Theoretical Investigations on Petunidin

By

Mohanapriya S

(15PPH005)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore – 641 043

In partial fulfilment of the requirements for the Degree of

Master of Science in Physics

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CERTIFIED AS A BONAFIDE RESEARCH WORK

10.4.17
Signature of the Guide

10/4/17
Signature of the Head of the Department

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Mohanapriya S

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INTRODUCTION

CHAPTER-I

INTRODUCTION

1.1 INTRODUCTION

The plant kingdom is a treasure house of potential drugs and there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, rarely have side effects and efficient. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds.

According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. However, such plants should be investigated to better understand their properties, safety, and efficiency. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These compounds are synthesized by primary or secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with unclear function. It has been used in the human therapy, veterinary, agriculture, scientific research and countless other areas [1].

1.2 PLANT METABOLITES

Metabolites are intermediate or end products that are regarded as the readouts of developmental or physiological status of an organism. The total number of plant metabolites is estimated to exceed 200000 reflecting the extraordinary diversity of functions that natural products may serve. Plant metabolites play essential roles in growth, cellular condition and whole plant resource allocation as well as in their interactions with the environment. In addition, they provide indispensable resources for human nutrition, energy and medicine. Plant metabolites can be conceptually divided into two categories.

- Primary metabolites
- Secondary metabolites

1.2.1 PRIMARY METABOLITES

Primary metabolites such as carbohydrates, amino acids, fatty acids, and organic acids are involved in growth, respiration, photosynthesis, protein synthesis and hormone. Primary metabolites are found across all species within broad phylogenetic groups and produced using the same biochemical pathways.

1.2.2 SECONDARY METABOLITES

Secondary metabolites such as flavonoids, carotenoids, sterols, phenolic acids, alkaloids, and glucosinolates determine the colour of vegetables, protect plants against herbivores and microorganisms, attract pollinators, seed dispersing animals and act as signal molecules under stress conditions [2, 3].

1.3 POLYPHENOLS

Polyphenols are phytochemicals, this compounds found abundantly in natural plant food sources that have antioxidant properties. Polyphenols or phenolic compounds constitute one of the most numerous and widely distributed groups of substances in the plant kingdom and more than 8000 phenolic structures are currently known. Phenolic compounds structurally differ from simple molecules, such as phenolic acids, and from highly polymerized compounds, such as proanthocyanidins (tannins), which occur in plants, common in many foods (fruits, vegetables, cereal grains) and beverages (wine, beer, teas).

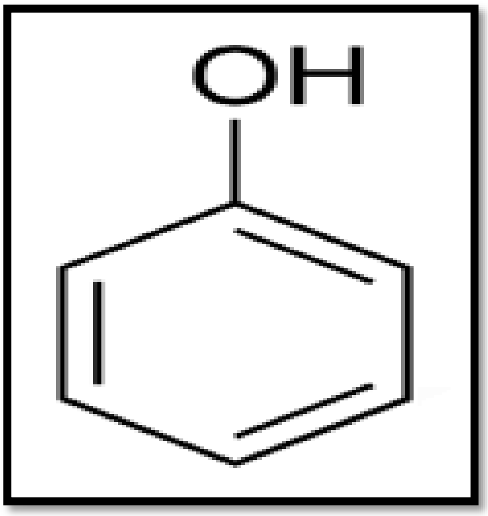


Fig.1.1 Structure of Phenolic compound

The most common phenolics in human diet are phenolic acids, flavonoids, stilbenes and tannins. Phenolic compounds have at least one aromatic ring with one or more hydroxyl groups (Fig.1.1) and may be classified as flavonoids and non-flavonoids. Polyphenols provides health benefits by several mechanisms, including the elimination of free radicals, the chelating of pro-oxidant metals, the protection and regeneration of other dietary antioxidants. The nature and content of phenolics varies dramatically among plants, which are mainly esterifies or glycosylated. Some studies evidenced an improvement of wound healing by these phytochemicals [4,5]

1.3.1 BIOACTIVITY OF NATURAL POLYPHENOLS

The reactive oxygen species (ROS) play an important role in many diseases, such as cardiovascular diseases, cancer, ageing, neurodegenerative diseases and diabetes. Natural polyphenols are the biggest group of phytochemicals, and have attracted more and more attention as potential agents for prevention and treatment of oxidative stress-related diseases. Natural polyphenols are secondary metabolites of plants, and many of them have been found in plant-based foods. Polyphenols have effects on the bitterness, astringency, colour, flavour, odour and oxidative stability in food. Natural polyphenols have been widely studied, and found to possess many important bioactivities. This is summarized current knowledge of natural polyphenols, including resources, bioactivities, bioavailability and potential toxicity. Special attention has been made to the bioactivities, such as antioxidant, cardio protective, anticancer, anti-ageing, anti-inflammation and antimicrobial properties.

1.3.2 BIOAVAILABILITY OF POLYPHENOLS

Bioavailability is usually defined as the fraction of an ingested nutrient or compound that reaches the systemic circulation and the specific sites where it can exert its biological activity. The conclusive evidence for the effectiveness of dietary polyphenols in disease prevention and human health improvement, it is useful to better define the bioavailability of polyphenols. The health effects of polyphenols in human and in animal models depend on their absorption, distribution, metabolism and elimination. The chemical structure of polyphenols determines their rate and extent of absorption as well as the nature of the metabolites present in the plasma and tissues. The most common polyphenols in human diet are not necessarily the most active within the body, either because they have a lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated [7].

1.4 FLAVONOIDS

Flavonoids are the most common and widely distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesising plant cells. Flavonoids consist of both antioxidant and anti-inflammatory properties, found in fruits, vegetables, legumes, red wine, and green tea. More than 4000 flavonoids have been identified in plants. Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and C) linked via a heterocyclic pyrane ring (B) (Fig.1.2) [8]. Flavonoids can be divided into 6 subclasses. They are

- Flavonols
- Flavones
- Flavanones
- Isoflavones
- Anthocyanidins
- Flavanols

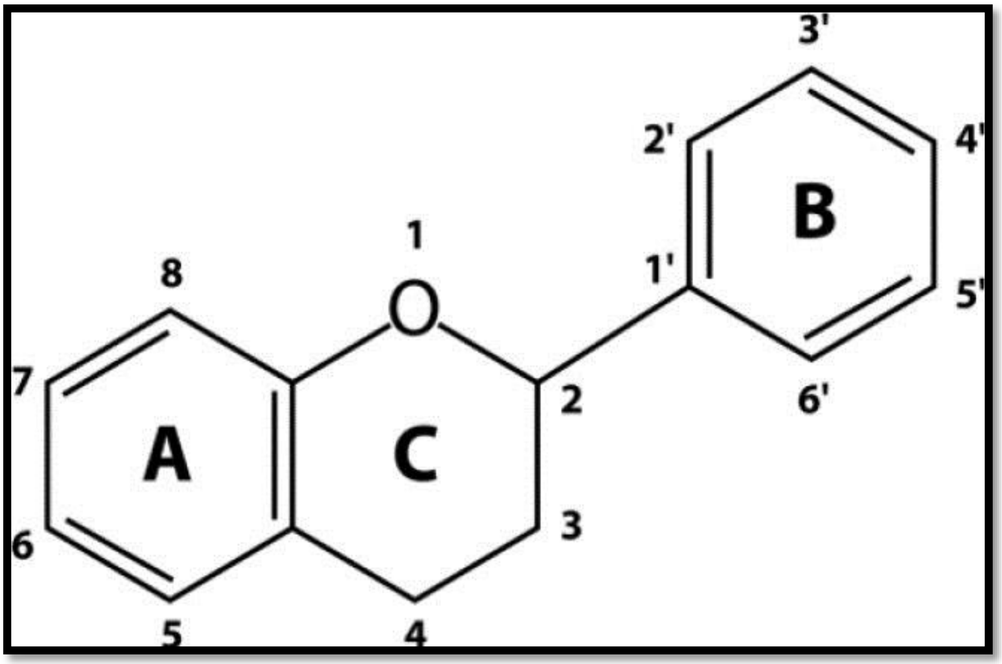


Fig.1.2 Basic Flavonoid Structure

1.4.1 THE BIOLOGICAL SIGNIFICANCE OF FLAVONOIDS

The flavonoid pigments which impart colours to flowers and fruits are associated with the attraction of agents of pollination and fruit dispersal. The significance of the pigments and colourless flavonoids found in stems, leaves and roots is less clear although some, e.g. pisatin in peas, have been shown to be phytoalexins-compounds which are produced in response to attack by pathogenic fungi and which may inhibit the growth of the invading organism. In a number of cases changes in the developmental stage or nutritional status of a plant have been found to be accompanied by changes in flavonoid content.

1.4.2 BIOLOGICAL FUNCTIONS OF FLAVONOID

The biological functions of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation. Some flavonoids provide stress protection, for example, acting as scavengers of free radicals such as reactive oxygen species (ROS), as well as chelating metals that generate ROS via the Fenton reaction. Flavonoids are also involved in the resistance to aluminum toxicity in maize. Roots of maize plants that were exposed to aluminum exuded high levels of phenolic compounds such as catechin and quercetin. Indicating their ability of chelating metals can be an in vivo mechanism to ameliorate aluminum toxicity. Auxins probably have a role in the stress response by controlling stomatal opening and by allocating resources under poor growth conditions [9].

