CHAPTER-1

Introduction

“Somewhere, something incredible is waiting to be known.”
~Carl Sagan
1.1 MEDICINAL PLANTS

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants have been the subjects of man’s concern since time immemorial (Constable, 1990). Just about every civilization has a history of medicinal plant use (Ensminger et al., 1983). Approximately 80% of the people in the world’s developing countries put your faith in traditional medicine for their primary health care needs, and about 85% of traditional medicine involves the use of plant extracts (Vieira and Skorupa, 1993). Recent estimates suggest the over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth et al., 1991)

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crores. Presently, Indian contribution is less than Rs.2000 crores. Indian export of raw drugs has steadily grown at 26% to Rs.165 crores in 1994-95 from Rs.130 crores in 199-92. The annual production of medicinal and aromatic plant’s raw material is worth about Rs.200 crores. This is likely to touch US $1150 by the year 2000 and US $5 trillion by 2050. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world.
These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

More than 80,000 plants are medicinal out off the 2,50,000 higher plant species on earth. India is one of the world’s 12 biodiversity centers with the presence of over 45000 different plant species. India’s diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities.

1.2 INDIA: REPOSITORY OF MEDICINAL PLANTS

Among primeval civilizations, India has been known to be rich depository of medicinal plants and principal repository of large number of aromatic and medicinal plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in Ayurveda. The Rigveda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda (4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs and these are still used in the classical formulations, in the Ayurvedic system of medicine (Thomas, 1997).

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceuticals research. Approximately 3000 plants species are known to have medicinal properties in India (Prakash et al., 2010). Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Phytomedicines are also being used increasingly in Western Europe. Recently the US Government has established the
“Office of Alternative Medicine” at the National Institute of Health at Bethesda and its support to alternative medicine includes basic and applied research in traditional systems of medicines such as Chinese, Ayurvedic, etc. with a view to assess the possible integration of effective treatments with modern medicines. The development of systematic pharmacopoeias dates back to 3000 BC, when the Chinese were already using over 350 herbal remedies. Ayurveda, a system of herbal medicine in India, Sri Lanka and South-East Asia has more than 8000 plant remedies and using around 35,000-70,000 plant species. China has demonstrated the best use of traditional medicine in providing the health care. China has pharmacologically validated and improved many traditional herbal medicines and eventually integrated them in formal health care system.

Many Westerner countries have long regarded the Indian systems of medicine as a rich source of knowledge (Subhose et al., 2005). Around 20,000 medicinal plants have been recorded in India however traditional communities are using only 7,000-7,500 plants for curing different diseases (Dev, 1997; Perumal et al., 2000). Pharmaceutical industries depend on the plant products for the preparation of Ayurvedic medicines. Right from its beginning, the records of conventional knowledge especially on the medicinal uses of plants, has provided many important drugs of modern day. Even today this area holds much more hidden treasure as almost 80% of the human population in developing countries is dependent on plant resources for healthcare. In the interior areas of western Himalaya plants become the only source of medicine and well being. The vacant impression would negate the infallibility of this nobel laureate, by giving examples of novel activities of various medicinal plants.

1.3 THERAPEUTIC PROPERTIES OF PLANTS

Medicinal plants have always been considered a healthy source of life for all people. Therapeutic properties of medical plants are very useful in healing various
diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombarded with thousand of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. There are many therapeutic properties of plants and their active constituents /Phytochemicals.

1.3.1 Antimicrobial properties of medicinal plants

Indian traditional medicine system depends on plants and their parts to treat various infectious diseases. Major of herbs are well known to be used for various diseases including many infectious diseases. Later on essential oil, crude extracts as well as pure active constituent from many of such plants and herbs have been screened by investigators in seek for potential and safer antibacterial agents (Nair et al., 2005; Khond et al., 2009). Many review articles have been published on the role of natural products in the discovery of antibacterial agents (Singh et al., 2000; Nussbaum et al., 2006; Shahid et al., 2009). The success of natural products as guideposts to new drugs is most obvious in antibacterials. Over 75% of new chemical entities submitted between 1984 and 2004 were based on natural product lead structures (Newman and Cragg, 2003).

