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Biological Evaluation and Comparison of *Nigella sativa* (Kalonji) and *Trachyspermum ammi* (Ajwain)

by

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Thesis is dedicated to Allah Almighty, Hazrat Muhammad (SAW) and to my great father, beloved mother, my elder brothers, adorable sisters, teachers and all those friends who have supports me since the beginning of this thesis.

CERTIFICATE OF APPROVAL

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Abstract

Trachyspermum ammi and *Nigella sativa* are mostly used traditional medicinal plants to cure different disease. Both of *N. sativa* and *T. ammi* contain essential constituents of human diet and also contain active compounds. Active compounds play important role to cure many kind of diseases both human and animal models. These spices used frequently to cure the different diseases like headache, fever, asthma, migraine, obesity, stomach problems, respiratory, Cardiovascular diseases, liver and kidney system. Presences of variety of diverse active compounds are responsible for a wide range of biological properties. These active compounds present in aqueous extracts are responsible for antimicrobial action. Various bioassays were performed to check the therapeutic efficacy of both these plants which are antimicrobial assays (antibacterial and antifungal), cytotoxic assay or brine shrimp lethality assay and antioxidant (DPPH assay). It was observed that both of these extracts showed significant antibacterial activity against gram negative bacteria which can be attributed to the absence of peptidoglycan layer. In antifungal assay, *T. ammi* showed highest percentage inhibition as compared to *N. sativa* against *Aspergillus flavus*. Antioxidant assay was performed using DPPH method and it was observed that *T. ammi* have more free radical scavenging activity as indicated by low IC50. Cytotoxic assay was performed by following brine shrimp lethality assay (BSLA) and it was observed that *T. ammi* has more cytotoxicity.

Keywords: *Nigella sativa*, *Trachyspermum ammi*, Antimicrobial, Antibacterial, Antifungal, Antioxidant, Cytotoxic, Cardiovascular Disease.

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Abbreviations

AE	Aqueous Extract
CVD	Cardiovascular Disease
Cu	Copper
DMH	Dimethylhydrazine
Fe	Iron
GIT	Gastro Intestinal Tract
I	Iodine
Min	Minutes
mL	Milliliter
Mn	Manganese
N. sativa	*Nigella sativa*
p	Phosphorus
T. ammi	*Trachyspermum ammi*
TQ	Thymoquinone
WHO	World Health Organization
Zn	Zinc

Chapter 1

Introduction

1.1 Background

The term "Medicinal plant" comprises different kinds of plants used in Herbalism "herbology or herbal medicine. It is the study of uses of plants for various medicinal purposes. The Latin word herb is originated from Herba and Herbe is an old French word. Recently, in these days' herb refers to several parts of plant like seeds, stem, bark, blossom, leaves, or roots and consist of non-woody plants. Earlier the expression of herb was just linked with non-woody plants, they may include those that begin from non-trees and bushes [1].

Plants are good friends of human being. They fulfill life needs such as medicine, fuel, food, and to build houses. World Health Organization has recorded 21, 000 plant species have the prospective for being used as medicinal plants. Plants contain bioactive constituent including group of chemicals like that tannins, lignins, coumarone, quinones, stilbenes, xanthones, phenolic acids, flavones, catechins, anthocyanins, and proanthocyanins. These bioactive compounds have the potential to be used in treatment of various disorders and boost lifespan. Throughout the world, the major reason of mortality is contiguous diseases and every day 50,000 people die due to these lethal diseases. In plants, bioactive constituents play important roles in modern drug development [2].

Indigenous societies, for example, Rome, Egypt, Iran, Africa and America and additionally Pakistan utilized herbs in their restoration way of life, at the same time as others created customary restorative frameworks, for example, Unani, Ayurveda and Chinese Medicine in which home grown treatments were utilized methodically. Ayurveda, Unani, Siddha and Society (intrinsic) drugs are the genuine structures of indigenous pharmaceuticals. Among these structures, Ayurveda and Unani medicines are made and extensively practiced in India. Treatment with medicinal plants is seen very safe as there is no or negligible side effect. The good fact is that, usage of herbal medications is free of any age group and the sexual base. Restorative plants, for example, aloe vera, tulsi, neem, turmeric, kalonji, ajwain and ginger used to treat ordinary infections. Herbal plants are rich resources of constituents, which can be used in drug development pharmacologically, non-pharmacologically or modern drugs. Beside the remedial uses, herbs are moreover used in trademark shading, bug control, sustenance, smell, tea and so on [1].

In Pakistan, about 400–600 medicinal plants are recognized to be use in the usual health-care system [3]. Major research activities in the study of medicinal plants are on the recording level in Pakistan. This research is mostly conducted in universities that also include botanical listing of resources. In different regions of Pakistan population has ancient information about plants, uses of the plants, and presence of medicinal plants in their area. From generation to generation, the knowledge about plants has been transferred. These plants are used for any kind of disease from headache to stomachache or cut and wound [4].

Some people believe that plants are created to give medical treatment and other effects. 80% people of the world live in the underdeveloped countries and world Health Organization proved that almost 80% of these people are fully dependent on traditional medicine. It means that in an underdeveloped country, public use therapeutic drugs on regular basis. Herbal plants are the “backbone” of habitual medicine. In the world, there are 2000 cultural groups and almost every group has its own traditional medical awareness and experiences. Obtained Information from local public may use as a guideline for drug development under the assumption.

Some Plants have an allopathic application because of wide used for longer period of time. In the world, the most common herbal remedies are used for the treatment of respiratory infections [5].

Rural area of Pakistan (Sindh and Baluchistan provinces) has the best representation of herbal medicine rich history of use. These are the native homes of medicinal plants, famous for giving primary health care in traditional system. In native community, plants were collected and sold. For health problems different species of plant has been used. With respect to plants, approximately 54% taxa belong to xerophytes and halophytes 40% and remaining few of them are glycophytes 6%. In this study, it is noted herbs (43%), shrub (31%), tress (19%) and some grasses (7%). Some of plants were reported on the basics annuals and perennials. Annuals are(28%) and perennials are (72%). Different parts of the plants have been used for making the medicine such as full plant (24%), fruits (23%), leafs (21%) these are more commonly parts of plant used. At this time root (9%), stem (10%), seeds (5%) and flowers (3%) also used. In different situations bark, raisin, latex, legume and gum also used [6]. Now a day, herbal therapy has gained, the significant role in human health care [7].

About 25% of the entire prescriptions dispensed in municipal pharmacies include drugs that are extracted from the plants and 64% population depends on herbal medicines for cure and treatments. About 7500 medicinal plants are traditionally used in India. On the other hand, many of the countries like Korea, China, and Malaysia and some southeast Asian countries are leading the world for the uses of herbal medicines. Vegetation of Pakistan consist of 7000 species and less than 10%medicinal plants are used for different diseases treatment [8]. Vitis vinifera (Angir) belong to family Caprifoliaceae. The fruit of V. vinifera use for treating the dental diseases it gave the pain relief and this is more effective [9].

Natural products play a vital role in the field of new drugs research and development. New research has introduced the use of therapeutic plants for the cure and inhibition of different diseases like that of diabetes, cardiovascular diseases, arthrosclerosis, cancer, and neurological disorder. Alternations in redox state is

the most beneficial effects of medicinal plants. It is noted that utmost diseases which are treated with different medicinal herbs [10].

Herbal medicines have been introducing the new products of plants as chemotherapeutic agents. The use of medicinal plant in Pakistan had been increased during the last few years and have able for the collection of significant information. Medicinal plants have been acknowledged from different regions of KPK of Pakistan. In Northern Himalaya ranges district Abbottabad (KPK) medicinal plants are used for wound healing [11].

Last few decades ago most people believe in the use of medicinal plants. people believe that herbal medicine when there were no modern medicine and no information about the molecular and cellular functions of the body. some herbs and spices are useful for lowering the blood glucose and useful for the treatment of type 2 diabetes, as reported in literature. Medicinal plants were used for different types of diseases.Different plant constituents help in blood glucose lowering before the discovery of insulin and glucose-lowering agents. A large number of pharmacological researches on the antidiabetic effects of medicinal plants resulted in an increase in the number of people who use these natural compounds to control their disease [12].

Medicinal plants are more effective and reasonable. Medicinal plants have active constituents that are helpful for the treatment of different human diseases. As antimicrobial substance plant extract has been used in rural areas, many of plants are used as medicine because they are easily available and less in price than latest medicine [13]. Many plant parts are used as the raw herbs and they have many herbal activities. For chronic and infectious diseases many herbal plants are used. Throughout the medicinal plants like (*N. sativa*) that is native to south Asia and have vast historical and cultural heritage, due to wide range of medicinal activities are mostly use in Iran. In the Middle East and South Asia, *N. sativa* seeds have been used particularly to fight against the diseases and for health promotion. In south Asia, *N. sativa* is known as kalonji in Arabic as Habbat-ul-sauda and commonly known as black cumin in English. For the usage of numerous diseases

like rheumatoid, arthritis, asthma, inflammatory diseases, diabetes and digestive disorders *N. sativa* seed and oil also have been used in Southeast Asia, Northern Africa, and the Middle East. The grains syrup of *N. sativa* seeds more effective for the digestion, dysmenorrheal, loss of appetite, treatment of worms and skin rashes [14].

T .ammi seeds have variously culinary used in households like pickles, dishes and different types of biscuits. Different parts of *T. ammi* plant like root and leaves are used for different purposes. The Root that contains febrifuge, carminative and diuretic properties that are useful in stomach troubles. For the treatment of abdominal pain, abdominal tumor and piles it is also used. Leaves of ajwain plants have antihelmintic properties that are useful for helminth infections that occur in animals. The oil of ajwain seeds also has been used to treat cough and work against pulmonary disorders e.g asthma. The seeds of *T. ammi* have many medicinal property they may contain anti-vomiting, diuretic, anti-asthma and antidysponea effect. *T. ammi* cannot be ignored in medicinal usage. *T. ammi* contains of active components that are used for the cure of different human diseases. *T. ammi* plant used as a medication that encompasses an immense assortment of components used for the cure of contagious and chronic diseases. In India, medicative herbs practiced in ancient folk's medication were screened for the presence of antimicrobial activity. additionally, *T. ammi* used for the cure of (GIT) and abdomen issues, [15].

1.2 Problem Statement

Synthetic drugs have been used since long but there are a lot of side effects of these drugs and also different microorganisms are becoming resistant to these medicines. So the need for hours is to develop such medicine which has less adverse effects on health.

1.3 Aims and Objective

Medicinal plants have an extensive history of safe use in human. Not only added the flavor in the food and helpful in the treatment of various disorders. Medicinal plants prove to be friendly alternative of synthetic drugs as they are devoid of side effect and toxicity. The current study was designed for exploration of drugs from the medicinal plants.
Thus in the exploration of drugs from medicinal plants current study was designed.

