



Original Research Article

PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY FROM MEDICINAL PLANT EXTRACTS OF *DANIELLA OLIVERI* ON CLINICAL ORGANISMS

ABSTRACT



Daniellaoliveri (Caesalpiniaceae) is a plant found in the Amazon region and other parts of South America and Africa. The tree may reach a height of 100 feet and trunk diameter of 4 feet. *Daniellaoliveri* (Rolfe) Fabiaceae is commonly known as Ilorin balsam (eepoiya) or *copaihuaficana*. It is an indigenous African tree found extensively in Benin, Cameroon, Gambia and Nigeria. The basic objective of this project is to determine the antimicrobial activity of *Daniellaoliveri*. The leaf and bark of the *Daniellaoliveri* were obtained from a location in the south western part of Nigeria, in the tropical rainforest of IkareAkoko, Ondo state and Ile Ife, Osun state, Nigeria. The plant were authenticated by a certified botanist at the herbarium unit of Department of Plant science and Biotechnology, AdekunleAjasin University, AkungbaAkoko, Ondo State, Nigeria and ObafemiAwolowo University, Ile Ife, Osun state, Nigeria. The leaf and bark were prepared using ethanol. The antimicrobial assay were carried out on leaf and bark using Agar diffusion method and the zone of inhibition was measured. The test organisms used for this research work are *Escherichia coli*, *Pseudomonas aeruginosa* (Michie, 1992) *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus viridians*, *Shigella**ysenteriae*, *Bacillus subtilis* and *Salmonella typhi*. All the extract (leaf and bark) of the highest concentration (60mg/ml) has the highest zone of inhibition while the lowest concentration (7.5mg/ml) has the lowest zone of inhibition. The phytochemical analysis revealed the presence of alkaloids and phenol, tannin, phylate, oxalate, saponin and steroid. The presence of these compounds were inferred as being responsible for the anti-microbial properties of the extracts. elemental composition content present in the plant are sodium (Na), potassium (K), calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Lead (Pb), therefore, the use of medicinal plants should be studied in detail and encouraged for use worldwide to reduce the level of resistance of microorganisms to drugs and make drugs available for the populace.

Keywords –Antimicrobial activity, phytochemical activity, proximate composition, elemental constituent.



INTRODUCTION

Daniellaoliveri(Caesalpinaceae) is a plant found in the Amazon region and other parts of South America and Africa. The tree may reach a height of 100 feet and trunk diameter of 4 feet (Mordi and Erah 2006). It produces liquid oleoresin which has been used as medicine by indigenous people for more than 400 year Cowan MM (2005). The oleoresin is produced in the tree's trunk, stem, and leaves and it consists of large but varying amounts of volatile oils (primarily composed of sesquiterpene hydrocarbons usually including caryophyllene), non volatile resinous substances and small quantities of acids. The oleoresin is traditionally used as an anti-inflammatory agent and in the treatment of a variety of genito-urinary tract diseases and skin ailments. Moreover, it is used as an anti-rheumatic, antiseptic, antibacterial, diuretic, and hypotensive agent, and also as an expectorant, laxative, purgative, vermifuge and vulnerary.Finer IL (2006).The leaves are also used in folk medicine as an anti-diabetic agent. Modern scientific studies have authenticated some of these medicinal uses of oleoresin such as its effectiveness as an antibacterial, anti-inflammatory, and anti-oxidant agent. (Bassileet al., 1988).

Daniellaoliveri(Rolfe) Fabiaceae is commonly known as Ilorin balsam (eepoiya) or *copaihuaficana*. It is an indigenous African tree found extensively in Benin, Cameroon, Gambia and Nigeria. (Onwekaema and Udorh, 1999).The leaves are used traditionally to treat diabetes, yellow fever and as aphrodisiac (Onwekaema and Udorh, 1999).The leaves were found to contain quercitrin, quercameritrin, rutin and the rare flavoured glycoside quercitin-3-methoxy 3-o-rhamnosylpranosyl (1,6)- β - d- Glycopyranoside (Narssine) isolated from n- butanol extract (Onwekaema and Udorh, 1999).

The roots, stem, bark and leaves of *Daniellaoliveri* are used in the treatment of scrotal elephantiasis, dysentery, ring worms, syphilis, typhoid fever, eye sore and ear ache and the stem bark of *Daniellaoliveri* was used in the treating fever, boil and back ache. The plants are found in both temperate and tropical regions of the world with 630 genera and species. Substances derived from medicinal plants remain the basis for a large proportion of commercial medications for the treatment of various diseases like heart diseases, high blood pressure, pains, asthma, malaria, typhoid fever, snake bites, arrow poison and other problems. These substances are available in a variety of forms. (Finer IL 2006).

In Nigeria, herbal therapies occupy a very special position in health delivery, especially among the rural populace. Easy accessibility and low cost of treatment enhance patronage. Diabetes mellitus is the commonest noncommunicable endocrine disease and is considered one of the leading causes of death all over the world; affecting over 100 million people worldwide¹. Many traditional plant treatments for

diabetes mellitus are used throughout the world, and some of these plants have been scrutinized while a good number of them are yet to receive scientific scrutiny. (Pushparaj *et al.*, 2000).

The use of plant parts in the treatment of human disease is as old as the disease themselves, and herbal medicine was the major form of medicine in Nigeria. Infectious diseases are major cause of death in developing countries and today according to WHO as many as 80% of world population depends on traditional medicines for their primary health care needs and that 25% of the drugs are based on plants and their derivatives. (Azu and Onyeagba, 2007).

Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics which increase the likelihood of being hospitalized and increase the length of stay in the hospital and increased use of a particular antibiotic could lead to increased bacterial resistance. Despite advance orthodox medicines availability, there has been an increased interest in the complementary and alternative medicines particularly by those who have apprehension concerning toxicity and safety of modern drugs, hence, discovering and identifying new safe drugs without severe side effects has become an important goal of research in biomedical science. Cowan MM (2005).

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 Moreover, it is used as an anti-rheumatic, antiseptic, antibacterial, diuretic, and hypotensive agent, and also as an expectorant, laxative, purgative, vermifuge, and vulnerary. The leaves are also used in folk medicine as an anti- diabetic agent. Modern scientific studies have authenticated some of these medicinal uses of oleoresin such as its effectiveness as an antibacterial, anti- inflammatory, and anti-oxidant agent. (Balogun E.A and Adebayo J.O., 2000).

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1.10 USES

UNDER PEER REVIEW

The wood, traded as 'West African gum copal' and 'daniella' is used for light flooring, joinery, interior trim, furniture, boat building, toys, novelties, cattle-troughs, drums, bowls, rice-mortars, packing cases, draining boards, carvings, venner, plywood, hardboard, and particle board. However, the gum makes it rather too gummy for high-quality joinery and carvings. It is popular as firewood, as it has a fragrant smoke and is slow burning. It is also made into charcoal. (Onwekaema and Udorh, 1999).

The wood has been used for paper making. The wood ash is sometimes used in soap production.

