ANTI- *SALMONELLA* POTENTIAL OF INDIVIDUAL AND COMBINED CRUDE EXTRACTS OF *Cassia occidentalis, Citrus sinensis* AND *Eucalyptus camaldulensis*

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DECLARATION

I hereby declare that this research work entitled "anti-*Salmonella* potential of individual and combined crude extracts of *Cassia occidentalis, Eucalyptus camaldulensis* and *Citrus sinensis*" is the product of my research effort undertaken under the supervision of Dr. Muhammad Yusha'u and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write-up by USMAN ADAMU with registration number SPS/13/MMB/00036 where carried out under my supervision.

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APPROVAL

This dissertation has been examined and approved for the award of Master of Science in Microbiology (medical).

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DEDICATION

This work is dedicated to all those that believe in the potentials of natural products found in herbal medicine, with the hope that this work will spark a change for greatness and fulfillment in their endeavors.

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ABSTRACT

Cassia occidentalis, Eucalyptus camaldulensis and *Citrus sinensis* were extracted separately and successively with ethanol, water and methanol using soxhlet apparatus. The extracts were tested *in vitro* for activity against clinical isolates of *Salmonella typhi and Salmonella paratyphi* using agar well diffusion and broth dilution methods. The zones of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The *in vitro* antimicrobial screening revealed that the extracts exhibited varying activities against the different isolates with zones of inhibition ranging from 7mm-25mm, MIC ranging from 62.5µg/ml - 125µg/ml for *C. occidentalis*, 62.5µg/ml - 125µg/ml for *E. camaldulensis,* 125µg/ml for *C. occidentalis* root,62.5µg/ml for the combined extracts and MBC of 125µg/ml -500µg/ml for *C. occidentalis* leave, 125µg/ml -500µg/ml for *E. camaldulensis,* 250µg/ml -500µg/ml for *C. occidentalis* root and 62.5µg/ml -500µg/ml for combined extract for the sensitive organisms at the tested concentrations. The highest activity was observed in *Cassia occidentalis* and *Eucalyptus camaldulensis* leaves extracts with MIC of 62.5 µg/ml and MBC of 125µg/ml against *Salmonella typhi, S. paratyphi A* and *S. paratyphi B*. The activities observed were due to the presence of the secondary metabolites like, alkaloids, anthraquinones, sterols, glycosides, saponins, terpenes and flavonoids detected in the plant. The gas chromatography mass spectrometry revealed the identity of compounds when matched with NIST library which is in line with the result of the thin layer chromatography that revealed the presence of multiple compounds in plant extracts. The toxicity study carried out revealed that the highest value for LD_{50} of 1308.872 which shows non toxic property was in *Eucalyptus camaldulensis* leaves aqueous extract while the lowest was LD_{50} of 3.085 which shows high toxic property was observed in *C. occidentalis* root methanolic extract against hatched brine shrimps. All extracts shows activity against the test organism in accordance with extract concentration with the exception of the *Citrus sinensis* extract which shows inactivity against the test organism.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Approximately 80% of the world's population relies on traditional medicines for the treatment of common illnesses. Presently, there exist a wide range of antimicrobial drugs derived from microbial and synthetic sources available for the treatment of infectious diseases, at least for those in developed countries and for urban elites of developing countries. In resource poor communities, ignorance of good hygienic practices, poverty coupled with high cost of synthetic drugs and the circulation of drugs of questionable qualities and counterfeit pharmaceuticals combine to worsen the plight of the less privileged, forcing many to seek for the medicines of their ancestors. Herbs have been used as source of food and medicinal purposes for centuries and this knowledge has been passed on from generation to generation. (Adedepo *et al.*, 2005). Even today, significant proportion of the populace, particularly in the developing world depends on herbal medicines. This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few. In Nigeria like other part of the African continent, practitioners of traditional system of medicines are still being consulted as a first choice before seeking for orthodox health care facility. This is particularly due to the fact that traditional medicine blends perfectly into the socio-cultural life of the people, and easily available at minimal cost.

Cassia occidentalis Linn*,* usually grows by the roadside in the northern part of Nigeria which is known as, *Akidi agbara (Igbo,) Abo rere (Yoruba,) mazam fari* or *rai-rai i*n Hausa and Coffee Senna in English. The plant belongs to *Caesalpiniaceae* family. The roots, leaves and seeds are the parts of the plant used. The root is believed to have depurative properties. Infusion or

decoction is taking in Gabon to cleanse the blood and also used to clean the body after parturition for Trinidad citizens (Burkill, 1995).

The roots are considered as adiuretic, a tonic, dysmenorrhea (menstrual problem),tuberculosis, anaemia, liver complaints and for fever. The leaf-sap is used in eye troubles in young and old as well as a febrifuge and laxative in The Gambia and Ijo area of Nigeria (Burkill 1995). The leaf is recognized as anti- neuralgic, purgative (in treatment of diarrhea and dysentery) and vermifuge. In Yoruba land, the preparation with palm oil is used to cure convulsion in children. It is an erect herb, commonly found by road sides, ditches and waste dumping sites. *Cassia occidentalis* has been widely used as traditional medicine. Entire parts of the plant have medicinal values (Mohammed *et al.,* 2012).

Citrus sinensis belongs to *Rutaceae* family and it is commonly known as sweet orange. It is the most commonly grown tree fruit in the world (Miami and Morton 1987). The sweet orange is an evergreen flowering tree generally growing to 9–10m in height. Its fruit is strengthening, cardiotonic, Laxative, anthelmintic and removes fatigue (Kirtikar and Basu 1984). It possesses anti inflammatory, antibacterial and antioxidant properties (Ramachandras *et al.,* 2002). Its leaves are shiny and leathery, arranged alternatively.

E. camaldulensis is a relatively large riparian tree, commonly growing to 20 m in height, but rarely exceeding 50 m. In open woodlands it usually has a short, thick bole and a large, spreading crown with heavy branching. In plantations it can have a clear bole up to 20 m with a lightly-branched crown. Those that grow predominantly in northern and southern forms of *E. camaldulensis* are generally recognised: *E. camaldulensis* var. *camaldulensis* refers to the southern form and *E. camaldulensis* var. *obtusa* is the northern form. *E. camaldulensis* var.

camaldulensis has a basal stocking of rough bark for the first 1–2 m of the trunk. Above this, the bark is smooth, creamy to white, pale grey or buff with grey and reddish patches. Leaves are non-glaucous and the flower buds form a beaked cap. *E. camaldulensis* var. *obtusa* has smooth bark to ground level. Bark is white to cream, sometimes with reddish brown patches. Leaves are glaucous (with a white waxy bloom) and flower buds are more rounded in shape. *E. camaldulensis* is generally fast growing. Tree form is variable but is typically poor in southern Australia. *Eucalyptus camaldulensis Dehnh* Linn.is one of such medicinal plants belonging to the family *Myrtaceae* which is frequently seen occupying open waste spaces and grasslands, road sides, along river banks and wetlands. Of the more than 700 species that comprise this genus, most are native of Australia, though they are also widely cultivated throughout the tropics, especially in Asia and Central America as well as Africa (Jacobs, 1955; Stone and Bacon, 1994; Brooker *et al.,* 2002).

1.2 Justification

Casssia occidentalis, Citrus sinensis and *Eucalyptus cameldulensis* plants have been extensively used in indigenous and folk-lore medicine system. Combinations of these plants or single (individual) plants have been used in the treatment of convulsion, cough, constipation, anti-malaria and other ailments.

This work intends to explore the activity of these plants extract against bacterial agents of typhoid fever (*Salmonella typhi* and *Salmonella paratyphi*). Though it is known in the treatment of earlier stated, the recent use of these plants in the treatment of typhoid fever by the people is the prime motivator of this research work.

1.3 AimandObjectives

The aim of this study is to evaluate the in vitro antibacterial properties of crude extracts of*Cassia occidentalis* (leaves and root), *Citrus sinensis* and *Eucalyptus camaldulensis* against*Salmonella typhi* and *Salmonella paratyphi* from clinical isolates. The specific objectives include;

1. To extract and determine the presence of phytochemicals in the plant.
2. To determine the anti-*salmonella* potentials of the extracts.
3. To determine the acute cytotoxicity of the various extracts.

1.4 Hypothesis

1. The plant extracts have no activity against clinical isolates of *Salmonella.*
2. The anti-*Salmonella* activity of the plants extracts did not differ significantly with the standard used.
3. There is no significant difference in the anti-*Salmonella* activity of the different extracts used.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Cassia occidentalis*

Cassia species has been well known for laxative and purgative properties and for the treatment of skin diseases in traditional medicine (Dalziel, 1956). *Cassia occidentalis* Linn.has been used as a folklore medicine for hepatotoxicity treatment (Sheebarini *et al*., 2010). There is now an increasing body of scientific evidence demonstrating that the plant possess many other beneficial properties.

Phytochemical screening of the plant showed the presence of carbohydrates, saponins, sterols, flavonoids, resins, alkaloids, terpenes, anthraquinones, glycoside and balsam. Presence of these metabolites strongly concluded the great potential of the plant as a source of phytomedicines. As the flavonoids and resins are present, it might be responsible for its anti-inflammatory properties. Chinese folkloric medicine contains flavonoids which has anti-inflammatory effect on both acute and chronic inflammation (Kunle *et al.,* 2009 and Sadique *et al.,* 1987). Alkaloids are known for decreasing blood pressure, balancing the nervous system in case of mental illness and antimalarial properties (Ronan *et al,*. 2009). Tannins help in wound healing and anti-parasitic. Presence of terpenes suggests possessing anti-tumor and anti-viral properties.

Eudesmane sesquiterpenes have been reported to contain antibacterial properties. Saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties. The anthraquinones, emodin and chrysophanone have been reported to possess wound healing properties.

Pharmacognistic analysis of the plant showed 10% moisture thus less sensitive for microbial attack and 7.4% total ash value indicates the low amount of inorganic substance. It contained 5.3% of acid insoluble ash value suggested that the soluble inorganic component is small. The alcohol and water extractive values were 7.7% and 15.1% respectively showed that water is a better solvent of bulk extraction than alcohol.

A study was carried on *Cassia occidentalis* antimicrobial properties (Vedpriya *et al,*. 2010). Test was conducted with four different extracts such as methanol, aqueous, benzene, petroleum ether and chloroform extract. Among which methanol extract showed positive against *P. aeruginosa, K. pneumoniae, P. mirabilis, E. coli, S. aureus* and *S. epidermidis*; aqueous extract was effective against *P. vulgaris, K. pneumoniae* and *P. aeruginosa*; benzene and petroleum ether extracts was active against *P. mirabilis* and *E. coli;* chloroform extract was found to be very inactive against all tested strains. Another study (Sadiq *et al.,* 2012) reported maximum activity against *Salmonella typhi* and minimum with *Shigella spp.* This study concluded that antibacterial activity of *Cassia occidentalis* leaves of ethanol and water extract were increase with higher concentration. A report (Daniyan *et al.,* 2011) with *Cassia occidentalis* flower extract showed maximum inhibition against *Klebsiella pneumonia and* no activity against *Staphylococcus aureus, Streptococcus pneumoniae,* and *Pseudomonas aeruginosa. Thus the* flower extract of *Cassia occidentalis* can be used to treat *Klebsiella* associated ailment such as pneumonia, bronchitis and other diseases known to be caused by *K. pneumoniae.* A report (Saganuwa *et al.,* 1998) states that the *E. coli* was sensitive to methanol, hexane, chloroform and aqueous extract of leaves of *C. occidentalis*. Similarly, Jain and his coworkers (Jain *et al.,* 1998) observed that the metabolite rich fraction of (anthraquinones) leave, pods, flowers and callus were effective against *E. coli*. Yet other study showed that the petroleum ether and ethanolic extract of leaves of

C. occidentalis was active against *E. coli*. With Chloroform and aqueous extract the inhibition was not observed against *E. coli*. Based on these experiments we can clearly say that changes in the activities of plant extracts might be due to spatial and temporal variations. *P.aeruginosa* showing multidrug resistance is highly challenging to treat by conventional antibiotics. A study (Mohammed *et al*., 2012) tested the efficiency of leaf extract of *C. occidentalis* against the growth of *P.aeruginosa* and found that the microbial growth was highly inhibited. And the crude extracts was effective onsome microbes such as *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Candida albicans* which was a common causative agent of urinary tract infection and diarrhea diseases (Basri *et al*., 2005). As this plant has potential antimicrobial activity but invivo studies with the extract should be carried out to confirm that the zone of inhibition is not only by the sensitivity of the microbes also the concentration is highly essential when using for treatment.