The antimicrobial actions of “carqueja” (Baccharis trimera Less.) decoction on Gram positive (Staphylococcus aureus and Streptococcus uberis) and Gram-negative (Salmonella gallinarum and Escherichia coli) bacterial strains were evaluated and it was found that the former microorganisms are more sensitive to this herb than the latter, which corroborates previous studies (Avancini et al., 2000). Similarly, antimicrobial assays with plant extracts used in Asia (Ruta graveolens and Zingiber officinale) revealed an inhibitory capacity against Bacillus cereus strains (Alzoreky et al., 2003).
More et al. (2008), studied extracts from eight South African plants frequently used against human oral cavity pathogens (Actinobacillus actinomycetemcomitans, Actinomyces naeslundii, Actinomyces israelii, Candida albicans, Porphyromonas gingivalis, Prevotella intermedia and Streptococcus mutans). He found that six of the eight plants (Annona senegalensis, Englerophytum magalismontanum, Dicerocarym senecioides, Euclea divinorum, Euclea natalensis, Solanumpanduriforme and Parinari curatellifolia) had antimicrobial effect against those microorganisms, of which gram-negative ones were more resistant (More et al., 2008). Rosmarinus officinalis Linn. (rosemary) hydroalcoholic extract was assayed against Streptococcus mitis, Streptococcus sanguinis, Streptococcus mutans, Streptococcus sobrinus and Lactobacillus casei standard strains, and its antimicrobial activity was proven in all tests, except against S. mitis (Silva et al., 2008). Zampini et al. (2009) examined multiresistant bacteria under the influence of ethanol extracts from 11 Argentinean plant species (Baccharis boliviensis, Chliotrichiopsis keidellii, Chuquiraga atacamensis, Fabiana bryoides, Fabiana densa, Fabiana punensis, Frankenia triandra, Parastrephia lucida, Parastrephia lepidophylla, Parastrephia phyliciformis, and Tetraglochin cristatum). They observed growth inhibition in at least one of the following tested strains: Staphylococcus aureus, Escherichia faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, Morganella morganii and Pseudomonas aeruginosa (Zampini et al 2009).

1.3.2 Anticancer properties of medicinal plants

Plants have a long history of use in the treatment of cancer (Hartwell, 1982). However, many of the claims for the efficacy of such treatments should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine (Cragg et al., 1994). Of the plant derived anticancer drugs in clinical use, the best known is the so-called vinca alkaloids, which include vinblastine and vincristine. These alkaloids are
isolated from the Madagascar periwinkle, *Catharanthus roseus* (Linn.) G. Don, Apocynaceae, known in Thailand as Phaeng phuai farang. These plants possess a long history of medicinal use by early American and Asian cultures, including the treatment of skin cancers and warts (Cragg *et al.*, 1994). From the time of Galen (about AD180), the juice expressed from woody nightshade (*Solanum dulcamara* L. Family Solanaceae) has been used to treat cancers, tumors and warts. The active tumor-inhibitory principle has been identified as the steroidal alkaloid glycoside β-solamarine. Various lichens (e.g. species of Cladonia, Cetraria and Usnea) also have a history of use in folk medicine against cancer since about AD 970.

Native American tribes used various parts of *Taxus brevifolia* Nutt. and other *Taxus* species (e.g., *canadensis, baccata*) (Cragg *et al.*, 1994) for the treatment of some non-cancerous conditions. The leaves of *Taxus baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system (Kapoor, 1990), with one reported use in the treatment of cancer (Hartwell, 1982). Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species and the ready semi-synthetic conversion of the relatively abundant baccatins to paclitaxel as well as active paclitaxel analogs such as docetaxel (Cortes and Pazdur, 1995) has provided a major renewable natural source of this important class of drugs. Ethanolic extracts from *Rhinacanthus nasutus* roots used in anticancer preparations showed high cytotoxic activity against prostate, lung and normal skin (Saetung *et al.*, 2005). Many *Rhinacanthus nasutus* Linn. derived compounds were effective against P388, HL-60, KB, HT-29 and A549; all compounds killed all cells although no specific cytotoxic activity was observed (Wu *et al.*, 1998). Ethanolic extracts of *Curcuma zedoaria* Roscoe showed specific activity against lung cancer cell lines, but were less cytotoxic against prostate cancer cells and normal fibroblast cells using the SRB assay (Saetung *et al.*, 2005). There is one report on cytotoxic compounds from *Curcuma zedoaria* Roscoe against human ovarian cancer cells, but no report on lung and prostate cancers (Syu *et al.*, 1998).
Numerous types of bioactive compounds have been isolated from plant sources. Several of them are currently in clinical trials or preclinical trials or undergoing further investigation. Two novel alkaloids, schischkinnin and montamine have been isolated from the seeds of Centaurea schischkinii and Centaurea montana (Shoeb et al., 2005; Shoeb et al., 2006). Both of the alkaloids exhibited significant cytotoxicity against human colon cancer cell lines. Betulinic acid, a pentacyclic triterpene, is a common secondary metabolite of plants, primarily from Betula species (Betulaceae) (Cichewitz et al., 2004). Betulinic acid was isolated from Zizyphus species, e.g. mauritiana, rugosa and oenopia (Pisha et al., 1995; Nahar et al., 1997) and displayed selective cytotoxicity against human melanoma cell lines (Balunas et al., 2005).

