Shendi University

College of Graduate studies

In-Vitro Antibacterial Activity of crude Garlic (*Allium Sativum* Extract Against Clinical Isolate of Methicillin Resistant *Staphylococcus aureus* from Elribat university hospital,Sudan.

A dissertation Submitted in Partial Fulfillment for the requirment of M.Sc in Medical Laboratory Science (Microbiology).

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الأيه

قَالَ تَعَالَىٰ:

﴿ ٱلۡحَمۡدُ لِلَّهِ ٱلَّذِيٓ أَنزَلَ عَلَىٰ عَبۡدِهِ ٱلۡكِتَٰبَ وَلَمۡ يَجۡعَل لَّهُۥ عِوَجَاۜ ١ قَيِّمٗا لِّيُنذِرَ بَأۡسٗا شَدِيدٗا مِّن لَّدُنۡهُ وَيُبَشِّرَ ٱلۡمُؤۡمِنِينَ ٱلَّذِينَ يَعۡمَلُونَ ٱلصَّٰلِحَٰتِ أَنَّ لَهُمۡ أَجۡرًا حَسَنٗا ٢ ﴾

صدق الله العظيم

سورة الكهف.. الأيه2

Dedication

This research is lovingly dedicated to my mother and my father who have been my constant source of inspiration, without their love and support this project would not have been made possible.

To my sister Sana, and Sahar for their encouragement continuously.

To my brother Mohammed and my husband Mohammed Mohieldein , who helped me with their love.

To my colleagues and friends who supported me with their confidence.

To those who help me even with a ward.
To my supervisor Dr. Nadir abuzeid who help me very much in every step needed to knowledge and all thankfull for his support.

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ABSTRACT

Background: Methicillin resistant Staphylococcus aureus (MRSA) continues to be one of the commonest pathogens encountered in clinical as well as laboratory practice. It has become a major health problem worldwide. Newer antimicrobials/agents are urgently needed to combat this problem. MRSA strains developed resistance to almost all current antibiotics. Medicinal plans have been used as the main source of remedies and pharmaceutical drugs from the past civilizations. Numerous plants exhibited effective antibacterial activity against MRSA strains and competitor to current antibiotics, some of them have a potential to restore the effectiveness of antibiotics on MRSA.

Methods: The aqueous and 70% ethanol crude garlic (Alllium.sativum) extract was prepared. Disc diffusion method was performed to assess the antibacterial activity for100clinical isolates MRSA 54 male and 46 female collected , *Staphylococcus aureus*(ATCC 25923) was used as the standard reference strain , methanol and distilled water were used as negative control.

Results: All tested strains of MRSA were sensitive to70% *ethanolic* extract at a concentrations range of 200% – 25%. (exhibited inhibitory effects Against clinical Isolates and *Staphylococcus aureus* (ATCC 25923) with the means of inhibition zones ranging from 17.76- 14.35mm and 15-13mm in length , while the aqueous extract were less active than ethanol in both clinical isolates and *Staphylococcus aureus* (ATCC 25923) ranging from 11.93-8.62 mm and11-8mm respectively, methanol and distilled water were not effect on growth.

Conclusion: The results indicated that 70% ethanol crude Allium.*sativum* has inhibitory effect on Methicillin resiatant staphylococcus aureus better than aqueous extract.

مستخلص الاطروحه

الخلفيه: ان المكورات العنقوديه الذهبيه المقاومه للميثيسيلين لا تزال واحده من اكثر مسببات الامراض اكلينيكيا ولذا اصبحت مشكله كبيره في جميع انحاء العالم مما يتوجب الحوجه الماسه لوجود بدائل جديده اكثر فعاليه لمكافحة هذه المقاومه التي بها ضمنت الانتشار. ولأنها تجاوزت مقاومة الميثيسلين واصبحت مقاومه لأغلبية المضادات الحيويه المتوفره حاليا لذلك تم استخدام النباتات الطبيه كمصدر رئيسي للعلاج في الحضارات السابقه وفي هذا السياق اظهرت العديد من النباتات فعاليتها المضاده لهذه البكتريا المقاومه واصبحت منافسه للمضادات الحيويه الموجوده ولديها القدره علي استعادة فعاليتها علي تلك السلالات البكتيريه.

الطرق: تم تحضير مستخلص الثوم بالماء والايثانول المخفف مائيا بنسبة70% بطريقة العطن ثم تم تقييم هذا المستخلص بالاختبار علي 100 عينه معزوله اكلينيكيا من(54 ذكور و 46 اناث تم جمعها من مسحات الجروح و العين وكذلك الدم والتفاف وأخرى بكتريا مرجعيه قياسيه للمكورات العنقوديه الذهبيه) عن طريق انتشار القرص ، واستخدام الماء والميثانول في اذابة نواتج الاستخلاص الجاف مرة اخري لذا تم وضعهم للتحكم السلبي في هذا الاختبار ورصد النتائج.

النتائج: تأثرت جميع السلالات بناتج مستخلص الثوم الايثانولي المخفف في جميع التراكيز التي تراوحت بين 200% الي 25% تأثيرا مثبطا ضد كل السلالات المعزوله و القياسيه بقراءة كل متوسط تثبيط علي حده وتراوح من 17.76 الي 14.35مم للمعزوله و من 15 الي 13مم للقياسيه في الطول ، في حين ان المستخلص المائي كان اقل تثبيطا من الايثانول المخفف مائيا في كل المعزولات الاكلينيكيه والمكورات العنقوديه الذهبيه المرجعيه التي تراوح متوسط تثبيطها بين 11.93 الي 8.62مم و 11 الي 8 مم علي التوالي.

الخلاصه: أشارت النتائج الي ان مستخلص الثوم الخام بالايثانول المائي المخفف بنسبة تركيز 70% عن طريق التعطين له تأثيرا مثبطا اكثر فعالية من المستخلص المائي لهذه المكورات العنقوديه الذهبيه المقاومه للميثيسلين

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List of Abbreviation:

Number	abbreviation	Refer to
1	MRSA	Methicillin Resistant Staphylococcus aureus.
2	A.Sativum	Allium Sativum.
3	DNA	Deoxyribo nucleic acid.
4	ATCC	American type culture collection
5	MDR	Multi drug resistant.
6	TB	Tuberculosis.
7	AIDS	Acquired immune deficiency syndrome.
8	HIV	Human immune deficiency virus.
9	S.Aureus	Staphylococcus aureus.
10	DNAse	Deoxy Ribo Nuclease.
11	DW	Distilled water.
12	KHZ, ug	Kilohertz, microgram.
13	BA, Ch BA	Blood agar , chocolate blood agar.
14	MHA	Muller Hinton agar.
15	NCCLS	National Committee for Clinical Laboratory standards Guidelines.
16	CFU	Colony forming unit.

CHAPTER ONE
Introduction

1.1-Introduction:

Antibiotic resistance is an important threat to public health on a global scale as it reduces the effectiveness of treatment and increases morbidity, mortality and health care costs (Grundmann *et al.,* 2006). Evolution of highly resistant bacterial strain has compromised the use of new generations of antibiotics (Davies and Davies, 2010). Antibiotic resistance is due to an inherent ability of microorganisms to form surface-attached communities of cells within the extracellular polymeric matrix called biofilms (Hall-Stoodley *et al*., 2004). Methicillin-resistant *Staphylococcus aureus* (MRSA) presents a significant threat to public health in many areas in the world and causing a significant morbidity and motility worldwide (El-Kalek and Mohamed, 2012). The frequencies of infectious and outbreaks due to MRSA have continued to increase (Boucher et al., 2009). *MRSA* is often multidrug resistant and therapeutic options are limited (Lewis and Jorgensen, 2005). MRSA normally possesses a multidrug-resistant genotype which causes it resistant to βeta lactams, aminoglycosides, fluoroquinolones and macrolides (Zhanel et al., 2010). There is an urgent need to develop anti -MRSA agents with novel mechanisms of action to address this problem (Rivers and Mancera, 2008). With a growing incidence of infections resistant to antibiotics, an arsenal of either new agents of the supplementation of current antibiotics is needed. Natural products from plants could be interesting alternatives (Taylor, 2013).

