Antibacterial Activity of Mentha Piperita Leaf Extract Against Staphylococcus Aureus Induced Septicemia In Albino Rats: A Pharmacological Evaluation

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Abstract: Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development (Newman et al, 2007). Many plants in Africa are medicinal. The use of medicinal natural plants predates the establishment of antibiotics and other modern drugs into the African continent. The antibacterial activity of Mentha piperita and a synthetic antibiotic (Gentamycin) on a clinical isolate of Staphylococcus aureus was evaluated in vivo using albino rats. Thirty two (32) male and female rats with weight ranging from 60 – 150g were purchased, housed, fed and nurtured for 14days of acclimatization before experiment commenced. Sixteen (16) albino (4 groups containing 4 rats each) was used for toxicological study (LD 50) which was determined to be 56568.54 mg/kg. Then the other 16rats were grouped into 4groups having 4rats each for in vivo antibacterial testing and were inoculated with 0.1 ml growing broth culture of Staphylococcus aureus. Blood was collected from the rats prior, during and after inoculation and treatment for septicemia. Treatment was done by administering 1ml of Gentamycin (equivalent to 4mg) to rat group D and 25980.75 mg/kg of purified aqueous plant extract to group B rats, Group C rats weren't given any treatment while group A rats were only fed with feed and water. Results indicated that the extract of Mentha piperita leaves possesses moderate antibacterial activity but lesser than the antibiotic Gentamycin popularly used in antibacterial therapy. This result has shown the relative potency of Mentha piperita against Staphylococcus aureus bacteria that causes Septicemia in vivo. Hence cannot substitute for antibiotics but their treatment maybe useful to intensify and strengthen the effects of antibiotics on pathogenic Staphylococcus aureus.

1. INTRODUCTION

1.1 Background study

Plants are the key to life on the earth as they directly or indirectly supply approximately 80% of human caloric and protein intake, the remainder being derived from animal products. They are economically important to man due to their multiple applications, such as pharmaceuticals, flavors, fragrance, insecticides, dyes, food additives, toxins etc (Mehta *et al*., 2012). *Mentha piperita* (Peppermint) is a perennial, glabrous and strongly scented herb belonging to family Lamiaceae. It thrives well in humid and temperate climate and most widely cultivated in temperate region of Europe, Asia, United States, India and Mediterranean countries (Mehta *et al*., 2012). The peppermint (*Mentha piperitta*), as a representative of the large family Lamiaceae, belongs to the oldest and traditional medicinal and culinary herbs (Sustrikova and Salamon, 2004). Peppermint is a non-native herbaceous plant, it is a perennial, which can reach 100 cm in height (40 inches) has four-sided stem. It is commonly known as Peppermint, Brandy mint, Candy mint, Lamb mint, Balm mint, Vilayati pudina or Paparaminta (Punit and Mello, 2012).

Peppermint oil is used in daily life. It is used in Pepperment candy, Chewing gum, Candy care, Ments chocolate, Shampoo, Insects repellants and used for flavoring pharmaceuticals and oral preparations, such as toothpastes, dental creams, and mouth washes. Higher and aromatic plants have traditionally been used in folk medicine as well as to extend the shelf life of food, showing inhibition against bacteria, fungi and yeast (Ebenezer *et al*., 2011). Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches, and migraines (Fatiuh *et al*., 2002). Peppermint oil or peppermint tea is often used to treat gas and indigestion; it may also increase the flow of bile from the gall bladder. Peppermint oils relaxing action also extended to tropical use, when applied tropically it acts as counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area (Sureshkumar *et al*., 2007).Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram negative bacteria. Peppermint extracts are bacteriostatic against *Streptococcus pyrogens*, *Staphylococcus aureus*, *Serratia marcescens*, *E.coli* and *Mycobacterium avium*. Peppermint is also found to have antiviral and

fungicidal activity. Menthol is virucidal against influenza, herpes and other viruses. Aqueous extracts of peppermint leaves were antiviral against influenza A, Newcastle disease virus in egg and cell culture system. Menthol and peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and Dermatophytic fungi and verity of parasites (Sureshkumar *et al*., 2007; Alexander and Heinz, 2004; Katikia *et al*., 2011). This essential oil dilates blood vessels and inhibits bacteria; especially menthol has a broad spectrum antibacterial activity. Peppermint oil is found to be strongly effective against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecium*, *Klebsiella pneumoniae* and *E. coli*. Peppermint is also found to have antiviral and fungicidal activity. It is virucidal against influenza, herpes and other viruses.

Antifungal activity of the essential oil of *M. piperita* was also reported (Ebenezer, *et al*., 2011; Beata, 2007). The study confirms that both aqueous as well as organic solvent leaf extracts possess strong antibacterial properties against various pathogens viz., *Bacillus substillus*, *Pseudomonas aureus*, *Pseudomonas aerogenosa*, *Serratia marcesens* and *Streptococcus aureus* (Sureshkumar *et al*., 2007: Pramila *et al*., 2012). *Mentha piperita*, the peppermint (mint) plant is an aromatic perennial herb cultivated in most part of the world, have traditionally been used in folk medicine. Leaves of mint plant are frequently used in herbal tea and for culinary purpose to add flavour and aroma. The distinctive smell and flavour, a characteristic feature of *Mentha spp.* is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain (Patil *et al*., 2007). The mint plants are rich sources of iron and magnesium, which play important role in human nutrition (Arzani *et al*., 2007). A large volume of literature is available on the medicinal properties of essential oils present in *Mentha* spp. (Gulluce *et al*., 2007). However, no much study has been directed toward the antioxidant and antimicrobial properties of the mint leaves which are locally available. Hence, the objective of the study was to assess the phytochemical contents, antioxidant and antimicrobial properties of the locally grown mint plant leaves.

S. aureus is a gram-positive bacterium that appears in clusters on Gram-stain. It is catalase positive and, unlike other staphylococcal species, coagulase positive. There are several methods to identify *S. aureus*, e.g., polymerase chain reaction and peptide nucleic acid fluorescence in situ hybridization, although the best known and most used is the bacterial culture (Prober, 2012).*S. aureus* has a variety of virulence factors that singly and in combination, can result in severe infection. Catalase, produced by *S. aureus*, is an enzyme that allows intracellular survival of this bacterium by breaking down hydrogen peroxide, a host defense mechanism (Wulf, 2007).Surface proteins of *S. aureus* include coagulase (the catalyst that generates fibrin from fibrinogen) and clumping factors (which cause clotting). Toxins and extracellular substances include hemolysins (which destroy erythrocytes), leukocidins (which cause skin necrosis), and exfoliative toxin and enterotoxins B and C (which propagate the systemic inflammatory response) (Prober, 2012). Panton-Valentine leukocidin is a toxin that can do all the above. These virulence factors allow *S. aureus* to cause the variety of clinical syndromes for which this bacterium is known, including the development of abscesses. *S. aureus* can acquire new genetic elements. Local environmental stressors, such as low pH, low oxygen, poor availability of nutrients, extremes in temperatures, and antibiotic use, may force altered genetic expression through regulatory mechanisms. In total, intrinsic and acquired genetic material expands the ability of *S. aureus* to affect the patient while surviving in harsh conditions. *S. aureus* has proved adept at developing antibiotic resistance. This is accomplished through acquisition of mobile genetic elements that transfer resistance and virulence from other bacterial species and staphylococcal strains. External pressures (namely, overuse of antibiotics) can also force a previously susceptible isolate to become resistant.

The most well-known story of *S. aureus* resistance follows the introduction of penicillin. *S. aureus* developed resistance via production of β-lactamase, which prevents the antibiotic from binding to the penicillin-binding protein (PBP) on *S aureus.* This lack of binding to PBP obstructs the ability of the penicillin drug to inhibit bacterial cell wall synthesis (Prober, 2012). This β-lactamase was not native to *S. aureus*, it was acquired via a plasmid-encoded penicillinase. The evolution of methicillin resistance should be familiar. Semi-synthetic penicillins (the prototype being methicillin) were developed by scientists to circumvent the problem of *S. aureus* β-lactamase resistance. However, shortly after the introduction of these compounds, some *S. aureus* strains became methicillin resistant.

Methicillin resistance was acquired via the *mecA* gene encoding for PBP2a, which decreases the binding affinity of antibiotics to the target bacterium. It is proposed that *S aureus* acquired the *mecA* gene from other staphylococcal species (Taylor, 2009).An ominous example of acquisition of resistance pertains to vancomycin, regarded by medical providers as a workhorse against gram-positive pathogens. Vancomycin-intermediate *S aureus* (VISA) was identified in the 1990s. Although the resistance mechanisms of VISA are not completely understood, the result is a minimum inhibitory concentration (MIC) of vancomycin greater than or equal to 4 μg/mL to overwhelm *S aureus* (the usual MIC is <2 μg/mL). Vancomycin-resistant *S. aureus* (VRSA) is defined as possessing a MIC for vancomycin that is greater than or equal to 16 μg/mL. The first case of VRSA was reported in 2002. The resistance of VRSA is attributed to the *VanA* gene, which was acquired from enterococcus, an entirely different bacterial genus (Prober, 2012).

Sepsis (Septicaemia) is a life-threatening condition that arises when the body's response to infection causes injury to its own tissues and organ. Common signs and symptoms include fever, increased heart rate, increased breathing rate, and confusion. There also may be symptoms related to a specific infection, such as a cough with pneumonia, or painful urination with a kidney infection (Jui, 2011).In the very young, old, and people with a weakened immune system, there may be no symptoms of a specific infection and the body temperature may be low or normal, rather than high. Severe sepsis is sepsis causing poor organ function or insufficient blood flow. Insufficient blood flow may be evident by low blood pressure, high blood lactate, or low urine output. Septic shock is low blood pressure due to sepsis that does not improve after reasonable amounts of intravenous fluids are given (Delinger, 2013).Sepsis is caused by an immune response triggered by an infection. Most commonly, the infection is bacterial, but it may also be from fungi, viruses, or parasites. Common locations for the primary infection include lungs, brain, urinary tract, skin, and abdominal organs. Risk factors include young or old age, a weakened immune system from conditions such as cancer or diabetes, major trauma, or burns. An older method of diagnosis was based on meeting at least two systemic inflammatory response syndrome (SIRS) criteria due to a presumed infection. In 2016, SIRS was replaced with qSOFA which is two of the following three: increased breathing rate, change in level of consciousness, and low blood pressure. Blood cultures are recommended preferably before antibiotics are started, however, infection of the blood is not required for the diagnosis. Medical imaging should be used to look for the possible location of infection (Delinger, 2013).Other potential causes of similar signs and symptoms include anaphylaxis, adrenal insufficiency, low blood volume, heart failure, and pulmonary embolism, among others. Sepsis is usually treated with intravenous fluids and antibiotics. Typically, antibiotics are given as soon as possible. Often, ongoing care is performed in an intensive care unit. If fluid replacement is not enough to maintain blood pressure, medications that raise blood pressure may be used. Mechanical ventilation and dialysis may be needed to support the function of the lungs and kidneys, respectively (Jui, 2011). To guide treatment, acentral venous catheter and an arterial catheter may be placed for access to the bloodstream. Other measurements such as cardiac output and superior vena cava oxygen saturation may be used. People with sepsis need preventive measures for deep vein thrombosis, stress ulcers and pressure ulcers, unless other conditions prevent such interventions. Some might benefit from tight control of blood sugar levels with insulin (Delinger, 2013).The use of corticosteroids is controversial (Patel, 2012). Activated drotrecogin alfa, originally marketed for severe sepsis, has not been found to be helpful, and was withdrawn from sale in 2011 (Cardona, 2012).

1.2 Justification

The burden of *S. aureus* bacteremia, particularly methicillin-resistant *S. aureus* bacteremia, in terms of cost and resource use is high. The risk of infective endocarditis and of seeding to other metastatic foci increases the risk of mortality and raises the stakes for early, appropriate treatment. The incidence of *S.aureus* bacteremia and its complications has increased sharply in recent years because of the increased frequency of invasive procedures, increased numbers of immunocompromised patients, and increased resistance of *S. aureus* strains to available antibiotics. This changing epidemiology of S. aureus bacteremia, in combination with the inherent virulence of the pathogen, is driving an urgent need for improved strategies and better antibiotics to prevent and treat S. aureus bacteremia and its complications. A variety of bioactive compounds that are present in different parts of a peppermint plant has spurred a

renewed interest in developing an alternate therapy. The traditional herbal medical system has been practiced globally from ancient times; consequently, a great volume of literature is available on the antimicrobial activity of a variety of plant species. Multidrug resistance among the microbial pathogens has been a great concern world over. Phytochemicals from plants have shown great promise in the treatment of intractable infectious human diseases including viral infections (Cowan, 2009).The aqueous extracts of *Camelia sinensis* and *Trachyspermum ammi* were found to be effective against *Salmonella* isolated from curry samples (Thanes *et al*., 2011). Methanolic leaf extract of *Coleus amboinicus* leaves showed remarkable antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) (Sahgal *et al.*, 2009). It is presumed that drugs developed from plant sources may have minimal and very slow to induce drug resistance among the pathogens. From this perspective, it is imperative to screen a variety of plants with potential antimicrobial activity for periodical introduction to manage the drug resistance among the human pathogens like *S.aureus* that causes septicemia.

1.3 Aim and Objectives

The aim of this research is to investigate the in vivo antibacterial activity of *Mentha piperita* leaf extract against *Staphylococcus aureus* causing Septicemia in an albino rat.

Objectives:

1. To compare an antibacterial in vivo comparative study of *Mentha piperita* and commercially prepared antibiotic (gentamycin) via colonial counts during and after treatment.

