MODELLING OF REACTION BETWEEN ANTIOXIDANTS AND FREE RADICALS

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Dedicated

to

My Mother (late) Rajamani

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Abstract

Radical scavenging ability (RSA) of the polyphenols was determined experimentally by kinetic parameters (rate constants, k and activation energy E_a) in different solvents using the stopped-flow technique and computationally by the molecular parameter, OH bond dissociation enthalpy (OH BDE) using density functional theory/ B3LYP method in Gaussian 98. Kinetic study on the model phenolic compounds reveals that rate of radical scavenging reaction of polyphenols depend not only the number and position of OHs but also the presence of electron donating groups (EDGs) in the structure. Computational study reveals that the presence of intramolecular hydrogen bond (IHB), which decreases the OH BDEs of phenols. Epigallocatechin gallate (EGCG), a tea polyphenol, showed the greater RSA (E_a = 60.9 kJ mol^{-1} against DPPH$\bullet$).

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ABBREVIATIONS

AAPH	2,2'-azobis(2-amidino-propane) dihydrochloride
ABAP	2,2'-azobis-(2-amidino propane) dihydrochloride
ABTS$^{\bullet+}$	2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate
AEAC	Ascorbic acid equivalent antioxidant capacity
AO	Atomic orbital
ArO$^{\bullet}$	Antioxidant derived free radical
ArOH	Phenolic antioxidant
BDE	Bond dissociation enthalpy
DFT	Density functional theory
DNA	Deoxyribo nucleic acid
DPPH$^{\bullet}$	2,2-diphenyl-1-picrylhydrazyl radical
DTNB	5,5'-diphenyl picryl hydrazyl radical
FRAP	Ferric reducing / antioxidant power
GAE	Gallic acid equivalents
GTF	Gaussian type functions
HAT	Hydrogen atom transfer
LCAO	Linear combination of atomic orbitals
ORAC	Oxygen radical absorption capacity.
ROOH	Hydroperoxide
ROS	Reactive oxygen species
SET	Single electron transfer
STO	Slater type orbital
TAA	Total antioxidant activity.
TAC	Total antioxidant capacity.
TEAC	Trolox equivalent antioxidant capacity
TRAP	Total radical absorption power
TROLOX	6-hydroxy-2, 5,7,8-tetramethyl-2-carboxylic acid
TST	Transition state theory

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1. GENERAL INTRODUCTION

While manufacturers of nutritional supplements have been fortifying foods with traditional vitamins and minerals, branding them as “nutraceuticals’, future trends seems to be pointing to the direction of neutraceuticals being supplemented with ingredients that can also help maintain wellness and prevent diseases. United States alone spent approximately $22.6 billion on nutritional supplements with an expected market value of $35.4 billion by year 2006. Within the nutraceutical category are antioxidants, essential compounds needed for controlling degenerative oxidation reactions caused by reactive oxygen and nitrogen species (ROS & RNS) in living tissues as well as in the inhibition of lipid peroxidation in foods. In recent years, there has been a growing interest in identifying potentially important antioxidants against free radicals, especially those from naturally occurring substances. Recently, harmless natural plant products have been an important source for the search of new antioxidants, by both nutraceuticals and pharmaceutical companies.

1.1 Free radicals

A free radical is any species that contains one or more unpaired electrons and is capable of independent existence. Such a radical can attach itself to a stable molecule within the body and damaging the surrounding cells. Most of the radicals are reactive oxygen species (ROS) formed during normal cell aerobic respiration (Gutteridge and Halliwell, 2000). ROS are oxygen derived chemically reactive molecules (Fridovich, 1999; Betteridge, 2000; Halliwell, 1999; Halliwell, 1996). The major ROS present in the cells are superoxide, hydrogen peroxide, hydroxyl radical, and nitric oxide. Superoxide anions

are formed by an electron addition to the molecular oxygen. Superoxide anions are not as reactive as other ROS. The hydroxyl radical is very reactive compared with other radicals. The hydroxyl radical is formed from hydrogen peroxide in a reaction known as Fenton reaction that is catalyzed by metal ions (Fe^{2+} or Cu^{2+}) (Halliwell, 1999 & 1987).

