Evaluation of the Antioxidant, Antimicrobial and Anti-inflammatory Activities of *Cyphostemma cyphopetalum* (Fresen) Descoings ex-Wild & Drummond and *Cyphostemma junceum* (Webb) Descoings ex-Wild & Drummond

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A Dissertation Presented to the Department of Microbial, Cellular and Molecular Biology in Fulfillment of the Partial Requirements for the Degree of Doctor of Philosophy (Biology: Biomedical Sciences)

Addis Ababa University

Addis Ababa, Ethiopia

February, 2018
Evaluation of the Antioxidant, Antimicrobial and Anti-inflammatory Activities of *Cyphostemma cyphopetalum* (Fresen) Descoings ex-Wild & Drummond and *Cyphostemma junceum* (Webb) Descoings ex-Wild & Drummond

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February, 2018
ACKNOWLEDGEMENTS

First of all, things I would like thank an Almighty God and his and our mother Saint virgin Mary. My deepest gratitude and heartfelt thanks go to my advisor Prof. Yalemtehay Mekonnen: for her support and constructive criticism. She devoted her precious time to guide me throughout my Ph.D. research work from the inception through proposal preparation to the dissertation defense. My appreciation and thanks go to Prof. Ariaya Hymete for providing me reading materials and his significant comments during the development of the research proposal and spend his precious time in reviewing the manuscript.

I would like to extend my sincere thanks to Dr. Fasil Assefa for his vital comments. My special thanks also go to Prof. Ermias Dagne and Mr. Mesfin Getachew for isolating and identifying the reported compounds. My appreciation and thank also go to Mrs. Amelework Eyado for her guidance in biological activity determinations and Dr. Shihun Shimelis for his support in data analysis.

I would like to thank the Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, AAU and Dr. Gurja Belay Head of the Department in particular and AAU in general for sponsoring my Ph.D. study and providing me all required facilities throughout the study period. I would like to thank staffs of the National Herbarium, AAU for identification of the plant species and Ethiopian public health institute for providing me the bacterial and fungal test organisms.
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<tr>
<td>ATCC</td>
<td>American type culture collection</td>
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<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>DPPH</td>
<td>2, 2 diphenyl-1-picrylhydrazyl hydrate</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum bactericidal concentration</td>
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<tr>
<td>MHA</td>
<td>Mueller Hinton agar</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<tr>
<td>NMR</td>
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<tr>
<td>OECD</td>
<td>Organization of economic cooperation and development</td>
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<td>SEM</td>
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ABSTRACT

*Cyphostemma cyphopetalum* and *Cyphostemma junceum* (Family Vitaceae) are known for their various medicinal uses in different parts of Ethiopia. This study was therefore conducted to evaluate the antioxidant, antimicrobial and anti-inflammatory activities of the leaves and roots of *C. cyphopetalum* and *C. junceum*. The leaves and roots of the two plants were extracted using 80% methanol and distilled water with maceration technique and filtered using Whatman No 1 filter paper and the filtrate of 80% methanol extracts were concentrated using rotary evaporator and that of water extracts were stored at (-20°C) and lyophilized to dryness. The extracts were further fractionated with methanol, ethanol, acetone and distilled water. Acute toxicity of 80% methanol extracts were evaluated on female Swiss albino mice up to a dose of 5000mg/kg body weight and the secondary metabolites present in these extracts were identified. Compounds were isolated from eight gram ethanol extract of the leaves of *C. cyphopetalum* using column chromatography. The column was eluted with n-hexane, chloroform, ethyl acetate and methanol to afford 24 fractions A-X. Structure of the compounds was elucidated using nuclear magnetic resonance (NMR) and compared with the available database. The antioxidant activities of the water crude extracts and methanol solvent fractions of the leaves and roots of the two plants were evaluated using 2, 2 diphenyl-1-picrylhydrazyl hydrates (DPPH) radical scavenging assay. Antimicrobial activities of 80% methanol extracts and methanol, ethanol, and acetone solvent fractions were evaluated against pathogenic gram-negative bacteria; *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Shigella boydii* (ATCC9207), *Shigella flexineri* (ATCC12022), *Klebsiella pneumonia* (ATCC70603) and Gram-positive bacteria; *Staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC12228), *Listeria monocytogens* (ATCC19115) and the fungal test organism, *Candida albicans* (ATCC10231).
using agar disc and agar well diffusion methods. The minimum inhibitory concentration (MIC) of the methanol solvent fraction was determined using broth dilution method, which was followed by conducting the minimum bactericidal concentration (MBC). Ciprofloxacin and fluconazole were used as positive control for the antibacterial and antifungal test respectively. The anti-inflammatory effect of the crude methanol, water fractions and β-sitosterol isolated from the ethanol leaves extract of *C. cyphopetalum* was evaluated using carrageenan-induced mouse paw edema. The acute toxicity study indicated that 80% methanol leaves and roots extracts did not cause death or induce other toxicity symptoms on mice to a dose of 5000mg/kg body weight. Alkaloids, flavonoids, catechol tannins, saponins, and terpenoids were the secondary metabolites identified from 80% methanol extracts of the two plants. β-sitosterol, diterpenoid, luteolin, sitosterol glucoside, resveratrol dimer, salicylic acid, a mixture of flavonoid glycosides and sucrose were isolated from ethanol leaves extract of *C. cyphopetalum*. The methanol solvent fraction of the leaves of *C. cyphopetalum* and *C. junceum* showed respective inhibition of 92.2% and 87.1% at 12.5mg/mL while ascorbic acid showed inhibition of 90.9% at 12.5mg/mL on DPPH assay. The highest inhibition zone was exhibited against *S. aureus* by 80% methanol leaves extract of *C. cyphopetalum* with a diameter of 13.0±0.0mm at 500mg/mL and methanol solvent fractions of the leaves of *C. cyphopetalum* with a diameter of 15.0±0.0mm at 500mg/mL whereas, the acetone solvent fractions of the leaves and roots showed the lowest inhibition zone against *K. pneumonia* with inhibition diameter of 7.0±0.0mm. The methanol solvent fraction of the roots of *C. junceum* showed the highest inhibition zone (9.7±0.3mm) against *S. aureus* at 500mg/mL while the methanol, ethanol, and acetone solvent fractions of the leaves of *C. junceum* exhibited the lowest against *K. pneumonia* (6.7±0.3mm). The methanol solvent fractions of the leaves of *C. cyphopetalum* exhibited the highest antifungal activity against *C. albicans* with inhibition diameter of 12.3±0.3mm when compared with ethanol and acetone solvent fractions.
The methanol solvent fractions of the leaves of *C. cyphopetalum* showed the lowest MIC (15.63mg/mL) and MBC (31.25mg/mL) against *S. aureus* and *S. epidermidis*. The highest MIC (125mg/mL) and MBC (250mg/mL) were depicted by methanol solvent fractions of the leaves of *C. cyphopetalum* and roots of *C. junceum* against *K. pneumonia*. On the anti-inflammatory evaluation, 80% methanol leaves extracts and water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* markedly reduced the swelling. The percentage reduction on mice paw thickness was 95.83% for 80% methanol leaves extracts of *C. cyphopetalum* (500mg/kg), 97.40% for distilled water fractions of the leaves of *C. cyphopetalum* (300mg/kg) and 97.40% for β-Sitosterol (25mg/kg) at six hours. Likewise, the percentage reduction on mice paw thickness was 88.54% and 93.23% for 80% methanol leaves extract of *C. junceum* (500mg/kg) and distilled water fractions of the leaves of *C. junceum* (300mg/kg) respectively. The leaves of *C. cyphopetalum* had a significant anti-inflammatory effect compared with the indomethacin (10mg/kg) (*P* < 0.05) that showed percentage paw thickness reduction of 93.23%. It can be concluded that the leaves and roots of *C. cyphopetalum* and *C. junceum* have antioxidant, antimicrobial and anti-inflammatory activity at the tested doses. Thus, these plants could be used for possible drug development.

*Keywords*: acute toxicity, anti-inflammatory, antimicrobial, antioxidant, *Cyphostemma cyphopetalum*, *Cyphostemma junceum*, phytochemical screening
CHAPTER 1: INTRODUCTION

Oxidative stress is essentially an imbalance between the production of free radicals and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (Rahman et al., 2012). It is associated with the mechanisms of the pathogenesis of several diseases including atherosclerosis, neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or aging processes (Rahal et al., 2014; Egbuna and Ifemeje, 2017).

Amongst the factors which contributed to oxidative stress are free radicals, which can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence (Phaniendra et al., 2015). Once these highly reactive radicals are created, they can initiate a chain reaction which leads to the development of the non-infectious disease. As reviewed by Nisha and Deshwal (2011), the principal risk of free radicals comes from the damage they inflict when they react with vital cellular components including DNA and the cell membrane, leading to a dysfunction of the cells and death. To prevent the damage which is caused by free radicals the body has a defense system of antioxidants (Phaniendra et al., 2015).

Antioxidants are molecules which can interact with free radicals and cease the chain reaction before fundamental molecules are damaged. Even if there are several enzyme systems within the body that prevent free radicals, the most important ones are the standard vitamin antioxidants like vitamin E, beta-carotene, and vitamin C. The body cannot produce these micronutrients so they must be supplied in the diet (Uttara et al., 2009; Prejeena et al., 2017).
Health is affected by different infectious and non-infectious agents that contribute to morbidity and mortality of human beings. The major causative agents that cause infectious disease are bacteria, fungi, virus, protozoa and parasitic worms. There are different drugs that are administered to heal infectious disease in humans. These include ciprofloxacin and fluconazole which used to control bacterial and fungal infections respectively. However, the frequent and unwise use of antimicrobial drugs in the treatment of infectious diseases, induce drug resistance by pathogens and has become a global problem (Kuthar et al., 2017). In addition to this problem, antibiotics are sometimes having side effects on the host including hypersensitivity, immune-suppression and allergic reactions (Goh et al., 2017). As reported by Digge et al. (2015), there is a need to develop alternative new and effective antimicrobial drugs from medicinal plants for the treatment of infectious diseases (Anyanwu and Okoye, 2017).

Since the beginning of the 19th century, a huge number of biologically active secondary metabolites of plant origin are reported to have commercial relevance as drugs. In the last few decades, there has been an increase of interest in the use of plants with their medicinal status as sources of potentially useful compounds (Kingston, 2011).

According to recent Kew royal botanical garden report, an estimated 369,000 species of flowering plants are known to Science of which in 2015, 2034 vascular plant species are newly known to Science and logged in the international plant names indexed by March 2016. At least 31,128 plant species have been used for different purposes. The number of plant species in each use category is summarized as follows: medicine (17,810), human food (5,538), fuels (1,621), animal food (3,649), invertebrate food (683) and poisons (2,503) (Kathy and Steve, 2016).
Medicinal plants are the wealthiest bio-resources of traditional medicines, food, pharmaceutical-grade and standardized nutrient and chemical entities for synthetic drugs. Traditional medicines are prepared from a single plant or combinations of a number of plants (Kalimuthu and Prabakaran, 2013). The efficacy depends on the use of the right plant part and its biological effectiveness which in turn depends upon the presence of required quantity and nature of secondary metabolite in a new drug (Vinoth et al., 2011). There is an increase in awareness in associating the phytochemical constituents of a medicinal plant with its pharmacological property (Turkey and Usta, 2008).

Several screening studies have been carried out in different parts of the world. Likewise, there are a number of reports on the antimicrobial activity of different herbal extracts in different regions of the world (Dahiya and Purkayastha, 2012). Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer (Sheeja and Kuttan, 2007).

Diverse compounds can be extracted and characterized from plants, which can offer a broad spectrum of therapeutic properties. Herbal products seem to be a promising basis, having diverse substances to be investigated, including alkaloids, curcuminoids, terpenoids, flavonoids, etc, which have been frequently used in folk medication and have efficacy against many diseases (Jaradat et al., 2017). The search for plants with reported traditional use as antioxidant, antimicrobial and anti-inflammatory agents should be considered as a fruitful and rational research strategy in the search for new drugs.
The Ethiopian flora is estimated to have between 6,500 and 7,000 species of higher plants of which about 12% are endemic (Wolde-Mariam et al., 2015). Ethiopia is also a home for many languages, cultures, and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, which, among others include the use of medicinal plants. Regardless of its significant contribution to society, traditional medicine has received slight awareness in modern research and development and less effort has been rewarded to improve the traditional health practices in the country (Teklay, 2015). But, the long history of the use of medicinal plants in Ethiopia and its vast biotic resources can be a dominant importance in future research and drug discovery.

Among the traditionally claimed many Ethiopian medicinal plants, *Cyphostemma cyphopetalum* (Gindosh in Amharic language) is used for the treatment of skin eruption, enlarged glands, diarrhea, wound, itchy rash, angina, phagedenic ulcer, nephritic colic, adenopathy, snake-bite and rabies in different parts of Africa (Lindsay and Hepper, 1978; Kayonga and Habiyaremye, 1987; Teklehaymanot et al., 2007). *Cyphostemma junceum* (Etsezewe in Ge’ez language) is traditionally used to treat snake-bite, diarrhea and venereal disease (Gelfand et al., 1985; Teklay et al., 2013). The biological activities of *C. digitatum* (Khan et al., 2016), *C. crotalarioides* (Ducrot et al., 1998), *C. adenocaule* (Chouna et al., 2016), *C. natalitium* (Lin et al., 1999), *C. hildebrandtii* (Mueller and Kanfer, 2011), *C. glaucophilla* (Eleojo et al., 2012), *C. greveana* (Cao et al., 2011), *C. bainesii* (Nitta et al., 2002), *C. serpens* (Ochwang’i et al., 2014), *C. flaviflorum* and *C. lanigerum* (Opoku et al., 2000) have been well known. From their medicinal value, it might be advisable to evaluate the chemical constituent and some selected biological activities of *C. cyphopetalum* and *C. junceum*. 
1.1. Statement of the Problems

Free radicals of different forms are generated at a low level in cells to help in the modulation of several physiological functions. However, if free radicals are produced in excess amount they can be destructive leading to inflammation, lung damage and degenerative diseases among others. Drug resistance caused due to the frequent and unregulated use of antimicrobial drugs for the treatment of infectious diseases and anti-inflammatory drugs have a symptomatic effect. Unless the root cause is tackled the symptoms will reappear on discontinuation of the anti-inflammatory drugs.

