Experimental and Theoretical Elucidation on the Antioxidant Mechanism of *Nigella sativa*

Thesis submitted in

Partial Fulfilment of the

Degree of Master of Philosophy (M.Phil)

By

S.BHUVANA

18MPPHF001

DEPARTMENT OF PHYSICS

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN

COIMBATORE – 641 043

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CERTIFIED AS A BONAFIDE RESEARCH WORK

31/7/2019

Signature of the Head of the department

31/7/19

Signature of the Guide

DECLARATION

I declare that the dissertation entitled **Experimental and Theoretical Elucidation on the Antioxidant Mechanism of *Nigella sativa*** submitted by me for the degree of Master of Philosophy (M.Phil) is the record of work carried out by me during the period from August 2018 to July 2019 under the guidance of **Mrs. S. Anitha,** M.Sc., M.Phil., Assistant Professor, Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, Titles in this University or any other University or other similar institution of Higher Learning.

G. Bhuvana

Signature of the candidate

CERTIFICATE

I certificate that the dissertation entitled **Experimental and Theoretical Elucidation on the Antioxidant Mechanism of *Nigella sativa*** submitted for the degree of Master of Philosophy (M.Phil.) by **S.BHUVANA** is the record of research work carried out by her during the period from August 2018 to July 2019 under my guidance and supervision, and that this work has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, or other Titles in this University or any other University or institution of Higher Learning.

S. [illegible] 31/7/19
Assistant Professor in Physics

Signature of the Supervisor with designation

ACKNOWLEDGEMENT

I owe my sincere thanks to **Lord Almighty** and **My Loveable Parents** for showering their generous blessings upon me in all endeavours.

I wish to express my gratitude to **Dr. P.R. Krishnakumar,** Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the facilities to conduct this study.

I extend my thanks to **Dr. (Tmt.) Premavathy Vijayan,** M.Sc., M.Ed., Dip. Spl. Edn., M.Phil., Ph.D., Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing flamboyant help towards the completion of the study.

I record my deep sense of gratitude and indebtedness to **Dr. (Tmt.) S. Kowsalya,** M.Sc., M.Phil., Ph.D., Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing adequate help for the study.

I gratefully record my sincere thanks to **Dr. (Mrs.) K. Udhaya Chandrika**, M.Sc., M.Phil., Ph.D., Dean, School of Physical sciences and Computational sciences, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for timely help rendered throughout the course of this work.

I whole heartily thank **Dr. (Mrs.) Rajeswari,** M.Sc., M.Phil., M.C.A., SLET, Ph.D., Assistant Professor-SG and Head (i/c) of the Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her encouragement and generous help which was of great value.

I express my heartiest help to my guide **Mrs. S. Anitha,** M.Sc., M.Phil.,Assistant Professor, Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her inspiring guidance, patience, help, encouragement and motivation.

I express my sincere thanks to **Dr. R. Shankar**, Assistant Professor, Department of Physics, Bharathiar University, Coimbatore for the help to carry out theoretical work in his lab.

I express my sincere gratitude to all the staff members of the Department of Physics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for their help and support.

I would like to express my special thanks to **my parents, my friends** and all **my well-wishers** for their constant encouragement, support and help in carrying out this work successfully.

BHUVANA.S

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INTRODUCTION

CHAPTER – I

INRODUCTION

"Health" is a state of complete physical, mental, social and spiritual well- being and not just the non existence of illness of infirmity" is the primary criteria for leading the happy life. Diseases on the other hand is " A pathological or pathophysiological state of a single part, whole organ or complete system of an organism consequentially developing from a variety of reasons such like contagion, hereditary defect or ecological stress and branded by a exact set of signs or sympotoms". Human being on this planet suffers from various catagories of diseases naming infectious diseases, Autoimmune diseases, mutational diseases, drug induces diseases which will be cured by the application of therapeutic agents. During early age, man learned the method of using plants and its different parts are medicine, later with the advent of science and technology individual molecules were separated and tested for the therapeutic potency which propelled mankind towards better future.

Although various allopathic medicines like insulin the future runners in the treatment of diabetes and pyrexia, but on the other hand they have serious side effects and gives rise to complications. As the intensity of this disorders increases, there is need to look for drug with satisfying results and less side effects.

1.1 NATURAL PLANTS

Plants that possess vast medicinal properties that show beneficial pharmacological effects on the human body are generally designed as medicinal plants [1]. Medicinal plants are used in pharmaceutical industries, medicines nutraceuticals and food supplements. Herbal medicine from different plants and their parts are used tradicinally in medicine to treat various diseases and disorders [2].

Bio active constituents of plants known as phytochemical components such as tannins, carbohydrates, alkaloids, terpenoids, phenolic compounds, steroid sand flavonoids are responsible for various pharmacological activities of the plants. V Phytochemical compounds are synthesized by primary or secondary metabolism of living organisms [3]. Plants are important to the global economy involved in formulation of drugs with

cheaper resources without side effect [4]. The present research is aimed to study the following medicinal plants with their phytochemical screening and antioxidant potential.

1.2 FREE RADICALS

A free radical is a molecular species that contains an unpaired electron in an atomic orbital. An unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive, it can donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants [5]. A type of unstable molecule which contains oxygen and reacts with other molecule in all leads to cell damage and cause all death. Reactive oxygen species (ROS) are free radicals formed due to oxidative stress. Oxidative stress can damage human cells, leads to wide range of diseases like aging, cancer, cardio vascular and neurological disorder. An exits of oxidative stress leads to change in structure of protein and lipids. The most frequently encountered oxygen-centered free radicals, known as reactive oxygen species (ROS), include superoxide ($O_2^{\cdot -}$), Hydrogen peroxide (H_2O_2), hydroxyl ($^{\cdot}HO$), nitric oxide ($NO^{\cdot}$) and peroxyl ($ROO^{\cdot}$).

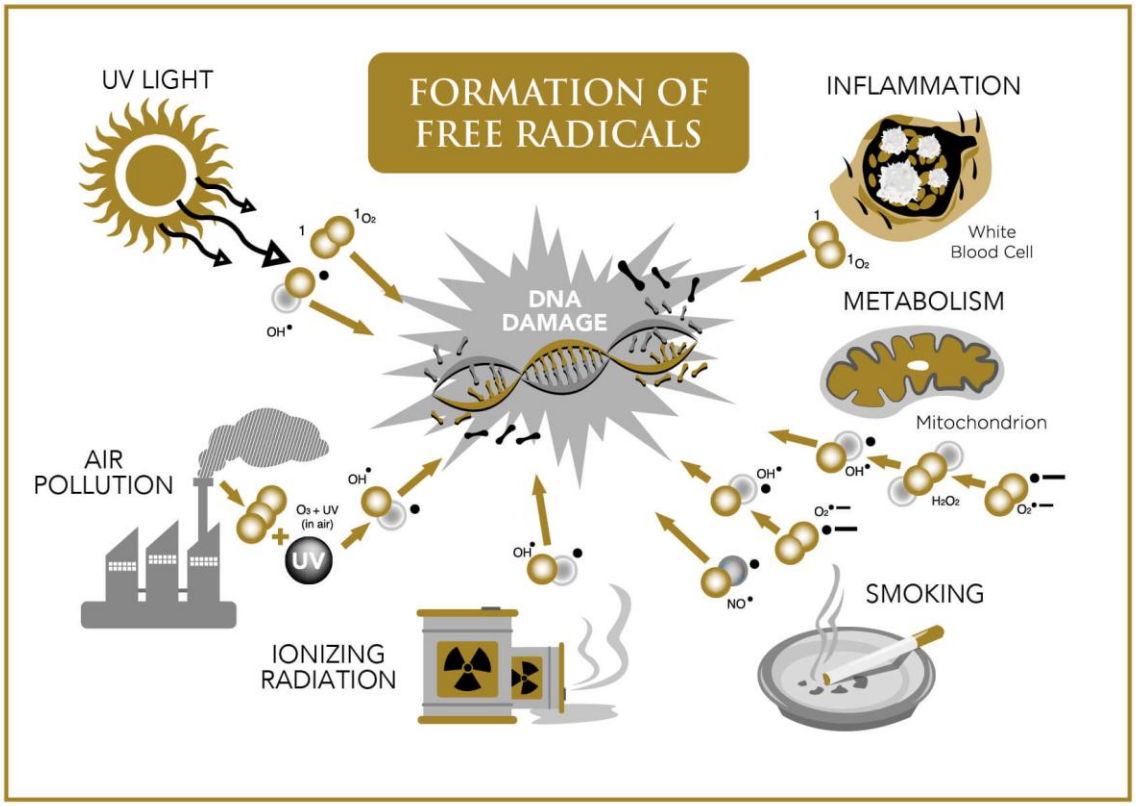


Fig 1.1. Formation of Free radicals

1.2.1 SUPEROXIDE RADICAL

Superoxide is an anion radical produced by one electron reduction of molecular oxygen. The reactive oxygen anion superoxide is the product of one electron reduction of dioxygen O_2. It is diradical containing two unpaired electrons, addition of an electron fills one of the two molecular orbitals, learning unpaired electron with charge of -1.

1.2.2 HYDROGEN PEROXIDE RADICAL

The hydroperoxyl radical, which is not so effective an oxidant as the hydroxyl radical, does not attack aliphatic alcohols; accordingly aldose is obtained from the higher aldonic acid. In the presence of an excess of hydrogen peroxide, however the accumulation of ferrous ions in solution catalyzes the production of hydroxyl radicals.

1.2.3 HYDROXYL RADICAL

The hydroxyl radical ($HO^{\cdot}$), the three electron reduction product of molecular oxygen, is the most reactive species of oxygen. It has a half life in biological system of about 1 ns and reacts with organic molecules. This high reactivity of $HO^{\cdot}$ Limited to no more 50 molecular diameters from the site of formation.

1.2.4 NITRIC OXIDE

The generation of Nitric oxide is frequently coupled to activation of the NMDA class of glutamate receptor. Once produced, no diffuses rapidly across membranes an to act on neighboring cells, its principle receptors being specialized gyranylyl cyclase-coupled proteins. The receptors are tuned to detect subnanomolar concentrations of Nitric oxide and rapidly transduce them into micromolar concentrations of cyclic GMP. This pathway participates in numerous physiological functions, including synaptic plasticity. Though other mechanism, Nitric oxide contribute to neuropathology.

1.2.5 PEROXYL RADICAL

Peroxyl radical are formed due to attack by molecular oxygen, which can abstract a hydrogen atom from a double bond in fatty acid site chains, producing a lipid hyroperoxide or lipid endoperoxide [6].

Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets. In order to prevent the propagation of radicals, need to powerful protective scavengers like synthetic and natural antioxidants are necessary. Hence the use of natural antioxidants from plant sources which acts as defense system in the human body are free from any side effects has gained much attention nowadays.

1.3 MODULATION OF FREE RADICALS BY NATURAL ANTIOXIDANTS

Two types of antioxidant namely the enzymatic antioxidants and nonenzymatic antioxidants modulate the free radicals reactions. Body protects itself from ROS by using enzymatic antioxidant mechanisms. The antioxidant enzymes reduce the levels of lipid hydroperoxide and H_2O_2 , thus they are important in the prevention of lipid peroxidation and maintaining the structure and function of cell membranes. SOD's located in the cytosol and mitochondria, catalytically convert the O_2.- into oxygen and H_2O_2 in presence of the metal ion cofactors such as copper (Cu), Zinc (Zn), or manganese (Mn). The enzyme CAT present in the peroxisome, converts H_2O_2 to water. The enzyme GSHPx has strong activity towards both H_2O_2 and fatty acid hydroperoxides. The enzyme peroxyredoxin catalyze the reduction of H_2O_2 organic hydroperoxides and the peroxynitrite ($ONOO^-$). [7] The different expression profiles, subcellular locations, and substarte of the antioxidant enzymes reveal the complex nature of the ROS biology. Clearly, the antioxidant enzymes play a major role in the prevention of oxidative damage. A demonstrated in the Fig 3. CAT, GSHPx and SOD show synergistic effect in the scavenging of $O_2^{\cdot -}$.

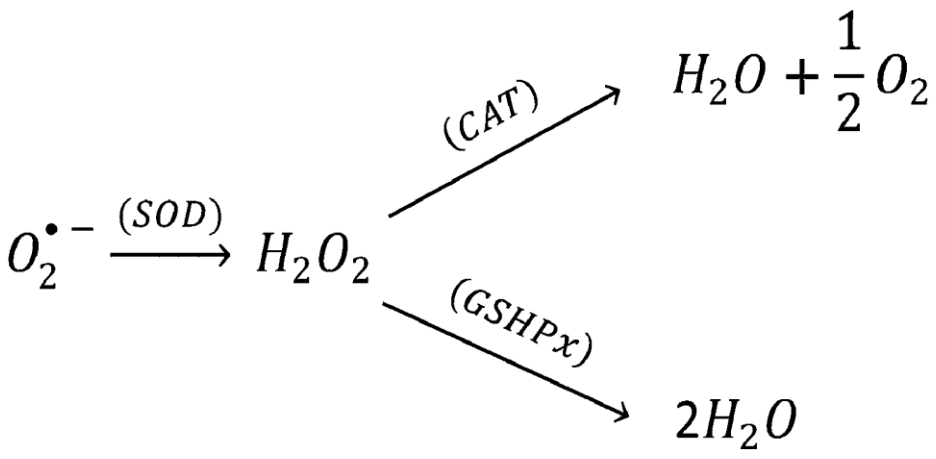


Fig 1.2 CAT, GSHPx and SOD show synergistic effect in the scavenging of $O_2^{\cdot -}$.

1.4 NIGELLA SATIVA

Nigella sativa is an indigenous herbaceous plant commonly known as fennal flower plant and belongs to Buttercup or Ranunculaceae family [8]. It grows in countries bordering the Mediterranean Sea, Pakistan and India, which widely distributed in native to Arab Countries and other parts of the Mediterranean region [9]. It is used as food preservative as well as a protective and health remedy in traditional folk medicine for the treatment of numerous disorders [10].

Nigella sativa is one of the most popular plants around the world. The seeds are very rich and diverse in chemical composition [11]. The seeds of *N.sativa* is traditionally used in food as well as "medicine" [12]. The fixed oils of *Nigella sativa* seeds have many pharmacological properties and are considered as antioxidant, anti-inflammatory, immunomodulatory, antitumor, antidiabetic agent and it plays a significant role in the cardiovascular and gastrointestinal systems. We focused our research on the search for biologically active molecule in *Nigella sativa* seeds, studying their phenolic fraction [13].

1.4.1 MORPHOLOGY OF NIGELLA SATIVA

The flowers grow terminally on its branches. *Nigella sativa* reproduces with itself and forms a fruit capsule which consist of many white trigonal seeds, once the fruit capsules has matured, it opens up and the seeds contained within are exposed to the air becoming black in colour that is reason it also called as black seeds, seeds are triangular in shape, black in colour

and possess a severe pungent smell, contains considerable amount of oil [14]. This plant is known by numerous names, for example black cumin (English), Black caraway seeds (USA), Shonaiz (Pakistan) and Kalajira (Bangali) [15].

Nigella plant

Nigella flower

Nigella pod

Nigella seeds

Fig 1.3. Different parts of *Nigella sativa*

1.4.2 CHEMICAL CONSTITUTE AND ACTIVE PRINCIPLES IN *NIGELLA SATIVA* SEEDS

The seeds are very rich and diverse in chemical composition, which contain amino acids, carbohydrates, fixed and volatile oil [16]. *Nigella sativa* also contain a good amount of various vitamins and minerals like Cu, P, Zn, and Fe. Many active compounds have been identified in *Nigella sativa* is attributed to Quinone constituents in the seed. The most important active

compounds of *Nigella sativa* are thymoquinone, thymohydroquinone, dithymoquinone (nigellone), carvacrol and thymol. *Nigella sativa* is also contain other compounds such as carvone, limonene, citronellol in trace amounts and two varieties of alkaloids (e.g. nigellidine and nigellicimine-N-oxide) and pyrazole alkaloids (e.g.nigellidine and nigellicine). The pharmacological properties of *Nigella sativa* are mainly attributable to its quinine constituents, TQ being the most abundant [17]. The *Nigella sativa* seeds contain fatty acids, constituting linoleic acid, eicosadienoic acid and saturated fatty acids. Most of the studies on the biological effects of Nigella sativa have dealt with its crude extracts in different solvents; however some studies used its active principles. Among the components isolated from the crude extract of Nigella sativa, thymoquinone (TQ) has been shown to be the principle active ingredient and thus is the most studied of all [18].

1.5 QUINONES

Quinones belongs to the group of quinoid compounds that are widely distributed in nature. Quinones have had two carbonyl group in the para-position of a ring, represent an important class of versatile organic compounds endowed with rich and fascinating chemistry. In the biological system like respiration and photosynthesis incorporate isoprenoid quinones as the integral part of the electron and energy transport chain in mitochondria and chloroplast [19]. Quinone can be formed from phenolic compounds either by acetate, affording a catechol or quinol system [20].

Quinones are secondary metabolites isolated from plants with structure of aromatic (hexacyclic saturated) di-one or di-ketone system, which derived from the oxidation of hydroquinones. Naturally occurring quinones are widely distributed and include;

(i) Benzoquinones
(ii) Naphthoquinones
(iii) Anthraquinones
(iv) Polyquinones.

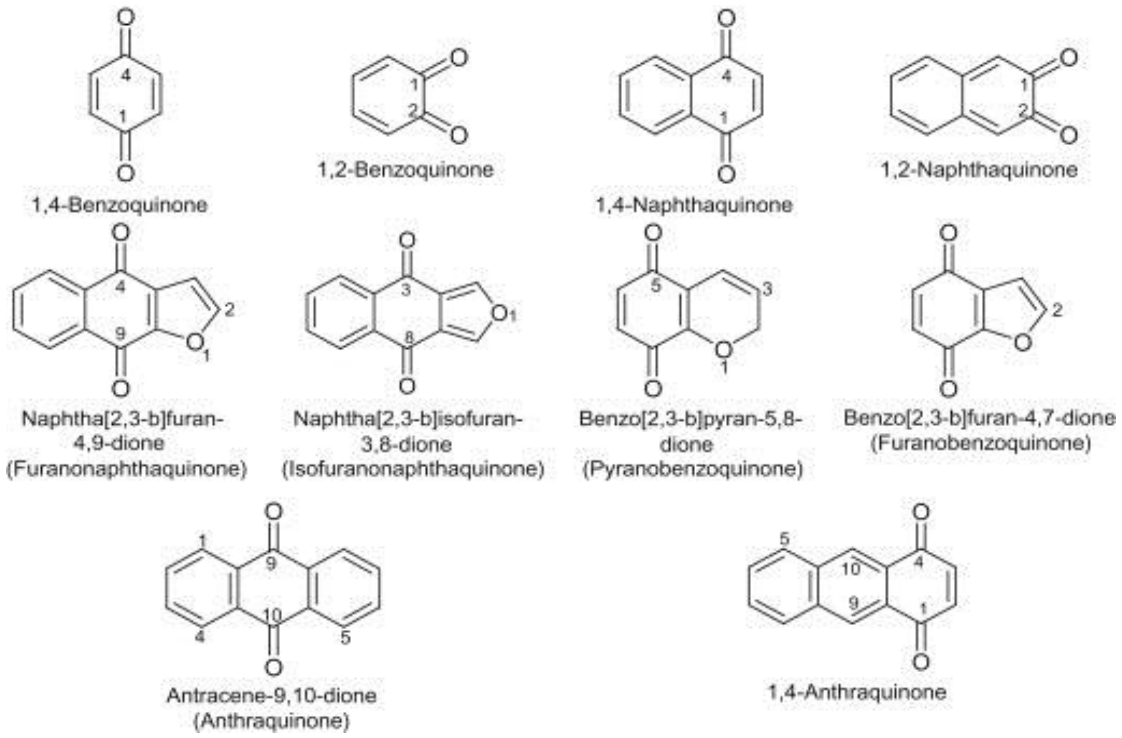


Fig 1.4. Schematic diagram of types of Quinones

1.5.1 BENZOQUINONES

Benzoquinones are groups of compounds containing two carbonyl groups on a saturated hexacyclic aromatic ring system (benzene ring), at ortho or para positions (monocyclic).

