

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Sudan University of Science and Technology
College of Graduate Studies



**Antimicrobial Activity of *Nigella sativa* Seeds Extracts Against
Staphylococcus aureus Nasal Isolates among Sudan University of Science
and Technology Students**

فعالية نشاط مستخلص بذور الحبة السوداء ضد المكورات العنقودية الذهبية المعزولة من الأنف
لدى طلاب جامعة السودان للعلوم والتكنولوجيا

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Medical Laboratory Science (Microbiology)

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الآيَة

قال تعالى

{ وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ }

صدق الله العظيم

سورة هود (الآية 88)

Dedication

My humble effort is dedicated to

My loving Father and mother

My sweet brothers and sisters

To teachers everywhere in the world those teach so other minds can grow

.

Acknowledgments

First and for most thanks to Almighty Allah for giving me strength, health and determination to accomplish this research work.

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Abstract

Nigella sativa has been used since ancient times as nutritional supplement and for the treatment of various infections and chronic ailments. *Staphylococcus aureus* is one of the important resistant pathogenic bacteria extremely adaptable to antibiotics pressure. It becomes a major health problem; so that use of new antimicrobial agents is the best choice to overcome this problem.

This was a descriptive cross sectional study conducted in Khartoum State-Sudan, during the period from October to December 2017. The study aimed to determine the antibacterial activity of *Nigella sativa* seed. The methanolic and aqueous extracts obtained using maceration extraction. Both extracts were tested in varying dilutions against *Staphylococcus aureus* isolated from nasal carriers. The antibacterial effect of *Nigella sativa* was determined through cup-plate agar diffusion technique under standard laboratory conditions.

One hundred nasal swabs were collected. 38/100(38%) showed bacterial growth. From which 11/38 (29%) *S.aureus* was isolated and identified using Gram stain, biochemical reactions and tested for their susceptibility to number of antibiotics. In this study percentage of *S.aureus* were found 7/38(64%) in males and 4/38(36%) in females .The results showed that 9% (1/11) of *S. aureus* isolates were resist to oxacillin while 91%(10/11) were resistant to amoxicillin. All strains were sensitive to Clindamycin, Vancomycin, and Fuscidin.

Methanolic and aqueous extracts of *Nigella sativa* inhibits the growth of *S.aureus* dose dependently. A significant correlation was observed between zone of inhibition and concentration of extract this result confirm the antibacterial activity of *Nigella sativa*. The minimum inhibitory concentration of *Nigella sativa* methanol and water extracts obtained by agar diffusion method for *S.aureus* isolates was 12.5 mg/ml and 25 mg/ml respectively.

In conclusion both *Nigella sativa* seeds aqueous and methanolic extract have activity against *Staphylococcus aureus*. This result authenticates the antibacterial activity of *Nigella sativa* seeds and support the traditional use of the plant in therapy of bacterial infection.

المستخلص

حبة البركة استخدمت منذ العصور القديمة كمكمل غذائي ولمعالجة الإصابات المختلفة والأمراض المزمنة. المكورات العنقودية الذهبية هي واحدة من أهم البكتيريا الممرضة المقاومة القابلة للتكيف بشده مع ضغط المضادات الحيوية ، وقد أصبحت مشكلة صحية كبيرة، لذلك استخدام عوامل جديدة مضادة للبكتيريا هو الخيار الافضل للتغلب على هذه المشكلة.

هذه دراسة وصفية مسحية أجريت في ولاية الخرطوم - السودان ، خلال الفترة من أكتوبر إلى ديسمبر 2017. هدفت الدراسة لتحديد النشاط المضاد للبكتيريا من بذور الحبة السوداء ز المستخلصات الميثانولية والمائية تم الحصول عليها باستخدام الإستخلاص بالنقع . كلا المستخلصين تم اختبارهم في تخفيفات مختلفة ضد المكورات العنقودية الذهبية المعزولة من ناقلي الأنف. التأثير المضاد للبكتيريا لحبة البركة تم تحديده من خلال طريقة الإنتشار عن طريق الحفر علي لوحات الأجار تحت ظروف المختبر القياسية.

مائة عينة من المسحات الأنفية تم جمعها 100/38 (38 %) أظهرت نمو بكتيري. منها 38/11 (29 %) مكورات عنقودية ذهبية تم عزلها وتحديدتها باستخدام صبغة غرام ، التفاعلات الكيميائية الحيوية وإختبار قابليتها لعدد من المضادات الحيوية. في هذه الدراسة تم العثور على نسبة من المكورات العنقودية الذهبية في كل من الذكور والإناث، 38/7 (64 %) في الذكور و 38/4 (36 %) في الإناث ، أظهرت النتائج أن 9 % (1/11) من عزلات المكورات العنقودية الذهبية كانت مقاومة للأوكسيسيلين في حين كانت 91 % (10/11) مقاومة لأموكسيسيلين، جميع السلالات كانت حساسة للكلينداميسين، الفانكوميسين و الفيسوسيديين.

المستخلصات الميثانولية والمائية لحبة البركة تثبط نمو المكورات العنقودية الذهبية اعتمادا" على الجرعة. ارتباط قوي لوحظ بين منطقة التثبيط وتركيز المستخلص هذه النتيجة تؤكد النشاط المضاد للبكتيريا من حبة البركة. التركيز المثبط الأدنى لمستخلص حبة البركة الميثانولي والمائي الذي المتحصل عليهم بواسطة طريقة الإنتشار علي لوحات الأكار لعزلات المكورات العنقودية الذهبية كانت 12.5 ملجم/مل و 25 ملجم/مل على التوالي.

الخلاصة أن كلا من المستخلصين الميثانولي والمائي لحبة البركة لهما نشاط ضد المكورات العنقودية الذهبية ؛ هذه النتيجة تؤكد النشاط المضاد للبكتيريا لبذور حبة البركة وتدعم الاستخدام التقليدي للنبات في علاج العدوى البكتيرية.

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List of Abbreviations

CA-MRSA	Community-associated MRSA
DNase	Deoxyribonuclease
<i>E.coli</i>	<i>Escherichia coli</i>
HA-MRSA	Healthcare-associated MRSA
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MSA	Manitol Salt Agar
MSSA	Methicillin sensitive <i>Staphylococcus aureus</i>
<i>N.sativa</i>	<i>Nigella sativa</i>
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
<i>S.aureus</i>	<i>Staphylococcus aureus</i>
SFD	<i>Staphylococcal</i> food borne diseases
SUST	Sudan university for technology and science
THQ	Thymoquinone
TQ	Thymoquinone
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1. Background

Appearances of multi drug resistance in human and animal pathogens as well as side effects of antibiotics are immense interest to search for new antimicrobial source (Namita and Mukesh 2012). The development of new antibiotics has become useless; because it is expensive and time-consuming process. Although microbes rapidly develop resistance to these new antibiotics. These led to an increasing awareness in searching for valuable alternatives for the current antibiotics with different mode of action on pathogens. Hence, medicinal plants appeared to be the best alternative source for new antimicrobial drugs (Abdallah, 2017).

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Among various medicinal plants, *Nigella sativa* (*N. sativa*) (Family Ranunculaceae) is a herbaceous plant found in the Middle East, Europe and Western and Middle Asia (Islam *et al.*, 2017).

Pharmacological effects of *Nigella sativa* seeds involved anti-inflammatory, anti parasitic, anti bacterial, anti fungal, and anti cancer. Also have ability to cure from bronchial asthma, dysentery, headache, gastrointestinal problem, eczema, hypertension and obesity (Anjum *et al.*, 2015).

Staphylococcus aureus is both a human commensal and a frequent cause of most important bacterial infections. Studies have revealed that the anterior nares are the most frequent carriage site for *S. aureus*. The relation between *S. aureus* nasal carriage and Staphylococcal disease was first reported by Danbolt, in 1931, who studied Furunculosis. *Staphylococcus aureus* causes superficial skin lesions such

as boils and furunculosis, acute infections such as pneumonia and urinary tract infections, and deep-seated infections such as osteomyelitis and endocarditis. Since methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961, it has been a major health problem worldwide. Therefore, the prevention of staphylococcal infections and reduction their spread and emergence are essential (Onyeagwara *et al.*, 2014).

N. sativa was reported as powerful antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa* & *Escherichia coli*) species. Also can fight against many multi-drug-resistant Gram positive and Gram negative bacterial infection (Islam *et al.*, 2017) .

1.2. Rationale

Antibiotics misuse increase the drug resistance bacterial strains, as a result patient with antibiotic resistant infection require powerful antibiotics that can cause severe side effect increasing the cost and hospital stay (Al-Salihy *et al.*, 2017).

Solving this problem is testing the biologically active compounds of plant origin. *Nigella sativa* seeds have a great medicinal importance and have been reported to exhibit antimicrobial activity. Seed diethyl ether extract, methanol and chlorophyll extracts well as seed oil were found to have antibacterial effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and pathogenic yeast *Candida albicans* (Datta *et al.*, 2012).

