

Antioxidant and Immunomodulatory Properties of Herbal Drug

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ABSTRACT

The *Nigella sativa* L. popularly referred to as black seeds are widely used as a form of traditional nutrition and medicine. *N. sativa* seeds were used for the extraction of their oil by way of extraction (SFE) and cold press (CP) to determine the physicochemical properties, antioxidant activity, and thermal behavior. Owing to the fact that plants have been accepted as an important part of traditional medicine due to their miraculous phytoconstituents. *Tinospora cordifolia* (Guduchi/Giloyis) an herb which has pharmacological functions and medicinal qualities because it contains several components like terpenes, glycosides, alkaloids, flavonoids and steroids. Therefore, this is the main reason it is texted as "Amrita" in old textbook. It belongs to family Menispermaceae. This herb is used in the treatment of fever, urinary problem, dysentery, skin diseases, leprosy, diabetes, and many more diseases. The latest analysis shows its pharmacological value as antioxidant, antimicrobial activity, antibacterial activity, antifungal activity, anti-diabetic activity, anti-stress activity.

key words: *N. sativa*, Guduchi, Immunomodulatory, Antioxidants.

I. INTRODUCTION

A) Antioxidants

It is an evident from the human history that medicinal plants have been the treatment regimen to cure a variety of diseases, including diseases caused by insects, fungi, bacteria, and viruses. The effects shown by the plants are due to the chemicals present in them and they work in the same manner as the conventional drugs. (1).

The metabolite of aspirin, and this bark has been used from ancient times as a pain killer and antipyretic commercial drugs, for example, bark of willow tree is very rich in salicylic acid, which is also an active metabolite of aspirin, and this bark has been used from ancient times as a pain killer and antipyretic substance. Some of the drugs which are frequently used by the physicians are also derived from plant sources, for example,

aspirin, digoxin, quinine and opium, etc. They have a long history of use as herbal drug. Currently, there is much growing interest to use these medicinal plants as modulators of the complex immune system. Through a number of researches conducted in the area have explored that many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties. The current research in the area to develop plant-derived natural products as potent and safer leads to act as immunomodulators, is gaining much interest. (2).

Antioxidant, the word itself is magic suggesting some type of all-encompassing protection against cellular wear and damage. The supply of oxygen is absolutely essential for the existence of higher organisms. As the saying goes too much of even the best is best. Very high concentrations of oxygen are found to be toxic, and can damage tissues. The present day concept of oxygen toxicity is due to the involvement of oxygen free radicals or reactive oxygen species (ROS).

Definition

An antioxidant maybe widely defined as a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals which start chain reactions that damage cells. Antioxidants mainly terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reaction by being oxidized themselves (3).

Sources and Origin of Antioxidant

Antioxidants are abundant in fruits and vegetables and other foods including nuts, grains and some meats, poultry and fish. Common antioxidants include Green leafy vegetables, including collard green, spinach etc. Rich in beta-carotene. beta-carotene is found in many foods that are orange in colour, including sweet potatoes, pumpkins, mangoes etc. Lycopene is potential antioxidant found in tomatoes, watermelons, guava etc.

B) Immunomodulators

Definition

The term immunity defines body's natural defense system against a vast array of diseases and disorders. Remarkably sophisticated and advanced among vertebrates, the complex immune system is capable to generate a limitless variety of cells and molecules to arrest enormous spectrum of infections and undesirable substances.

Immunomodulators

Immunomodulators refer to those substances capable of inducing, amplifying, and inhibiting any component or phase of the immune system. Immunostimulators and immunosuppressant are two types of immunomodulators are known for use.

Immunopharmacology

immunopharmacology is a newer branch of pharmacology concerned with immunomodulators(4). Administration of immunostimulators as in the case of AIDS and use of immunosuppressor in cases of an exaggerated response of an immune system is appreciating to reconstitute the normal immune system and increase the longevity of life. Immunomodulator intake along with antigen, the process is meant to boost the immune system, and the modulator is known as immune adjuvant(5).

Immunology

Immunology is one of the rapidly developing fields of biomedical research, holds great promises concerning various diseases and disorders. The two ways of defense of an immune mechanism involving short-term mechanism which is the first line of defense and the other highly advanced adaptive immune response marked by complexity, diversity, and memory(6). An adaptive immune response also consists of two subtypes of immune responses, humoral immune response concerned with β -lymphocytes and cell-mediated cytotoxic response mediated by T-cells. Well, all the component cells of the immune system originate from bone marrow through hematopoiesis from bone marrow-derived stem cells. They are either develop into mature cells or migrate to other peripheral sites for migration. Besides a vast range of specialized cells of immune cells, certain molecules called cytokines which are one of the important mediators of the immune system mediate the cross talk between the specialized cells of the immune system, thereby completely integrating the behavior and action responses of the cells(7).

Cytokine Modulation Caused by Herbal Plants

Through a number of in vitro and in vivo studies conducted to see the effect of the herbal medicine on cytokines have shown that they

influence a large number of multiple cytokines. By nature, cytokines are a group of soluble extracellular proteins or glycoproteins in the form of interleukins (ILs), interferons, chemokines, etc., and are crucial to both innate and acquired types of immunity. These cytokines through intermolecular cross talks maintain physiological stability through their secretions in all nucleated cells through inducible response to some injury. In fact, it is evident from knowledge of the medical literature of various diseases that these disease conditions are in connect with cytokine secretions. In diseases of the central nervous system, these cytokines have a predominant role as in the variety of psychiatric disorders, and abnormal secretions of these chemicals have been demonstrated. Various neurochemicals, neuroendocrine, and neuroimmune substances have appeared at the command of cytokines. Their role has been marked in cases of depression(8), Alzheimer's disease, and schizophrenia(9); various behavioral shifts, positive and negative emotions, stress, infection, etc., have all been demonstrated to stimulate cytokine secretion(10).

