Sudan University of Science and Technology

College of Graduate Studies

Antibacterial Activity of *Nigella Sativa* Extract against Pathogenic Bacterial Isolate

النشاط البكتيرى المضاد لمستخلص الحبة السوداء على العزلات البكتيرية الممرضة

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

By:

Hanaa Awad Alkareem Ahmed Mnan

B.Sc (Honors) Medical Laboratory Science, Shendi University, 2015

Supervisor:

Dr. Omer Mohamed Khalil

2018

الآيـــــــة

أعوذ بالله من الشيطان الرجيم

قال تعالى:-

﴿ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ﴾

صدق الله العظيم

سورة النمل – الآية (21).

DEDICATION

****

..To my father

To my mother

… To my family …

To My Friends and

…. Teachers ….

Acknowledgement

****

First to ALMIGHTY ALLAH for giving me the power and self-confidence to complete this research

To my prophet Mohamed who recommend us to search for science

Thanking you is not enough to express the gratitude that should be bestowed upon you, but my respect which is there for you ever since you accepted me as your student .Very grateful to you my supervisor **Dr. Omer Mohamed Khalil**

Thanks to the Sudan University of science and technology, teaching staff College of Medical Laboratory Science for their help.

My thanks extend to The University of Khartoum Faculty of science, department of botany. Those who did not spare any effort to give me their experience and skills while conducting my study **Us: Souhanda Ahmed Musa**

My thanks also extend to all staff of Medicinal and Aromatic Plants and Traditional Medicine Research Institute, Department of chemistry.

Special thanks to all my friends those always support me and stand by my side. Thanks To my all teachers those gave me the light of knowledge.

Abstract

There are many reports about usage medicinal plants and their potential as possible therapeutic agent against human pathogens. The aim of this study was to determine the antibacterial activity of different concentrations of petroleum ether, methanol and aqueous extracts of the medicinal plant Nigella *sativa* (seeds) using disc diffusion method on reference standard and selected pathogenic isolates of bacteria.

The study was conducted in Khartoum state, Sudan, during the period from May to November 2018. Fife Reference strain and forty isolated pathogenic bacteria were tested for their sensitivity to *Nigella sativa* extracts. The Reference strain includes *P. aeruginosa* ATCC27853 *K. pneumonia* ATCC 53657, *E. coli* ATCC 25922, *B. subtilis* NCTC8236. While the selected pathogenic strain included *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Proteus species, staphylococcus epidermidis, bacillus cerus and Pseudomonas aeruginosa.*

Petroleum ether and methanol extract of *Nigella sativa* showed pronounced dose dependant antibacterial activity against the Reference strains and clinical isolates of *Staphylococcus aureus, staphylococcus epidermidis, B. subtilis* NCTC8236, *bacillus cerus, E. coli* ATCC 25922 *and* clinical isolates of *Escherichia coli*. But showed no activity against *Klebsiella pneumoniae* ATCC 53657 and *Proteus species.* Petroleum ether extract was partially active against the reference strain of Pseudomonas *aeruginosa* ATCC27853, while the isolated pathogenic strains of Pseudomonas aeruginosa were *non sensitive to the extract.*

The aqueous extract showed no activity to all tested Grams negative organism it just showed activity against *Staphylococcus aureus, staphylococcus epidermidis, bacillus subtilis NCTC8236 and bacillus cerus.*

The study concluded that the greatest activity of Nigella sativa extract were against Gram positive bacteria.

Further studies using large sample size and advance techniques are required to validate the results of this study.

مستخلص الاطروحة

هناك العديد من التقارير عن استخدام النباتات الطبية وإمكاناتها العلاجية الممكنة ضد مسببات الأمراض البشرية. وكان الهدف من هذه الدراسة تحديد النشاط المضاد للبكتيريا من تركيزات مختلفة من إيثر البترول، الميثانول و المستخلص المائي للنبات الطبي الحبه السوداء (البذور) ضد البكتريا البكتريا القياسيه والبكتريا المعزولة من عينات طبية باستخدام طريقة الانتشار عن طريق القرص.

اجريت هذه الدراسة في ولاية الخرطوم في السودان من الفترة مايو الى نوفمبر2018 .

اختبر النشاط المضاد للبكتريا لمستخلص ايثر البترول والميثانول والمستخلص المائي للكمون الاسود لخمسه انواع من البكتريا القياسيه واربعين نوعا من البكتريا الممرضه المعزوله من عينات طبيه

شملت البكتريا القياسيه الزائفة الزنجارية القياسية.27853 الكلبسيلا الرئوية القياسية53657 و الاشريكيه القولونيه القياسية 25922 والعصوية الرقيقة القياسية 8236 .بينما شملت البكتريا المعزوله من عينات طبية المختبره الكلبسيلا الرئوية، الاشريكيه القولونيه،العنقودية الذهبية، المتقلبة الاعتيادية،العنقودية البشروية ،العصوية الشمعية والزائفة الزنجارية.

مستخلصي ايثر البترول والميثانول للكمون الاسود اظهرا فعالية ملحوظة حسب الجرعة المستخدمة ضد السلالات القياسية والمعزولة من عينات طبية وهى العنقودية الذهبية،العنقودية البشروية،العصوية الشمعية،الاشريكية القولونية.بينما لم يظهرا فعالية ضد الكلبسيلا الرئوية ،المتقلبة الاعتيادية .اظهر مستخلص ايثر البترول حساسية جزئية ضد الزائفة الزنجارية القياسية فقط بينما لم يظهر تاثير على الزائفة الزننجارية الممرضه المعزولة من عينات طبية.

المستخلص المائى لم يظهر فعالية ضد كل البكتريا سالبة الجرام المختبرة. فقد اظهر فعالية فقط ضد العنقودية الذهبية،العنقودية البشروية، العصوية الشمعية .

خلصت الدراسة الى ان الاثر الاعظم لمستخلص الكمون الاسود ضد البكتريا موجبة الجرام.

أوصت الدراسة باجراء مزيد من الدراسات و زيادة عدد العينات و استخدام طرق تشخيصية متقدمة للتأكد من صحة نتائج هدة الدراسة.