1.2. Justifications

To overcome the problem related with free radicals, drug resistance and the side effects of anti-inflammatory drugs people use different kinds of medicinal plants and most of the traditional uses of various plants have been based on personal beliefs. Although research has been done and is going on to investigate a number of plant secondary metabolites, many questions still remain unanswered. The composition, concentration, and bioactivity of the secondary metabolites present in the medicinal plants may be of some benefit if further investigated to validate the medicinal use of such plants. Traditionally, *C. cyphopetalum* and *C. junceum* are used for the treatment of different ailment but, to date, there is no report on the biological activities and chemical composition of these traditionally used medicinal plants in some parts of Ethiopia.
1.3. Hypothesis

The traditional medicinal uses of *C. cyphopetalum* and *C. junceum* suggest that their secondary metabolites are bioactive for antioxidant, antimicrobial and anti-inflammatory activities.

1.4. Objectives

1.4.1. General objective

✓ The overall objective of the present study was to determine the major chemical constituents and to evaluate the bioactivity of the leaves and roots of *C. cyphopetalum* and *C. junceum*.

1.4.2. Specific objectives

The specific objectives were:

- To evaluate the acute toxicity of 80% methanol leaves and roots extracts of *C. cyphopetalum* and *C. junceum*.
- To screen the phytochemical constituents of 80% methanol extracts of the leaves and roots of *C. cyphopetalum* and *C. junceum*.
- To evaluate the antioxidant activity of water extracts and methanol solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*.
➢ To evaluate the antimicrobial activities of 80% methanol extracts and methanol, ethanol and acetone solvent fractions using disc and agar well diffusion, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

➢ To evaluate the anti-inflammatory activity of 80% methanol extracts and the water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* using carrageenan-induced mice inflammatory model.
2.1. Antioxidants

Antioxidants are molecules that inhibit the formation of free radicals and the resultant cell injury and death (Wahlqvist, 2013). Free radicals are molecules with an unpaired electron. Due to the presence of a free electron, these molecules are highly reactive. Free radicals are important intermediates in natural processes involved in cytotoxicity, control of vascular tone and neurotransmission. Production of free radicals in the body is a continuous process (Bergendi et al., 1999; Phaniendra et al., 2015). The basic source of free radicals as reviewed by Sarma et al. (2010) and Phaniendra et al. (2015) are immune system cells that deliberately create oxy-radicals and reactive oxygen species. During energy-production cells generate continuously and abundantly oxy-radicals and reactive oxygen species as toxic waste. The cells perform a number of metabolic processes, each of which can produce different free radicals. Thus, even a single cell can produce many different kinds of free radicals. Abheri et al. (2010), reported air pollutants, chemical solvents, water pollutants, food containing farm chemicals, metabolism and stress as a source of free radicals.

Generally, free radicals of diverse forms are produced at a low level in cells to help the modulation of numerous physiological functions and controled by an integrated antioxidant system of the body. Nevertheless, when free radicals are produced in surplus quantity they can be unhelpful and lead to inflammation and lung damage (Hadi et al., 2000). Free radical reactions, particularly through the involvement of oxidative radicals, have been shown to be implicated in many biological processes that cause damage to lipids, proteins, membranes and nucleic acids, thus giving rise to a range of non-infectious diseases. This include various pathological
circumstances such as diabetes, cardiovascular diseases, cancer, asthma and inflammation (Demirtas et al., 2017).

When the generation of free radicals and active intermediates surpasses the body's ability to neutralize and eliminate them a condition known as oxidative stress occurs. To overcome this problem cells of animals and humans have developed antioxidant defense system comprising several molecules. Some of the antioxidants are produced in the body (endogenous) while others obtained from diet (exogenous) (Rahman et al., 2012). Based on their source the types of antioxidants includes natural antioxidant, synthetic antioxidant and dietary antioxidants (Yadav et al., 2016).

2.2. Test Organisms Used for Antimicrobial Assay

The bacterial test organisms used in this study were eighth in number five from gram negative and three from gram positive whereas, one fungus was used for the antifungal test.

2.2.1. Bacterial test organisms

2.2.1.1. Escherichia coli

*Escherichia coli* are Gram-negative rods in the family Enterobacteriaceae. The majority *E. coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their ownership of virulence factors such as exotoxins (Bardiau et al., 2010).
2.2.1.2. *Klebsiella pneumonia*

*Klebsiella pneumoniae* is an encapsulated Gram-negative bacterium that is frequently found in the mouth, skin, and intestines as well as in natural environments. It is the most significant clinical affiliate of the genus *Klebsiella* and the third most frequently isolated microorganism in blood cultures from sepsis patients. *K. pneumoniae* can cause severe epidemic and endemic rigorous hospital-acquired infections including septicemia, pneumonia, urinary tract infection and soft tissue infection in injured individuals (Stahlhut *et al.*, 2012).

Immunocompromised individuals have a significantly increased chance of having *K. pneumonia* in the lung, urinary tract, blood, liver and other organs. Globally, *K. pneumoniae* is a commonly encountered hospital-acquired opportunistic pathogen that typically infects patients with indwelling medical devices (Guo *et al.*, 2012). In this environment, it is often severe, persistent and difficult to eradicate. This bacterium is rapidly developing resistance to multiple antibiotics, including broad-spectrum cephalosporins and β-lactams.

2.2.1.3. *Listeria monocytogenes*

*Listeria monocytogenes* is a Gram-positive, rod-shaped bacterium considered as the causative agent of a severe foodborne illness in humans contacted through the fecal-oral route and characterized by localized infections (Manyi-Loh *et al.*, 2016). It is a facultative anaerobic bacterium, capable of surviving in the presence or absence of oxygen. It can grow and reproduce inside the host's cells and is one of the most virulent foodborne pathogens.
2.2.1.4. *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is Gram-negative rod belongs to the family *Pseudomonadaceae*. More than half of all clinical isolates generate the blue-green pigment pyocyanin; this pigment is supportive in the identification of the organism and accounts for the species name “*aeruginosa*” (Harris, 1999).

*P. aeruginosa* has a strong link for growth in moist environments, which is most likely a manifestation of its natural survival in soil and water. These natural properties of the bacterium contribute to its ecological success as an opportunistic pathogen. These also help to explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen. These infections are difficult to manage, in part because of the natural resistance of the bacterium to antibiotics and ultimately lead to pulmonary failure and death (Stover *et al.*, 2000).

2.2.1.5. *Shigella boydii*

The genus *Shigella* is found in the family *Enterobacteriaceae*. It is Gram-negative, facultatively anaerobic, non-sporulating, non-motile and rod-shaped bacteria. *S. boydii* inhabits the intestine and rectum of humans and other primates (Ansaruzzaman *et al.*, 2005). It can survive in feces and soil or food and water contaminated with fecal matter. *Shigella* bacteria cause diarrhea and shigellosis through the oral-fecal transmission. Once ingested, the *Shigella* makes its way through the gastrointestinal tract until it reaches the epithelial cells of the intestinal mucosa, where it infects, causing inflammation (Lee *et al.*, 2006).
2.2.1.6. *Shigella flexneri*

*Shigella flexneri* is a Gram-negative bacterium which causes the most infectious of bacterial dysenteries, shigellosis. As reviewed by Jennison and Verma (2003), the high incidence of *Shigella* in developing countries is generally attributed to the lack of clean water, poor sanitation, malnutrition and cost of antibiotic treatment. *S. flexneri* is increasingly developing antibiotic resistance (Kotloff *et al.*, 1999; Jennison and Verma, 2003).

2.2.1.7. *Staphylococcus aureus*

*Staphylococcus aureus* is facultatively anaerobic, Gram-positive, non-motile cocci, ferment glucose and has large, round, golden-yellow colonies. The golden appearance is the etymological root of the bacterium's name; *aureus* means "golden" in Latin. Cell division occurs in more than one plane; so that cells form irregular clumps resembling bunches of grapes. *S. aureus* is catalase and coagulase-positive which are used as unique characteristics. It is mesophile with a growth temperature range between 7 and 48°C (Ebrahimi and Akhavan, 2009). According to the study conducted by Reiß *et al.* (2011), with the emergence of multi-resistant strains, antimicrobial therapy of *S. aureus* infections has to turn into an escalating challenge.

2.2.1.8. *Staphylococcus epidermidis*

*Staphylococcus epidermidis* is Gram-positive which belongs to the group of coagulase-negative *staphylococci*, which is discriminated from coagulase-positive *staphylococci* such as *S. aureus* by its lack of the enzyme coagulase (Otto, 2009). *S. epidermidis* represents the most frequent
causative agent involved with infections which include any kind of medical devices, such as peripheral or central intravenous catheters. Specifically, catheter-related infections are associated with increased mortality and contribute to an increased length of hospital stay and higher healthcare costs, which are problematic in limited-resource settings (Rogers et al., 2009).

2.2.2. Fungal test organism

A number of *Candida* species are commensal and colonize the skin and mucosal surfaces of humans. According to the report of Kusuma et al. (2017), seriously ill or immunocompromised patients are more susceptible to develop both superficial and life-threatening *Candida* infections.

An increase in the number of yeasts that are resistant to antifungal drugs is documented worldwide. The ability of *Candida* species to form drug-resistant biofilms is a vital factor in their contribution to human disease. As reported by Sardi et al. (2013), the development of drug resistance in *Candida* biofilms has been related to a parallel increase in the maturation process. In addition, some studies have also shown that biofilms of *Candida* extend statistically in the presence of a minimal matrix and exhibit the same level of resistance to drugs (fluconazole and amphotericin B) as cells grown in a shaker and exhibiting large amounts of the matrix (Jasim et al., 2016). The increase in resistant strains necessitates a search for new targets for new antifungal agents.
2.3. Antimicrobial Drug Resistance

Use of antibacterial drugs has become widespread over several decades and these drugs have been widely misused for humans medicine and prophylactic agents for food-producing animals in ways that favor the selection and spread of resistant bacteria. As a result, antibacterial drugs have become less successful or even ineffective, which is disputing for the frequency of resistance outpace the discovery of new drugs (Ventola, 2015). The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection.

According to WHO (2014), *K. pneumonia* is resistance to third-generation cephalosporins and carbapenems. Likewise, *Shigella* species is resistance to fluoroquinolones. *E. coli* is resistance to third-generation cephalosporins and fluoroquinolones, whereas *S. aureus* is resistance to the same antibiotics and carbapenems.

2.4. Anti-inflammatory Drugs

Inflammatory conditions such as arthritis are associated with increased risk of cardiovascular diseases (Maradit-Kremers et al., 2005). Evidence suggests that common proinflammatory mediators are associated with the development and/or progression of both cardiovascular complications and arthritis.
The majority of clinically important medicines used for the treatment of various inflammatory diseases belong to the steroidal and non-steroidal chemical groups (Choi and Hwang, 2003; Iwalewa et al., 2007).

In addition, the non-steroidal anti-inflammatory drugs that are used to treat inflammation and pain also increase both cardiovascular and renal risks (Harirforoosh et al., 2013). The greatest disadvantage of the potent synthetic anti-inflammatory drugs is the reappearance of symptoms after discontinuation.

2.5. Therapeutic Potential of Plants as Sources of Antioxidants, Antimicrobials and Anti-inflammatory Drugs

Plants serve as a source of various medicinal compounds which are used to treat infectious and non-infectious diseases. There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments (Kupeli et al., 2007). Medicinal plants for treatment of various diseases is getting more and more popularity (Juneja et al., 2007; Rakotoarivelo et al., 2015).

Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring substances in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary metabolites include
terpenoid, alkaloids and phenolic compounds. Plant phenolics have multiple biological functions such as antioxidant, anti-inflammatory, anti-cancer and anti-microbial activities (John and Grohmann, 2001). Alkaloids are applied in medicine to reduce pain and fever as well as remedy for gout, tumor and hypertension (Babbar, 2015). Flavonoids have protective effects against bacterial, viral and degenerative diseases such as cancers, cardiovascular and other age-related diseases (Kumar and Pandey, 2013). Saponins possess antibacterial and antifungal activities (Khanna and Kannabiran, 2008) and anti-inflammatory activity as well (Patel and Patil, 2012). Tannins are very important in that they facilitate healing of wounds and inflammed mucous membranes (Yadav et al., 2014). Terpenoids constitute antimicrobial, antiviral, anti-allergenic, antihyperglycemic and anti-inflammatory properties (Wagner and Elmadfa, 2003).

2.5.1. Antioxidant activities of medicinal plants

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products (Pizzino et al., 2017). Supplementing the natural defense mechanism with dietary antioxidants might offer better protection against the risk of certain cancers, inflammation and other degenerative diseases (Madhujit and Shahidi, 2008). The addition of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in lipid containing foods is often discouraged due to their safety and perceived carcinogenicity. On the other hand, the use of plant-based antioxidant compounds in foods and preventive medicine are gaining a great deal of interest because of their potential health benefits (Hussain et al., 2008). It is well accepted that plants are the richest source of antioxidants. Among plants, cereals and legumes are prominent because they contain a wide
array of phenolics. In view of the beneficial effects and vital role that natural antioxidants can play in human health, a greater demand currently exists for their isolation from more and more plants and agro waste materials using some effective extracting techniques.

2.5.2. Antimicrobial activities of medicinal plants

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency (Neogi et al., 2008). In addition to this problem antibiotics are sometimes associated with adverse side effects on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions (Al-Jabri, 2005). There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection (Sung and Lee, 2007).

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans et al., 2002). Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed (Hussain and Gorsi, 2004; Saklani et al., 2013).
The use of botanical medicines is generally on the rise in many parts of the world. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new microbial agents (Costa et al., 2008; Atanasov et al., 2015; Anyanwu and Okoye, 2017). To date, numerous experiments have been carried out to screen natural products for antimicrobial property (Akinyemi et al., 2005; Kuete et al., 2007; Aslim and Yucel, 2008; Ndhlala et al., 2009; Anyanwu and Okoye, 2017).

