PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF CRUDE *FICUS NATALENSIS (KRAUS)* LEAF EXTRACTS

BY

SHEYIN, FORTUNE

DEPARTMENT OF CHEMI

FACULTY OF PHYSICAL SCIENCE

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

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BY

SHEYIN Fortune, B.Sc. (Hons) Chemistry

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ZARIA, NIGERIA

MARCH, 2019

DECLARATION

I declare that the work in this desertation entitled ‘Phytochemical screening and antimicrobial activity of *Ficusnatalensis (Kraus)*leaf extracts’ was carried out by me in the department of chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This thesis titled 'Phytochemical screening and antimicrobial activity of *Ficusnatalensis (Kraus)*leaf extracts' by SHEYIN, Fortune meets the regulations governing the reward of the degree of Masters of Science in Chemistry (Organic) of the Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof G. I. Ndukwe Chairman, Supervisory Committee	_____ Signature	_____ Date
Dr O. R. A. Iyun Member, Supervisory Committee	_____ Signature	_____ Date
Prof A. Uzairu Head of Department	_____ Signature	_____ Date
Prof S. Z. Abubakar Dean, School of Postgraduate Studies	_____ Signature	_____ Date

DEDICATION

This work is dedicated to the God Almighty the maker of Heaven and earth (the field of chemistry) and my parents Rev and Mrs Titus Sheyin.

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I am grateful to God Almighty for provision, sustenance, preservation especially health wise, knowledge and wisdom to make this work a success.

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ABSTRACT

Plants have been used by traditional healers to cure and prevent ailment. These Plants contain medicinal principle that can be the reason for their medicinal properties. Cold extraction was carried out using three solvents; n-hexane, ethylacetate and methanol. Phytochemical screening, antimicrobial activity of the crude extract, isolation, characterization of the compound that was iolated and its biological study was carried out using standard techniques.The result of the phytochemical screening of the crude n-hexane, ethyl acetate and methanol extracts showed the presence of carbohydrates, flavonoids, alkaloids, saponnins, cardiac glycosides, tannins, and anthraquinones. The antimicrobial*in vitro* screening was carried out on ten microorganisms.Methicillin resistant *Staphylococcusaureus*, Vancomycin resistant *enterococci, Campylobacter jejuni,* and *Shigelladysenteriae* were resistant to the three extract*Escherichia coli, Streptococcus pyogene,Escherichia coli, Helicobacter pylori,Candida krusei*and *Candida tropicalis*showed sensitivity to the extract. The Minimum Inhibitory Concentration (MIC) andMinimum Bactericidal Concentration/Minimum Fungicidal Concentration of the crude extracts were determined. The purification of the ethylacetate extract using a column of silica gel gave a compound that was named F35 after characterization; it was 3-hyroxylanost-7-en-29-carboxylic acid. The structure of the isolated compound was established based on the spectral data (IR, 1D and 2D NMR) in comparison with literature.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Herbal product medicine

Herbal medicine can be seen as the use and study of properties of plants and their activities on disease cauing agents (Mahalingam*et al.,* 2008).Medicinal plants have extensively been explored for centuries as agents to combat diseases. Their use dates back to man's origin (Okorondu *et al.,*2015) and the occurrence of natural products with medicinal properties (Gyawali, 2010). They have been known to contain a number of effective antibacterial, antioxidants and anticancer properties which provide an alternative means of therapy to various infections caused by drug resistant bacteria, oxidative stress and dreadful diseases like cancer and other physiological disorders (Kambli *et al.,* 2014). There is a growing need for herbal medicines due to a sudden increase in the demand and collection of some rare endangered herbs from wild populations in many parts of the world (Manukyan, 2011) and owing to the fact that all plants produce chemical compounds as part of their normal metabolic activities (Patrick, 2005).Herbs have been used to treat many diseases throughout the globe before the synthesis or production of modern clinical drugs. The use of medicinal plants still plays a vital role as the basic health system in the developing countries and the developed world. These role of plants as therapeutic agents can be traced to the use, extraction and development of many drugs from plants (Shrikumar and Ravi, 2007).

Natural products obtained from plants represent an attractive and excellent source of antimicrobial agents because they are natural with minimal side effects and inexpensive, especially for many people in the developing countries (Ghosh *et al.,*2008). Medicines ofplant

origin provide an alternative form of healthcare system because they stand as potential sources of novel antibiotic prototypes. Moreover, these compounds may have different mechanisms or pathway of action than conventional drugs and could be of tremendous importance to stepping up the health care system (Rabe and Van, 1997; Koduru *et al.,* 2006; Okeke *et al,* 2001). World health organisation (WHO) has made clear its stand on traditional medicine by encouraging its use because of its minimal side effects. Sinceancient times, people have been exploringnature, particularly plants in search of new drugs (Savithramma *et al.,* 2011).

Some of the phytochemical compounds, e.g., glycoside, saponin, tannin, flavonoids, and terpenoid, in plants have been known to contain components that are bioactive which are regarded as phytomedicines. These constituents in plants can be obtained from any part of the plant like the leaves, bark, flowers, roots, fruits, and seeds (Parekh and Chanda,2007).

1.2 Problems of modern clinical drugs

In the recent years, there is an increase in the resistance of pathogens to antibiotics which has become a serious issue caused in part by the indiscriminate use of modern antibiotics (Rahman *et al.,* 2008).The discovery of effective antibiotics, vaccines, and other products or methods decreased the frightening impact of infectious diseases and improved quality of life. However, the effectiveness of many antibiotics is being threatened by the appearance of microbial resistance to existing chemotherapeutic agents (Cowan *et al.,* 1999). And the uses of some antibiotics are also linked to allergy, immune suppression, and hypersensitivity as side effects (Ahmad *et al.,* 1998).

Many populations who live in developing countries are deprived of the advantages of modern medicine because of the high costs; therefore, poor people lack the means to explore than to

combat infectious diseases. Besides these, co-infection with multiple diseases is a serious problem to infection prevention and treatment. For all these reasons above, there is therefore an urgent need to identify new, safe, and low cost antimicrobial agents that would help to reduce these challenges of infectious diseases (Muhammed *et al.,* 2013).The search and screening of medicinal plants have become very vital because this may serve as potential sources of antimicrobial prototypes which arecontrary to synthetic drugs (Torres *et al.,* 2007). The antimicrobial activities of plants generally are attributed to the variety of chemical substances synthesized by plants (Stafford *et al.*, 2004). These chemical substances synthesized by plants include alkaloids, saponins, tannins, flavonoids, glycosides, anthraquinones, terpenoids, and many others.

1.3 Herbal medicine today

There is a growing need for herbal medicine due to a sudden increase in the demand and collection of some rare endangered herbs from wild populations in many parts of the world (Manukyan, 2011). This demand is as a result of the potential harmful side effects, addiction, immune suppressions of some modern drugs and resistance of some pathogen to some of our regular modern drugs leading many to search for alternative treatment via herbal medicines. These problems have also led physicians to seek new remedies for the treatment of common diseases. Therefore they too are revisiting traditional medicine by using herbs to treat their patients and as a result, making herbal medicine to gain popularity (NCPM, 1998).World Health Organisation (WHO) encourages traditional medicine due to lesser side effects (Savithramma *et al.,* 2011). For these growing demands for herbs and with no structure in place to grow these herbs, in some years to comesome plants and herbs may go into extinction in Africa especially Nigeria due to the harvesting from the wild only. From the encouragement

given by World Health Organisation (WHO) on traditional medicine ithas made medicinal plants to be the surest means to achieve total healthcare coverage of the world's population and has called the attention of governments towards healthcare thereby opening it to an area for research by scientist and investments in developing countries (Cunningham, 1993). With the knowledge that medicinal plants contain some organic compounds which produce physiological action in the human body and the bioactive constituents which can also be known as secondary metabolites (Okwu, 2001).

1.4 Statement of Research Problem

The use of some antibiotics is linked to allergy, immune suppression, and hypersensitivity as side effects (Ahmad *et al.,* 1998). Hence, the need to develop more drugs friendly to the human body. This cannot be carried out without thorough research on the natural sources of these active principles that are contained in plants making it of medicinal value. These active principles can serve as substitute for synthesized drugs that have side effects.

1.5 Aim of the Study

The aim of this research was to screen the extracts of *Ficus natalensis* for its phytochemical constituents and *in vitro* biological activity on selected human pathogens.

1.6 Objectives of the research

The objectives of this research were to:

i. Screen for the presence of phytochemicals in the crude extracts of these solvents used.
ii. determine the antimicrobial activity of the different extracts on some pathogens.

iii. carry out analytical separations involving several conventional steps of chromatographic techniques and purificationof the isolated.

iv. structurally elucidate and characterize of the possible isolated compounds using available spectral techniques such as ^{1}H-NMR, ^{13}C-NMR and FT-IR.

Traditional healers have successfully used several species of the genus *Ficus* to cure several ailments such as; diarrhea, dysentery, cuts, wounds, mumps, cholera, and jaundice. *Ficus natalensis* is of the genus ficus, therefore there is theneed for a scientific investigation of the bioactive components.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Moraceae Family

The Moraceae often called the mulberry family or fig family — is a family of flowering plants having numerous species. Most are widespread in tropical and subtropical regions, less so in temperate climates. The only synapomorphy within Moraceae is presence of laticifers and milky sap in all parenchymatous tissues, but generally useful field characters include two carpals sometimes with one reduced, compound inconspicuous flowers, and compound fruits.

Moraceae, the mulberry family of the rose order (Rosales), with about 40 genera and some 1,000 species of deciduous or evergreen trees and shrubs, distributed mostly in tropical and subtropical regions. Plants of the family contain a milky latex and have alternate or opposite leaves and small, petalless male or female flowers. The fruits of many species are multiple because fruits from different flowers become joined together. Some genera produce edible fruits, such as the mulberry (Morus), fig (Ficus carica), breadfruit and jackfruit (Artocarpus), and affon, or African breadfruit (Treculia). Others, such as Antiaris, Ficus, and Castilla, are important for their timber and latex. The latex of the upas tree (Antiaris toxicaria) of Java is used as an arrow poison; the latex of the cow tree (Brosimum utile) of tropical America is sweet and nutritious. *Ficus*, the largest genus in the mulberry family, contains the banyan and the India rubber tree. The bark of the paper mulberry (Broussonetia) has been used for the manufacture of cloth and paper products. Among the ornamentals in the family are paper mulberry and Osage orange (Judd *et al.*, 1999).

2.2*Ficus natalensis*

*Ficus natalensis*is a tree in the family Moraceae. It is commonly called the Natal fig. *Ficus natalensis* is one of the 800 species under the genus Ficus (Woodland 1997). In Uganda, *F. natalensis* is commonly used as a natural fabric for the formation of bark cloth, an environmentally-friendly, renewable material. Skilled artisans incorporate this unique fabric into many modern uses, including fashion, accessories; house wares, interior design, and art. It is widely propagated in many areas of South Western Uganda where it is an important agro-forestry tree and highly appreciated for other purposes besides bark cloth (Reitzenstein 2003). Toxicologically, *Ficusnatalensis* is one of the oldest known human foods owing to the fact that figs as a fruit have a very high safety profile (Chelminska., 2004).

Kingdom - Planteae

Order - Rosales

Family - Moraceae

Genus - Ficus

Species - *Ficus natalensis*

Local names - Mutuba(Hausa) and Ndabele (Igbos)

Plate 1. Image of the *Ficus natalensis* leaves (http//en.org/wiki/file)

The ethnomedicinal and traditional uses of *Ficus* species in the treatment of diarrhea, dysentery, cuts, wounds, mumps, cholera, jaundice etc. suggest that the plant must have antimicrobial effect. Several studies on other species of *Ficus* have shown the potential

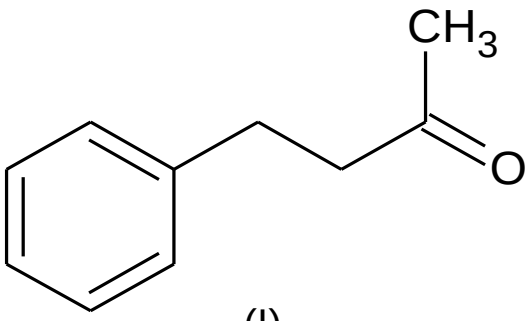
antioxidant and antimicrobial activity (Hazra *et al.,* 2010). Stem barks of *Ficus auriculata* have been used in Nepal as folk remedy in the form of juice to treat diarrhea, dysentery, cuts and wounds (Bhakta *et al.,* 2011).

