Sudan University of Science and Technology
College of Graduate Studies

Antimicrobial Activity of *Solenostemma argel* and Cymbopogon *proximus* Against Carbapenem Resistant Gram Negative Bacteria Isolated from Different Hospitals in Khartoum State

نشاط الحرجل والمحريب ضد البكتريا سالبة الجرام المقاومة للكاربابينم المعزولة من مستشفيات الخرطوم المختلفة

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DEDICATION

This Force was dedicated with Great love

TO:

My Father and Mother

My Husband

My Brothers and Sisters

all my friends and colleagues

Acknowledgement

Frist and all thanks to the ALMIGHTY ALLAH for giving me strength to do this research , great thanks to my supervisor professor.Yousif Fadlalla Hamed Elnil for his valuable adevice and great effort ,to all Members of Department of Microbiology in Sudan University for Science and Technology , to National Center of Research for the assistance , to my husband Nader Bakhit Rakhis for support and assistance , to my Mother for traditionally guideline , to my colleague Umkalthoum Mohammed and other colleagues for helping , to everybody encourage assist and support me in this study without you all the work could not be and became impossible .

ABSTRACT

Use of plant based drugs and chemicals for curing various ailments as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. *Cymbopogon proximus* and *Solenostemma argel* from medicinal plant which widely used especially in Sudan.

The study was aimed to investigate the antimicrobial activity of ethanolic extract of *Solenostemma argel* and *Cymbopogon proximus* against carbapenem resistant bacteria during the period from February 2018 to December 2018. Agar disc diffusion method was used to determine the antimicrobial activity of of *Solenostemma argel* and *Cymbopogon proximus*, the ethanolic extract was examined against carbapenem resistant *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumoniae* isolated from different hospitals in Khartoum State from urine, wound swab, blood and body fluid from 80 clinical isolates.

The ethanolic leaves extract of *Cymbopogon proximus* show activity against tested bacteria , the activity reach to (76%),at high concentration decrease by low concentration, gave best result with *Proteus mirabilis*(87.5) *then Escherichia coli* (80.9%), *Klebsiella pneumonia*(75%) and *Pseudomonas aeruginosa* (61.5%). And *Solenostemma argel* less in activity just gave (12%).

Ethanol extract of *Cymbopogon proximus* was found to be effective as antibacterial against bacteria under test, providing the scientific basis for its traditional application in Sudanese folk medicine against many bacterial diseases, extract had an *in vitro* antibacterial activity against carbapenem resistant bacteria, and Ethanol extract *Solenostemma argel* poor in activity further studies are required to confirm this result to identify active ingredient and toxicity.

المستخلص

استخدام العقاقير والمواد الكيميائيه النباتية لعلاج الامراض المختلفة قديم قدم الحضارة الانسانية النباتات والادوية المستندة الى النباتات وهي اساس العديد من الادوية الحديثة التى نستخدمها اليوم لامراضنا المختلفة. الحرجل والمحريب من النباتات الطبيه التى تستخدم على نطاق واسع وخاصه فى السودان.

تهدف هذة الدراسة للتحقق من فعالية مستخلص الحرجل والمحريب الكحولي كمضاد للبكتريا المقاومة للكاربابينم في الفترة من فبراير 2018 الى ديسمبر 2018.

اتبعت طريقة الانتشار الطبقي في الاجار للتقصي من فعالية المستخلص الكحولي للحرجل والمحريب كمضاد للبكتريا المقاومة للكاربابينم وتشمل (الاشريكية القولونية ،الزائفة الزنجبارية ،الكلبسيلا الرئوية ، ,المتقلبة الاعتيادي،) ، المعزولة من مستشفيات مختلفة في لاية الخرطوم من البول ، الدم، مسحة الجلد للجروح وسوائل الجسم من ثمانون عينة.

مستخلص الاوراق الكحولي للمحريب ظهرت فعاليته ضد البكتريا المختبره ،حيث فعاليته تصل الى 76% فى التركيز العالى وتقل مع نقصان التركيز.اعطى افضل نتيجه مع المتقلبة الاعتيادي، الاشريكية القولونية، الكلبسيلا الرئوية و،الزائفة الزنجبارية.

تمحضت هذة الدراسة عن ان مستخلص المحريب الكحولي المستخدم في هذة الدراسة ذو فعالية كمضاد حيوي للبكتريا تحت الاختبار والتي تبرر استخدامة في الفلكلور الشعبي ضد الامراض البكتيرية ، والمستخلص الحرجل الكحولي قليل الفعاليه مزيد من الدراسات لتاكيد هذة النتائج وتحديد المركبات النشطة وسمية النبات .

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CHAPTER ONE

INTRODUCTION

1.1Introduction

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem; to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue to studies develop new drugs, either synthetic or natural(Gislene and Nascimen, 2000).

Increasing rates of bacterial resistance among common pathogens and serious ones are threatening the effectiveness of even the most reliable potent antibiotics. With the ever increasing spread of multidrug resistance pathogens in our daily lives it becomes imperative to find a way out to suppress this menace because sooner or later the spread will eventually become a serious public concerns(Adekunle,2012).

Medicinal plants have been widely used in traditional medicine for several centuries for the treatment of many health-related ailments. According to the World Health Organization (WHO), the majority of the world's population depends on traditional medicine for primary healthcare. There has been An increasing interest in medicinal plants and their active ingredients because of their potency and negligible adverse side effects (Hashim, 2017).

Medicinal plants are of worldwide distribution. The use of such plants was directed to overcome the drug resistance especially against bacteria and avoid the side effects of synthetic drugs. (Abdelhady,1994).

The medicinal value of plants lies in some chemical substances due to secondary metabolites where most of them are bioactive constituents

such as alkaloids, terpenoids, volatile oil, flavonoids and phenols that producea definite physiological action in human body(Malin *etal*.,2018). Hargel is deep rooted in the Sudanese primary health care as it is used as fumes for eye infection remedy or as aqueous extract for gastro-intestinal tract.