Table of Contents

List of Tables

Table of Figures

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Undoubtedly, antibiotics, the marvelous drugs of the 20th century, have successively reduced the human mortality and morbidity during their golden period (from 1950s to 1970s) (Abdallah, 2011). However, pathogens have gradually developed resistance to these miracle drugs. Recently, the antibiotics resistance has become a serious global health concern, with a huge economic burden on the community by increasing the cost of the treatment and raises the rates of hospitalization, particularly in the developing countries which already suffers from economic crises, poor sanitation and misuse of antibiotic drugs (Abdallah, 2017). So far, it turns out that the development of new antibiotics, which are costly and time-consuming process, has become useless, as pathogens rabidly develop resistance to these new antibiotics. This has led to an increasing interest in searching for effective alternatives for the current antibiotics with different mode of action on microbes. Hence, medicinal plants appeared to be the best alternative source for new antimicrobial drugs. In literature, numerous studies reported that some plant's phytochemical compounds have potent antimicrobial activity, such as Phenolics, flavonoids, alkaloids, terpenoids, saponins, tannins, anthraquinones, among others; which may kill the bacteria or fungal cells by inhibiting the growth, affect on cellular membrane permeability, interference with some metabolic processes and modulating the signal transduction or gene expression . (Omojate *et al*., 2014).

Accordingly, with the urgent need for new antimicrobial drugs, the efforts toward innovate new antibiotics must behaves different approaches and get benefit from the hidden treasures of medicinal plants. A plethora of scientific research has been published on the bioactivity and medicinal properties of the seeds of *Nigella sativa*; this mini-review

highlights the significance of this plant product as an alternative and promising source for new antimicrobial drugs (Abdallah, 2011). Nigella sativa is a spice plant of family Ranunculacea, commonly known as black cumin or black seed. It is an erect herbaceous annual plant. It grows in Mediterranean countries and Asian countries including India, Pakistan, Indonesia, Italy and Afghanistan (Desai et al,2015). Nigella sativa is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of N. sativa have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Tibb-e-Nabwi (Prophetic Medicine). It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders. Extensive studies on N. sativa have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties(Aftab Ahmad et al,.2013)

1.2 Rational

In the last decades, the antibiotics-resistance phenomenon has become a global health crisis, due to the rapid emergence of multi-drug resistant pathogens. Novel approaches in designing drugs able to overcome these resistant microbes are persistent need. With the suggestion of looking at nature for solutions, exploring medicinal plants may lead to develop new antimicrobial drugs (Abdallah, 2017). Seeds of *Nigella sativa* are mentioned and used since ancient great civilizations and until now in many regions as a part of their traditional health care systems. This study highlights the potential use of this distinguished plant product as an effective antimicrobial drug.

1.3 Objectives

1.3.1 General objective

To study the antibacterial activity of *Nigella sativa extract* against pathogenic bacterial isolates.

1.3.2 Specific objectives

1- To study the antibacterial activity of *Nigella sativa e*xtracts against selected reference and isolated pathogenic bacterial strains using disc diffusion method.

2- To compare the antimicrobial activity of aqueous, methanol, and petroleum ether extracts of *Nigella sativa* against selected reference and isolated pathogenic bacterial strains.

3- To determine the Minimum Inhibitory Concentration (MICs) of the selected extracts against the selected bacterial strains.

4. To carry out the phytochemicals screening for the plant- seeds methanolic extract.

CHAPTER TWO

LITERATURE REVIEW

LITERATURE REVIEW

2.1. Nigella sativa

Nigella is a genus of about 14 species of annual plants in the family Ranunculaceae, native to southern Europe, North Africa and Southwest Asia. The plant is indigenous to the Mediterranean region but now found widely in India (Jammu, Kashmir, Himachal Pradesh, Bihar, Assamand Punjab). The herb is also cultivated in Bengal and north-east India (Ziya and tambe, 2013).

The seeds of *Nigella sativa* are known as black seed or black cumin (English), black-caraway seeds (US), Al-habba Al-sawda (Arabic), shonaiz (Persian), kalonji (India and Pakistan), kalajira (Bangladesh) and krishnajirika (Southeast Asia). Seeds of *Nigella sativa* are initially considered as spices, it has a distinctive aroma and taste and used in some bread recopies in some regions such as Pakistan **(**Abdallah (2017). Nigella sativa is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds of Nigella sativa used in pickles as spice have also been traditionally used in treatment of many diseases including diabetes and hypertension. Among many activities exhibited by Nigella sativa and its constituents in animal experiments, ant diabetic property is most important. Nigella sativa Seeds and oil have a long history of folklore usage in various systems of medicines and food. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Tibb-el-Nabwi. Extensive studies on Nigella sativa have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include ant diabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastro-

protective, antioxidant properties. The present review is focused on its phytochemical analysis and ant diabetic property (Desai *et al*., 2015).

2.1.1. History of Nigella Sativa

The black seed 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(Ibrahim et a.,l 2000). It is also famous drug from the Islamic civilization and well known in the Islamic heritage, in what is called Prophetic medicine, as Muslims believe that the black seed is an effective remedy for all diseases except death, based on some Prophetic statements (Haddith) in the Islamic religion. IbnSina (Avicenna) mentioned the black in his distinguished book "Al-Kanon fit-tib" or the canon of medicine, he prescribed it to stimulate the body's energy and for treatment of fatigue and dispiritedness (Hussain, 2016). Black seed also prescribed in the Indian traditional system of medicine (Ayurveda and Siddha) which used to treat jaundice, fever dyspepsia, paralysis, piles, and skin diseases(Paarakh, 2010). It is also widely used in different traditional health systems as antihypertensive, appetite stimulant, analgesic, anthelmintic, liver tonic, diuretic, and anti-diarrheal (Gilani *et al*., 2004). On the other side, huge number of studies has been carried out revealing the medicinal, pharmacological, and therapeutic properties of the seeds of *Nigella sativa* and a wide spectrum of its curative power have been explored (Abdallah, 2017).