2.5.3. Anti-inflammatory activities of medicinal plants

Several medicinal plants have been reported with anti-inflammatory activity (Adegbola et al., 2017). Classic examples of herbs traditionally used to treat inflammation in Western medicine are *Matricaria chamomilla* L. and *Arnica montana* L. (Asteraceae), *Salix alba* (Salicaceae) and *Glycyrrhiza glabra* (Fabaceae). Other well-known plant products with anti-inflammatory activity are the distillate of *Hamamelis virginiana* (witch hazel; Hamamelidaceae), *Echinacea* species including *Echinacea angustifolia* (purple coneflower; Asteraceae), *Ananas comosus* (pineapple; Bromeliaceae), *Abelmoschus esculantus* (bhindi, Malvaceae) (Shah and Seth, 2010).

The number of new, pharmacologically active herbal ingredients, in particular that of anti-inflammatory compounds, rises continuously and very important plant-derived anti-inflammatory compounds: curcumin, colchicine, resveratrol, capsaicin, epigallocatechin-3-gallate and quercetin were isolated (Robert Fürst and Ilse Zündorf, 2014). The screening and development of drugs for anti-inflammatory activity is still in progress and there is much hope for finding anti-inflammatory drugs from indigenous medicinal plants (Dongmo et al., 2003; Kupeli et al., 2007; Juneja et al., 2007; Hossain et al., 2012; Kanagasanthosh et al., 2015; Ben Salem et al., 2017).
2.6. Description of the Study Plants

2.6.1. Botanical description of Cyphostemma

The genus *Cyphostemma* belongs to the family *Vitaceae* which includes 250 species, it is erect, trailing or climbing perennial herbs, rarely woody climbers or succulent shrubs; tendrils is opposite to the leaves or absent. Leaves are digitately 3-7(-11)-foliolate, rarely simple or pedate. Flowers are 4-merous, bisexual, in leaf-opposed, axillary or terminal umbelliform or corymbiform cymes, ultimately in small divaricately branched cymules. Buds cylindric to lageniform, constricted near the middle, ± square at apex; calyx subentire; petals usually yellowish to cream with red tips and edges, dropping separately or as a hood; a disc of 4 free glands, adnate to ovary; style long, stigma bifid or entire. Fruit 1(-2)-seeded, ripening through red to blackish; seed with a central dorsal ridge and 2 lateral crests, sides variously sculptured to almost smooth (Gebre Egziabher *et al*., 1989).

*C. cyphopetalum* (gindosh in Amharic language) a tropical Africa climber, herbaceous, scrambling succulent leaves, creamy yellow flower (Gebre Egziabher *et al*., 1989). *C. cyphopetalum* is distributed in Ethiopia, Eritrea, Chad, Cameroon, Central African Republic, Sudan, South Sudan, Congo, Uganda, Rwanda, Burundi, Kenya, Tanzania, Northern Zambia, Malawi and Yemen (Hassler, 2017).

Locally *C. junceum* is called etse zewe in Ge’ez language. *C. junceum* is a tropical Africa herb, erect, thick woody rhizome, succulent leaves, and flowers deep yellow. It can grow in a mixed

2.6.2. Bioactive compounds from *Cyphostemma*

Phytochemical investigation of the methanol extract of *C. adenocaula* liana (bark and wood) led to the isolation of two new ceanothane-type triterpenoids, cyphostemmic acid A 1 and cyphostemmic acid B 2, together with the known triterpenoids, β-sitosterol and its glucoside (Chouna et al., 2016). Bioassay-guided fractionation of the ethanol extracts obtained from a plant identified as *C. greveana* Desc. (*Vitaceae*) led to the identification of one macrolide, lasiodiplodin, three sesquiterpenoids and diterpenoid (Cao et al., 2011).

Six antifungal constituents were isolated from the roots of *C. crotalaroides* (*Vitaceae*): a new product, cis-ε-viniferin along with five known compounds trans-resveratrol and its oligomers: trans-ε-viniferin, gnetin C, pallidol and gnetin E (Bala et al., 2000). By the same author resveratrol and its oligomers: ε-viniferin, gnetin C, pallidol and gnetin E, as well as three new dehydrodimers, cyphostemmins A-C, have been isolated from the roots of *C. crotalaroides* (Bala et al., 1999).
2.6.3. Ethnobotanical studies and biological activities of *Cyphostemma* species

Most of the plant species which belongs to the genus *Cyphostemma* are used in traditional medicine including: *C. cyphopetalum* (Megersa *et al.*, 2013), *C. digitatum* (Khan *et al.*, 2016), *C. junceum* (Teklehaymanot and Giday, 2007) and *C. vogelii* (Udegbunam *et al.*, 2013).

According to the study of Kipkore *et al.* (2014), the tuber of *C. cyphopetalum* is used to eradicate all insects. It is crushed, water added and the resultant concoction is used against all insects. When used to kill household insects like mites, the concoction is left standing in the room for one day and after that, the room left for at least two days before occupation since the plant is very poisonous. It is also employed as a poison to kill unwanted animals.

In the study reported by Teklehaymanot *et al.* (2007), people around the monastery of Debre Libanos in Ethiopia, use the stem of *C. cyphopetalum* for the treatment of snake bite by chewing and oral administration of the infusion of the roots for rabies treatment. As reported by Lulekal (2008), in Southeastern Ethiopia (Mana Angetu District) people used the fresh root of *C. cyphopetalum* to treat diarrhea, cough in horse and donkey and inability to walk properly by crushing and drinking.

In Kenya, Lindsay and Hepper (1978), reported the use of the leaves of *C. cyphopetalum* to treat skin eruption, chicken-pox, and adenopathy by pounding the leaves at the site of local application. As reported by Kokwaro (1976), the pounded leaves of *C. cyphopetalum* used for the treatment of itchy rash and enlarged glands. In Kenya, people used the leaves of *C. cyphopetalum* for the treatment of rash, dermatitis, and itch by applying the crushed leaves on the infection site (Kokwaro, 1987).
In Rwanda, the leaves of *C. cyphopetalum* used to treat phagedenic ulcer (the crude water extract paste on the wound), sciatic (pounded, decoction with water taken orally), pain in the joints (pounded), angina (pounded, juice taken orally), nephritic colic (the crude water extract administered orally) and kwash (maceration taken orally) (Kayonga and Habiyaremye, 1987). In Zimbabwe; the tuber powder of *C. junceum* is applied on blister, hygroma, edema, brucellosis, and leprosy; the tuber infusion is used to treat diarrhea, colic and venereal diseases; the tuber powder with porridge is applied for easier delivery and against painful menstruation (Gelfand *et al.*, 1985)

As reported by Teklay *et al.* (2013), in Ethiopia; the root bark of *C. junceum* is crushed and is eaten with honey to treat a snake bite, the plant’s whole part is applied for a toothache and spider bite through chewing and swallowing the juice, and the leaf is used for the evil eye by placing it on fire for fumigation. The studies of Teklehaymanot and Giday (2007) show that the root of *C. junceum* is effective in the treatment of snake bite when the crushed fresh root is taken orally.

According to the study conducted by Khan *et al.* (2016), *C. digitatum* stem and roots extracts were investigated for antioxidant, antimicrobial, urease inhibition potential and phytochemical analysis was also done. As they reported, the methanolic extracts of the roots displayed highest antioxidant activity (93.518%) against DPPH while the crude methanolic extract of the stem showed highest antioxidant activity (66.163%) at 100 μg/mL concentration. Based on their finding they concluded that the methanolic extracts of both stem and roots were moderately active or even found to be less active against the selected bacterial and fungal strains.
According to the study reported by Bala et al. (2000), one of the extracts, obtained from *C. crotalarioides* exhibited a very promising antifungal activity against *Fusarium nivale*. In their previous report, they have described the isolation and the identification of resveratrol and some of its known natural oligomers from the roots of *C. crotalarioides*. This type of compounds were known to be produced by numerous plant species and proved to exhibit cancer chemopreventive, antifungal, and antibacterial activities. Compounds of *C. greveana* showed antiproliferative activities (Cao et al., 2011). As reported by Eleojo et al., (2012) *C. glaucophilla* may serve as a cheap alternative in maintaining liver and kidney disorders.

2.6.4. Distribution of *C. cyphopetalum* and *C. junceum* in Ethiopia

*C. cyphopetalum* and *C. junceum* distributed in different parts of Ethiopia including Tigray, Welo, Shewa, Gonder, Gojam, Welega, Afar, Harerge, Sidamo, Ilubabor, Bale, Arsi and Gamo gofa (Gebre Egziabher et al., 1989).
CHAPTER 3: MATERIALS AND METHODS

3.1. The Plant Collection Site

The plant collection sites for *C. cyphopetalum* were the town of Adama (located at 8.50°N 39.25°E at an elevation of 1654 m, 88.8 km southeast of Addis Ababa) and Sebeta 19.3 km far from Addis Ababa (located at 8.91°N 38.63°E at an elevation of 2207m and while for *C. junceum* were Debre Libanos monastery (located at 9.71°N and 38.85°E, 108 km from Addis Ababa in the North Shewa Zone of Oromia State at 2398 m altitude) and Huruta (located at 8.15°N and 39.35°E, at an altitude of 2040m and 132km far from Addis Ababa). The reason for collecting the two plants from a different location is due to their use for different purposes.

![Figure 1](image_url)

**Figure 1:** The sample collection sites captured from google map (accessed on September 16, 2017)
3.2. Plant Collection and Identification

The leaves and roots of *C. cyphopetalum* and *C. junceum* were collected between July and November 2015. The plants were identified by a taxonomist and voucher specimens (T09 and T10) representing the plants were deposited at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia. The leaves and the roots were washed with tap water and allowed to dry at room temperature (22-25°C) in the shade of biomedical sciences stream and the dried specimens were manually grounded to powder form using a mortar and pestle. The powder then, sieved through the 250µm mesh to obtain a fine material. The powdered fine plant materials were transferred into closed amber colored bottles until use.

3.3. Preparation of Crude Extract and Solvent Fractionation

In this study, 500g of each air dried leaves and roots of *C. cyphopetalum* and *C. junceum* were processed for extraction and fractionation of active compounds according to Pereira *et al.*, 2015 and Zazouli *et al.*, 2016. At first the leaves and roots samples were separately macerated in 80% (V/V) methanol for 72 hours. After filtration, the extracts were then concentrated under vacuum in a rotary evaporator from which 42g of residue from the leaves of *C. cyphopetalum*, 40.5g from the leaves of *C. junceum*, 32g and 30.4g were obtained from the roots of *C. cyphopetalum* and *C. junceum* respectively (Figure 2a).
Figure 2a: Methods of extraction of the plant samples with 80% methanol and fractionation using the solvent methanol, ethanol and acetone.
Similarly, 500g of the leaves and roots of *C. cyphopetalum* and *C. junceum* separately were macerated in distilled water for 72 hours. Following filtration and storage in the deep freeze (at -20°C) for 24 hours, the distilled water extracts of the leaves and roots of *C. cyphopetalum* and *C. junceum* was lyophilized using lyophilizer (Alpha 2-4 LD plus) and 40g of powdered residue from the leaves of *C. cyphopetalum* and 38.6g of powdered residue from the leaves of *C. junceum* were obtained and residues from the roots of *C. cyphopetalum* and *C. junceum* were 34g and 32.8g respectively (Figure 2b).
Figure 2b: Methods of extraction of the plant samples with distilled water and fractionation using methanol, ethanol, and acetone.
Additionally, air-dried and powdered leaves and roots (500g each) of *C. cyphopetalum* and *C. junceum* separately were macerated in 80% (V/V) methanol for 72 hours. After filtration, the extracts were then being concentrated under vacuum in a rotary evaporator and 41.8g of residue from the leaves of *C. cyphopetalum* and 40g from the leaves of *C. junceum* was obtained and residues from the roots of *C. cyphopetalum* and *C. junceum* were 31.6g and 30g respectively (Figure 2c).

**Figure 2c:** Methods of extraction of the plant samples with 80% methanol and fractionation using distilled water.
3.4. Isolation of Compounds from the Leaves of C. cyphopetalum

The isolation and characterization of the compounds were done in the Department of Chemistry of the College of Natural and Computational Sciences, AAU. The shade dried and powdered (100g) leaves of C. cyphopetalum were extracted with ethanol by placing on a shaker for 12 hours. These were filtered and concentrated to give 8 g of ethanol extracts. The ethanol extracts (8 g) of the leaves of C. cyphopetalum was applied on the column after adsorbed on silica gel and eluted with n-hexane, chloroform, ethylacetate and methanol to afford 24 fractions A-X. Fraction C was recrystallized from chloroform and methanol to afford a β-sitosterol. Fraction E was applied on preparative TLC and gave a diterpenoid. Fraction M was applied on Sephadex to give salicylic acid and mixtures of flavonoid glycosides. The solution containing fraction F was concentrated to give luteolin. After repeated washing fraction K with chloroform, it gave sitosterol glucoside. Fraction L was applied on preparative TLC and gave a resveratrol dimer. Fraction N was left in solution for a week and gave a sugar crystal. Due to economic reason the rest of the fractions (i.e. fractions A,B,D,G,H,I,J,O,P,Q,R,S,T,U,V,W,X) were not identified.

Nuclear magnetic resonance (NMR) was used to identify the structure of the fractions. $^1$D spectrum ($^1$HNMR and $^{13}$CNMR) was used to get the number of proton and carbon respectively. The structure of each compound was constructed using Chem-Draw software and identified based on an available literature.
3.5. Acute Toxicity Test

The toxicity test was performed on Swiss albino female mice (weighing 20-30g) at the age of 8 - 12 weeks, which were obtained from the Department of Biology Addis Ababa University. All of them were acclimatized to the animal house of the department prior to use for two weeks. Mice were kept in cages in animal house and they were fed on standard pellets (layers feed) and tap water ad libitum.