Large strips of bark are made into beehives. The resin or gum, is fragrant and used as torch or as incense in religious ceremonies and to fumigate houses and clothes to chase insects. The gum is furthermore used to fix spearheads to the shaft. The powdered gum is applied to cloth to make it shiny, and it is applied to furniture as a polish or vanish, to make it shiny and more resistant to termites. It is not suitable for commercial furniture polish as it is not soluble in turpentine or alcohol. However, it can be esterified to produce a lacquer. In Nigeria, drums are soaked in a solution of the gum, a treatment which deepens the tone. The gum and stem bark, and to a lesser extent the roots and leaves are widely used in traditional medicine. The gum, bark and leaves are burnt and the smoke inhaled to treat headache and migraine; the smoke is also used as mosquito repellent. The gum is chewed and swallowed as a purgative to treat diarrhoea (Finer IL 2006).

In cote d'Ivoire, the gum is considered aphrodisiac and diuretic, and it is chewed to treat cough, headache, tachycardia and painful menstruation. The gum is externally applied to itching skin and skin diseases. The gum and bark are taken in various preparations, internally and externally, and sometimes with other plant parts, to treat venereal diseases, ulcers and sores, circumcision wounds, leprosy, dysentery, colic, menstrual problems, cough, cold, angina bronchitis, tuberculosis, kidney problems, appendicitis, headache, backache, rheumatism, fever pains, hernia, tooth-ache, and snakebites (Finer IL 2006).



The root is considered diuretic and a decoction is taken to treat venereal diseases, absence of menses, anxiety, insanity, food poisoning, and skin diseases. Leafy twigs are put in baths to treat fever and jaundice, and also as a tonic. A decoction of the leafy twigs with salt is taken as a purgative, to treat constipation and stomach-ache.



A leaf bud infusion is taken to treat migraine and feverish pain in teething children. Pounded young leaves are applied to wounds, burns and abscesses to ripen them, they are also applied to painful parts of the body. A leaf decoction is gargled to treat toothache and drunk to treat colic.

The large stipules are used to cover sores, ulcers and injuries, and are also used as tampon for menstruating women. In Burkina Faso sheep and goats are given stem bark decoction to treat intestinal worms. In Nigeria, the gum is applied to the skin of horses to treat mange. The young leaves are eaten with condiments or cooked in soup, as famine food. The seeds and fruits are eaten occasionally as well.

Cattle browse the leaves readily, and the leaves are used as fodder. From the roots, excellent chewing



stick are made. The gum is sometimes chewed for its sweetness, or used as a filling in dental practices. It is also made into false amber beads. The bark is added to accelerate fermentation in brewing beer (Edeoga 2005).



Grilled branches are added to palmwine to improve digestability. The burnt bark is used to blacken the interior of pots. The tree is used in reforestation programmes, and is also planted as a windbreak and avenue tree. The leaves are used for mulching. The flowers are much visited by honey bees for nectar. In Ghana, *Daniellaoliveri* is considered an indicator of underground water. Children wear a piece of gum around their neck as a charm against bad luck (Onwekaema and Udorh, 1999).

1.11 CHEMOTHERAPEUTIC USE OF *Daniellaoliveri* PLANT



An antimicrobial is an agent that kills microorganisms or inhibits their growth (Cowan, 1999). Chukwujekwu *et al.*, (2005) reported that plant extracts and their products are used in many parts of the world as the active principles in herbs remedies. They are used locally in treatment of infections, many centuries before scientific studies were discovered. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterial are used against bacteria and antifungal are used against fungi.



They can also be classified according to their function. Agents that kill microbes are called *microbicidal*, while those that merely inhibit their growth are called *microbiostatic*. The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living

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organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones (Tona et al., 1999).

 The term also used to be restricted to antibacterial (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth. Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection (Doughari et al., 2009).

 More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine. In 1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as *penicillium rubens*. He named the substance extracted from the fungus penicillin and in 1942 it was successfully used to treat a *streptococcus* infection. Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhea, strep throat and pneumonia, which were potentially fatal to patients up until then. Many antimicrobial agents exist, for use against a wide range of infectious diseases (Burkill, 1997).

Materials and methods

2.5 COLLECTION AND IDENTIFICATION OF SAMPLES

 The leaf and bark of the *Daniellia* were obtained from a location in the south western part of Nigeria, in the tropical rainforest of Ikare Akoko, Ondo state and Ile Ife, Osun state, Nigeria. The plant were authenticated by a certified botanist at the herbarium unit of Department of Plant science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria and Obafemi Awolowo University, Ile Ife, Osun state, Nigeria. The leaves, bark and stem were washed thoroughly with distilled water, stored in air tight containers and kept at room temperature prior to use (Vats and Sardana 2011).

2.6 TEST ORGANISMS

The test bacteria used in this study were *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Bacillus cereus*, *Streptococcus viridians*, *Proteus vulgaris*, *Bacillus subtilis*. They were obtained from Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria and Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. They were isolated on sterile nutrient agar slants and taken to the microbiology laboratory of the Adekunle Ajasin University, Akungba Akoko, Nigeria. All slants of test organisms were kept at -4°C prior to bioassay of the extracts. Extensive biochemical tests were carried out to further confirm all the test bacterial strains (Osuntokun & Olajubu 2014), (Barrow 1995).

2.7 PREPARATION OF PLANT EXTRACTS FOR EXTRACTION

All the plant materials obtained were first washed thoroughly with sterile distilled water and air dried at room temperature for about two weeks to ensure that the samples lose most of their moisture content. The following extractions were carried out: aqueous and Ethanol. For each extraction, 400g of each dried plant material was weighed separately into conical flasks containing 1200ml each of distilled water and ethanol. The mixtures were initially shaken rigorously and left for 9 days. All mixtures were filtered using sterile Whatman filter papers and the filtrates were collected directly into sterile crucibles. All filtrates obtained were introduced into sterile reaction tubes and heated continuously in water bath at the following temperatures: 78°C for ethanol extraction, and 105°C for distilled water. The residues obtained were kept at room temperature (Osuntokun 2015).

2.8 STANDARDIZATION OF EXTRACTS

Using aseptic condition, the extract is reconstituted by adding 1.2g of each extract with 5ml of dimethylsulphoxide (DMSO) and 15ml of sterile distilled water making it 60mg/ml. For each extract, 7.5ml of distilled water is measured into three sterile bijou bottle. In bijou bottle A 7.5ml from 60mg/ml extract was added and in bijou bottle B 2.5ml from 60mg/ml extract was added and bijou bottle C 2.5ml from bijou bottle A was added. A is 30mg/ml, B is 15mg/ml, C is 7.5mg/ml respectively (Osuntokun 2014).

2.9 STANDARDIZATION OF INOCULUM

 Slants of the various organisms were reconstituted using an aseptic condition. Using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, transfer 0.1ml of the isolated colony using a sterile needle and syringe into 9.9ml of sterile distilled water contained in each test tube and then mixed properly. The liquid now serve as a source of inoculum containing approximately 10^6 cfu/ml of bacterial suspension. (El Astal 2005).