2. The effect of the extract on the body weights of the rats across the groups.

3. To determine LD_{50} of *Mentha piperita* aqueous Leaf extract in milligram per kilogram body weight.

2. LITERATURE REVIEW

2.1 PEPPERMINT (*Mentha piperita*)

2.1.1 Description

Peppermint was first described in 1753 by Carl Linnaeus from specimens that had been collected in England; he treated it as a species (Linnaeus, 1753), but it is now universally agreed to be a hybrid(Harley, 1975*)*. It is a herbaceous schizomatous perennial plant that grows to be 30–90 cm (12–35 in) tall, with smooth stems, square in cross section. The rhizomes are wide-spreading, fleshy, and bear fibrous roots. The leaves can be 4–9 cm (1.6–3.5 in) long and 1.5–4 cm (0.59–1.57 in) broad. They are dark green with reddish veins, and they have an acute apex and coarsely toothed margins. The leaves and stems are usually slightly fuzzy. The flowers are purple, 6–8 mm (0.24–0.31 in) long, with a four-lobed corolla about 5 mm (0.20 in) diameter; they are produced in whorls (verticillasters) around the stem, forming thick, blunt spikes. Flowering season lasts from mid- to late summer. The chromosome number is variable, with 2n counts of 66, 72, 84, and 120 recorded (Blamey, 2001). Peppermint is a fast-growing plant; once it sprouts, it spreads very quickly.

Image of *Mentha piperita*
Source: Wikipedia

2.1.2 Ecology

Peppermint typically occurs in moist habitats, including stream sides and drainage ditches. Being a hybrid, it is usually sterile, producing no seeds and reproducing only vegetatively, spreading by its runners. If placed, it can grow almost

anywhere(Blamey,2001).Outside of its native range, areas where peppermint was formerly grown for oil often have an abundance of feral plants, and it is considered invasive in Australia, the Galápagos Islands, New Zealand, and the United States in the Great Lakes region, noted since 1843.

2.1.3 Classification

The scientific name for peppermint (*Mentha* x *piperita*) is derived from the name Mintha, a Greek mythological nymph who transformed herself into the plant, and from the Latin piper meaning "pepper."

Domain: Eukarya (Eukaryotic)
Kingdom: Plantae (Plant)
Subkingdom: Tracheophyta (Vascular Plant)
Superdivision: Spermatophyta (Seed Plant)
Phylum: Angiospermophyta (Flowering Plant)
Class: Magnoliopsida (Dicotyledon)
Order: Lamiales
Family: Lamiaceae (Mint Family)
Genus: Mentha (Mint)
Species: *Mentha* x *piperita* (Peppermint)

Domain Eukarya: The peppermint plant falls under the domain Eukarya because it is a multicellular organism with membrane-bound organelles including a nucleus.
Kingdom plantae: The plant kingdom is characterized by cell walls made of cellulose and a chloroplast that goes through the process of photosynthesis.

Phylum Angiospermophyta: Peppermint is an angiosperm, meaning it produces flowers. They bloom from July through August, sprouting tiny purple flowers in whorls and terminal spikes. However, the plant is typically pruned to prevent flowering and increase leaf production.

Class Magnoliopsida: Dicotyledons or 'Dicots' are characterized by six characteristics. The first characteristic is the seed. A dicot seed has two cotyledons or seed leaves in the embryo. Dicot flowers are found in multiples of four or five. The stem of a dicot is found having a ring shape compared to being scattered. Pollen has three pores in a dicot plant, while a monocot has only one. Dicot roots are known to have a main root. Lastly, the leaf veins of dicots are branching, not parallel.

Order Lamiales: This order is classified using the following characteristics: an ovary composed of two fused carpels, five petals fused into a tube, bilateral symmetry, and four (or less) fertile stamens (the male organ of a flower).

Family Lamiaceae: The mint family share characteristics including square stems, leaves that are usually opposite but sometimes whorled, are equal in size and shape and lack stipules (the appendages at the base of the leaves), the edges of leaves may be smooth, toothed, scalloped, and characteristic scents when crushed (oils in leaves) distinctive flavors of the particular species.

Genus Mentha: Mentha refers to the true mints (fertile and not a hybrid). Some examples are *Mentha aquatica*, *Mentha arvensis*, *Mentha longifolia*, and *Mentha suaveolens*.

Species: *Mentha piperita* is a hybrid plant derived from the crossing of *Mentha aquatic* (water mint) and *Mentha spicata* (spearmint).

2.1.4 Distribution

Peppermint can be found over much of the world; indigenous to Europe and Asia, it has been naturalized in North America. In the United States *Mentha piperita* can be found practically everywhere, however; it is commonly found near streams and other wet areas. Following is a map of the distribution of peppermint in the United States and Canada.

Distribution of Peppermint in the United States and Canada of *Mentha piperita* can survive in just about any condition in any location. All this plant needs is water and good drainage. In warm climates peppermint can grow in partial shade or full sun. This particular plant can colonize an entire flower bed, which is why most people choose to control the growth by confining it to a pot/container. It is recommended to move a peppermint plant to a new place periodically because they have a tendency to swindle if kept in one spot too long. *Mentha piperita* needs more water than any of the other *Mentha spp*. especially when it is grown in full sun, where it's flavor and oil content will be higher.

2.1.5 Phytochemical and Uses

Peppermint has a high menthol content. The oil also contains menthone and carboxyl esters, particularly menthyl acetate (Thomson, 2007). Dried peppermint typically has 0.3–0.4% of volatile oil containing menthol (7–48%), menthone (20–46%), menthyl acetate (3–10%), menthofuran (1–17%) and 1,8-cineol (3–6%). Peppermint oil also contains small amounts of many additional compounds including limonene, pulegone, caryophyllene and pin ene (Leung,2000*)*.

Peppermint contains terpenoids and flavonoids such as eriocitrin, hesperidin, and kaempferol 7-O-rutinoside (Dolzhenko, 2010).

CARDIOVASCULAR PROPERTIES:

Peppermint has traditionally been used as a rubefacient (Hawthorn, 2008). It is said to have vasodilating properties on some animals. It has a lowering effect on the heart rate and the systolic pressure. Relaxation of bronchial smooth muscles, increase in the ventilation are also other cardiovascular effects of peppermint oil(Robbers, 2009).

PULMONARY PROPERTIES:

Inhalation of peppermint oil increases the nasal air force and thus supplies more air into the lungs. In a few studies it was also claimed that it makes the lung surfactant more efficient enabling better pulmonary function. In a study where the effect of peppermint on athletic activity was tested it showed that the subjects had a faster breathing rate and as a result a lower arterial PCO2 , decreased end tidal carbon dioxide tension and fractional end tidal carbon dioxide concentration. Lung function test are said to increase after intake of peppermint (Andreoli, 2009). Also the inspiratory muscle strength will be increased with the intake of peppermint. Menthol stimulates the same reflex inhibition of respiration in humans. Peppermint and menthol do not have nasal decongestant properties; however, menthol does cause subjective improvement in nasal breathing.

DENTAL HEALTH:

Pepper mint is used in making oral dentifrices as it can provide over all freshness in breath and also keep away bad breath. More studies are being done as to whether or not it directly contributes to preventing caries and plaque, however it is confirmed that it does create an un-favorable environment for bacteria.

GASTROINTESTINAL BENEFITES:

Peppermint is used for treatment of non-obstructive dyspepsia without any known side effects. It improves the gastric emptying rate. There is a significant antiemetic effect of peppermint in reducing postoperative nausea for patients with very sensitive gag reflexes (Paula, 2000).

NEURO-PSYCHIATRIC EFFECTS:

Some studies have suggested that peppermint is a central nervous system stimulant. Studies have been conducted on the effectiveness of aromas on cognitive performance, perceived physical workload, and pain responses were conducted based on possible changes in the brain activity.

ENDOCRINE EFFECTS

Certain researches have proved that there was a statistically significant increase in the secretion of endocrine hormones (Robbers, 2009). In one study there was a noted segmental maturation arrest in the seminiferous tubules however, the effects of *M.spicata* extended from maturation arrest to diffuse germ cell aplasia in relation to the dose. Other than this there are not many significantly known effects on the human endocrine system.

EFFECT ON SKIN AND MUCOUS MEMBRANE:

Peppermint is said to be a good analgesic to be applied topically and also a coolant for the skin. Peppermint oil stimulates cold receptors on the skin and dilates blood vessels, causing a sensation of coldness and an analgesic effect (Robbers, 2009). Menthol is a topical vasodilator that enhances the absorption of other topical skin medications. It is said that menthol enhances the absorption of cortisone, mannitol, indomethacin, morphine hydrochloride, and propranolol (El-Kady, 2003). Menthol moderates oral sensations of warmth and coldness (Moleyar, 2002). In low concentrations, topical application of menthol causes a cooling sensation, while in

high concentrations it causes irritation and local anesthesia (Raudenbush, 2002). It also increases cutaneous blood flow, muscle temperature, and skin temperature after topical application of the oil. Some studies have claimed that menthol has reduced histamine induced irritation and itching.

IMMUNE MODULATION:

Menthol has anti-inflammatory effects when applied topically. In one study it was claimed that it could suppress antigen induced allergies. Menthol also has a property of inhibiting cutaneous anaphylaxis that's mediated by IgE antibody.

ANTIMICROBIAL EFFECT:

Menthol is virucidal against Influenza, Herpes and other viruses in vitro(Eccles, 2004). Aqueous extracts of peppermint. Peppermint oil and menthol have mild antibacterial effects against both Gram-positive and Gram-negative bacteria (El-Kady, 2003). Peppermint extracts are bacteriostatic against *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Pattnaik, 2007). Menthol is bactericidal against strains like *Staphylococcus pyogenes*, *S. aureus*, *Streptococcus pyogenes*, *Serratia marcescens*, *Escherichia coli*, and *Mycobacterium avium* (Pattnaik, 2007). Menthol and peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and dermatophytic fungi

(El-Kady, 2003). Its use for the treatment of certain types of cancer and viral infections has also been reported (Abascal *et al.*, 2003). Plants have formed the basis of sophisticated traditional medicine system and natural products make excellent leads for new drug development (Newman *et al*., 2007).

ANTISPASMODIC EFFECT OF PEPPERMINT OIL:

Previous studies have shown that various kinds of mint were effective in reducing muscle pain, muscle relaxation, and reduce fatigue. Until now, many researchers have been done on the effectiveness of various kinds of natural products in the improvement of sport performances. Mint is a herb which is well known for its antispasmodic, painkilling (Eccles, 2004), anti-inflammatory, antispasmodic, decongestant, and antioxidant effects (Hoffman, 2006). Peppermint is one of the mentha species (*i.e.*, *Mentha piperita*, peppermint oil, *Mentha arvensis*, cornmint oil (Bove, 2006). Menthol and menthone are the major components of the peppermint essential oil. External application of peppermint extract raised the pain threshold in human (Blumenthal, 2008).Peppermint aroma was also effective on perceived physical workload, temporal workload, effort, and anxiety (Fleming, 2008). According to certain in vitro studies conducted on the antispasmodic effect of peppermint oil, peppermint relaxes gastrointestinal smooth muscle spasm by reducing calcium influx in both guinea pig large intestine and rabbit jejunum.

2.1.6 Cultivation

Peppermint generally grows best in moist, shaded locations, and expands by underground rhizomes. Young shoots are taken from old stocks and dibbled into the ground about 1.5 feet apart. They grow quickly and cover the ground with runners if it is permanently moist. For the home gardener, it is often grown in containers to restrict rapid spreading. It grows best with a good supply of water, without being water-logged, and planted in areas with part-sun to shade. The leaves and flowering tops are used; they are collected as soon as the flowers begin to open and can be dried. The wild form of the plant is less suitable for this purpose, with cultivated plants having been selected for more and better oil content. They may be allowed to lie and wilt a little before distillation, or they may be taken directly to the still.

2.2.0 *Staphylococcus aureus*

2.2.1 Classification

Domain: Bacteria

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Baccilli

Order: Bacillales

Family: Staphylococcaceae

Genus: *Staphylococcus*

Species: *Staphylococcus aureus*

Subspecies: Methicillin-resistant *Staphylococcus aureus*

2.2.2 Distribution

Distribution of *Staphylococcus aureus* infection in Africa

Africa is a prosperous continent. Despite the uneven distribution of wealth, the lack of translation of economic growth to productive jobs, and poor governance structures in some countries, we are facing a continent with the higher birth rates and rates of economic growth in some countries than in the rest of the world.However, the links to global markets, particularly the shipment of goods and passenger traffic, are still loose; therefore, interest in the dissemination of emerging pandemic pathogens might have been low in Africa. For instance, the picture of the spread of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) in Africa is unclear, in contrast to the rest of the world. To date, research in infectious diseases in Africa has been focused on the ‘big three’ (malaria, human immunodeficiency virus (HIV)/AIDS, and tuberculosis), but is now being expanded to ‘neglected tropical diseases’ as promoted by the Millennium Development Goals (Hotez,2011) However, major bacterial pathogens are not yet sufficiently considered in future research agendas, although the burden of infection by, for instance, *S. aureus*, *Streptococcus pneumoniae* or extended-spectrum β-lactamase-producing Enterobacteriaceae is high in Africa (Herrmann,2013).This is urgently needed, as HIV/AIDS, malaria, malnutrition, crowded living conditions, high temperatures and humidity increase the risk of other bacterial infections (Panchanathan, 2013).Research on bacterial infections in Africa

should enter a phase where the focus of our attention is not limited to the high burden of disease and medical need. Phylogenetic analyses might also help us to understand the origin of major pathogens, considering Africa as the 'cradle of man'. Indeed, the spread of some pathogens is linked to human migration, suggesting Africa as the origin of a number of bacteria, such as *Helicobacter pylori* (Schaumburg, 2011*),* *Mycobacterium tuberculosis* complex (Allix-Beguec *et al.,* 2008), *Mycobacterium leprae* (Monot,2001), and *Salmonella enterica* (Roumagnac,2006*).* This might also apply to *S. aureus* (Schaumburg, 2011).