The current study explains following objectives.

1 . Preparation of aqueous extracts of *T. ammi* seeds and *N. sativa* seeds

2 . Determination of antimicrobial and antioxidant properties of these seeds extracts

3 . Determination of cytotoxic potential of these seeds extracts

1.4 Scope of the Research

As drugs originated from herbs prove to be cheap, non-toxic and friendly for biological system as compared to other synthetic drugs, thus plants under study can be explored further as potential therapeutic agents.

Chapter 2

Literature Review

Plants medicine and phytomedicine, they may be called herbal medicine, is the use of plants like seeds, leafs, roots, blossoms and bark for the purposes of humans and animal's healthcare. Since the prehistoric time they have been used by the people worldwide to cure, stop and manage different variety of diseases. In the 19th century starting with the morphine that is separated from the opium and now a day's active compounds are isolated from plants. Different drugs discovered from plants and large number of drugs isolated like that codeine, cocaine, quinie, digitoxin and morphine also. Still some of these drugs are used [16].

The traditional medicine practice is well-known in Japan, Pakistan, China, India, Sri Lanka and Thailand. In china about 40% the medicinal use is recognized to herbal medicines. Now the development of human culture therapeutic herbs played vital character, for example optimisms and different ceremonies. Among the variety of modern drugs, many of them are produced indirectly from herbs, for example aspirin. Garlic a food crop has many medicinal effects. The study of herbal plants helps to understand herb as well poisonous herb and how to protect human as well as animals from the natural poison. The medicinal properties of herbs are due to secondary metabolite production of the plants [17].

In many communities, for primary health care system herbal drugs are still used. For their medical purposes, world population use medicinal plants more than 60%

and in developing countries 80% [18]. People depend on medicinal plants because of so many reasons, like affordability, ease of access and low-priced [19].

Medicinal plants are used to cure the many kinds of diseases. Different parts of plants like leaves, stem, bark, root, seeds etc. are used to prevent allay symptoms, and abnormalities into normality's. The herbal remedies' practice does not adhere strictly to facts accrued using scientific approaches. However, the most of the pharmaceutical products currently dispensed by physicians and have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine. Active compounds are obtained from higher plants and these compounds are utilized to develop modern medicine, and about 80% of these active compounds show a positive correlation between their modern therapeutic use and the traditional uses. Now a day, Use of herbal medicines and dietary supplements are obtained from plants and plant sources increasing day by day. [20].

2.1 Ajwain (*T. ammi*)

Ajwain botanical name is *T. ammi* is anuual herbaceous plant. It belongs to the family Apiaceae. It has an erect and striate stem and grows up to 90 cm tall as shown in figure 2.1. *T. ammi* plant have active constituents that are helpful for the treatment of different human diseases. As antimicrobial substance, plant extract has been used in rural areas, many of plants like tulsi, neem, turmeric is used as medicine because they are easily available [21].

2.1.1 Taxonomic Classification

Class: Magnoliopsida

Subclass: Rosidae

Order: Apiales

Family: Apiaceae

Genus: *Trachyspermum*

Species: *ammi*

Botanical name: *T. ammi* [22].

FIGURE 2.1: *T. ammi*

2.1.2 Morphology of the Plant

T. ammi grow Up to 70-80cm in height that is annual herb, stem is striate, distant leafs, and linear segments. Flowers are white in color; seeds are smaller in size and yellowish brown in color. Owa, omum, Bishop's weed, carom and Ethiopian cumin many others name of ajwain.

2.1.3 Origin

Firstly *T. ammi* originated from India and Egypt. In India *T. ammi* is native but in semiarid and other regions of world like Pakistan, Iran, Egypt and Afghanistan. In northern part of India in the states like Rajasthan, Gujarat, Uttar Pradesh, Andhra Pradesh and Punjab ajwain broadly spread [23].

2.1.4 Habitat

In arid and semi-arid regions *T. ammi* grows. These earths have abundant amount of salts [24].

2.1.5 Culinary Uses

Ajwain seeds are almost exclusively used kitchen. In pulse dishes such as dhal, as well as vegetable dishes and pickles, they are mainly found. The severe flavor of ajwain has the capacity to change the rich flavors and densely spiced foods [25].

2.1.6 Traditional Medicinal Uses of *T. ammi*

Aqueous extract of *T. ammi* is use for diarrhea. *T. ammi* seed have many activities such as antidiarrhoeal, antiseptic, antispasmodic, carminative, stimulant, stomachic, and tonic. The other beneficial effect of *T. ammi* seed are also helpful to cure from bronchitis, atonic dyspepsia and gassiness, dipsomania, hysteria, sore throat, plaster or poultice applied to abdomen in cramp. In bronchial pneumonia and other, respiratory disorders *T ammi* beneficial [26]. The proper functioning of respirational system and the kidney they play important role. By the existence of a phytochemical, thymol clear the pungent taste, thymol is strong bactericide, fungicide, anti-spasmodic and for the throat irritation, also used for mouthwash [27].

2.1.6.1 Abdominal Pain

For abdominal pain, homemade remedies work properly. For different problems like digestive infections, asthma, colic griping pain, cramps muscle spasms, edema and rheumatism. 2 teaspoons of grind soybean seeds and 1 teaspoon of grind *T. ammi* seeds are mixed. Take 570mL water in a pan. All the things are mixed and boil for 2 minutes give no more extra time. After boiling, the mixture drink

regularly it gives relief. Externally the act as an antiseptic. Mostly used to clean the wounds and treat the skin infections [28].

Rural people use *T. ammi* for the preservation of flatulence and gas. They took half kilogram *T. ammi* seeds and each of 20 g of black salt, rock salt and table salt all salts and seed are mix properly. After mixing these are added into half kilogram of lemon juice for several days to be dry its own. One tablespoon of this mixture taking with warm water, this is best home remedy for the cure of nausea and vomiting [29].

2.1.6.2 Gastro Protective Activity

T. ammi play vital role in digestion. It can be taken with buttermilk to alleviate digestion linked other issues. It acts as anti-acidic agent. *T.ammi* is beneficial in improving spasmodic pains of the stomach and intestines, in young's on the other hand of children's. Abdomen pain due to flatulence, indigestion and intestinal infection can easily be comforted by taking one spoon of *T.ammi* with 2-3 pinches of salt in warm water [30]. *T. ammi* seed indicated antiulcer activity via different ulcer models. Animals pretreated with extract play major role decrease in ulcer index and percentage ulcer protection in all models as well human beings [31].

2.1.6.3 Chest Problem

For the bronchitis and other chest infection in child the paste of *T. ammi* seed apply over chest to relief pain. Leafs of *Pergularia daemia* (milky weeds) are mixed with *T. ammi* seeds are boiled in water and the aqueous extract is obtained. The extract is taken orally for 7 days against cough and asthma for quick relief [32].

2.1.6.4 Respiratory Disorders

T. ammi seed mixed with clove and a pinch of salt are chewed for the pharyngitis and influenza treatment. A tablespoon of *T. ammi* seeds crushed and hold it in

a cloth and inhaled for the cure of common cold and nasal congestion; during the sleeping hours' patients cover with blanket. Infusion of seeds with salt is useful for the treatment of acute pharyngitis, sore and congested throat and hoarseness of the voice due to colds. *T. ammi* seeds are tied in the cotton cloth, heated in a frying pan and applied on the chest and neck when still warm in case of bronchial asthma.

2.1.6.5 Migraine

To get the cure from migraine seeds of *T. ammi* can be smoked or sniffed normally [33].

2.1.7 Chemical Constituents

Major resources of ajwain are protein, moisture, fiber, carbohydrate, flavonoids, nicotinic acid, thiamine, riboflavin and mineral includes Ca, P, Fe, Cu, I and Mn [34]. Different chemical compounds found in *T. ammi*. The oil *T. ammi* seeds contain 96.3% active compounds. The most important compound is thymol (39%), along with p-cymene (30.8%), γ-terpinene (23.2%), γ-pinene (1.7%) and terpinene-4-ol (0.8%). In acetone extract of *T. ammi* seeds 18 chemical constituents are found and the entire percentage is 68.8%. The most important constitute thymol is (39.1%) remaining other like oleic acid is (10.4%), linoleic acid is (9.6%), p-cymene is (1.6%), palmitic acid is (1.6%) and xylene is (0.1%) [35]. Chemical structure of thymol shown in figure 2.2. In aqueous extract of T.ammi seeds different constituents are present like Monoterpenoid, 3, 7-Dimethyloct-3(10)-ene-1, 2, 6, 7-tetrol; and Glucide namely (3R)-2-Hydroxymethylbutane- 1,2,3,4-tetrol. Other glucosides such as 1-Deoxy- L-Erythritol and 1-Deoxypentitol and also nucleosides as adenosine and uridine were obtained from the T.ammi seeds.

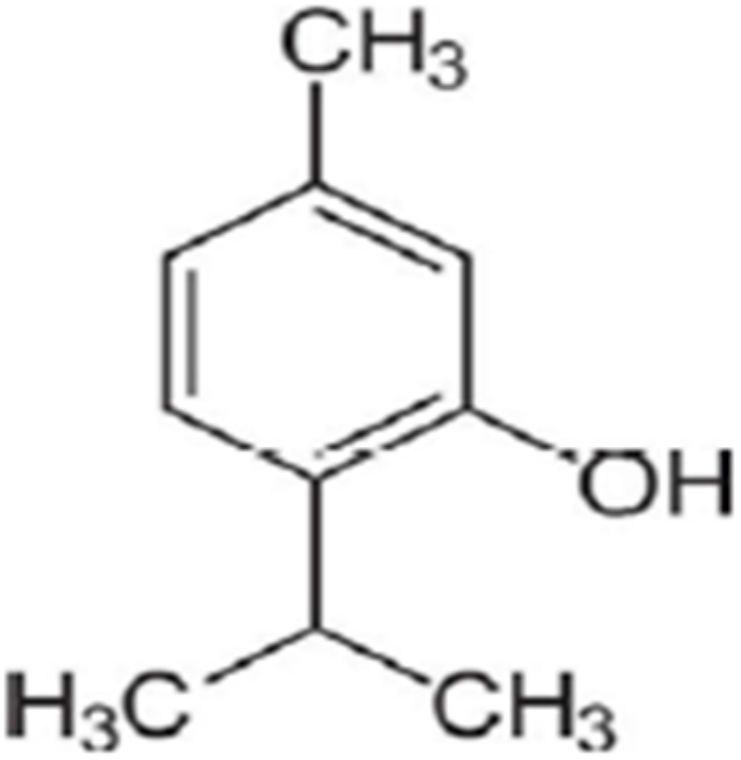

FIGURE 2.2: Chemical Structure of Thymol [36].