Staphylococcus aureus is a most important nosocomial pathogen causing health care associated infections all over the world. A casual relationship between *S.aureus* nasal carriage and subsequent infection is supported by the fact that nasal and infecting strain share the same genotype. Colonizing strains serve as endogenous reservoirs for clinical infection and can spread to population (Maroof, *et al.*, 2016)

In Sudan many studies were done examine antimicrobial activity of *Nigella sativa*. The result of this studies showed that *N.sativa* extracts posses antimicrobial activity as Petroleum ether extract (Ali, 2015) Ethanolic extract (Kabbashi *et al.*, 2015), methanolic extract (Fadailallah *et al.*, 2012), and oil (Ayoub, 2015). Therefore, the main objective of this study was to examine the *in vitro* antimicrobial activity of *Nigella sativa* seeds extracts and to compare the effect of different solvents in the extraction method for antimicrobial activity.

1.3. Objectives

1.3.1. General objective

To determine antimicrobial activity of *Nigella sativa* seeds extracts against *Staphylococcus aureus* isolated from nasal isolates among Sudan University of Science and Technology students.

1.3.2. Specific objective

- 1- To isolate and identify *Staphylococcus aureus* from nasal carriage among university students.
- 2- To determine the antimicrobial susceptibility of *Staphylococcus aureus* to selected antibiotics.
- 3- To determine antimicrobial activity of *Nigella sativa* methanolic and aqueous extract against *Staphylococcus aureus*.
- 4- To determine the minimum inhibitory concentration of methanolic and aqueous extract *Nigella sativa* against *Staphylococcus aureus* isolated from nasal carriers.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Medicinal plants

Human beings have depended on nature for their simple requirements as being the sources for treatment, shelters, preparation of food, perfumes, made clothes, flavors, fertilizers and transports throughout the ages (Dar *et al.*, 2017).

Use of plants in medicinal purposes is very old. The writings indicate that the use of plants in therapy is as old as 4000 - 5000 B.C. Chinese community are the first users of natural herbal preparations as treatment source. In India, however, earliest references of use of plants in medicine appear in Rig-Veda, which is said to be written between 1600 - 3500 B.C. later on the medicinal plants properties and therapeutic uses were studied in detail and recorded empirically by the ancient physicians an indigenous system of medicine (Hosseinzadeh *et al.*, 2015).

WHO indicates that over 80% of the world population use medicinal plants as their main source of health care. The problem of microbial resistance is increase day by day and the outlook of the use of antimicrobial drugs in the future is still undecided (Karateek *et al.*, 2012). In addition to this problem antibiotics are sometimes have adverse outcome on host which include hypersensitivity, depletion of beneficial gut and mucosal flora ,immunosuppression and allergic reaction (Namita *et al.*, 2012) so that action must be taken to overcome this problem.

Antimicrobial ingredients that extracts from plants were used for centuries in food preservation. Egyptians, Chinese, and Indians used spices and their essential oils since ancient time. Spices, herbs, and their constituents are reported as safe by GRAS (General Recognized As Safe) and approved by many regulatory agencies such as US Food and Drug Act, the European Union standards, Codex Alimentaris, and Food Safety and Standards Authority of India (Dhiman *et al.*, 2015).

2.2. Plant extraction

Medical herbs are the richest bio-resource of drugs of traditional systems of medicine, folk medicines, modern medicines, food supplements, pharmaceutical intermediates and chemical entities (Ijaiya *et al.*, 2014).

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues by using selective solvents from the inactive/inert components. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Pandey & Tripathi, 2012).

The products so obtained from plants are usually mixtures of the plant metabolites, in liquid or semisolid condition or (after removing the solvent) in dry powder form, and are intended for oral or external use (Tiwari *et al.*, 2011). Secondary plant metabolites that have medicinal importance are alkaloids, terpenoids, glycosides, Flavonoids and lignans. For successful extraction of biologically active ingredients from plant material is largely dependent on the type of solvent used during the extraction procedure. The characteristics of a good solvent in extraction of herbs include; little toxicity, ease of evaporation at low heat, encourage the rapid physiologic absorption of the extract, act as preservative, lack of ability to cause the extract to complex or dissociate. Solvents that commonly used for herbal extraction are: water, ethanol, methanol, chloroform, ether, and acetone. The basic principle is to crush the plant material (dry or wet) finer, this process will increase the surface area for extraction thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal (Pandey & Tripathi, 2012).

Extractions techniques for plant metabolites can be called conventional (long been used) these are: Decoction, Infusion, Soxhlet extraction, Maceration and Hydrodistillation. Conventional techniques use organic fluid (hexane, acetone, methanol, and ethanol) or water and are carried out generally at atmospheric pressure. New techniques (developed more recently) using pressure and / or

elevated temperatures they are also call ‘Green extraction’ related to the discovery and design of extraction processes that reduce energy consumption, by use of alternative solvents and renewable natural products which ensure a safe and high quality extract. They are Ultrasound assisted extraction (UAE) Microwave-assisted extraction (MAE) Supercritical fluid extraction (SFE) (Njla *et al.*, 2017).

2.3. Plants extract as potential antibacterial agents

Globally there are over 3, 00,000 plant species and just about 2% of plants have been checked for their antimicrobial activities. More than 157 plant families have potential antimicrobial activities (Narayan *et al.*, 2010). Over usage of antibiotic is destructive to human health, ecosystem, and environment. It could also enlarge the incidences of drug-resistant pathogens (Masoumian and Zand, 2017). Many medicinal herbs contain many ingredients that exhibit antibacterial activity (Khan *et al.*, 2011). Ingredients such as emetine, berberine and quinine which are extract from plants are very effective for the infectious bacteria (Ahmad and Wajdi, 2013) Medicinal plants are rich in a many variety of secondary metabolites that posses antimicrobial activities such as saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Abdallah, 2011).

2.4. *Nigella sativa*

The genus *Nigella* is relatively small and contains about 18 species with several sub-species and several genotypes. All *Nigella* species are therophytes (annuals); that mean complete their life cycle in a short favorable time and survive harsh periods as seeds. *N. sativa*, perhaps the most well known member of the genus *Nigella*, it is about 8-35 inches (20-90 cm) in height and has finely divided, somewhat threadlike leaves. This species has pale-blue to pale-purple flowers that flourish in the spring and produce seed capsules (fruit) that contain many black seeds (Engels and Brinckmann, 2017).

2.4.1. Taxonomic classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Ranunculaceae

Genus: *Nigella*

Species *N. sativa* (Sultana *et al.*, 2015)

2.4.2. Habitat

Nigella sativa, is annual flowering plant, it is grown in many parts of the world (Abdallah, 2017). *N.sativa* is found growing wild in regions of northern Africa, Turkey, Syria, Iraq, and Iran. The species is also cultivated on a commercial scale in northern Africa (Egypt, Tunisia, Sudan), eastern Africa (Ethiopia), western Asia (Iraq, Israel, Jordan, Lebanon, Syria, Turkey, Yemen), and southern Asia (India, Iran, Pakistan) (Engels and Brinckmann, 2017).

2.4.3. Common names

The name *N. sativa* comes from the Latin word, nigellus, meaning black, it is also known around the world by many other names because of its ancient popular history and medicinal value. The Arabic name of *N.sativa* is ‘Al-Habbatus Sauda’ mean black seed or ‘Habbat al Baraka’ that mean the seed of pleasing, while in old Latin it was called „Panacea“ meaning „cure all“ (Hussain and Hussain, 2016) . The common English name for *N.sativa* is Love in a Mist. It is known as „Kalijeera“ (Bangladesh), „Kalonji“ (in India) and „Hak Jung Chou“ (China), carvi (French), schwarzkummel (German), kalonji (Hindi/Urdu), kezah (Hebrew) chernushka (Russian), corek-out (Turkish), siyahdaneh (Persian) (Abdallah, 2017).

2.4.4. History of the black seed

The traditional uses of *Nigella sativa* one of the earliest cultivated plants in human history. Originating from ancient Egypt, Greeks and the Romans (Al-Jaafary *et al.*, 2016). The black seeds have been prescribed by ancient Egyptian and Greek physicians to treat headache, nasal congestion, toothache and intestinal worm, as well as a diuretic to promote menstruation and milk production (Khan *et al.*, 2011). The seed and oil of *N. sativa* was commonly used in ancient remedies in Unan, Ayurveda, China, Middle-East, Arabic and Asian countries (Islam *et al.*, 2017). They have been reported to be used in folk traditional medicine as remedy for asthma, hypertension, diabetes and cough (Emeka *et al.*, 2015).

2.4.5. In Prophetic Medicine (Tibb-el-Nabwi)

The last Prophet of the religion Islam, the Holy Prophet Muhammad (peace be upon him) he said in hadith narrated by Bukhari “It is a remedy (cure) for every disease except death.” (Javed *et al.*, 2012). The Prophet never spoke a single lie in his entire lifetime of 63 years; that is why he earned the title „Al-Amin“ meaning truthful from everyone irrespective of their religious faiths and tribes. One wonders how an unlettered man of the desert without having any pen and paper, could make such a wonderful statement on medical science? About Muhammad (peace be upon him) God (Allah) says in the Holy Qur’an, “He does not speak anything of his own desire...” (Surah An-Najm) (Hussain and Hussein, 2016).