Mahareb used traditionally as a diuretic to inhibit kidney stone formation, an anti-infectious agent in urinary tract infections, an antibacterial and an antifungal.

The use of plant extract and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic of the traditional medicinal plant in Sudan.

1.2. Justification and Rationale

The continuous emergence of multidrug resistant bacteria is a major threat to the lives of human around the global. Antibiotics and synthetic chemotherapeutic agents were thought of as miracle tablets that cure all infectious diseases as they marked the end of the pre-antibiotic era; however, this fact was shortly found to be incorrect as methicillin resistant strains emerged science the 1960s the spread of resistant genes from one antibiotic to another through extra chromosomal elements (plasmids),the use of antibiotics in the fisheries and poultry, in addition the sale of antibiotics over the counter has all contributed in reaching the post antibiotic era. Plants produce antimicrobial substances that protect the plant against Plant pathogens. The research has proved the antimicrobial substances produced by plants could produce the same effects on human Pathogens (Tagwa and Thuwaiba, 2015).The *Solenostemma argel* and *Cymbopogon proximus* possesses antibacterial activity used in rural medical care for treatment of many infectious and chronic diseases, thus, to verify the antibacterial activity of those plants against resistant bacteria isolated from different clinical isolates.

This was done attempt to solve problem of resistant, found good treatment for bacterial disease.

In Sudan, with high percentage of multidrug resistant bacteria, we in urgent need to develop new drug from our traditional medicine.

1.3. Study Objectives

1.3.1. General objective

To study the antimicrobial activity *of Solenostemma argel and Cymbopogon proximus* against selected Carbapenem resistant Gram negative bacteria isolated from different hospitals in Khartoum State.

1.3.2.Specific objectives

1-To isolate and identify Gram negative bacilli from clinical specimens using convential methods.

2-To determine antimicrobial susceptibility pattern of the isolated bacteria against carbapenem antibiotic.

3-To determine the antimicrobial activity of Alcoholic extract of against *Solenostemma argel* and Cymbopogon *proximus* carbapenem resistant bacteria.

4-To determine antimicrobial susceptibility pattern of the isolated bacteria against carbapenem antibiotic and other antibiotics.

CHAPTER TWO

LITERATURE REVIEW

2.1. Conventional Antibiotics and the Problem of Microbial Resistance:

Infections caused by multidrug-resistant (MDR) organisms are associated with increased mortality compared to those caused by susceptible bacteria and they carry an important economic burden, estimated at over 20 billion dollars per year in the US only. The Center for Disease Control and Prevention conservatively estimates that at least 23,000 people die annually in the USA as a result of an infection with antibiotic-resistant organisms. Moreover, according to a recent report, antibiotic resistance is estimated to cause around 300 million premature deaths by 2050, with a loss of up to$100 trillion (£64 trillion) to the global economy. This situation is worsened by a paucity of a robust antibiotic pipeline, resulting in the emergence of infections that are almost untreatable and leaving clinicians with no reliable alternatives to treat infected patients (Munita and Arias, 2016).Infections have been the major cause of disease throughout the history of human population. With the introduction of antibiotics, it was thought that this problem should disappear. However, bacteria have been able to evolve to become resistant to antibiotics. The increase in antibiotic resistance has been attributed to the combination of microbial characteristics, the selective pressure of antibiotic use and social and technical changes that enhance the transmission of resistant organisms. The growing threat from resistant organisms calls for concerted action to prevent the emergence of new resistant strains and the spread of existing ones (Dzidic *et al*., 2008).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality.

Increasing rates of bacterial resistance among common pathogens and serious ones are threatening the effectiveness of even the most reliable potent antibiotics. With the ever increasing spread of multidrug resistance pathogens in our daily lives it becomes imperative to find a way out to suppress this menace because sooner or later the spread will eventually become a serious public concerns(Gislene,2000).

Resistance mechanisms allow bacteria to survive in the presence of toxic conditions that can result from acquired or intrinsic cell changes. Bacteria may be intrinsically resistant to antimicrobial products, or may acquire resistance by denovo mutation or via the acquisition of resistance genes from other microorganisms. Acquisition of new genetic material by antimicrobial susceptible bacteria from those resistant counterparts may occur through gene transfer, by conjugation (via plasmids and conjugative transposons), transformation (via bacteriophages), or transduction (via incorporation into the chromosome of chromosomal DNA or plasmids). Once acquired, resistance genes are not easily lost. Instead, they become a relatively stable part of a genome. Additional resistance determinants may join those already prevailing, broadening the multidrug resistance phenotype (Umkalthom, 2019).

Infections remain one of the most serious concerns in the critical care setting, where multidrug resistant organism (MDRO) outbreaks can

jeopardize the chances for effective therapy. An organism is considered multi-drug resistant when in vitro drug susceptibility testing shows resistance to one or more classes of antimicrobial agents recommended as first line therapy. Multidrug resistance has been demonstrated for a variety of organisms that are more common in ICUs than In other hospital wards, and the risk of infection increases with duration of hospitalization (Magiraa *et al., 2018*).

The increasing prevalence of infections due to multidrug resistant organisms (MDROs) represents a worldwide public health problem and is not only of importance in acute care hospitals. Long term care facilities (LTCFs), such as nursing homes (NHs), have been identified as important reservoirs of methicillin resistant *Staphylococcus aureus* (MRSA) and extended-spectrumβ-lactamase producing enterobacteriaceae (ESBLE) byprevalenceandincidencestudiesconductedindifferentEuropeancountries.

Recently, there have also been several reports of infection and/ or colonization by other MDRO s like carbapenemase producing enterobacteriaceae (CPE) and vancomycin-resistant enterococci (VRE) among its residents(Latour *et al*., 2019).