1.2 Effect of free radicals on biological system

The high reactivity of reactive oxygen species (ROS) induces damage in lipids, DNA, and proteins (Blokhina *et al*., 2003; Werns and Lucchesi, 1989; Auroma, 1994; Kirkinezos and Moraes, 2001; Lee and Wei, 2001). They oxidize important components of cell and cause permanent damage. Radicals are capable of reacting with any biomolecule in the living cell (Halliwell, 1989). ROS are found to be mutagenic. Free radical also induces structural changes in DNA leading to cancer and other diseases (Marnett. 2000; Mates *et al.*, 1999). Free radical reactions mainly contribute to atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS, and severe degenerative diseases in humans (Halliwell, 1997; Giblin, 1985; Keller, 1998; Halliwell, 1999; Prasad, 1999 and Pratico, 2000). Lipid peroxidation is another process that produces many pathological events in the cells (Halliwell and Gutteridge, 1999b; Noguchi and Niki, 1999; Drueke *et al*., 2001 and Spiteller, 2001). This process causes damage to unsaturated fatty acids, tends to decrease membrane fluidity and lead to many other pathological events.

1.3 Effect of free radicals on food

One of the most common causes of off-flavors and odors in many foods is lipid oxidation (Eriksson, 1987). Lipid oxidation occurs through either an enzymatic or non-enzymatic

mechanism. Both mechanisms yield hydroperoxides, which then break down to form a number of volatile compounds that are responsible for off-flavors and odors. Enzyme-catalyzed oxidation, as the name implies, must be initiated by an enzyme, such as lipoxygenase, acyl hydrolase, or hydroperoxide lyase (Nawar, 1996). Autoxidation, however, does not require enzymatic catalysis. Once the process is initiated, it is "self catalyzing" as long as there is molecular oxygen present. The mechanism of autoxidation of lipids involves the three stages: initiation, propagation and termination.

Initiation	RH	→	$R\bullet$	Eqn 1. 1
	$R'\text{-}CH{=}CH\text{-}R'' + O_2$	→	$ROOH$	Eqn 1. 2
Propagation	$R\bullet + O_2$	→	$ROO\bullet$	Eqn 1. 3
	$ROO\bullet + RH$	→	$ROOH + R\bullet$	Eqn 1. 4
	$ROOH$	→	$RO\bullet + OH\bullet$	Eqn 1. 5
Termination	$R\bullet + R\bullet$	→	*Non radical products*	Eqn 1. 6
	$ROO\bullet + ROO\bullet$	→	*R1-CO-R2 + R1-CHOH-R2 + O2*	Eqn 1. 7

The initiation step is the most intriguing aspect of this chemical process. The spontaneous abstraction of a hydrogen atom from an organic material by molecular oxygen **(equation 1.1)** is an endothermic reaction which demands large activation energy and although it might occur to a certain extent, it is probably too slow to be of practical importance. Alternatively, the direct addition of an oxygen molecule to a double bond to generate hydroperoxide (ROOH) compounds is prevented by the spin conservation rule due to the triplet state character of the ground state oxygen. Therefore, either the organic molecule or the oxygen should be activated before reaction. Many foods are now being packed in

plastic containers that have significant oxygen permeability. This can lead to an increase in autoxidation as oxygen migrates into the container. In addition, foods that have high concentrations of fatty acids, especially polyunsaturated fatty acids, are more susceptible to lipid oxidation.

1.4 Antioxidants

Halliwell (1995) defined an antioxidant as any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. The term oxidizable substrate here refers to biological molecules that are found in the body or fats that are present in food. Antioxidants can be classified in a different manner according to their activity. Based on the mechanism of reactions, antioxidants are classified into primary antioxidants and secondary antioxidants.