An acute toxicity study was carried out according to the organization of economic cooperation and development (OECD, 2001) guidelines 420 for both 80% methanol extracts of the leaves and roots of *C. cyphopetalum* and *C. junceum* using female Swiss albino mice that were fasted for 4 hours. Thirteen groups of healthy mice each consisting of 5 mice were divided randomly into a control and twelve treatment groups which was left without food for 4 hours and weighted. The experimental groups orally received 80% methanol extracts of the leaves and roots of *C. cyphopetalum* and *C. junceum* at a dose of 1000, 2000 and 5000mg/kg body weight. Control group received only distilled water. The mice were then kept under close observation for 6 hours after administering the extract and then they were observed daily for fourteen days for any change in the general behavior and/or other physical activity(Jothy et al., 2011). These include hair erection, ability to feed, general appearance, weight loss (weekly) and death. Attention was also given to signs of salivation, diarrhea, convulsion, sleep, and coma (OECD, 2001).
3.6. Qualitative Phytochemical Analysis

The types of different secondary metabolites exist within the leaves and the roots of *C. cyphopetalum* and *C. junceum* were determined as per the method described by Linga Rao and Savithramma (2011). The detection of secondary metabolites was carried out on 80% methanol leaves and roots extracts. Accordingly, each extract (100 mg) was dissolved in 100 mL of methanol and filtered through Whatman No.1 filter paper. Thus, the filtrate obtained was used for the preliminary phytochemical screening tests (Appendix 2).

3.7. Evaluation of the Antioxidant Activity

The radical scavenging properties of the water extracts and methanol solvent fractions of the leaves and roots of the two plants were determined using 2, 2 diphenyl-1-picrylhydrazyl hydrates (DPPH) radical scavenging activity as described by Adedapo *et al.* (2009). Sample stock solutions (25mg/mL) were diluted to final concentrations of 12.5, 6.25, 3.125, 1.563, 0.78, 0.39 and 0.195mg/mL in methanol. 2mL samples were added to 2 mL of freshly prepared methanolic DPPH (0.5 mM). The mixture was shaken and left to stand at room temperature in the dark. After 30 min, absorbance was measured at 517 nm against a blank containing methanol using the UV-Vis spectrophotometers. Ascorbic acid was used as the positive control with similar concentration of the plant test sample. Assays were carried out in triplicate.
The percentage of inhibition of DPPH was calculated using the following equation:

\[ I\% = \left(\frac{A_0 - A}{A_0}\right) \times 100 \]

Where,

\[ A_0 = \text{the absorbance of the blank solution (methanol + DPPH), and} \]

\[ A = \text{the absorbance of the water extracts or methanol solvent fractions +DPPH.} \]

3.8. Evaluation of the Antimicrobial Activity

3.8.1. Preparation of test solution

500mg of 80% methanol extracts, and methanol, ethanol, and acetone solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* were measured and dissolved in 1mL of 3% of Tween 80.

3.8.2. Bacterial and fungal strains

The standard bacterial and fungal test micro-organisms used in this investigation were the Gram-negative bacteria; *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Shigella boydii* (ATCC9207), *Shigella flexineri* (ATCC12022), *Klebsiella pneumonia* (ATCC70603) and the Gram-positive bacteria; *Staphylococcus aureus* (ATCC29213), *Staphylococcus epidermidis* (ATCC12228), *Listeria monocytogens* (ATCC19115) and the fungal test organism; *Candida albicans* (ATCC10231). The bacterial and fungal test organisms were obtained from Ethiopian Public Health Institute (EPHI) Addis Ababa.
3.8.3. Standard antibiotic and antifungal drugs

Ciprofloxacin and fluconazole were used as positive control for the antibacterial and antifungal susceptibility test respectively whereas 3% Tween 80 served as a negative control for both tests.

3.8.4. Inoculum preparation

In order to obtain pure bacterial culture the standard test organisms with a mixed colony were streaked on differential or selective media, that is, Mannitol salt agar for *S. aureus*, *Pseudomonas* isolation agar for *P. aeruginosa* and MacConkey agar for *E. coli*. A pure colony of bacteria was cultivated on nutrient agar for 24 hours. The inoculums were standardized by inoculating the pure colony of the bacteria from the nutrient agar in normal saline and compared with 0.5 McFarland turbidity standard (that was prepared by adding a 0.5mL aliquot of 0.48 mol/L BaCl₂ (1.175% w/v BaCl₂. 2H₂O) added to 99.5 mL of 0.18 mol/L H₂SO₄ (1%v/v).

In order to activate and obtain a pure colony the fungal test organism, *C. albicans* was streaked using sabouraud’s dextrose agar and incubated at 37°C for 24 hours then the pure colony of *C. albicans* was inoculated in potato dextrose broth medium at 37°C for 24 hours.
3.8.5. Antibacterial susceptibility test

3.8.5.1. Agar well diffusion method

Agar well diffusion assay (AWD) was done following the procedure described by Adegoke et al. (2010). 1mL culture of each test bacteria (10^8/mL) was inoculated separately on petri dishes containing solidified Mueller Hinton agar (MHA), spread using rod spreader and allowed to set. Then, wells of 5mm diameter were made in the solidified agar using a sterile cork borer. About 30µL of 80% methanol extracts, methanol, ethanol and acetone solvent fractions at the concentration of 500mg/mL (dissolved in 3% Tween 80) were dispensed into the wells and allowed to stand for about 15 min for diffusion. As a positive control, 30µL of ciprofloxacin at a concentration of 1 mg/mL and 3% Tween 80 (negative control) were also loaded into respective wells of each agar plates. The plates were then incubated at 37ºC for 24 h. The sensitivity of the test bacteria to the 80% methanol extracts and solvent fractions was determined by measuring the diameters of the zone of inhibition surrounding the wells in millimeter (mm) using vernier caliper. All tests were performed in triplicate.

3.8.5.2. Agar disc diffusion method

The inhibitory activity of 80% methanol extracts and solvent fractions against the test organisms was determined using agar disc diffusion method as described by Valgas et al. (2007), accordingly, 1mL of inoculums from the overnight growing and activated bacteria were mixed with 9mL of sterile normal saline and compared with the turbidity standard 0.5 McFarland solution which is equivalent to 10^6CFU/mL. Then, 30µL of each bacterial inoculum was
transferred and uniformly spread on the Mueller Hinton agar (MHA) using a sterile glass rod spreader. The inoculated plates were left at room temperature for 3-5 minutes to allow for any surface moisture to be absorbed before applying the test sample. The 80% methanol extract, methanol, ethanol, and acetone solvent fractions were dissolved in 3% Tween 80 at a concentration of 500mg/mL. Ten microliters of 80% methanol extract and solvent fractions and ciprofloxacin were transferred onto a sterile filter paper disc (Whatman No.1; 6 mm in diameter) and allowed to dry for 30 minutes. With precaution, the disc with positive control ciprofloxacin (1mg/mL), 80% methanol extracts, and methanol, ethanol, and acetone solvent fractions were applied on the Mueller Hinton agar and left for 15 minutes to allow diffusion. The plates were then incubated at 37°C for 18-24 hours and the inhibition zone was measured using vernier caliper. All tests were performed in triplicate.

3.8.5.3. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by the broth dilution method. A 2-fold serial dilution of the methanol solvent fraction of the leaves of *C. cyphopetalum* and roots of *C. junceum* (7.81mg/mL, 15.63mg/mL, 31.25mg/mL, 62.5mg/mL, 125mg/mL, 250mg/mL, 500mg/mL), was prepared in glass tubes using distilled water. Standardized suspension of bacteria(1mL) was added to 4.5 mL of Mueller Hinton broth containing diluted methanol solvent fractions in glass test tubes. Positive controls were made of broth and inoculum only. The contents of each tube were mixed on a vortex and then incubated at 37°C for 24 hours. The lowest concentrations of the test tubes which did not show any visible growth after the physical observation was considered as MIC (Oskay *et al.*, 2009).
3.8.5.4. Determination of minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined after the MIC test was completed. The broth without growth was sub-cultured on Mueller Hinton agar at 37°C for 24 hours. The lowest concentration of the sub-cultured plates which did not show any visible growth after the macroscopic evaluation was considered as MBC (Mathur, 2013). All the antibacterial parameters were determined by triplicate assays.

3.8.6. Antifungal susceptibility test

3.8.6.1. Agar well diffusion method

Antifungal activities of 80% methanol extracts, and methanol, ethanol, and acetone solvent fractions at the dose of 500mg/mL (dissolved in 3% Tween 80) were tested according to Nejad et al. (2014). One hundred microliters of C. albicans was uniformly spread onto Mueller Hinton agar using glass rod spreader. Then four wells of 5mm diameter were punched by a cork borer into the Mueller Hinton agar and filled with 30 µL of each plant extracts and solvent fractions as well as 3% Tween 80 prepared with sterile distilled water as a negative control and 30 µL of fluconazole at a dose of 1mg/mL. The plates were incubated at 37°C for 24 hours. Antifungal activity was determined by measuring the zone of inhibition using vernier caliper. Each experiment was carried out three times.
3.8.6.2. Agar disc diffusion method

The antifungal activities of 80% methanol extracts, and methanol, ethanol, and acetone solvent fractions at the concentration of 500mg/mL (dissolved in 3% Tween 80) were tested according to Masomi and Hassanshahian (2016). *C. albicans* was grown in potato dextrose broth medium at 37°C for 24 hours, from which one mL *C. albicans* was added on the surface of each plate containing Mueller Hinton agar and swabbed using a sterile cotton swab and allowed to remain in contact for 1 minute. The disc prepared from sterile 6mm filter paper was placed into 500mg/mL concentration of each plant extracts and solvent fractions for 1 hour. The disc was placed for 30 minutes at room temperature and transferred to Mueller Hinton agar. The disc soaked in 3% Tween 80 was used as negative control and the disc soaked in fluconazole (1mg/mL) was used as positive control. To determine the antifungal activity, the inhibition zone was measured in millimeter using vernier caliper and the assay was carried out three times for each test sample.

3.9. Evaluation of Anti-inflammatory Activity

*In vivo* anti-inflammatory activities of 80% methanol extracts and water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* and β-sitosterol isolated from the ethanol leaves extracts of *C. cyphopetalum* were evaluated on the basis of inhibition of carrageenan-induced mice hind paw edema as previously described by Winter *et al.* (1962) and Singh *et al.* (2010). Thus, the mice were divided into 11 groups (*n* = 6) as shown in Table 1.

The paw edema was induced thirty minutes after administration of distilled water, indomethacin, 80% methanol extracts and water fractions of the leaves and roots of *C. cyphopetalum* and *C.
Junceum and β-sitosterol isolated from the ethanol leaves extracts of C. cyphopetalum. To induce acute inflammation, all groups of mice (0.1 mL of freshly prepared 1 % w/v suspension of carrageenan in distilled water) were injected subcutaneously to the plantar surface of the left hind paw. Mice paw thickness was measured just before carrageenan injection (0 hours) and at 1, 2, 3, 4, 5 and 6 hours after administration of carrageenan using a micrometer. Percentage reduction of edema was calculated as described by Kanagasanthosh et al. (2015).

\[
\text{Mean increase in paw diameter} - \text{Mean increase in paw diameter} \\
\quad \text{in vehicle group} \quad \text{in drug-treated group} \\
\quad \frac{\text{Mean increase in paw diameter in vehicle group}}{\text{Mean increase in paw diameter in drug-treated group}} \times 100
\]

Where,

Vehicle group = received only the distilled water and carrageenan

Drug-treated group = received 80% methanol extracts, water fractions, β-sitosterol, and indomethacin
Table 1: Group of mice treated with 80% methanol extracts and water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment which was given to mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>80% methanol leaves extracts of <em>C. cyphopetalum</em> (500mg/kg)</td>
</tr>
<tr>
<td>II</td>
<td>Water fractions of the leaves of <em>C. cyphopetalum</em> (300mg)</td>
</tr>
<tr>
<td>III</td>
<td>80% methanol roots extracts of <em>C. cyphopetalum</em> (500mg/kg)</td>
</tr>
<tr>
<td>IV</td>
<td>Water fractions of the roots of <em>C. cyphopetalum</em> (300mg/kg)</td>
</tr>
<tr>
<td>V</td>
<td>80% methanol leaves extracts of <em>C. junceum</em> (500mg/kg)</td>
</tr>
<tr>
<td>VI</td>
<td>Water fractions of the leaves of <em>C. junceum</em> (300mg/kg)</td>
</tr>
<tr>
<td>VII</td>
<td>80% methanol roots extracts of <em>C. junceum</em> (500mg/kg)</td>
</tr>
<tr>
<td>VIII</td>
<td>Water fractions of the roots of <em>C. junceum</em> (300mg/kg)</td>
</tr>
<tr>
<td>IX</td>
<td>β-sitosterol (25mg/kg)</td>
</tr>
<tr>
<td>X</td>
<td>Indomethacin (10mg/kg)</td>
</tr>
<tr>
<td>XI</td>
<td>Distilled water (10mL/kg)</td>
</tr>
</tbody>
</table>

3.10. Ethical Consideration

The ethical clearance was obtained from College of Natural and Computational Science Institutional Review Board (CNS-IRB) in its meeting held on 26/03/2015 minute number IRB/015/2015. Swiss albino mice used for the acute toxicity test were kept in cages in animal house. They were provided with tap water *ad libitum* and fed on standard pellets (layers feed).
3.11. Data Analysis

The data were analyzed using window SPSS version 20 and descriptive values were expressed as mean± SEM (standard error of the mean). Data on the antimicrobial activity test were analyzed using oneway ANOVA followed by Tukey-HSD post hoc test whereas results of the anti-inflammatory property were analyzed using one way ANOVA followed by Dunnet’s test. Differences were considered as statistically significant at $P < 0.05$ when compared with control.
CHAPTER 4: RESULTS AND DISCUSSION

4.1. Acute Toxicity of the Leaves and Roots of C. cyphopetalum and C. junceum

In this study, an oral acute toxicity of the leaves and roots of C. cyphopetalum and C. junceum were investigated on mice model with the objective of detecting any possible adverse effect. However, all mice in both the experimental and control group neither died nor showed any physical sign of acute toxicity. Also, C. vogelii, a plant within the same genus, did not show any toxicity and mortality in all treatment groups (Udegbunam et al., 2013). Furthermore, in their report symptoms such as convulsion and diarrhea that are suggestive of toxicity were not observed. Thus, the leaves and roots of C. cyphopetalum and C. junceum did not constitute sufficient amount of toxic phytochemicals that can kill mice or induce signs of acute toxicity up to the dose of 5000mg/kg body weight.