1.3.3 Anti-inflammatory and analgesic properties of medicinal plants

Herbal therapy is used to treat a large variety of ailments and symptoms e.g., inflammation, fever, and pain. Plants which have been used traditionally as analgesics or have yielded compounds which are used in pain relief include Cannabis sativa (Cannabinaceae), Mandragora officinarum (Solanaceae), Papaver somniferum (Papaveraceae) and Conium maculatum (Umbelliferae). Aloe vera barbedensis is used as gel in pain healing or analgesic effect. The aloe vera gel is used in reducing pain during dental treatments. Aloe vera is also used for pain healing purposes in the treatment of piles and hemorrhoids (Amrit et al., 2008). Andrographis paniculata, a popular medicine, is commonly used for treating infection, inflammation and pain (Lin et al., 2009). Bunus iongifolia (aerial) the results, with regard to the analgesic activity of the ethanolic extract showed a highly significant effects at all the three doses tested in mouse tail immersion method. The onset and duration of action is much better than standard drugs (Ahmad et al., 2003).

Ethanolic extract of the aerial part of Cissampelos pareire Linn. Hirusta (Menispermaceae) was tested for anti-inflammatory and analgesic activity
(abdominal writhes and hot plates) in rat and mice, respectively. Oral administration of extract exhibited significant and dose dependent anti-inflammatory activity (Amresh et al., 2007). Oral administration of Cissus quadrangularis is significantly decreases the painful stimulus in both phases of test which confirms central and peripheral effects of the drugs (Priyanka et al., 2010). Daphne retusa and Jasminum amplexicaule extracts possesses anti-inflammatory and analgesic properties (Qiang et al., 2008). Fumaira vaillantii is another very promising Turkish medicinal plant which showed highly significant analgesic effects in this study (Ahmad et al., 2003). Seeds and samples of the stem of two medicinal plants, Lactusa scariola and Artemisia absinthium respectively showed potent analgesic and anti-inflammatory activity (Ahmad et al., 1992). Morinda citrifolia Pterocephalus hookeri Punica granatum Urtica dioica also showed significant analgesic effect (Younos et al., 1990; Ahmad et al., 2003; Rezazadeh et al., 2005; Chakrobarty et al., 2008).

1.3.4 Hepatoprotective properties of medicinal plants

Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor (Nadeem et al., 1997). Among many diseases that can affect liver, the most common is viral hepatitis (Inflammation of liver caused by viral infection). A large number of medicinal plants have been tested and found to contain active principles with curative properties against a variety of diseases (Lewis, 1977). Liver protective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes (Sharma, 2002). Recent experience has shown that plant drugs are relatively non-toxic, safe and even free from serious side effects (Momin, 1987).
Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of phytodrugs (Agrawal, 2007) and some medicinal herbs have proven hepatoprotective potential. Silymarin (a flavonol lignan mixture) extracted from the milk thistle (*Silybum marianum*) is a popular remedy for hepatic diseases. Today every herbal company is marketing formulations for liver disorders but the actual scene is that only selected medicinal herbs have been tested for hepatoprotective activity (Bruneton, 1999) Traditionally *Taraxacum officinale* has been used as a remedy for jaundice and other disorders of the liver and gallbladder, and as a remedy for counteracting water retention. Oral administration of extracts from the roots of *Taraxacum officinale* has been shown to act as a cholagogue, increasing the flow of bile. Bitter constituents like taraxecerin and taraxcin are active constituents of the medicinal herb (Kokate *et al.*, 2007). *Cichorium intybus* is a popular Ayurvedic remedy for the treatment of liver diseases. It is commonly known as *kasni* and is part of polyhedral formulations used in the treatment of liver diseases. *Cichorium intybus* was found to be effective against chlorpromazine-induced hepatic damage in adult albino rats. *Solanum nigrum* in Ayurveda, the drug is known as *kakamachi* prescribed by herbal vendors for liver disorders (Chang-Chi *et al.*, 2008). *Glycyrrhiza glabra*, commonly known as licorice has potential for therapeutic use in liver disease (Trease and Evans, 2010). *Curcuma longa* Like silymarin, turmeric has been found to protect animal livers from a variety of hepatotoxic substances, including carbon tetrachloride, galactosamine, pentobarbitol, 1-chloro-2, 4-dinitrobenzene, 7 4-hydroxy-nonenal, and paracetamol. Diarylhepatonoids including Curcumin is the active constituent of the plant (Kapoor, 1990).

### 1.3.5 Neuroprotective properties of medicinal plants

Although very few drugs are currently approved by regulatory authorities for treating multi-factorial ailments and disorders of cognition such as Alzheimer's disease, certain plant-derived agents, for example, galantamine and rivastigmine (a semi-synthetic derivative of physostigmine) are finding an application in modern
medicine. However, in Ayurveda, the Indian traditional system of medicine which is more than 5000 years old, selected plants have long been classified as 'medhya rasayanas', from the Sanskrit words 'medhya', meaning intellect or cognition, and 'rasayana', meaning 'rejuvenation'. These plants are used both in herbal and conventional medicine and offer benefits that pharmaceutical drugs lack. Ginkgo biloba, St John's wort, Kava-kava, Valerian, Bacopa monniera and Convolvulus pluricaulis, are widely used for their reputed effectiveness in central nervous system (CNS) disorders (Kumar, 2006).