It is cultivated for its ornamental value. Fig trees have a unique form of fertilization, each species relying on a single, highly specialized species of wasp that is itself very dependent on that fig species to breed.

Its bark, aerial roots and leaves have great medicinal uses as it is used in the treatment of colds, cough, sore throat, dysentery, wounds, acne and pimples, cataracts, worms, cholera and stomachulcers (Monik, 2005). Phytochemical studies of a number of Ficus species have led to identification of over 100 compounds. A good number of these compounds are alkaloids, several coumarins, flavonoids and triterpenoids (Ephraim *et al*, 2008).

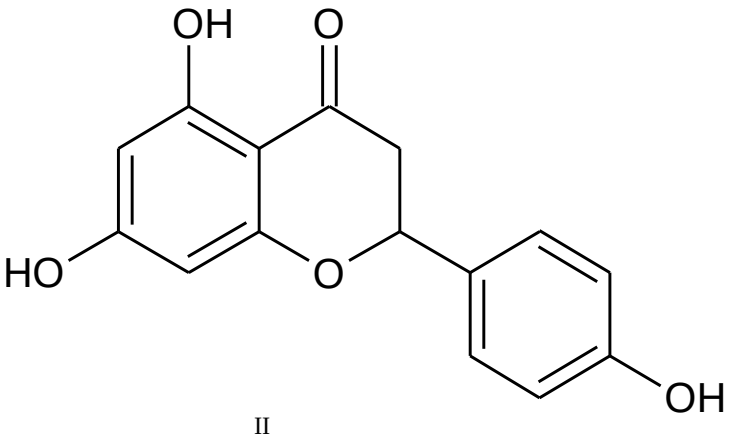
2.3 Compounds isolated from *Ficus* species

Plants have the natural ability to produce or synthesize a wide variety of chemical compounds that are used for important biological activities or functions and serve as a defense against several attacks from predators such as herbivorous mammals, insects, and fungi.(Mahalingam*et al.,* 2008). Ficus benjamina was subjected to isolation and characterization where cinnamic acid (I), naringenin (II), quercetin (III), caffeic acid (IV) and stigmasterol (V) were isolated andcharacterized (Almahy*et al*., 2003). Mehtab *et al*(2014)reported the isolation of four compounds stigmast-5-en3-yl acetate (VI), 3-acetyl-2H-chromen-2-one (VII), 3-(2-hydroxyphenyl)-1-(piperidin-1-yl) propan-1-one (VIII), and 1-isopentyl-3,4-dioxomethylene-2-phenol (IX)from *Ficus rumbphii*β-sitosterol was isolated from the leaves , bark and heartwood of *Ficus palmate*.



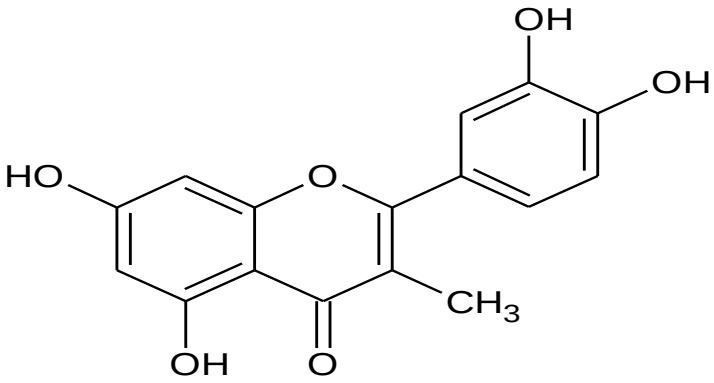
(I)

Cinnamic acid (I)



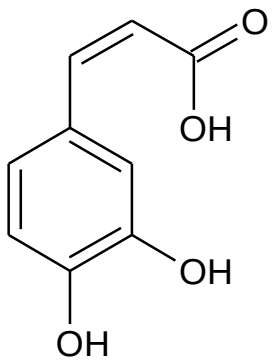
II

Naringenin (II)



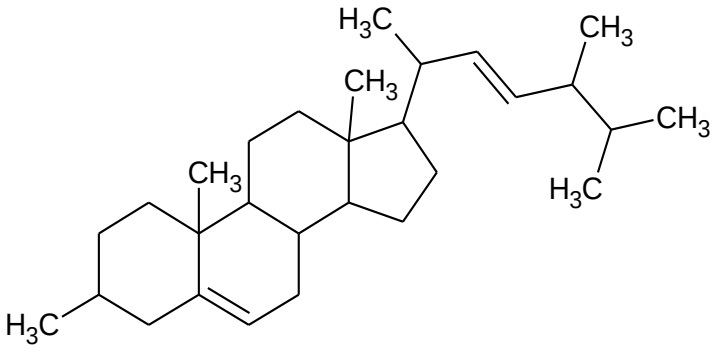
(III)

Quercetin (III)



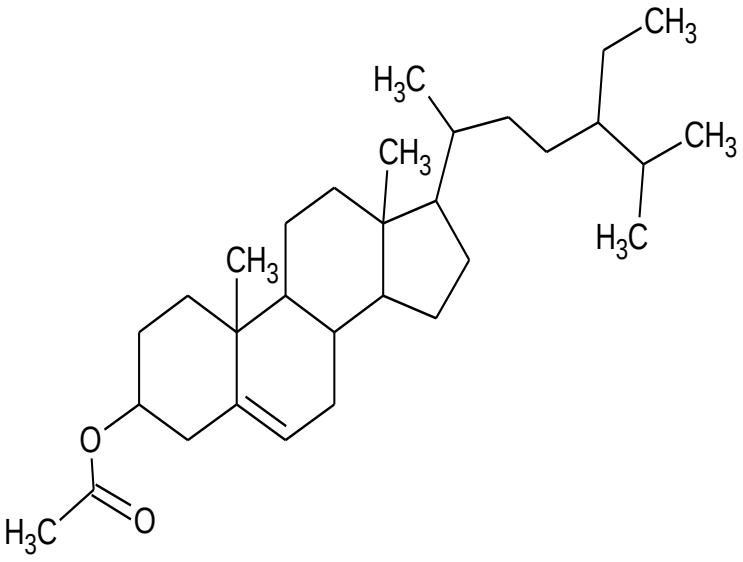
(IV)

Caffeic acid (IV)

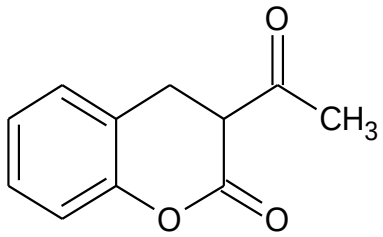


(V)

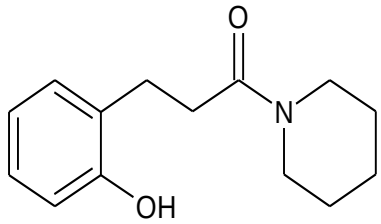
Stigmasterol (V)



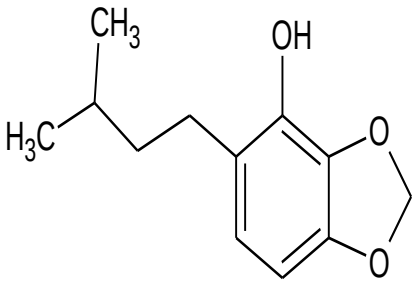
stigmast-5-en3-yl acetate (VI)



3-acetyl-2H-chromen-2-one (VII)



3-(2-hydroxyphenyl)-1-(piperidin-1-yl) propan-1-one (VIII)



1-Isopentyl-3,4-dioxomethylene-2-phenol (IX)

2.4 Medicinal Uses of Ficus Species

Several species of *Ficus* have been known to be of great health importance traditionally.*Ficus* species has been reported by several researchers one of which is Dighe *et al*(2009) who reported that the *Ficus* species have been used in treating female infertility. *Ficus auriculata*is also known by traditional healers for its usefulness in the treatment of diarrhea, dysentery, cuts, wounds, mumps, cholera, and jaundice(Gairola and Biswas 2008). Another specie of the Ficus genus; *Ficus racemosa L*. Fresh juice (50-100 ml) of the leaves is given with water for 10 days to treat gastrointestinal disorder (Rout*et al.,*2009). *Ficus religiosa*, the Leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhoea, haematuria, ear-ache and toothache, migraine, eye troubles, gastric problems and scabies; leaf decoction has been used as an analgesic for toothache; fruits for the treatment of asthma, other respiratory disorders and scabies; the stem bark is used in treating gonorrhea, bleeding, paralysis, diabetes, diarrhea, bone fracture, antiseptic, astringent and antidote (Damanpreet and Rajesh, 2009; Hamed 2011).

Adding to the medicinal uses of ficus species, *Ficus mucoso* is used locally to treat insanity, generalized edemas and leprosy, diarrhea and dysmenorrheal. These diseases can be cured by taking the bark and leaf decoctions of the plant (Burkill, 1997). *Ficus carica* is considered one of the most important medicinal plants worldwide. It is used in the nutritive value of the human beings it also showed the presence of different types of bioactive compounds which makes the plant medicinally very important for the human being. One of these compounds is Psoralen. It has been used to cure several human diseases like heart, skin, kidney, memory, cancer, fungal, viral diseases etc(Manik *et al.,* 2017).

Ficus sp(*sur, sycomorus*)ethanol and petroleum ether crude extracts were subjected to phytochemical screening and antimicrobial activity. Phytochemicals such as carbohydrate, alkaloids,phenol,flavanoids, saponins, phytosterols, diterpenes, protein and tannins were reported to be present (Devi *et al.,* 2015).

The ethanolic extract of *Ficus glomerata* fruit posseses antioxidants effect thus protects the gastric mucosa from the deleterious effects of free radicals. *F. glomerata* serves as a gastroprotective. The phenolic compounds (gallic acid and ellagic acid) in the ethanol extract of *F.glomerata* fruit serves as antioxidants and hence exhibits gastroprotective activity. These phenolic compounds serve as anti cancerogenic,anti ulcerogenicand antimutagenic agents (Rao *et al.,* 2008).The anti inflammatory activity of *Ficus benghalensis* was reported to be due to flavanoids and tannins present in the methanol extract. They are also responsible for the reduction of the effects of oxidative stress and they serve as membrane stabilization agents (Vishnu *et al.,* 2010 and Patil *et al.,* 2009). The methanol extract of*Ficus carica* leaveswas reported to have showed the presence of steroids/triterpenoids, their glycosides and coumarins.It was speculated that these constituents of *Ficus carica* could be responsible for protective effects against lipid rise in rats (Krishna *et al.,* 2007). Scientific studies indicated that *F. racemosa* possesses various biological effects such as hepatoprotective (Mandal *et al.,* 2014), chemopreventive (Khan and Sultana, 2005), anti-diabetic (Rao *et al.,* 2002), anti-inflammatory (Mandal *et al.,* 2000), antipyretic (Rao *et al.,* 2002), and antidiuretic (Ratnasooriya *et al.,* 2003). Bark of *Ficus arnottiana* and *F.hispida* shows hypoglycaemic activity (Papiya*et al.,* 2009). Roots of *Ficus bengalensis* showed anthelmintic activity. The extracts also reported to inhibit insulinase activity from liver and kidney. Fruit extracts exhibits anti-tumour activity (Manoj*et al.,* 2008). Various pharmacological actions such as anti-ulcer,

anti-diabetic, lipid lowering and antifungal activities have been described for *Ficus exasperata*. Ethanol leaf extract of *F.exasperata* shows anti-bacterial activity (Odunbaku*et al.,* 2008). Leaves exhibit hypotensive activity (Buniyamin*et al.,* 2007). Ethanolic and aqueous wood extracts of *F.glomerata* shows anti-HIV- 1 integrase activity (Kingkan *et al.,* 2009).

