PHYTOCHEMICAL, NUTRITIONAL AND ANTIMICROBIAL STUDIES OF *CORCHORUS OLITORIUS* LINN.

BY

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CERTIFICATION

We hereby certify that the thesis "Phytochemical, Nutritional and Antimicrobial Studies of *Corchorus olitorius* Linn" was carried out by Ekene Afam Ikwuegbu and it is a record of his own research work, submitted to the postgraduate school, in partial fulfillment of the requirements for the award of Master of Science (M.Sc) degree in biotechnology.

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DEDICATION

This project is dedicated to the Almighty God, my family, friends and to all lovers of science.

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ABSTRACT

The phytochemical analysis, proximate and mineral analysis, extraction of active principles and antimicrobial susceptibility tests were carried out on *Corchorus olitorius Linn*. The phytochemical analysis of the plant extracts (aqueous and ethanol) revealed the presence of alkaloids (53.50±0.05 mg), flavonoids (81.38±0.07 mg), tannins (71.00±0.02 mg), saponins (54.70±0.03 mg) and phenols (200.03±16.07 mg). The proximate analysis of whole plant showed that the plant contains moisture (29.69 ±0.01 mg), ash (4.20±0.03 mg), crude fat (7.14±0.01 mg), protein (28.20±0.27 mg), fiber (23.86 ±0.02 mg) and carbohydrate (74.30±0.70 mg). The mineral analysis also showed varied quantities of calcium (616.33±4.73 mg), magnesium (157.50±42.50 mg), iron (1.21±0.01 mg), sodium (306.00 ±6.00) and potassium (233.17±1.61 mg) in the different parts (root, stem, seed and leaves) respectively. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Microsporum canis, Candida albicans, Aspergillus niger,* and *Penicillium chrysogenum* were the organisms used as challenge organisms in the antimicrobial susceptibility tests. The results of the antimicrobial susceptibility tests showed that the extracts and active principles exhibited inhibitory and bactericidal but not fungicidal activities against the challenge organisms in various degrees. The zones of inhibition of the extracts and active principles on the test bacteria ranged from 4.50±0.1 mm – 10.00±0.07 mm while those of the test fungi were 10.00±0.1 mm. The minimum inhibitory concentrations of the extracts and active principles on the challenge organisms ranged from 100 mg/ml – 250 mg/l while the minimum bactericidal concentration of the extracts and active principles on the test bacteria was 250 mg/ml. This study has justified the traditional use of this plant for the treatment of many bacterial and fungal infections and also as an agent for wound healing whose causative agents are some of these organisms. It also lends credence to the need for further research on this plant so as to fully explore its pharmacological benefits.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

For centuries, people have used plants for healing. Until recently, plants were important sources for the discovery of novel pharmacologically active compounds, with many drugs being derived directly or indirectly from plants (Cordell, 2000). Many modern drugs have their origin in the ethno-pharmacology (Badami *et al.,* 2003). Information based on long-term use of plants by humans (ethno-medicine) likely helps to isolate safer active compounds from plants than isolating active compounds from plants with no history of human use (Lamidi *et al.,* 1995). Thus, instead of relying on trial and error, as in random screening procedures, traditional knowledge helps scientists to target plants that may be medicinally useful (Cordell, 2000). Traditional medicine is a comprehensive term used to refer to both traditional systems such as traditional Chinese medicine and to various forms of indigenous medicine (WHO, 2002). Historically, plants have provided a good source of anti-pathogenic agents in the fight against microbial infections. Seeking remedies for human ailments from the environment has formed the basis for therapeutics (Potier *et al*., 1990). Indeed, traditional medicine is an agent to potential source of new drugs and as a source of cheap raw materials for the synthesis of known drugs. Some examples include resperpine from *Rauwolfia serpentina*, viablastine from *Catharanthus rosesis* etc (Sofowora, 1993). There is an ever continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase that has been witnessed in the incidence of both new and

reemerging infectious diseases. A further big concern is the development of resistance to the antibiotics in current clinical use.

Corchorus olitorius Linn is an annual herb with slender stems. *C. olitorius* (Jute) is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, in tropical Asia in such countries as the Philippines and Malaysia, as well as in tropical Africa, Japan, South America, the Caribbean and Cyprus. In West African countries, particularly Ghana, Nigeria and Sierra Leone, where staple diets consist of starchy food-stuffs such as rice, cassava, maize and yams, leafy vegetables are used to complement such staple foods (Tulio *et al*., 2002). It is cultivated to provide bark for the production of fibers and its mucilaginous leaves are used in food as a vegetable (Meikle, 2007 and Abouzied, 2002). Jute is commonly known as long-fruited jute, Tossa jute, Jute mallow, Jew's mallow, Bush okra and West African sorrel. It is also called Moroheiya in Japan, Molehiya in Cyprus and Saluyot in the Philippines (Tulio *et al*., 2002). The crop is an excellent source of vitamins A and C, fiber, minerals including calcium and iron and other micronutrients. *C. olitorius* L. is extensively consumed as a healthy vegetable in Japan, because it contains abundant carotenoids, vitamin B1, B2, C and E, and minerals (Matsufuji *et al*., 2001). The leaves have been variously used in folk medicines for ascites, pain, piles, tumors cystitis, dysuria, fever, and gonorrhea while the cold infusion is said to restore the appetite and strength. Jute contains high levels of all essential amino acids except methionine which is at marginal concentrations (Tulio *et al*., 2002). It has high protein levels and is, along with other leafy species, the main source of dietary protein in many tropical countries (Tulio *et al*., 2002). The seeds are used as a purgative and leaves as demulcent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria. On preliminary analysis, seeds have been found to contain cardenolide glycosides (Gupta

et al., 2003). The methanol extracts of *C. olitorius* seeds have shown a broad spectrum of antibacterial activity (Pal *et al*., 2006). The cardenolide glycosides of *Corchorus* sp. have already been reported; erysimoside, olitoriside, corchoroside A and coroliside were isolated as constituents of the seeds of bush okra by Nakamura *et al.* (1998).

In Nigeria, especially amongst the Yorubas, it is commonly used in a stew known as Ewedu, a condiment to other starch-based foods such as Amala. The Hausa people and their Fulani neighbours call it Rama. They use it to produce soup (taush) or boil the leaves and mix it with kuli-kuli (groundnut cake) to form a dish known as kwado in Hausa. *C. olitorius* is believed to have prophylactic effect against enteric-fever and gastroenteritis (Adegoke and Adebayo, 2009). The plant is also used as a tonic against fever, toothache, dental carries, septic mouth and diarrhea (Lamidi *et al*., 1995). Many commonly proven drugs used in modern medicine were initially used in form in traditional or folk healing practices, or for other purpose that suggested potentially useful biological activity. The primary benefits of using plant derived medicine being that they are relatively safer than synthetic alternatives, offering profound benefits and more affordable treatment (Adegoke and Adebayo, 2009).

1.2 STATEMENT OF THE PROBLEM

Bacterial and fungal infections contribute basically to general health problems of man and have been reported to be responsible for over 50% of deaths recorded in developing countries. This challenging threat posed by bacterial and fungal species appears not to have an explanation in view as some conventional antibacterial and antifungal drugs have been unsuccessful in their activity against the pathogens due to the development of drug resistance. Multiple drug resistance in pathogenic microorganisms have been frequently

reported in current years throughout the world, mainly in developing countries, due to undiscriminating use of commercially available antibiotics in the treatment of infectious diseases. Though the resistance development by microbes cannot be clogged, suitable action will reduce the death and health care costs by using antibiotic resistant inhibitors of plant origin. Moreover, traditional remedies utilizing plants still occupy a central place among rural communities of developing countries for curing various diseases in the absence of an efficient primary health care system. The search for antimicrobials of plant origin has been mainly stimulated by the fact that some of the major antibacterial and antifungal agents have considerable drawbacks in terms of limited antimicrobial spectrum. Till today resistance in bacteria and fungi is most prevalent. This increasing resistance to antibiotics has therefore resulted in the search for leads for new organic molecules from plants with antimicrobial properties.

1.2 AIM OF THE STUDY

The aim of the study is to determine the bioactivity of the aqueous and ethanolic extracts of *Corchorus olitorius* Linn against some pathogenic microbes.

1.2.1 OBJECTIVES OF THE STUDY

The objectives of the study are as follows

- To extract the active phytochemicals
- To evaluate the phytochemical constituents of the extracts (qualitative and quantitative).
- To determine the proximate composition of the plant.
- To determine the mineral composition of the plant.
- To determine the antimicrobial sensitivity of the extracts and active principles

- To determine the minimum inhibitory concentrations (MIC) of the extracts and active principles against the challenge organisms.
- To determine the minimum bactericidal concentrations (MBC) of the extract and active principles against the challenge organisms.
- To determine the minimum fungicidal concentrations (MFC) of the extracts and active principles against the challenge organisms.

1.3 JUSTIFICATION OF STUDY

C. olitorius Linn is acclaimed traditionally to be a highly potent ethno-medicinal plant. It is therefore necessary to have scientific evidence for this. If found to be potent, it may be incorporated into the health care system of our country. The study may lead to the discovery of new sources of raw materials for the industrial manufacture of new drugs of plant origin. Furthermore, the scientific characterization of the ethno-botanical claim of this plant will help in the codification of this plant as well as its documentation into the ethno-medical data of medicinal plants. This study can offer the possibility of discovering new organic substances that will be useful against diseases for which suitable cures are not yet available. The active ingredients discovered from this work will lead to an increase in the available raw materials for the industrial production of drugs and other useful products. Finally, there is need to promote the use of medicinal plants as a sources of materials for drug manufacturing. We have to utilize our country's natural resources to solve our health problems. Furthermore, the paucity of knowledge on the phytochemical constituents, nutritional and antimicrobial properties of *Corchorus olitorius* has resulted to its neglect and underutilization. It is envisaged that the result of this study will initiate the exploitation of the pharmacological, nutraceutical and therapeutic potentials of the plant.

1.4 SCOPE OF THE STUDY

This research tends to assess the phytochemical constituents and the bioactivities of *C. olitorius* Linn through:

i. Collection and preparation of the plant materials.

ii. Extraction of the phytochemical from the plant materials.

iii. Phytochemical screening of the plant extracts (qualitative and quantitative).

iv. Proximate testing of the whole plant.

v. Mineral analysis of the whole plant.

vi. Collection and preparation of test organisms.

vii. Antimicrobial sensitivity testing of the plant extracts and isolation of active principles.

viii. Determination of the minimum inhibitory concentrations (MIC) of the plant extracts and the active principles against the test microbes.

ix. Determination of the minimum bactericidal concentrations (MBC) of the plant extracts and the active principles against the test organisms.

x. Determination of the minimum fungicidal concentrations (MFC) of the plant extracts and the active principles against the test organisms.

1.5 SIGNIFICANCE OF THE STUDY

The outcome of this study will increase the body of knowledge available on the antimicrobial, phytochemical and nutritional qualities of *Corchorus olitorius* Linn. It will also inform health policy makers on the need to improve on the traditional medicine practice. Also, it will lend credence to the primary health benefits in using plant-derived medicine being that they are relatively safe and affordable than synthetic alternatives. The

outcome of this study will also create a source of income for farmers who would propagate this plant as the country now relies on agriculture for revenue rather than oil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE PLANT "*Corchorus olitorius* Linn"

2.1.0 TAXONOMY

Kingdom: Plantae

Division: Eudicots

Sub-Division: Anthophyta

Class: (Dicotyledon) Magnoliate

Order: Malvales

Family: Malvaceae

Sub family: Grewiodeae

Genus: *Corchorus*

Species: *C. olitorius*

Binomial Name: *Corchorus olitorius*

Common Names

English: Jute Mallow, Bush Okra

Igbo: Krin Krin

Yoruba: Ewedu

Hausa: Rama

2.1.1 MORPHOLOGY AND BOTANICAL DESCRIPTION

The plants are erect, annual herbs, reaching a height of 2-4 meters, unbranched or with few lateral branches. The leaves are alternate, simple, lanceolate, 5 - 15cm long with an acuminate tip and a finely seriated or lobed margin. The flowers are small (2-3cm diameter) and yellow, with five petals. The fruit is a many-seeded capsule. It thrives almost anywhere, and can be grown year-round. They have a capsule–dehiscent, spindle-shaped

fruit-which is pentalocular and dehisce longitudinal, with 5 valves. They measure from 2 to 8cm long, to the summit, present a long thick beak between 5 to 10mm. Every capsule contains 25 to 40 seeds per chamber, 140 to 200 in each fruit, pyramidal, with colour varying from grayish – blue or green to brownish-black. The stem and the leaves are glabrous.

Figure 2.1 *Corchorus olitorius* Courtesy: (Adegoke, 2009).

2.1.2 ECOLOGY AND DISTRIBUTION

C. olitorius spread out in all tropical regions. It seems not to have any particular preferences of soils, as long as humidity is sufficient. It thrives well in soils with good fertility, of which the ratio of sand, silt and clay is proper. On the other hand, it is rare on degraded soils with a sandy top horizon and weak fertility. This plant may be only 30cm tall, quick maturing, and profusely branched.

2.1.3 PROPAGATION

C. olitorius is propagated by seeds.

2.1.4 ECONOMC IMPORTANCE

C. olitorius has the following uses;

i. Fiber: The plant is most widely cultivated for its vegetable fiber after cotton (*Gossypium hirsutum*) (Adegoke and Adebayo, 2009).

ii. Food: The leaves are consumed in various countries, especially Southern Asia, the Middle East, North Africa and West Africa. It has a mucilaginous texture similar to okra (*Abelmoschus esculentus*), when cooked (Adegoke and Adebayo, 2009).

iii. Ethno-Medicine: Rural dwellers in Nigeria use the plant to treat a number of diseases like malaria, peptic ulcer, urinary tract infections (Adegoke and Adebayo, 2009) etc.

2.2 PHYTOCHEMICALS

Phytochemicals are chemical compounds that occur as secondary metabolites in plants. Some are responsible for colour and other organoleptic properties of plants. Phytochemicals may have biological significance, but are not established as essential nutrients (USFDA, 2014). The medicinal value of plants are also directly connected to the vast array of organic chemical compounds (Phytochemicals) synthesized by their various pathways. Phytochemicals are considered as secondary metabolites since they are not directly related to the primary functions in plant survival. There are over 4000 known phytochemicals some of which include alkaloids, glycosides, saponins, colouring matters (USFDA, 2014) etc.

2.2.1 ALKALOIDS

Alkaloids are a group of naturally occurring organic chemical compounds that contain basic nitrogen atoms. They were among the earliest isolated pure compounds with

expressed biological activity. Alkaloids have been described in so many ways but close to actuality, an alkaloid is a plant – derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring, has a complex structure and is of limited distribution in the plant kingdom (Sneden, 2005). Alkaloids sharing several characteristics have bitter tastes, and most physiological effect on animals is on the nervous system (Akpuaka *et al*., 2003). They are usually very poisonous but in small quantity are used medically due to their physiological effects. Alkaloids are usually colourless, crystalline, non-volatile solids, few examples such as nicotine and hygriene are liquid, and few are coloured like herberine which is yellow in colour (Akpuaka *et al*., 2003). Alkaloids form insoluble precipitates with solutions of phosphotugistic acid, phosphomolybolic acid, picric acid, potassium mercuric iodide (Meyer's regent), Wagner's regent, Dragendorf's reagent etc. Many of these precipitate have definite crystalline shapes and so may be used to help in the identification of an alkaloid (Finar, 2006). They are usually found in the seeds, roots, leaves or barks of the plant and generally occur as salt of various plant acids e.g. acetic, oxalic, malic and tartaric acid. Alkaloids are classified according their physiological activities as Narcotics, Analgesics, Central Nervous System stimulants and psychedelics (Sneden, 2005).

Pharmacological Uses of Alkaloids

Alkaloids are very poisonous substances but are used medicinally in very small quantities. Alkaloids have been utilized as medicines and poisons. It should also be noted that the difference between medicinal or a toxic effects of many alkaloid (or any drug) is often the dosage (Fellows, 1991). Examples are;

a. Quinine, the active alkaloid in the bark of *Cinchona officinalis*, has been used for centuries in the treatment of malaria (Sneden, 2005).

b. In 1952, the alkaloid reserpine was isolated from roots of *Rauwolfia serpentine*, a plant used by Hindu healers for thousands of years. Although first used as a tranquilizer, reserpine has their greatest use in the treatment of hypertension (Fellows, 1991).

c. The alkaloids vinblastine and vincristine, isolated from Madagascar periwinkle (*Catharanthus roseus*) are remarkably effective in the treatment of certain forms of leukemia (Fellows, 1991).

d. The most additive drug in widespread use is nicotine (*Nicotiana tabacum*), the major alkaloid in tobacco. The abusive smoking of tobacco increases risks of certain cancers of the lung and coronary heart disease.

e. Erythrine alkaloids were used as a substitute for curare, which has been used therapeutically against tetanus and other convulsions. This began in 1935 (Fellows, 1991).

f. Certain herbs that contain the tropane alkaloids have been associated with the practice of witchcraft in medieval Europe. *Atropa belladonna*, a branching herbaceous perennial plant native to Europe and Asia, basically due to its ability to be absorbed through the skin, has the following effects, relax smooth muscles, dilate the pupil of the eye, dilate blood vessels, increase heart rate and body temperature, induce sleeping and can even stimulate the central nervous system. The atropine is used medically today by ophthalmologists (Estelle and McMahon, 1999).

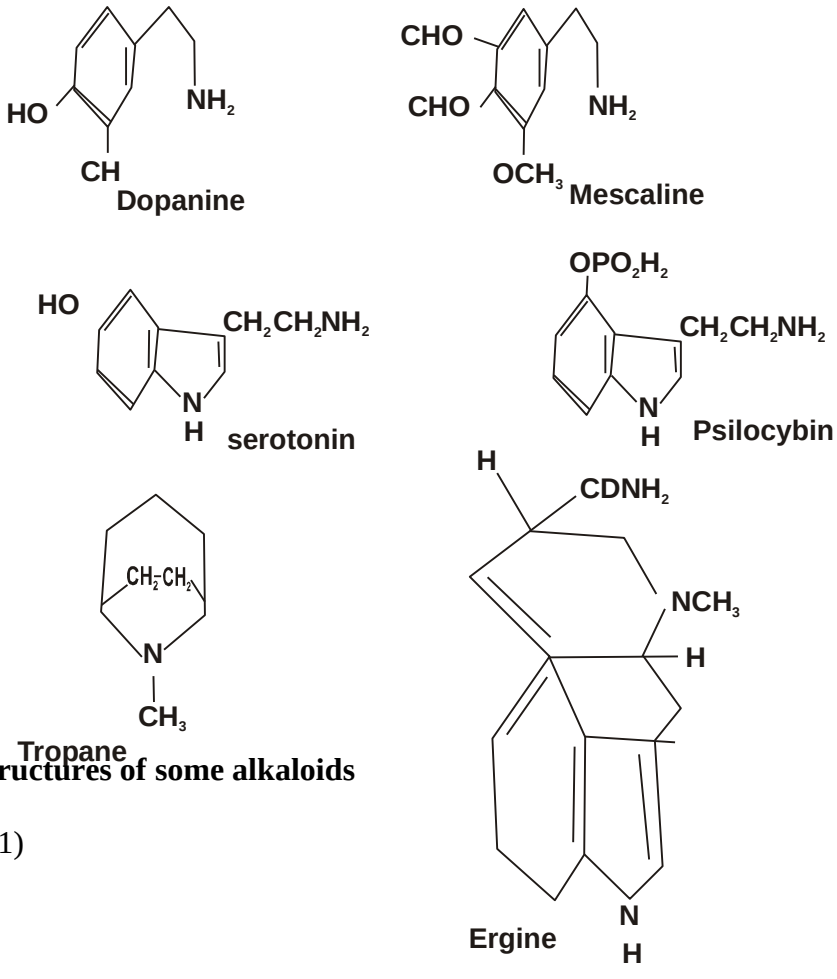


Figure 2.2 Structures of some alkaloids

(Fellows, 1991)

Properties of Alkaloids

1. Most alkaloids contain oxygen in their molecular structure; those compounds are usually colorless crystals at ambient conditions. Oxygen-free alkaloids, such as nicotine or coniine are typically volatile, colorless, oily liquids.
2. Some alkaloids are colored, like berberine (yellow) and sanguinarine (orange).
3. Most alkaloids are weak bases, but some, such as theobromine and theophylline, are amphoteric.
4. Many alkaloids dissolve poorly in water but readily dissolve in organic solvents, such as diethyl ether, chloroform or 1, 2-dichloroethane. Caffeine, cocaine, codeine and nicotine are slightly soluble in water (with a solubility of ≥1g/L), whereas

others, including morphine and yohimbine are very slightly water-soluble (0.1–1 g/L) (Sneden, 2005).