1.5 ANTHOCYANIDIN

Anthocyanidins are a class of natural polyphenol based coloured compounds that are largely responsible for the vibrant colours in most fruits and flowers [10]. Anthocyanidins, derivatives of the 2-phenylbenzopyrylium cation are a class of natural polyphenol which are often found in cranberries and many other brilliantly coloured fruits and vegetables. Up to now, there are reports of 23 anthocyanidins [11], among which only six are the most common in vascular plants. They are

- Pelargonidin
- Cyanidin
- Delphinidin
- Malvidin
- Peonidin
- Petunidin

The colour properties and disease-preventing virtues of anthocyanidins make them particularly attractive candidates to replace artificial food colorants and the colour intensity of these compounds can be enhanced by the intermolecular co pigmentation between anthocyanidins and other colourless compounds. Antioxidative activity is an important property of anthocyanidins, many experimental studies have proved that the antioxidative capacities of pigmented vegetables and fruits highly correspond with the content of anthocyanidins and its glycoside form, i.e. anthocyanins [12]. Anthocyanidins have exhibited even more excellent antioxidative activity than vitamins C, E, and have outstanding radical-scavenging capacity. The antioxidative activities have significant contribution to the other various bioactivities, such, prevention of cancer, diabetes, and cardiovascular and neurological disease, as well as anticarcinogenic activity [13].

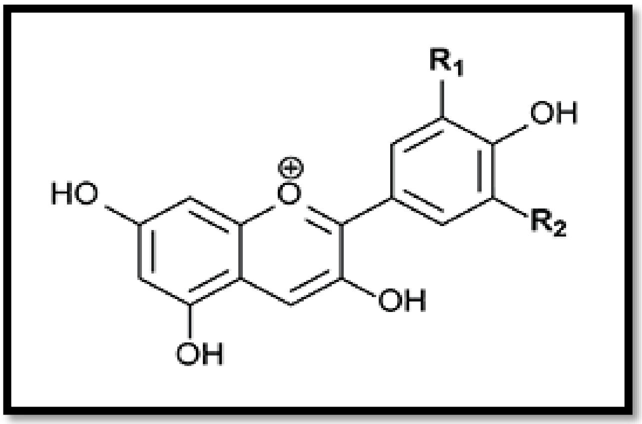
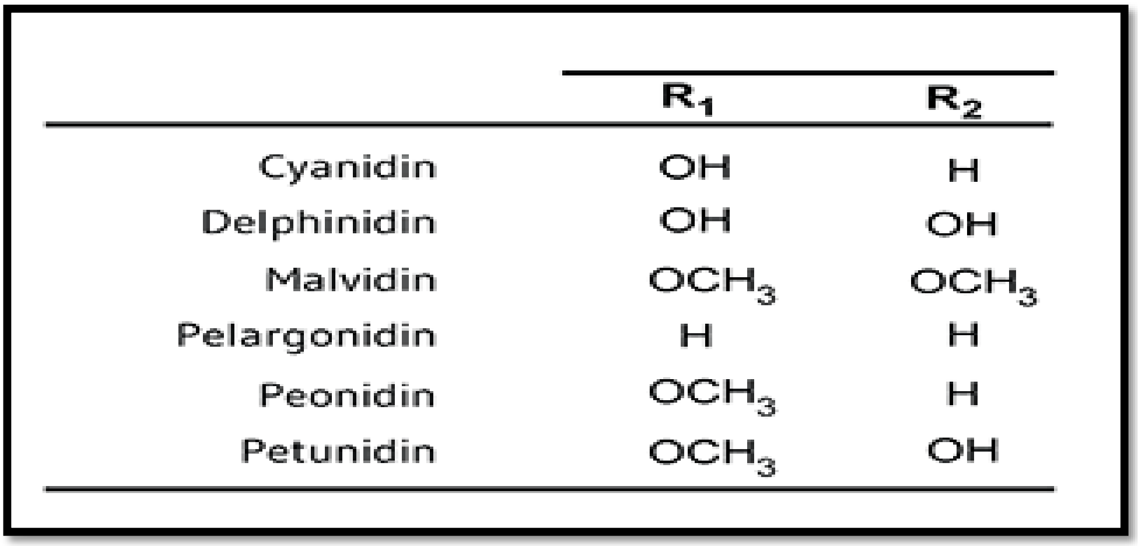


Fig.1.3 Basic structure of anthocyanidin



	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Pelargonidin	H	H
Peonidin	OCH_3	H
Petunidin	OCH_3	OH

Fig.1.4 Different types of anthocyanidins

1.5.1 THE CHEMICAL FEATURES OF ANTHOCYANIDINS

- The salt derivative of the cation flavylium is anthocyanidin.
- At the 2-position of the phenyl group different substituent's can be placed to make anthocyanidins.
- The formula for flavyliumcation is $C_{15}H_{11}O^+$
- The molar mass of the anthocyanidin is 207.24g.mol
- They have a slight astringent taste. Anthocyanins are also found in stems, flowers, leaves and roots of higher plants.

1.5.2 THE HEALTH BENEFITS OF ANTHOCYANIDINS

- The human brain is prone to oxidative damages. Peroxynitrite nitration caused by tyrosine residues present in enzymes and proteins are responsible for brain damage. Nerve receptor sites are blocked by nitrated tyrosine. Active ingredient pelargonidin in Anthocyanidin prevent the nitration of tyrosine and restrict occurrence of any neurological damage caused to the nervous system.
- Anthocyanidin can prevent atherosclerosis in large blood vessels. It can prevent the oxidation of low density lipoproteins in the blood vessels. Anthocyanidins present in bilberries are stronger antioxidants that vitamin C or even BHT (used as preservative). Anthocyanidins are also very effective in reducing stress on the blood vessels. Micro-capillary integrity is maintained by Anthocyanidins. The capillary walls are stabilized by Anthocyanidins. It is very effective in fighting "ischemia-reperfusion". Reduced oxygen supply creates oxidants that cause the white blood cells to stick to the capillary walls and increase the wall permeability which further causes capillary damage. This condition can be improved by taking fruits with high Anthocyanidin content.
- Diabetes retinopathy can cause severe vision loss or even blindness. It occurs when the body itself tries to repair damaged capillaries, but in order to do so it produces excess of proteins. Anthocyanidins prevent leaking along with abnormal protein proliferation.
- Regular consumption of fruits and vegetables rich in Anthocyanidins improves night-vision.

1.6 PETUNIDIN

Petunidin is subclass of flavonoids, known as O-Methylated anthocyanidin of the 3-hydroxy type. These are flavonoids with methoxy groups attached to the C_3 atom of the flavonoid backbone. It is a natural organic compound, a dark-red or purple water-soluble pigment found in many red berries including chokeberries, Saskatoon berries or different species of grape. A part of the pigments responsible for the petal colours in many flowers and gives the indigo rose tomatoes, the majority of their deep purple colour are due to fruits are exposed to sunlight.

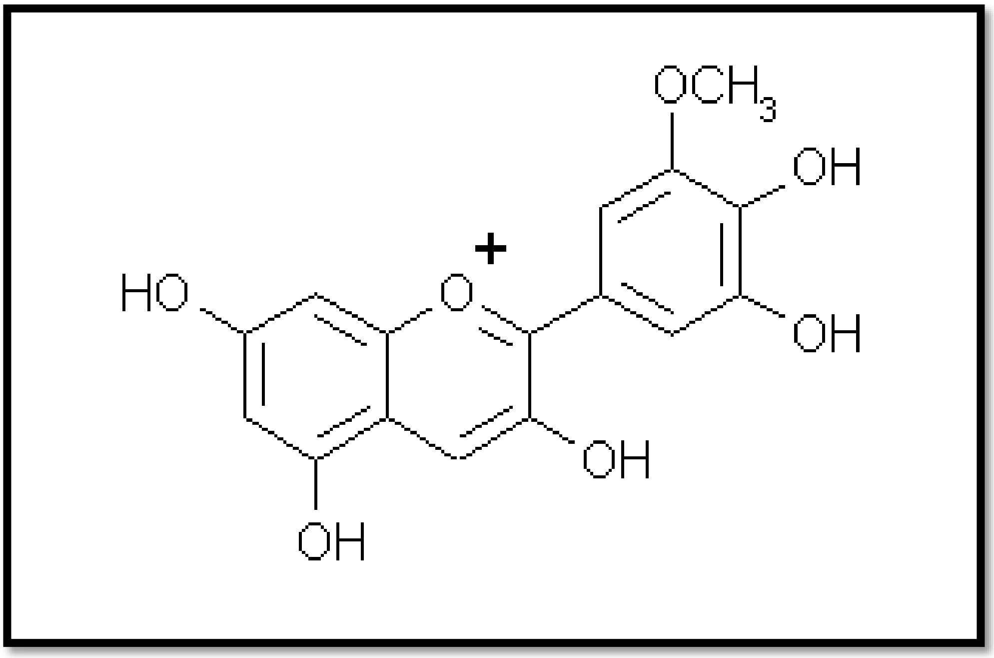


Fig.1.5 Structure of Petunidin

1.7 REACTIVE OXYGEN SPECIES

Reactive oxygen species (ROS) are produced as a normal product of plant cellular metabolism. The environmental stresses lead to excessive production of ROS causing progressive oxidative damage and ultimately cell death. The destructive activities are well-described second messengers in a variety of cellular processes, including conferment of tolerance to various environmental stresses. ROS would serve as signalling molecules or could cause oxidative damage to the tissues depend on the delicate equilibrium between ROS production, and their scavenging. Efficient scavenging of ROS produced during various environmental stresses requires the action of several no enzymatic as well as enzymatic antioxidants present in the tissues. The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS [14].