2.5 Antimicrobial activity of some Ficus species

Ficus sp ethanol and petroleum ether extracts showed antimicrobial activity on the following microbes *Escherichia coli, Staphylococcus aureus, Streptococcus pylori,* some *Pseudomonas* species *(sp), Proteus sp, Bacillus sp, Aspergillus sp and Candida sp* as reported by Devi *et al.*(2015).The leaf and root extracts of *Ficus bengalensis* had no antimicrobial activity on *P. aeruginosa* and *K. pneumonia*. But it showed activity againstS.aureus, *B. subtilis, E.coli*(Ogunlowo *et al.*,2013). The extracts of *Ficus auriculata* were tested against two micro organisms*E. coli* and *S. aureus* which are known to cause diarrhea, dysentery, abscesses, wounds and many other infections to humans. Methanol extract showed greater zone of inhibition for *E. coli* than that of hexane extract, whereas chloroform extract showed the least antibacterial effect. In case of *S. aureus*, hexane extract showed greater zone of inhibition than that of chloroform extract whereas methanol extract showed least antibacterial activity(Gaire *et al.,* 2011).

2.6Phytochemical screening

Medicinal plants possess numerous properties like, antimicrobial, aroma and wound healing properties attributed to the presence of several complex chemical substances with different composition. Phytochemicals can be divided into two constituents: primary and secondary constituents, depending on their role in plant metabolism. Primary constituents include the

common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glycosides (Alapati and Shaik, 2015). Phytochemistry has greatly developed into a unique or distinct discipline in recent years, so in between natural product organic chemistry, plant biochemistry and other related fields. It deals with the great variety of organic substances that are largely accumulated by plants and is concerned with the chemical structures, biosynthesis, turnover and metabolism, natural distribution and the biological function of these substances (Harborne, 1973).

Phytochemical screening helps to evaluate the antibacterial properties which may be of great importance in the discovery of new therapeutic agents, especially at a time like this when thequest foralternative treatment to fight theincreasing threat of drug resistant microorganisms has been the driving force of the scientific community (Aliyu*et al.*,2011).Some phytochemical compounds like glycoside, saponin, tannin, flavonoids, and terpenoid have been known to contain components that are bioactive which are regarded as phytomedicine. These constituents in plants can be obtained from any part of the plant like the leaves, bark, flowers, roots, fruits, seeds etc (Pareck *et al.*, 2007). The search and screening of medicinal plants have become very vital because this may serve as potential sources of antimicrobial prototypes which are contrary to synthetic drugs (Torres *et al.*, 2007). The antimicrobial activity of plants generally is attributed to the variety of chemical substances synthesized by plants (Stafford *et al.*, 2004). These chemical substances synthesized by plants include alkaloids, saponins, tannins, flavonoids, glycosides, anthraquinones, terpenoids, and many others.

Phytochemical investigations of some Ficus species showed that phenolic compounds as their major components (AbdelHameed, 2009;Basudan *et al.,* 2005;Salem, 2005). Taking into account, the vast potentials of plants as the sources for antimicrobial (bacteria and fungi) drugs, an efficient investigation was undertaken to monitor the antibacterial activity of diverse *Ficus* species. Antimicrobial (bacteria and fungi) activity and antiviral activity was also reported for many species of Ficus (Aref *et al.,* 2011; Mahmoud *et al.,* 2010). Over the years, researchers have been able to examine and identify phytochemicals with unknown pharmacological activities but having adequate antibacterial, antifungal and antiviral effects. Many Ficus species have long been used in folk medicine and various pharmacological actions (Trivedi *et al.,* 2006).

2.6.1 Triterpenoids

Triterpenoids are compounds with a carbon skeleton based on six isoprene units and which are derived biosynthetically from the acyclic C-30 hydrocarbon, squalene. They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids. They are colourless, crystalline, often high melting point, optically active substances, which are generally difficult to characterize because of their lack of chemical reactivity. A widely used test is the Liebermann-Burchard reaction (acetic anhydride- Conc. H_2SO_4), which produces a blue-green colour with most triterpenes and sterols. Triterpenoids can be divided into at least four groups of compounds:

i. Triterpenes,
ii. Steroids,
iii. Saponins and

iv. Cardiac glycosides.

The latter two groups are essentially triterpenes or steroids which occur mainly as glycosides. There are also the steroidal alkaloids. Many triterpenes are known in plants and new ones are regularly being discovered and characterized. So far only a few are known to be of widespread distribution. This is true of the pentacyclic triterpenes α- and β-amyrin and the derived acids, ursolic and oleanolic acids. These and related compounds occur especially in the waxy coatings of leaves and on fruits such as apple and pear and they may serve a protective function in repelling insect and microbial attack. Triterpenes are also found in resins and barks of trees and in latex (Euphorbia, Hevea, etc.). Certain triterpenes are notable for their taste properties, particularly their bitterness. Limonin, the lipid soluble bitter principle of citrus fruits, is a case in point. It belongs to series of pentacyclic triterpenes which are bitter, known as limonoids and quassinoids. They occur principally in the Rutaceae, Meliaceae, Simaroubaceaeand are also of chemotaxonomic interest. Another group of bitter triterpenes are the cucurbitacins(Harborne, 1973).

2.6.2 Sterols

Sterols are triterpenes which are based on the cyclopentane perhydrophenanthrene ring system. At one time, sterols were mainly considered to be animal substances (as sex hormones, bile acids, etc.) but in recent years, an increasing number of such compounds have been detected in plant tissues. Indeed, three so-called 'phytosterols' are probably ubiquitous in occurrence in higher plants: sitosterol, stigmasterol and campesterol. These common sterols occur as both free and as simple glucosides. A less common plant sterol is α-spinasterol, an isomer of stigmasterol found in spinach, alfalfa and senega root. Certain sterols are confined to lower

plants; one example is ergosterol, found in yeast and many fungi. Others occur mainly in lower plants but also appear occasionally in higher plants, e.g. fucosterol, the main steroid of many brown algae and also detected in the coconut(Harborne, 1973).

2.6.3 Saponins

Saponins are glycosides of both triterpenes and sterols and have been detected in over seventy families of plant. They are surface active agents with soap-like properties and can be detected by their ability to cause foaming and to haemolyse blood cells. The search in plants for saponins has been stimulated by the need for readily accessible sources of sapogenins which can be converted in the laboratory to animal sterols of therapeutic importance (e.g. cortisone, contraceptive estrogens, etc.). Compounds that have so been used include hecogenin from Agave and yamogenin from Dioscorea species. Saponins are also of economic interest because of their occasional toxicity to cattle (e.g. saponins of alfalfa) or their sweet taste (e.g. glycyrrhizin of liquorice root). The glycosidic patterns of the saponins are often complex; many have as many as five sugar units attached and glucuronic acid is a common component(Harborne, 1973).

2.6.4 Cardiac glycosides

Another group of triterpenoids are the cardiac glycosides or cardenolides; here again there are many known substances, with complex mixtures occurring together in the same plant. A typical cardiac glycoside is oleandrin, the toxin from the leaves of the oleander, Nerium oleander, Apocynaceae. An unusual structural feature of oleandrin and many other cardenolides is the presence of special sugar substituents, sugars indeed which are not found elsewhere in the plant kingdom. Most cardiac glycosides are toxic and many have

pharmacological activity, especially as their name implies on the heart. Rich sources are members of the Scrophulariaceae, Digitalis, Apocynaceae, Nerium, Moraceae and Asclepiadaceae, Asclepias(Harborne, 1973).

2.6.5 Phenolic compound

The term phenolic compound is concern with a wide variety of plant substances which possess in common an aromatic ring bearing one or more hydroxyl substituents. Phenolic substances tend to be water-soluble, since they most frequently occur combined with sugar as glycosides and they are usually located in the cell vacuole. Among the natural phenolic compounds, of which over a thousand structures are known, the flavonoids form the largest group but simple monocyclic phenols, phenylpropanoids and phenolic quinones all exist in considerable numbers. Several important groups of polymeric materials in plants - the lignins, melanins and tannins - are polyphenolic and occasional phenolic units are encountered in proteins, alkaloids and among the terpenoids (Harborne, 1973).

2.6.6Flavonoids

Flavonoids are all structurally derived from the parent substance flavone, which occurs as a white mealy farina on plants, and all share a number of properties in common (Harborne, 1973). Flavonids in a clearer sense of the term are universal plant pigments, which are almost alwayssoluble in water. They are responsible for the color of flowers, fruits and sometimesleave of plants.Examples are the yellow flavonoids; chalcones, aurones and yellow flavonoids (Kursinszki, 2015). Flavonoids are mainly water soluble compounds. They are phenolic and hence change in colour when treated with base or with ammonia; thus they are easily detected on chromatograms or in solution. Flavonoids contain conjugated aromatic

systems and thus show intense absorption bands in the UV and visible regions of the spectrum. Finally, flavonoids are generally present in plants bound to sugar as glycosides and anyone flavonoid aglycone may occur in a single plant in several glycosidic combinations. For this reason, when analysing flavonoids, it is usually better to examine the aglycones present in hydrolysed plant extracts before considering the complexity of glycosides that may be present in the original extract. Flavonoids are present in all vascular plants but some classes are more widely distributed than others; while flavones and flavonols are universal, isoflavones and biflavonyls are found in only a few plant. The following are classes of flavanoids.

I. Anthocyanins
II. Leucoanthocyanidins
III. Flavonols
IV. Flavones
V. Glycoflavones
VI. Biflavonyls
VII. Chalcones and Aurones
VIII. Flavanones
IX. Isoflavones

Flavonoids are present in plants as mixtures and it is very rare to find only a single flavonoid component in a plant tissue. In addition, there are often mixtures of different flavonoid classes (Harbourne, 1973).

2.6.8 Anthocyanins

The anthocyanins are the most important and widespread group of colouring matters in plants. These intensely coloured water-soluble pigments are responsible for nearly all the pink, scarlet, red, mauve, violet and blue colours in the petals, leaves and fruits of higher plants. The anthocyanins are all based chemically on a single aromatic structure, that of cyanidin, and all are derived from this pigment by addition or subtraction of hydroxyl groups or by methylation or by glycosylation (Harborne, 1973).

2.7 Phytochemicals reported for Ficus species

The Ficus *sp*ethanol and petroleum ether crude extracts were subjected to phytochemical screening and phytochemicals such as carbohydrate, alkaloids,phenol,flavanoids, saponins, phytosterols, diterpenes, protein and tanninswere presentas reported by Devi *et al.*(2015).Gaire *et al.*(2011) reported the presence of alkaloids, carbohydrates, saponins, phenols, proteins and amino acid in the n-hexane, chloroform and methanol extracts of *Ficus auriculata* (Lour.) stem bark. Flavonoids, diterpenes, resin, phytoterols, were present in only the chloroform and methanol extracts. And tannins were present in the n-hexane and methanol extracts.The hot (soxhlet) and cold extracts of *Ficus racemosa* stem bark showed toxicity effect (Kambli *et al.*, 2014).The preliminary phytochemical screening of *F. sycomorus* sterm bark showed the presence of flavonoid, tannins, saponins, in the aqueous stem bark, n- hexane, ethylacetate, chloroform and methanol extracts. Alkaloid was present in theall extracts except n-hexane, reducing sugar was present in all extracts except ethylacetate and n-hexane extracts, tannin is present in all extracts except chloroform extract, cardiac glycoside is present in only chloroform and the n-hexane extracts and resins present in only the ethylacetate extract (Bello

et al,. 2013). The methanol extract of *F. pandurata Hance* leaves was subjected to phytochemical screening. The result showed the presence of carbohydrates, glycosides, cardenolides, unsaturated steroids/ triterpenes, tannins, flavonoids, and phenolic compounds. Saponins and coumarins were present in trace amount and alkaloids/ basic nitrogenous substances and anthraquinonesare absent (Amgad*et al.,* 2015).Kevwe*et al,* (2018) reported in a reviewed, the presence of tannins, terpenoids, alkaloids, flavonoids, cardiac glycosides and reducing sugars, with steroids and anthraquinones absent in the water extract of the leaves and bark of *Ficus capensis*. Saponin was present in the bark but absent in the leaves. Terpenoids, flavonoids, steroids, cardiac glycosides and reducing sugars were present in ethanol extract of the leaves and bark, while anthraquinones were absent. Alkaloids were present in the ethanol extracts of the bark but was absent in the leaves.