2.10 ANTIMICROBIAL ASSAY OF PLANTS EXTRACTS USING AGAR WELL DIFFUSION METHOD.

 All antibacterial assays for the plant extracts were carried out by well diffusion technique. All the test organisms were sub-cultured onto sterile Mueller Hinton Agar plates and incubated at 37°C for 18-24h. Five distinct colonies for each organism were inoculated onto sterile Mueller Hinton broth and incubated for 3-4h. All inocula were standardized accordingly to match the 0.5 McFarland standard and this standard was used for all susceptibility tests. All the extracts were reconstituted accordingly into the following concentrations:60, 30, 15 and 7.5mg/ml; using the Dimethyl Sulphoxide (DMSO) (Osuntokun & Ayodele 2014).

 The susceptibility testing was investigated by the Agar well diffusion method. A 0.1 ml of 1:10,000 dilutions (equivalent to 10^6 cfu/ml) of fresh overnight culture of the clinical isolates grown in Muller Hilton agar was seeded into 40 ml of Mueller Hilton agar, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork borer of 4 mm diameter, equidistant wells were made in the agar. Drops of the re-suspended, (2ml per well) extracts with concentrations between 60 to 7.5 mg/ml were introduced into the wells till it was filled. Ciprofloxacin (2mg/ml) was used as the control experiment.

 The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37°C for 24 hours. The zones of inhibition were measured to the nearest millimetre (mm) using a standard transparent meter rule. All experiments were performed in duplicates (Osuntokun 2015 & Osuntokun 2014).

2.11 DETERMINATION OF THE PHYTOCHEMICAL SCREENING, MINERALS, ANTI-NUTRIENT AND PROXIMATE COMPOSITION OF PLANT EXTRACTS.

QUALITATIVE METHOD OF ANALYSES

PRELIMINARY TEST / PREPARATION TEST

 Plant filtrates were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrate were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides. (Ahmedulla 1999).

(I) TEST FOR ALKALOIDS

 About 0.2 gram was warmed with 2% of H_2SO_4 for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the presence of Alkaloids.

(II) TEST FOR TANNINS

 One milliliter of the filtrate were mixed with 2 ml of $FeCl_3$. A dark green colour indicated a positive test for the tannins. (Edeoga 2005).

(III) TEST FOR SAPONINS

 One milliliter of the plant filtrate were diluted with 2 ml of distilled water; the mixture were vigorously shaken and left to stand for 10 min during which time, the development of foam on the surface of the mixture lasting for more than 10 min, indicates the presence of saponins. (Edeoga 2005).

(IV) TEST FOR ANTHRAQUINONES

 One milliliter of the plant filtrate was shaken with 10 ml of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test (Edeoga 2005).

(V) TEST FOR ANTHOCYANOSIDES

One milliliter of the plant filtrate were mixed with 5 ml of dilute HCl; a pale pink colour indicates the positive test.

(VI) TEST FOR FLAVONOIDS

One milliliter of plant filtrate were mixed with 2 ml of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 ml of the plant filtrate were mixed with 2ml of dilute NaOH; a golden yellow colour indicated the presence of flavonoids. (Ekpo 2009).

(VII) TEST FOR REDUCING SUGARS

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars (Ekpo 2009).

(VIII) TEST FOR CYANOGENIC GLUCOSIDES

This was carried out subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil (Ekpo 2009).

(IX) TEST FOR CARDIAC GLUCOSIDES

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydride plus H_2SO_4 .

2.12 QUANTITATIVE METHOD OF ANALYSES

(I) SAPONINS

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixture were heated using a hot water bath. At about $55^{\circ}C$, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about $90^{\circ}C$. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether were added and then shaken vigorously. The aqueous layer were recovered while the ether layer was discarded. The purification process was repeated three times. 60 ml of n-butanol were added.

The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution were heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material. (Guevarra 2005).

(II) FLAVONOIDS

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution were filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content were weighed to a constant weigh. (Guevarra 2005).

(III) CARDIAC GLUCOSIDES

Legal test and the killer-kilianjwwas adopted, 0.5g of the extract were added to 2ml of acetic anhydrate plus H_2SO_4 .

(IV) TANNINS

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle, 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance were measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract. (Mahato 1997).

(V) ALKALOIDS

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture were covered and allowed to stand for 4 hour. These were filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass (Mary 2008).

(VI) PHLOBATANNINS

About 0.5grams of each plant extracts were dissolved in distilled water and filtered. The filtrates were boiled in 2% HCl, red precipitate show the present of phlobatannins (Sofowora 1982).

2.13 TOTAL PHENOL (SPECTROPHOTOMETRIC METHODS)

2 g each of the samples were defatted with 1mL of diethyl ether using a soxhlet apparatus for 2 hours. The fat free samples were boiled with 50 mL of ether for the extraction of the phenolic components for 15 minutes. 5 mL of the extracts were pipetted into 5 mL flask and then 10 mL distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was measured at 505 nm (Nweze 2004).

2.14 DETERMINATION OF PROXIMATE ANALYSIS OF *DANIELLA OLIVERI*

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods. (Trease and Evans 1983)

1. Determination of moisture content was done by drying samples in oven (WiseVen, WON-50, Korea) at 110 °C until constant weight was attained (Horwitz 2003).
2. Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360, Switzerland) method with some modification. (Hussainet al.,2011).
3. The crude proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25 (Hussainet al.,2011). The energy value estimation was done by summing the multiplied values for crude protein,
4. Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fats were determined by Soxhlet apparatus using *n*-hexane as a solvent.
5. The ash values were obtained by heating samples at 550 °C in a muffle furnace (Wise Therm, FHP-03, Korea) for 3 h (Hussainet al.,2011).

6. The carbohydrate content was determined by subtracting the total crude protein, crude fibre, ash content and crude fat from the total dry matter (Horwitz 2003).

7. Crude fibre was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25% NaOH (w/v) solutions (Al-Harrasiet al., 2012)

3.0 RESULT

The leaf and bark of *Daniellaoliveri* was soaked with ethanol and left for 7 days. Table 1 shows the yield of the extracts after separation and evaporation.

Table 2 shows the antimicrobial activities of ethanolic leaf extract of *Daniellaoliveri* at concentrations 60, 30, 15 and 7.5mg/ml tested against the some selected clinical isolates. There was a greater zone of inhibition when tested against *Shigelladysenteriae*(15mm), *Proteus vulgaris*(14mm), and *pseudomonas aeruginosa*(13mm)at 60mg/ml.

Table 3 shows the antimicrobial activity of ethanolic bark extract of *Daniellaoliveri* at concentrations 60, 30, 15 and 7.5mg/ml tested against the same clinical isolates. The extract has greater inhibition zones against *Staphylococcus aureus* (14mm), *Shigelladysenteriae* (14mm) and *Bacillus subtilis* (14mm) at 60mg/ml.

Table 4 shows the Minimum Inhibitory Concentration of *Daniellaoliveri* plant extract at concentrations 60, 30, 15, 7.5, 3.75, 1.88 and 0.94mg/ml. It was observed that the organisms grow at concentrations 7.5, 3.75, 1.88 and 0.94mg/ml indicating that the plant is not effective at those concentrations, while at concentration 15, 30, and 60mg/ml there was no growth. This indicates that the plant is effective at higher concentrations.