The aqueous–ethanolic extract of leaves of *C. occidentalis* was tested for hepatoprotective activity on liver damage in rat which was induced by paracetamol and ethyl alcohol by monitoring serum transaminase, alkaline phosphatase, serum cholesterol, serum total lipids and histopathological alterations. They found that the leaf extract had shown significanthepatoprotective activity (Jafri *et al,*. 1999). Some other observations had found that the seed extracts of *C. occidentalis* limits the DNA degradation caused by iron (II)-driven Fenton reaction. It is notable that inhibition of DNA damage may be due to their capability of strong ferrous ion chelation. Further, they proposed that the scavenging activity towards free radicals might be the reason. *C. occidentalis* is an ingredient in Himoliv, a polyherbal ayurvedic formulation. It is also proved that it prevents the carbon tetra chloride induced hepatotoxicity in rats (Bhattacharyya *et al,.*2003). Based on the observation they suggested that Himoliv increases

the protective enzymes superoxide dismutase (SOD) and catalase in liver homogenate of rats (Kolhapure *et al,.*2004). It is also present in other polyherbal formulation Liv.52 tablet and syrup extensively used for Hepatitis A (HA). For the preparation of this syrup, other plants included *Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Achillea millefolium* and *Tamarix gallica* etc along with *C. occidentalis* are present. A study with 50 clinical samples over 30 years with 4490 patients was performed to identify the efficacy with short and long term safety of Liv.52 in Hepatitis A (Tona *et al,*. 2001). This study concluded that Liv.52 tablets and syrups are potential and safer for hepatitis A.

C. occidentalis plant extract was proved to have effective antimalarial activity (Tona *et al,*. 1999). A study with ethanolic, dichloromethane and lyophilized aqueous extracts of *C. occidentalis* root bark was tested for antimalarial activity against *Plasmodium berghei* ANKA. They tested its toxicity by treating the orally and found that there was no toxic effect or mortality in mice with a single dose, of 500 mg/kg of body weight, or same dose given twice weekly for 4 weeks. The extracts produced significant chemo suppressions of parasitemia with 200 mg/kg dose when administered orally. *C. occidentalis* was found to be potential with 60% chemo suppression. They also found that the ethanolic extract is more active than the lyophilized aqueous extract. *C. occidentalis* leaf extract with ethanol and chloroform was found to possess better antimalarial activity. When tested with 6µg/ml concentration more than 60% inhibition was observed against the parasite.

The larvicidal and pupicidal potential of *Cassia Occidentalis* was analyzed in a study (Abirami *et al,*. 1993) against the larvae of *Anopheles Stephensi.* The ethanol extract of *Cassia Occidentalis* were found to be more effective against larva and pupa respectively. The smoke toxicity study was also conducted and identified that it was more effective against the *Anopheles stephensi*.

Smoke exposed gravid females oviposited fewer eggs when compared to those that were not exposed. Yet another study (Lienard *et al,.*1993) reveal that seed oil creates increase in mortality of *C. maculatus* eggs. Based on numerous trials with pure compounds suggested that fatty acids (linoleic, oleic and stearic) are responsible for *C. occidentalis* toxicity. The oviposition of *C. maculatus* was not reduced by *C. occidentalis* seed oil at 10 ml/kg seed.

The cyclophosphamide (CP) -exposed animals administered with plant extract and showed better humoral responses. The plaque forming cells were found to be more in CP-treated animals after *C. occidentalis* administration. In QHS assay, also *C. occidentalis* showed protection in CP-treated animals. They also found out that the bone marrow cell counts were much higher in plant extract treated animal which were reduced in CP-treated animals. They suggest that modulating the hepatic drug metabolizing enzymes might be the mechanism for hematotoxic and immunotoxic responses of cyclophosphamide.

Cassia occidentalis leaf powder was tested for anti-inflammatory activity and *Cardiospermum halicacabum* aerial parts with ethanol extract was assayed in male albino rats using carrageenan-induced rat paw edema. The efficiency was tested in cotton pellet granuloma assay and observed that the transudative, exudative and proliferative components of chronic inflammation were suppressed by these drugs. Lipid peroxide content and γ-glutamyl transpeptidase and phospholipase A2activity in the exudate of cotton pellet granuloma was lowered with the usage of these drugs. In normalized cotton pellet granulomatous rats, increased alkaline phosphatase activity with decreased A/G ratio of plasma were found after the treatment. *C. occidentalis* powder and *C. halicacabum* extract were able to stabilize the human erythrocyte membrane against hypotonicity-induced lysis. It is likely that these drugs may exert their anti-inflammatory activity by inhibition of phospholipase A2, resulting in the reduced availability of arachidonic

acid, a precursor of prostaglandin biosynthesis, and/or by stabilization of the lysosomal membrane system (Sadique *et al,.* 1987).

Acute toxicity test was conducted in a report with *Cassia occidentalis* and found that this plant did not show any hazardous symptoms or death (Tanimu, 2012). With the sub acute treatment, the *Cassia occidentalis* doesn't change body weight gain, consumption of food and water and the profiles of hematological and biochemical. Also, no changes were seen in macroscopical and microscopical aspect of organs in the animals. Thus they conclude that acute or sub acute administration of *Cassia occidentalis* is not toxic. Histopathological analysis showed no cell death, necrosis or inflammation of the liver and kidney. The leaves of this plant are thus found to be safe with no adverse effect on the liver and kidney functions at the doses administered. Another study had investigated the effects of *Cassia occidentalis* oral administration during pregnancy in female Wistar rats. They found that there was no statistically significant changes between control and test groups with respect to fetuses, placentae and ovaries weights; number of implantation and resorption site number of corpora lutea in the ovaries and pre- and post-implantation loss rates (Avagae *et al,.* 2009).

Around 5% of world's population was affected by anxiety and depression a widespread psychiatric disorder. Previously, plants and formulations were used to treat anxiety and depression over decades. A recent report has studied the antianxiety and antidepressant activity of ethanolic and aqueous extracts of *Cassia occidentalis* leaves in rodents. Exposing the rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer antianxiety activity was tested. Less aversion fear elicits antianxiety activity. Antidepressant activity was analyzed by despair swim test and tail suspension test. Reduced immobility time elicits antidepressant activity. They conclude that ethanolic and aqueous extracts of *Cassia*

occidentalis leaves possess antianxiety and antidepressant activity. Ethanolic extract of *Cassia occidentalis* leaves showing more significant activity over the aqueous extract (Saba *et al,*. 2012).

Cassia occidentalis Linn was screened for analgesic and antipyretic activity (Sini *et al,*.2011). Ethanol and water extracts of *Cassia occidentalis* leaves were screened in mice which was induced by acetic acid and tested for hot plate and tail immersion assay, and also in yeast induced pyrexia method in rats. They found that the ethanol and water extracts of *Cassia occidentalis* possess antinociceptive and antipyretic properties. The report clearly mentioned that both the ethanolic and water extracts of *Cassia occidentalis* showed significant effect on pyrexia induced by yeast.

The aqueous extract of *C. occidentalis* was tested for antidiabetic activity and the study (Laxmi *et al,*. 2010) proved that there was a significant reduction in fasting blood glucose levels in the normal and alloxan-induced diabetic rats. They also tested for other extracts include petroleum ether and chloroform extracts and concluded that activity from day 14 and activity from 7 days respectively. Specific variations were seen in serum lipid profiles (cholesterol and triglyceride), serum protein, and changes in body weight by aqueous extract treated-diabetic animals, when compared with the diabetic control and normal animals. Histopathological studies have also revealed that pancreas of the animals showed regeneration by extract which were necrosed earlier.

2.2 *Citrus sinensis*

Oranges are said to lower cholesterol and aid in the digestion of fatty foods (Cesar *et al.,* 2010). The vitamin C in Oranges is concentrated mainly in the peel and the white layer just under the peel. The peel contains citral, an aldehyde that antagonizes the action of vitamin A. Therefore,

anyone eating quantities of orange peel should make certain that their dietary intake of vitamin A is sufficient (Audery ,1983) .Consumption of fruits such as *Citrus sinensis* is beneficial to health and contributes to decrease of the mortality rate of Cardiovascular and other diseases (Faulks and Southon 2001).This positive influence is attributed to some natural antioxidant phytonutrients (Rice Evans and Miller 1996). The majority of antioxidant capacity of *Citrus sinensis* has been attributed to the presence of vitamin C and flavonoids.

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids, essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material (Di Majo *et al.,* 2005). Increase in fruits and vegetables consumption protects against degenerative pathologies such as cancer and therosclerosis (Keys, 1995); as epidemiological surveys had shown an inverse relationship between dietary flavonoid intake from citrus and cardiovascular diseases (Hertog *et al*., 1993; Di Majo *et al.,* 2005). Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits. Moreover, it is now appreciated that other biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibres are known to be helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity and coronary heart diseases (Crowell, 1999).

The biological activity and the healthy effects of citrus flavonoids as antioxidants have been reported (Tripoli *et al.,* 2007). Also, they are present in dietary fruits and vegetables (Macheix *et al.,* 1990), and exercise their antioxidant activity in several ways,including the activities of metal chelation (Bombardelli and Morazzoni, 1993). Studies indicate that flavonoids are excellent

radical-scavengers of the hydroxyl radical (Cillard and Cillard, 1988; Darmon *et al.,* 1990), due to their ability to inhibit the hydroxyl radical and donate hydrogen atom (Di Majo *et al.,* 2005, Tripoli *et al.,* 2007). Oranges as excellent source of vitamin C, contain powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases (Ejaz *et al.,* 2006). Consumption of foods rich in vitamin C improves body immunity against infectious agents and scavenging harmful, pro-inflammatory free radicals from the blood. Sweet orange contains a variety of phytochemicals like hesperetin and narigenin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator.

Citrus flavonoids contain compounds with anti-inflammatory activity due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxygenase, and cyclooxygenase) that control the formation of the biological mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation. Flavonoid inhibition of the immune and inflammation responses can be associated with their inhibition of these enzymes (Tripoli *et al,.* 2007). Indeed, citrus flavonoids are able to inhibit the kinases and phosphodiesterases essential for cellular signal transduction and activation. They also affect the activation of a number of cells involved in the immune response, including T and B lymphocytes (Manthey *et al.,* 2001). Citrus flavonoids also prevent atherosclerosis, inhibiting the formation of atheroma (Hertog *et al.,* 1993). Tripoli *et al.,* (2007) reported that hesperidin obtained from citrus cultures may have a potential therapeutical use as a mild anti-inflammatory agent, being also useful as a precursor of new flavonoids endowed with this activity (Da Silva *et al.,* 1994). Studies using mouse macrophage cells also show that hesperidin has an inhibitory effect on lipopolysaccharide (LPS)-induced over expression of cyclooxygenase-2, inducible nitric oxide

synthase (iNOS), over-production of prostaglandin E2 and nitric oxide (NO) (Sakata *et al.,* 2003).

Citrus flavonoids can prevent câncer through selective cytotoxicity, antiproliferative actions and apoptosis (Elangovan *et al.,* 1994; Hirano *et al.,* 1994). Flavonoids are antimutagenic, thus protects the DNA from damage by their ability to absorb ultraviolet light (Stapleton and Walbot, 1994). They neutralize free radicals that promote mutations when they are generated near DNA. This hás been shown in mice body irradiated with c-ray (Shimoi *et al.,* 1994). Flavonoids can also protect the DNA by interacting directly with the tumoral agents, as in the induced chromosomal aberrations by bleomycin (Heo *et al.,* 1994). The inhibitory effect of citrus flavonoids on tumoral development and cell proliferation by rat malignant cells, in cardiac and hepatic tissue of syngenetic rats have been reported (Bracke *et al.,* 1989). The ability to function as such by citrus flavonoids are based on cell mobility inhibition (Bracke *et al.,* 1989). Oranges are also rich in iron, chlorine, manganese, zinc, sodium, phosphorous, iodine, calcium, folic acid, potassium, pectin, beta-carotene and amino acids and fibre. A single orange is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, blood clot inhibiting and antioxidant properties. All these properties help to promote overall health (Cha *et al.,* 2001).

Sweet orange contains low calories and no saturated fats or cholesterol, but is rich in dietary fibre, pectin which is very effective in persons with obesity. Pectin as bulk laxative protects the mucous membrane from exposure to toxic substances, as well as by binding to cancer causing chemicals in the colon. Pectin has also been shown to reduce blood cholesterol levels by decreasing its re-absorption in the colon by binding to bile acids in the colon (Walton *et al.* 1945). Orange peels contain the alkaloid synephrine, which reduces the production of cholesterol

in the liver. The antioxidant elements in oranges combat oxidative stress that oxidizes the LDL (low-density lipoprotein) in the blood.