5. Alkaloids and acids form salts of various strengths. These salts are usually freely soluble in water and ethanol and poorly soluble in most organic solvents. Exceptions include scopolamine hydrobromide, which is soluble in organic solvents, and the water-soluble quinine sulfate.
6. Most alkaloids have a bitter taste or are poisonous when ingested. Alkaloid production in plants appeared to have evolved in response to feeding by herbivorous animals; however, some animals have evolved the ability to detoxify alkaloids. Some alkaloids can produce developmental defects in the offspring of animals that consume but cannot detoxify the alkaloids. One example is the alkaloid cyclopamine, produced in the leaves of corn lily (Sneden, 2005).

2.2.2 FLAVONOIDS

Flavonoids, also known as bioflavonoids, are a class of plant secondary metabolites commonly known by their yellow colour in nature. They are responsible for the colour of the plants. Chemically, they have the general structure of a 15-cabon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C) (McNaught, 1997). Flavonoids consist of six major subgroups, Chalcone, Flavone, Flavonol, (or – 3 – hydroxyl flavones), Flavonone, Anthocyanins and Flavans. Together with carotenes, flavonoids are responsible for the colouring of fruits, vegetables and herbs (Estelle and McMahon, 1999). Flavonoids are found in most plant materials. The most important dietary sources are fruit tea and soybean. Green and black tea contains 25% flavonoids. Other important sources are Apple (*Malus domestica),* Citrus fruits as well as red wine which contain high level of flavonoids, mainly quercetin and rutin 20 (McNaught, 1997).

Epidemiological studies and scientific experiments suggest a protective function of flavonoids against cardiovascular diseases. Also, many studies have confirmed that one or two glasses of red wine daily can protect against heart disease. Tea flavonoids equally have many health benefits like reducing the oxidation density of lipoprotein, lowers the blood level of cholesterol, prevents osteoporosis, and ease menopausal symptoms. Flavonoids are well known for their health benefits but they may have adverse effects on a very high intake such as anti-nutritional effects, reduces glucose or minerals, thyroid toxicity, carcinogenic and drug interaction (Estelle and McMahon, 1999).

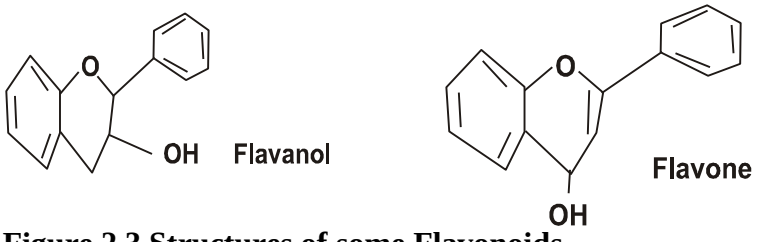


Figure 2.3 Structures of some Flavonoids

(McNaught, 1997).

2.2.3 TANNINS

Tannins are a group of plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. They are colourless and non-crystalline substances which form colloidal solution in water. They have astringent, homeostatic, antiseptic and invigorating properties (Harbone, 1973). The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Katie *et al*., 2006). The astringency from the tannins is responsible for the dry and puckery feeling in the mouth following the consumption of unripe fruit or red wine (Katie *et al*., 2006). Likewise, the destruction and/or modification of tannins with time play an

important role in the ripening of fruit and aging of wine. Tannins have molecular weights ranging from 500 to over 3,000 (Gallic acid esters). Three groups of tannins exist;

a. Hydrolysable tannins e.g. Glycogallin, Gallic acid, Ellagic acid.
b. Non-hydrolysable tannins e.g. Catechin whose structures are resistant to breaking.
c. Tannins of unclassified nature.

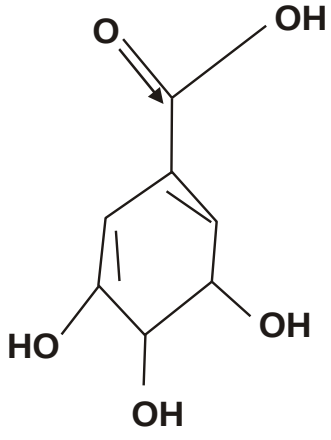


Figure 2.4 Structure of Gallic Acid

2.2.4 GLYCOSIDES

Glycoside is a general term which embraces all the many varied combination of sugars and glycine. Glycosides are widespread in the plant kingdom and are second in importance as medicines or toxins (Estelle and McMahon, 1999). The common feature they share is the presence of sugar molecule attached to the active components. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. They are classified according to the type of glucosidic bond they have. For the purpose of pharmacology and biochemistry, classification according to the chemical nature of the aglycone is the most useful. There are Anthraquinone, Alcoholic and Thio, Flavonoid, Saponins, Cumarin, Cynogenic. Glycosides are very active structures in human body. The glycosidic units are frequently found in antibiotics (Roger-Pamplona, 1999).

The physiological effects depend on the active component e.g. cardio-active glycosides which affect the contraction of heart muscle. Therapeutically, also glycosides strengthen weaken parts (Roger-Pamplona, 1999).

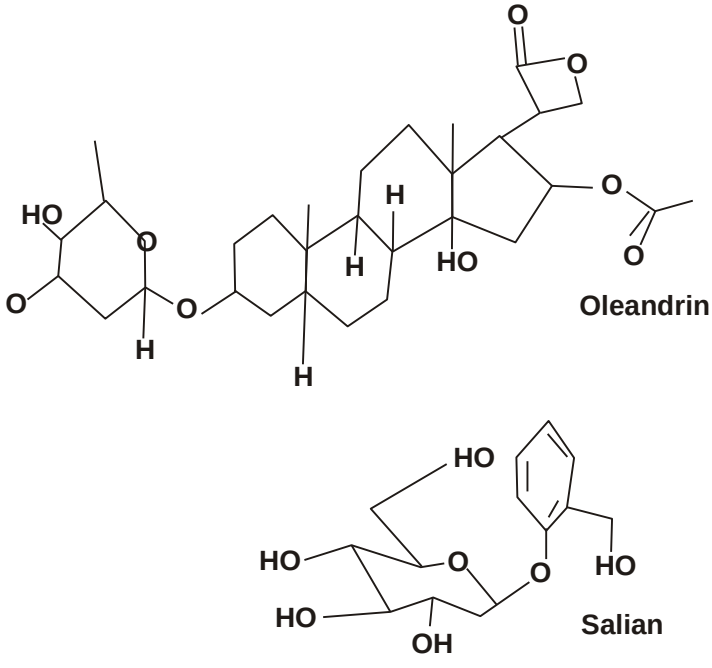


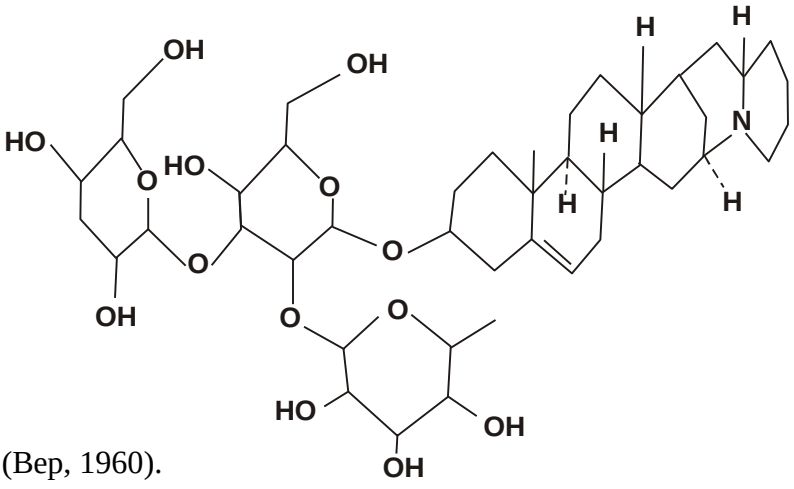
Figure 2.5 Structures of Some Glycosides

(Roger-Pamploner, 2006)

SAPONINS

Saponins are a class of organic chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with lipophlic triterpene derivative (Hoslettman, 1995). Saponins are flavour additives, which are found in legumes e.g beans, groundnut etc. Saponins boost the immune system, lower the cholesterol level in the blood and reduce the rate of getting intestinal cancer. When taken orally, saponins are

comparatively harmless. For example, sarsaparilla is rich is saponins but is widely used in the preparation of non-alcoholic beverages (Bep, 1960).



(Bep, 1960).

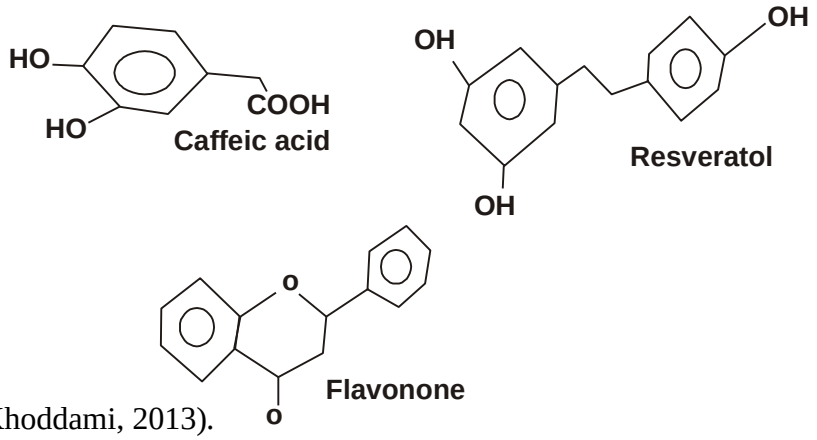
Solanine

Figure 2.6 Structures of Saponins

2.2.5 PHENOLS

Phenols, sometimes called phenolics, are a class of organic chemical compounds consisting of a hydroxyl group (-OH) bond directly attached to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid (C_6H_5OH). Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). The most important role of phenols may be in plant defense against pathogen and herbivore predators, and thus are applied in the control of human pathogenic infections. They are classified into (i) phenolic acids, (ii) flavones, (Xanthenes and Catechin) and (iii) Non-Flavonoid polyphenolics. Caffeic acid is regarded as the most common of phenolic compound distributed in the plant kingdom followed by chlorogenic acid known to cause allergic dermatitis, among humans *(*Khoddami, 2013). Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples (*Malus domestica*), green tea and red wine for their enormous ability to

combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents.

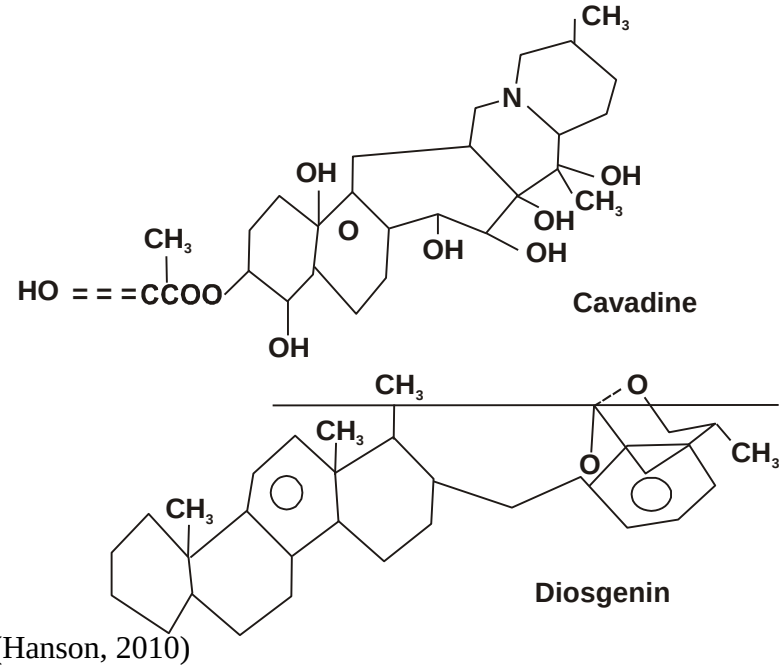


(Khoddami, 2013).

Figure 2.7 Structures of some phenols

2.2.6 STEROIDS

Plant steroid (or steroid glycosides) also refered to as cardiac glycosides are one of the most naturally occurring plant phytochemicals that have found therapeutic applications as arrow poison or cardiac drugs (Hanson, 2010). The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness (Hanson, 2010). Caution should be taken when using steroidal glycosides as small amounts would exhibit the much needed stimulation on a diseased heart, whereas excessive dose may even cause death. Diosgenin and Cavadine (from *Veratrum veride*) are examples of plant steroids.



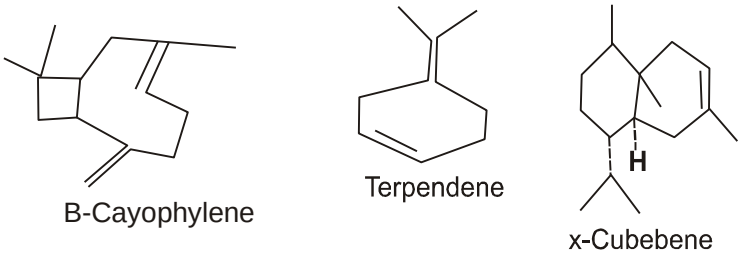
(Hanson, 2010)

Figure 2.8 Structures of some steroids

2.2.7 TERPENES

Terpenes are among the most widely spread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form, commonly found in essential oils, resins or oleoresins (Hanson, 2010). Terpenoids includes hydrocarbons of plant origin with general formular $(C_5H_8)_n$ and are classified as mono-,di-,tri- and sesquiterpenoids depending on the number of carbon atoms. Examples of common important monoterpenes include terpinen-4-ol, thupene, camphor, eugenol and menthol. Diterpenes (C_{20}) are classically considered to be resins and taxol, the anticancer agent, is the common example. The triterpenes (C_{30}) include steroids, sterols and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes; amyrins, ursolic acid and deanic acid sesquiterpene (C_{15}) like monoterpenes, are major components of many essential oils (Hanson, 2010). The sesquiterpene acts as irritants when applied externally and when consumed internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and

broadly they have antimicrobial and neurotoxic action. Terpenoids are classified according to the number of isoprene units involved in the formation of those compounds.

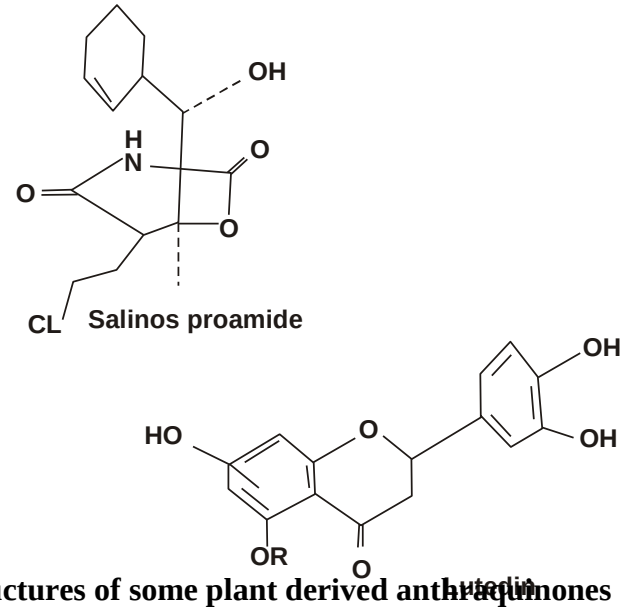


(Hanson, 2010).

Figure 2.9 Structures of some pharmacological important plant derived terpenes

2.2.8 ANTHRAQUINONES

Anthraquinones (also known as anthraquinonoids) are a class of naturally occurring phenolic compounds based on the 9,10-anthraquinone skeleton. They are derivatives of phenolic and glycosidic compounds. They are solely derived from anthracene, giving variable oxidized derivatives such as anthrones and anthranols (Hanson, 2010). Other derivatives such as chrysophanol, aloe-emodin, rhein, salinosporamide, lutedin and emodin have in common a double hydroxylation at positions C-1 and C-8.

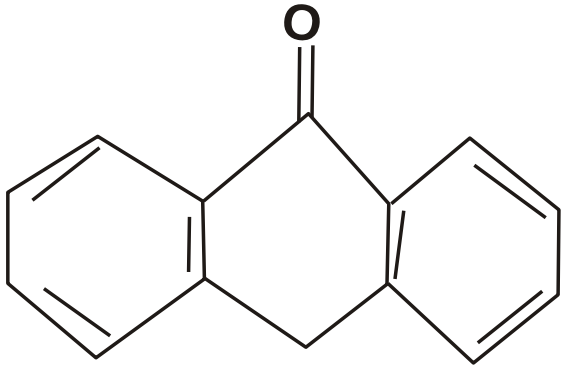


(Hanson, 2010).

Figure 2.10 Structures of some plant derived anthraquinones

2.2.9 ANTHRANOID

Anthranoids are a form of tricyclic aromatic ketone. They are used for popular cellulose assay in the calorimetric determination of carbohydrates (Fellows, 1991). They are used in pharmacy as laxative. They stimulate the motion of the colon and are responsible for less water reabsorption. They may only be used for a short amount of time, because long time use may lead to loss of electrolytes.



(Fellows, 1991).

Figure 2.11 Structure of Anthranoid

2.3 AN OVERVIEW OF THE TEST ORGANISMS

2.3.1 *Escherichia coli*

E. coli are gram negative, facultatively anaerobic, rod-shaped bacteria of the genus *Escherichia.* They are commonly found in the lower intestine of warm blooded organisms (Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Vogt, 2005). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Benthy, 1982) and preventing colonization of the intestine with pathogenic bacteria (Hudault *et al*., 2001). *E. coli* and other facultative anaerobes constitute about 0.1% of gut flora (Eckburg *et al*., 2005) and fecal-oral transmission is the major route through which pathogenic strains of the

bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Vogt, 2005). The bacterium can be grown and cultured easily and in expensively in a laboratory setting and has been intensively investigated for over 60 years. *E. coli* are the most widely studied prokaryotic model organism, and an important species in the field of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favourable conditions, it takes only twenty (20) minutes to reproduce. *E. coli* can live on a wide variety of substrates and uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to below, as is the case when *E. coli* lives together with hydrogen consuming organisms, such as methanogens or sulphate-reducing bacteria (Madigan and Martinko, 2006). Optimum growth of *E. coli* occurs at 37^0C (98.6^0f), but some laboratory strains can multiply at temperatures of up to 49^0C (Vogt, 2005). *E. coli* have the ability to transfer DNA via Bacterial conjugation, transduction, or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *shigella* to *E.coli* carried by a bacteriophage (Brussow *et al*., 2004). Most *E. coli* strains do not cause disease but virulent strains can cause gastroenteritis, urinary tract infections and neonatal meningitis. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes fever. In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic–uremic syndrome (HUS), peritonitis, mastitis, septicemia and gram negative pneumonia (Vogt, 2005). There is one strain, *E. coli* #0157.117, which produces a toxin called shiga toxin (classified as

bioterrorist agents).This toxin causes premature destruction of the red blood cells which then clog the body's filtering system, the kidneys, causing hemolytic-uremic syndrome (HUS). This in turn causes strokes due to small clots of blood which lodge in capillaries in the brain. This causes the body parts controlled by this region of the brain not to work properly. In addition, this strain causes the building up of fluid (since the kidneys do not work) leading to edema around the lungs, legs and arms. This increase in fluid building especially around the lungs impedes the functioning of the heart, causing an increase in blood pressure. Uropathogenic *E. coli* (UPEC) are one of the main causes of urinary tract infections. It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacterium into the male urethra and in switching from anal to vaginal intercourse; the male can also introduce UPEC to the female urogenital system. Due to its long history of laboratory culture and ease of manipulation, *E. coli* play an important role in modern biological engineering and industrial microbiology. *E. coli* is frequently used as a model organism in microbiology study. Cultivated trains (e.g *Escherichia coli* K12) are well adapted to the laboratory environment, and unlike wild-type strains, have lost their ability to thrive in the intestine. These features protect wild-type strains from antibodies and other chemical attacks, but require a large expenditure of energy and material resources.