Table 5 shows the quantitative analyses of the phytochemicals present in the plant. It shows the presence of phytochemicals such as alkaloid, saponin, tannin, glycoside, flavonoid, phenol, steroid present in the leaf and shows that alkaloid, steroid and flavonoid is not present in the bark of the plant.

Table 6 shows all the minerals present in the leaf and bark extract and they include sodium, potassium, calcium, magnesium, zinc, iron, lead, copper, manganese and phosphorus. The result shows that there is a large amount of Potassium, magnesium and sodium in both leaf and bark while both has a low amount of iron.

Table 7 shows the analysis of the anti-nutrient present in the plant extract calculated in percentage (%). Saponin (10.54%) and flavonoid (7.92%) has the highest value for leaf while alkaloid (1.98%) and oxalate (3.92%) has the lowest anti-nutrient composition. The result indicates that saponin is more present in the bark (9.34%) and leaf (10.54%) of the plant.

Table 8 shows the proximate nutrient composition of bark and leaf which has their highest nutrient composition in carbohydrate with 42.59 and 45.29 for bark and leaf respectively. They have their lowest value for the two extract in their fat with 6.59 and 7.21 respectively.

TABLE 1: PERCENTAGE YIELD OF *Daniellaoliveri* EXTRACTS

PERCENTAGE YIELD	INITIAL WEIGHT OF THE SAMPLE (g)	VOLUME USED (ml)	PERCENTAGE YIELD OF FINAL WEIGHT (g)
Percentage yield of <i>Daniellaoliveri</i> leaf extract	400	1200	8.7
Percentage yield of <i>Daniellaoliveri</i> bark extract	400	1200	8.2

TABLE 2: ANTIMICROBIAL ACTIVITY OF LEAF ETHANOLIC EXTRACTS OF *Daniellaoliveri*

CLINICAL ORGANISM	CONCENTRATION 60mg/ml	CONCENTRATION 30mg/ml	CONCENTRATION 15mg/ml	CONCENTRATION 7.5mg/ml	CONCENTRATION CIPROFLOXACIN 30mg/ml
<i>Escherichia coli</i>	12.0	7.0	3.0	1.0	20.0
<i>Salmonella typhi</i>	11.0	7.0	2.0	0.0	19.0
<i>Proteus vulgaris</i>	14.0	9.0	4.0	1.0	20.0
<i>Klebsiella pneumonia</i>	12.0	10.0	2.0	0.0	28.0
<i>Bacillus cereus</i>	12.0	7.0	2.0	0.0	19.0
<i>Staphylococcus aureus</i>	10.0	6.0	1.0	0.0	20.0
<i>Pseudomonas aeruginosa</i>	13.0	6.0	1.0	0.0	22.0
<i>Streptococcus viridians</i>	12.0	7.0	4.0	1.0	18.0
<i>Bacillus subtilis</i>	10.0	5.0	3.0	0.0	19.0
<i>Shigelladysenteriae</i>	15.0	8.0	4.0	1.0	20.0

ZONE OF INHIBITION IS MEASURED IN mm

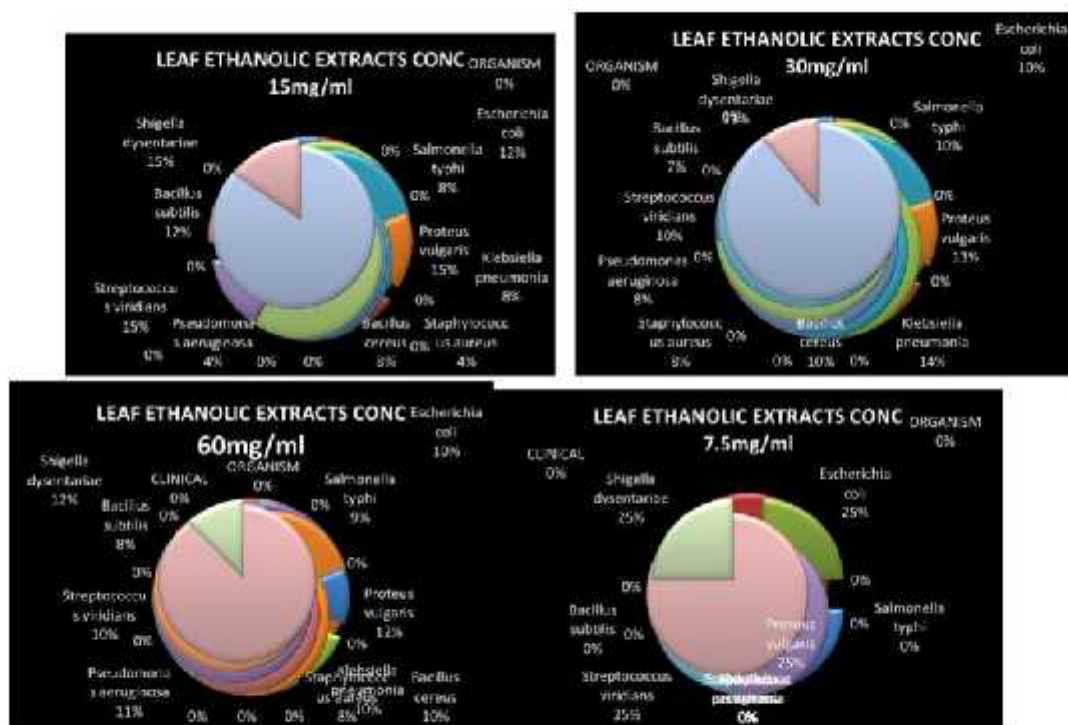


Fig: A graphical representation showing the activity of *Daniellia verticillata* leaf extract on some selected clinical isolates

TABLE 3: ANTIMICROBIAL ACTIVITY OF ETHANOLIC BARK EXTRACTS OF *Daniellaoliveri*

Selected Clinical Organism	Concentration 60mg/ml	Concentration 30mg/ml	Concentration 15mg/ml	Concentration 7.5mg/ml	Ciprofloxacin 30mg/ml
<i>Escherichia coli</i>	11.0	5.0	3.0	1.0	20.0
<i>Salmonella typhi</i>	12.0	4.0	1.0	0.0	19.0
<i>Proteus vulgaris</i>	11.0	6.0	2.0	1.0	20.0
<i>Klebsiella pneumonia</i>	10.0	9.0	4.0	0.0	28.0
<i>Bacillus cereus</i>	8.0	5.0	1.0	0.0	19.0
<i>Staphylococcus aureus</i>	14.0	8.0	3.0	1.0	20.0
<i>Pseudomonas aeruginosa</i>	13.0	7.0	3.0	0.0	22.0
<i>Streptococcus viridians</i>	13.0	6.0	2.0	1.0	18.0
<i>Bacillus subtilis</i>	14.0	6.0	3.0	1.0	19.0
<i>Shigelladysenteriae</i>	14.0	7.0	4.0	1.0	20.0