Oranges also contain very good amount of vitamin A, and other flavonoid antioxidants such as alpha and beta carotenes, beta-cryptoxanthin, zeaxanthin and lutein, compounds that have antioxidant properties. Vitamin A is rnecessary for maintaining healthy mucus membranes, skin and essential for vision. It is also a very good source of B-complex vitamins such as thiamin, pyridoxine and folates. These vitamins are essential in the sense that body requires them from external sources to replenish. Orange fruit also contains a very good amount of minerals like potassium and calcium. Potassium in an important component of cell and body fluids helps control heart rate and blood pressure. Vitamin A also required for maintaining healthy mucus membranes and skin and is also essential for vision. Consumption of natural fruits rich in flavonoids helps body to protect from lung and oral cervical cancers. Orange fruit also contains a very good amount of minerals like potassium and calcium. Potassium is an important component of cell and body fluids and helps to control heart rate and blood pressure. The alkaline properties in the orange stimulate the digestive juices, thus, reliving constipation. Regular intake of orange juice reduces the chances in the formation calcium oxalate which causes kidney stones. Polyphenols present in oranges prevents viral infections. Oranges protect the skin from damage caused by free radicals, thereby helping you look young and keeps the skin fresh and glowing (Tsuda *et al.*, 2004).

Oranges can be processed into juice, which can be consumed directly or further processed into concentrate, both used in numerous soda and cocktail drinks, punches, orangeades, and liqueurs (although many orange liqueurs are made from sour, rather than sweet, oranges, or from a combination). Orange fruits and peels are used in numerous desserts, jams and marmalades,

candied peels, as well as cookies, cakes, and candies. Oil derived from orange peels, as well as flowers, leaves, and twigs is used as an essential oil in perfumes; orange seed oil may also be used in cooking or as a component in plastics. The leaf extract of *Citrus sinensi* shows activity against *Pseudomonas aerogenosa, S .aureus* and *K. pneumonae* only they are found to be inactive againstorganism like *S. typhi, S. faecals , S. pyogenes, E. coli, M . catahalis* and *Proteus spp.* (Nada *et al.,* 2014). The peels of *C. sinensis* has remarkable activity against *Proteus spp, S. typhi P. aerogenosa, S. pyogenes* and *K. pneumonae.* Interestly the juice of *C. sinensis* have activity against *S. typhi* and *P. aerogenosa.*(Nada *et al.,* 2014).

2.3 *Eucalyptus camaldulensisSynonym/s*:*Eucalyptus camaldulensis var. camaldulensis, Eucalyptus rostrata*

This plant originated from Australia and its common names include; Murray Red Gum, Red Gum, River Gum, River Red Gum.Their name originates from the Greek word "eucalyptol" which means "well covered". Eucalyptus trees thrive in environments that maintain average temperatures of about 60^0C.

A hardy, fast growing gum that is tolerant of salinity, water logging, drought and frost, with a range of amenity and wood uses. As the eucalypt with the widest natural distribution, provenance variation for many traits is large, so selection of stock is important when planting. It is grown extensively, so much of its silvicultural and pest information is known. Due to its naturally spreading crown, close spacing and good management are required to develop a desirable form for timber production. The wood is hard, heavy and durable; care is needed in drying, but it is sought after for a range of uses and is prized for use in heavy furniture. It is regarded as excellent firewood. *E. camaldulensis*Leaves contain 0.1–0.4% essential oil, 77% of which is cineol There is some cuminal, phellandrene, aromadendren (or aromadendral), and some valerylaldehyde,

geraniol, cymene, and phellandral . Leaves contain 5–11% tannin. The kino contains 45% kinotannic acid as well as kino red, a glucoside, catechol, and pyrocatechol. Leaves and fruits test positive for flavonoids and sterols. The bark contains 2.5–16% tannin, the wood 2–14%, and the kino 46.2–76.7(Watt and Beyer-Brandwijk 1962)

The medicinal usefulness of the redgum tree has been the subject of numerous studies. Some of the reported phytoconstituents of the tree included essential oils, sterols, alkaloids, glycosides, flavonoids, tannins and phenols.The tree is widely used in traditional medicine to treat a variety of diseased conditions including colds, asthma, coughs, diarrhea and dysentery, hemorrhage, laryngalgia, laryngitis, sore throat, spasm, trachagia and vermifuge (Duke and Wain, 1981). Traditional Aboriginal society in Australia used a wide range of Eucalyptus species in medicines to treat gastrointestinal symptoms, arrest bleeding, open wounds and cuts as well as the drinking of the decoctions for the relief of aches and pains in muscles, joints and even tooth. In some cases, the leaves are burnt and the smokes inhaled to treat fever.

Commonly called “zaity” in Nigeria, the resinous exudates from the trunk is taken orally to cure bladder infections (Lassack and MacCarthy, 2006) and a decoction of the plant is used to treat enteric infections including diarrhea and dysentery, constipations and other stomach problems, asthma, oral thrush, boils, sores, skin and wound infections, asthma, bronchitis, eczema and athletes foot (Bala, 2006; Duke and Ayensu, 1985). There is still little evidence on the antimicrobial properties of the plant under investigation against majority of the economically significant bacteria that cause infections.

Some tropical *E. camaldulensis* leaf oil are rich in 1,8-cineole and they are potential commercial sources of medicinal-grade *Eucalyptus* oil (Doran and Brophy, 1990). *Eucalyptus* spp. essential oils are widely used in medicine, pharmaceutics, cosmetics and food industries. Many species of

the *Eucalyptus* have been used widely in folk medicine for a variety of medicinal applications (Silva *et al*., 2003; Marzoug *et al*., 2011). Moreover, essential oil from *E. camaldulensis* has been reported to have a variety of beneficial efficacies and contains different bioactive ingredients capable to display antibacterial activity (Ghalem and Mohamed, 2008), antifungal activity (Falahati *et al*., 2005), larvicidal activity (Cheng *et al*., 2009), antioxidative and antiradical activities (Siramon and Ohtani, 2007). In addition there are many reports on the cytotoxic effects of essential oils belong to Myrtaceae plants as described by Ashour (2008) and Schnitzler *et al*., (2008). . *Eucalyptus* oil is believed to possess a wide variety of healing properties. It works very effectively as an antibiotic that is particularly successful against some strains of bacteria. The oil also possesses anti-inflammatory properties. It can help stimulate the flow of blood and works to ease muscle and joint pain. *Eucalyptus* oil also acts as an antiseptic and works well in treating sore throats, mouth sores, gum disease and gingivitis. The essential oil from the leaves is used as a disinfectant and in medicinal applications. Although *Eucalyptus* oil has been used orally to treat some conditions, the oil is toxic when taken by mouth and must be diluted. The oil was used in traditional aboriginal medicines to heal wounds and fungal infections. Teas made of *Eucalyptus* leaves were also used to reduce fevers. *Eucalyptus* is used in many medicines to treat coughs and the common cold. It can be found in many lozenges, cough syrups, rubs, and vapor baths throughout the United States and Europe. Herbalists often recommend using fresh leaves in teas and gargles to soothe sore throats and treat bronchitis and sinusitis. Ointments containing eucalyptus are also applied to the nose and chest to relieve congestion. Cancer diseases have been treated with a number of bioactive agents mostly being chemicals, but the naturally occuring and derived anticancer agents have increased recently.

Since these plant-derived agents have shown lesser adverse effects than synthetic drugs (Kinghorn *et al*., 2003; Newman and Cragg, 2007).

E. camaldulensis have shown high antibacterial activities against organisms like, *S. typhi, S. aureus , B. mirabilis* etc.

CHAPTERTHREE

3.0 MATERIALS AND METHODS

3.1 Ethanobotanical Survey

The information on the uses and practices of using *Cassia occidentalis, Citrus sinensis* and *Eucalyptus camaldulensis* by traditional herbalist to cure ailments was gathered through structured questionnaire (appendix 11) by means of in-depth interview with the local herbalist around Hadejia and Kafin-Hausa Local Government areas of Jigawa State, Nigeria who claimed to have effective medications for common infectious diseases.

3.2 Collection, identification and authentication of Plant Material

The plants were collected in the month of April 2015 in different locations of Hadejia Jigawa state Nigeria. It was identified and authenticated at the Herbarium of the Department of Plant Biology, Bayero University, Kano where a voucher specimen was deposited at the herbarium of the Department. The whole plants were rinsed with clean water and air-dried for six days under shade, and then pulverized and homogenized using a mechanical grinder. The pulverized plant was kept in an air-tight cellophane bag until used.

3.3 Extraction of the Crude Extracts

The powdered samples of the plants were extracted following the method of Gupta *et al*. (2009). One hundred grams (100g) each of the dried powder of the leaves of the plant were weighed into 3 different glass containers and sequentially extracted with 500ml each of methanol, ethanol and distilled water by percolation method for three days during which the sealed bottles were undergoing vigorous shaking at regular intervals. The mixtures thus obtained were filtered through Whattman's filter paper No. 1. The filtrates were concentrated by complete evaporation

of solvent using rotary evaporator at room temperature to yield the crude extracts with the exception of the aqueous extract, which was evaporated on the water bath at 45°C. The extracts were subsequently transferred into clean sterile airtight glass containers, weighed and stored in the refrigerator at 4°C until use.

The percentage yield of each extract was calculated from the respective weights of the extracts using the formula below:

$$\text{Percentage yield} = \frac{\textit{weight of the extract}}{\textit{Total weight of sample extracted}} \times 100\%$$

Other physical parameters such as colour and texture of the extracts were also recorded.

3.4 Preparation of Extract Stock Concentration for Antimicrobial screening

A test stock concentration of 30mg/ml, 60mg/ml, 90mg/ml and 120mg/ml for aqueous, methanol and ethanol extracts were prepared by dissolving 0.3g, 0.6g, 0.9g and 1.2g respectively of each extract in 10mls of distilled water in separate test tubes. The same concentration was made for amoxicillin which serves as the control.

3.5.0 Phytochemical screening

The presence of some basic secondary metabolites in the pulverized plant material was determined using standard methods (Sofowora 2008; Evans 2002).

3.5.1 *Test for steroids and terpenoids*

A small amount of sample was dissolved in 2ml of chloroform taken in a dry test tube. Equal volume of concentrated sulphuric acid was added. The tube was shaken gently. The presence of steroids and terpenoids was indicated by the upper layer of chloroform turning red and lower layer showing yellow green fluorescence (Khandelwal, 2002).

3.5.2 *Test for tannins*

i.) One millilitre (1ml) of freshly prepared 10% KOH was added to 1ml of the extract. A dirty white precipitate indicated the presence of tannins.

ii.) Powdered leaves and root of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl3 were then added. Production of greenish precipitate indicated the presence of tannins (Harborne, 1978).

3.5.3 *Test for flavonoids*

A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange colour (Khandelwal, 2002).

3.5.4 *Test for alkaloids*

A fraction of the extract was treated withconcentrated sulphuric acid and observed for the formation of orange colour (Khandelwal, 2002).

3.5.5 *Test for saponins*

In a test tube, about 5ml of extract was added and a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3minutes. The formation of a honey comb like froth showed the presence of saponins (Khandelwal, 2002).

3.5.6 *Test for glycosides*

Coarsely powdered leaves and root (1 g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for

3 min. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1978).

3.5.7 *Test for anthraquinones (Borntrager's test)*

To 5ml of the extract 10ml of water was added, boiled and allowed to cool. Then 2ml of the solution was shaken with 5 ml of chloroform. The chloroform layer seperated and concentrated to about 2ml. 2-3ml ammonia solution was then added. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone (Ogbonnia *et al.*,2008).

3.5.8 *Test for reducing sugars*

To 2ml of Fehling's reagent (copper sulphate/sodium potassium tratrate in water) in an empty test tube, three drops of extract was added and boiled in a water bath at 60ºC. Green suspension brick-red precipitate indicates a reducing sugar (Ogbonnia *et al.,*2008).

3.6 Antimicrobial Screening

3.6.1 Organism Source

The clinical isolates were obtained from the Department of Medical Microbiology Aminu Kano Teaching Hospital (AKTH) and Department of medical Microbiology, Rasheed Shakoni specialist Hospital Dutse, Nigeria.The test organisms were characterized using the methods of Cheesebrough, (2002) by observing their cultural growth characteristics each. Biochemical confirmatory tests were performed to further confirm the identity of each of the test organisms All the organisms were checked for purity and maintained at 4°C in slants of nutrient agar.

3.6.2 Preparation of the Inoculum

A loopful of the test organism was taken from their respective agar slants and sub-cultured into test tubes containing nutrient broth for the test-tubes were incubated for 24hrs at 37°C. The obtained microorganisms in the broth were standardized using normal saline to obtain a

population density, equivalent to a 0.5 McFarland standard. Approximately 99.5ml of 1% $BaCl_2$ was added to 0.5ml of 1% H_2SO_4 in order to obtain 100ml of $BaSO_4$ which corresponded to 0.5 McFarland's turbidity standard equivalent to 1.0 X 10^8 cfu/ml population for bacterial isolates.

3.6.3 Preparation of Media

The medium was prepared according tomanufacturer's instruction (AVONCHEM limited, Wellington House waterloo, west Macclesfield Cheshire, England). Fourty grams (40g) of BloodAgar and 28g of nutrient Agar were weighed into a conical flask1000ml of distilled water was added and capped with cotton wool. The media were boiled to dissolutionand then sterilized at 121ºC for 15mins. The mediawere allowed to cool to45ºC and 20ml of thesterilized medium was poured into sterile Petri-dishesand allowed to cool and solidify. The plates werelabeled with the test microorganism (each plate with atest microbe).