The objective of this review is to report the epidemiology of *S. aureus* colonization and infection in Africa, considering the geographical and lifestyle differences between North Africa and sub-Saharan Africa. We also point out the challenges in combating *S. aureus*-related infections.

2.2.3 Epidemiology of *S. aureus* Colonization

S.aureus colonization is a risk factor for subsequent infection caused by the colonizing clone (VonEiff, 2001).Therefore, knowledge about colonizing isolates is key to understanding *S. aureus* infection. The colonization pattern in different age groups is similar in Africa and Europe, with high colonization rates immediately after birth and in teenagers. This holds true not only for urban and semi-urban populations, but also for remote Pygmy populations (Schaumburg, 2011). *Staphylococcus aureus* nasal colonization rates in Africa. Community-associated *S. aureus* nasal carrier studies (1985-2013) performed in Africa are included in this diagram (Schaumburg,2011).The trend-line was fitted by use of a polynomial regression (order 4).Confirmed risk factors for *S. aureus* colonization in Africa are HIV infection (Kinabo,2011), frequent hand-washing (more than three times daily) , living in rural areas, and being hospitalized on surgery wards . A risk factor for *S. aureus* colonization in infancy is *S. aureus* colonization of the mother. In contrast, age of <5 years, higher educational level of parents and male sex seem to be protective against *S. aureus* colonization. As in other parts of the world, *S. aureus* also colonizes animals, such as pets (e.g. dogs), livestock (e.g. donkeys, pigs, and sheep) or wild animals (e.g. monkeys, chimpanzees, gorillas, and bats) (Schaumburg,2011). The zoonotic risk for humans has not yet been sufficiently studied, but might be lower than that associated with the emerging burden of livestock-associated MRSA(Schaumburg,2011)some parts of Europe (Mellmann *et al.,*2012). So far, only MRSA colonization in pigs (12.5% in South Africa and 1.3% in Senegal) might point towards future problems. However, colonization rates are still lower than porcine colonization rates in Belgium (40%), Germany (43.5%), and Spain (46.0%) (Richard *et al.,* 2012). Only isolates belonging to sequence type (ST)5-MRSA-IV and ST88-MRSA-IV, and not ST398-MRSA, have so far been found in African livestock (Turck *et al*,2012).Not only livestock but also wild animals, such as chimpanzees and gorillas, can be affected by human-associated *S. aureus* lineages, causing both asymptomatic colonization (Peck *et al.,*2012*)* and fatal infection (Turck *et al.,*2012*)* This represents a high risk for endangered species such as the great apes (Peck*et al.*,2012). It is noteworthy that monkeys can be colonized with a highly divergent *S. aureus* subclade comprising isolates belonging to multilocus sequence types ST1874, ST2058, and ST2071, and others, showing almost no antibiotic resistance. One isolate belonging to this divergent clade has so far been found only once in humans, in Gabon (Kock*et al*., 2012). Transmission of this highly divergent subclade between animals and humans is therefore possible, but seems to be rare. The spectrum of *S. aureus*-related infections differs from that in other parts of the world, with a higher proportion of pyomyositis, of up to 27%, among cases with bone, skin and soft tissue infection (Pouillot *et a.l,*2011), and up to 21.7% among all *S. aureus*-related infections (Dromigny,2002) Multifocal lesions are frequently encountered, and occur mostly in immunocompromised patients, but might also affect immunocompetent individuals (Adjei,2011). In addition, some studies have suggested a higher proportion of *S. aureus* in urinary tract infections: 6.3–13.9% of urinary tract infections are caused by *S. aureus* in Senegal (Dromigny,2002), Ghana (Adjei,2004), and Nigeria (Otajevwo,2013), as compared with 1.06% in Europe and Brazil (Naber,*2008*). In general, *S. aureus* is a major pathogen in bloodstream infections (9.5–39.0%), skin and soft tissue infections (62.8–90.0%), ear, nose and throat infections (16.7–29.0%), and surgical site infections (20.4–32.0%).

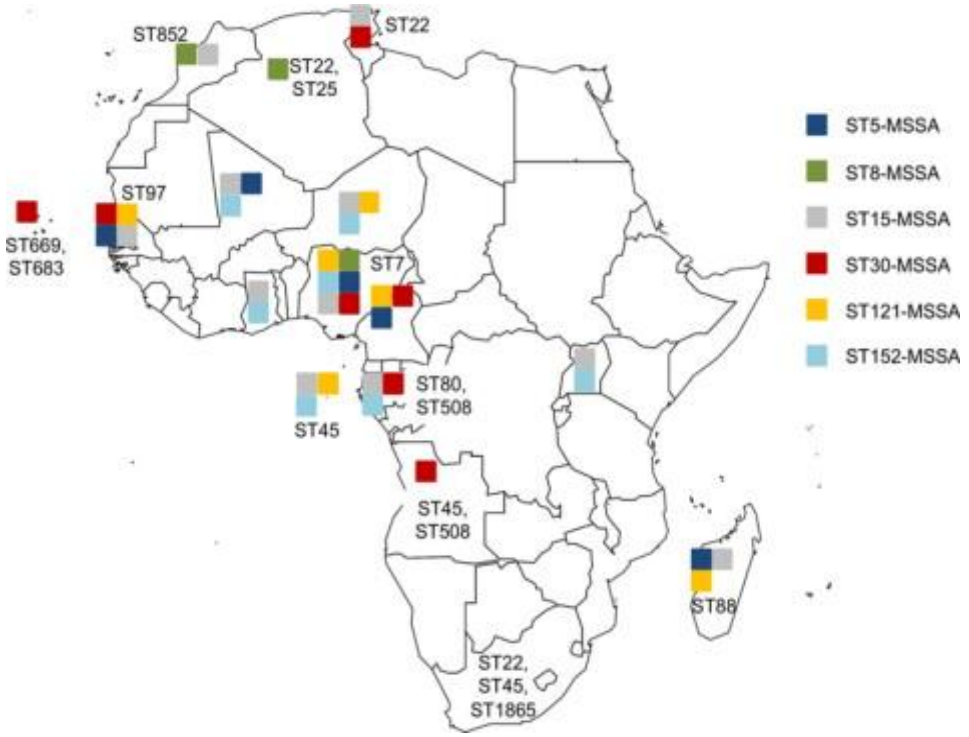
A few prospective studies have revealed a higher incidence of *S. aureus* infection in Africa than in industrialized countries. The annual incidence of *S. aureus* bacteraemia was 3.28 cases per 1000 hospital admissions (South Africa) or between 101 and 178

cases per 100 000 child-years (Mozambique), with the highest incidence in children aged <5 years (Sigauque *et al.*,2009). For comparison, the annual incidence rates of community-acquired *S. aureus* bloodstream infection in the USA were 2.3 cases per 100 000 person-years for methicillin-sensitive *S. aureus* (MSSA) and 1.5 cases per 100 000 person-years for MRSA (Landrum,2010).

Annual incidence rates were high for skin and soft tissue infections (1572 cases per 100 000 children aged <12 months) and mastitis (452 cases per 100 000 mothers), as a study from Gabon revealed (Schaumburg,2011).Detailed molecular characterization of clinical *S. aureus* isolates from Africa has been largely neglected in the past. Only cases of *S. aureus*-related infections in travelers returning from Africa have suggested that African *S. aureus* might have a different genetic background and might be more virulent than isolates from Europe. Reports of fatal *S. aureus* pneumonia and complicated skin and soft tissue infection in travelers returning from Africa were frequently associated with isolates producing Panton–Valentine leukocidin (PVL) (Beilouny,2008). PVL can lyse granulocytes, and is associated with skin and soft tissue infection (Grundmeier *et al.*,2014).Field studies performed in Africa in the last two decades have showed PVL-positive *S. aureus* infection in travellers: Africa is now considered to be a PVL-endemic region with high rates of PVL-positive isolates, mainly MSSA, ranging from 17% to 74% . This is in stark contrast to Europe, where the prevalence of PVL-positive isolates is low (0.9–1.4%) (Oosthuysen, 2013). Studies from Gabon and South Africa further support the association of PVL with abscesses and skin and soft tissue infection (Oosthuysen, 2013). The reasons for the high prevalence of PVL are unknown, but might be related to the host (i.e. altered C5a receptors, which have been identified as PVL targets), so far unidentified virulence factors of *S. aureus* that facilitate dissemination (similarly to *sasX*), and the humid environment of tropical Africa (Van-Rooijen *et al.,* 2013).

2.2.4 Population Structure

The distribution of major African MSSA clones is heterogeneous. ST5-MSSA and ST15-MSSA are highly prevalent in West Africa (including Cameroon). Isolates belonging to ST5 rarely encode PVL; however, the proportion of PVL-positive isolates in this lineage can be up to 33% (Breurec*et al.*,2011). ST15 isolates frequently harbour PVL (25.9–90%) and enterotoxin A (22–74.6%) (Breurec *et al.*,2011) ST8-MSSA isolates are mainly found in the Maghreb, including Nigeria, and only rarely carry the PVL-encoding genes (up to 9% in Morocco) (Breurec *et al.*,2011). In Central and West Africa, *S. aureus* isolates mainly belong to ST30, ST121, and ST152. These are the major PVL-positive clones, with high proportions of PVL-positive isolates in ST30 (9.1–100%), ST121 (50–93%), and ST152 (97–100%) (Schaumburg *et al.*,2011). A similar genetic background of MSSA and MRSA among the major clones is evident for ST5 and ST8. Whereas ST5-MRSA and ST5-MSSA are highly prevalent in the same geographical region (Central and West Africa), ST8-MRSA (Central and South Africa) and ST8-MSSA (Maghreb) clearly differ in their geographical distribution. This might suggest that African ST5-MRSA evolved in Africa from ST5-MSSA through acquisition of the SCCmec element, whereas this might not be true for ST8-MRSA. It is noteworthy that some ST8-MRSA isolates that are closely related to the hypervirulent CA-MRSA clone USA300 (ST8, t008, PVL-positive, and arginine catabolic mobile element [ACME]-positive) were recently reported from Gabon and Ghana (ST8, t121 and t112, PVL-positive, and ACME-positive) (Egyir*et al.*,2014) There is one reported case of a severe invasive infection by this USA300-related clone, causing bacteraemia, pneumonia and pericarditis in an otherwise healthy person in Gabon (Risk *et al.*,2013*)*



Abdulgader, 2015

FIG.1. Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) clones in Africa.

The three major MRSA multilocus sequence types (STs) of each study were identified (Armand-Lefevre *et al.*,2011) . Of these, the five most widely distributed clones in Africa are shown. Sporadic clones are indicated by letters. STs from Uganda were derived from spa types.ST80-MRSA-IV is mainly found in the Maghreb, and frequently carries PVL (91.7–100%) (Ben,2013) . Whereas ST80-MRSA-IV from Egypt did not show any resistance to tetracycline and fusidic acid (Enany,2010), this resistance was detected in isolates from Algeria (tetracycline, c. 75%) and Tunisia (tetracycline, 4.7%; fusidic acid (intermediate), 3.1%) (Ben, 2013). ST80-MRSA-IV is the major community-acquired MRSA clone in Europe that is always PVL-positive and usually resistant to tetracycline and intermediately resistant to fusidic acid (Tristan*et al.*,2007). The geographical proximity, the phylogenetic relatedness and the first report of the clone in North Africa in 2006 (Tristan*et al.*,2007)suggest that this MRSA clone spread from Europe to the Maghreb. However, whole genome analyses are warranted to assess this hypothesis. ST88-MRSA is predominant in West, Central and East Africa (Fig. 1), where the prevalence of PVL and other virulence factors is very low (Schaumburg *et al.*,2011). This clone has been only sporadically reported around the globe, except for the Far East, where the prevalence rates among MRSA isolates are 5.3–10% (China) and 12.5% (Japan) (Yao *et al.*, 2010). This proportion is markedly lower than that Africa, where ST-88-MRSA accounts for 24.2–83.3% of all MRSA isolates (Schaumburg *et al.*,2011). As the origin of this clone is unclear, we suggest naming ST88-MRSA-III/IV the 'African clone'. ST239/241-MRSA-I/III/IV is prevalent on the whole African continent, and this is mirrored by the global spread of this Iberian–Hungarian–Brazilian clone.

2.2.2 Diseases Caused by Staphylococci

Staphylococci cause disease by

- Direct tissue invasion
- Sometimes exotoxin production

Direct tissue invasion is the most common mechanism for staphylococcal disease, including the following:

- Skin infections
- Pneumonia
- Endocarditis
- Osteomyelitis
- Septic arthritis

Multiple **exotoxins** are sometimes produced by staphylococci. Some have local effects; others trigger cytokine release from certain T cells, causing serious systemic effects (eg, skin lesions, shock, organ failure, death). Panton-Valentine leukocidin (PVL) is a toxin produced by strains infected with a certain bacteriophage. PVL is typically present in strains of

community-associated methicillin-resistant *S. aureus*(CA-MRSA) and has been thought to mediate the ability to necrotize; however, this effect has not been verified.Toxin-mediated staphylococcal diseases include the following:

- Toxic shock syndrome
- Staphylococcal scalded skin syndrome
- Staphylococcal food poisoning

2.2.2.1 Staphylococcal bacteremia

S. aureus bacteremia, which frequently causes metastatic foci of infection, may occur with any localized *S. aureus* infection but is particularly common with infection related to intravascular catheters or other foreign bodies. It may also occur without any obvious primary site. *S. epidermidis* and other coagulase-negative staphylococci increasingly cause hospital-acquired bacteremia associated with intravascular catheters and other foreign bodies because they can form biofilms on these materials. Staphylococcal bacteremia is an important cause of morbidity (especially prolongation of hospitalization) and mortality in debilitated patients.