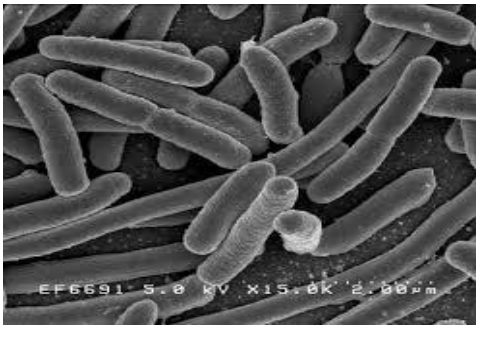


Figure 2.12 Micrograph of *Escherichia coli* (Brooks *et al*., 2004)

2.3.2 *Salmonella typhi*

Salmonella typhi is a rod shaped flagellated facultative anaerobic, gram-negative bacterium and a member of the genus salmonella (*Murray et al., 2009).* A number of its serovars are serious human pathogens. S. typhi is found only in humans. According to the Robert Koch institute, Germany (1999), the number of disease caused by this organism can be greatly reduced by improving hygienic conditions. *S. typhi* pass through fecal contaminated food or drinking water in the gastrointestinal tract. Also, raw chicken eggs and goose eggs can harbor *salmonella* initially in the egg white, although most eggs are not infected. *S. typhi* has killed over 600,000 people annually all over the world. It is a deadly bacterium that causes typhoid fever.

S. typhi usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of high fever, headache and nausea. Other common symptoms include loss of appetite, diarrhea and enlargement of the spleen. Some individuals who are infected with *S. typhi* become life-long carriers that serve as the reservoir of these pathogens. *S. typhi* has an endotoxin (which is typical of gram negative organisms), as well as the antigen, which increases virulence. It also produces a protein called invasin that allows non-phagocytic cells to take up the bacterium and allows it to live intracellularly.

S. typhi is a strong pathogen for humans due to its resistance to the innate immune response system (Fallow, 2007). Recently, strains of MDR (Multi-drug resistance) *Salmonella* have been identified and grouped together in a single halotype named H58. It has also been found that these strains are now resistant to nalidixic acid and have reduced susceptibility to fluoroquinolenes. This strain has been recently found in Morocco, which shows that the MDR strain has reached far in Africa (Murray *et al.,* 2009).

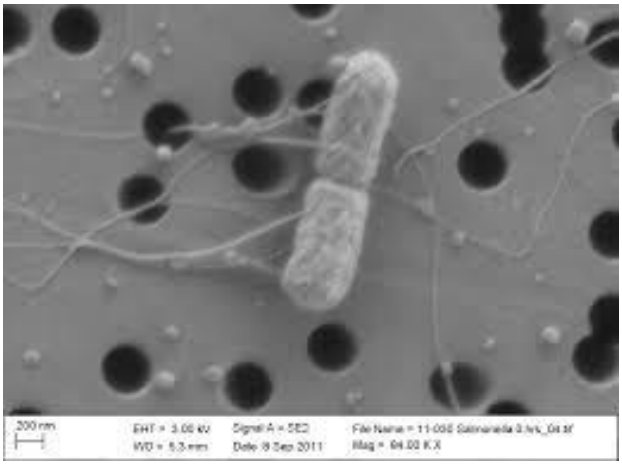


Figure 2.13 Micrograph of *Salmonella typhi* (Brooks *et al*., 2004)

2.3.3*Staphylococcus aureus*

Staphylococcus aureus is a gram-positive coccal bacterium that is commonly found in the respiratory tract and on the skin. It is often positive for catalase and nitrate reduction. Although, *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses and respiratory infections such as sinusitis and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell surface proteins that bind and inactivate antibodies. The emergence of antibiotic–resistant forms of *S. aureus* such as MRSA (Methicillin-resistant *Staphylococcus aureus*) is a worldwide problem in clinical medicine. *Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by the surgeon, sir Alexander Ogston in pus from a surgical abscess in a knee joint (Cole *et al.*, 2001). This name was later appended to *Staphylococcus aureus* by Fredrick Julius Rosenbach, who was credited by the official system of nomenclature at the time. An estimated 20% of the human populations are long-term carriers of *S. aureus* (Kluytmans, 1997) which can be found as part of the normal skin flora and in the nostrils (Cole *et al.*, 2001).

S. aureus is the most common species of *Staphylococcus* to cause staph infections and is a successful pathogen due to a combination of nasal carriage and bacterial immunoevasive

strategies (Cole *et al.,* 2001). *S. aureus* can cause a range of illnesses, from minor skin infections such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scaled skin syndrome and abscesses, to life-threatening disease such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis (Bowersox, 1999). Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of hospital-acquired infection and often the cause of post surgical wound infections. Each year, about 500,000 patients in United States Hospital contract a Staphylococcus infection (Bowersox, 2000).

In as much as *Staphylococcus aureus* is responsible for many infections, it may also occur as a commensal. The presence of *S. aureus* does not always indicate infection. It can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain (Cimolai, 2008). *S. aureus* can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infection, including boils and carbuncles (a collection of boils). *S. aureus* infection can spread through contact with pus from an infected wound, skin to skin contact with an infected person by producing hyalurondase that destroys tissues, and contact with objects such as towels, sheets, clothing or athletic equipment used by an infected person. Deeply penetrating *S. aureus* infections can be severe. *S. aureus* can survive on dogs (*Cole et al.,* 2001), cats (Hanselman *et al.,* 2009) and horses, (Burton *et al.,* 2008) and can cause bumblefoot in chickens.

The most common treatment of choice for *S. aureus* infection is penicillin. Spread of *S. aureus* generally is through human-to-human contact, although recently some veterinaries have discovered that the infection can be spread through pets, with environmental contamination thought to play a relatively unimportant part. Emphasis on basic and hand washing techniques are, therefore, effective in preventing its transmission.

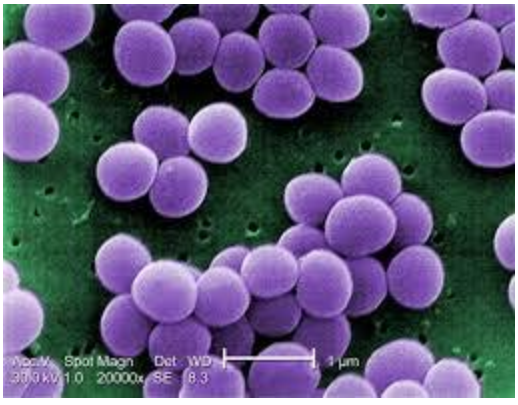


Figure 2.14 Micrograph of *Staphylococcus aureus* (Brooks *et al*., 2004)

2.3.4 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram negative, non motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Although found in the normal flora of the mouth, skin and intestines (Ryan and Ray, 2004). It can cause destructive change to human and animal lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum. In the clinical setting, it is the most significant member of the Klebsiella genus of Enterobacteriacea. *K. oxytoca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens.

In recent years, Klebsiella have become important pathogens in nosocomial infections. It naturally occurs in the soil, and about 30% of the strains can fix nitrogen in anaerobic conditions (Postgate, 1998). As a free living diazotroph, its nitrogen fixation system has been much studied, and is of agricultural interest, as *K. pneumoniae* has been demonstrated to increase crop yields in agricultural steads (Riggs *et al.,* 2010). Members of the genus typically express two types of antigen on their cell surfaces. The first, "O" antigen is a component of the lipopolysaccharide (LPS), of which 9 varieties exist. The second is Klebsiella antigen, a capsular polysaccharide with more than 80 varieties (Podschun and ullmann, 1998). Both contribute to pathogenicity and form the basis for serogrouping. It is closely related to *K. oxytoca* from which it is distinguished by being indole-negative and

by its ability to grow on both melizitose and 3-hydroxybutyrate. *K. pneumoniae* can cause destructive changes to human lungs via inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum (currant jelly sputum). These bacteria gain access typically after a person aspirates colonizing oropharyngeal microbes into the lower respiratory tract. As a general rule, *K. pneumoniae* infections are seen mostly in people with a weakened immune system. The most common condition caused by *K. pneumoniae* outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitations, and plural adhesions. It has a high death rate of about 50%, even with antimicrobial therapy. The mortality rate can be nearly 100% for people with alcoholism and bacteremia. In addition to pneumonia, *K. pneumoniae* can also cause infections in the urinary tract, lower respiratory tract and surgical wound site. The range of clinical disease includes pneumonia, thrombophlebitis, urinary tract infection, diarrhea, upper respiratory tract infection, would infection, osteomyelitis, meningitis, and bacteremia septicemia.

Klebsiella ranks second to *E. coli* for urinary tract infections in older people. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy and rhinoscleromatis. New antibiotic-resistant strains of *K. pneumoniae* are appearing (Prescott *et al*., 2005). Klebsiella organisms are often resistant to multiple antibiotics. Current evidence implicates plasmids as the primary source of the resistance genes (Prescott *et al*., 2005). Klebsiella with ability to produce extended spectrum beta-lactamases (ESBL) are resistant to many classes of antibiotics. The most frequent resistances include resistance to aminoglycosides, fluoroquindone, tetracycline, chloromphenical and trimethoprim/ sulfamethoxazole (Prescott *et al*., 2005).

As with many bacteria, the recommended treatment has changed as the organism has developed resistances. The choice of a specific antimicrobial agent or agents depends on local susceptibility patterns and on the part of the body infected. For patients with severe infections, a prudent approach is the use of an initial course (48-72hr) of combination therapy, followed by a switch to a specific monotherapy once the susceptibility pattern is known for the specific patient. If the specific Klebsiella in a particular patient does not show antibiotic resistance, then the antibiotics used to treat such susceptible isolates include ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clarulanate, ceftazidime, cefepim, lerofloxacin, and ertapenem. Some experts recommend the use of meropenem for patients with ESBL producing Klebsiella. The claim is that meropenem produces the best bacterial clearing. To prevent spreading Klebsiella infections between patients, health care personnel must follow specific infection-control precautions (Prescott *et al*., 2005).

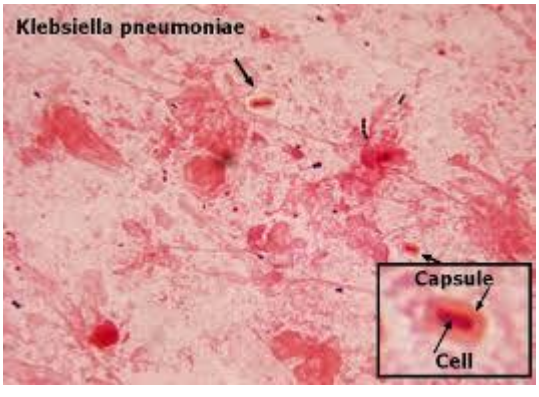


Figure 2.15 Micrograph of *Klebsiella pneumoniae* (Brooks *et al*., 2004)

2.3.5 *Candida albicans*

Kingdom: Fungi

Division: Ascomycota

Class: Saccharomycetes

Order: Saccharomycetales

Family: Debaryomycetaceae

Genus: *Candida*

Species: *C. albicans*

Candida albicans is a dikaryotic fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans (Ryan and Ray, 2004). Fungal infections including those caused by *C. albicans* have emerged as important causes of morbidity and mortality in immuno-compromised patients (e.g AIDs, Cancer, organ or bone marrow transplantation). *C. albicans* biofilms may form on the surface of implantable medical devices. In addition, hospital acquired infections by *C. albicans* have become a cause of major health concerns. *C. albicans* is a commensal and a constituent of the normal gut flora comprising microorganism that live in the human mouth and gastro-intestinal tract. Overgrowth of the fungus results in candidaisis. One of the most important features of the *C. albicans* genome is the occurrence of numeric and structural chromosomal rearrangements as means of generating genetic diversity, named chromosome length polymorphisms, reciprocal translocations, chromosome deletions and trisomy of individual chromosomes. These karyotypic alterations lead to changes in the phenotype, which is an adaptation strategy of this fungus. The genome of *C. albicans* is highly dynamic, and this variability has been used advantageously for molecular epidemiological studies and population studies in this species. The genome sequence has allowed for identifying the presence of a parasexual cycle (no detected meiotic divisions) in *C. albicans* (Ryan and Ray, 2004). Treatment for infections caused by *C. albicans* commonly includes Nystasin, amphotericin B, echinocandin or fluconazole for systemic infections, oral or esophageal infections, topical azole for vaginal infections (Ryan and Roy, 2004).

Figure 2.16 Micrograph of *Candida albicans* (Brooks *et al*., 2004).

Morphological Description of *Candida albicans*

C. albicans grow as both single cells and filamentous microscopic structures called hyphae. Many interconnected hyphae form a mycelium. They produce a large number of asci during sexual reproduction. The asci are often contained in a multicellular, occasionally readily visible fruiting structure called the ascocarp. Ascocarps come in a very large variety of shapes; cup-shaped, club-shaped, potatoe-like etc. They can appear either solitary or clustered. Ascocarps come in multiple colours such as red, orange, yellow, brown, black etc. The cell walls of *C. albicans* contain chitin and β-glucans.

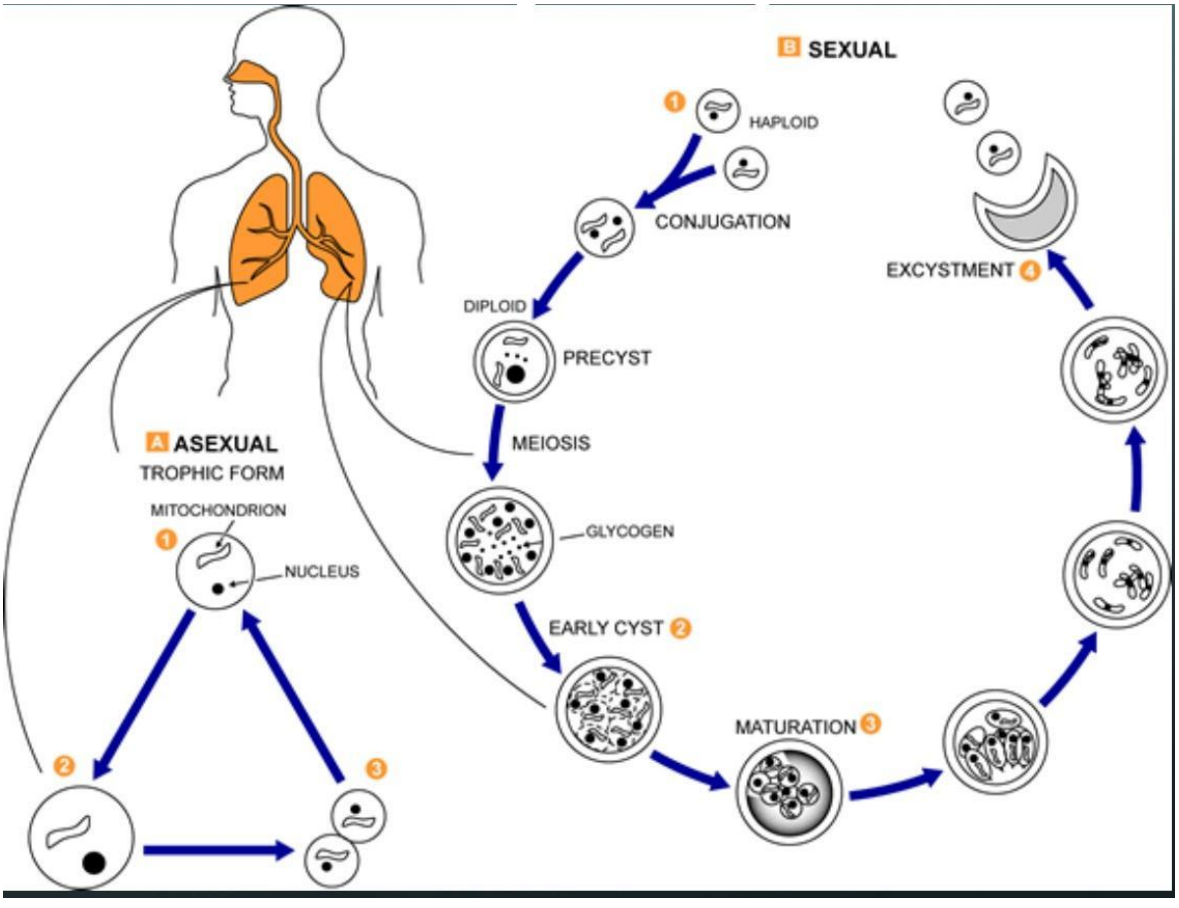


Figure 2.17 Life Cycle of *Candida albicans* (Brooks *et al*., 2004).

Cultural Characteristics and Ecology of *Candida albicans*

When cultured in a standard yeast extract medium, *Candida albicans* grows as ovoid yeast cells. However, mild environmental changes in temperature and pH can result in a morphological shift to pseudophyhal growth (Ryan and Ray, 2004). *C. albicans* cells, where grown in a medium that mimics the physiological environment of a human host, they grow as "true" hyphae. Its ability to form hyphae has been proposed as a virulence factor, as these structures are often observed invading tissues, and strains that are unable to form hyphae are defective in causing infection. *C. albicans* can also form chylamydospores (Ryan and Ray, 2004). On Sabouraud's dextrose agar colonies of *C. albicans* are white to cream colored, smooth, glabrous and yeast-like in appearance. Ecologically, *C. albicans* can be classified as a normal microbial flora of man.

Candida albicans is the fifth most common cause of blood stream infections and fourth common cause of nosocomial infections (Ryan and Ray, 2004). *C. albicans* has been historically documented as the predominant cause of Candidiasis (Ryan *et al*., 2004). *C. albicans* is an important cause of morbidity and mortality in immuno-compromised patients (Ryan *et al*., 2004). *C. albicans* manifest in pathogenic state causing painful mucosal infections presenting as oral thrush, and vaginal infections among other clinical manifestations (Ryan and Ray, 2004). They are also a major cause of both mucosal and deep tissues infections (Ryan and Ray, 2004).

2.3.6 *Aspergillus niger*

Kingdom: Fungi

Phylum: Ascomycota

Class: Eurotiomycetes

Order: Eurotiales

Family: Trichocomaceae

Genus: *Aspergilllus*

Species: *A. niger*

Aspergillus niger one of the most common species of the genus *Aspergillus*. It causes a disease called black mould on certain fruits and vegetables such as grapes (*Vitis vinifera*), apricots (*Prunus amerniaca*), onions (*Allium cepa*) and peanuts (*Arachis hypogaea*) and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould") (Ryan and Ray, 2004). *A. niger* is less likely to cause human disease than some other species. However, it is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss and in severe cases, damage to the ear canal and tympanic membrane. *A. niger*

has a wide range of industrial and research uses. It is often used as a challenge organism for clearing validation studies performed within sterile manufacturing facilities.

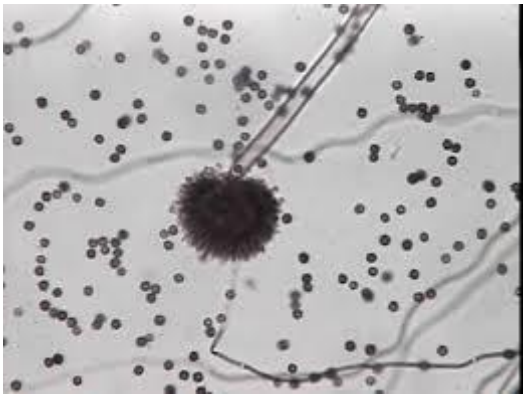


Figure 2.18 Micrograph of *Aspergillus niger* (Ryan and Ray, 2004).

Morphological Description of *Aspergillus niger*

Morphology provides a reasonable means of classification and delimitation of *A. niger*, however, it is not a reliable means of identifying a given isolate from the field. The major distinction currently separating *A. niger* from the other species is the production of carbon black or very dark brown spores. Other features include the smooth and generally colorless conidiophores and spores that have conspicuous ridges or spines not arranged in rows. *A. niger* isolates grow slowly on Czapek agar (Brooks *et al*., 2004). These physical characters such as spore color and rate of growth on a defined media are subject to change, especially under extended pure culture or selection and mutation. Though *A. niger* is relatively stable to spontaneous mutation compared to other aspergilli, variation in morphology may still be a problem with some strains (Brooks *et al*., 2004). Thus this species may be misidentified with other *Aspergillus* spp.