The use of *N. sativa* seeds had been mentioned by many Arabian scientist . Ibne-Sina (980–1037) in his famous book *Al-Qanoon fitt-Tibb* (Islam *et al.*, 2017) ‘The Canon of medicine’ regarded as one of the most famous book in the history of medicine, refers to black seed as the seed that stimulates the body’s energy and helps recovery from fatigue and dispiritedness and several therapeutic effects on digestive disorders, gynaecological diseases and respiratory system have been ascribed to it (Salman *et al.*, 2016).

2.4.6. Chemical composition of black seeds

Nigella sativa seeds contain fixed oil, proteins, alkaloid, saponin and essential oil. It is composed of unsaturated fatty acid that includes (arachidonic, eicosadienoic, linoleic and linolenic acid). The saturated fatty acid present in the oil are (palmitic, stearic and myristic acid). The pharmacologically active constituents of volatile oil are (thymoquinone, dithymoquinone, thymol and thymohydroquinone) (Khan *et al.*, 2016). Interestingly, these tiny seeds are rich in bioactive compounds of pharmacological benefits are nigellicine, nigellicimine, nigellicimine N-oxide, carvone, thymoquinone, thymol and many more (Parrakah, 2010)

2.4.7. Medicinal uses

Nigella sativa has long remarkable history in traditional medicine, it is well known since the ancient civilizations such as ancient Egyptian and Greek to promote menstruation and increase milk production beside its use to treat headache, toothache, nasal congestion and many more (Abdallah, 2017)

The seeds of *N.sativa* are used in food as flavor like in the breads and pickles because it has very low level of toxicity. *N. sativa* are also used in the treatment of various diseases like bronchitis, asthma rheumatism, and skin disorders. It acts as a liver tonic, anti-diarrheal, appetite stimulant, digestive disorders, and to strengthen immune system. Seeds are useful in the treatment of worms and skin eruptions. Oil is used as an antiseptic and local anesthetic externally. Roasted black seeds are given internally to stop the vomiting (Sultana *et al.*, 2015).

2.4.8. Pharmacological activities

The Black seed have been thoroughly studied scientifically in the last 3-4 decades and reported to possess a number of medicinal properties like immunomodulatory (Alshatwi, 2014) antioxidant activity, antimicrobial activity, and antitumor activity (Raja and Dewangan 2016, Hasan *et al.*, 2013).

The decoction is helpful in the ailments like skin disorders, jaundice, piles, and intermittent fever. The mixture of kalonji and butter milk provides a solution for

hiccups and vomiting. Different mixtures of Black seed are useful in the treatment of obesity. If Black seeds are regularly consumed after frying, the conditions like catarrh and cold can be prevented. It is also effective in preventing migraine and persistent headache (Nasir and Saba, 2015).

2.4.9. Antimicrobial activity

2.4.9.1 Antibacterial activity

Different crude extracts of *N. sativa* exhibited effective antimicrobial activity against different bacterial strains either Gram negative or Gram positive bacteria. The most effective extracts of *N. sativa* were the crude alkaloid and water extracts. Gram negative isolates were more susceptible than the Gram positive ones (Sultana *et al.*, 2015).

Thymoquinone, Thymol and other active compounds the Black seed contribute to have its medicinal properties (Thilakarathna *et al.*, 2018). Black seed's antimicrobial activity effect on Gram-negative and Gram-positive bacteria, viruses, parasites, Schistosoma, and fungi pathogens (Tavakkoli *et al.*, 2017).

The antimicrobial activities of thymoquinone were determined against different bacterial strains. Among sixteen oral strains, seven strains including *S. aureus* were found to be sensitive to thymoquinone (Nasir and Saba, 2015). Methanol and water extract of *Nigella sativa* reported to have effective antibacterial activity towards *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Proteus vulgaris*, the greater antibacterial effect was against the Gram-positive bacteria (Hasan *et al.*, 2013, Abdallah 2017).

2.4.9.2 Antifungal activity

Nigella sativa seeds exhibited high inhibitory effect against fungi. Thymoquinone one that isolated from *Nigella sativa* posses high antifungal activity against *Aspergillus niger*, *Fusarium solani* and *Scopnlariopsis brevicaulis*; and this activity was comparable to the antifungal drug amphotericin-B (Alakloby *et al.*, 2015).

N. sativa and its isolated compounds have activity against *Candida albicans* and *Madurella mycetomatis*. TQ possess antifungal effect against *Aspergillus niger*, *Fusarium solani* and *Scopulariopsis brevicaulis*. The activity of TQ was reported more effective than amphotericin-B and griseofulvin. The TQ also have activity against *Trichophyton spp.*, *Epidermophyton spp.*, and *Microsporum spp.* In addition TQ, thymohydroquinone and thymol are also demonstrated an antifungal effect against many clinical isolates, including dermatophytes, molds and yeasts (Alakloby *et al.*, 2015). Black seed oil (10-200µg/ mL), it is also effective against *Saccharomyces cerevisiae* and *C. utilis* (Nadaf *et al.*, 2015).

2.4.9.3. Antiviral activity

N. sativa was proven as a good inhibitor to the human immunodeficiency virus (HIV) protease and murine cytomegalovirus. Otherwise, *N. sativa* enhance helper-T-cell (T4) and suppressor-T-cell (T8) ratio and increased natural killer (NK) cell activity in human. In the latter case, it was found to increase in number and function of CD4+ve T cells with the production of interferon-gamma (INF-γ) was reported (Amin and Hosseinzadeh, 2016) .

2.4.9.4. Antiparasitic activity

N. sativa was shown to have anti-leishmaniasis, anti-miracidia, anti-cercariae and anti-*Schistosoma mansoni* potentials. In the latter case the oil of the black seed showed powerful activity as compared to a well-known anti-schistosomal and anthelmintic treatment (Alakloby *et al.*, 2015). Ethanol extract of *N. sativa* (0.5-8%) produced significant anti-*Ascaris suum* activity (Simalango and Utami, 2014).

2.5. *Staphylococcus aureus*

In 1880 *S. aureus* was discovered by the surgeon Sir Alexander Ogston, he named them staphylé, which means in Greek a bunch of grapes (Rao *et al.*, 2015). In 1884, Rosenbach named them *Staphylococcus aureus*, “aureus” means golden in Latin. *Staphylococcus aureus* belongs to the family *Micrococcaceae* and is part of the genus *Staphylococcus*, *S. aureus* is by far the most virulent and pathogenic

staphylococci for humans it is 1 μm in diameter, Gram-positive bacteria. Under microscope observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as catalase positive and coagulase, non-motile, non-spore-forming and as facultative anaerobic. It grows in yellow colonies on nutrient rich media and is referred to as the yellow staphylococci (Stark, 2013).

2.5.1. *Staphylococcus aureus* Infection

S.aureus is one of the most common pathogens causing nosocomial infections, due to its virulence and resistance to the usual antibiotics, this bacterium occupies great importance in human pathology (Ouidri, 2018). *S.aureus* is a leading cause of superficial skin lesions such as boils and furunculosis, acute infections such as pneumonia and urinary tract infections, and deep-seated infections such as osteomyelitis and endocarditis (Onyeagwara *et al.*, 2014). *S.aureus* is a predominant cause of bloodstream infections, with an associated mortality of up to 20% (Salas *et al.*, 2017).

S. aureus is also a leading cause of hospital-associated (HA) and community-associated (CA) bacterial infections in humans, associating with numerous mild skin and soft tissue infections, as well as life-threatening pneumonia, bacteraemia, osteomyelitis, endocarditis, sepsis and toxic shock syndrome (David and Daum, 2010).

Methicillin resistant *S. aureus* (MRSA), classified as one of the most nosocomial pathogen of major worldwide importance infections due to its ability to spread from patient to patient, also it's an increasingly frequent cause of community-acquired infections that cause considerable morbidity and mortality (Behzad *et al.*, 2015).

2.5.2. *Staphylococcus aureus* carriers

S. aureus may colonize the human body as a part of the normal flora. Around 30% of populations are inhabited by *S. aureus*, mostly in the anterior nares (Akmatov *et*

al., 2010). The anterior nares are one of the favored carrier sites of this bacterium, and the rate of skin portage depends on nasal carriage (Ouidri, 2018) .

The relationship between *S.aureus* nasal carriage and Staphylococcal disease was first reported by Danbolt, in 1931, who studied Furunculosis. MRSA nasal carriage has been identified as a major risk factor for surgical site infections, when MRSA untreated can cause mucopurulent crusting and discharge in patients, thereby affecting postoperative outcomes in hospitalized Otorhinolaryngology patients (Onyeagwara *et al.*, 2014).

2.6. Antimicrobial activity of *Nigella sativa* against *Staphylococcus aureus*

N.sativa have been reported to exhibit many pharmacological properties that include antimicrobial action against bacteria (Khan *et al.*, 2013). *N.sativa* oil as well as methanolic extract are active against multidrug resistant *S.aureus* strains (Salman *et al.*, 2016). Also petroleum ether extract of *N.sativa* have been reported to possess antimicrobial activity against *S.aureus* and some pathogenic clinical isolates (Ali, 2015). Aqueous extract of *N.sativa* were posses bacteriostatic activity against *S.aureus* and some clinical isolates; were observed as compared with control aqueous extract can slow down the multiplication of the selected clinical isolates after overnight incubation (Anjum *et al.*, 2015).