The herbs like Celastrus paniculatus, Acorus calamus and Piper longum are claimed as brain tonics. Clitoria ternatia is used as memory stimulant (Venkata Rao, 2003). The herbs Eugenia caryophyllus, Glycyrrhiza glabra and Tinnospora cordifolia exhibit their activity in mental disorders by acting on acetalcholine content. Lawsonia inermis, Nardostachys jatamansi are also important herbs used in mental disorders (Lakshami Chandra Mishra, 2004).
1.4 NIGELLA SATIVA LINN: A THERAPEUTIC PLANT


<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Ranunculales</td>
</tr>
<tr>
<td>Family</td>
<td>Ranunculaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Nigella</td>
</tr>
<tr>
<td>Species</td>
<td>sativa</td>
</tr>
</tbody>
</table>

1.4.2 Description

_Nigella sativa_ L. commonly known as _kalonji_ belongs to the buttercup family, _Ranunculaceae_. It is an annual flowering plant, native to Southwest Asia. The dry roasted seeds are used in flavoring curries, vegetables and pulses. The black seeds taste like oregano and have bitterness them like mustard-seeds. It can be used as a "pepper" in recipes with pod fruit, vegetables, salads and poultry. _Nigella_ is a genus of about 14 species of annual plants in the family Ranunculaceae, native to Southern Europe, North Africa and Southwest Asia. The plant grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a capsule composed of several united follicles, each containing numerous seeds. Several species are grown as ornamental plants in gardens, popular for their seed capsules, which are used in dried flower arrangements. Karayal are used exclusively for dried arrangements. The flowers are the best to add texture to any dried flower arrangement. The delicate purple striped pods are used in several arrangements for an airy effect.
Plate 1.1: Plants and flowers of *N. sativa*.  Plate 1.2: Immature fruit of *N. sativa*.

Plate 1.3: Mature fruit with seed of *N. sativa*.  Plate 1.4: Seeds of *N. sativa*.

Source of figures: https://www.google.co.in/search/images/nigella+sativa
1.4.3 Vernacular names of *N. sativa* L.

Habbat-al-Sauda (Hadith) Shoniz, Habbat-al-Barakah (Arabic name), Black Cumin, Black seed (English), Nielle (French, German), Melanthion (Greek), Shoniz, Siah Dana (Persian), Ketzach, Ketyach (Hebrew), Kalaunji (Hindi and Urdu), Krishn Jirak (Sans), Kalijira (Bengla), Kalaonji Jiram (Gujarati), Nilajirakira (Telegue.), Kalijira (Marathi), Karaunji Rigam (Tamil), Karun Chiragam (Malyalam).

1.4.4 Habitat and distribution

The plant is found wild in Southern Europe, Northern Africa, Asia Minor and in the Mediterranean region, but has been cultivated into other parts of the world including Saudi Arabia, Mediterranean countries, northern Africa and parts of Asia. The plant is indigenous to the Mediterranean region but now found widely in India (Jammu, Kashmir, Himachal Pradesh, Bihar, Assam and Punjab). The herb is also cultivated in Bengal and north-east India (Domestication of plants in the Old World, 2000).

1.4.5 Appearance and texture of *N. sativa* L.

*N. sativa* (Black Seed) are small matte black grains with a rough surface and an oily white interior, similar to onion seeds. The seeds have little bouquet, though when rubbed, their aroma resembles oregano. They have a slightly bitter, peppery flavor and a crunchy texture. The seeds may be used whole or ground and are usually fried or roasted before use (they are easily crushed in a mortar).

1.4.6 History of *N. sativa* L.: Folk medicine

According to Zohary and Hopf, archeological evidence about the earliest cultivation of *N. sativa* "is still scanty", but they report that *N. sativa* seeds have been found in several sites from ancient Egypt, including Tutankhamun's tomb. Although
its exact role in Egyptian culture is unknown, it is known that items entombed with a pharaoh were carefully selected to assist him in the afterlife (Dwivedi, 2004).

*N. sativa* has been used for medicinal purposes for centuries, both as a herb and pressed into oil, in Asia, Middle East, and Africa. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support, and for general well-being. In Islam, it is regarded as one of the greatest form of healing medicine available. It is an important medicine of Tibb-e-nabwi (prophetic medicine).

As per the saying of the Prophet Muhammad (Peace Be upon Him) about black seed quoted in *Al-Bukhari* and *Sahih Muslim*.