Staphylococcus aureus is a major human pathogen responsible for severe morbidity and mortality worldwide. It is one of the leading causes of human infections in the skin and soft tissues, bones and joints, Abscesses and normal heartvalves (Bartlett, 2008). *Staphylococcus aureus* is an opportunistic pathogen often carried asymptomatically on the human body. MRSA strains have acquired a gene that makes them resistant to nearly all

βeta-lactam antibiotics (Uhlemann et al., 2014). Resistance to other antibiotics is also common, especially in hospital-associated MRSA. These organisms are serious nosocomial pathogens, and finding an effective treatment can be challenging. Community-associated MRSA strains, which originated outside hospitals, are also prevalent in some areas (Rivera and Boucher, 2011). While these organisms have generally been easier to treat, some have moved into hospitals and have become increasingly resistant to drugs other than βeta-lactams. Animals sometimes become infected with MRSA from humans, and may either carry these organisms asymptomatically or develop opportunistic infections(Rennie, 2012). Most of the MRSA found in dogs and cats seem to be lineages associated with people. Colonization of dogs and cats is often transient and tends to occur at low levels ; however, these organisms can be transmitted back to people, and pets might contribute to maintaining MRSA within a household or facility (Wieler et al., 2015). MRSA can also be an issue in settings such as veterinary hospitals, where carriage rates can be higher, especially during outbreaks in pets , horses and other animals (Leonard and Markey, 2008).

Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure forms or crude extracts to treat many diseases (Parekh and Chanda, 2007).

Garlic (Allium sativum L.) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases (Onyeagba et al., 2004). The taxonomic position of garlic and related genera had been a matter of controversy for long period. The most recent classification scheme of garlic was class Liliopsida, subclass Liliidae, super order Liliianae, order Amary-llidales, family Alliaceae, subfamily Allioideae, tribe Allieae and genus Alliums which is mainly based on the sequences of nuclear ribosomal DNA (Friesen et al ., 2006).

The early Egyptians used garlic to treat diarrhea and its medical power was described on the walls of ancient temples and on papyrus dating to 1500 BC (Bradley,1992). It was used by Greek physicians Hippocrates and Galen to treat intestinal and extra-intestinal diseases; ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections (Jaber and Al-Mossawi, 2007). In Europe and India, it was used to treat common colds, hay fever and asthma. Garlic is nicknamed as Russian penicillin for its widespread use as a topical and systemic antimicrobial agent; it is commonly used in many cultures as an excitement and reputation of healing power (Timbo et al., 2006).

1.2 Rationale:

Antibiotics were introduced in the 1940s and they were thought off as the cure for all emerging diseases. However; resistant strains emerged only two decades after the introduction of penicillin. Even with the introduction of new classes of antibiotics and synthetic drugs, the problem of resistance continues as bacteria develop resistance to almost all classes of antibiotics. In recent years pharmaceutical companies has almost stopped producing new antibiotics which has led researchers to look for alternative antimicrobials. Herbs were used for treatment of infectious diseases for many centuries before the introduction of antibiotics and the emergence of resistant strains has renewed the in herbs and medicinal plants to serve as novel antimicrobial agents. The use of plant extracts with known antimicrobial properties, can be of great significance in therapeutic treatments. Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Pseudomonas, Shigella, Salmonella, Proteus, and Helicobacter pylori (Tsao and Yin, 2001). According to this aiming of recent study to highlighting search the *in vitro* antibacterial activity of crude extract extract from garlic against isolated *Staphylococcus aureus* that resistant to Methicillin, also as natural component and least side effect rather than synthetic and chemical antibiotic.

1.3 Objectives:

1.3.1 General Objective:

To estimate the *In vitro* antimicrobial activity of Garlic extracts against Clinical isolate of *Methicillin Resistant Staphylococcus aureus* From Elribat university hospital, Sudan.

1.3.2 Specific Objectives:

1. To determine the antibacterial activity of aqueous and 70%ethanol crude A.*sativum* extracts on selected MRSA strains by Disc diffusion method test.
2. To compare the susceptibility of aqueous and 70%ethanol crude A.*sativum* garlic extracts on random selected isolated MRSA strain from male, female and standard *staphylococcus aureus* American type culture collection (ATCC, 25923) .

CHAPTER TOW
LITERATURE REVIEW

LITERATURE REVIEW

2.1. Herbal medicine.

2.1.1Definition.

Herbal medicine is sometimes referred to as herbalism or traditional medicine. It is the use of herbs for their therapeutic or medicinal value. A herb is a plant or a plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body (Ahmed et al, 1998).

2.1.2. History.

Herbs have been used for many centuries by many cultures to enhance the flavor and aroma of food. Early cultures also recognized the value of using herbs in preserving food and for their medicinal value. Scientific experiments since the late 19 century have documented the antimicrobial properties of some herbs and their components (Zakia, 1988).

2.1.3. Antimicrobial Properties of Medicinal Plants.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body.

Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cough, cold, cholera, fever and bronchitis (Saran raj and Sivasakthi, 2014).

2.2. Potentially Active Chemical Constituents of Garlic.

Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids to be found in garlic: lysine, histidine, arginine, aspartic acid threonine, swine, glutamine, proline, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine (Josling, 2005). It has a higher concentration of sulfur compounds than any other Allium species which are responsible than both any other Allium species which are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfinateor diallyldisulfide). The most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 and 30 mg/g in fresh and dry garlic, respectively (Lawson, 1998). Typical garlic food preparation such as chopping, mincing and crushing disturbs S-allyl cysteine sulfoxide and exposed it to the allinase enzymes, then quickly converted it to diallyl thiosulfinate, which give off garlic's characteristic aroma. The allinase enzyme responsible for diallyl thiosulfanate conversion becomes inactivated below a pH of 3.5 or with heating (Pedrazza-Chaverri et al., 2006). Although allicin is considered the major antioxidant and scavenging compound, recent studies showing that other compounds may play stronger roles; such as polar compounds of phenolic and steroidal origin, which offer various pharmacological properties without odor and are also heat stable (Lanzotti, 2006).

2.3. Role of Garlic in Health.

Garlic can rightfully be called one of nature's wonderful plants with healing power. It can inhibit and kill bacteria, fungi, lower (blood pressure, blood cholesterol and blood sugar), prevent blood clotting, and contains anti-tumor properties. It can also boost the immune system to fight off potential disease and maintain health (Abdullah et al., 1988). It has the ability to stimulate the lymphatic system which expedites the removal of waste products from the body. It is also considered an effective antioxidant to protect cells against free radical damage. It can help to prevent some forms of cancer, heart disease, strokes and viral infections. Garlic alone can provide us with over two hundred unusual chemicals that have the capability of protecting the human body from a wide variety of diseases. The sulfur containing compounds found in garlic afford the human body with protection by stimulating the production of certain beneficial enzymes (Mansell and Reckless, 1991).