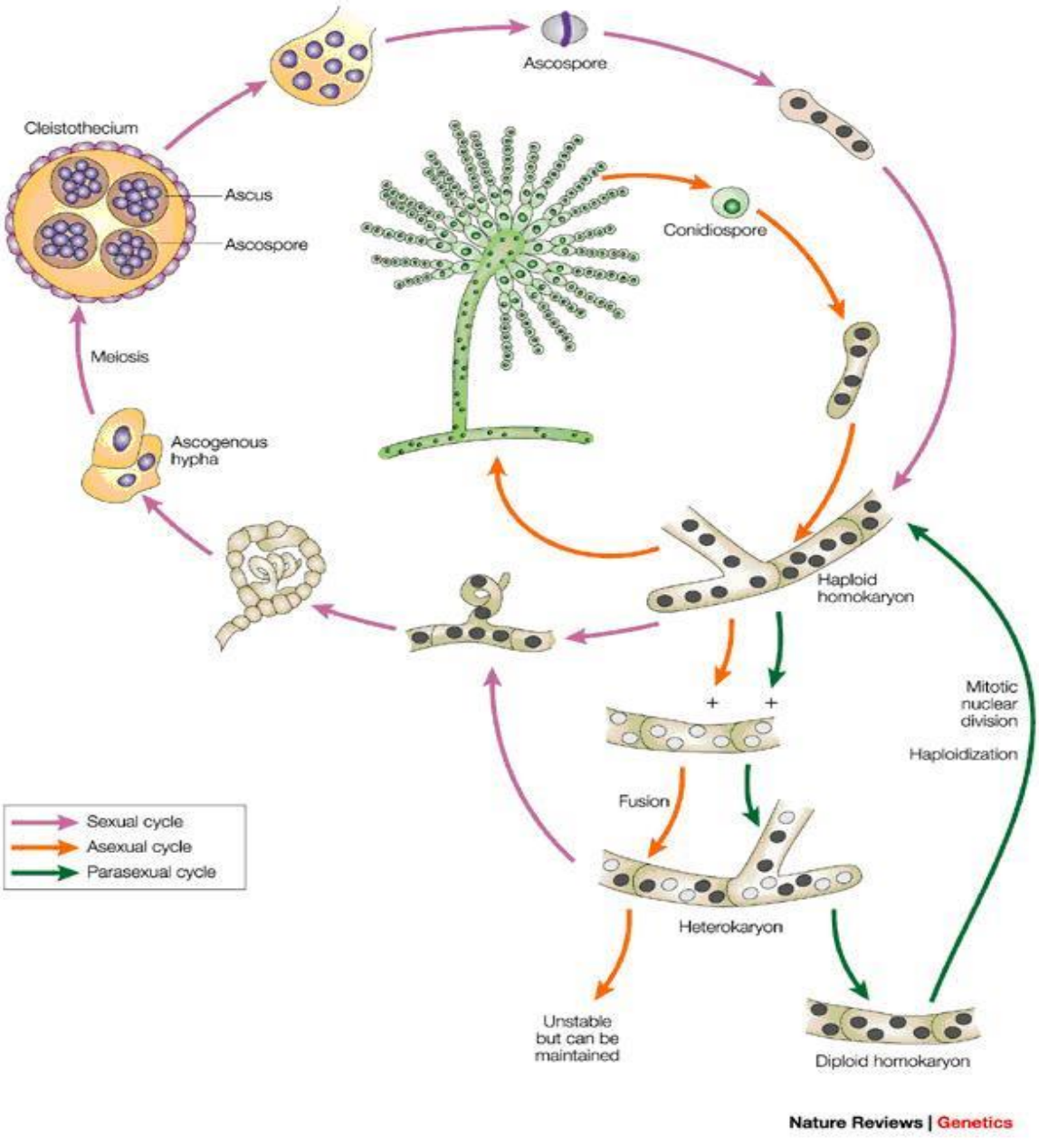


Figure 2.19 Life Cycle of *Aspergillus niger* (Brooks *et al*., 2004)

Cultural Characteristics and Ecology of *Aspergillus niger*

A. niger is a ubiquitous fungus that grows very quickly. Strains can be isolated from many different ecological habitats such as soil, plant debris, rotting fruit, and even indoor air environments. They can be identified growing on substrates producing colonies of felt like yellow to white hyphae, turning black with the formation of conidia. Microscopically, *A. niger* can be identified by its hyaline, septate hyphae. Asexual conidiophores can be

identified by being long and globose at the tip, with what appears to be a hymenial layer of structures, each "ejecting" its own spore (Brooks *et al*., 2004). Aspergilli are ubiquitous in nature. They are geographically widely distributed, and have been observed in a broad range of habitats because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The primary use of *A. niger* is for the production of enzymes and organic acids by fermentation. It is also used for the production of organic acids such as citric acid and gluconic acid. It is a common cause atomycosis (fungal ear infections), which cause pain, temporary hearing loss and in severe cases, damage to the ear canal.

2.3.7 *Microsporum canis*

Kingdom: Fungi

Division: Ascomycota

Class: Eurotiomycetes

Order: Onygenales

Family: Arthrodermataceae

Genus: *Microsporum*

Species: *M. canis*

Microsporum canis is a pathogenic asexual fungus in the phylum Ascomycota that infects the upper, dead layers of skin on domesticated cats, and occasionally dogs and humans (Shaifee *et al*., 2014). *M. canis* forms a white, coarsely fluffy spreading colony with a distinctive "hairy" or "feathery" texture. On the underside of the growth medium, a characteristic deep yellow pigment develops due to the metabolites secreted by the fungus. The intensity of this yellow pigmentation peak on the 6^{th} day of colony growth fades gradually making the identification of older colonies difficult. Some strains of *M. canis* fail to produce yellow pigment altogether, exhibit abnormally slow colony growth and form

undeveloped macro conidia. Cultivation on polished rice tends to reestablish the typical growth morphology and is helpful for identification (Shaifee *et al*., 2014). *M. canis* reproduces asexually by forming macro conidia that are asymmetrical, spherically shaped and have cell walls that are thick and coarsely roughened. *M. canis* produces infections of scalp and body sites, creating highly inflammatory lesions associated with hair loss (Shaifee *et al*., 2014). Infection by this species can often be detected clinically using wood's lamp which causes infected tissues to fluoresce bright green (Shaifee *et al*., 2014). Fluorescence is attributed to metabolic pteridine, which is produced by the fungus in actively growing hairs. Infected hairs remain fluorescent for prolonged periods of time (over the years), even after the death of the fungus.

Culture of the fungus is most commonly used to evaluate morphological and physiological parameters of growth and contain the identity of the agent. Microscopic examination of the growth can show the presence of the typical warted and spindle – shaped macro conida confirming the identity of the isolate as *M. canis*. *M. canis* has no specific growth factor or nutrition requirements; hence it grows well on most commercially available media. In addition, *M. canis* exhibits rapid colony growth at 25^{o}C. Two growth media that help distinguish *M. canis* from other Microsporum species (notably the morphologically similar species, *Microsporum audouinni*) are specifically polished rice and potatoe dextrose agar. On potatoe dextrose agar, *M. canis* produces a lemon-yellows pigment that is easily visualized, due to the presence of aerial hyphae, while on the polished rice, most isolates (even a typical strain) produces yellow pigment. *M. canis* has been identified as a causal agent of a ringworm infection in pets, *tinea capitis* and *tinea corporis* in humans, children in particular (Shaifee *et al*., 2014). Humans become infected as a result of direct or indirect contact with infected pets. *M. canis* infections can be easily managed using topical antifungal agents. However, severe cases may necessitate systemic therapy with

griseofulvin, itraconazole or terbinafine. Treatment of human cases also requires the identification and elimination of the infections reservoir, which typically involves the investigation and treatment of colonial animal and the elimination of infected bedding and other environmental reservoirs.

Figure 2.20 10 days old *Microsporum canis* on Sabouraud agar (Ryan and Ray, 2004).

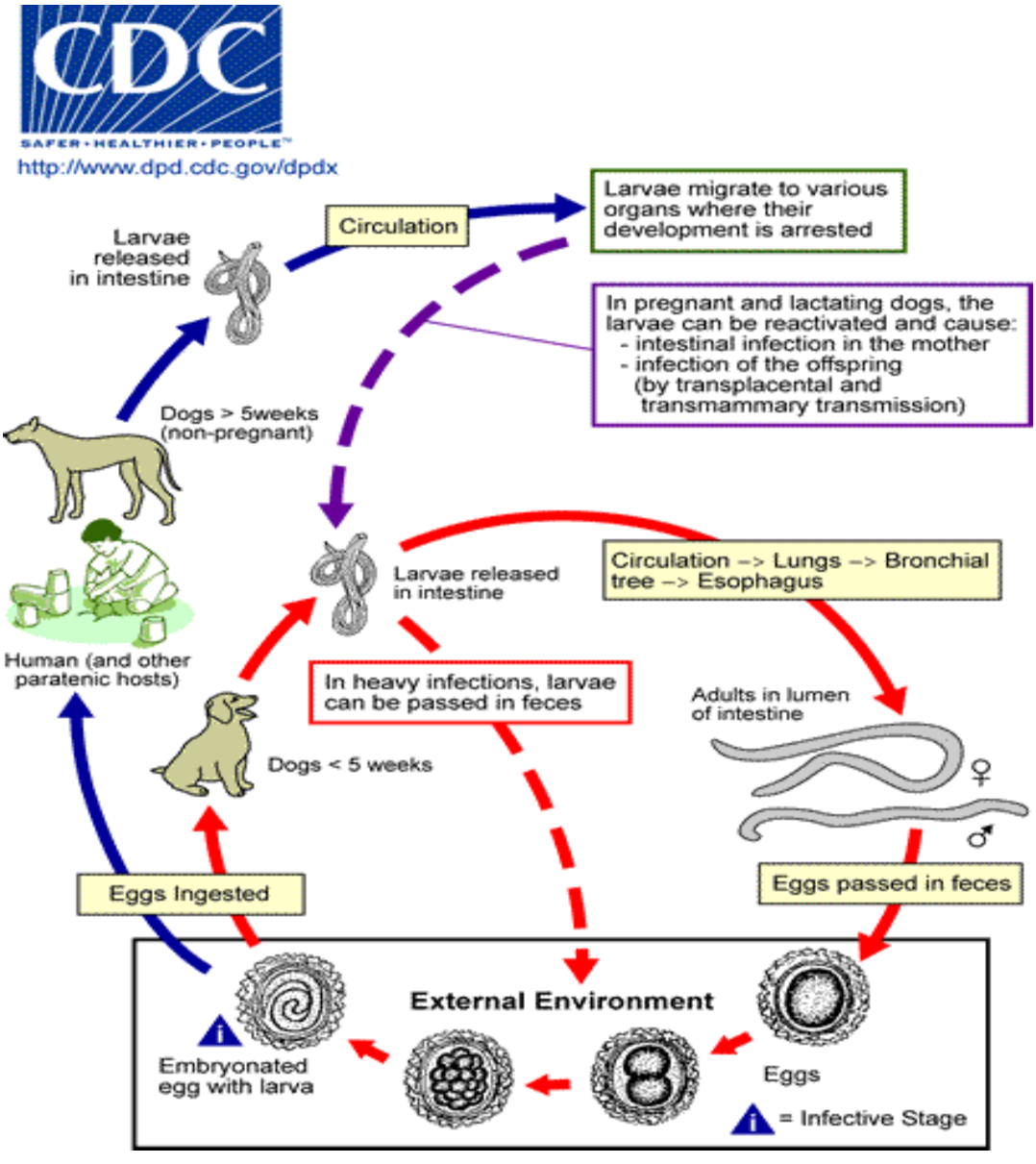


Figure 2.21 Life Cycle of *Microsporum canis* (Ryan and Ray, 2014).

Cultural Characteristics and Ecology of *Microsporum canis*

Microsporum canis produces infections of scalp and body sites, creating highly inflammatory lesions associated with hair loss. Infection by this species can often be detected clinically using Wood's lamp, which causes infected tissues to fluoresce bright green (Shaifee *et al*., 2014). Fluorescence is attributed to pteridine, which is produced by the fungus in actively growing hairs. Infected hairs remain fluorescent for prolonged periods of time (over the years), even after the death of the fungus (Shaifee *et al*., 2014).

Despite the frequent use of Wood's lamp in the clinical evaluation of ringworm infections, diagnosis of *M. canis* requires the performance of additional tests given the potential for false positives. Culture of the fungus is most commonly used to evaluate morphological and physiological parameters of growth, and confirm the identity of the agent. Growth of the fungus on Sabouraud's agar (4% glucose), Mycosel or rice medium characteristically yields the bright yellow pigment. Microscopic examination of the growth can show the presence of the typical, warted and spindle-shaped macroconidia, confirming the identity of the isolate as *M. canis* (Shaifee *et al*., 2014). The *In vitro* hair perforation test, commonly used to differentiate many dermatophytes, is not particularly useful for this species as it reveals the formation of "pegs" that penetrate into hair shafts - a characteristic shared widely among many zoophilic species. Despite its species name ("canis" implies dogs), the natural host of *M. canis* is the domestic cat. However this species can colonize dogs and horses as well. In all cases, it resides on the skin and fur. *M. canis* may also persist as dormant spores in environmental for prolonged periods. *M. canis* causes dermatophytosis in dogs and cats as well as a common pathogen of human infection.

2.3.8 *Penicillium chrysogenum*

Kingdom: Fungi

Division: Ascomycota

Class: Eurotiomycetes

Order: Eurotiales

Family: Trichocomaceae

Genus: *Penicillium*

Species: *P. chrysogenum*

Penicillium chrysogenum is a species of fungus in the family Trichocomacea. It is common in temperate and subtropical regions and can be found on salted food products (Brooks *et*

al., 2004) but it is mostly found in indoor environment especially in damp water or damaged buildings (Brooks *et al*., 2004). It has only been reported as a cause of human diseases on a few occasions. It is the source of several β-lactam antibiotics, mostly significant, penicillin. Other secondary metabolites of *P. chrysogenum* include roquefortine C, meleagrin, chrysogine, xanthocillins, secalonic acids and PR-toxin (Brooks *et al*., 2004). *P. chrysogenum* usually reproduces by forming dry chains of spores from brush-shaped conidiophores. The conidia are typically carried by air currents to new colonization sites. In *P. chrysogenum*, the conidia are blue to blue-green, and the mold sometimes exudes a yellow pigment. However, *P. chrysogenum* cannot be identified based on colour alone. Observations of morphology and microscopic features are needed to confirm its identify and DNA sequencing is essential to distinguish it from closely related species. The airborne asexual spores of *P. chrysogenum* are important human allergens. Vaculor and alkaline serine proteases have been implicated as the major allergenic proteins (Shen *et al.*, 2003). *P. chrysogenum* has been used industrially to produce penicillin and xanthocillins to treat pulp mill waste and to produce the enzymes polyamine oxidase, phosphogluconate dehydrogenase and glucose oxidase (Bohm *et al.*, 1994).

Figure 2.22 *Penicillium chrysogenum* Culture (Ryan and Ray, 2004).

Morphological Description of *Penicillium chrysogenum*

Like most members of the *Penicillium* genus, *P. chrysogenum* is a filamentous fungus. It usually sports a wooly to cotton-like appearance which starts out as a whitish color and over time changes to different shades of blue/green, yellow, pink or grey.

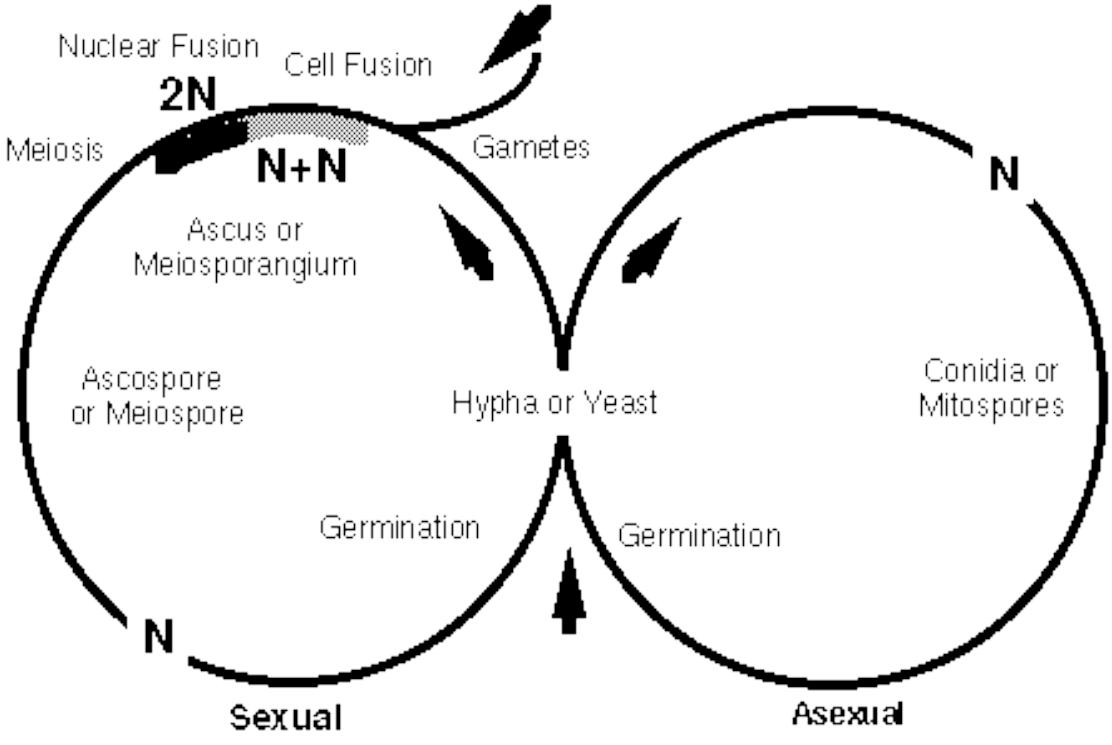


Figure 2.23 Life cycle of *Penicillium chrysogenum* (Brooks *et al*., 2004).

Cultural Characteristics and Ecology of *Penicillium chrysogenum*

P. chrysogenum is a heterotrophic organism. It does not depend on light to survive. This characteristic allows the organism to live in multiple habitats. Thus, *P. chrysogenum* is less likely to adapt to its environment, but instead flourish in an environment which is adapted to it. *Penicillium* species are found in a variety of niches. However, they prefer areas which are dark and damp. Moist conditions are favored among most fungi to avoid drying out. This is a common problem fungi face because they like to have maximum surface area to increase the amount of nutrition they can ingest. Wind is a large help in spreading the

reproductive spores of *P. chrysogenum*. Thus, it is most common to find the fungus in temperate areas. Common natural habitats of *P. chrysogenum* include soil, decaying vegetation, cultivated land, and temperate forest areas. *Penicillium chrysogenum* has been used industrially to produce penicillin and xanthocillins X, to treat pulp mill waste, and to produce the enzymes polyamine oxidase, phosphogluconate dehydrogenase, and glucose oxidase (de Hooz *et al*., 2000). Also, the airborne asexual spores of *P. chrysogenum* are important human allergens.

2.4 ANTIMICROBIALS

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterials are used against bacterial and antifungals are used against fungi. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. The main classes of antimicrobial agents are disinfectants ("non selective 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 tissues and help reduce infection during surgery) and antibiotics (which destroy microorganism within the body). Antimicrobial drugs are the greatest contribution of the present century to therapeutics. They are one of the few curative drugs. They are very important where infective diseases predominate, especially in developing countries like Nigeria. Antimicrobial drugs are designed to inhibit or kill the infecting organism and to have a minimal effect on the recipient. This type of therapy is called chemotherapy which has come to mean treatment of systematic infections with specific drugs that selectively suppress the microorganism without significantly affecting

the host (Tripathi, 1999). These chemical substances (chemotherapeutic agents) are prepared in the chemical laboratories or obtained from microorganisms, some plants and animals (Bep, 1960).

2.4.1 ANTIBIOTICS

These are substances being produced by microorganisms which suppress the growth of or kill microorganisms at very low concentrations. However, it will be more meaningful to use the term antimicrobial agent to designate synthetic as well as naturally obtained drugs that attenuate microorganisms. Antimicrobial drugs are classified based on the type of organisms against which it is primarily active (Tripathi, 1999) as:

1. Antibacterial e.g penicillin, aminoglycosides, erythromycin
2. Antifungal e.g griseofulvin, amphotericin B, ketoconazole
3. Antiviral e.g acyclovir, amantadine
4. Anti protozoal e.g chloroquine, pyrimthamine, metronidazole

Antimicrobial drug administration goes with some problems like toxicity, hypersensitivity reactions like rashes and drug resistance either natural or acquired due to use of antimicrobial agent or drugs over a period of time.