2.1.8 Reported Therapeutic Activities

Ajwain have aromatic smell and bitter taste is commonly use in spice. These seeds used for flavoring numerous foods, as preservatives, in medicine and use for the manufacturing of essential oil in perfumery [37]. In system of medicine ajwain seed play significance role for the treatment of stomach disease, for the relieving colic pain the paste of crushed seed is applied externally [38]. *T. ammi* seeds have antimicrobial, digestive stimulant, antispasmodic, antihypertensive, anti-inflammatory, antifilarial, gastro protective, and detoxification of aflatoxins effects. *T. ammi* fruits has therapeutic activities include; antimicrobial, stomachic, carminative and expectorant. Seeds are soaked in lemon juice with *Prunusa mygdalus* (badam) are given in curing amenorrhea and it is used as antipyretic, febrifugal and in the treatment of typhoid fever [39]. *T. ammi* contain various other therapeutic like that antibacterial, antifungal, antioxidant, anthelmintic, antiflatulent, antiplatelet-aggregatory, and other shown in figure 2.3

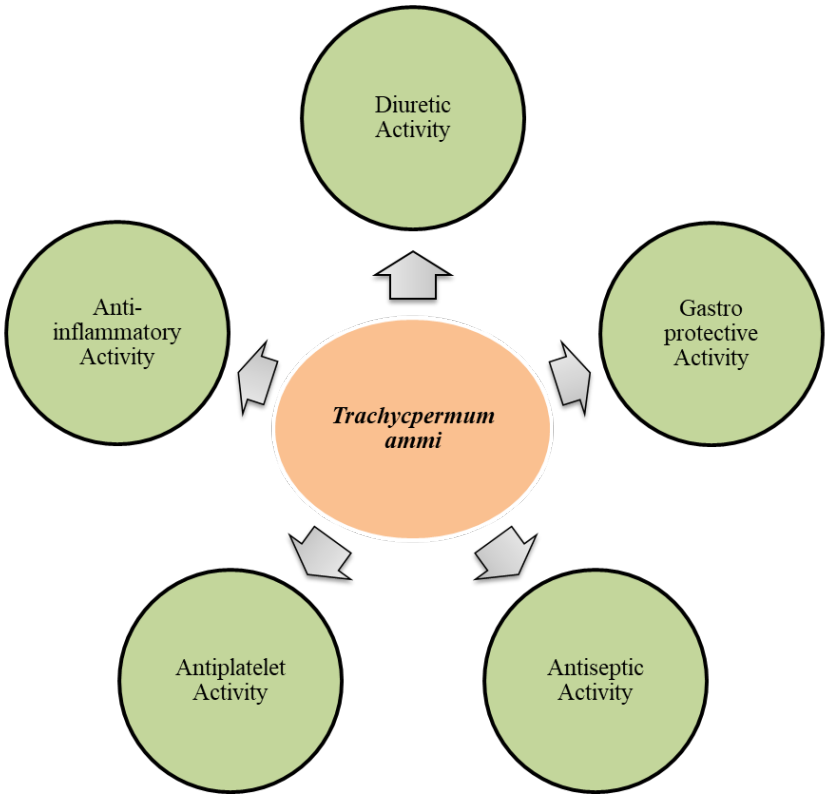

FIGURE 2.3: Theraputic Activities of *T. ammi* [40].

2.1.8.1 Antimicrobial Actions

The antimicrobial properties of *T. ammi* protect foodstuff against microbial spoilage. By conducting laboratory assay of antimicrobial efficiency in vitro and it's also investigated by using antimicrobial in human. The chief compounds that are present in *T. ammi* are thymol and carvacol. These are responsible for the antimicrobial property and by the presence of thymol bacteria destroy. Thymol resists to prevalent third generation antibiotic. Multi drug resistant microbial pathogens and thus they work as a plant based 4th generation herbal antibiotic formulation.

2.1.8.2 Antifungal Activity

T. ammi extract play vital role against the fungal strains. Antifungal activities are tested and they found that 72-90% control the growth of all fungal tests due to phenolic compounds. The active compounds (thymol and carvacol) are known to be also bactericidal or bacteriostatic agents depend upon concentration used.

2.1.8.3 Anti-inflammatory Potential

Anti-inflammatory properties of total aqueous extract of *T. ammi* seed were reported. The extract has efficiency to show anti inflammatory properties both the animal models. In animal the weight of adrenal glands was found to be significantly increased to treat animals with aqueous extract. Total aqueous extract of *T. ammi* seed show the significant anti inflammatory action.

2.1.8.4 Antihypertensive, Antispasmodic and Bronchodilating Studies

For the traditional uses the aqueous extract of *T. ammi* show antihypertensive, antispasmodic and bronchodilating activities. They studied that the extract of *T. ammi* is dose dependent. The fall in blood pressure, inhibit the broncho constriction and show hepatoprotective effects [41].

2.1.8.5 Antioxidant Activity

In food or pharmaceutical industry it is uses as natural antioxidants. *T. ammi* extract show more antioxidant activity against DPPH [42].

2.2 Kalonji (*N. sativa*)

N. sativa usually known as black seed. It belongs to the family Rananculacea. Annual herbs and bushy almost grow in various part of the world. In Pakistan and India mostly cultivated, it is amazing herb containing historical and religious background. Black seeds are the foundation of active components of the plants. Its grow 50 to 60 cm in height as shown in figure 2.4. Leafs are divide into linear segment long 2-3 cm. Many plant parts are used as the raw herbs and they have many herbal activities. For the chronic and infectious diseases, many herbal plants also used [43].

2.2.1 Taxonomic Classification

Class: Magnoliopsida

Subclass: Magnoliidae

Order: Ranunculales

Family: Ranunculaceae

Genera: *Nigella*

Species: *sativa*

Botanical name: *N. sativa*.

FIGURE 2.4: *N. sativa* [44]

2.2.2 Morphology of the Plant

Small shrub grows to 20-90cm tall that bear different flowers are white, yellow, pink, purplish and flower consist 5-10 petals [45]. The fruit have large capsule which bears large number of *N. sativa* seed. *N. sativa* seeds also as Black seed. In English *N. sativa* seeds known as black cumin. In Arabic Habbat el Baraka or Habbahsawda.In India and Pakistan commonly called kalonji [46].

2.2.3 Origin

Firstly *Nigella sativa* originated in India and Arab. Native to the geographic area linked with Southern Europe, North Africa and Southwest Asia and in many countries of Middle Eastern Mediterranean region, south Europe, and also in India, Pakistan, Turkey and Saudi Arabia *N. sativa* is cultivated [47].

2.2.4 Habitat

It cannot grow in the shade. Dry or moist soil is suitable for growing these plants [48].

2.2.5 Culinary Uses

In food like flavoring additive in the breads and pickles black seeds are also used the reason is that it has very low level of toxicity. Black seeds also use for the spice and food preservative [49].

2.2.6 Traditional Medicinal uses of *N. sativa*

Thousands of years, in starting millions of people in the Mediterranean area and East countries use the oil of *N. sativa* seeds daily as a natural defending and therapeutic remedy. Generally, it is prove that *N. sativa* seeds also prescribed by ancient Egyptian and Greek physicians for the cure of headache, nasal congestion, toothache and intestinal worm in addition diuretic to promote menstruation and production of milk.

The seed of *Nigella sativa* have been used in obesity and dyspnoea. Black seeds have antibilious property and controlled the sporadic fever. By the inhalation of deep-fried seeds of *N. sativa* relieve the cold and mucus. These seeds have many other effects like effect on cardiovascular system, obesity, respiratory system,

gastro-intestinal tract, nervous system, reproductive system and so many others [50].

By the presences of biological active Constituents, they work against the anti-cancer activity and cardiovascular diseases and these two diseases are the leading reasons of death in low on the other hand increase the income countries. CVD'S are the main reason of worldwide [51]. In developing countries, it is estimated that over 80% of the CVD death occur [52]. According to the worldwide the second major reason of death is cancer [53].

N. sativa seeds use for the treatment of all diseases except death [54].*N. sativa* has carminative, diaphoretic, digestive, abortifacient, anodyne, anthelmintic, appetizing, diuretic, curative effects and some other health benefits shown in figure 2.5 [55].

N. sativa seeds use as herbal medicine for the cure of different diseases like fever, jaundice, diarrhea, dysentery, dyspepsia, asthma, bronchitis, cough, paralysis, piles and so many other disorders related to the cardiovascular, digestive, immune, liver respiratory and kidney system [56].

N. sativa contain active constitute Thymoquinone play important role in anti-cancer activity that have been established for colon, blood, breast, live, pancreatic, lung, kidney, skin and prostate and cervix cancer cell lines. In animal models of kidney, skin, colon, breast also lungs cancer [57].For the treatment and prevention against different disease and morbidity conditions in human *N. sativa* used as shown in figure 2.5 .

N. sativa Capable to reduce the symptoms of or treat patients by numerous diseases, they may contain diabetes, asthma, hypertention, dyslipidemia, and metabolic syndrome, natural and chemical toxicities. Thymoquinone present in *N. sativa* TQ use to prevent some disorders as well as liver, neurobehavioral and kidney disorder [58].