2.2.2.2 Staphylococcal skin infections

Skin infections are the most common form of staphylococcal disease. Superficial infections may be diffuse, with vesicular pustules and crusting (impetigo) or sometimes cellulitis, or focal with nodular abscesses (furuncles and carbuncles). Deeper cutaneous abscesses are common. Severe necrotizing skin infections may occur. Staphylococci are commonly implicated in wound and burn infections, postoperative incision infections, and mastitis or breast abscess in breastfeeding mothers.

2.2.2.3 Staphylococcal neonatal infections

Neonatal infections usually appear within 6 wk after birth and include

- Skin lesions with or without exfoliation
- Bacteremia
- Meningitis
- Pneumonia

2.2.2.4 Staphylococcal pneumonia

Pneumonia that occurs in a community setting is not common but may develop in patients who have influenza, are receiving corticosteroids or immunosuppressants, and those who have chronic bronchopulmonary or other high-risk diseases. Staphylococcal pneumonia may be a primary infection or result from hematogenous spread of *S. aureus* infection elsewhere in the body (e.g, IV catheter infection, endocarditis, soft-tissue infection) or from injection drug use. However, *S. aureus* is a common cause of hospital-acquired pneumonia, including ventilator-acquired pneumonia. Staphylococcal pneumonia is occasionally characterized by formation of lung abscesses followed by rapid development of pneumatoceles and emphysema. CA-MRSA often causes severe necrotizing pneumonia.

2.2.2.5 Staphylococcal endocarditis

Endocarditis can develop, particularly in IV drug abusers and patients with prosthetic heart valves. Because intravascular catheter use and implantation of cardiac devices have increased, *S. aureus* has become a leading cause of bacterial endocarditis. *S. aureus* endocarditis is an acute febrile illness often accompanied by visceral abscesses, embolic phenomena, pericarditis, subungual petechiae, subconjunctival hemorrhage, purpuric lesions, heart murmurs, and heart failure secondary to cardiac valve damage.

2.2.2.6 Staphylococcal osteomyelitis

Osteomyelitis occurs more commonly in children, causing chills, fever, and pain over the involved bone. Subsequently, the overlying soft tissue becomes red and swollen. Articular infection may occur; it frequently results in effusion, suggesting septic arthritis rather than osteomyelitis. Most infections of the vertebrae and inter-vertebral disks in adults involve *S. aureus*.

2.2.5.7 Staphylococcal toxic shock syndrome

Staphylococcal toxic shock syndrome may result from use of vaginal tampons or complicate any type of *S. aureus* infection (eg, postoperative wound infection, infection of a burn, skin infection). Although most cases have been due to methicillin-susceptible *S. aureus* (MSSA), cases due to MRSA are becoming more frequent.

2.2.2.8 Staphylococcal scalded skin syndrome

Staphylococcal scalded skin syndrome, which is caused by several toxins termed exfoliatins, is an exfoliative dermatitis of childhood characterized by large bullae and peeling of the upper layer of skin. Eventually, exfoliation occurs. Scalded skin syndrome most commonly occurs in infants and children < 5 yr.

2.2.2.9 Staphylococcal food poisoning

Staphylococcal food poisoning is caused by ingesting a preformed heat-stable staphylococcal enterotoxin. Food can be contaminated by staphylococcal carriers or people with active skin infections. In food that is incompletely cooked or left at room temperature, staphylococci reproduce and elaborate enterotoxin. Many foods can serve as growth media, and despite contamination, they have a normal taste and odor. Severe nausea and vomiting begin 2 to 8 h after ingestion, typically followed by abdominal cramps and diarrhea. The attack is brief, often lasting < 12 h.

2.2.2.10 Sensitivity

This study had in view to establish and test the sensitivity of *Staphylococcus aureus* bacteria, because of its multiple effects on skin, nasal lining, pharynx and the urine culture. Staphylococcus can cause a wide range of infections. Their severity and location vary from superficial skin infections, to infections which endanger our lives like septicemia and meningitis. Mostly, the Staphylococcus produces penicillinase. Some people can even resist without any kind of problems to analogues of penicillin. The three most common types of Staphylococcus are: *Staphylococcus aureus, Staphylococcus epidermidis* (resistance of the nasal vestibule and teguments, accidental pathogen, found in sub-acute bacterial endocarditis, followed by surgery and cardiovascular explorations at the level of the infected acne) and *Staphylococcus saprophyticus* (external environment saprophyte may contaminate the lining and teguments).*Staphylococcus aureus* is a bacteria widely spread in the environment, like in air, dust and on the household items. Healthy people have it, in a percentage of 30% on their nasal lining. The percentage is even higher at people, who work in the hospital, at patients who need dialysis, those who have diabetes and those who use intravenous drugs. *Staphylococcus aureus* is also a pathogen species and presents superficial skin infections from simple pimples to infections that endanger our lives, like an abscess, furuncle septicemia, meningitis, toxic food, urinary tract infection at sexually active young women.

It can cause different angina, a bronchitis and lung infection which, in case of children, forms bronchopneumonia and is often fatal (Andreoli,2009).

Staphylococcus aureus can be treated with a wide range of antibiotics. Efficient and inefficient antibiotics were enlisted. In specialized literature, we identified 10 groups of antibiotics: penicillin, oxacilin, gentamicin, tobromicin, tetracycline, erythromycin, clindamycin, vancomycin, rifampicin, linezolid and ciprofloxacin.

Taking into consideration the last years, branches of *Staphylococcus aureus* and *Staphylococcus epidermidis* have been developed. These are resistant to methicillin, to all kind of penicillin, to almost every cephalosporin, to streptomycin and tetracycline. Penicillin, represented by ampicillin and amoxicillin, is an efficient antibiotic from this species. Oxacillin can be used as a mono therapy in the treatment of staphylococcus infections. Oxacilin might be useful in case of dangerous staphylococcus infections, endocarditis and septicemia in association with active aminoglycoside. Gentamicin and tobromicin have a large antimicrobial spectrum. The tetracycline is formed of Gram positive and Gram negative, aerobe and anaerobe bacteria. Brand new and resistant branches were selected. This is the reason why they are rarely used as antibiotics. Gram positive cocci are inhibited by small concentrations. The MIC for *Staphylococcus spp, Streptococcus spp* and *Pneumococcus spp* is 0.2 and 3.1 mcg/ml (Stroescu,2002).Patients who are allergic to penicillin can use erythromycin. These patients have slight infections of staphylococcus, streptococcus, pneumococcus, anthrax and syphilis. Recently, it has been discovered that the eritromicin can be used with success in vulgar acne therapy (Stroescu,2002).Clindamycin is useful in sever staphylococcus infections, like in case of staphylococcus infections resistant to G penicillin, but sensitive to clindamycin. Several branches of *Staphylococcus aureus* are inhibited at a concentration of 0.4 mcg/ml (Cinca,2006). Vancomycin and teicoplanina have a major effect on staphylococcus infections resistant to methicillin (Appelbaum, 2006).

Linesolid is used in Staphylococcus infections (resistant to methicillin), pneumonia (caused by *Staphylococcus aureus*), community pneumonia, skin infections, soft tissue infections and bacterial endocarditis with sensitive germs (Cinca,2006).

Staphylococcus has a great sensitivity. For *Staphylococcus aureus* the MIC is 0.005-0.001 mcg/ml. The antibiotic is active also in the case of poli resistant branches - *Staphylococcus* resistant to penicillin G and methicillin and/or vancomycin. It may have a synergistic reaction, when the penicillin is resistant to penicillinaze and vancomycin (Stroescu, 2002).Ciprofloxacin presents a high efficiency against

bacteria resistant to aminoglycoside, penicillin, cephalosporine, tetracycline and other antibiotics. Infections of the middle ear (middle otitis) and para-nasal sinuses (sinusitis) may appear, especially if these are generated by Pseudomonas and Staphylococcus(Stroescu,2002*)*.The efficacy of chlorhexidine digluconate was determined against some strains of collected and clinically isolated bacteria and fungi. The efficacy was evaluated either by calculating a minimum inhibitory concentration (MIC). The MIC values of chlorhexidine for *Staphylococcus aureus*, *Microsporum gypseum*, *Microsporum canis* and *Trichophyton mentagrophytes* were 0.625 mg/ml, 12.5 mg/ml, 50 mg/ml and 6.25 mg/ml, respectively. The *in vitro* efficacy of chlorhexidine was higher against the strains of *Staphylococcus aureus*and *Pseudomonas aeruginosa* (0.5 mg/ml for 5 min and 0.5 mg/ml for 10 min) than against clinical isolates (Odore,2000*)*.It is generally accepted that methicillin-resistant *Staphylococcus aureus*is also resistant to aminoglycoside antibiotics. We investigated trends of gentamicin and arbekacin susceptibilities and the prevalence of the genes encoding aminoglycoside modifying enzymes (AMEs) for a total of 218 strains of MRSA isolated from blood specimens obtained from 1978 through 2002 in one hospital. The minimum inhibitory concentrations of gentamicin at which 50% of the strains that were inhibited (MIC50) are 0.5 μg/ml for the isolates obtained from 1990 to 2002. The MIC of gentamicin was consistently 128 μg/ml. Our results imply that gentamycin resistant and arbekacin-resistant MRSA's have consistently decreased for the past 25 years (Barada,2002).Thirty persons with staphylococcus infections have been studied, 19 women and 11 men, between the age of 2 and 79. Patients have been examined. Nasal and pharynx secretion, acne secretion and urine culture has been collected. It's compulsory to establish the sensitivity of antibiotics and chemotherapy of every bacterial branch in order to determine the right treatment. Usually, a branch can be considered sensitive if the germs are efficiently affected by the antibiotic. The therapeutic effect may be obtained with the help of "usual" doses and administration. The branch may be considered slightly sensitive if the germs are little affected. The therapeutic effect cannot be obtained but in special conditions (like, to recommend a higher doses than as usual, the use of some special administration: intravenous injection and intrarahidiane injection).Bacteria sensitivity towards antibiotics is tested "in vitro", assuring them standard culture condition (culture environment and incubation time) in the presence of a smaller quantity of antibiotic. Basically, a branch is resistant if the result of the sensitivitytested "in vitro" is negative. The antibiogram can be made by a test "in vitro". It represents the lab method, showing the antibiotic sensitivity of germs, collected from patients with bacterial infections. These are cultivated in special environments (for instance on the Mueller-Hinton agar). We have to use pure cultures for antibiograms (a single bacterial branch), even in case of multibacterial infections. The isolation of *Staphylococcus aureus*is based on its growth in a simple environment and in a hyperchorinate environment. On the agarose, the blood is developed in colonies of 2-3 mm. The colonies have a creamy consistence. Usually,

it contains beta hemolysis (Vaida, 2002*)*. We submitted a disk with antibiotics, on the surface of a solid environment, planted with a bacterial culture. The active antimicrobial substance will run into the environment, having a small concentration, started from the edge of the microtablet. After a certain time of incubation, there are two different ways to follow: the first one, because here the microbial growth is inhibited by a concentration of antimicrobial substance. Second, a growing area, where the antibiotic concentration is too small for a growing inhibition.

If the diameter of the inhibitive area is bigger, the germ is more sensitive. The quantity of the antibiotic necessary to the inhibition of the tested bacteria (minimum inhibited concentration=MIC), is smaller. There is an inversely proportional relation between the diameter of the inhibited area and MIC (Gan-Bularda, 2000).Staphylococcusfrequently produces infections which can be treated only in hospital. This is the reason why it is recommended to test their sensitivity to antibiotics for a right treatment. In this order, there are: pharynx and nasal perspire, acne secretion and urine culture than the so called antibiograms. As concerning the pharynx perspire, a group of 10 people were tested: 4 men and 6 women with ages between 20 and 49. This test was made on 7 children (4 girls and 3 boys) between the ages of 2 and 14.The nasal perspires has been made on a group of 10 people (4 men and 6 women), between the age of 18 and 52. Only one nasal perspire has been made on children. In order to test the resistance of *Staphylococcus aureus*on antibiotics, the acne secretion has been made on a single woman (aged 25).The urine culture, has been

made on a single woman, too (aged 79). In the case of antibiograms, the following antibiotics have been used: penicillin, oxacilin, gentamicin, tobromicin, tetracycline, erythromycin, clindamycin, vancomycin, rifampicin, linezolid and ciprofloxacin. The concentration/microtablets of the antibiotics were already known. After the antibiograms were made (based on the above mentioned method), and after the inhibition was established, meaning the minimum inhibitive concentration (MIC), the obtained results were statistically processed, permitting to achieve the following figure. Using the statistical tests, we established 4 most efficient antibiotics: oxacilin, erythromycin, rifampicin and ciprofloxacin. These antibiotics may be recommended and used in different kind of disease caused by *Staphylococcus aureus*. Different kind of disease caused by *Staphylococcus aureus*may be treated and used with other antibiotics, like the linezolid .As concerning the tested antibiotics, we established that penicillin is less efficient (63.33% cases) in order to treat *Staphylococcus aureus*.

