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TECHNOLOGY, ISLAMABAD



Comparison of The Biological Evaluation of Two Different Varieties of Cumin Seed Extract

by

Rabbia Ahmed Awan

A thesis submitted in partial fulfillment for the
degree of Master of Science

in the

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Dedicated to **Allah Almighty, Hazrat Muhammad (S.A.W.W)** and my father who is not with me physically but his prayers from heaven have always enlightened my way throughout my life. It's also dedicated to my mother who taught me that the best kind of knowledge to have is that which is learnt for its own sake. She also taught me that even the largest task can be accomplished if it is done one step at a time.



CERTIFICATE OF APPROVAL

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Abstract

The seeds of cumin (*Cuminum cyminum* and *Bunium persicum*) used as spice for their distinctive aroma. These plants have significant medicinal, antimicrobial, cytotoxic and antioxidant properties which indicates it high potential for use in medicine and food industry. In traditional medicines these plants are used to treat and improve some cases i.e. digestive and urinary disorders, diabetes, obesity and increasing breast milk. A comprehensive literature review was conducted by searching different studies in all relevant scientific databases. These studies support their uses either independently or as adjunct along with conventional drugs in metabolic syndromes, diabetes mellitus, respiratory problems, dyspepsia, inflammatory diseases, allergic rhinitis and all different types of human cancers.

Various bioassays were performed to check the therapeutic efficacy of plants which are antimicrobial assays (antibacterial and antifungal), cytotoxic assays or brine shrimp lethality assay and antioxidant (DPPH assay). It was observed that both of this plant extract showed significant antibacterial activity against gram negative bacteria (*AT-10* and *E. aerogenes*). In antifungal assays, *B. persicum* showed highest percentage inhibition as compared to *C. cyminum* against *Aspergillus-niger*. Antioxidant was performed using DPPH method and it was observed that *C. cyminum* have more free radical scavenging activity as indicated by low IC50 value. Cytotoxic assays were performed by following brine shrimp lethality assay (BSLA) and it was observed that *C. cyminum* showed more toxicity as compared to *B. persicum*.

Keywords: *Cuminum cyminum* , *Bunium persicum* , Antibacterial, Antifungal , Cytotoxic , Antioxidant.

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Abbreviations

<i>B. perdicum</i>	<i>Bunium perdicum</i>
<i>C. cyaminum</i>	<i>Cuminum cyminum</i>
C.O.I	Counter Osteoporotic Impact
C.P.D	Carpel Passage Disorder
CDV	Cardiovascular
DPPH	1,1-Diphenyl-2-Picrylhydrazyl
E.O	Essential Oil
Mg	Magnesium
MS	Menstrual Stream Stimulant
W.H.O	World Health Organization

Chapter 1

Introduction

1.1 Background

Since from long decades people used natural products, for example, plants, creatures, microorganisms, and marine living beings in prescriptions to diminish or control their belongings and treatment of various diseases. Plants are rich wellsprings of regular items which are having significant principle in therapeutic Industry for integrating medications, for example, hostile to malignant medications, calming operators against malarial medications and so on. Plants are playing very affective role in human welfare in various parts of life, for example, giving nourishment, incredible wellspring of fuel, and furthermore play significant standard in therapeutic industry for planning drugs. Plants keep up our climate by creating oxygen and retaining carbon dioxide during photosynthesis. As we realize that oxygen is fundamental for cell breath for all living life form, also keeping up the ozone layer, that shields us from UV radiations originating from the sun. Lion's share in Pakistan relies upon therapeutic plants for minor, even in some significant maladies. Wild plants are currently generally utilized against ephedra, Artemisia, Hippophae. In the present, an exertion is made to check the status of medicinal plants in Pakistan. [1]

Plants are utilized for various purposes. The historical backdrop of characteristic item is moderately goes back to old ages ended up aware about condition. For decades ago man has faced many environmental challenges for his survival. Man has suffered by different diseases, and helped out against these by different types of plants which were beneficial for his survival, these plants having supreme qualities of drugs in this modern age. From decades, mankind has used herbs for purpose of medication. [2]

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 plants species have the prospective for being used as medicinal plants. Plant contains bioactive constituents including group of chemicals like that tannins, lignins, coumarone, quinones, stilbenes, xanthones, catechism and anthocyanins. These compounds have the potential to be used in treatment of different disorders and boost life span. Through the world, the major reason of mortality is contiguous diseases and about 60,000 people die on daily basis due to lethal diseases. These tremendous compounds play vital role in drug development.

From historical background of mankind; the people of Assyrian, Egypt, Indies valley and, China have used therapeutic plants to cure different ailments. Different old writings palm leaves, past compositions, therapeutic bargains, and fables contain information about these herbs and those old communities, all are even saved today. The plants present in the grave were later known to have numerous therapeutic properties. Herbs grown on grave, are also having therapeutic properties.

During the most recent decade the utilization of conventional drugs has consumed all around and has picked up appeal. These are utilized not just for essential social insurance of needy individuals in the creating nations, but at the same time are utilized in the nations where regular prescription is dominating in the National Health Care System. [3] As indicated by WHO natural prescriptions serve the wellbeing needs of about 80% of the total populace, particularly for millions of individuals in the huge rustic territories of creating nations.(Over half

of the considerable number of drugs in clinical use have a characteristic item cause Of the universes 25 top of the line pharmaceutical specialist, 12 are regular items inferred.[4] In excess of 600 organic things have been perceived in different release of the United State Pharmacopeia. [5] The most significant components for the proceeded with utilization of the conventional drugs are its prepared availability and affordability. The huge numbers of therapeutic agents remedy are practically sheltered yet logical toxicological preliminaries are as yet fundamental. [6]

Mostly the plants part having substances that are used for curing different illnesses, and these are known as therapeutic plants.[7] Medications got from plants are broadly well known because of their wellbeing, simple accessibility and ease.[8] Natural drugs are given through orally, breathed and injected through skin. [9]. Therapeutic herbs are increasing critical soundness and individual network. Bioactive phytochemical component which produces specific physiological response on the human body. [10]

Phenolics, tannins, alkaloids, saponins, and basic oils are as most important phytochemicals [11].These normal mixes shaped the establishments of present day professionally prescribed medications as we probably am aware today. [12]

Utilization of plants based medications for restoring different diseases is as old as human development and is utilized in all societies since the beginning. The crude man began to recognize helpful and unsafe are noxious plants by trail and mistakes. Natural medicines has been categorized which area mentioned below

1. Chinese herbalism
2. Western herbalism
3. African herbalism
4. Ayurveda herbalism

In hot regions of world, therapeutic plants are found abundantly. Present day medication from high plants either legitimately or in a roundabout way determined

is assessed 25%. [13] The advancement and presentation of new medications like anti-toxins, immuno-stimulants and hostile to tumor specialists have prompted emotional achievement responsible for some ailments. The medications got from plants, notwithstanding, still from the pillar of medicinal treatment in the creating nations. Characteristic items and the restorative specialists got there from, are additionally a basic component in the social insurance arrangement of the staying of the populace dwelling for the most part in created nations, more than half of all medications in clinical use have a characteristic item birthplace. [14] Common items keep on assuming a significant job in tranquilize disclosure projects of the pharmaceutical business and other research associations. [15] In excess of 600 natural things have been perceived in different releases of the United States Pharmacopeia. [16] The reality highlights that the greater part of plants on earth has been used for curing different infections for long centuries. These plants having greater efficiency to overcome on numerous diseases. [17]

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 microorganism are becoming resistant to these medicines. So the need for hours is to develop such medicines which have less adverse effect on health.

1.3 Aims

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

1.4 Objectives

This study entails the following objective:

1. To prepare the aqueous extract of cumin plants.
2. To assess the therapeutic potential by performing antimicrobial, antifungal, antioxidant and Cytotoxicity assays.
3. To compare the biological activity of both plants (white cumin, bitter cumin).

1.5 Scope

As drugs originated from herbs to be cheap, no- 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

2.1 Medicinal Plants

The term medicinal plant is having different types of plants in Herbalism” herbology or herbal medicines” and it highlighted 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 herbs refers to several part of plants like seeds, stem, bark, blossom, leaves or roots and consist of non-woody plants, they may include those that begin from non-tree and bushes.

2.2 History

There are more than 10000 plus well known natural products having vital role in human’s life are discovered till now. Drugs which are discovered and isolated from natural sources are having Effective poisons which also belong to natural products and Active therapeutic agents and pharmaceutical objects.

Cancer is worldwide disease and recognized as the world health issue which is causing for round about 7.5 million deaths on this planet, almost which is supposed to

increase 13.2 million by 2030. Developed countries have grip on this deadly disease, and still working to reduce the cancer by developing anti-cancer therapeutic agents.

The separation of several alkaloids such as morphine is obtained from the *Papaver-somniferum L. Morphine* is important drug that was reported in 1803. The plant *P. somniferum*, boiled under presence acetic anhydride to produce heroin which then change into painkiller (codeine). Opium poppy is compelling drug which is synthesized from the juice of the opium poppy, consumed illicitly as a narcotic and in medicine as an analgesic.

Plants are well known from centuries in their use in medicine. They have acquired insects, bacteria, fungi, and also produce secondary metabolites. From early ages, due to supreme characteristics of plants, are used in medicine as important source. With reference to W.H.O more than 80-85% human rely on these plants base medicine for initial health care Pakistan is introducing several types of plants which are medicinally having supreme qualities about drugs and have priority to all over the world. In Pakistan there is variety of different environments and also having diversity. Round about 72% population is living in rural areas of Pakistan. This rural population is mostly depended on these plants for treatment of diseases. About 700 plus species of different plants are utilized as medicine for various diseases by the people of rural areas. With the passage of time, now improvements are made in medicine for synthesizing better quality of drugs. Always human beings depended on plants for food and health. [18]

About 1500 species of different plants are grown in Pakistan, are very important for synthesizing drugs. Among these plants, 300 species are capitalized by Hakims for treatment of various ailments in rural areas. Large amount of plants must be grown In Pakistan for treating different diseases and there must be research done to extract medicinally compounds from these plants species. [19]

2.3 Plants History in Pakistan

Worldwide, Pakistan is presenting a few kinds of plants which are restoratively having incomparable characteristics about medications and have need to everywhere throughout the world. In Pakistan there is wide range of environment and furthermore having assorted variety. Almost 72% population is living in rustic zones of Pakistan, and these are relied upon plants for reason for various ailments. Around 500 or more types of various plants are utilized as medication for different ailments by the people of country regions. With the progression of time, presently enhancements are made in medicine for better quality of drugs. It's a fact that human creatures totally dependent upon plants for nourishment and wellbeing. [20]

Around 1500 types of various plants are developed in Pakistan, are significant for synthesizing drugs. Among these plants, 300 species are capitalized by Hakims in country regions for treatment of various infections. Huge measure of plants must be developed In Pakistan for treating various ailments and there must be research done to extract therapeutic compounds from these plants species. [21]

2.4 Examples of Medicinal Plants

Some supreme examples are mentioned below.

2.4.1 Ajwain

Scientific name	<i>Trachyspermumammi</i>
Family:	Apiaceous
Major Phytochemicals:	linoleic acid, γ -terpinene, P-cymene, and Oleic acid
Medicinal Uses:	Anti flatulence, Anti thelminitic, and Anti tussive.

2.4.2 Cumin

Scientific name	<i>Cuminum cyminum</i>
Family:	Apiaceae
Major Phytochemicals:	Cuminaldehyd, β -pinene, γ -terpinene
Medicinal Uses:	Anti microbial, antifungal, chemo protective and GTI.

2.4.3 Coriander

Scientific Name	<i>Coriandrum sativum</i>
Family	Apiaceae
Major Phytochemicals	Linalool, Geranial, Limonene, Borneol, Camphor, Elemol, Carvone
Medicinal uses	Tonic, Diuretic, Analgesic and Antioxidant

2.4.4 Dill

Scientific Name	<i>Anethum graveolens</i>
Family	Apiaceae
Major Phytochemicals	Limonene, Carvone, Anethofuran
Medicinal uses	Pain killer, Reproductive disorders and Antipyretic.

2.4.5 Kalonji

Scientific Name	<i>Nigella sativa</i>
Family	Ranunculaceae
Major Phytochemicals	Nigellimine, Nigellimine N-oxide., Nigellicine and Nigellone.
Medicinal uses	Anti-inflammatory, Antidiabetic, Diuretic, Analgesic and Liver tonic.