2.7. Previous studies

Petroleum ether extract of *N.sativa* possess antibacterial activity against some pathogenic bacteria that showed resistance to number of antibiotics. The petroleum ether extract was found to be more effective in Gram positive more than Gram negative bacteria (Ali, 2015).

Ethanollic seeds extract of *N.sativa* produce antimicrobial activity for two Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative bacteria (*Escherichia coli* , *Pseudomonas aeruginosa*) as well as two fungi

(*Aspergillus niger*, *Candida albicans*), this study observe that *N.sativa* has useful antimicrobial properties (Kabbashi *et al.*, 2015).

Both Petroleum ether and methanolic extracts of *N.sativa* have antimicrobial activity against *Rhodococcus rhodochrous*, *Mycobacteria phlei*, *Nocardia otidiscaviarum* and *Gordonia bronchialis*, organisms were inhibited at different concentrations petroleum ether extract is more effective than methanolic extract (Fadailallah *et al.*, 2012).

Green tea extracts (both water and methanol) shows antibacterial activity against *S.aureus* nasal carriage isolates from healthcare workers, methanolic extract of green tea is effective in inhibition bacterial growth more than aqueous extract (Abdurahim, 2015) .

N.sativa and its derived callus have a potential to produce active compounds with antimicrobial activities, *N.sativa* callus extracts show higher activity than seeds methanolic extracts against *Escherichia coli* (Mohammed *et al.*, 2015).

Antimicrobial studies on Black cumin show that cumin oil has some effect on radial growth of *Aspergillus niger*, but could not inhibit it completely. On the other hand, the black cumin oil show clear inhibition zone of both *Escherichia coli* and *Staphylococcus aureus*, but they more effective on *Escherichia coli* (Ayoub, 2015).

Antibacterial activity of *N.sativa* seeds extracts; N.hexane, chloroform, methanol, fixed oil, volatile and crude oil were tested against 10 bacterial species (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella* species, *Klebsiella* species, *Proteus species*, *Neisseria catarhalis*, *Streptococcus faecalis*, *Brucella* species). The antibacterial activity of volatile oil and hexane extracts were more pronounced than the rest of investigated extracts. The fixed oil extract of *N.sativa* showed no significant activity against the examined organisms, while methanol and chloroform extracts show inhibitory activity against five or more of tested organisms (Abdalmoneim *et al.*, 2002).

Three different forms of *N.sativa* seeds; aqueous extract, methanolic extract, and oil; have been investigated against different clinical isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Micrococcus luteus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *klebsiella pneumonia*). Aqueous extract observed to have bacteriostatic activity rather than bactericidal, the methanolic extract are effective against *Streptococcus pyogenes* and *Micrococcus luteus* while other strains are resistant, *N.sativa* oil are more effective than methanolic extract against the clinical isolates (Anjum *et al.*, 2015).

N.sativa cold water, hot water and methanolic extracts have high zone of inhibition against Gram negative bacteria (*Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*) comparing to Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus faecalis*). Methanolic extract show no activity against *S.aureus*. The result of agar well diffusion method is clear more than disc diffusion method (Khan *et al.*, 2013)

N.sativa oil as well as methanolic extract are active against multi drug resistant strains of *S.aureus*. Methanolic extract showed the highest antibacterial activity (Salman *et al.*, 2016)

Multidrug resistant *S.aureus* isolated from diabetic wounds were susceptible to different concentrations of *N.sativa* oil. The oil showed pronounced dose dependant antibacterial activity against the isolates (Emeka *et al.*, 2015).

Using maceration *N.sativa* seeds were extracted by four different solvents (water, methanol, ethanol, and cyclohexan). Antibacterial and antifungal effects of extracts and essential oil are investigated against (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella abony*, *Candida albicans*), all extracts and essential oil showed varying degree of inhibition (Benlafya *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

This was descriptive cross sectional study.

3.2. Study area and duration

This study was conducted in Sudan University of Science and Technology (SUST), in Khartoum State, Sudan between ‘October 2017 to December 2017’.

3.3. Study population

100 students studying at Sudan University of Science and Technology (SUST) were recruited in this study.

3.4. Inclusion criteria

Healthy male and female students studied in Sudan University of Science and Technology (SUST).

3.5. Exclusion criteria

Students on antimicrobial therapy.

3. 6. Ethical considerations

Ethical clearance for this study was obtained from the research committee of the College of Medical Laboratories Science/ Sudan University and Science and Technology. The aim of the study was explained to the participants and a verbal consent was obtained from the students before collection of specimens.

3.7. Sampling

Non –probability sampling.

3.8. Sample size

A total of 100 samples (n= 100) were randomly collected .After explaining the study and its goal. A verbal consent was taken from the study recruits before proceeding with the study and collecting the samples.

3.9. Study variables

Screen on *S.aureus* nasal carriage (dependent variable). Age and gender taken by members as (independent variables).

3.10. Data collection

The data were collected by using a direct interviewing questionnaire.

3.11. Specimen processing

3.11.1. Collection of the specimens

Nasal swabs were taken from healthy students using sterile cotton wool swabs moistened with sterile normal saline. These swabs rotated inside each anterior nares of each member. Each specimen was labeled with the same serial number given to the person whom undergoing swabbing. Then specimens were immediately transported to the laboratory and processed within two hours.

3.11.2. Cultivation of the specimens

Swabs were cultured on manitol salt agar using sterile wire loop, inoculated plates were incubated aerobically at 37°C for 18-24 hours.

3.12. Identification of the isolates

3.12.1. Cultural characteristics

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

3.12.2. Gram stain

From cultured growth pure single colony was selected to prepare smear on slide using sterile loop, air dried and fixed by flame. The smear was covered with crystal violet for 30-60 seconds. Then washed by clean tap water and covered with Lugol's iodine for 30-60 seconds, washed by tap water and decolorized by alcohol for 20-30 seconds. Finally the smear was covered by saffranine for 2 minutes, then washed by clean tap water, dried by blotting on a filter paper and examined by using oil immersion lens (Cheesbrough, 2006) .

3.12.3. Biochemical tests

3.12.3.1. Catalase Test

Organisms were tested for catalase production by bringing it into contact with hydrogen peroxide use wooden stick. Active air bubbles of oxygen are released indicate; positive catalase test, no air bubbles; negative (Cheesbrough, 2006).

3.12.3.2 Coagulase Test

On clean slide a drop of distilled water was placed and emulsified a colony of tested organism. Then a loopful of plasma was added to the suspension and mixed gently for 10 seconds. Clumping within 10 seconds indicate; positive, no clumping in more than 10 seconds; negative coagulase test (Cheesbrough, 2006).

3.12.3.3 Deoxyribonuclease test (DNase)

The tested organisms were cultured on media which contains DNA (HiMedia laboratories Pvt, Ltd, India). After overnight incubation at 37°C, the colonies were tested for DNase production by flooding the plate with a weak hydrochloric acid solution to precipitate the unhydrolyzed DNA in the media and waited for minutes until clear zone appear around colonies which considered as positive result, no clear zone; negative (Cheesbrough, 2006).

3.12.3.4. Manitol Salt Agar (MSA)

The tested organisms were streaking on MSA media (Hi-Media laboratories Pvt, Ltd, India) after overnight incubation at 37°C observed the change of the color to yellow (mannitol fermenter colonies); MSA test positive, red colonies (non manitol fermenter colonies); MSA test negative (Cheesbrough, 2006)

3.12.4. Antibiotics Sensitivity Testing (Modified Kirby–Bauer Method)

Under aseptic condition the suspension from all growth culture media were prepared by using normal saline. 2-3 colonies were emulsified from each isolate in separate tube and compared with turbidity standard (McFarland standard 0.5=10 cfu/ml) in a good light for adjustment. Then using sterile swab immersed in suspension in the surface of the tube to remove the excess. Muller Hinton (Hi-

Media laboratories Pvt, Ltd, India) surface was inoculated by swabbing. After that application of antimicrobial disc by using sterile forceps to the medium, the distances were at least 24 mm between two disc on the inoculated plate and 15mm from the edges of the plate (Cheesbrough, 2006)

Antibiotic discs (Hi Media laboratories PV + Ltd, India) were applied on inoculated plates: amoxicillin, vancomycin, clindamycin, vancomycin and fuscidin. Plates were left at room temperature and then incubated at 37° C for 18-24 hrs .Zone of inhibition were measured in mm and results interpreted according to standardized chart.

3.13. Storage

Isolated organisms were kept in nutrient agar slope at 4°C for subsequent susceptibility tests. Nutrient glycerol broth used for long stage preservation of isolates at -20°C refrigerator.

3.14. Preparation of the extracts

3.14.1. Collection and Preparation of *Nigella sativa*

Specimen of *N.sativa* was obtained from Khartoum central market from "alteman atara". The dried *N.sativa* sample was cleaned from dust and grass.