*Abu Huraira (Radi Allah Anhu) reported that he heard Allah's Messenger as saying: “Nigella seed is a remedy for every disease except death”. (Sahih Muslim, 5490)*

*Narrated Khalid bin Sa’d R.A: We went out and Ghalib bin Abjar R.A was accompanying us. He fell ill on the way and when we arrived at Medina he was still sick. Ibn Abi ’Atiq came to visit him and said to us, 'Treat him with black cumin. Take five or seven seeds and crush them (mix the powder with oil) and drop the resulting mixture into both nostrils, for 'Aisha has narrated to me that she heard the Prophet saying, 'This black cumin is healing for all diseases except As-Sam.' ‘Aisha said, 'What is As-Sam?' He said, 'Death.' (Sahih Bukhari, 591)*

*Ibn Sina (980-1037), most famous for his volumes called "The Canon of Medicine," refers to black seed as the seed "that stimulates the body's energy and helps recovery from fatigue or dispiritedness." *N. sativa* seeds and their oil have a long history of folklore usage in Arabian and Indian civilization and are used in food as well as medicine. The seeds are used as flavoring, to improve digestion and produce warmth, especially in cold climates. They are sometimes scattered in the
fields of woolen fabrics to preserve them from insect damage (Sinha et al, 1998; Kurion et al, 2003; Khare et al, 2004) references

1.4.7 Chemical composition of *N. sativa* L. seeds

Seeds contain numerous esters of structurally unusual unsaturated fatty acids with terpene alcohols (7%), furthermore, traces of alkaloids are found which belong to two different types: isochinoline alkaloids are represented by nigellimine and nigellimine-N-oxide and pyrazol alkaloids include nigellidin and nigellicin (Rittel et al., 1953).

In the essential oil (average 0.5%, maximum 1.5%), thymoquinone was identified as the main component besides p-cymene, pinene, dithymoquinone and thymohydroquinone. Other terpene derivatives were found only in trace amounts: carvacrol, carvone, limonene, 4-terpineol, and citronellol. Furthermore, the essential oil contains significant (10%) amount of fatty acid ethyl esters. The seeds also contain fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. Also contain parts of the essential oil, mostly thymoquinone, by which it acquires an aromatic flavour. The seeds give on steam distillation a yellowish brown volatile oil with an unpleasant odor. The oil contains carvone, d-limonene, and a carbonyl compound nigellone (Rittel et al., 1953).
Table 1.1: Chemical composition of \textit{N. sativa} volatile oil (Rittel \textit{et al}., 1953; Chopra \textit{et al}., 1958)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percentage</th>
<th>Compound</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerol</td>
<td>1.3</td>
<td>α–Phellandrene</td>
<td>0.6</td>
</tr>
<tr>
<td>Estragole</td>
<td>1.9</td>
<td>α-Thujene</td>
<td>2.4</td>
</tr>
<tr>
<td>Carvone</td>
<td>2.0</td>
<td>3-Methyl Nonane</td>
<td>0.6</td>
</tr>
<tr>
<td>Dihydrocarvone</td>
<td>0.3</td>
<td>α-Pinene</td>
<td>1.2</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>1.8</td>
<td>β-pinene</td>
<td>1.3</td>
</tr>
<tr>
<td>Anisaldehyde</td>
<td>1.7</td>
<td>Sabinene</td>
<td>1.4</td>
</tr>
<tr>
<td>Trans-Anethole</td>
<td>27.1</td>
<td>Myrcene</td>
<td>0.6</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>3.7</td>
<td>n-Decane</td>
<td>0.4</td>
</tr>
<tr>
<td>Longifolene</td>
<td>5.7</td>
<td>(p)-Cymene</td>
<td>9.0</td>
</tr>
<tr>
<td>Uvidine</td>
<td>1.3</td>
<td>Limonene</td>
<td>4.3</td>
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<tr>
<td>Myristicin</td>
<td>1.4</td>
<td>1-Methyl-3-propyle benzene</td>
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<tr>
<td>n-Hexadecane</td>
<td>0.2</td>
<td>(γ)-Terpinene</td>
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<td>Apiole</td>
<td>1.0</td>
<td>1-Ethyl-2,3-dimethyl benzene</td>
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<td>n-Tetradane</td>
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<td>2(1H)-Naphthalenone</td>
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<td>α-longipinene</td>
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<td>Fenchone</td>
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<td>(p)-Cymene-8-ol</td>
<td>0.4</td>
<td>Terpinen-4-ol</td>
<td>0.7</td>
</tr>
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</table>

1.4.8 Organoleptic characteristics and physicochemical constants of \textit{N. sativa}

(Sameera \textit{et al}., 2010)

- **Appearance:** Solid powder
- **Colour:** Grayish black
- **Odour:** Spicy
- **Taste:** Hot
Solubilities (%)  
Alcohol solubility: 34.80-36.80  
Water solubility: 12.00-13.00  

Ash values (%)  
Total ash: 4.50  
Water soluble ash: Nil  
Acid-insoluble ash: 0.33  

pH values  
pH of 1% solution: 6.14  
pH of 10% solution: 5.97  

Table 1.2: Phytochemicals and active constituents of *N. sativa*. (Khan, 1999; Bahman Nickavara *et al.*, 2013)  