2.5 Natural Products

As definition, the word natural is referring to specific substances which are generated by naturally and not artificially. The term Natural Product in this moderate era is used for herbs, herbal concoction, dietary supplements, and traditional medicine [22]. The chemical substances which are synthesized by the series of enzymatic metabolic pathways by the body of living organisms such as plants, fungi and animals are known as natural products [23]. These chemical substances or compounds which are derived from living organism are in form of primary and secondary metabolites. These natural products are important

- Herbal concoctions
- Dietary supplements
- Traditional and alternative medicinal material
- Drugs which are discovered and isolated from natural sources
- Effective poisons which also belong to natural products
- Active therapeutic agents and pharmaceutical objects etc.

Primary metabolites are obtained from living organism (plants), these are involved in growth, development, reproduction, of a plant. Primary metabolites are referring to be central metabolites which are present in growing cell. The natural products are also known as bio-molecules as these are linked to natural sources of many chemical substances. Natural products play vital rule in survival. Ahead of 1800s secondary metabolites became important constituent of medicine. Secondary metabolites brings out from primary metabolites in different forms. In 21th century, it is now possible to separate, and regulate the active compounds obtain from crude extracts. [24]

There are more than 10000 plus well known natural products having vital role in human's life are discovered till now. Drugs which are discovered and isolated from

natural sources are having Effective poisons which also belong to natural products and Active therapeutic agents and pharmaceutical objects.

Main sources of natural products are plants, animals and microorganisms but land, sea and air can be sources of natural products [25]. Large quantity of medicinal plants is being utilized as a tool for natural products especially novel drugs which lead to synthetic drugs. Synthetic drugs are derived from natural products:

1. Form Chinese, Indian, Korean and North Africans civilization, Plants are the major source of phyto medicines and has provided written evidences of the applications of natural products for treatment of the infectious ailments. [26]
2. About 4000 years ago a written record on phenomenal use of natural products for treatment of the diseases is a clay tablet. For example rawgarlic is prescribed for circulatory disorders and witloof plant to treat the gall bladder's disorders. [27] *Artemisia annua* L plant can be used to treat more than 52 diseases [28]. Since ancient times, plants and their materials are being used to treat the diseases for examples to cure the urinary tract infections juices of cranberry and bear berry are used . Some plant species like balm, tea, lemon and garlic are used as anti-microbial agents. Plant oil is also to cure the infectious diseases like gastrointestinal, respiratory, skin diseases and urinary tract's disorders and circulatory diseases.
3. More than 65% officially drugs are synthesized from natural products. Natural products are the main origin of many synthetic drugs. Many biological potential agents are discovered from natural products. It is said that about 90 plant species are source of more than 115 well known bioactive compounds and drugs especially anticancer drugs [29]. Drugs originated from plants sources are mostly antibacterial drugs and some are effective anti-cancer drugs .[30]
4. Some plants and marine organisms have been screened to evaluate the high potential biological active compounds because several infectious agents have

obtained the resistance against various antibiotics. So there is an alarming need to found and introduce novel chemo types which can give right path for the discovery of new antibiotics. [31]

5. More than 1000 bioactive compounds are discovered from marine sources from the mid-1970s to 2010. [32] The first official accepted drug was based on marine source is Ziconotide [33]. Yondelis is an effective anticancer drug discovered from marine sources. [34] Yondelis is an analgesic drug and 1000 times more powerful than Morphine. .It is used to treat two soft tissue sarcomas i.eliposarcoma and lieomyosarcoma.
6. Many important pharmaceutical compounds such as lovastatin (drug which is used to control the blood cholesterol) and cephaloxin are obtained from marine sources. [35] To discover many novel drugs more than 7000 plant's species are evaluated .This evaluation leads to discovery of several bioactive substances which can be served to cure many serious disorders with least side-effects. [36]
7. Phytomedicinal system based on medicinal plant and these medicinal components are obtained through the extraction from seeds, leaves, fruits, bark, roots and floors etc. [37]. Medicinal plant are the sources of many compounds the can play an important role in improving of health. Alkaloids, diuretic phenolic compounds, antioxidants, antibacterial and anti-inflammatory substances are compounds obtained from medicinal plants. Phenolic substances work as natural antibiotics and alkaloids which give the sense of wellbeing. [38]
8. Unani medicine is also derived from herbs. In Asia and its surrounding regions numerous quantity and high quality medicinal plants have been used by Hakims from ancient times. [39] The use of natural products to cure of diseases is common practice in India, Pakistan and surrounding areas.[40] Characteristic items can be characterized as the results of normal foundations. Normal items include:
 - (a) A total creature (e.g., an organism, or a creature),

- (b) Some portion related to living being (a detached creature organ, blooms or leaves of a plant),
- (c) Some portion of a life form, exudates and a life form concentrate and
- (d) Unadulterated mixes

Ideas of optional digestion comprise of results of flood digestion because of supplement impediment, shunt digestion created during idiophase, resistance instrument controller particles, and so forth. Regular items may be from earthbound or aquatic sources and creatures. [42]

As per 39% of the 520 novel acknowledged prescriptions during 1983 and 1994 were from normal items or their branches and 60–80% of anticancer and antibacterial meds have regular item birthplaces. As indicated by around 60% medications of normal inceptions were in exploratory preliminaries for the proliferation of tumors. In "common" pharmaceutical industry the characteristic items have an immediate use, which is expanding quickly in North America and Europe separated from regular item determined present day prescription. The utilization of regular items in conventional drug is being fused into the essential social insurance frameworks of creating nations. In consumption of natural medication is fresh bringing increasingly prominent as integral and elective prescription, nourishment supplements and nutraceuticals. [43]

The broadest meaning of a characteristic item is whatever is created by life, and incorporates biotic materials (for example wood, silk), bio-based materials (for example bio plastics, cornstarch), organic liquids (for example milk, plant exudates), and other common materials that were once found in living beings (for example soil, coal). A progressively prohibitive meaning of a characteristic item is any natural intensify that is integrated by a living life form. The study of natural science, indeed, has its birthplaces in the investigation of common items, and has offered ascend to the fields of manufactured natural science where researchers make natural atoms in the lab, and semi-engineered natural science where researchers adjust existing characteristic items to improve or change their exercises. [44]

2.5.1 Current Status of Natural Products

Common items have been assuming an indispensable job in medicinal services for quite a long time. Since antiquated occasions, normal items speak to the fundamental wellspring of mixes utilized in medication revelation and improvement. From a huge number of years nature has been a rich asset of gainful organic specialists and exceptional number of late medications has been coming about because of regular sources grounded for their conventional prescription worth. Characteristic items have had focal influence in curing and avoidance of mankind infections between huge numbers of years. Nature providing roads for curing extraordinary assortments related ailments through furnishing mankind by decent varieties concerning active compounds.

2.6 Classification of Natural Products

The broadest meaning of regular item is whatever is delivered by life [45] and incorporates any semblance of biotic materials (for example wood, silk), bio-based materials (for example bio plastics, cornstarch), natural liquids (for example milk, plant exudates), and other regular materials (for example soil, coal). A progressively prohibitive meaning of a characteristic item is a natural exacerbate that is orchestrated by a living organism. The rest of this article confines itself to this increasingly restricted definition. [46] Regular items might be arranged by their natural capacity, biosynthetic pathway, or source as depicted underneath.

2.6.1 Metabolites

All the organic compounds which are produced or modified and used by plant body are called metabolites. These metabolites are known as intermediates as these are the products of enzyme catalyzed series of metabolism. Metabolites include carbohydrates, proteins, lipids, alcohol, trepan, alkaloids, flavonoids, lignin, phenols, pigments and steroids etc.

2.6.2 Types of Metabolites

Metabolites are categorized into two major classes

1. Primary metabolites
2. Secondary metabolites

2.6.2.1 Primary Metabolites

Primary metabolites are branch of metabolism in which normal growth, development, and reproduction is involved. Break down products of proteins fats nucleic acid are compulsory for living organism growth. Primary metabolites are present in numerous living organism or cells. Some common examples of primary metabolites are lactic acid, amino acids. [47] Those products which are prepared from primary metabolism are useful for different aspects of life. Primary metabolites play the physiological function in living organism. These are synthesized in high quantities and directly involved in growth. Extraction of primary metabolites is very easy and basic part of molecular structure of living organism. Examples are ethanol, lactic acid, nucleotides, and vitamins.

TABLE 2.6: List of Primary Metabolites

Primary Metabolites	
Alcohols	Propanol ethanol, and Methanol.
Amino acids	Alanine, glutamic acid and aspartic acid
Organic acids	Acetic acid and Lactic acid
Anti-oxidants	carotenoids , vitamin E and xanthophylls
Nucleotides	Purines and pyrimidines(Nitrogenous basis)
Poly-ols	Glycols

2.6.2.2 Secondary Metabolites

Secondary metabolites are obtained from primary metabolites. These are not part of growth, development, and reproduction directly. Derivatives of primary metabolites play important role in ecological functions, generated in small quantities and extraction is very complex, not a basic part of molecular structure of a living organism. Most secondary metabolites are synthesized from mevalonic acid, Shikimic acid and Acetyl CoA. These are capable for preparation natural products under specific chemical reactions e.g., Schiff base formation and alkylation. [48] Secondary metabolites are supposed to be building blocks, uses skimate, amino acids. [49] Examples are pigments, antibiotics, and drugs.

2.6.2.3 Phytochemicals

The earth is rich in common and different therapeutic plants. In this modern age of medical science, therapeutic plant are under studies more than other common plants and also having ability of creating supreme advantages for society and field of medicine. The phytochemical constituents of therapeutic plant are having pharmacological activities on human body. Alcohols, saponins, flavonoids, glycosides tannins are most common active compounds present in plants [50].

These common mixes structure the establishment of present day physician recommended sedates as we probably am aware today. [51] The characteristic items present in plants are phytochemicals e.g. organic products provide supplements for strengthening filaments to overcome on sicknesses. These are further categorized into two types.

1. Essential
2. Optional

Essential comprise of proteins, sugars and amino acids. Optional involve tannins, alkaloids, Phenolics and much more. [52]

2.6.3 Classification of Phytochemicals

2.6.3.1 Alkaloids

The largest group of secondary metabolites is alkaloids. It is class of organic compound which contain basic nitrogen. The biggest gathering of concoction weapons as like alkaloid is synthesized by natural plants. These alkaloids have greater pharmaceutical importance as the important drugs such as morphine and quinine and poisons such as atropine and strychnine. Alkaloids can be the first point of drug discovery. The metabolic results obtained from amino acids and incorporate a large numbers of mixtures of harsh nitrogenous. More than 10,000 distinct alkaloids are obtained from various species of 300 plant families. [53] Major sources of alkaloids are plants, animals, fungi and bacteria. An herbaceous plant Rosy Periwinkle *Catharanthus roseus* is a source of 75 different alkaloids. [54] Alkaloids can be used as anticancer, antibacterial, anti-malarial, anesthetic and antihypertensive effect. Alkaloids are necessary for plant's protection and survival as these are antibiotics and have bitterness in taste.

Morphine is the first alkaloid which is extracted from opium poppy in 1804. Its molecular formula is $C_{17}H_{19}NO_3$. Morphine decreases the sensation of pain and can be effective in acute pain or chronic pain.

Caffeine is extracted from tea plants and has stimulant property. [55] *Catharathus roseus* species are sources of vincristine (anticancer drug). Vincristine has market name Oncovin and used as anticancer in radiotherapy medication. This drug can treat the acute lymphocytic leukemia and acute myeloid leukemia. Some alkaloids are also important pharmaceutical compounds such as Vinblastine (an anticancer drug), Bebeerines (an antibiotic) and Scopolamine (sedative). [56]

About 1 ring of carbon iotas is contained by alkaloids and there is most part of nitrogen molecule ring. The nitrogen molecule in carbon ring shifts its position in various alkaloids present in various families of plants. In specific alkaloid as like mescaline nitrogen iota is not present in C ring. Truth to be told that nitrogen molecule influences the specialties of alkaloids. [57] These alkaloids having

amazing impact on people has prompted the development of ground-breaking torment executioner prescriptions, profound medications, and genuine addictions by individuals who are uninformed of the properties of these amazing synthetics [58]

2.6.3.2 Glycosides

These are alkaloids which contain a glycon (sugar group) and a glycon (non-sugar group) which are linked by glycosidic linkage due to the replacement of hydroxyl group in sugar group. Many plants store their chemicals in the form of inactive glycosides i.e. drugs and poisons etc. The first glycoside isolated in 1830 is Amygdalin. Glycosides act as protecting substances. [59]

2.6.3.3 Phenolic

Phenols are the organic compounds belong to aromatic class having phenol basic group (C_6H_6O) for example simple phenol, phenolic acid, nutraceutical, caffeic acid, hydroxyl cinnamic acid and its derivatives. Phenols are abundantly present in nature. These are the largest group of secondary metabolites. Phenolic compounds are associated with sensory functions. Phenolic compounds are present in vegetables, apples, red vine, green tea and other plants. They exist in simpler to complicated forms. [60] Phenol can lower cholesterol level, improve the bile secretion and show antimicrobial activities. [61]

Phenols might be separated into further categories. Among these simpler are therapeutic phenols. Another phenolic aldehyde called vanillin, present in unready new products of vanilla. Various products of capsicum contain capacin. These phenolic are utilized for inner part [62].