3.14.2. Extraction of *Nigella sativa*

Extraction was carried out according to maceration method (Azwanida, 2015).

3.14.2.1. Methanolic extract

Hundred grams of the plant seeds were coarsely powdered using mortar and pestle. Then successively extracted with methanol by soaking plant materials (powdered) in a stoppered container with a solvent and allowed to stand at room temperature for a period of 3 days with frequent agitation, after that the stopper of container is opened to allow the solvent to evaporate.

3.14.2.2. Aqueous extract

Hundred grams of *N.sativa* seeds were coarsely powdered using mortar and pestle. Then soaked in 500 ml distilled water in a stoppered container and allowed to

stand at room temperature for a period of 3 days with frequent agitation. Extract was then filtered and freeze-dried in a deep freezer at -20°C . Freeze-dried extract was dried using a freeze-dryer -40°C till powder extract was obtained.

3.14.3. Preparation of bacterial suspension

New subcultures of the selected bacterial strains of *S.aureus*, including bacterial isolates, and standard bacteria ATCC25923 which were brought from the microbiology department of National Institute for Research, were inoculated into 3.0 ml of sterile normal saline. Inoculum density was compared with McFarland standard solution.

3.14.4. Agar well diffusion method

Sterile cotton swab was dipped into the bacterial test suspension matched with 0.5 McFarland standard to inoculate entire surface of Mueller Hinton agar plate.

Wells or cups of 8 mm were made with a sterile cork borer in the inoculated agar plates. 100 μl volumes of methanolic extract from different concentrations were poured directly into the wells.

The plates were allowed to stand for 1 hour in refrigerator for diffusion of the extract to take place and incubated at 37°C for 24 hours. After incubation inhibition zone diameters were measured in millimeter (Mohammed, 2015).

3.14.5 Determination of Minimum Inhibitory Concentration (MIC)

Determination of inhibition zones and MIC of *N.sativa* extracts were assessed using Agar diffusion dilution method as described by (Mohammed, 2015)

Two grams from each extract was dissolved in 10 ml 100% methanol for alcohol extract and distilled water for water extract. Then serially diluted two fold to obtain final concentration (50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml), 60 microliters of each prepared concentration were added in to the corresponding well. The plates were left for 1 hour in refrigerator (4°C), and then incubated at 37°C for 24 hours. Inhibition zone around each well were measured using a ruler in millimeter. MIC is the lowest concentration of plant extract that did not permit any visible growth of the inoculated test organism.

3.14.6. Interpretation of results

After 24 hours incubation antibacterial activity results were expressed in diameters of inhibition zones in millimeter where < 9 mm zone was considered as inactive; 9-12 mm as partially active while 13-18 mm as active and > 18 mm as very active (Mhkhtar and Ghori, 2012).

3.15. Control of susceptibility testing method

3.15.1. Reference strain quality control

The quality control *S. aureus* strain ATCC 25923, those reference strains were recommended for controlling the susceptibility test as described in NCCLS document M7-A7. The stock culture was stored at -20°C in 10% glycerol broth and sub cultured on to agar plate to obtain freshly isolated colonies. Control strain was suspended for testing according to the recommended inoculums preparation procedures.

3.16. Batch quality control

Each new batch plates were tested with the reference strain to determine if zone diameter obtained with the batch fall within the expected range or not. Also one uninoculated agar plate from each batch was incubated overnight to ensure the medium's sterility.

3.17. Data analysis

SPSS version 21 was used for data analysis.

CHAPTER FOUR

RESULTS

4.1. The frequency and percentage of bacterial growth

Hundred samples investigated 38/100(38%) showed bacterial growth while 62/100(62%) showed no bacterial growth presented in Table .1

Table. 1: The frequency and percentage of bacterial growth

Result of culture	Frequency	Percentage (%)
Growth	38	38%
No growth	62	62%
Total	100	100%

4.2: Percentage of *Staphylococcus aureus* growth

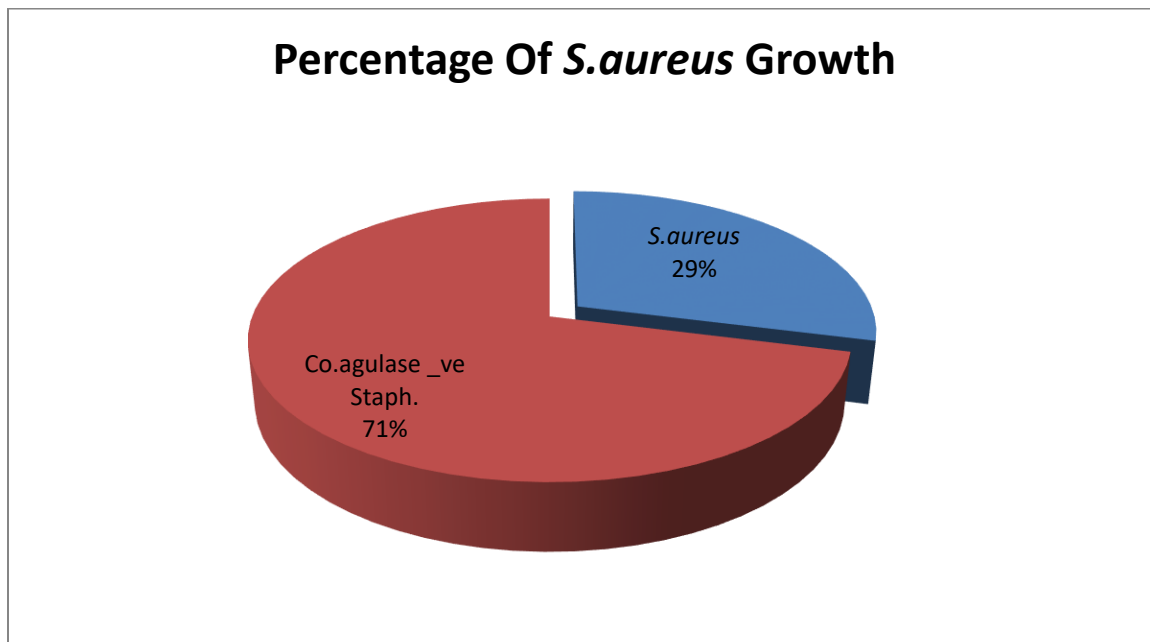


Figure 1: Percentage of *Staphylococcus aureus* growth

4.3. Percentage of *Staphylococcus aureus* growth in both male and female

Percentage of *S.aureus* growth isolated from nasal carriers in both males and females presented in table .3

Table .2: Percentage of *Staphylococcus aureus* growth in both male and female

Gender	Frequency	Percentage
Males	7	64%
Females	4	36%
Total	11	100%

4.4. Antibacterial Susceptibility Test

The antibacterial susceptibility test of isolates and standard organism were determined using standard disk diffusion method, the result presented in table .4

Table .3: Sensitivity of *Staphylococcus aureus* isolates to selected antibiotics

Antibiotic	Sensitive	Resistance
Oxaxillin	10 (91%)	1(9%)
Amoxicillin	1(9%)	10(91%)
Clindamycin	100(100%)	0(0%)
Vancomycin	100(100%)	0(0%)
Fuscidin	100(100%)	0(0%)

4.5. Antibacterial activity of *Nigella sativa*

Methanolic and aqueous extracts with different concentrations against *S.aureus* isolated and standard showed in table 5, 6

Table 4: Mean inhibition zones of *Nigella sativa* methanolic extract in (mm)

Extract concentration in mg/dl	Inhibition zones in mm	
	<i>S.aureus</i> ATCC 25923	<i>S.aureus</i> isolates
100%	20 mm	19.64 ±2.3 mm
50%	17 mm	16.63±2.4 mm
25%	15 mm	13.72±2.5 mm
12.5%	12 mm	11± 2.7 mm
6.25	10 mm	9.27± 1.6 mm

Table 5: Mean inhibition zones of *Nigella sativa* aqueous extract in (mm)

Extract concentration in mg/dl	Inhibition zones in mm	
	<i>S.aureus</i> ATCC 25923	<i>S.aureus</i> isolates
100%	16 mm	14.36 ±4 mm
50%	14 mm	12.27±3.6 mm
25%	12 mm	10.36±2.7 mm
12.5%	8 mm	8.7± 1.6 mm
6.25	8 mm	8.18± 0.6 mm

Diameter of inhibition zone include diameter of well (8 mm).

Values are represented as mean ± SD

Zone < 9 mm zone was considered as inactive; 9-12 mm as partially active while 13-18 mm as active, and > 18 mm as very active

P. value= .04

4.6 Comparison between antibacterial activity of aqueous extract and methanolic extract

The antibacterial action of seeds extracts were investigated by the agar diffusion method, methanolic extract showed highest antibacterial activity while aqueous extract gave the least activity

Table .6: Comparison between antibacterial activity of aqueous extract and methanolic extract

Extract Concentration	Inhibition zones in mm		P. value
	Aqueous extract	Methanolic extract	
100%	14.36 mm	19.6 mm	.01
50%	12.27 mm	1.63 mm	
25%	10.36 mm	13.70 mm	
12.5%	8.70 mm	11.00 mm	
6.25%	8.18 mm	9.27 mm	

Values are represented as mean

4.8. Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of *Nigella sativa* methanol and water extracts obtained by agar diffusion method for *S.aureus* isolates was 12.5 mg/ml and 25 mg/ml respectively.