1.4.1 Primary antioxidants

Free radicals can attach themselves into an oxidizable substrate and cause the damage. After the stable molecule (substrate) loses its electron it becomes a free radical and begins a chain reaction. Primary antioxidants are the ones that inhibit the chain initiation, and break chain propagations. They delay or prevent oxidation of the substrate from acting as a chain-propagating radical, hence called chain breaking antioxidants. For example, secondary aromatic amines are primary antioxidants that react with peroxyl radicals to form stable hydroperoxides by donating their hydrogen atom.

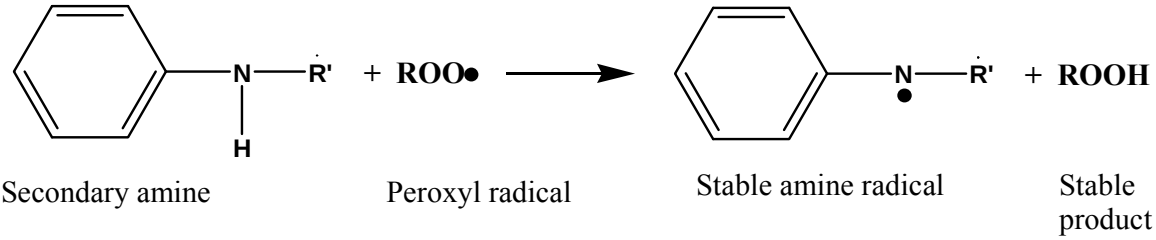


Figure 1.1: An illustration of primary antioxidant mechanism

Among many classes of primary antioxidants, polyphenols are probably the most widely studied antioxidants in biochemical systems (Halliwell, 1999; Burton, 1986; Noguchi, 2000; Denisov, 2000). The term polyphenols embraces a wide range of plant substances that possess an aromatic ring bearing one or more hydroxyl substituents (Lazarus *et al*., 2001). Literatures report that the lipid oxidation can be effectively quenched by polyphenols (Periera da Silva *et al*., 2000; Czinner *et al*., 2001 & Lodovici *et al*., 2001), which are also known to be scavengers of various oxygen species, even as toxic as the HO• radical and singlet oxygen (Croft, 1998 & Morton *et al*., 2000). Phenolic antioxidants (ArOH) have recently attracted increasing interest in pharmaceutical and the food industries (Richelle *et al*., 2001). The flavanoids are the largest group of phenolic compounds. As shown in Figure 1.2, flavonoids are divided into sub classes; they are flavones, flavanones, isoflavones, flavonols, flavanols, and anthocyanins (Rice-Evans and Miller, 1997). Examples of natural phenolic antioxidants that belongs to flavonoids are 3, 4-dihydroxychalcones (*e.g.* butein, okanin), flanones (*e.g.* luteolin, isovitexin), anthocyanins (*e.g.* cyanidin-3-glucoside, malvidin-3-glucoside), isoflavones (*e.g.* daidzein, genistein), dihydroflavonols (*e.g.* dihydroquercetin), flavonols (*e.g.* gossypetin), cinnamic acids, ferulic acid, and caffeic acid.

1.4.2 Secondary antioxidants

Secondary antioxidants are different from chain-breaking antioxidants in that they react with lipid peroxides. While chain-breaking antioxidants react with radicals and donate an electron or hydrogen atom to reduce the radicals, secondary antioxidants are not involved in reaction with radicals or donation of electrons. Secondary antioxidants react with lipid peroxides (LOOH) through non-radical processes like reduction or hydrogen donation and convert them into stable end products like alcohols. Thiols (RSH) such as cysteine and gluthathione, sulphides (R-S-R) such as methionine and 3,3'-thiodipropionic acid and free amine groups of proteins ($R\text{-}NH_2$) react with lipid peroxides and form stable products as given by equations below (Yanishlieva-Maslarova, 2001).