Role of Cytokines

It is apparent from the vast literature on cardiovascular diseases and the role of cytokines as these are abundantly prevalent in the liver, heart, vessels, adipose tissues, etc., and these tissues contribute to inflammatory nature of cardiovascular diseases. Growing realization of the fact, the usefulness of cytokines, roles, alterations in cytokine expression, and targeting their receptors may offer a novel approach to their use as a therapeutic target.

A number of pharmacological agents are needed in the form of an antagonist, agonist, and initiator at stimulation. Interferon agonist has been approached by the Food and Drug Administration in the year 1986, for hairy cell leukemia(11). Similarly, for rheumatoid arthritis treatment, antigens of tumor necrosis factor- α (TNF- α) have been approached as target(12). In periodontal diseases, IL-1 β and TNF- α have been targeted. Inhibition of TH cell-derived cytokines, use of IL-2 and IL-12, and TNF- α also provide potential benefits therapeutically in neuroblastomas. Cytokines manifest diverse and pleiotropic characters, play a promising role also for other disorders not related to immune system(13). The use of interferon produces flu-like symptoms, depression, fatigue, etc., in patients(13).

Effect of Medicinal Plants on Innate And Acquired Immune Components

Various herbal medicines have been found to modulate various components of innate and acquired immune system. In fact, based on proper understanding of various immunomodulatory activities of herbal plants, plants derived the secondary metabolites in natural products can be the lead molecules for the future development of immunomodulators for therapeutic use. Various immunomodulators have been suggested in various allergic diseases including asthma, allergic rhinitis, and eosinophilic esophagitis on the basis of experiments performed on various animal models.

Effect on Innate Immune System

Although most of these products are not up to the mark in the human trial, which warrants for the careful understanding of the mechanism of various phenotypes with the goal to decrease excessive TH2 cells through blocking critical TH2 cytokines activity. Inhibition of cytokine involved in the synthesis of TH2 cells, blocking TH2 effector molecules, and inhibition of various cell types involved in TH2 induction(14). Assessment of plant activity should be conducted against immunoglobulin E (IgE) (main effector molecule in allergic response), for targeting IL-4/IL-13 receptors and for hiking in the ratio of TH1/TH2 balance. These aforesaid sites are promising targets for immunomodulatory therapy in allergic reactions(15). Halwani R, et al. have reported the reduction in eosinophil infiltration of lungs and inhibition of airway hyperresponsiveness among ovalbumin level of IgE and associated cytokines IL-5, IL-4, and IL-13 when they were treated with *Ganoderma lucidum*, *Glycyrrhiza uralensis*, and *S. flavescens*(16). Components of innate immunity involved in immunomodulation are array of cells including natural killer (NK) cells, NKT-cells, T-cells, macrophages, granulocytes (neutrophils, eosinophils, and basophils), and dendritic cells while B-cells naïve CD4+ T-cells, differentiated CD4+ T-cells including helper T- cells (TH1, TH2, and TH17 cells), induced regulatory T-cells, and natural regulatory T-cells(17).

Effect on Acquired Immune System

This plant is known by numerous names, for example black cumin (English), black caraway seeds (USA), shonaiz (Persian) and kalajira (Bangali)(21)

Chemical Constituents Of *Nigella Sativum*

Extensive studies were done to identify the composition of the black cumin seed, the ingredients of *N. sativa* seed includes: fixed oil, proteins, alkaloid, saponin and essential oil.

Patil et al. explained that ethanolic extract of *Ficus carica* produces stimulatory effect on humoral and cell-mediated immune response in experimental animals and suggested its therapeutic use in immunological disorders(18). *Chlorophytum borivilianum* root extract, an effective immunomodulator, not only potentiates non-specific immune response but also improves humoral as well as cell-mediated immunity. It may use in infection condition, enhancement of immunological response against foreign particles or antigens, and improving defensive response under normal circumstances(19).

C) Antioxidant, Immunomodulatory properties of *Nigella Sativa*



Nigella sativa flower

Figure 1. *Nigella Sativa* Flower

It is an annual flowering plant. It grows to 20–30 cm (7.9–11.8 inch) tall and has linear lanceolate leaves. The delicate flowers have 5-10 petals and the colors are usually yellow, white, pink, pale blue or pale purple. The fruit of plant is large and inflated capsule composed of 3-7 united follicles, that each of them has numerous seeds. The black colored seeds are flattened, oblong and angular, funnel shaped, with the length of 0.2 cm and 0.1 cm wide(20).

The fixed oil (32-40 %) contains: unsaturated fatty acids which includes: arachidonic, eicosadienoic, linoleic, linolenic, oleic, almitoleic, palmitic, stearic and myristic acid as well as beta-sitosterol, cycloeucaenol, cycloartenol, sterol esters and sterol glucosides(22)

The volatile oil (0.4-0.45 %) contains saturated fatty acids which includes: nigellone that is the only component

of the carbonyl fraction of the oil, Thymoquinone(TQ), thymohydroquinone(THQ), dit

hymoquinone, thymol, carvacrol, α and β -pinene, d-limonene, d-citronellol, p-cymene volatile oil of the seed also contains: p-cymene, carvacrol, t-anethole, 4-terpineol and longifoline(23)

Black cumin seed have two different forms of alkaloids: isoquinoline alkaloid that includes: nigellicimine, nigellicimine n-oxide and pyrazol alkaloid that includes: nigellidine and nigellicine(24)

By sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) the fractionation

❖ **Traditional uses of folk remedies**

N. sativa seeds have been used traditionally in middle eastern folk medicine as a treatment for various diseases for more than 2000 years ago .The seeds were used as pungent appetizer, aromatic, thermogenic, diuretic, expectorant, purgative, stimulant, sudoriferous, sedative and carminative anti-cancer , antibacterial and antifungal activity (27).