Nigella sativa has historical as well as religious background. The Islamic prophet Muhammad(PBUH)once stated that the black seed can heal every disease except death(Ziya i and Tambe,2013). Black cumin is also mentioned in Holy Bible as "Curative black cumin" and is described as 'Melancthon of Hippocrates and Dioscorides' and as'Glitch of Plin (*Hira Ijaz et al,.2017).*

2.2 Traditional uses of *Nigella sativa*

In the traditional system of medicine practiced in the Arabian Gulf region, Black Seed is recommended for a wide range of ailments, including fever, cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegic, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea. It has been used as a stimulant, diuretic, emmenagogue, lactagogue, anthelmintic, and carminative. Black Seed has also been used externally where it is applied directly to abscesses, nasal ulcers, orchitis, eczema, and swollen joints (Ziya and tambe, 2013).

The results of extensive pharmacological studies justify the broad, traditional therapeutic value of Black Seeds. These studies found black seed to have analgesic, anti-lipemic, post coital contraceptive, diuretic and anti hypertensive bronchodilator and calcium antagonist, histamine release inhibitor, hepatoprotective, anthelmintic, antifungal, antimicrobial against a (Ziya and tambe, 2013) wide range of organisms), anticancer, and anti-inflammatory activities

2.3. Morphology:

It is small prostrate annual herb; the plant grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are pale blue or pale purple, with 5-10 petals. The Fruit is a capsule composed of several united follicles, each containing numerous seeds, the seeds trigonometric and black in color. The plant has a rather stiff, erect, branching stem, bears deeply-cut grayish green leaves and terminal grayish blue flowers, followed by odd, toothed seed vessels, filled with small compressed seeds, usually three-cornered, with two sides flat and one convex, black or brown Externally white and oleaginous, strong agreeable aromatic odor, like that of nutmegs, and a spicy, pungent

taste. The flowers are delicate, and usually colored pale blue and white, with 5–10 petals .The fruit is a large and inflated capsule composed of 3–7 united follicles, each containing numerous seeds. It has a pungent bitter taste and a faint smell of strawberries (Ziya and tambe, 2013).

2.4. Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Order: Ranunculales

Family: Ranunculaceae

Genus: *Nigella*

Species: *sativa*

2.5. Chemical Composition

Natural products are believed to be an important source of new chemical substances with potential therapeutic applicability. Therefore phytochemicals evaluation of plant is essential to find out the relationship between the biological activity and the chemical structure of the biologically active phytochemicals (Desai *et al*., 2015).

The seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%); furthermore, traces of alkaloids are found in the seeds. The seeds also contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50 - 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%) which is characteristic for the genus. Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. Commercial nigella oil ("Black Seed Oil", "Black Cumin Oil")

May also contain parts of the essential oil, mostly thymoquinone, by which it acquires an aromatic flavor (Ziya and tambe, 2013).

The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol

(6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α-pinene and thymol etc. Black seeds also contain some other compounds in trace amounts. Seeds contain two different Types of alkaloids; that is isoquinoline alkaloids e.g. nigellicimine and nigellicimine- N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Nigella sativa seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent other compounds like carvone, limonene, citronellol were also found in trace amounts. Most of the pharmacological properties of Nigella sativa are mainly attributed to quinine constituents, of which Thymoquinone is the most important. On storage, Thymoquinone yields dithymoquinone and higher oligocondensation products (Ziya and tambe, 2013).

The seeds of Nigella sativa contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and Total ash (4.8 %). The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid .The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50- 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α-sitosterol is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigma sterol (6.57-20.92% of total sterols). In some studies it is reported that the other components includes nigellone, avenasterol-5-ene, avenasterol- 7(Ziya and tambe, 2013).

2.6. Black seed as antibacterial agent

A large number of scientific articles refers to the antibacterial activity of the black seed have been published in journals of PubMed/Medline, Science Direct, Scopus and Google Scholar; and many publishers. In summary, Oils extracted from Nigella sztiva showed significant antibacterial effect against multidrug-resistant *Staphylococcus aureus* isolated from wounded diabetic patients from Southeast Nigeria(Emeka et al,.2015) .Oil of *Nigella sativa* revealed effective antibacterial activity against considerable number of methicillin resistant and coagulase negative *Staphylococcus aureus*, safety of that oil was examined, and there was no cytotoxic influence on the proliferation of gingival fibroblasts(Ugur et al,.2016). The black seed oil was recommended to be used as an antimicrobial agent in food production to prevent spoilage. Based on the results that showed that this oil at 2.0% concentration was able to inhibit the growth of twenty-four pathogenic, spoilage and lactic acid bacteria (Arici et al,. 2005).

Ethanol and n-hexane extracts of the black seeds recorded remarkable dose dependant antibacterial effects against different gram-positive and gram-negative strains, namely *Bacillus cereus, Bacilllus subtilis, Escherichia coli, Staphylococcus epidermidis, Klebsiella pneumonia* and *Salmonella typhmurium*. However, no antibacterial activity detected against *Pseudomonas aeruginosa* and *Enterobacter aerogens* (Khan and kou 2016).

The black cumin seeds exhibited antibacterial activity against *Salmonella typhi* (Utami et al,. 2016). Methanol and water extract of the black seed reported remarkable antibacterial efficacy towards *Streptococcus pyogene, Pseudomonas aeruginosa*, *Klebseilla pneumonia*, and *Proteus vulgaris*, the greater antibacterial effect was against the Gram-positive bacteria (Hasan *et al,. 2013)*. An active principle isolated from seeds of

Nigella sativa called thymoquinone showed a broad spectrum of activity against different Gram-positive and Gram-negative bacteria, namely *Bacillus cereus* ATCC 14579, *Listeria monocytogene* ATCC 19115, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* NCIMB 8166, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218 *Salmonella enteric*, serovar typhimurium ATCC 14028, *Vibrio lginolyticus* ATCC 33787 and *Vibrio paraheamolyticus* ATCC 17802, thymoquinone was able to prevent bacterial biofilm formation (Chaieb *et al*., 2011).The potential antibacterial activity of the black seed was also evaluated in-vivo, a groups of male mice were infected with *Staphylococcus aureus* and *Escherichia coli,* and subjected to varied doses of methanol, chloroform and essential oil of the black seed (Abdallah EM, 2017) .The petroleum ether and methanol extract of *Nigella sativa* were screened for their antibacterial activity against standard and clinical isolates. Petroleum ether extract of *Nigella sativa* showed pronounced dose dependant antibacterial activity on standard strains and clinical isolates, while methanolic extract showed no activity (Elgassim,2015)

CHAPTER THREE

MATERIALS AND METHODS

MATERIALS AND METHODS

3. 1. Study design

This was analytical and experimental study.