The emergence of "pan-resistant" Gram-negative strains, notably those belonging *to Pseudomonas aeruginosa* and *Acinetobacter baumanii,* occurred more recently, after most major pharmaceutical companies stopped the development of new antibacterial agents. Hence, there are almost no agents that could be used against these strains, in which an outer membrane barrier of low permeability and an array of efficient multidrug efflux pumps are combined with multitudes of specific resistance mechanisms. Multidrug resistance in bacteria occurs by the accumulation, on resistance (R) plasmids or transposons, of genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux

pumps, each of which can pump out more than one drug type(Nikaido, 2009).

The control of infectious diseases is badly endangered by the rise in the number of microorganisms that are resistant to antimicrobial agents. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. The primary cause of antibiotic resistance is genetic mutation in bacteria. Inappropriate and irrational use of antimicrobial medicines provides favorable conditions for resistant microorganisms to emerge, spread and persist. The greater the duration of exposure of the antibiotics, the greater the risk of the development to resistance, irrespective of the severity of the need for the antibiotic. As resistance towards antibiotics becomes more common a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies there has been a continued decline in the number of newly approved drugs. Antibiotic resistance therefore poses a significant problem (Odonkor and Addo, 2011).

2.2. Plant Derived Antimicrobials

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries.

Natural products perform various functions, and many of them have interesting and useful biological activities. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. Researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. More than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicine in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants accumulated in areas where the use of plants is still of great importance. The medicinal value of plants lays in some chemical substances that body. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds (Zain *et al*., 2011).

The escalating interest in herbal therapies and its expansive involvement in the health sector are not surprising and are un doubtfully capturing positive reception. Foliage and plant parts like roots, leaves, flowers, etc. have been continually used to promote health or treat diseases and are typically marketed as herbal remedies or phyto pharmaceuticals.(AbouShoer *et al*., 2011).

2.3. Medical uses of natural products:

The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, skin, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the

living organisms .Human is mainly dependent on raw plant materials in order to meet medical needs to maintain health and cure diseases.

Medicinal plants are used for treatment because they have certain properties, including synergistic actions. The constituents of the plant may interact with each other, and this interaction can be beneficial for both or adverse to either of them or eliminate the harmful effects of both. Plant-derived compounds can dramatically improve hard-to-treat illnesses, such as cancer. Plant components are also characterized by their ability to prevent the development of certain diseases. The toxicity and adverse effects of conventional and allopathic medicines have also been important factors in the sudden increase in population demands and increase in the number of herbal drug manufactures as well as a reduction in the use of chemical drugs(JamshidiKia *et al*.,2018).

Herbal medicines have provided the world's population with safe effective and low cost medicines for centuries. They have a rich and extensive historical basis in use and study which can be referenced in ancient medical writing. More importantly modern research has validated many of these traditional uses. When integrated into medical care with other medications, herbal medicines can provide Consumers and patients with the best chance for maintaining a high quality of life and in some cases, increase their chance for survival. They can also fill therapeutic niches that are not adequately addressed through conventional therapies.

The main problem facing the use of herbal medicines is the proof requirement that the active ingredients contained in medicinal plant are useful, safe and effective. This is highly important requirement to get the approval of health authorities and to assure the medical staff and the public with regard to the use of medicinal plants as drug alternatives. The proofs of pharmacological activity that are available at present are mostly based on empirical experience. The scientific and clinical proofs then become the

most important priority in order to eliminate the concern of using medicinal plants as drugs for alternative treatment. Therefore it is of vital importance to conduct research or provide scientific proof of pharmacology international collaboration is important for utilization of these herbal medicines as it would enhance the development of drugs obtained from medicinal plants for the benefit of all (Rayan *et al*., 2016).

Alternative treatments have been sought especially from herbal medicines, in conjunction with the resurgence of interest in phyto therapy and medicinal plants, as sources of effective, safe, cheap, and socially accepted treatments In addition, there are several anti urolithic. herbal remedies provided by many traditional systems of medicine all over the world (Warrag *et al*., 2014).

Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system. The doubts about the efficacy and safety of the oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes. A wide variety of the traditional herbal remedies are used by diabetic patients, especially in the third world countries and may therefore, represent new avenues in the search for alternative hypoglycemic drugs (Mansour *et a l*., 2002).

The use of medicinal plant products to treat various ailments is a common practice in many developing countries. However, a lack of information on the adverse effects of these plants raises questions on their safety and possible adverse side effects (Abdurrahman *et al*., 2017).

2.4. Phytochemicals in plants

Pharmacologically active constituents of plants are mostly, chemically distinct, but often overlapping, classes of constituents are mainly terpenoids (such as sesquiterpenes, saponins, iridoids, carotenoids and steroids), phenolics (such as tannins, quinones, salicylates and lignin's), and their glycosides (such asflavonoids, glucosinolates and cyanogens), alkaloids,

polysaccharides (such as gums and mucilage's) and peptides. Also of interest are essential oils and resins, which often contain several of the above constituent classes. Secondary metabolites were originally considered as peripheral to the essential metabolism
of the cell and often as merely waste products of metabolism. They are now believed to full many important plant functions, although the full function of most is not completely understood (Wills *et al*.,2000).

2.5. *Solenostommaargel*

2.5.1. Classification:

Class: Magnoliopsida

Order: Gentianales

Family: Asclepiadaceae

Genus: *Solenstomma*

Species: *Solenstomma argel*

2.5.2. Description:

It is an erect perennial shrub that reaches up to 1.5-2 feet in height with numerous branches carrying opposite decussate leaves. The leaves are lance late to oblong-ovate, with acute or sub–acute apex and cuneate base. The leaf petiole is thick. Fruits are solitary follicles, ovoid, lance late, acuminate at the apex and they are very hard with dark purple color. Seeds are turgid, ovoid and they are channel down at one face; they are minutely tuberculation bearing an apical tuft hair.(Aesha,2012).