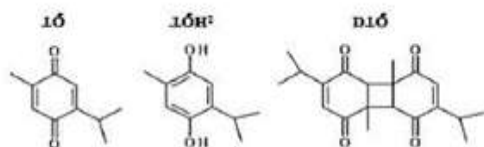


Figure 2. Structural formulas of thymoquinone, thymohydroquinone and dithymoquinone.

Black cumin seeds have a history of use in traditional Arabic herbal medicine to treat many diseases such as skin diseases, jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic hemorrhage, paralysis, amenorrhea, anorexia, asthma, cough, bronchitis, headache, fever, influenza and eczema(28).

Pharmacological properties

In recent years huge number of studies have been carried out, acclaimed medicinal properties emphasized on different pharmacological effects of N. sativa seeds such as antioxidant , anti-tussive , gastroprotective , anti-anxiety , anti-ulcer , antiasthmatic anti-cancer, anti-inflammatory, immunomodulatory and anti-tumor properties(29).

of whole N. sativa seeds was done which shows the bands ranged from 94 to 100 kDa molecular mass(25)

Black cumin seeds also have saponin and alpha hederine and in trace amount has carvone, limonene and citronellol,

Most of the pharmacological effects are due to quinine constituent, of which TQ is the mainly abundant. TQ possess anticonvulsant activity(26) , antioxidant , anti-inflammatory



figure 03. Pharmacological Properties of Nigella Sativa

a) Antioxidant Properties of N. Sativa (Black Cumin Seed)

It has been revealed that N. sativa extracts and essential oils possess strong antioxidant activity. The antioxidant effect of TQ has been found in different diseases, including diabetes, asthma, carcinogenesis and encephalomyelitis. TQ preserves the activity of a variety of antioxidant enzymes such as glutathione peroxidase, glutathione-S-transferase and catalase and also acts as free radical and superoxide scavenger. It has been reported that TQ acts as a nephroprotective and decreases SSAT and CYP3A1 gene expression via antioxidant mechanisms. It reacts with GSH, NADH and NADPH and forms glutathionyl-dihydro-thymoquinone, offering evidence for potent free radical scavengers. Influential chemo preventive action of TQ has been shown against MC-induced fibrosarcoma tumours due to the antioxidant activity and its interference with DNA synthesis. Treatment with N. sativa extract

prevented liver damage induced by lipid peroxidation. The favourable safety profile of herbal medicines is one of the reasons people often favour herbal medicines. It has been reported that a mixture of *N. sativa* with honey exhibits protection against methylnitrosourea-induced oxidative stress and carcinogenesis. Antioxidant properties of *N. sativa* ethanol extract exhibit protection from diabetes by improving antioxidant enzyme glutathione peroxidase and decreasing blood glucose levels, lipids levels.

Biological Activity of N

Sativa Biological activity of *N. Sativa* and TQ Much of the biological activity of *N. sativa* has been shown to be due to TQ, which is now considered the active component of its essential oil. Beneficial medicinal effects of NSO and TQ have been attributed to their radical scavenging (anti-oxidative) activity and their ability to inhibit the production of 5-lipoxygenase products during inflammation .

Additionally, an important mechanism clarifying the anti-toxic effects of NSO and TQ is directly linked to its potent antioxidant effects. Oxidative stress requires different lipids (e.g., triglycerides, cholesterol and LDL-cholesterol), which are transported into cells alter binding to LDL receptors [28].

As a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen. The strong antioxidant properties of TQ may be related to the redox properties of the quinone structure of the TQ molecule, and its unrestricted crossing of morphological barriers, thus gaining easy access to sub cellular compartments and facilitating the ROS scavenging effect [23].

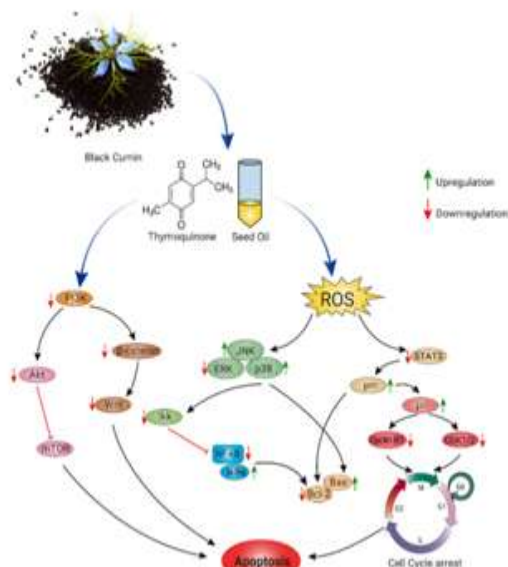


figure 04. Antioxidant Properties of *N. Sativa* (Black Cumin Seed)