They are

- Superoxide
- Hydrogen peroxide
- Hydroxyl radical
- Hydroxyl ion
- Nitric oxide

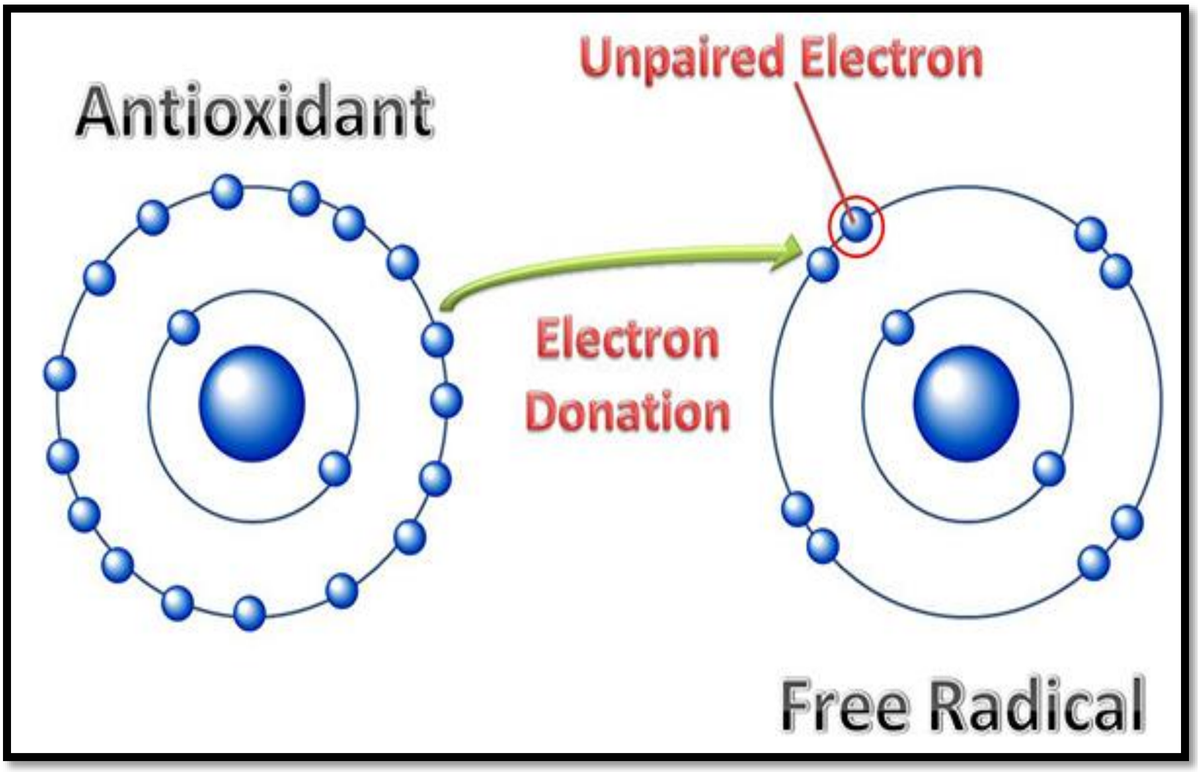


Fig.1.6 Processes of Reactive Oxygen Species

1.8 ANTIOXIDANTS

Antioxidants are the molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidant compounds have the ability to capture, deactivate and repair the damaged caused by free radicals. Antioxidant compounds from plants can minimize the generation of free radicals and alleviate diseases caused by oxidative stress. Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. There are two major groups of antioxidants in living organism: enzymatic antioxidants and non-enzymatic antioxidants. These two groups are divided into several subgroups. The enzymatic antioxidants are divided into primary and secondary enzymatic antioxidants [15]. The primary enzymatic antioxidant is composed of three important enzymes that prevent the formation of and neutralize free radicals. The secondary enzymatic antioxidant includes glutathione reductive and glucose-6-phosphate dehydrogenase. Glutathione reductase reduces to continue neutralizing more free radicals. These two enzymes support the primary enzymatic defense antioxidants and do not neutralize free radicals directly. The group of non-enzymatic antioxidants contains several subgroups, the main ones being: vitamins (A, E, C), enzyme cofactors, minerals, peptides, phenolic acids, and nitrogen compounds [16].

Even though our body is safeguarded by natural antioxidant defence, there is always a demand for antioxidants from natural sources. Antioxidants occurring naturally in leafy vegetables and seeds such as ascorbic acid, vitamin E and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases [17].

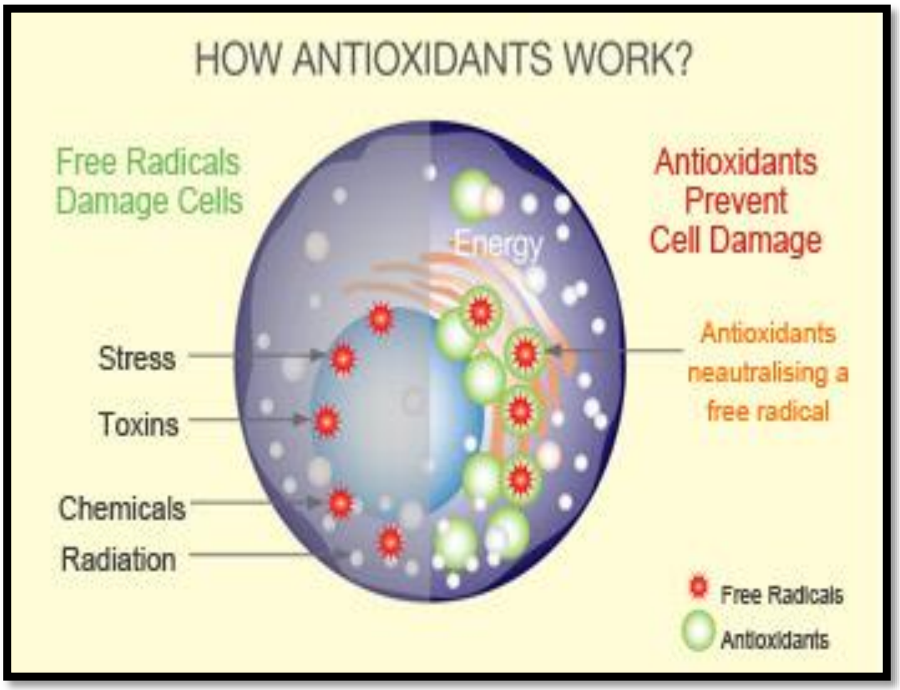


Fig.1.7 Working of Antioxidants

1.9 OBJECTIVE OF THE PRESENT STUDY

- To optimize the structure of Petunidin by using B3LYP/6-311 (d,p) level of theory.
- IR and Raman spectra were analysed by the same level of theory.
- The O-H bond dissociation enthalpies, ionisation potential and proton dissociation enthalpies of Petunidin are obtained by HAT, SET, and SEPT mechanism.
- The energy gaps of the Petunidin were calculated.

REVIEW OF LITERATURE

CHAPTER-II

REVIEW OF LITERATURE

2.1 INTRODUCTION:

Review of the related literature is an essential part of research project. The research carried on the topics related to the theoretical investigation of anthocyanidins.

2.2 LITERATURE OF REVIEW

Jeffrey N. Woodford, (2005) have studied a theoretical analysis of anthocyanidins, a class of natural plant pigments using density functional theory at the B3LYP/6-31G (d) level. It is found that these compounds are non-planar, with the 2-phenyl ring twisted relative to the benzopyrylium rings. TD-DFT calculations reveal a first excited state dominated by two orbital transitions, including the HOMO–LUMO transition. From a comparison of these molecular orbital's with those of related compounds and from the known chemistry of these compounds, it is suggested that anthocyanidins should be regarded as natural, stable carbocations [21].

Lilin Lu et al., (2014) have explored the antioxidant properties of anthocyanidins by density functional theory calculations. The three main antioxidative mechanisms which included H atom transfer (HAT), single electron transfer (SET) and sequential proton loss electron transfer (SPLET), have been investigated at B3LYP/6-311G (d,p) level of theory. The O-H bond dissociation enthalpies (BDEs), ionization potentials (IPs), electron affinities (EAs), proton affinities (PAs) and electron transfer enthalpies (ETEs) are investigated in gas phase and aqueous solution. Results show 3-OH and 4′-OH posses lower BDE as compared to other OH groups, the substituents in B-ring influence 4′-OH BDE, but exhibit negligible influence on other OH BDEs. Among all investigated anthocyanidins, pelargonidin has the highest IP and EA in gas phase, substituent in ortho position of 4′-OH leads to remarkable decrease in IP and EA. OH substituent in B-ring almost show no influence on PAs and ETEs of 3-, 5-, and 7-phenolate anions, but OCH_3 substituent influence them significantly. For PAs and ETEs of 4′-phenolate anion, two types of substituent both exhibit remarkable influence. Base on the simplest anthocyanidins, pelargonidin, molecular simplification has been performed to explore the necessary pharmacophores responsible for the antioxidative activity of anthocyanidins [22].

Rosa Guzman et al., (2009) have studied a density functional theory (DFT) using the B3LYP/6-31G (d,p) method, was performed in a attempt to understand the antioxidant properties of some anthocyanidins. This study is based on the H-atom transfer mechanism, which implicates the evaluation of the bond dissociation enthalpy (BDE) of all OH substituent in each structure. The electronic structures studied in this paper are aurantinidin, cyanidin, delphinidin, malvinidin, pelargonidin and peonidin. Analysis of the computed results suggests that the antioxidant capacity of those structures is in the following order: cyanidin > malvidin > aurantinidin > delphinidin > peonidin > pelargonidin [23].

Ken Sakata et al., (2006) were studied the structural and electronic characters of four types of hydroxyl group-substituted anthocyanidins (pelargonidin, cyanidin, delphinidin, and aurantinidin) using quantum chemical calculations. For these cationic molecules, both the planar and nonplanar structures in the electronic ground state were determined at the B3LYP/D95 level of theory. They revealed that the planar structure is slightly more stable than the non-planar structure for each molecule. For the optimized planar structures, single excitation–configuration interaction (SE–CI) based on the restricted Hartree Fock (RHF) wave function was evaluated and the electronic character in the low-excited states was discussed in terms of the MO theory. Symmetry adapted cluster (SAC)/SAC-CI calculations were also carried out to estimate the excitation energies precisely. The results showed that hydroxylation of the phenyl group causes a change in the excitation energies without taking the solvent effects into account. The results are in agreement with spectral experiments and previous MO calculations [24].