2.3.1 Antimicrobial.

The antimicrobial properties of garlic were first described by Pasteur (1958), and since then, many researches had demonstrated its effectiveness and broad spectrum antimicrobial activity against many species of bacteria, viruses, parasites, protozoan and fungi (Jaber and Al-Mossawi, 2007). Garlic is more effective with least side effects as compared to commercial antibiotics; as a result, they are used as an alternative remedy for treatment of various infections (Tepe et al., 2004). Out of the many medicinal plants, garlic has an antimicrobial property which protects the host from other pathogens highlighting the importance of search for natural antimicrobial drugs (Bajpai et al ., 2005 ; Wojdylo et al., 2007).

Previously conducted researches confirmed that garlic is not only effective against Gram positive and Gram negative bacteria but also possess antiviral and antifungal activities (Tsao and Yin, 2001).

2.3.2. Antiviral.

Garlic and its sulfur constituents verified antiviral activity against coxsackievirus species, herpes simplex virus types 1 and 2, influenza B, parainfluenza virus type 3,vaccinia virus, vesicular stomatitis virus, human immunodeficiency virus type 1and human rhinovirus type 2. The order of compounds found in garlic for virucidal activity was, ajoene > allicin > allyl methylthiosulfanate > methyl allyl thiosulfanate; no activity was found for the polar fractions, alliin, deoxyalliin,diallyldisulfide, or diallyl trisulfide. Several laboratory tests have shown that garlic is an effectual treatment for both the influenza B virus and herpes simplex virus. Two independent researchers in Japan and Romania have found that garlic is able to protect living organisms from the influenza virus (Tsai et al., 1985). Most recently, a double blind placebo controlled study has shown significant protection from the common cold virus. As conducted by The Garlic Centre, published in Advances in Therapy, this is the first serious work to show prevention, treatment and reduction of reinfection benefits from taking Allimax Powder capsules once daily (Josling, 2001).

2.3.3. Antibacterial.

Garlic extract inhibits the growth of Gram positive and Gram negative bacteria,such as Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia, Klebsiella, Pseudomonas, Shigella, Salmonella,Proteus, and Helicobacter pylori (Tsao and Yin, 2001). Its antibacterial activity is mainly due to the presence of allicin produced by the enzymatic activity of allinase on alliin. Allicin is considered to be the most potent antibacterial agent in crushed garlic extracts, but it can be unstable, breaking down within 16 h at 23°C (Hahn, 1996). However, the use of a water-based extract of allicin

stabilizes the allicin molecule due to the hydrogen bonding of water to the reactive oxygen atom in allicin or there may be water soluble components in crushed garlic that destabilize the molecule (Lawson, 1996). The disadvantage of this approach is that allicin can react with water to form diallyl disulphide, which does not exhibit the same level of antibacterial activity of allicin (Lawson and Wang, 1996).

2.3.4. Antifungal.

Ajoene is an active compound found in garlic which plays a great role as topical antifungal agent (Ledezma and Apitz-Castro, 2006). Garlic has Been shown to inhibit growth of fungal diseases as equally as the drug ketoconazole,when tested on the fungi Malassezia furfur, Candida albicans, Aspergillus, Cryptococcus and other Candida species (Shams-Ghahfarokhi et al., 2006). A report from a Chinese medical journal delineates the use of intravenous garlic to treat a potentially fatal and rare fungal infection of the brain called Cryptococcus meningitis. In the report, the Chinese compared the effectiveness of the garlic with standard medical treatment which involved a very toxic antibiotic called Amphotericin-B. The study revealed that, intravenous garlic was more effective than the drug and was not toxic regardless of its dosage (Lemar et al., 2007).

A study found that Candida colonies were substantially reduced in mice that had been treated using liquid garlic extract. The study also revealed that garlic stimulated phagocytic activity. This implies that infections such as Candida may be controlled because garlic stimulates the body's own defenses. Garlic oil can be used to treat ringworm, skin parasites and warts if it is applied externally. Lesions that were caused by skin fungi in rabbits and guinea pigs were treated with external applications of garlic extract and began to heal after seven days (Sabitha et al., 2005).

2.3.5. Antiparasitic.

Many herbalists worldwide recommend garlic as a treatment for intestinal parasites. In some cultures, children infested with helminthes are treated with enemas containing crushed garlic. One of the traditional Chinese medical treatments for intestinal diseases is an alcoholic extract of crushed garlic cloves. Allicin exhibits Antiparasitic activity against major human intestinal parasites such as Entamoeba histolytica, Ascaris lumbricoides and Giardia lamblia (Kalyesa et al., 1975). Entamoeba histolytica, the human intestinal protozoan parasite, is very sensitive to allicin, as only 30 μg/ml of allicin totally inhibits the growth of amoeba cultures (Mirelman et al., 1987). Moreover, researchers have found that at lower concentrations (5 μg/ml), allicin inhibited 90% the virulence of trophozoites of
E. histolytica as determined by their inability to destroy monolayers of tissue cultured mammalian cells in vitro(Ankri et al., 1997).

2.3.6. Role of garlic against multidrug resistant bacteria.

Garlic is active against microorganisms that are resistant to antibiotics and the combination of garlic extracts with antibiotics leads to partial and total synergism (Didry et al., 1992).The emergence of multi-drug resistant strains of Gram negative (Pseudomonas, Klebsiella, Entero-bacter, Acinetobacter, Salmonella species, etc…)and Gram positive (Staphylococcus, Enterococcus, Streptococcus species, etc….) bacteria is troubling for human and animals. The emergence of epidemic MRSA resistant to mupirocin has led many authors to suggest that the use of mupirocin should be controlled more strictly, especially as there is a lack of alternative agents. Consequently, garlic is an alternative agent for the treatment of MRSA and in a great demand (Sharma et al., 2005).

2.3.7. Role of garlic against multi-drug resistant tuberculosis (MDR-TB).

Scientific evidence from randomized clinical trials supports the use of garlic and enhances access for MDR-TB infected people, through the public health system. Its use can allow an effective MDR-TB management, due to its affordability and the absence of toxic effects (Catia et al., 2011). In view of the increased incidence of MDR-TB, the research of new anti-tubercular drugs based on affordable and more effective treatments has already begun. Studies on innovative alternative plant extracts of medicinal values need to be emphasized, as plants are an important source of new antimicrobial agents, with little toxicity, able to replace drugs to which Mycobacterium resistance has occurred (Amin et al., 2009). As garlic is concerned, the *in-vitro* tests undertaken about the inhibitory effect on MDR-TB are at an advanced stage whereas few researches *in-vivo* have been conducted. The concentration of garlic extract re-quired was in the range of 1.34 to 3.35 mg/ml suggesting that there is only a slight variation in the susceptibility of the strains to allicin (Delaha and Garagusi, 1985).

The anti-tuberculosis activity *in-vivo* of garlic oil preparation was demonstrated in a study of guinea pigs which were given an intra-peritoneal dose of 0.5 mg/kg. However, when garlic oil was used, a reduced causative process was noted in the organs involved, indicating that garlic oil administration causes less marked lesions in the viscera of the animals inoculated with tubercle bacilli (Jain, 1998). The high potential of garlic extract was revealed to inhibit the growth of Mycobacterium tuberculosis H37RV and M.Tuberculosis TRC-C1193, susceptible and resistant to isoniazid (first-line anti-tuberculosis medication), respectively. The minimum inhibitory concentration (MIC) of garlic was between 80and 160 μg/ml for the susceptible strain and 100 and 200 μg/ml for the resistant strain. In addition, water extract of garlic was proven to inhibit the

incorporation of 14C glycine into the whole cells, indicating that the primary mechanism of action is by inhibition of protein synthesis (Ratnakar and Murthy, 1996). An interesting *in-vitro* test about the anti-tubercular activity of garlic was performed in Nigeria using disc diffusion method and compared with standard antibiotics.