b) Immunomodulatory Activities of *N. Sativa*

The immunomodulatory effects are one of the most valuable properties of *N. sativa*. Active constituents of *N. sativa* augment the immunomodulatory properties through T cells and NK cells . Significant effects were shown with treatment of *N. sativa* oil in most of the participating subjects by a 55% increase in CD4 and CD8 T cell ratios and improving the function of NK cells. *N. sativa* oil has a strong potentiating effect on the cellular immunity mediated by T cells, whilst suppressor activity on immunity mediated by B cells has been reported by other constituents. *N. sativa* stimulatory properties on cellular immunity are linked to the nature of the immune response. The effects of *N. sativa* and TQ on the cellular and humoral immunity have been compared and documented in . In vitro results of black seed soluble fractions on human peripheral blood mononuclear cells response to various mitogens were observed. It was found that major stimulatory results were not exhibited by components on peripheral blood mononuclear cells response to T cell mitogens phytohemagglutinin while components showed a stimulatory outcome on the peripheral blood mononuclear cells response to pooled allogeneic cells. Moreover, *N. sativa* fractionated proteins illustrated stimulatory activity in lymphocyte cultures (30).

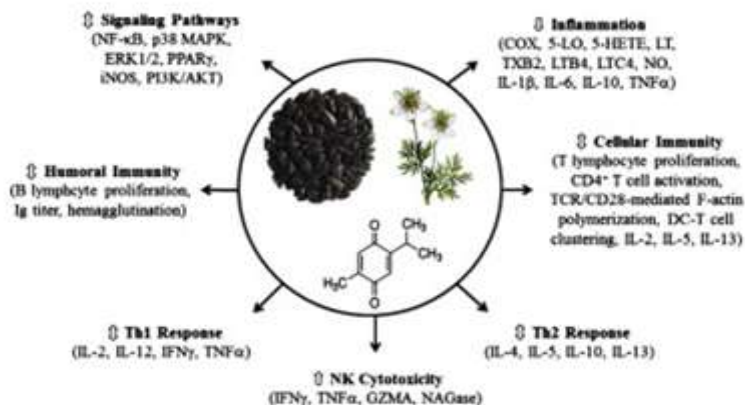


figure 05. Immunomodulatory Properties of N. Sativa(Black Cumin Seed)

| Activity | N.Sativa | Thymoquinone | Reference |
|-------------------|--|---|--|
| Cellular Immunity | Improvement of the proliferative capability of T lymphocytes and splenocytes | Improvement of number of circulating and thymus-homing CD4+ and CD8+ T lymphocytes | (Badr et al., 2011; Majdalawieh et al., 2010; Swamy and Tan, 2000) |
| | Increase secretion of IL-3 from PBMCs | Increase IL-2 serum level | Badr et al., 2011; Haq et al., 1995 |
| | Enhanced CD4 + T cell count acts as therapeutic role against HIV infection | Suppression of IL-13 and IL-5 release by mast cells | (El Gazzar, 2007; Onifade et al., 2013) |
| | Stimulation of CD4 + T lymphocytes | Increase total leukocyte count, chemokine expression, phagocytic action, chemotaxis | (Onifade et al., 2013; Salem and Hossain, 2000) |
| | Increase peripheral lymphocyte and monocyte counts | Inhibition of DC survival, maturation, and cytokine secretion (IL-10, IL-12, TNFα) | Fararh et al., 2004; Islam et al., 2004; Xuan et al., 2010) |

| | | | |
|------------------|---------------------------------|-------------------------------|---|
| Humoral immunity | Decrease serum IgA, IgM levels. | Increase immunoglobulin total | Ebaid et al., 2011; Mohany et al., 2012; Sapmaz et al., 2016) |
|------------------|---------------------------------|-------------------------------|---|

Table 02. Comparative studies of immunomodulatory activities of *N. sativa* Linn and Thymoquinone

c) Black Seed as an Additive To Honey

Honey
 Honey is a part of traditional medicine thanks to its dietary and pharmaceutical properties since ancient times. Honey is a thick, viscous, liquid or crystallized product of the honey plants nectar or secretion of living parts of plants. The composition of honey and its properties primarily depend on botanical origin, but there is also influence of geographic position, climatic conditions, types of bees, processing conditions and further handling of honey. Honey has a very variable composition, so specifying the average composition of honey is limited. Some of the honeys components depends of honey bees, some of the honey plants, while some are formed or changed during the maturation of honey. Components that make up the majority of honey are carbohydrates and water, while, in smaller quantities, are present organic acids, mineral substances, pigments, vitamins, proteins (including enzymes), the components of the aroma and phenolic components thanks to which honey has and antioxidant property. In most cases, in honey is a larger amount of fructose than glucose, but there are some types of honey in which glucose prevails. The relationship of fructose and glucose is one of the parameters identification of honey and its value is usually greater than 1. Research has shown that honey has a bactericidal and bakteriostatic effect on the many bacteria, many of which are pathogens. Last researches suggest that the consumption of honey alone or with other antioxidant beverages significantly increases the capacity of antioxidants in human serum



figure 06. *N. Sativa*(Black Cumin Seed) with Raw Honey

Possible Mechanism of Antioxidative Affect

Experimental data show that thymoquinone has a dual nature, and that it can act as electron – acceptor and as the H-atoms donor. As an electron donor, thymoquinone acts as the other biological quinones, such as CoQ0 and CoQ10, so the main activity of the superoxides „capturing“ is done via an electron-transfer mechanism. Antioxidant properties of honey and preventive effects against different diseases, such as tumors, are mostly credited to phenolic components, precisely, flavonoids, phenolic acids and phenolic acid derivatives, depending on their concentrations or ratios.