$$RSH + LOOH \rightarrow R\text{-}S\text{-}S\text{-}R + LOH + H_2O \quad \text{Eqn 1. 8}$$

$$R\text{-}S\text{-}R + LOOH \rightarrow R\text{-}SO\text{-}R + LOH \quad \text{Eqn 1. 9}$$

$$R\text{-}NH_2 + LOOH \rightarrow R\text{-}N\,(OH)\,L + H_2O \quad \text{Eqn 1. 10}$$

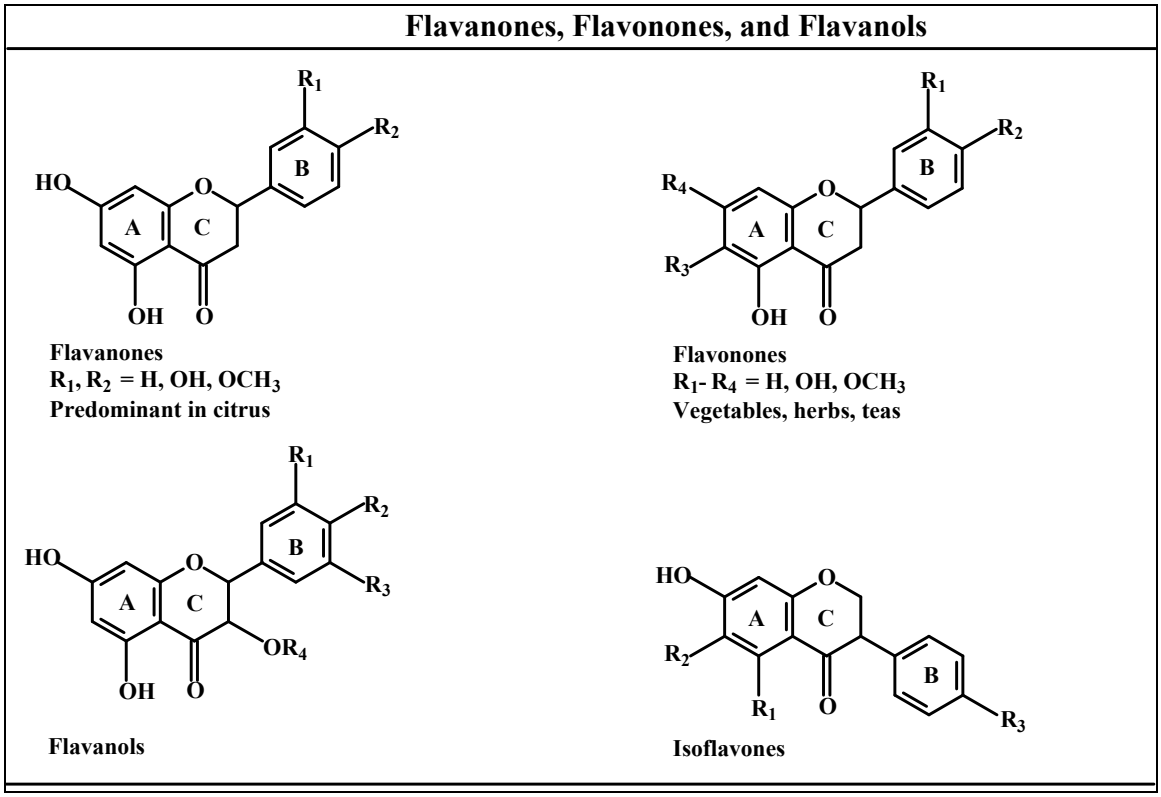
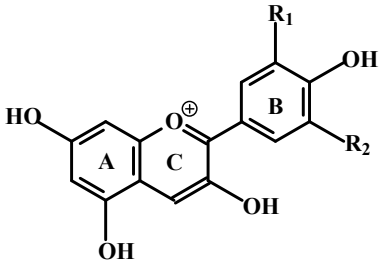