Caffeic acid is a phenolic compound used as medicine to treat dermatitis allergic. Nutraceutical is anticancer chemotherapeutic agent and decrease the heart diseases. [63] Many degenerative diseases (cancer) can be treated by phenolic compounds such as hydroxycinnamic acid. [64]

2.6.3.4 Steroids

Steroids are the secondary metabolites isolated from plants, animals and fungi. Steroid is basic unit of steroid family and it contains 17 carbons atoms bonded with four fused six member rings and one five member ring. Testosterone is a male sex hormone which is responsible for male reproductive tissue such as testes as well as promotes secondary sexual characteristics such as beard.

2.6.3.5 Flavonoids

Flavonoids are plant pigments for flower and petals coloration to attract pollinator animals. These flavonoids function as cell cycle retarders and it also inhibit the activities of organisms that cause plant's diseases [65]. Flavonoids such as Flavopiridol can stop progression of cell cycle .Its source are Amorohituka plan. Polyphenol are known as and different organs of plants are sources of flavoids [66] such as quercetin, luteolin and 5, 7-dimethoxyflavone which show anti-inflammatory abilities. More than 5000 bioflavonoid are identified and isolated from various plants. [67]

2.6.3.6 Terpenes

The unstable fluid which is obtained from pine tree is disconnected from turpentine is known as terpenes. The term terpene has been used nowadays through certain creator to highlight Terpenoids. [68] These are the very diverse classes of natural products isolated from plants. Terpenes are made up of isoprene unit with 5 carbon unit in each. These may be simple or complex like squalene and lanosterol found in animals .Some naturally occurring terpenes are varied in structure and their complexity level is also variable. These terpenes are eventually formed by the repetition of isoprene units.

2.6.3.7 Terpenoids

There are various forms of compounds present in nature. Terpenoids are evaporative substances. Terpenoids are structure, a gathering of normally happening mixtures which are found in natural plants. Terpenoids are unsaturated compounds which are unstable and provide aroma to plants and their blossoms, as like eucalyptus, conifers and citrus. [69] Organic molecules as like Terpenoids are produced naturally in mostly leaves like cannabis plant. Terpenoids are obtained from tree gums, plants and gums. Carotenoids are tetra terpenoid structures obtained from various compositions of mixtures. Terpenoids which are consisting of hydrocarbons are obtained from natural plants. They form isoprene unit on thermal decomposition. The essential oils are obtained from various tissues of trees and plants. "Terpenoids are the hydrocarbons of plant source of the general recipe $(C_5H_8)_n$ just as their oxygenated, hydrogenated and dehydrogenated subordinates." Thermal role of isoprene is disintegration of terpenoids. The unit of isoprene is joined with terpenoids by head to

2.6.3.8 Polyketides

Polyketides are the largest and diverse class of natural products obtained from bacteria, animals, and fungi. These contain compounds which are characterized by more than two carbonylgroup's single intervening carbon atom. For example polyketides antibiotics and antifungals like erythromycin and tetracycline. There are following type of polyketides.

1. Type 1 Polyketides.
2. Type 2 Polyketides
3. Type 3 Polyketides

2.6.3.9 Tannins

Tannins are the polymeric phenolic compounds which are used in tanning leathers. They can also inactivate and kill the microorganisms which can cause local tumors. [71]

2.7 Natural Products from Plants As a Source of Drugs

Plants are well known from centuries in their use in medicine. They have acquired insects, bacteria, fungi, and also produce secondary metabolites. From early ages, due to supreme characteristics of plants, are used in medicine as important source. With reference to W.H.O more than 80% people rely on plants base medicine for initial health care.

2.7.1 Paclitaxel Drug

The treatment of chest cancer is done by paclitaxel drug which is obtained from the bark of *Taxusbrevifolia*. The USAD first accumulated the bark by plant screening scheme at national cancer institute. [72] The bark of old matures tree supply 1gram of paclitaxel drug which is required for treatment of breast cancer. About 50,000 treatments required 100-200kg per annum. Paclitaxel is present in very small quantity in natural plants. From 1940 to 2010 in Japan round about 175 anti-cancer drugs were accepted in which about 80 plus drugs were naturally synthesized. [73]

2.7.2 Baccatin III Drug

Baccatin is obtained from Yew tree and is precursor of anti-cancer drug (paclitaxel). The substance which may lead to synthesize baccatin III is the expression

of endophytic fungal gene and reported in 2014 by researchers. It is present in higher quantities in needles of *T.brevifolia*. [74]

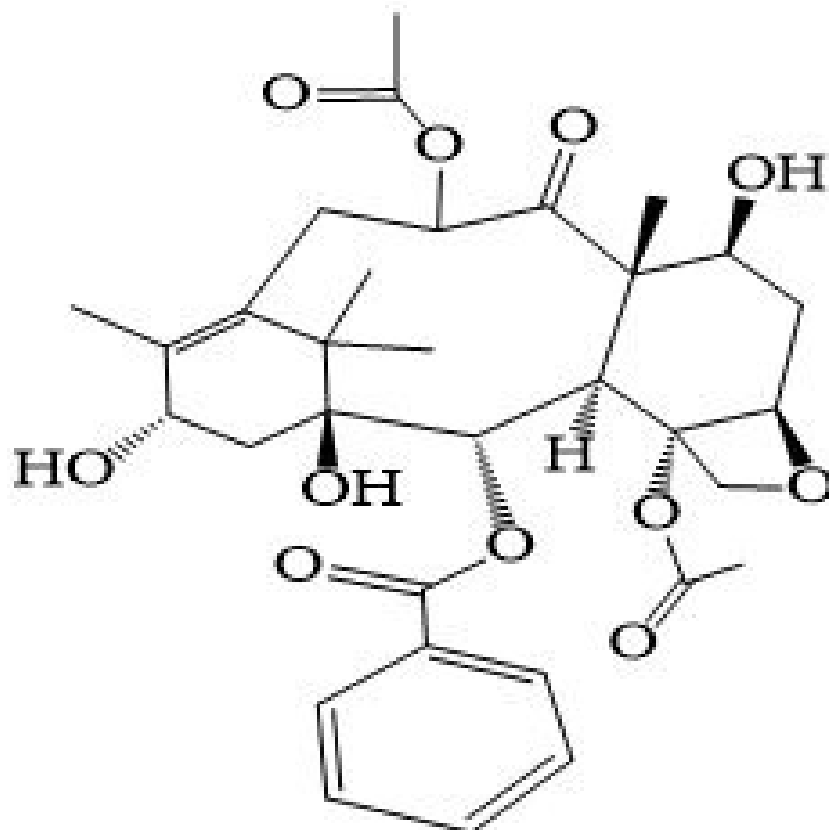
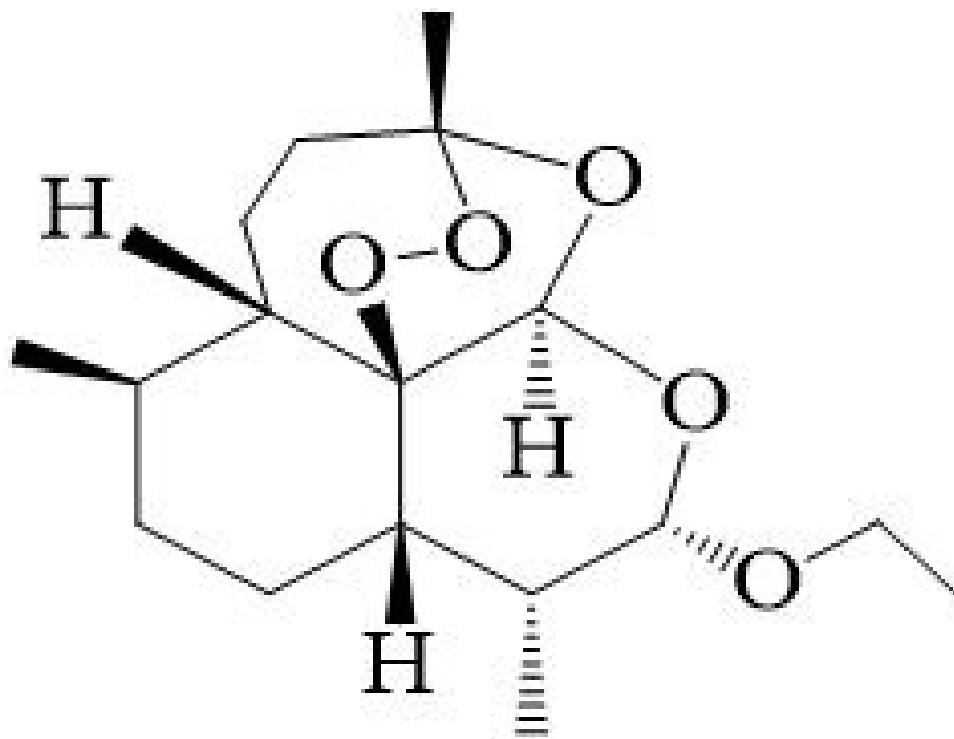


FIGURE 2.1: Chemical Formula $C_{32}H_{40}O_{11}$

2.7.3 Artemotil

From the plant *Artemisia annua*, Artemotil was isolated (artemisnim) and used as anti-malarial drug. In past, that plant was served for curing the chill and fever. Patient with diagnosed case of malaria is given intra muscular injection of Artemether. During first three month of pregnancy, artemether is not given to malarial patient because it can be harmful for fetus.

FIGURE 2.2: Chemical Formula C₁₇H₂₈NO₅

2.7.4 Codeine

Name codeine is given from the Ancient Greek *κωδεία* (kodeia, "poppy head"). Codeine is served for curing pain such as anti-tussive, diarrhea or cough medicine. [75] From the start of the 19th century, raw opium utilized as diverse synthesis known as laudanum. [76] It is obtained from plant opium poppy, which is cultivated for decades for synthesizing medicine such as morphine, codeine and papaverine. About 1% to 3% of codeine is found in this plant and most in common opium alkaloid at 4% to 23%.

2.7.5 Cocaine

In past, over thousand year, the people of South America, chewed the plant *Erythroxylon coca-coca*, which having supreme nutrients and also alkaloids such as cocaine. Cocaine is local anesthetic drug which is obtained from *Erythroxylon*

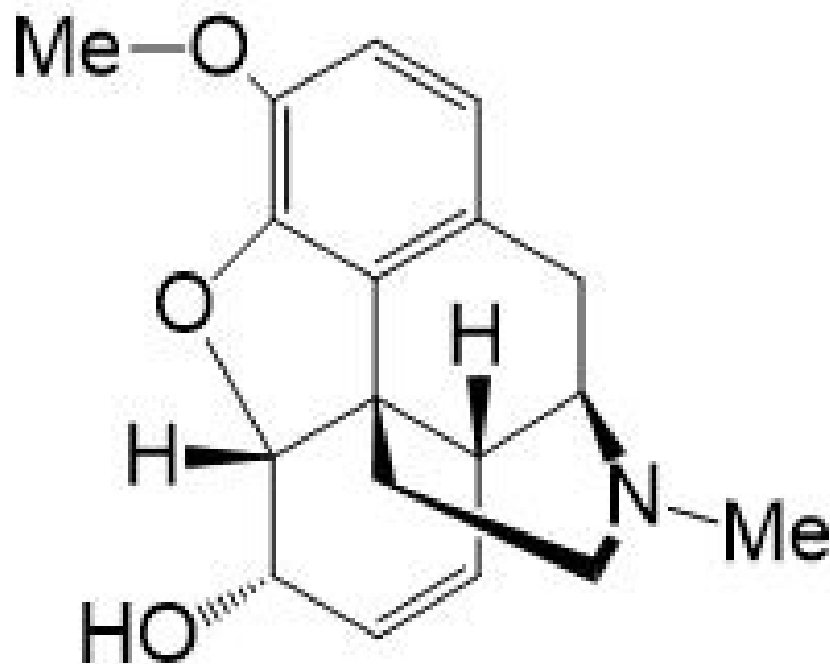
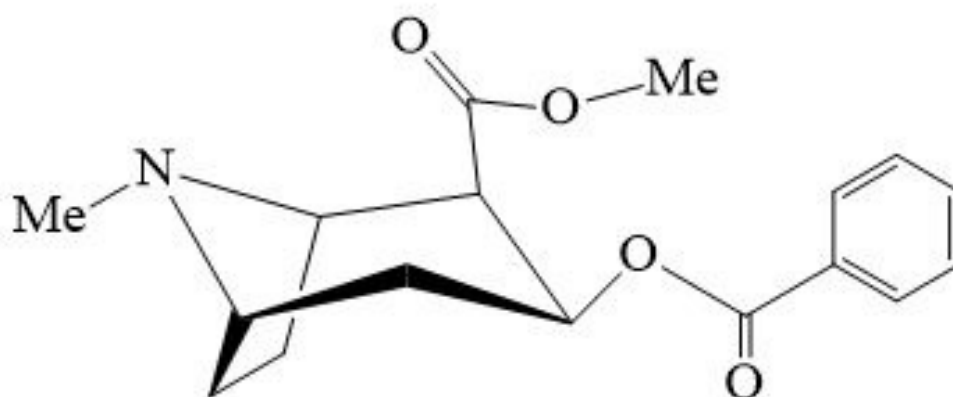