Monica Leopoldini et al., (2004) were performed to evaluate the antioxidant activity of molecules commonly present in many Mediterranean foods using density functional theory (DFT). A series of interesting systems, including tyrosol, hydroxytyrosol, gallic, and caffeic acids, belonging to the family of phenols, resveratrol of the stilbenes family, epicatechin, kaempferol, and cianidin as examples of flavonoids and a simplified model of R-tocopherol (6-hydroxy-2,2,5,7,8-pentamethylchroman (HPMC)) were studied. Conformational behaviour was examined at the B3LYP/6-311++G (3df,2p) level of theory, in the gas phase and in two solvents with different polarity (water and benzene), with the aim to compute the bond dissociation enthalpy (BDE) for the O-H bonds and the adiabatic ionization potentials (IP). BDE and IP for these systems do not follow the same trends in gas and solution phases, the major differences with respect to vacuum are found as when water computations are performed. On the basis of the computed BDE and IP values, the most active systems able to transfer an H-atom seem to be R-tocopherol followed by hydroxytyrosol, gallic acid, caffeic acid, and epicatechin. Instead, kaempferol and resveratrol appear to be the best candidates for an electron-transfer mechanism [25].

Raluca pop et al., (2012) carried out a theoretical study regarding the evaluation of the antioxidant character of three of the most wide-spread anthocyanidins (cyanidin, delphinidin and malvidin) at ab initio level. Different parameters including bond dissociation enthalpy, ionization potential, proton affinity, and electron transfer enthalpy were computed for each OH group of the compounds in order to predict their antioxidant capacity. Several molecular descriptors based on frontier molecular orbital theory (hardness, electrophilicity, frontier charge density) were also calculated, as well as the atomic charges corresponding to the O atoms of the hydroxyl groups [26].

Esteves L et al., (2010) have analyzed the radical-scavenging property attributed to anthocyanidins on the basis of Quantum chemical calculations. The three main antioxidative mechanisms which included hydrogen atom transfer (HAT), stepwise electron-transfer-proton-transfer (ET-PT), and sequential proton loss electron transfer (SPLET). They found that the activity of anthocyanidins and the mechanism through which they react are pH-dependent, because the diverse colourful forms in which anthocyanidins may exist in prototropic equilibria (cationic, neutral, and anionic) are susceptible to experience each of the mechanisms proposed. According to redox parameters calculated, HAT is always the most favoured of the generally accepted mechanisms to scavenge reactive oxygen species (ROS) by the three coloured forms. Nevertheless, only neutral and anionic forms are found to be able to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH.) radical through HAT and SPLET mechanisms from a thermodynamical point of view, whereas ET-PT is only feasible for anions. Sequential proton loss hydrogen atom transfer (SPL-HAT) is proposed as the only pathway for the reaction between anthocyanidin cations and the (DPPH.) radical. It should be viable according to our quantum mechanical calculations and even competitive with typical HAT, ET-PT, and SPLET [27].

M. Wolniak et al., (2008) have successfully carried out the density functional theory of anthocyanidins which are beneficial to human health. They attract considerable attention owing to their strong antioxidant and radical scavenging properties. However they are unstable in solution and available in small amounts only. ^{13}C CP MAS NMR spectra were recorded to characterize solid-state conformation of nine anthocyanidins such as apigenidin, pelargonidin, cyanidin, delphinidin, peonidin, malvidin robinetidin, luteolinidin and diosmetinidin chlorides. For some carbons, the solid-state chemical shifts were different from those obtained for solutions, indicating differences in conformation and intermolecular interactions. The principal elements of the ^{13}C chemical shift tensor were measured for pelargonidin, cyanidin, delphinidin and malvidin chlorides using PASS-2D NMR technique. DFT GIAO calculations of shielding constants were performed for apigenidin and several geometric isomers of pelargonidin [28].

Y. El Kouari et al., (2015) have calculated a simulation of UV-VIS spectra and microscopic third order nonlinear optical (NLO) coefficients at 532nm wavelength of molecules based of anthocyanidin structure in gas phase environment using semi-empirical PM3 method. One-photon absorption characterizations of the investigated molecules have been theoretically obtained by configuration interaction method with doubly occupied molecular orbitals. Estimated theoretical spectra show a sensibility to the different functional groups such as hydroxyl and methoxyl substitution in radicals [29].

Laura Estevez et al (2009) have studied the acid/base and prototropic equilibria of anthocyanidins using density functional theory (DFT) and polarizable continuum solvation model (PCM) calculations. They have used the B3LYP/6-31++G** basis set. The results indicate that, excluding pelargonidin, the most favored neutral tautomers in solution are deprotonated at C4′. With the same exception, deprotonations at C5 and C4′ characterize the most stable anionic tautomers in solution. The equilibrium population of tautomers is mainly modified along the series by the substitution pattern on the B-ring. Electron densities, analyzed within the formalism of the quantum theory of atoms in molecules (QTAIM), indicate that the electron distribution of cations, neutral forms and anions is not well described by the Lewis structures usually employed to represent them [30].

El Hassane Anouar et al., (2012) has studied the UV/Visible spectra of natural polyphenols. In addition to their numerous biological activities, natural and semi synthetic polyphenols contribute to the large variety of colors (from red to violet) in nature (e.g., fruit, vegetables, leaves and petals). In order to understand the color variation attributed to the multitude of chemical structures of this wide class of compounds, time-dependent density functional Quantum-chemical calculations at the B3P86/6-311+G (d,p) level of theory appears as a relevant and efficient tool. The UV/Vis properties of 33 polyphenols were systematically investigated, including mainly flavonoids, isoflavonoids and flavonolignans. On the basis of molecular orbital analysis they established the structure property relationship, inter alia showing the role of π orbital delocalisation, mesomeric (+M) effects of hydroxyl groups and structural modification of the molecular backbone. The results might help in the future, for example, for the prediction of novel hemisynthetic compounds [31].

Laura Rustioni et al., (2013) found that anthocyanins are the main grape pigments. Due to their aromatic cyclic arrangements, they are able to absorb the radiation in the low energy range of the visible spectrum. In the fruit of Vitis vinifera L, the five main anthocyanidins (cyanidin, peonidin, delphinidin, petunidin and malvidin) are present as 3-O-glucosides, as well as their acetyl, p-coumaroyl and caffeoyl ester forms. Despite the huge number of experimental studies dedicated to the anthocyanin profile analysis of grapes and wines, the complete theoretical elucidation of the optical properties of grape anthocyanins is missing. The present work carried out this task through quantum chemistry calculations based on time-dependent density functional theory (TD-DFT), compared to experimental spectra. The differences in visible absorption spectra between the most common grape anthocyanins were rationalized according to B-ring substitution, glucosylation and esterification. A particular attention was given to the intra-molecular copigmentation effect, demonstrating the existence of an intra-molecular charge transfer excited state for the p-coumaroyl and caffeoyl ester forms [32].

Weirong Cai et al., (2014) have studied the inhibition of free radicals using quercetin, hyperin and rutin is examined to determine their antioxidant effects and the structure–activity relationships of flavonoids. Two species of the free radicals are used, including hydroxyl radical (·OH) and superoxide anion radical ($O_2^{-}\cdot$). Density functional theory calculations under the level of B3LYP/6-311G (d) have been utilized to explore the structure, molecular properties and antioxidant abilities of the three flavonoids. Bond dissociation enthalpy (BDE) and frontier molecular orbital energy gap are investigated. They are compared with the experiment results assayed by the spectrophotometric. All of the flavonoids show a high activity on inhibiting ·OH and $O_2^{-}\cdot$ radicals. Scavenging activity determined by half maximal inhibitory concentration (IC_{50}) values of the three flavonoids decreases in the order: quercetin > hyperin > rutin. The calculations show that quercetin owns the lowest BDE values, which agree well with the experimental results of antioxidant activity determined by IC_{50} values [33].

METHODOLOGY

CHAPTER-III

METHODOLOGY

3.1 INTRODUCTION

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 and processes us to predict, confirm, or reject experimental observations and measurements. Hence 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 [34]. In a similar way, Computational Chemistry simulates chemical structures and reactions numerically based on the fundamental laws of Physics. It allows chemists to study chemical phenomena by running calculations on computers rather than by examining reactions and compounds experimentally [35].

3.2 THEORETICAL METHODS

A theoretical model or method is a way to model a system using a specific set of approximations. These approximations are combined with a calculation algorithm and are applied to atomic orbitals, defined by the basis set, in order to compute molecular orbitals and energy. In general the methods can be separated into four main types. They are

1. Ab initio methods
2. Density functional methods
3. Molecular Mechanics methods
4. Semi empirical methods

3.3 AB INITIO METHODS

The Ab initio means “from the beginning” or “from the first” in Latin. It is the first principle for polyatomic molecule. The most common type of ab initio calculation is called Hartree fock calculation (HF), in which the primary approximation called central field approximation. The electronic structure methods are based upon Quantum Mechanics and therefore provide the most accurate and consistent predictions for chemical systems. Ab initio calculations use the true Molecular Hamiltonian and not the empirical data in the calculation except for the fundamental constants of nature such as the mass of the electron, planck's constant etc that are required for the arrival numerical prediction [36].

The main uses of the ab initio method are calculating molecular geometries, energies, vibrational frequencies, spectra, ionisation potential, electron affinities. The good side of Ab initio methods is that they eventually converge to the exact solution, once all of the approximation are made sufficiently small in magnitude. The bad side of Ab initio methods is that they are expensive. These methods often take enormous amounts of computer CPU time, memory and disk space. Ab initio calculations give very good qualitative results and can give increasingly accurate results as the molecules become smaller [37].