The anti-tubercular activity of garlic on multiple-drug resistant Mycobacterium was investigated among Nigerian HIV-infected-persons and it exhibited maximal activity against all isolates even at reduced concentrations. Only two of the standard anti-tubercular antibiotics used, streptomycin and rifampicin, showed significant activity against isolates tested (Dibua, 2010).

2.3.8. As natural immunity booster.

With the arrival of frightening viral diseases like /AIDS, boosting immunity system is receiving a new attention. Because these types of diseases have no effective cures or treatments, strengthening the body's ability to fight off infection has become even more important. Garlic has abundant sulfur containing amino acids and other compounds that seem to initiate increased activity in the immune system (Lau et al., 1991). It is one of the impressive conductors of the body's immune system; which stimulates immune function by making macrophages or killer cells more active. We are constantly beaten by inadequate nutrition, cigarette smoke, physical injury, mental tension and chemical pollution. In light of the enormous pressures, which our immune systems sustain, supplemental nutrients like garlic are clearly needed (Salman et al., 1999). Its remarkable content of germanium alone offers excellent immune stimulation. In addition to germanium, garlic contains thiamine, sulfur, niacin, phosphorous, and selenium (Morioka et al., 1993). Preliminary studies in humans, using an alliin standardized garlic powder preparation, have demonstrated positive effects on immunoreactions and phagocytosis. In aged subjects, the administration of

600 mg garlic powder per day for 3 months induced significant ($p<0.01$) increases in the percentage of phagocytosing peripheral granulocytes and monocytes when tested ex vivo for their ability to engulf Escherichia coli bacteria. Another human study was conducted with an unrefined garlic extract (5to 10 g/day) which was given to HIV/AIDS patients. For the seven patients who completed the 12 weeks study, there was a major increase in the natural killer cells activity from a seriously low mean value (Abdullah et al., 1988).

In USA, trials in HIV/AIDS patients have demonstrated enhancement of natural killer cells activity using garlic extracts; and Chinese studies with viral infections in bone marrow transplant patients have demonstrated a "potent antiviral activity". A double blind placebo controlled survey using a 100% allicin yielding supplement has reported that allicin can reduce the occurrence of the common cold and recovered from symptoms (Josling, 2001).

2.3.9. Reduces high blood pressure/hypertension.

Garlic has probably been most popularized as a complementary therapy for blood pressure control (Capraz et al., 2006). A recent in vitro study has confirmed that, the vasoactive ability of garlic sulfur compounds whereby red blood cells convert garlic organic polysulfide's into hydrogen sulfide, a known endogenouscardio-protective vascular cell signaling molecule (Benavides et al., 2007). Using 2400 mg garlic tablet containing 31.2 mg allicin has high dose reduced diastolic pressure by 16 mmHg after 5 h of administration (McMahon and Vargas, 1993). A meta-analysis made on pooled data from 415 patients showed also reduction of 7.7 mmHg diastolic pressure (Silagy and Neil, 1994).

2.3.10. Treat cardiovascular disease.

Disorders of the heart and the circulatory system claim more lives than any other diseases. It is the obstruction or clogging of the coronary arteries which causes more deaths than any other factors. The arteries, which supply the heart with blood and oxygen, become increasingly narrower as plaque builds up over time. When blood supply becomes restricted, a certain portion of the heart is deprived of oxygen and leads to heart attack. The two greatest means of heart disease are high blood pressure and high blood serum cholesterol levels; which are directly impacted by the therapeutic action of garlic. The relevant role of garlic in coronary heart disease was done on rabbits and found that even pre-existing athero-sclerotic deposits and lesions could actually be reversed if garlic was consistently consumed (Bordia,1981). From a study conducted in India, 432 coronary artery patients were randomly grouped into two groups and half of them were supplied with garlic juice in milk, whereas the other group patients were not supplied with garlic juice. The result showed that within the three years of the study time, nearly twice as many patients had died in the group not supplied with garlic juice (Yeh et al., 2006). It is well reported to scavenge oxidants, increase superoxide dismutase, catalase, glutathione peroxidase, glutathione levels, inhibit lipid peroxidation as well as it reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA. It has been shown to reduce platelet aggregation, arterial plaque formation, decrease homocysteine, lower blood pressure, and increase microcirculation. It may also help prevent cognitive decline by protecting neurons from neurotoxicity and apoptosis, thereby preventing ischemia or reperfusion-related neuronal death and by improving learning and memory retention (Borek, 2006).

2.3.11. Antioxidant.

Whole garlic and aged garlic extract exhibit direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, catalase and glutathione peroxidase (Prasad et al., 1995). Garlic extract, allicin is efficiently scavenged exogenously generated hydroxyl radicals in a dose dependent fashion, but their effectiveness was reduced about 10% by heating to 100°C for 20 min. Other garlic constituents, such as S-allyl cysteine, also confirmed significant antioxidant effects. The sulfur compounds found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude, aged garlic extract. Garlic (both the homogenate of 10% in physiological saline solution and its supernatant) was able to reduce the radicals present in cigarette smoke (Torok et al., 1994).

2.3.12. Drug toxicities and pharmacokinetics.

Glutathione is a compound necessary for liver to facilitate detoxification of substances. It has been hypothesized that garlic organo-sulfur compounds may be able to prevent glutathione depletion. Patients who experience increasing in reactive oxygen induced stress on liver function may be protected by garlic ingestion (Sabayan et al., 2006). It was found in E.Coli cultures that aged garlic extract, S-allyl cysteine, diallyl sulfide and diallyl disulfide do not interfere with the antibiotic activity of gentamycin but may improve gentamycin induced nephrotoxicity (Maldonado et al., 2005). Aged garlic has also been shown to reverse oxidant effects of nicotine toxicity in rat studies. More researches are required in the future garlic may be a unique choice to help minimize the toxic effects of therapeutic drugs (Sener et al., 2005).

2.3.13. Reduces stress.

Among The many uses of garlic, it appears to have the fortunate capacity for protecting against the negative effects of stress that affects the autonomic nervous and neuroendocrine system. Rats that were trained with endurance exercises to physical fatigue enjoyed improved parameters of aerobic glucose metabolism, attenuated oxidative stress, and vasodilatations, when given garlic at a dosage of 2.86 g/kg for 30 min before exercise (Morihara et al., 2006). In rats exposed to psychologically stressful situations, aged garlic extracts significantly prevented the decreases in spleen weight seen in control animals. Additionally, the garlic significantly prevented the reduction of hemolytic plaque forming cells in spleen cells Moreover, garlic was able to block the lipopolysaccharide induced immune cytokine and plasma corticosterone and catecholamine changes following cold water immersion stress (Nance et al., 2006). Aged garlic extract is also effective to prevent adrenal hypertrophy, hyperglycemia and elevation of corticosterone in hyperglycemic mice induced by immobilization stress. Given the extreme chronic stress many people now facing their daily life, garlic may prove useful to counter the negative impact of this stress on human physiology (Kasuga et al., 1999).