1.5.2 NAPHTHOQUINONES

Naphthoquinones contain the naphthalene nucleus with two carbonyl groups on one nucleus, usually at the ortho or para position (bicyclic) and occur at fungi and in plants.

1.5.3 ANTHRAQUINONES

The compounds containing the anthracene nucleus with two carbonyl groups, usually on ring B at para positions (tricyclic).

1.5.4 POLYQUINONES

polyquinones are dimers which had inter- or intramolecular oxidative coupling with formation of carbon–carbon or carbon–oxygen bonds [21].

1.6 QUINONES IN *NIGELLA SATIVA*

Quinones are found in a wide variety of plant families such as Ranunculaceas, Aphodelaceas, Fabaceae, Ebenaceae and Rhamnaceae. Fig 3.3 Shows the quinones types of compound identified in *Nigella sativa* (Ranunculaceas family). Many active compounds have been isolated, identified and reported in different varieties of black cumin seeds. The most important active compounds are thymoquinone, thymohydroquinone, dithymoquinone , thymol, and carvacrol Black seeds also contain some other compounds in trace amounts. Seeds contain two different alkaloids i.e isoquinoline alkaloids e. g. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine [22].

1.6.1 THYMOHYDROQUINONE

Thymohydroquinone (1,4-benzenediol, 2-methyl-5-(1-methylethyl) is a component of Black cumin seed oil and thyme. It is also a reduction product of thymoquinone. Which is a phytochemical compound found in the plant *Nigella sativa*. Thymoquinone exhibits antioxidant and analgesic properties.

1.6.2 DITHYMOQUINONE

Dithymoquinone(4b,8b-Dimethyl-3,7-di(propan-2-yl)-4a,8a-dihydrobiphenylene-1,4,5,8-tetrone) is a bioactive isolate of *Nigella sativa*. Chemically, it is a dimer of thymoquinone.

1.6.3 THYMOL

Thymol (2-isopropyl-5-methyphenol 5-methyl-2-isopropylphenol) is a phenol and had cymene derivative from natural.

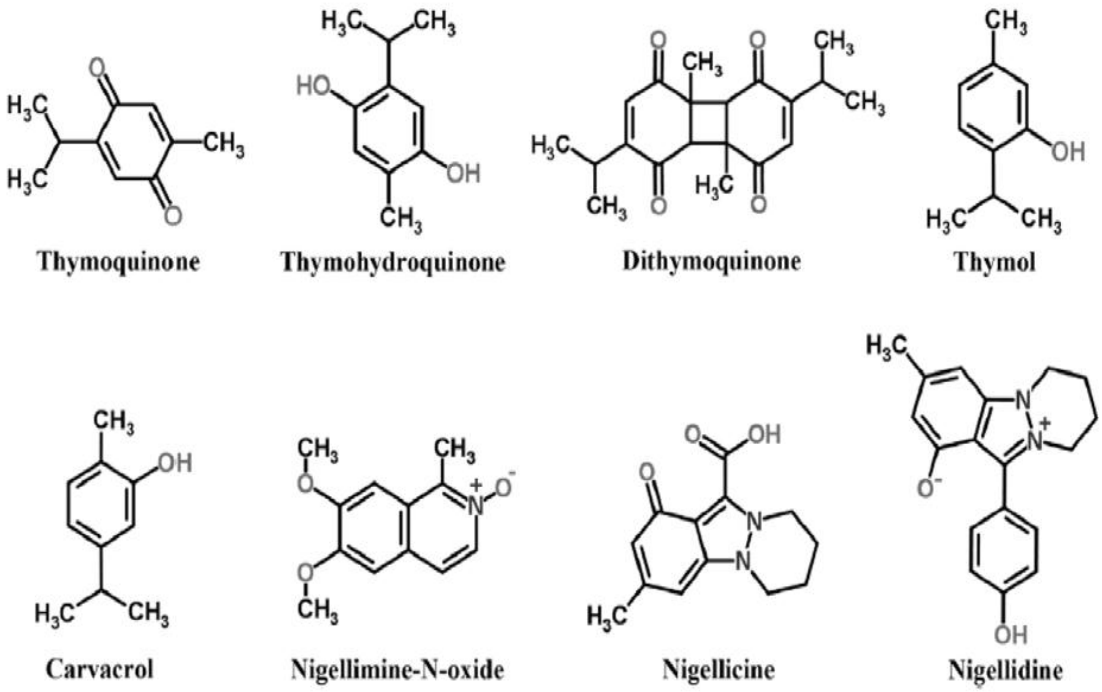


Fig 1.5. Chemical constituents found in *N. sativa seed* extracts

1.6.4 CARVACROL

Carvacrol (5-isopropyl-2-methylphenol) is a phenol and natural monoterpene derived from cymene. Bacterial growth is used a food additive to inhibit.

1.6.5 NIGELLIMINE-N-OXIDE

Nigellimine N-oxide (6,7-dimethoxyl-1-methyl-2λ^5-isoquinolin-2-one) belongs to the class of organic compounds known as isoquinolines which had aromatic polycyclic compounds containing an isoquinoline moiety, consists of a benzene ring fused to a pyridine ring and forming benzo pyridine.

1.6.6 NIGELLICINE

Nigellicine (9-hydroxy-7-methyl-5-pyridazino [1,2-a] indazol-11-ylium-10-carboxylate) is found in herbs and spices. Nigellicine is an alkaloid from the seeds of *Nigella sativa* [23].

1.6.7 NIGELLIDINE

Nigellidine(6,7,8,9-Tetrohydro-1-hydroxyl-11-(4hydroxyphenyl)-3methylpyridazino[1,2-a]indazol-5-ium inner salt,9cl) which is found in *Nigella sativa* seeds.

1.6.8 THYMOQUINONE

Thymoquinone (TQ) (2-isopropyl- 5-methyl-1,4-benzoquinone), which has the chemical formula $C_{10}H_{12}O_2$ and a molecular weight of 164.2 g/mol, with major phytochemical bioactive ingredient in *Nigella sativa* oil and extracts [24]. The chemical structure of TQ is shown in **Fig. 1.6**.

Table 1.1 Physicochemical properties of TQ

IUPAC Name	**2-isopropyl-5-methylbenzo-1, 4-quinone**
Chemical formula	C10H12O2
Molecular formula	164.20g/mol
Appearance	TQ is dark yellow crystalline powder
Melting Point	45-47^{0}C

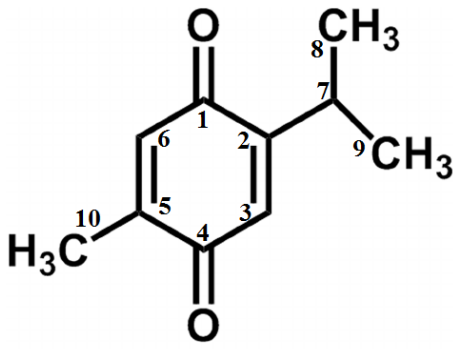


Fig 1.6. Basic Thymoquinone structure

Herbal medicine has attracted great attention in the recent years and is increasingly used as alternatives to chemical drugs. Several lines of evidence support the positive impact of medicinal plants in the prevention and cure of a wide range of diseases. TQ is the most abundant

constituent of the volatile oil of *Nigella sativa* seeds and most properties of *N sativa* are mainly attributed TQ. A number of pharmacological actions of TQ have been investigated including anti-oxidant, anti-inflammatory, immunomodulatory, anti-histammic, anti-microbial and antitumor effects. It has also gastroprotective, hepatoprotective, nephroprotective and neutroprotective activities. In addition, a large body of data shows that TQ has very low adverse effects and no serious toxicity. More recently, a great deal of attention has been given to this dietary phytochemical with an increasing interest to investigate it in preclinical and clinical researches for assessing its health benefits. The analyze of numerous properties of the active ingredient of *Nigella sativa* seeds, TQ in the context of its therapeutic potentials for a wide range of illnesses.

TQ is known to be the primary active constituent on *Nigella sativa* seeds responsible for its medicinal effects and also showing promise for treatment of cancer. tq is the bioactve compound derived from black seed (*Nigella sativa*). the seed is reportedly associated with diverse therapeutic beneffits to bronhial asthma, dysentery, headache, gastrointensial problems, eczema, hypertension and obesity [25]**.**

1.6 FREE RADICAL SCAVENGING AND ANTIOXIDANT POTENTIAL OF THYMOQUINONE (TQ)

TQ is a short-chain ubiquinone derivative that potentially acts as a pro-oxidant it has been hypothesized that inflammation and pro-oxidant milieu is actually something that is our bodies own creation, an adverse by product effect of the essential metabolism and inflammatory system that protects us against diseases.

Its broad spectrum antioxidant potential is associated with its potential to alter “redox state” and its scavenging ability against free radicals, including reactive oxygen species (ROS; superoxide anion radical, hydroxyl radical, hydrogen peroxide, peroxynitrate) through modulation of hepatic and extra hepatic antioxidant enzymes which as superoxide dismutase, catalase and GPx [26].

OBJECTIVE OF THE PRESENT WORK

- To extract the crude from *Nigella sativa* using Methanol.
- To evaluate the antioxidant activity of the crude extract
- To characterize the crude extract using spectroscopic techniques.
- To study the structural properties of TQ by DFT method at B3LYP/6311G ++ (2d,2p) using Gaussian software.
- To investigate the antioxidant properties of TQ through HAT mechanism.
- To analyze electronic properties of TQ.
- To analyze the spin density and charge transfer of TQ.

CHAPTER – II

REVIEW OF LITERATURE

2.1 INTRODUCTION

This chapter deals with review of literature on the extraction and oil from *Nigella sativa* seed using different methods and characterization of the samples. And the geometrical parameters and antioxidant properties of TQ were investigated using DFT theory.

2.2 OVERVIEW OF LITERATURE

1. Mohammed Shariq Iqbal et.al., (2018) have investigated that *Nigella sativa* pharmacological properties are mainly ascribed to its volatile oil of which TQ was an important bioactive component. The standard formulation or TQ rich *Nigella sativa* extract was under clinical use probably due to its poor extraction and lesser stability. The investigation solubility, extraction, percent composition and total antioxidant activity from the seeds of *Nigella sativa*. The presence of TQ in the respective extracts by absorbance spectra analysis and compared with pure TQ. Additionally total antioxidant activity of *Nigella sativa* extracts has been evaluated using ascorbic acid as standard. Absorbance spectra analysis confirms the presence of TQ peak in the benzene, hexane and methanol extracts. Then the optimization and standardization of bioactive rich formulation of *Nigella sativa* [27].

2. Nurulain Syamimi Mohamad et.al., (2018) have investigated the efficiency of two different extraction method namely was aqueous and methanol extraction was successfully developed through TQ screening analysis and characterization through HPLC analysis. The *Nigella sativa* seed contain a bioactive compound is TQ which was prominent for its pharmacological properties such as antioxidant, anti-microbial, antibacterial and anti-inflammatory and its aim to extract and characterize TQ from *Nigella sativa* based products. TQ was isolated from raw *Nigella sativa* seeds using methanolic and aqueous extraction methods. Extracts were subjected to qualitative and quantitative analysis using High-Performance Liquid Chromatography [28]..

3. RCN Thilakarathna et.al., (2018) have investigated the phenolic content and antioxidant activities of methanolic extracts of *Nigella sativa* seeds relative to Ethiopian and

Indian *Nigella sativa* were examined. Ferric thiocyanate test was carried out to find the reducing power of both types of *Nigella sativa* seeds. This test was utilized in explaining antioxidant activity of methanolic extract of seeds. The phenolic composition of the methanolic extract was estimated by Folin-Ciocalteu spectrophotometric method. Significantly higher amount of total phenolic content was exhibited by Ethiopian *Nigella sativa* seeds compared to Indian type with a mean value of gallic acid. Similarly DPPH radical scavenging activity to gallic acid equivalents per ml respective Ethiopian and Indian *Nigella sativa* Seeds were observed. In addition ABTS radical scavenging assay showed and were recoreded in Ehiopian and Indian *Nigellasativa* seeds respectively [29].

4. Omar Mechraoui et.al., (2018) have studied the phenolic extracts of the seeds of *Nigella sativa* from the point of view of the chemical composition and the antioxidant activity. The extraction of polyphenols was carried out using two different solvent systems, namely methanol: water and acetone: water. The qualitative and quantitative analysis of methanolic and acetonic extracts by HPLC (High Performance Liquid Chromatography) revealed the presence of gallic acid, apigenin, naringenin, rutin, quercetin and kaempferol. The quantification of total phenols by Folin-Ciocalteu colorimetric method and flavonoids by the AlCl3 method gave higher values with the methanolic extracts compared to the acetonic extract. The evaluation of the antioxidant power of the phenolic extracts was made by two chemical tests namely the DPPH test and the β-carotene bleaching test and we finished this work by studying the antibacterial and antifungal activity of these phenolic extracts [30].

5. Kausar H (2017) have reported the study of organic solvent and extraction method for the isolation of TQ from *Nigella sativa* seeds used different extraction techniques such as maceration, soxhlet, reflux and ultrasound assisted extraction were employed for extraction of TQ from the seeds by using three solvents of different polarity like petroleum ether, hexane and methanol. The TQ was qualified in each sample extract of seeds by simple high performance liquid chromatography technique and its found to be higher in methanolic extract among the various techhniques and solvents tried. Ultrasound assisted extraction technique was observed the best for extraction of TQ. And this study concluded on the basis HPLC finding that ultrasound assisted extraction technique was the best for extraction of TQ and will be preferred in academia for extraction of TQ from *N.sativa* seeds [31].

6. Muthu K.Shanumugam (2017) have studied that (TQ) is the main bioactive constituent of black seed essential oil extracted from the seed of *Nigella sativa*. TQ has been shown to have promising effects against a variety of inflammatory diseases and cancer. Drugs that interfere with this process are considered potential anti-cancer therapeutics, which may ultimately result in their clinical usage. It has been reported that modulate several major signaling pathways and key oncogenic molecules that play a prominent role in cancer initiation, progression, invasion, metastasis, and angiogenesis. Various studies have reported that TQ can enhance the anti-cancer potential when co-administered with several chemotherapeutic agents while reducing their toxic side effects [32].

7. Wesam Kooti et.al., (2016) were studied that *N. sativa* have been considered worldwide as an important medicinal herb. Its use in pharmaceutical, food and ornamental industries displays considerable commercial value. *N.sativa* and its compounds, particularly TQ, have various pharmacological effects on different body parts. "Black seed", "*Nigella sativa*", "therapeutic effect", and "medicinal plant". The results showed that *N.sativa* has many biological effects such as anti-inflammatory, anti-hyperlipidemic, anti-microbial, anti-cancer, anti-oxidant, anti- diabetic, anti-hypertensive, and wound healing activities. It also has effects on reproductive, digestive, immune and central nervous systems, such as anticonvulsant and analgesic activities. In summary, it can be used as a valuable plant for production of new drugs for treatment of many diseases [33].

8. Abdurohman Mengesh Yessuf (2015) have studied that natural products and their derivatives an important drugs and the pharmaceutical industries and traditional medicine as a source of bioactive agents can be used in the preparation of synthetic medicine and helpful also as nutritional values. The investigation was directed to the detection of the bio active *Nigella sativa* which is one of the miraculous plant having a phytochemical constituents and nutritional value. In this manner the result of investigation of qualitative phytochemical analysis conducted on the crude *Nigella sativa* seed extracts revealed the presence of bioactive compounds in petroleum ether, ethyl acetate and methanol extract revealed medicinal as well as physiological activities. Thus can scientifically conclude that the *Nigella sativa* seed extract could be seen as an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit [34].

9. **Zeinab Solati et.al., (2014)** were prepared *Nigella sativa* seeds were extracted using supercritical carbon dioxide (SC-CO_2) and Soxhlet. Chemical characteristics, fatty acids composition, antioxidant activity and TQ content *N.Sativa* extracts obtained through different methods were investigated and compared. It was revealed that antioxidant activity and TQ content could be significantly different for SC-CO_2 and soxhlet extracts. The result for fatty acids composition indicated that linoleic acid, palmitic acid and oleic acid were the main fatty acids in both extracts. The SC-CO_2 extraction could provide an extract with higher quality and antioxidant activity compared to soxhlet extraction method and can be considered a more appropriate method and can be considered a more appropriate method for attaining a high-quality extract [35].

10. Shabina Ishatiaq et.al., (2013) have investigated to find in vitro antibacterial activity of *Nigella sativa* extracts against seven clinical isolates identified by ribotyping. Crude extracts of *Nigella sativa* in eight organic solvents (Aqueous, Methanol, Ethanol, Chloroform, Butanol, Diethyl ether, n-Hexane and Acetone) were evaluated at 5 different concentrations 100 mg/ml , 50mg/ml , 25mg/ml , 10mg/ml and 5mg/ml by using disk diffusion method against human pathogenic bacterial strains including gram positive bacteria and gram negative bacteria. All the organic *N.Sativa* extracts with minimum concentrations of 5mg/ml showed effective growth inhibitation aginst the tested pathogenic bacterial strains. Out of the eight extracts showed maximum antibacterial activity. The study will provide an insight to characterize bioactive compounds from these extracts, which can acts as strong bacterial growth inhibitor against wide range of infectious disease caused by pathogenic bacteria [36].

11. Prawez Alam et.al., (2013) have develop a sensitive and accurate high-performance thin layer chromatography method and to determine the quality of TQ in two different *N.sativa* extracts and marketed formulations. TQ was separated on aluminium backed silica gel with n-hexane-ethyl acetate as mobile phase. A compact band was obtained for TQ calculated R_f value. This study concluded that to developed high-performance liquid chromatography densitometric method was sound cheap, selective, precise and accurate and can be used for routine analysis of *N.sativa* extracts and marketed formulations [37].

12. Muzaffar Iqbal et.al (2013) have estimated a simple, economic, robust, reproducible, selective, and precise high-performance liquid chromatography (HPLC) method developed and validated for the estimation of TQ in two different extract and marketed formulations. The mobile phase composed of 20-mM KH2PO4 buffer and acetonitrile at a ratio of 60:40 eluted at a flow rate of 1 mL/min. TQ was monitored using florescence detector set at 274 excitation and 340 emission wavelength, having column oven temperature of 40°C and sample cooler temperature of 8°C ± 0.2°C. The linear regression analysis data for the calibration curve shows good linear relationship with correlation coefficient of 0.998 in the concentration range of 0.07-12 μg/mL. The limit of detection and limit of quantification were 0.023 and 0.07 μg/mL, respectively. The developed method was validated for accuracy, precision, reproducibility, and robustness as per ICH guidelines. The proposed method with high degree of precision and accuracy is employed for the estimation of TQ in methanolic and petroleum extract of *Nigella sativa* as well as in formulation. Statistical analysis proved that the method is precise, reproducible, selective, and accurate for the estimation of TQ for quality control purpose [38].