The most efficient antibiotic used in the case of *Staphylococcus aureus*.

ciprofloxacin > 76.66%
oxacilin >3.33%
Rifampicin >13.33%
Erythromycin > 6.66%

Other antibiotics which can be used in the case of *Staphylococcus aureus*.
linezolid>13.33%
ciprofloxacin> 23.33%
erythromycin> 26.66%
rifampicin> 13.33%
oxacilin> 6.66%
others> 16.66%

Inefficient antibiotics in the case of *Staphylococcus aureus*.
Erythromycin > 63.33%
Tobramicin > 13.33%
Penicillin > 6.66%
Linezolid > 3.33%
Tetracycline > 3.33%
Ciprofloxacin > 6.66%
Depending on every person, on penicillin and on every person's clinical picture, there are other inefficient antibiotics, but on a lower rate. People had a certain clinical picture, a kind of specificity in disease initiated by *Staphylococcus aureus.* This is the reason why some antibiotics appear in both groups, as efficient and inefficient, but of course, on a lower rate.Infections caused by methicillin-resistant strains may be more difficult to manage or more expensive to treat, because vancomycin is inherently less efficacious. The increasing prevalence of MRSA will inevitably increase vancomycin use, adding further to the problem of antibiotic-resistant gram-positive bacteria (Conterno,2008).Antimicrobial resistance to penicillin, methicillin, or vancomycin is an unavoidable consequence of the selective pressure of antibiotic exposure. Although the details of the epidemiology of staphylococcal drug resistance may change, the fundamental forces driving it are similar. The question is not whether resistance will occur, but how prevalent resistance will become. Minimizing the antibiotic pressure that favors the selection of resistant strains is essential to controlling the emergence of these strains in the hospital and the community, regardless of their origins (Cinca,2006).Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram positive antibiotics such as linezolid because of its availability as an oral drug (Blot,2002).Vancomycin-resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has become resistant to the glycopeptides. The first case of vancomycin intermediate *S. aureus* (VISA) was reported in Japan in 1996 (Hiramatsu,2007) but the first case of *S. aureus* truly resistant to glycopeptide antibiotics was only reported in 2002 (Chang, 2003). Three cases of VRSA infection have been reported in the United States as of 2005(Blot,2002).The treatment of choice for *S. aureus* infection is penicillin; but in most countries, penicillin-resistance is extremely common and first-line therapy is most commonly a penicillinase-resistant penicillin (for example, oxacilin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections like endocarditis, but its use is controversial because of the high risk of damage to the kidneys. The duration of treatment depends on the site of infection and on severity (Bayer,2017).

Antibiotic resistance in *S. aureus* was almost unknown when penicillin was first introduced in 1943 observed the antibacterial activity of the penicillium mould was growing a culture of *S. aureus*. By 1950, 40% of hospital *S. aureus* isolates were penicillin resistant; and by 1960, this had risen to 80% (Chambers,2001).

Patients with affections determined by *Staphylococcus aureus*and which are under standard therapy with antistaphylococcal penicillin or vancomycin, were administred initial low dose gentamicin, in order to

increase the treatment`s efficiency. By studying the the administration of a standard dose of gentamicin as a part of therapy for *Staphylococcus aureus*bacteremia, but also for native valve infective endocarditis, is proven that it is nephrotoxic and should not be used routinely (Gentry,2007).

2.3.0 Septicemia

Septicemia is a serious bloodstream infection. It's also known as bacteremia, or blood poisoning. Septicemia occurs when a bacterial infection elsewhere in the body, such as in the lungs or skin, enters the bloodstream. This is dangerous because the bacteria and their toxins can be carried through the bloodstream to your entire body(Martin, 2012). Septicemia can quickly become life-threatening. It must be treated in a hospital. If it's left untreated, septicemia can progress to sepsis. Septicemia and sepsis aren't the same. Sepsis is a serious complication of septicemia. Sepsis is when inflammation throughout the body occurs. This inflammation can cause blood clots and block oxygen from reaching vital organs, resulting in organ failure. The National Institutes of Health (NIH) estimates that over 1 million Americans get severe sepsis each year. Between 28 and 50 percent of these patients may die from the condition. When the inflammation occurs with extremely low blood pressure, it's called septic shock. Septic shock is fatal in many cases. Septicemia can also be defined as life-threatening organ dysfunction due to a dysregulated host response to infection (Aziz *et al.*,2013).Septic shock is associated with particularly profound circulatory, cellular and metabolic abnormalities, with a greater risk of mortality than with sepsis alone. Patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L in the absence of hypovolaemia. This combination is associated with hospital mortality rates greater than 40%.The Surviving Sepsis Campaign (SSC) was established to raise awareness of severe sepsis and to improve its management. The SSC is collaboration between several groups worldwide and its aim is to reduce the mortality from sepsis.

2.3.2 Mechanism of Pathogenicity

The ability to up-regulate virulence factors under stressful stimuli (e.g., host immune response or circulating antibiotics) is a key factor in the enabling of *S. aureus* to persist in the bloodstream, to seed deep tissues, and to form secondary foci of infection. *S. aureus* strains have been effectively able to adhere to and colonize the skin and mucosa of nares, to invade the bloodstream, to evade host immunological responses, to form protective biofilms, and to develop resistance to several antibiotics (Amagai,2000). Consequently, despite the availability of many antibiotics with activity against wild-type strains, *S. aureus* is a highly successful and increasingly clinically important gram-positive pathogen (Aziz *et al.*,2013).

Adhesion and colonization: *S. aureus* can up-regulate a variety of virulence factors, enabling it to adhere to and colonize the nares and damaged skin or the surfaces of implanted devices or prostheses and to cause serious bloodstream infections. Teichoic acid, a polymer on the surface of *S. aureus* , is essential for this purpose (Weidenmaier *et al.*,2004).

Invasion: *S. aureus* can disrupt the skin barrier by secreting exfoliative toxins (Amagai,2000), hemolysins (including α-hemolysin [α-toxin], which forms pores in skin cell membranes), and various enzymes that destroy tissue . Invasion may be triggered when the immune system is compromised, when there is a break in the physical integument, and/or when localized inflammation occurs (Otto,2000).

Evasion: *S. aureus* evades the host immune response by secreting anti-opsonizing proteins (e.g chemotaxis inhibitory protein), which prevent phagocytosis by neutrophils (Haas*et al.*,2004*)*. Protein A, located on the surface of *S. aureus* cells, also has antiphagocytic properties. Furthermore, *S. aureus*secretes leukotoxins (e.g., Panton-Valentine leukocidin), which lyse leukocytes , and expresses superantigens (e.g enterotoxins and toxic shock syndrome toxin), which subvert the normal immune response by inducing strong, polyclonal stimulation and expansion of T cell receptor Vβ-specific T cells (followed by the deletion or suppression of these T cells to an anergic state) (Proctor, 2000).

Biofilms: *S. aureus* quorum sensing may regulate gene expression to form slimy biofilms on damaged skin, fitted medical devices, and healthy or damaged heart valves. The depletion of nutrients and oxygen causes bacteria to enter a non growing state in which they are less susceptible to some antibiotics. In particular, small-colony variants of *S. aureus*, when adherent and in the stationary phase, demonstrate almost complete resistance to antimicrobial agents. The biofilm matrix provides protection against immune cells and may restrict the penetration of some antibiotics (Patel, 2008).

Antibiotic resistance: Strains of *S. aureus* have developed resistance to antibiotics, including penicillin, cephalosporins, methicillin, vancomycin, and linezolid. *S. aureus* abrogates the effects of penicillin by producing β-lactamase, and MRSA strains have acquired the *mec* gene, which encodes penicillin-binding protein 2a, and the *fem* gene, which confers resistance to methicillin, penicillinase-resistant penicillins, and cephalosporins (Jansen,2006). True vancomycin resistance in *S. aureus* appears to rely on acquisition of the *vanA* gene, whereas reduced vancomycin susceptibility in vancomycin-intermediate *S. aureus* and heteroresistant vancomycin-intermediate *S. aureus* has been linked to a different mechanism: mutations in structural or regulatory genes associated with the accessory gene regulator pathway (Tenover, 2007). Linezolid resistance is conferred by a mutation in *S. aureus* ribosomal RNA (Peeters,2008). In addition to clear resistance, there is the phenomenon of "MIC creep," which is best recognized with respect to the glycopeptide class of antibiotics and refers to insidious, numerically small increases in MICs over time that, nevertheless, appear to reflect a clinically significant reduction in susceptibility (Gould,2007).

2.3.3 Pathophysiology of septicaemia

Sepsis is not a random occurrence and is usually associated with other conditions, such as perforation, compromise, or rupture of an intra-abdominal or pelvic structure (Merrell, 2005). Intrarenal infection (pyelonephritis), renal abscess (intrarenal or extrarenal), acute prostatitis, or prostatic abscess may cause urosepsis in immunocompetent hosts. Urosepsis has also been reported after prostatic biopsy (Levy, 2003). An abdominal wall abscess is depicted on the CT scan depicted below. A right lower quadrant abdominal wall abscess and enteric fistula are observed and confirmed by the presence of enteral contrast in the abdominal wall. Sepsis or septic shock may be associated with the direct introduction of microbes into the bloodstream via intravenous (IV) infusion (e.g., IV line infections and other device-associated infections). Meningococcemia from a respiratory source may also result in sepsis, with or without associated meningitis. Bacteremia due to bacteriuria (urosepsis) may complicate cystitis in compromised hosts, and sepsis may be caused by overwhelming pneumococcal infection in patients with impaired or absent splenic function (William, 2007).The pathophysiology of sepsis is complex and results from the effects of circulating bacterial products, mediated by cytokine release, caused by sustained bacteremia. Cytokines are responsible for the clinically observable effects of the bacteremia in the host (Bone, 2004). Impaired pulmonary, hepatic, or renal function may result from excessive cytokine release during the septic process. Current research is focusing on such issues as the immunosuppressive phase of host immune responses, mitochondrial dysfunction and the individual reactions between pathogen and the host immune system (Opal, 2013).

2.3.4 Epidemiology

Sepsis is a leading cause of morbidity and mortality worldwide and particularly in Africa where awareness is low and resources are limited. There are limited reports on the epidemiology, management and outcomes of the sepsis syndromes from Africa. However, this region is likely to account for a significant proportion of the global burden of sepsis which goes unrecognized. It is imperative to address this through research, increased awareness, capacity building and introduction of practical clinical guidelines. Infections are responsible for an estimated 300 million annual deaths worldwide, the majority from developing countries (Melville, 2013). Sepsis can be triggered by almost any infection and is responsible for an estimated 8 million annual deaths worldwide (Melville, 2013). In the United Kingdom (UK), sepsis is the third most important cause of death in hospital with an average management cost of £20,000 per admission (Jacob, 2009). Given the high incidence of Human Immunodeficiency Virus (HIV) and other infections in the African continent, it is likely that the burden of sepsis is at least equal if not higher than estimates from Europe and North America. Why are sepsis related cases and deaths under-reported? firstly, health care professionals often miss the diagnosis or fail to document it in the clinical notes. Secondly, the World Health Organisation (WHO) Global Burden of Disease Report (GBDR) does not include sepsis as a cause of death. The GBDR is one of the leading information sources for healthcare decision-making worldwide. Although deaths from infections occur most commonly as a result of sepsis, the GBDR lists only the underlying infections as cause of death. It is therefore not surprising that sepsis occurs only as "neonatal" sepsis and is ranked 16th place, despite about 60% of deaths in children under five being due to severe infections. Thirdly, guidelines for coding of sepsis are often

difficult to use especially in under resourced and busy developing world healthcare settings.

2.3.5 Risk factors

There is usually an abscess or nidus of infection, which may be occult. Risk factors for developing sepsis include the following: Age - the elderly (over 75 years) and very young (< 1 year) are at risk, instrumentation or surgery (including illegal abortion occurring in unhygienic circumstances), Indwelling line or catheter, Alcohol abuse, Diabetes mellitus, breach of skin integrity e.g., burns, Immunocompromised, Medications – e.g., high-dose corticosteroids, chemotherapy, Males are more prone than females to develop severe sepsis, although the mortality in females is higher. The reasons for this are not known (Angus, 2013), intravenous drug misuse and Pregnancy.

2.3.6 Causes Septicemia

Septicemia is caused by an infection in another part of your body. This infection is typically severe. Many types of bacteria like *Staphylococcus aureus* can lead to septicemia (McPherson, 2013). The exact source of the infection often can't be determined. The most common infections that lead to septicemia are: urinary tract infections, lung infections, such as pneumonia, kidney infections and infections in the abdominal area bacteria from these infections enter the bloodstream and multiply rapidly, causing immediate symptoms. People, who are already in the hospital for something else, such as a surgery, are at a higher risk of developing septicemia. Secondary infections can occur while in the hospital. These infections are often more dangerous because the bacteria may already be resistant to antibiotics.

2.3.7 Symptoms of Septicemia

The symptoms of septicemia treatments that weaken your immune system, such as chemotherapy or steroid injections. It usually starts very quickly. Even in the first stages of the illness, a person can look very sick. They may follow an injury, surgery, or another localized (confined to one location) infection, like pneumonia. The most common initial symptoms are: chills, elevated body temperature (fever), very fast respiration and rapid heart rate more severe symptoms will begin to emerge as the septicemia progresses without proper treatment. These include the following: confusion or inability to think clearly, nausea and vomiting and red dots that appear on the skin (Martin, 2012).

2.3.8 Presentation

Early recognition is essential. Presenting features may be nonspecific and vague. A high degree of vigilance is therefore required at all times. Patients may have presented a few days earlier with a focus of infection. Patients may then deteriorate rapidly despite having the appropriate oral antibiotics. Nonspecific symptoms are common – e.g., lethargy, nausea and vomiting, abdominal pain and diarrhoea, also enquire about symptoms relating to a possible focus of infection – e.g., cough, urinary symptoms, recent travel and to ask about frequency of micturition in the past 18 hours.

2.3.9 Complications of Septicemia

Septicemia has a number of serious complications. These complications may be fatal if left untreated or if treatment is delayed for too long Sepsis. Sepsis occurs when your body has a strong immune response to the infection. This leads to widespread inflammation throughout the body. It's called severe sepsis if it leads to organ failure. People with chronic diseases, such as HIV or cancer, are at a higher risk of sepsis. This is because they have a weakened immune system and can't fight off the infection on their own (Vincent, 2013).