3.3.1 ADVANTAGES OF Ab INITIO METHOD

- Ab initio method is useful for broad range of systems.
- It does not depend on experimental data.
- It is capable of calculating transition states and excited states.

Ab initio method is best for:

- Small systems (ten of atoms).
- Systems involving electronic transition.
- Molecules without available experimental data.
- Systems requiring rigorous accuracy.

3.3.2 HARTREE-FOCK METHOD

The most rigorous method for the determination of orbitals in many electron atoms is provided by the Hartree Fock theory. The Hartree method had been formulated in 1928 and approximated an N-electron wave function as a simple product of N one electron functions

$$\Phi_1 (1)\, \Phi_2 (2) \ldots\ldots\ldots \Phi_N (N) \longrightarrow (1)$$

The electron spin have not been included in this theory. The Hartree wave function does not obey the antisymmetry principle. A couple of years later Fock modified the Hartree theory by including electron spin and resulting formulation is known as the Hartree Fock theory. The aim of the HF theory is to determine the one electron function or orbitals which minimize the energy expectation value of the Slater's determinant (antisymmetrized product) constructed from the orbitals. Therefore, a Hartree-fock calculation is an ab initio (as is an SCF) calculation that gives only an approximation to the Hartree-fock wave function [38].

3.3.3 LIMITATION OF THE HF METHOD

Hartree-fock approximation solves equations of the behaviour of each electron in the average field on the remaining electrons. Unfortunately electrons respond to each other in an instantaneous manner through the coulomb's law. The motion of each pair of electron is correlated and this electron correlation is neglected in the HF model which forms its drawback. The correlation energy is defined as

$$E_{corr}=E_{exact} - E_{HF} \longrightarrow (2)$$

i.e the difference between the (non-relativistic) exact energy and the HF limit energy. Great deal of modern work in the field of electronic structure calculation is aimed at taking electron correlation into account. This method also has another disadvantage that it does not give pure spin state.

3.4 DENSITY FUNCTIONAL THEORY (DFT)

Density Functional theory are often considered to be ab initio methods for determining the molecular electronic structure, even though many of the most common functional use parameters derived from empirical data, or from more complex calculations. This means that they could also be called semi-empirical methods. In DFT, the total energy is expressed in terms of the total electron density rather than the wave function. In this type of calculations, there is an approximate Hamiltonian and an approximate expression for the total electron density. DFT methods can be very accurate for little computational cost. The drawback is that unlike ab initio methods, there is no systematic way to improve the methods by improving the forms of the functional [39].

Density functional theory based methods are ultimately derived from Quantum Mechanics research, especially from the Thomas-Fermi-Dirac model in 1920's and from the Slater's fundamentals work in Quantum Chemistry in the year 1950's. The DFT approach is based upon a strategy of modelling electron correlation of the electron density.

Density functional theory are proposed solely in terms of the one electron density and based upon the Hohenberg-Kohn vibrational principle which provide no general procedure for accurately calculating relatively small energy difference such as excitation energies, ionisation potentials and electron affinities. A power full method inspired by the Hohenberg-Kohn vibrational principle has been used with great success in the calculation of such quantities [40]. Following on the work of Khon and Sham, the approximate functional employed by current DFT methods partition the electronic energy into several terms:

$$E = E^T + E^V + E^J + E^{XC} \xrightarrow{\qquad} (3)$$

Where

E^T = Kinetic energy terms (arising from the motion of electrons)

E^V = The potential energy of the nuclear- electron attraction and of the repulsion between pairs of nuclei.

E^J = Electron-electron repulsion term (i.e. coulomb self-interaction of the electron density)

E^{XC} = Exchange correlation term and includes the remaining part of the electron-electron interactions.

3.4.1 KOHN SHAM EQUATION

In order to obtain an electron density from DFT, some assumptions are made that are analogous to those made in solving the Schrodinger equation. For the Schrodinger equation, LCAO-MO approximation used to obtain a trial wave function. In DFT, the similar approximation for the trial electron density,

$$\rho_t(x,y,z) = \sum_i \left|\Phi_i^{ks}(x,y,z)\right|^2 \xrightarrow{\qquad} (4)$$

Φ_i^{ks} , are Kohn Sham orbitals, which are similar to the molecular orbitals that appear in the approach for solving the Schrodinger equation approximately using the Hartree- Fock method. Each Kohn- Sham orbitals holds two electrons and can be represented using a linear combination of atomic orbital basis function.

$$\Phi_i^{ks}(x,y,z) = \sum_{\mu=0}^{ks} c_{\mu i} f_\mu(x,y,z) \xrightarrow{\qquad} (5)$$

In this equation, $c_{\mu i}$ corresponds to numerical coefficients and the functions $f_\mu(x,y,z)$ are the atomic orbital basis function. The coefficients $c_{\mu i}$ are obtained by solving a set of equations called the Kohn-Sham equations. These are analogous to the Hartree- Fock equations employed when obtaining a solution of the Schrodinger equation [41].

3.4.2 ADVANTAGES OF DFT METHOD

- Density functional theory is computationally very efficient.
- DFT is conceptually simple.
- DFT can be easily combined with Molecular Dynamics of the nuclei.
- In the basis set limit, the Hartree-fock equations would not give the correct energy, but the Khon-Sham equations would correct energy by knowing the exact exchange correlation energy functional.
- In wave function theory, Hartree-fock level results are improved by using perturbation or configuration interaction treatments of electron correlation, but in DFT theory there is as yet no systematic way of improving the exchange-correlation energy functional.
- The solution to the Hartree-fock equation may be viewed as exact solution to the approximate description, while the solutions to the kohn-sham equations are approximation to an exact description.

3.4.3 APPLICATION OF DFT

- DFT is a general- purpose of computational method,, and it can be applied to most of the systems.
- Like all the computational methods, DFT methods are more useful for some types of calculations than others.
- Some DFT methods are specifically designed for specific applications, such as the MPW1k hybrid method, designed for determination of kinetic problems.
- Application of modern DFT calculations has been extended from small molecules for testing the accuracy to transition metal complexes.

3.5 MOLECULAR MECHANICS

In many cases, large molecular system can be modelled successfully while avoiding Quantum Mechanical calculation entirely. For example Molecular Mechanics simulations use a single classical expression for the energy of a compound for instance the harmonic oscillator. All constants appearing in the equations must be obtained beforehand from experimental data or ab initio calculations. The database of compounds used for parameterization is crucial to the success of Molecular Mechanics calculations. A force field parameterized against a specific class of molecules, for instance proteins, would be expected to only have any relevance when describing other molecules of the same class [42].

3.5.1 ADVANTAGES OF MOLECULAR MECHANICS

- It requires less of a computer than Quantum Mechanical methods.
- It can be used for molecules as large as enzymes.
- An important advantage of Molecular Mechanic method is their efficiency.

3.6 SEMI-EMPIRICAL MODELS

The evolution of Molecular Orbital (MO) methods has resulted in two main methods, ab initio and semi empirical. Semi empirical methods are simplified versions of Hartree-fock theory. The semi empirical calculations are much faster than the ab initio calculations. Such methods are currently very slow and routine application at any reasonable degree of accuracy to systems of even a few tens of atoms is still not practical. In contrast, semi empirical methods employ empirically determined parameters and less attractive to the theoretical purist. Unlike ab initio methods, the accuracy of any empirical method is limited to the accuracy of the experimental data used in obtaining the parameters. However, semiempirical methods are fast enough and accurate enough for routine application to quite large systems [43].

Semi empirical methods are based on three approximation, they are

- The elimination of core electrons from the calculation.
- The use of minimum number of basis sets.
- The reduction of the number of two electron integrals.

Semi empirical calculations have been most successful in the description of organic chemistry, where only a few elements are used extensively and molecules are of moderate size. However, semi empirical methods were also applied to solid and nanostructure but with different parameterization.

3.6.1 ADVANTAGES OF SEMI EMPIRICAL METHOD

- Less demanding computationally than ab initio method.
- Capable of calculating transition state and excited state.

Semi empirical methods are best for:

- Medium sized systems.
- Systems involving electronic transition.

3.7 BASIS FUNCTIONS

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 of function $\{\Phi_v\}$. In principle, for high frequency, a complete basis set should be used. In practice a finite basis set of reasonable dimension is used. The functions are generally so chosen as to create least difficulty in integration.

3.8 BASIS SETS

A basis set is a mathematical description of the orbitals with in a molecular system used to perform Quantum Chemical calculations. Larger the basis set, more accurately the approximate orbitals, by imposing a fewer restrictions to the electron in space.

3.8.1 CLASSIFICATION OF BASIS SETS

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

➢ MINIMAL BASIS SETS (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 sets 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) [2s1p] respectively. The integers should not be confused with the principal quantum numbers.

Commonly used minimal basis sets are

- STO-3G
- STO-4G
- STO-6G
- STO-3G*

➢ EXTENDED BASIS SETS

There are several types of extended basis sets:

- Double zeta and triple zeta basis sets
- Split- valence basis sets
- Polarized basis sets
- Diffuse sets

➢ DOUBLE-ZETA AND TRIPLE-ZETA BASIS SETS

The double zeta valance basis sets form molecular orbitals from the linear combinations of two sets of functions for each atomic valence orbital. Similarly, triple split valence basis sets such as 6-311G, use three sets of contracted functions for each valence orbital type.

➢ SPLIT VALENCE BASIS SETS

Split valence basis set is one of way to increase the size of a basis set is to take more basis functions per atom. Split valence basis sets, such as 3-21G and 6-31G basis sets, have two or more size of basis function for each valence orbital. It has only the valence part of the basis set is doubled.

