indicates the presenc e of alkaloids.

ii. **Mayer's Reagent:** A creamy-white colored precipitate indicates the presence of alkaloids.

iii. **Wagner's Reagent:** A reddish-brown precipitate indicates the p resence of alkaloids.

iv. **Picric Acid (1%):** A yellow precipitate indicates the presence of alkaloids.

3.2.1.2 Determination for Flavonoids

A quantity (0.2g) each of the extracts was heated with 10ml of ethyla cetate in boiling water for 3 minutes. The mixture was filtered differently an d the filtrates used for the following tests:

i. **Ammonium Test:** A quantity (4ml) each of the filtrates was shaken wi th 1ml of dilute ammonia solution (1%). The layers were allowed to se parate. A yellow coloration was observed at the ammonia layer, which indicates the presence of flavonoids.

ii. **Aluminum Chloride Test:** A quantity (4ml) each of the filtrates was s haken with 1ml of 1% aluminum chloride solution and observed for lig ht yellow coloration. A yellow precipitate indicates the presence of fla vonoids.

3.2.1.3 Determination for Glycosides

Dilute sulphuric acid (5ml) was added to 0.1g each, of the extracts in

a test tube and boiled for 15 minutes in a water bath. It was then cooled an d neutralized with 20% potassium hydroxide solution. A mixture, 10ml of eq ual parts of Fehling's solution A and B was added and boiled for 5 minutes. A more dense red precipitate indicates the presence of glycoside.

3.2.1.4 Determination for Steroids and Terpenoids

A quantity (9ml) of ethanol was added to 1g each of the extracts and refluxed for a few minute and filtered. Each of the filtrates was concentrate d to 2.5ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 hour and the waxy matter was filtered off. Each of the filtrates was extracte d with 2.5ml of chloroform using a separating funnel. To 0.5ml each of the c hloroform extracts in a test tube was carefully added 1ml of concentrated s ulphuric acid to form a lower layer. A reddish-brown interface shows the pre sence of steroids.

To another 0.5ml each of the chloroform extract was evaporated to d ryness on a water bath and heated with 3ml of concentrated sulphuric acid f or 10 minutes on a water bath. A grey colour indicates the presence of terp enoids.

3.2.1.5 Determination for Saponins

A quantity (0.1g) each of the extracts (aqueous and Chloroform) was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered whi le still hot and the filtrates used for the following tests:

i. Emulsion Test

A quantity (1ml) each of the filtrates was added drops of olive oil. Th

e mixture was added to another two drops of olive. The mixture was shaken and observed for the formation of emulsion.

ii. Frothing Test

A quantity (1ml) of the different filtrates was diluted with 4ml of distil led water. The mixture was shaken vigorously and then observed on standin g for a stable froth.

3.2.1.6 Determination for Tannins

A quantity (2g) each, of the extracts (Chloroform and water) was boil ed with 5ml of 45% ethanol for 5 minutes. Each of the mixtures was cooled and filtered. The different filtrates were subjected to the following tests.

i. Lead Sub-acetate Test

To 1ml of the different filtrates was added 3 drops of lead sub-acetate solu tion. A cream gelatinous precipitate indicates the presence of tannins.

ii. Ferric Chloride Test

A quantity (1ml) each of the filtrates was diluted with distilled water a nd added 2 drops of ferric chloride. A transient greenish to black color indic ates the presence of tannins.

3.2.2 Antimicrobial Screening

3.2.2.1 Disc Diffusion Method

A suspension of the organism was added to sterile nutrient agar med ium at 450 C. The mixture was transferred to sterile petri dishes and allowe d to solidify. Sterile discs, 5mm in diameter (made from Whatmann filter pa per sterilized in UV lamp) was dipped in solutions of different concentration

s of test, standard and blank and were placed on the surface of agar plate. The plates were allowed to stand for 1 hour at room temperature as a perio d of pre-incubation diffusion to minimize the effect of variation in time betw een the applications of the different solutions. Then the plates were incubat ed for 24 hours at 370 C ± 1 0 C and observed for antibacterial activity. The diameter of zone of inhibition was observed.

3.2.2.2 Preparation of Plates

10µg/ml stock solution of test compound was prepared using dimet hylformamide (DMF) as the solvent. From this stock solution, required quan tities of drug solutions were mixed with known quantities of molten sterile a gar media aseptically to provide the following concentrations of 12,13,14,1 5,16,17,18,19,20,21,22,23,24µl/ml. About 20ml of the media containing the drug was dispensed into each sterile petri dish (diameter about 10cms). Th en the media was allowed to solidify. Procedure

3.2.2.3 Agar Streak Dilution Method

Microorganisms were streaked one by one on the agar plates aseptically. A fter streaking, all the plates were incubated in the incubator, set at 370 C for 24 hours and then observed for growth of microorganisms. The lowest con centration of test compound showing no growth of the given bacteria has b een reported as MIC of the test compound against the bacteria.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Result

Table 4.1 Results of phytochemical screening

Constituents	Inference
Flavonoids	-
Steroids	+
Glycosides	+
Alkaloids	+
Tannin	+
Saponins	+

Key

+ = PRESENT

- = Negative

Table 4.2 Result of Anti-microbial screening

Organism used	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
Gram negative organisms	Standard	25µl	50µl	100µl
E.coli	36	18	21	24
k.pneumoniae	37	16	20	22
p.aeruginosa	36	15	19	23
Gram positive organisms S.aureus	38	17	20	25