The phytochemicals present in n-hexane extract of *F. mucoso* leaves revealed the presence of steroids, glycosides, terpenoids, carbohydrates and fat and oil. Ethylacetate extract of *F. mucoso* leaves showed the presence of steroids, flavonoids and glycosides, while methanol extract confirmed the presence of saponins, terpenoids, steroids, flavonoids, alkaloids, glycosides, anthraquinone and tannins (Oguntoye *et al.,* 2016).Banyan (*Ficus benghalensis*) showedthe presence of flavonoids, sapponins and alkaloids in methanol and distilled water extracts also,phenols and tannins were present in the leaf extracts of banyan(Alapati *et al.,* 2015; Sharma*et al.,* 2010).*Ficus glomerata* fruitshowed the presence of phenolic compounds (gallic acid and ellagic acid) which serves as antioxidants and hence exhibits gastroprotective activity. The phenolic agents serve as anti ulcerogenic, antimutagenic and anti cancerogenic compounds (Rao *et al.,* 2008).The leavesof *Ficus hispida* Linn possess antioxidant and antiperoxide activities Thus it can be used in the treatment of liver ailments(Shanmugarajan

and Devaki, 2008). The methanol extract of*Ficus carica* leaveswas reported to have showed the presence of steroids, triterpenoids, their glycosides and coumarins(Krishna *et al.*, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Reagents

The solvents used were all of analytical grade and were distilled at suitable temperatures before use. The solvents were hexane, ethylacetate and methanol.

3.1.2 Equipment

I. NMR spectra were obtained on aBruker ADVANCE spectrometer(400MHz for ^{1}H and 125 MHz for ^{13}C) using (tetramethylsilane) TMS peak as standard.

II. Fouier Tranformed Infra-Red Spectroscopy (FTIR)

III. The melting point of the compound was determined using Ernst Leitz Wetzlar melting point apparatus.

3.1.3 Test organisms

I. *Staphylococcus aureus*

II. *Streptococcus pyogenes*

III. *Escherichia coli*

IV. *Helicobacter pylori*

V. *Candida tropicalis*

VI. *Candida krusei*

VII. Methicillin resistant *Staphylococcusaureus*

VIII. Vancomycin resistant *Enterococci*

IX. *Campylobacter jejuni*

X. *Shigella dysenteriae*

3.1.4 Microbiological media

Mueller Hinton Agar

Sabouraud dextrose agar

Nutrient broth

Sodium chloride (saline)

3.2 Methods

3.2.1 Collection and identification of plant materials

The plant sample, *Ficus natalensis* was collected atArea BZ, Ahmadu Bello University Staff Quarters Zaria. The plant was authenticated by Namadi Sanusi of the Herberium unit, Department of Botany, Ahmadu Bello University Zaria- Nigeria by comparing with an existing specimen with voucher number 021116. The plant was air-dried and pulverized into powder using mortar and pestle.

3.2.2 Extraction

3.2.2.1 Cold Maceration

The powdered plant (550g) was introduced into a Winchester bottle (2.5L) and extracted in the cold for 48 hours with n-hexane (1.5L) then it was filtered and concentrated *in vacuo*at 40˚C. Extraction was continually repeated until the solvent appeared colourless after which itwas

repeated using ethyl acetate and methanol was used as the extraction solvent. Residues after concentration were respectively referred to as n-hexane, ethylacetate and methanol crude extracts. The concentrated extracts were allowed to dry in a fume hood.

3.3.0 Preliminary Phytochemical Screening

The crude extracts were screened for the presence of various chemical constituents such as alkaloids, carbohydrates, proteins, cardiac glycosides, steroids, saponins, flavanoids, terpenoid, tannins, and phenols using standard method described by Sofowora, (1993) and Trease and Evans, (2002).

3.3.1 Test for alkaloids (Wagner's reagent)

A fraction of the extracts was treated with a few drops of Wagner's reagent (iodine (1.27g) and potassium iodide((2g) in 100 ml of water) and observed for the formation of reddish brown precipitate.

3.3.2 Test for carbohydrates (Molisch's test)

Few drops of Molisch reagent (1- naphthol (15g)) was dissolved in or chloroform (100 ml)) were added to a portion of the extracts (2 ml). This was followed by addition of 2 ml of conc. H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 min. Formation of a red or dull violet color at the interphase of the two layers was a positive test.

3.3.4 Test for cardiac glycosides (Keller Kelliani's test)

The extract/fraction, (0.5g) was treated with conc. H_2SO_4 (5 ml) and boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions.

Few drops of ferric chloride solution well added to one of the portions, and a green to black precipitate indicated phenolic aglycone as a result of hydrolysis of glycoside.

3.3.5 Test for steroids (Salkowski test)

To the extract, (2 ml) of chloroform, and concentrated sulphuric acid (2 ml) were added and shaken, red color at lower layer indicated the presence of steroids.

3.3.6 Test for saponins

The extract(0.5g) was shaken with water in a test tube. Frothing was observed which indicates the presence of saponins.

3.3.7 Test for Flavonoids

To the extract (1 ml), a few drops of dilute sodium hydroxide were added. An intense yellow color was produced in the plant extract, which become colorless on addition of a few drops of dilute acid indicates the presence of flavonoids.

3.3.8 Detection of terpenoids

The extract (5ml)each was mixed in chloroform (2ml) and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown colour at the interface indicates the presence of terpenoids (Singh and Saxena, 2011).

3.3.9 Test for phenols and tannins

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration was observed.

3.4.0 Antimicrobial screening

The antimicrobial activities of*Ficus natalensis* extractswere determined using some pathogenic microbes, obtained from the Department of Microbiology, Ahmadu Bello University Teaching Hospital,Zaria (ABUTH). All microbes were screened for purity and maintained in slants of nutrient agar for bacteria and sabouraund dextrose agar for fungi.

3.4.1 Bacterial Susceptibility Testing

Standard inoculums (0.1 ml) 10 ml colony forming unit per milliliter (CFU/ml) was introduced onto the surface of a sterile agar plate and allowed to settle for 20 min. Reservoirs (wells) were made using a sterile cork borer, on the surface of the agar plate and 3 drops of a known concentration of the plant extract was introduced in the wells. A control was set withDMSO (10%)as solvent used in dissolving the crude extract. The plates were incubated at 37°C for fungi; the zone of inhibition was examined after 24 hr for bacteria and for 48 hr for fungi. The zone of inhibition was measured using a pair of dividers in millimeter (mm) and compared with the control(Wayne, 2016).

3.4.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the extracts on the test organisms were determined using the broth dilution method. Prepared Muller Hinton broth (10ml) was dispensed into test tubes and the broth was sterilized at 121 °Cfor 15min, it was then allowed to cool. A 0.5 McFarland's turbidity scale number was prepared to give turbid solution. Normal saline was prepared, and10ml was dissolved into sterile test tube and the test microbe was inoculated and incubated at 37 °C for 6 hr. The dilution of test microbe was done in the normal saline until the turbidity reached that of the McFarland's scale by visual comparism. At this point the test

microbe had a concentration of $1.5x10^8$CFU/ml. Two fold serial dilutions of the extracts in broth were performed to obtain the concentration of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.63mg/ml respectively. The initial concentration was obtained by dissolving 0.1g of the extracts in 100ml of sterile broth. Having obtained the different concentrations of the extract in the broth, 0.1ml of the standard inoculum of the test was made at 37 ℃for 24 hr after which each broth was observed for turbidity. The lowest concentration of the extracts in the broth which showed no turbidity was recorded as the minimum inhibition concentration (Wayne, 2016).

3.4.3 Minimum Bactericidal Concentration/Minimum Fungicidal Concentration (MBC/MFC)

The minimum bactericidal concentration/minimum fungicidal concentrations(MBC/MFC)were carried out to determine if the test microbes were bactericidal or bacteriostatic. Mueller-Hinton agar was prepared and sterilized at 121 ℃ for 15 min, transferred into Petri dishes and allowed to cool and solidify. The content of MIC in the serial dilution was sub-cultured onto the prepared medium and incubation was done at 37 ℃ for 24 hr. Thereafter each plate of the medium was observed for colony growth was recorded as MBC/MFC (Wayne, 2016).

Using the pour plate method, (0.1ml)of the sample was introduced into sterile petri dishes and freshly prepared Sabouraud Dextrose Agar was then poured aseptically into the plates swirled gently and left to set. The plates were then incubated at room temperature (37 ℃)for 3-5 days. The MFC was carried out and results were obtained for both the extract and isolated compound (Anibujuon *et al.,* 2015).

3.5.0 Chromatographic Purification of Extracts

3.5.1 Thin layer chromatography (TLC)

Thin layer chromatography was carried out using precoated silica gel analytical plated to determine the components of the three extracts. The extracts were dissolved in the smallest volume of their extracting solvent, spotted on the TLC plates and allowed to dry. The plates were developed in a chromatographic tank with suitable solvent system that will move the spots for effective separation. The plates were then sprayed with H_2SO_4 (10 %).

3.5.2Visualization of spots

The spots were viewed under UV light (254-366 nm) and by spraying with H_2SO_4(10 %) and also by heating at 110˚ C for few minutes.

3.5.3 Purification and isolation of compounds

A small portion of the ethylacetate fraction (EA) was dissolved in ethylacetate the solution was spotted on TLC plates which were run by specific solvent systems and were viewed individually under UV light and also using vanillin- H_2SO_4 reagent. Following a number of pilot experiments, it was observed that the ethylacetate fraction was separated fairly well by the solvent system of n-hexane and ethylacetate (4:1). The ethylacetate fraction (10g) was then subjected to column chromatography on a silica gel (60-120 mesh) stationary phase with gradient elution using n-hexane: ethylacetate (Srivastave and Srivastave, 1987).

The fractions were found to be homogeneous on TLC plate by using n-hexane: ethylacetate (4:1) solvent system

CHAPTER FOUR

4.0RESULTS

4.1 Phytochemical screening

The preliminary phytochemicals screening of the plant *Ficus natalensis* showed the presence of triterpens in the n-hexane extract but carbohydrate, alkaloid, tannins, saponins, flavonoids, steroids, cardiac glycoside and anthraquinones were abent. The ethylacetate extract had a number of phytochemicals present which are: carbohydrate, alkaloid, saponins, flavonoids, steroids and triterpens while cardiac glycosides and anthraquinones were absent. The methanol extract showed the presence of carbohydrates, tannins, saponins, flavonoid, steroids, triterpens, cardiac glycosides but alkaloids and anthraquinones were absent (Table 4.1).

4.2 Antimicrobial Screening of Extracts

The result of antimicrobial activity and zones of inhibition of the n-hexane, ethylacetateand the methanol extracts showed resistanceand sensitivity by the microbes on the extracts. Methicillin resistant *Staphylococcusaureus,* Vancomycin resistant *Enterococci, Campylobacter jejuni,* and *Shigella dysenteriae*were resistant to the extracts while,*Staphylococcusaureus,Streptococcus pyogenes, Escherichia coli, Helicobacter pylori,Candida krusei*and*Candida tropicalis*weresensitive to the three extractsas seen in Table 4.2 and 4.3 respectively.

Then-hexane, ethyl acetate and methanol extracts showed minimum inhibitory concentration (MIC) measured in mg/mlagainst *Staphylococcus aureus,Streptococcus pyogenes, Escherichia coli, Helicobacter pylori,Candida krusei*and*Candida tropicalis*.From the result of the antimicrobial activity and zone of inhibition the following microbes showed resistance

Methicillin resistant *Staphylococcusaureus*, Vancomycin resistant *enterococci*, *Campylobacter jejuni,* and *Shigella dysenteriae*and as such no MIC was tested on them as seen in Table 4.4 respectively.

The minimum bactericidal/fungicidal concentration (MBC/MFC) for n-hexane, ethyl acetate and methanol extracts was carried out at the following concentrations 0.63, 1.25, 2.5, 5.0, 10.0 and 20.0 mg/ml respectively. The MBC/MFC of the three extracts showed the minimum concentration against the following microbes *Staphylococcus aureus,Streptococcus pyogene, Escherichia coli, Helicobacter pylori,Candida krusei*and*Candida tropicalis*as seen in Table 4.4 respectively.