ZONE OF INHIBITION IS MEASURED IN mm

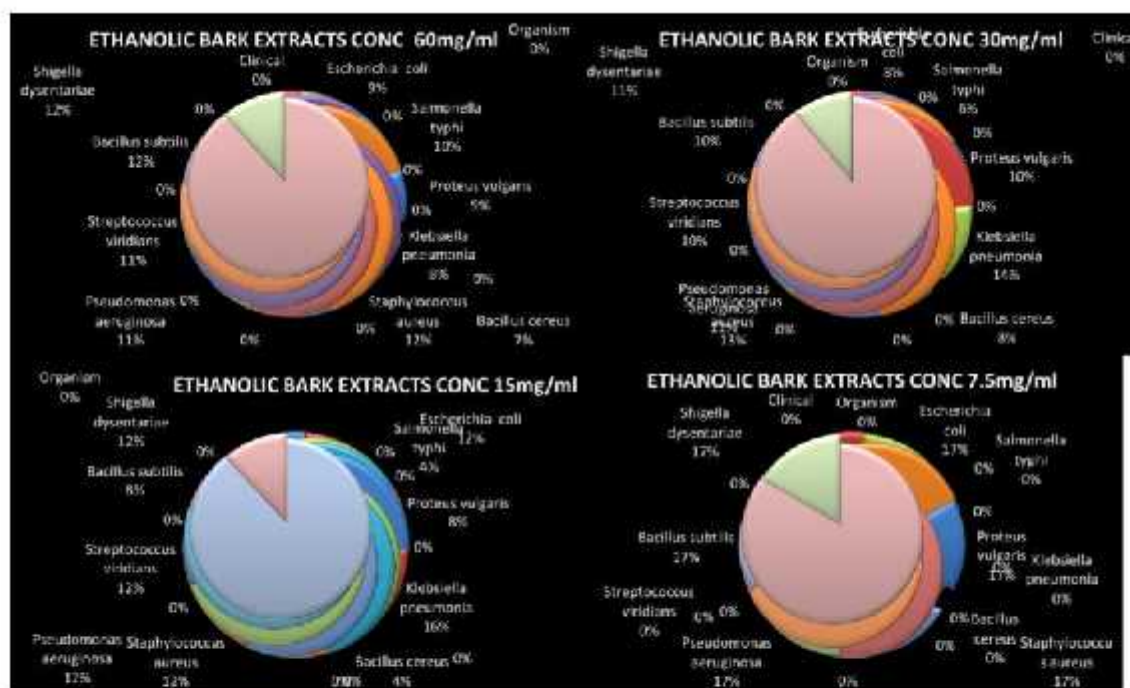


Fig: A graphical representation showing the activity of *Daucylactone* extract on some selected clinical isolates.

TABLE 4: MINIMUM INHIBITORY CONCENTRATION OF *Daniellaoliveri* EXTRACTS

Concentration mg/ml	Leaf Ethanol	Bark Ethanol
60	NG	NG
30	NG	NG
15	NG	NG
7.5	G	G
3.75	G	G
1.88	G	G
0.94	G	G

KEY

G	GROWTH
NG	NO GROWTH

NOTE: At 60mg/ml, the minimum inhibitory concentration is recorded while the minimum bactericidal concentration is recorded at 15mg/ml.

TABLE 5: QUALITATIVE ALYSIS OF THE PHYTOCHEMICAL SCREENING OF *DANIELLA OLIVERI* EXTRACTS

SAMPL E	Alkaloi d	Cardia c Glycosi de	Steroid	Anthra quinone	Pheno l	Tannin	Saponi n	Flavo noids
BARK <i>Daniellaoliveri</i>	- ve	+ ve	-ve	+ ve	+ ve	+ ve	+ ve	- ve
LEAF <i>Daniellaoliveri</i>	+ve	+ ve	+ve	+ ve	+ ve	+ ve	+ ve	+ ve

TABLE 6: QUANTITATIVE ANALYSES OF ELEMENTAL COMPOSITION PRESENT IN *DANIELLA OLIVERI* EXTRACTS (mg/100g)

Plant sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn
BARK <i>Daniellaoliveri</i>	20.33	41.24	15.53	25.38	18.71	4.38	ND	ND	15.34
LEAF <i>Daniellaoliveri</i>	23.47	42.46	17.47	21.43	16.92	5.20	ND	ND	16.00

TABLE 7: QUANTITATIVE ANALYSES OF ANTI –NUTRIENT PRESENT IN *DANIELLA OLIVERI* EXTRACTS RESULT IN PERCENTAGE (%)

Parameters	BARK <i>Daniellaoliveri</i>	LEAF <i>Daniellaoliveri</i>
Tannin	2.34	2.73
Phenol	3.21	3.94
Phylate	2.97	2.03
Oxalate	2.31	3.92
Saponin	9.34	10.54
Flavonoid	8.43	7.92
Alkaloids	1.87	1.98

TABLE 8: QUANTITATIVE ANALYSES OF PROXIMATE NUTRIENT COMPOSITION OF *DANIELLA OLIVERI* EXTRACTS IN PERCENTAGE.

PLANTS	Ash	MC	CP	Fat	Fibre	CHO
BARK <i>Daniellaoliveri</i>	10.56	9.12	14.45	6.59	10.33	42.59
LEAF <i>Daniellaoliveri</i>	10.21	8.24	13.34	7.21	11.32	45.24

KEY

ASH- ash content

MC-mineral content

CP- crude protein

FAT- fat content

FIBRE- fibre content

CHO- carbohydrate content

4.0 DISCUSSION

Several investigations had reported that *Daniellaoliveri* and other medicinal plants contain antimicrobial substances (Lewis, 1980; Akujobiet *al.*, 2004). The result of this present study agrees with the reports of these previous workers, who showed wide range of some active plant substances against certain organisms. Plant extracts have been used for many thousands of years in food preservation (Sofowora, 1996), pharmaceuticals, alternative medicine and natural therapies (Jones, 1996; Reynolds, 1996).

It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against *Escherichia coli*, *Pseudomonas aeruginosa* (Michie, 1992) *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus viridians*, *Shigelladysenteriae*, *Bacillus subtilis* and *Salmonella typhi* (Michie, 1992; Aboaba and Efuwape, 2001; Aboaba *et al.*, 2006).

Antimicrobial properties of plants are desirable tools in the control of undesirable microorganisms especially in the treatment of infectious diseases which correlates with research of Aboaba and Efuwape, (2001). The active components usually interfere with growth and metabolism of microorganisms in a negative manner (Forbes *et al.*, 1997).

Preliminary phytochemical screening showed that ethanolic extracts contain most of the phytochemicals like alkaloids, tannins, saponins, quinine and anthraquinone. It was discovered that the presence of alkaloid, saponin, steroid and phenol explain why the leaves can be used in the treatment of malaria, ring worms, syphilis, typhoid fever, eye sore and ear ache, head ache and other forms of fever and

the stem bark of *Daniellaoliveri* was used in the treating fever, boil and back ache while the tannin explain why it is used for treatment of dysentery according to (Matanet *al.*, 2006) which explain the antimicrobial efficacy of saponin and steroid against clinical organisms.