Table .7: Minimum inhibitory concentration (MIC) of *Nigella sativa*

Bacterial species		ATCC 25923	<i>S. aureus</i> isolates
Aqueous extract	Zone of inhibition	12 mm	12 mm
	Ex. Conc. %	25 %	12.5 %
	Ex. Conc. in mg/ml	50 mg/ml	25 mg/ml
Methanolic extract	Zone of inhibition	12 mm	12 mm
	Ex. Conc. %	12.5 %	6.25 %
	Ex. Conc. in mg/ml	25 mg/ml	12.5 mg/ml

Ex.: Extract

Conc.: Concentration

CHAPTER FIVE

5. DISCUSSION

The present study obtained *Nigella sativa* seed extracts using maceration extraction by various solvents (water, and methanol); to demonstrate the in vitro antibacterial activity of *N.sativa* against *S.aureus* isolated from nasal carriers and standard *S.aureus* ATCC25923.

The antibacterial action of *N.sativa* seeds aqueous and methanolic extracts were investigated by the agar diffusion method against *S.aureus* isolated from nasal carriers. All types of bacteria isolates showed different sensitivity for the *N. sativa* extracts. Both extracts showed varying degree of inhibition, methanolic extract of *N.sativa* showed remarkable antibacterial activity against standard strain and isolated bacteria. The maximum inhibition zone in high concentration was observed is (25 mm) with MIC 12.5 mg/ml. This results agreed with study of (Anjum *et al.*, 2015) showed that methanolic extract have activity against *S.aureus* with maximum zone of inhibition (22.3 mm). Variation in MIC and zone of inhibition may be due to the variation in the nature and combination of phytochemicals present in extract due to environment or type of soil. The results disagree with (Kakil, 2013) and (Ali, 2015) who found that methanolic extract showed no antibacterial activity. This variation may be due to the difference in method of extraction that used.

Study revealed that aqueous extract of *N.sativa* performance in inhibition of bacterial growth, the maximum inhibition zone at high concentration was observed (20 mm) and MIC 25 mg/ml. This result agreed with (Khan *et al.*, 2013), who found that aqueous extract of *N.sativa* has activity against *S.aureus* with maximum zone of inhibition (21 mm). Also agreed with study of (Anjum *et al.*, 2015) with maximum zone of inhibition (15 mm). The study disagreed with (Ramli *et al.*,

2014). They report that aqueous extract have no activity against *S.aureus*. The variation in the results may be due to the variation in the method of antibacterial activity, extraction method and the difference of environment and soil.

Methanolic extract showed highest antibacterial activity and lowest MIC whereas aqueous extract gave the least activity this is may be due to that *N.sativa* active ingredients have propensity to dissolve in methanol more than water. This result agreed with study of (Mohammed, 2015). He found that alcoholic extract showed lowest MIC compared to water extract.

The means diameter of growth inhibition zone of standard and clinical isolates of bacteria increased with the increased in extract concentration. This result is in agreement with report of (Suleiman, 2013) .

More research work is required to validate these results and to determine the role of the other remaining compounds of *N.sativa* in antimicrobial activity using advanced techniques.

Conclusion

- ✓ 9% (1/11) of *S. aureus* isolates were resist to oxacillin, while 91 %(10/11) were resistant to amoxicillin. All strains were sensitive to Clindamycin, Vancomycin, and Fuscidin.
- ✓ Methanolic and aqueous extracts of *N.sativa* inhibits the growth of *S.aureus* dose dependently confirming the antibacterial activity of *N.sativa*
- ✓ Methanolic extract of *N.sativa* is more effective in inhibiting bacterial growth than water extract.

Recommendations

1. Extracts of *Nigella sativa* using different solvents such as petroleum ether & chloroform is necessary since other solvents extract other active ingredients that were not extracted with methanol and water.
2. Pharmacological, toxicological studies should be carried out to assess their safety, therapeutic efficiency and potential for commercial utilizations
3. Further research to advocate its activity in systemic infection.
4. Isolate and purify the active ingredients in the extract responsible for antibacterial activity.

References

- Abdallah, E. M.** (2017). Black seed (*Nigella sativa*) as antimicrobial drug: A Mini-Review. *Novel Approaches In Drug Designing Development* , **3** (2), 1-4.
- Abdallah, E. M.** (2011). Plants: An alternative source for antimicrobials. *Journal of Applied Pharmaceutical Science* , **1** (6), 16-20.
- Abdalmoneim, M.**, Elniema, I. E., & Gumaa, S. A. (2002). Antibacterial activity of *Nigella sativa* linn. *Journal of Science and Technology* , **3** (2), 78-86.
- Abdurahim, M. M.** (2015). Antibacterial activity of Green tea extract against *Staphylococcus* nasal carriage among health care workers in Omdurman teaching hospital. Khartoum-Sudan. M.Sc. Thesis. Sudan University for Science and Technology.
- Ahmad, M.**, & Wajid, M. (2013). Plants as potential source of antimicrobial agents. *Journal of Pharmacy and Alternative Medicine* , **2** (3), 18-25.
- Akmatov, M. K.**, Mehraj, J., Gatzemeier, A., Stroömpl, J., Witte, W., Krause, G., Pessler, F. (2010). Serial home-based self-collection of anterior nasal swabs to detect *Staphylococcus aureus* carriage in arandomized population-based study in Germany. *International Journal of Infectious Disease* , **25**, 4-10.
- Ali, M. E.** (2015). Antibacterial activity of *Nigella sativa* extract against selected bacterial clinical isolates from khartoum state. Sundan-khartoum. M.Sc. Thesis. Sudan university of science and technology.
- Alakloby, O.M.**, Aljabre, S.H., and Randhawa, M.A. (2015). Dermatologic effects of *Nigella sativa*. *Journal of Dermatology & Dermatologic*. **10**(2). 92-98.
- Al-Jaafary, M.**, Al-Atiyah, F., Al-Khamis Ebtesam, E., Al-Sultan, A., & Badger, E. L. (2016). In-vitro studies on the effect of *Nigella sativa* Linn.,seed oil extract on Multidrug resistant Gram positive and Gram negative bacteria. *Journal of Medicinal Plants Studies* , **4** (2), 195-199.

- Al-Salihy, S. R.,** & Rashid, I. M. (2017). Use and miss use of antimicrobial agents in healthcare settings of Diyala Province. *Diyala Journal Of medecine* , **12** (2), 30-33.
- Alshatwi, A.A.** (2014). Bioactivity-guided identification to delineate the imunomodulatory effects of methanolic extract of *Nigella sativa* seed on human peripheral blood mononuclear cells *China Journal Of Integrative Medecine*. <https://doi.org/10.1007/s11655-013-1534-3>.
- Amin, B.,** Hosseinzadeh, H. (2016). Black cumin (*Nigella sativa*) and its active constituent, Thymoquinone: An overview on the analgesic and anti-inflammatory effects. *Planta Medica* , **82** (1-2), 8-16.
- Anjum, K.,** Abbas, T., Kazim, M. R., Subhan, S. A., Abdul Wahab, Mehmood, N., Mujahid, T., Hasan, N. (2015). Antimicrobial activity of *Nigella sativa* aqueous extract, methanolic extract and oil of clinical isolates. *International Journal of Current Research* , **7** (2), 12294-12299.
- Ayoub, A.O.** (2015). Biological activities and some physiochemical characteristics of the Black cumin (*Nigella sativa*) seed oil. M.Sc.Tthesis .Al-Gezira, Sudan: University of Gezira- faculty of engineering and technology.
- Azwanida, N.N.** (2015). Areview on the extraction methods use in medicinal plants, principle, strength and limitation . *Medicinal and aromatic plants*. **4**(3)
- Behzad, M.N.,** Akhi, M.T., Alizadeh, M., Saleh, P., Jafarzadeh, S., Navi, Z.S., Bagheri, M.M., Barband, S., Sadeghi, G., Asghari, B. (2015). *Staphylococcus aureus*: resistance pattern and risk factors. *Journal of Analytical Research in Clinical Medicine* , **3** (1), 43-50.
- Benlafya, K.,** Karrouchi, K., Charkaoui, Y., El Karbane, M., & Ramli, Y. (2014). Antimicrobial activity of aqueous, ethanolic, methanolic, cyclohexanic extracts and essential oil of *Nigella sativa* seeds. *Journal of Chemical and Pharmaceutical Research* **6** (8), 9-11.

Cheesbrough, M. (2006). District Laboratory practice in Tropical Countries. Second edition. Part 2. P. 64, 65, 67, 70, 137, 138, 157,395, 396.

Dar, R. A., Shahnawaz, M., & Qazi, P. H. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology* , **6** (6), 349-351.

Datta, A. K., Saha, A., Bhattacharya, A., Mandal, A., Paul, R., & Sengupta, S. (2012). Black Cumin (*Nigella sativa L.*)_ Areview. *Journal Of Plant Development Science* , **4** (1), 1-43.