3. 2. Study area

The study was conducted in Sudan University of science and technology, college of medical laboratory science Khartoum state.

3. 3. Study duration

This study was conducted from May to November, 2018.

3. 4. Study population

Clinical isolates from males and females of different ages with different bacterial Infection.the isolated bacteria include *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Proteus species, staphylococcus epidermidis, bacillus species and Pseudomonas aeruginosa.*

3.5 . Re-identification of the clinical isolates:

Forty clinical isolates were re-identified in the lab by standard microbiology procedures (Cheesbrough, 2006). Biochemical tests including Kliglar Iron Agar test (KIA), indole, urease, citrate, motility test, esculin hydrolysis, catalase test, DNAse test, as well as inoculation on differential selected media such as Mannitol salt agar (MSA) was used to distinguished between the clinical isolates. The principle of the tests started with indole test, the tested organism was cultured in medium contain tryptophan; indole production is detected by kovac‘s reagent which contain 4dimethylaminobenzaldehyde. This reacts with indole to give red color compound (Cheesbrough, 2006). Then urease test was done, the tested organism was cultured in medium contains urea and indicator phenol red. If there is change in color from yellow to pink color was recorded (Cheesbrough, 2006), then citrate test, the tested organism was cultured in a medium contain sodium citrate, ammonia salt and indicator bromo-thymol blue showing turbidity. The change in color of

the indicator from green to blue was considered as positive (Cheesbrough, 2006). The motility test also done that depend on the properties of bacteria structure which contain flagella or no, also Bile esculin test was done, the tested organism was cultured in esculin agar (Cheesbrough, 2006). While catalase test was used, detect the break down of hydrogen peroxide to give oxygen and water, indicated by production of air bubble. Then Kligler Iron Agar test (KIA) was used for detection of H2S, gases production and sugar fermentation (lactose), to help in identification of *Enterobacteriasae* (Cheesbrough, 2006). Finally special media were used for more identification of the isolated organisms. Mannitol salt agar was used to differentiate between the *Staphylococcus* species. DNase test also was used to differentiate between *Staphylococcus* species, the tested organism was cultured on media contain DNA, after inoculation and incubation for overnight HCL was used, which precipitates the un hydrolyzed DNA to give clear zone around the colonies (Cheesbrough, 2006).

3. 5. 2. Preservation and storage of isolated organisms

Isolated organisms were kept in nutrient agar slope at 4 C for further identification and susceptibility tests. Glycerol (20 ml) with peptone water (80 ml) (20% v/v) was used for long preservation of the isolates at -20 C.

3. 6. Collection and identification of plant material

Nigella sativa seeds were procured from Bahri market, Khartoum. They were freed of dust and crushed in a domestic grinder and then soaked. The plant was taxonomically identified by taxonomist in Medicinal and Aromatic plants and traditional Medicine Research Institute National Center, Khartoum, Sudan.

3.7. Preparation of the extracts:

For aqueous extract, 10 g of dry powder were mixed with distilled water and boiled on slow heat for 2 h. It was then filtered and centrifuged at 5000 g for 10 min. The supernatant was collected. This procedure was repeated twice more and stored at 4oC. (Khalil, 2014).

Alcoholic Extraction was carried out according to method described by (Sukhdev *et. al.* 2008). One hundred and fifty Gram of the plant sample was coarsely powdered using mortar and pestle. Coarsely sample was successively extracted with petroleum ether and methanol using soxhelt extractor apparatus. Extraction carried out for about four hours for petroleum ether and six hours for methanol till the color of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air till complete dryness and the yield percentages were calculated as followed:

Table (3.1) Weight of extract obtained / weight of plant sample X100

NO	Name of extract	Weight of plant in gm	Weight of extract in gm
1	Petroleum ether	150	6.7 (4.47) %
2	Methanol	150	4.2 (2.8) %

3.8.Phytochemical screening

Phytochemical screening for the active constituents was carried out on methanolic extract using the methods described by (Martinez & Valencia (1999), Sofowora (1993), Harborne (1984) and Wall et al (1952)) with few modifications.

Phytochemical screening:

1- Identification of tannins:

In this test 0.5 g of the extract was washed three times with petroleum ether, dissolved in 10 ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride was added and to the other one 2 – 3 drops of gelatin salts reagent were also added. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins (Wall et al, 1952).

2- Test of sterols and triterpenes:

In this test 0.5 g of the extract was washed three times with petroleum ether and dissolved in 10ml of chloroform. To 5 ml of the solution, 0.5 ml acetic anhydride was added and then 3 drop of conc. Sulphuric acid at the bottom of the test tube were added . At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

3: Test for Alkaloids: -

In this test 0.5 g of the extract was heated with 5 ml of 2N HCL in water bath and stirred for about 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent were added while to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanken as presumptive evidence for the presence of alkaloids (Sofowora, 1993).

4: Tests for Flavonoids: -

In this test 0.5 g of the extract was washed three times with petroleum ether, dissolved in 30 ml of 80% ethanol. The filtrate was used for following tests: -A/ to 3 ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution in methanol was added. Appearance of a

yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

B/ to 2 ml of the filtrate 0.5 ml of 10 % lead acetate was added. Appearance of creamy turbidity was taken as an evidence of flafonoids.

5- Test for Saponins: -

In this test 0.3 g of the extract was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins (Sofowora, 1993).

6 - Test for Cumarins: -

In this test 0.2 g of the extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KOH put on it. Then the filter paper was inspected under UV light, the presence of Cumarins was indicated if the spot have found to be adsorbed the UV light (Martinez et al,. 1999).