The presence of these bioactive components in the crude extracts has been linked to their activities against disease causing microorganisms and also offering the plant themselves protection against infection by pathogenic organisms (De and Ifeoma, 2002).

The high percentage of saponin among plant parts used leaf has the highest antinutrient which is (10.54), and the phytochemicals result also suggest the activities present in *Daniellaoliveri* extract of leaf and bark may be due to presence of tannin and this finding correlate with the observation of Hatanoet *al.*, (2005) who reported the antibacterial efficacy of flavonoids and tannin against clinical organisms and these findings relate with the observations of Mattanaet *al.*, (2010) who related that these bioactive components are known to show medicinal activity as well as exhibiting physiological activity (Edeoga et al., 2005) and exhibit anti-inflammatory, anti-oxidant, and membrane stabilizing property (Perenz et al.,1995).

The presence of terpenoid has been reported to be useful in herbal medicines (Baker *et al.*, 1983). These phytochemicals also have some strong antimicrobial significance against some potential enteric pathogens; the presence of Alkaloids in significant quantities may be used as anti malaria, analgesics and stimulants (Duke and Ayensu, 1985).

The other phytochemicals present in the plant are known to inhibit tumour growth, treatment of intestinal disorder like diarrhea and dysentery, Tannins are used in treating wounds, sprains, bruises and arresting bleeding (Akinpelu and Onakoya)

In general, the levels of medicinal plants and their compounds necessary to inhibit microbial growth are higher in foods than in culture media. This is due to interactions between phenolic compounds and the food matrix (Parekh and Chanda, 2007; Mitchell and Ahmad, 2006) and should be considered for commercial applications.

The plant extracts presented positive antimicrobial activity for *Escherichia coli*, *Pseudomonas aeruginosa* (Michie, 1992) *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus viridians*, *Shigelladysenteriae*, *Bacillus subtilis*and *Salmonella typhi* (Michie, 1992; Aboaba and Efuwape, 2001; Aboabaet *al.*, 2006). The ethanolic extracts are very potent in terms of activity because

bioactive components leading to the incomplete extraction of the components are more soluble in ethanol (De and Ifeoma, 2002).

Several phenolic compounds like tannins, flavonoids present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant organisms. Other preformed compounds like Saponins also have antifungal properties. Many plants contain non-toxic glycosides that can get hydrolyzed to release Phenolics that are toxic to microbial pathogens (Facey *et al.*, 1999). Therefore, the compounds detected may be responsible for the antibacterial activity according to (Michie, 1992).

Similarly various other compound present in several plants showed such antimicrobial activity against disease causing organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus cereus*, *Proteus vulgaris*, *Streptococcus viridians*, *Shigelladysenteriae*, *Bacillus subtilis* and *Salmonella typhi* (Leonard and Sheratt, 1961; Chhabra *et al.*, 1984).

Providing effective health care is a challenge under the best of economic circumstances. In the world's poorest countries, where infectious diseases are rife and resources limited, that challenge can assume over-whelming proportions, hence the resurgence in the use of herbal preparations to treat diseases.

4.1 CONCLUSION

After the experiment, it was concluded that *Daniellaoliveri* plant can be effective in the treatment of Dysentery, typhoid fever, headache and series of infections caused by microorganisms. This can be used in the production of more antibiotics in Nigeria and Worldwide to avoid and combat resistance of microorganisms to antibiotics. It also shows that the use of local herbs or medicinal plant should be encouraged by Government agencies and individuals worldwide.

4.2 RECOMMENDATION

It is thereby recommended that crude extract of *Daniellaoliveri* which is a medicinal plant is safe for the treatment of diarrhea and dysentery and should be encourage for use and research purposes.

Onsent: It is not applicable.

Ethical Approval: It is not applicable.

REFERENCE



- 1) Aboaba, O. and Efuwape, B. (2001) "Antibacterial properties of some Nigerian species", *Biology Research Communication*. 13: 183-188.
- 2) Aboaba, O., Smith, S. and Olide, F. (2006) "Antimicrobial Effect of Edible Plant Extract on *Escherichia coli* 0157:H7". *Pak. J. Nutr.* 5(4): 325-329.
- 3) A.C., Basile, J.A.A. Sertie, P.C.D. Fratas and A.C. Zanini. N. Anti-inflammatory activities of xanthone from Brazilian *Copaifera*. *J. Ethnopharmacol.* 22:101-109 (1988).
- 4) Ahmedulla M., Nayar M. P., 1999 Red data book of Indian plants: Calcutta: Botanical Survey of India, p. 4. Arora D. S., Kaur J., 1999 Antimicrobial activity of spices. *Int J Antimicrobial Agents* 12(3): 257-262.
- 5) Al-Harrasi A, Al-Rawahi A, Hussain J, Rehman N, Ali L, Hussain H (2012). Proximate analysis of the resins and leaves of *Boswellia sacra*. *J Med Plants Res*; 6(16):3098-3104.
- 6) Akinpelu D.A. and T.M. Onakoya, 2006. Antimicrobial properties of medicinal plants used in folklore remedies in south western Nigeria. *Afr. J. Biotechnol.*, 6(22): 2502-2505.
- 7) Akujobi, C., Anyanuwa, B., Onyerie, G. and Ibekwe, V. (2004). Antibacterial activities: a preliminary phytochemical screening of four medicinal plants. *Journal of Applied Science* 7 (3).
- 8) Aureli, P., Costantini, A. and Zolea, S. (1992) "Antibacterial activity of some plant essential oils against *Listeria monocytogenes*". *Journal of Food Protection*. 55:344-348.
- 9) Azu NC and Onyeagba RA. (2007). Antimicrobial properties of Extract of *Allium cepa* (onion) and *Zingiber officinale* (ginger) on *E. Coli*, *Salmonella typhi* and *Bacillus subtilis*. *The internet journal of tropical medicine*. 3(2) 246-9.
- 10) Balandrin, M., Klocke, J., Wurtele, E. and Bollinger, W. (1985). Natural plant chemical: Sources of industrial and medicinal materials science. 228:1154-1160.
- 11) Bao J. Y., Cai M., Sun G., Wang and H. Corke, (2005). Anthocyanins, Flavonoid and Free Radical Scavenging Activity of *Myrica bayberry* (*Myrica rubra*) extracts and their colour properties and stability. *Journal of Agric Food Chem.* 53: 2327-2332.
- 12) Baker CN, Thornsberg CH (1983). Inoculum standardization in antimicrobial susceptibility tests: Evaluation of overnight age culture. *J. Clin. Microbiol.* (17): 140-457.