2.3.14. Prevents Diabetes.

A number of animal studies support the effectiveness of garlic in reducing blood glucose in streptozotocin-induced as well as alloxan-induced diabetes mellitus in mice. Most of the studies showed that garlic can reduce blood glucose level in diabetic mice and rabbits (Ohaeri, 2001). A study was conducted to evaluate oral administration of garlic extract for 14 days on the level of serum glucose, total cholesterol, triglycerides, urea and uric acid, in normal and streptozotocin-induced diabetic mice. The result of the study showed significant decrease ($p<0.05$) in serum glucose, total cholesterol, triglycerides, urea, acid, aspartate amino transferase and alanine

amino transferase levels, while increased serum insulin in diabetic mice, but not in normal mice. From a comparison study made between the action of garlic extract and glibenclamide, it was shown that the ant diabetic effect of the garlic was more effective than the glibenclamide (Eidi et al., 2006).

2.4. General techniques for extraction of medicinal plants.

The general techniques of medicinal plants extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter current extraction, microwave-assisted extraction, ultrasound techniques (water distillation, steam extraction (sonication), supercritical fluid extraction, and distillation, phytonic extraction (with hydro fluorocarbon solvents) .The basic parameters influencing the quality of an extract are plant part used as starting material, solvent used for extraction and extraction procedure (Amita and Shalini, 2014).

2.4.1. Extraction procedures.

Plant tissue homogenization Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5-10 minor left for 24 hours after which the extract is filtered. The filtrate then may be dried under reduced pressure and dissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract (Das et al., 2010).

2.4.2. Serial exhaustive extraction.

It is another common of extraction, which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. Some researchers employ soxhlet extraction of dried

plant material using organic solvent. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds (Das et al., 2010).

2.4.3 Soxhlet extraction.

Soxhlet extraction is only required where the desired has a compound limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds (Sutar et al., 2010).

2.4.4 Maceration.

In maceration (for fluid extract), whole or coarsely powdered plant drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs(Amita and Shalini, 2014).

2.4.5 Decoction.

This method is used for the extraction of the water soluble and heat stable constituents from crude drug by boiling it in water for 15minutes, cooling, straining and passing sufficient cold water through the drug to produce the required volume (Bimakr, 2010).

2.4.6. Infusion.

It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period with either cold or boiling water (Bimakr, 2010).

2.4.7. Digestion.

This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstruum is increased thereby (Bimakr,2010).

2.4.8. Percolation.

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for the specified menstruum and allowed to stand for approximately 4hours in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24hours. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting (Cowan, 1999).

2.4.9. Sonication.

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfia root, its large scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but

known deleterious effect of ultrasound energy (more than 20kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules(Cowan, 1999).

CHAPTER THREE
MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Study design.

Descriptive cross study.

3.2. Study area.

This study was conducted at Elribat University Hospital, in Khartoum State, Sudan, during the period from April to June 2018.

3.3.3. Study population.

patients male and female were studied as known clinical isolate MRSA after full microbilogy identification methods were done from different specimens , wound swab, eye swab , sputum , and blood culture according to their symptoms, hundred clinical isolate were collectd.

Also Standard bacterial strains include American type culture collection (ATCC), *S.aureus* (ATCC 25923) used as control positive. And Sudanese garlic bulbs with small sizes and strong Odor obtained from Khartoum bahri market to determine its antibacterial activity .

3.4. Inclusion criteria.

Clinical isolates that show resistance pattern to oxacillin.

3.5. Exclusion criteria.

Clinical isolates that show sensitive pattern to oxacillin.

3.6. Sample size.

A total of hundred Isolates (n= 100) were collected randomly, 73 MRSA isolated from wound , 3 eye swabs, 21 MRSA from blood culture , and 3 MRSA isolated from sputum samples.

3.7. Ethical considerate.

This study was approved by College of High graduate studies ethical committee/Shendi university, also permission issued by microbiology lab administration at Elribat Hospital university,and national center for research , Khartoum.

3.8. Laboratory methods.

3.8.1. Plant materials collection.

The *Garlic bulb* (seeds) was collected from Central Sudan, Khartoum Bahri market in April 2018. The plant was identified and authenticated by the taxonomists of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.

(Figure 1). Garlic bulb (seeds) was air dried under the shadow with good ventilation , then cleaned with distilled water for its ground finely until their use for extracts prepration as homogenous dry mater after present to sun light.

3.8.2. Preparation of crude extracts.

Extraction was carried out for the seeds of garlic plant 100 g by using maceration techniques after weighting the grounded material by sensitive balance about 34g round material was macerated equal quantity for both aqueous and 70% ethanol extract.

3.8.3. Aqueous extraction.

Extraction was carried out according to method descried by Sukhdev et al.(2008):100 g of the sample was extracted by soaking in 100 ml hot distilled water for about four hours with continuous steering. After cooled, extract was filtered using filter paper and the solvents were evaporated using freeze drier. And the yield percentage were calculated as followed

Weight of extract obtained / weight of plant sample X100

Table (3.1): Aqueous extraction.

Sample No	Name of plant	Weight of plant in g	Weight of extract in g	Yield %
1	Garlic	100 g	6.8 g	6.8 %

3.8.4. 70% ethanol extraction.

Extraction was carried out according to method descried by Sukhdev *et al.* (2008):100g of the plant sample was grounded using mortar and pestle and extracted by soaking in 70 % ethanol for about five days with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness and the yield percentages were calculated as followed:

Weight of extract / weight of sample * 100

Table (3.2) 70% Ethanol Extraction.

Sample No	Name of plant	Weight of plant in g	Weight of extract in g	Yield %
2	garlic	100 g	7.2 g	7.2 %

Each residue was weighed and the yield percentage was calculated and then stored at 4°C in tightly sealed glass vial ready for use.

3.8.5. Bacteria strains.

A total of 100 clinical isolates of MRSA and ATCC 25923 were obtained from Elribat hospital university-Microbiology lab and National Center for Research, Khartoum, Sudan.

Under a septic conditions all the isolates collected pastly from wound swab, sputum , eye swab , and blood culture and preserved to me after

cultivations and cultured in suitable media agar as Blood agar , chocolate blood agar , and MacConky agar using sterile wire loop,inculated plates were incubated aerobically at 37^0C for 18-24hours.

Identification was done on the basis of morphology, cultural characteristics, biochemical reactions and susceptibility to Oxacillin discs (1 µg) using Mueller-Hinton agar supplemented with 4% NaCl.

3.8.6. Cultural characteristics.

After the incubation period the plates were examined for the size, colour, edges, side views, odor and surface of the colonies.

3.8.7. Gram stain.

smears from the growth were prepared and stain by Gram stain as follow:
fixed by heat, after cooling covered by crystal violet stain for 30 60seconds,washed off stain by clean water, covered with Iogl's iodine for 30-60seconds,washed with cleaned water, covered with safranine stain for 2 minutes, then washed and let to air dry and microscopically examined using oil immersion objective (100X) to observe morphological appearance, Gram positive reaction and Gram negative. The results of Gram's stain were reported.

3.8.8. Biochemical tests.

3.8.8.1. Catalase test.

A pure of 2-3ml of hydrogen peroxide solution was added in a test tube, by sterile wooden stick several colonies of test organisms were put in hydrogen peroxide solution. The positive results indicated by immediate bubbling (Cheesbrough, 2000).

3.8.8.2. Coagulase test.

A drop of physiological saline was placed on each end of slide, a colony of the test organism was emulsified in each of the drops to make two thick suspensions and adrop of plasma was added to one of the suspensions and

mixed gently by rotating The positive results indicated by producing clump within 10seconds (Cheesbrough,2000).