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>Nigellidine, nigellicine and nigellimine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>A number of quercetin and kaempferol glycosides have been isolated</td>
</tr>
<tr>
<td>glycosides</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>The seeds have been reported to contain many saponin, melanthin and melanthigenin.</td>
</tr>
<tr>
<td>Sterols</td>
<td>Cholesterol, campesterol, stigmasterol, β-Sitosterol and a-spinastero.</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>Thymoquinone, thymol, carvone dithymoquinone, monoterpenes including d-limonene, p-cymene and (+) citronellol have been isolated.</td>
</tr>
<tr>
<td>Fix oil</td>
<td>Linoleic acid, palmitic acid, stearic acid and oleic acid are present.</td>
</tr>
</tbody>
</table>
1.4.9 Clinical/Pharmacological activities of *N. sativa* L.

**Antimicrobial activity**

*N. sativa* exhibited strong antimicrobial activity against *Salmonella typhi*, *Pseudomonas aeruginosa* and others. The essential oil has been shown to have activity against Gram-positive and Gram-negative bacteria. However, sensitivity against Gram-positive bacteria such as *Staphylococcus aureus* and *Vibrio cholerae* was found to be stronger. Bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus viridans* are more susceptible to *N. sativa*.

In an *in vitro* study, volatile oil showed activity comparable to ampicillin. The activity of the volatile oil also extended to many drug-resistant strains and was found to have a synergistic action with streptomycin and gentamycin (Ferdous, 1992). *In vivo* studies showed that the diethyl ether extract successfully eradicated localized infections of *Staphylococcus aureus* in mice (Nair *et al*., 2005). The activity of the volatile oil also extended to drug-resistant strains of *Shigella spp*, *Vibrio cholerae* and *Escherichia coli* and was found to have a synergistic action with streptomycin and gentamycin (Salman *et al*., 2007). *N. sativa* oil may potentially be useful for inhibition of *Listeria monocytogenes* in food as it showed strong antibacterial activity against 20 strains of the bacteria with the oil producing inhibition zones that were significantly larger than that of gentamicin (Nair *et al*., 2005).

**Antioxidant activity**

The essential oil of black cumin seeds, *N. sativa* was tested for a possible antioxidant activity. A rapid evaluation for antioxidants, using two TLC screening methods, showed that thymoquinone and the components carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging property. These four
constituents and the essential oil possessed variable antioxidant activity when tested in the diphenylpicrylhydrazyl assay for non-specific hydrogen atom or electron donating activity (Burits et al., 2000). *N. sativa* oil is used for the treatment of inflammatory diseases and has antioxidant properties (Abdel-Wahhab et al., 2005). Study of Al-Othman suggest that *N. sativa* and thymoquinone partly protected rat gastric mucosa from acute ethanol-induced gastric mucosal damage, with the gastroprotection mediated by their antiperoxidative, antioxidant and antihistaminic effects (Al-Othman et al. 2006; Al-Khalaf et al., 2013).

**Hepatoprotective activity**

*N. sativa* and thymoquinone (one of the active constituents of *N. sativa*) were reported to have hepatoprotective activity. An *in vitro* study showed the protective effect against tert-butyl hydroperoxide (TBHP) induced oxidative damage to hepatocytes (El-Gazzar *et al.* 2006). Significant hepatoprotective effects of *N. sativa* in carbon tetrachloride induced hepatotoxicity (Mastour *et al.*, 2003), D-Galactosamine (Gani, 2011) and turpentine oil induced liver damage (Subodh *et al.*, 2011) were noted.

**Anti diabetic activity**

Significant hypoglycemic activity has been reported and is thought to be due to the essential oil present. Clinical studies have confirmed these results and suggest that the plant extract has anti-diabetic activity. (Al-Hader *et al.*, 1993). Study of Benhaddou-Andaloussi *et al.*, (2010) suggested that use of *N. sativa* seed oil is beneficial in treatment of diabetes and suggests potential uses of this product, or compounds derived thereof against obesity and the metabolic syndrome (Benhaddou-Andaloussi *et al.*, 2010).
Anti-inflammatory and analgesic activity

Asthma and arthritis are chronic inflammatory disorders involving a variety of inflammatory mediators and different pathways. The fixed oil and thymoquinone from the seeds were found to inhibit eicosanoid generation in leucocytes and membrane lipid peroxidation and a significant reduction in rat paw oedema and a reduction in granuloma pouch weight were also observed. Nigellone in low concentration is effective in inhibiting the histamine release from the mast cells, which supports an anti asthmatic role of the plant (Khan et al., 1999). The anti-inflammatory activity of Black cumin seed oil has also been evaluated using carrageenan-induced paw edema in rats and croton oil-induced ear edema in mice by Hajhashemi and colleagues in 2004. The aqueous and methanolic extracts of *N. sativa* showed analgesic effect in mice as it produced significant increases in reaction times in the hot plate and pressure tests (Al-Naggar et al., 2003). The aqueous extract also has an anti-inflammatory effect as demonstrated by its inhibitory effects on carrageenan-induced paw edema in mice (Al-Naggar et al., 2003). Al-Shebani investigated the antinociceptive activity of the watery suspension of powdered *N. sativa* seeds in mice (Al-Shebani et al., 2009). In a recent study of Islam et al., (2013) it was reported *N. sativa* extracts from germination phases possesses significant analgesic and anti-inflammatory effect against kaolin induced oedema.