Figure 1.2: Chemical Struture of different types of polyphenols

Anthocyandins



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

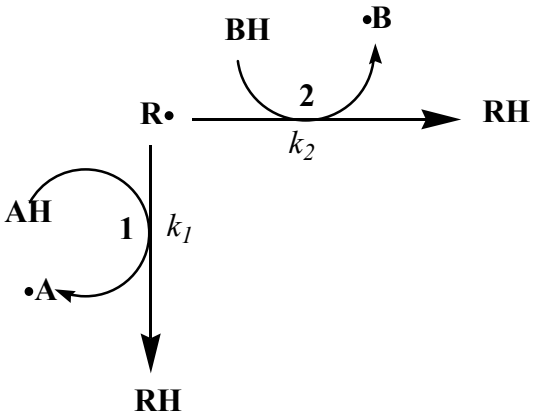
Figure 1.3: Chemical Struture of different types of polyphenols

1.5 Effect of antioxidants on free radicals in food and biological system

Free radicals initiate oxidation of lipids in food systems and leads to the development of rancidity, protein damage, and oxidation of pigments causing a loss of sensory properties, nutritive value, and shelf life of food products (Madhavi *et al*., 1996). The antioxidants in foods increase their shelf-life by preventing lipid peroxidation, thereby maintaining freshness in foods for a long time. They can be incorporated into dairy products, and other food products. Natural antioxidants currently used include ascorbic acid, citric acid, and α-tocopherol. Some synthetic antioxidants that are commonly used are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propyl gallate (PG) (Nawar, 1996). In recent times there has been an

increase in the use of antioxidants in the food industry, not only to increase the shelf life of foods but also as dietary supplements.

In principle, the antioxidant is to prevent the biological molecules from food or *in vivo* depends on its ability to scavenge the radical before it has the opportunity to react with them (Figure 1.3). For example, α-tocopherol (α-TOH), the most effective lipid-soluble chain-breaking antioxidant, reacts with peroxyl radical at a rate constant of about 10^6 $M^{-1}s^{-1}$, which is much faster than the reaction of peroxyl radicals with lipid substrate, typically 10^1 $M^{-1}s^{-1}$ (Ou, 2002).



BH = Biologically important molecule
AH = Antioxidant
R• = Reactive radical
k_1, k_2 =Rate constants for reaction **1** and reaction **2**
$[AH]k_1 >> [BH]k_2$

Figure 1.4: Schematic representation of antioxidant mechanism in food and biological systems.

There is a continuous search for foods rich in antioxidants. Antioxidant phytochemicals in foods especially in vegetables, fruits, and grains are found to have human disease prevention abilities, and may improve food quality (Yu *et al*., 2002). Endogenous antioxidants, such as glutathione present in living cells, alone cannot completely prevent the damaging effects of free radicals (Simic, 1988). Therefore, there is a need for exogenous antioxidants (*e.g.* antioxidants from natural sources) that are widely available from food. Polyphenols are natural antioxidants.

The importance of antioxidants in prevention of diseases and as promoters of good health is widely recognized and studied. Antioxidants are effective in prevention of degenerative illnesses, such as cancers, cardiovascular and neurological diseases, cataracts, and oxidative stress disfunctions (Riemersma *et al*., 1991; Ames *et al*., 1993; Riemersma, 1994; Halliwell, 1996; Schwartz, 1996). Vitamin E, a natural antioxidant shows anticarcinogenic properties because it prevents lipid oxidation and scavenges radicals (Gaby & Machlin, 1991).

1.6 Mechanism of phenolic antioxidants

Phenolic antioxidants are excellent candidates as free radical chain terminators because their radical intermediates, ArO• are relatively stable due to delocalization of the unpaired electron into the aromatic ring (Shahidi and Wanasundara,1992). There are arguments in the literature over the specific ways by which antioxidant mechanism of polyphenols follow. Several researchers claim that phenols donate hydrogen atoms from the phenolic group (Barclay and Vinqvist, 2000; Khopde *et al*., 1999; Masuda *et al*.,

1999; Sun *et al*., 2002). Antioxidants react with radicals by two major mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET) (Nagaoka *et. al*, 1992, Jovanovic *et. al*, 1999, Wright, 2001).