➢ POLARISED BASIS SET

Split valence basis set could be improved by adding orbitals with different shapes. Polarized basis sets add orbital's with angular momentums going beyond of requirement for the proper description of the ground state of each atom at the HF level. For example, polarised basis sets add d-functions to carbon atoms and some of them add p-function to a hydrogen atom.

Examples for polarised basis sets are

- 6-31G (d)
- 6-311G (d,p)

➢ DIFFUSE BASIS SET

Basis sets with additional diffuse functions are large by size versions of and s and p-type split valence basis sets. Diffuse orbitals occupy a larger region of space. Basis sets with diffuse functions are important for systems where electrons may be far from the nucleus. One example for diffuse basis function is the 6-311+G (d,p) basis sets [41].

3.9 OPTIMIZATION

The energy of the molecular system which varies with small changes in its structure is specified by its potential energy surface. A potential energy surface is a mathematical relationship linking molecular structure and the resultant energy. Geometry optimization is usually attempt to locate minima on the potential energy surface, thereby predicting equilibrium structures of molecular systems. All successful optimizations locate a point on the potential energy surface where the force are zero is called stationary point.

3.10 FREQUENCY ANALYSIS

Frequency calculations can serve a number of different purposes:

1. To predict the IR and Raman spectra of molecules (frequencies and intensities).
2. To compute force constants for a geometry optimization.
3. To identify the nature of stationary point on the potential energy surface.
4. To compute zero point vibration and thermal energy corrections to total energies as well as other thermo dynamical quantities of interest such as the enthalpy and entropy of the system.

3.11 COMPUTATIONAL DETAILS OF THE PRESENT STUDY

- The structure of Petunidin is optimized by using DFT method with B3LYP level using 6-311G (d,p) basis set.
- Using the optimized structure, geometrical parameters like Bond length, Bond angle and Dihedral angle of Petunidin is determined by using same level of theory.
- The energy values of Petunidin have been computed by the same level of theory.
- To predict the IR and Raman spectrum using DFT method.

RESULTS AND DISCUSSION

CHAPTER-IV

RESULTS AND DISCUSSION

4.1 INTRODUCTION

Anthocyanidins are a class of natural polyphenol based coloured compounds that are largely responsible for the vibrant colours in most fruits and flowers. Anthocyanidins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) cation and are usually classified in terms of the number and position of the hydroxyl substituents. Petunidin is a class of flavonoids and O-Methylated anthocyanidin of 3-hydroxy type. Flavonoids are present in substantial amounts in fruits and vegetables. They have a high induced antioxidant activity associated with the presence of several aromatic cycles with hydroxyl constituents in their structure. Petunidin is a natural organic compound and found in different types of berries. Their health beneficial effects are related to antioxidant and radical scavenging properties. Petunidin is characterized by the presence of five hydroxyl groups in positions 3, 5, 7, 4′ and 5′.

In the present study, optimized and structural parameters of Petunidin have been investigated using density functional theory with 6-311G (d,p) using Gaussian 09 software. Antioxidant activities, spectroscopic analysis and electronic properties of Petunidin have been performed using same level of theory. It is based on two prevalent radical scavenging mechanisms. They are

- H atom transfer mechanism (HAT)
- Single electron transfer (SET)
- Sequential proton loss electron transfer mechanism (SPLET)

The phenolic OH bond dissociation enthalpies (BDEs), ionisation potentials (IPs), and proton dissociation enthalpies (PDE) were calculated to elucidate the radical scavenging capacity of Petunidin [50].

4.2 MOLECULAR GEOMETRY

The structure of Petunidin have been optimized by the theory of Density Functional theory with B3LYP/6-311G (d,p) and shown in fig(4.1)

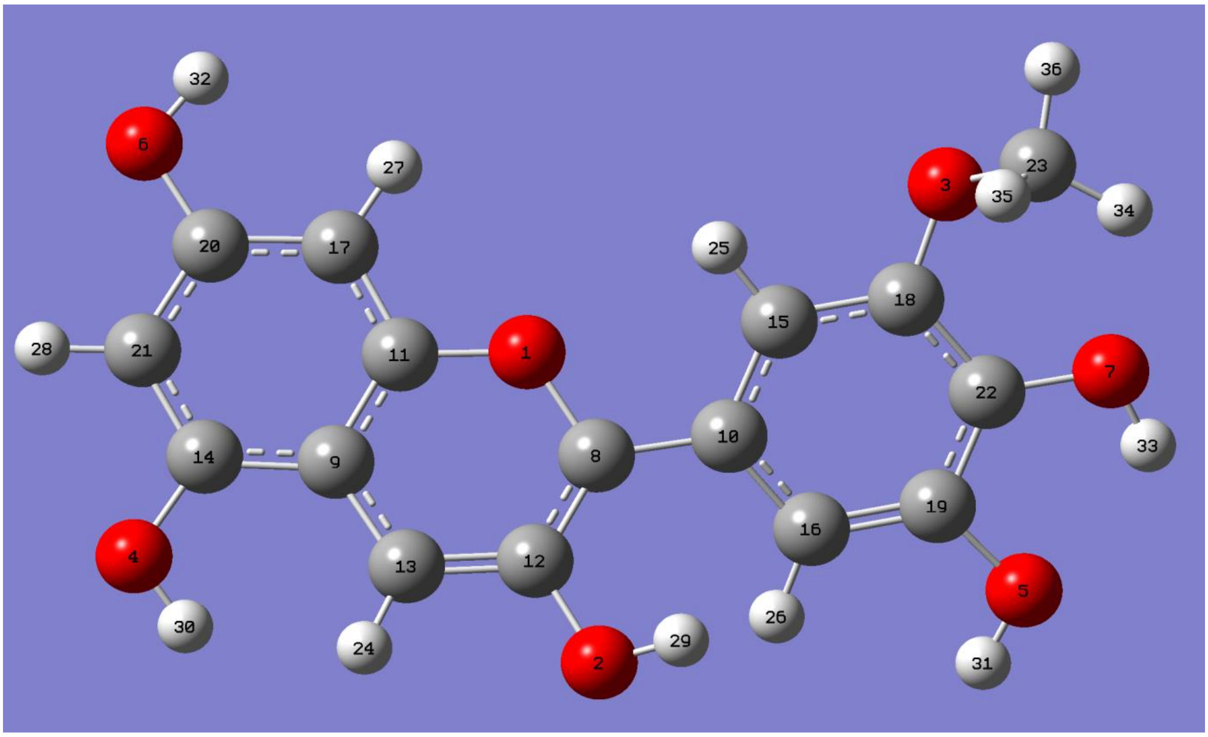


Fig.4.1 Optimized structure of Petunidin

Optimized structure is confirmed to be real minima by frequency calculation at the same level of theory. From the optimized geometry, the geometrical parameters like Bond angles, Bond lengths and Dihedral angles of Petunidin were determined by using the same level of theory. From the optimized structure, the energy value of Petunidin in gas phase is -1144.3323 (a.u). Table 4.2, 4.3 and 4.5 shows the Bond length, Bond angle and Dihedral angle of Petunidin respectively.

TABLE.4.1 Bond length (Å) of Petunidin optimized by using B3LYP/6-311G (d,p)

Parameters	**Bond length(Å)**
O1-O2	3.66009
O2-03	6.54611
O3-O4	9.68871
O4-O5	9.67225
O5-O6	10.71693
O6-O7	10.71807

C8-C9	2.80982
C9-C10	4.25112
C10-C11	3.65987
C11-C12	2.77772
C12-C13	1.38598
C13-C14	2.52461
C14-C15	6.37205
C15-C16	2.41872
C16-C17	5.97943
C17-C18	6.13624
C18-C19	2.41002
C19-C20	8.35841
C20-C21	1.39614
C21-C22	9.10822
C22-C23	3.02561
H24-H25	5.90008
H25-H26	4.32258
H26-H27	6.50039
H27-H28	4.31686
H28-H29	7.71450
H29-H30	5.40602
H30-H31	8.71956
H31-H32	10.67556
H32-H33	11.00018
H33-H34	3.18003
H34-H35	1.78929
H35-H36	1.78192

TABLE.4.2 Bond angle (deg) of Petunidin optimized by using B3LYP/6-311G (d,p)

Parameters	**Bond angle(deg)**
O1-O2-O4	63.613
O4-O1-O6	58.726
O1-O3-O7	106.126
O5-O3-O7	28.938
O2-O3-O7	76.915
O3-O5-O2	84.99
C8-C12-C13	121.262
C13-C9-C11	118.298
C9-C13-C12	198.183
C9-C11-C17	122.784
C11-C17-C20	118.457
C20-C21-C14	119.686
C8-C10-C16	121.895
C16-C10-C15	117.986
C15-C18-C22	119.954
C18-C22-C19	118.559
C16-C19-C22	121.849
C23-C18-C22	102.801
C8-C10-C15	120.114
C13-C18-C15	166.403
C15-C19-C10	30.537
C11-C13-C8	59.256
C8-C15-C16	61.566
C18-C23-C22	26.875
C20-C14-C11	59.642
C11-C21-C14	60.093
C8-C18-C19	71.371
H32-H27-H28	54.430
H28-H30-H24	123.879

H24-H30-H27	76.704
H25-H26-H31	113.702
H35-H36-H34	60.239
H33-H35-H34	38.894
H29-H26-H31	128.696
H24-H29-H27	61.171

TABLE.4.3 Dihedral angle (deg) of Petunidin optimized by using B3LYP/6-311G (d,p)