Antifertility activity

The antifertility activity of *N. sativa* in male rats has been established, shown by an inhibition of spermatogenesis and a significant reduction in sialic acid content of the testis, epididymis, seminal vesicles and prostate (Vohora et al.1973).
Anticonvulsant effects

Thymoquinone (active constituent of *N. sativa*) may have anticonvulsant activity in petit-mal epilepsy probably through an opioid receptor-mediated increase in GABAergic tone (Ilhan *et al.* 2005). The use of *N. sativa* oil could be a potential approach for arresting or inhibiting seizure genesis caused by excitotoxic agents (Büyüköztürk *et al.* 2005). Study of Akhondian *et al.* (2007) suggested water extract of *N. sativa* has antiepileptic effects in children with refractory seizures. Anticonvulsant effects of thymoquinone, the major constituent of *N. sativa* seeds was also reported in mice (Hosseinzadeh *et al.*, 2004).

Anticancer activity

Cytotoxic and immunopotentiating effects of *N. sativa* have been established. The long chain fatty acids are thought to contribute to the antitumor activity. The extract shows a modulatory effect in cisplatin-induced toxicity in mice and a protective effect against cisplatin-induced falls in haemoglobin levels and leukocyte counts. The anti-tumor effects of *N. sativa* oil was attributed to the volatile oil obtained from the seed, the major active components of which were thymoquinone and dithymoquinone (Shoieb *et al.* 2003). Few authors have reviewed the medicinal properties of *N. sativa*, giving some description of the anticancer effects (Randhawa and Alghamdi, 2002; Ali and Blunden, 2003; Salem, 2005; Padhye *et al.*, 2008). The literature search revealed that a lot more studies have been carried out since then on the anticancer activities of *N. sativa* and its active principles. Recently, the molecular and therapeutic potential of thymoquinone in cancer have also been reviewed (Banerjee *et al.*, 2010), but it does not include studies on the anticancer activities of *N. sativa* seed, its oil and various extracts.
Anthelmintic activity

*N. sativa* was found to have an anthelmintic activity against tapeworm comparable to that of piperazine (Chowdhury *et al.*, 1998). Hot water extract or seeds of *N. sativa* were used as oral anthelmintic in human adults (Al-Yahya, 1986; Zagari, 1993). Larvicidal activity has been reported against *Culex pipiens* (Gayar & Shazli, 1968). It is found to be highly effective against *Entamoeba histolytica* (Dhar *et al.*, 1968).

Hematological effects

*N. sativa* may have a beneficial role as a hypoglycaemic agent with protective effects against pancreatic β-cell damage from alloxan-induced diabetes in rats by virtue of its ability to decrease oxidative stress and to preserve pancreatic β-cell integrity (Meral *et al.* 2004). In another study *N. sativa* showed inhibitory effects on arachidonic acid-induced platelet aggregation and on blood coagulation (Al-Jishi & Hozaifa, 2003). It induced transient changes in the coagulation activity of rats according to the study of Mansi, (2005). Treatment of alloxan-induced diabetic rabbits with *N. sativa* resulted in lowering of elevated glucose concentrations, and an increase in the lowered serum triiodothyronine concentration (Meral *et al.* 2004).

*N. sativa* oil may also play a role in modulating the balance of fibrinolysis/thrombus formation by modulating the fibrinolytic potential of endothelial cells (El-Dakhakhny *et al.* 2000). Study of Awad (2003) reported that *N. sativa* increased the depressed red and white blood cells count, the packed cell volume and neutrophil percentage but decreased the elevated heart rate in the alloxan-induced diabetic rabbits.
**Immunomodulatory effect**

Black seed as a natural immune enhancer revealed that the majority of subjects who took black seed displayed a 72% increase in helper to suppressor T-cells ratio, as well as an increase in natural killer cell functional activity. These findings may be of great practical significance since a natural immune enhancer like the black seed could play an important role in the treatment of cancer, AIDS and other disease conditions associated with immune deficiency states (El-Kadi and Kandil, 1986). Haq et al. (1999) reported stimulatory effect of whole *N. sativa* and fractionated proteins was also noticed on the production of TNF-alpha either using non-activated or mitogen activated cells. The oil and certain active ingredients of *N. sativa* showed beneficial immunomodulatory properties, augmenting the T cell- and natural killer cell-mediated immune responses (Saleem, 2005).