Septic shock

One complication of septicemia is a serious drop in blood pressure. This is called septic shock. Toxins released by the bacteria in the bloodstream can cause extremely low blood flow, which may result in organ or tissue damage. Septic shock is a medical emergency. People with septic shock are usually cared for in a hospital's intensive care unit (ICU). You may need to be put on a ventilator, or breathing machine, if you're in septic shock (Czura, 2011).

Acute respiratory distress syndrome (ARDS)

A third complication of septicemia is acute respiratory distress syndrome (is a life-threatening condition that prevents enough oxygen from reaching your ARDS). This lungs and blood. According to the National Heart, Lung, and Blood Institute (NHLBI), ARDS is fatal in about one-third of cases. It often results in some level of permanent lung damage. It can also damage your brain, which can lead to memory problems (Czura, 2011).

2.3.10 Diagnosis

Diagnosing septicemia and sepsis are some of the biggest challenges facing doctors. It can be difficult to find the exact cause of the infection. Diagnosis will usually involve a wide range of tests (Popa, 2004).The doctor will evaluate the symptoms and asked about ones medical history. They'll perform a physical examination to look for low blood pressure or body temperature. The doctor may also look for signs of conditions that more commonly occur along with septicemia. These conditions include pneumonia, meningitis, and cellulitis (Singer, 2016). Multiple types of fluids to help confirm a bacterial infection using culture method. These may include the following:

- urine
- wound secretions and skin sores
- respiratory secretions
- blood

Your may look for your cell and platelet counts and also order tests to analyze blood clotting. Your may also look at the oxygen and carbon dioxide levels in your blood if septicemia is causing you to have breathing issues. If signs of infection are not obvious, your doctor may order test to look more closely at specific organs and tissue, such as: X-ray, MRI, CT scan and Ultrasound.

2.3.11 Treatment for septicemia

Septicemia that has started to affect your organs or tissue function is a medical emergency. It must be treated at a hospital. Many people with septicemia are admitted to a hospital's ICU for treatment and recovery (Fiusa, 2015).Your treatment will depend on several factors, including: age, overall health the extent of health condition and tolerance for certain medications.

Antibiotics are used to treat the bacterial infection that's causing septicemia. There isn't typically enough time to figure out which type of bacteria is causing the infection. Initial treatment will usually use "broad-spectrum" antibiotics. These are designed to work against a wide range of bacteria at once. A more focused antibiotic may be used if the specific bacterium is identified. You may get fluids and other medications intravenously to maintain your blood pressure or to prevent blood clots from forming. You may also get oxygen through a mask or ventilator if you experience breathing issues as a result of septicemia.

2.3.12 Prevention

Bacterial infections are the underlying cause of septicemia. See a doctor right away if you think you have this condition. If your infection can be effectively treated with antibiotics in the early stages, you may be able to prevent the bacteria from entering your bloodstream (Martin, 2012). Parents can help protect children from septicemia by ensuring they stay up to date with their vaccinations.

3. MATERIALS AND METHODS

3.1 Experimental Design

32 rats were used for the whole study. 16 rats were used for toxicological study, 4 rats were shared into Group 1 (G_3, G_6, G_{17} and G_{18}), Group 2 (G_{20}, G_2, G_{12}, G_{10}), Group 3 (G_5, G_{13}, G_{16}, G_9), and Group 4 (G_8, G_4, G_{11}, G_{14}) each. Then other 16 rats were used for the experiment of which 4 rats were used for Group A (negative control group) having experimentally assigned no of G_{29}, G_{30}, G_{31} and G_{23}; 4 rats were used for Group B(Test control group) having experimentally assigned no of G_{22}, G_7, G_{28} and G_{31}; 4 rats were used for Group C (positive control group) having experimentally assigned no of G_2, G_{15}, G_{19} and G_{21}; and then 4 rats were used for Group D (Treatment control group) having experimentally assigned no of G_1, G_{20}, G_{32} and G_{24}.

3.2 Toxicological Study

16 albino rats (G_3, G_6 G_{17}, G_{18}, G_{20}, G_2, G_{12}, G_{10}, G_5, G_{13}, G_{16}, G_9, G_8, G_4, G_{11} and G_{14}) whose weights range from 60 to 150g and whose age range from 10 to 14 weeks were used for the study. The aim is to determine the lethal doses for 50 percent of the rat (LD_{50}) and was determined to be 34641mg/kg after having death of G_{11} and G_{14} and curative dose of aqueous *Mentha peperita* leaf extract that will have a curative effect on the albino rat determined as 25980.75mg/kg. The subjects were divided into 3 groups of four albino rats each (group 2,3 and 4) while group 1 was taken as negative control group. Each of the test group of rat was given 2500mg/kg, 5000mg/kg, 10000mg/kg doses of extract orally using feeding tube. These extracts were given once daily after the animals had fasted for 18hrs for LD_{50}determination.The number of death in each group within 48hrs was recorded. Besides, delayed mortality up to 24hrs was considered as lethal dose (WHO, 2002). This was done by observing the mice for toxicity signs. The dose increment was limited to 50000mg/kg because all most all the rats died at this dose.

3.3 STERILIZATION OF MATERIALS

The work bench was first disinfected by swabbing with cotton wool soaked in 70percent alcohol. The glass wares used were washed with detergent, rinsed properly in several changes of tap water and further rinsed with distilled water. They were then dried and wrapped with aluminum foil and thereafter sterilized in the autoclave at 121^0c for 15mins.Aseptic techniques were applied in the working environment by ensuring that all work was not done near the naked flame of Bunsen burner (Ochei and Kolhatkar, 2008).

3.4 Sample Collection

After the inoculation of *Staphylococcus aureus* isolate, blood collection was collected from group B, C, D after 7days in a sterile EDTA container and it is immediately transported immediately to the laboratory for urine microscopy, culture, viable count and sensitivity testing.

3.5 Preparation of Media

Each of the medium namely Nutrient agars, Blood agar, Mac Conkey and CLED were prepared according to manufacturers' instruction. The media were then autoclaved at 121^0c for 15mins.The media were allowed to cool for 45mins and poured into the sterile petri dishes (Cheesebrough *et al.*,2006).

3.6 Collection of Isolate

Staphylococcus was gotten from blood of patient attending Emergency clinic at FMC Owerri and was confirmed by the Department of Medical Laboratory Science, Imo State University. It was cultured in Nutrient broth for 24hrs at 37^0c prior to inoculation.

3.7 Viable Count

For each media in duplicated form for Nutrient agar, Blood agar, Mac Conkey and CLED, number of colonies was counted each for two plates and added together and divided by two to obtain the viable count.

3.8 Enumeration of Total Viable Bacteria Count

The total viable counts of bacteria were estimated using the pour plate method. For samples with more bacteria.0.1ml of undiluted sample(dilution factor = 10) was used to estimate the bacterial load, this means adding the sample without diluting it to the petri dishes containing the molten agar (Nutrient, blood, CLED and Mac Conkey agar) using a sterile pipette. 1ml or 0.1ml of the 100x dilution was added to the petri dishes containing NA, BA, MAC and CLED respectively. The plates were gently mixed well and allowed to solidify. The plates were then inverted and incubated at 37^0c for 24hrs to 48hrs.

The number of colonies that developed in the plates for each location was counted and the average recorded. Discrete colonies were collected aseptically and subcultured on nutrient agar plate (for bacteria purification) and incubated at 37^0c over night. Pure colonies were later stored in McCartney bottles containing nutrient agar slants and put into the fridge as stock cultures for further biochemical tests.

3.9 Isolation and Identification

Before being used, the isolates were re-identified from the slope culture by inoculating it on solid media such as Nutrient agar, Blood agar, Mac Conkey and CLED. The pure isolates were identified using their morphological and microscopic features. The identities of the organism were confirmed by employing some biochemical tests.

3.9.1 Gram Staining Reaction

Principle of Gram staining Gram staining reaction differentiate gram negative from gram positive bacteria due to differences in their cell wall, the positive form possess high content of peptidoglycan layer which enable them to retain the primary stain, crystal violet and resist decolourisation by an acetone while gram negative possess low amount of peptidoglycan to retains the colour of counterstain.

Staining procedure Gram staining method was adopted for the determination of gram staining reactions of bacterial isolates (Onyeagba *et al.,* 2004).With the aid of a wire loop, the isolate was smeared onto a clean and grease free slide also air dried. Primary stain, crystal violet was poured on the smear and allowed for 30seconds it was washed in the gentle running tap water for 5secs ,covered with lugol's iodine and rinsed in distilled. The stained smeared was thereafter decolorized using 95 percent alcohol, rinsed with tap water and then counterstained using neutral red for 60secs.The smear was finally rinsed in clean tap water, air dried and then a drop of immersion oil was placed on the stained slide. It was viewed under the microscope.

3.9.2 Biochemical Test

Coagulase Test (slide method)

1. A drop of distilled water was placed on each end of a slide or on two separate slides.
2. A colony of the test organism (previously checked by Gram staining) was emulsified in each of the drops to make two thick suspensions.
3. A loopful of plasma was added to one of the suspensions and mixed gently.

Clumping of the organisms was observed within 10secs.

No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping (Cheesbrough, 2006).

Catalase Test

Required

Hydrogen peroxide (H_2O_2)

Method

1. 2ml of hydrogen peroxide solution was added into the test tube.
2. Using a sterile glass rod several colonies of the test organism were immersed in hydrogen peroxide solution.
3. It was examined immediately for bubbles (Cheesbrough, 2006).

Mannitol salt agar Test

1. Plates were allowed to warm to room temperature and the agar surface was dried before inoculating.

2. Isolate was heavily inoculated and streaked as soon as possible after collection.

3. The specimen was cultured on a swab, and was rolled on the swab over a small area on the agar surface.

4. It was Streak for isolation with a sterile loop and incubated aerobically at 37ºC for 24hrs.

5. The colonial morphology was then observed.

Results:

After incubation, yellow colonies of growth were observed (Chapman, 2005).

Motility Test

1. A semisolid agar medium was prepared in a test tube.
2. Using a straight wire loop, stab inoculates the motility medium with the test organism.
3. It was incubated at 37^0c for 48hrs (Ochei and Kolhatkar,2008).

Results: Hold the tube up to the light and look at the stab line to determine motility.

Motile bacteria typically give diffuse hazy growths that spread throughout the medium rendering it slight opaque. After incubation of organisms, the culture plates were stained using gram staining technique to differentiate gram positive from gram negative organisms.

3.10 Inoculation of Media

Broth culture of Staph was cultured using nutrient agar and blood agar at 37^0c overnight for 24hrs to obtain a pure isolate of Staph colony. It was then adjusted according to MacFarlane standard of 0.5Cfu/ml, Staph saline suspension was prepared using 0.5Cfu of *Staphylococcus aureus* to 0.5 of 0.85 percent of saline solution prior to inoculation. It was done via intraperitoneal once daily using 1ml calibrated insulin syringe. The same colony forming unit per ml was given to group B,C,D.

3.11 Urinalysis

For the urinalysis, test strip for the rapid determination of blood, urobilinogen, bilirubin, protein, nitrite, ketone, ascorbic acid, glucose and pH value in urine produced by Macherey – nagel was used. The urine microscopy was carried out by collecting the urine into a centrifuge tube and centrifuging for 5mins at 12,000rpm.After this, the supernatant is decanted and the sediment is placed on a clean and grease free slide before covering with coverslip and viewed the microscope using 40X objective lens (Ochei, 2008*).*

3.12 Preparation of *Mentha peperita* Aqueous Extract (Infusion Method)

Leaves of *Mentha piperita* was gotten from Nekede market, Owerri and was identified by the department of Plant science and Biotechnology, Imo state university Owerri. The plant leaf was sun dried for 3days.After drying the leaf was manually crushed using a clean small plastic pestle and mortar to obtain a powdered particle of the *Mentha piperita* leaf after it has been sieved.

Procedure:
The procedure used in this study was as described by (Arhoghro *et al.*, 2009).
The leaves was sun dried, manually pulverized using a clean plastic mortar and pestle and sieved.400g of the sieved *Mentha x piperita* leaf was dissolved in 1000ml of boiled water, it was stirred for 30seconds.After 30mins it was sieved twice using filter paper and the filtrate was collected under strict aseptic conditions in fresh sterilized bottles and stored at 4^0c until it needed to be given to the rats.

3.13 Statistical analysis
Arithmetic mean and standard error of mean were calculated and all the data obtained were analyzed

statistically using Analysis of Variance (ANOVA). Statistical analyses were made by a SPSS for Windows version 13.0 packaged statistics program.

RESULTS

4.1 Acclimatization Study:

The acclimatization study was conducted over two weeks, after which 25 subjects (labeled G1 to G25) were assessed based on sex, agility, and weight before and after acclimatization. The results are summarized in Table 4.1a.

Table 4.1a: Weights of the rats before and after the two weeks acclimatization in addition to their various sexes and numbers assigned to them.