2.5.3. Distribution:

Solenostemma argel is a desert plant, which is of wide spread in central and North's parts of the Sudan, Egypt, Libya, Chad, Algeria, Saudi Arabia and Palestine. However, Sudan is regarded as the richest source of this plant (Hanadi, 2014).

2.5.4. Uses

Is a desert plant of traditional medical used in folk medicine in different places in the world especially in African country. Argel belongs to the Asclepiadaceous family. Argel is considered to be medicinally important in Sudan, Libya and Chad Argel leaves are used in herbal medicine for the treatment of some liver and kidney diseases and some allergies. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica. Also, it is used as incense in the treatment of measles and sometimes crushed and used as remedy for supporting wounds. The leaves are infused to treat gastrointestinal cramps, stomach ache, colic, and cold and urinary tract infections and are effective as anti-syphilitic if used for prolonged period of 40-80 days. Phytochemicals of medicinal properties from Argel shoots had been reported by many workers reported that the aqueous extracts of Argel have antifungal and antibacterial properties. Argel leaves nowadays are used as traditional medicine in Yemen for prevention of diabetes and the leaves are consumed as tea. Since a literature search indicated the absence of information regarding biological and investigations of the effect of the leaves of Argel as hypoglycemic agent (Tajaldeen and AlNaqeb, 2014).

2.5.5. Active ingredients present in *S.argel*:

These plants are known to contain secondary metabolites such as alkaloids, cardinolides flavonoids etc., which are needed in manufacturing important pharmaceuticals. having remedial properties, *S. argel* was also reported to contain various percentages of minerals, carbohydrates and proteins together with a number of organic compounds including flavonoids, kaempferaol, quercetin, rutin, flavornols, flavanones, chalcones and alkaloids.(Osman *et al*., 2014)..

S.argel leaves, stems and flowers reveal the presence of numerous biochemical ingredients such as pyrgene glycosides, flavonoids,

kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids. In report on *S.argel*, showed the presenceof kaempferol and steroidal glycosides in leaves of hargel also they found that the flavanoids can bedetected. *Solenostemma argel* contain flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids in *S.argel*. Also they contain pregnane ester glycosides in *S.argel* extracts. *S.argel* was found to include some flavonoids saponins alkaloids. Moreover there are 2000 flavonoid found in *S.argel* found in as methoxil or hydroxile group, further studies .were needed to investigate this flavonoid. *S.argel* can be used medically in kidney disease, liver, respiratory system. Leaves of *S.argel* can be used an anti-inflammatory, antiseptic, vasodilatory and hypotensive.(Rayan *et al*., 2016).

2.6.*Cymbopogon proximus*

2.6.1.Classification

Kingdom: Plantae

Order: Poales

Family:Poaceae.

Genus: *Cymbopogon*

Species: *Cymbopogon proximus*

2.6.2. Description:

Cymbopogon proximus(locally named, MaharEb) from Family: Poaceae (Graminae), is a great interest due to its commercially valuable essential oils and widely used in traditional medicine, and thus thepotentiality of *Cymbopogon proximus* essential oil which could be the alternative approach for the treatment of chronic diseases such as chronic kidney disease and failure .The Essential oil of *C. proximus* has a strong aromatic odor and has great medicinal value such that traditionally it is widely used as antispasmodic, a protection against fever, anti-intestinal ailment problems, anti-malarial, and anti-helminthic (especially against Guinea

worms). Here in Sudan used traditionally as a diuretic to inhibit kidney stone formation, an anti-infectious agent in urinary tract infections, an antibacterial and an antifungal. According to different researches done it was founded to be effective renal antispasmodic and diuretic agent. Furthermore, is used in the treatment of colds, epilepsy, abdominal cramps and pains, as well as in culinary and perfume products. (Malin *et al*., 2018).

2.6.3. Biological activities:

Many biological activities have been reported bioactivity assisted fractionation of the *C. proximus* extracts led to the isolation of an active sesquiterpene, proximadiol (cryptomeridiol) which was found to have anti-diabetic activity. In addition, *C. proximus* essential oil was found to possess a bronchodilator activity mediated via antagonizing both histamine and serotonin receptors. Furthermore, it has a significant ganglionic blocking action and a mildanti-inflammatory activity (Elnezhawy *et al.*, 2014).

2.7. Carbapenems

Carbapenem: Antimicrobials carbapenems are bactericidal B-lactam antimicrobials with proven efficacy in severe infections caused by extended spectrum B-lactamase (ESBL) producing bacteria. There are a few examples, namely imipenem, meropenem, doripenem, ertapenem, panipenem and biapenem, in use world wideas a result of the rising resistance to cephalosporin antimicrobials in the Enterobacteriaceae group. Recent emerging mechanisms of resistance accumulate through the spread of carbapenem-destroying B-lactamases leaving narrow therapeutic options. The search for carbapenem agents was initially from diverse sources. Among these carbapenem agents, selection for treatment depends on the pathogen present.(Codjoe and. Donkor,2018).

2.7.1. Uses

This agent is most appropriately used for the treatment of severe community-acquired infections. However, the agent should not be used as first-line empirical therapy, except in certain specific circumstances. also be used in a few specific instances for nosocomial infections where *Pseudomonas spp*. are not deemed important pathogens, such as early nosocomial pneumonia acquired out of the intensive care unit (ICU), ideal for directed therapy based on the results of microbiological testing, and especially for the treatment of infections with isolates demonstrating ESBLs, well suited for the treatment of chronic and recurrent or persistent infections in cases in which cultures are most likely to demonstrate resistant Enterobacteriaceae or that are poly microbial in nature; however, it is not effective against *Pseudomonas* and *Acinetobacter*s pp.