13. Nejdet Sen et.al., (2010) have studied the antioxidant activity of the methanolic extracts of the black cumin (*Nigella sativa*) seeds collected from Konya, Isparta, Çorum, Burdur, Afyon and Samsun was evaluated using three different methods, specifically the b-carotene and linoleic acid system, 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging activity and reducing power assays. Among the six *Nigella sativa* samples of different origins, the *Nigella sativa* sample origins from Konya city showed the most potent radical scavenging activity in each assay, showing 94.59% (at 1 mg/mL) in the b-carotene bleaching method and 44.44% in the DPPH radical scavenging method (at 0.5 mg/mL). Positive correlations were found between the total phenolic content in the black cumin extracts and their antioxidant activities. The values of their antioxidant activity were either equivalent to or higher than those of positive BHA and BHT controls [39].

14. **Erkan Karacabey (2016)** have investigate that the high potential of thymoquinone as an ingredient and/or additive in the food, pharmaceutical and cosmetic industries has been well established. However, its extraction from natural sources was considered in the limited studies and none of them included the microwave-assisted ex-traction (MAE) of a thymoquinone-rich

extract and process optimization. In this study, this high-value-added bioactive was aimed to extract from its well-known natural source, black cumin seed (*Nigel-la sativa*), using methanol as a solvent for all of the studied extraction methods. For extraction of a compound of interest, microwave-assisted extraction system having temperature controlling function was used and its performance was compared with common extraction methods, Soxhlet and conventional solid/liquid extraction. The results indicated that the MAE system provided a rich extract containing thymoquinone, which was 2 and 7 times higher than those produced by conventional solid/liquid extraction and Soxhlet, respectively. Influences of temperature, time and solvent/solid ratio on thymoquinone yield were investigated for MAE. The solvent/solid ratio was found to have the main effect on extraction performance, whereas an interaction effect of temperature and time was significant. Variables of MAE were optimized by response surface methodology to produce a thymoquinone-rich extract. Optimal conditions for the highest yield of thymoquinone were determined as 10 minutes extraction at 30 °C, using 30 ml solvent per gram of *Nigella sativa*. The estimated thymoquinone yield of the extract was 628 mg/kg *Nigella sativa* seed. It could be concluded that the currently optimized MAE with temperature con-trolling function is a promising technique to produce a thymoquinone-rich extract from *Nigella sativa* seeds [40].

THEORETICAL REVIEW

15. Keivan Akhtari et.al., (2015) have studied the mechanism of TQ for scavenging peroxide radical was explicated in gas phase and in liquid phase using density functional theory approach and calculated bond dissociation enthalpy, ionization potential and proton dissociation enthalpy revealed that H-atoms at position 2-isopropyl and 5-methyl groups can be readily abstracted. Analysis of spin density distribution indicates that H-atom abstraction in 2-isopropyl site generates more stable radical and the plots of highest occupied molecular orbital of TQ demonstrate that the whole molecule can be easily attacked by electrophilic agents like radicals. So This study interaction of TQ with hydroxyl, hydroperoxyl and superoxide anion radicals using potential energy scans and natural orbital analysis and this modeling of the reaction between TQ and lipid peroxyl radical [41].

16. A.B. Raschi et.al., (2010) have investigated the molecular structure of 2-isopropyl-5-methyl-1,4-benzoquinone, $C_6O_2H_2$ $(CH_3)_3CH$, have been optimized using density functional theory (DFT) and Moller - Plesset second order perturbation theory (MP2). As regards $C_6O_2H_2$ $(CH_3)_3CH$, two populated conformations with C_1 (trans) and C_2 (cis) symmetric are obtained. The theoretical data indicated that although both anti and cis conformers are possible by rotation about C-C bond, the preferred conformation is trans. The effects governing the torsion barriers and preferred conformations were analyzed at B3LYP/6-311++G^{++} level. The atoms in molecules (AIM) theory and natural bond orbital (NBO) analysis to the cis and trans conformers. Furthermore the infrared spectra for the gas and solid phases and the Raman spectrum for the solid one, were recorded and the observed bands assigned to the vibrational modes [42].

17. Fazlul Huq et.al., (2010) have investigated the TQ induces apoptosis, disrupts mitochondrial membrane potential and triggers the activation of caspases 8, 9 and 3 in myeloblastic leukaemia HL-60 cells. Although TQ acts as antioxidant and is found to inhibit inflammation in animal models and culture systems, it can also cause glutathione depletion thus acting as a pro oxidant. It has been suggested that TQ may be metabolized to reactive species and increase oxidative stress that contributes to the depletion of antioxidant enzymes and damage to DNA in hepatocytes treated with high concentrations of the compound. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that TQ and its metabolic products have LUMO-2 HOMO energy differences ranging from 3.8 to 5.4 eV indicating that the compounds would be moderately inert kinetically with THY being most inert. The molecular surfaces of TQ and DTQ are found to possess significant amounts of positively charged electron-deficient regions so that they may be subject to nucleophilic attacks by glutathione and nucleobases in DNA, thus causing cellular toxicity due to glutathione depletion and DNA damage due to oxidation of nucleobases [43].

18. Bun Chan et.al., (2007) have studied the uncatalyzed transfer hydrogenation between a range of hydrogen donors and acceptors, in the gas phase and in solution and the study shows in the reliable condensed-phase transition structures, it is necessary to perform geometry optimization in the presence of a continuum. In addition, the use of a free energy of solvation obtained with the UB3-LYP/6-31+G(d,p)/IEF-PCM/UA0 combination, in conjunction with UMPWB1K/6-311+G(3df,-2p)//B3-LYP/6-31+G(d,p) gas-phase energies, gives the best

agreement with experimental barriers. In condensed phases, the geometries and energies of the transition structures are found to relate to one another in a manner consistent with the Hammond postulate. There is also a correlation between the barriers and the energies of the radical intermediates in accord with the Bell-Evans-Polanyi principle and find that in the gas phase, all the transfer-hydrogenation reactions examined proceed via a radical pathway. The calculations indicate that the detection of radical adducts by EPR does not necessarily indicate a predominant radical mechanism, because of the possibility of a concurrent ionic reaction and also find that the transition structures for these reactions do not necessarily have a strong resemblance to the intermediates, and therefore one should be cautious in utilizing the influence of polar effects on the rate of reaction as a means of determining the mechanism [44].

19. Bun Chan et.al., (2007) have studied the uncatalyzed transfer hydrogenation between a range of hydrogen donors and acceptors, in the gas phase and in solution and the study shows in the reliable condensed-phase transition structures, it is necessary to perform geometry optimization in the presence of a continuum. In addition, the use of a free energy of solvation obtained with the UB3-LYP/6-31+G(d,p)/IEF-PCM/UA0 combination, in conjunction with UMPWB1K/6-311+G(3df,-2p)//B3-LYP/6-31+G(d,p) gas-phase energies, gives the best agreement with experimental barriers. In condensed phases, the geometries and energies of the transition structures are found to relate to one another in a manner consistent with the Hammond postulate. There was also a correlation between the barriers and the energies of the radical intermediates in accord with the Bell-Evans-Polanyi principle and find that in the gas phase, all the transfer-hydrogenation reactions examined proceed via a radical pathway. The calculations indicate that the detection of radical adducts by EPR does not necessarily indicate a predominant radical mechanism, because of the possibility of a concurrent ionic reaction and also find that the transition structures for these reactions do not necessarily have a strong resemblance to the intermediates, and therefore one should be cautious in utilizing the influence of polar effects on the rate of reaction as a means of determining the mechanism [45].

20. A.P.Avdeenko et.al., (2006) have investigated the direction of halogen addition to N-arylsulfonyl-1, 4-benzoquinone monoimines dialkyl-substituted in the quinoid ring is governed by the steric factors: the size and position of the substituent, the halogen volume, and the position

of the substituent at the nitrogen. The first stage of halogenation of N-arylsulfonyl 1-4-aminophenols with two alkyl substituents in the phenylsulfony ring largely occurs as electrophilic substitution [46].

21. Hala Gali-Muhtasib et.al., (2006) have reported that there has been growing interest in naturally occurring compounds with anti-cancer potential. TQ is the bioactive constituent of the volatile oil of black seed. It has been shown to exert anti-neoplastic and anti-inflammatory effects. The molecular pathways of TQ action are not clear. Nevertheless, TQ is known to induce apoptosis by p53-dependent and p53- independent pathways in cancer cell lines. Growth inhibition is associated with induction of cell cycle arrest. TQ also acts on the immune system by modulating the levels of inflammatory mediators. To date, the chemotherapeutic potential of TQ in the clinic has not been tested, but numerous studies have shown its promising anti-cancer effects in animal models. The combination of TQ with clinically used anti-cancer drugs has led to improvements in their therapeutic index and prevents non-tumor tissues from sustaining chemotherapy-induced damage [47].

22. Himansu Mohapatra et.al., (2002) have investigated that Time-resolved resonance Raman spectroscopy (TR3) and density functional calculations have been used to study the effect of asymmetric substitution of methyl group on the structure and vibrational spectra of the radical anion of benzoquinone. The asymmetrically substituted benzoquinones that have been studied are methyl-1,4-benzoquinone (MBQ) and 2,6-dimethyl-1,4-benzoquinone (2,6 DMBQ). Specific vibrational mode assignments have been made to all the vibrational frequencies recorded in the experiment. The coupling between the CdO bonds has been analyzed on the basis of the (DFT results) shift in the vibrational frequency upon isotopic substitution of carbonyl carbon and oxygen atoms. Comparison of the (18O) isotopic shift and shift in their CdO stretching frequencies between MBQ and 2,6 DMBQ suggests in the case of 2,6 DMBQ that coupling of the CdO modes with other internal coordinates is considerable for the symmetric CdO stretch compared to asymmetric CdO stretch. Further studies based on site-specific isotopic substitution of the carbonyl carbon and oxygen atoms suggest that symmetric CdO stretch is predominantly from the proximal carbonyl bond and the extent of contribution from the proximal carbonyl bond

increases in case of 2,6 DMBQ. The asymmetric CdO stretch has slightly more contribution from the distal carbonyl stretch than the proximal [48].

23. Mohamed A. El-Mahdy et.al., (2003) have reported that TQ, the major biologically active component isolated from a traditional medicinal herb, *Nigella sativa*, was a potential chemopreventive and chemotherapeutic compound. Despite the promising antineoplastic activities of TQ, the molecular mechanism of its pharmacologic effects was poorly understood. Here, we report that TQ exhibits antiproliferative effect, induces apoptosis, disrupts mitochondrial membrane potential and triggers the activation of caspases 8, 9 and 3 in myeloblastic leukemia HL-60 cells. The apoptosis induced by TQ was inhibited by a general caspase inhibitor, z-VAD-FMK; a caspase-3-specific inhibitor, z-DEVD-FMK; as well as a caspase-8-specific inhibitor, z-IETDFMK. Moreover, the caspase-8 inhibitor blocked the TQ-induced activation of caspase-3, PARP cleavage and the release of cytochrome c from mitochondria into the cytoplasm. In addition, TQ treatment of HL-60 cells caused a marked increase in Bax/Bcl2 ratios due to upregulation of Bax and downregulation of Bcl2 proteins. These results indicate that TQ-induced apoptosis is associated with the activation of caspases 8, 9 and 3, with caspase-8 acting as an upstream activator. Activated caspase-8 initiates the release of cytochrome c during TQ-induced apoptosis. Overall, these results offer a potential mechanism for TQ-induced apoptosis in p53-null HL-60 cancer cells [49].

24. Osama A. Badary et.al., (2003) have studied the antioxidant and pro-oxidant effects of TQ , a natural main constituent of the volatile oil of *Nigella sativa* seeds, and a synthetic structurallyrelated tert-butylhydroquinone (TBHQ), were examined in vitro. Both TQ and TBHQ efficiently inhibited iron-dependent microsomal lipid peroxidation in a concentration-dependent manner with median inhibitory concentration (IC50) values of 16.8 and 14.9 mM, respectively. TBHQ was stronger than TQ as a scavenger of 2,20-diphenyl-p-picrylhydrazyl radical (DPPH) (IC50¼5 mM, 200 times more active than TQ) and as a scavenger of hydroxyl radical (OH.) with an IC50 of 4.6 mM (approximately 10 times more active than TQ). TQ was more active than TBHQ as a superoxide anion scavenger with IC50 of 3.35 mM compared to 18.1 mM for TBHQ. Only TBHQ significantly promoted DNA damage in the bleomycin-Fe(III) system. The results suggest that both TQ and TBHQ have strong antioxidant potentials through scavenging

ability of different free radicals. Moreover, the data indicate that TQ is acting mainly as a potent superoxide anion scavenger [50].

25. Mahmoud A. Mansour et.al., (2002) have investigated focused on the effect of oral administration of TQ on antioxidant enzyme activities, lipid peroxidation and DT – diaphorase activity in hepatic, cardiac and kidney tissues of normal mice. Superoxide dismutase, catalase, glutathione peroxidase, glutathione treatment of mice with the different doses of TQ produced significant reductions in hepatic SOD, CAT and GSH-Px activities. In addition cardiac SOD activity was markedly inhibited with the higher doses of TQ. moreover TQ significantly reduced hepatic and cardiac lipid peroxidation as compared with the respective control group. Kinetic parameters for the reduction of TQ to dihydrothymoquinone indicated that DT- diaphorase of different tissues can efficiently reduces TQ to dihydrothymoquinone. The results revealed that TQ and DHTQ acted not only as superoxide anion scavengers but also as general free radical scavengers. The IC_{50} for TQ in biochemical and photochemical assays were in the nanomolar and micromolar range respectively. And its reported beneficial in vivo protective effects of TQ through the combined antioxidant properties of TQ and its metabolite [51].

26. Laila I. Abou Basha et.al., (1995) have studied a simple quantitative TLC method for the determination of TQ in commercially available *Nigella sativa* seed oil obtained from *Nigella sativa* (Ranunculaceae) using a scanning densitometer was described. Also the identification of thymol, dithymoquinone in this sample was established. The Rf values forTQ , thymol and dithymoquinone are 0.77, 0.37 and 0.52 respectively. The identification of the TQ spot, obtained from the methanol extract of oil was confirmed by GCMS which was essentially identical to TQ standard. The solvent system consisted of benzene: isopropyl ether (1:l). All the spots were visualised and quantitated at 254 nm. The method proposed was simple, reproducible with a lower limit of detection of 100 nmoles/ml and can be used in routine analysis of TQ in *Nigella sativa* oil for quality control purposes [52].

27. Daniel M. Chipman et.al., (1986) have investigated the Properties of the ground electronic states of neutral p-benzoquinone and the related p-benzosemiquinone radical anion are determined by ab initio calculations. The structures, vibrational frequencies, and frequency

assignments of the neutral are found to be in good agreement with the most recent experimental determinations. Good agreement is also found for the few anion frequencies known experimentally. The calculations provide new information on the structure of the anion as well as on its other vibrational modes. Significant differences between the neutral and anion can be explained on the basis of weakening of the C=O and C=C bonds and strengthening of the C-C neutral bonds upon electron attachment [53].

CHAPTER – III

EXPERIMENTAL AND THEORETICAL METHODOLOGY

3.1 INTRODUCTION

This chapter deals the both Experimental and Theoretical methods implemented in the present work. The extraction of crude and oil from the *Nigella sativa* (NSE) were carried out. The antioxidant activity of NSE extract is analyzed. The FT-IR spectra of NSE and Thymoquinone (TQ) molecule were recorded. The experimental and theoretical wavenumbers were assigned and compared. UV-vis spectra were simulated in liquid phase using TD-DFT calculations and compared with the experimentally observed absorption spectra. The relation between structure and electronic properties of TQ is investigated using molecular descriptors. Thermodynamical mechanism mainly Hydrogen atom Transfer (HAT) are employed to compute C-H bond dissociation enthalpies (BDE) act both gas and solvent phases. The Natural Bond Orbitals (NBO) analysis was carried out to evaluate intramolecular stabilization energy of the molecule. The Frontier Molecular Orbitals and Fukui function analysis, which reveals the charge transfer mechanism in the molecule.

3.2 EXPERIMENTAL METHODOLOGY

3.2.1 MATERIALS AND METHODS

3.2.1.1 Materials

- *Nigella sativa*
- Hexane
- Methanol
- Thymoquinone – Sigma Aldrich

3.2.1.2 EXTRACTION OF *NIGELLA SATIVA* SEED OIL

In the present study, *Nigella sativa* is collected from OTR Organic shop, Coimbatore. The *Nigella sativa* seeds were cleaned with fresh water to remove dust, then dried for four days

at room temperature. After that the seed crushed into powder using grinder and store in refrigerator for further use [53].

3.2.1.3 ULTRASOUND ASSISTED EXTRACTION

The *Nigella sativa* seed powder (25 gm) was mixed with 200 ml of hexane in a beaker. The beaker is immersed into another beaker containing ice-bath. The probe submerged into the mixture and worked at (30 Hz) frequency and (50 w) power for 30 mints. After extracted, the mixture was filtered under vacuum through whatman No.1 paper and the solvent was removed.

Nigella sativa* seeds** ***Nigella sativa seed **Powder** **Hexane**

***Nigella sativa* seed oil**

Fig 3.1 Ultrasound assisted Extraction from *Nigella sativa* seed

3.2.1.4 EXTRACTION FROM DEFATTED NSE

The 23 gram of *Nigella sativa* seed powder defatted by ultrasonic assisted extraction are mixed with 230 ml of Methanol and kept in beaker submerged in ice-bath and sonicated at (30 Hz) frequency with 50w power for 15 mints. The Extraction was filtered through whatman no.1 filter paper and solvent was removed by rotary evaporator at 50^0C. The extraction was kept at freeze dryer at 300^0 C for 20 days. The powder BCS extract is stored at 4^0C for further use [54].

***Nigella sativa* seed Powder** **Methanol** **Methanol extract**

Fig 3.2 Extraction from *Nigella sativa* seed powder

3.3 ANTIOXIDANT ACTIVITY

3.3.1 DPPH RADICAL SCAVENGING ACTIVITY

The determination of antiradical activity of *Nigella sativa* seed extract were assessed by (2,2-diphenyl-1-Picryl hydrazyl) and the ability of Scavenging fraction towards the relatively stable Nitrogen centered free radical were measured by the method [55].

PRINCIPLE

Antioxidants reacts with DPPH (2,2-diphenyl-1-picryl hydrazyl) and convert it to α,α' -diphenyl- β-picryl hydrazine by donating its OH group. This can be identified by the conversion of purple colour to yellow colour, which can be qualified at 518nm.

REAGENTS

1. **DPPH -** 2,2-diphenyl-1-picryl hydrazyl
2. **Methanol**

PROCEDURE

Free radical scavenging activity of the NSE extract was measured in terms of radical scavenging ability using the stable free radical DPPH [56]. The NSE extract was diluted in pure methanol in five different concentrations of 20µl, 40µl, 60µl, 80µl and 100µl. A methanolic solution of 0.3Mm of DPPH (0.5ml) was added to NSE extract. The mixture was allowed to react at room temperature for 30 minutes in the dark. Methanol was used as a blank and DPPH in methanol without *Nigella sativa* seed extract was used as a control. After 30 minutes of incubation, the decolourization of the purple to yellow colour. Then the antiradicals are measured by using UV-Spectrophotometer at 517 nm [57]. A lower IC_{50} value corresponds to the higher antioxidant activity. The ability to scavenge DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect} = \frac{\text{control} - \text{sample}}{\text{control}} \text{X}100$$

3.4 SPECTROSCOPY ANALYSIS

3.4.1 FTIR- SPECTROSCOPY

INTRODUCTION

Infrared Spectroscopy is nondestructive technique for materials analysis and used in the laboratory for over seventy years. Infrared absorption spectroscopy is the study of interaction of infrared radiation with matter as a function of photon frequency. Fourier Transform Infrared Spectroscopy (FTIR) provides specific information about the vibration and rotation of the chemical bonding and molecular structures, making it useful for analyzing organic materials and certain inorganic materials.