4.3 Antimicrobial Screening of Isolate

F35 showed activity against Methicillin resistant *Staphylococcusaureus,Staphylococcus aureus, Escherichia coli, Streptobacter pylori,Shigella dysenteriae, Candida krusei* and *Candida tropicalis*respectively. However, *Streptococcus pyogenes* andVancomycin resistant *enterococci*were resistant to F35. This activity was tested in comparison to that of three (3) standard drugs: Ciprofloxacin, Sparfloxacin and Fluconazole as seen in Table 4.5 respectively.

The minimum inhibitory concentration (MIC) of the isolate was observed on the following microbes;Methicillin resistant *Staphylococcusaureus,Staphylococcus aureus, Escherichia coli, Helicobacter pylori,Shigella dysenteriae, Candida krusei*,and *Candida tropicali*s in mg/ml respectively. From the result of the zone of inhibition *Streptococcus pyogenes* andVancomycin resistant *enterococci* were resistant to the isolate and as such were not tested for MIC as recorded in Table 4.6 respectively.

The minimum bactericidal/fungicidal concentration (MBC/MFC) of F35 was observed in mg/ml against Methicillin resistant *Staphylococcusaureus,Staphylococcus aureus, Escherichia coli, Helicobacter pylori,Shigella dysenteriae, Candida krusei,*and *Candida tropicali*s in mg/ml respectively.*Streptococcus pyogenes* and Vancomycin resistant *Enterococci*were not determined for MBC/MFC due to the same reason in MIC as recorded in Table 4.6 respectively.

4.4 FT- IR spectrum of Isolate

The FT- IR spectrum of F35 showed a very broad absorption peak at 3406.8cm^{-1}, 2922.2cm^{-1}, A sharp peak at 2855.1cm^{-1}, an intense but sharp absorption at 1707.1cm^{-1}, at 1401.1cm^{-1}, at 1379.1cm^{-1} and a peak at 1170.4cm^{-1}.

Table 4.1; Phytochemicals Screening of the Extracts of *Ficus natalensis* Crude extracts

Phytochemicals/ Test	Hexane fraction	Ethylacetate Fraction	Methanol Fraction
Carbohydrate	_	+	+
Alkaloids	_	+	_
Tanins	_	_	+
Saponin	_	+	+
Flavonoids	_	+	+
Steriod	_	+	+
Triterpens	+	+	+
Cardiac glycoside	_	_	+
Anthraquinones	_	_	_

Key; - = Negative (absent) and + = Positive (present)

Table 4.2: Antimicrobial Activity of Ficus natalensis Crude Extracts (mg/ml)

Test organism	HE	EA	ME	CIP	SPF	FLU
Methicillin resistant *Staphylococcusaureus*	R	R	R	R	S	R
Vancomycin resistant *enterococci*	R	R	R	S	R	R
Staphylococcus aureus	S	S	S	S	S	R
Streptococcus pyogenes	S	S	S	S	R	R
Escherichia coli	S	S	S	S	S	R
Campylobacter jejuni	R	R	R	S	R	R
Helicobacter pylori	S	S	S	R	S	R
Shigeila dysenteriae	R	R	R	S	S	R
Candida krusei	S	S	S	R	R	S
Candida tropicalis	S	S	S	R	R	S

Key; S= Sensitivity, R= Resistance, HE= n-Hexane Extract, EA= Ethylacetate Extract, CIP= Ciprofloxacin, SPF=Spafloxacin, FLU= Floconazole.

Table 4.3: Zone of Inhibition of the Extracts on Test Organisms in (mm)

Test organism	HE	EA	ME	CIP	SPF	FLU
Methicillin resistant *Staphylococcusaureus*	NA	NA	NA	30	30	NA
Vancomycin resistant *enterococci*	NA	NA	NA	32	0	NA
Staphylococcus aureus	21	25	23	30	32	NA
Streptococcus pyogenes	22	26	24	NA	NA	NA
Escherichia coli	20	28	24	39	35	NA
Campylobacter jejuni	NA	NA	NA	32	NA	NA
Stelicobacter pylori	20	26	22	NA	30	NA
Shigeila dysenteriae	NA	NA	NA	37	37	NA
Candida krusei	18	25	23	NA	NA	35
Candida tropicalis	20	26	22	NA	NA	32

Key; HE= n-Hexane Extract, EA= Ethylacetate Extract, ME= Methanol Extract, CIP= Ciprofloxacin, SPF=Spafloxacin, FLU= Floconazole, NA= No Activity.

Table 4.4 Minimum Inhibitory Concentration/ Minimum Bacterial Concentration (mg/ml)

Test organism	MIC			MBC/MFC		
	HE	EA	ME	HE	EA	ME
Methicillin resistant*Staphylococcusaureus*	-	-	-	-	-	-
Vancomycin resistant *enterococci*	-	-	-	-	-	-
Staphylococcus aureus	5	2.5	5	20	10	10
Streptococcus pyogenes	5	2.5	2.5	20	10	10
Escherichia coli	5	1.25	2.5	20	5	10
Campylobacter jejuni	-	-	-	-	-	-
Stelicobacter pylori	5	2.5	5	20	10	20
Shigeila dysenteriae	-	-	-	-	-	-
Candida krusei	10	2.5	5	20	10	-
Candida tropicalis	5	2.5	5	20	10	-

Key; HE= hexane extract, EA= ethylacetate extract, ME= methanol extract, MIC= Minimum Inhibition Concentration, MBC/MFC= Minimum Bacterial Concentration/Minimum Fungicidal Concentration, - = Not tested

Table 4.5 Zone of Inhibition of the Isolated Compound F35 (mm)

Test organism	F35	CIP	SPF	FLU
Methicillin resistant *Staphylococcusaureus*	26	30	30	NA
Vancomycin resistant *enterococci*	NA	32	NA	NA
Staphylococcus aureus	29	35	32	NA
Streptococcus pyogenes	NA	32	NA	NA
Escherichia coli	28	38	35	NA
Campylobacter jejuni	NA	31	NA	NA
Stelicobacter pylori	27	NA	30	NA
Shigeila dysenteriae	30	38	37	NA
Candida krusei	26	NA	NA	35
Candida tropicalis	27	NA	NA	32

Key; F35= Isolated Compound, CIP= Ciprofloxacin, SPF= Sparfloxacin, FLU= Fluconazole, NA= No Activity.

Table 4.6 Minimun Bactericidal Concentration/ Minimum Fungicidal Concentration (mg/ml) of F35

Test organism	MIC	MBC
Methicillin resistant S*taphylococcusaureus*	25	50
Vancomycin resistant *enterococci*	-	-
Staphylococcus aureus	12.5	25
Streptococcus pyogenes	-	-
Escherichia coli	12.5	50
Campylobacter jejuni	-	-
Stelicobacter pylori	12.5	50
Shigeila dysenteriae	12.5	12.5
Candida krusei	25	50
Candida tropicalis	12.5	50

Key; F35= isolated compound, CIP= ciprofloxacin, SPF= sparfloxacin, FLU= fluconazole, - =Not tested

Table 4.7 Infra Red Spectra data

Serial No	Frequency cm^{-1}	Bond	Functional Group
1	3406.8	O-H	Alcohols
2	2922.2	C-H	Alkanes
3	2855.1	C-H	Alkanes
4	1707.1	C=O	Carbonyl
5	1401.1	C-H	Alkanes
6	1379.1	C-O	Alcohols
7	1170.4	C-C	Alkanes

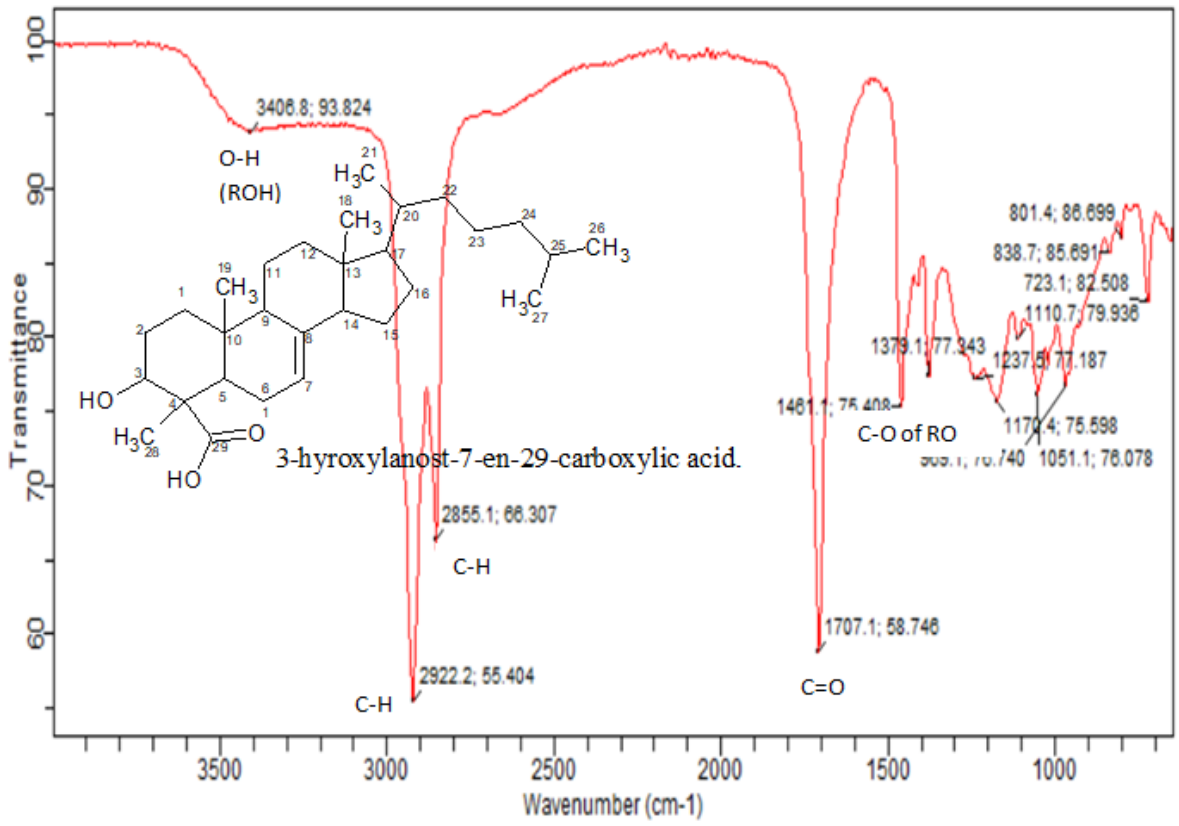


Figure 2.0 FT-IR spectrum of compound F35

4.5 Chemical test on F35

A violet blue color which turned green was observed when Liebermann – Burchard reaction was carried out on F35. Also Salkowski test was carried out on F35, a reddish brown coloration was observed in the upper chloroform layer which is indicative of presence of a steroidal nucleus (Harborne 1973).

4.6 Proton Nuclear Magnetic Resonance (^{1}H-NMR) SpectrumData of F35

The^{1}H-NMRspectrum should 48 proton signals (Figure 3.0). The signals within the range 0-2.0ppm are due to methine (-CH-), methylene (-CH_2-) and methyl (- CH_3-) protons. The signal observed at 3.54ppm is characteristics of an oxymethine proton while the signal at 5-6ppm corresponds to olefinic protons. Therefore these three regions of signals indicate that the spectrum is of a steroidal nucleus.