Direct hydrogen atom transfer (HAT)

$ArO\text{-}H + ROO\bullet \rightarrow ROOH + ArO\bullet$ Eqn 1. 11

HAT reactions are solvent and pH independent and are usually quite rapid, typically completed in seconds to minutes.

Single electron transfer (SET)

$ArO\text{-}H + ROO\bullet \rightarrow ROO^{-} + ArOH^{+} \rightarrow ROOH + ArO\bullet$ Eqn 1. 12

In SET mechanism, the reactivity is based primarily on deprotonation (Lemanska, 2001) and ionization potential (IP) (Wright, 2001). In general, IP values are pH dependent and decrease with increasing pH, reflecting increased electron donating capacity with deprotonation. So SET reactions are also pH dependent. SET reactions are usually slow and can require long times to reach completion, so antioxidant capacity calculations are based on percent decrease in product rather than kinetics. HAT and SET reactions may occur in parallel, and the mechanism dominating in a system will be determined by antioxidant structure and properties, solubility and partition coefficient, and system solvent. SET and HAT mechanisms almost always occur together in all samples.

However, the net result of the two mechanisms is the same, *i.e*. a hydrogen atom is transferred from the phenolic antioxidant to the free radical.

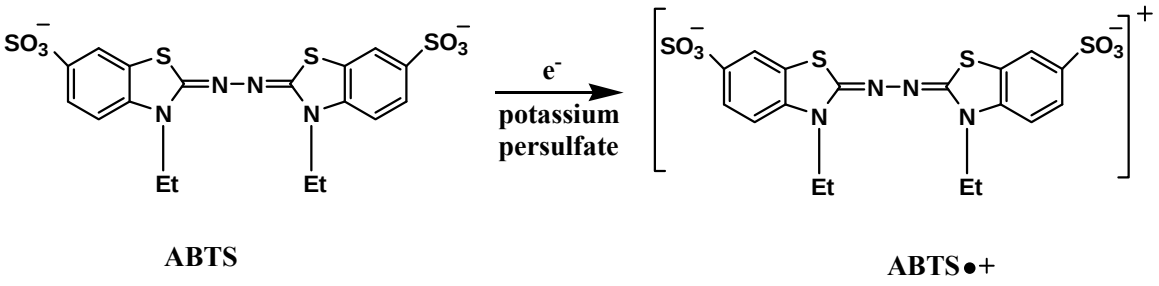
1.7 Experimental methods for antioxidant analysis

Analytical methods must be selected in relation to the specified questions being asked. The characteristic (benefits and disadvantages) of a particular method, such as targeting information (total antioxidant capacity, chemical parameters), sensitivity, and cost, should be considered to determine the most useful methods for a specific situation. Antioxidants can be categorized according to their antioxidant activity. Although the terms antioxidant activity and antioxidant capacity are often used interchangeably in the literature, their meanings are quite distinct. The antioxidant activity corresponds to the rate constant of a single antioxidant against a given free radical. The antioxidant capacity is the measure of the moles of a given free radical scavenged by the sample solution, independently from the antioxidant activity of any antioxidant present in the mixture. As known, free radical generation is directly related to oxidation in foods and biological systems. The radical assay methods to determine antioxidant activity (or free radical scavenging ability) should be more evident.

1.7.1 ABTS radical cation scavenging assay

This method involves the generation of 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) by oxidation (Figure 1.4). According to Cano *et al*. (2002), $ABTS^{\bullet+}$ can be generated by either chemical reaction [*e.g*., manganese dioxide (Miller *et al*., 1996), potassium persulfate (Re *et al*., 1999)] or enzyme reactions [*e.g*., metmyoglobin (Miller *et al*., 1993), hemoglobin, or horseradish peroxidase (Arnao,

1996, Cano *et al*., 2002)]. Generally, ABTS$\bullet^+$ radical generation requires a long time (*e.g*., up to 16 h for potassium persulfate generation), whereas enzyme generation is faster and the reaction conditions are milder.