7- Test for Anthraquinone glycoside: -

In this test 0.5 g of the extract was boiled with 10 ml of 0.5N KOH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color (Harborne, 1984).

3.9. Antibacterial susceptibility testing

Modified Kirby-Bauer Method.

In this method, 6 mm sterilized filter papers discs (Whatmann No. 1) are saturated with filter sterilized (Salie et al., 1996) plant extract of desired concentration. The impregnated discs are then placed into the surface of a

suitable solid agar medium like Mueller Hinton (Mueller and Hinton, 1941).

Isolated organisms were tested against different concentration of black seed extract by using Kirby-Bauer disk-diffusion method with the bacterial suspension .in which 3-5 selected colonies were touched by sterile standard loop then emulsified into sterile normal saline and adjusted to 0.5 McFarland standards. A sterile cotton swab was inserted into the bacterial suspension, rotated and then compressed against wall of the test tube to expel any excess fluid. The swab was then streaked on the surface of MHA plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface (Cheesbrough, 2006). *Nigella sativa* extract were assessed using disc diffusion method, One gram from methanolic extract was dissolved in 10 ml (99.9%) methanol, 1ml of DMSO(di methyl sulphate) with 1ml of nigella sativa oil, and then serially diluted two fold to obtained final concentrations of (50 (%w/v), 25 (%w/v), 12.5 (%w/v), 6.25 (%w/v) and 3.125 (%w/v)). Hundred µl of each prepared concentration was added into corresponding sterile discs. The plates were left for 1 hour in refrigerator (4 C) for diffusion of effective compounds of nigella sativa in the media and then incubated at 37 C for 24 hours. Inhibition zone around each disc were measured using a ruler in millimeters. MIC considered as the lowest concentration of extract that prevent visible bacterial growth (Anejaetal, 2009).

CHAPTE RFOUR

Results

Results

4.1. Antimicrobial activity results

Antibacterial activity of *N. sativa* extracts against selected pathogenic and reference stander strains of bacteria were summarized in Table (4.1.) petroleum ether, methanol and aqueous extract were prepared .Every extract was tested in triplicate against three strains of the selected bacteria and reference stander bacteria .after incubation the diameters of the resultant growth inhibition zones were measured and the mean values were tabulated (Table 4.1*). S. aureus, S. epidermidis, B.cerus, bacillus subtilis NCTC8236 and Escherichia coli* were high active to the petroleum ether extract, moderate activity to the methanolic and aqueous extract was observed except to *Escherichia coli.* In general all Gram negative isolates showed resistant to the aqueous extract.

Minimum inhibitory concentration of petroleum ether and methanol extract was found to be 6.25 mg/mL against *S. aureus, S. epidermidis, B. subtilis NCTC8236 and B.cerus* strains, while it is 12.5 mg/mL against *E.coli.*. In this study methanolic extract of *Nigella sativa* showed *no activity against P. aeruginosa ATCC27853 K. pneumonia ATCC 53657, Proteus species*, Petroleum ether extract showed antimicrobial activity against strains of *S. aureus, E. coli, S. epidermidis, B. subtilis* NCTC8236, *B.cerus, E. coli* ATCC 25922 and partially active against *P. aeruginosa* ATCC27853 and no activity against *Klebsiella pneumoniae* ATCC 53657, *Proteus* species. While the aqueous extraction show activity against *S. aureus, S. epidermidis, B.subtilis* NCTC8236 *and no activity against whole tested Gram negative bacteria.*

Results were expressed as mean SD on table (4.1) **.** The result revealed that petroleum ether extract was the most effective extract followed by methanol and aqueous extract respectively.

Table **(4.1)** the mean SD of *Nigella sativa inhibition zone of* petroleum ether, methanol and aqueous extract against pathogenic bacterial strains .

Bacteria	Petroleum ether extract (The oil)	Methanolic	Aqueous
Escherichia coli ATCC25922	16	15	7
Escherichia Coli	15	14	5
*K. pneumonia*e ATCC53657	9	8	5
*K.pneumonia*e	8	8	5
Proteus species	7	6	5
P.aeruginosa ATCC27853	11	8	5
P. aeruginosa	9	8	5
Bacillus subtilis NCTC8236	17	20	13
B.Cereus	17	19	12
S. aureus	15	17	12
S. epidermidis	30	22	12