4.7 Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) spectrum data of F35

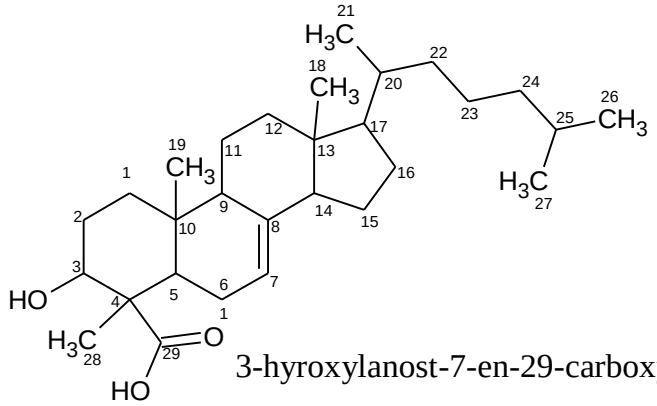
The ^{13}C NMR spectrum (Figure 3.0) showed 29 carbon signals which correspond to the regions of signals of a steroidal nucleus (Chaturvedula and Indra, 2012). The signals from 0–56.89ppm correspond to methine (-CH-), methylene (-CH_2-), and methyl (-CH_3-) carbons. The signal at 72.00ppm corresponds to an oxymethine carbon and the signals at 121.89, 140.78ppm are typical of olefnic carbons (Chaturvedula and Indra, 2012). A sequential position and signals of each carbon in ppm with the type of carbon is recorded in Tables 4.9 and 4.10 respectively.

4.8 Heteronuclear Multiple Bond Correlation (HMBC) Spectrum in F35

The sprectrum showed the following information: position 29 based on HMBC correlation of δH 1.6 ppm (H- 28) and δC 178.08 ppm (C–29) and δH 2.2 ppm (H–5) and δC 178.08 ppm (C–29). Another crucial HMBC correlation was observed between δH 2.2 ppm (H–5) and δC 121.48 ppm (C–7) thus supporting the placement of the olefinic bond.This showed correlation between carbon and proton atleast two to three bonds away. The proton signal at 0.98ppm showed correlation with carbon signals 50.30, 36.31, and 141.06ppm. The proton signal at 0.86ppm (H-28) showed correlation with various carbon signals 22.89 ppm (C-26) and 32.16ppm. Also at 2.32ppm (proton signal) it was observed that there was correlation with carbon signals at 24.87 and 29.31ppm.

4.9 Heteronuclear Single Quantum Correlation (HSQC) in F35

The HSQC spectrum of F35 showed carbon and proton correlation at 34.17ppm and 2.34ppm, carbon signal at 34.24ppm showed correlation with proton signal at 2.32ppm, 34.29and 2.29ppm, at 29.87ppm and 1.23ppm, 19.55ppm and 0.98ppm respectively.



3-hyroxylanost-7-en-29-carboxylic acid.

Table 4.8 Comparion of ^{13}CNMR and ^{1}HNMR spectra data of F35 with literature (Habila *et al.*, 2018)

Carbon Position	CH_n	δc (Reference)	δ_c (ppm) F35	δ_H (ppm) F35
1	CH_2	37.3	37.1	1.31
2	CH_2	36.3	36.1	1.72
				1.48
3	CH	71.8	72.0	3.51
				3.54 (m, 1H)
4	C	45.9	46.0	-
5	CH	42.9	42.8	1.43
6	CH_2	24.3	24.3	2.00
				1.80
7	CH	121.7	121.9	5.35 (t, 1H)
8	C	140.8	140.8	-
9	CH	50.2	50.2	1.99
10	C	39.8	39.9	-
11	CH_2	25.4	25.5	1.42
				1.17
12	CH_2	36.5	36.5	1.81
				1.00
13	C	42.3	42.3	-
14	CH	56.1	56.2	2.15
15	CH_2	24.3	25.0	1.64
				1.39
16	CH_2	28.2	29.4	1.60
				1.29
17	CH	56.8	56.9	1.48
18	CH_3	12.2	12.1	1.08
19	CH_3	18.8	18.8	0.85
20	CH	37.3	37.1	1.64
21	CH_3	19.0	19.1	0.84
22	CH_2	36.5	36.5	1.25
23	CH_2	24.7	24.8	1.25
24	CH_2	40.5	40.2	1.25
25	CH	29.1	29.2	1.62
26	CH_3	22.6	22.5	0.87
27	CH_3	23.1	23.5	0.87
28	CH_3	21.1	19.9	0.89
				0.81 (d, 3H, J=4.0Hz)
29	COOH	178.1	179.8	