FIGURE 2.3: Chemical Formula $C_{18}H_{21}NO_3$

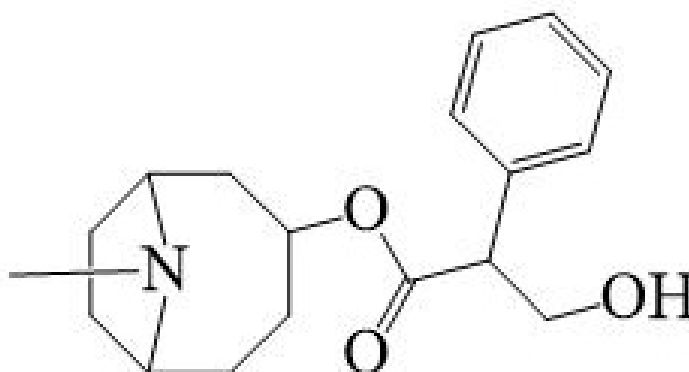
coca-coca plant which is present in South America. In past, people of south America cultivated coca-coca plants for cocaine. They use leaves stems and flowers because these things were full of nutrients such as alkaloids and cocaine. In moderate age, it is used for decreasing bleeding during Nasal surgery. [77] Its use increases the blood infection as well as increase the heart attack risk. Cocaine is second most illegal drug after cannabis worldwide.

2.7.6 Atropine

Atropine was first obtained from night shade plant family such as Jimson weed, Mandrake in 1833. It is class of anti-muscarinic drugs and its duration of action is about 30 to 60 minutes. In B.C, atropine was isolated from Egyptian plant Henbane for purpose of curing pupils. Most common atropine used in medicinal industry is atropine sulfate (monohydrate) which is enantiomer of Alpha hyoscyamine and I-hyoscyamine. Atropine is injected through intravenous, by oral, by rectal,

FIGURE 2.4: Chemical Formula $C_{32}H_{21}NO_{11}$

or in form of eye drops. It decreases the excretion of saliva during the surgery. It is used as an antidote for organo phosphate poisoning. Over dose of atropine can cause dryness of mouth, dilated pupils (mydriases) urinary retention, constipation, tachycardia, nausea, and photophobia. The contraction of the circular pupillary sphincter is barricade due to bad effects of atropine, and stimulated due to acetylcholine. [78]

FIGURE 2.5: Chemical Formula $C_{18}H_{25}NO_3$

All living organism required transformed and inter converted number of organic compounds to achieve them to live, grow, and reproduce. They required energy ion form of ATP, to build their tissues. Network of enzymes and specific chemical Reactions are involved for this purpose, these path ways are called as metabolic pathways.

2.8 Cumin

Cumin is spreading in local regions of Middle East and extending to east India. Every seed of cumin is having natural products, which are dried under sunlight and serve as food purposes in various societies. In spite of the fact that cumin is thought to have utilizes in customary prescription, there is no great proof that it is protected or viable as a helpful specialist.



FIGURE 2.6: Cumin plant and its seeds

2.8.1 Etymology

The term cumin has been obtained from the latin *cuminum*, which is Greek word. It is related to Hebrew.

2.8.2 Description

The dried seeds of herb cumin *C.cyminum*, relate to parsley family individual. The plant cumin develop about 40-50 cm in length and cutted by hands. It does

not have woody structure. It having soft green stem which is slim, fanned and reaching to 25 -35 cm in length and also spreading about 4-7 cm. Cumin plant is having a lot of sub branches. Every branch of cumin is having similar structure which makes it specific umbrella. It has dim green stem. The length of leaves is about 4-8 cm long, bipinnate. It contains pinkish tiny flowers which are grown in umbel. Each umbel is consisting on 5-6 umbellets.

2.8.3 Taxonomic Classification

There are two different varieties of cumin.

- Bitter cumin
- White cumin

2.9 Systematic Bitter Cumin

Kingdom	Planteae
Division	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	<i>Bunium</i>
Species	<i>Persicum</i>

2.10 Systematic of White Cumin

Kingdom	Planteae
Division	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	<i>Cuminum</i>

Table 2.8 continued from previous page

Kingdom	Planteae
Species	<i>C.cuminum</i>

2.11 History

From past ages it is local to Egypt region and mostly grown in India, and Mediterranean nations. Since the beginning, cumin has assumed a significant job as a nourishment and prescription and has been a social image with changed traits. Greeks use cumin powder while having dinner. In roman cooking, cumin was used vigorously. Cumin has been used for centuries as in various flavors in India.

It becomes referenced in Bible not just flavoring for soup and bread, yet in addition as cash served to pay tithes to the clerics. In antiquated Egypt, cumin was not just utilized as a culinary zest; it was likewise a fixing used to preserve pharaohs. Seeds of cumin had used as flavoring in old fashioned kitchen of Roman and Greek. Cumin was known for due to its two restorative and corrective properties. Its application to actuate a colorless appearance was much of the time utilized by numerous understudies attempting to persuade their instructors that they had pulled "dusk 'til dawn affairs" reading for their classes. Despite the fact that a much prized price, cumin turned into an image of cheapness and insatiability in antiquated Rome. Marcus Aurelius and Antonius Pius, heads with notoriety for insatiability, were provided monikers that the reference to cumin.

The most well-known flavor in the middle era of Europe was cumin. At that time, cumin adopted another credit to its collection. Cumin would perceived as an image of admiration and devotion. Individuals conveyed cumin in their pockets when going to wedding services. Cumin's utilization for sustaining affection is likewise spoken to in certain Arabic customs where in a clue of cumin, pepper and nectar is supposed to have Spanish fly characteristics. While regardless it kept up a significant job in various styles of cooking in Middle East and India. Nowadays, cumin is encountering recharged acknowledgment inferable from freshly discovered

FIGURE 2.7: *Bunium persicum*FIGURE 2.8: *Cuminum cyminum*

valuation for its culinary and helpful properties. Cumin is a little bristly, tarnish in shading, sweet fragrance and amazing marginally harsh somewhat bitter and impactful flavor. [79]

2.11.1 Cultivation and Productivity

The major cultivation of cumin has harvested by India and china round about 65-70% of the world supply. The ratio of consumption of India and china is 90%. Mexico also included in this list of cultivating cumin. The cultivation of cumin is about 350000 tons per year. [80]

2.11.2 Climatic Requirements for Productivity

It is dry seasonal plant. Its growth period is about 110-130 days. It require temperature range about 27-32 °C. Growth period of cumin is long term. It requires unique atmospheric conditions. If temperature altered, it harms the growth. It is cultivated in middle of Oct until start of December and cutting start in the month of February. [81]

2.11.3 Breeding

Cumin is having 14 chromosomes and also known as diploid organism. These chromosomes are having various genetic information containing morphological resemblance and length. Cumin is belonging to flowering family. From the moderate technology genetic soft cumin plant is further crossed for better varieties of cumin plants. Nowadays these different varieties are achieved because of specific collections. Breeding consisting on three methods;

1. Invitro regeneration
2. DNA technology
3. Gene transfer technique

The identical production of plants is done through invitro regeneration, as like cultivation of cumin. Leaves, cotyledons, root, internodes, shoot and embryo are medium for invitro cultivation of cumin plant. [82]

2.11.4 Composition

Cumin is organic product containing amino acids, fats, aldehydes, flavonoids and oils; these are major constituents of cumin seeds. Oil or fluids obtained from seeds is highly unsaturated and unstable. It contains cumin aldehyde, o-and p-cymene- and γ -trepanned, 8-cineoleand linalool. Cumin seeds contains fixed oils which

having cellulose, sugar, minerals and protein components. The well-known fundamental oils of cumin are anis liquor, ascorbic, vanillin, octanol, benzoic corrosive and limonene. Natural acids also found in cumin seeds e.g. tartaric, maleic, citrus and aspartic. Oils of cumin are used as an aroma part for beautifying agents. [83]

2.11.5 Constituents of Cumin

Significant level of Phenolics mixes is present in basics oils of cumin seeds. There are two standard dynamics components of cumin e.g. cumin aldehyde is utilized to ensure liver against oxidative pressure. Cumin aldehyde is free smelling monoterpenoids, which is unstable and consisting on basic oils of cumin. Cumin aldehydes is having subatomic recipe $C_{10}H_{12}O$. The weight of subatomic cumin aldehyde is 146.5gmol⁻¹. [84]

B.persicum also contains other compounds belonging to different classes of natural products such as Caryophyllene, β -Pinene, Eugenol, carvacrol, Safrole, 1,8-Cineole, S-3-Carene, cuminyl acetate, pinocarvyl acetate, α -methyl-benzene methanol, croweacin, Bornyl acetate, p-mentha-1, 3-dien-7-al, p-mentha-1, 4-dien-7-al and p-menth-3-en-7-al, Sabinene, Myrcene, Caranone, α -Thujene, Camphene and Terpinolene. [85]

2.11.6 Chemical Constituents

Seeds of Cumin are nutritionally rich. They give large quantity of fat such as mono unsaturated fat and dietary fiber. Several dietary minerals as such vitamins B, E and, especially iron, are also present in cumin seeds. The major volatile components of cumin seed extracts are Cumin aldehyde, cymene and terpenoids. [86] Cumin has a peculiar strong flavor. Due to the presence essential oil content, cumin is having warm aroma. Cumin aldehyde and cumin alcohol is the main component of its aroma. The other especial aroma compounds of roasted cumin are the substituted 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-sec-butylpyrazine, and 2-methoxy-3-methylpyrazine. More other constituent of cumin are γ -terpinene,

safranal, p-cymene, and β -pinene [87]. *Nigella sativa* seeds are having protein (26.4%), fat (27.5%), carbohydrates (23.9%), crude fiber (8.7%), and total ash (4.1%). *Nigella sativa* seeds also having a good quantity of several vitamins and minerals like Cu, P, Zn, and Fe. Numerous active compounds have been recognized in *N. sativa*. The most active compounds of *N. sativa* are thymoquinone (TQ) (35%–45%) thymohydroquinone, dithymoquinone, p-cymene (11%–14%), carvacrol (7%–13%), 4-terpineol (3%–6%), t-anethole (2%–5%), sesquiterpene-longifolene (2%–7%), α -pinene, and thymol. [87] *Nigella sativa* also having other substances as such carvone, citronellol, limonene in small amounts, and two types of alkaloids, i.e. isoquinoline alkaloids and pyrazole alkaloids. *Nigella sativa* seeds also contain α -hederin, a water soluble pentacyclitriterpene. [88]

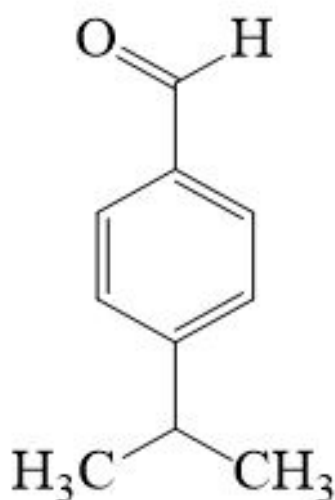


FIGURE 2.9: Chemical Formula C₁₀H₁₂O

The pharmacological abilities of *N. sativa* are mainly quinone constituents, TQ being the richest. The *N. sativa* seeds extract are having fatty oil rich in unsaturated fatty acids, linoleic acid (55%–62%), oleic acid (22%), eicosadienoic acid (4%), and dihomolinoleic acid (11%), and saturated fatty acids contribute up to 30 percent. α -Sitosterol is the major sterol, accounting for 44%–54% of the total sterols in *N. sativa* oils, followed by stigmasterol (7.57%–21.9% of total sterols) [89]. Bitter cumin (Shahijeera) seeds have calcium, vitamin A, potassium, sodium, iron,

magnesium, and phosphorus. *B. persicum* has 0.6 to 1.6% essential oil, especially carvone (45%–55%), limonene, and p-cymene. Bitter cumin looks like brownish to yellowish green. As we know that there is not enough scientific knowledge on the health effects of bitter cumin, this is limited review to *C. cyminum* (cumin seeds) and *N. sativa* (black seeds).