ANTIBACTERIAL DRUGS

Antibacterial drugs are the group of antimicrobials used in the treatment and prevention of bacterial infection. They may either kill or inhibit the growth of bacteria. Antibacterial can be used equally in the treatment of protozoa infection e.g metronidazole and bactrim which are effective against several parasites. They can also be used for immunomodulation e.g tetracycline which is effective in periodontal inflammation, and dapsone, which is effective in autoimmune disease such as oral mucous membrane perphigoid (Tripathi, 1999). Antibacterial drugs are also utilized as non operative resource for patient who have non-complicated acute appendicitis as well as in prevention of infection in surgical wound,

dental antibiotic prophylaxis (Tripathi, 1999) and in conditions of neutropenia e.g cancer-related. Antibacterial drugs are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Those that target bacterial cell wall (penicillin and cephalosporin) or the cell membrane (polymyxins) or interfere with essential bacterial enzymes (rifamyacins, lipiarmyans, quinolones and sulfonamides) have bactericidal activities. Those that target protein synthesis (macrolides, lincosamides and tetracycline) are usually bacteriostatic (with the exception of bactericidal aminoglycosides) (Tripathi, 1999). Further categorization is based on their target specificity. Narrow spectrum antibacterial target specific type of bacteria such as gram negative or gram positive bacteria whereas Broad-spectrum antibacterial affect a wide range of bacteria.

ANTIFUNGAL DRUGS

Antifungal drugs are pharmaceutical fungicides used to treat and prevent mycoses such as Athlete's foot, Ringworm, Candidaisis (thrush), serious systemic infections such as Cryptococcal meningitis and others. They are classified as follows;

1. Polyene antifungals e.g Amphotericin B, Candicidin, Filigin, Hamcyin, Natamycin, Nystatin etc.
2. Imidazole, Triazole and Thiazol antifungals e.g. Bifonazole, Econazole, Eficanazole, Fluconazole, Abafungin etc.
3. Allylamines e.g Amorolfin, Butenafin, Terbinafine etc.
4. Echinocandins e.g. Anidulafungins, Caspofungin, Micafugin etc.

Antifungal drugs work manly by exploiting differences between mammalian and fungal cells to kill the fungal organism with reduced adverse effects to the host.

2.4.2 MECHANISM OF ACTION OF ANTIMICROBIAL DRUGS

Antimicrobial drugs work in one of several ways; by selective toxicity, by addition of cell membrane function or by inhibition of nucleic acid synthesis.

Selective Toxicity: This means that the drug is harmful to a pathogen without being harmful to the host. Selective toxicity is relative than absolute, meaning that a drug in a concentration tolerated by the host may damage an infecting microorganism. This action may be a function of a specific receptor required for drug attachment or in the inhibition of biochemical events essential to the pathogen but not to the host.

Inhibition of Cell Wall Synthesis: An ideal antimicrobial that inhibit bacteria cell wall synthesis act on the formation of the peptidoglycan layer. Bacteria have a rigid outer layer which is the cell wall. The inhibition of the cell wall formation may lead to the lysis of the cell. The cell wall contains a chemically distant complex polymer mucopeptide (peptidoglycan) consisting of polysaccharides and highly cross-lined polypeptide. All β-lactam drugs are selective inhibitors of bacterial cell wall synthesis and therefore act against growing bacteria.

Inhibition of Cell Membrane Function: The cytoplasm of all living cells is bounded by the plasma membrane, which serves as selective permeability barrier, carries out active transport functions and thus controls the internal content of the cell. Thus, if the functional integrity of the plasma membrane is disrupted, cell damage or death ensues (Tripathi, 1999).

Inhibition of Protein Synthesis: It has been established that erythromycins, tetracycline, aminoglycides and chloromphenical can inhibit protein synthesis in bacteria. They do so by interfering with transfer of the elongating peptide chain to a newly attached amino acyl-tRNA at the ribosome RNA complex (Tripathi, 1999). Bacteria have 70s ribosome, whereas mammalian cells have 80s ribosome, as the subunits of each ribosome. The chemical composition and their functional specificities are sufficiently different to explain why the antimicrobial drugs can inhibit protein synthesis in bacterial ribosome without having major effects on mammalian ribosome (Tripathi, 2007).

Inhibition of Nucleic Acid Synthesis: Some antimicrobials like Quinolnes, Pyrimethamine, Rifamgin etc. act by inhibiting nucleic acid synthesis. Rifamgin inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase of bacteria. Thus, it inhibits bacterial RNA synthesis.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION AND PREPARATION OF THE PLANT MATERIALS

The test plant, *Corchorus olitorius* Linn, was collected from the Crop Science Garden at the Federal University of Technology, Owerri, Imo State, Nigeria. The plant was identified by Dr. C. M. Duru, a Plant Taxonomist at the Department of Biology, Federal University of Technology, Owerri, Imo State. The plant was thereafter, air-dried and ground to fine power using an electric blender (Binatone, BLG 699), milled and sieved with 0.5µm wire mesh to obtain fine powder.

3.2 PREPARATION OF EXTRACTS

3.2.1 HOT ETHANOL EXTRACTION

Ethanol extraction was carried out using the soxhlet apparatus. Ten grams (10g) of the ground plant materials were folded in a filter paper, fed into a thirutle and placed alongside a condenser and 130ml of ethanol in a conical flask. The set up was then placed on a heating mantle and the extracts were collected in a bottle and stored at room temperature for later use (Harbone, 1973).

3.2.2 AQUEOUS EXTRACTION

Fifty grams (50g) of the ground test plant sample were dissolved in 300ml of distilled water. It was mixed properly and allowed to stand for 48 hours after which it was filtered using Whatman No. 42 (125mm) filter paper and then stored at room temperature for later use (Harbone, 1973).

3.3 PHYTOCHEMICAL SCREENING

3.3.1 TEST FOR ALKALOIDS

Five milliliter (5ml) of 1% aqueous hydrochloric acid was added to 2ml of each of the extracts and placed in a water bath for 3 minutes. Thereafter, 3 drops of Mayer's reagent was added. A white precipitate indicates a positive test (Trease and Evans, 1989).

3.3.2 TEST FOR FLAVONOIDS

Aqueous ammonia (1ml) was added to 2ml of each extract in a test tube and shaken vigorously. The layer was allowed to separate. Formation of a yellow colouration in the extract indicates a positive test (Harborne, 1973).

3.3.3 TEST FOR TANNINS

Two milliliters (2ml) of 1% ferric chloride was added to 1ml of each of the extracts. A clear colour change to greenish blue-black indicates a positive test (Harborne, 1973).

3.3.4 TEST FOR CARDIAC GLYCOSIDES

The Salkowski test was employed in this test. To about one milliliter (1ml) of the extracts, two milliliters (2ml) of chloroform was added and then two milliliters (2ml) of concentrated Tetraoxosulphate (vi) acid was added to form lower layer. A reddish brown colour at the interphase is an indication of a positive test (Harborne, 1973).

3.3.5 TEST FOR PHENOLS

Five milliliters (5ml) of each of the extracts was mixed with eight milliliters (8ml) of distilled water in a test tube and six milliliters (6ml) of ferric chloride was added to the mixture. A colour change to light brown indicates a positive test (Harborne, 1973).

3.3.6 TEST FOR STEROIDS

The Salkowski test was employed in this test. Two milliliters (2ml) of each extract was placed in a clear dry test tube, 5 drops of Conc. Tetraoxosulphate (vi) acid (H_2SO_4) was

added down the test tube. Formation of red or reddish-brown colouration indicates a positive test (Harborne, 1973).

3.3.7 TEST FOR SAPONINS

Ten milliliters (10ml) of distilled water was added to about two milliliters (2ml) of each of the extracts in a test tube and shaken vigorously. Persistent frothing even after heating is an indication of the presence of saponins (Hosttetman, 1991).

3.3.8 TEST FOR ANTHRAQUINONES

Five milliliters (5ml) of 10% ammonia was added to two milliliters (2ml) of each of the extracts and shaken vigorously. Thereafter, two milliliters (2ml) of benzene was added. A colour change to pink, red or violet indicates a positive test (Trease and Evans, 1989).

3.3.9 TEST FOR ANTHRANOIDS

To about two milliliters (2ml) of each of the extracts, five milliliters (5ml) of 0.5% potassium Hydroxide was added and mixed properly. Then six (6) drops of acetic acid was added followed by two milliliters (2ml) of toluene. To the upper layer formed, two milliliters (2ml) of 0.5% potassium hydroxide was added. A change in colour of the mixture to reddish brown is an indication of a positive test (Trease and Evans, 1989).

3.3.10 TEST FOR PHYLOBATANNIS

Two milliliters (2ml) of 1% aqueous hydrochloric acid was added to two milliliters (2ml) of each of the extracts and boiled for 5 minutes. The presence of white precipitate indicates a positive test (Trease and Evans, 1989).

3.4 PHYTOCHEMICAL ANALYSIS

3.4.1 DETERMINATION OF PHENOLIC CONTENT

Half a milliliter (O.5ml) of the extracts was added to a twenty-five (25ml) volumetric flask filled with ten milliliter (10ml) distilled water and two and a half (2.5ml) of 0.2N Folin-

coicalteau phenol reagent. Reagent blank using distilled water instead of the sample was also prepared. After 5minutes, 2ml of 2% sodium carbonate (Na_2CO_3) solution was added and mixed. The solution was diluted to the 25 volume with distilled water and then allowed to stand for 90minutes after which the absorbance was measured at 780nm against the prepared blank. Quercetin was used as standard for the calibration curve. Total phenolic content was calculated as mg quercetin g^{-1} dry weight of the sample (Harborne, 1973).

3.4.2 DETERMINATION OF FLAVONOID CONTENT

Ten grams (10g) of the plant sample was repeatedly extracted with one hundred milliliters (100ml) of 90% ethanol at room temperature. The whole solution was then filtered using filter paper and the filtrate was later on transferred into a water bath after which the solution was evaporated to dryness until a constant weight (Bohm and Kocipai, 1994).

3.4.3 DETERMINATION OF ALKALOID CONTENT

Five grams (5g) of the plant sample was poured in a beaker followed by 200ml of 10% ethanol. The mixture was then covered and allowed to stand for 4 hours after which it was filtered and the extract was concentrated in a water bath at 45^{o}C till it reaches a quarter (¼) of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (NH_4OH) and then filtered. The residue, alkaloid which was then air dried and weighed (Harborne, 1973).

3.4.4 DETERMINATION OF TANNINS CONTENT

Five hundred milligrams (0.5g) of the plant sample was weighed into a fifty milliliter (50ml) plastic bottle. Fifty milliliter (50ml) of distilled water was added and stirred for one hour. The sample was then filtered into a fifty milliliter (50ml) volumetric flask and made up to mark. Five milliliters (5ml) of the filtered sample was then pipetted out into a test

tube and mixed with two milliliters (2ml) of 0.1ml iron (iii) chloride ($FeCl_3$) in 0.1m hydrochloric acid (HCl) and 0.008m potassium ferrocyanide trihydrate ($K_4Fe(CN)_6$) .$3H_2O$. The absorbance of the sample was measured with a spectrophotometer (PD-303 S) at 395nm wave length within 10minutes (Harborne, 1973).

3.4.5 DETERMINATION OF SAPONINS CONTENT

Twenty grams (20g) of the plant sample was put into a conical flask and 100ml of 20% ethanol was added to the plant sample. The sample was heated over a hot water bath for 4hours with continuous stiring at about 55^oC. The mixture was then filtered and the residue re-extracted with another 200ml of 20% ethanol. The extract was reduced to 40ml over a water bath at about 90^oC. The concentrate was then transferred into a 250ml separating funnel and 20ml of diethyl ether [$(CH_3CH_2)_2O$] was added to the extract and vigorously shaken. The aqueous layer was recovered while the diethyl ether [$(CH_3CH_2)_2O$] layer was discarded and the purification process was repeated. 60ml of normal butyl alcohol [n-C_4H_9OH] was added and the communed normal butyl alcohol [n-C_4H_9OH] extracts was washed twice with 10ml of 5% NaCl. The remaining solution was then heated in a water bath at 50^oC and after evaporation; the sample was dried in the oven to a constant weight (Obadoni and Ochuko, 2001).

3.5 PROXIMATE ANALYSIS

3.5.1 DETERMINATION OF MOISTURE CONTENT

Two grams (2g) of the plant sample was weighed into already dried and weighed crucible. The sample was then put into an oven at 105^oC and heated for 3 hours. Afterwards, the dried sample was transferred into a desiccator where it was cooled and the weight checked again. The process was continued until a constant weight was obtained. The difference in weight was calculated as percentage of the original sample. Thus,

$$\%\text{Moisture} = \frac{(\text{Weight of Crucible + Sample}) - (\text{Weight of crucible})}{\text{Weight of Sample}} \times \frac{100}{1}$$

(Pearson, 1976).

3.5.2 DETERMINATION OF TOTAL ASH CONTENT

Two grams (2g) of the dried plant sample was accurately weighed into a crucible and was completely dried in an oven at 100^{o}C for 1hour. The sample was charged on low flame and then heated at 600^{o}C in a furnace until a white ash was obtained with constant weight. The crucible was then cooled in a desiccator and weighed again. The ash content will then be determined using the formular,

$$\%\ \text{Ash} = \frac{\text{Weight of the Sample after Ashing (g)}}{\text{Weigh of sample taken (g)}} \times \frac{100}{1}$$

(Pearson, 1976).

3.5.3 DETERMINATION OF CRUDE FAT CONTENT

Two grams (2g) of the plant sample was loosely rapped with a filter paper and kept in the thimble of the soxhlet apparatus which was fitted to a clean round bottom flask which was dried and weighed, and containing petroleum either. The sample was heated with a heating mantle and then allowed to reflux for 5 hours after which the heating stops and the thimble with the refluxed sample was weighed again. The difference in the weight was taken as the mass of fat in the sample which will be expressed in percentage of the sample as;

$$\%\ \text{Fat} = \frac{(\text{Weight of Flask + Oil extracted}) - (\text{Weight of empty extraction flask})}{\text{Weight of Sample}} \times \frac{100}{1}$$

(Pearson, 1976).

3.5.4 DETERMINATION OF CRUDE PROTEIN CONTENT

Two grams (2g) of the plant sample was put in a Kjedahl flask followed by 5g of anhydrous sodium sulphate and 1g of copper sulphate as well as 25ml of concentrated

H_2SO_4. The Kjedahl flask was heated in a fume cupboard very gently at first and then heat was increased with occasional shaking till solution assumes a green colour (temperature of digest is above 420^oC for about 30 minutes). The flask was cooled and neck of the flask was washed down with distilled water. It was then heated gently at first until the green colour disappeared. Then, it was allowed to cool. After cooling, the digest was transferred into a 250ml volumetric flask and the content of the flask was made up to mark with water. This was transferred into Kjedahl distillation flask and the apparatus was assembled. 20% of NaOH was added into the receiving flask. The content of the flask was distilled until about 150ml of the distillate was obtained. After which the collection flask was removed and the content titrated with 0.2m NaOH using phenolphthalein as indicator. Crude protein was the calculated as;

$$\%\text{Crude protein} = \frac{(\text{Titre valve of sample}) - \text{blank}) \times 0.01 \times 14.007 \times 6.25}{\text{Weigh of sample}} \times \frac{100}{1}$$

(Pearson, 1976).

3.5.5 DETERMINATION OF CRUDE FIBRE CONTENT

Two grams (2g) of the plant sample was weighed and put into a clean and dried conical flask. It was boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. The solution was filtered through linen cloth on a fluted funnel. It was washed with boiling water until the washings were no longer acidic. The residue was transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. The sample was dried in an oven and weighed. It was then incinerated, cooled and weighed. The percentage crude fibre was calculated thus;

% Crude Fibre = (Weight of Sample before incineration) – (Weight of sample after incineration) x 100

Weight of Original Sample 1

(Pearson, 1976).

3.5.6 DETERMINATION OF CARBOHYDRATE CONTENT

The carbohydrate content of each of the samples was calculated as the dry weight by difference between 100 and the summation of other proximate parameters.

Nitrogen free extract (NFE) = percentage carbohydrate

= 100 – (M + P + F1 + F2 + A)

Where;

M = Moisture content

P = protein content

F1 = Fat content

F2 = Fibre content

A = Ash content

(Pearson, 1976).

3.6 MINERAL ANALYSIS

The ground plant samples were sieved with a 2µm rubber sieve and 2g of each sample was subjected to dry ashing in a well-cleaned porcelain crucible at 550^0C in a muffle furnace. The resultant ash was dissolved in 5ml of HNO_3/H_2O_2 (1:1) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100ml volumetric flask and the volume was made up to mark with deionized water. Calcium, Magnesium and Iron

contents were quantified using atomic absorption spectrophotometer while Sodium and Potassium were determined using a flame photometer (AOAC, 1990).

3.7 ANTIMICROBIAL SUSCEPTIBILITY TESTING OF THE PLANT EXTRACTS (ACTIVE PRINCIPLES)

3.7.1 COLLECTION AND PREPARATION OF THE CHALLANGE ORGANISMS

The clinical isolates of *Escherichia coli* (0157:H7), *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (MTC 455), *Candida albicans* (HLC54), *Aspergillus niger* (ATCC 10949), *Microsporum canis* (MTCC 144) and *Penicillium chrysogenum* (MTCC 135), were collected from the Microbiology unit of the Medical Laboratory Department, Federal Medical Centre, Owerri and identified by Mr. Michael Obi, the laboratory technologist. The identified isolates were then sub-cultured on sterile Nutrient agar and Sabouraud Dextrose agar slants for the Bacteria and Fungi respectively. The isolates were transported to the laboratory using Staurt transport medium, which is a non-nutrient soft agar gel containing a reducing agent to prevent oxidation and charcoal to neutralize. The microbial culture was afterwards diluted with peptone water until the final suspension that contained 1.0×10^8 cfu/ml of the isolates was obtained. The cell densities were obtained in accordance with 0.5 McFarland's Standard. The McFarland's standard was prepared by adding 0.1ml of 1% $BaCl_2$ into 9.9ml of 1% Tetraoxosulphate (vi) acid.

3.7.2 PREPARATION OF CULTURE MEDIA

Nutrient agar and Sabouraud Dextrose agar were weighed according to the manufacturer's specifications and dissolved in water and shaken for proper mixing. Sterilizing of constituted medium was carried out by autoclaving at 121^0C for 15 minutes. The sterilized media were dispensed into sterile Petri dishes (about 20ml) volume aseptically at

temperature of about 50^0C (to avoid excessive evaporation and condensation). The dispensed media were allowed to cool and solidify in the plates at room temperature.

3.7.3 INOCULATION AND INCUBATION OF THE CHALLANGE ORGANISMS

Two milliliters (2ml) of each of the extracts was dropped on sterile filter paper discs of 6mm in diameter and allowed to get absorbed before they were placed into the prepared nutrient agar plates and Sabouraud dextrose agar inoculated onto each of the test organisms and appropriately labeled. Discs impregnated with Chloramphenicol for bacteria and Nystatin for fungi were used as control in each case. The nutrient agar plates and Sabouraud dextrose agar plates were incubated at 37^0C for 24 hours and 25^0C for 72 hours respectively.

3.7.4. DETERMINATION OF ZONES OF INHIBITION

The zones of inhibition were measured using a meter rule.

3.7.5 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE PLANT EXTRACTS (ACTIVE PRINCIPLES)

Each of the plant extract was concentrated by evaporation and about 1g of each of the extract was added into 4ml of nutrient broth to give 250mg/ml. Also, 0.8g of the same extract was placed in 4ml of peptone water to obtain the concentration of 200mg/ml. Thereafter, two fold serial dilutions was carried out from the 200mg/ml concentration by transferring 2ml of the 200mg/ml concentration to 2ml of peptone water, contained in a test tube and homogenized properly. This procedure of transferring 2ml of peptone water contained in the subsequent test tubes was continued until the fourth test tube to give the following concentrations; 250mg/ml, 200gm/ml, 100mg/ml and 50mg/ml. Having obtained the different concentrations and dilutions, three drops of overnight broth cultures of the test organism was inoculated into the dilutions in each of the test tubes. Afterwards, the tubes

were incubated at 37^0C for 24 hours and 25^oC for 72 hours for bacteria and fungi respectively. The lowest concentration of each extract that inhibited the growth of the test organism was recorded as the minimum inhibitory concentration (Nethathe and Ndip, 2011).

3.7.6 DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF THE PLANT EXTRACTS (ACTIVE PRINCIPLES)

The minimum inhibitory concentration was serially diluted in 0.9% saline after which a loop full was taken from each test tube and inoculated into the nutrient agar plates and incubated for 24hours at 37^0C. The minimum bactericidal concentration was recorded as the lowest concentration of the extract or active principle that gives complete inhibition of colony formation of the test bacteria (Nethathe and Ndip, 2011).