CHAPTER FIVE

5.1 Discussion

Photochemical study of the coconut fiber (Mesocay) ethanolic extrac t revealed that the phenols, tannin, steroids, alkaloids, glycosides, saponins are presents while flavonoids was absent. Notably compound, flavonoids ha ving antioxidant action are widely distributed in edible vegetable, fruit and c ondensed tannins are reported to possessed antihelminthic activity by bindi ng to prevent in the cuticle oral activity the esophagus and cloaca of nemat odex thus intensing the chemical damage in helminth The antimicrobial resu lts in table 2 shows that the best microbes resistance action of ethanol extr act from coconut root at various diameter zones of inhibition (mm). Pseudo monas Aeruginosa, E coli. Pneumoni and candida spp. Are not susceptible t o inhibition at any zone of inhibition; E.coli, flavonoids S.Aureus are suscepti ble to be inhibited but only at the high oncentration and low concentration r espectively. In other words, patients suffering from E.coli and staphylococc us Aureus can be treated with ethanol extract (Krauss, 2014).

5.2 Conclusion

In conclusion, the result obtained from the phytochemical analysis of coconut nucifera root shows the presences of tannins, alkaloids, steroids, gl ycosides, saponins were present while flavonoids is absent. This justify the use of cocos nucifera root is good in the treatment of many deliberating ail ments like diabetes, heart diseases and infectious due to microorganisms. However, the medicinal and pharmacological usage of coconut root is as a r

esult of the compound present in it and its non-nutrient (phytochemical) con tent which act as an antioxidant against dangerous free radicals in the body system.

5.3 Recommendations

It is recommended that more research should be done on coconut ro ot because of its medicinal and economical valves to the economics. There are wide ranges of chemical detected in ethanol extract of coconut which p ossess important industrial, medicinal, physiological and antibiotic properti es. Further research is therefore required to isolate the individual chemical c omponents in cocos nucifera root and examine their biochemical effects in the treatment of some debilitating ailments such as cancer, diabetes, ulcer, heart disease, obesity and microbial infections. (Enig, 2008)

REFERENCES

Abdulhameed S, and Zafar J. (2011):*Chemical Composition of Meat (Kernel) and Nut Water of Major Coconut (Cocos NuciferaL.).*

Banzon, G and Velaseo, (1982): *Coconut Production and Utilization.*

Chan E, and Elevitch CR. (2006): *Species Profiles for Pacific Island Agrofore stry.*

Chan. E, and Elevitch CR. (2006): *Cocos Nucifera (Coconut), Ver.2.1. In Elevit ch, C.R. (Edn).*

Child R. (1964). *Coconut Hangman Green and Corporation, Hondon Pp. 73 – 79.*

Clemk, Campbell – Falck D, Thomas T. (2000):*The Intravenous Use of Cocon ut Water.*

Eiseman, B; R.E, Lozano and T. Hager, (1954):*Clinical Expenences in Intraven ous Administration of coconut Water.*

Ewansinal C.J et al (2012):*Proximate and Mineral Composition of Coconut (Cocos Nucifera) Shell. International Jornal of Pure and Applied Scien ce and Technology.*

Falck Ton, Tutuo N, Clem K. (2000): *The Intravenous Uses of Coconut.*

George E.F. (1993):*Plant Propagation by Tissue Culture. The Technology, an d Edn. London Exegeticis Limited. Pp.*

Jackson J.C et al; (2004): *Change in Chemical Composition of Coconut (Co cos Nucifera L.)*

Jackson J.C, et al. (2004): *Change in Chemical Composition of Cocont (Coc os Nucifera L.) Water during Maturation of the Fruit.*

Jeron E., Emojevwe Victor.(2012). *Antiohabetic Effect of the Cocos Nucifer a (Coconut) Husk Extract Journal of Medical and Applied Bioscienece s.*

Kaunitz, H, (1986). *Medium Chanin Trygly Cenles (MCT) Inagingand Arterios derosis and in Ritro LDL Oxidation.*

Onyeike and Acheru (2002). *Investigation of Fruit Pell Extract as Sources for Compound with Antioxidant and Anti Prociferative Activities Against Human Cell Lines.*

NMCE; *Report on Copra. National Multi – Commodity Exchange of India Lim ited:* (*2007).*

Onifade AK, and Jeff – Agboola YA. (2003). *Effect of Fungal Infection on Pr oximate Nutrient Composition of Coconut (Cocos Nucifera Zinn).*

Onyeike and Acheru (2002). *Investigation of Fruit Pell Extract as Sources for Compound with Antioxidant and Anti Prociferative Activities Against Human Cell Lines.*

Popenoe, J. (1969): *Onifade and Jaff – Agboola 2003.Coconut in Hand Boo k of North America Nut Trees.*

Rinaldi, S. Silva, D. O. Bello F. Alviano C.S (2009). *Characterization of the Anti nocIceptive and Anti – Inflammatory Activities from Cocos NuciferaL. (Palmae).*

Tejano, E.A, (1984): *State of the Art of Coconut Oil and Husk Utilization (Gen eral Overview).*

Tutuo N, (2000). *The Intravenous Use of Coconut Water.*

Vestlund L; (2010). *The Healing Power of Organic Virgin Coconut Oil.*