2.12 Uses of Cumin

Numerous therapeutic effects are addressed in traditional medicine through the world for treatment of various disorders such as gastrointestinal, urinary tract and use as a diuretic, gynecologic, anticonvulsants, anti-helmentic, anti-asthma and dyspnea. [90]

2.12.1 Common Uses

Seasonal nourishment as like flavor is synthesized by using cumin seeds. Cumin segments also having cancer prevention agent as like anticancer, antibacterial.

2.12.2 Classical Uses

Cumin seeds additionally severed to lower the glucose level and decreases fortify bones, Conventional employments of cumin incorporate lessening irritation, expanding pee, avoiding gas and smothering muscle fits. It is likewise utilized as a guide for acid reflux, jaundice, the runs, and tooting. Cumin is used to synthesize toppings mixed refreshments and treats. Cumin is major part of different moisturizers, scents, creams and various fragrances. For distinctive customary frameworks, cumin is said to be carminative furthermore, fruitless, clean, laxative also having helpful impacts on GTI, reproductive and respiratory issue, and furthermore to cure tooth problems, looseness of the bowels and epilepsy.

2.12.3 Therapeutic Uses

Cumin's dynamics parts are utilized as mitigating, antibacterial, CDV, CPD, eye illness, waterfalls holes, assimilations, GTI problems, elevated cholesterol, low glucose, MSS, decrease in RBC, weight loss, production of milk in females, creep crawly repellent insecticidal and cancer preventing agents. Further medicinal practices of cumin are portrayed underneath.

2.12.4 Antioxidants

Because of presence of different active compounds like flavonoids, alcohols, terpenes and Phenolic, cumin oil is considered as highly antioxidant.

2.12.5 Microbicidal

The antibiotic activity of cumin oil and fluid has evaluated verses significant scope and pathogenic effect on gram positive and gram negative strains. Alcoholic cumin seed oil concentrates trained development of klebsielappneumonia. And reason to cause development in cell structure. The counter parasitic movement against nourishment creatures, pathogens, yeast, a flotoxins and mycotoxin maker are exemplified by due to cumin.

2.12.6 Anti-carcinogenic

The dietary enhancements of cumin have forestalled the event of rodent colon malignancy instigated by a colon-explicit cancer-causing agent and furthermore decline the action of β -glucuronidase and mucinase proteins. The nearness about phytoestrogens in cumin has demonstrated by COI, decreasing Calcium level of urine and retarding growth of calcium content in bones.

2.12.7 Antidiabetic

Cumin lowered the blood sugar. Diabetic medication also causes to lower the blood sugar. Taking cumin with diabetic medicine may cause to lower the level of blood sugar. Monitor your blood sugar closely. Some medications served for diabetes include glimepiride, glyburide, insulin, pioglitazone, rosiglitazone, and others.

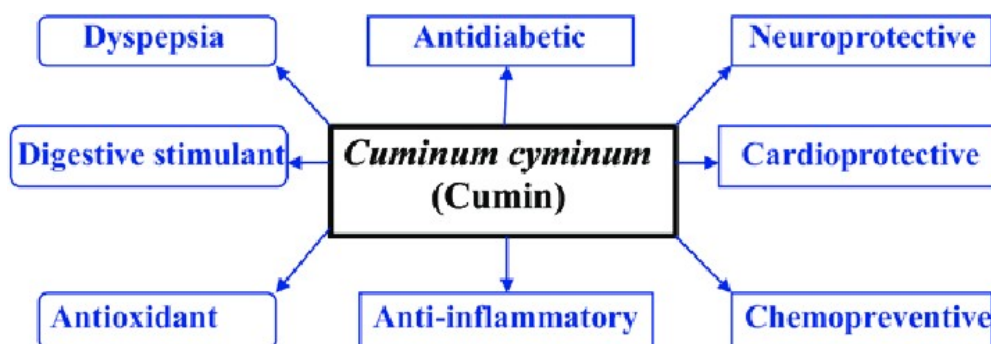


FIGURE 2.10: Medicinal Importance of Cumin.

2.13 Side Effects and Safety

The safe way through cumin is taken is mouth and a balanced doze of cumin is given to the patient with maximum safety. The side effects of cumin or draw backs of cumin for health are not known yet.

2.13.1 Precautions & Warnings

2.13.1.1 Breast-feeding

There is no any authentic and reliable knowledge about the safety of cumin while someone is in pregnancy or in breast feeding.

2.13.1.2 Bleeding Disordersng Disorders

Cumin can provide a source to slow down the phenomenon of blood clotting by causing the retardation in platelets formation on wounded blood vessel.

2.13.1.3 Diabetes

Due to cumin some time the sugar level of some people decreases. If the sugar level of a patient increases he must be given cumin which may cause to slow down the sugar level of that patient.

2.13.1.4 Surgery

While doing surgery, cumin lowers blood sugar levels. Few experts suggested that it can disturb the blood sugar control during and after surgery. Need to stop having cumin at least one week before a scheduled surgery.

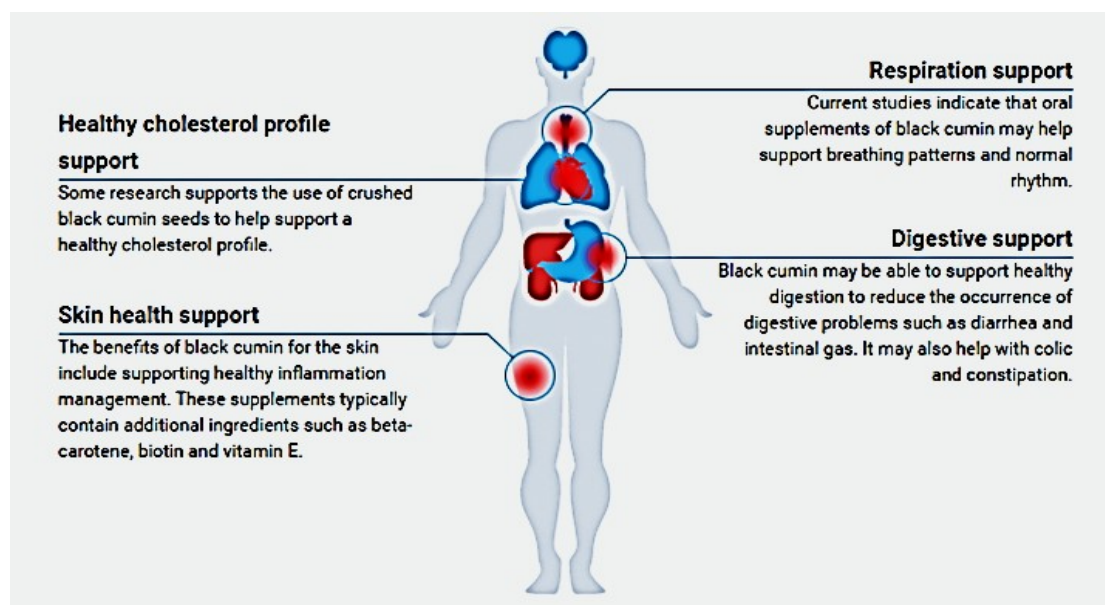


FIGURE 2.11: Cumin Medicinal Use

2.14 Research Questions and Aims of The Present Study

As in our knowledge that 65-70% Pakistan's live in rural areas, depending on plants for food, health, and also for medicine. These 70% people of Pakistan are utilizing traditional methods for curing the various ailments. So, it is need of time now to study these plants for better aspects in drug formation. Due to poverty, the people of Pakistan use homeopathic medicine instead of allopathic medicines. As a result for need of age, I have to select Pakistani plants; *Cuminum cyminum* and *Bunium persicum* to highlight it for its supreme and noval bioactive substances.

Chapter 3

Materials and Methods

Research work of my thesis was carried out in wet lab, Department of Biosciences and Bioinformatics, Faculty of Health and Life Sciences, Capital University of Sciences and Technology, Islamabad.

3.1 Materials

Following material was utilized for research work:

3.2 Chemicals

TABLE 3.1: Chemicals

Chemical name	Company from which Chemical Bought
Nutrient agar	Sigma- Aldrich
Sabouraud dextrose agar	Sigma- Aldrich
Brine shrimps eggs	Sigma- Aldrich
Sea salt	Sigma- Aldrich
Ascorbic acid	Sigma- Aldrich

Table 3.1 continued from previous page

Chemical name	Company from which Chemical Bought
Ethanol	Sigma- Aldrich
Tetracycline	Sigma- Aldrich
Distilled water	Sigma- Aldrich
DPPH reagent (2,2-diphenyl-1-picrylhydrazyle)	Sigma- Aldrich
Terbinafine	Sigma- Aldrich

3.2.1 Apparatus and Equipments

Beakers 500ml, 100 ml, petri plates, test tubes, glass vials, micropipette, micropipette tips, cotton swabs, cotton plugs, falcon tubes 15 ml, 50 ml, aluminium foil, ependroftubes, parafilm wax or masking tapes, test tube racks, discs, foeceps.

3.2.2 Microorganism

Different bacterial and fungal strains are used.

3.2.2.1 Bacterial Strains

- *Micrococcus luteus*
- *Enterobactoraergenes*
- *Staphylococcus aureus*
- *Agrobacterium tumefaciens*
- *Bacillus subtilis*
- Gram positive (*M.luteus*, *S.aureus*, *B.subtilis*)
- Gram negative (*A. tumefaciens*, *S. setubal*, *E. aerogenes*)

3.2.2.2 Fungal Strains

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

3.2.3 Overview of Research Methodology

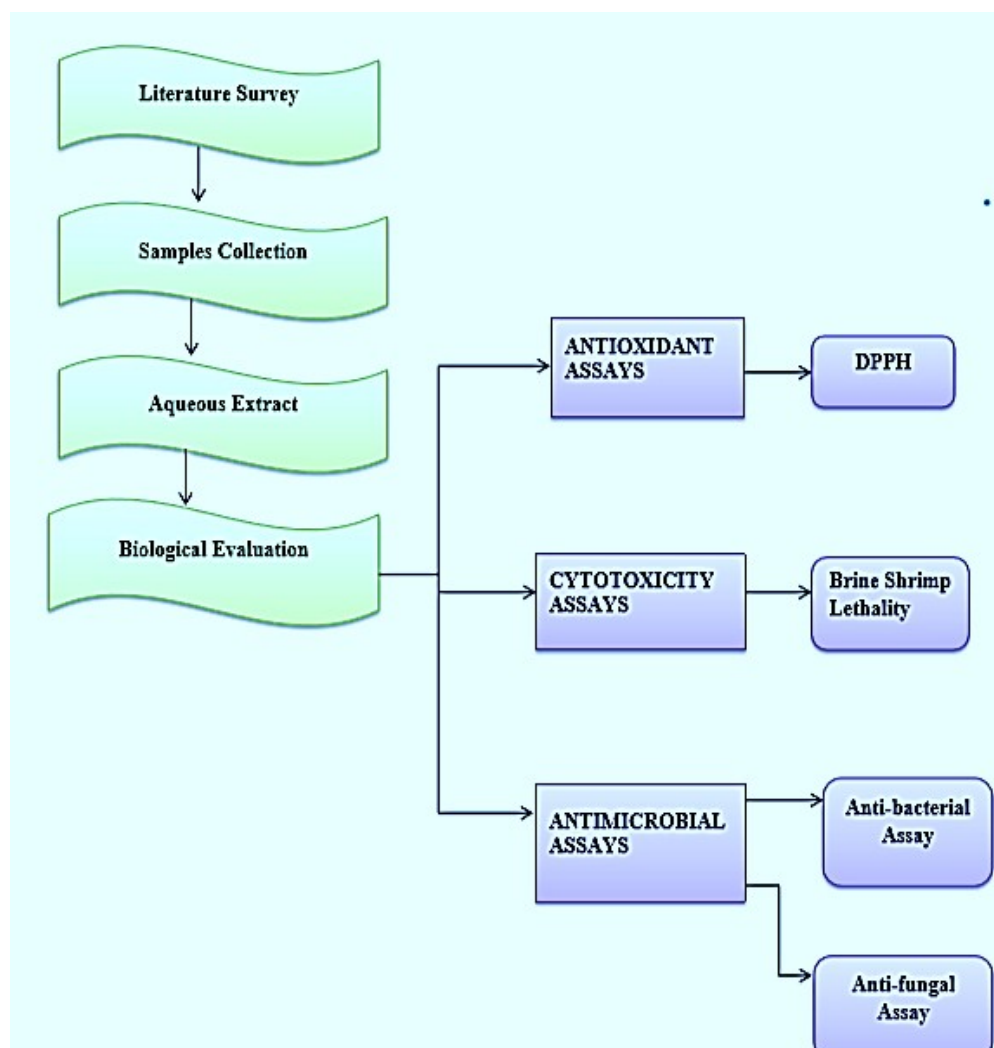


FIGURE 3.1: Overview of Methodology

3.2.4 Sample Collection

C. cyminum (white cumin) and *B. percicum* (bitter cumin) seeds were purchased from grocery shop of local market of Soan garden Islamabad on 1st November.