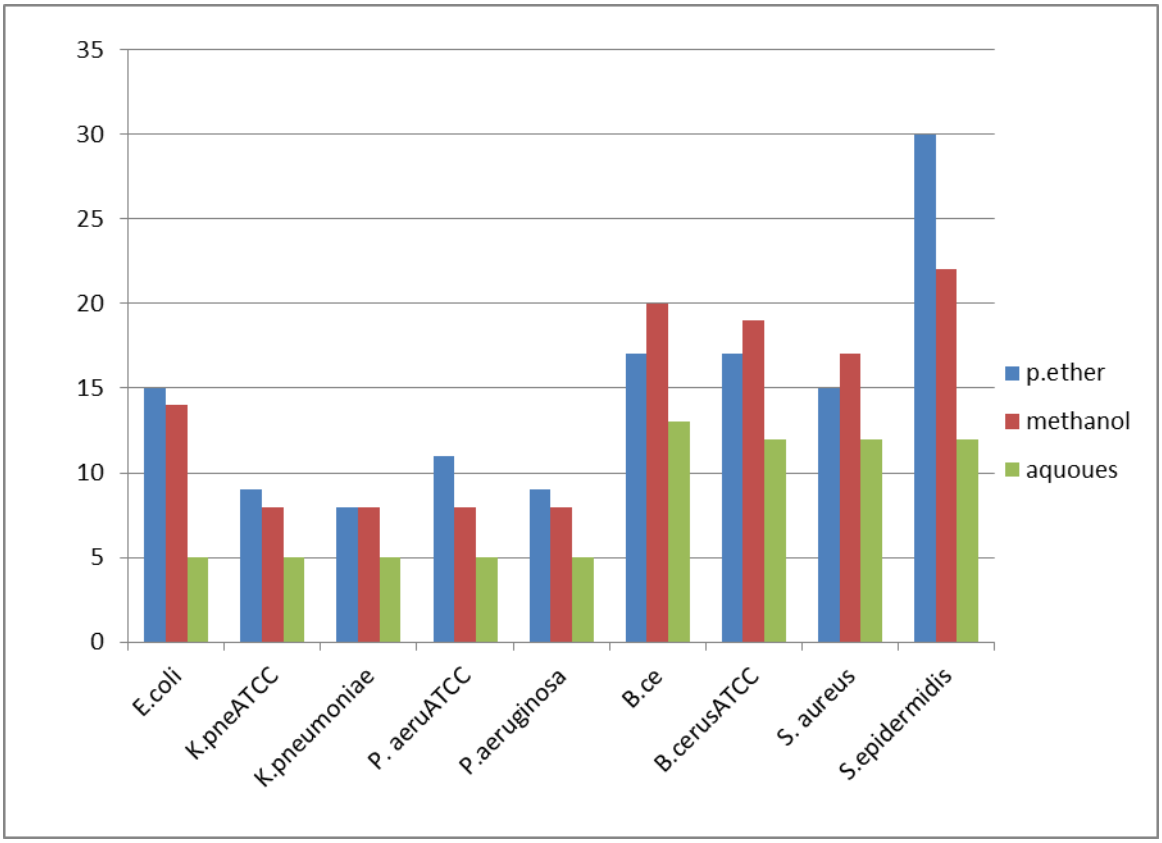

Figure (4.1) the mean SD of *Nigella sativa inhibition zone of* petroleum ether, methanol and aqueous extract against pathogenic bacterial strains .

4.2. Interpretation of results

After 24 hours incubation antibacterial activity result were expressed in diameters. Inhibition zones were measured in millimeters 9mm zones was considered as inactive; 9-12mm as partially active while 13-18 mm as active and 18 mm as very active (Mukhtar and Ghori, 2012).

Table (4.2) *Nigella sativa* Petroleum ether and methanolic extract minimum inhibitory concentration *against* selected reference and clinical bacterial strains.

Bacteria	Petroleum ether extract (The oil)	methanolic
Escherichia coli ATCC25922	12.5	12.5
Escherichia coli1	12.5	12.5
Bacillus subtilis NCTC8236	6.25	6.25
Bacillus cereus	6.25	6.25
Staphylococcus aureus	6.25	6.25
Staphylococcus epidermidis	6.25	6.25

4.3 .Phytochemical screening results:

Phytochemical screening for the active constituents was carried out on methanolic extract using the methods described by (Martinez & Valencia (1999), Sofowora (1993) and Wall et al (19527/89) with many few modifications.

Table (4.3) Phytochemical screening results of Nigella sativa methanolic extract.

The test	saponin	cumarins	Alkaloids	flavonoids	Tannins	steroids	Triterpens	Anthraquinone
result	+	-	-	+++	++	+	+++	-

Key:

+ Trace, ++ Moderate, +++ High, -Negative

CHAPTER FIVE

DISCUSSION

Discussion, Conclusions and recommendations

5.1 DISCUSSION

There are many reports about usage medicinal plants and their potential as possible therapeutic agent against human pathogen. Many of these studies report the antimicrobial potential based on selected clinical isolates which make the comparison impossible between various studies (abdallah,2017). Reference strains used for screening antimicrobial activity could minimize or limit the variations between different studies. In vitro studies in this work showed that the extract inhibited bacterial growth but in varying degrees. The current study was carried out to screen the antimicrobial activity of *Nigella sativa* methanolic, petroleum ether and aqueous extract against Selected reference and clinical isolate strains. The results showed high activity of the petroleum ether against *epidermidis* and *S. aureus* that is agreed with (Emeka, 2015), who reported in study carried out that the oil extract showed activity against multidrug resistance *staphylococcus aureus* .the oil extract also showed prounced activity against reference and clinical isolates of *Pseudomonas aeruginosa* .*Bacillus* and *E.coli* but no activity against the clinical isolate. This results agree with that reported by (khan and kou2016) ,whose result revealed that Ethanol and n-hexane extracts of the black seeds recorded remarkable dose dependant antibacterial effects against different Gram-positive and Gram-negative strains, namely *Bacillus cereus, Bacilllus subtilis, Escherichia coli, Staphylococcus epidermidis, Klebsiella pneumonia* and *Salmonella typhmurium*. However, no antibacterial activity detected against *Pseudomonas aeruginosa.* In contrary in this study the oil extract of Nigella sativa showed no activity against *Klebsiella pneumonia. Nigella sativa oil (petroleum ether* extract) was the most active against reference and clinical isolate strains of *staphylococcus* bacteria. This results agree with (Ugur et al,.2016) whose

reported that the Oil of *Nigella sativa* revealed effective antibacterial activity against considerable number of methicillin resistant and coagulase negative *Staphylococcus aureus*, safety of that oil was examined, and there was no cytotoxic influence on the proliferation of gingival fibroblasts. The active principle isolated from seeds of *Nigella sativa oil* called thymoquinone showed a broad spectrum of activity against different gram-positive and gram-negative bacteria reported by (Chaieb et al,. 2011),whose conducted a broad spectrum of activity against different Gram-positive and Gram-negative bacteria, namely *Bacillus cereus* ATCC 14579, *Listeria monocytogene* ATCC 19115, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* NCIMB 8166, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli ATCC 35218 Salmonella enteric, serovar typhimurium ATCC 14028, Vibrio lginolyticus ATCC 33787 and Vibrio paraheamolyticus ATCC 17802*, thymoquinone was able to prevent bacterial biofilm formation. The difference in the activity of Nigella sativa oil in this study against *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* ,This may be because of the reason that N. sativa oil obtained from different commercial sources or isolated by different methods from the same seeds have been shown to vary significantly in their content of Thymoquinone, which has antibacterial activity and various storage conditions are expected to make a difference in the amounts of the quinone constituents of the oil, especially if the seed oil samples are exposed to heat and light (Burits *et al*.,2000). Additional studies conducted that the antimicrobial activity of petroleum ether extract of *Nigella sativa* may be attributed to the presence of thymoquinone and thymol (Kahsai, 2002), **(**El-Fatatry, 1975), (Randhawa *et al*, 2002), (Karapinar *et al*, 1987).