- 13) Barrow GI, Feltham RKA (1995) Cowan and Steel's Manual for Identification of Medical , 3rd edn. Cambridge, Cambridge University.
- 14) Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). LWT- Food Science Technology.263-268 .
- 15) Brown, J. and Arthur, J. (2001). "Selenium, selenoproteins and human health: a review". *Public health nutrition* (2B): 593-9.
- 16) Burkill, H. (1997). The useful plants of Tropical Africa, 2nd edition. Vol.4. Chhabra, S., Usio, F. and Mshiu, E. (1984). "Phytochemical Screening of Tanzanian Medicinal plants" *Journal of Ethnopharmacology*. 1984, 11: 157-179.
- 17) Chukwujekwu, J., Staden, J. and Smith, P. (2005). Antimicrobial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants. 71:315-325.
- 18) Colombo, M. and Bosisio, E. (1996). Pharmacological activities of *Chelidonium majus*. 33:127-134.
- 19) Cowan, M.M. (2005). Plants products as antimicrobial agents. (Clinical microbiology review). 12:546-582.
- 20) Daziel, J. (1973) : The useful plants of West Africa. 1st edition. Pp.403-404.
- 21) Diplock, A., Charleux, J., Crozier-Willi, G., Kok, F., Rice-Evans, C., Roberfroid, M., Stahl, W. and Vina-Ribes, J. (1998). Functional food science and defense against reactive oxidative species. *Brazilian Journal of Nutrition*. 80: S77-S112.
- 22) De N, Ifeoma E (2002). Antimicrobial effects of components of the bark extract of *Ocimum gratissimum* L. (Basil) and *Xylopias aetiopica* A. Rich (Dunal). *Nig. J. Appl. Sc.* (11): 165-175.
- 23) Doughari, J., Human, I. Bennade, S. and Ndakidemi, P. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*. 3(11): 839-848.
- 24) Edeoga, H., Okwu, D. and Mbaebie, B. (2005). *Phytochemical constituents of some Nigerian medicinal Plants African Journal of Biotechnology* 4 (7): 685- 686.
- 25) Ekpo M. A., Etim P. C., 2009 Antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections. *J Med Plant Res* 3(9):621-624. *Encyclopedia Britannica, 2010 Anthelmintic. Encyclopedia Britannica Ultimate Reference Suite, Chicago.*
- 26) El Astal, Z.Y., Ashour, A.E. and Kerit, A.A. (2005). *Antimicrobial activity of some medicinal plant extracts in Palestine. Pak. J. Med. Sci.* 21(2):187-193

27. Ernst, E (2000). "Location bias in controlled clinical trials of complementary/alternative therapies". *International Journal of Epidemiology* 53 (5): 485–489.
28. Fabricant, D. and Farnsworth, N. (2001). "The value of plants used in traditional medicine for drug discovery". *Environmental Health Perspectives* . 109: 69–75. doi: 10.1289/ehp.01109s169.PMC 1240543 . PMID 11250806 .
29. Facey, P., Pascoe, K., Porter, R. and Jones, A. (1999). "Investigation of plants used in Jamaican folk medicine for anti-bacterial activity". *J Pharm Pharmacol*. 51: 1455–60.
30. Finer IL (2006). *Organic chemistry. Stereo chemistry of Natural products*. 5th edition.
31. Dorlingkindersely, Delhi. Pp 710-807.
32. Firn, R. (2010). *Nature's Chemicals*. Oxford University Press, Oxford. Pp 74-75.
33. Guevarra B. Q., 2005 A guidebook to plant screening phytochemical and biological. UST Publishing House, Philippines. Hawkins E. B., Ehrich S. D., 2006 Goto Kola. University of Maryland Medical Center, Baltimore, USA.
34. Horwitz W, Editor. *Official methods of analysis of AOAC international*. 17th ed. USA: Association of Official Analytical Communities; 2003.
35. Hussain J, Rehman N, Al-Harrasi A, Ali L, Ullah R, Mabood F, et al. Nutritional prospects and mineral compositions of selected vegetables from Dhoda Sharif- Kohat. *J Med Plants Res*. 2011; 5(29):6509–6514.
36. Harbone, J. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd Edition. Chapman and Hill, London. 279pp.
37. Hertog, M. and Feskens, E. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly study. *Lancet*. 342: 1007-1011.
38. Hatano, T, Kusuda, M, Inada, K, Ogawa, T.O, Shiota, S, Tsuchiya, T (2005). Effects of tannins and related polyphenols on methicillin-resistant *Staphylococcus aureus*. *Phytochemistry*. ;66:2047–55.
39. Indu, M., Hatha, A., Abirosh, C., Harsha, U. and Vivekanandan, G. (2006) "Antimicrobial activity of some of the south-Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonashydrophila*". *Braz. J. Microbiol*. 37: 153-158.
40. Iwu, M., Duncan, A. and Okinyi, C. (1999). New antimicrobial of plant origin. Pp. 457-462.
41. Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A. and Sharma, S. (2010). Cinnamon: a pharmacological review. *Journal of Advanced Scientific Research*. 1(2): 19-23.

41. Janick, J. (1999). Perspective on new crops and new uses. ASHS press, Alexandria VA. Pp.457-462.
42. Jones, F.(1996). "Herbs – useful plants. Their role in history and Today" Euro J GastroenterolHepatol 8:1227-123.
43. Kar, A. (2007). Pharmaocgnosy and Pharmacobiotechnology (Revised-Expanded Second Edition). New Age International Limited Publishers New Delhi. pp 332-600
44. Krishnaraju, A., Sundaraju, D., Vanisree, M., Tsay, H. and Subbaraju, G. (2005). *Assessment of bioactivity of Indian medicinal plants using Brime shrimp (Artemiasalina) lethality assay*. International Journal Applied science. 3:125-134.
45. Kroemer G., 2012 Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. SciTransil Med 4(143):ra99. doi: 10.1126/scitranslmed.3003807.
46. Lai, P. and Roy, J. (2004). "Antimicrobial and chemopreventive properties of herbs and spices". Curr. Med. Chem. 11(11): 1451–60.
47. Leonard, B. and Sherratt, H.(1961). "Pharmacology of the alkaloidal fraction from the bark of the Jamaican shade tree Pithecolobiumsamath Benth". 191: 287.
48. Lewis, H. (1980). Plants used in chewing sticks. *J. Prer. Dent.* 6:71-73.
49. Madziga, H., Sanni, S. and Sandabe U.(2010). Phytochemical and Elemental Analysis of Acalyphawilkesiana Leaf. Journal of American Science. 6(11): 510-514.
50. Martinez, M., Lazaro, R., Olmo L. and Benito, P.(2008) Anti-infectious activity in the anthemideatribe. In: Atta-ur- (Ed.) *Studies in Natural Products Chemistry*, Vol. 35. Elsevier. pp 445-516.
51. Mahato S. B., Sen S., 1997 Advances in triterpenoid research: 1990-1994. Phytochemistry 44:1185-1236.
52. Mattana C.M, Sattores S.E, Sosa A, Fusco A, Fusco M, Alcaraz L.E (2010). Antibacterial activity of extracts of acacia aroma against methicillin-resistant and methicillin sensitive *Staphylococcus*. *Braz J Microbiol.* ;41:581–7.