3.2.5 Preparation of Seed Extract

About 20 g of each *C. cyminum* and *B. percicum* seeds were taken and properly cleaned by hands and then washed thoroughly to remove other dust particles, then boiled at 100ml of distilled water round about 15-20 minutes at room temperature. After boiling kept for some time to cool down and then refine with Whatmann No. 1 filter paper and seed extract was kept in refrigerator for further use. That extract act as reducing and stabilizing agent.

3.3 Biological Evaluation of Samples

3.3.1 Antimicrobial Assays

For evaluating biological activity of prepared seed extracts two types of antimicrobials assays were performed which are as under

- Antifungal Assay
- Antibacterial Assay

3.3.2 Antibacterial Assay

Five bacterial strains were used in this assay. Four strains are opportunistic or mostly human pathogenic except *Anterobactor tumefaciens*. Disc diffusion processes was used to know bactericidal activity as described by Ruparelia et al.

Different microorganisms are responsible for causing different diseases in human ,plants as well as animals. These infectious diseases are transmitted by water, contaminated food and by any other infectious material containing organisms. They infect mostly those having weak immune system. Examples includes HIV , ageing malnutrition etc.

3.3.3 Bacterial Strains Used

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

3.3.3.1 Gram Positive Strains

B. subtilis is a bacterium which was found by Christine Gottfried in 1835. Its name was changed in 1872. It is very common model organism used in different laboratory studies. It is also known as Hay bacillus, is a catalase positive, rod shaped, gram positive and Spore forming bacterium. *B. subtilis* generally found in gastrointestinal tract of ruminants and people and soil. It is considered as one of the bacterial champions, used on industrial scale by many biotechnologists. Their survival range is 30-39°C.



FIGURE 3.2: *B.subtilis* Gram Positive Strains [87].

B. subtilis nourishment harming includes a fast onset and with acute vomiting commonly taken after by runs.

S. aureus is a gram positive, anaerobic, catalase positive round shaped bacterium. It is member of fermicutes. *S. aureus* is generally found on skin and in upper respiratory tract. It was found by Sir Alexander Ogston in 1880. Its name represents staph meaning a cluster of grapes and the coccus portions representing the circular shape of microorganism and the aureus because of its colorful appearances. It is an opportunistic pathogen and responsible for causing common skin infections like abscesses and respiratory illnesses like sinusitis. In about 30 % of population it is considered as a part of l skin flora. Its survival range is 20- 40°C.

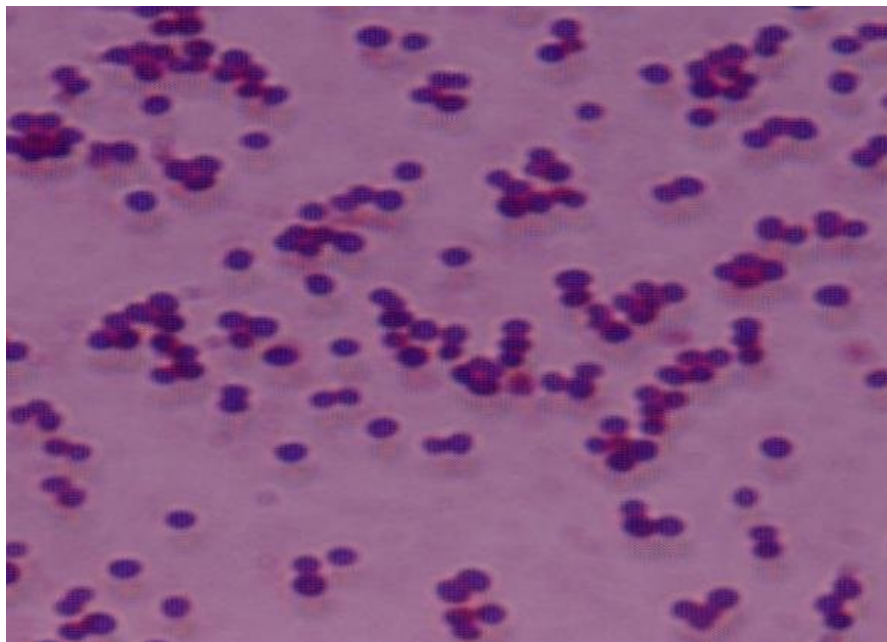


FIGURE 3.3: *S. aureus* Gram Positive Strains [88].

M. luteus is gram variable, coccus, gram positive, nonmotile bacterium belongs to Micrococcaceae family. It can form irregular clusters. It is catalase positive aerobic bacterium. It is commonly found in air, water bodies and in mammalian skin. It was discovered by Sir Alexander Fleming before the discovery of penicillin in 1928.

It was sequenced in 2010 and considered as one of the smallest genome of free-living. Its name stands for tiny (micro), of round shaped (coccus) and yellow

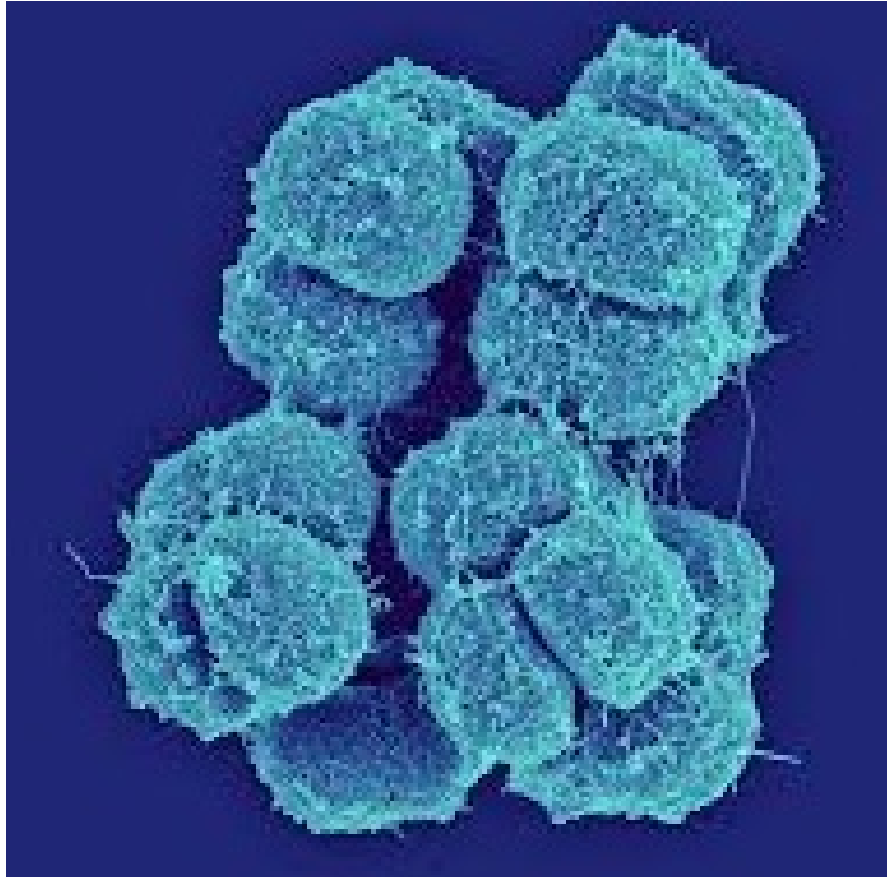


FIGURE 3.4: *M.luteus* Gram Positive Strains [89].

(luteus). *M. luteus* is responsible for odors in people by breaking components of sweat. It is considered as contaminants in patients. In immune compromised people it is responsible for septic stuns. It is a climatic microorganism and is one of the most common contaminants of lab societies. *M. luteus* is associated with different illnesses like septic arthritis, meningitis endocarditis etc.

3.3.3.2 Gram Negative Strains

A.tumefaciens is motile, none spring, rod shaped, gram negative bacterium belonging to family Rhizobiaceae, which have nitrogen fixing knobs on different leguminous plants. Agrobacterium is the main agent for causing crown gall disease in over 140 species. Unlike nitrogen fixing, Agrobacterium are serious pathogens and responsible for various tumor formation in stone fruits, nut trees, sugar beets, walnuts and rhubarb, most importantly they are harmful to perennial crops.

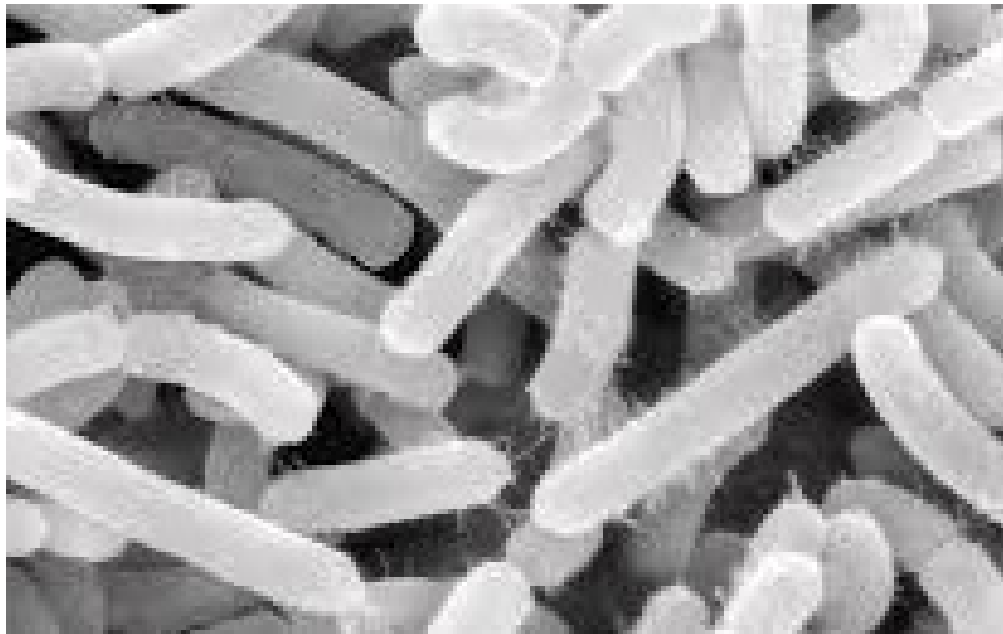


FIGURE 3.5: *AT-10* Gram Negative Bacteria [90].

E. aerogenes is *Klebsiella aerogenes*, whose previous name is *Enterobacter aerogenes*; it is indole negative, gram negative, citrate positive, rod shaped, motile, catalase positive bacterium. Its size ranges in about 1-3 microns in length. It is considered as multiresistant opportunistic pathogen for humans.