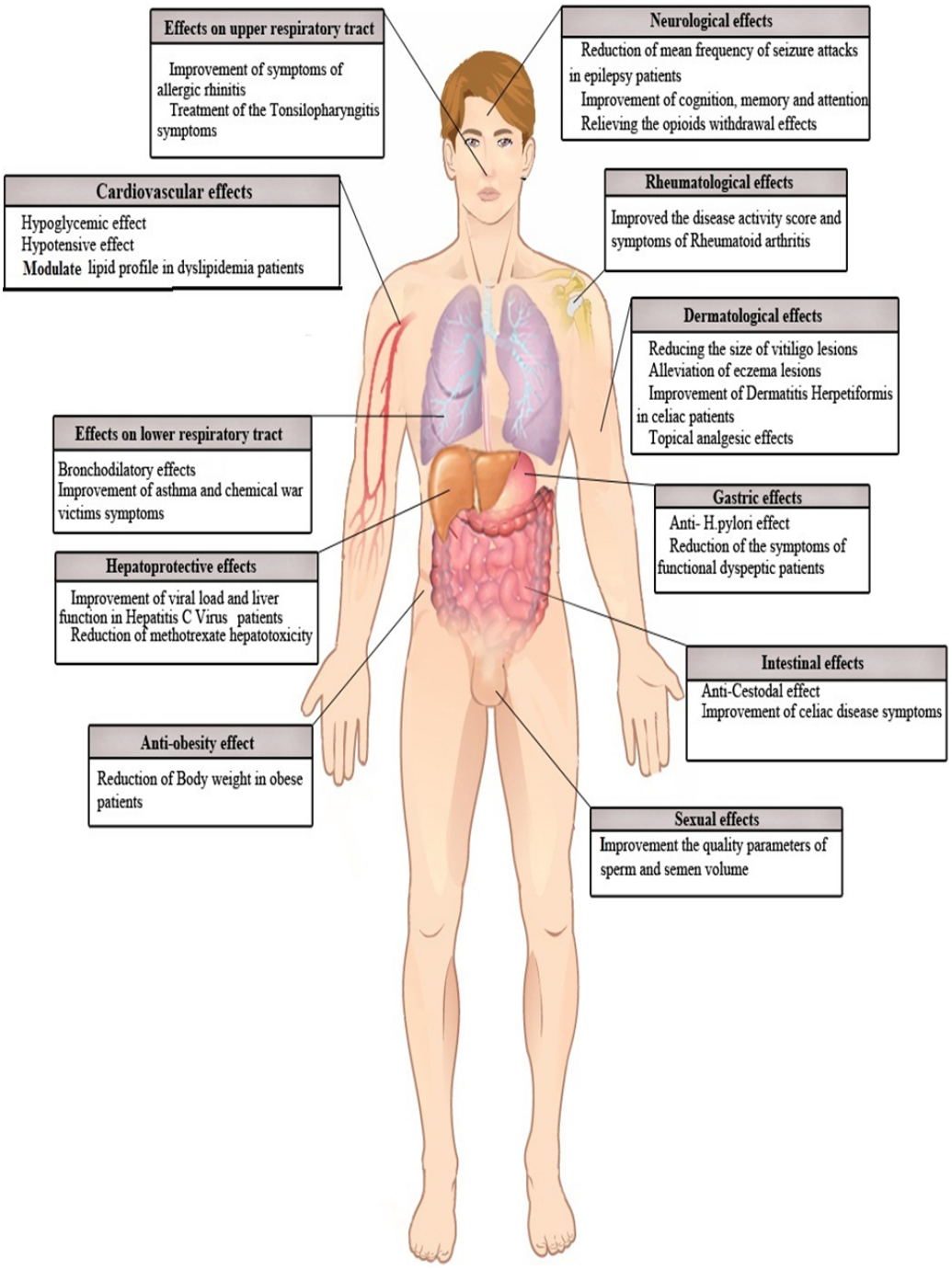

FIGURE 2.5: Effects of *N. sativa* in Different Parts of the Human Body [59]

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2.2.6.1 Obesity

Obesity is particularly additional fat in belly. The major possibility factor is heart disease [60]. *N. sativa* seed contain thymoquinon that show anti-obesity effect and on the other hand protection of cardiovascular, insulin sensitivity, anticancer and

immune modulatory effect. Intake of black seed (3g/day) can reduce the body weight, waist circumference, and systolic blood pressure. The higher dose for longer time period of black seed intake will gave better result in case of obesity as well as no side effects occur [61].

2.2.7 Chemical Constituents

Black seeds have chemical compounds that contain carbohydrates, protein, fats, oils, fibers, vitamins, minerals (Cu, Fe, P and Zn etc.), alkaloids, saponins and many others biologically active compound [62].
N. sativa contain wide range of biologically active compounds that consist of thymoquinone (TQ), thymohydroquinone, dithymoquinone (DIM), 4- terpineol, carvacrol, carvone, t-anethol, α-pinene, thymol, α-hederin, limonene and small amount of other compounds also found [63]. Oleic acid, linolenic acid, linoleic acid, eicodadienoic acid, arachidic acid, palmitoleic acid, palmitic acid, stearic acid and myristic acid these fatty acid present in black seeds [64, 65].

2.2.8 Reported Therapeutic Activities

These seeds contain Therapeutic Characteristic that are anti-fungal, antimicrobial, antioxidant, Anti-asthmatic effects, gastro protective, antidiabetic, anticancer, anti- inflammatory and anti-histamatic effect different activites shown figure 2.6 [66].

2.2.8.1 Antibacterial Activity

The extract of black seeds show antimicrobial activities against different bacterial strains. They may be Gram positive and Gram negative. Extract of *N. sativa*. *N. sativa* show resistances against antibiotic, and gram negative particularly. Black seeds extract show strong antibacterial potential against some of the test organisms. Most valuable extract is alkaloid and aqueous extract. The extract show

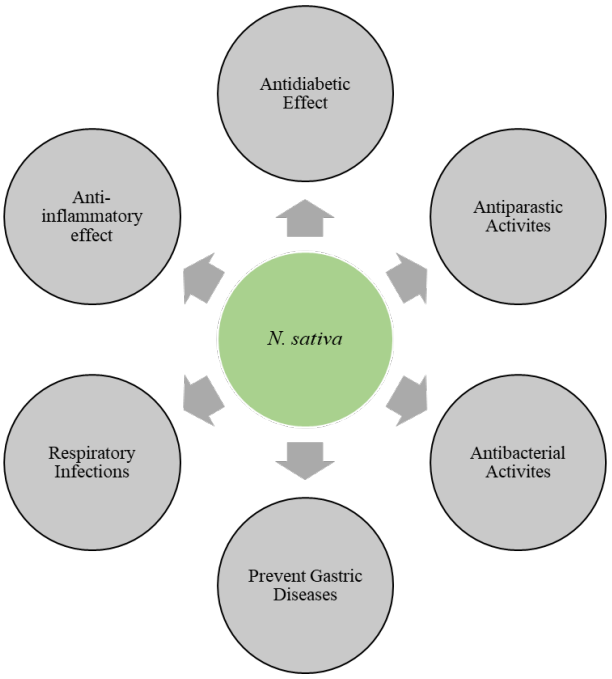

FIGURE 2.6: Therapeutic Activities of *N. sativa*

more antibacterial potential and more effective in gram negative as compare to gram positive [67].

2.2.8.2 Antifungal Activity

N. sativa seeds extract show very strong antifungal potential against different antifungal strains. *N. sativa* aqueous extract use to inhibitory effect against the candidiasis in mice [68]. In the extract of *N. sativa* they found that two novel defenses known as Ns-D1 and Ns-D2. Ns-D1 and Ns-D2 both of showed different antifungal properties toward the phytpathogenenic fungi [69].

2.2.8.3 Antioxidant Activity

N. sativa extract showed strong antioxidant potential using the oxygen free radicals absorbance capacity method. In Wistar rats TQ suppress the Fe-NTA-induce oxidative stress hyperproliferative response and renal carcinogenesis [70]. Black seed can be taken as dietary supplement. In rats the crushed from of *N. sativa*

seeds control the oxidative stress due to oxidized com oil. TQ show modulatory effect on erythrocytes lipid peroxidation and antioxidant status during 1, 2-dimethylhydrazine- (DMH-) reduce colon carcinogenesis after initiation in male Wistar rats were studied [71].

2.2.8.4 Anti-asthmatic Effects

TQ and nigellone show antispasmodic effect and they used for trachea and respiratory clearance. By the prescence of Ba2+ carbachol and leukotriene that induce trachea construction. Both are concentration dependent, Nigellone and TQ show inhibitory effects on trachea when contracted start by the depolarizing effect of Ba2+. Trachea contractions reduce by the leukotriene-d (4) LT4 has been inhibiting by TQ and nigellone. Nigellone show more antispasmodic effect as compare to TQ. TQ does not have these effects and nigellone helpful for the mucociliary clearance. Thus it is reported that nigellone but not TQ may also be use to treatment of many respiratory diseases [72].

2.2.8.5 Gastro-Protective Activity

Aqueous extract of *N. sativa* show anti-ulcer activities that reduce the gastric ulcers and basal gastric secretion in rats. Aqueous extract also use as Unani and herbal medicine practitioners. Due to different chemical noxious Wistar albino rats are infected with acute gastric ulceration.

Anti secretory observes undertake the individually group of rats. It is estimated that gastric wall have mucus content and nonprotein sulfhoydryl concentration as well as gastric tissue were observed histopathologically. *N. sativa* have anti-ulcer potential probably prostaglandin-mediated or through its anti-secretory and antioxidant properties [73].

2.2.8.6 Anticancer Activity

Anticancer potential of *N. sativa* has ability the natural killer cell properties were examines in cancer effectd person that reciving multimodality immunotherapy program [74]. TQ show antineoplastic activity that helpful in mouse keratinocytes, papilloma (SP-1) and spindle- 17 carcinoma cells. In SP-1 cells, TQ induce G0/G1 cell cycle and the active role for TQ as a chemopreventive agent, generally near the beginning stages of tumorigenesis [75].

Antitumor activity of thymoquinone and thymohydroquinone was also demonstrated using tumor cell lines and fibrosarcoma, and murine and squamous cell carcinoma [76].

In a mouse xeno-graft model, mixture of thymoquinone with diosgenin significantly reduced tumor volume, mass as well as increased apoptosis [77].

2.2.8.7 Antidiabetic Activity

In rats *N. sativa* play important role in glucose lowering effect. Due to the inhibition of hepatic gluconeogenesis, *N. sativa* exposed the blood glucose lowering effect. The extract is use as therapeutic agent for the treatment of non-insulin dependent diabetes mellitus [78, 79]. *N. sativa* use to the lower density lipoprotein cholesterol, or fasting blood glucose indicating effective as .an add-on therapy in patients of insulin resistance syndrome [80].

2.3 Bioassay

"The procedure which are used for the biological evaluations, concentration or purity of material by determine the impact on cell tissue, organisms and enzyme or receptor preparation linked to a standard ground work are known as bioassay". Bioassays play important role in the development of drugs and investigation of pollutants in the environment.

2.3.1 Why We Use Bioassay?

- Bioassays used to find out the drug toxicity.
- To calculate the unknown substance concentration.
- To determine the pharmacological potential of substance.
- To retrieve the amount of pollutants that are released by a specific source [81].

2.3.2 Antibacterial Assay

Pathogenic microorganisms for example fungi, bacteria and algae are causing different diseases in plant, animals and as well as human. The major reasons of death are bacterial and fungal infections. In all over the world the best and most significant discovery is penicillin discovery. Penicillin is use as antibiotic. From the natural sources and organic products different new antibiotics were isolated and after the synthesized they used in medical practices.

Human work against the bacteria and fungi is unluckily far from over because of different causes. One of the major causes is that with the passage of time new species of pathogens are discovered and their incredible abilities gain the resistance against antibiotic. This is continuing process [82]. Different bacterial strains are selected in this study were based on the significance as human pathogens.