**Other activities**

Other reports include hypocholesterolaemic (Al-Awadi and Gumma, 1987; Eskandar *et al.*, 1995), antihypertensive, antihypotensive and galactagogue (Zawahry, 1964; Dhar *et al.*, 1968; Agrawala, 1968) effects of *N. sativa* seeds extract and oil.

**1.5 Germination and biochemical changes during germination of seed**

The seed is more than just a plant waiting to happen. It is a complex marvel of evolution, a miniature life-support system that responds to environmental cues in order to give the embryo nestled within the best chance of survival. Physiological condition of a viable seed that prevents germination is called dormancy. During dormancy, the seed's metabolic rate is extremely slow and it uses very little energy to stay alive. Germination is the reactivation of metabolic activity in the seed, an awakening from dormancy. It culminates with the emergence of the radicle.
(embryonic root) and finally, the plummule (first shoot) Germination can be divided into three main phases (Koning, 1994):

**Activation**

This phase commences with imbibition, the uptake of water by the seed. This causes the seed to increase in volume. Respiration increases and various enzymes involved in endosperm digestion and other functions are manufactured. The cells of the embryo begin to elongate, and the radicle begins to grow.

**Digestion and Translocation**

During this phase metabolic activity increases dramatically. Protein synthesis begins and endosperm is metabolized. Enzymes soften and loosen the cell walls, in preparation for further cell elongation and volume increase. Nutrients from the endosperm (sometimes located in the cotyledons) are mobilized and transported to areas of growth.

The changes in chemical composition, respiratory gas exchange, and enzymatic activities were followed through the germination of seeds. First phase of germination associated with the earlier absorption of water and activation of the enzymes, lipase and amylase in seeds. The higher enzyme activity in turn was reflected in a more efficient utilization of oil reserves as indicated by a more rapid drop in respiratory quotient values following planting and by the absence of free fatty acids in either the endosperm or the embryo oil of the stratified seeds until the seedling had reached the stage preceding dissolution of the endosperm. Increased respiration leads to increased ATP synthesis. Endosperm becomes rich in soluble carbohydrates, peptides, lipids and glucose transform to sucrose. Sucrose and amides are translocated to embryonic axis for energy and new proteins causing growth. Starch and lipids degrade to form sucrose in cotyledons; whereas proteins degrade to
form amides compounds translocated to embryonic axis for growth and enzyme formation during germination (Gilbert et al., 1957).

As soon as the dry-stored seeds sprout off, free fatty acids are found, and the quantity increases rapidly as germination proceeds. The formation of saturated intermediates during the process of oil utilization is evident earlier in the oils of dry-stored seeds than in those of the more efficient stratified seeds. One end product of oil utilization is starch, which increased rapidly in the developing seedling while the oil of the endosperm decreases at a corresponding rate. At all stages of germination, irrespective of treatment, the embryo oil has a lower percentage of unsaturated glycerides than the endosperm oil. This indicates that the fatty acid radicals remain essentially unchanged in molecular weight prior to utilization with no detectable accumulation of lower molecular weight intermediates during their utilization (Gilbert et al., 1957; Miller, 2003).

1.6 AIMS AND OBJECTIVES

Keeping the foregoing account in view, the present investigation has been planned on the following aspects:

- To standardize the germination of Nigella sativa L. seeds.
- To estimate the antimicrobial effects of Nigella sativa L. extracts in different germination stages on different bacterial strains.
- To study hepatoprotective activity of Nigella sativa L. extracts in different germination stages of seed.
- To study Anti-inflammatory and analgesic activities of extracts.
- To study Neurobehavioral activities: such as locomotor activity, antiepileptic activity and antidepressant activity of different extracts.

The data and the observations are reported in the subsequent chapters, supported by relevant literature on the subject and other possible interpretations.
1.7 SIGNIFICANCE OF THE PROPOSED STUDY

In seeds, the activity of various metabolites is suppressed and primary and secondary metabolites are not produced in optimum concentrations. There are many studies that reported that germinating seeds especially sprouts are known to contain high concentrations of various compounds/metabolites of nutritional and medicinal value and several biochemical constituents and enzymes. To the best of our knowledge, present study is the first study on pharmacological activities of *N. sativa* during its germination. It will give an insight into the medicinal profile of this seed at various germination stages. No earlier study has been performed on pharmacological properties of *N. sativa* in different germination phases such as anticancer, anti inflammatory, analgesic, hepatoprotective and neuroprotective activities. This study will give a path to use *N. sativa* in germination stage for these effects with its additional benefits without any adverse effects reported in chemical drugs for these diseases.
1.8 REFERENCES


- Sahih Muslim, Book 26, Kitab As-Salam, Number 5490.
• Sahih Bukhari, Book 7, Volume 71, Hadith 591.


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