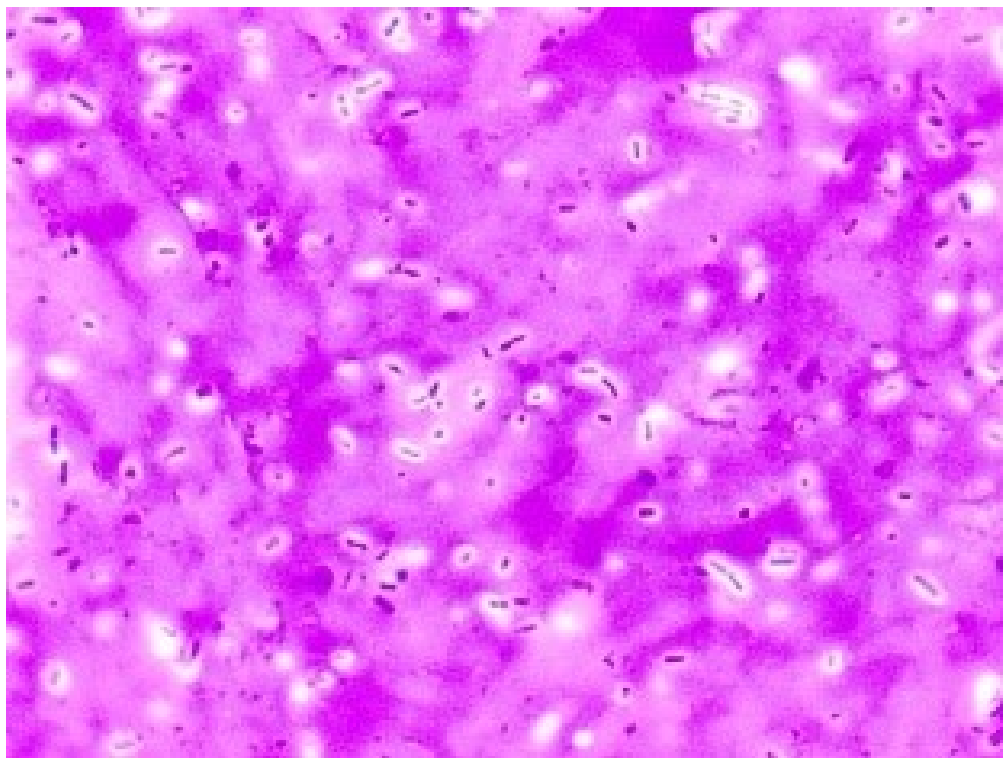


FIGURE 3.6: *E. aerogenes* Gram Negative Bacteria [91].

It can be found in dairy items, water bodies, in humans and animals. They can also be found in GIT, soils and in hygienic chemicals. Enterobacter aerogenes are responsible for causing nosocomial infections like UTIs and respiratory tract infections and various bloodstream infections. These can be transmitted during different surgical cross contamination issues.

3.3.4 Media for Bacterial Growth

For bacterial growth in petri plates Luria broth agar was used. Its composition is as following:

- | | |
|-------------------|---------------|
| 1. NaCl | 5.00g/ 500ml |
| 2. Yeast | 2.50g / 500ml |
| 3. Agar | 7.50g / 500ml |
| 4. Bacto-tryptone | 5.50g / 500ml |

3.3.5 Experimental Procedure

Firstly all material like petri plates, cotton swabs, media, forceps each and every thing autoclaved at 121 for 15 minutes. As we know disc diffusion method was used so disc were prepared by punching the Whatmann No. 1 filter paper. The average size taken for disc is 4mm measured using scale.

After this all equipment's and material were transferred to culture room and allowed to stand under the UV radiation for 15-20 minutes to avoid contamination. Luria broth agar was poured in petri plates in equal quantity and leaves 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 five discs, three for extract and other two for positive and negative control. Tetracyclin (100ppm) was used for positive control. Distilled water (10ppm) was used as

negative control. Then petri plates sealed with parafilm and incubate at 37 for 24 hours. After 24 hours bacterial growth was observed and zone of inhibition were identified around each disc. Experiment was done in triplicate.

The plant extracts antimicrobial activity may not be due to the action of a single active compound, but the synergistic effect of many compounds which are in less proportion in a plant. [91] Crude plant extracts are generally consisting on a mixture of active and non-active compounds, and MICs of less than 100 μ gmL⁻¹, suggested well antimicrobial activity. [92] Some studies have addressed the antifungal activities of essential oil, ethanolic and aqueous extracts of *S. mole*. [93]

3.4 Antifungal Assay

Antifungal activity of cumin plant extract was known by following tube dilution method

3.4.1 Fungal Strains

The fungal strains were used which are as follows:

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

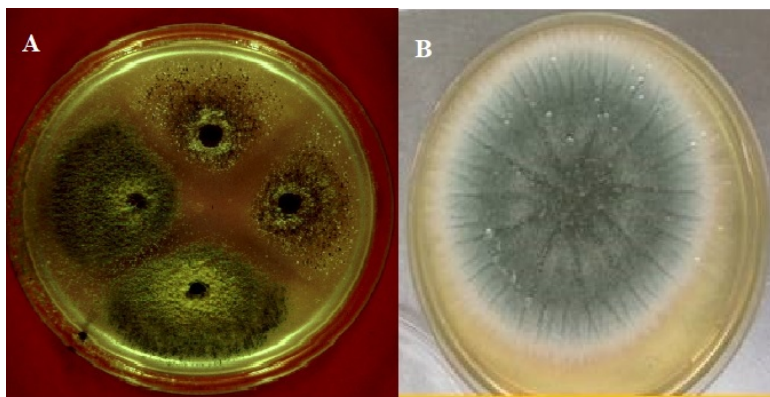


FIGURE 3.7: A: *Aspergillus flavus* B: *Aspergillus fumigatus* [92].

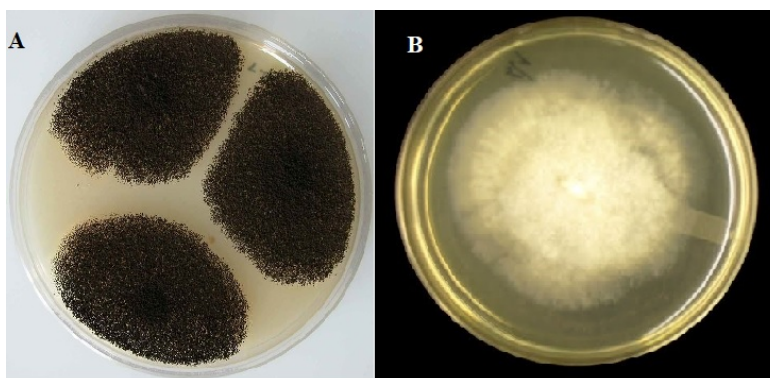


FIGURE 3.8: A: *Aspergillus fumigatus* B: *Aspergillus niger* [93].



FIGURE 3.9: *Fusarium solani* [94].

3.4.2 Media Preparation

Sabouraud dextrose agar for the purpose of growth of fungal strains was prepared. Its composition is given below:

Sabouraud dextrose agar	6.50g/100ml distilled water
-------------------------	-----------------------------

3.4.3 Experimental Procedure

First of all material was autoclaved at 121 for 20 minutes. After this test tubes were marked to 10 cm with help of scale. 5 ml of sabuoured dextrose agar was included to test tubes and cotton plugs used to cover the test tubes. Then (100ppm, 50ppm, 150ppm) of different solutions were added in test tubes. Turbinafine and distilled water both were used as positive control and negative control respectively; a slant was made to 10 cm mark at room temperature and leaves it to solidify.

After solidification test tubes were inoculated with fungal strains and covered with cotton plugs. The whole procedure was carried out in triplicate for different extracts. Test tubes were incubated at 37 for 4 days. By a quantitative microspectro photometric assay the antifungal activity was measured [95]. A microplate process was utilized with little update to know minimal inhibitory concentration (MIC) values of plant extracts. [96] The fungicidal activity (MFC) was known and explained by Espinel-Ingroff. [97]

Readings were taken by measuring the fungal growth in slanting position. Percentage was calculated with reference to growth inhibition. The growth inhibition percentage was calculated by using following formula:

$$\%I = \left[\frac{(\text{Linear growth in } - \text{ive control}) - (\text{Linear growth in samples})}{\text{Linear growth in } - \text{ive control}} \right] \times 100$$

3.5 Antioxidant Assay

Antioxidant activity of cumin plant extracts was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method as explained by Gyamfi et al.

3.5.1 Sample Preparation

Stock solution (200 mg/mL) was prepared by making further different (200, 300, 150 ppm) concentrations.

3.5.2 Preparation of DPPH Solution

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

3.5.3 Experimental Procedure

Glass vials were taken and about 200ppm of different extract was added along with 2.8ml of reagent (DPPH) solution. The whole procedure was carried out in duplicate. For positive and negative control, ascorbic acid along with reagent and ethanol was used respectively. Then vials were placed in dark for 45 minutes. After 45 minutes, absorbance of the sample measured at 516nm by using distilled water as blank reference. Water was used to check whether the water has an individual antioxidant activity or not because water is used as solvent in this assay, and best result was selected.

Following formula used to calculate the radical scavenging.

$$\%Scavenging = \left[\frac{Control\ absorbance - Nanoparticle\ sample\ absorbance}{Control\ absorbance} \right] \times 100$$

3.6 Cytotoxicity Assay

Cytotoxic potential of seed extracts were determined by using brine shrimp lethality method.

3.6.1 Sample Preparation

Stock solution (200 mg/mL) was prepared and used for analysis of different (100, 200, 300ppm) concentrations.

3.6.2 Sea Salt Preparation

Sea salt was prepared using following concentration;

Sea salt	34g/L
----------	-------

3.6.3 Hatching of Eggs

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

3.6.4 Experimental Procedure

‘ Different vials were prepared with extract sample (300, 200, 100ppm) to make final volume of 5ML. Sea water was added in vials. Distilled water was used for negative control. After 1 day, shrimp eggs were hatched and seen floating in sea salt water. These shrimps were transferred to each vial (about 15 shrimps each vial) vials are kept under the light at room temperature.

After 1 day brine shrimps were counted by pasture pipette (3 x magnifying glasses) and process was carried out in duplicated, then best result was selected. Percentage viability was calculated by formula:

$$\%age Viability = \left[\frac{(No. of AS in - ive control) - (No. of AS in test)}{No. of AS in - ive control} \right] \times 100$$

Chapter 4

Results and Discussion

Biological evaluation of prepared seed extracts (*C. cyminum* and *B. persicum*) was carried out by performing different biological assays; Antibacterial, Antifungal, Cytotoxic and Antioxidant activities results are given below here.

4.1 Antibacterial Assay

Especially plants extracts are well known as antibacterial agents due to their inhibitory properties against a wide range of pathogens, gram-positive and gram-negative bacteria. [98] By using disc diffusion technique antibacterial activity of *C. cyminum* and *B. persicum* were determined. Five bacterial strains were used in this assay i.e. three gram positive (*B. subtilis*, *M. luteus*, *S. aureus*) two gram negative (*A. tumefaciens*, *E. aerogenes*). Results highlighted in table no 4.1. The antimicrobial effect of plant extracts was known by comparing with control treatments. Minimum inhibitory concentration was determined against different concentrations of *C. cyminum* and *B. persicum* 30ppm, 60ppm and 90ppm. Minimum inhibitory is the lowest concentration of any chemical usually a drug that retards bacterial growth. After incubation period of about 24 hours, clear inhibition zone was observed and measured.

Growth of all bacterial strains, both gram positive (*S. aureus*, *B. subtilis*, *M. luteus*) and gram negative (*A. tumefaciens*, *E. aerogenes*) was inhibited at 90ppm concentrations. At 90ppm Concentration, in gram positive *B. persicum* shows antibacterial activity 1.2cm while *C. cyminum* showed 1.0cm against *B. subtilis*. At same concentration *B. persicum* showed 1.7cm while *C. cyminum* showed 1.1cm against *S. aureus* strain. In case of gram negative strains *B. persicum* showed least activity as compared to *C. cyminum*, such as at 90ppm *B. persicum* showed 1.2cm while *C. cyminum* showed 1.9cm against *E. aerogenes*. It was also observed that there was rapid decline in bactericidal activity when concentration was decreased.

Among all the five strains used, four are human pathogenic and are responsible for causing variety of human infection or we may say that opportunistic pathogen. Therefore these four pathogens are not harmful for plants species but the strain *Agrobacterium tumefaciens* which is causing agent for crown gall disease, beside this it is host soil bacterium and also known as biotechnological tools, here we concluded that *Agrobacterium tumefaciens* is the only strains which is responsible for causing various diseases in different woody shrubby plants Worldwide.