3.6.4 Zone of Inhibition - Well Diffusion Method

A standard cork borer of 5mm in diameter was used to cut well. 10µl of the text solution (extract) was then introduced into the well. The plates were incubated at 37ºC for 24hrs, and observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Equal concentration of amoxycilin was used as control.

3.6.5 Minimum Inhibitory Concentration - Broth Dilution Method

MIC of the extracts was also carried out using broth dilution method as described in Ibekwe *et al*, 2001. The nutrient broth were prepared according to the manufacturer's instruction (10ml of each broth was dispensed into separate test-tube and was sterilized at 121ºC for 15mins and then allowed to cool. Two-fold serial dilution of the extract in the broth were made from the stock concentration of the extract to obtain 10, 5, 2.5, 1.25, 0.625mg/ml (1000µl, 500 µl, 250 µl, 125

µl, 62.5µl) 0.1ml of the standardized inocula of the microbes were then inoculated into the different concentrations of the extracts in the broth. The tubes containing the test solution were then incubated at 37^{o}C for 24hrs and observed for turbidity of growth. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

3.6.7 Minimum Bactericidal Concentration Broth Dilution Method

Blood agar was prepared, sterilized at 121^{o}C for 15mins and was poured into sterile Petri-dishes and left to cool and solidify. The contents of the tubes without growth were then sub-cultured onto the blood agar plates and incubated at 37ºC, and observed for colony growth. The MBC was the plate with the lowest concentration of extract and without colony growth.

3.6.8 Determination of activity index

The activity index of the crude plant extract was determined using the relation;

$$\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

3.6.9 Determination of proportion index

The proportion index was determined using;

$$\text{Proportion index (P.I.)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

3.7 Quantitative and qualitative analysis of the extract using GC-MS technique

The different extracts were subjected to quantitative analysis using the GC-MS analyser to quantify the compounds contained in each of the plants extracts and determine the proportion as well as to identify the chemical constituent of the extract. This plant extract were analysed using GC-MS while the Mass spectra of the compounds found in the extract were matched with the National Institute of Standard and Technology (NIST) Library.

3.8.5 Analytical Thin Layer Chromatography

Analytical Thin Layer Chromatography was carried out by developing TLC glass using silica gel which was suspended in distilled water. Plates were made by coating a rectangular glass sheet of 20cm X 10cm sizes to fractionate each extract into its various components using the respective solvent systems. A pencil was used to draw a horizontal line 2cm from the base and 1cm at the other extreme end of each plate. 0.5mg/ml of each extract was prepared in sterile glass vials.

3.8.2 Spotting, development and visualization of plates

The solutions of each extract were spotted closely on the 10cm side of the plate. The plates were allowed to dry for 30mins for the solvents to evaporate before putting them in the TLC tanks containing 200mls of the respective solvent systems and allowed the solvents to move and carry the extract upward along the TLC plates for the separation to take place, after which the plates were dried for 24hours then stained in iodine tank they were then air dried before viewed under UV light. The bands for each plate were marked and their RF values were calculated and recorded.The Rf value is given by following equation:

$$\text{Rf value} = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

3.8.3 Sensitivity disc making

Each band was scraped into a sterile beaker and dissolved in methanol solvent, then filtered with Whatman's filter paper, the filtrate was used to make a "sensitivity disc" by sucking a punched filter paper and allowed to evaporate completely.

3.8.4 Determination bioactive component in each extract.

The prepared sensitivity disc was used to test the antimicrobial activity of each fraction on the test organisms using the normal Kirby Bauer' agar disc diffusion method. The activity was evaluated by the presence or absence of zone of inhibition around each disc.

3.9.0 Brine shrimps lethality assay

3.9.1 Test sample preparation for Brine shrimp bioassay

Test samples were dissolved in DMSO (Dimethyl sulfoxide) to obtain stock solution from which various concentrations of 10, 100, and 1000 µg/ml were made by serial dilution after dissolving 1g of the extract in 100ml of the DMSO. Pure DMSO and artificial seawater were used as negative control.

3.9.2 Hatching of Brine shrimp eggs

Brine shrimps eggs were obtained from Chemistry Department Bayero University Kano. The cysts were hatched in a tank containing artificial seawater made through dissolving a commercial marine salt 38g/L in distilled water (mineral water). The tank was well aerated and the proper light source was also provided. The nauplii were hatched within 24-36 h.

3.9.3 Brine shrimp lethality test

The toxicity of extracts was tested at various concentrations viz. 10, 100, and 1000 µg/ml in seawater. About 0.5 ml of diluted test solution was added to the pre marked test tubes containing 4.5ml of artificial sea water. Finally 10 active shrimps were added into each test tube. A vial containing 50µl DMSO diluted to 5 ml was used as control After 24 h, survivors were counted using a dissection microscope (hand lens) and the percentage of the mortality (%M) of each dose calculated.

3.9.4 Gas Chromatography Mass Spectrometry Analysis (GC-MS)

Extracts of these plants were analyzed using Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library.

3.10 Statistical analysis

Using probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} of less than 100 ppm was considered as potent (active) Gupta *et al.,* 1996). As mentioned by (Meyer *et al.,* 1982), LC_{50} value of less than 100µg/mL is toxic while LC_{50} value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

Standard deviation (SD) to determine the significant difference in activity of the extracts was also determined by One-way Analysis of Variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test. The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance. If the value of q is greater than 5.249 then the P value is less than 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Ethanobotanical information on the practice of using *Cassia occidentalis,Citrus sinensis* and *Eucalyptus camaldulensis*

Root Infusion of (10–20g) is considered beneficial in obstruction of stomach and incipient dropsy. Roots are also used as veterinary medicines for animal diseases, and as antidote in case of poison Roots of *C. occidentalis* were also used against gastric complaints, to increase lactation, in whooping cough etc. In Nigeria, the roots of this plant were boiled with water and taken as tea for constipation and against white vaginal discharge.

Leaves paste is externally applied on healing wounds, sores, itch and cutaneous diseases. Leaves are also used on bone fracture, fever, ringworm, skin diseases, throat infection and wounds. Twigs are used as tooth brushes. Leaves are burnt and the soot obtained is mixed with coconut oil and applied on eye-lids for cooling sleep.

4.2 Physical Characteristics and the percentage yield of the extracts

The physical characteristics and the percentage yield of the aqueous extract, ethanolic extract and methanolic extract are shown in (Table 4.1). During the extraction, the filtrates appeared brownish and greenish in colour depending on the plant, whereas the extracts appeared as deep brown, deep green, brown or light green in colour with soft, crystalline and solid textures. The highest percentage yield of the extract was observed in aqueous extract of *E.camaldulensisi* which was 18.1% of the total sample extracted, followed by the methanolic extract which has a

percentage yield of 14.18% and least in terms of percentage yield was ethanolic extract of *Citrus sinensis* which was 7.2% as shown in (table 4.1).

4.3 Preliminary Qualitative Phytochemical Screening Tests of the various extracts

Phytochemical screening for the bioactive components present in the aqueous extract, ethanolic extract and methanolic extract of *Cassia occidentalis* leaves and root, *Citrus sinensis* leaves, and *Eucalyptus camaldulensis* revealed that the extracts were very rich in secondary metabolites including of alkaloids, saponin, terpenoid, flavonoid, anthraquinone, tannins, glycosides and steroids as shown in (Table 4.2). The methanolic extract has the highest number of phytochemical in all the plants extract followed by the ethanolic extract the least which is the aqueous extract. Tannin was detected in all the plants extracts, while saponins, glycosides and anthraquinines were not detected in all the *Citrus sinensis* extract. Table 4.2 shows the distributions of the bioactive phytochemicals in each of the plants extracts.

4.4 Antimicrobial Activity of the plants extracts against clinical isolates

The antimicrobial activities of the methanolic, ethanolic, and aqueous extract and that of Amoxycillin antibiotic at four different concentrations (120mg/ml, 90mg/ml, 60mg/ml, and 30mg/ml for each extract) against the test organisms are indicated in (Table 4.3-4.7). Combined methanolic extracts produced higher zones of inhibition against all the test organisms, even at the lowest concentration, no resistance was observed. It produced higher zones of inhibition with mean standard error values of 25±0.00mm for *S. typhi*, 25±0.00mm for *S. paratyphi A*, and 25±0.00mm for *S. paratyphi B* at 120mg/ml concentration each. This is followed the ethanolic extract in which also no resistance was observed even at the lowest concentration used. Its higher zones of inhibition were 24±0.00mm for *S. typhi,* 25±0.00mm for *S. paratyphi A* and

25±0.00mm for *S. paratyphi B* at 120mg/ml concentration each. The third in bioactivity is the aqueous extract which produces higher zones of inhibition of 23±0.00mm for *S. typhi* and 21±0.00mm for *S.paratyphi B* and 20±0.00mm *S. paratyphi A* at 120mg/ml concentration each. Extracts of *Citrus sinensis* shows no activity against the test organism at all concentration tested. The susceptibility pattern of all the test organisms against the various extract is generally very high as resistance was only observed at lower concentration 30mg/ml of some of the extract. *Salmonella typhi* appeared the most susceptible organism which showed no resistance to the extracts.

4.5 Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the plants Extracts.

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the extracts of *C. occidentalis* leaves and roo*t, C. sinensis* leavesand *E. camaldulensis* leavesagainst the test organisms are presented in (Table 4.8). The MIC of the combined extracts against all the test isolates had range of values of (6.25 – 125µg/ml). The *Cassia occidentalis* leaves extracts, *Eucalyptus camaldulensis* leaves extracts, *C. occidentalis* root extracts and Amoxycillin, however, had MIC range of (62.5 – 125µg/ml), (62.5 – 125µg/ml), (125 – 250µg/ml) and (62.5 - 125µg/ml) respectively against all the test organisms. *C. occidentalis* root extract has the lowest MIC range *Citrus sinensis* have no activity against the entire test organisms,

Similarly, the minimal bactericidal concentration (MBC), generally do not exceed the minimal inhibitory concentration (MIC) by more than a factor of 2 (Table 4.8).

Table 4.1: Physical characterisrics of the various extracts of *Cassia occidentalis, Citrus sinensis* and *Eucalyptus camaldulensis* leaves.

Extracts	Methanol			Ethanol			Aquous		
	Colour	Texture	% yield (g)	Colour	Texture	% yield (g)	Colour	Texture	% yield (g)
Cassia occidentalis leaves	Deep green	Soft	7.9%	Deep green	Soft	7.7%	Green	Soft	15.1%
Citrus sinensis leaves	Green	Soft & gummy	7.9%	Green	Soft	7.2%	Green	Soft	14.2%
Eucalyptus camaldulensis leaves	Deep brown	Crystalline	23.1%	Deep brown	Crystalline	19.6%	Brown	Crystalline	25.1%
Cassia occidentalis root	Deep brown	Crystalline	12.5%	Brown	Crystalline	10.6%	Brown	Crystalline	9.2%

Table 4.2: Phytochemical constituents of *Cassia occidentalis (*leaves and root), *Eucalyptus camaldulensis,(*leaves) and , *citrus sinensis (* leaves) extract.

S/N		*C. occidentalis* leaves extract			*Citrus. sinensis* leaves extract			*E.camaldulensis* leaves extract			*C. occidentalis* root extract		
		Met	Eth	Aqu	Met	Eth	Aqu	Met	Eth	Aqu	Met	Eth	Aqu
1	Tannins	+	+	+	+	+	+	+	+	+	+	+	+
2	Saponins	+	+	+	-	-	-	+	+	+	+	+	+
3	Alkaloids	+	+	+	+	+	+	-	+	-	-	+	+
4	Flavanoids	+	-	-	+	+	+	-	-	-	+	+	-
5	Glycosides	+	+	+	-	-	-	-	-	+	+	+	+
6	Steroids	+	+	-	+	+	+	+	+	+	+	-	-
7	Terpenoids	+	+	-	+	+	+	+	-	-	+	+	-
8	Anthraquinones	+	-	+	-	-	-	-	-	+	+	+	+

KEYS met =methanol, Eth= ethanol Aqu=aqueous

Table 4.3: Antimicrobial activity of *Cassia occidentalis* leaves extract against the test organisms by agar well diffusion method.