3.8.8.3. Deoxyribonuclease (DNAse)test.

The test organism was cultured on a medium which contains DNA. After overnight incubation, the colonies were tested for DNAse production by flooding the plate with a weak hydrochloric acid solution. The acid precipitated hydrolyzed DNA. DNAse producing colonies were therefore surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2000).

3.8.8.4. Manitol Salt Agar (MSA).

This medium was used to differentiate *S.aureus* from other *Staphlylococci* species. Aportion of colony was inoculated on manitol salt agar containing 75g/1sodium chloride and incubated aerobically at 37oC for 18-24hours. *S.aureus* ferments manitol producing yellow colonies (Cheesbrough, 2000).

3.8.9. Detection of Methicillin Resistance

MRSA identification was carried out using oxacillin screen plates following the guidelines of NCCLS. Briefly, a suspension equivalent to 0.5 McFarland standards, prepared from each strain, was inoculated homogenously on the entire surface of the Mueller-Hinton agar plate (Oxoid-UK) containing 4% NaCl and 6 μg/mL oxacillin, with the help of sterile swabs. All the plates were incubated at 35 ^{o}C for 24 hrs. Indication of growth (>1 colony) identified the isolates as oxacillin/methicillin-resistant (Genç et al., 2008) ,when zone of inhibition present above 12mm was identified as sensitive and 11-12mm identified as intermediate.

3.8.10. Antibacterial susceptibility testing for extract.

3.8.10.1. Disc diffusion method.

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^{8}

CFU/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) by oven at 80 ^{o}C for 30 minutes were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts with different concentration diluted serial dilution as 200%, 100% , 50% , and 25% (2mg/ml , 1mg/ml , 0.5mg/ml , 0.25mg/ml) respectively ,after resuspended by methanol for 70% ethanolic extract and the same concentration used after resuspended by sterile distilled water for aqueous extract.

Addition to this used methanol and DW soaked filter paper as control for both extracts.The inoculated plates were incubated at 37 °C for 24 hrs in the inverted position. The diameters (mm) of the inhibition zones were measured.

3.9. Data analysis.

All data was analyzed using Statistical Packaged for Social Science (SPSS) software version 21 and Graph Pad Prism Version 5.

CHAPTER FOUR
RESULT

4. Result

4.1Distributions of 100 clinical isolates according to types of specimens:-

A total of 100 clincal isolates collected Included wound swab(73%) , eye swab (3%) , sputum (3%) , and blood for culture(21%). As show in figure 2.

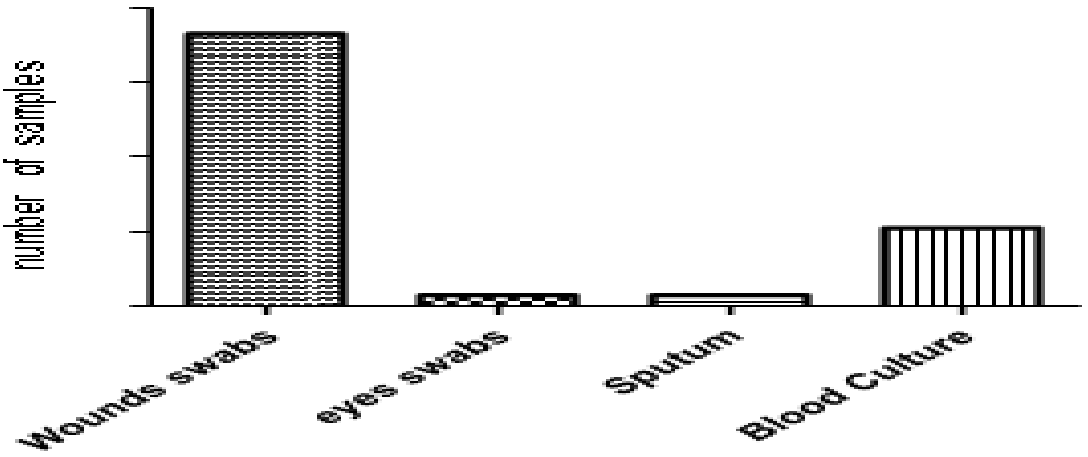


Figure (4.1) :- Demographics distribution of isolates according to their sources in Khartoum state.

Table (4.1) :

Different concentrations of 70% ethanol extract and their inhibition effect related to sex, There is no significant

P. value≥ 0.05 association between sex with different concentrations of ethanol extracts and their inhibitions effect.

Con%	Sex	N	Mean±Std.deviation	P.V
200%	M	54	17.59 ± 0.880	0.74
	F	46	17.96 ± 1.095	
100%	M	54	17.96 ± 1.095	0.388
	F	46	16.54 ± 0.836	
50%	M	54	15.41 ± 0.714	0.971
	F	46	15.41 ± 0.805	
25%	M	54	14.33 ± 0.614	0.798
	F	46	14.37 ± 0.771	

*N= number , Con= concentration , Std.deviation= standard deviation. , P.V= P.value.

Table (4.2):

Different concentrations of aqueous extract and their inhibition effect related to sex, There is no significant P. value≥ 0.05 association between sex with different concentrations of water extracts and their inhibitions effect.

Con%	sex	N	Mean ±Std.deviation	P.V
200%	Male Female	54 46	11.91 ± 0.293 11.96 ± 0.206	0.329
100%	Male Female	54 46	10.665 ±0.555 10.70 ± 0.465	0.642
50%	Male Female	54 46	9.59 ± 0.659 9.46 ± 1.501	0.571
25%	Male Female	54 64	8.57 ± 0.716 8.67 ± 0.474	0.422

*N= number , Con= concentration , Std.deviation= standard deviation. , P.V= P.value

4.2. Gram stain result:-

Gram positive cocci in cluster.

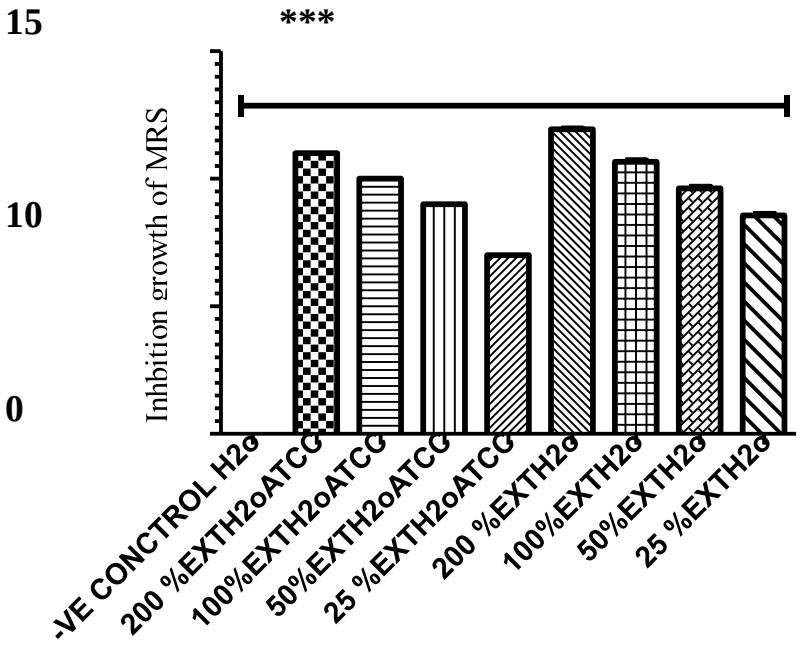
4.3. Biochemical reactions:-

Table (4.3) Results of biochemical reactions for gram positive pathogens:-

Biochemical tests	Reactions results
Catalase	+
Coagulase	+
DNase	+
Manitol	+
Lactose ferment	+

Note:+: *Positive*.