Antioxidant Activity of Natural Honey, Black Cumin Oil

In this study, the antioxidant activity of natural honey, black cumin oil, black cumin oil and honey mixture (in which the honey was dominant component) were investigated and determined. Also, in this paper was determined the content of active components: gallic acid, thymoquinone and total phenols in all samples. The goal was to show that the black seed could be used as an additive to honey that will improve the antioxidant properties of honey. The relative antiradical activity of all samples is determined by using a stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) which is often used in the assessment of the antioxidative activities. Also, FRAP method was used, which is

based on the antioxidants ability that by donating electrons in acidic medium (pH 3.6) reduces the yellow ferric complex iron (Fe^{3+}) with TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) into blue colored complex of Fe^{2+} -TPTZ. Intensity of resulting blue color was measured spectrophotometrically at 593 nm(31).

D) Antioxidant and Immunomodulatory Properties of Guduchi

Guduchi



figure 07. stem of Guduchi

Tinospora Cordifolia(32)(willd) Mires ex Hook. F & Thoms. (Family: Menispermaceae) commonly known as “Amrita” or “Guduchi” is an important drug of Indian system of medicine and used since time immemorial. It is a perennial climber found throughout Tropical India, drug is used in fresh form. The drug is well known Indian bitter (33) and prescribed in fevers, Diabetes, Dyspepsia, Jaundice, Urinary problems, skin diseases, chronic diarrhoea and dysentery (34). It has been also indicated in the treatment of leprosy, Helmenthiasis and rheumatoid arthritis(35). “Sat Giloe” is incorporated in the preparations. “Arq Giloe” Prepared from the fresh plant is considered a febrifuge, while “Arq Maul Laham Mako-Kashiwala” is a general tonic(36).

The anti-oxidant capacity of *Tinospora cordifolia* stem methanol extracts administered orally increased the erythrocytes membrane lipid peroxide and catalase activity. It also decreased the activities of SOD, GPx in alloxan-induced diabetic rats. *Tinospora cordifolia* Willd. (Menispermaceae) extracts possess possible inhibitors of aldose reductase and anti-oxidant agents, established by different spectroscopic studies [23, 24, 25]. Some of the essential constituents reported and major constituents in Fig. 2 whereas the structure of the active chemical constituent for *Tinospora cordifolia*. Stem and root part of *T. cordifolia*. Some of the essential constituents reported and

major constituents in Fig. 2 whereas the structure of the active chemical constituent for *Tinospora cordifolia*. Stem and root part of *T. cordifolia* contain alkaloids as active constituents.

Chemical Constituents of Guduchi

The chemical constituents of *T. cordifolia* belong to different classes such as alkaloids, glycosides, steroids, phenolics, aliphatic compounds, polysaccharides, leaves are rich in protein (11.2%), calcium and phosphorus [22].

The stem contains clerodane furone diterpene glucoside (amritoside A, B, C, and D) and the structure has been

These are tembetarine, choline, magnoflorine, berberine, tinosporin, isocolumbin, palmetine, jatrorrhizine, aporphine alkaloids, tetrahydropalmatine which showed anti-cancer, anti-diabetes, anti-viral, anti-inflammatory, anti-psychiatric.

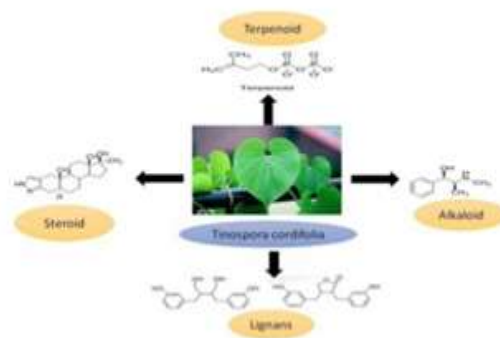


figure 08. Chemical Constituents of Guduchi

a) Immunomodulator Properties of Guduchi

Immunomodulatory action is seen because of non-specific immune mechanism stimulation. The association with immunomodulatory intervention, a polysaccharide component is attributable as monomer groups, high in glucose, fructose, and arabinose. Other active immunomodulatory components that are present are: 11-hydroxymustakone, N-methyl-2-pyrrolidone, and N-formylannonine, cordifolioside A, tinocordiside, magnoflorine, and syringing. In the present review the immunomodulatory behaviour of multiple forms of *Tinospora cordifolia* stem fractions and extracts were assessed. Studies of the phagocytic role of polymorphonuclear neutrophils (PMN) of *Tinospora cordifolia*, were also evaluated. Using this herb with cisplatin in the murine model, for the treatment of visceral leishmaniasis, instead of Th2

(T helper cell 2), it confirms the answer to type Th1(T helper cell 1) as well as also improved the adverse outcome of cisplatin . Giving therapy along with this herb raises NADH-oxidase activity, NADPH-oxidase and myeloperoxidase that lead to activation of macrophages.

In CCl4-intoxicated rats, in which macrophage function was attenuated (reduced bacteria-killing capability, reduction phagocytosis and reduction in the development of NO,etc.) is restored by the administration of this extract of the plant .