In this study the methanolic extract of *Nigella sativa reported activity against staphylococcus aureus , Bacillus cereus, B. subtilis* NCTC8236, *Escherichia coli, and Staphylococcus epidermidis* these results are similar to a study that carried out by Abdallah EM (2017) whose evaluated The potential antibacterial activity of the black seed in-vivo in a groups of male mice were infected with *Staphylococcus aureus* and *Escherichia coli*, and subjected to varied doses of methanol, chloroform and essential oil of the black seed. But disagreed to study carried out by Elgassim (2015) in Sudan university whose reported no activity of methanolic extract . The aqueous extract didn't have activity to the most strains except the selected gram positive bacteria *(Staphylococcus aureus*, *Staphylococcus epidermidis and Bacillus cereus)* The study in disagreement with (Hassan *et al, 2013)* whose reported that the Methanol and water extract of the black seed reported remarkable antibacterial efficacy towards *Streptococcus pyogene, Pseudomonas aeruginosa*, *Klebseilla pneumonia*, and *Proteus vulgaris*.

The greatest antibacterial effect was against the Gram-positive bacteria The results are similar to many studies that carried out by (Elgassim,2015) (Abdallah, 2016), (Hassan,2015) , (Amir *et al*,2016), all reported the extract was found to be more effective on Gram positive than Gram negative bacteria. so a number of compounds derived from plants often showed considerable activity against Gram positive bacteria but not against Gram negative species .Gram negative bacteria have an effective permeability barrier, comprised of outer membrane which restricts the penetration of amphipathic compounds and multidrug resistance pumps that extrude toxins across this is barrier. It is possible that the apparent effectiveness of plant anti bacterial is largely due to the permeability barrier (Tegos *et al*., 2012). The variability in the performance of Mueller- Hinton agars from different manufacturers has

been shown to be statistically significant, the size of inoculums used, depth of medium in the plates, inoculation technique and time period between inoculation and application of discs, incubation temperature and time of incubation will also cause differences in the results obtained. (Barry *et al*,1974).

5.2 Conclusions

In conclusion Nigella sativa has high activity against some bacteria, which justify its use in traditional and folk medicine The *Nigella sativa oil (petroleum ether* extract) showed highest antibacterial activity while the methanolic extract showed relatively high to medium activity. The aqueous extract showed resistant to most bacterial strains. The highest concentrations of Nigella sativa were more effective than lower concentrations which mean that the antimicrobial activity of Nigella sativa extract is concentration dependent. The *petroleum ether* extract was the most potent extract giving MICs of 6.25 g/ml against four reference bacterial strains.

5.3 Recommendations

Further studies with reference strains as well as clinical isolate is required to verify these results and confirm the antimicrobial activities of, *petroleum ether*, methanolic, aqueous extract, and other extracts of *Nigella sativa*. Identify the active compounds of different extracts of *Nigella sativa* and the toxicity of these compounds.

It is recommended to design and develop novel antibacterial drugs from *Nigella sativa* seeds.

CHAPTER SIX

References

References

Abdallah EM. (2011). Plants As alternative source for antimicrobials. *J Applied Pharm Sci*1; (6): 16-20.

Abdallah EM. (2017). Black Seed (*Nigella sativa*) As Antimicrobial Drug. *novel approaches in drug designing and development ;* 3(2): 1-3 .

Aftab Ahmad,(2013) Asif Husain,Mohd Mujeeb, Shah Alam Khan, Abul Kalam Najmi, Nasir Ali Siddique, Zoheir, Damanhouri, Firoz Anwar,A review on therapeutic potential of Nigella sativa: A miracle herb, *Asian Pac J Trop Biomed* 3(5): 337-352,

Amir R. Khan and KirandeepKour. **(2016).** Wide spectrum antibacterial activity of Nigella *Sativa L.* seeds. *IOSR Journal Of Pharmacy; 6: 12-16.*

Aneja K and Joshi R. **(2009).** Evaluation of antimicrobial proprieties of the fruit extracts of *Terminaliachebula* against dental caries pathogens. *Jun. Jou. Micro;* **2**:105-111.

Arici M, Sagdic O and Gecgel U. **(2005)**. Antibacterial effect of Turkish black cumin (Nigella sativa L.) oils. *Grasas y Aceites*; 56(4): 259-262.

Arunkumar and MuthuselvamM**. (2009)**. Analysis of Phytochemical constituents and antimicrobial activities of *Aloe vera*L against clinical pathogens. *World J. Agric. Sci;* 5: 572–576.

Barry AL and Effinger LJ**. (1974)**. Evaluation of two standardized disk methods for testing antimicrobial susceptibility of Pseudomonas aeruginosa and of the Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*; 6(4):452-459

Burits M and Bucar F**. (2000).**Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res*; 14: 323-328.

Chaieb K, Kouidhi B, Jrah H, Mahdouani K and Bakhrouf A**. (2011).** Antibacterial activity of Thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation. *BMC Complement Altern Med*;11: 29.

Cheesbrough M. (2006). District Laboratory Practice in Tropical Countries. Second edition. United States of America by Cambridge University Press, (Part 2). P. 64, 65, 67, 70, 137, 138, 157, 395, 396.

Das KSR, Tiwarib and Shrivastava DK. **(2010).** Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research ; 4(*2): 104-111.

Desai SD, ShaikH, Kusal K and Haseena S**. (2015)**. Phytochemical Analysis of *Nigella sativa* and it`s Antidiabetic Effect. J. *Pharm. Sci. & Res*. Vol. 7(8): 527-532.

El-Fatatry HM. (1975). Isolation and structure assignment of an antimicrobal principle from the volatile oil of *Nigella sativa* seeds.,*Pharmazie;* **30** (2): 109-111.

Elgassim Mahi. (2015). Antibacterial Activity of *Nigella sativa* extract against selected bacterial clinical isolates from Khartoum State, Sudan University of Science and Technology master's theses.