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the

material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition the size of the peaks in the spectrum is a direct induction of the amount of material present. With modern software algorithms, infrared is an excellent tool for qualitative analysis [5,6].

The IR region is commonly divided into three smaller areas. Near – IR (400-10 cm^{-1}) mid – IR (4000 – 400 cm^{-1}) and far –IR (14000-4000cm^{-1}). Infrared spectroscopy have enough energy to cause groups of atoms to vibrate with respect to the bonds that connect them. Like electronic transitions, these vibrational transition correspond to distinct energies, and molecules absorb infrared radiation only at certain wavelength and frequencies. Chemical bonds vibrate at characteristic frequencies and when exposed to infrared radiation, they absorb the radiation at frequencies that match their vibration modes. Measuring the radiation absorption as a function of frequency produces a spectrum that can be used to identify functional groups and compounds. Some impurities produce their own characteristics bands in infrared region. Spectral measurements of these bands are used to determine concentration of the impurities and their bonding with the host materials. In order to make identification the measured interferogram signal cannot be interpreted directly. A means of "decoding" the individual frequencies is required. This can be accomplished via a well-known mathematical technique called for Fourier transformation.

From this spectroscopy, we study about electromagnetic radiation interacts with matter which are dealing with the transition. From these transitions a molecules undergoes between higher and lower energy levels. H_2, O_2, N_2 are those molecules which does not contain IR spectrum. Likewise HCL, H_2O, NO_2 molecules contain IR spectrum during transition change in vibrational quantum numbers can only be +- 1.

The IR spectroscopy is also known as vibrational rotational spectroscopy because each vibrational state contain several rotational energy states. There are two modes of fundamental vibrations such as

- Stretching vibrations
- Bending vibrations

STRETCHING VIBRATIONS

This type of vibrations contain atoms that are move along the bond axis. At regular interval of time the bond length increases or decreases but the atom remain in the same bond axis. Such a mode of vibration does not cause any change in dipole. O=C=O is the symmetrical molecules and therefore stretching vibration is nor IR active. Further stretching vibration is classified into two types

- Symmetrical stretching
- Asymmetrical stretching

SYMMETRICAL STRETCHING

This type of stretching with respect to a particular atom, other two atom in a molecule move in the same direction. For example methylene group, H-C-H, the two hydrogen atoms move away from the central carbon atom without change in the bond angle.

ASYMMETRIC STRETCHING

In this type of stretching one atom departs, from the central atom, while the other atom approaches the central atom.

BENDING VIBRATIONS

Bending vibration consist of change in bond angle between two bonds. Bending vibrations are also called Deforming vibrations. Various types of bending vibration are scissoring, rocking, wagging and twisting.

a) **Scissoring** : Two atoms move towards and away from each other with deformation of the valency angle (in plane of bending).
b) **Rocking** : In rocking the structural units swings back and forth in the plane of the molecule (in plane bending).
c) **Wagging** : In this type, the structural units swings back and forth in the plane of the molecule (out of plane bending).
d) **Twisting** : In this type one the atoms moves up the plane while the other moves down the plane with respect to the central atom (out of plane bending).

CONSTRUCTION

These are three basic spectrometer components in an FTIR system a radiation source, an interferometer and a detector.

Fig 3.3. FT-IR instrument

Infrared divides radiant beams, generated an optical path difference between the beams, and then recombines them in order to produce repetitive interference signals measured as a function of optical path difference by a detector. As its name implies, the interferometer produces interference signals, which contain infrared spectral information generated after passing through a sample. The most commonly used interferometer is a Michelson interferometer. It consists of three active components a moving mirror, a fixed mirror, and a beam splitter. The two mirrors are perpendicular to each other. The beam splitter is a semi-reflecting device and is often made by depositing a thin film of germanium onto a flat KBr substrate. Radiation from the broadband IR Source is collimated and directed into the interferometer, and impinges on the beam splitter.

WORKING

The normal instrumental process is as follows:

1. **The source** : infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and ultimately to the detector).

2. **The interferometer**: the beam enters the interferometer where the "spectral encoding" takes place. The resulting interferogram signals then exits the interferometer.
3. **The detector**: The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.
4. **The Computer** : The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation
5. **The sample** : To determine the infrared spectrum of a compound, one must place the compound in a sample holder or cell. Glass and plastics absorb strongly throughout the infrared region of the spectrum. Cells must be constructed of ionic substances typically sodium chloride or potassium bromide widely sodium chloride plate is used.

LIQUIDS

A drop of a liquid organic compound is placed between a pair of sodium chloride or potassium bromide plates which is called salt plates. When the plate is squeezed gently, a thin liquid film forms between them. A spectrum determined by this method is referred to as a neat spectrum since no solvent is used. Salt plate easily breaks and it is soluble in water. The pair of plates in inserted into a holder that fits into the spectrometer.

SOLIDS

There are three common methods used for preparing a solid sample for spectroscopy. The first method involves mixing the finely ground solid sample with powered potassium bromide and pressing the mixture under high pressure. Under pressure, the potassium bromide melts and seals the compound into a matrix. The result is a KBr pellet that can be inserted into a holder in the spectrometer.

WORKING

The Michelson interferometer, which is the core of FTIR spectrometers. It is used to split one beam of light into two so that the paths of the two beams and conduct them into the detector where the differences of the intensity of these two beams is measured as a function of the difference of the paths.

It consists of two perpendicular mirrors and a beam splitter. One of the mirrors is a stationary mirror and another one is a movable mirror. The beam splitter is designed to transmit half of the light and reflect half of the light. Subsequently, the transmitted light and the reflected light strike the stationary mirror and the movable mirror respectively. when reflected back by mirrors, two beams of light recombine with each other at the beam splitter. The beam which emerges from the interferometer at 90^0 to the input beam is called the transmitted beam and beam is detected in spectroscopy [58].

APPLICATIONS

- Identification of sample mixture of organic and inorganic both as solids or liquids.
- Identification of polymers and polymer blends.
- Indirect verification of trace organic contaminants on surfaces.
- Routine qualitative & qualitative FTIR analysis.
- Thin film analysis.
- Identification of rubber and filled rubber.

STRENGTH

The FT-IR spectrometer has several major advantages over the dispersive instrument.

- Its sensitivity is better because it measures all frequencies simultaneously rather than scanning through the individual frequencies.
- Less energy is needed from the source and less time (typically 1 to 2 seconds) is needed for a scan.
- Several scans can be completed in a few seconds and averaged to improve the signal.
- The light beam is a precise frequently references that keep the spectrometer accurately calibrated.

LIMITATIONS

Few limitations of FT-IR spectrometer are listed below:

- Minimal elemental information is given for most samples.

- Background solvent or solid matrix must be relatively transparent in the spectral region of interest.
- Molecule must be active in the IR region i.e., when exposed to IR radiation, a minimum of one vibrational motion must alter the net dipole moment of the molecule in order for absorption to be observed [59].

3.4.2 UV-VISIBLE SPECTROPHOTOMETER

Ultraviolet-visible spectrometer or ultraviolet-visible spectrophotometer (UV-Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. Thus means it uses light in the visible and adjacent(near-UV and near- infrared [NIR]) ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemical involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transition from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state[60].

PRINCIPLE OF ULTRAVIOLET-VISIBLE ABSORPTION

Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons (i.e. lower energy gap between the HOMO and the LUMO), the longer the wavelength of light it can absorb.

INSTRUMENTATION

Instruments used for measuring the absorption of UV or visible radiation are made up of following components which is arranged systematically.

- A Source of radiation
- Monochromator
- Sample container
- Detector

- Recorder

A SOURCE OF RADIATION

It is important that the power of the radiation source does not change abruptly over its wavelength range. The electrical excitation of deuterium or hydrogen at low pressure produces a continuous UV spectrum. Both Deuterium and Hydrogen lamps emit radiation in the range 160-375nm. Hydrogen discharge lamp consist of two electrode contains in deuterium filled silica envelope. Selection of lamps made by moving lamp mounting or mirror to cause the light falls on monochromatic. For Deuterium lamp emits radiation up to 3- 5 times when compared to hydrogen discharge lamps. For xenon lamp is stored under pressure in 10-30 atmosphere.

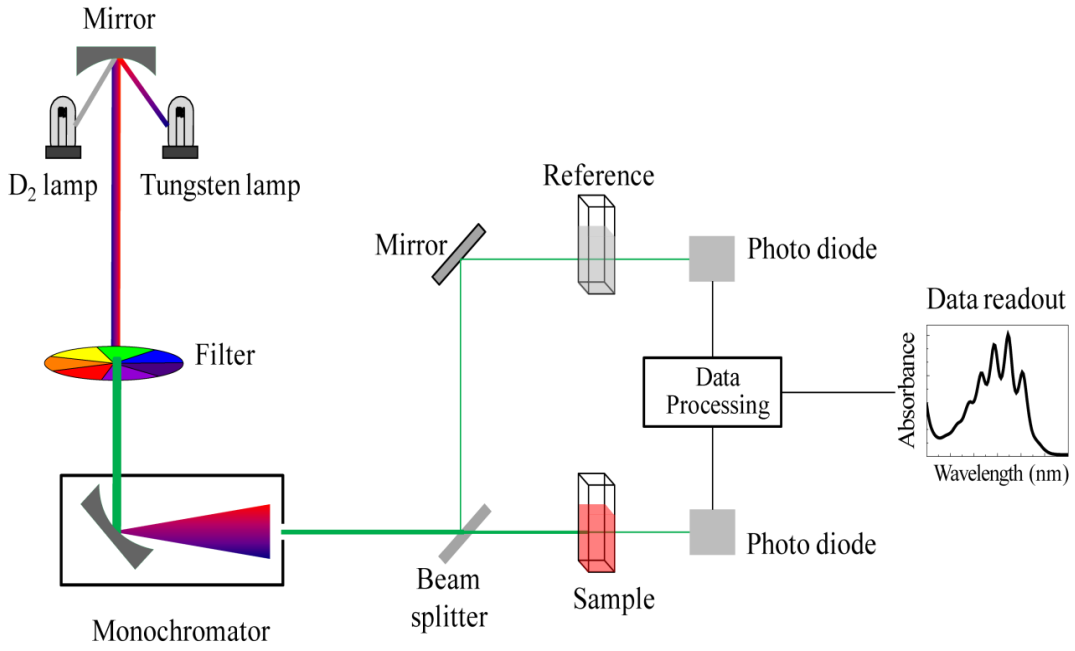


Fig 3.4 UV instrument

MONOCHROMATOR

All Monochromators contain the following components parts:

- An entrance slit
- A Collimating lens

- A dispersing device (a prism or a grating)
- A focusing lens
- An exit slit

FILTERS

The filters are classified into two types:

(i) **glass filters**- made from pieces of colored glass which transmit limited wavelength range of spectrum. Wide band width 150nm.

(ii) **Gelatin Filters**- Consist of mixture of dyes placed in gelatin and sandwiched between glass plates. Band width 25nm.

(iii) **Inter Ferro metric filters**- Band Width 15nm.

PRISMS

Prism bends the monochromatic light. Amount of deviation depends on wavelength**.** They produce nonlinear dispersion.

SAMPLE CONTAINER

A variety of sample cells available for UV region. The choice of sample cell is based on

- The path length, shape, size
- The transmission characteristics at the desired wavelength
- The relative expense.

The cell holding the sample should be transparent to the wavelength region to be recorded. Quartz or fused silica is required for spectroscopy in the UV region. Silicate glasses can be used for the manufacture of cuvettes for use between 350 and 2000nm. The thickness of the cell is generally 1cm. cells may be rectangular in shape or cylindrical with flat ends.

DETECTOR

They are three types of photosensitive devices, to detect the radiation such as,

a. Photo emissive Tubes

b. Photomultiplier Tubes

c. Photo-voltaic cells or barrier-layer cell

A) **PHOTOEMISSIVE** tubes consist of an evacuated glass tube fitted with a semi-cylindrical cathode and a wire anode. The cathode is made of cesium or a metal coated with cesium or a metal coated with light of suitable wave length. These electron flows to the wire anode and result photocurrent is produced. The number of electrons ejected from the photo emissive surface is directly proportional to the radiant energy. The current is a measure of the quantity of light falling on a photocathode. Such tubes are generally operated at 90-100 volts.

B) **PHOTOMULTIPLIER** Tubes are used to increase the sensitivity of a photo emissive cell. Photomultiplier tubes are also photoemissive cells. It consists of an electrode of a photoemissive material and series of positively charged plates, each charged at a successively higher potential. The photomultiplier tubes also contain an additional electrode called dynode. The dynode is an electrode with a coating of cesium or antimony or some such material. Dynode 1 is maintained at a potential of 90v, more positive than the cathode and electrons are accelerated towards dynode 2 which is 90v more positive than dynode 1.Again many an electrons are emitted . If the number of dynodes in tubes is nine, then106 -107 electrons are formed for each photon. As a result the electron current gets amplified and the output current called pulse at the anode is as much as a million times greater than the current admitted by photocathode.

C) **PHOTO-VOLTAIC CELLS** are mainly used for detection of radiation is Visible region. These cells have maximum sensitivity of about 550nm. It consists of flat copper or iron electrode is coated with semi-conducting material such as selenium or Cu oxide is deposited. A thin transparent film of sliver is spread over the selenium layer which acts as collector of electrons called collector electrode. Finally iron base acts as the outer electrode. This generates e.m.t by its own and does not need any external source for operation.

RECORDING DEVICE

Computer is used as a recording device. This computer stores all the generated data and the spectrum is produced for desired compound.

WORKING

The functioning of this instrument is relatively straightforward.

A beam of light from a visible and/or UV light source is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. One beam, the sample beam, passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference beam, passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as I_0. The intensity of the sample beam is defined as I. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm [61].

3.5 COMUTATIONAL DETAILS

3.5.1 QUANTUM CHEMISTRY

Quantum chemistry has long promised to become a major tool for the study of molecular properties and reaction mechanisms. The fundamental methods of quantum chemistry date back to the earliest days of quantum mechanics in the decades of the twentieth century. Quantum mechanics is the theoretical frame work within which it has been found possible to describe, correlate and predict the behavior of vast range of physical systems, from elementary particles, through nuclei, atoms and radiation, to molecules and solids. Modern molecular physics includes the quantum quantum mechanical explanation of the several kinds of chemical bonding between the atom in a molecules, the quantization of the vibrational, rotational and electronic motion of the molecules, the phenomena arising from intermolecular forces. Quantum chemistry has developed into an indispensable part of modern chemistry and molecular physics over the last 50 years. It provides the concepts and ideas that are central to our modern understanding of chemical systems. Quantum chemistry is considered to be a quantitative tool, capable of producing numerical results that agree quantitatively with the true or exact results, to within some estimated uncertainty.

There are mainly four methods in quantum chemistry to calculate the molecular properties of polyatomic molecules. They are,

- Semi empirical Methods
- Ab initio Methods
- Molecular mechanics Methods
- Density Functional Methods

3.5.1.1 SEMI – EMPIRICAL METHODS

Semi-empirical quantum chemistry methods are based on the Hartree-Foack formation, but make many approximation and obtain some parameters fro empirical dataand are very important in computational chemistry for treating large molecules where the full Hartree Fock method without the approximation is too expensive. The use of empirical parameters appears to allow some inclusion of correction effects into the methods. Semi-empirical methods are called empirical methods where the two electrons of the part of the Hamiltonian is not explicitly included.

HARTREE – FOCK EQUATION

The starting point of the Hartree-fock procedure for helium is as the two-electron wave function as a product of orbitals, i.e.,

$$\Psi (r_1, r_2) = \phi(r_1)\, \phi (r_2) \longrightarrow (3.1)$$

According to eqn 1, the probability distribution of electron 2 is $\phi^*(r_2)\, \phi(r_2)\, dr_2$. We interpret this probability distribution classically as a charge density, and so the potential energy that electron 1 experiences at the point r_1 due to electron r_2.

$$U_1^{eff}(r_1) = \int dr2\ \ \phi^*(r_2) \frac{1}{r12} \phi (r_2) \longrightarrow (3.2)$$

Where the subscript of eff emphasizes that $U_1^{eff}(r_1)$ is an effective or average potential. We can define an effective one-electron Hamiltonian operator by

$$H_1^{eff}(r_1) = -\frac{1}{2} \nabla_1^2 - \frac{Z}{r1} + U_1^{eff}(r_1) \longrightarrow (3.3)$$

The schrodinger equation corresponding to this effective Hamiltonian is

$$H_1^{eff}(r_1)\, \phi(r_1) = \phi_1 \phi(r_1) \longrightarrow (3.4)$$

In equation 3.4, is the Hartree Fock equation for the Helium atom. The solution to equation 3.4 gives the best orbital wave function for helium.

Although we have deduced Equation 4, by a physical argument and possible to derive directly by applying the variational principle to the energy of the helium atom.

$$E = \iint dr1\,dr2\ \phi^*(r_1)\,\phi^*(r_2)\,\hat{H}\,\phi(r1)\phi(r2) \longrightarrow \quad (3.5)$$

Substituting $\hat{H}$ into Eq 3.5, We get

$$E = I_1 + I_2$$

And

$$J_{12} = \iint dr1\,dr2\ \phi^*(r_1)\,\phi^*(r_2)\,\frac{1}{r12}\,\phi(r_1)\,\phi(r_2) \longrightarrow \quad (3.6)$$

The integral J_{12} here is called a coulomb integral. Equation 3.4 can be obtained by minimizing E with respect to ϕ .

Because $\hat{H}_1^{\text{eff}}$ in equation 4 is spherically symmetric, we write

$$\Phi(r) = R(r)\,Y_l^m(\theta,\phi) \longrightarrow \quad (3.7)$$

Hence R(r) is a radial function to be determined and $Y_l^m(\theta,\phi)$is a spherical harmonic. If Equation 3.7 is substituted into equation 3.4 and obtain as

$$\left[-\frac{1}{2r}\frac{d}{dr1}\left(r_1^2\frac{d}{dr1}\right)-\frac{z}{r1}+\frac{l(l+1)}{2r1}+U_1^{\text{eff}}(r_1)\right]R(r_1) = \varepsilon\,R(r_1) \longrightarrow \quad (3.8)$$

The method solving an equation 8 is the self- consistent field method. We first guess the form of $\Phi(r)$ and using $\Phi(r)$ to evaluate $U_1^{\text{eff}}(r_1)$ by equation (2). And then solve equation (8)for a new $\Phi(r_1)$. Usually, afterone cycle, the $\Phi(r)$is used as input and $\Phi(r_1)$ obtained as output differ. $\Phi(r)$ is used as input by calculating $U_1^{\text{eff}}(r_1)$ with this new $\Phi(r)$ and then solve the $\Phi(r)$ obtained from eqn 8 are sufficiently close,or are self-consistent. The orbitals obtained by this method are the Hartree-Fock orbitals.

In practice, one uses linear combinations of slater orbitals Φ (r), varying the parameters in each Slatar orbital and the number of Slater orbitals used until convergence is obtained. The method using linear combination of Slater orbitals in Hartree-Fock calculations was developed by Roothan procedure is widely used for the calculation of atomic orbitals.