		DATA ON ARRIVAL		DATA AFTER ACCLIMATIZATION	
EXPERIMENTAL ASSIGNED S/N	SEX	WEIGHT BEFORE ACCLIMATIZATION	AGILITY	WEIGHT AFTER ACCLIMATIZATION	AGILITY
G_1	F	65	AGILE	60	AGILE
G_2	M	60	AGILE	75	AGILE
G_3	F	70	AGILE	65	AGILE
G_4	M	70	AGILE	90	AGILE
G_5	F	78	AGILE	85	AGILE
G_6	F	58	AGILE	70	AGILE
G_7	M	85	AGILE	70	AGILE
G_8	M	75	AGILE	85	AGILE
G_9	F	70	AGILE	80	AGILE
G_{10}	F	80	AGILE	80	AGILE
G_{11}	F	60	AGILE	90	AGILE
G_{12}	M	60	AGILE	78	AGILE
G_{13}	F	50	AGILE	90	AGILE
G_{14}	M	65	AGILE	80	AGILE
G_{15}	F	70	AGILE	80	AGILE
G_{16}	F	70	AGILE	90	AGILE
G_{17}	F	55	AGILE	75	AGILE
G_{18}	F	60	AGILE	80	AGILE
G_{19}	F	55	AGILE	70	AGILE
G_{20}	F	70	AGILE	70	AGILE
G_{21}	F	70	AGILE	60	AGILE
G_{22}	F	80	AGILE	100	AGILE
G_{23}	M	60	AGILE	90	AGILE
G_{24}	M	80	AGILE	85	AGILE
G_{25}	M	90	AGILE	100	AGILE

Table 4.1a shows the weights of the rats before and after the two weeks acclimatization in addition to their various sexes and numbers assigned to them. The data showed that acclimatization positively affected the weight of most rats, especially males. Although a few female rats (e.g., G1, G21) lost weight, the majority gained weight, indicating that the acclimatization conditions (diet, environment, etc.) were overall conducive to weight gain or maintaining health. The consistent agility across all rats indicates that the acclimatization period was not stressful or detrimental to their overall activity levels.

Table 4.1b: Urinalysis Results of the Rats to ascertain the Total Body Status of all the Rats.

EXPERIMENTALLY ASSIGNED S/N	Glucose	Protein	Nitrite	Blood	Ascorbic acid	Urobilinogen	PH	Ketone	Bilirubin
G_1	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_2	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_3	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve

G_4	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_5	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_6	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_7	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_8	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_9	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{10}	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{11}	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{12}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{13}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{14}	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{15}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{16}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{17}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{18}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{19}	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{20}	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{21}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{22}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{23}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve
G_{24}	-ve	-ve	-ve	-ve	-ve	Normal	5	-ve	-ve
G_{25}	-ve	-ve	-ve	-ve	-ve	Normal	6	-ve	-ve

Table 4.1b: Shows Urinalysis done on the arrival of the rats to ascertain the total body status of all the rats. All parameters of the urinalysis revealed normal results, indicating no significant health issues among the rats.

Table 4.1c: Microscopic and biochemical characteristics of *Staphylococcus auerus.*

Gram staining reaction	positive (+)
Motility	Positive (-)
Mannitol salt	Positive (+)
Catalase	Positive (+)
Coagulase	Positive (+)

Table 4.1c shows the microscopic and biochemical characteristics of *Staphylococcus auerus.* The positive reaction for the Gram staining, combined with the positive reaction for motility Mannitol salt fermentation, indicates that the organism is likely *S. aureus*. The catalase and coagulase-positive results further support the identification *of S. aureus* organism, distinguishing it from other coagulase-negative *Staphylococcus species*.

Table 4.2: Mean viable count during infection and after infection (after treatment with both the prepared aqueous extract and Gentamycin)

Groups	Mean viable count during infection	Mean viable count after infection
B	93.00±14.14	59.50±19.09
C	114.00±1.41	82.25±12.37

D	141.75±6.01	0.00±0.00

Table 4.2 shows the mean viable count during infection and after infection (after treatment with both the prepared aqueous extract and Gentamycin) The mean viable count in group B, group C and group D are 93.00±14.14, 114.00±1.41 and 141.75±6.01 respectively. This shows that the mean viable count of group B (59.50±19.09) after infection is significantly lower when compared with the mean viable count of group C(82.25±12.37) at P<0.05 and also higher when compared with group D (0.00±0.00) at P < 0.05.

4.3 Effect of the *Mentha piperita* leaf extract on the body weight across groups at the doses of 2500mg/kg, 5000mg/kg and 10000mg/kg on day one.

Table 4.3a: Effect of *Mentha piperita* aqueous leaf extract on the body weight across groups at 5000mg/kg, 10000mg/kg, and 20000mg/kg on day two

Day 1	Treatment (mg/kg)	Mean initial Weight ±SD	Mean final Weight ±SD	Mean Weight change ±SD
Group 1	-	56.25 ± 4.79	65.00 ± 0.00	8.75 ± 4.79
Group 2	2500	75.75 ± 4.35	81.75± 2.36	6.00 ± 1.99
Group 3	5000	86.25 ± 4.79	93.75 ± 4.79	7.50±0.00
Group 4	10000	86.25 ± 4.79	111.25 ± 13.15	25.00 ± 8.36

Table 4.3a shows the weight effect of the extract on day 1, this revealed that the Mentha piperita extract was administered at varying concentrations across the groups. The mean weight change ±S.D of groups 2, 3 and 4 at varying doses of the extract 2500mg/kg, 5000mg/kg and 10000mg/kg of the extract were 6.00±1.99, 7.50±0.00 and 25.00±8.36 respectively, indicating a significant increase in weight compared with the control (8.75±4.79) at P< 0.05.

Table 4.3b: Effect of *Mentha piperita* aqueous leaf extract on the body weight across groups at 5000mg/kg, 10000mg/kg, and 20000mg/kg on day two.

Table 4.3b shows the effect of *Mentha piperita* aqueous leaf extract on the body weight across groups at 5000mg/kg, 10000mg/kg, and 20000mg/kg on day two. The mean weight change ±S.D of groups 2,3 and 4 at the concentrations of 20000mg/kg, 30000mg/kg and 40000mg/kg of the extract are 7.00±0.14,8.75±0.21 and 84.25±6.16 respectively, indicating a significant increase in weight compared with the control(87.75±4.79) with P<0.05.

Day 2	Treatment (mg/kg)	Mean initial Weight ±SD	Mean final Weight ±SD	Mean Weight change ±SD
Group 1	-	65.00 ± 0.00	69.00 ± 1.15	4.00 ± 1.15
Group 2	5000	81.75 ± 2.36	88.75 ± 2.50	7.00 ± 0.14
Group 3	10000	93.75 ± 4.79	102.50 ± 5.00	8.75 ± 0.21
Group 4	20000	85.00 ± 5.77	109.25 ± 11.93	84.25 ± 6.16

Table 4.3c: Effect of *Mentha piperita* aqueous leaf extract on the body weight across groups at 10000mg/kg, 20000mg/kg, and 30000mg/kg on day three.

Table 4.3c: shows the weight effect of the extract on day 3, the *Mentha piperita* extract was administered at varying concentrations across the groups. The mean weight change ±S.D of groups 2,3 and 4 at the concentrations of 10000mg/kg,20000mg/kg and 30000mg/kg are 7.50±2.29,13.75±2.50 and 3.25±3.07 respectively, indicating a significant increase in weight across the groups when compared with the control(2.00±0.91) P<0.05.

Day 3	Treatment (mg/kg)	Mean initial Weight ±SD	Mean final Weight ±SD	Mean Weight change ±SD
Group 1	-	67.00 ± 2.45	69.00 ± 1.54	2.00 ± 0.91
Group 2	10000	88.75 ± 2.50	96.25 ± 4.79	7.50 ± 2.29
Group 3	20000	88.75 ± 2.50	102.50 ± 5.00	13.75 ± 2.50
Group 4	30000	109.25 ± 11.93	112.50 ± 15.00	3.25 ± 3.07

Table 4.3d: Effect of *Mentha piperita* aqueous leaf extract on the body weight across groups at 20000mg/kg, 30000mg/kg, and 40000mg/kg on day four.

Table 4.3d: shows the body weight effect on day 4, the *Mentha piperita* extract was administered at varying concentrations across the groups. The mean body weight change ±S.D of groups 2,3 and 4 at the concentrations of 20000mg/kg,30000mg/kg and 40000mg/kg are 14.25±7.36, 5.75±6.75 and 2.00 ± 3.28 respectively, indicating a significant increase in weight across the groups compared with the control(6.25±4.10) P<0.05.

Day 4	Treatment (mg/kg)	Mean initial Weight ±SD	Mean final Weight ±SD	Mean Weight change ±SD
Group 1	-	69.00 ± 1.15	75.25 ± 5.25	6.25 ± 4.10
Group 2	20000	96.25 ± 4.79	110.50 ± 12.15	14.25 ± 7.36
Group 3	30000	105.5 ± 6.40	111.25 ± 13.15	5.75 ± 6.75
Group 4	40000	112.50 ± 15.00	114.50 ± 18.28	6.25 ± 3.28

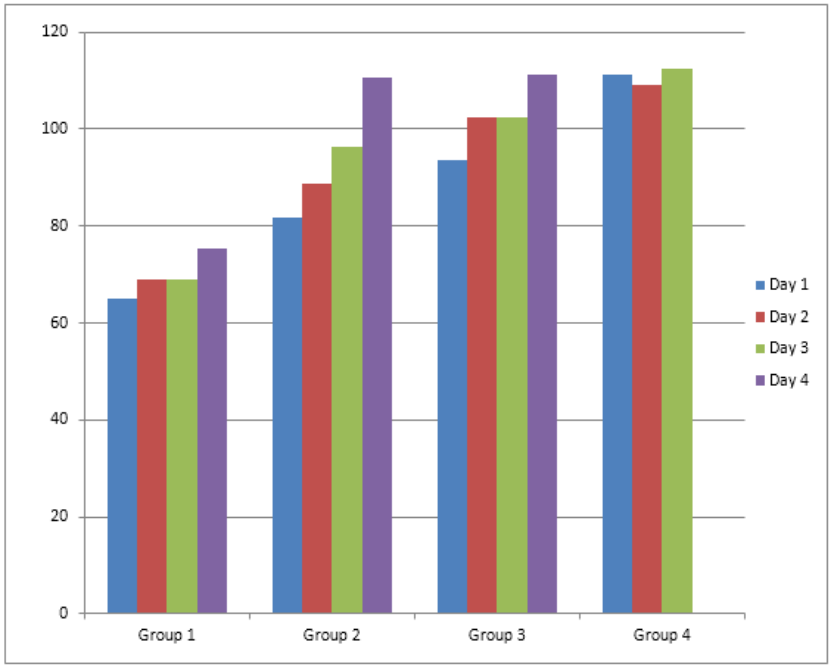


Figure 2: Chart representing the effect of *Mentha piperita* aqueous leaf extract on the ±SD mean body weight across groups on Day 1, 2, 3 and 4.

Table 4.4: LD_{50} result obtained at the dose of 40000mg/kg in group 4 and on the day 4.

Day	Dose (mg/kg)	Mortality
Three	30000	No
Four	40000	Yes

Table 4.4 presents the LD50 result obtained at a dose of 40,000 mg/kg in Group 4 on Day 4, which was then calculated using the dose from Group 3, following the formula provided below.

Therefore, LD50 =$\sqrt{(D0\ xD)}$

Where D_0 = Last non-lethal dose = 30000mg/kg

D = Lethal dose = 40000mg/kg

Hence LD_{50} = $\sqrt{30000\ x\ 40000}$ =$\sqrt{1200000000}$ = 34641mg/kg

Actual LD50 = 34641mg/kg.

Treatment of the subjects in group C of the experimental study (G_5, G_{13}, G_{16}, G_9):

This was done using 75 percent of the LD_{50} of the prepared extract thus,

75/100 x 34641.0mg/kg = 2598075/100 = 25980.75 mg/kg.

5. DISCUSSION AND CONCLUSION

5.1 Discussion

The aim of this research is to investigate the in vivo antibacterial activity of *Mentha piperita* leaf extract against *Staphylococcus aureus* causing Septicemia in an albino rat. *M. piperita* plant with several other compounds that have antimicrobial activity (Sureshkumar, 2007). The antimicrobial activities of medicinal plants are qualified due to the presence of alkaloids, and flavonoids (Burapedjo and Bunchoo 2005). These reports and presence of flavonoids, alkaloids in different extract of *M. piperita* confirm it's prospective against all selected pathogens bacterial strain. Results of this study showed that the potential usefulness of *M. piperita* in the treatment of infection like Septicemia may help in the innovation of new chemical classes of antibiotics or drugs. In addition to these properties, pepermint leaf has also been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported (Abascal *et al*, 2003).

The extensive use of *Mentha piperita* aqueous leaf extract in traditional medicine for the treatment of various infectious diseases and the reported in vitro activity of the extract against a *Staphylococcus aureus* causing Septicemia suggest that *Mentha piperita* aqueous leaf extract has a great potential as an antibacterial agent. However, all the antimicrobial bioassays that have been carried out on this plant hitherto by Rajinder (2010) and Ahmet (2011) been in vitro studies using the hole-in-plate agar diffusion and the agar dilution methods. These in vitro studies show that *Mentha piperita* aqueous leaf extract is indeed active as an antibacterial agent but these studies have not given any insight into the ability of the plant to exert its antibacterial effect in vivo. This omission is important because it is not always certain that the degree of in vitro activity shown by a plant extract is matched by its activity in vivo. In the first instance, the antibacterial components of plant extracts may be metabolized in vivo to yield inactive metabolites, which result in the loss of antibacterial activity in vivo.

In addition, in vivo tests establish the toxicity or safety of agents that have shown activity in vitro. Since *Mentha piperita* is often taken orally in the form of a decoction, in vivo tests are of particular importance because such tests simulate the actual condition of use of the plant materials. In vivo studies to determine toxicity are also of importance when considering the future formulation of *Mentha piperita* leaf extract or any other plant of medicinal value into dosage forms for human or veterinary use.

From our study of acute toxicity, the oral administration during LD50 of the aqueous extract of *Mentha piperita* of 34641mg/kg was recorded as being toxic. Based on the result obtained in this present determination, the dose for treatment of infected mice was chosen up to a maximum of 25980.75mg/kg.