2.3.2.1 *Bacillus subtilis*

"*Vibrio subtilis*, is a bacterium which was found by Christian Gottfried Ehrenberg in 1835. It was renamed in 1872 by Ferdinand Cohn". *B. subtilis* could be a Gram-positive, high-impact bacterium. It is catalase-positive, rod-shaped and generally found in soil and the gastrointestinal tract of ruminants and people

FIGURE 2.7: *B. subtilis* [83]

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shown in figure 2.7. The survival range of this bacterium is within 30-39°C. It is warm resistance and its spores can survive the warm connected amid cooking. It has been embroiled in nourishment harming caused by poor quality pastry kitchen items among others. *B. subtilis* nourishment harming includes a fast onset and with acute vomiting, commonly taken after by the runs.

2.3.2.2 *Staphylococcus aureus*

S. aureus Found by Sir Alexander Ogston in 1880. *S. aureus*, is a Gram-positive circular microscopic organisms that commonly causes surgical and skin diseases, bacteraemia and nourishment poisoning. They begin with portion of the title,

"*staph*", may be a reference to clusters of "grapes" that the life form shapes. The "coccus" portion alludes to the circular shape of the microscopic organisms.

The '*aureus*' is from its brilliant colour and suvive between 18 - 40°C.Up to 20% of the human populace may be a carrier of this bacteria.

It is one of the foremost common causes of healing center procured diseases and is one of the five most common causes of contamination after damage or surgery. It can be transmitted by a number of implies, for case through discuss beads or pressurized canned products, coordinate contact with objects that are sullied (nourishment, water, lifeless objects) or bites.

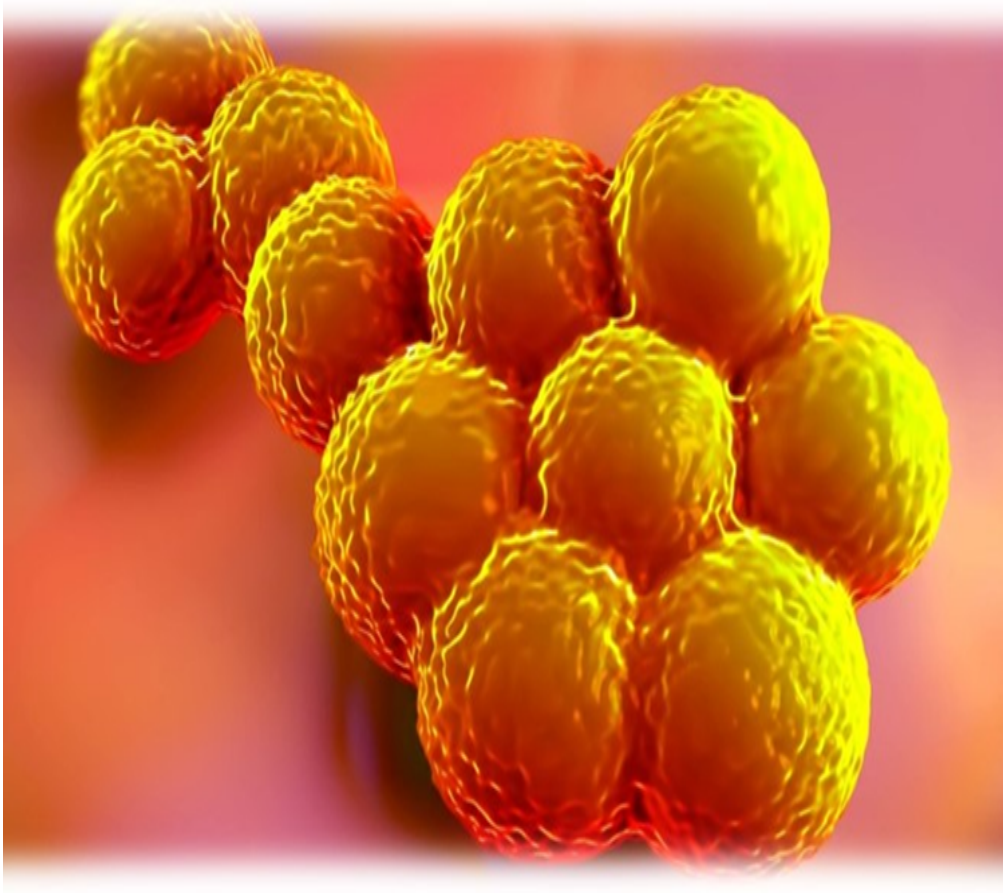

FIGURE 2.8: *S. aureus*[84]

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Infections run from mellow to life debilitating. It can cause abscesses, bubbles and other contaminations of the skin, but can moreover deliver disease in any organ of the body. The foremost common shape of nourishment harming is caused by *S.*

aureus toxins. One of the poisons created by this microscopic organisms is capable for Poisonous Stun Syndrome. Practicising great hand cleanliness will minimize the chance of infection.

2.3.2.3 *Micrococcus luteus*

M. luteus is a Gram-positive, non-motile, coccus, saprotrophic bacterium. It can shape in tetrads or unpredictable clusters but not in chains and its belongs to the family Micrococcaceae. *M. luteus* was known as Micrococcus lysodeikticus and was found by Alexander Fleming in 1928.

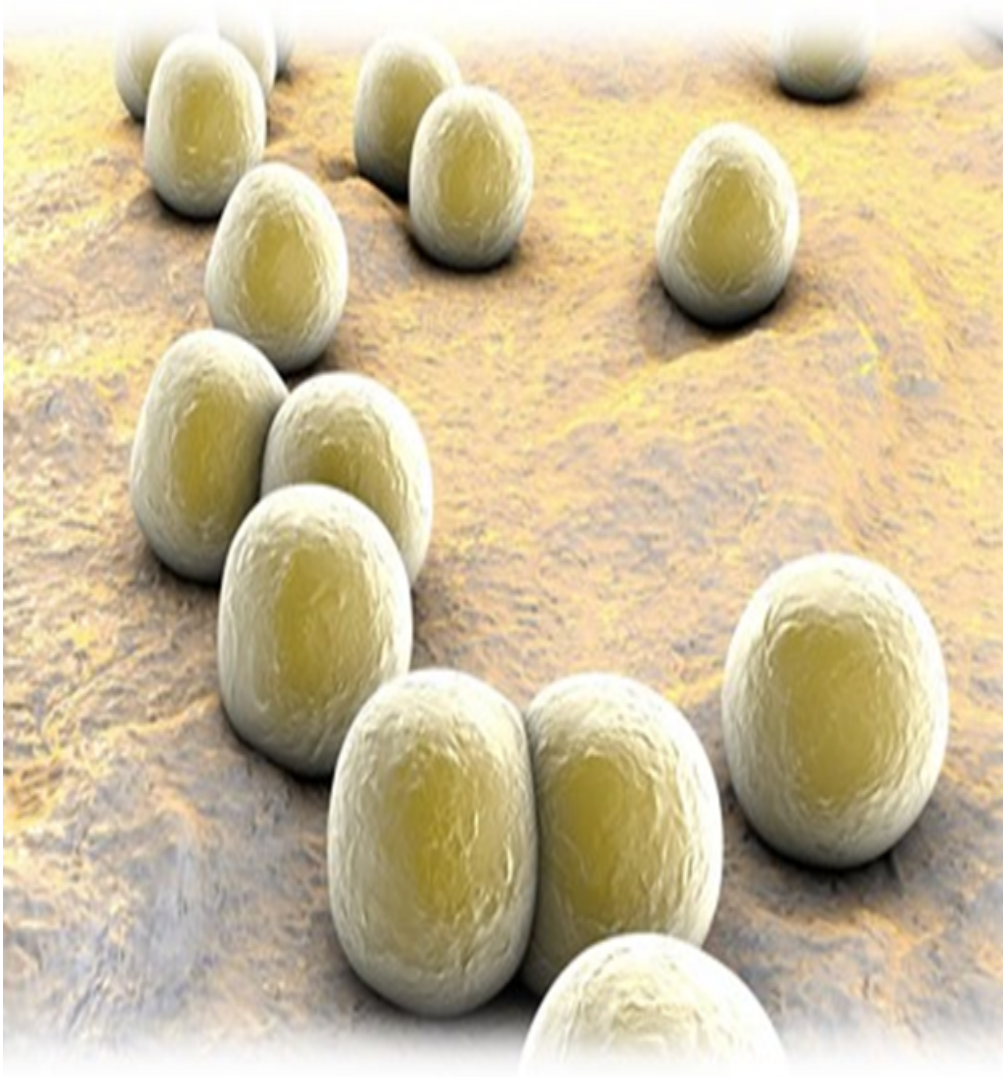

FIGURE 2.9: *M. luteus*[85]

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Its title stands for: tiny (small scale), of round shape (coccus), and yellow (luteus). *M. luteus* is found in soil, clean, water, and in human skin vegetation. It has too been confined from nourishments such as drain and goat's cheese. *M. luteus* causes odors in people when breaking down the components of sweat. *M. luteus* can cause skin diseases. This bacterium can be transmitted due to destitute hand-washing practices.

M. luteus can cause septic stun in immunocompromised people. M. luteus is an climatic microorganism commonly found on natural checking plates and it is one of the foremost common contaminants of lab societies. It is regularly watched on agar plates from bioburden testing of pre-sterilisation restorative devices.

2.3.2.4 *Salmonella* Species

Salmonella is a rod-shaped, Gram negative, non-motile microbes, which does not produce spores. This bacterium was found in 1880s and named Bacterium suipestifer. Afterward it was renamed *Salmonella* after the researcher who found it, Dr Daniel Salmon. Most reptiles and amphibians carry *Salmonella*.

Salmonella illnesses are zoonotic, spreading from creatures to people, conjointly from human to human. *Salmonella* move through their host's guts by means of flagella.

They have roughly 2,500 distinctive strains of *Salmonella*. *Salmonella* infections have been found to be more in summer than in winter. Even after symptoms of *Salmonella* disease have ceased, it is still conceivable to contaminate others.

That's why it is advisable to hold up another 48 hours after indications have vanished to go back to work or school. *Salmonella* isn't murdered within the solidifying process. *Salmonella* infections are known as salmonellosis. It takes 12 to 72 hours for indications .

FIGURE 2.10: *Salmonella* Species [86]

2.3.2.5 *Enterobacter aerogenes*

Enterobacter was to begin with proposed by Hormaeche and Edwards (1960a). In any case, the history of a few species presently set within the sort *Enterobacter* can be followed, but with a few perplexity, to the conclusion of the 19th century. "*Bacillus lactis aerogenes*" was confined by Escherich (1885) from drain and renamed "*Bacillus aerogenes*" by Kruse (1896) and "*E. aerogenes*" by Beijerinck (1900).

This microbes is Gram-negative, rod-shaped, and radially encompassed by flagellum. It can be found in dairy items, soil, and the gastrointestinal tract of creatures. *Enterobacter* species are found in environment such as water, sewage, vegetables, and soil.