It is indicated for the treatment of the following infections,

with specific indications: pneumonia, surgical infections including intra-abdominal, skin and soft-tissue and gynecological infections(Brink., *et al* 2014).

2.8.Carbapenem - resistant Enterobacteriaceae (CRE) or carbapenemase - producing Enterobacteriaceae (CPE)

Are Gram negative bacteria that are resistant to the carbapenem class of antibiotics, considered the drugs of last resort for such infections. They are resistant because they produce an enzyme called a carbapenemase that disables the drug molecule. The resistance can vary from moderate to severe, Enterobacteriaceae are common commensals and infectious agents (Umkalthoum, 2019).

CRE are among the most challenging antibiotic resistant pathogens emerged in the clinical setting, due to their ability to spread rapidly in healthcare environments and to cause infections associated with high

morbidity and mortality, for which very limited treatment options are available(Ambretti *et a*l.,2019).

2.9. Previous study

In study investigated the antimicrobial activity of harjal aqueous extracts against two Gram negative bacteria *Escerichiacoli* and *Salmonella typhi*. The effect of harjal leaves extract on the two bacteria (*E. coli*, *S. typhi*) was evaluated by the inhibition zone and dilution methods. A clear zone of inhibition was shown by the extracts against both bacteria, although the effect was less against *E. coli*. The results of the dilution plate method, showed that the log number of colonies of both bacteria was highly decreased with harjal extracts, however, *S.typhi* was more susceptible and greatly affected (Sulieman *et al*.,2009).

In this study showed that the essential oil of *C. proximus* strongly inhibited the growth of the test bacteria studied, while the methanol extract had moderate antibacterial, *Bacillus cereus and Salmonella cholerasuis* were proven to be the most susceptible against essential oil (Selim .,2011).

Other study of *Cymbopogon proximus* in Sudan. In leaves extracts to determine the phytochemical composition of the leaves extracts of *C.proximus*, Its Vitro effects against *Aspergillusniger, Candida albicans, Escherichia coli, Klebsiella pneumonia,Pseudomonas aeruginosa, Staphylococcus aureus* showed high activity on both Gram-positive, Gram negative bacteria and the two tested fungus however no activity observed against *Pseudomonas aeruginosa* (Malin *eta.l*,2018).

In study investigated the antimicrobial activity of harjal aqueous extracts against two Gram negative bacteria *Escerichia coli* and *Salmonella typhi*. The harjal aqueous extracts were found to inhibit mycelial radial growth of both fungi. . The effect of harjal leaves extract on the two bacteria (*E. coli*, *S. typhi*) was evaluated by the inhibition zone and dilution methods. A clear zone of inhibition was shown by the extracts against both bacteria,

although the effect was less against *E. coli*. The results of the dilution plate method, showed that the log number of colonies of both bacteria was highly decreased with harjal extracts, however, *S.typhi* was more susceptible and greatly affected (Sulieman, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This was a descriptive cross sectional study.

3.2. Study population

Hospitalized patients from different hospitals in Khartoum State with bacterial infection.

3.3. Study area and duration

Military hospital, Omdurman Teaching Hospital, Fedial hospital, Ultra laboratory, Alacadimy Hospital and Police Education Hospital, From February 2018 to December 2018.

3.4. Sample size

The study done on fifty clinical isolates from eighty isolates that identified.

3.5. Inclusion criteria

All isolates that were Gram negative carbapenem resistant bacteria were included.

3.6. Exclusion criteria

All isolates that were non Gram negative bacteria and carbapenem susceptible bacteria were excluded

3.7. Data collection:

Data were collected for the isolates using data collection form containing all study variables (Appendix 2).

3.8. Ethical consideration

Permission was issued by the Medical Laboratory Science Collage's Ethical Committee, Sudan University of Science and Technology. The consent was taken from Laboratory Manager to collect specimens with

insuring all ethical consideration for conducting the research in a way that protect the patient's privacy.

3.9. Collection of plant samples

The plant extracts were collected and authenticated at the Medicinal and Aromatic plant Research Institute (MAPRI).

3.10. Preparation of the extract

Extraction was carried out according to method described by (Sukhdev *et al*., 2008).

Hundred gram of the plant samples were grounded using mortar and pestle and extracted by soaking in 80 % ethanol for about five days with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness and the yield percentages were calculated.

3.11. Collection of sample

Various clinical samples including (wound swab, urine, blood and fluid) were collected, inoculated in basic media and selective media then identified and preserved in glycerol peptone water, the initial collection and processing of the specimen was done in hospital then further investigation and sensitivity was done at Research Laboratory in Sudan University for Science and Technology.

3.12. Identification

3.12.1. Gram stain:

Smears from the growth were prepared and stains by Gram's stain as follow: fixed by heat, after cooling covered by crystal violets stain for 60 second, washed off stain by cleaned water, covered with Iogl's iodine for 60 second, washed with clean water, covered with safranine stain for 2 minutes, then washed and left to air dry and microscopically examined using oil immersion objective (100X) to observed morphological

appearance, Gram-positive reaction and Gram-negative. The result of Gram's stain was reported (Carter and Cole, 2012)**.**

Biochemical test for Gram's negative rods were carried out according to Cheesbrough, 2000.

3.12.2. Indole test:

The test colony was inoculated in sterile peptone water using a sterile wire loop and then incubated at 73°C aerobically overnight. Few drops of Kovac's reagent were added to medium and shaken gently to test for indole. A positive result was indicated by the production of red color in the surface layer within 10 minutes.

3.12.3. Citrate utilization test:

Slopes of Simmon's citrate agar medium were prepped, by using sterile straight wire loop the slope was streaked the butt was stabbed with a small part of the test colony. Then the slops of medium were incubated overnight at 35°C. A positive reaction was indicated by the change in the color of the medium into blue color while the negative reaction was indicated by no change in the color.