4.4. The antibacterial activity of *A. sativum* aqueous extract tested on clinical isolates and ATCCC (25923)*Staphylococcus aureus* reference strain after re-suspended with distilled water as negative control:



Figure(4.2):-Explain mean of growth inhibition of aqueous extract present in ATCCC strain was little effect from (11-8) mm in length than clinical isolates (11.9-8.9), and distilled water was no effect.

4.5. The antibacterial activity of A.sativum 70% ethanol extract tested on clinical isolates and ATCCC (25923)*Staphylococcus aureus* reference strain after re-suspended with methanol as negative control:-

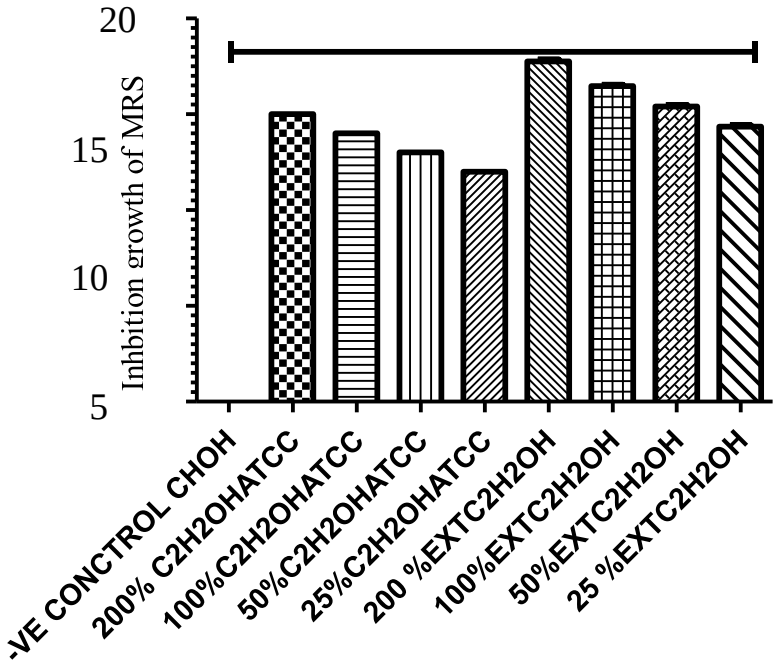


Figure (4.3) :- Explain mean of inhibition growth 70% ethanol extract present in ATCCC strain was little effect from (15-13) mm in length than clinical isolates (17.76- 14.35), and methanol was no effect.

4.6.The anti-bacterial activity of A.sativum aqueous extract opposite to 70% ethanol extract against clinical isolates MRSA:-

The 70% ethanol extracts in all concentrations were sensitive when matches with oxacillin susptibility test on MRSA strain , intermediate only in 200% aqueous extract and other concentrations were resistant.

The activity of *A.sativum* in both extracts increased with the concentration of it.

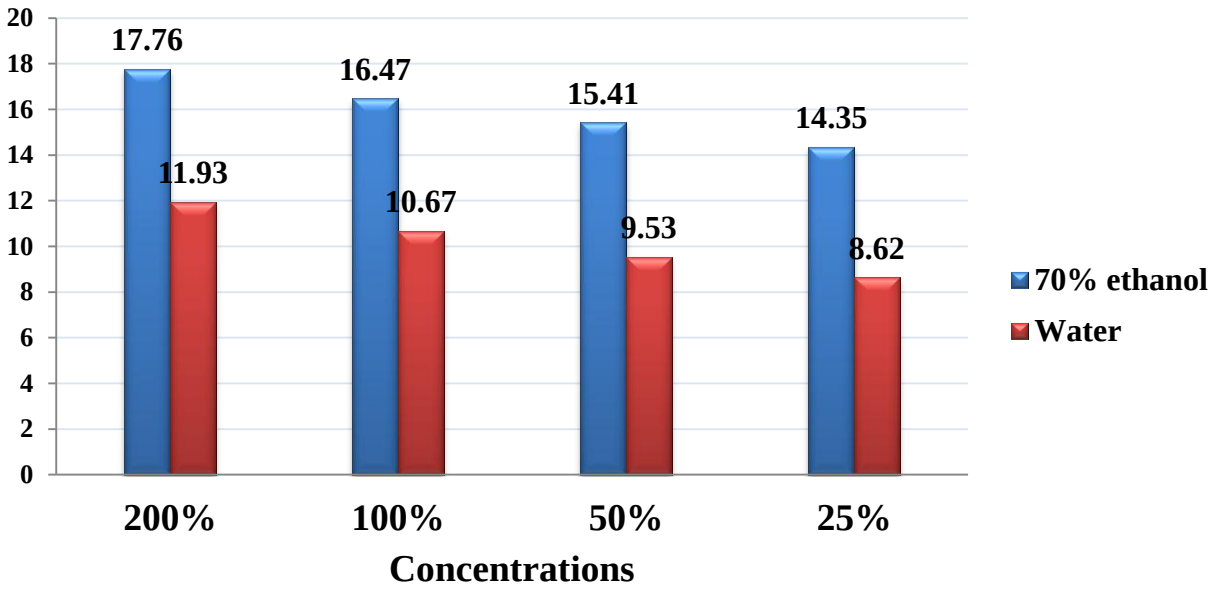


Figure (4.4): The anti-bacterial activity of A.sativum aqueous extract opposite to 70% ethanol extract against clinical isolates MRSA

CHAPTER FIVE
DISCUSSION

5. Discussion

5.1 Discussion:

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains and this became a major cause of failure of the treatment of infectious disease (Ibrahim et al., 2011).

In this study 70 % ethanol extract of crude A.*sativum* showed remarkable antibacterial activity against standard strains of *S.aureus* and clinical isolates methicillin resistant that agreed with Janan et al., (2016), While aqueous extract showed little activity, these were different with that obtained by Hadir et al. (2013), may be refer to using homogenization aqueous extraction technique,.

However, Opposite results extract do not indicate the less activity of bioactive constituents, since active compound (s) may be present in insufficient quantities , but the use of boiled water in the maceration method of extract for garlic may be effect on stability of allicin and other bioactive components that agreed with Strika et al , (2017). Also This may be due to two factors: the hydrogen bonding of water to the reactive oxygen atom in allicin can reduce its instability; and/or there may be water-soluble components in crushed garlic that destabilize the molecule. Lawson ,(1996).

The disadvantage 0f this approach is that allicin can react with water to form diallyl disulphide, Block , (1992). , And Lawson and Wang , (1995). Which does not exhibit the same level of antibacterial activity as does allicin.

The yield percentage of ethanolic extract was noted 7.2 % slightly increased than aqueous 6.8 % , that due to the time of extract according to methodology of technique , in aqueous 4 hours only needed and then filerated the final product in one day, but ethanolic extract needed 5 days

with daily filtration. this effect of time on yield and in the sufficient quantity has role of antibacterial activity.

The most probable explanation for these differences results of zone inhibition mm in this study compare with other studies may be due to different bacterial strains tested, and antibacterial activity method, method of extract and time, in addition to the amount composition of organo sulfar compounds which vary with different species of garlic around the world Arora and Kaur , (1999). A study by Lawson et al. (1991) established that the garlic cropped in China may have twice allicin as much as in Europe or United States. However, one of the disadvantages in evaluating the antibacterial activity of Garlic Extract is lack of standardization in techniques being used by the scientists. This gives rise to marked difference in results obtained. So it is important to develop guidelines for all procedure adopted in evaluating antibacterial activity of Garlic Extract. One significant advantage of garlic is that the bacteria do not seem to evolve to build up a resistance to it as they do to many modern antibiotics; "garlic does not seem to produce such resistant strains" Erdogrul , (2002). This also makes it potentially effective against hospital superbugs. Rangan and Barceloux , (2009) - or at least less likely to contribute to their evolution.