4.2. Phytochemical Constituents of the Leaves and Roots of C. cyphopetalum and C. junceum

4.2.1. Qualitative phytochemical screening

In this work, the qualitative phytochemical screening of the 80% methanol leaves and roots extracts of the two plants revealed the presence of alkaloids, catechol tannins, saponins, flavonoids and terpenoids (Table 2). The existing reports also disclosed that plants that belong to the genus Cyphopetalum constitute different phytochemicals. For example Khan et al. (2015) reported the presence of flavonoids, alkaloids, saponins, terpenoids, tannins, reducing sugars,
carbohydrates and phenolic compounds in the methanol extract of the roots of *C. digitatum*. In addition, they noted the existence of flavonoids, alkaloids, saponins, terpenoids, betacyanins, carbohydrates, phenolic, free reducing sugars and coumarins in the stem extract of *C. digitatum*. Also, the presence of flavonoids, cardiac glycoside, tannins and saponins in the aqueous extract of the leaves of *C. glaucophilla* was also confirmed by Ojogbane *et al.* (2015).

**Table 2**: The preliminary phytochemical screening of secondary metabolites from 80% methanol leaves and roots extracts of *C. cyphopetalum* and *C. junceum*

<table>
<thead>
<tr>
<th>Types of secondary metabolites</th>
<th>Type of plant extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. cyphopetalum</em></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Catechol tannins</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
</tr>
</tbody>
</table>

Note: + shows moderate presence; ++ shows strong presence

4.2.2. Distribution of active compounds from the ethanol leaves extract of *C. cyphopetalum*

The column chromatography of the ethanol leaves extracts of *C. cyphopetalum* led to the isolation of different compounds as shown in Figure 3. In brief, β-sitosterol (60 mg), salicylic
acid (20 mg), mixtures of flavonoid glycosides (30 mg), luteolin (15 mg) and sugar crystal (300 mg) were obtained.

**Figure 3:** Group of compounds isolated from the leaves of *C. cyphopetalum*

### 4.3. Antioxidant Properties of the Leaves and Roots of *C. cyphopetalum* and *C. junceum*

On radical scavenging test using DPPH, both the crude water extracts and the methanol solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* showed dose-dependent increase in activities ranging from 23.1% to 92.2% at concentrations varying from 0.195–12.5 mg/mL. A highest antioxidant activity was produced by crude water extracts of both plants with a percent inhibition of 86.1% for the leaves, 81.0% for the roots of *C. cyphopetalum* and 79.5% for the leaves and 78.0% for the roots of *C. junceum* at 12.5 mg/mL. The crude water extracts of the
leaves and roots of both plants showed a lowered DPPH scavenging activity compared with ascorbic acid at each concentration tested (Table 3).

The methanol solvent fractions of the leaves of *C. cyphopetalum* exhibited the highest radical scavenging activity (92.2%) at 12.5 mg/mL concentration. In addition, the methanol solvent fractions obtained from the roots of *C. cyphopetalum* exhibited a raised scavenging activity of 91.3% at 12.5 mg/mL higher than that of ascorbic acid (90.9%) as shown in Table 4. To sum, the methanol solvent fractions of the leaves and roots of *C. cyphopetalum* exhibited highest antioxidant activity at each tested concentration in comparison to ascorbic acid and the methanol solvent fractions of the leaves and roots of *C. junceum* (Table 4).

The antioxidant activity of the crude water extracts and methanol solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* were higher than that of the aqueous leaves extract of *Cyphostemma glaucophilla* extending from 25.44% to 38.23% at 0.5 to 1.25mg/mL concentration (Ojogbane *et al.*, 2015). However, their activity was lower than the reported radical scavenging activity of *C. digitatum* roots methanol extract that displayed the highest antioxidant activity of 93.518 % at 100µg/mL concentration (Khan *et al.*, 2016).
Table 3: The antioxidant activity of the water extracts of the leaves and roots of *C. cyphopetalum* and *C. junceum*

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Type of plant extract</th>
<th>Percent inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. cyphopetalum</em></td>
<td><em>C. junceum</em></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td>0.195</td>
<td>36.0</td>
<td>23.1</td>
</tr>
<tr>
<td>0.39</td>
<td>43.6</td>
<td>35.2</td>
</tr>
<tr>
<td>0.78</td>
<td>54.2</td>
<td>43.0</td>
</tr>
<tr>
<td>1.563</td>
<td>61.6</td>
<td>62.5</td>
</tr>
<tr>
<td>3.125</td>
<td>71.4</td>
<td>71.4</td>
</tr>
<tr>
<td>6.25</td>
<td>77.0</td>
<td>74.1</td>
</tr>
<tr>
<td>12.5</td>
<td>86.1</td>
<td>81.0</td>
</tr>
</tbody>
</table>
Table 4: The antioxidant activity of the methanol solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Percent inhibition (%)</th>
<th>Type of plant fractions</th>
<th>C. cyphopetalum</th>
<th>C. junceum</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td>0.195</td>
<td>50.0</td>
<td>50.0</td>
<td>48.3</td>
<td>35.7</td>
<td>47.1</td>
</tr>
<tr>
<td>0.39</td>
<td>64.6</td>
<td>55.3</td>
<td>52.0</td>
<td>40.3</td>
<td>53.1</td>
</tr>
<tr>
<td>0.78</td>
<td>68.4</td>
<td>62.5</td>
<td>63.2</td>
<td>49.8</td>
<td>60.3</td>
</tr>
<tr>
<td>1.563</td>
<td>76.0</td>
<td>70.7</td>
<td>69.2</td>
<td>58.7</td>
<td>67.2</td>
</tr>
<tr>
<td>3.125</td>
<td>84.5</td>
<td>77.0</td>
<td>77.6</td>
<td>67.9</td>
<td>76.4</td>
</tr>
<tr>
<td>6.25</td>
<td>86.4</td>
<td>83.0</td>
<td>81.5</td>
<td>74.0</td>
<td>86.0</td>
</tr>
<tr>
<td>12.5</td>
<td>92.2</td>
<td>91.3</td>
<td>87.1</td>
<td>81.3</td>
<td>90.9</td>
</tr>
</tbody>
</table>

4.4. Antimicrobial Activities of the Leaves and Roots of *C. cyphopetalum* and *C. junceum*

In this investigation, antibacterial and antifungal activities of 80% methanol extracts, and methanol, ethanol, and acetone solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* were evaluated using agar disc and agar well diffusion methods. The 80% methanol extracts and solvent fractions of these two plants showed varying degrees of inhibitory activities.
against *E. coli, K. pneumonia, L. monocytogens, P. auruginosa, S. aureus, S. boydii, S. epidermidis, S. flexineri* and *C. albicans* at a concentration of 500mg/mL.

4.4.1. Antibacterial activities of methanol (80%) extracts and methanol, ethanol and acetone solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*

In both agar well and agar disc diffusion methods, the 80% methanol crude extracts and methanol, ethanol and acetone solvent fractions of the leaves and roots of *C. cyphopetalum* exhibited a range of inhibition zones varying in diameter from 7.0±0.0mm to 15.0±0.0mm whereas that of *C. junceum* were between 6.7±0.0mm and 9.3±0.3mm at a concentration of 500mg/mL (Table 5, 6, 7, 8, 9 and 10).

An inhibition zone ranging from 7.7±0.3mm to 13.0±0.0mm and 7.0±0.0mm to 11.0±0.0mm using agar well diffusion method 7.0±0.0mm to 11.3±0.3mm and 6.7±0.3mm to 10.0±0.0mm with agar disc diffusion test against the standard test bacteria were produced by 80% methanol crude extracts of the leaves and roots *C. cyphopetalum*, respectively. While, 80% methanol extracts of the leaves and roots of *C. junceum* depicted an inhibition zone diameter of 7.0±0.0mm–8.7±0.3mm and 7.0±0.0mm–9.0±0.0mm by agar well diffusion test and 6.7±0.3mm–8.3±0.3mm and 7.0±0.0mm–8.7±0.3mm using agar disc diffusion method, sequentially (Table 5 and 6).

The result from agar well diffusion method indicated that the highest inhibition zone against *S. aureus* (13.0±0.0mm) was produced by 80% methanol extracts of the leaves of *C. cyphopetalum* while the least inhibition zone was observed on *K. pneumonia, S. boydii* and *S. flexineri*
(7.7±0.3mm) by the leaves and against *K. pneumonia* (7.0±0.0mm) by the roots extracts (Table 5). In the agar disc diffusion assay, *K. pneumonia* was the least susceptible bacteria with 7.0±0.0mm mean inhibition for roots and 6.7±0.3mm for the leaves extracts whereas the most susceptible bacteria was *S. aureus* with an inhibition diameters of 11.3±0.3mm followed by *S. epidermidis* (10.0±0.0mm) for 80% methanol extracts of the leaves of *C. cyphopetalum* (Table 6). On comparison, the roots methanol (80%) extracts of *C. cyphopetalum* exhibited lower mean inhibition values against the standard test bacteria compared with the leaves extracts.

With regard to *C. junceum*, 80% methanol extracts of the roots produced the highest antibacterial activity against *S. aureus* with inhibition zone of 9.0±0.0mm followed by *E. coli* with 8.0±0.0mm. The lowest being on *K. pneumonia* (7.0±0.0mm) using agar well diffusion method. Similarly, *S. aureus* was the most susceptible bacteria while *K. pneumonia* being the least susceptible with inhibition zone of 8.7±0.3mm and 7.0±0.0mm, respectively in agar disc diffusion method (Table 5 and 6). Unlike with *C. cyphopetalum*, the roots 80% methanol extracts antibacterial activity exceeded that of the leaves. To sum, the extracts of both the leaves and roots of the two plants demonstrated a significantly higher antibacterial activity against *S. aureus* and the least towards *K. pneumonia* (*P* < 0.05) in both testing methods. Nonetheless, the antibacterial activity produced by the crude extracts of either of plants and their parts was found to be significantly lower than that of ciprofloxacin used as a positive control antimicrobial drug (*P* < 0.05).
Table 5: The antibacterial activity of 80% methanol leaves and roots extracts of *C. cyphopetalum* and *C. junceum* at 500mg/mL concentration using agar well diffusion method

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Type of plant extracts</th>
<th>Inhibition zones in mm (Mean ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. cyphopetalum</em></td>
<td><em>C. junceum</em></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td>E. coli</td>
<td>8.7±0.7<em>a</em></td>
<td>7.3±0.3<em>a</em></td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>7.7±0.3<em>a</em></td>
<td>7.0±0.0<em>a</em></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>9.0±0.0<em>b</em></td>
<td>7.7±0.3<em>ab</em></td>
</tr>
<tr>
<td>P. auruginosa</td>
<td>9.3±0.7<em>ab</em></td>
<td>8.0±0.0<em>ab</em></td>
</tr>
<tr>
<td>S. aureus</td>
<td>13.0±0.0<em>c</em></td>
<td>11.0±0.0<em>c</em></td>
</tr>
<tr>
<td>S. boydii</td>
<td>7.7±0.3<em>a</em></td>
<td>7.7±0.3<em>ab</em></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>11.0±0.0<em>b</em></td>
<td>8.7±0.3<em>ab</em></td>
</tr>
<tr>
<td>S. flexineri</td>
<td>7.7±0.3<em>a</em></td>
<td>7.3±0.3<em>ab</em></td>
</tr>
</tbody>
</table>

Note: The concentration of ciprofloxacin was 1mg/mL; Means labeled with different letters are statistically significant (*P* < 0.05); *statistically different from the control (*P* < 0.05)
Table 6: The antibacterial activity of 80% methanol extracts of the leaves and roots *C. cyphopetalum* and *C. junceum* at 500mg/mL concentration using agar disc diffusion method

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Type of plant extracts</th>
<th>C. cyphopetalum</th>
<th>C. junceum</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>7.7±0.3^ab*</td>
<td>7.0±0.0^ab*</td>
<td>8.0±0.0^ab*</td>
<td>7.7±0.3^ab*</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>7.0±0.0^a*</td>
<td>6.7±0.3^a*</td>
<td>6.7±0.3^a*</td>
<td>7.0±0.0^a*</td>
</tr>
<tr>
<td><em>L. monocytogens</em></td>
<td>8.7±0.3^bc*</td>
<td>7.3±0.3^ab*</td>
<td>7.3±0.3^ab*</td>
<td>7.3±0.3^ab*</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>9.0±0.0^cd*</td>
<td>7.7±0.3^ab*</td>
<td>7.7±0.3^ab*</td>
<td>7.7±0.3^ab*</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>11.3±0.3^e*</td>
<td>10.0±0.0^e*</td>
<td>8.3±0.3^b*</td>
<td>8.7±0.3^b*</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>7.3±0.0^a*</td>
<td>7.7±0.3^ab*</td>
<td>7.0±0.0^ab*</td>
<td>7.3±0.3^ab*</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>10.0±0.0^d*</td>
<td>8.0±0.0^b*</td>
<td>7.7±0.3^ab*</td>
<td>7.7±0.3^ab*</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>7.7±0.3^ab*</td>
<td>7.3±0.3^ab*</td>
<td>7.3±0.3^ab*</td>
<td>7.3±0.3^ab*</td>
</tr>
</tbody>
</table>