Parameters	**Dihedral angle(deg)**
O4-O1-O2-O5	161.549
O4-O6-O2-O1	175.194
O1-O3-O5-O7	174.533
C12-C20-C14-C17	179.402
C14-C17-C9-C11	178.385
C9-C11-C13-C12	175.373
C8-C10-C16-C19	179.664
C10-C16-C15-C18	179.836
C15-C18-C19-C22	179.931
C21-C11-C13-C12	178.054
C8-C10-C16-C19	179.664
C14-C9-C13-C12	176.976
C23-C18-C22-C19	154.530
H28-H30-H32-H27	175.794
H28-H30-H27-H24	162.318
H24-H29-H26-H25	88.420
H29-H26-H25-H31	128.045
H33-H35-H34-H36	143.330

4.3 O-H BOND DISSOCIATION ENTHALPY

H atom transfer (HAT) is a major mechanism by which phenolic antioxidant exerts its antioxidative capacity, H atom directly transfer from antioxidant to active free radical to break chain reaction. The reactivity of an antoxidant can be estimated by the O-H bond, where the lower BDE value to give the higher expected activity [50]. BDEs values were calculated according to the formula

$$\mathrm{BDE} = \mathrm{H}\,(\mathrm{ArO}^{\cdot}) + \mathrm{H}\,(\mathrm{H}^{\cdot}) - \mathrm{H}\,(\mathrm{ArOH}) \longrightarrow \quad (1)$$

Where

$\mathrm{H}\,(\mathrm{ArO}^{\cdot})$ = Enthalpies of the phenoxy radical

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

H (ArOH) = Enthalpy of parent molecule

The computed BDE values for the anthocyanidin compound of Petunidin is shown in table (4.4) and we can see that lowest BDE value obtained for Petunidin is at 3–OH at 54.400 kcal/mol with BDE sequence of : 3–OH < 5–OH < 7–OH < 4′–OH < 5′-OH. It clearly confirms that H-transfer from C-ring (3–OH) is much easier than from A-ring (5–OH and 7–OH) and B-ring (4′–OH and 5′-OH). Effect of H-transfer from C3–OH will have high antioxidant property to scavange free radicals compare to other hydroxyl group present in the ring [48].

TABLE.4.4 BDE values for Petunidin

Parameters	BDEs (kcal/mol)
3-OH	54.400
5-OH	56.793
5′-OH	63.145
7-OH	59.843
4′-OH	60.597

4.4 IONIZATION POTENTIAL

In this mechanism electron can transfer from antioxidant to free radical, or transfer from free radical to antioxidant, to break the radical chain reaction. Ionization potential of antioxidant is often used to predict the antioxidative capacity through SET pathway [51]. The IP values were calculated according to the formula,

$$IP = H\,(ArOH^{+\cdot}) + H\,(e^{-}) - H\,(ArOH) \longrightarrow (2)$$

Where

$H\,(ArO\,H^{+\cdot})$ = Enthalpy of the cation radical

$H\,(ArOH)$ = Enthalpy of parent molecule

$H\,(e^{-})$ = Enthalpy of electron

The lower ionization potential implies that the antioxidant is a better electron donor. The substituent effect of methoxyl group is more significant than that of the hydroxyl group; this could be attributing to the stronger electron donating capacity of methoxyl, which enhances the electron density on the molecular skeleton to favour electron transferring to free radical. This is also confirmed by IP of Petunidin 125.912 (kcal/mol).

4.5 PROTON DISSOCIATION ENTHALPY

PDE represents the reaction enthalpy of the second step in SET-PT mechanism. SET-PT mechanism involves two steps: electron absorption from ArOH, followed by proton transfer from the radical cation $H\,(ArO\,H^{+\cdot})$. In this case PDE is the most significant energetic factor for the scavenging activity evolution. Molecules with low PDE are expected to have high activity [51]. The PDE values were calculated according to the formula,

$$PDE = H\,(ArO^{\cdot}) + H\,(H^{+}) - H\,(ArOH^{+}) \longrightarrow (3)$$

Where

$H\,(ArO^{\cdot})$ = Enthalpies of the phenoxy radical

$H\,(H^{+})$ = Enthalpy of the proton

$H\,(ArO\,H^{+\cdot})$ = Enthalpy of the cation radical

All PDEs of investigated Petunidin were calculated and the results are listed in table [4.5]

TABLE.4.5 PDE values for Petunidin

Parameters	PDE(kcal/mol)
3-OH	244.410
5-OH	246.803
5′-OH	253.155
7-OH	249.853
4′-OH	250.607

4.6 IR AND RAMAN FREQUENCIES

Spectroscopy involves the study of the interaction of electromagnetic radiation with matter. The amount of energy absorbed by the molecule gives information about the structure of the molecule. The frequency of the absorbed radiation reveals the information on the nature of the bond, types of bond and molecular groups present [53]. The modes of vibration are of two types.

They are

- Stretching
- Bending

4.6.1 STRETCHING

In stretching vibrations, the atoms move along the bond axis, so that the bond length increases or decreases at regular intervals. But the atoms remain in the same bond axis. There are two types of stretching.

(a) Symmetric stretching

In symmetric stretching two attached atoms move away and towards the central atom at the same time.

(b) Asymmetric stretching

In asymmetric stretching two attached atoms do not move away and towards the central atom at the same time.

4.6.2 BENDING

Bending vibrations may consist of a change in bond angle between bonds with a common atom. There are four types of bending vibrations.

(a) Scissoring

Two atoms move away and towards each other is called scissoring.

(b) Rocking

It is like the motion of pendulum in which the atom is pendulum and there are two instead of one.

(c) Wagging

Two atoms swing back and forth out the plane of molecule.

(d) Twisting

Two atoms rotate about the bond which joins it to the remainder of the molecule.

The strongest peak for IR vibrational spectrum is at 1209 cm^{-1} and for Raman spectrum is at 1635.8 cm^{-1}. The second highest peak of IR spectrum is 1373 cm^{-1} and for the Raman spectrum is 1428 cm^{-1}. Vibrational spectral analysis of Petunidin was performed by DFT method. The finger print region from the graph is 1500 cm^{-1} to 450 cm^{-1}. The calculated IR spectra of Petunidin are shown in fig (4.2) and a raman spectra of Petunidin is shown in fig (4.3). Table (4.6) shows the frequency mode of vibration of Petunidin using DFT/6-311G (d,p) level of theory

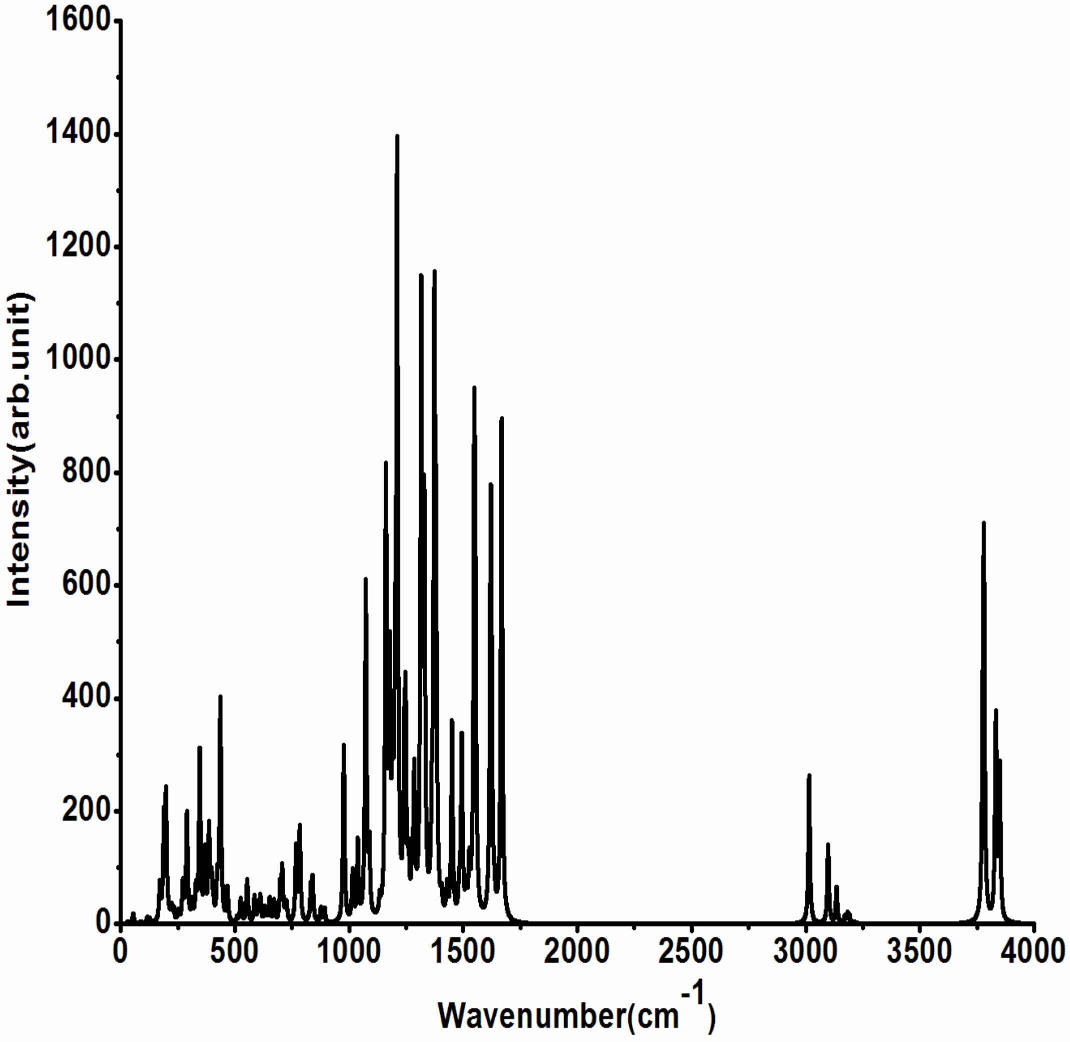


Fig.4.2 IR spectra for Petunidin using B3LYP/6-311G (d,p)