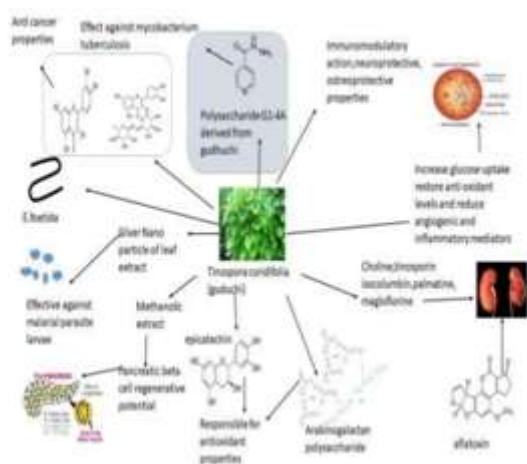


figure 09. Immunomodulatory Properties of Guduchi (*Tinospora cordifolia*) Guduchi Powder

b) Antioxidant activity of Guduchi

It has been evaluated that the oxidative status of the heart, liver, kidney, and brain after using *T. cordifolia* extract; such extracts were concluded to be more effective than insulin and glibenclamide. Moreover, *T. cordifolia* has been used to attenuate ischemic brain damage via preventing ROS production, with consequent amelioration of oxidative stress-mediated cell injuries caused by oxygen/glucose deprivation through direct effects and modulation of gene expression . However, concluded that the ameliorative effect of *T. cordifolia* against rat hippocampal slices exposed to low glucose and oxygen levels was due to its strong free radical-scavenging effect. Moreover, oral administration of *T. cordifolia* methanolic extract to alloxan-induced diabetic rats resulted in the upregulation of lipid peroxide and catalase activity on the erythrocyte membrane and downregulated SOD and GPx activity.

The antioxidative activity of *T. cordifolia* is credited to its alkaloids and diterpenoid lactones such (-epicatechin, tinosporin, isocolumbin and palmatine [48,49]. Consistently, *T. crispa* also possesses antioxidant activity. The antioxidant potency of aqueous extract of *T. crispa* stem is equivalent to establish antioxidants such as butylhydroxy toluene and vitamin C [50]. It was reported that methanol extract of the stem has the strongest antioxidant potential between aqueous, methanol and chloroform extracts of *T. crispastem*, which potentially due to the phenolic compounds presented in the extract [51].

The arabinogalactan polysaccharide derived from *T. cordifolia* possessed an acceptable protective property against iron-induced lipid peroxidation in brain homogenates from rats. The hydroalcoholic extract of *T. cordifolia* aerial roots has been reported to exert potent beneficial effects on the liver contents of antioxidant enzymes, GSH, and lipid peroxidation in Swiss albino mice . The leaf extract of *T. cordifolia* has been concluded to have potent antioxidant and ROS-scavenging activity.

II. METHODS AND MATERIAL
Description of Nigella sativa plant



Description of Guduchi:



figure 11. Fresh Stem of Guduchi Guduchi Powder

Description: *Nigella sativa* L. is an edible, annual flowering plant, native to south and southwest Asia, middle Europe, middle and west region of Saudi Arabia. *N. sativa* grows to 20–30 cm tall, with finely divided, linear leaves. The fruit is a large capsule consisting of three to seven united follicles, and in the follicles are located small black seeds of a strong aromatic scent and flavour.

Scientific Classification:

Kingdom: Plantae
Clade: Tracheophytes
Order: Ranunculales
Family: Ranunculaceae
Genus: *Nigella*
Species: *N. Sativa*

Description: *Tinospora Cordifolia* (32) (Willd.) Miers ex Hook. & Thoms. commonly known as “Amrita” or “Guduchi” is an important drug of Indian system of medicine and used since time immemorial. It is a perennial climber found throughout Tropical India, drug is used in fresh form. The drug is well known Indian bitter (33) and prescribed in fevers, Diabetes, Dyspepsia, Jaundice, Urinary problems, skin diseases, chronic diarrhoea and dysentery (34).

Scientific Classification:

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperem
Order: Ranunculales
Family: Menispermaceae
Genus: *Tinospora*
Species: *T. cordifolia*

Method of Extraction of Nigella seed (By Maceration Method)

Maceration Method

This is an extraction procedure in which coarsely powdered drug material, either leaves or stem bark or root bark, is placed inside a container; the menstruum is poured on top until completely covered the drug material. The container is then closed and kept for at least three days. [(37), (38), (39), (40), (41)]. The content is stirred periodically, and if placed inside bottle it should be shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration or decantation. Subsequently, the micelle is then separated from the menstruum by evaporation in an oven or on top of water bath. This method is convenient and very suitable for thermolabile plant material (41).

Properties of solvent Extraction

Water.

It is the most polar solvent and is used in the extraction of a wide range of polar compounds. [(42), (43)] Advantages. It dissolves a wide range of substances; it is cheap, nontoxic, nonflammable, and highly polar

Disadvantages. It promotes bacterial and mold growth; it may cause hydrolysis, and a large amount of heat is required to concentrate the extract. (43)

Alcohol.

It is also polar in nature, miscible with water, and could extract polar secondary metabolites. Advantages. It is self-preservative at a concentration above 20%. It is nontoxic at low concentration, and as small amount of heat is required for concentrating the extract. Disadvantages. It does not dissolve fats, gums, and wax; it is flammable and volatile.

Chloroform.

It is a nonpolar solvent and is useful in the extraction of compounds such as terpenoids, flavonoids, fats, and oils. Advantages. It is colorless, has a sweet smell, and is soluble in alcohols. It is also well absorbed and metabolized in the body. Disadvantages. It has sedative and carcinogenic property.

Ether.

It is a nonpolar solvent and is useful in the extraction of compounds such as alkaloids, terpenoids, coumarins, and fatty acids. Advantages. It is miscible with water, has low boiling point, and is tasteless in nature. It is also a very stable compound and does not react with acids, bases, and metals. Disadvantages. It is highly volatile and flammable in nature (44).

Ionic liquid (green solvent)

This is a unique solvent of extraction and is highly polar and extremely heat stable. It can remain in a liquid state even at 3,000°C and usable where high temperature is applicable. It has extreme miscibility with water and other solvent and is very suitable in the extraction of polar compounds. Advantages. It has excellent solvent that attracts and transmits microwave, and hence it is suitable for microwave-assisted extraction. It is nonflammable and is useful for liquid-liquid extraction and highly polar. Disadvantage. It is not ideal for preparation of tinctures (44).