S/N	Test organisms	Mean zone of inhibition in (mm) produced by various concentration of each extract against the test organism																ACTIVITY INDEX		
		Methanolic extract (mg/ml)				Ethanolic extract (mg/ml)				Aqueous extract (mg/ml)				Amoxicillin (mg/ml)						
		120	90	60	30	120	90	60	30	120	90	60	30	120	90	60	30	Met	Eth	Aqu
1	*Salmonella typhi*	20	18	15	10	18	13	12	09	17	15	09	08	20	17	15	10	1.01	0.83	0.79
2	*Salmonella paratyphi A*	23	19	15	11	21	17	15	9	20	18	15	11	21	18	15	13	1.09	1.00	1.03
3	*Salmonella paratyphi B*	2 3	18	12	09	20	18	15	9	23	19	15	11	21	17	15	15	0.91	0.91	1.00

P.I : MET=1, ETH=1, AQU=1

S.D:

MET vs SA p>0.05	ETH vs SA p>0.05	AQU vs SA p>0.05
MET vs ETH p>0.05	ETH vs MET p>0.05	AQU vs MTH p>0.05
MET vs AQU p>0.05	ETH vs AQU p>0.05	AQU vs ETH p>0.05

Table 4.4: Antimicrobial activity of the combined *Cassia occidentalis, Citrus sinensis* and, *Eucalyptus camaldulensis* leaves extracts against the test organisms by agar well diffusion method.

S/N	Test organisms	Mean zone of inhibition in (mm) produced by various concentration of each extract against the test organism																		
		Methanolic extract (mg/ml)				Ethanolic extract (mg/ml)				Aqueous extract (mg/ml)				Amoxicillin (mg/ml)				AC TIVITY INDEX		
		120	90	60	30	120	90	60	30	120	90	60	30	120	90	60	30	Met	Eth	Aqu
1	*Salmonella typhi*	23	19	14	11	22	18	12	8	20	18	15	09	20	17	15	10	1.08	0.96	1.00
2	*Salmonella paratyphi A*	24	18	15	12	24	19	14	8	20	18	15	10	21	18	15	13	1.02	0.97	0.94
3	*Salmonella paratyphi B*	24	19	16	12	24	19	14	8	21	19	15	10	21	17	15	15	1.04	0.91	0.95

P.I: MET=1, ETH=1, AQU=1

S.D: MET vs SA $p<0.05$ ETH vs SA $p<0.05$ AQU vs SA $p<0.05$
MET vs ETH $p<0.05$ ETH vs MET $p<0.05$ AQU vs MTH $p<0.05$
MET vs AQU $p<0.05$ ETH vs AQU $p<0.05$ AQU vs ETH $p<0.05$

Table 4.5: Antimicrobial activities of the various *Cassia occidentalis* root extract against the test organisms by agar well diffusion method.

S/N	Test organism	Mean zone of inhibition in (mm) produced by various concentration of each extract against the test organism																		
		Methanolic extract (mg/ml)				Ethanolic extract (mg/ml)				Aqueous extract (mg/ml)				Amoxicillin (mg/ml)				ACTIVITY INDEX		
		120	90	60	30	120	90	60	30	120	90	60	30	120	90	60	30	Met	Eth	Aqu
1	*Salmonella typhi*	20	17	13	8	18	13	12	9	17	15	9	8	20	17	15	10	0.93	0.83	0.68
2	*Salmonella paratyphi A*	18	14	8	7	17	14	0	0	15	8	0	0	21	18	15	13	0.70	0.34	0.34
3	*Salmonella paratyphi B*	18	15	13	8	17	13	0	0	13	8	0	0	21	17	15	15	0.79	0.29	0.33

P.I: MET=1, ETH=0.67, AQU=0.67

S.D

MET vs SA $p>0.05$
MET vs ETH $p>0.05$
MET vs AQU $p>0.05$

ETH vs SA $p<0.05$
ETH vs MET $p>0.05$
ETH vs AQU $p>0.05$

AQU vs SA $p<0.05$
AQU vs MTH $p>0.05$
AQU vs ETH $p>0.05$

Table 4.6: Antimicrobial activity of the various *Eucalyptus camaldulensis* leaves extract against the test organisms by agar well diffusion method.

S/N	Test organism	Mean zone of inhibition in (mm) produced by various concentration of each extract against the test organism																		
		Methanolic extract (mg/ml)				Ethanolic extract (mg/ml)				Aqueous extract (mg/ml)				Amoxicillin (mg/ml)				ACTIVITY INDEX		
		120	90	60	30	120	90	60	30	120	90	60	30	120	90	60	30	Met	Eth	Aqu
1	*Salmonella typhi*	21	18	13	10	19	15	12	8	17	15	09	0	20	17	15	10	1.00	0.87	0.66
2	*Salmonella paratyphi A*	24	19	15	8	20	17	13	9	18	15	10	0	21	18	15	13	0.98	0.74	0.64
3	*Salmonella paratyphi B*	22	19	13	8	20	18	15	9	19	15	10	0	21	17	15	15	0.91	0.77	0.64

P.I : MET=1, ETH=1, AQU= 0.75.

S.D

MET vs SA $p<0.05$	ETH vs SA $p<0.05$	AQU vs SA $p<0.05$
MET vs ETH $p>0.05$	ETH vs MET $p>0.05$	AQU vs MTH $p<0.05$
MET vs AQU $p<0.05$	ETH vs AQU $p<0.05$	AQU vs ETH $p<0.05$

Table 4.7: Antimicrobial activity of the various *Citrus sinensis* leaves extract against the test organisms by agar well diffusion method.

S/N	Test organism	Mean zone of inhibition in (mm) produced by various concentration of each extract against the test organism																		
		Methanolic extract (mg/ml)				Ethanolic extract (mg/ml)				Aqueous extract (mg/ml)				Amoxicillin (mg/ml)				ACTIVITY INDEX		
		120	90	60	30	120	90	60	30	120	90	60	30	120	90	60	30	Met	Eth	aqu
1	*Salmonella typhi*	0	0	0	0	0	0	0	0	0	0	0	0	23	19	15	15	0.00	0.00	0.00
2	*Salmonella paratyphi A*	0	0	0	0	0	0	0	0	0	0	0	0	21	19	15	13	0.00	0.00	0.00
3	*Salmonella paratyphi B*	0	0	0	0	0	0	0	0	0	0	0	0	21	17	15	15	0.00	0.00	0.00

P.I: MET=0, ETH=0, AQU=0

Table 4.8: Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of *Cassia occidentalis* leaves extract.

S/N	Test organisms	Methanolic extract		Ethanolic extract		Aqueous extract		Amoxycillin	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	*Salmonella typhi*	62.5	250	125	500	125	500	62.5	250
2	*Salmonella paratyphi A*	125	500	125	250	125	500	125	500
3	*Salmonella paratyphi B*	62.5	125	62.5	500	62.5	1000	125	500

Table 4.10: Minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of *Eucalyptus camaldulensis* leaves extract.

S/N	Test organisms	Methanolic extract		Ethanolic extract		Aqueous extract		Amoxycillin	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	*Salmonella typhi*	62.5	250	125	500	125	500	62.5	250
2	*Salmonella paratyphi A*	125	500	6.25	250	125	500	125	500
3	*Salmonella paratyphi B*	125	500	125	500	125	500	125	500

Table 4.11: Minimun inhibitory (MIC) and minimum bactericidal concentrations (MBC) of *Cassia occccidentalis* root extract.

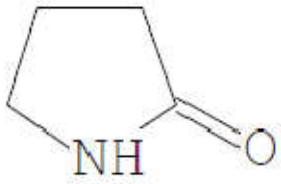
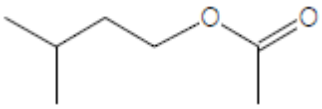
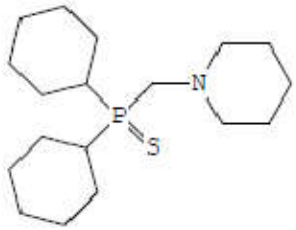
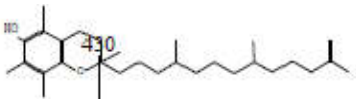
S/N	Test organisms	Methanolic extract		Ethanolic extract		Aqueous extract		Amoxycillin	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	*Salmonella typhi*	125	500	250	1000	250	1000	62.5	250
2	*Salmonella paratyphi A*	125	500	250	1000	250	1000	125	500
3	*Salmonella paratyphi B*	125	500	250	1000	250	1000	125	500

Table 4.12: Minimum inhibitory concentration (MIC) and minimmun bactericdal concentration (MBC) of combined extracts of *Cassia.occidentalis* , *Citrus.sinensis* and, *Eucalyptus camaldulensis*.

S/N	Test organisms	Methanolic extract		Ethanolic extract		Aqueous extract		Amoxycillin	
		MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1	*Salmonella typhi*	62.5	250	62.5	250	6.25	250	62.5	250
2	*Salmonella paratyphi A*	62.5	250	125	500	125	500	125	500
3	*Salmonella paratyphi B*	62.5	250	125	500	125	500	125	500

4.7.1 GC-MS analysis on *Eucalyptus camaldulensis* water extract

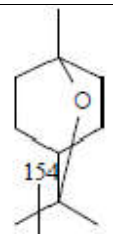
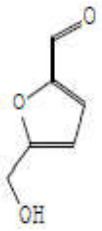
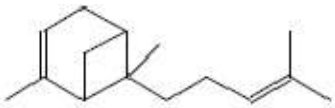
Nineteen (19) compounds were identified in the preliminary GC-MS of this extract and the major compounds include (appendix 1);

S/N	Retention time	Area %	IUPAC nomenclature	Molecular formula	Structural formula
1	5.631	23.24	2-pyrrolidinone (Aminobutyrolectam)	C_4H_7NO	
2	6.511	24.82	1- butano, 3 methyl acetate	$C_7H_{14}O_2$	
3	9.098	8.73	1-((Diclohexylphosphorothioyl)methyl) piperidine	$C_{18}H_{34}NPS$	
4	25.790	6.28	Trifloroacetic acid, n-octadecyl ester	$C_{20}H_{37}F_3O_2$	
5	20.586	5.96	9-octadecenoic acid (oleic acid)	$C_{18}H_{34}O_2$	
6	26.680	1.45	Vitamin E	$C_{29}H_{50}O_2$	
7		29.52	OTHERS (remaining 13 compounds)		

Other important compounds identified from the extract includes; Piperdine, Acetic acid, linalool.

4.7.2 GC-MS analysis on *Eucalyptus camaldulensis* leaves methanolic extract

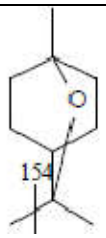
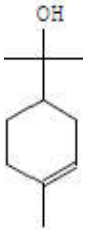
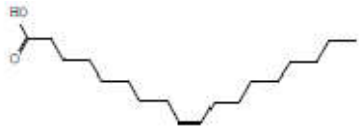
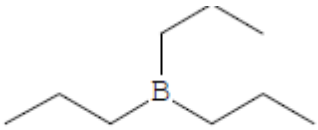
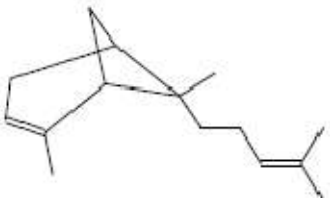
Twenty two (22) compounds were identified in the preliminary GC-MS analysis with the major ones being (appendix 2);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	21.00	4.669	2-oxabicyclo(2.2.2) octane (Eucalptol)	$C_{10}H_{18}O$	
2	23.44	7.555	2-Furancarboxaldehye, 5-(Hydroxymethyl)	C_9H_{12}	
3	9.38	20.497	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$	
4	5.93	10.310	Trans-alpha-bargomotene	$C_{15}H_{24}$	
5	5.43	6.877	2-Methyl-6-methylene-7-octen-2-ol (Mrycenol)	$C_{10}H_{18}O$	
6	34.82		OTHERS (remaining 17 compounds)		

Other important compounds identified from the *E. camaldulensis* methanolic leaves extract include; Ledol, Globulol, Erucic acid and Squalene etc

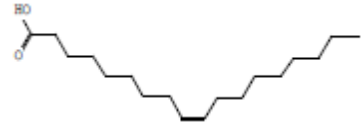
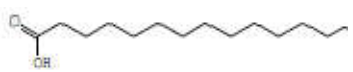
4.7.3 GC-MS analysis on *Eucalyptus camaldulensis* leaves ethanolic extract

Twenty six (26) compounds were identified in the preliminary GC-MS analysis of this extract and major ones include (appendix 3);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	22.67	4.693	2-oxabicyclo(2.2.2) octane (Eucalptol)	$C_{10}H_{18}O$	
2	7.44	6.903	p-menth-1-en-8-ol (alpha Terpeneol)	$C_{10}H_{18}O$	
3	6.51	20.545	9-octadecanoic acid (Oleic acid)	$C_{18}H_{34}O_2$	
4	6.23	7.500	Tripropylborane	$C_9H_{21}B$	
5	5.51	10.333	2- Norpinine,2,6-dimethyl-6-(4-methyl-3-pentenyl), alpha Bargamotene	$C_{15}H_{24}$	
6	41.11		OTHERS (remaining 20 compounds)		

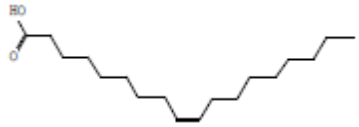
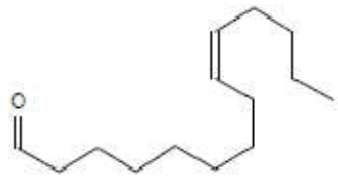
4.7.4 GC-MS analysis on *Cassia occidentalis* leaves aqueous extract