THE HARTREE-FOCK LIMIT

It should be apparent that different choices of basis set will procedure different SCF wave functions and energies. If we do an SCF calculation on some molecule, using a minimal basis set and obtain a total electronic energy E_1 and a double-ζ basis and do a new SCF calculation, we will obtain an energy E_2 that normally will be lower than E_1. If we now add polarization functions and repeat the SCF procedure and find E_3 to be lower than E_2. In continuation, adding new functions in bonds and always increasing the capabilities of our basis set, but always requiring that the basis describe MO, in a single determinantal wave function. The electronic energy will decreases with each basis set and become very slight for any improvement. i.e., the energy will approach a limiting value is the lowest that are achieved for a single determinantal wave function called the Hartree- Fock energy. The MO, that correspond to this limit are called Hartree-Fock orbitals, and the determinant is called HF wave function.

Sometimes the term restricted Hartree-Fock (RHF) is used to emphasize the wave function is restricted to be a single determinantal function for a configuration wherein electrons of a spin occupy the same space orbitals as do the electrons of β spin. When this restricted is relaxed , and different orbitals are allowed for electrons with different spins, and have an unrestricted Hartree-Fock (UHF) calculation. This refinement is must likely to be important when the numbers of α-and β-spin electrons differ.

3.5.1.2 KOHN-SHAM THEORY

The foundation for the use of DFT methods in computational chemistry is the introduction of orbitals as suggested by Kohn-Sham. The main flaw in orbital free models is the poor representation of the kinetic energy, and the idea in the KS formation is to split kinetic energy functional into two parts, one which is calculated exactly, and a small correction term. The orbital re-introduced, thereby increasing the complexity form 3 to 3N variables, and that

electron correction re-emerges as a separate term. The KS model is closely related to the HF method, Sharing identical formulas for the kinetic, electron-nuclear and coulomb electron – electron energies.

The division of the electron kinetic energy is into two parts , with the major contribution being equivalent to the HF kinetic energy. Assume for the moment a Hamiltonian operator of the form in equation (1) with $0 \leq \lambda \leq 1$.

$$H_\lambda = T + V_{ext}(\lambda) + \lambda V_{ee} \longrightarrow \quad (3.9)$$

The external potential operator V_{ext} is equal to the + V_{ee} for $\lambda=1$, but for intermediate λ values, it is assumed that $V_{ext}(\lambda)$ is adjusted such that the same density is obtained for $\lambda=1$ (the real system). For $\lambda=0$ case, the electrons are non-interacting and the exact solution to the schrodinger equation is given as a slater determinant composed of (molecular) orbitals, ϕ_i and the exact kinetic energy functional is given in equation (3.10).

$$T_s = \sum_{i=1}^{N} elec \; \langle i|-\frac{1}{2}\nabla^2|i \longrightarrow \quad (3.10)$$

The subscript S denotes that it is the kinetic energy calculated from the slater determinant. The $\lambda=1$ case corresponding to interacting electrons, and eqn (10) is an approximation to the total kinetic energy, but a substantial improvement over the TF formula.

$$K\rho[\rho] = -C_X \int \rho^{4/3}(r)\, dr$$

$$C_F = \frac{3}{10}(3\pi^2)^{2/3}$$

Another way of justify the use of equation (3.10) for calculating the kinetic energy is by refernces to natural orbitals. The exact kinetic energy can be calculated from the natural orbitals arising from the exact density matrix.

$$T[\rho_{exact}] = \sum_{i=1}^{\infty} n_i \langle \frac{N}{i}|-\frac{1}{2}\nabla^2|\frac{N}{i}\rangle$$

$$\rho_{exact} = \sum_{i=1}^{\infty} n_i \left|\frac{N}{i}\right|^2 \longrightarrow \quad (3.11)$$

$$N_{elec} = \sum_{i=1}^{\infty} n_I \longrightarrow \quad (3.12)$$

The orbital occupation numbers n_i (eigen values of the density values) will be between 0 & 1, corresponding to the number of electrons in the (spin) orbitals. Representing the exact density will require an infinite number of natural orbitals, with the N_{elec} first having occupation numbers close to 1, and the remaining close to 0. Since the exact density matrix is not known, an (approximate) density can be written in terms of a set of auxiliary one-electron functions. i.e., orbitals.

$$P_{approx} = \sum_{i=1}^{N} |n|^2 \longrightarrow \quad (3.13)$$

This corresponding to equation (3.11) with occupation numbers of exactly 1 or 0. The "missing" kinetic energy from equation (3.10) is due to the occupation numbers deviating from being exactly 1 or 0, the missing kinetic energy can also be considered as the (kinetic) correlation energy.

The key of kohn-sham theory is to calculate the kinetic energy under the assumption of non-interacting electrons. The reality the electrons are interacting and does not provide the total kinetic energy. However, just as HF theory provides 99% of the correct answer, the difference between the exact kinetic energy and that calculated by assuming non-interacting orbitals is small. The remaining kinetic energy is absorbed into an exchange-correlation term, and a general DFT energy expression can be written as in eqn (3.13)

By equating E_{DFT} to the exact energy, this expression defines Exc, is the part that remains after subtraction of the non-interacting kinetic energy, E_{ne} and J potential energy terms as

$$E_{XC}[\rho] = (T[\rho] - T_S[\rho]) + (E_{ee}[\rho] - J[\rho]) \longrightarrow \quad (3.14)$$

The first parenthesis in eqn (3.14) are considered as the kinetic correlation energy, which the last contains both potential correlation and exchange energy.

The task in developing orbital-free models is to derive approximation to the kinetic, exchange and correlation energy functionals, while the corresponding task in kohn-sham theory is to derive approximation to the exchange-correlation energy function only. The exchange correlation energy is roughly a factor of 10 smaller than the kinetic energy, kohn-sham theory is much less sensitive to the inaccuracies in the functionals than orbital free theory. While orbitals- free theory is a true density functional theory, khon-sham methods are independent particle models,

analogous to Hartree-fock theory, but are still much less complicated than many particle wave function models [62].

3.5.1.3 AB INITIO METHODS

Over the past three decades, Ab initio quantum chemistry has become as essential toolis the study of atoms and molecules also in modeling complex systems in biology and materials science. It has the first principle for polyatomic molecules. Ab initio calculation uses the true molecular Hamiltonian $\widehat{H}$ and does not use empirical data in the calculation. This type of computation is based only on theoretical principles, using no experiment data. The numerous methods have the same basic approach, but differ in the mathematical approximations used. These are the most popular type of models, despite the fact the calculations take unbelievably long time.

Hartree-Fock is the basis ab initio model. It uses the approximation that coulomb electro-electron repulsion can be averaged, instead of considering explicit repulation interactions (i.e., Central field approximation) [63].

On Ab initio calculations involves the following steps:

- Working down Hamiltonian operator $\widehat{\widehat{H}}$
- Selecting a trial function ψ
- Minimizing

$$< E >= \int \frac{\Psi_* \hat{H} \Psi \, d\tau}{\Psi_* \Psi \, d\tau} \longrightarrow \quad (3.15)$$

3.5.1.4 MOLECULAR MECHANICS THEORY

Molecular mechanics simulation use the laws of classical physics to predict the structures and properties of molecules, they are many different molecular mechanics methods. Each one is characterized by a force field. A force field has the following components:

- A set of equation defining how the potential energy of a molecule varies with the location of its component atoms.

- A series of atom types, defining the characteristics of an element within a specific chemical context. The atom type prescribes different characteristics and behavior for an element depending upon its environment. The atom type depends on hybridization, charge and the types of the other atoms to which an atom is bonded.
- One or more parameters sets that fit the equations and atom types to experimental data. Parameter sets define force constants, which are values used in the equations to relate atomic characteristics to energy components, and structural data such as bond lengths.

Molecular mechanics calculations do not explicit treat the electron in a molecular system. Instead, they perform computation based upon the interaction among the nuclei. Electronic effects are implicit included in force and its parameterization. This approximation makes molecular mechanics computation quite in expansive in computation time, and allows them to be used for very large systems containing thousands of atoms. However, it also carries several limitations:

- Particular force fields achieve good results only for a limited class of molecules, related to those for which it was parameterizes. No force field can be generally used for all molecular systems.
- Neglect of electrons means that molecular mechanics methods cannot treat chemical problems where electronic effects predominate.

The potential functions generated by molecular mechanics have no absolute meaning but are simply for comparing different configuration of a molecule. Molecular mechanics is useful for large molecules such as proteins [62].

3.5.1.5 DENSITY FUNCTIONAL METHODS

The wave function Ψ for an n-electron molecule is a function 3n-spatial co ordinates and n-spin coordinates. From Ψ we can produce the molecule's spin-free electrons density function ρ(1), by integrating Ψ^* Ψ overall of the spin coordinates and all the space coordinates except those for our of the electrons:

$$\rho(1) = \int | \Psi (1,2,\ldots\ldots\ldots\ldots N)|^2 \, d\omega_1 \, d\tau_2 \ldots\ldots d\tau_n \longrightarrow \quad (3.16)$$

which is a function of only the three spatial coordinates.

In 1964, Hohenberg and Kohn made a connection between the ground state energy (E_0), and the ground state density function (ρ_0) for a system.

For a system having n electrons and N-nuclei, the Hamiltonian operator for the electronic energy is,α

$$H=-\frac{1}{2}\sum_{i=1}^{n}\nabla_i^2+\sum_{i=1}^{n}\sum_{i=1}^{N}-\frac{z\alpha}{r\,i\alpha}++\sum_{i=1}^{n-1}\sum_{j=i+1}^{n}rij \longrightarrow (3.17)$$

The first and last term are written if there is known number of electrons present, but the middle term depends on , which is a function of nuclear charges and locations. This quantity is called the external potential, symbolized V_{ext} (r), because it results from the presence of fields produced by particles not included in the group of electrons.

Hohenberg and Khon were able to prove that there is a uniqueness relation between ρ_0 and the external potential: No two external potential could give the same ρ_0. This raises the possibility that to work backwards from ρ_0 to find V_{ext} (r) and E_0 . Using ab initio methods, we get an accurate E_0 and Ψ_0, and from Ψ_0 we calculate T_0, V_{neo}, V_{eeo} and all the other properties of interest for the system.

There is no generally applicable procedure known for getting from ρ_0, V_{ext} (r). V_{ext} (r) is a functional of ρ_0 , which we symbolize V_{ext} (r) but don't know the functional relationship. Then Hamiltonian operator by uniqueness theorem have led to the current goal of density functional theory.

A subsequent relation proved by Hohenberg and Kohn indicated a way to proceed and proved that an approximation density function, when subjected to the (unknown) procedure that relates the exact ρ_0 to the exact E_0: $E_{0,approx} \geq E_0$, so a variational bond exists. Unknown process referred to here is that assumes V_{ext} (r) to be the same for the analysis of ρ_0 and $\rho_{0,approx}$ which means that the same nuclear frame work applies in both cases.

If a procedure were known for finding E from ρ, then the existence of a variational bound would allow a variational procedure analogous to what applied earlier. One would start with a trail ρ, calculate its energy and vary ρ to locate the ρ that gives the lowest energy.

In analogy to wave function methods, the functional that connects E to ρ, E(ρ) are separated into an electronic kinetic energy contribution, T(ρ), a contribution due to nuclear –electron attractions, E_{ne} [ρ] and the electron repulsion , E_{ee} [ρ]. The latter term is further decomposed into coulomb and exchange terms, J[ρ] and K[ρ]. Both the nuclear-electron attractions and the interelectronic coulomb terms are easily written in terms of the density using their classical expressions as in wave function methods. For an accurate treatment of the electronic kinetic energy term, differentiate a wave function, and led to the practice first proposed by kohn and sham of expressing density in terms of one-electron orbitals ϕ. These orbitals serve two purpose and allow us to calculate a value of the kinetic energy within a single slater determinant framework similar to Hartree-Fock theory [62].

$$T_s = \sum_{i=1}^{n} < i| -\frac{1}{2}\nabla^2|i> \longrightarrow \quad (3.18)$$

And to obtain the electron density, defined in terms of these Kohn-sham orbitals as

$$\rho_s = \sum_i^n |i|^2 \longrightarrow \quad (3.19)$$

The final DFT energy expression is then written as,

$$E_{DFT}[\rho] = T_s[\rho] + E_{ne}[\rho] + J[\rho] + E_{XC}[\rho] \longrightarrow \quad (3.20)$$

3.6 BASIS FUNCTION

The calculation of molecular integrals is the most cumbersome step in the calculation of electronic wave function. There are numerical difficulties which depend on the choice of the basis set function {ϕv}. In principle, for high frequency, a complete (infinite) basis set should be used. In practice a finite basis set of reasonable dimension is used. The functions are generally chosen as to create least difficulty in integration.

3.6.1 BASIS SET

A basis set is a mathematical description of the orbitals within a molecular system used to perform Quantum chemical calculations. Larger the basis set, more accurately the approximate orbitals, by improving a fewer limit to the electron in space [63].

3.6.2 CLASSIFICATION OF BASIS SET

The basis sets can be broadly classified in to the following types.

Minimal basis set (STO-NG)

In minimal basis set each AO is represented by an STO which in turn is a linear combination of N GTF S (Gaussian type function). Once the coefficients of expansion and the Gaussian orbital exponents are determined and are fixed throughout the calculations. The most commonly used minimal basis is STO-3G. A Gaussian basis set is often denoted by the notation (A) [B] , or (A/B) , where A is a listing of the primitive Gaussian and B is a listing of the contracted gaussians. The second notation is more convenient to use.

STO-3G basis set for H and Li are (3s) [1s] or (3s/1s) and (6s3p) [2p1p] or (6s 3p/2s1p) respectively. the integers should not be confused with the principal quantum number.

Extended Basis set

- **Split valence double zeta (SVDZ) basis set**

  SVDZ basis set result when each AO in the minimal basis set is replaced by two AO's with different zetas (exponents). The example of (SVDZ), basis set is 4-31G. The numbers 4,3 and 1 in the notation 4-31 G identify the contraction lengths. Since there are P-type orbitals (p_x, p_y and p_z) the two p CGTF's included six p orbitals.

- **Split valance double zeta plus polarization (SVDZP) basis set**

  An SVDZP basis set results when a set of polarization functions are added to the SVDZP basis set. When a set of polarization functions are added on all the atoms in the molecule the resulting basis st is denoted by 4-31 G*.

- **Split valnce double zeta plus polarization plus disuse (SVDZPD) basis set**

  This basis set result when an s-type diffuse GTF's are included in the SVDZP basis set. This basis set 4-31+G*, 4-31+G** and 4-31++G** are of this type. On hydrogen only an s-type diffuse function is added.

- **Split valance triple zeta plus polarization plus diffuse (SVDZPD) basis set**

  A triple-zeta (TZ) basis set replaces each STO of a minimal basis set by three STOs that differ in their exponents. The 6-311+G*, 6-311G** and 6-311++G** basis set belongs to this type

3.7 GEOMETRICAL PARAMETERS

3.7.1 BOND LENGTH

The distance between centres of bonded atoms are called bond lengths, or bond distances. Bond length vary depending on many factors, in general they are very consistent. The bond orders the bond length, and of the same order for the same pair of atoms in various molecules are consistent. The bond length ranges from the shortest of 74pm for H-H to 200pm for long atoms, and the bond energies depends on bond order and lengths.

3.7.2 BOND ANGLE

The average angle between the orbitals of the central atom containing the bonding electrons pair in the molecules is known as bond angle between the atoms. The unit of bond angle is either degree or minute or second. The gives the distribution of orbital's around the central atom in a molecule. Therefore bond angle determines the shape of a molecule.

3.8 BOND DISSOCIATION ENTHALPY (BDE)

Hydrogen atom transfer (HAT) is a major mechanisms by which phenolic antioxidant exerts its antioxidative capacity, H atom directly transfers from antioxidant to active free radical to break chain react. The mechanism occurs when an antioxidant compound quenches free radical species by donating hydrogen atom. The reactivity of an antioxidant can be estimated by the O-H bond. BDE value calculated according to the formula [64]

$$BDE = H(Ar\,O^{\cdot}) + H(H^{\cdot}) - H(ArOH) \longrightarrow \quad (3.21)$$

Where,

$H(Ar\ O^{\cdot})$ = Enthalpies of the phenoxy radical

$H(H^{\cdot})$ = Enthalpy of hydrogen atom

H(ArOH) = Enthalpy of neutral molecule

In HAT, the reactivity of an ArOH can be estimated by calculating the O-H bond dissociation enthalpy (BDE), where the lower BDE value the higher the expected antioxidant activity. In order to calculate BDE value enthalpies of H-atom, in gas phase and solvents are needed. The gas phase enthalpy of a hydrogen atom is -0.49987 hartree [65]. Hydrogen atom solvation enthalpies taken from [66,67].

3.9 MOLECULAR DESCRIPTORS

The molecular descriptors are computed and the validity of koopman's theorem is verified by calculating both E_0 (energy orbital) and E_V (energy vertical) of the species. The ionization potential is calculated as the energy difference between the energy of the compound derived from electron-transfer (radical anion) and the respective neutral compound [68].

$$IP_E = E_{cation} - E_n\ ;\ IP_O = -\ E_{HOMO} \longrightarrow \quad (3.22)$$

The electron affinity is computed as the energy difference between the neutral molecule and anion molecule.

$$EA_E = E_n - E_{anion}\ ;\ EA_O = -E_{LUMO} \longrightarrow \quad (3.23)$$

Calculations the other parameters electronegativity (χ), hardness (η), Softness (S) and electrophilicity index (ω) have been calculated.

Electronegativity (χ) , describes the ability of a molecule to attract electrons towards itself in a covalent bond; and the global hardness, measure the resistance towards the deformation or polarization of the electron cloud of theatoms, ions or molecules under small perturbation of chemical reaction, through the following relation :

$$\text{Electronegativity } (\chi) = \frac{(IP + EA)}{2} \longrightarrow \quad (3.24)$$

Chemical potential (μ) = $-\frac{(IP+EA)}{2}$ ⟶ (3.24 a)

Hardness (η) = $\frac{(IP-EA)}{2}$ ⟶ (3.25)

Global softness (S) is defined as:

Softness (S) = $\frac{1}{2\eta}$ ⟶ (3.26)

Global electrophilicity index is a combined descriptor involving chemical potential and hardness which expresses propensity of a species to accept electron. This index is expressed as,

Electrophilicity index (ω) = $\frac{\mu}{2\eta}$ ⟶ (3.27)

3.10 NATURAL BOND ANALYSIS (NBO)

Natural bond orbital (NBO) analysis is a useful tool for understanding delocalization of electron density from occupied (donor) to unoccupied (acceptor) with in the molecule. The stabilization of orbital interaction is proportial to the difference energy between interacting orbitals. Therefore, the interaction having strongest stabilization takes place between effective donors and acceptors. The interaction between bonding and antibonding molecular orbitals can be quantitately expressed by second order perturbation interaction energy E(2). The stabilization energy E(2) associated with i (donor)→j (acceptor) delocalization is determined from second order perturbation as,

$$E(2) = \Delta E_{ij} = q_i \frac{F(i,j)}{(\varepsilon i, \varepsilon j)} \longrightarrow (3.28)$$

Where q_i is the donor orbitals occupancy, $\varepsilon i \; and \; \varepsilon j$ are diagonal elements (orbital energies) and F(i,j) is the off-diagonal fock matrix element [69].

3.11 LOCAL REACTIVITY PARAMETERS

Local reactivity parameters describe the relative reactivity and the selectivity of atoms in a molecule. The nucleophilic attack at a particular site of a system represents the sites with maximum values of Fukui function, (f_k^+) and/or local philicity, ω_k^+. Similarly, electrophilic

attack at a particular site of a system represents the sites with maximum values of Fukui function, f_k^- and/ or local philicity, ω_k^-.