Factors to be considered in selecting solvents of extraction

Various factors enumerated below should be taken into consideration when choosing a solvent of extraction. [(39)(45)]

Selectivity. The ability of a chosen solvent to extract the active constituent and leave the inert material.

Safety. Ideal solvent of extraction should be nontoxic and nonflammable.

Cost. It should be as cheap as possible.

Reactivity. Suitable solvent of extraction should not react with the extract.

Recovery. The solvent of extraction should be quickly recovered and separated from the extract.

Viscosity. Should be of low viscosity to allow ease of penetration.

Boiling temperature. Solvent boiling temperature should be as low as possible to prevent degradation by heat. [(39)(45)]

Sample preparation

Black cumin and sesame seeds extract. To prepare extracts of cumin, material was cleaned and selected whole seeds in good condition, washed with running water, drained. Black cumin seeds are soaked in 1% tempeh culture solution (1:2) for 24 hours, dried by oven blower for 12 hours at 50°C, grounded using a seed grinder, obtained the black cumin powder. Black cumin powder were extracted at twice by maceration method with shaker for 20 hours at room temperature: first extraction using 96% ethanol (black cumin: ethanol = 1:6 w/ v) and second extraction with the same solvent (black cumin: ethanol = 1:4 w/v). Extraction was carried out for 20 hours by maceration method. The filtrate was separated from the solvent with rotary evaporator at 40°C until a thick extract was obtained.

A. Selection of Ingredient

Raw material such as black cumin seed were procured from authorised local shop of Nashik City.

B. Extraction of Oil

The oil of *N. sativa* seeds was obtained by Soxhlet extraction method A.O.A.C. (1990) 25 g seeds were crushed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed for the extraction. The oil was extracted for 2 days by using ethanol (96%) as a solvent. After extraction the solvent was removed by hot air oven. The same method was repeated by using acetone as extract agent.

The efforts were made to study the possible utilization of black cumin oil in

incorporation into various value added food products. In search of getting maximum yield of oil, the seeds were extracted by using different solvents by Maceration Method while other parameters viz. amount of black cumin seeds (10 g) and quantity of solvent (60 ml) were kept constant. The results pertaining to yield of black cumin oil were presented.

Each value represents the average of three determinations. On the basis of observed result the ethanol (96%) extract yield 33.0% oil and acetone extract yield 31.4%. It could be concluded that black cumin contains significantly higher amount of fat (33.0 %) which defines its oilseed value. Findings of present investigation were in close conformity with values described in literature of Bertrand Matthaus and Mehmet Musa Ozcan (2011).

Materials and Methods for Preparation of Guduchi Ghanavati

Ayurveda is science which deals with diseases management and promotion of health. Many ayurvedic formulation have been explained in classics which includes herbal, herbo-mineral and mineral. In present era herbal formulation are widely used and promoting globally for the treatment of many diseases (46). Considering these points, the Standardization of herbal formulations is crucial for the assessment of drug quality. It is also important to know the active principles and its chemical constituents of the herbal formulation. The acceptability and safety of the drug depends on the quality the formulation (47). So, it is important to do quality assessment of the formulation. One of the major problems faced by Ayurveda physicians is the lack of unique quality control parameters for herbal medicines and their formulations. In India, the Department of AYUSH Government of India has launched a central scheme to develop standard operating procedures for the manufacturing process in order to develop pharmacopoeial standard for ayurvedic preparations (48). In present era formulation needs to be standardized so it can get acceptance globally. This formulation has been explained in classics for the management of *sthoulya* (33). In this present study, Guduchi-Badamustradi Ghanavati was prepared.

Collection Plant Material

Musta (*Cyperus Rotundus*. Linn), Guduchi (*Tinospora Cordifolia*. Thumb), Amalaki (*Embllica officinalis*. Gaertn), Vibheetaki (*Terminalia Bellerica*. Gaertn), Haritaki (*Terminalia Chebula*.

Retz) were purchased from authenticated resources at Nashik.

Identification and Authentication of Raw Drugs

Raw drugs identification and authentication was done by the literature survey and Internet resources.

Method of preparation Guduchi Ghanavati

The ingredients of Guduchi Ghanavati (Table 3) were taken in equal quantity and

converted into coarse powder. The coarse powder was well mixed in a mass mixer until a homogenous mixture was obtained. The Kashaya was prepared by obtained mixture and transformed into Ghana according to the guidelines given in Sharangdhar Samhita (49). Prepared Ghana was kept in a hot oven for 3 days until it was completely dried(50).

Table 01. Showing Ingredients of Guduchi Ghanavati

| Sr No | Name of Drug | Botanical Name | Family | Quantity |
|-------|--------------|-----------------------------|----------------|----------|
| 1. | Guduchi | Tinospora Cardifolia Thunb | Menispermaceae | 2 gm |
| 2. | Amlaki | Embllica officinalis Linn | Phyllanthaceae | 2 gm |
| 3. | Hartaki | Terminalia bellerica Gaertn | Combretaceae | 2 gm |
| 4. | Vibheetaki | Terminalia bellerica Gaertn | Combretaceae | 2 gm |
| 5. | Musta | Cyperus rotundus Linn | Cyperaceae | 2 gm |
| 6. | Water | | | Q.S |

Methods of evaluation of Guduchi Ghanavati

Guduchi Ghanavati was analyzed by using standard qualitative and quantitative parameters. All the procedures were conducted at G.M.P certified Laboratory.