Seventeen (17) compounds were identified in the preliminary GC-MS analysis and the major ones include (appendix 4);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	33.09	23.190	9-Octadecenoic acid (Oleic acid)	$C_{18}H_{34}O_2$	
2	13.73	18.972	Tetradecanoic (Neo Fat 14)	$C_{14}H_{28}O_2$	
3	8.74	22.663	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{36}O_2$	
4	9.81	28.111	E-13-Docosenoic acid (Brassidic acid)	$C_{22}H_{42}O_2$	
5	5.54	13.277	2-Butene, 1,4-diethoxy	$C_8H_{16}O_2$	
6	29.07		OTHERS (remaining 12 compunds)		

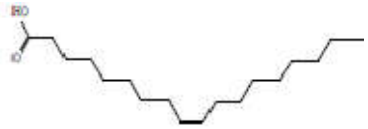
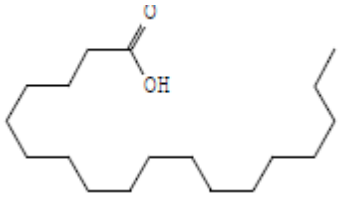
4.7.5 GC-MS analysis on *Cassia occidentalis* leaves ethanolic extract

Twenty two (22) compounds were identified in the preliminary GC-MS analysis with major ones being (appendix 5);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formular	Structural formula
1	29.54	19.622	9-Octadecenoic acid (Oleic acid)	$C_{18}H_{34}O_2$	
2	14.88	17.003	n-Pentadecanoic acid	$C_{15}H_{30}O_2$	
3	8.87	19.985	n-Heptadecanoic acid (Margaric acid)	$C_{17}H_{34}O_2$	
4	9.09	26.416	Z-9-Tetradecen al	$C_{14}H_{26}O$	
5	5.31	14.812	1-Octadecyne	$C_{18}H_{34}$	
6	32.31		OTHERS (remaining 17 compounds)		

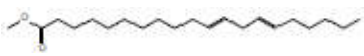
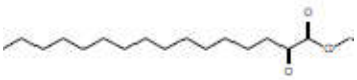
4.7.6 GC-MS analysis of *Cassia occidentalis* leaves methanolic extract

Twenty (20) compounds were identified in the preliminary GC-MS analysis and the major ones include (appendix 6);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	30.13	20.712	9-Octadecenoic acid (Oleic acid)	$C_{18}H_{34}O_2$	
2	13.00	17.837	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	
3	8.64	20.958	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	
4	8.25	26.317	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	
5	4.97	15.182	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	
6	35.01		OTHER remaining 12 compounds		

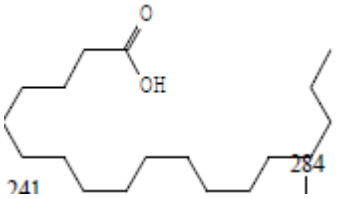
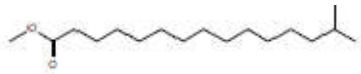
4.7.7 GC-MS analysis of *Cassia occidentalis* root aqueous extract

Ten (10) compounds were identified in the preliminary GCMS, with major ones being (appendix 7);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	38.12	22.675	Pentanoic acid, 10-undecenyl ester	$C_{16}H_{30}O_2$	
2	22.23	23.911	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	
3	9.42	14.612	11-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	
4	5.15	18.325	2-Octtlcyclopropene-1-heptanol	$C_{18}H_{34}O$	
5	5.31	16.245	Methyl 2-oxohexadecanoate	$C_{17}H_{32}O_3$	
6	19.77		OTHERS (remaining 5 compounds)		

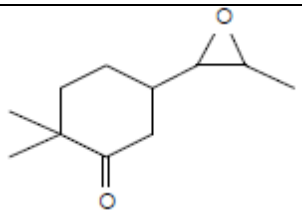
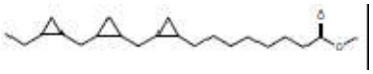
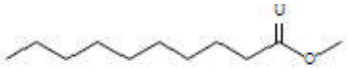
4.7.8 GC-MS analysis of *Cassia occidentalis* root ethanolic extract

Nine (9) compounds were identified in the preliminary GC-MS analysis and the major ones include (appendix 8);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	39.84	19.442	9,12-Octadecadienoyl chloride, (Z,Z) (Linoleic acid	$C_{18}H_{31}ClO$	
2	20.16	21.144	9-Octadecynoic acid methyl ester	$C_{19}H_{34}O_2$	
3	8.01	13.243	15 –Tetracosenoic acid methyl ester	$C_{25}H_{48}O_2$	
4	7.97	16.765	Stearic acid	$C_{18}H_{36}O_2$	
5	4.26	15.704	Methyl 14-methylpentadecanoate	$C_{17}H_{34}O_2$	
6	20.63		OTHERS (remaining 4 compounds)		

4.7.9 GC-MS analysis of *Cassia occidentalis* root methanolic extract

Thirteen (13) compounds were identified in the preliminary GC-MS analysis with the major ones being (appendix 9);

S/N	Area %	Retention time	IUPAC nomenclature	Molecular formula	Structural formula
1	34.54	20.646	2,2-Dimehtyl-5-(3-methyl-2-oxiranyl)cyclo hexanone	$C_{11}H_{18}O_2$	
2	17.86	21.180	Cyclopropaneoctanoic acid methyl ester	$C_{22}H_{38}O_2$	
3	7.87	13.484	15-Tetracosenoic acid, Methyl ester	$C_{25}H_{48}O_2$	
4	7.60	17.765	Tetradecanoic acid (Univol U316S)	$C_{14}H_{28}O_2$	
5	5.35	16.751	Capric acid Methyl ester	$C_{11}H_{22}O_2$	
6	26.78		OTHER remaining 8 compounds		

4.8.1 Thin layer chromatographic separation of individual plants extracts component.

Extract band	Distance moved by compound (cm)	Distance moved by solvent (cm)	Rf value	Antimicrobial property
E. camaldulensis	17.0	4.5	0.26	Resistance
Methanol	17.0	5.7	0.33	Active
	17.0	7.0	0.41	Active
	17.0	7.9	0.46	Resistance
	17.0	9.2	0.54	Resistance
	17.0	10.3	0.61.	Active
E. camaldulensis	17.0	4.7	0.27	Resistance
Ethanol	17.0	6.5	0.38	Active
	17.0	8.8	0.51	Active
	17.0	12.6	0.74	Resistance
	17.0	13.3	0.78	Resistance
E. camaldulensis	17.0	4.5	0.26	Resistance
Aqueous	17.0	6.9	0.40	Active
	17.0	11.7	0.68	Resistance
	17.0	13.8	0.81	Active
	17.0	16.0	0.94	Resistance
C. occidentalis	17.0	2.2	0.12	Active
Methanol	17.0	5.9	0.34	Resistance
	17.0	7.9	0.46	Active
	17.0	13.4	0.78	Resistance
	17.0	15.8	0.92	Active
C. occidentalis	17.0	2.0	0.11	Active
Ethanol	17.0	5.6	0.32	Active
	17.0	7.0	0.41	Resistance
	17.0	13.7	0.80	Resistance
	17.0	15.8	0.92	Resistance
C. occidentalis	17.0	2.9	0.17	Active
Aqueous	17.0	6.4	0.37	Resistance
	17.0	8.5	0.5	Active
	17.0	14.6	0.85	Resistance
C. occidentalis	17.0	5.7	0.33	Active
Root methanol	17.0	8.3	0.48	Resistance
	17.0	11.0	0.64	Resistance
	17.0	14.1	0.82	Active
C. occidentalis	17.0	5.2	0.30	Resistance
Root ethanol	17.0	7.9	0.46	Resistance
	17.0	10.5	0.61	Resistance
	17.0	12.8	0.75	Active
C.occidentalis	17.0	4.9	0.28	Resistance
Root aqueous	17.0	7.1	0.41	Resistance
	17.0	9.9	058	Resistance
	17.0	11.5	0.67	Active

4.8.2 Brine Shrimp cytotoxicity Assay of the individual and combined extracts

Plant Extract	Organic solvent	Concentration (ppm or µg/ml)	No. of Shrimps	No. of Survivors	% Mortality	LC_{50} (µg/mL) Brine Shrimp Lethality
C. occidentalis leaves extract	Aqueous	1000	10	3	70	
		100	10	6	40	191.639
		10	10	8	20	
	Methanol	1000	10	2	80	
		100	10	4	60	30.765
		10	10	6	40	
	Ethanol	1000	10	2	80	
		100	10	5	50	71.427
		10	10	7	30	
E. camaldulensis leaves extract	Aqueous	1000	10	6	40	
		100	10	7	30	1308.872
		10	10	10	00	
	Methanol	1000	10	4	60	
		100	10	7	30	469.630
		10	10	8	20	
	Ethanol	1000	10	4	60	
		100	10	7	30	472.221
		10	10	9	10	
C. occidentalis rootextracts	Aqueous	1000	10	1	90	
		100	10	3	70	11.243
		10	10	5	50	
	Methanol	1000	10	0	100	
		100	10	1	90	3.087
		10	10	3	70	
	Ethanol	1000	10	0	100	
		100	10	2	80	6.615
		10	10	5	50	
Combined extracts (1:1:1 ratio)	Aqueous	1000	10	4	60	
		100	10	7	30	472.221
		10	10	9	10	
	Methanol	1000	10	2	80	
		100	10	5	50	100.000
		10	10	8	20	
	Ethanol	1000	10	3	70	
		100	10	5	50	140.004
		10	10	8	20	

CHAPTER FIVE

5.0 DISCUSSION

5.1 DISCUSSION

Water is the best solvent of extraction of these leaves extract as it gives higher yield of the extract, however methanol gives more yield of the phytochemical compounds, with the exception of *C. occidentalis* root. Tannins was found in all the extract and, all the phytochemical tested were found in *Cassia occidentalis.* Saponin, glycoside and anthraquinones were absent in all the *Citrus sinensis* extract. Babayi *et al.,* 2004, in his work also report the presence of the phytochemicals identified in this study. The inactivity of *Citrus sinensis* leaves extract on the test organism as seen in these work confirms earlier finding by (Nada *et al*., 2014). This may be due to absence of important phytochemicals like saponins which have antibacterial property and the nature of the gram negative cell wall. The antibacterial activity of *Eucalyptus camaldulensis* concur with earlier studies conducted by Ayopola *et al.,* 2008. The result of antibacterial potential of *E. camaldulensis* recorded contradicts earlier findings by Babayi *et al.,* 2004. Yadav *et al.*, (2010) have studied the antimicrobial potential of *C. occidentalis* leaves with similar result. From the study the combined extract have more activity than individual extract with activity more than the control antibiotic (Amoxycillin). *Cassia occidentallis* and combined extract have proportional index of 1, while other extract have P.I less than 1. *Citrus sinensis* extracts have proportional index of 0. *Cassia occidentalis* have activity index more than 1 against *Salmonella typhi.* Combined extract have activity index of 1 on *S. paratyphi A.* The result

of *Cassia occidentalis* root extract conform with earlier study by (Krishna *et al.*, 2010). In addition, the result of thin layer chromatography TLC conducted reveals that the *E. camaldulensis* methanolic extract have more bands under the UV light. Root extract of *C. occidentalis* have the least band under UV light with just four bands seen.The gas chromatography mass spectrometry (GC- MS) reveals the compounds present as matched with the NIST library. Extracts of *E. camaldulensis* contains more compounds as reveal by the preliminary TLC conducted during the GCMS analysis. The brine shrimp test represents a rapid, inexpensive and simple bioassay for testing the plant extract lethality which in most cases correlates reasonably well with cytotoxic properties.Most often, a desired biological response is not due to one component but rather due to a mixture of bioactive plant components. Therefore, crude extracts must be screened for biological activity. The brine shrimp lethality assay has been proved to be a convenient system for monitoring biological activities of natural products. In brine shrimp lethality bioassay, % mortality increased gradually with increase in concentration of the test samples. An LC_{50} (concentration killing fifty per cent of the brine shrimp larvae), value greater than 100µg/ml is considered to present a non-toxic compound or extract (Moshi *et al.,* 2010). The brine shrimp toxicity assay showed that six extract (66.6%) out of the 12 extracts tested had LC_{50} values less than 100µg/ml; the cut-off point. Among these 1 extract had LC_{50} value greater than 1000µg/ml, while the remaining had LC_{50} value between 100 and 700µg/ml. only six extract (66.6%) showed LC_{50}<100µg/ml, and therefore classified as toxic. *Cassia occidentalis*root methanol extract was the most toxic with LC_{50} = 3.087µg/mL, followed by the extracts of same plant ethanol leaves (6.615µg/mL), (Table 4.8). The high toxicity of *C. occidentalis* leaf extract on brine shrimp larvae may be due to the effect of saponins. Previous studies revealed that the leaves of *C. occidentalis*are rich in triterpenoidal saponins and these

compounds were reported to have high antilipemic, hemolytic and capacity to lower the serum chlesterol level (Muhammed *et al*., 2012). *Cassia occidentalis* shows no significant standard deviation with the standard antibiotics and between the various extracts of the plant. *E. camaldulensis, C. occidentalis* root extracts shows significant standard deviation with the standard antibiotics and between the various extracts of the plants.