Note: The concentration of ciprofloxacin was 1mg/mL; Means labeled with different letters are statistically significant (P < 0.05); *statistically different from the control (P < 0.05)
In this study, the antibacterial activity of the solvent fractions of the leaves of *C. cyphopetalum* using agar well diffusion method was between 8.0±0.0mm and 15.0±0.0mm, 7.3±0.3mm and 11.0±0.0mm, and 7.0±0.0mm and 9.3±0.3mm for methanol, ethanol and acetone, respectively. In contrast, with agar disc diffusion method the antibacterial activity for methanol, ethanol and acetone solvent fractions of the leaves extended from 7.7±0.3mm to 14.7±0.3mm, 7.0±0.0mm to 10.7±0.3mm, and 6.7±0.3mm to 9.0±0.0mm, in that order. The methanol solvent leaves fraction was found to be the most potent antibacterial agent. *S. aureus* was the most susceptible bacteria for the methanol solvent fraction of the leaves of *C. cyphopetalum* with inhibition diameters of 15.0±0.0mm whereas *K. pneumonia* was the least (8.0±0.0mm). *S. epidermidis* was the second sensitive bacteria with inhibition diameter of 11.0±0.0mm. The lowest inhibition zone was exhibited by acetone solvent leaves fraction of *C. cyphopetalum* against *K. pneumonia* with inhibition diameter of 6.7±0.3mm. The inhibition zones of methanol, ethanol and acetone solvent fraction of the leaves of *C. cyphopetalum* showed a statistically significant difference (*P* < 0.05). Nevertheless, their antibacterial activities were significantly lower than that of ciprofloxacin (*P* < 0.05).
Table 7: The antibacterial activity of the methanol, ethanol and acetone solvent leaves fractions of *C. cyphopetalum* at 500mg/mL using agar well and agar disc diffusion methods.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Agar well diffusion</th>
<th>Agar Disc diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>10.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>8.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>L. monocytogens</em></td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. auruginosa</em></td>
<td>10.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>15.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>10.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>11.0±0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. flexineri</em></td>
<td>10.3±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Note:** The concentration of ciprofloxacin was 1mg/mL; Means labeled with different letters are statistically significant (*P* < 0.05); *statistically different from the control (*P* < 0.05)
With regard to the antibacterial activity, the methanol, ethanol and acetone solvent roots fractions of *C. cyphopetalum* showed lower activity than that of the corresponding leaves fractions. The methanol solvent fractions from the roots of *C. cyphopetalum* showed a range of antibacterial activity varying from 8.0±0.0mm to 9.3±0.3mm using agar well diffusion test and from 7.7±0.3mm to 9.0±0.0mm on agar disc diffusion. The highest inhibition diameter was exhibited by roots methanol solvent fractions against *S. aureus* with 9.3±0.3mm followed by *L. monocytogenes, P. aeruginosa* and *S. epidermidis* with 9.0±0.0mm diameter using agar well diffusion test. Likewise, with agar disc diffusion method *S. aureus* was the most susceptible bacteria with 9.0±0.0mm inhibition followed by *L. monocytogenes, P. aeruginosa* and *S. epidermidis* with 8.7±0.3mm inhibition.

The methanol solvent fractions of the roots of *C. cyphopetalum*, showed the highest inhibitory activity towards *S. aureus* with an inhibition zone of 9.3±0.3mm while the acetone solvent fraction showed the least activity for *K. pneumonia* with 7.0±0.0mm. In general, the methanol solvent fractions showed the highest antibacterial activity. In contrast, to the methanol and ethanol solvent fractions of the leaves of *C. cyphopetalum*, the acetone solvent fraction of the roots of *C. cyphopetalum* was less active. Besides, a raised antibacterial activity was obtained in the agar well diffusion method than in that of agar disc diffusion test. Alike the leaves fractions, the antibacterial activity of the roots fractions were significantly lower than that of the activity of ciprofloxacin (*P* < 0.05).
Table 8: The antibacterial activity of the methanol, ethanol and acetone solvent roots fractions of *C. cyphopetalum* at 500mg/mL using agar well and agar disc diffusion methods

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Agar well diffusion</th>
<th>Disc diffusion</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
<td>Acetone</td>
</tr>
<tr>
<td>E. coli</td>
<td>8.7±0.3^{ab*}</td>
<td>8.3±0.3^{ab*}</td>
<td>7.3±0.3^{a*}</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>8.0±0.0^{a*}</td>
<td>7.7±0.3^{a*}</td>
<td>7.0±0.0^{a*}</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>9.0±0.0^{ab*}</td>
<td>8.7±0.3^{ab*}</td>
<td>7.7±0.3^{a*}</td>
</tr>
<tr>
<td>P. aurginosa</td>
<td>9.0±0.0^{ab*}</td>
<td>8.7±0.3^{ab*}</td>
<td>8.3±0.3^{a*}</td>
</tr>
<tr>
<td>S. aureus</td>
<td>9.3±0.3^{b*}</td>
<td>9.0±0.0^{b*}</td>
<td>8.3±0.3^{a*}</td>
</tr>
<tr>
<td>S. boydii</td>
<td>8.3±0.3^{ab*}</td>
<td>8.0±0.0^{ab*}</td>
<td>7.3±0.3^{a*}</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>9.0±0.0^{ab*}</td>
<td>8.7±0.3^{ab*}</td>
<td>7.7±0.3^{a*}</td>
</tr>
<tr>
<td>S. flexineri</td>
<td>8.7±0.3^{ab*}</td>
<td>8.0±0.0^{ab*}</td>
<td>7.3±0.3^{a*}</td>
</tr>
</tbody>
</table>

Note: The concentration of ciprofloxacin was 1mg/mL; Means labeled with different letters are statistically significant (*P* < 0.05); *statistically different from the control (*P* < 0.05)
The methanol solvent fractions of the leaves of *C. junceum* showed the highest inhibition diameter against *S. aureus* (9.3±0.3mm) while the least inhibition zone was exhibited towards *K. pneumonia* (6.7±0.3mm) for the acetone, ethanol, and methanol solvent fraction. The leaves of *C. junceum* compared with the leaves of *C. cyphopetalum* and showed lower antibacterial activity. The second sensitive bacterial test organisms for the methanol solvent fraction of the leaves of *C. junceum* were *E. coli*, *L. monocytogens* and *P. auruginosa* with an inhibition zone of 8.7±0.3mm. The ethanol and methanol solvent fractions of the leaves of *C. junceum* exhibited the same inhibition diameter against *S. epidermidis* (8.3±0.3mm). The methanol solvent fraction of the leaves of *C. junceum* showed inhibition zones ranging from 6.7±0.3mm – 9.3±0.3mm. On the agar disc diffusion, the acetone solvent fraction showed the lowest inhibition zones against *K. pneumonia* and *S. boydii* with inhibition diameter of 6.7±0.3mm whereas the highest inhibition zone was exhibited against *S. aureus* (9.0±0.0mm) for methanol solvent fraction.
Table 9: The antibacterial activity of the methanol, ethanol and acetone solvent leaves fractions of *C. junceum* at 500mg/mL using agar well and agar disc diffusion methods

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Agar well diffusion</th>
<th>Disc diffusion</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
<td>Acetone</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>8.7±0.3b^*</td>
<td>8.0±0.0b^*</td>
<td>7.3±0.3a^*</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>6.7±0.3a^*</td>
<td>6.7±0.3a^*</td>
<td>6.7±0.3a^*</td>
</tr>
<tr>
<td><em>L. monocytogens</em></td>
<td>8.7±0.3b^*</td>
<td>8.0±0.3b^*</td>
<td>7.3±0.3a^*</td>
</tr>
<tr>
<td><em>P. auruginosa</em></td>
<td>8.7±0.3b^*</td>
<td>8.3±0.0b^*</td>
<td>7.3±0.3a^*</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>9.3±0.3b^*</td>
<td>8.7±0.3b^*</td>
<td>7.7±0.0a^*</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>8.3±0.3b^*</td>
<td>8.0±0.3b^*</td>
<td>7.0±0.0a^*</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>8.3±0.3b^*</td>
<td>8.3±0.3b^*</td>
<td>7.7±0.3a^*</td>
</tr>
<tr>
<td><em>S. flexineri</em></td>
<td>8.3±0.3b^*</td>
<td>7.7±0.3ab^*</td>
<td>7.0±0.0a^*</td>
</tr>
</tbody>
</table>

Note: The concentration of ciprofloxacin was 1mg/mL; Means labeled with different letters are statistically significant (*P* < 0.05); *statistically different from the control (*P* < 0.05)
The methanol solvent roots fractions of *C. junceum* showed a range of inhibition zone from 7.0±0.0mm–9.7±0.3mm. When the highest inhibition zone was exhibited against *S. aureus* (9.7±0.3mm), the least inhibition diameter was observed from *K. pneumonia* (7.0±0.0mm). In agar well diffusion, the inhibition zone exhibited against *K. pneumonia* was similar by methanol ethanol, and acetone solvent fractions from the roots of *C. junceum* (7.0±0.0mm). On the agar disc diffusion, the acetone solvent fraction of the roots of *C. junceum* exhibited the lowest zone of inhibition against *K. pneumonia* (6.7±0.3mm) while the highest inhibition zone was observed from methanol solvent fraction against *S. aureus* (9.3±0.3mm). The acetone solvent fractions of the roots of *C. junceum* did not show statistically significant difference but, compared to the methanol and ethanol solvent fraction among the test organisms, a significant difference (*P* < 0.05) was observed.

The results of this study are comparable with the work of Khan *et al.* (2016) who reported that the methanolic roots extract of *C. digitatum* (Vitaceae) exhibited antibacterial activity against *B. subtilis, S. aureus, E. coli, S. typhimurium* and *S. epidermidis* with maximum zone of inhibition in the range of 12mm–16 mm. Also, the methanolic extracts of the stem of *C. digitatum* showed activity against *S. epidermidis, B. subtilis, E. coli, S. typhimurium* and *S. aureus* with inhibitory zone in the range of 10mm–14mm. Methanolic extract of whole plant of *Cissus quadrangularis* Linneaus (Vitaceae) exhibited 14mm–18mm diameter of zone of inhibition against *E. coli* at a concentrations of 400–1000μg/mL (Mishra *et al.*, 2009). Deshmukh Omraj (2017) has reported an inhibitin zone of 17mm against *S. aureus* and 18mm towards *E. coli* for ethanol extracts and 16mm against *E.coli* for acetone extracts of the roots of *Parthenocissus quinquefolia* (L.) Planch (Vitaceae) at 100 μg/disc. The ethanol extract of the stems of *Cissus quadrangularis* at
concentration from 100–500µg/µl produced a zone of inhibition between 15mm–20mm against 
*E. coli* and 13mm–18mm towards *S. aureus* using disc diffusion method (Selvamaleeswaran et
al., 2016). Antimicrobial activity of ethanol extract of the stem of *C. quadrangularis* was reported to be 12mm for *S. aureus* and 10mm for *E. coli* (Srivastava et al., 2013).

In contrast, the antibacterial activity observed in this study is lower than that of hot water extract of *Vitis vinifera* (Vitaceae) leaf extracts against both standard and isolated strains of *Enterococcus faecalis* (28.9mm), *Staphylococcus aureus* (30mm), *Pseudomonas aeruginosa* (23.7mm) and, *Escherichia coli* (28mm) using agar disc diffusion method (Ahmad et al., 2014).

In general, it is apparent that plants belonging the family Vitaceae including *C. cyphopetalum* and *C. junceum* own moderate antibacterial activity.

The lower antibacterial activity of crude extracts and solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* observed in agar disc diffusion compared to agar well diffusion in this study could be due to a reduced diffusion of the test sample impregnated on filter paper discs (Uche-Okereafor et al., 2016).
Table 10: The antibacterial activity of the methanol, ethanol and acetone solvent roots fractions of *C. junceum* at 500mg/mL using agar well and agar disc diffusion methods

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Agar well diffusion</th>
<th>Disc diffusion</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol</td>
<td>Ethanol</td>
<td>Acetone</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>7.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>L. monocytogens</em></td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. auruginosa</em></td>
<td>9.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>9.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>8.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>9.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.7±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. flexineri</em></td>
<td>8.7±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.0±0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: The concentration of ciprofloxacin was 1mg/mL; Means labeled with different letters are statistically significant (P<0.05); *statistically different from the control (P<0.05)
The minimum inhibitory concentration assay was also employed to evaluate the effectiveness of the methanol solvent fractions to inhibit the growth of the bacterial test organisms. The methanol solvent fractions of the leaves of *C. cyphopetalum* exhibited the lowest minimum inhibitory concentration against *S. aureus* and *S. epidermidis* with a MIC of 15.63mg/mL whereas the highest minimum inhibitory concentration was obtained for *K. pneumonia* (MIC of 125mg/mL). Similar minimum inhibitory concentration was exhibited against *E. coli*, *P. aeruginosa*, *S. boydii* and *S. flexineri* (MIC of 31.25mg/mL). The methanol solvent roots fractions of *C. junceum* showed the lowest minimum inhibitory concentration against *S. aureus* (MIC of 15.63mg/mL) while the highest MIC was exhibited towards *K. pneumonia* (MIC of 125mg/mL). The fraction exhibited the same minimum inhibitory concentration of 62.5mg/mL against *E. coli*, *L. monocytogens*, *S. boydii*, *S. epidermidis* and *S. flexineri*.

As reported by Ojogbane *et al.* (2014), the aqueous extract of the leaves of *C. glaucophilla*, a plant with in the same genus, showed MIC of 20mg/mL against *K. pneumonia*, *P. aeruginosa* and *S. aureus* with in the same study this plant extract exhibited MIC of 10mg/mL towards *E. coli*.

Kashikar and George (2006) determined the MIC of the methanol exracts of the stem of *Cissus quadrangularis* Linn (Family: Vitaceae) agianst *S. aureus*. The MIC of methanol extracts of the stem of *C. quadrangularis* against *S. aureus* was reported as 0.465mg/mL. The MIC of the methanolic extract of the whole plant of *C. quadrangularis* towards *E. coli* was determined as 400μg/mL (Mishra *et al.*, 2009).
The minimum bactericidal concentration test was conducted to evaluate the efficacy of the methanol solvent fractions of the leaves of *C. cyphopetalum* and roots fractions of *C. junceum* to kill the bacterial test organisms. The solvent fractions of the two medicinal plants were subjected to the concentrations ranging from 15.63 mg/mL up to 500 mg/mL.