3.7.7 DETERMINATION OF MINIMUM FUNGICIDAL CONCENTRATION (MFC) OF THE PLANT EXTRACTS (ACTIVE PRINCIPLES)

After 72 hours of incubation, 2ml of the organism was subcultured from each well that showed no visible growth from the last positive well and from the growth control onto the Sabouraud dextrose agar plates. The plates were then incubated at 27^0C until growth was seen in the control subculture. The minimum fungicidal concentration was recorded as the lowest extract or active principle that did not yield any fungal growth on the medium used (Espinel-Ingroff *et al.,* 2002).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 PHYTOCHEMICAL CHARACTERISTICS OF THE ETHANOLIC AND AQUEOUS EXTRACTS

Tables 4.1 and 4.2 below showed the results of the phytochemical screening of the ethanol and aqueous extracts of *Corchorus olitorius Linn* respectively. The result showed that different parts of the plant are rich in various phytochemicals including alkaloids, flavonoids, steroids, anthraquinone, cardiac glycosides, tannins, phenol, saponins, and phylobatannins. However, some of the extracts were abstract in different parts of the plant.

Table 4.1 PHYTOCHEMICAL DISTRIBUTION IN THE ETHANOL EXTRACTS

PHYTOCHEMICAL COMPONENTS	ROOT	STEM	SEED	LEAVES
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	-	-	+	+
Cardiac glycoside	+	+	+	-
Phenol	-	-	-	+
Steroid	+	+	+	+
Saponins	+	+	-	+
Anthraquinone	+	+	+	+
Anthranoid	+	-	+	-
Phylobatannins	-	+	+	+

Legend: (+) = Present, (-) = Absent

Table 4.2 PHYTOCHEMICAL DISTRIBUTION IN THE AQUEOUS EXTRACTS

PHYTOCHEMICAL COMPONENTS	ROOT	STEM	SEED	LEAVES
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	-	+	+	+
Cardiac glycoside	+	+	+	+
Phenol	-	-	+	+
Steroid	+	+	+	+
Saponins	-	+	+	+
Anthraquinone	-	+	-	+
Anthranoid	+	+	+	+
Phylobatannins	+	+	+	+

Legend: (+) = Positive, (-) = Negative

4.1.2 PHYTOCHEMICAL CHARACTERISTICS OF THE ETHANOL AND AQUEOUS EXTRACTS

Tables 4.3 and 4. 4 below showed the results of the phytochemical analysis of the ethanol and aqueous extracts of *C. olitorius Linn*. The result showed that the extracts contain varied quantities of phenols, flavanoid, alkaloid, tannins and saponins. However, some of the phytochemicals has zero values. This means that their presence could not be quantitatively determined in the extracts.

Table 4.3 PHYTOCHEMICAL ANALYSIS OF THE ETHANOLIC EXTRACTS

PHYTOCHEMICAL COMPONENTS	ROOT (mg/g)	STEM (mg/g)	SEED (mg/g)	LEAVES (mg/g)
Phenol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	200.03 ± 16.07^{b}
Flavonoids	28.03 ± 0.03^{a}	54.30 ± 0.05^{b}	45.50 ± 0.02^{c}	81.38 ± 0.07^{d}
Alkaloids	26.00 ± 0.02^{a}	29.50 ± 0.02^{a}	33.00 ± 0.06^{a}	53.50 ± 0.05^{b}
Tannins	38.00 ± 0.06^{a}	0.00 ± 0.00^{b}	28.00 ± 0.02^{c}	71.00 ± 0.02^{d}
Saponins	3.00 ± 0.01^{a}	5.00 ± 0.01^{a}	0.00 ± 0.00^{b}	54.70 ± 0.03^{c}

Legend: Values are expressed as Mean ± Standard Deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.4 PHYTOCHEMICAL PROPERTIES OF THE AQUEOUS EXTRACTS

PHYTOCHEMICAL COMPONENTS	ROOT (mg/g)	STEM (mg/g)	SEED (mg/g)	LEAVES (mg/g)
Phenol	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	15.08 ± 0.02^{b}	150.03 ± 15.08^{c}
Flavanoids	15.09 ± 0.03^{a}	38.30 ± 0.04^{b}	25.50 ± 0.05^{b}	63.51 ± 0.05^{c}
Alkaloids	21.00 ± 0.02^{a}	17.56 ± 0.02^{a}	27.00 ± 0.04^{a}	45.00 ± 0.03^{b}
Tannins	0.00 ± 0.00^{a}	15.31 ± 0.03^{b}	14.51 ± 0.02^{b}	51.03 ± 0.02^{c}
Saponins	0.00 ± 0.00^{a}	3.04 ± 0.01^{b}	15.32 ± 0.04^{c}	28.01 ± 0.02^{d}

Legend: Values are expressed as Mean ± Standard Deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.3 PROXIMATE CHARACTERISTICS OF THE WHOLE PLANT

Table 4.5 below showed the results of the proximate analysis of the whole plant (*Corchorus olitorius* Linn). The results showed that the plant contained a relatively appreciable quantity of the proximate components including fat, fiber, protein, moisture and carbohydrate.

Table 4.5 PROXIMATE CHARACTERISTICS OF THE WHOLE PLANT

PROXIMATE COMPONENTS	ROOT (mg/g)	STEM (mg/g)	SEED (mg/g)	LEAVES (mg/g)
Moisture content	29.69±0.01[a]	28.18±0.01[a]	28.40±0.01[a]	27.50±0.20[a]
Total ash content	3.14±0.02[a]	3.29±0.04[a]	4.20±0.03[a]	4.15±0.07[a]
Crude fat content	4.15±0.05[a]	3.23±0.04[a]	7.14±0.01[b]	5.25±0.04[b]
Crude protein content	6.91±0.01[a]	9.51±0.01[b]	10.25±0.01[b]	28.20±0.27[c]
Crude fiber content	23.86±0.02[a]	21.73±0.03[a]	3.21±0.02[b]	9.20±0.12[c]
Carbohydrate content	67.75±0.13[a]	65.94±0.11[a]	53.20±0.08[a]	74.30±0.70[b]

Legend: Values are Mean ± Standard Deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.4 MINERAL CHARACTERISTICS OF THE WHOLE PLANT

Table 4.6 below showed the results obtained from the mineral analysis of the root, stem, seed and leaves of *C. olitorius* Linn. The results showed that the various parts of the plant contained appreciable amount of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) with iron (Fe) being the least in quantity.

Table 4.6 MINERAL CONTENTS OF THE WHOLE PLANT

MINERAL	ROOT (mg/g)	STEM (mg/g)	SEED (mg/g)	LEAVES (mg/g)
Calcium	616.33 ± 4.73^{a}	52.35 ± 0.30^{b}	314.32 ± 0.50^{c}	1.27 ± 0.01^{d}
Magnesium	157.50 ± 42.50^{a}	98.20 ± 0.15^{b}	115.20 ± 0.30^{c}	0.60 ± 0.01^{d}
Iron	1.11 ± 1.00^{a}	0.09 ± 0.01^{b}	1.21 ± 0.01^{c}	0.05 ± 0.34^{d}
Sodium	306.00 ± 6.00^{a}	259.05 ± 0.76^{b}	5.01 ± 0.01^{c}	0.34 ± 0.21^{d}
Potassium	233.17 ± 1.61^{a}	151.21 ± 0.52^{b}	156.01 ± 0.40^{b}	3.76 ± 0.02^{c}

Legend: Values are Mean ± Standard Deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.5 ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF THE PLANT EXTRACTS (ACTIVE PRINCIPLES).

Table 4.7 below showed the zones of inhibition (mm) of the ethanol extracts of *C. olitorius* Linn on the bacterial and fungal isolates used as challenge organisms. The results obtained confirmed the antibacterial as well as the antifungal potentials of the plant. The extract that expressed the highest activity against the bacterial isolates was obtained from the leaves which inhibited the growth of *E. coli* at 10.00±0.07 mm on while the least was obtained from the seeds which inhibited the growth of *E. coli* at 4.50±0.01 mm. Most of the extracts at the concentration used did not express activity against some of the fungal isolates. Activities were only seen on *C. albicans, A. niger* and *P. chrysogenum* with the highest being 11.00±0.06mm (Leaf extract on *A. niger*) and the lowest 9.00±0.02mm (Leaf extract on *P. chrysogenum*).

Table 4.8 below showed the zones of inhibition (mm) of the aqueous extracts of *C. olitorius* Linn on the bacterial and fungal isolates used as challenge organisms. The results obtained confirmed the antibacterial and antifungal potentials of the plants. However, not all the extracts expressed activity against the bacterial isolates. The most active extract was obtained from the leaves which inhibited the growth of *E. coli* (9.50±0.7 mm) while the least active extract was obtained from the seeds which inhibited the growth of *E.coli* (3.50±0.01 mm). The result also revealed that only the root extracts expressed activity against *C. albicans* and *A. niger* (8.00±0.01mm and 7.00±0.05mm) respectively.

Table 4.7 ZONES OF INHIBITION OF THE ETHANOL EXTRACTS ON THE CHALLANGE ORGANISMS.

CHALLANGE ORGANISM	ROOT (mm)	STEM (mm)	SEED (mm)	LEAVES (mm)	CH/NY (mm)
Escherichia coli	9.00±0.7[a]	8.50±0.4[a]	4.50±0.1[b]	10.00±0.7[c]	12.50±0.1[d]
Staphylococcus aureus	8.00±0.5[a]	9.00±0.6[a]	6.50±0.2[b]	8.50±0.4[c]	10.50±0.1[c]
Klebsiella pneumoniae	10.00±0.7[a]	8.10±0.39[b]	NA	8.00±0.5[b]	12.00±0.1[c]
Salmonella typhi	7.00±0.2[a]	8.50±0.3[a]	8.50±0.5[a]	NA	11.50±0.1[b]
Microsporum canis	NA	NA	NA	NA	15.00±0.1
Candida albicans	10.00±0.1[a]	NA	NA	NA	14.00±0.1[b]
Aspergillus niger	NA	NA	NA	NA	12.50±0.1
Penicillium chrysogenum	10.00±0.1[a]	NA	NA	NA	12.00±0.1[b]

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as Mean ± Standard Deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.8 ZONES OF INHIBITION OF THE AQUEOUS EXTRACT ON THE CHALLANGE ORGANISMS.

CHALLANGE ORGANISM	ROOT (mm)	STEM (mm)	SEED (mm)	LEAVES (mm)	CH/NY (mm)
Escherichia coli	NA	4.00±0.1[a]	3.50±0.1[b]	9.50±0.7[c]	12.00±0.1[d]
Staphylococcus aureus	9.00±00.7[a]	5.50±0.1[b]	4.50±0.1[c]	7.50±0.5[d]	10.00±0.1[e]
Klebsiella pneumoniae	8.00±0.5[a]	NA	NA	7.00±0.5[b]	10.00±0.1[c]
Salmonella typhi	7.00±0.4[a]	NA	6.00±0.4[b]	10.00±0.7[c]	12.00±0.1[d]
Microsporum canis	NA	NA	NA	NA	12.00±0.1
Candida albicans	8.00±0.6[a]	NA	NA	NA	10.00±0.1[b]
Aspergillus niger	7.00±0.5[a]	NA	NA	NA	12.50±0.1[b]
Penicillium chrysogenum	NA	NA	NA	NA	10.00±0.1

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as Mean ± Standard Deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.6 ZONES OF INHIBITION (MM) OF THE ACTIVE PRINCIPLES ON THE BACTERIAL AND FUNGAL ISOLATES.

Tables 4.9, 4.10, 4.11, 4.12 and 4.13 below showed the zones of inhibition of the active principles extracted from *C. olitorius* Linn on the challenge organisms. The results revealed that alkaloids expressed antibacterial activity against all the bacteria except for the seed extracts on *K. pneumonia*. It further showed that they have antifungal activity on *A. niger* and *P. chrysogenum*. Again, the results revealed that saponins expressed antibacterial activity against all the bacteria except for the seed extracts. It further showed that they have antifungal activity against *C. albicans*, *A. niger* and *P. chrysogenum*. Also, results revealed that only the phenols from the leaves expressed antibacterial activity against the bacteria isolates and antifungal activity against *P. chrysogenum*. Flavonoids have strong antibacterial potency as it expressed antibacterial activity against all the bacteria isolates. However, it only expressed antifungal activity against *C. albicans* and *P. chrysogenum*. Tannins expressed antibacterial activity against all the bacteria except for the stem extracts. It further showed that they have antifungal activity on *C. albicans, A. niger* and *P. chrysogenum*.

Table 4.9 ZONES OF INHIBITION OF ALKALOIDS ON THE CHALLENGE ORGANISMS.

CHALLA-NGE ORGANI-SM / EXTRAC-TS	*E. coli (mm)*	*S. aureus (mm)*	*K. pneumoniae (mm)*	*S. typhi (mm)*	*M. canis (mm)*	*C. albicans (mm)*	*A. niger (mm)*	*P. chrysogenum (mm)*
ROOT	8.00±0.7[a]	10.01±0.4[b]	7.50±0.5[c]	7.00±0.5[d]	NA	NA	NA	8.00±0.1[e]
STEM	7.50±0.4[a]	8.95±0.5[b]	8.50±0.5[c]	7.05±0.25[d]	NA	NA	NA	NA
SEED	3.01±0.1[a]	5.05±0.2[b]	NA	7.00±0.5[c]	NA	NA	NA	NA
LEAVES	10.00±0.7[a]	9.50±0.7[b]	8.50±0.5[c]	7.00±0.5[d]	NA	NA	10.00±0.1[e]	10.00±0.1[e]
CH/NY	12.50±0.1[a]	10.50±0.1[b]	12.00±0.1[c]	11.50±0.1[d]	15.00±0.1[a]	14.00±0.1[b]	12.50±0.1[c]	12.00±0.1[d]

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as mean ± standard deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.10 ZONES OF INHIBITION OF SAPONINS ON THE CHALLENGE ORGANISMS.

CHALLA-NGE ORGANI-SM EXTRAC-TS	*E. coli (mm)*	*S. aureus (mm)*	*K. pneumoniae (mm)*	*S. typhi (mm)*	*M. canis (mm)*	*C. albicans (mm)*	*A. niger (mm)*	*P. chrysogenum (mm)*
ROOT	7.05±0.7[a]	10.05±0.6[b]	6.55±0.5[c]	7.50±0.5[d]	NA	10.05±0.3[e]	NA	9.00±0.3[f]
STEM	8.60±0.4[a]	7.50±0.5[b]	NA	7.90±0.3[b]	NA	NA	NA	NA
SEED	NA	NA	NA	NA	NA	NA	NA	NA
LEAVES	11.00±0.2[a]	9.03±0.3[b]	8.90±0.3[b]	10.00±0.2[c]	NA	9.05±0.4[d]	10.00±0.1[e]	8.50±0.5[f]
CH/NY	13.00±0.1[a]	13.05±0.2[a]	11.25±0.4[b]	11.45±0.1[b]	15.00±0.1[a]	13.50±0.4[b]	10.50±0.2[c]	12.00±0.4[d]

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as mean ± standard deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.11 ZONES OF INHIBITION OF PHENOLS ON THE CHALLANGE ORGANISMS

CHALLA-NGE ORGANI-SM EXTRAC-TS	*E. coli (mm)*	*S. aureus (mm)*	*K. pneumoniae (mm)*	*S. typhi (mm)*	*M. canis (mm)*	*C. albicans (mm)*	*A. niger (mm)*	*P. chrysogenum (mm)*
ROOT	NA	NA	NA	NA	NA	NA	NA	NA
STEM	NA	NA	NA	NA	NA	NA	NA	NA
SEED	NA	NA	NA	NA	NA	NA	NA	NA
LEAVES	11.04 ± 0.2^{a}	9.50 ± 0.2^{b}	10.00 ± 0.1^{c}	10.50 ± 0.5^{d}	NA	NA	NA	10.00 ± 0.5^{e}
CH/NY	12.50 ± 0.1^{a}	10.55 ± 0.3^{b}	12.05 ± 0.2^{c}	11.50 ± 0.1^{d}	15.00 ± 0.1^{a}	14.00 ± 0.1^{b}	12.50 ± 0.1^{c}	12.00 ± 0.1^{d}

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as mean ± standard deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.12 ZONES OF INHIBITION OF FLAVONOIDS ON THE CHALLANGE ORGANISMS.

CHALLA-NGE ORGANI-SM EXTRAC-TS	*E. coli (mm)*	*S. aureus (mm)*	*K. pneumoniae (mm)*	*S. typhi (mm)*	*M. canis (mm)*	*C. albicans (mm)*	*A. niger (mm)*	*P. chrysogenum (mm)*
ROOT	7.00±0.3[a]	8.50±0.01[b]	8.00±0.1[c]	9.30±0.02[d]	NA	6.05±0.1[e]	NA	NA
STEM	9.20±0.4[a]	8.90±0.5[a]	7.05±0.25[b]	9.50±0.3[c]	NA	NA	NA	NA
SEED	9.00±0.1[a]	10.0±0.2[b]	8.00±0.5[c]	9.10±0.05[d]	NA	NA	NA	NA
LEAVES	12.00±0.2[a]	10.00±0.1[b]	12.00±0.2[c]	10.50±0.3[d]	NA	8.50±0.02[e]	NA	10.03±0.02[f]
CH/NY	12.50±0.2[a]	10.50±0.1[b]	12.60±0.1[c]	11.50±0.1[d]	15.00±0.1[a]	13.50±0.4[b]	10.50±0.2[c]	12.00±0.1[d]

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as mean ± standard deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.13 ZONES OF INHIBITION OF TANNINS ON THE CHALLANGE ORGANISMS.

CHALLA-NGE ORGANI-SM / EXTRAC-TS	*E. coli (mm)*	*S. aureus (mm)*	*K. pneumoniae (mm)*	*S. typhi (mm)*	*M. canis (mm)*	*C. albicans (mm)*	*A. niger (mm)*	*P. chrysogenum (mm)*
ROOT	10.02±0.3[a]	11.00±0.1[b]	8.00±0.7[c]	7.60±0.3[d]	NA	8.00±0.1[e]	NA	NA
STEM	NA	NA	NA	NA	NA	NA	NA	NA
SEED	9.50±0.2[a]	8.40±0.2[b]	7.00±0.1[c]	7.00±0.1[c]	NA	NA	NA	NA
LEAVES	10.00±0.1[a]	9.50±0.7[b]	10.00±0.7[c]	10.50±0.3[d]	NA	NA	10.00±0.2[e]	9.00±0.1[f]
CH/NY	12.50±0.1[a]	12.00±0.1[b]	12.00±0.1[b]	11.50±0.1[c]	12.00±0.1[a]	10.00±0.1[b]	12.50±0.1[c]	12.00±0.1[d]

Legend: NA = No Activity. CH = Chloramphenicol. NY = Nystatin. Values are expressed as mean ± standard deviation of triplicate data. Mean followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.7 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE EXTRACTS (ACTIVE PRINCIPLES).

Tables 4.14 and 4.15 below showed the results of the minimum inhibitory concentration (MIC) of the ethanol and aqueous extracts of *Corchorus olitorius Linn* on the challenge organisms respectively. The results showed that *E. coli* is the most susceptible bacteria to the extract with MIC 200mg/ml while *Klebsiella pneumoniae* is the least susceptible to the extract with MIC 250mg/ml. It further showed that *A. niger* is the least susceptible fungi with MIC 100mg/ml. On the aqueous extracts, the growth of the bacteria was least inhibited at 100mg/ml for *E. coli*, *S. aureus* and *S. typhi*, while the growth *K. pneumoniae* was least inhibited at 200mg/ml. Also, the growth of C. *albicans* and *A. niger* was inhibited at 200mg/ml by the root and leaf extracts.

Table 4.14 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE ETHANOLIC EXTRACTS ON THE CHALLANGE ORGANISMS

CONCENTRATION(mg/ml) CHALLENGE ORGANISMS	**ROOT**	**STEM**	**SEED**	**LEAVES**
Escherichia coli	50^{a}	50^{a}	50^{a}	100^{b}
Staphylococcus aureus	R	100^{a}	100^{a}	50^{b}
Klebsiella pneumoniae	R	R	R	R
Salmonella typhi	R	R	250^{a}	200^{b}
Microsporum canis	R	R	R	R
Candida albicans	200	R	R	R
Aspergillus niger	R	R	R	100
Penicillium chrysogenum	250^{a}	R	R	200^{b}

Legend: R = Resistant. Values followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.15 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE AQUEOUS EXTRACTS ON THE CHALLANGE ORGANISMS.