Sometime recently the broad utilize of anti-microbials, *Enterobacter* species were once in a while found as pathogens, but these living beings are presently progressively experienced, causing nosocomial infection such as urinary tract contaminations and bacterium.

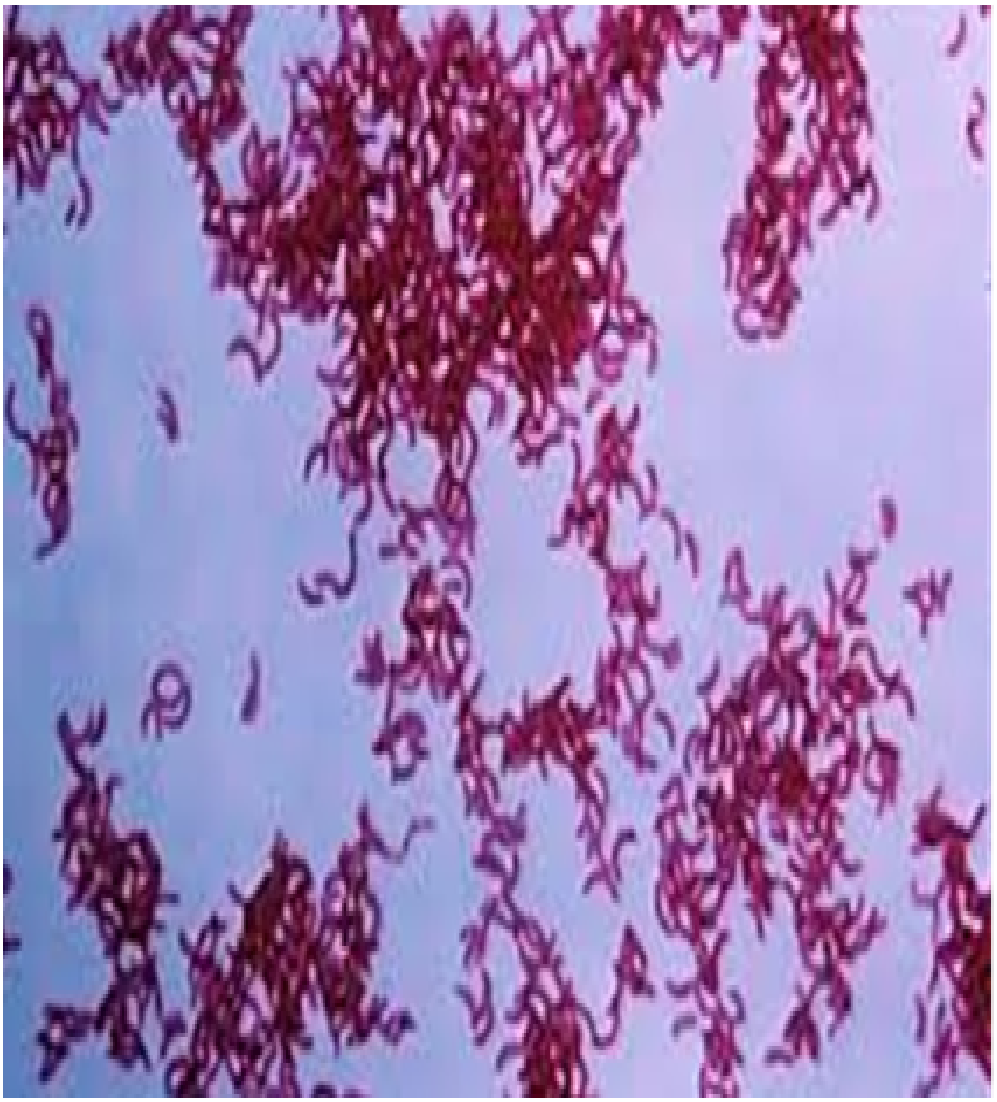

FIGURE 2.11: *E. aerogenes* [87]

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2.3.2.6 *Agrobacterium tumefaciens*

A. tumefaciens is a Gram-negative, non-sporing, motile, rod-shaped bacterium, closely related to Rhizobium which has nitrogen-fixing knobs on clover and other leguminous plants. *A. tumefaciens* causes crown gall infection of a wide run of dicotyledonous (broad-leaved) plants, particularly individuals of the rose family such as apple, pear, peach, cherry, almond, raspberry and roses. A partitioned strain, named biovar 3, causes crown gall of grapevine.

FIGURE 2.12: *A. tumefaciens* [88]

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2.3.3 Antifungal Assay

Fungal diseases in humans can be classified into (a) allergic reactions to fungal proteins, (b) toxic reactions to toxins present in certain fungi and (c) infections. Fungal infections are the most adaptable infections. Intensive therapy was starting to manage the cancer therapy, HIV infections and organ transplantation. For antifungal control and the new drugs discovery fungal pathogens are used.

In market the less number of drug availability and multidrug resistant fungal strains are used to make fresh module of antifungal drugs. In a recent study, it is reported Candida albicans had developed increased resistance against fluconazole (an antifungal drug). Hence it is needed to develop novel drugs that have the potential to cope with the harmful effects of fungal pathogens [89]. Different fungal

strain used in antifungal assay.

Fungal strain	**Diseases**
Fusarium solani	Osteomyelitis and skin infection [**?**]
Aspergillus fumigatus	Aspergillosis [**?**]

2.3.4 Cytotoxic Assay

To evaluate the toxicity of heavy metals, pesticide or medicine mainly natural plant extracts brine shrimp lethality bioassay is generally used. Firstly Michael et al was proposed cytotoxic assay in 1956. Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organisms brine shrimp [90].

2.3.5 Antioxidant Assay

In human body and food system free radical reactions occur. Normal physiology free radical presents in the form of reactive oxygen and nitrogen species. Some time these reactive species occur over production due to oxidative stress brought about the imbalance of the bodily antioxidant system and free radical formation. These species react with biomolecules causing cellular injury and death. They lead the chronic diseases like that cancer and cardiovascular systems. The free radical DPPH (2, 2 diphenyl-1-picrylhydrazyl) have been used more for the determination of primary antioxidant activity that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts and food materials. The assay measures the reducing ability of antioxidants toward the DPPH radical [91].

FIGURE 2.13: Brine Shrimp Eggs

Chapter 3

Materials and Methods

Following research work of my thesis was carried out in wet lab, Departmentof Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad.

3.1 Material

Material utilized for the research work is given below:

3.1.1 Chemicals

Chemicals	Company Name
Nutrient agar	**Sigma-Aldrich**
Sabouraud dextrose agar	”
Brine shrimps egg	”
Sea salt	”
Ascorbic acid	”
Ethanol	”
Terbinafne	”
Streptomycin	”
Distal water	”

3.1.2 Apparatus and Equipment

Petri plates, test tubes, vials, micropipette, cotton plugs, cotton swabs, aluminum foil, falcon tubes 15 mL, 50 mL, eppendorf tubes, beakers 100 mL, 500 mL, 1000 mL, test tubes racks, discs, para film or masking tape, forceps.

3.1.3 Microorganism Used in Different Assay

Different bacterial and fungal strains are used

Bacterial Strains

- *Micrococcus luteus*
- *Salmonella Setubal*
- *Enterobacter aerogenes*
- *Staphylococcus aureus*
- *Agrobacterium tumefaciens*
- *Bacillus subtilis*

Bacterial strains use in antibacterial assay (Gram positive: *M.luteus*, *S.aureus*, *B.subtilis*)
(Gram negative: *A.tumefaciens*, *S.setubal*, *E.aerogenes*)

Fungal Strains

- *Fusarium solani*
- *Mucor species*
- *Aspergillus niger*

- *Aspergillus fumigates*
- *Aspergillus flavus*

Fungal strains ues in antifungal assay

3.1.4 Overview of Research Methodology

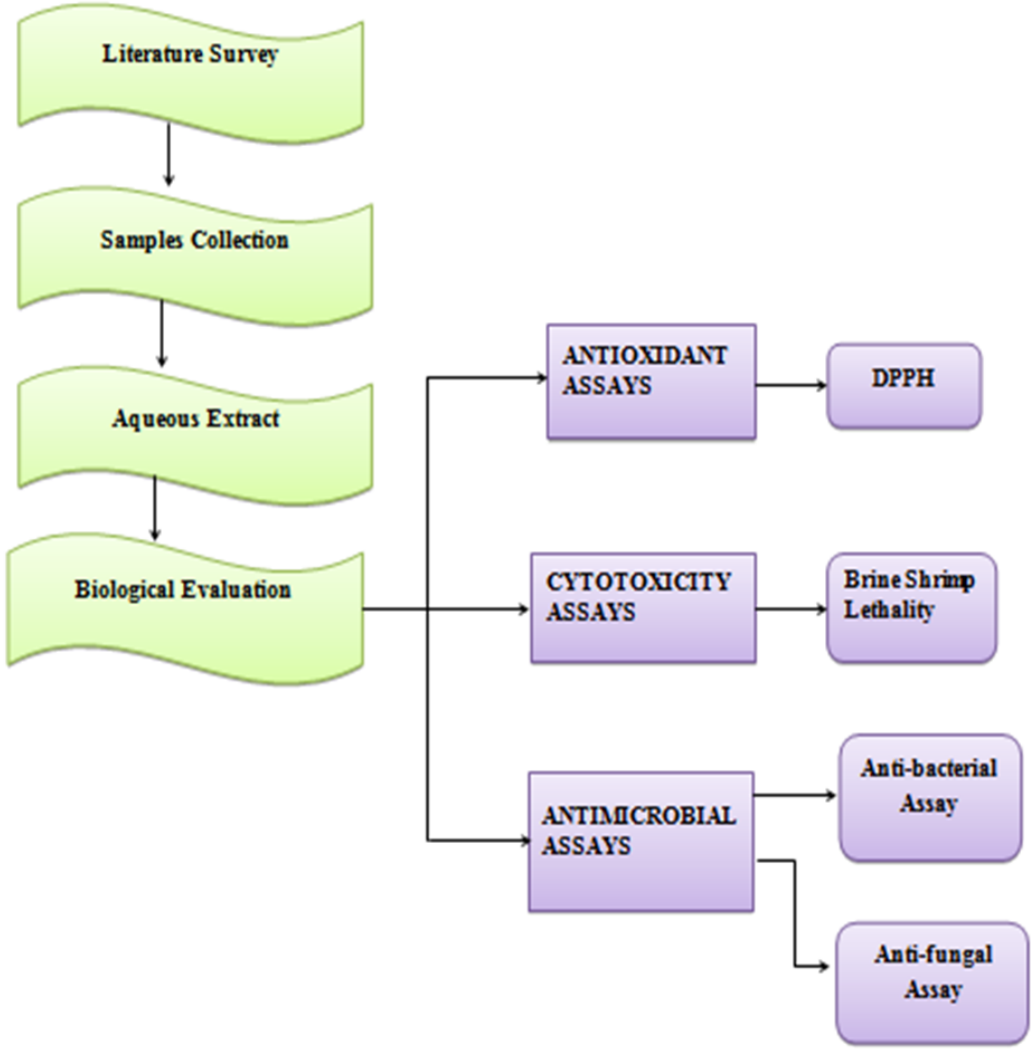

FIGURE 3.1: Overview of Methodology

3.1.5 Media Preparation

3.1.5.1 Sample Collection

T. ammi (Ajwain) and *N. sativa* (Klonji) seeds were purchased from a grocery shop of Islamabad 1st April 2019.

3.1.5.2 Preparation of Seeds Extract

About 100g of each *T. ammi* and *N. sativa* seed were taken and seeds were washed thoroughly to remove the dust particles. 500mL distilled water was taken in the flask. Boiling was done for 15-20 minutes at room temperature and the flask was removed from the flame and allowed to cool down. Filtration was done using Whatmann No. 1 filter paper and seed extracts were kept in the refrigerator for further use [92].