5.2 CONCLUSION

Methanol, ethanol and water have the ability of extracting phytochemical from *E. camaldulensis,Citrus sinensis* leaves and *C. occidentalis* leaves and root. All extract showed activity against the test organisms in accordance with the extract concentration with exception of the *Citrus sinensis* extract which shows inactivity against the test organism.

The result from MIC and MBC indicates the bacteriostatic property against the test organism, in accordance with the extract concentration. *Cassia occidentalis* (methanolic, ethanolic and aqueous extract) shows high P.I similar to control antibiotic Amoxycillin. Similarly, the combined extract of *Cassia occidentalis* leaves and root, *Eucalyptus camaldulensis* and *Citrus sinensis* shows higher activity with P.I value of 1.00 similar to control antibiotic showing synergism. Statistical analysis reveals no significant difference between all the *C. occidentalis* extract, and standard antibiotics. However, significant difference was analyzed with other plants extracts. From the GC-MS analysis carried out it reveals compounds of antimicrobial property from the various extracts. Toxicity study carried out on the plants extract revealed that aqueous extracts of *C. occidentalis* leave and *E. camaldulensis* leave were non toxic, similarly all combined extracts were non toxic.

5.3 RECOMMENDATIONS

From the result obtained

I. Water is the best solvent of extraction of these leaves extract as it gives more percentage yield, with the exception of *C. occidentalis* root. However, methanol gives more phytochemical components.

II. The plants should be combined for treatment of infections caused by *Salmonella* as it is more active and safer than individual extract.

III. Further studies research on compound identified through GC-MS from these extract may reveal vital breakthrough, considering important compound like vitamin E and emodin identified.

IV. Brine shrimp lethality assay conducted shows greater percentage of the extract tested are toxic, contrary to the claims by traditional medicine practitioners. Prolong administration of such plants should be avoided especially by community that patronize such plants as herbal cure.

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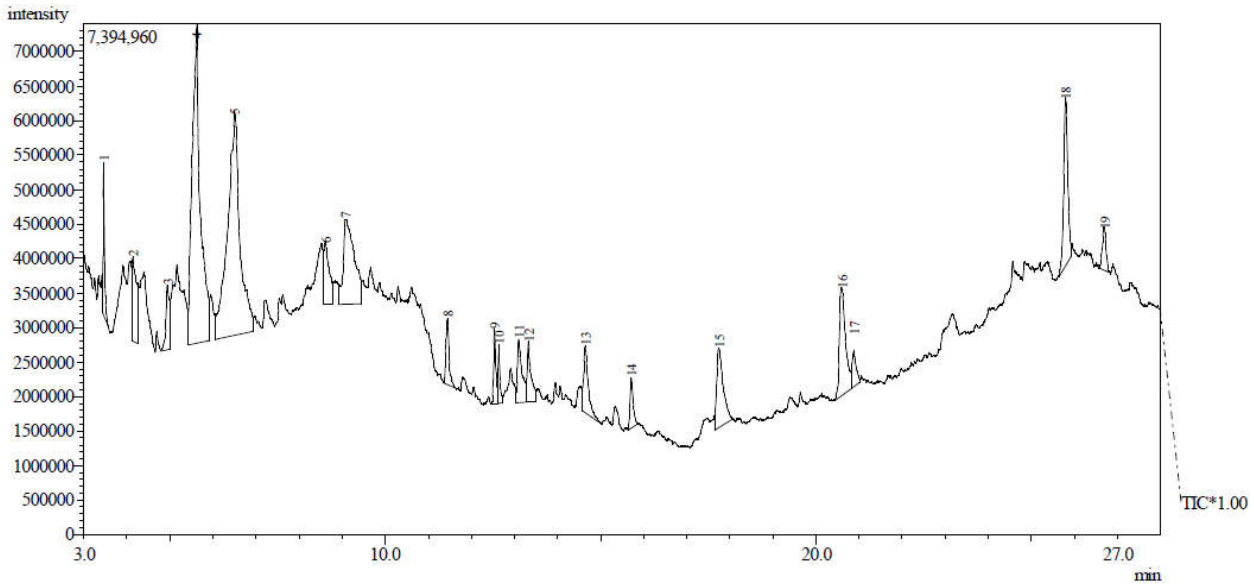
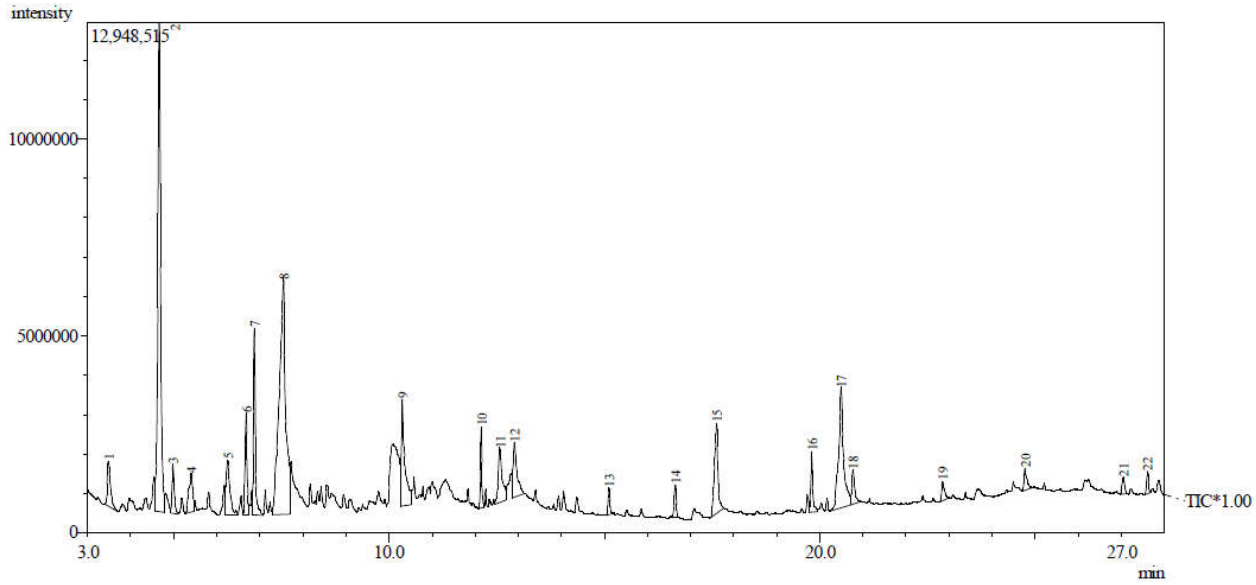
APPENDIX

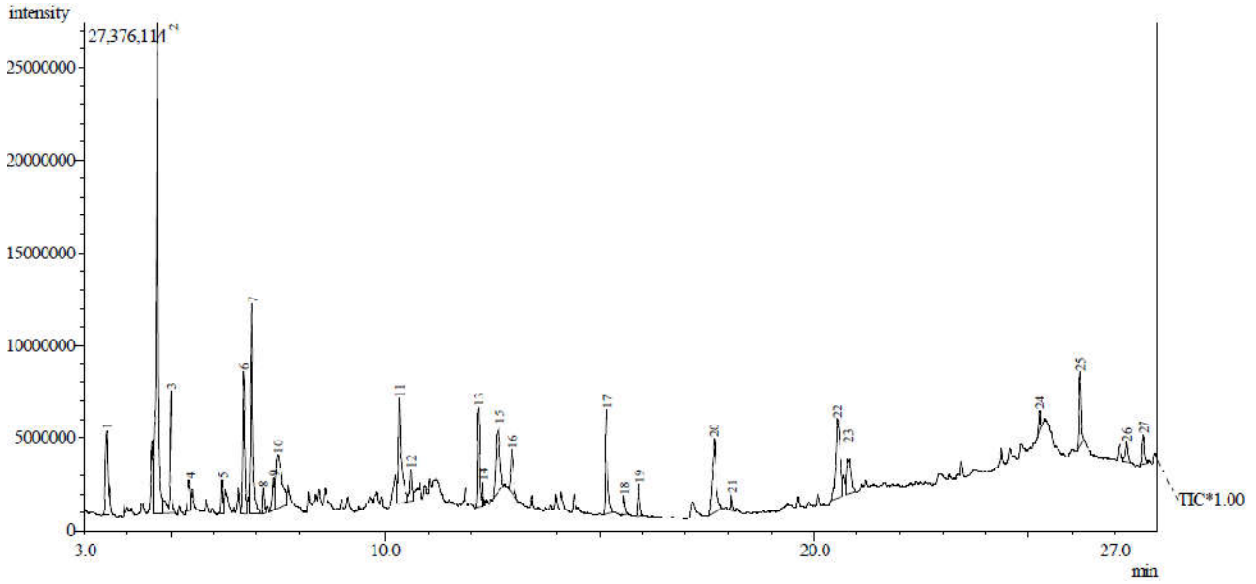
Appendix 1:GC-MS analysis on *E. camaldulensis* leaves water extract



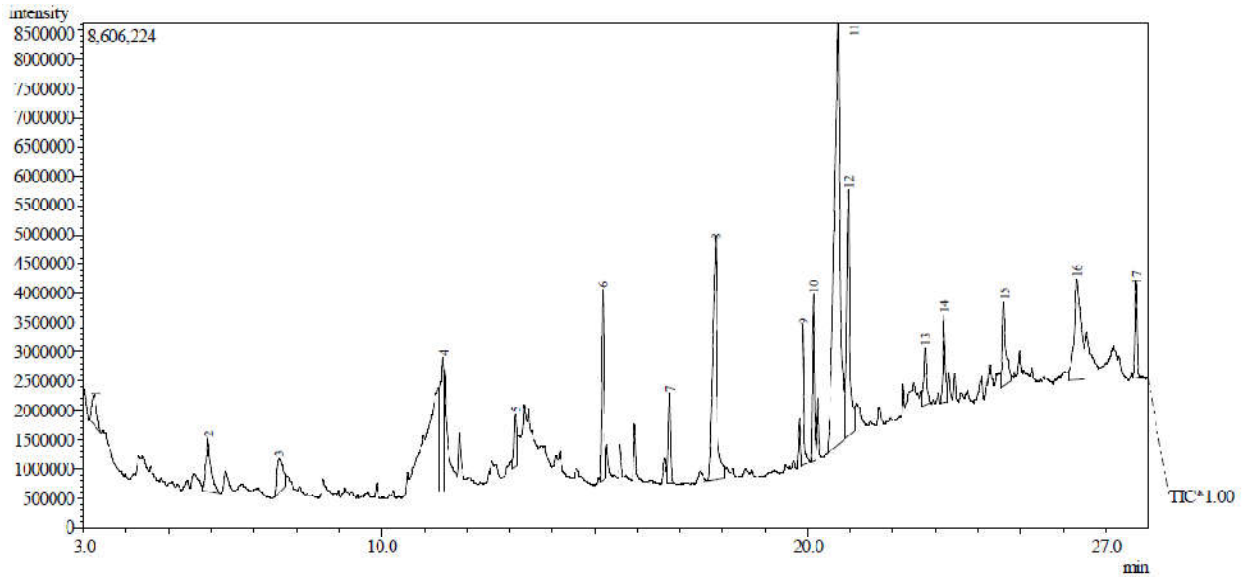
Appendix 2: GC-MS analysis on *Eucalyptus camaldulensis* leaves methanolic extrac



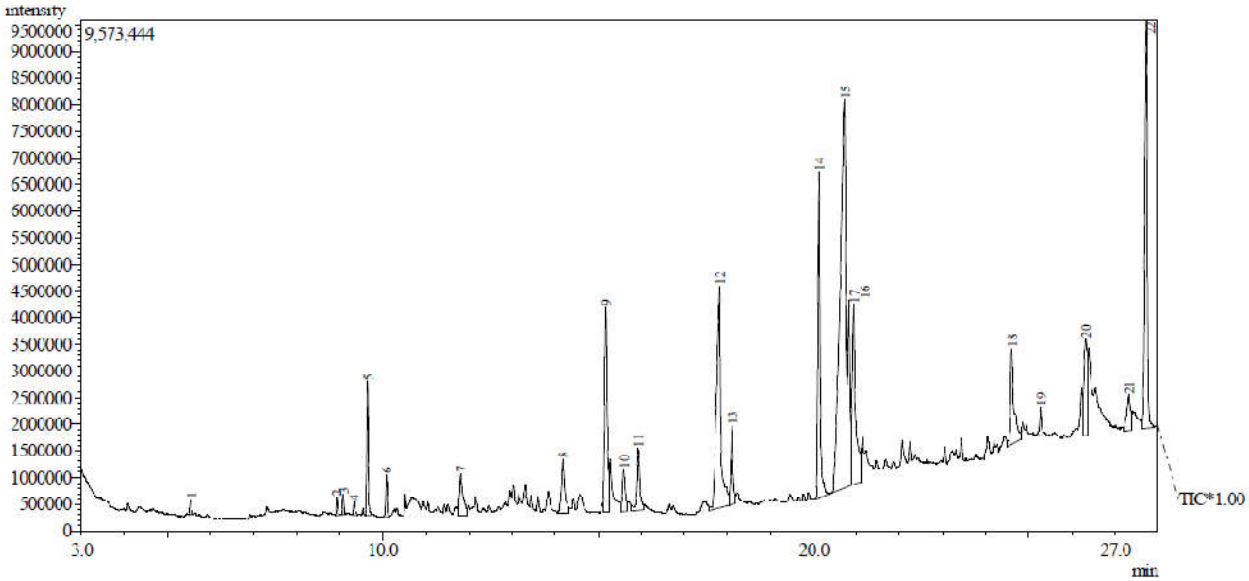
Appendix 3:GC-MS analysis on *Eucalyptus camaldulensis* leaves ethanolic extract