David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiolgy Reviews* , **23**, 616–687.

Dhiman, R., Aggarwal, N., Aneja, K.R., & Kaur,M. (2015). In vitro antimicrobial activity of spices and medicinal herbs against selected microbes associated with juices. *International Journal of Microbiology*, **2016**, 1-9.

Emeka, L. B., Emeka, P. M., & Khan, T. M. (2015). Antimicrobial activity of *Nigella sativa L.* seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds. *Paistan of Pharmceutical Science.* , **28** (6), 1985-1990.

Engels, G., & Brinckmann, J. (2017). *Nigella - Nigella sativa*. *Journal Of The American Botonical council* , **8** (114), 8-16.

Fadailallah, A. F., Mahgoub, A., & Hamid, M. E. (2012). In vitro sensetivity of some mycolic acid-containing Actinomycetes to *Nigella stiva* extracts. *American Journal of Medicine and Medical Science* , **2** (6), 111-113.

Hasan, N. A., Nawahwi, M. Z., & Ab Malek, H. I. (2013). Antimicrobial Activity of *Nigella sativa* Seed Extract. *Sains Malaysiana* , **42** (2), 143–147.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine:A Review of *Thymus vulgaris*. *International Journal of Clinical Medicine* , **6**, 635-642.

Hussain, D. A., & Hussain, M. M. (2016). *Nigella sativa* (black seed) is an effective herbal remedy for every disease except death – a Prophetic statement which modern scientists confirm unanimously: A review. *Advancement in Medicinal Plant Research* , **4** (2), 27-57.

Ijaiya, I. S., Arzika, S., & Abdulkadir, M. (2014). Extraction and phytochemical screening of the root and leave of *Annona senegalesis*. *Academic Journal of Interdisciplinary Studies* , **3** (7), 9-15.

Islam, M. T., Riaz, T. A., Shahadat, S., Sousa, L. d., Shahadat, S., Sousa, L. d., Leonardo .R., Santos, J. V., Junior, J. J., de Lima, R. M., Baraga, A. L., dos Reis, A.C., Barros, A., Marcus V.O., Melo-Cavalcante, A. (2017). Nigellalogy: A review on *Nigella sativa*. *MOJ Bioequivalence & Bioavailability* , **3** (6), 1-15.

Javed, S., Shahid, A.A., Haider, M.S., Umeera, A., Ahmad, R., & Mushtaq, S. (2012). Nutritional, phytochemical potential and pharmacological evaluation of *Nigella sativa* (Kalonji) and *Trachyspermum Ammi* (Ajwain). *Journal of Medicinal Plants Research* , **6** (5), 768-775.

Kabbashi, S.A., Garbi, M., E. Osman, E., M.Dahab, M., S.Koko, W., & Abuzaid, N. (2015). In vitro antimicrobial activity of ethanolic seed extract of *Nigella sativa* (Linn) in Sudan. *African Journal of Microbiological Research* , **9** (11), 788-792.

Kakil, S. (2013). Antibacterial activity of *Zingiber officinal*, *Matricaria chamomilla* and *Nigella sativa* extracts on growth of pathogenic bacteria isolated from different clinical specimens. khartoum, Sudan. M.Sc. Thesis. Sudan University of Science and Technology.

Karateek, P., Jahnavi, V., Kerthii, D., & Chaitanya, K. (2012). Evaluation of antibacterial activity of herbs. *International Research Journal of Pharmacy* , **3** (8), 230-232.

Khan, A. M., Qureshi, R.A., Gillani, S.A., & Ullah, F. (2011). Antimicrobial activity of selected medicinal plants of Margalla hills. *Journal of Medicinal Plants Research* , **5** (18), 4665-4670.

- Khan, A. U.**, Ali, S., Ur Rehman, A., Ali, H., Ahmed, T., Wagar, M., Niaz, Z. (2013). Antibacterial activity of *Nigella sativa* and *Piper nigrum*. *Asian Journal for Natural and Applied Science* , **2** (4), 173-179.
- Khan, Z.**, Hasan, N., Ahmad, N., & Khalid, M. (2016). Pharmacological Activity of *Nigella sativa*: A review. *World Journal of Pharmaceutical Sciences* , **4** (5), 234-241.
- Mhkhtar, S.**, Ghori I. (2012). Antibacterial activity of aqueous and ethanolic extracts of Garlic and Cinnamon and Turmeric against *Escherichia coli* ATCC25922 and *Bacillus subtilis*. *International Journal of Applied Biology and Pharmaceutical Technology*, **3** (2), 131-136.
- Maroof, P.**, Nasir, R., Bali, N., Farhan, A., Amin, M., & Kanth, f. (2016). *Staphylococcus aureus* nasal-carriage in health care workers and in patients with special refrence to MRSA. *British Microbiology Research Journal* , **13** (4).
- Masoumian, M.**, & Zand, M. (2017). Antimicrobial activity of some medicinal plant extracts against multidrug resistant bacteria. *Journal of Research in Medical Sciences* , **19** (11), 1-8.
- Mohammed, H. A.** (2015). Antibacterial activity of *Lawsonia inermis* (Sudaneese Henna) leaves extracts against *Staphylococcus aureus*, *Esherichia coli* and *Pseudomonas aeruginosa* among recurrent urinary tract infection patients in Omdurman millitary hospital. Khartoum, Sudan . M.Sc. Thesis. Sudan University of Science and Technology.
- Mohammed, M. E.**, Mahmoud, F. Z., & Yagoub, S. O. (2015). In vitro callus induction and antimicrobial activities of callus and seeds extracts of *Nigella sativa*. *Research Journal of Biology* , **3** (3), 21-28.
- Nadaf, N.H.**, Gawade, S.S., Muniv A.S., Waghmare S.R., & Jadhav. (2015). Exploring anti-yeast activity of *Nigella sativa* seed extracts. *Industrial Crops Production*, **77**, 624-630.

- Namita, P., & Mukesh, R.** (2012). Medicinal plants used as antimicrobial agents : A review. *international research journal of pharmacy* , **3** (1), 31-40.
- Narayan, G., Kartik, V., Manoj, P., Singh, P., & Alka, G.** (2010). Antibacterial activities of ethanolic extracts of plants used in folk medicine. *International Journal of Research in Ayurveda & Pharmacy* , **1** (2), 529-535.
- Nasir, U., & Saba, I.** (2015). Evaluation of the antimicrobial activity of aqueous, ethanolic and methanolic extracts of *Nigella sativa* (kalonji) against Gram positive and Gram negative bacterial isolates. *Paistank Journal of Biochemistry and Molecular Biology.* , **48** (4), 92-96.
- Njla, M. I., Mahdi, E., Lembe, D. M., Zacharie, N., & Nyonseu, D.** (2017). Review on extraction and isolation of plant secondary metabolites. *7th International Conference on Agricultural, Chemical, Biological and Environmental Sciences* . Kuala Lumpur (Malaysia). (pp. 67-72).
- Onyeagwara, N., Obasuy, O. i., Ottih, I., & O. Akerele, J.** (2014). Nasal carriage of methicillin-resistant *Staphylococcus aureus* among hospitalized otorhinolaryngological patients in Benin City of Nigeria. *Archives of International Surgery* , **4** (2), 99-103.
- Ouidri, M. A.** (2018). Screening of nasal carriage of methicillin-resistant *Staphylococcus aureus* during admission of patients to Frantz Fanon Hospital. *New Microbes and New Infections* , **23**, 52–60.
- Pandey, A., & Tripathi, S.** (2012). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry* , **2** (5), 115-119.
- Parrakah, P. M.** (2010). *Nigella sativa* - A comprehensive review. *Indian journal for natural Products and resources* , **1** (4), 409-429.
- Raja, W., & Dewangan, T.** (2016). Determination of antibacterial activity of *Nigella sativa* seed extract against some human pathogenic bacteria. *American Journal of Biological and Pharmaceutical Research* , **3** (2), 65-69.