3.2 Biological Evaluation of Samples

It is reported in the literature extracts of these samples that microbial, antioxidants and cytotoxic properties. The biological evaluation process of these samples undergoes different assay according to different reported references. These are

- Antimicrobial Assay
- Antioxidant Assay
- Cytotoxic Assay

3.2.1 Antimicrobial Assay

Two kinds of antimicrobial assays are uses to evaluate the biological activity of these extracts.

- Antibacterial assay
- Antifungal assay

3.3 Antibacterial Assay

For the antibacterial evaluation six bacterial strains were used. Antibacterial potential of these extract was analyzes by mean of disc diffusion method [93].

3.3.1 Bacterial Strains are Used

To determine the antimicrobial potential of these extract following Bacterial strains were used.

These identified strains were obtained from Quaid-i-Azam University Islamabad.

- *Micrococcus luteus*
- *Salmonella Setubal*
- *Enterobacter aerogenes*
- *Staphylococcus aureus*
- *Agrobacterium tumefaciens*
- *Bacillus subtilis*

3.3.2 Sample Preparation

Stock solution (200mg/mL) was prepared and used for this analysis at 100μg/mL concentration.

3.3.3 Media for Bacterial Growth

Composition of luria broth agar.

a) Yeast	5g/500mL
b) NaCl	2.5g/500mL
c) Agar	7.5g/500mL
d) Bacto-tryptone	5g/500mL

For the bacterial growth Luria broth agar was used in petri plates.

3.3.4 Experimental Procedure

Firstly all materials like the Petri plates cotton swabs and media were autoclaved at 121°C for 20 minutes. Disc diffusion test was used to check the antimicrobial activity. Discs were prepared by punching the Whatmann No. 1 filter paper. The average size of disc was 4mm measured using the scale.

After this, all things were transferred into the culture room and allowed to stand under the UV radiation for 5-10 minutes to kill the germs that cause the contamination. Luria broth agar was poured in petri plates in equal quantity and leave it to solidify. After media solidification, the bacterial strains were streaked by using the cotton swabs and discs were put in sequences.

Each petri plate contains 4 discs, two for extracts of *N. sativa* and *T. ammi* and the other two for positive and negative control. The positive control was streptomycin 100μg/mL and negative control that was distilled water. These Petri plates were sealed with parafilm and incubate at 37°C for 24 hours.

After 24 hours bacterial growth was observed and zones of inhibition were identified around each disc. By using the vernier caliper zone of inhibition was measured. The experimental procedure was done in triplicate.

3.4 Antifungal Assay

To determine the antifungal activity of plant extracts tube dilution method was used [25, 94].

3.4.1 Fungal Strains are Used

To determine the antifungal potential of these extracts following Fungal strains were used.

These identified strains were obtained from Quaid-i-Azam University Islamabad.

- *Fusarium solani*
- *Mucor species*
- *Aspergillus niger*
- *Aspergillus fumigates*
- *Aspergillus flavus*

3.4.2 Sample preparation

Stock solution (200mg/mL) was prepared and used for this analysis at 100μg/mL concentration..

3.4.3 Preparation of Media for Fungal Growth

For the fungal growth, Sabouraud dextrose agar was prepared. Its composition is given below

Sabouraud dextrose agar , 26g / 400 mL of distilled water

3.4.4 Experimental Procedure

With the help of scale test tubes were marked to 10 cm. Sabouraud dextrose ager (5mL) was added and these test tubes and cotton plugs were already autoclaved at 121°C for 20 minutes. After this test tubes were filled with 100μg/mL of different extracts. Terbinafine was used for positive control and distilled water was used for negative.

After filling test tubes make a slant to the marked position at room temperature. The media was solidified after a few minutes and the test tubes were inoculated with fungal strains and cover with cotton plugs. The whole procedure was carried out in triplicate for different extracts. These test tubes were incubated for 4 days at 37°C.

Reading was documented by measuring the fungal growth in a slanting position. By using the vernier caliper linear growth of inhibition was measured the process is carried out triplicate but best one result selected. According to the reference to the negative control linear growth, growth inhibition was calculated. The growth inhibition percentage was calculated by this formula.

$$\%\text{age Inhibition} = \frac{\text{Linear growth in -ve control - linear growth in sample} \times 100}{\text{Linear growth in -ve control}}$$

3.5 Antioxidant Assay

Antioxidant Capacity of these extracts was determined by using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging that was described by this method [95].

3.5.1 Sample Preparation

Stock solution (200mg/mL) was prepared and used for this analysis at (250, 500, 1000μg/mL) concentration.

3.5.2 Preparation of DPPH Solution

The reagent solution was prepared by adding about 12mg of DPPH in 100mL of ethanol.

3.5.3 Experimental Procedure

200mL of the solution of these extracts along with 2.8mL of reagent (DPPH) was in different vials. The whole procedure was carried out in triplicate. . For positive control, ascorbic acid along with reagent was used and negative control, ethanol was used. These vials were placed at the dark place for 45 minutes.

After 45 minutes, the absorbance of samples was measured at 517nm by using distilled water as a blank reference. Water was used as a blank reference in order to check whether water has an individual antioxidant activity or not because water is used as a solvent in this assay. The process is carried out triplicate but best one result selected. Following formula used to calculate the free radical scavenging.

$$\% \text{ Scavenging} = \frac{\text{Control absorbance - Extract samples absorbance} \times 100}{\text{Control absorbance}}$$

3.6 Cytotoxic Assay

Brine shrimps lethality cytotoxicity assay was performed to determine the level of toxicity of these extracts reported by the method [96].

3.6.1 Preparation of sample

tock solution (200mg/mL) was prepared and used for this analysis at (250, 500, 1000μg/mL) concentration.

3.6.2 Sea Salt Preparation

Sea salt water was prepared according to the given concentration:
Sea salt , 34g / L

3.6.3 Hatching of Eggs

Sea salt water was used for the hatching of brine shrimp eggs (34g/L).

3.6.4 Experimental Procedure

Different vials were prepared with the extracts sample (250, 500, 1000μg/mL) were added in each vial and the volume of 5mL was made by adding the seawater. For negative control, distilled water was used. After 1-day shrimps eggs were hatched and shifted to the vials. 15 shrimps were added in each vial and vials are kept under the light at room temperature 25°C.

After 24 hours, alive shrimps were counted by pasture pipette (3x magnifying glass) and the process is carried out by triplicate but best one result selected. Percentage viability was calculated by this formula.

$$\%\text{age Viability} = \frac{\text{No. of alive shrimps in -ve control - No. of alive shrimps in test} \times 100}{\text{-ve control}}$$

Chapter 4

Results and Discussion

Biological evaluation of prepared extracts (*N. sativa* and *T. ammi*) was carried out by performing different biological assay; Antibacterial, Antifungal, Cytotoxic and Antioxidant activities results are given below here:

4.1 Antibacterial Assay

By using disc diffusion method antibacterial activity of *N. sativa* and *T. ammi* were determined. In this assay six bacterial strains are used i.e three gram positive and three Gram negative. Gram positive (*M. luteus*, *S. aureus*, *B. subtilis*) and Gram negative (*A. tumefaciens*, *S. Setubal*, *E. aerogenes*). Results are shown in table 4.1. After incubation period about 24 hours clear inhibition zone were observed and vernier caliper used to measure the inhibition zone.

For control treatment, positive control streptomycin dissolved in water and for negative distilled water was used. Growth of all bacteria strains gram positive (*M. luteus*, *S. aureus*, *B. subtilis*) and Gram negative (*A. tumefaciens*, *S. Setubal*, *E. aerogenes*) was inhibited at concentration of 100μg/mL. In this concentration, bacterial strains both aqueous extracts *N. sativa* and *T. ammi* showed least activity.

Growth of all bacteria strains both Gram positive (*M. luteus*, *S. aureus*, *B. subtilis*) and Gram negative(*A. tumefaciens*, *S. Setubal*, *E. aerogenes*) was inhibited at concentration this. About 100μg/mL concentration in Gram positive strains *T. ammi* show antibacterial activity against the *B. subtilis* 1 cm ± 0.4 cm and *N. sativa* show antibacterial activity against the *S. aureus* 0.6 cm ± 0.36 cm. Gram negative, both *N. sativa* and *T. ammi* showed antibacterial activity. *T. ammi* show antibacterial activity against the *A. tumefaciens*s 0.8 cm ± 0.72 cm and *N. sativa* show against *E. aerogenes* 0.6 cm ± 0.12 cm. The control discs did not show any zone of inhibition.In Gram positive *T. ammi* showed least antibacterial activites as compared to *N. sativa* and in Gram negative bacteria *N. sativa* more effective as compare to *T. ammi*.

TABLE 4.1: Antibacterial Activity of Ajwain, (*T.ammi*) and Kalonji (*N.sativa*) Against Different Bacterial Strains by Using Disc Diffusion Method

	Zone of Inhibition (cm ±S.E)					
	Gram Positive Strains					
	M. luteus		***S. aureus***		***B. subtiles***	
	T. ammi	***N. sativa***	***T. ammi***	***N. sativa***	***T. ammi***	***N. sativa***
Extracts Conc 100μ g/mL	0.7±1.5	0.6±2.1	0.8±0.7	0.6±0.3	1±0.4	0.7±0.5
Distilled Water (Negative Control)	0	0	0	0	0	0
Streptomycin (Positive Control) conc 100μg/mL	1.2	1.2	1.8	1.8	2	2

Trachyspermum ammi, Nigella sativa, Micrococcus luteus, Staphylococcus aureus, Bacillus subtiles, Agrobacterium tumefaciens, Salmonella Setubal, Enterobacter aerogenes.