KEY; - = Quaternary carbon

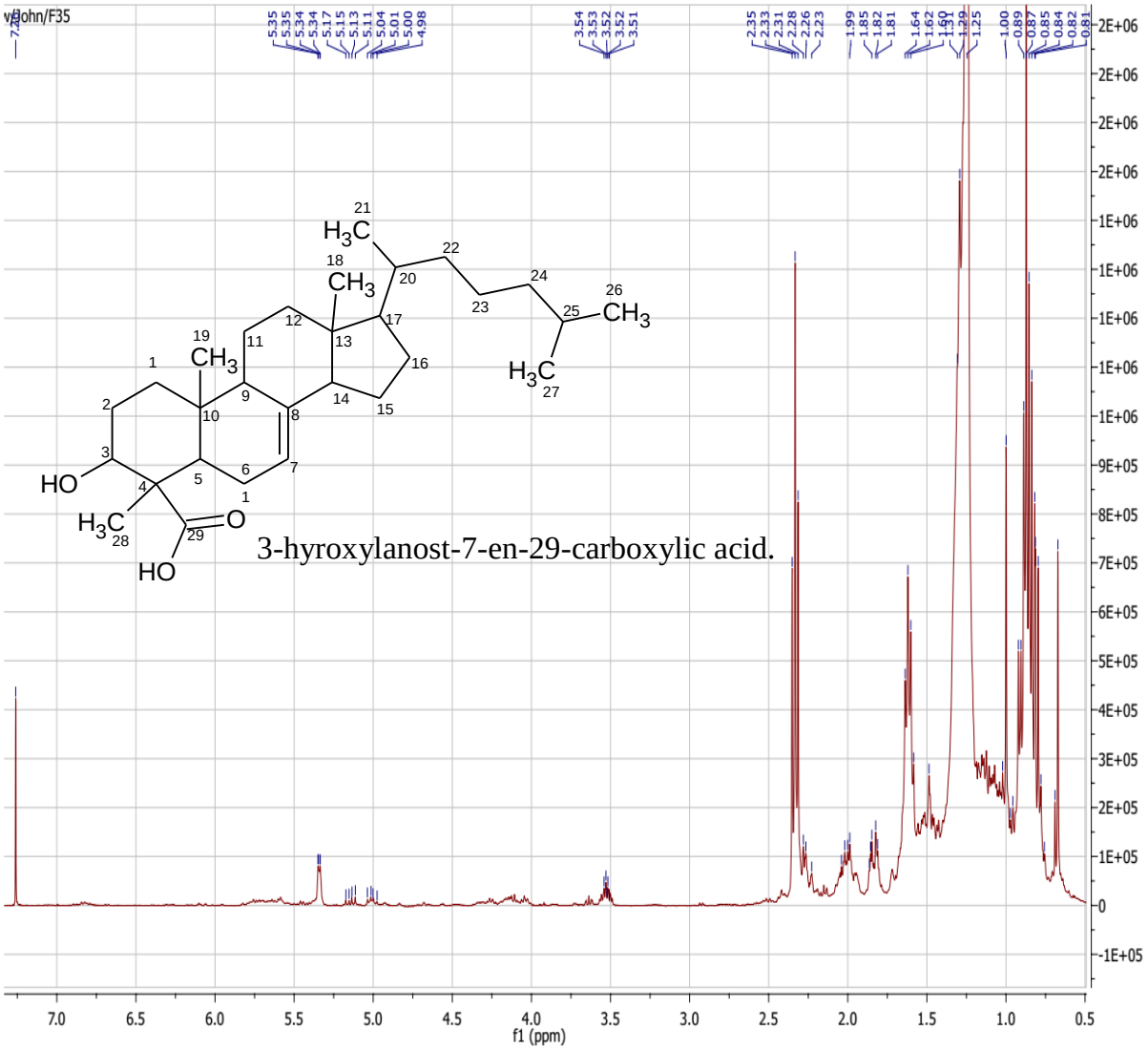


Figure 3.0 ^{1}H-NMR Spectrum of Compound F35

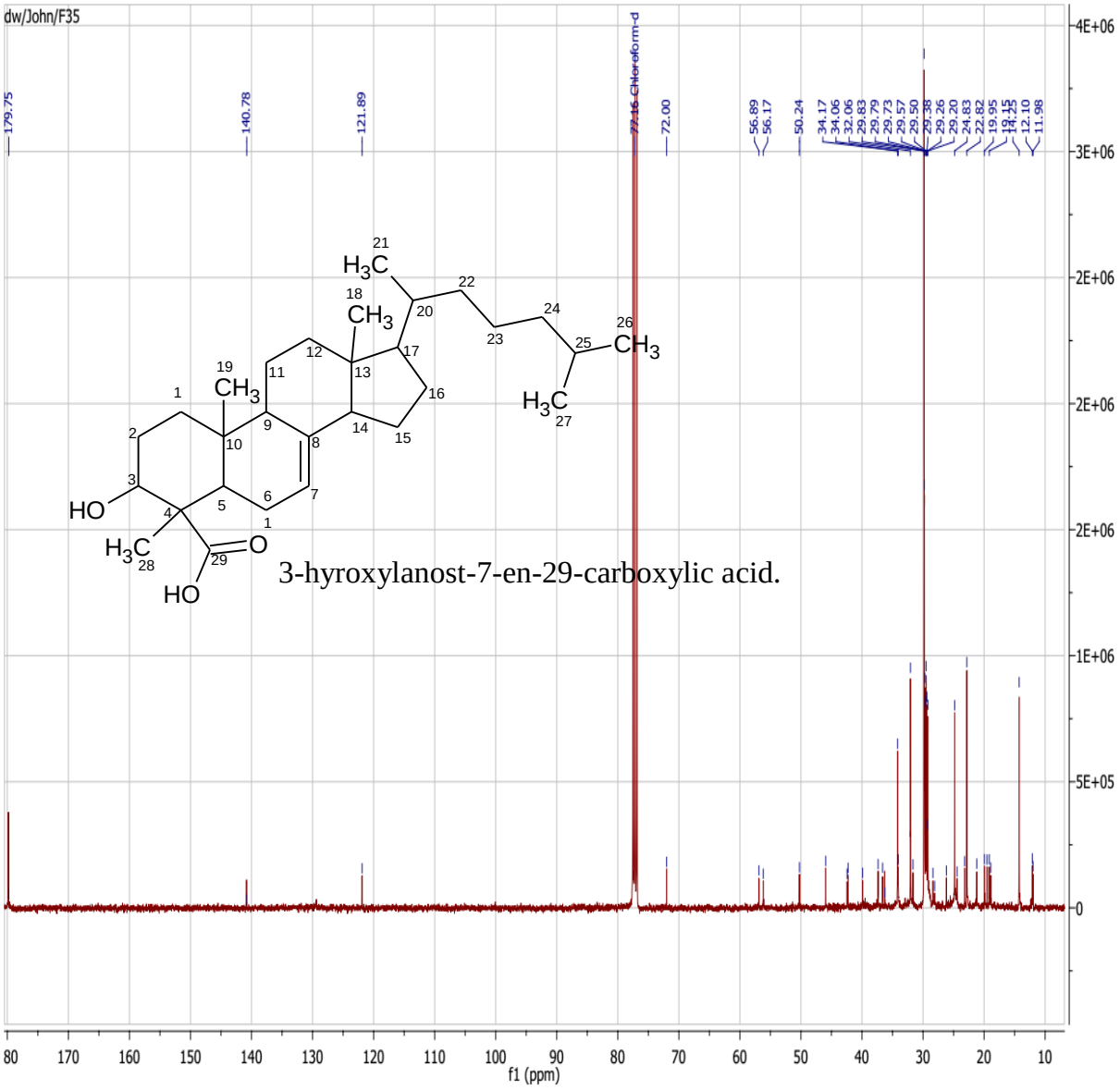


Figure 4.0^{13}C-NMR Spectrum of Compound F35

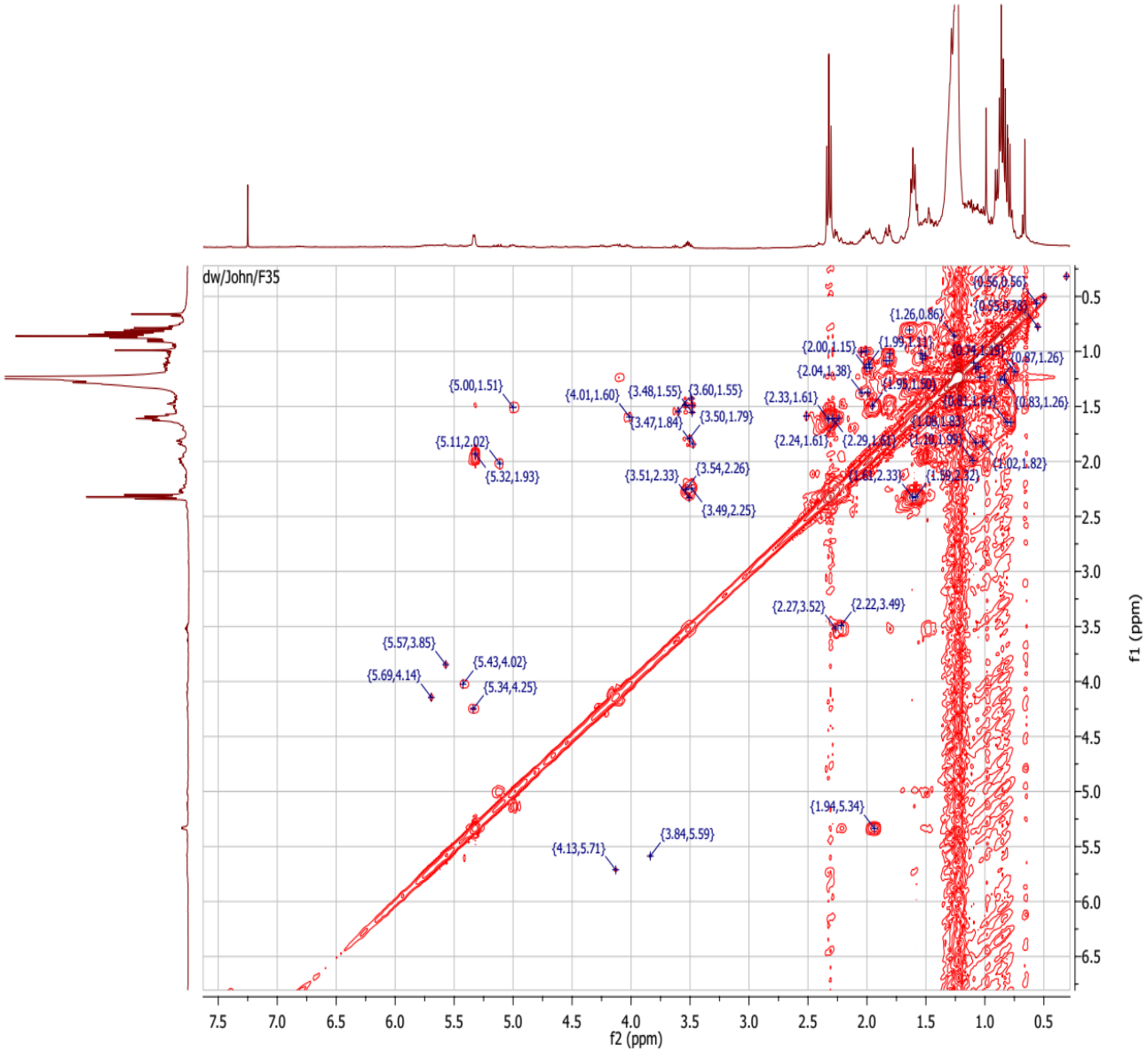


Figure 5.0 NOESY Spectrum of Compound F35

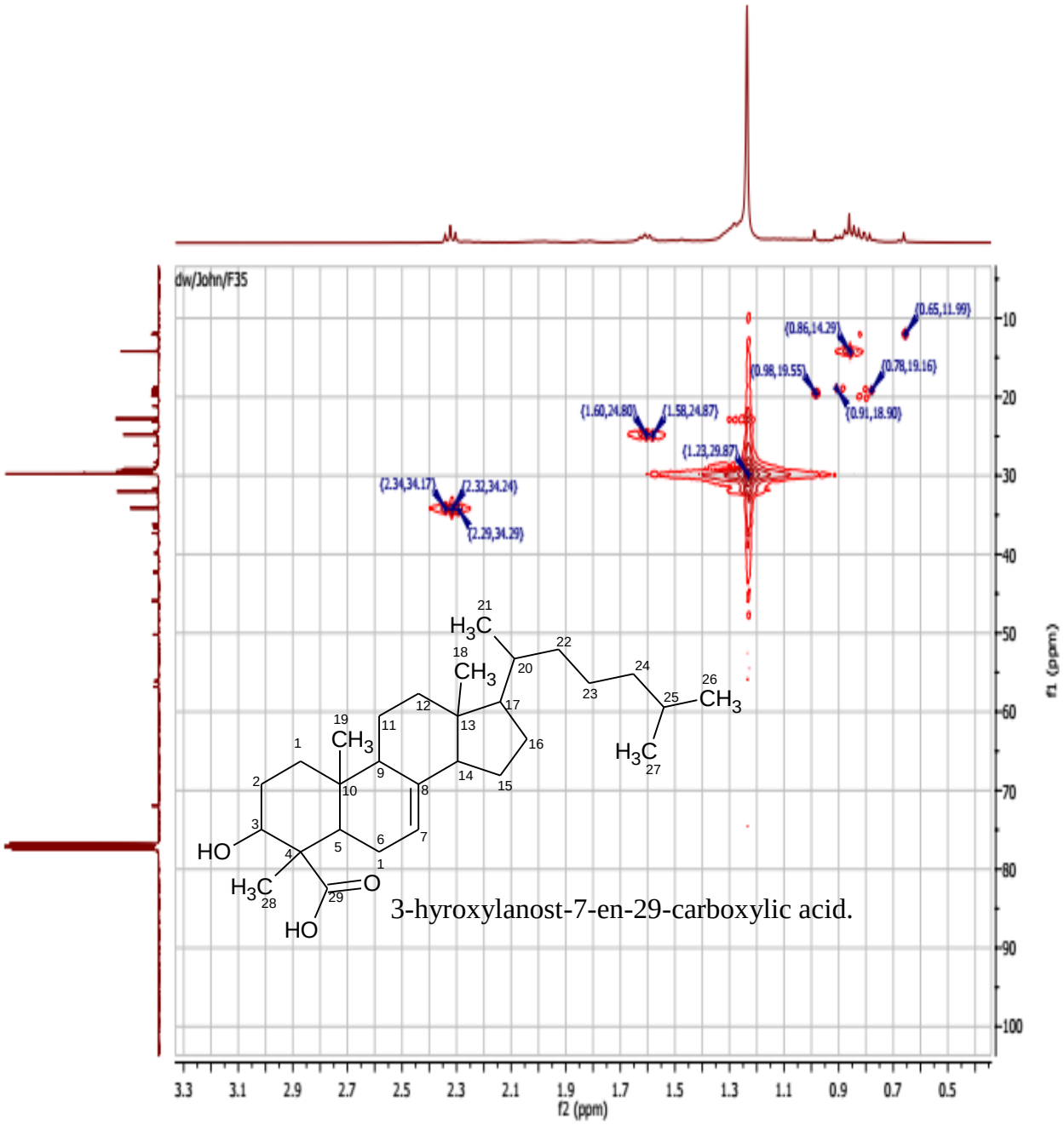


Figure 6.0 HSQC Spectrum of compound F35

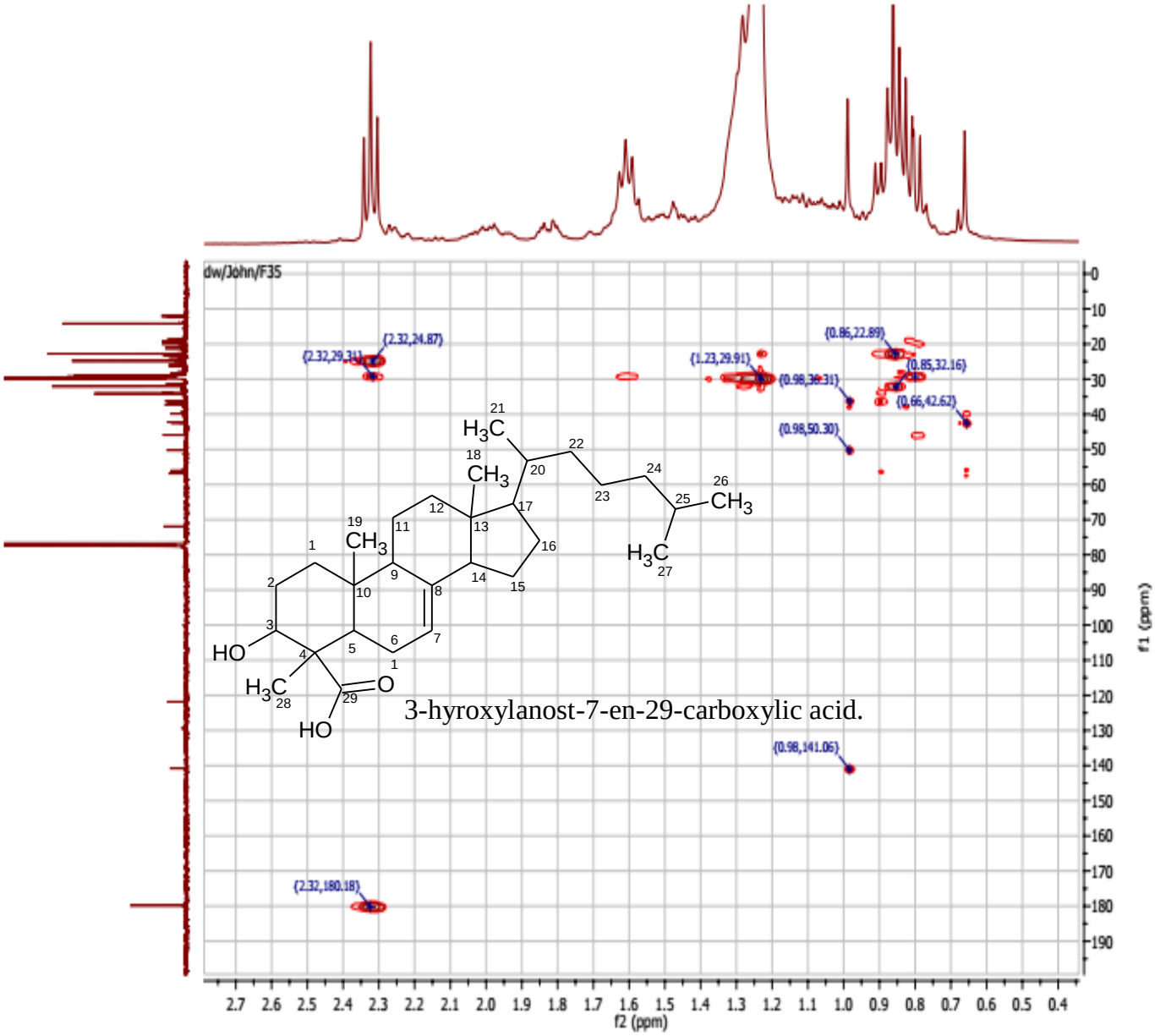


Figure 7.0 HSQC spectrum of Compound F35

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phytochemical Screening of Extracts

Plants have the natural ability to produce or synthesize a wide variety of chemical compounds that are used for important biological activities or functions (Mahalingam*et al.,* 2008) and serve as a defense against several attacks from predators such as herbivorous mammals, insects and fungi (Manoj *et al.,* 2008). Phytochemical screening of *Ficus natalensis* showed positive test for carbohydrates and steroids and negative for alkaloid, tannin, saponin, flavonoid, triterpenes, cardiac glycosids and anthraquinones in the hexane extract. In the ethylacetate extract, the screening showed the presence of carbohydrates, alkaloids, saponins, flavonoids, steroids, and triterpenes while tannins, cardiac glycosides, and anthraquinones were absent. Therefore carbohydrates and steroids are present while cardiac glycosids, tannins and anthraquinones were absent in both the hexane and ethylacetate extracts. Many of these secondary metabolites or phytochemicals have beneficial effects on long-term health when consumed by humans, and can be use to effectively treat human diseases (Manoj *et al.,* 2008).