3.12.4. Urease test:

The test colony was inoculated on the surface of the slope of Christensen's urea agar medium by a sterile straight loop in zigzagging manner and then incubated over night at 37°C aerobically. The positive reaction was indicated by the color change in the indicator (phenol red) to pink color and negative reaction as indicated by no change in the color.

3.12.5. Motility test:

The tested colony was taken by a sterile straight loop and inoculated by stabbing a semi-solid media, then incubated aerobically at 37°C overnight. The motility was shown by spreading turbidity from the stab- line or turbidity throughout the medium.

3.12.6. Kliger Iron Ager

A small part of the tested colony was picked off using a straight loop and inoculated in KIA medium. First stabbing the butt, then streaking the slope in the zigzag pattern, and then incubated at 37°C aerobically overnight. Then the results were interpreted as following:

A yellow butt red –pink slope indicated the fermentation of glucose only.

A yellow slope and butt indicated the fermentation of lactose and glucose.

A red-pink slope and butt indicated no fermentation of glucose and lactose.

Blacking along the stab line or throughout the medium indicated H_2 S production. Cracks and bubbles in the medium indicated gas production from glucose fermentation.

3.12.7. Oxidase test

A piece of filter paper was placed on a clean glass slid and three to four drop of freshly prepared oxidase reagent (tetra methyl para phenylene diamine dihydrocholoride) were added using sterile Pasteur pipette, a wooden stick was used to pick a colony of the test organism and placed on filter paper. The positive reaction was indicated by the production of blue-purple color within 10 seconds

NOTE: must use for every test control positive and negative to ensure the reliability of the result.

Then sub cultured in CLED agar plate to do the sensitivity by the extracted plant.

Additionally, the following data will be obtained from the hospitals information systems in order to identify possible risk factors: (1) basic patient characteristics (gender, residence and age), (2) antibiotic usage (during current admission and up-to six months before admission), and (3) admission information (during current admission and up to 1 year before admission).

3.13. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of Gram negative bacilli isolates were performed on Muller-Hinton Agar (MHA) plate (Oxoid, UK) by the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standards Institute guidelines (CLSI.,2011). The antimicrobial agents which were tested from different categories including: extended spectrum cephalosporins [ceftazidime (30 μg), antipseudomonalpenicillins with β-lactamase inhibitors carbapenems [imipenem (10 μg), meropenem (10 μg)], aminoglycosides [gentamicin (10 μg), amikacin (30 μg)], fluoroquinolones [ciprofloxacin (5 μg), folate pathway inhibitor [co-trimoxazole (25 μg)], and polymyxin [colistin (10 μg)] (Oxoid, UK). *E. coli* ATCC 25922 were used as control strains and tested each time when susceptibility testing were performed, zone diameters of each of the antibiotics will be interpreted as per CLSI recommendations (CLSI ,2011).

3.13.1. Sensitivity testing

Muller Hinton media susceptibility of common and rapid growing bacteria using antimicrobial by Kirby – Bauer method , antimicrobial to be included in sensitivity test well depend on pathogen and rang of locally available antimicrobial (Cheesbrough, 2005)

3.13.2. Preparation of bacterial suspension

The inoculum density was compared with McFarland standard solution of $BaSO_4$ (0.1ml of 1% $BaCl_2$ + 9.9ml of 1% H_2SO_4). The suspension was stored in the refrigerator at 4ºC until used.

3.13.3. Modified Kirby Bauer method

Three to five colonies of similar appearance were touch and emulsified in 3 to 4 ml of normal saline or nutrient broth , in good light the turbidity of the suspension were matched with turbidity of McFarland standard against piece of paper Muller Hinton agar was seeded by using sterile cotton swab and the surface of the media allow to dry , then by sterile forceps was

applied the disc about 15mm from the edge and 25mm, then incubate the plate in incubator at $37C^0$ for 18-24 hour interpretation of zone size by interpretative chart either to be sensitive ,intermediate and resistant . (cheesbrough , 2005)

3.13.4. Determination of ethanolic extract of *Solenostemma argel* and Cymbopogon *activity*

By Disc diffusion method with some modifications; In aseptic conditions, 20 ml of warm Mueller Hinton agar (Watin-Biolife, KSA), was poured on sterile disposable plates (Jalil Medicals) and left at room temperature to solidify, Then turned upside down and kept in the refrigerator for about 30minutes. 100 µl of the bacterial suspensions (previously adjusted) were swapped onto the Mueller Hinton plates, using sterile cotton swaps. Sterile blank discs of 6 mm were previously prepared from Whatman No.1 filter paper (Sigma-Aldrich) and saturated with 100,50 and 25 mg/ml to trap about 8 and 4 mg/disc, respectively (Pre-experimental measurements showed that the 6 mm disc absorb about 20 µl). Saturated discs were placed onto inoculated plates, the plates were allowed to stand for a while at room temperature, and then incubated at 37°C for 24 hrs. The susceptibility of the tested bacteria to the extract was indicated after incubation by zones of growth inhibition in millimeter (mm) using a transparent ruler. Gentamicin discs (10 µg/disc) (Oxoid), were used as standard antibacterial (positive control), another discs saturated with the solvent (ethanol) were loaded in a separate inoculated plates and served as negative control (Abdalla.,2016).

3.14. Phenotypic detection of carbapenemase

All isolates resistant to meropenem and imipenem in Kirby-Bauer disk diffusion method would be confirmed for carbapenemase production by Modified Hodge Test (MHT). In the test, inoculum of *E. coli* ATCC 25922 (comparable to 0.5 McFarland standard), were inoculated on MHA. Two discs of meropenem and imipenem (10 µg) were placed on the surface of

MHA 30 mm opposite to each other in a straight line, the test organisms will be streaked from the edge of one disk meropenem to edge of the other imipenem disk. The plates would be incubated at 37 °C for 24 h. They would be examined for a clover leaf type indentation or flattening at the intersection of the test organism and *E.coli* ATCC 25922 within the zone of inhibition of the carbapenem susceptibility disc as described by (Anderson et al., 2007).