It has been known that gram positive bacteria is having less resistant to plant extracts as compared to gram negative bacteria. The resistant of gram positive bacteria to antibacterial substances is due to lipophilic substances that pass across the membranes and walls. Interaction b/w extracts and with phospholipids and polysaccharides increases the permeability of cellular content and bacterial membrane, causing the death of cell. [99] Also, the activity of the proton pump leakage of cell contents and reduction of membrane clotting can cause cells death. [100] These compounds can be the reason of denaturing of cytoplasm proteins and inactivation of cellular enzymes. [101]

For new antimicrobial compounds especially against bacterial pathogens plant extracts are potential sources. These extracts have hydrophobicity characteristics. Therefore, they can degrade the lipids of the bacterial cell wall and the mitochondria and subsequently destroy the bacterial structures. Phenolic substances are having an antimicrobial activity such as γ -terpinene, carvacrol, φ -cymene and

thymol. [102] Essential Oils of *B.persicum* have a greater inhibitory effect on gram-positive bacteria than gram-negative bacteria. [103]

In another study, the GC-MS analysis of the cumin essential oils showed 15-17 parts in its composition with the predomination of cumin aldehyde and γ -terpinen, α -terpinen, β -pinene and p-cymen. This finding was consistent with the previous studies.

Study on the inhibitory impacts of *C. cuminum* EO on the fibrillation of α -SN showed that the little rich natural compound, cumin aldehyde can balance α -SN fibrillation, showed that such regular active aldehyde have capability of remedial applications. [104] In the current study we found that *C. cuminum* was effective in elimination of the tested microorganisms in the ZOI (Zone of inhibition). Partially in line with our findings, a previous research on Tunisian cumin reported that the MIC of cumin essential oil were about 78-150 μ g/mL versus panel of gram positive and gram negative microorganisms including *E. faecalis*.

TABLE 4.1: Results of antibacterial assay of *B.persicum* and *C. cuminum* against different bacterial strains.

Plant extract	Zone of inhibition (cm) \pm S.D									
	Gram Positive Strains						Gram Negative Strains			
	<i>M. luteus</i>		<i>S. aureus</i>		<i>B. subtiles</i>		<i>AT-10</i>		<i>E. aerogenes</i>	
Conc (ppm)	Bp	Cc	Bp	Cc	Bp	Cc	Bp	Cc	Bp	Cc
90	1.1	1.3	1.7	1.1	1.2	1.0	1.0	1.0	1.2	1.9
	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
	0.05	0.1	0.05	0.05	0.1	0.1	0.1	0.1	0.1	0.1
60	0.5	0.9	1.2	0.8	0.9	0.7			0.6	1.7
	\pm	\pm	\pm	\pm	\pm	\pm	-	-	\pm	\pm
	0.1	0.1	0.1	0.1	0.1	0.05			0.05	0.05
30						0.3				
	-	-	-	-		\pm	-	-	-	-
						0.05				

Table 4.1 continued from previous page

Plant extract	Zone of inhibition (cm)±S.D									
Distilled H ₂ O (Negative Control)	-	-	-	-	-	-	-	-	-	-
Tetracycline (Positive Control)	2	2.2	2	2.1	2.5					

4.2 Antifungal Assay

The word antifungal is a medication used for treatment and also retardation from mycosis, athlete foot and ring worm. Antifungal species are fruitful agents for variety of diseases and give resistance to pathogenic species of fungi. There is requirement of age to assemble such alternatives which can be useful against the problems of mankind. For this motive, medicines are synthesized from natural plants which are having lots of antibiotics properties as like antioxidant, antibacterial and antifungal activities which are significantly useful. Plants are great source of variety of secondary metabolites with antifungal, anti-oxidant and antifungal properties. The phytochemicals which are obtained from primary and secondary metabolites of the natural plants, are studied for the determination of structure and activity correlation, the most recognized seven volatile constituents namely liane, p-cymene, β -pineene, cumenal, and myrrh.

In these components, azuldehyde and p-benzyl isopropyl anisole have the strongest antifungal activity against *Fussarium oxysporum*, which recommended that they play a vital role in Bitter cumin antifungal activity. Similarly *B.persicum* and *C.cyminum* both are natural plants having antifungal activities against various fungal strains and pathogens. The main antifungal agent of *B.persicum* and *C.cyminum* is cumin aldehyde which is obtained from the extracts of these plants.

There is great inhibitory effect on *Candida Albicans* growth by *Carum cari* aqueous extract. [107] The outstanding and remarkable antifungal agent is cumin which explains the promise as a potential therapeutic agent for treatment of superficial and mucosal Candidiasis, including vaginal candidiasis. Thus seed oil of cumin plant can be useful as natural retarder to overcome on growth of most significant pathogenic candida species and choices treatments for candidiasis.

4.3 Cytotoxic Assay

To observe the cytotoxic effect of both plants extract, brine shrimps lethality assay was performed. Different concentrations for plant extracts are i.e. 300ppm, 200ppm and 100ppm were used and showed significant toxic effects. It was observed that toxicity decreases with decrease in concentrations. Results are shown in table 4.3 and 4.4. Highest activity of *Bunium persicum* is 53%, 40% and 46% showed at 300ppm concentration in triplet experiment performance, similarly *Cuminum cyminum* highest activity was 33%, 40% and 40% at 300ppm concentration. The least activity of *B. persicum* was 13%, 6% and 6% at 100ppm conc.

Similarly for *C. cyminum* was 0%, 6% and 6% at 100ppm highest concentrations. Higher concentration had more mortality rate then lower concentration of plant extracts, average results are shown in tables 4.3 and 4.4. Results were also found significant because IC₅₀ value for *B. persicum* is 300ppm and *C. cyminum* is 240ppm. With the help of this study it was evaluated that *C. cyminum* is more significant as compared to *B. persicum* .

It is fact that *B.persicumicum* is widely used for people's diet due to its flavoring properties, there is no any major concern about the harmful effects of this plant. It was observed that *C. cyminum* aqueous extract showed enhances cytotoxic potential as compared to *Bunium persicum*.

TABLE 4.3: Analysis Variances for Factors Affecting the Toxicity of Brine Shrimps

% Toxicity						
Source of variation	D.f Value	Sum of square	Mean Square	F-value	P- Value	Significant
Interaction	4	1711	4276	49.98	<0.0001	Yes
Plant extract	2	24370	12180	1424	<0.0001	Yes
Conc.	2	3258	1629	190.4	<0.0001	Yes
Residual	18	154.0	8.556			
Missing Values	9					

TABLE 4.4: cytotoxic assays of *B.persicum* and *C.cyminum* effecting toxicity of brine shrimps

Sr. No	Sample	Concentration (ppm)	% Toxicity	IC ₅₀
1	<i>B. persicum</i>	300	50%	300ppm
		200	23%	
		100	10%	
2	<i>C. cyminum</i>	300	60%	240ppm
		200	43%	
		100	21%	

4.4 Antioxidant Assay

The antioxidant ability of Cumin plants extract was evaluated by DPPH assay. The free radical scavenging activity was exhibited by both plants extracts. In this study *C. cyminum* exhibited high free radical scavenging activity as compared to *B. persicum*, which means that *C. cyaminum* is more effective as compared to

B. persicum. At highest concentration (300ppm) plant extract (*C. cyminum*) showed 69% whereas *B. persicum* showed 52%. At 200ppm *C. cyminum* showed 42% while *B. persicum* showed 31% free radical scavenging activity.

At lowest concentration of 100ppm *C. cyminum* showed 31% whereas *B. persicum* showed 24% free radical scavenging activity (table 4.5). These results are significant as ($p < 0.0001$) and IC₅₀ value for *Cuminum cyminum* is 150ppm and for *Bunium persicum* is 299ppm (table 4.6). The less IC₅₀ value of *Cuminum cyminum* shows that it shows more significant free radical scavenging activity as compared to *Bunium persicum*. It was also found that free radical scavenging activity of cumin plant extract increases in dose dependent manner and similarly there is gradual decline in scavenging activity by decreasing concentrations.

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical and has ability to give hydrogen atoms by reacting with different compounds. This method is used to measure the reducing ability of different antioxidants by DPPH free radical. In addition this method is additionally practical because of its short response time. It was reported earlier that *C. cyminum* showed a strong antioxidant activity against DPPH, superoxide's and phenylehydrazyl assays.

Several researches have been performed to carry out in field of antioxidant properties of extracts and essential oil of black Caraway which all highlighted high antioxidant effects and scavenging ability of free radicals for this plant [108]. Essential oil and phenolic extract of black caraway showed a good antioxidant and DPPH scavenging properties as compared to BHT and BHA. [109] Caraway is having high antioxidant activity due to mono terpenes, alcohols, linalool, carvacrol, carvone, limonene, anethole, estragol, flavonoids and other polyphenolic compounds. [110]. It was addressed that daily use of *B. persicum* in diet will be efficacious, cost-effective as a good antioxidant with no side effects [111]. The results of peroxide value (PV) and tiobarbituric acid (TBA) tests highlighted that *B. persicum* could be served as a natural alternative instead of synthetic antioxidants [112]. *B. persicum* seeds extract prevent the hemolysis of human erythrocytes that can be due to the presence of bioactive compounds which has a radical-scavenging activity. [114] Caraway

supplementation had a modulatory role in tissue lipid peroxidation, antioxidant profile and prevented 1, 2-dimethylhydrazine-induced histopathological lesions in colon cancer of rats. [115]. It has been reported that *Cuminum cyminum* showed enhanced antioxidant activity because of presence of certain bioactive compounds such as terpenes, phenols, and flavonoids [116]. It was also observed that *Bunium persicum* is even better in controlling oxidative stress and AGE formation, which is implicated in pathogenesis of diabetic micro vascular complications.

TABLE 4.5: Analysis Variance for factors affecting the free radical scavenging activity of these plant extract

Source of variation	D.f	Value	Mean Square	F-value	P- Value	Significant
Interaction	4	898.4	224.6	31.34	<0.0001	Yes
Plant extract	2	20180	10090	1408	<0.0001	Yes
Conc.	2	1760	880.2	122.8	<0.0001	Yes
Residual	18	1290	7.166			
Missing Values	9					

TABLE 4.6: % Scavenging of Plant Extract Against DPPH

Sr. No	Sample	Concentrations (ppm)	% scavenging	IC 50
1	B. persicum	100	24%	299ppm
		200	31%	
		300	52%	
2	C. cyminum	100	31%	150ppm
		200	42%	
		300	69%	

Chapter 5

Conclusions and Recommendations

In traditional medicine, *B. percicum* and *C. cyminum* is served to cure gastrointestinal and urinary disorders, bloating and dyspepsia. Mixture of caraway is utilized as cumin formulation for the analysis of dyspepsia and bloating in children. According to this research, *B. percicum* and *C. cyminum* are most valuable drugs that have antimicrobial, cytotoxic and antioxidant effects.

The most significant effects of *B. percicum* and *C. cyminum* seeds extract are antimicrobial and antioxidant properties. According to this research work of biological evaluation and comparison of *C. cyminum* and *B. percicum*, it is concluded that the aqueous extracts of *C. cyminum* is having best inhibitory effect on bacteria such as *S. aureus*, *E. aerogenes*, and *M. luteusas* compared to *B. percicum* at different concentrations. While in case of antifungal activity *B. percicum* has shown more inhibitory effect as compared to *C. cyminum* against different fungal strains such as *Aspergillus niger*, *Aspergillus flavis*, *solani* and *mucor spp* In upcoming days, there will be the plant's extensive utilization in food industry or by combining with other agents and it can be used in food industry because it increases the shelf-life of food and food safety.

It was explained that *B. percicum*, *C. cyminum* and their derivatives in combination with other food preservatives can be used to control spoilage and pathogenic microorganisms. It avoids chemical spoilage in foods such as in cheeses, fish and meat products. *B. percicum* due to its unique and phenomenal properties it is widely used in people's diet without having any toxic effects. However, the study on this plant seems necessary for use as auxiliary treatment and application in food industry.

Also, according to various researches which are carried out on the anti-carcinogenic and anti-mutagenic properties of this plant, it seems that *B. percicum* and *C. cyminum* both have the abeyant for use as adjunct therapy in the treatment and obstruction of seizure and cancer. The contrast reported in the EO composition of these plants, it is suggested that upcoming research is done on different effects and the aspects of its compounds and utilizing a standard essential oil with distinct compounds.

These extracts can be studied in nanoformulations in nanobiotechnology. Further research is needed to know the chemical identity of the bioactive compounds for various biological activities. Natural plant derived fungicides is a new source of alternative active compounds, which is specific with antifungal, antioxidant activity.

The greater proportion of active extracts in assayed species, elected according to available ethnobotanical data, corroborates the efficiency of this path for the selection of plant species in the search for unique activity.

However, more proper phytochemical analysis, biological and pharmacological experiments are needed to announce *B. percicum* and *C. cyminum* as a wonderful profitable food preservative. More research is suggested to highlight the unique cellular and molecular targets of different constituents of *C. cyminum* and *B. percicum*, particularly cuminaldehyde and TQ.

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