The methanol solvent leaves fractions of *C. cyphopetalum* exhibited the lowest minimum bactericidal concentrations against *S. aureus* and *S. epidermidis* (MBC of 31.25 mg/mL) whereas the highest MBC was showed towards *K. pneumonia* (MBC of 250 mg/mL). The fraction showed similar MBC against *E. coli*, *P. auruginosa*, *S. boydii* and *S. flexineri* (MBC of 62.5 mg/mL) likewise, the fraction exhibited MBC of 125 mg/mL against *L. monocytogens*.

The methanol solvent roots fractions of *C. junceum* exhibited the lowest minimum bactericidal concentration against *S. aureus* (MBC of 31.35 mg/mL) while the highest MBC was showed towards *K. pneumonia* (MBC of 250 mg/mL). The fraction showed similar MBC against *E. coli*, *L. monocytogens*, *S. boydii*, *S. epidermidis* and *S. flexineri* (MBC of 125 mg/mL) also, the fraction exhibited MBC of 62.5 mg/mL against *P. auruginosa*. 
Table 11: The minimum inhibitory concentration (MIC) and the Minimum bactericidal concentration (MBC) of methanol solvent fractions of the leaves of *C. cyphopetalum* and roots of *C. junceum* against the standard test bacteria

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>Leaves of <em>C. cyphopetalum</em></th>
<th>Roots of <em>C. junceum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/mL)</td>
<td>MBC (mg/mL)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>31.25</td>
<td>62.5</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>125.0</td>
<td>250.0</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>62.5</td>
<td>125.0</td>
</tr>
<tr>
<td><em>P. auruginosa</em></td>
<td>31.25</td>
<td>62.5</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>15.63</td>
<td>31.25</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>31.25</td>
<td>62.5</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>15.63</td>
<td>31.25</td>
</tr>
<tr>
<td><em>S. flexineri</em></td>
<td>31.25</td>
<td>62.5</td>
</tr>
</tbody>
</table>
4.4.2. Antifungal activities of methanol (80%) extracts and methanol, ethanol and acetone solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*

Using agar well diffusion method, 80% methanol crude extracts of the leaves and roots of *C. cyphopetalum* revealed the highest mean inhibition zones of 9.0±0.0mm and 8.7±0.3mm against *C. albicans*, respectively. Nonetheless, using agar disc diffusion test, 80% methanol crude extracts of the leaves and roots of both plants depicted equal 8.0±0.0mm mean inhibition zone. With regard to the solvent fractions, the methanol, ethanol and acetone solvent fractions of the leaves of *C. cyphopetalum* showed the highest mean inhibition zone towards *C. albicans* followed by the solvent fractions of its roots in both the agar well and agar disc diffusion tests (Table 12).

In agar well diffusion, 80% methanol extracts of the leaves of *C. cyphopetalum* exhibited the highest inhibition zone of 9.0±0.0mm against *C. albicans* while the lowest inhibition was shown from 80% methanol extracts of the leaves of *C. junceum* (7.7±0.3mm). Among the solvent fractions used in this antifungal test, the methanol solvent fraction of the leaves of *C. cyphopetalum* showed the highest inhibition zone of 12.3±0.3mm with agar well diffusion and 12.0±0.0mm using agar disc diffusion methods. The antifungal test revealed that solvent fractions in order of their antifungal activity: methanol > ethanol > acetone. The crude extracts and solvent fractions of the leaves and roots of *C. cyphopetalum* had higher antifungal activity than that of *C. junceum*. With regard to *C. cyphopetalum* the crude extracts and solvent fractions of the leaves showed better activity while in *C. junceum* it was the root crude extracts and solvent fractions which depicted raised antifungal activity. An increased antifungal activity was observed in agar well diffusion test than in agar disc diffusion method. Even though, the crude extracts and solvent
fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* demonstrated moderate antifungal activity towards *C. albicans* this was significantly lower than that of fluconazole (*P* < 0.05).

There were no reports on the antifungal activities of *C. cyphopetalum* and *C. junceum*, however, there are studies that revealed the inhibitory activity of other plants. With in the same family, an ethanol extract of the stem of *C. quadrangularis* showed 8mm inhibition zone against *C. albicans* (Srivastava *et al.*, 2013). In vitro study had demonstrated that the 70% ethanol leaf extracts of *Murraya paniculata* had antifungal activity against *C. albicans* with inhibition zone of 12.123±0.0339 at concentration of 80mg/mL (Kusuma *et al.*, 2017).

As reported by Masomi and Hassanshahian (2016), the ethanol and methanol extracts of *Trachyspermum ammi, Teucrium polium, Piper nigrum, Pistachia vera* and *Camelia sinensis* had ample inhibitory effect against *C. albicans* but, the extracts of *P. vera* have the best inhibitory effect on *C. albicans* with inhibition diameter of 40 mm. The methanolic extracts of these five herbal plants were better than ethanolic extracts. The lowest antifungal effect between these five plants related to *P. nigrum* inhibition zone of 13mm. Their report also indicated that the results of agar well diffusion was higher than the agar disc diffusion method in all tested plants; our study was also in agreement with their study.

According to the study reported by Nejad *et al.* (2014), hydroalcoholic extracts of *Heracleum persicum* fruits exhibited the strongest antifungal activity against *C. albicans* with a range of inhibition zones (12mm - 21 mm); the ethanolic extract of the tested plant has more anti-*Candida* effects at 0.625 µg/µL compared to the methanolic extract at 2.5 µg/µL.
Table 12: The antifungal activities of 80% methanol extracts and methanol, ethanol and acetone solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* using agar well and disc diffusion methods.

<table>
<thead>
<tr>
<th>Test sample (500mg/mL)</th>
<th>Method</th>
<th>Type of and part plant</th>
<th>Mean Inhibition zones in mm (Mean ±SEM)</th>
<th>Fluconazole (1mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>C. cyphopetalum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>Crude extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80% Methanol</td>
<td>AWD</td>
<td>9.0±0.0&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.7±0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>ADD</td>
<td>8.0±0.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Solvent Fractions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>AWD</td>
<td>12.3±0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.3±0.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>AWD</td>
<td>10.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.0±0.0&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.3±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetone</td>
<td>AWD</td>
<td>9.3±0.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>8.3±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol</td>
<td>ADD</td>
<td>12.0±0.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>ADD</td>
<td>10.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.7±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.3±0.3&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetone</td>
<td>ADD</td>
<td>9.0±0.6&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>8.0±0.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.7±0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: The concentration of fluconazole was 1mg/mL; Means labeled with different letters are statistically significant (P<0.05); *statistically different from the control (P<0.05)
4.5. Anti-inflammatory Activities of the Leaves and Roots of *C. cyphopetalum* and *C. junceum*

The result of the anti-inflammatory test carried out on 80% methanol leaves extracts and water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* showed a significant effect on inflammation and markedly reduced the swelling against carrageenan-induced paw mice edema. The percentage reduction in paw thickness at six hours in mice treated with 80% methanol leaves extracts of *C. cyphopetalum* (500mg/kg), for the water fractions of the leaves of *C. cyphopetalum* (300mg/kg) and β-sitosterol (25mg/kg) were 95.83%, 97.40% and 97.40%, respectively. Also, the percentage reduction in the paw thickness in the group of mice treated with 80% methanol leaves extracts of *C. junceum* (500mg/kg) was 88.54% while for the water fractions of the leaves of *C. junceum* (300mg/kg) was 93.23%. The 80% methanol extracts and water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* had a statistically significant anti-inflammatory effect when compared with the negative control (*P* < 0.05). The indomethacin 10mg/kg showed percentage paw thickness reduction of 93.23 % as indicated in Table 13.

The local injection of carrageenan induces inflammatory reactions in two different phases (Dongmo *et al.*, 2003; Taesotikul *et al.*, 2003; Panthong *et al.*, 2004; Ilic *et al.*, 2014). The initial phase, after the injection of the carrageenan, has been attributed to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability (Yonathan *et al.*, 2006 ; Ilic *et al.*, 2014). It has been reported that histamine and serotonin are mainly released during the first 1.5 h while bradykinin is released within 2.5 h after carrageenan injection (Panthong *et al.*, 2004). The late phase is correlated with the production of inducible cyclooxygenase enzymes and elevated
production of prostaglandins and may occur from 2.5 to 6 hours post-carrageenan injection (Bounihi et al., 2013). This late phase appears to be the most interesting phase in terms of inflammatory processes. Thus, the maximal vascular response as determined with leukocyte migration to the inflamed area, also reaches its maximum level in this phase. It is well established that prostaglandins, by virtue of their activity as modulators of inflammatory responses, have a major role in the inflammatory mechanism (Panthong et al., 2004).

Based on the results of this study, both the 80% methanol crude extracts and water fractions of the leaves and roots of the two plants were able to effectively inhibit the increase in paw thickness during both phases of carrageenan-induced inflammation. This indicates that the active component(s) of the plant may suppress both early and late phases of inflammation perhaps by inhibiting the release and/or activity of the inflammatory mediators involved in carrageenan induced paw edema. The strong anti-inflammatory activity of the leaves and roots of C. cyphopetalum and C. junceum, thus, might be by interfering with histamine, serotonin and prostaglandins synthesis.

In the present study the phytochemical investigation of the 80% methanol crude extracts of the leaves and roots of C. cyphopetalum and C. junceum and the ethanol leaves extracts of C. cyphopetalum has revealed the presence of different types of biologically active phytochemicals which was responsible for the antimicrobial, antioxidants and anti-inflammatory activities of the two plants. In line with this study, different kinds of secondary metabolites and biologically active compounds were isolated from different medicinal plants which exhibited antioxidant, antimicrobial and anti-inflammatory activities. For instance, alkaloids are applied in medicine to reduce pain and fever as well as remedy for gout, tumor and hypertension (Babbar, 2015).
Flavonoids have protective effects against bacterial, viral and degenerative diseases such as cancers, cardiovascular, oxidative stress and other age-related diseases (Kumar and Pandey, 2013). Saponins possess antibacterial and antifungal activities (Khanna and Kannabiran, 2008) and anti-inflammatory activity as well (Patel and Patil, 2012). Tannins are very important in that they facilitate healing of wounds and inflammed mucous membranes (Yadav et al., 2014). Terpenoids constitute antimicrobial, antiviral, anti-allergenic, antihyperglycemic and anti-inflammatory properties (Wagner and Elmadfa, 2003). β-sitosterol exhibits antioxidant (Gupta et al., 2011), anti-inflammatory (Loizou et al., 2010), antibacterial and antifungal activity (Kiprono et al., 2000). Luteolin has antioxidant, anti-inflammatory, anti-allergy and anticancer activities (Lin et al., 2008). Salicylic acid reveals antioxidant and anti-inflammatory properties (Randjelović et al., 2015). These different compounds, thus, may be responsible for the antimicrobial, antioxidant and anti-inflammatory activities of the leaves and roots of C. cyphopetalum and C. junceum.
Table 13: Anti-inflammatory activity of the 80% methanol extracts and water fraction of the leaves and roots of *C. cyphopetalum* and *C. junceum* against carrageenan-induced paw edema in mice model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percent Inhibition of paw edema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; hour</td>
</tr>
<tr>
<td>I 80% methanol leaves extracts of <em>C. cyphopetalum</em></td>
<td>2.44</td>
</tr>
<tr>
<td>II Water fractions of the leaves of <em>C. cyphopetalum</em></td>
<td>0.81</td>
</tr>
<tr>
<td>III 80% methanol roots extracts of <em>C. cyphopetalum</em></td>
<td>8.94</td>
</tr>
<tr>
<td>IV Water fractions of the roots of <em>C. cyphopetalum</em></td>
<td>4.07</td>
</tr>
<tr>
<td>V 80% methanol leaves extracts of <em>C. junceum</em></td>
<td>4.88</td>
</tr>
<tr>
<td>VI Water fractions of the leaves of <em>C. junceum</em></td>
<td>0.0</td>
</tr>
<tr>
<td>VII 80% methanol roots extracts of <em>C. junceum</em></td>
<td>5.69</td>
</tr>
<tr>
<td>VIII Water fractions of the roots of <em>C. junceum</em></td>
<td>0.0</td>
</tr>
<tr>
<td>IX β-sitosterol (25mg/kg)</td>
<td>5.70</td>
</tr>
<tr>
<td>X Indomethacin (10mg/kg)</td>
<td>8.94</td>
</tr>
</tbody>
</table>
CHAPTER 5: CONCLUSIONS

The results of this study have given a pioneering insight into the toxicity and biological activities of *C. cyphopetalum* and *C. junceum*. The leaves and roots extracts of the two plants were devoid of acute toxicity on mice. Secondary metabolites were found in abundance in the leaves of *C. cyphopetalum*. Several compounds including the useful β-sitosterol were isolated from the ethanol extracts of the leaves of *C. cyphopetalum*. Methanol solvent fractions of the leaves and roots of *C. cyphopetalum* exhibited higher antioxidant activity superior to ascorbic acid at 12.5mg/mL concentration. The crude extracts and solvent fractions of the root and leaves of the *C. cyphopetalum* and *C. junceum* showed moderate antibacterial activity predominantly against *S. aureus* and the least towards *K. pneumonia*. Methanol solvent fraction of the leaves of *C. cyphopetalum* revealed moderate antifungal activity against *C. albicans*. β-sitosterol, water fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum* and 80% methanol extracts of the leaves and roots of *C. cyphopetalum* had anti-inflammatory effect exceeding that of indomethacin. The observed antioxidant, antibacterial, antifungal and anti-inflammatory activities may be due to the presence of bioactive secondary metabolites that were more abundant in the leaves of *C. cyphopetalum* whose methanolic extracts and solvent fractions showed the highest activities.
CHAPTER 6: RECOMMENDATIONS

In light of the above conclusion, the following recommendations are forwarded:

- The toxicity profile of each plant requires checking for the sub-acute, sub-chronic and chronic toxicity in an animal model.