3.15. Data analysis

The Data analyzes was carried out through statistical package for the social science (SPSS) version 20 (one sample T-test and other statistical method eg mean and stander division percentage and frequency.

CHAPTER FOUR

THE RESULTS

4.1. Results

Most predominant clinical isolates type contain carbapenem resistant bacteria was urine 45 (56%) then followed by swab sample 17 (21%), and blood sample represent 13 (16 %) and fluid sample 5 (6 %). (Table 2) and table (1) contain total clinical isolates.

The total bacteria that isolated and identified were 80 and 50 were carbapenem resistant bacteria ,*E.coli* 35(44%) were most abundant , followed by *Proteus mirabilis* 20(25%) followed by *Pseudomonas aeruginosa* 15(19%), *Klebsiella pneumonia* were 10(12%) (Table 2).

The result of antimicrobial agents showed that most effective antibiotic against carbapenem resistant isolated bacteria was Amikacin 22/80(28%) %), followed by cotrimoxazole15/80 (18%) Imipenem 2/50(4%), ciprofloxacin 20/80(25%) and ceftazidime 8/80(10%) and more resistant antibiotic was ceftazidime 72 /80(90%) followed by ciprofloxacin 70/80(87%) and Amikacin 58/80 (72%), (Table3).

The activity of *Solenostemma argel* the extract at100 mg/ml concentration show Sensitive 6/50 (12%) and resistant 44/50(88%), 1/50(2%) for 50mg/ml resistant represent 49/50 (90%) and 0/50(100%) and 0/50(100%) for 25 mg/ml (table 4).

The activity of *Cymbopogon proximus* the extract at100 mg/ml concentration show sensitive 38/50 (76%) and resist 12/50(24%), 16/50(32%) for 50mg/ml resistant represent 34/50 (68%) and 0/50(100%) and 0/50(100%) for 25 mg/ml (table 5).

Bacterial isolate and *Cymbopogon proximus* plant concentration sensitive and Resistant Table (6).

Table (1) Frequency and percentage of clinical isolates

Sample	Frequency	Percentage %
Urine	45	56%
Swab	17	21%
Blood	13	16%
Fluid	5	6%
Total	80	100

Table (2) Percentage and frequency of isolated bacteria

Percentage %	**Frequency**	**Percentage %**
E.coli	35	44%
Proteus mirabilis	20	25%
Pseudomonas aeruginosa	15	19%
Klebsiella pneumonia	10	12%
Total	80	100

Table (3) frequency and Percentage of drug sensitivity

Antibiotic	Sensitive	Resistant
Cotrimoxazole	15/80 (19%)	65/80 (81%)
Ciprofloxacin	10/80 (13%)	70/80 (87%)
Imipenem	20/80 (25%)	60/80 (75%)
Amikacin	22/80 (28%)	58/50 (72%)
Ceftazidime	8/80 (10%)	49/50 (90%)

Table (4) Antimicrobialactivity of *Solenostemma argel* against isolated bacteria

***S. argel* concentration**	**Sensitive**	**Resistant**	**Mean ± Stander Deviation**	***p.value***
100%	6/50 (12%)	44/50 (88%)	10.9± 2.7	.000
50%	1/50 (2%)	49/50 (98%)	9.0 ±2.2	.000
25%	0/50 (0%)	0/50 (0%)	7.3± 1.7	.000
Total	50			

Table (5) Antimicrobial activity of *Cymbopogon proximus* against isolated bacteria

***Cymbopogon proximus* concentration**	**Sensitive**	**Resistant**	**Mean±Stander Deviation**	***p.value***
100%	38/50 (76%)	12/50 (24%)	14.6± 2.9	.392
50%	16/50 (32%)	34/50 (68%)	12.3± 3.1	.000
25%	0/50 (0%)	0/50 (0%)	9.5± 2.5.	.000
Total	50			

Table (6) Bacterial isolate and *Cymbopogon proximus*plant concentration sensitive and Resistant

Isolates	**Sensitive/ Concentration**			**Resistant/ Concentration**		
	100%	50%	25%	100%	50%	25%
E.coli	17/21 (80.9%)	6/21 (28.5%)	0/21 (0%)	4/21 (19.1)	15/21 (71.5%)	0/21 (0%)
Klebsiella spp	6/8 (75%)	2/8 (25%)	0/8 (0%)	2/8 (25%)	6/8 (75%)	0/8 (0%)
Pseudomonas spp	8/13 (61.5%)	3/13 (23%)	0/13 (0%)	5/13 (38.5%)	10/13 (77%)	0/13 (0%)
Proteus spp	7/8 87.5%)(	3/8 (37.5%)	0/8 (0%)	1/8 (12.5%)	5/8 (62%)	0/8 (0%)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion:

Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments (Malin *et al.*, 2019).

The study from February 2018 to December 2018 done todetermine the possible antimicrobial activity of *Cymbopogon proximus* and *Solenostemma argel* by disc diffusion method show The activity of *Solenostemma argel* the extract at100 mg/ml concentration show Sensitive 6/50 (12%) and resistant 44/50(88%), 1/50 (2%) for 50mg/ml resistant represent 49/50 (90%) and 0/50(100%) and 0/50(100%) for 25 mg/ml .

The activity of *Cymbopogon proximus* the extract at100 mg/ml concentration show sensitive 38/50 (76%) and resistant 12/50(24%), 16/50(32%) for 50mg/ml resistant represent 34/50 (68%) and 0/50(100%) and 0/50(100%) for 25 mg/ml.