AQUEOUS EXTRACTS CONCENTRATIONS(mg/ml) CHALLENGE ORGANISMS	ROOT	STEM	SEED	LEAVES
Escherichia coli	R	200[a]	R	100[b]
Staphylococcus aureus	200[a]	250[b]	250[b]	200[c]
Klebsiella pneumoniae	200[a]	R	R	200[a]
Salmonella typhi	200[a]	R	200[a]	100[b]
Microsporum canis	R	R	R	R
Candida albicans	200	R	R	R
Aspergillus niger	200[a]	R	R	200[a]
Penicillium chrysogenum	R	R	R	R

Legend: R = Resistant. Values followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.8 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE ACTIVE PRINCIPLES ON THE CHALLANGE ORGANISMS.

Tables 4.16, 4.17, 4.18, 4.19 and 4.20 below showed the minimum inhibitory concentration (MIC) (Mg/ml) of the active principles on the bacterial and fungal isolates. Alkaloids inhibited the growth of *E. coli*, *S. aureus* and *S. typhi* at 100mg/ml while that of *A. niger* and *P. chrysogenum* was inhibited at 200mg/ml. Saponins inhibited the growth of *E. coli* and *S. aureus* as well as *P. chrysogenum* was inhibited at 50mg/ml. Phenols inhibited the growth of *E. coli*, *S. aureus* and *K. pneumoniae* was inhibited at 100mg/ml while that of *P. chrysogenum* was inhibited at 200mg/ml. For flavonoids, *E. coli* has the lowest MIC at 50mg/ml while that of *C. albicans* and *P. chrysogenum* was inhibited at 200mg/ml. Tannins inhibited the growth of *S. aureus* was inhibited at 50mg/ml while that of *A. niger* and *P. chrysogenum* was inhibited at 200mg/ml.

Table 4.16 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF ALKALOIDS ON THE CHALLANGE ORGANISMS.

ALKALOID CONCENTRATIONS(mg/ml) CHALLENGE ORGANISMS	**ROOT**	**STEM**	**SEED**	**LEAVES**
Escherichia coli	200[a]	200[a]	200[a]	100[b]
Staphylococcus aureus	100[a]	100[a]	250[b]	100[c]
Klebsiella pneumoniae	250[a]	250[a]	250[a]	250[a]
Salmonella typhi	250[a]	250[a]	250[a]	100[b]
Microsporum canis	R	R	R	R
Candida albicans	200	R	R	R
Aspergillus niger	R	R	R	200
Penicillium chrysogenum	200[a]	R	R	200[a]

Legend: R = Resistant. Values followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.17 MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF SAPONINS ON THE CHALLENGE ORGANISMS

SAPONINS	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	100[a]	100[a]	R	50[b]
Staphylococcus aureus	100[a]	200[b]	R	100[c]
Klebsiella pneumoniae	200[a]	R	R	100[b]
Salmonella typhi	200[a]	250[b]	R	200[c]
Microsporum canis	R	R	R	R
Candida albicans	200[a]	R	R	100[b]
Aspergillus niger	R	R	R	R
Penicillium chrysogenum	100[a]	R	R	100[a]

Legend: R = Resistant. Values followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.18 MINIMUM INHIBITORY CONCENTRATION (MIC) OF PHENOLS ON THE CHALLENGE ORGANISMS

PHENOL	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	R	R	R	100
Staphylococcus aureus	R	R	R	100
Klebsiella pneumoniae	R	R	R	200
Salmonella typhi	R	R	R	100
Microsporum canis	R	R	R	R
Candida albicans	R	R	R	R
Aspergillus niger	R	R	R	R
Penicillium chrysogenum	200	R	R	200

Legend: R = Resistant.

Table 4.19 MINIMUM INHIBITORY CONCENTRATION (MIC) OF FLAVONOIDS ON THE CHALLENGE ORGANISMS

FLAVONOID CONCENTRATIONS(mg/ml) CHALLENGE ORGANISMS	ROOT	STEM	SEED	LEAVES
Escherichia coli	100[a]	100[a]	250[b]	50[c]
Staphylococcus aureus	200[a]	200[a]	100[b]	100[b]
Klebsiella pneumoniae	100[a]	250[b]	250[b]	100[c]
Salmonella typhi	100[a]	250[b]	200[c]	100[d]
Microsporum canis	R	R	R	R
Candida albicans	200[a]	R	R	200[a]
Aspergillus niger	R	R	R	R
Penicillium chrysogenum	R	R	R	200

Legend: R = Resistant. Values followed by the same letter(s) within the same row are not significantly different at $p<0.05$

Table 4.20 MINIMUM INHIBITORY CONCENTRATION (MIC) OF TANNINS ON THE CHALLENGE ORGANISMS

TANNIN CONCENTRATIONS(mg/ml) CHALLENGE ORGANISMS	ROOT	STEM	SEED	LEAVES
Escherichia coli	R	R	200[a]	100[b]
Staphylococcus aureus	200[a]	R	200[a]	50[b]
Klebsiella pneumoniae	200[a]	R	200[a]	100[b]
Salmonella typhi	250[a]	R	250[a]	200[b]
Microsporum canis	R	R	R	R
Candida albicans	200[a]	R	250[b]	250[b]
Aspergillus niger	R	R	R	200
Penicillium chrysogenum	R	R	R	200

Legend: R = Resistant. Values followed by the same letter(s) within the same row are not significantly different at $p<0.05$

4.1.9 RESULTS OF MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE EXTRACTS AND ACTIVE PRINCIPLES.

Tables 4.21 and 4.22 below showed the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the ethanol and aqueous extracts. For the ethanol extracts, the result showed that only the leaf demonstrated expressed bactericidal activity against *E. coli* (100 mg/ml) and *S. aureus* (100 mg/ml). No activity was seen in other organism. It also revealed that the extracts did not express any fungicidal activity.

For the aqueous extracts, the result showed that only the leaf extracts exhibited bactericidal activity against *S. aureus* at MBC 250mg/ml. There was no activity in the others. Also, the extracts did not elicit any fungicidal activity against the challenge fungi.

Table 4.21 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE ETHANOL EXTRACTS.

ETHANOL EXTRACT	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	R	R	R	250
Staphylococcus aureus		R	R	250
Klebsiella pneumoniae				R
Salmonella typhi	R	R	R	R
Microsporum canis				
Candida albicans	R			R
Aspergillus niger				R
Penicillium chrysogenum	R			R

Legend: R = Resistant.

Table 4.22 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE AQUEOUS EXTRACTS.

AQUEOUS EXTRACT CONCENTRATIONS(mg/ml)	**ROOT**	**STEM**	**SEED**	**LEAVES**
CHALLENGE ORGANISMS				
Escherichia coli		R		R
Staphylococcus aureus	R	R	R	250
Klebsiella pneumoniae	R			R
Salmonella typhi	R		R	R
Microsporum canis				
Candida albicans	R			
Aspergillus niger	R			R
Penicillium chrysogenum				

Legend: R = Resistant.

4.1.10 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE ACTIVE PRINCIPLES

Tables 4.23, 4.24, 4.25, 4.26 and 4.27 below showed the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the active principles. The result showed that alkaloids demonstrated bactericidal activity against *E. coli* (250 mg/ml) and *S. aureus* (200 mg/ml) but did not express any fungicidal activity against the challenge fungi. Saponins demonstrated bactericidal activity against all the challenge bacteria at different concentrations but no fungicidal activity against any of the challenge fungi. Phenols demonstrated bactericidal activity against all the challenge bacteria, *E. coli* (200 mg/ml), *S. aureus* (200 mg/ml), *K. pneumoniae* (250 mg/ml) and *S. typhi* (200 mg/ml) at but no fungicidal activity against any of the challenge fungi. Flavonoids demonstrated bactericidal activity against the entire challenge bacteria, *E. coli* (100 mg/ml), *S. aureus* (100mg/ml), *K. pneumoniae* (100 mg/ml), *S. typhi* (100 mg/ml) but no fungicidal activity against any of the fungal isolates. Tannins demonstrated bactericidal activity against all the challenge bacteria at different concentrations but no fungicidal activity against any of the challenge fungi.

Table 4.23 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE ALKALOIDS

ALKALOID	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	250	250	250	250
Staphylococcus aureus	200	R	R	200
Klebsiella pneumoniae				R
Salmonella typhi	R	R	R	R
Microsporum canis				
Candida albicans				
Aspergillus niger				R
Penicillium chrysogenum	R			R

Legend: R = Resistant.

Table 4.24 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE SAPONINS

SAPONIN	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	100	R		100
Staphylococcus aureus	200	200		200
Klebsiella pneumoniae	250			250
Salmonella typhi	R			200
Microsporum canis				
Candida albicans	R			R
Aspergillus niger				
Penicillium chrysogenum	R			R

Legend: R = Resistant.

Table 4.25 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE PHENOLS.

PHENOL	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	ND	ND	ND	200
Staphylococcus aureus	ND	ND	ND	200
Klebsiella pneumoniae	ND	ND	ND	250
Salmonella typhi	ND	ND	ND	R
Microsporum canis	ND	ND	ND	ND
Candida albicans	ND	ND	ND	ND
Aspergillus niger	ND	ND	ND	ND
Penicillium chrysogenum	ND	ND	ND	R

Legend: R = Resistant. ND= Not Detected

Table 4.26 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE FLAVONOIDS.

FLAVONOID	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	100	250	ND	100
Staphylococcus aureus	200	200	200	100
Klebsiella pneumoniae	100	R	R	100
Salmonella typhi	100	250	250	100
Microsporum canis	ND	ND	ND	ND
Candida albicans	R	ND	ND	R
Aspergillus niger	ND	ND	ND	ND
Penicillium chrysogenum	ND	ND	ND	R

Legend: R = Resistant. ND = Not Detected

Table 4.27 MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) AND MINIMUM FUNGICIDAL CONCENTRATIONS (MFC) OF THE TANNINS.

TANNIN	**ROOT**	**STEM**	**SEED**	**LEAVES**
CONCENTRATIONS(mg/ml)				
CHALLENGE ORGANISMS				
Escherichia coli	200	ND	R	200
Staphylococcus aureus	200	ND	ND	100
Klebsiella pneumoniae	R	ND	250	200
Salmonella typhi	R	ND	250	250
Microsporum canis	ND	ND	ND	ND
Candida albicans	R	ND	R	R
Aspergillus niger	ND	ND	ND	R
Penicillium chrysogenum	ND	ND	ND	R

Legend: R = Resistant. ND = Not detected

4.2 DISCUSSION

4.2.1 PHYTOCHEMICAL SCREENING (QUALITATIVE AND QUANTITATIVE) OF THE ETHANOL AND AQUEOUS EXTRACTS OF *CORCHORUS OLITORIUS* LINN.

Phytochemical screening revealed that both the ethanol and aqueous extracts of the different plant parts were rich in various phytochemicals including Alkaloids, Flavonoids, Phylobatannins, Anthraquinone, Steroid, Tannins, Cardiac glycosides, Phenols, Saponins and Anthranoid which may have been responsible for the antimicrobial and wound healing activities of the plant. The results further showed that tannins were absent in the ethanolic extract of the root and stem as well as in the aqueous extract of the root. Phenols were absent in the ethanol extract of the root, stem and seeds as well as the aqueous extract of the root, stem and seeds. The observed results supports the view that the plant is important in traditional/folk medicine as it is a promising source of potential antimicrobial, antioxidant and medical compound (Abouzad, 2002). Phytochemicals act as antioxidants, suppressors of tumor growth, anti-mutagens, enzyme modulators, chemical inactivators and free radical scavengers. Flavonoids, tannins and saponins have been reported to have medicinal properties such as antimicrobial and antioxidant properties (Stephen *et al.*, 2009). Alkaloids on the other hand, are heterocyclic indole compounds which have proved to have pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antimicrobial and antimalarial activities (Mallikharjuna *et al*., 2007). They also have pronounced physiological effects particularly on the nervous system (Stephen *et al*., 2009). Plants have the limitless ability to synthesize phenols or their derivatives. These phenolic compounds give the vegetable an astringent taste. They also bind proteins and may lower protein digestibility and quality as well as reducing the risk of heart disease and certain types of cancers. The presence of alkaloid in the extracts has been attributed to the

inhibition of growth of *Staphylococcus aureus* and *Escherichia coli* as well as use as an antimycotic and in the treatment of stomach pains (Adegoke, 2009). Flavanoids may also be responsible for diuretics and antibacterial activity; they are antioxidants (Bohm and Kocipai, 1994). They also help in healing of wounds and in the treatment of skin disease due to its ability to neutralize the acidity of wounds and inflammations (Ajiwe *et al.,* 2007). Gills (1992) reported that tannins are anti-inflammatory, effective in the treatment of gastric and irritating bowel disorder and as well contribute to antimicrobial power which heals wounds and stops bleeding. Tannins ensure the inhibition of growth in organisms by coagulating their micro protoplasm (Ajiwe *et al.,* 2007). Saponins detected in the plant have been found to be an antibacterial and antimycotic substance on the cell wall of many organisms. They neutralize the acidity of inflammation due to the presence of hydroxyl bond in their benzene ring (Ajiwe *et al.,* 2007). They also lower the cholesterol level in man. The presence of steroids in the plant reveals the importance of the plants in pharmacy due to their relationship with sex hormones (Fellows, 1991). Cardiac glycosides in the plants are also of optimal importance pharmaceutically because they have the properties of stimulating the muscles of the heart. Again, saponins inhibit Na^{+} efflux, leading to higher Na^{+} in cells, thereby activating a Na^{++} Ca^{2+} antiport (Sneden, 2005). This effect produces elevated cytosolic Ca^{2+} which strengthens the contraction of the heart muscle and thereby reducing congestive heart failure (Sneden, 2005). The presence of these secondary metabolites such as alkaloid, saponins, tannins, flavanoid and phenols in the plant contributes to its medicinal and physiological activities. Suggesting they can be used as starting materials in the synthetic or production of new drugs today (Adegoke and Adebayo, 2009). Some of these compounds have also been documented to exhibit hypoglycemic activities in animals (Shaaf and Hegm, 2005).

Tables 4.3 and 4.4 showed the results of the phytochemical analysis of the plant. Qualitative evaluation of phytochemicals was aimed simply to identify the presence of individual groups of phytochemical compounds. The results obtained showed that the extracts (ethanolic and aqueous) contain varied quantities of phenols, flavanoids, alkaloids, tannins and saponins. The extracts of the leaves contain a higher quantity of the phytochemicals than other parts (Phenol; 200.03±0.05, Flavanoid; 81.38±0.07, Alkaloid; 53.50±0.05, Tannins; 71.00±0.02, Saponins 54.70±0.03). The result also showed that there is no significant difference in the quantity of alkaloids obtained from the root, stem and seeds but leaves at $p<0.05$. The same also goes for phenols, tannins and saponins. Generally, the leaves contained the highest quantity of the phytochemicals having subjected the result to a test of significance at 0.05 confidence level. The result of the quantitative phytochemical analysis of the aqueous extracts is relatively the same with that of the ethanol extracts in terms of statistical significance but with different values. Hence, the leaves also contain the highest quantity of the phytochemicals in the aqueous extracts. These findings lend credence to the work of Adegoke *et al.* (2009) which states that the leaves of *Corchorus olitorius* are rich in various phytochemicals which account for its antibacterial activities. The quantity of tannins found in some parts of the plant accounts for the healing properties of the plant since tannins have been found to hasten the healing of wounds and inflamed mucous membrane (Parr and Bowell, 2000). The quantity of flavonoids found in the extracts supports the findings of Okwu (2003) which states that *Corchorus olitorius* has antimutagenic, antibacterial, anti-inflammatory, anti-allergic, antiviral, anti-neoplastic, antithrombotic and vasodilatory activity, all of which are activities associated with flavonoids. Also, the presence of various quantities of saponins accounts for the utilization of the plant in manufacturing shampoos, insecticides and various drug preparations and synthesis of steroid hormones (Okwu, 2003).

4.2.2 PROXIMATE ANALYSIS

Proximate analysis carried out on the plant showed that the different parts of the plant contained quite an appreciable quantity of the proximate qualities that were assessed. The results further showed that there is no significant difference at $p<0.05$ in the moisture and total ash content quantified. The crude fat obtained from the root and stem is significantly different from that of the seed and leaves. However, the carbohydrate content of the leaves is significantly different from that of the other parts of the plant. Nutrients are necessary for life and good health; these may be found in a number of different foods. The general function of nutrients includes energy supply, building materials for body structures and regulations as well as control of body processes. The proximate analysis showed that the plant is a good source of carbohydrate and protein especially the leaves. These findings agree with that of Edeoga (2005). The carbohydrate and protein present in this plant may be a conglomerate of bioactive sugars, glyco-proteins or proteins which gives most of vegetables their medicinal potency against certain diseases. Proteins are known to be needed for growth and body building. The relatively high protein content in the leaves of *C. olitorius* has highlighted the importance of the vegetable. Most rural communities in Nigeria rely on vegetables as sources of proteins; therefore *C. olitorius* could play a significant role in the provision of cheap and affordable source of protein for rural populations. The protein content of this plant is in consonance with the findings of Adegoke and Adeboye (2009) who reported similar variations in the protein content of the leaves and root. Also, crude fiber component in this plant could have application in the treatment of diseases such as obesity, cancer and gastrointestinal disorders. The ash content is an indication of mineral content. It implies that the plant will contain an appreciable amount of minerals due to its ash component. The differences in moisture content of the various plant parts are relatively insignificant. Crude fat content, which was not

significantly different in the various parts of the plant, is an indication that the plant is a good source of lipids. *C. olitorius* leaves appear to be nutritionally better than other parts of the plant. This could explain the widespread consumption of the leaves more than the other parts. It should be noted that the seed is rich in crude lipid while the stem has good fiber content; this makes the entire aerial part of the plant important when the issue of good nutrition is being discussed. This equally explains the classification and use of the plant as a fiber crop (Roy *et al*., 2006). *C. olitorius* was also found to be rich in fiber and this is beneficial nutritionally, since it has been reported that food fiber aids absorption of trace elements in the gut and reduces absorption of cholesterol. Besides, adequate intake of dietary fiber can lower the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al.,* 2000).

4.2.3 MINERAL ANALYSIS

The results obtained from the mineral analysis of the plant showed that the various plant parts contain quite an appreciable amount of calcium, magnesium, sodium, potassium, with iron being the least. There is no significant difference at $p<0.05$ in the minerals obtained from the different parts of the plant. The mineral contributions of vegetables to human nutrition are limited due to the presence of antinutrients which render some of the micronutrients unavailable to human nutrition (Akwaowo *et al*., 2000). The most common antinutrients in leafy vegetables are phytate, tannins, hydrocyanic acid and oxalic acid. Phytate is the major phosphorus storage compound in plants (Gupta *et al*., 2006). It has the strong ability to chelate multivalent metal ions especially zinc, calcium and iron. This leads to poor bioavailability of such ions as they are precipitated in the form of insoluble complexes (Gupta *et al.,* 2006). This study showed that *C. olitorius* can play a role in alleviating micronutrient deficiency especially iron and calcium which are recognized to be

a major worldwide nutritional problem. Diets with low calcium and iron status are correlated to decreased growth, poor pregnancy outcome, and impaired immune functions (Loornedal, 2002). While the presence of phytate could decrease mineral absorption in humans, this antinutrient is usually eliminated during cooking (Akwaowo *et al*., 2000). Therefore, it would be in order to state cooking *C. olitorius* leaves reduces its phytate content and so makes it nutritionally safe for consumption. All the minerals present in the plant are important in human and animal nutrition and hence, makes the plant an important dietary component. Again, the high composition of minerals is advantageous since certain inorganic mineral elements intervene in therapeutic aspects such as aiding normal functioning of immune system. They play important roles in the maintenance of normal glucose tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary and Bandyopadhyay, 1999). In addition, some minerals are important in connection with ischemic heart disease (Ishida *et al*., 2000), bone formation and control of salt balance in human tissues. On the other hand, children, women of reproductive age and pregnant women need food with high iron content since they are most vulnerable to micronutrient deficiency and anemia. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, protein and fats. Calcium was highest in the root (616.33±4.73mg) and least in the leaves (1.27±0.01mg). Calcium is required as a component of the human diet, and it is essential for the full activity of many enzymes, such as nitric oxide synthase, protein phosphatases, and adenylate kinase. Iron also acts as a cofactor in catalase, an enzyme that catalyzes the conversion of hydrogen peroxide to water and oxygen. Magnesium as well is a co-factor of pyruvate dehydrogenase, an enzyme which transforms pyruvate into acetyl-CoA used in the citric acid cycle to carry out cellular respiration to release energy. With respect to this, the consumption of *C. olitorius* should be encouraged to acquire these

important minerals. Again, Calcium is one of the minerals present in the largest quantity in the structure of the body and bones. Calcium levels in the blood and fluid surrounding the cells must be maintained within a very narrow range for normal physiological functioning. The physiological functions of calcium are so vital to survival that the body will demineralize the bone to maintain normal blood calcium levels when calcium intake is inadequate. Thus, adequate dietary calcium is a critical factor in maintaining a healthy skeleton (Weaver and Heaney, 1999). Also, the binding of calcium to the protein, calmodulin, activates enzymes that breakdown muscle glycogen to provide energy for muscle contraction (Weaver and Heaney, 1999). Calcium is also known to play a significant role in muscle contraction, bone and teeth formation and blood clotting (Weaver and Heaney, 1999). Magnesium was highest in the root (157.50± 42.50mg) and least in the leaves (0.60±0.01mg). Magnesium is needed as a cofactor in enzyme catalysis in the body (Weaver and Heaney, 1999). The metabolism of carbohydrates and fats to produce energy requires numerous magnesium-dependent chemical reactions. Magnesium is required by the adenosine triphosphate (ATP)-synthesizing protein in mitochondria. ATP, the molecule that provides energy for almost all metabolic processes, exists primarily as a complex with magnesium (Rude and Shils, 2006). Magnesium is also required for the active transport of ions like potassium and calcium across cell membranes (Rude and Shils, 2006). Sodium and potassium were highest in the root (306.00±6.00mg and 233.17±1.61mg) respectively and least in the leaves (0.34±0.21mg and 3.76±0.02mg). The presence of sodium and potassium in the intracellular and extracellular fluid helps to maintain electrolyte balance and membrane fluidity. Iron, which is the least in quantity, is known to be a component of some mettaloenzymes, myoglobin and haemoglobin (Rude and Shils, 2006), which is needed in the transport of oxygen and carbondioxide during respiration or cellular metabolism. This haemoglobin (containing iron) also serves as

buffer to regulate changes in blood pH (Rude and Shils, 2006). It is also known that inorganic mineral elements such as potassium, calcium play important roles in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of langerhans (Rude and Shils, 2006). Iron is an essential trace element for haemoglobin formation and normal functioning of the central nervous system. The amount of iron in *C. olitorius* is of particular importance considering the fact that 21% of children in Africa suffer from anaemia, a condition caused by iron deficiency (van Vuren, 2006).