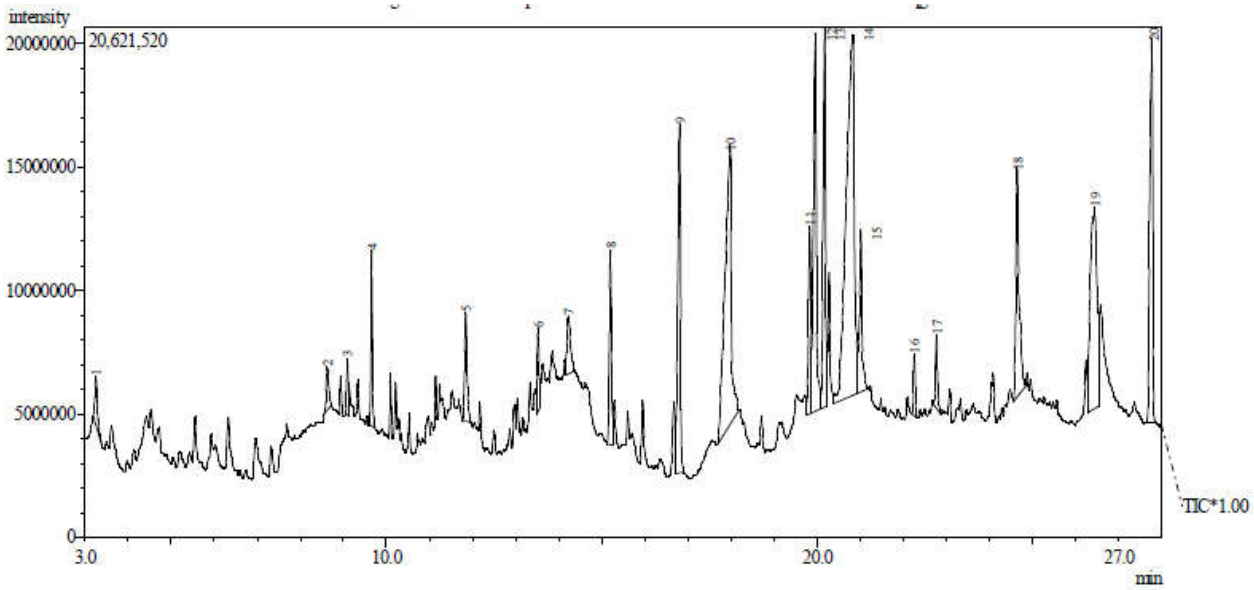
Appendix 4: GC-MS analysis on *cassia occidentalis* leaves aqueous extract



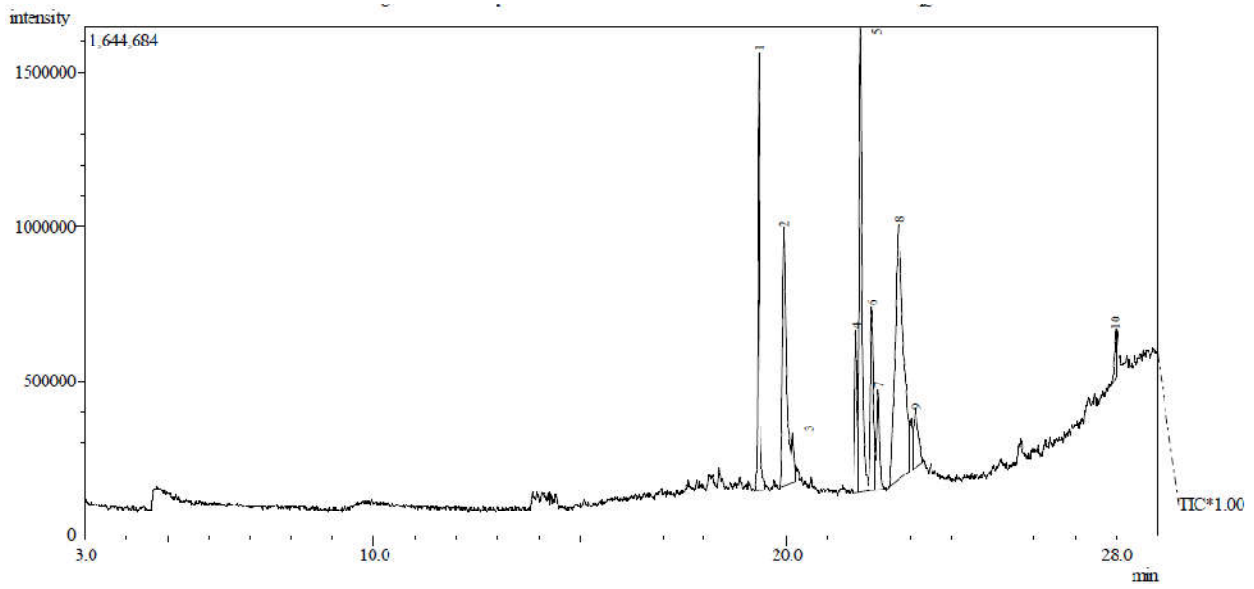
Appendix 5:GC-MS analysis on *Cassia occidentalis* leaves ethanolic extract



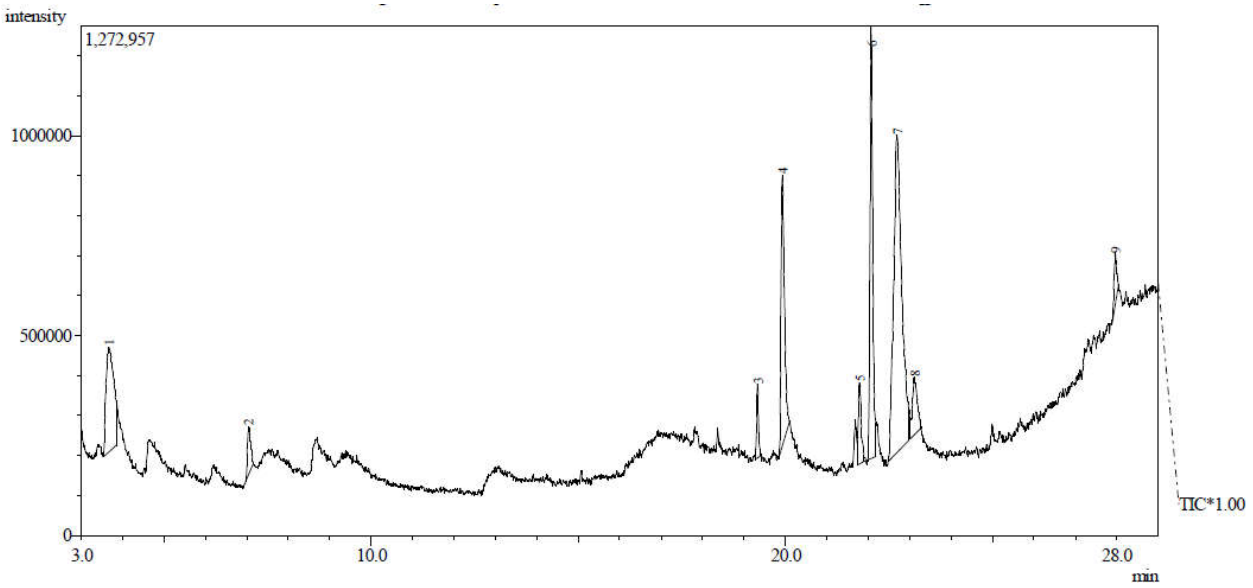
Appendix 6: GC-MS analysis of *Cassia occidentalis* leaves methanolic extract



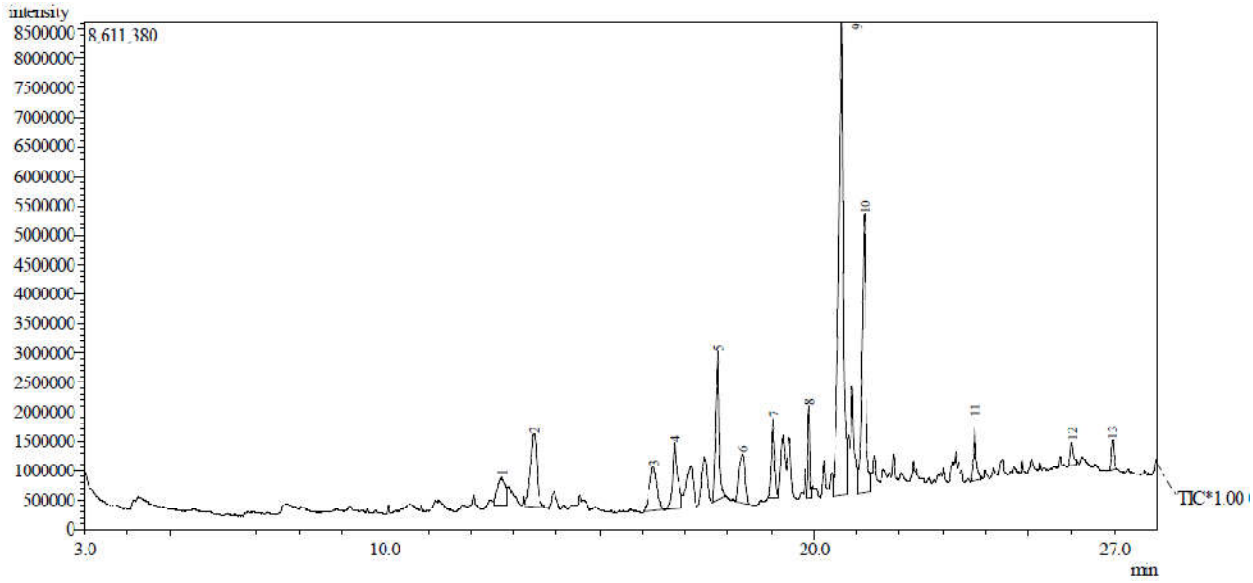
Appendix 7:GC-MS analysis of *Cassia occidentalis* root aqueous extract



Appendix 8:GC-MS analysis of *Cassia occidentalis* root ethanolic extract



Appendix 9:GC-MS analysis of *Cassia occidentalis* root methanolic extract



APPENDIX 10

Department of Microbiology,

Bayero University, Kano.

LETTER OF INTRODUCTION

Dear sir./Ma/Alh./Mal.

I' am a student of Bayero University Kano, Department of Microbiology, currently undertaking a research on **"anti-*Salmonella* potential of individual and combined crude extracts of *Cassia occidentalis, Citrus sinensis,* and *Eucalyptus camaldulensis."***

You are requested to please fill this questionnaire which will be used as source of information. Your cooperation is highly needed towards this study please.

Yours faithfully

USMAN ADAMU

SPS/13/MMB/00036

APPENDIX 11

QUESTIONNAIRE

Where as: SA is strongly agree, A is agree, D is disagree and SD is strongly disagree

S/N	Variable
1.	Sex : Male [ ] Female [ ]
2.	Age : Less than 30 [ ] 30-40 years [ ] 41-60 years [ ]

	Above 60 years [ ]
3.	Educational qualification: Non formal [ ] Primary [ ] W.A.S.S.C.E./NECO [ ] HND/Diploma [ ] B.Sc/BA [ ]
4.	Local government: Hadejia [ ] Kafin hausa [ ]

S/N	Variable	SA	A	D	SD
5.	Did you agree with the use of natural plant product in treatment of ailment?				
6.	Did you agree with the use of *Cassia occidentalis* in treatment of typhoid fever?				
7.	Did you agree with the use of *Citrus sinensis* in treatment of typhoid fever?				
8.	Did you agree with the use of				

	Eucalyptus camaldulensis in treatment of typhoid fever?				
9	Did you agree in the effectiveness of these above mentioned plants in treatments of other ailments?				
10.	Did you agree in the safety of these above mentioned plants?				