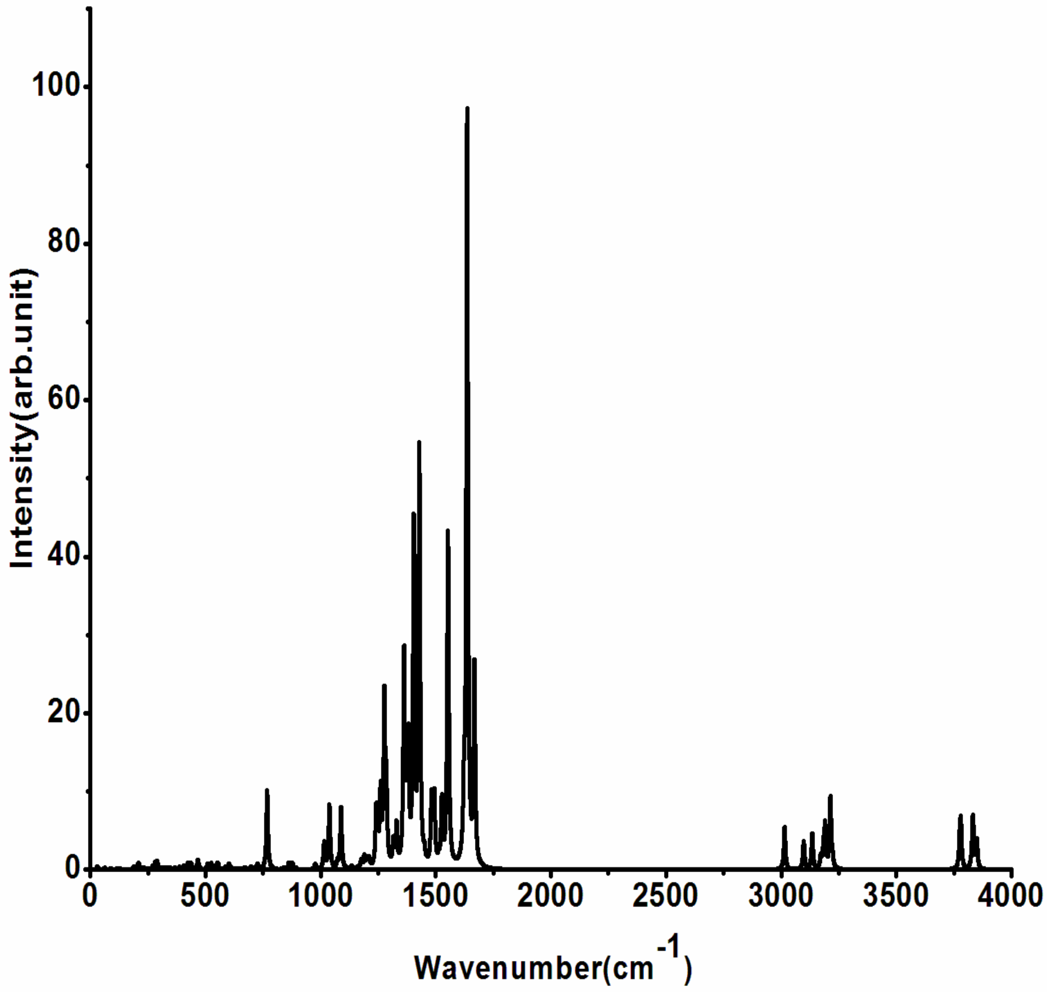


Fig.4.3 Raman spectra for Petunidin using B3LYP/6-311G (d,p)

4.7 ELECTRONIC PROPERTIES HOMO-LUMO ANALYSIS

The frontier molecular orbitals are very much useful for studying the electric and optical properties of organic molecules. The stabilization of the bonding molecular orbital and destabilization of the antibonding can increase, when the overlap of two orbitals increase. In the molecular interaction, there are two important orbitals that interact with each other. One of the highest energy occupied molecular orbital called HOMO, represents the ability to donate an electron. The other one is the lowest energy unoccupied molecular orbital called LUMO, as an electron acceptor. These orbitals are sometimes called the frontier orbitals.

The total energy and energy gap affect the stability of a molecule. The conjugated molecules are characterized by a highest occupied molecular orbital-lowest unoccupied molecular orbital (HOMO–LUMO) separation, which is the result of a significant degree of intermolecular charge transfer (ICT) from the end capping electron-donor to the efficient electron acceptor group through p-conjugated path. The HOMO is the orbital that primarily acts as an electron donor and the LUMO is the orbital that largely acts as the electron acceptor, and the gap between HOMO and LUMO characterizes the molecular chemical stability. The energy gap between the HOMO and the LUMO molecular orbitals is a critical parameter in determining molecular electrical transport properties because it is a measure of electron conductivity. The energy of the highest occupied molecular orbital is -0.15002 eV and energy of the first lowest unoccupied molecular orbital is -0.02786 eV. The energy gap value of HOMO and LUMO is 0.12216ev. The alpha HOMO and LUMO for Petunidin molecule is shown in fig (4.9).

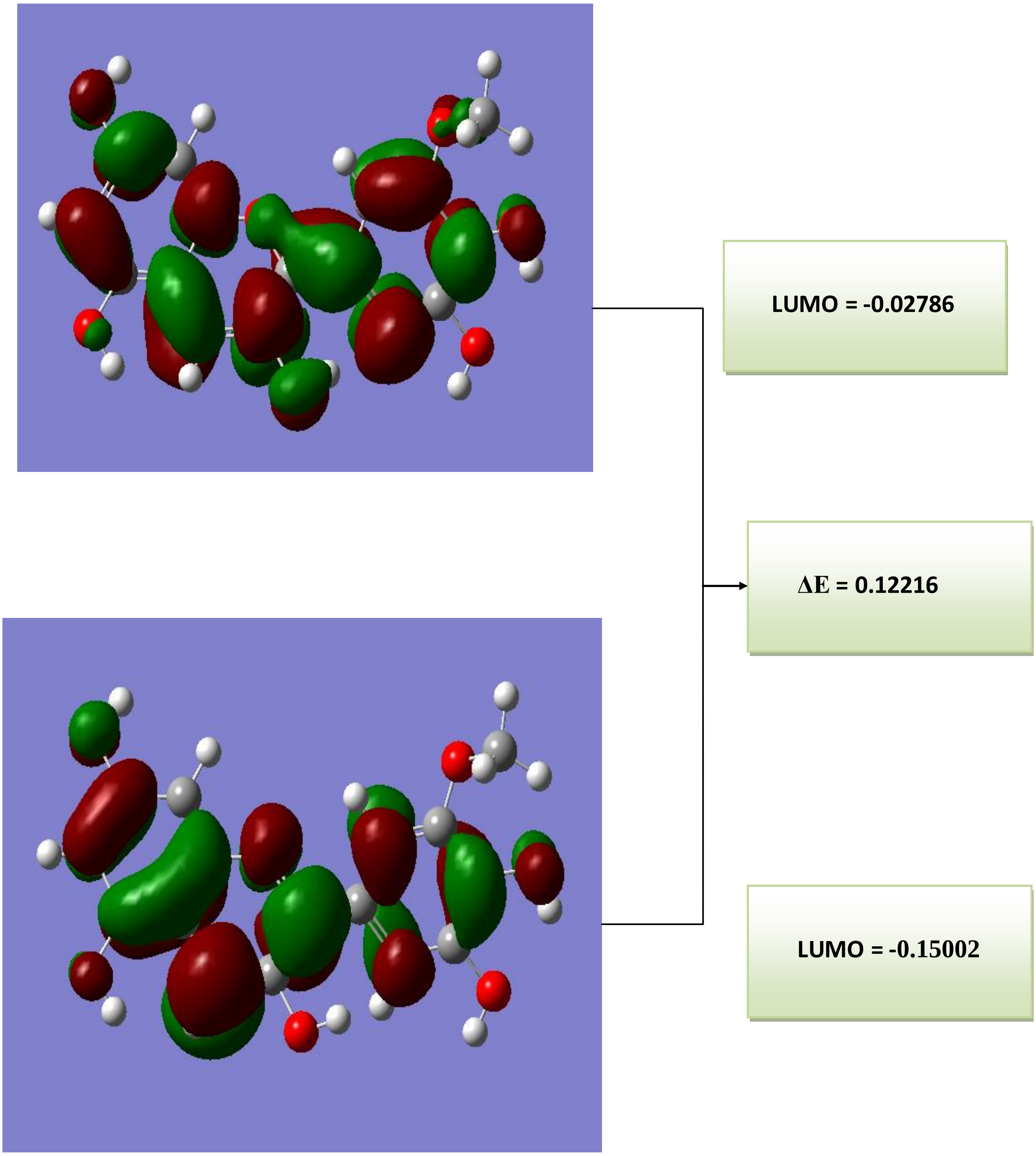


Fig.4.4 HOMO and LUMO for Petunidin

SUMMARY AND CONCLUSION

CHAPTER-V

SUMMARY AND CONCLUSION

The antioxidative activities of Petunidin in gas phase have been investigated by density functional theory at B3LYP/6-311G (d,p) level of theory. The minimum energy structure of Petunidin is obtained by using same level of theory. The O-H bond dissociation enthalpies, ionisation potential and proton dissociation enthalpies of Petunidin are obtained using HAT, SET and SPLET mechanism. Using the optimized structure, geometrical parameters like Bond length, Bond angle and Dihedral angle of Petunidin is determined by using same level of theory. Absorption spectra for Petunidin are also obtained by DFT method. Frontier molecular orbit of Petunidin were analyzed and compute the energy gap between the HOMO-LUMO.

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