Emeka LB, Emeka PM and Khan TM. **(2015)**. Antimicrobial activity of Nigella sativa L. seed oil against multi-drug resistant Staphylococcus aureus isolated from diabetic wounds. *Pak J Pharm Sci*2; 8(6):1985-1990.

Gilani AH, Jabeen Q and Khan MA. **(2004).** A review of medicinal uses and pharmacological activities of Nigella sativa. *Pakistan J BiolSc*i 7(4): 441-451.

Gupta, C., Amar, P., Ramesh, G., Uniyal, C. and Kumari, antimicrobial activity of some herbal oils against common food-borne pathogens. *African J Microbiol Res*. **2**: 258-261.

Hasan NA, Nawahwi Z and Malek HAB. **(2013).** Antimicrobial Activity of Nigella sativa Seed Extract. *Sains Malaysiana*; 42(2): 143-147.

Hira Ijaz, Ume Ruqia Tulain1, Junaid Qureshi, Zeeshan DanishSamina Musayab, Muhammad Furqan Akhtar, Ammara Saleem, Khanzada Atta-

Ur-Rehman Khan, Muhammad Zaman, Imran Waheed, Imran Khan and Mohamed Abdel-Daim1,**(2017),** Nigella sativa prophetic medicine, *Pakistan journal of pharmaceutical sciences* ,(30) 229.

Hussain DAS and Hussain MM. **(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 *Med Plant Res*; 4(2): 27-57.

Ibrahim ZS, Ishizuka M, Soliman M, ElBohi K, Sobhy W, et al. **(2000)** Protection by Nigella sativa against carbon tetrachloride-induced down regulation of hepatic cytochrome P450 isozymes in rats. Jpn J Vet Res; 56(3): 119-128.

Islam SK, Ahsan M, Hassan CM and Malek MA**. (1989).** Antifungal activities of the oils of Nigella sativa seeds. *Pak J Pharm Sci; 2(*1): 25-28.

Kahsai AW, (2002). Isolation and Characterization of ActiveIngredients from *Nigella sativa* for Antibacterial Screening. MasterThsis, Department of Chemistry, East Tennessee State University.

Kapil A, (2005). The challenge of antibiotic resistance: Need to contemplate. *Indian J Med Res*; 121(2): 83-91.

KarapinarM and Aktu SE. **(1987).** Inhibition of food borne pathogens by thymol, eugenol, menthol and enethole. *Int J Food Microbiol; 4* (2): 161-166

Khalil, (2014). in vitro screening of antibacterial activity of ferrula ass-afoetida extracts, *Asian Jr. of Microbiol. Biotech.Env. Sc;* 16(3): 859-862.

Khan AR and Kou K**, (2016).** Wide spectrum antibacterial activity of Nigella sativa L seeds. *IOSR Journal of Pharmacy*; 6(7): 12-16.

Krishnapura Srinivasan,(2017). Traditional uses chemical Constituents and nutraceutical effects. f*ood quality and safety; 2: 1-16.*

Martinez A, Valencia G: Marchafitoquimica. **(2003)**. In Manual de prácticas de Farmacognosia y Fitoquímica: 1999.1.st edition. Medellin: UniversidaddeAntioquia;Phytochemical screening methods 59-65.

MuellerHinton. (1941). A protein-free medium for primary isolation of the gonococcus and meningococcus.*Proc. Soc. Exp. Biol. Med.* 48: 330.

Mukhtar S. and Ghori I. **(2012)**. Antibacterial Activity of aqueous and ethanolic extract of Garlic, Cinnamon and Turmeric against *Escherichia coli* ATCC25922 and *Bacillus subtilis. Inte. J Appl. Biol Pharm;* **3**:131-136

Paarakh PM. (2010). Nigella sativa Linn.- A comprehensive review. *Indian J Nat Prod Res*; 1(4): 409-429.

RandhawaMA and Al-GhamdiMJ. **(2002).** A review of pharmaco the rapeutic effects of *Nigella sativa*. *Pak Jr of Med Res;* **41**(2): 77-83.

Salama RH. (2011). Hypoglycemic effect of lipoic acid, carnitine and Nigella sativa in diabetic rat model. *Int J Health Sci (*Qassim); 5(2): 126-134.

Salie F, Eagles PFK and Lens HMJ**, (1996)**. Preliminary antimicrobial screening of four South African *Asteraceae*species. *J. Ethnopharmacol*; 52(1): 27-33.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicines in Africa.*Chichester John, Willey & Sons Ltd. New York* .256.

Sudha P., Zinjarde S., Bhargava S. and Kumar M. (2011). Potent a-amaylase inhibitory activity of Indian Ayurvedic medicinal plants. *Inter. soci. Comp. Med. Res*. **11**:5.

Sukhdev. SH, SumanP, Gennaro L and DevDR**, (2008).** Extraction technologies for medicinal and aromatic plants.United Nation Industrial Development Organization and the International Center for Science and High Technology. 116-118.

Tegos, G., Stermitz, S.R., lomovskaya, O. and Lewisk**. (2012)** Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobials. *Antimicrob Agents Chemother*. **46**(10). 3133.

Ugur AR, Dagi HT, Ozturk B, Tekin G and Findik D**, (2016).** Assessment of In vitro Antibacterial Activity and Cytotoxicity Effect of Nigella sativa oil. *Pharmacogn Mag*; 12(S4): S471-S474.

Umar S, Zargan J, Umar K, Ahmad S, Katiyar CK, et al. **(2012)**. Modulatsion of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. *ChemBiol Interact*; 197(1): 40-46.

Utami AT, Pratomo B and Noorhamdani. **(2016)**. Study of Antimicrobial Activity of Black Cumin Seeds (Nigella sativa L.) Against Salmonella typhi In Vitro. *J Med SurgPathol*; 1: 127.

Wall ME, EddyCR, McClennaML and KlumpME. **(1952)**. Detection and estimation of steroid and sapogenins in plant tissue. *Analytical Chemistry*; 24:1337-1342.

Ziya Ansari and Tambe Satish (2013)**,** Traditional uses of *Nigella sativa,* in Malegaon region of Nashik, *International Journal of Pure & Applied Bioscience*, 1 (2): 19-23.