4.2.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING OF THE PLANT EXTRACTS AND ACTIVE PRINCIPLES

Antimicrobial susceptibility testing is usually carried out to determine the ability of any antimicrobial agent to inhibit the growth of a microorganism or to determine which antimicrobial agent will be most successful in treating a microbial infection in vivo. Some antimicrobials actually kill the organism while some just prevent the multiplication of the organism. The analysis carried out confirms the antibacterial and antifungal potentials of the plant. Both the ethanol and aqueous forms of the extract, and the alkaloids as well exhibited varying degrees of antimicrobial activities against the test organisms. The results obtained showed that the zones of inhibition of the extracts (ethanol and aqueous) are statistically significant at $p<0.05$. On a general note, the ethanolic extract and the active principle (Alkaloids, Saponins, Phenols, Flavonoids and Tannins) exhibited higher degrees of antibacterial and antifungal activities than the aqueous extracts. This variation or difference in the degree of activity expressed by the different plant parts/extracts may be due to the method of extraction. The most active extract was that obtained from ethanol and this extract inhibited the growth of all the bacterial strains tested, specifically *Escherichia coli* (9.00mm, 8.50mm, 4.50mm and 10.00mm) for the root, stem, seed and

leaves respectively. However, among the fungal strains studied, only *Candida albicans*, *Aspergillus niger* and *Penicillium chrysogenum* showed susceptibility to the aqueous extracts while the rest were resistant. The active principles tested displayed varying degrees of activity against the test organisms. However, they expressed more activity against the bacterial isolates than the fungi. Hence, it can be said that most of the active principles are antibacterial agents. Flavonoids expressed a high degree of antimicrobial activity against both bacteria and fungi with the highest being (leaves; 12.00±0.2 on *E. coli)* and the least being (leaves; 6.03±0.03) on *C. albicans*. Alkaloids and tannins equally expressed activity against the bacteria. Saponins displayed both antibacterial and antifungal activities. However, phenols are the least active against the organisms. The ethanolic extracts of the investigated species had an invitro antimicrobial activity against almost all the bacteria. The result revealed that the extracts from the leaves are the most active. The result further indicates that ethanolic extracts of the leaves and active principles have the strongest inhibitory effect on *Aspergillus niger*. It should however be noted that the presence of coumarin compounds in the leaves of the plant may be responsible for the inhibitory activity (Abouzied, 2002). Also, the inhibitory activities of the extracts on the test organism can be adduced to the presence of numerous phytochemical constituents, which can equally be of prophylactic importance (Adegoke *et al.*, 2009). Alkaloids are endowed with diuretic biological activities, being already used in therapy as pharmacological tools. Among the reported biological effects, they present antitumour (Sneden, 2005) anticholinergic (Sneden, 2005), diuretic (Adegoke and Adebayo, 2009) and anti-inflammatory properties (Adegoke and Adebayo, 2009). There are also reports of toxic effects of alkaloids to humans. Thus, it is necessary to use different experiential models to understand the exact mechanism of the molecules under study in order to have the real knowledge of their effect (Rude and Shils, 2006). Again, the significant display of

antimicrobial activity (especially antibacterial) by the plant can be attributed to the presence of tannins, flavonoids and saponins in the plant since they both possess antimicrobial potency. Furthermore, the results obtained justifies the utilization of the plant in traditional/folk medicine for the treatment of various bacterial and fungal infections such as pneumoniae, cellulitis, carbuncles, enteric fiver, mycosis, diarrhea, tooth ache, gastroenteritis etc (Adegoke *et al.,* 2009).

MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF THE PLANT EXTRACTS AND ACTIVE PRINCIPLES

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Okwori *et al.* (2002) stated that minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganism to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that patients will receive but also the type of antibiotic to be used which, in turn, lowers the opportunity for microbial resistance to specific antimicrobial agents. Applying MIC testing to a number of bacterial strains in the some species provides an estimate of the concentration than inhibits 50% (MIC_{50}) and 90% (MIC_{90}) of bacterial isolates and can indicate shifts in the susceptibility of bacterial populations to antibiotics (Davison *et al.,* 2010). MICs are therefore often the starting point for larger preclinical evaluations of novel antimicrobial agents (Obadoni and Ochuko, 2001). The results of the minimum inhibitory concentration displayed on the tables shows that most of bacterial isolates were not inhibited by the ethanolic extracts at the test concentrations used. However, *Escherichia coli* are the most susceptible to the ethanol extracts and active principles followed by *Staphylococcus aureus* and *Salmonella typhi*. *Klebsiella pneumoniae* is the least

susceptible. Also, the growths of some of the bacterial isolates were inhibited by the aqueous extracts, although the ethanolic extracts displayed a higher inhibitory activity. Flavonoids, alkaloids, saponins and tannins inhibited the growth of most of the bacterial isolates and a few of the fungi (*C. albicans*, *A. niger* and *P. chrysogenum*). Phenols expressed the least inhibitory activity against the test organisms. The most susceptible bacteria to the aqueous extracts were *Escherichia coli* and *Staphylococcus aureus* at MIC 100mg/ml, followed by *Klebsiella pneumoniae* and *Salmonella typhi* at MIC 200mg/ml and MIC 250mg/ml respectively. Furthermore, the results show that both of ethanolic and aqueous extracts, as well as the active principles, exhibited minimal inhibitory activity on the fungal isolates. *Microsporum canis* showed no activity on both extracts. However, *Candida albicans, Aspergillus niger* and *Penicillium chrysogenum* showed varying degrees of inhibitory activity to both extracts ranging from 200 – 250mg/ml. *Penicillium chrysogenum* showed no inhibitory activity to the aqueous extracts. From these results obtained, it can again be said that the ethanolic extracts exhibit more inhibitory activity on the bacterial and fungal isolates than the aqueous extracts. Hence the plant has more antibacterial potency than antifungal. The plant will therefore be more effective in the treatment of bacterial infections (Adegoke, 2009). This further lends credence to the utilization of this plant by traditional medicine practitioners in the treatment of common bacterial diseases especially those associated with *E.coli* and *S. aureus*. It is obviously interesting to observe the result of high antibacterial effects to the common bacteria of public health importance. *Staphylococcus aureus* is no doubt frequently connected to cases of bacteremia, septicemia, endocarditis, osteomyelitis, furunculosis etc. It is also frequently involved in both nosocomial and community-acquired infections. Considering the successful inhibition of these bacteria and its contemporary aetiology, and the long record of resistance to conventional antibiotics, the plant can therefore pass for prophylactic use

by people living with HIV/AIDS against infections involving the two isolates (*E.coli*, *S. aureus*). These findings further justify the prophylactic and therapeutic applications of the plant. It could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs.

MINIMUM BACTERICIDAL CONCENTRATION (MBC) AND MINIMUM FUNGICIDAL CONCENTRATION (MFC)

Minimum bactericidal concentration is the lowest concentration of an antibacterial agent required to kill a particular bacterium. Minimum fungicidal concentration is the lowest concentration of an antifungal agent required to kill a particular fungus. The results obtained reveal that only the ethanol extracts and active principles expressed bactericidal activity against the test bacteria. The extracts were only able to kill *E.coli* and *S. aureus* at MBC 100mg/ml - 250 mg/ml. This justifies the effectiveness of *C. olitorius* extracts in the treatment of bacterial infections especially those caused by *E.coli* and *S. aureus* as stated by Adegoke (2009). The results obtained shows that the extracts did not express fungicidal activity against the test fungi. Hence, it can be said that the plant does not possess fungicidal ability.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

From the results and discussions, the phytochemical analysis reveals that bioactive natural constituents like saponins, alkaloids, tannins, flavonoids, phenols, anthraquinone, anthranoids, steroids and cardiac glycosides have basic contributions to the medicinal values of the plant. The mineral and proximate analysis carried out reveals that the plant contains some essential elements like calcium, magnesium, iron, sodium and potassium, and nutrients like carbohydrate, protein, fiber etc all of which contributes to the high nutritional value of the plant. The antimicrobial activities of the different parts of the plant against the test organisms shows that the plant could be very useful in the cure and treatment of diseases caused by these organisms. It also shows that the plant has a significant pharmaceutical importance. The inhibitory activity of these extracts confirmed their potential application in the treatment of microbe induced diseases.

5.2 RECOMMENDATIONS

Few researchers have worked on the plant; *Corchorus olitorius* Linn, but the full potentials of the plant has not been fully exploited. It is recommended that the plant should be deeply studied and harnessed for its antibacterial potency. *C. olitorius* thrives well and should be planted in homes to avoid local extinction in order to benefit from its medicinal applications. Genetic engineering could also be used to improve the yield of secondary metabolites of this plant.

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APPENDIX 1

Chemicals/Reagents: The following chemicals/reagents were used to carry out the study;

Acetic acid

Ammonia

Ammonium Hydroxide

Amyl Alcohol

Barium Chloride

Benzene

Chloramphenicol

Chloroform

Diethyl Ether

Distilled water

Ethanol

Ether

Fehling Solution I & II

Ferric Chloride

Folin-Denis Reagent

Hydrochloric acid

Mayer's reagent

Naphthylethylenediamine Dihydrocholride

n-butanol

Nutrient agar

Nystatin

Phosphate Buffer Saline

Potassium Hydroxide

Sabouraud Dextrose agar

Sodium Chloride

Sodium Nitroprusside

Sulfanilic Acid

Tetraoxosulphate (vi) acid

Toluene

Apparatus: The following apparatus were used to carry out the work;

FTIR- 8400s Fourier Transform Infrared Atomic Absorption Spectrophotometer (AAS)

Beakers

Condenser

Conical flasks

Cotton wool

Crucibles

Electronic Blender

Electronic weighing balance

Fitter paper (Whatman No.42)

Funnel

Manual Grinder

Heating mantle

Hot Air Oven

Incubator

McCartney Bottles

Meter rule

Paper clipper

Paper discs

Petri dishes

Pipettes

Soxhlet Apparatus

Spatula

Stirrer

Swab Sticks

Syringes

Test tubes

Thermometer

Universal Indictor Paper

Volumetric flasks

Water bath

Wire loop

Test Organisms: The following test organisms were used for the study;

Escherichia coli

Salmonella typhi

Staphylococcus aureus

Klebsiella pneumonia

Candida albicans

Aspergillus niger

Microsporum canis

Penicillium chrysogenum

APPENDIX II

STATISTICAL ANALYSIS

PHYTOCHEMICAL ANALYSIS OF THE ETHANOLIC EXTRACTS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0	3	200.03	66.67667	13337.33
28.03	3	181.18	60.39333	349.6901
26	3	116	38.66667	168.0833
38	3	99	33	1279
3	3	59.7	19.9	914.53

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4538.566	4	1134.641	0.353501	0.835927	3.47805
Within Groups	32097.27	10	3209.727			
Total	36635.84	14				

PHYTOCHEMICAL ANALYSIS OF THE AQUEOUS EXTRACTS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0	3	165.11	55.03667	6824.652
15.09	3	127.31	42.43667	374.024
21	3	89.56	29.85333	194.3445
0	3	80.85	26.95	435.0448
0	3	46.37	15.45667	155.8892

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2773.371	4	693.3428	0.43421	0.781215	3.47805
Within Groups	15967.91	10	1596.791			
Total	18741.28	14				

PROXIMATE ANALYSIS OF THE WHOLE PLANT

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
29.69	3	84.08	28.02667	0.220133
3.14	3	11.64	3.88	0.2617
4.15	3	15.62	5.206667	3.823433
6.91	3	47.96	15.98667	112.011
23.86	3	34.14	11.38	89.3119
67.75	3	193.44	64.48	112.9012

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7795.878	5	1559.176	29.36951	2.47E-06	3.105875
Within Groups	637.0588	12	53.08823			
Total	8432.936	17				

MINERAL ANALYSIS OF THE WHOLE PLANT

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
616.33	3	367.94	122.6467	28206.29
157.5	3	214	71.33333	3824.653
1.11	3	1.35	0.45	0.4336
306	3	264.4	88.13333	21914.83
233.17	3	310.98	103.66	7490.768

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	26430.12	4	6607.529	0.537749	0.711668	3.47805
Within Groups	122874	10	12287.4			
Total	149304.1	14				

ZONES OF INHIBITION (MM) OF THE ETHANOL EXTRACT ON THE CHALLANGE ORGANISMS.

Groups	*Count*	*Sum*	*Average*	*Variance*
9	4	35.5	8.875	11.22917
	0	0		
8	4	34.5	8.625	2.729167
	0	0		
10	3	28.1	9.366667	5.203333
	0	0		
7	3	28.5	9.5	3

	0	0		
	1	15	15	
10	1	14	14	
	1	12.5	12.5	
10	1	12	12	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65.82778	11	5.984343	0.616078	0.769956	4.027442
Within Groups	58.28167	6	9.713611			
Total	124.1094	17				

ZONES OF INHIBITION (MM) OF THE AQUEOUS EXTRACT ON THE CHALLANGE ORGANISMS.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
	4	29	7.25	17.41667
9	4	27.5	6.875	5.895833
8	2	17	8.5	4.5
7	3	28	9.333333	9.333333

	1	12	12
8	1	10	10
7	1	12.5	12.5
	1	10	10

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	51.51348	11	4.683044	0.251495	0.973637	4.703967
Within Groups	93.10417	5	18.62083			
Total	144.6176	16				

ZONES OF INHIBITION (MM) OF ALKALOIDS ON THE CHALLENGE ORGANISMS.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
8	4	32.5	8.125	1.729167
7.5	3	24.5	8.166667	0.985833
3.01	2	12.05	6.025	1.90125
10	5	45	9	1.625
12.5	7	87	12.42857	2.702381

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	98.21839	4	24.5546	12.36435	8.98E-05	3.006917
Within Groups	31.7747	16	1.985919			
Total	129.9931	20				

ZONES OF INHIBITION (MM) OF SAPONINS ON THE CHALLENGE ORGANISMS.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
7.05	5	43.15	8.63	2.44325
8.6	2	15.4	7.7	0.08
11	6	55.48	9.246667	0.379667
13	7	86.75	12.39286	2.397857

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	63.4195	4	15.85488	9.098585	0.000615	3.055568
Within Groups	26.13848	15	1.742565			
Total	89.55798	19				

ZONES OF INHIBITION (MM) OF PHENOLS ON THE CHALLANGE ORGANISMS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
11.04	4	40	10	0.166667
12.5	7	87.6	12.51429	2.292262

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16.09143	1	16.09143	10.16046	0.01105	5.117355
Within Groups	14.25357	9	1.58373			
Total	30.345	10				

ZONES OF INHIBITION (MM) OF FLAVONOIDS ON THE CHALLANGE ORGANISMS.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
7	4	31.85	7.9625	1.912292
9.2	3	25.45	8.483333	1.630833
9	3	27.1	9.033333	1.003333
	0	0	#DIV/0!	#DIV/0!
12	5	51.03	10.206	1.57218
12.5	7	85.6	12.22857	2.665714

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	61.23946	5	12.24789	5.886957	0.00285	2.852409
Within Groups	33.28821	16	2.080513			
Total	94.52768	21				

ZONES OF INHIBITION (MM) OF TANNINS ON THE CHALLENGE ORGANISMS.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
10.02	4	34.6	8.65	2.49
	0	0	#DIV/0!	#DIV/0!
9.5	3	22.4	7.466667	0.653333
10	5	49	9.8	0.325
12.5	7	82	11.71429	0.654762

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	47.10424	4	11.77606	11.77165	0.000212	3.11225
Within Groups	14.00524	14	1.000374			
Total	61.10947	18				

MINIMUM INHIBITORY CONCENTRATION (MIC) (Mg/ml) OF THE ETHANOL EXTRACTS ON THE CHALLANGE ORGANISMS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
50	3	200	66.66667	833.3333
	3	250	83.33333	833.3333
	2	450	225	1250
200				
	1	100	100	
250	1	200	200	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	41416.67	7	5916.667	2.581818	0.307435	19.35322
Within Groups	4583.333	2	2291.667			

MINIMUM INHIBITORY CONCENTRATION (MIC) (Mg/ml) OF THE AQUEOUS EXTRACTS ON THE CHALLANGE ORGANISMS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
	2	300	150	5000
200	3	700	233.3333	833.3333
200	1	200	200	
200	2	300	150	5000
200	0	0		
200	1	200	200	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12222.22	7	1746.032	0.14966	0.963788	236.7684
Within Groups	11666.67	1	11666.67			

MINIMUM INHIBITORY CONCENTRATION (MIC) (Mg/ml) OF ALKALOIDS ON THE CHALLANGE ORGANISMS.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
200	3	500	166.6667	3333.333
100	3	450	150	7500
250	3	750	250	0
250	3	600	200	7500
200				
	1	200	200	
200	1	200	200	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17619.05	7	2517.007	0.411874	0.864498	4.206658
Within Groups	36666.67	6	6111.111			
Total	54285.71	13				

MINIMUM INHIBITORY CONCENTRATION (MIC) (Mg/ml) OF SAPONINS ON THE CHALLENGE ORGANISMS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100	2	150	75	1250
100	2	300	150	5000
200	1	100	100	
200	2	450	225	1250
200	1	100	100	
100	1	100	100	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	27500	7	3928.571	0.52381	0.790449	236.7684
Within Groups	7500	1	7500			

MINIMUM INHIBITORY CONCENTRATION (MIC) (Mg/ml) OF FLAVONOIDS ON THE CHALLENGE ORGANISMS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
100	3	400	133.3333	10833.33
200	3	400	133.3333	3333.333
100	3	600	200	7500
100	3	550	183.3333	5833.333
200	1	200	200	
	1	200	200	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13035.71	7	1862.245	0.203154	0.972365	4.206658
Within Groups	55000	6	9166.667			
Total	68035.71	13				

MINIMUM INHIBITORY CONCENTRATION (MIC) (Mg/ml) OF TANNINS ON THE CHALLENGE ORGANISMS

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
	2	300	150	5000
200	2	250	125	11250
200	2	300	150	5000
250	2	450	225	1250
200	2	500	250	0
	1	200	200	
	1	200	200	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	24166.67	7	3452.381	0.613757	0.731414	6.094211
Within Groups	22500	4	5625			
Total	46666.67	11				