The results of the antibacterial study shows that the aqueous extract of the leaves of *Mentha piperita* possesses a demonstrable level of in vivo antibacterial effects against Septicemia infection in mice at doses which are much smaller than the dose at which 50% of the test animals are killed by the extract of the leaves. The leaf extract produced a noticeable prolongation of the lives of multiply antibiotic-resistant *S. aureus*-infected albino rat beyond those of the infected untreated mice in a dose-dependent manner ($p < 0.05$). Statistically significant prolongation of survival time

was shown at a dose a slow as 25980.75mg/kg of the animals when compared with the infected untreated animals.

Comparing the antibacterial property of antibiotic and extract, it was found that the commonly used Gentamycin antibiotics has a higher antibacterial activity (0.00±0.00) as shown in table 4.2 though the extract has a significant antibacterial effect 59.50± 19.09) against *Staphylococcus aureus* induced Septicemia infection.
Also when comparing with the positive control (group C) of 82.25±12.37 with the *Mentha piperita* induced group B (59.50±19.09) showed a moderate decrease in its therapeutic activity towards Septicemia which shows that it's effective against *Staphylococcus aureus.*

We can deduce that there was increase in body weight during oral administration of the aqueous leaf extract (from day till the fourth day) might due to its high nutritive benefits to the body system of the rats. The results from the present study are very encouraging and indicate that this herb should be studied more extensively to explore its potential in the treatment of Septicemia. The antibacterial activity can be associated with the phytochemical constituents which include terpenoids and flavonoids such as eriocitrin, hesperidin, and kaempferol 7-O-rutinoside (Dolzhenko, 2010).
It was established that the test group control group (Group C) were given orally leaf extract which inhibited the growth of microorganisms and results were compared with antibiotic gentamycin commonly used therapeutically.

5.2 Conclusion

Hence it can be deduced that *Mentha piperita* have moderate antibacterial effect but lesser when compared with commonly used antibiotic (gentamycin), hence cannot be used alone but can have a higher synergistic effect as an antibacterial drug with other drugs.

5.3 Recommendations

These findings thus provide scientific bases for the use of *Mentha piperita* in ethnomedicine.
Further research is necessary to establish the actual constituents responsible for the anti-inflammatory activity. There is also need for toxicological studies of the extract for possible histopathological damages to the vital organs using enzyme markers. Also further work may be extended to isolate the active constituents for their biological potential.

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APPENDIX I

WORKING METHODOLOGY

The rats arrived at Imo State University Owerri Anatomy department animal house from Ibadan, Oyo state on the 1^{1ST} of JUNE, 2018 and acclimatization started on that day. The animals were weighed using the analogue kitchen scale and sent into cages for acclimatization, noting their sexes. During weighing, identification numbers/tags were attached to each rat. The physical agility of the animals was noted. The room temperature was measured using the room thermometer and the temperature ranged from 26 – 29^{0}c. Acclimatization lasted for one week and at the end of acclimatization, it was noted beyond every doubt that the rats gained weight and they became very agile.

After acclimatization, toxicological studies commenced in three (3) groups which were aimed at determining the toxicity dose of the *Menthax piperita* leaf extract. The LD_{50} was then deduced.

The leaves of the plant were purchased at Relief market owerri and left to dry under room temperature for 7days. The dried leaves were weighed and a bulk preparation was made and refrigerated. This preparation was made using 400grams of the powdered extracts and mixing with 1000mililitres of freshly boiled water.

Toxicological study calculations

400g of *Mentha x piperita* leaf powder was dissolved in 1litre of steamed water to obtain a ratio of *Mentha x piperita* water suspension of 400/1000ml =1:2.5(0.4)

Normal daily dose for man = 500mg/kg

Rat is not up to a kilogram, so mg/kg to gram/milligram = 500mg/kg/1000 = 0.5mg/g.

Therefore, rats weighing 1g will require 0.5mg of the extract of aqueous.

For rats weighing 100g = 100 x 0.5= 50mg.

This means that 100g rat requires 50mg of the extract as the daily dose.

Toxicologically, each of the group 2, 3, 4 will receive different doses of the aqueous extract as an increment of 5, 10 and 20.

Group 2

5 x 50mg = 250mg or 0.25g (2500mg/kg)

Vol needed = 0.25 ÷ 1/2.5 = 0.25 x 2.5 = 0.625ml for 100g.

For rats weighing 70g, =====> 70 x0.625/100 = 0.4375ml

For rats weighing 75g, =====> 75 x0.625/100 = 0.469ml

For rats weighing 78g, =====> 78 x0.625/100 = 0.488ml

For rats weighing 80g, =====> 80 x0.625/100 = 0.5ml

Group 3

10 x 50mg = 500mg or 0.5g (5000mg/kg)

Vol required

0.5/1 x 2.5 = 2.5 = 1.25ml of extract 100g in this group.

For rats weighing 80g, =====> 80 x 1.25/100 = 1.0ml

For rats weighing 85g, =====> 85 x 1.25/100 = 1.06ml

For rats weighing 90g, =====> 90 x 1.25/100 = 1.13ml

Group 4

20 x 50mg = 1000mg or 1g(10000mg/kg)
Vol required
1g x 2.5 = 2.5ml of the aqueous extract.

For rats weighing 100g, =====> 80 x0.625/100 = 0.5ml
For rats weighing 115g, =====> 80 x0.625/100 = 0.5ml
For rats weighing 120g, =====> 80 x0.625/100 = 0.5ml

After day 1 administration, the rat were observed for 30mins, 1hr, 4hrs, 24hrs, and 48hrs for mortality recorded.
On day 2 ,the dosages were further increased in each of the group 2 by 10x,group 3 by 20x and group 4 by 40x respectively.
Day 2:
Group 2: 50mg x10 = 500mg (0.5g =5000mg/kg)
0.5/1 x 2.5 = 1.25ml for rats weighing 100g,
For rats weighing 82g, =====> 82 x1.25/100 = 1.03ml
For rats weighing 80g, ======>80 x1.25 /100 = 1.0ml
For rats weighing 85g, ======>85 x1.25 /100 = 1.06ml

Group 3: 50mg x 20= 1000mg or 1g(10000mg/kg)
Vol required
0.5/1 x 2.5 = 2.5ml of extract for rats weighing 100g.

For rats weighing 100g, =======> 100 x 2.5/100 = 2.5ml
For rats weighing 90g, =======> 90 x 2.5/100 = 2.25ml
For rats weighing 95g, =======> 95 x 2.5/100 = 2.37ml

Group 4: 50mg x 40 = 2000mg or 2g(20000mg/kg)
Vol required
2/1 x 2.5 = 5ml for rats weighing 100g.
For rats weighing 120g, ======> 120g x 5ml/100 = 5.5ml
For rats weighing 115g, =======>115 x 5ml/100 = 6.0ml

After day 2 administration, the rats were observed for 30mins, 1hr, 4hrs, 24hrs, and 48hrs for mortality recorded.
On day 3 ,the dosages were further increased in each of the group 2 by 20x,group 3 by 40x and group 4 by 60x respectively.

Day 3
Group 2: 50mg x 20 = 1000mg or 1g (10000mg/kg)
Vol required
1/1 x 2.5 = 2.5ml for rats weighing 100g.
For rats weighing 85g ====> 85g x 2.5ml/100 = 2.13ml
For rats weighing 90g =====>90 x 2.5ml/100g = 2.25ml

Group 3: 50mg x 40 = 2000mg 0r 2g(20000mg/kg)
Vol required
2/1 x 2.5 = 5ml for rats weighing 100g
For rats weighing 100g====> 5ml
For rats weighing 110g=====>110g x 5ml/100g = 5.5ml

Group 4: 50mg x 60 = 3000mg or 3g(30000mg/kg)
Vol required
3/1 x 2.5 = 7.5ml for rats weighing 100g.\
For rats weighing 112g====> 112g x 7.5ml/100g = 8.4ml
For rats weighing 125g====> 125g x 7.5ml/100g = 9.35ml

After day 3 administration , the rat were observed for 30mins,1hr,4hrs,24hrs, and 48hrs for mortality recorded.
On day 4 ,the dosages were further increased in each of the group 2 by 40x,group 3 by 60x and group 4 by 80x respectively.

Day 4
Group 2 : 40 x 50mg = 2000mg or 2g(20000mg/kg)
Vol required
2/1 x 2.5 = 5ml for rats weighing 100g
For rats weighing 100g====> 5ml
For rats weighing 90g===> 90g x 5ml/100g = 4.5ml
For rats weighing 95g===> 95g x 5ml/100g = 4.75ml

Group 3 50mg x 60 = 3000mg or 3g(30000mg/kg)
Vol required
3/1 x 2.5 = 7.5ml for rats weighing 100g
For rats weighing 112g===>112g x 7.5ml/100g = 8.4ml
For rats weighing 110g===>110 x 7.5ml/100g = 8.25ml

Group 4: 50mg x 80 = 4000mg or 4g(40000mg/kg)
Vol required = 4/1 x 2.5 = 10ml for rats weighing 100g
For rats weighing 120g===> 120g x 10ml/100g = 12ml
For rats weighing 130g===>130g x 10ml/100g = 13ml

After day 4 administration, the rat were observed for 30mins,1hr,4hrs,24hrs, and 48hrs for mortality recorded.
On day 5 ,the dosages were further increased in each of the group 2 by 60x,group 3 by 80x and group 4 by 90x respectively.

Day 5
Group 2: 50mg x 60 = 3000mg or 3g(30000mg/kg)
Vol required
3g/1 x 2.5 = 7.5ml for rats weighing 100g.
For rats weighing 120g ===> 120g x 7.5ml/100g = 9ml
For rats weighing 122g===>122g x 7.5ml/100g =9.15ml

Group 3: 50mg x 80 = 4000mg or 4g(40000mg/kg)
Vol required
4/1 x 2.5 = 10ml for rats weighing 100g
For rats weighing 125g===> 125g x 10ml/100g = 12.5ml
For rats weighing 120g===> 120g x 10ml/100g = 12.0ml

Group 4: 50mg x 90 = 4500mg or 4.5g(45000mg/kg)
Vol required,

4.5/1 x 2.5 = 11.25ml for rats weighing 100g.
For rats weighing 120g===> 120g x 11.25ml/100g = 13.5ml.
For rats weighing 138g===> 138g x 11.25ml/100g = 15.53ml.
After 5hrs of administration, mortality was observed.

Therefore,
LD50 =$\sqrt{(D0\ xD100)}$

Where D_o = Highest dose that gave no mortality = 40000mg/kg
D_{100} = Lowest dose that produced mortality = 50000mg/kg
Hence LD_{50} = $\sqrt{50000\ x\ 40000}$ =$\sqrt{2000000000}$ = 44721.36mg/kg
To convert to mg/g, we divide by 1000
>>$\frac{44721.36\ mg/kg}{1000}$ = 44.72mg/g

75% of the LD_{50} for treatment= 75/100 x 44721.36mg/kg = 33541.0mg/kg

APPENDIX II
TABLES FROM TOXICOLOGICAL STUDY

Tables for toxicological study on *Mentha piperita* aqueous leaf extract
Day 1
Group 1

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_3	60	-	65	-	No
G_6	60	-	65	-	No
G_{17}	55	-	65	-	No
G_{18}	50	-	65	-	No

Group 2

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_{20}	70	2500	80	2500	No
G_2	75	2500	80	2500	No
G_{12}	78	2500	85	2500	No
G_{10}	80	2500	82	2500	No

Group 3

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_5	85	5000	90	5000	No
G_{13}	90	5000	100	5000	No
G_{16}	90	5000	95	5000	No

G_9	80	5000	90	5000	No

Group 4

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_8	85	5000	100	5000	No
G_4	90	5000	100	5000	No
G_{11}	90	5000	125	5000	No
G_{14}	80	5000	120	5000	No

Day 2

Group 1

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_3	65	-	68	-	No
G_6	65	-	70	-	No
G_{17}	65	-	68	-	No
G_{18}	65	-	70	-	No

Group 2

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_{20}	80	5000	90	5000	No
G_2	80	5000	90	5000	No
G_{12}	85	5000	85	5000	No
G_{10}	82	5000	90	5000	No

Group 3

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_5	90	10000	100	10000	No
G_{13}	100	10000	110	10000	No
G_{16}	95	10000	100	10000	No
G_9	90	10000	100	10000	No

Group 4

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_8	8	20000	100	20000	No
G_4	90	20000	100	20000	No
G_{11}	90	20000	125	20000	No
G_{14}	80	20000	112	20000	No

Day 3

Group 1

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_3	68	-	68	-	No
G_6	70	-	70	-	No
G_{17}	65	-	68	-	No
G_{18}	65	-	70	-	No

Group 2

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_{20}	90	10000	95	10000	No
G_2	90	10000	100	10000	No
G_{12}	85	10000	90	10000	No
G_{10}	90	10000	100	10000	No

Group 3

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_5	90	20000	100	20000	No
G_{13}	90	20000	110	20000	No
G_{16}	85	20000	100	20000	No
G_9	90	20000	100	20000	No

Group 4

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_8	100	30000	100	30000	No
G_4	100	30000	100	30000	No
G_{11}	125	30000	130	30000	No
G_{14}	112	30000	120	30000	No

Day 4

Group 1

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_3	68	-	80	-	No
G_6	70	-	78	-	No
G_{17}	68	-	68	-	No
G_{18}	70	-	75	-	No

Group 2

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_{20}	95	20000	100	20000	No
G_2	100	20000	120	20000	No
G_{12}	90	20000	100	20000	No
G_{10}	100	20000	122	20000	No

Group 3

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality
G_5	100	30000	100	30000	No
G_{13}	100	30000	100	30000	No
G_{16}	110	30000	125	30000	No
G_9	112	30000	120	30000	No

Group 4

Rat	Weight Before (g)	Dose in mg/kg	Weight after (g)	Total dose (mg/kg)	Mortality

G_8	100	40000	100	40000	No
G_4	100	40000	100	40000	No
G_{11}	130	40000	138	40000	No
G_{14}	120	40000	120	40000	No