- The leaves and roots of *C. cyphopetalum* could be a potential source of antioxidants for the treatment of oxidative stresses.

- The leaves and roots of *C. cyphopetalum* and *C. junceum* as well as β-sitosterol could be a potential source of anti-inflammatory drugs to reverse inflammation.

- Further study is recommended to isolate and identify other compounds from the different parts of the two plants and to test their biological activities.
REFERENCES


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APPENDICES

Appendix 1: Summarized and analyzed data

Table 1: The antioxidant test (UV mean absorbance of the water extracts of the leaves and roots of C. cyphopetalum and C. junceum)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean absorbance (Mean ±SEM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. cyphopetalum</td>
<td>C. junceum</td>
</tr>
<tr>
<td></td>
<td>Leaves Roots</td>
<td>Leaves Roots</td>
</tr>
<tr>
<td>0.195</td>
<td>0.9262±0.00*</td>
<td>1.1107±0.00*</td>
</tr>
<tr>
<td>0.39</td>
<td>0.8163±0.00*</td>
<td>0.9390±0.00*</td>
</tr>
<tr>
<td>0.78</td>
<td>0.6622±0.00*</td>
<td>0.8238±0.00*</td>
</tr>
<tr>
<td>1.563</td>
<td>0.5552±0.00*</td>
<td>0.5422±0.00*</td>
</tr>
<tr>
<td>3.125</td>
<td>0.4132±0.00*</td>
<td>0.4243±0.00*</td>
</tr>
<tr>
<td>6.25</td>
<td>0.3322±0.00*</td>
<td>0.3742±0.00*</td>
</tr>
<tr>
<td>12.5</td>
<td>0.2013±0.00*</td>
<td>0.2754±0.00*</td>
</tr>
</tbody>
</table>

Note: Ascorbic acid was used as positive control
Table 2: The antioxidant test (UV mean absorbance of the methanol solvent fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*).

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean absorbance (Mean ±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. cyphopetalum</em></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>0.195</td>
<td>0.7230±.00*</td>
</tr>
<tr>
<td>0.39</td>
<td>0.5120±.00*</td>
</tr>
<tr>
<td>0.78</td>
<td>0.4560±.00*</td>
</tr>
<tr>
<td>1.563</td>
<td>0.3470±.00*</td>
</tr>
<tr>
<td>3.125</td>
<td>0.2240±.00*</td>
</tr>
<tr>
<td>6.25</td>
<td>0.1960±.00*</td>
</tr>
<tr>
<td>12.5</td>
<td>0.1130±.00*</td>
</tr>
</tbody>
</table>

Note: Ascorbic acid was used as positive control.
Table 3: The anti-inflammatory activity of 80% methanol extracts and distilled water fractions of leaves and roots of *C. cyphopetalum* and *C. junceum* on carrageenan-induced paw edema in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>1st hour</th>
<th>2nd hour</th>
<th>3rd hour</th>
<th>4th hour</th>
<th>5th hour</th>
<th>6th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (80% methanol leaves extracts of <em>C. cyphopetalum</em>)</td>
<td>1.20±.08</td>
<td>1.00±.08*</td>
<td>0.78±.10*</td>
<td>0.58±.10*</td>
<td>0.32±.09*</td>
<td>0.08±.10*</td>
</tr>
<tr>
<td>II Water fractions of the leaves of <em>C. cyphopetalum</em></td>
<td>1.22±.08</td>
<td>0.97±.07*</td>
<td>0.77±.08*</td>
<td>0.57±.088*</td>
<td>0.32±.08*</td>
<td>0.05±.11*</td>
</tr>
<tr>
<td>III 80% methanol roots extracts of <em>C. cyphopetalum</em></td>
<td>1.12±.06</td>
<td>0.88±.08*</td>
<td>0.70±.08*</td>
<td>0.48±.09*</td>
<td>0.29±.11*</td>
<td>0.09±.11*</td>
</tr>
<tr>
<td>IV Water fractions of the roots of <em>C. cyphopetalum</em></td>
<td>1.18±.06</td>
<td>0.98±.06*</td>
<td>0.73±.07*</td>
<td>0.47±.08*</td>
<td>0.28±.08*</td>
<td>0.08±.08*</td>
</tr>
<tr>
<td>V 80% methanol leaves extracts of <em>C. junceum</em></td>
<td>1.17±.08</td>
<td>0.97±.08*</td>
<td>0.78±.09*</td>
<td>0.55±.09*</td>
<td>0.37±.10*</td>
<td>0.22±.12*</td>
</tr>
<tr>
<td>VI Water fractions of the leaves of <em>C. junceum</em></td>
<td>1.23±.06</td>
<td>1.02±.06*</td>
<td>0.78±.07*</td>
<td>0.56±.08*</td>
<td>0.36±.07*</td>
<td>0.13±.08*</td>
</tr>
<tr>
<td>VII 80% methanol roots extracts of <em>C. junceum</em></td>
<td>1.16±.08</td>
<td>1.04±.07*</td>
<td>0.84±.07*</td>
<td>0.63±.07*</td>
<td>0.38±.07*</td>
<td>0.14±.09*</td>
</tr>
<tr>
<td>VIII Water fractions of the roots of <em>C. junceum</em></td>
<td>1.23±.08</td>
<td>1.03±.08*</td>
<td>0.80±.09*</td>
<td>0.50±.09*</td>
<td>0.32±.10*</td>
<td>0.12±.10*</td>
</tr>
<tr>
<td>IX β-sitosterol 25mg/kg</td>
<td>1.16±.08</td>
<td>0.96±.08*</td>
<td>0.76±.10*</td>
<td>0.56±.10*</td>
<td>0.28±.10*</td>
<td>0.05±.11*</td>
</tr>
<tr>
<td>X Indomethacin (10mg/kg)</td>
<td>1.12±.08</td>
<td>0.92±.08*</td>
<td>0.73±.10*</td>
<td>0.48±.10*</td>
<td>0.33±.13*</td>
<td>0.13±.13*</td>
</tr>
<tr>
<td>XI Distilled water 10mL/kg</td>
<td>1.23±.07</td>
<td>1.33±.07</td>
<td>1.53±.07</td>
<td>1.62±.06</td>
<td>1.75±.04</td>
<td>1.92±.06</td>
</tr>
</tbody>
</table>

Values are Mean ±SEM of 6 individual mice; *Values are significant at P<0.05 over negative control (Group XI).
Compound 1

- **White solid with M.Pt: 265-267°C**
- **^1H-NMR:** Six methyls: at δ 0.65 (3H), 0.80 (9H), 0.90 (3H) and 0.95 (3H)
  - One olefinic proton: at δ 5.32 (1H, br s)
  - One oxygenated proton: at δ 3.65
  - Anomeric proton: at δ 4.22 (1H, d, J = 7.6)

![1H, Beta-sitosterol glucoside, DMSO](image-url)

β-Sitosterol glycoside
\(^{13}\text{C}-\text{NMR and DEPT-135:}\)

- 35 C-signals for 35 C-atoms
- 2 Olefinic carbons: at \(\delta\) 140.9 (C-5) & 121.6 (C-6)
- Oxygenated signals: b/n \(\delta\) 61.6 - 77.5
- Anomeric carbon: at \(\delta\) 101.3

\[
\begin{array}{cccc}
140.9 & 121.6 & 101.3 \\
\end{array}
\]

C-13, Beta-sitosterol glycoside, DMSO

\(\beta\)-Sitosterol glycoside
Compound 2

- white powder

$^1$H-NMR

- Four methine protons at $\delta$ 6.67 (2H, m), 7.20 (1H, td, $J = 8.0, 1.6$ Hz) and 7.6 (1H, dd, $J = 7.6, 1.2$ Hz).

$^1$H, Salicylic Acid, DMSO
$^{13}$C-NMR: and DEPT-135:

- **3 quaternary**: at $\delta$ 162.6 (C-2) and 120.0 (C-1) & carbonyl at $\delta$ 173.1
- **4 methine**: at $\delta$ 116.3 (C-3), 117.1 (C-5), 130.7 (C-6) & 132.5 (C-4)
Compound 4

- Yellow solid

- $^1$H-NMR: Ring A: at $\delta$ 6.19 (1H, d, $J = 2$ Hz); 6.44 (1H, d, $J = 2$ Hz); 6.68 (1H, s)
  Ring B: at $\delta$ 6.89 (1H, d, $J = 8$ Hz); 7.43 (1H, d, $J = 2$ Hz); 7.40 (1H, m)
  Ring C: at $\delta$ 6.75 (1H, s); Chelated OH: at $\delta$ 12.98 (1H, s)
$^{13}$C-NMR and DEPT-135:
six methine: $\delta$ 94.3 (C-8), 99.3 (C-6), 103.3 (C-3), 113.8 (C-2’), 116.5 (C-5’) & 119.4 (C-6’)
nine quaternary: $\delta$ 164.6 (C-7), 164.3 (C-2), 161.9 (C-9), 157.4 (C-5), 150.2 (C-4’), 146.2 (C-3’), 104.1 (C-10), 121.9 (C-1’) and 182.1 (C-4)
Compound 5

1H, Beta-sitosterol, CDCl₃
C-13, Beta-Sitosterol, CDCl3
Appendix 2: Laboratory procedures

A. Test for Flavonoids

The test solution of the extract was dissolved in one mL of alcohol and then subjected to the following tests:

1. Ferric chloride test: A few drops of neutral ferric chloride solution were added to one mL each of the test solutions. Formation of blackish red color indicates the presence of flavonoids.

2. Shinoda’s test: To one mL of alcoholic solution, a small piece of magnesium ribbon or magnesium foil and a few drops of concentrated HCl were added. Change in color (from red to pink) shows the presence of flavonoids.

B. Test for Tannins

The test solution of the extract was dissolved in minimum amount of water separately, filtered and the filtrate was then subjected to the following test:

1. Ferric chloride test: To the filtrate, a few drops of ferric chloride solution were added. The addition of FeCl$_3$ reagent to the filtrate production of blue, blue-black, green-blackish or blue-green coloration or precipitation was taken as evidence for the presence of tannins.

C. Test for Terpenoids

Dried plant material was treated with 5 mL of 1% aqueous hydrochloric acid. After 3-6 hours, the extract was treated with 1 mL of Trim-Hill reagent (10 mL of acetic acid, 1 mL of 0.2%
copper sulfate in water and 0.5 mL of concentrated hydrochloric acid) and heated on a water bath. The appearance of a blue color indicates the presence of diterpenoids (compound composed of four isoprene units) while green color indicates the presence of monoterpenoids (are classes of terpenes that consist of two isoprene units).

D. Test for Saponins

The test extracts were mixed with 20 mL of distilled water and then agitated in a graduated cylinder for 15 minutes. Foam formation indicates the presence of saponins.

E. Test for Alkaloids

The test extract was dissolved in chloroform and the solutions were extracted with dilute HCl or dilute H$_2$SO$_4$. The acid layer was tested for the presence of alkaloids.

1. Mayer’s test: 3mL of Mayer’s regent (Potassium mercuric iodide solution) was added. Cream colored precipitate indicates the presence of alkaloids.

2. Wagner’s test: Wagner’s reagent (iodine in potassium iodide) was added. Formation of reddish brown precipitate indicates the presence of alkaloids.

3. Dragendorff’s test: 2 mL of Dragendorff’s reagent and 2 mL of dilute HCl were added to the test solution. An orange-red colored precipitate indicates the presence of alkaloids.
Appendix 3: Laboratory work

Figure 1: Maceration of the leaves and roots of *C. cyphopetalum* and *C. junceum*

Figure 2: Concentration of solvent extracts and fractions

Figure 3: Lyophilization of water extracts and fractions of the leaves and roots of *C. cyphopetalum* and *C. junceum*

Figure 4: Result observation of phytochemical screening of the 80% methanol leaves and roots extracts of *C. cyphopetalum* and *C. junceum*
Figure 5: Preparation of antioxidant test of the water extracts and methanol solvent fractions of leaves and roots of *C. cyphopetalum* and *C. junceum*

Figure 6: Result observation of antioxidant test

Figure 7: Evaluation of the antimicrobial activity of the leaves and roots of *C. cyphopetalum* and *C. junceum*
Figure 8: Antibacterial activity of the methanol solvent fractions of the leaves of *C. cyphopetalum* against *S. aureus*. The middle shows the inhibition zone of ciprofloxacin, the negative sign shows the result of 3% of Tween 80 and the rest inhibition zones show the result of the methanol solvent fractions of the leaves of *C. cyphopetalum* at different concentration.

Figure 9: Antibacterial activity of the methanol solvent fractions of the leaves of *C. cyphopetalum* against *S. epidermidis*. The middle shows the inhibition zone of ciprofloxacin, the negative sign shows the result of 3% of Tween 80 and the rest inhibition zones show the result of the methanol solvent fractions of the leaves of *C. cyphopetalum* at different concentration.
Figure 10: Antibacterial activity of the methanol solvent fractions of the leaves of *C. cyphopetalum* against *P. auruginosa*.

The middle shows the inhibition zone of ciprofloxacin, the negative sign shows the result of 3% of Tween 80 and the rest inhibition zones show the result of the methanol solvent fractions of the leaves of *C. cyphopetalum* at different concentration.
Edematous (Inflamed) paw        Paw treated with water fraction of *C. cyphopetalum*

**Fig 11:** Anti-inflamatory Activity
Fig 12: Measuring the paw edema of mice using Micrometer
DECLARATION

I declare that the thesis entitled as Evaluation of the Antioxidant, Antimicrobial and Anti-inflammatory Activity of Cyphostemma cyphopetalum and Cyphostemma junceum is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

This thesis was prepared according to Addis Ababa University thesis writing, examination, and grading guidelines.

Prepared by: Name TEGENU GELANA TIKI
ID: GSR/1341/06
Date: March 2018
Signature: _______________________

Approved by: Name: Yalemtsehay Mekonnen (Professor)
Date: March 2018
Signature: ______________________