Physico-Chemical Analysis

It includes parameters like colour, taste, pH, Loss on Drying(51), total ash (52), acid insoluble ash(53), alcohol soluble extractive, water soluble extractive, uniformity of weight, disintegration time friability test , & hardness (54).

Qualitative analysis

The qualitative analysis of Guduchi Ghanavati was done for Glycoside Sugar, Alkaloids, Tannins, Flavonoids, Gallic acid, Ascorbic Acid, Saponin, Starch

III. RESULT

The extraction of Nigella sativa seed done by maceration method was obtained and the formulation Guduchi-Badramustadi Ghanavati was prepared following standard operating procedures in pharmacy lab was subjected for qualitative and quantitative analysis.

➤ **Organoleptic evaluation:**

Table 02. Organoleptic Characteristics of Guduchi Ghanavati

| Sr No | Characters | Observed |
|-------|------------|-------------------|
| 1. | Color | Black |
| 2. | Odor | Slightly Aromatic |
| 3. | Taste | Pungent |

The organoleptic parameters are the basic criteria for selecting raw ingredients and confirming the quality of the finished formulation. The texture of the finished formulation was found to be smooth, indicating surface uniformity without cracks. The color was black, the taste was pungent and the smell was slightly aromatic and

characteristic due to the special properties of the ingredients used.

Physio Chemical Analysis of Guduchi - Ghanavati Loss on drying

Drying between samples indicates that the samples are devoid of excess water content and that

there is no microbial overgrowth or insect infestation. In the sample of Guduchi - Ghanavati loss of drying was found to be 2.56 %, which means that the samples have a good shelf-life and will not decay when stored.

Total ash and acid insoluble ash

It provide information on contamination, substitution, adulteration. Low total ash and acid insoluble ash means low levels of inorganic matter and Contents of silica. In this sample, the value of Guduchi Ghanavati ash was 1.07%, which was slightly higher and may be due to the presence of fibers and sclereids in the ingredients.

Water and Alcohol soluble extracts

Water soluble extract and Alcohol soluble extract were 81 per cent and 3.86 per cent respectively in the sample of Guduchi Ghanavati. The high solubility of the sample in water indicates that the drug is best suited for extraction with water or water-based preparations. The negligible presence of Volatile oils is also in favor of thermal extraction with water.

pH

The pH is measured to detect the acidity or alkalinity of the aqueous solution of the drug, which helps to understand the pharmacological basis of drug absorption and metabolism. In this sample, Guduchi Ghanavati pH was 4.9 percent, therefore, it is clear that the drug tested was acidic in nature.

Uniformity of weight

It helps to distribute drugs and to fix the quantity of drugs. The average weight of the present sample of Guduchi- Ghanavati was 510 mg, which denotes the uniformity of the weight in relation to the planned weight of each Ghanavati i.e., 500 mg.

the ingredients used for the preparation of the medicinal product. The prepared drug, Guduchi-Ghanavati was pharmacologically subjected for physicochemical analysis, qualitative analysis. The ingredients of Guduchi Ghanavati are guduchi, musta, amalaki, haritaki and vibhitaki and it is an herbal formulation.

V. CONCLUSION

The above article hence bestows the hypothetical existence of Antioxidant and its all-encompassing protection against cellular wear and damage. The article mainly emphasises on the importance and the advantageous effects of **Natural Antioxidants**. The various sources and

Disintegration time

The disintegration time of this sample was reported to be 36 minutes. Disintegration time of the tablet is an important criterion for quality assessment, as it provides important clues as to the bioavailability of the contents of the tablet. In further research on the same trial drug, comparative criteria for assessing the quality of the formulation have become essential.

Hardness and friability

The hardness of the tablet should not be less than 3 kg/cm² whereas the friability is at an ideal level of 1%. The Guduchi Ghanavati sample had a hardness of 5.2 kg/cm² and was found to have a friability of 0.45 per cent, therefore both values were within the required range indicating the durability of the finished formulation.

IV. DISCUSSION

Black seed oil has shown a significant capacity for neutralization of free radicals, much more than pure natural honey, even more than oil and honey mixture. Therefore, it can be used as an antioxidant component in the form of the addition to the honey, and to prevent or decelerate oxidative stress caused by free radicals or as a potential natural antioxidant in the food industry instead of synthetic antioxidants. The Ayurvedic system of medicine is increasingly being relied on for various health issues, particularly lifestyle diseases. The ingredients have been pharmacognostically identified and authenticated and used for preparation. Any plant or formulation used medicinally requires a detailed study prior to its use, as the therapeutic efficacy depends on the quality of

the origin of various antioxidants have been enumerated. Also the advantage of natural antioxidant over the various synthetic sources have been stated. The toxic effects and the therapeutic disadvantages of synthetic antioxidants in today's genera has been detailed respectively. Herbal Antioxidant particularly has been discussed in detail. The herbs and spices associated with Herbal Antioxidants has been discussed and also its imperative role in the maintenance of health has been stated. Hence, the superiority of Natural antioxidants over the synthetic have been proven in terms of safety, tolerance and non-toxicity.

Many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to

cause alterations in the immunomodulatory

In most of the studies on thymoquinone obtained from black seeds and seeds; it is reported that black seed has many beneficial pharmacological activities and can show toxic effects only in very high doses. It is reported that thymoquinone maybe contact allergen. Since the reliability of oral and dermal exposure is not sufficient, it is not recommended to use it during pregnancy and breastfeeding. It has been shown by studies that black seed significantly increases the

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