The means diameter of growth inhibition zone of standard and clinical isolates of bacteria were increased with the increased in extract concentration. This result is in agreement with report of Suleiman (2013) .The best inhibition zone obtained by 70% ethanol extract of crude garlic was 20 mm in diameter against clinical isolates of MRSA strains at concentration of 4 mg\ 2ml , (200% conc). And the minimum inhibition zone was 13 mm in diameter at 0.5 mg/ml , (25% conc) also on aqueous.

5.2. Conclusion:

70% ethanol crude extracts of *Allium sativum* has inhibitory effect on methicillin resistant *staphylococcus aureus* is better than aqueous extract.

5.3. Recommendation:

- Isolate and purify the active ingredients (Allicin) compounds in the extract responsible for antimicrobial activity.
- Determination of minimum bactericidal concentrations for the allicin on each bacterium including those of multi drug resistant bacteria.
- Study the toxicity of the active ingredients.
- More research is required to verify these results and for the evaluate the combination of garlic with other commercial antibiotics.
- More broad extractions project for garlic plant components distribute geographically around the world and determine the best active ingredients by Ideal standard method to optimized strong wide spectrum drug to long period treatment without resistances.
- Alternative method to extracts processing by solvents must be more use and tested as squeezing technique to avoid the loss of bioactivity of component

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Appendix 1

Materials:

A-Equipment:

1-Autoclave.

2-Bunsen burner.

3-Hot air oven.

4-Incubator.

5-Rack.

5-Refrigerator.

6-waterbath.

7-Wire loops with handle.

8-sensitive balance.

9-pipete.

10-plain container.

11-light microscope

12-freezer dryer.

B-Glasswares:-

1-Petri dishes (plates).

2-Flask with different size.

3-Measuring cylinder.

4-Beakers.

5-Funneles.

6-Spoons.

7-Sterile containers (bijou bottles).

8-Test tubes.

9-Slides.

C-Disposable materials:

1-Disposable swabs.

2-blue and yellow tips.

3-Filter papers.

4-Disposible syrings.

D-Culture media:

1-Mueller Hinton agar

Typical formula g/L

Contents

Casein acid hydrolysate.....17.50

Beef heart infusion.....2.00

Starch,soluble.....1.50

Agar.....17.00

pH (at 25ºc) 7.3±0.1

Preparation

Suspend 38g of powder in 1000ml D.W mix well and heat to boiling to dissolve the medium completely. Sterilize by autoclave at 121ºc for 15mins.

2-Nutient agar

Typical formula in g/L

Contents

Peptone.....5.0

Meat extract.....3.0

Agar.....15.0

pH 7.0±0.2

Preparation

Suspend 23g of powder in 1L of D.W and heat to boiling.

Dispense into containers and sterilize in the autoclave at 121ºc for 15minutes.

DNAse agar

Typical formula g/L

Conents

Tryptose.....20

Deoxyribonucleic acid.....2

Sodium chloride.....5

Agar.....12

pH 7.2±0.2

Preparation

Suspend 3.9g in 1L of D.W. bring to boil to dissolvecompletely.

Sterilize by autoclave at 121ºc for 15minutes. Cool to 50ºc and pour into the petridishes. Dry the surface of the medium before inoculation.

Blood agar and chocolate blood agar

To make about 35blood agar plates:

Nutritious agar.....500

Sterile defibrinated blood.....25ml

Preparation

1-Prepare the agar medium as instructed by the manufacturer.

Sterilize by autoclaving at121C0for 15minutes. Transfer to a 50C0

water bath.

2-When the agar has cooled to 50C0, add aseptically the sterile blood and mix gently but well. Avoid forming air bubbles.

3-Dispense aseptically in 15ml amounts in sterile petri dishes.

4-Date the medium and give it a batchnumber.

5-Store the plates at 2–8C0, preferably in sealed plastic bags to prevent loss of moisture

pH of medium: 7.2–7.6 at room temperature.

CHOCOLATE (HEATED BLOOD) AGAR

When blood agar is heated, the red cells are lyzed and the medium becomes brown in colour.

prepration

1-Prepare as described for blood agar except after adding the blood, heat the medium in a70C water bath until it becomes brown in colour. This takes about 10–15 minutes during which time the medium should be mixed gently several times.

2-Allow the medium to cool to about 45C,remix and dispense in sterile petri dishes as described for blood agar.

3-Date the medium and give it a batch number.

Store the plates as described for blood agar.

pH of medium: 7.2–7.6 at room temperature.

Mannitol salt agar

Contents

Peptone, Lab-Lemco powder, mannitol, sodium chloride, phenol red, agar.

Preparation

1-Prepare the medium as instructed by the manufacturer. Sterilize by autoclaving at121C0for 15minutes.

2-When the medium has cooled to 50–55C0, mix well, and dispense it aseptically in sterile petri dishes. Date the medium and give it a batch number.

3-Store the plates at 2–8C0 preferably in plastic bags to prevent loss of moisture.

pH of medium:7.3–7.7at room temperature.

MacConkey agar

This medium is best prepared from ready to usedehydrated powder, available from most sup-pliers of culture media.

Contents:

Peptone, lactose, bile salts, sodium chloride, neutral red, agar.

The medium is usually used at a concentration of5.2 g in every 100 ml distilled water.

prepration

1-Prepare as instructed by the manufacturer.Sterilize by autoclaving at 121C for 15minutes.

2-When the medium has cooled to 50–55C,mix well and dispense aseptically in sterile petri dishes. Date the medium and give it abatch number.

3 Store the plates at 2–8C preferably in plasticbags to prevent loss of moisture.

Shelf-life: Up to 4 weeks providing there is no change in the appearance of the medium tosuggest contamination or an alteration of pH.

pH of medium:PH 7.2–7.6 at room temperature.

E-Chemicals and reagents

1-70% ethanol.

2-Sodium chloride (normal saline).

3-Methanol.

4-Mc ferland turbidity standard

5-DW

Mc ferland turbidity standard

Contents

Concentrated sulphricacid.....1ml

Dihydrate barium chloride.....0.5g

Distilled water.....150ml

Preparation

1-Prepare 1%(v/v) solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99ml of water and mix well.

2-Prepare 1.175% (w/v) solution of barium chloride by dissolving 2.35g of di-hydrate barium chloride (Bacl2.2H2o) in 200ml of distilled water.

3-Add .5ml of barium chloride solution to 99.5ml of sulphuric acid solution and mix.

Plate 1:- Antibacterial activity of 70% ethanol A.*sativum* extract on clinical isolates MRSA strains.

Plate 2:- Antibacterial activity of 70% ethanol *A.sativum* extract on STD ATCC strain.

Plate 3:- Antibacterial activity of aqueous extract A.sativum on clinical isolate MRSA strains.

Plate 4:-Antibacterial activity of aqueous extract *A.sativum* on STD ATCC strain by using disk diffusion method with different concentrations.

Plate 4:-Antibacterial activity of aqueous extract *A.sativum* on STD ATCC strain by using disk diffusion method with different concentrations.

The production of 70% ethanol extract after daily filtration

Scratching process by using spatula after well dried extract at room temperature