- Ramli, Y.**, Karrouchi, K., Benlafya, K., Charkoui, K., & Alkarbane, M. (2014). Antibacterial activity of aqueous, methanolic, ethanolic, cyclohexanic extract and essential oil of *Nigella sativa* extract. *Journal of chemical and pharmaceutical research*, **6** (8), 9-11.
- Rao, Q.**, Weilong, S., Xiaomei, H., and Xiancai, R. (2015). "*Staphylococcus aureus* ST121: a globally disseminated hypervirulent clone. *Journal of Medical Microbiology*, **64**, 1462–1473.
- Salas, D. E.**, Minejima, E., Joanna.W., Fang, C., Wang, J., Rosemary,S., Nieberg, P., Wong-Beringer, A. (2017). *Staphylococcus aureus* bacteremia in patients not meeting sepsis criteria: clinical features, host immune response and outcomes. *Journal of Clinical Medicine and Therapeutics*, **2**(4), 1-7
- Salman, M. T.**, Khan, R. A., & Shukla, I. (2016). Antibacterial activity of *Nigella sativa* Linn. seeds against multiple antibiotics resistant clinical strains of *Staphylococcus aureus*. *International Archives of BioMedical and Clinical Research*, **2** (3), 96-99.
- Simalango, D.M.**, Utami, N.V. (2014). In-vitro antihelminthic effect of ethanol extract of Black seeds (*Nigella sativa*) against *Ascaris suum*. *Procedia Chemistry*, **13**, 181-185.
- Stark, L.** (2013). *Staphylococcus aureus* - aspects of pathogenesis and molecular epidemiology P.78. in [http:// urn.kb.se/resolve?urn=urn :nbn:liu:diva-97343](http://urn.kb.se/resolve?urn=urn:nbn:liu:diva-97343)
- Suleiman, S.** (2013). Antibacterial activity of Guava (*Psidium guajava*) leaves and Safflower (*Carthamus tinctorius*) seeds extracts against wound infection bacteria. khartoum, Sudan. M.Sc Thesis. Sudan University of Science and Technology.
- Sultana, S.**, Asif, H.M., Akhtar, N., Iqbal, A., Nazar, H., & Rehman, R. U. (2015). *Nigella sativa*: Monograph. *Journal of Pharmacognosy and Phytochemistry*, **4** (4), 103-106.

Tavakkoli, A., Mahdian, V., Razavi, B. M., & Hosseinzadeh, H. (2017). Review on clinical trials of Black seed (*Nigella sativa*) and its active constituent, Thymoquinone. *Journal of Pharmacopuncture* , **20** (3), 179-193.

Thilakarathna, R., Mudhusankha, G., & Navaratne, S. (2018). Phytochemical analysis of Indian and Ethiopian Black cumin seeds (*Nigella Sativa*). *Agricultural Research And Technology* , **17** (1).

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A Review. *Internationale Pharmaceuticasciencia* , **1** (1), 98-106.

Appendixes

Appendix 1

Materials

A-Equipment

1. Autoclave.
2. Bunsen burner.
3. Cork borer.
4. Deep freezer.
5. Freezer dryer.
6. Hot air oven.
7. Incubator.
8. Light microscope with oil immersion lens.
9. Rack.
10. Refrigerator.
11. Water bath.
12. Sensitive balance

B-Glass wares and consumables

1. Flask with different size.
2. Measuring cylinder.
3. Beakers.
4. Funnels.
5. Spoons.
6. Test tubes.
7. Slides.
8. Filter papers.
9. Wooden sticks
10. Wire loops

11. Sterile cotton swab

D-Culture media

Different culture media were used for inoculation, isolation, and identification of organisms. These include:

1-Manitol salt agar

Contents

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

Preparation

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

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

Store the plates at 2–8 °C preferably in plastic bags to prevent loss of moisture .pH of medium: 7.3–7.7 at room temperature.

2-DNA agar

Typical formula g/L

Contents

Tryptose.....	20
Deoxyribonucleic acid.....	2
Sodium chloride.....	5
Agar.....	12

pH 7.2±0.2

Preparation

Suspend 3.9 g in 1L of D.W. bring to boil to dissolve completely. Sterilize by autoclave at 121°C for 15 minutes. Cool to 50°C and pour into the sterile petridishes. Dry the surface of the medium before inoculation.

3-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 25 °c) 7.3±0.1

Preparation

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

E-Chemicals and reagents

- 1- Sodium chloride (normal saline).
- 2- Methanol.
- 3-McFarland turbidity standard

McFarland turbidity standard

Contents

Concentrated sulphuric acid.....	1 ml
Dihydrate barium chloride.....	0.5 g
Distilled water.....	150 ml

Preparation

- 1- Prepare 1% (v/v) solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water and mix well.
- 2- Prepare 1.175 % (w/v) solution of barium chloride by dissolving 2.35 g of dihydrate barium chloride ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in 200 ml of distilled water.
- 3- Add .5 ml of barium chloride solution to 99.5 ml of sulphuric acid solution and mix.

Appendix 2

Result of *Nigella sativa* extraction

One hundred gram of *Nigella sativa* seeds was extracted using maceration method by methanol and water solvents, the methanolic extract yield was 3.13/100 g, while water extract yield 1.07/100 g.

Table .9: Weight and yield percentage of *Nigella sativa* extracts obtained using methanol and water solvents

Type of extract	Methanolic extract	Aqueous extract
Weight of sample	100 g	100 g
Yield	3.13 g	1.07 g
Percentage	3.13%	1.07%

Appendix 3

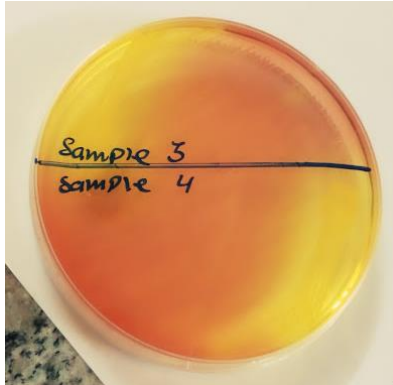


Figure 2: Growth of *S.aureus* on manitol salt agar show yellow ferment colonies



Figure 3: DNase test

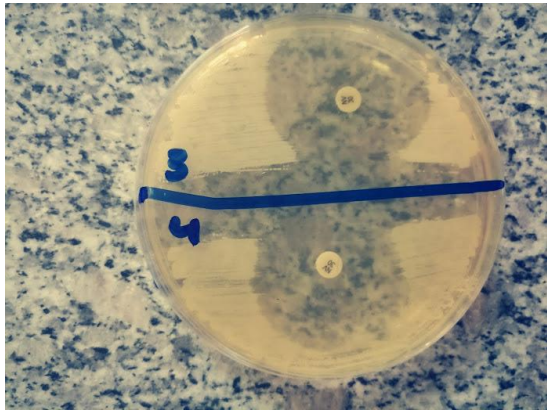


Figure 4: Novobiocin sensitivity test



Figure 5: Oxacillin sensitivity test

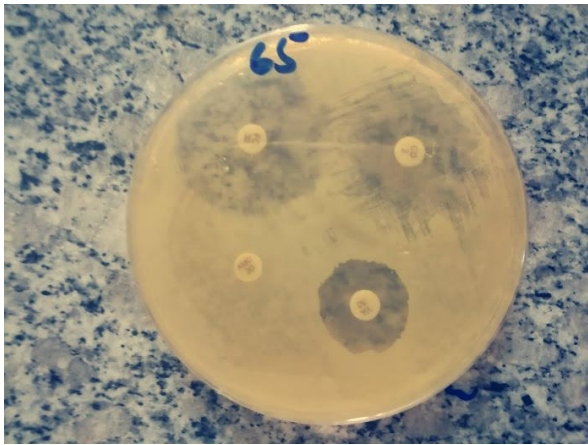


Figure 6: Antibiotic sensitivity test

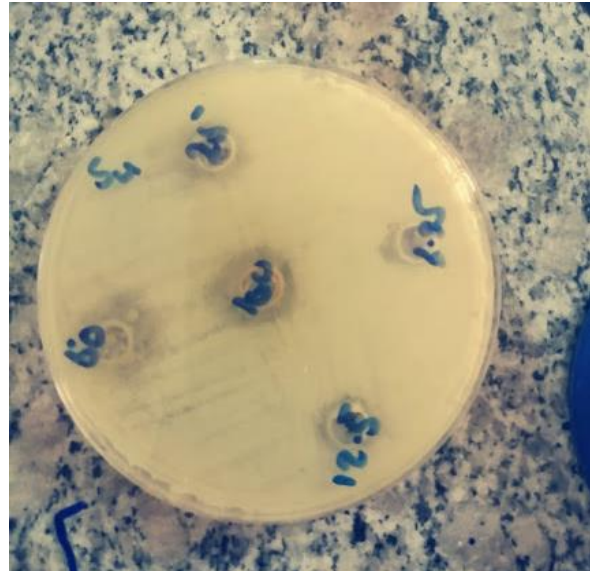
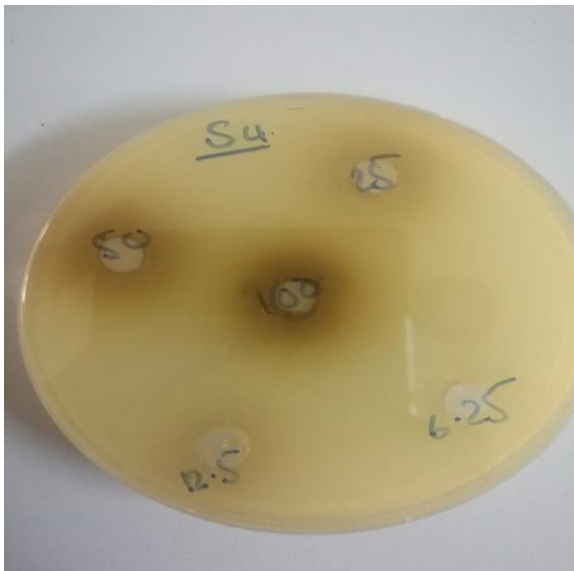


Figure 7: Antimicrobial activity of Aqueous and Methanolic extracts of *Nigella sativa* against *S.aureus* isolate