TABLE 4.2: Antibacterial Activity of Ajwain, (*T. ammi*) and Kalonji (*N. sativa*) Against Different Bacterial Strains by Using Disc Diffusion Method

	Zone of Inhibition (cm ±S.E)					
	Gram Negative Strains					
	A. tumefaciens		***S. setubal***		***E. aerogenes***	
	T. ammi	***N. sativa***	***T. ammi***	***N. sativa***	***T. ammi***	***N. sativa***
Extracts Conc 100μ g/mL	0.8 ± 0.72	0.6 ± 0.81	0.9 ± 0.96	0.7 ± 0.31	1 ± 0.62	0.6 ± 0.12
Distilled Water (Negative Control)	0	0	0	0	0	0
Streptomycin (Positive Control) Conc 100μg/mL	1.1	1.1	2.5	2.5	2	2

Trachyspermum ammi, Nigella sativa, Micrococcus luteus, Staphylococcus aureus, Bacillus subtiles, Agrobacterium tumefaciens, Salmonella Setubal, Enterobacter aerogenes.

Gram positive bacteria have less resistance to plants extract as compared with Gram positive it has been reported. The resistance of gram-negative bacteria to antibacterial substances is related to nature of lipopolysaccharides in their outer membrane. Generally, the extent of the inhibitory effects of the extracts could be attributed to their phenolic and chemical composition [97].

Periplasmic space Gram positive bacteria do not have and an outer membrane the periplasmic space present in Gram negative bacteria. In Gram positive the antibacterial substance disturb the cytoplasmic membrane and bacterial cell wall and leakage and coagulation present in cytoplasm. In Gram positive bacteria the outer membrane has lipopolysaccharide rich hydrophilic surface that work against many antibiotic molecules. Enzyme present in periplasmic space that break the molecules from outside [98].

It was reported earlier that *Nigella sativa* showed enhanced antimicrobial activity against *Staphylococcus aureus*, *Esherichia coli* and *Salmonella enteric*. This is due to the occurrence of bioactive compound (Thymoquinone) [99].

In other study it was observed that *Nigella sativa* aqueous extract was found effective against *Esherichia coli*, *Staphylococcus albus* and *Salmonella typhi*. The most significant antimicrobial effect was found against *Beslius subtilis* [100].

It has been studied earlier that *T. ammi* seeds showed higher antibacterial activity against *E. coli*, *Streptococcus mutans* and *S. bovis*. Some bioactive compounds present in *T. ammi* extract and those compounds (carvacol and thymol) are responsible for the microbial activity against different bacterial strains [101].

In other studied *T. ammi* showed more antibacterial activity against Gram positive and Gram negative bacterial strain. Aqueous extract showed more antibacterial activity due to the presence of phenolic compounds. Phenolic compounds exhibit the antibacterial activity against different Gram positive and Gram negative bacterial strains [102].

4.2 Antifungal Assay

The aqueous extract of *N. sativa* and *T.ammi* show antifungal property. Those extract show inhibition against different fungal strains (*Mucor* species, *F.solani*, *A. fumigates*, *A. flavus*, *A. niger*). Results are showed in (table 4.2.1). Antifungal drug Terbinafine was used as positive control and for negative control distilled

water was used. The highest activity of *T. ammi* was observed in the case of *Aspergillus flavis* 90% (growth inhibition). In contrast to this least activity was observed in the case of *Mucor* species 44% (growth inhibition). *T.ammi* extract showed considerable activity against *Solani*, *Mucor* species and *Fumigatus* which are 65%, 85% and 80% respectively. *N. sativa* show highest activity in case of *F. solani* 70% (growth inhibition).

In contrast to this least activity was observed in the case of *Mucor* species 30%. Whereas against *Fumigatus*, *Flavus* and *Mucor* species, considerable activities was observed which was 60%, 50% and 60% (Table 4.3). *T. ammi* showed highest activity against fungal strains as compare to *N. sativa*. *N. sativa* showed highest activity observed in case *F. solani* and *T.ammi* show highest activity observed in case of *Aspergillus flavis*. Least activity of both extracts was observed in *Mucor* species.

TABLE 4.3: Percentage Inhibition of Ajwain ,(*T.ammi*) and Kalonji (*N.sativa*) Against Fungal Species

Sr. No	**Samples**	**% age Inhibition Against Fungal Species**				
		Mucor. species	***F. solani***	***A. fumigatus***	***A. flavus***	***A. niger***
1	***T. ammi***	44	65	80	90	85
2	***N.sativa***	30	70	60	50	60
4	**Distilled Water (-ve Cont)**	0	0	0	0	0
5	**Terbinafine (+ve Cont) Conc 100 μg/mL**	100	100	100	100	100

Abbreviations: *A. fumigatus*: *Aspergillus fumigatus*, *A. niger*: *Aspergillus niger*, *Mucor.sp*: *Mucor species*, *F. solani*: *Fusarium solani*, *A. flavus*: *Aspergillus flavus*.

N.sativa has strong antimicrobial property against different pathogens, including bacteria, viruses and fungus. It is reported that in recent years *N. sativa* seed used as traditional medicine for the treatment of microbial diseases has been used without any side effects [103].It has been studied earlier that *T. ammi* showed strong antifungal activity against 10 fungal strains. The percentage inhibition was found 72-90%. Fungicidal activity was found significant against *A. niger* [104].

4.3 Antioxidant Assay (DPPH)

The antioxidant ability of these seed extracts were assessed by DPPH assay. Free radical scavenging activity was exhibited by both plant extracts. Antioxidant assay was performed different concentrations i.e (1000μg/mL, 500μg/mL and 250μg/mL). Results of antioxidant assay of both extract are shown in (table 4.3 and 4.4). At highest concentration (1000μg/mL) both *N. sativa* and *T. ammi* showed highest free radical scavenging activity. *N. sativa* show 55% free radical scavenging activity at 1000μg/mL concentration and *T. ammi* show 60% free radical scavenging at same concentration.

TABLE 4.4: % Scavenging of Plants Extract Against DPPH

Sr. No	**Sample**	**Concentrations**	**Percentage Inhibition**	**IC50**
1	***T. ammi***	1000 μg/mL	60%	685.3
		500 μg/mL	51%	
		250 μg/mL	35%	
2	***N. sativa***	1000 μg/mL	55%	836.4
		500 μg/mL	40%	
		250 μg/mL	28%	

The lowest activity (free radical scavenging) of *N. sativa* and *T. ammi* was observed at lowest concentration (250μg/mL), which is 28% and 35% respectively.

T. ammi show highest free radical scavenging as compare to *N. sativa*. The results are significant as IC50 of *T. ammi* is 658.3 and *N. sativa* is 836.4. It is observed that decreasing the concentration there is gradually decline in the free radical scavenging.

DPPH is a stable free radical and has the ability to give the hydrogen atom by reacting the various compounds. This assay is used to measure the reducing ability of various antioxidants by DPPH free radicals. It was reported earlier that *T.ammi* showed a strong antioxidant activity against DPPH, nitric oxide, superoxide and hydroxyl radical assay. Presence of caroteniods and flavonoids provide successful antioxidant action [105].

It has been reported that *N. sativa* showed enhanced DPPH activity because of presence of certain bioactive compound such as (TQ, carvacol, t-anethole and 4-terpineol) [106].

TABLE 4.5: Analysis of Variance for Factors Effecting the Free Radical Scavenging Activity of These Extract

Source of Var	**D.f.**	**% of Total Var**	**Sum of Squares**	**Mean Square**	**F Value**	**P Value**	**Sig.**
Interaction	2	3.08	82.11	41.06	3.079	0.0833	No
Plant Extracts	1	9.64	256.9	2569	19.27	0.0009	Yes
Conc	2	81.27	2165	1083	81.2	0.0001	Yes
Residual	12		160	13.13			
Missing Value	0						

4.4 Cytotoxic Assay

To evaluate the toxic effect of plant aqueous extracts brine shrimp cytotoxic assay was used. Different concentrations of plant extracts i.e 1000μg/mL, 500μg/mL and 250μg/mL were used and showed significant toxic effect. It was observed that viability of shrimps decrease by increasing the concentration. Results are shown in table 4.5 and 4.6. Highest activity of *T.ammi* is 80% at lowest concentration 250μg/mL and *N. sativa* activity is high 67% at 250μg/mL concentration. The least activity of *T.ammi* was 45% and *N. sativa* was 33% at (1000μg/mL) highest concentration.

Higher concentration had more mortality rate than lower concentrations of plant extract shown in table 4.5, 4.6. Results were also found significant because IC50 of *T.ammi* is 162 and *N. sativa* is 284.9. By this study it is observed that *T.ammi* is more significant as compare to *N. sativa* result shown in tables.

TABLE 4.6: Cytotoxic Assay of *T.ammi* and *N. sativa* Effecting the Viability of Brine Shrimps.

Sr. No	**Sample**	**Concentrations**	**Percentage Viability**	**IC50**
1	***T. ammi***	1000 µg/mL	45%	162
		500 µg/mL	67%	
		250 µg/mL	80%	
2	***N. sativa***	1000 µg/mL	33%	284.9
		500 µg/mL	45%	
		250 µg/mL	67%	

TABLE 4.7: Analysis of Variance for Factors Effecting the Viability of Brine Shrimps

Source of Var	**D.f.**	**% of Total Var**	**Sum of Squares**	**Mean Square**	**F Value**	**P Value**	**Sig.**
Interaction	2	1.69	96.44	48.22	4.769	0.0299	Yes
Plant Extracts	1	22.17	1089	1089	107.7	0.0001	Yes
Conc	2	73.4	3605	1803	178.3	0.0001	Yes
Residual	12		121.3	10.11			
Missing Value	0						

It was observed that *T.ammi* aqueous extract showed enhanced cytotoxic potential against human acute lymphoblastic leukemia cell [107].

It was found in a particular study that *N. sativa* showed enhanced activity against human epithelial cell lines (Hep-2). [108].

Chapter 5

Conclusions and Recommendations

According to biological evaluation and comparison of *N. sativa* and *T. ammi* it is concluded that *T. ammi* is more effective as compared to *N. sativa* regarding biological activities. Additionally, both *N. sativa* and *T. ammi* aqueous extract were found significant antimicrobial, cytotoxic and antioxidant agents giving us prospects further discover them in the field of herbal drug.

It is concluded that both *N. sativa* and *T. ammi* are the source of medicinally active constituents and has numerous pharmacological effect, hence it is encouraging to find its new therapeutics. Additionally, both were found significant antibacterial agents and hence can be used as an antibiotic. They have also showed significant antioxidant and cytotoxic potential and hence can be used to unravel their potential in the field of herbal medicine and cancer genetics.

In the future, these extracts can be subjected to isolation of bioactive compounds responsible for biological activities. Furthermore, these extracts can also be studied at the nanoformulations in nanobiotechnology.

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