- 52.Matan, N., Rimkeeree, H., Mawson, A., Chompreeda, P., Haruthaithanasan,V. and Parker, M.(2006). "Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions". *International Journal of Food Microbiology*. 107:180-185.
- 53.Maurya, R., Singh, G. and Yadav, P. (2008). Antiosteoporotic agents from Natural sources. In:Atta-ur-Rahman (Ed.) *Studies in Natural Products Chemistry*, Vol. 35. Elsevier, pp 517- 545.
- 54.Michie, C. (1992). "The use of herbal remedies in Jamaica". *Ann Trop Paediatr*. 12: 31-6.
- 55.Mitchell, S. and Ahmad, M.(2006). "A Review of Medicinal Plant Research at the University of the West Indies, Jamaica". *West Indian Med J* 55 (4): 243.
- 56.Mitscher, L., Drake, S., Gollapudi, S. and Okwute, S. (1987). "A modern look at folkloric use of anti-infective agents." *J. Nat. Prod.* 50:1025- 1040.
- 57.Mordi RM and Erah PO (2006). Susceptibility of common urinary tract isolates to the commonly usedantibiotics in a tertiary hospital in Nigeria. *Am. J. Biotechnol*.5(11):1067-1071.
- 58.Nascimento, G., Locatelli, J., Freitas, P. and Silva, G. (2000). Antibacterial activity of plant Extracts and phytochemical on antibiotic resistant bacteria. *Brazil Journal Microbiology*.31:247-256.
- 59.Nweze E. L., Okafor J. I., Njokn O., 2004 Antimicrobial activities of methanolic extracts of Tremaguinensis (Schumm and Thorn) and Morinda Lucida Benth used in Nigeria. *Biol Res* 2:39-46.
- 60.Osuntokun ,O., Ojo, R. and Ogundeyi S.(2014).Chewing sticks and oral health care in owo Local Government Ondo State. *Journal Archives of Biomedical Science and the Health Pon` Publishers Ekpoma Edo state Nigeria*. 2(1):68-74.
- 61.Osuntokun OludareTemitope (2015), Bioactivity and Phytochemical Screening of Nigerian Medicinal Plants Growing in Ondo and Ekiti State against Bacterial Isolates from Pediatrics Hospital,*Journal of Advances in Medical and pharmaceutical Sciences* 4(2),Pp;1-14,Article no JAMPS. 2015. 032, ISSN: 2394-1111, SCIENCE DOMAIN international.www.sciencedomain.org

- 62.Osuntokun OludareTemitope and AjayiAyodele .O.(2014), Antimicrobial, Phytochemical and Proximate Analysis of Four Nigerian Medicinal Plants on some Clinical Microorganisms, *Current Research in Microbiology and Biotechnology*, Vol. 2, No. 5 (2014): 457-461.ISSN:2320-2246.
- 63.Osuntokun.O.T &Olajubu F.A, (2014). Antibacterial and Phytochemical Properties of SomeNigerian Medicinal plant on *Salmonella typhi* and *Salmonella paratyphi* Isolated from Infected Human Stool in Owo local Government, *Journal of Scientific Research & Reports*, 4(5): 441- 449, 2015; Article no.JSRR.2015.046, ISSN: 2320-0227, SCIENCEDOMAIN international, DOI:10.9734/JSRR/2015/12021 Editor(s)
- 64.Papp, L., Lu, J., Holmgren, A. and Khanna, K. (2007). "From selenium to selenoproteins: synthesis, identity, and their role in human health". *Antioxidants & redox signaling*9(7): 775–806.
- 65.Parekh, J. and Chanda, S.(2007). "In vitro antimicrobial activity and Phytochemical analysis of some Indian medicinal plants". *Turkey Journal of Biology* 31: 53-58.
- 66.Perez, R.M., S. Perez, A.M. Zavala and N. Salazar, 1995. Anti-inflammatory activity of the bark of *Hippocrata excels.* *J. Ethol. Pharmacol.*, 47(1): 85-90.
- 67.Philipson, J. (2007). Phytochemistry and pharamacognosy.*Phytochemistry*. 68:2960-2972.
- 68.Pulido R., Bravo L., Saura-Calixto F. (2002)*Journal of Agric Food Chem.* 48: 3396-3402.
- 69.Puntel, R., Nogueira, C. and Rocha, J. (2005).Krebs cycle intermediates modulate Thiobarbituric Acid Reactive Species (TBARS) production in rat brain *in vitro*. *Neurochem. Res.*, 30: 225-235.
- 70.Pushparaj, P., Tan, C. H. and Tan, B. K. H. (2000). Effects of *Averrhoa bilimbin* leaf extract on blood glucose and lipids in Streptozotocin-diabetic rats. *J.Ethnopharmacol.* 72: 69 – 76.
- 71.Rao, A. and Rao, L. Carotenoids and human health.*Pharmacological Research*. 2007;55(3):207-16.
- 72.Reynolds, J.(1996) "Martindale – the Extra Pharmacopoeia". 31st edition. London. RoyalPharmaceutical Society of Great Britain.Pp 21-25.

- 73.Salle, A. (1974). Fundamental principles of bacteriology. 7th edition Tata McGraw-Hill publishing company limited , New Delhi.
- 74.Samy, R. (2005). "Antimicrobial activity of some medicinal plants from India". *Fitoterapia*.76:697-699.
- 75.Santos, P., Olivena, A. and Tomassini, T. (1995). Controlemicrobiogico de productos fitoterapicos.31: 35-38.
- 76.Sarker, S. and Nahar, L. (2007).Chemistry for Pharmacy Students General, Organic and NaturalProduct Chemistry. England: John Wiley and Sons. pp 283-359.
- 77.Sofowora A., 1982 Medicinal Plants and Traditional medicine in Africa. 1st ed., New York: John Wiley and Sons Ltd. pp. 168-171.
- 78.Sofowora, E. (1993). Medicinal plant and traditional medicine in Africa Chichester.Pp.256.
- 79.Sofowora, E. (1996). "Medicinal plants and traditional medicine in Africa" Spectrum books Ltd. John Wiley and sons. Nigera, 1992 pp: 134-156.
- 80.Stepp and John R. (2004). "The role ofweeds as sources of pharmaceuticals"*Journal of Ethnopharmacology*92 (2-3): 163-166.doi: 10.1016/j.jep.2004.03.002 .
- 81.Swain and Tony. (1968). Plants inthe Development of Modern Medicine .Harvard University Press. ISBN 0-674-67330-1 .
- 82.Talaro, K. and Talaro, A. (2002).Foundation in microbiology 2nd edition.Brown publisher USA.
- 83.Tapsell, L., Hemphill, I. and Cobiac L. (2006). "Health benefits of herbs and spices: the past, the present, the future".*Med. J. Aust.* 185 (4 Suppl): S4-24.PMID 17022438 .
- 84.Tona, L., Ngimbi, N., Tsakala, M., Totte, K.andVlietinck, A. (1999). Antimicrobial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. *Journal of Ethnopharmacology*. 68(13): 403-404.
- 85.Trease G. E., Evans M. C., 1983 Textbook of pharmacognosy, 12th ed. Tindall, London, pp. 343-383.
- 86.Vats M, Singh H, Sardana S (2011) Phytochemical screening and antimicrobial activity of roots of *Murayyakoenyii* (Linn) Spreng. (Rutaceae). *Br. Jour. Microbiol.* 42: 1569-73.

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87.Zaika, L. (1988). "Spices and herbs: their antibacterial activity and its Determination". *Journal of FoodSafety*. 23:97-118.