Fukui function is one of the widely used local density functional descriptors to model chemical reactivity and site selectivity. The atom with the highest Fukui function is highly reactive compared to the other atoms in the molecule. Fukui function is defined as the derivative of the electron density ρ(r) with respect to the total number of electrons N in the system, at constant external potential v(r) acting on an electron due to all the nuclei in the system where μ is the chemical potential of the system.

$$F(r) = \left[\frac{\partial \rho(r) \partial}{N}\right] \upsilon_{(r)} = \left[\frac{\delta \mu}{\delta \vartheta(r)}\right]_N \longrightarrow (3.29)$$

The electronic chemical potential is the derivative of the total energy E with respect to the electron density. It is more convenient to represent the Fukui function values around each atomic site into a single value that characterizes the atoms in a molecule. Depending on the electron transfer, three types of the fukui function are defined:

$$f_k^+(r) = \rho_{N+1}(r) - \rho_N(r) \longrightarrow (3.30)$$

$$f_k^-(r) = \rho_N(r) - \rho_{N-1}(r) \longrightarrow (3.31)$$

$$f^0(r) = \frac{1}{2}[\rho_{N+1}(r) - \rho_{N-1}(r)] \longrightarrow (3.32)$$

however, for studying the reactivity at the atomic level, a more convenient way of calculating the fukui function is through the condensed forms of the Fukui function for an atom k in a molecule which are expressed as [70],

$f_k^+(r) = q_k(N+1) - q_k(N)$ for nucleophilic attack, $\longrightarrow$ (3.33)

$f_k^-(r) = q_k(N) - q_k(N-1)$ for electrophilic attack, $\longrightarrow$ (3.34)

$f_k^0(r) = [q_k(N+1) - q_k(N-1)]/2$ for radical attack. $\longrightarrow$ (3.35)

In these equations, q_k is the atomic charge (evaluated from the Mulliken population, electrostatic derived charge) at the K^{th} atomic site in the neutral (N), anionic (N+1) or cationic (N-1) Chemical species.

CHAPTER – IV

RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

Nigella sativa seed which obtains extraction has been shown to protect cell membrane from oxidative damage. Among them, TQ has been identified as the major active compound of *Nigella sativa* seed. TQ is used to beneficial effect on the human body. The antioxidant activity of TQ is the hydroxyl radical scavenging activity.

In the present study experimental analysis of antioxidant activity is investigated. DPPH free radical scavenging activity is discussed to confirm their antioxidant activity. The various compounds which are present in the *Nigella sativa* seed extracts are characterized in terms of various functional groups by using IR spectroscopy. UV – visible spectroscopy are also used to confirm to presence of TQ.

In theoretical study, structure of TQ is optimized and their structural characteristics have been determined by using Density Functional Theory (B3LYP) method with 6-311G++ (2d,2p) as basis set using Gaussian 09. The optimized structure of TQ is same level of theory used to geometrical parameters such as bong length and bond angle is calculated. The radical site of TQ is obtained by abstracting hydrogen from hydroxyl group. The antioxidant mechanism of TQ is computed by using HAT through thermodynamical mechanism to measure bond dissociation enthalpy (BDE) at different sites of H-abstraction at gas and solvent phases. Global descriptors such as the quantum electron affinity, ionization potential, electronegativity softness, hardness and softness , E_{HOMO} (Highest Occupied Molecular Orbital), E_{LUMO} (Lowest Unoccupied Molecular Orbital), the energy difference between the E_{HOMO} and E_{LUMO} were calculated by using same method. Spin density distribution analysis for radical species for TQ have been computed and interpreted. The formation of intramolecular interactions sites are explained by using natural bond analysis (NBO) analysis. The fukui function analysis explains the charge transfer mechanism. The theoretically simulated FT-IR computed at same level of theory and compared with the experimental data. The UV-vis spectra was computed by the time dependent TD-DFT/B3LYP method with polarizable continuum model (PCM) using 6-311G ++(2d,2p) basis set and methanol as the solvent.

EXPERIMENTAL STUDY

4.2 ANTIOXIDANT ACTIVITY

4.2.1 DPPH RADICAL SCAVENGING ACTIVITY

Antioxidant moleucules as phenols, sterols, tocopherols and triterpenoids can help cells to neutralize these free radicals. The present study involved various methods to evaluate antioxidant activity of black cumin seed extract, namely DPPH radical scavenging activity, reducing and chelating power assays. The result of Antioxidant activity showed in table 4.1.

Table 4.1 Antioxidants activities of *Nigella sativa* seed Extraction

Concentration of extraction (µl)	% Scavenging of DPPH
20	27.27 ± 0.18
40	58.76 ± 2.38
60	64.92 ± 0.24
80	70.11 ± 0.56
100	86.40 ± 0.07

Antioxidant activity was assessed using DPPH radical scavenging assay. It is a free radical and accepts electron to become stable molecule. The spectrophotometric method was determined the scavenging activity of DPPH radical. NSE extraction was incubated at different concentration with DPPH. The colour change from violet to yellow was observed. It confirms that formation of complex between the compound present in the NSE extract with DPPH. This formation is used to quenching the free radicals. Fig 4.1 demonstrates the graph plotted against % scavenging vs different concentrations. The value of the inhibitory concentration (IC_{50}), defined as the amount of an antioxidant necessary to decreases the initial free radical concentration by 40%, was calculated. The value of IC_{50} is calculated for NSE was 58.76 µl [71]. This assay confirms the free radical quenching ability of NSE extraction.

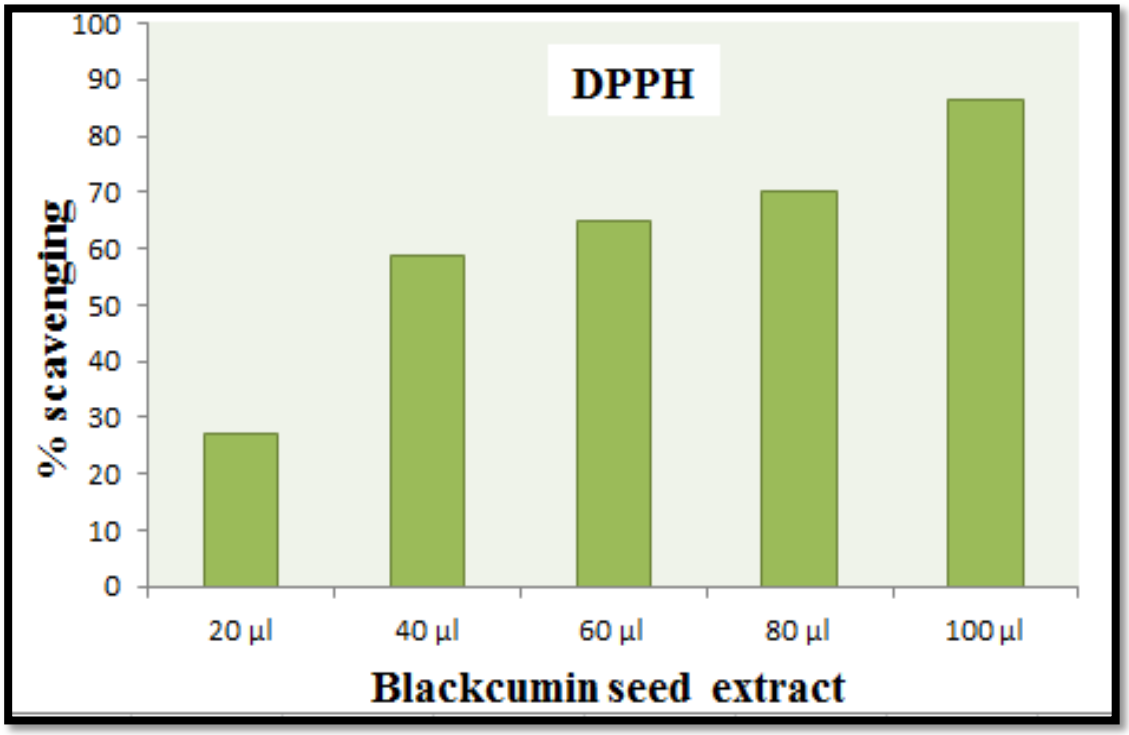


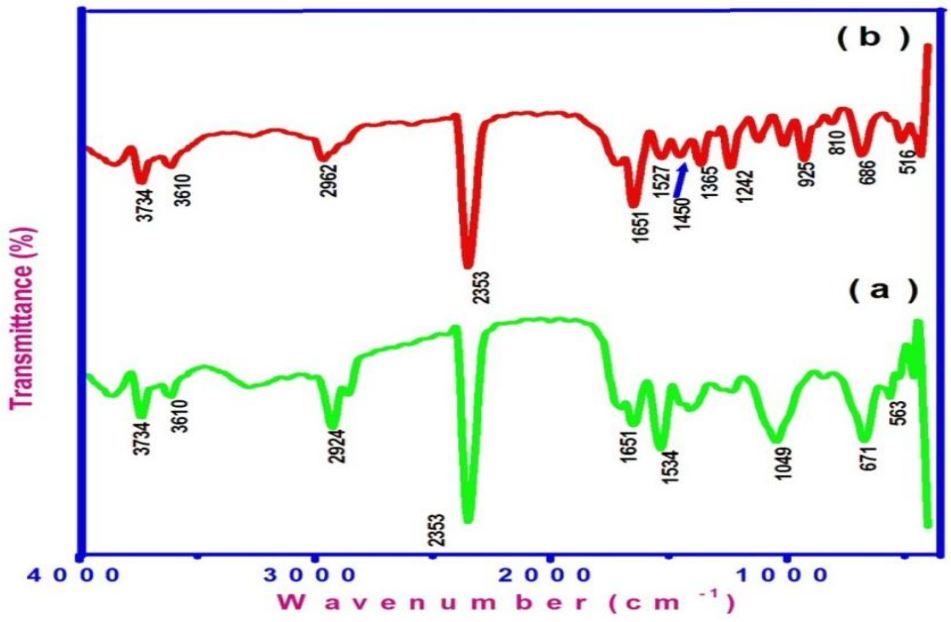
Fig 4.1 Illustrates IC_{50} value of NSE Extract

4.3 SPECTROSCOPIC ANALYSIS

4.3.1 FT-IR ANALYSIS

FT-IR spectra of both experimental and theoretical analysis plotted as wavenumber (cm-1) against transmittance (%) is represented in the Fig 4.2 ranging from 4000-400 cm-1.

(i)



(ii)

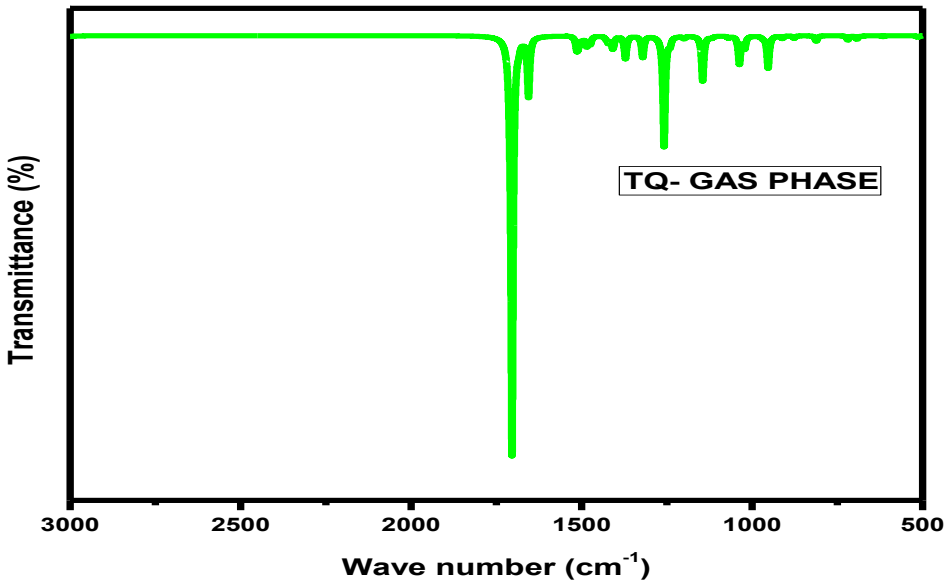


Fig 4.2 (i) FT- IR spectrum of (a) NSE extract (b) Standard TQ (ii) Theoretical IR spectrum of TQ in gas phase

Theoretical IR spectrum of TQ in gas phase is shown in fig 4.2 (ii). The interpretation of the IR spectrum involves the correlation of the vibrational bands with the functional groups present in the compounds of sample. In this way, the biomolecules are analyzed in NSE . In gas phase the DFT calculations are reported the normal frequencies of vibration followed by the B3LYP/6311G++ (2d,2p) with the basis set using same level of theory. The basis set were used used for the vibrational analysis to facilitate the comparison of the present results with those obtained before the related molecule. The vibrational frequencies are compared to the both experimental and theoretical analysis.

4.3.1.1 The C-H modes

The medium bands in the IR spectrum appear, at 3734 cm^{-1} the bands has a weak in both experimental and theoretical TQ compound in gas phase respectively. These features assigned to the C-H anti-symmetric stretching modes respectively. These presence of functional group of phenol.

The CH_3 asymmetric bending gives the band of 1450 cm^{-1}. In gas phase the spectrum are assigned to the C-H out of plane and C-H in plane rocking modes respectively [73].

4.3.1.2 The C-C modes

The band located at 1242 cm^{-1} in the gas phase infrared spectra are assigned to antisymmetric stretching mode C-C stretching mode, the bands is not far from that assigned to the C-C stretching in disopropyl-p-benzoquinone and dimethyl-p-benzoquinone , located at 1238 cm^{-1} and 1245 cm^{-1} , respectively.

The C(5)-C(12)H_3 and C2-C8 (ring-isopropyl) stretching modes are associated with the bands located at 1049 cm^{-1} [74].

4.3.2.2 The C=C and C=O modes

The band region is 1712 cm^{-1} is assigned to the expected C=O stretching mode. The band located at 1651 cm^{-1} the band occur in ester C=O stretching mode which is supported by reported for Thymoquinone [72].

4.3.2.3 The torsional and rocking methyl modes

The calculations show that the methyl rocking modes appear at 1100-900 cm^{-1} region. The bands are located between 1049 cm^{-1} and 925 cm^{-1} can be assigned to the CH_3 rocking modes. In fact, this is a complex spectral rgion in which the mentioned rocking modes overlap with the C-C stretching mode [74].

4.3.2.4 The methyl modes

The bands 2962 cm^{-1} and 2924 cm^{-1} corresponds to the CH_3 stretching mode of aliphatic group. They are assigned to the antisymmetric modes, predicted at almost the same frequency. The corresponding bands of dimethyl-p-benzoquinone.

The band occur at 1450 cm^{-1} should be assigned to the CH_3 antisymmetric bending modes. The band appears at gas phase in infrared spectrum are assigned to the anti-symmetric deformation $C-H_3$. These assigned in Table 4.2.

The band attribute to the 1365 cm^{-1} for C=O stretching mode. In gas phase infrared spectra was assigned to the CH_3 symmetric stretching where as the bands which appear to the C-H stretching [73].

Table 4.2 Observed frequencies for NSE extract , Standard TQ in methanol and TQ in gas phase

Wavenumber (cm^{-1})	**Peak assignments**
3734	Presence of functional group of phenol
3610	N-H stretching vibrations and the group of primary amines
2962 and 2924	C-H stretching vibrations and the group of aliphatic
2353	Attributed to the enhanced stretching mode.
1651	Ester C=O stretching vibrations which is supported by the value reported for Thymoquinone
1534 and 1527	C=C stretching vibrations in carboxylic

	group
1450	CH_3 asymmetric bending
1365	symmetric bending of tertiary carbon in the isopropyl group
1049 and 1242	attributed to the ester C-O, C-N, C-C stretching vibrations
925	the trans –CH-CH-
810	–C-C=C mode
563	C-X stretching vibrations and the group of alkyl halides

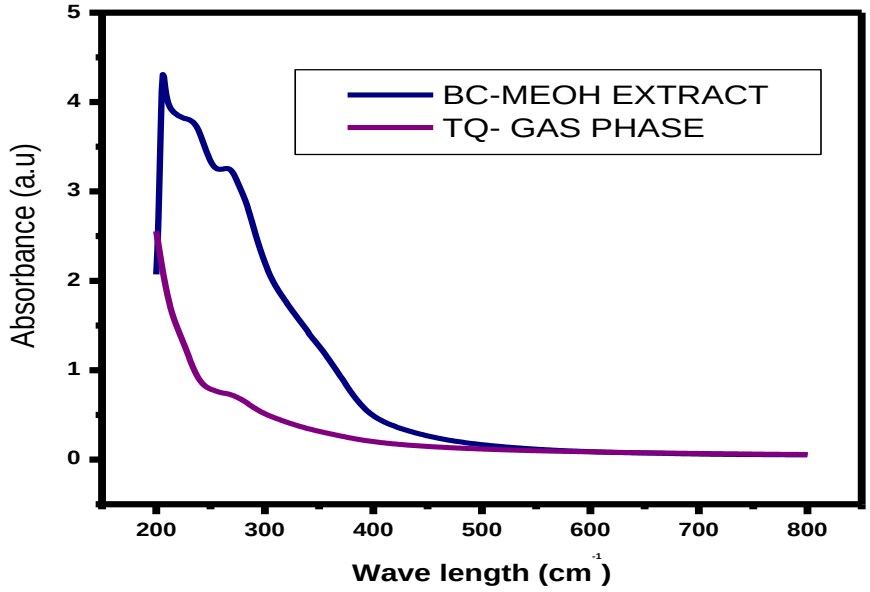
The theoretical computed vibrational assignments shows good agreement with recorded spectrum.

4.3.2 UV-VISIBLE SPECTRA OF TQ

UV visible spectra of *Nigella sativa* seed extract and standard TQ in methanol is represented in Fig. 4.4 (a). The absorption spectra of TQ in gas phase and methanol medium is simulated using TD/DFT/B3LYP 6311G++ (2d,2p) level of theory shown in fig 4.4 (b). The observed region is shown in Table 4.3.

In experimental analysis, UV spectrum of BCS extract was observed in the region is 267 nm (λ_{max}) and TQ in methanol was observed at 265 nm (λ_{max}) is assigned to π- π^* transition. In theoretical study, UV absorbed at the region of λ_{max} at 272 nm and 265 nm in the region of gas phase and methanol respectively. However, no absorption was observed in the visible region.