The results of this study indicate that the ethanol extract of *Cymbopogon proximus* contains active compounds capable of killing a range of bacteria types, including *E.coli ,Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa* ,which are carbapenem resistant bacteria .And the activity effective at the highest concentration of plant extract and lower at the lower concentration for all tested bacteria that agreement with study show high activity on both Gram positive, Gram negative bacteria *Escherichia coli, Klebsiella pneumonia*, *Pseudomona aeruginosa,*

Staphylococcus aureus (Malin *et al.*, 2018). The study done disagreement with(Selim,2011)Essential oil showed moderate in vitro antimicrobial activity against all tested bacteria, including Gram positive and Gram negative Whereas the methanol extract showed less antimicrobial activity. This disagree may be to use different solvent.

The study done on *Solenostemma argel* disagreement with (Sulieman , 2009).show active against *Escerichia coli* and*Salmonella typhi* and my study no activity to *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis.*

The study of *Solenostemma argel* extract with chloroform/methanol against *Staphylococcus aureus*; *Micrococcus;Streptococcus spp*; *Bacillus anthracis*; *E. coli, Klebsiella pneumoniae*; *Pseudomonas aeruginosa;* and *Proteus vulgaris*. A powerful effect was observed in case of *Streptococcus spp*. and moderate action against *E.coli*. *B. anthracis;* S. aureus*; Klebsiella pneumoniae* and *Proteus vulgaris*. There was no effect on Micrococcus and Pseudomonas my study agree with but differ in the solvent used and the bacterial type show no effect against *E. coli, Klebsiella pneumoniae;Pseudomonas aeruginosa;* and *Proteus mirabilis (*Abdelhady*et al*,1994).

Ethanol extract of *Cymbopogon proximu s*was found to be effective as antibacterial against different bacterial pathogens, and ethanol extract *Solenostemma argel* less effectively.

5.2. Conclusion:

Ethanolic extract of *Cymbopogon proximus* was found to be effective as antibacterial against, *Proteus mirabilis*, *E. coli*, *Pseudomonas aeruginosa, and Klebsiella pneumoniae* providing the scientific basis for its traditional application in Sudanese folk medicine against many bacterial diseases.

Good activity of ethanol extract of *Cymbopogon proximus* at highest concentration (100 mg/ml), Poor activity in contrast with lower concentration.

Ethanolic extract of *Solenostemma argel* was found to be no activity as antibacterial against different bacterial pathogens, providing the scientific basis for its traditional application in Sudanese folk medicine against many bacterial diseases after done other study.

5.3. Recommendation:

1-Further investigation into pharmacological properties of secondary metabolites of *Solenostemma argel* and Cymbopogon *proximus* More research is required to understand the mode of actions of these plants.

2-Further studies should be carried out for the isolation and characterization of the bioactive compounds.

3-Determination of minimum inhibition concentration (MICS) and toxicity for the active ingredients of each bacteria including in this study.

4-More studies about carbapenem resistance bacteria, and can be supported by molecular detection of resistant genes.

5-Further study on *Solenostemma argel* by using other solvents.

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APPENDICES

APPENDIX (1)

Material

A- Equipment

Autoclave

Bunsen burner.

1- Cork borer.

3- Freezer dryer

4- Hot air

5- Incubator

6- Light microscope with oil immersion lens.

7- Rack.

8- Refrigerator.

9- Soxhlet apparatus (round bottom, reflex, condenser).

10- Straight loops with handle

11- Water bath

12- Wire loops with handle

B- Glassware

1- Petri dishes (plates).

2- Flask with different size.

3- Measuring cylinder.

5- Funnels.

6- Spoons

7- Sterile containers (bijou bottles).

8- Test tubes.

9- Slides.

C- Disposable material

1- Disposable syringes.

2- Wound swab

APPENDIX 2

Sudan University of Science and Technology

College Of Graduate Studies

Antimicrobial Activity of Alcoholic Extract of *Solenostemma argel* and *Cymbopogon proximus* Resistant Gram Negative Bacteria Isolated from Different Hospitals in Khartoum Stat

Data collection form

Date of sample collection: …… Hospital name:

……

ID number:……Ward: ……

Age:……Sex:……

…….....Length of hospital stay: …… Place of Residence: …….....

Clinical diagnosis:

……

……Previous and current administration of antimicrobial agents:

Yes( ) No ( )

If yes, type(s) of antimicrobial agents:

……

……

Table (7) Characteristics and biochemical properties of tested Gram-negative bacteria

Character	*P. aeruginosa*	*Proteus. Spp*	*E. coli*	*K. Pneumoni*a
MacConkey' Agar	Yellow	Yellow	Red	Red
Blood agar	Large Haemolytic Colonies	Fishy odor and swarming	Large white, non-hemolytic colonies	Large mucoid colonies
Shape	Rod	Rod	Rod	Rod
Oxidase test	Positive	Negative	Negative	Negative
Catalase test	Positive	Positive	Positive	Positive
Citrate test	Positive	Positive/ Negative	Negative	Positive
Urease test	Negative	Positive	Negative	Positive
KIA Slop/butt	R/R	R/Y	Y/Y	Y/Y
Gas production	Negative	Positive	Positive	Positive
H2S production	Negative	Positive	Negative	Negative
Indole test	Negative	Positive/ Negative	Positive	Positive/ Negative
Lactose fermentation	Non	Non	Yes	Yes

Key: y (Yellow), R (Red), KIA (Kligler Iron Agar)

Table (8) Weight of extract / weight of sample 100

Sample No	**Sample**	**Weight of sample in gm**	**Weight of extract in gm**	**Yield %**
1.*Solenostemma argel* *2.Cymbopogon proximus*		100 gram	50.8 gram	50.8 %

Solenstomma argel leaves

Cymbopogon proximus leaves

Antimicrobial activity of *Cymbopogon proximus*

Antimicrobial activity of *Cymbopogon proximus* and *Solenstomma argel*