Several researchers around the world have evaluated somephytochemicals preent inFicus species from which they concluded that, the presence of different compounds such as flavonoids and steroids gives a clue on the activity against microbes (Zaman *et al.*, 2016). The antimicrobial activity of any plant is dependent on the type and nature ofsecondary metabolites that are contained therein. The need for phytochemical screening has become necessary, because many plants contain biologically active principles in their different tissues and parts (Amgad*et al.,* 2015).

5.2 Antimicrobial activity of crude extract

The antimicrobial activity of the n-hexane, ethylacetate and methanol fractions showed activity on some microbes and some of the microorganisms were resistant to the three extracts. Methicillin resistant *Staphylococcusaureus,* Vancomycin resistant *Enterococci, Campylobacter jejuni,* and *Shigella dysenteriea*were resistant to the three extracts while*Escherichia coli, Streptococcus pyogenes,Escherichia coli, Helicobacter pylori, Candida krusei* and*Candida tropicalis* were sensitive to the three extracts. Thezones of inhibition of the microbes in the n-hexane extract were21, 22, 20, 20, 18 and 20mm. While the ethylacetate extract showed higher zones of inhibition than those of n-hexane and methanol extracts having the following values 25, 26, 28, 26,25 and 26mm. The highest effect was seen against *Escherichia coli* with zone of inhibition at 28 mm and the lowest at 25 mm against *Staphylococcus aureus* and *Candida krusei.* The methanol extract showed zone of inhibition at 23, 24, 24, 22, 23 and 22mm, having the highest zone of inhibition to be 24 mm against *Staphylococcus aureus* and *Escherichia coli* respectively.

The minimum inhibitory concentration (MIC) in mg/ml for n-hexane was observed to be 5 mg/ml against *Streptococcus pyrogenes, Staphylococcus aureus, Escherichia coli, Stelicobacter pyleri,* and *Candida tropicalis* the highest at 10 mg/ml for *Candida krusei*. For ethylacetate extract, the MIC was observed for *Streptococcus pyogenes, Staphylococcus aureus, Stelicobacter pylori, Candida krusei,* and *Candida tropicalis* to be 2.5 mg/ml and 1.25 mg/ml for *Escherichia coli*. The methanol extract showed MIC at 5 mg/ml for *Streptococcus pyrogene, Helicobacter pylori, Candida krusei,* and *Candida tropicalis*. The MIC of 2.5 mg/ml for *Staphylococcus aureus*and *Escherichia coli*in the methanol extractrespectively.

The minimum bactericidal/fungicidal concentration (MBC/MFC) for n-hexane was observed to be 20 mg/ml for *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Helicobacter pylori, Candida krusei,* and *Candida tropicalis,* while the ethylacetate extract showed MIC to be at 10 mg/ml for *Streptococcus pyogenes, Staphylococcus aureus, Helicobacter pylori, Candida krusei,* and *Candida tropicalis,* 5 mg/ml for *Escherichia coli* while, the methanol extract had MIC of 10 mg/ml for *Streptococcus progene, Staphylococcus aureus* and *Escherichia coli* and 20 mg/ml for *Helicobacter pylori*respectively.

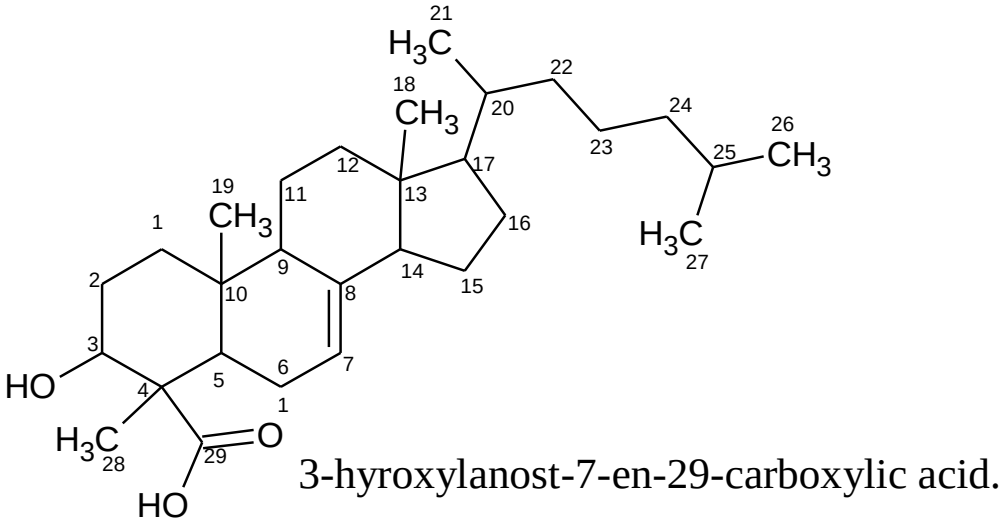
The n-hexane, ethyl acetate and methanol fractions of *F. natalensis* showed significant activities against these selected microorganisms. This result is in line with the result of other researchers that have worked on several Ficus species. They reported that Ficus species have antimicrobial activity, anticancer, antiulcer, antisickle cell,antidiarrheal, antimicrobial inhibitory andimmune-boosting activities (Esievo *et al.*, 2018).

5.3 Isolation and characterization of F35

The compound isolated and purified using standard purification techniqueof column chromatography, thin layer chromatography was a white crystal, which was coded F35. Compound F35 gave a positive test for the salkowski reaction, indicating the presence of a steroidal nucleus.

The broad absorption peak at 3406.8cm^{-1} is typical of a hydroxyl group, the band at 1707.18cm^{-1} istypical of a carbonyl functional group and the peak at 2922.28cm^{-1} is typical of a CH stretch. These regions of the functional groups are typical of steroidal nucleus having a carbonyl. Therefore F35 might be a steroid.

The ^{1}H-NMR spectrum for F35 revealed three regions typical of the steroidal nucleus at 0.5-2.5ppm representing overlapping methyl, methylene and methine protons. And an oxymethine proton signal at 5.22ppm. The ^{13}C NMR spectrum showed a total of 29 carbon signals. The signal between 11.8- 56.7 ppm are typical of the region of overlapping methyl, methylene, and methine carbon atoms, oxymethine carbon signal was seen at 72.0 ppm. The unsaturated carbon signal at 121.7 and 140.8ppm were assigned to a two carbon olefinic system and the signal at 179.8ppm indicated the presence of carboxylic group in the compound. This NMR data is similar to the data reported in literature for 3-hyroxylanost-7-en-29-carboxylic acid (Momoh *et al.,* 2015; Habila *et al.,* 2018). Therefore F35 was assigned to be 3-hyroxylanost-7-en-29-carboxylic acid as shown in the structure below.



3-hyroxylanost-7-en-29-carboxylic acid.

Figure 5.1:Structure of isolated compound F35

5.4 Antimicrobial activity F35

The result of the antimicrobial activity showed that Vancomycin resistant *enterococci,Streptococcus pyogenes*and *Campylobacter jejuni*were resistant to F35 but sensitive onMethicillin resistant *Staphylococcusaureus, Staphylococcus aureus, Escherichia coli,Helicobacter pylori, Shigella dysenteriae, Candida krussei,*and *Candida tropicalis.* It had zones of inhibition of26mm for Methicillin resistant *Staphylococcusaureus,* 29mm for *Staphylococcus aureus,* 28mm for *Escherichia coli,*27mm for*Helicobacter pylori,* 30mm for *Shigella dysenteriae,* 26mm for *Candida krusei*and 27mm for *Candida tropicalis.* Following the same order of microorganism, the minimum inhibitory concentration (MIC) in mg/ml of compound F35 was observed to be at 25, 12.5, 12.5, 12.5, 12.5, 25, and 12.5 mg/ml having the least concentration for *Staphylococcus aureus, Escherichia coli,Helicobacter pylori, Shigella dyenteriae, Candida tropicalis,* and the highest concentration for MIC to be 25 mg/ml against *Candida krusei*. Minimum inhibitory concentration (MIC) is defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth.

The minimum bactericidal/fungicidal concentration of F35 was observed to be 50, 25, 50, 50, 12.5, 50, and 50mg/mL following the same order of organisms as stated earlier. Having MBC/MFC of 50 mg/ml againstMethicillin resistant *Staphylococcusaureus, Escherichia coli, Helicobacter pylori,Candida krusei,* and*Candida tropicalis,*25 mg/ml against*Staphylococcusaureus,* and 12.5 mg/ml against *Shigella dysenteriae*.

The antimicrobial activity results show that 3-hydroxylanost-7-en-29-carboxylic acid can serve as leads for the development of agents that will be effective in treating infections caused by tested bacteriaand fungi this is in agreement with the finding of Habila *et al.* (2018) as they

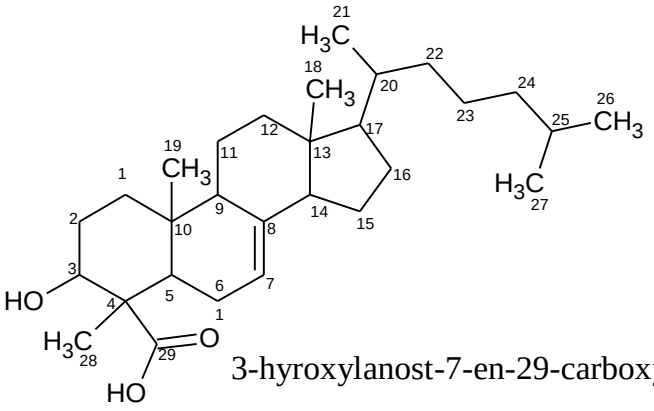
reported, 3-hydroxylanost-7-en-29-carboxylic acid was used to on*Mycobacterium bovis* (BCG) and it inhibited the growth of *Mycobacterium bovis* (BCG) with a minimum inhibitory concentration (MIC) of 250 mg/ml indicating that the compound has potential that can be explored in the search forantituberculosis agents.The compound also exhibitedantimicrobial activity (antibacterial and antifungal) against some microbes tested with the highest zone of inhibition was recorded against *Shigella dysenteriae* and Methicillinresistant *Staphillococus aureus*(MRSA). Other test organisms include:*Staphylococcus aureus,*Vancomycin resistant *enterococci* (VRE), *Bacillus subtillis, Pseudomonas aeruginosa, Proteus rettgeri, Streptococcus feacalis, Enterobacter species, Candida stellatoidea, Candida pseudotropicalis* and *Candida albicans*.The antimicrobial activity of plant can be traced to the presence of secondary metabolites (Amgad*et al.*, 2015).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Ficus natalensis possessed a number of phytochemicals which inferred that the plant is rich in phytochemicals andthe antimicrobial activity of plant can be linked to the presence of secondary metabolites. The plant extract inhibited the growth of some human bacterials; *Escherichia coli, Streptococcus pyogenes,Escherichia coli, Helicobacter pylori, Candida krusei* and*Candida tropicalis*but had no effect on Methicillin resistant *Staphylococcusaureus,* Vancomycin resistant *enterococci,* Campylobacter *jejuni,* and *Shigella dysenteriae.* This shows the plant extract possesses antimicrobial activity and can be used as a source of natural therapy in treating ailment that can be caused by some of the microbes used this experiment. The isolation process gave a compound that was characterized to be a steroid; 3-hyroxylanost-7-en-29-carboxylic acid. This compound also possessed antimicrobial activity on some microbes; Methicillin resistant *Staphylococcusaureus*, *Staphylococcus aureus*, *Escherichia coli,Helicobacter pylori, Shigella dysenteriae, Candida krusei,* and*Candida tropicalis*. Therefore, from the findings of this research, 3-hyroxylanost-7-en-29-carboxylic acid was isolated and characterized from *Ficus natalensis* and can be a window to developing new, side effect free drugs that can be used to combat some notorious ailments which has been a thing of concern.



3-hyroxylanost-7-en-29-carboxylic acid.

6.2 Recommendation

This research has touched certain areas but we believe there is still more areas to cover. Therefore, more solvents can use for extraction which can lead to a qualitative and quantitative study of the phytochemicals present in the plant and it will be of great advantage to the world of research. Also, isolation of more compounds should be focused on and it subjected to biological and pharmaceutical activity studies to explore other resistance strain human pathogens and their efficacy.

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