(a)



(b)

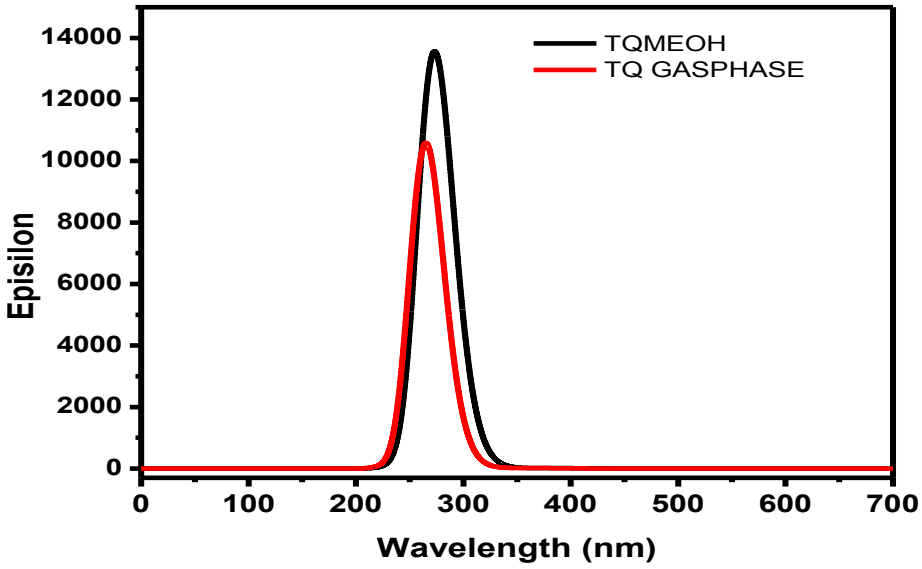


Fig 4.3 (a) Experimental UV spectra of NSE extract and TQ in methanol (b) Theoretical UV spectrum of TQ in gas and MeOH Phase

Table 4.3 Experimental and Theoretical analysis of UV- visible spectrum (λ_{max})

Experimental analysis of UV spectrum		Theoretical analysis of UV spectrum	
NSE MeOH extract (λ_{max}) nm	TQ in MeOH extract (λ_{max}) nm	TQ in MeOH (λ_{max}) nm	TQ in Gasphase (λ_{max}) nm
267	265	272	265

THEORETICAL STUDY

4.4 OPTIMIZED STRUCTURAL PROPERTIES

The structure of TQ is optimized using DFT method at B3LYP-6311G++ (2d,2p) level of theory .The existence of the ground state of the compound have been verified by absence of imaginary frequencies. The atom numbering and optimized structure of TQ is indicated in Fig 4.4.

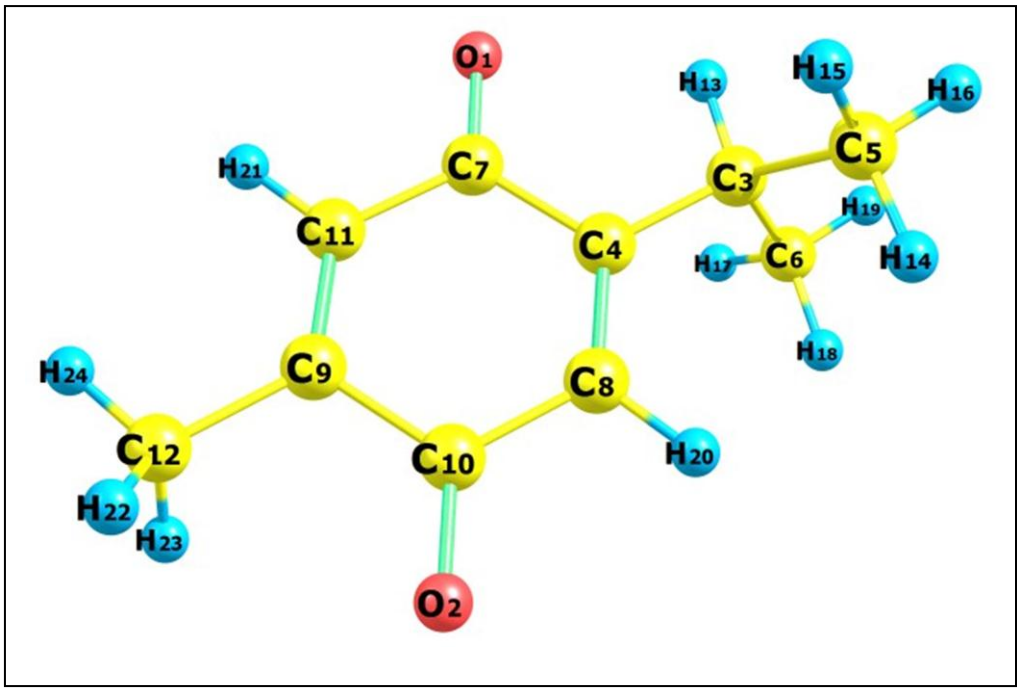


Fig 4.4 Optimized structure of TQ using DFT at B3LYP-6311G++(2d,2p) level of theory

Single point energy calculated using same level of theory is -538.8918 kcal/mol. Optimization of cationic and anionic calculated using same level of theory is -538.5546 kcal/mol and -538.9642 kcal/mol respectively. The complete optimized geometrical parameters such as bond length and bond angle are calculated for the neutral TQ compound and given in the Table 4.4.

TABLE 4.4 Bond length (Å) of TQ at B3LYP/ 6311G++ (2d,2p) level of theory

Atoms	Bond length (Å)
$C_5 - H_{15}$	1.08999
$C_5 - H_{16}$	1.09048
$C_5 - H_{14}$	1.09116
$C_5 - C_3$	1.53820
$C_5 - H_{13}$	1.08909
$C_3 - C_6$	1.53819
$C_6 - H_{19}$	1.09047
$C_6 - H_{18}$	1.09116
$C_6 - H_{17}$	1.08999
$C_3 - C_4$	1.51395
$C_4 - C_8$	1.34137
$C_4 - H_{20}$	1.08263
$C_8 - C_{10}$	1.47486
$C_{10} - O_2$	1.22126
$C_{10} - C_9$	1.49608
$C_9 - C_{11}$	1.34044
$C_{11} - H_{21}$	1.08249
$C_{11} - C_7$	1.47641
$C_7 - O_1$	1.22113
$C_7 - C_4$	1.50071
$C_9 - C_{12}$	1.49520
$C_{12} - H_{23}$	1.09074
$C_{12} - H_{22}$	1.09074
$C_{12} - H_{24}$	1.08774

TABLE 4.5 Bond angle (deg) of TQ at B3LYP/ 6311G++ (2d,2p) level of theory

Parameters	Bond Angle (deg)
H_{16}–C_5 -H_{14}	107.854
H_{16}–C_5 -H_{15}	107.961
H_{14}–C_5 -H_{15}	107.985
C_6–C_3 -C_5	111.368
C_6–C_3 –H_{13}	108.044
C_4–C_8–H_{20}	122.093
C_4–C_8–H_{20}	123.412
C_8–C_{10}–O_2	120.768
C_8–C_{10} –C_9	118.518
C_{10}–C_9–C_{11}	118.358
C_7–C_{11}–H_{21}	114.917
C_{11}–C_7–O_1	120.137
C_7–C_4 –C_3	118.072
C_9–C_{12}–H_{24}	111.106
C_9–C_{12}–H_{23}	110.535
H_{23}–C_{12} –H_2	106.161

4.5 BOND DISSOCIATION ENTHALPY (BDE) – HAT MECHANISM

The BDE of C-H group is a useful descriptor in structure activity analysis of antioxidants. The radical scavenging path of TQ antioxidant takes place through the single-step hydrogen atom transfer (HAT) to form radical ($ArO^{\cdot}$). In gas phase, optimization of TQ followed by optimization of radical species has been implemented after removal of H-atom from C_8, C_9 and C_{10} with out any geometrical constraints. 8-CH radical species is named after he removal of H-atom at C_8, other radical species are generated and named in same manner [75]. Numbering of atoms TQ to compute BDE at different radical site is shown in fig 4.5. The BDE value of C-H groups at various position of TQ is presented in Table 4.6.

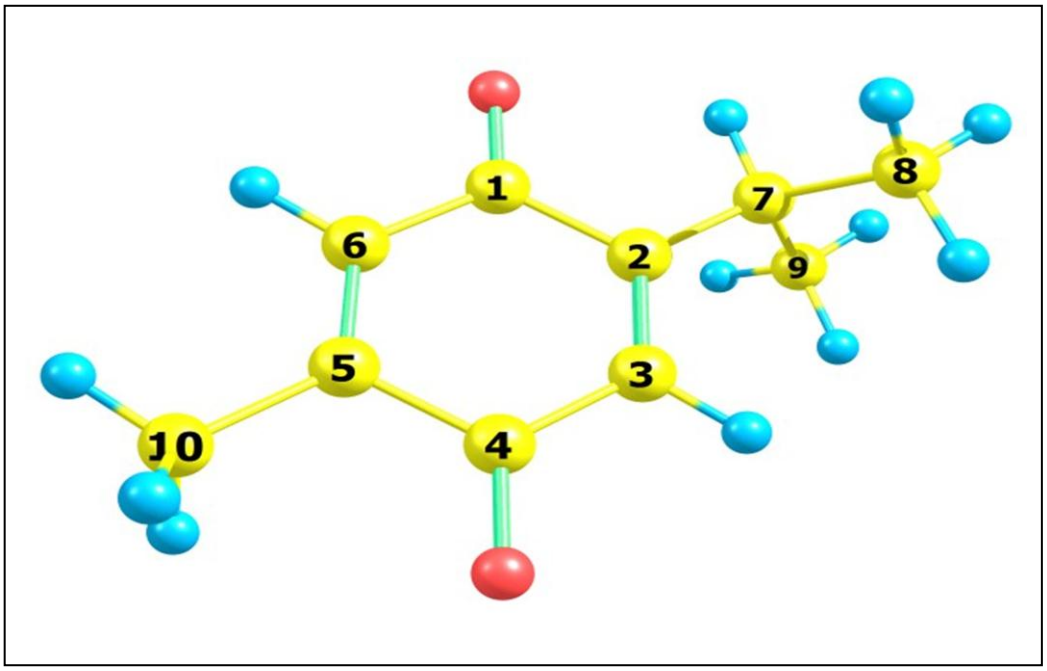


Fig 4.5 Numbering of atoms of TQ for BDE analysis

Table: 4.6 Bond dissociation enthalpy (BDE) values (kcal/mol) of TQ in the gas phase and solvents

Position	Gas phase	Benzene	Methanol	Water
8-CH	98.9036	100.5144	100.1967	98.0514
9-CH	98.9042	100.5138	100.2603	98.1217
10-CH	82.4359	83.7881	83.1544	81.0748

The BDE values of TQ at the site of 10-CH, 9-CH and 8-CH are 98.90 kcal/mol, 98.9042 kcal/mol, 82.4359 kcal/mol respectively. From the calculations, BDE value of C-H group at 10-CH found to be lower than other sites of 8-CH and 9-CH. This is because presence of 5-methyl which stabilizes the radical formed by H-atom abstraction. At 8-CH and 9-CH , BDE value found to be higher than 5-methyl site (10-CH). The higher BDE value at 8-CH and 9-CH is due to 2-isopropyl and delocalization of electrons over entire molecules, which may result in an unfavourable site for radical formation.

The BDE are computed in solvent phases with increasing polarity such as benzene, methanol and water to study the existence of solute solvent interactions which may alter the chemical reactivity of TQ. The order reactivity of C-H group in solvent phases slightly difference is gas phase in all the radicals. The order of reactivity of C-H groups in solvent phase is same as that of gas phase. However, there is slight difference existing between the BDE values calculated in gas and benzene phases, 1.6108 kcal/mol for 8-CH, 1.6096 kcal/mol for 9-CH and 1.3522 kcal/mol for 10-CH respectively. The difference in BDE values calculated in methanol and gas phase are 1.2931 kcal/mol for 8-CH, 1.3561 kcal/mol for 9-CH and 0.7185 kcal/mol in 10-CH. In methanol and water mediums, the higher BDE value observed 8-CH and 9-CH. This observed difference in gas phase and solvent phase BDEs is the resultant of solute-solvent interactions. The lower BDE value at all phases such as methanol (83.1544 kcal/mol) ,benzene (83.7881 kcal/mol) gas phase (82.4359 kcal/mol) and water (81.0748 kcal/mol) are observed at the site of 10-CH. The lower BDE value at 10-CH radical site indicates that H-atom transfer is much easier than other radical site and had high antioxidant activity at all phases.

4.6 MOLECULAR DESCRIPTORS

The value of molecular descriptors are tabulated in Table 4.7 for each variable is obtained both from the total energy denoted with subscript V and from the orbital energies through koopman's theorem which is represented by the subscript O. The first one is based on the difference of total electronic energies when an electron is added or removed with references to the neutral compound and is called energy vertical (Ev).

The antioxidant activity of TQ is characterized by analyzing global molecular descriptors namely Ionization potential (IP), Electron affinity (EA), Chemical hardness (η), electronegativity (χ), softness (S) and electrophilic index (ω). The above descriptors are computed and displayed in table 4.7. Each component is obtained by total energies: E_V by energy vertical method and Eo by koopman's theorem at same level of theory [76].

Table 4.7 Molecular descriptors of TQ calculated from E_v and E_o methods at B3LYP/6-311++(2d,2p) level of theory

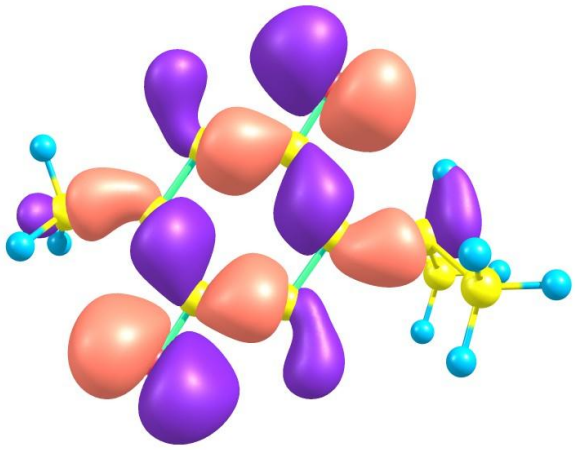
Molecular descriptors	E_o in eV	E_v in eV
Ionization Potential (IP)	7.4239	9.1763
Electron affinity (EA)	3.5508	1.9709
Hardness (η)	1.93655	3.6027
Softness (S)	0.2582	0.1388
Electronegativity (χ)	5.4874	5.5736
Electrophilic Index (ω)	7.7744	4.3113

The electronegativity is the tendency to attract electrons towards it while the chemical potential is the tendency of electrons to flow from region of higher to lower chemical potential. Hardness shows the resistance of the molecule to use electrons and its inverse gives softness. The hardness is an important stability index related to the HOMO-LUMO energy gap of the molecule. The large value for I_P by both vertical method and orbital method shows that it is easy to remove electrons from TQ. Electron affinity (EA) is small, it is difficult to gain electrons. The properties calculated clearly confirm that TQ act as electron donor which enhances its antioxidant property.

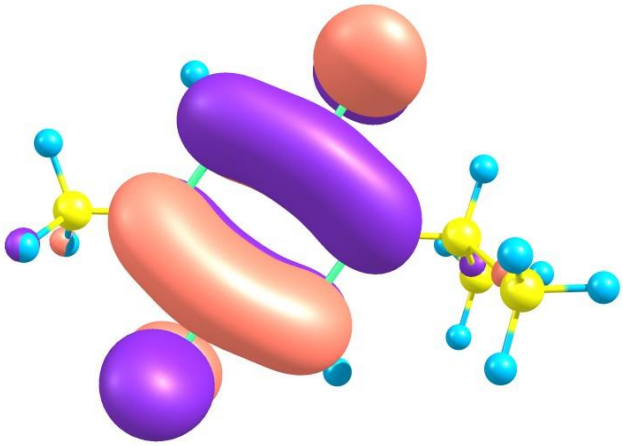
4.7 FRONTIER MOLECULAR ORBITALS (FMO)

It is important that electron distributions in the FMO's play a crucial role in a wide range of chemical reactions determining the reactivity of TQ. As the radical scavenging activity of TQ is governed by π electrons interations, a group of atoms associated with π ring for neutal and radical species as shown in fig 4.7. Analyzing FMO compositions, it is observed that charge delocalization is higher in 5-methyl site of neutral and 10-CH radical species. A decrease in charge delocalization of 8-CH and 9-CH are observed at2-isopropyl which favours formation of π- type electrons and hence electron donating capacity is difficult at this site. Further HOMO structures of 8-CH and 9-CH radical species reveal poor charge density concentration than 10-CH radical. This is in good agreement with the computed results of BDE value. It is interesting

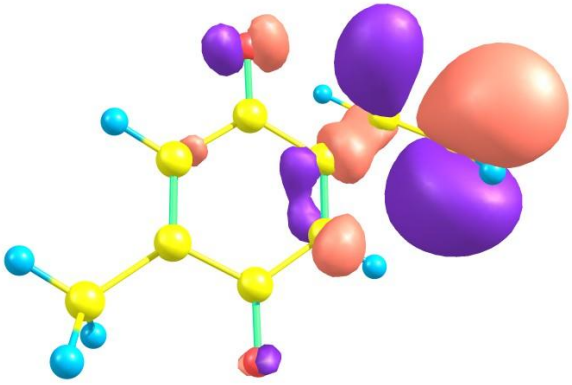
to observe that the distribution of charge density is more on 10-CH which denotes the formation of a stable radical center. It has been observed from the LUMO contours of neutral and radical species of TQ, the charge density is distributed on phenyl ring and does not contribute much towards the analysis of radical scavenging behavior. Higher HOMO eigen value, higher is the antioxidant activity. Hence, TQ exhibit good antioxidant activity.



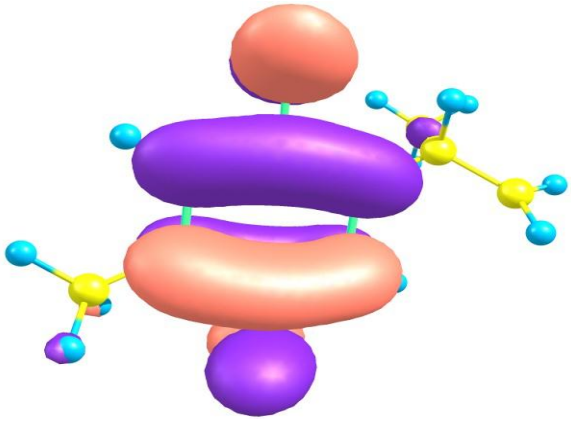
TQ- HOMO



TQ- LUMO



8-CH- HOMO



8-CH-LUMO

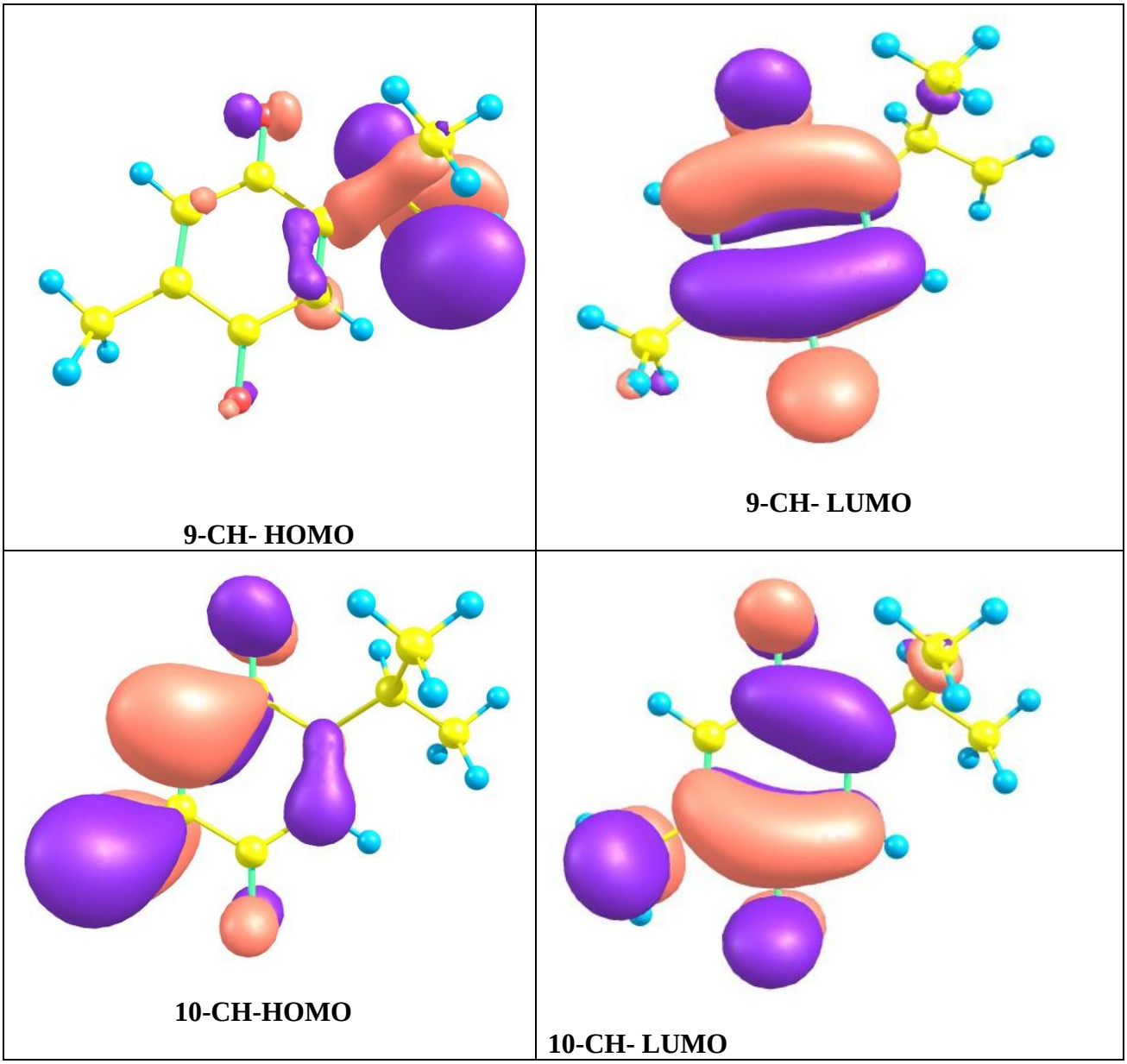


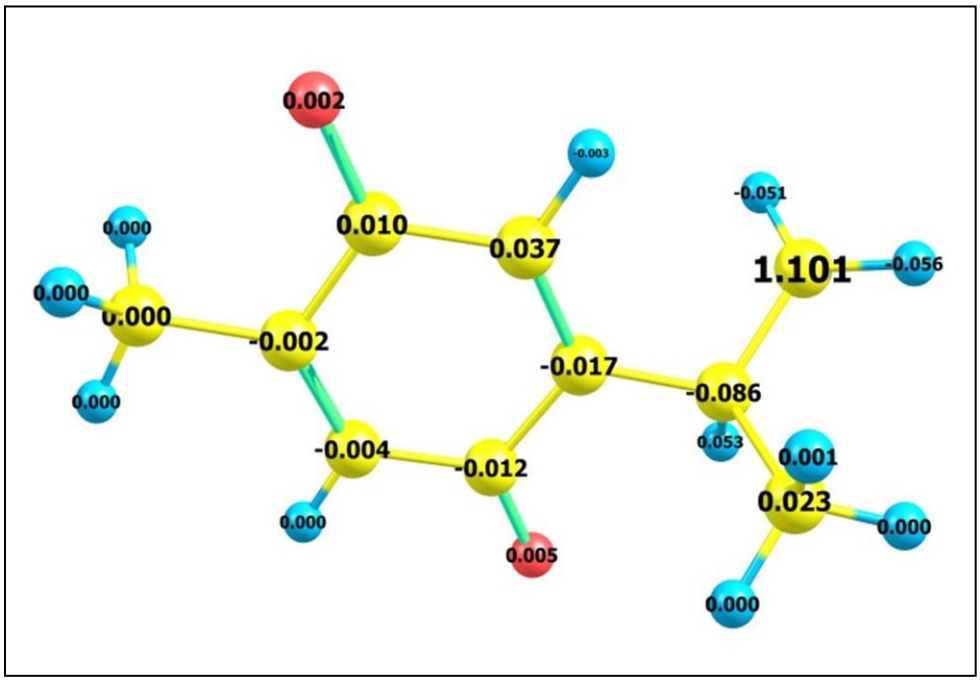
Fig 4.6 FMO distribution of TQ for neutral and radical species(8-CH,9-CH &10-CH)

Table 4.8 Frontier Orbital Energies of TQ

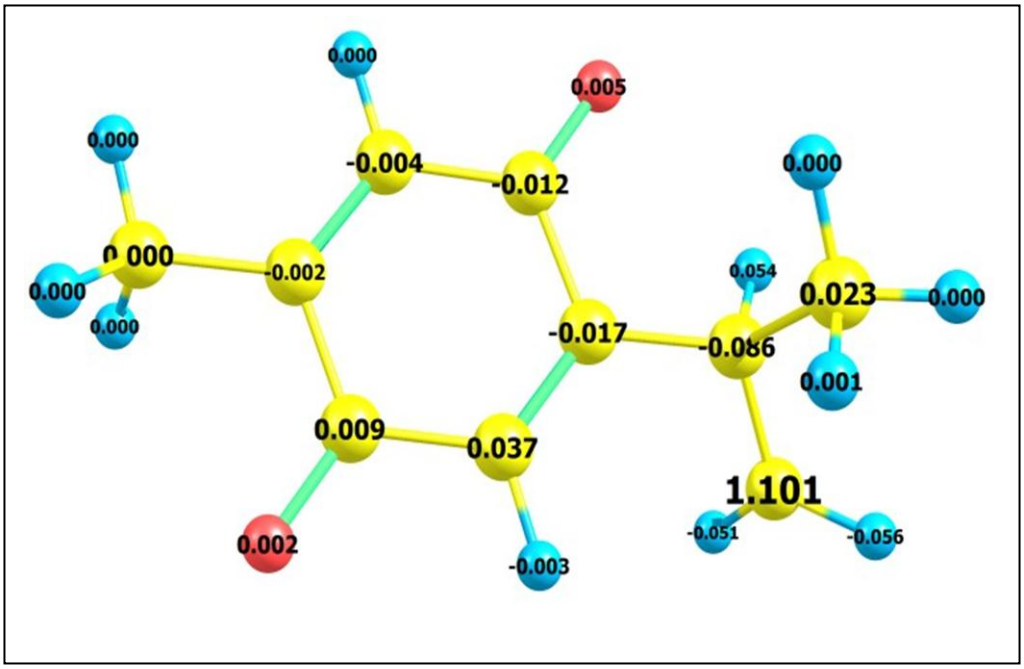
TQ compound	HOMO energy (eV)	LUMO energy (eV)	Energy gap (eV)
Neutral	0.2728	0.1305	0.1423

4.8 SPIN DENSITY DISTRIBUTION

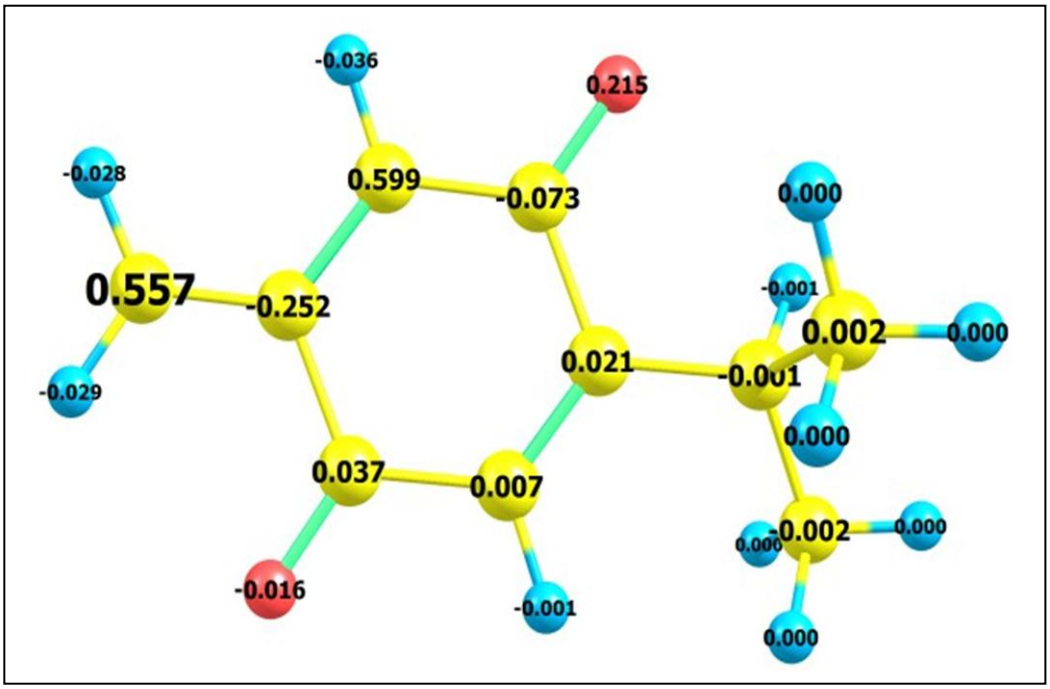
It is found that spin density at 8-CH, 9-CH and 10-CH is 1.101, 1.101 and 0.557 respectively. It is observed that 8-CH and 9-CH possess higher charge delocalization than radical 10-CH. Hence, more delocalized the spin density in the radical 10-CH, so it can easily donates H-atom and thus lower the BDE value. The atomic spin density distributions of the radical species of TQ are shown in Fig 4.8. The spin population appears to be slightly more delocalized for radicals 10-CH than radical site 8-CH and 9-CH. Thus delocalization with smaller spin density which indicates higher activity in scavenging free radicals.



TQ 8-CH Radical



TQ 9-CH Radical



TQ 10-CH Radical

Fig 4.7 Spin density distribution of TQ radical species

4.9 NATURAL BOND ANALYSIS (NBO)

NBO analysis gives the information about bonding and charge transfer interactions among bonds. It also gives information about delocalization of electrons between occupied lewis type (bond or lone pair) and unoccupied non-lewis type (anti-bond or Ryd bery). The obtained E(2) of each interaction measures intramolecular delocalization. Larger the value E(2), donating capacity from donors to acceptor is greater.

Table 4.9 Second-order perturbation theory analysis of the Fock matrix for TQ calculated by the NBO method using at B3LYP/6-311 ++G (2d, 2p) level

Donar NBO(i)	Acceptance NBO (j)	E(2) kcal/mol
σ (C 3 - C 6)	σ* (C 5 - H 15)	1.53
σ (C 7 - C 11)	σ* (C 3 - C 4)	2.04
σ (C 9 - C 12)	σ* (C 8 - C 10)	2.05
σ (C 5 - H 14)	σ* (C 3 - H 13)	2.3
σ (C 8 - C 10)	σ* (C 9 - C 12)	2.34
σ (C 3 - H 13)	σ* (C 5 - H 14)	2.95
σ (C 3 - H 13)	σ* (C 6 - H 18)	2.95
σ (C 5 - H 15)	σ* (C 3 - C 6)	3.08
σ (C 6 - H 17)	σ* (C 3 - C 5)	3.08
σ (C 9 - C 12)	σ * (C 9 - C 11)	3.09
σ (C 5 - H 16)	σ* (C 3 - C 4)	3.13
σ (C 6 - H 19)	σ* (C 3 - C 4)	3.13
σ (C 9 - C 12)	σ* (C 7 - C 11)	3.26
σ (C 3 - C 4)	σ* (C 8 - C 10)	3.28
σ (C 9 - C 10)	σ* (C 11 - H 21)	3.89
σ (C 8 - C 10)	σ* (C 3 - C 4)	4
σ (C 4 - C 7)	σ* (C 8 - H 20)	4.02
σ (C 7 - C 11)	σ* (C 9 - C 12)	4.5
σ (C 3 - H 13)	σ * (C 4 - C 8)	4.97
σ (C 11 - H 21)	σ* (C 9 - C 10)	5.39

π (O 1 - C 7	π* (C 9- C 11)	6.22
π (O 2 - C 10	π* (C 4- C 8)	6.28
π (O 1 - C 7)	π* (C 4 - C 8)	632
π (O 2 - C 10)	π* (C 9 - C 11)	6.36
π (C 9 - C 11)	π* (O 2 - C 10)	17.96
π* (C 4 - C 8)	π* (O 1 - C 7)	18.9
π* (C 4 - C 8)	π*(O 2 - C 10)	20.17
π (C 9 - C 11)	π* (O 1 - C 7)	20.41
π (O 2 - C 10)	π*(C 4 - C 8)	22.04
π (O 1 - C 7)	π* (C 4 - C 8)	22.72
π (O 1 - C 7)	π* (C 9 - C 11)	24.67
π (O 2 - C 10)	π* (C 9 - C 11)	25.89
LP(2) (O1)	π* (C 4 - C 7)	19.40
LP(2) (O 1)	σ* (C 7 - C 11)	18.45
LP(2) (O 2)	σ* (C 8 - C 10)	18.6
LP(2) (O 2)	σ*(C 9 - C 10)	19.63

The second order perturbation theory analysis of Fock matrix provides more intensive intramolecular hyperconjugate interactions between bonding C-C, C-H, C=O and antibonding C-C, C-H, C=O. The result reveals that charge transfer between orbitals causes stabilization of whole molecule. From 4.9 strong delocalization with greater stabilization occurs at π (C9 - C11) → π* (O 2 - C10) (17.96 kcal/mol) , π* (C 4 - C 8) → π* (O 1 - C 7) (18.9 kcal/mol) , π* (C 4 - C 8) → π*(O 2 - C 10) (20.17 kcal/mol), π (C 9 – C 11) → π* (O 1 - C 7) (20.41 kcal/mol), π (O 2 – C 10) → π*(C 4 - C 8) (22.04 kcal/mol), π (O 1 - C 7) → π* (C 4 - C 8) (24.72 kcal/mol), π (O 1 - C 7) → π* (C 9 - C 11) (25.89 kcal/mol) and π (O 2 - C 10) → π* (C 9 – C 11) (25.89 kcal/mol) respectively.

The magnitude of charges transferring from the lone pairs of lewis basis site LP(2) (O1), LP(2) (O 2) , LP(2) (O 1), LP(2) (O 2) to anti-bonding lewis acid sites π* (C 4 - C 7) , σ* (C 7 - C 11) and σ* (C 8 - C 10) , σ*(C 9 - C 10) with stabilization energy (19.40 kcal/mol), (18.45 kcal/mol), (18.6 kcal/mol) and (19.63 kcal/mol). The strong intramolecular interactions between

oxygen lone pairs and C-C antibonding orbitals which afford strong interactions and leads to describe the structure activity relationship in antioxidant activity.

4.10 LOCAL REACTIVITY DESCRIPTORS

The value of the Fukui function for Nucleophilic, Electrophilic and Radical attack condensed Fukui functions over the atoms of TQ are reported in Table 4.10. It was observed change in number of electrons involves addition or substraction of at least one electron in frontier orbitals. Thus , calculating fukui functions help us to determine the active sites of TQ, based on electron density charges experienced by it during reaction.

Fukui functions f^+ (Nucleophilic attack), f^- (electrophilic attack) and f^o (radical attack) are calculated at same level of theory using equations. It shows that most suspeptible site to nucleophilic attacks for TQ is located on C_{11}. In the case of an electrophilic attacks the most reactive site of radical attack is on C_{12}. The results shows that the most reactive site to donate electrons of TQ compound is at C_{11} and electron donating site is observed at the site of C_6. This implies that referred site of TQ by fukui functions which elucidate antioxidant activity relationship.

Table 4.10 Fukui function(f_+, f_-, f_0) of TQ using Mulliken population analysis at B3LYP/6-311 ++G (2d, 2p) level.

ATOM	N	N+1	N-1	f_+	f_-	f_0
O-1	-0.52582	-0.69866	-0.34465	-0.17284	-0.18117	-0.177005
O-2	-0.52605	-0.69737	-0.35337	-0.17132	-0.17268	-0.172
C-3	-0.26735	-0.25796	-0.25525	0.00939	-0.0121	-0.001355
C-4	-0.00496	-0.07929	0.08415	-0.07433	-0.08911	-0.08172
C-5	-0.55348	-0.55334	-0.55045	0.00014	-0.00303	-0.001445
C-6	-0.55347	-0.55334	-0.57439	0.00013	**0.02092**	0.010525
C-7	0.49609	0.37059	0.51632	-0.1255	-0.02023	-0.072865
C-8	-0.22886	-0.2626	-0.16142	-0.03374	-0.06744	-0.05059
C-9	-0.01838	-0.08092	0.0511	-0.06254	-0.06948	-0.06601
C-10	0.4923	0.3683	0.50527	-0.124	-0.01297	-0.068485
C-11	-0.22945	-0.26144	-0.18467	-0.03199	-0.04478	-0.038385
C-12	-0.60162	-0.58271	-0.60712	**0.01891**	0.0055	**0.012205**
H-13	0.22519	0.22029	0.25703	-0.0049	-0.03184	-0.01837
H-14	0.19239	0.1855	0.21094	-0.00689	-0.01855	-0.01272

H-15	0.2041	0.19898	0.21403	-0.00512	-0.00993	-0.007525
H-16	0.20289	0.17602	0.2332	-0.02687	-0.03031	-0.02859
H-17	0.2041	0.19898	0.21593	-0.00512	-0.01183	-0.008475
H-18	0.19239	0.1855	0.21221	-0.00689	-0.01982	-0.013355
H-19	0.20289	0.17601	0.2406	-0.02688	-0.03771	-0.032295
H-20	0.21548	0.18116	0.26895	-0.03432	-0.05347	-0.043895
H-21	0.21491	0.17979	0.2645	-0.03512	-0.04959	-0.042355
H-22	0.22936	0.20286	0.25983	-0.0265	-0.03047	-0.028485
H-23	0.22937	0.20287	0.26007	-0.0265	-0.0307	-0.0286
H-24	0.20798	0.18077	0.23721	-0.02721	-0.02923	-0.02822

CHAPTER V

SUMMARY AND CONCLUSION

The *Nigella sativa* seed (NSE) is extracted using methanol solvent. NSE has an ability to control DPPH free radicals. This result shows that extract have high antioxidant property to scavenge free radicals. The functional groups present in the NSE and presence of TQ are characterized using IR spectra. The compounds present in the NSE extract are identified by using UV absorbance spectra.

The geometrical optimization structure of TQ is obtained by using DFT method with B3LYP/6311G++(2d,2p) using Gaussian software. The quantum geometrical parameters namely, bond length and bond angle were obtained using same level of theory. The thermodynamical parameter namely C-H bond dissociation enthalpy have been computed in gas and solvent phases to evaluate the mechanism involved in the process of radical scavenging.

According to HAT mechanism, the sequence of hydrogen donating ability of the TQ compound is as follows: 8C-H, 9C-H and 10C-H, in which lower BDE value observed at the site of 10C-H radical species and had high antioxidant activity with strong donating ability of hydrogen atom. On comparing the computed BDEs of TQ in gas and solvent phase (Benzene, Methanol and Water), it is found that order of reactivity of O-H groups could not be altered. Global reactivity parameters like electron affinity (EA), ionization potential (IP), electronegativity (χ), hardness (η), softness (σ) are also analyzed to characterize the antioxidant activity of TQ. E_{HOMO} (highest occupied molecular orbital energy) and, E_{LUMO} (lowest unoccupied molecular orbitals), the energy difference between (ΔE) between E_{HOMO} and E_{LUMO}, also provides the information about electron donating nature of TQ. The spin density distribution of radical species of TQ is also analyzed using charge delocalization. The results of NBO analysis reflects that the charge transfer are mainly due to C-H and C=O groups. Preferred active site of electrophilic and nucleophilic attack observed at the site of C_6 and C_{12} respectively using fukui function. The theoretical results reveals that TQ had high electron donating capacity to scavenge free radicals present in our body.

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