ABTS **ABTS●+**

Figure 1.5: Formation of ABTS radical cation on oxidation by potassium persulfate

In ABTS assay, the sample containing antioxidants is added to the initially prepared ABTS$\bullet^+$ radical solution. Antioxidants donate electrons to ABTS$\bullet^+$ (Wolfenden and Willson, 1982) to form ABTS, leading to the decrease in absorbance. The drop in absorbance at 730 nm is directly proportional to the amount of ABTS$\bullet^+$ converted into ABTS, and this depends on the antioxidant capacity of the sample. The addition of oxidizing reagents in the ABTS assay to generate radical is considered as a major pitfall, because antioxidants can react with oxidizing agents themselves and, which results in the overestimation of antioxidant capacity (Arts *et al*., 2003 and Strube *et al*., 1997).

1.7.2 Ferric Reducing / Antioxidant Power (FRAP)

Ferric reducing method (Iris and Strain (1996) involves the preparation of a solution, containing Fe^{3+}-TPTZ (ferric 2,4,6-tripyridyl-*s*-triazine) complex in acetate buffer. The

reaction involves the donation of an electron from an antioxidant (AH) to Fe^{3+}-TPTZ complex and thereby reducing it into ferrous form, which is blue in colour. The increase in absorbance at 593 nm is monitored to find out the reducing ability of the sample (Iris and Strain, 1996). The change in absorbance is related to the total ferric reducing / antioxidant power.

$$Fe(III) + ArOH \rightarrow Fe(II) + ArOH^{+} \qquad \text{Eqn 1. 13}$$

FRAP assay has the following disadvantages (Ou *et al*., 2002); It was found that the redox potential of Fe (III) / Fe (II) is 0.77V. So any compound having redox potential lower than this can reduce Fe (III) to Fe (II) resulting in deceptively higher FRAP value. Antioxidant compounds such as polyphenols and thiol compounds reduce Fe (III) very slowly and the reaction does not reach steady state even after several hours of reaction. For such reactions this method is practically meaningless. So only fast-reacting phenols that bind the iron or break down to compounds with lower or different reactivity are best analyzed with short reaction times (Pulido *et al*., 2000).

1.7.3 Oxygen radical absorption capacity (ORAC)

In this assay, 2, 2'-azobis (2-amidino-propane) dihydrochloride (AAPH) radicals are produced by the loss of nitrogen. AAPH radicals so formed react with oxygen (O_2) and this reaction results in the formation of stable peroxy radicals ($ROO\bullet$).

$$R-N=N-R \xrightarrow{O_2} N_2 + 2ROO\bullet \qquad \text{Eqn 1. 14}$$

$$ROO\bullet + FL-H \rightarrow ROOH + FL\bullet \qquad \text{Eqn 1. 15}$$

$$ROO\bullet + ArOH \rightarrow ROOH + ArO\bullet \qquad \text{Eqn 1. 16}$$

Peroxy radicals react with fluorescein (FL-H) causing the loss of fluorescence. In the presence of antioxidants (ArOH), the peroxy radicals are scavenged thus protecting FL-H, and thus reflects classical radical chain breaking antioxidant activity by H atom transfer (Ou *et al., 2001*). The loss in fluorescence is monitored using the spectrometer. A graph is plotted between the fluorescence intensity and time. The area under the curve is proportional to the total antioxidant capacity of a particular sample. The difference in areas obtained without and with the addition of sample ($A_{sample} - A_{blank}$) is used for determination of antioxidant capacity of a sample. Finally, the results are compared with a standard known antioxidant and expressed in its Trolox equivalents (Glazer, 1990; Ou *et al*., 2002).

1.7.4 Total radical-trapping antioxidant parameter (TRAP) method

This method involves the generation of peroxy radicals by thermal decomposition of 2,2'-azobis-(2-amidino propane) dihydrochloride (ABAP) which oxidizes and damages R-pycoerythrin (R-PE), a fluorescent substance, thereby resulting in a decrease of fluorescence (Ghiselli *et al*., 1995). Hence, the basic reactions of the assay are similar to those of ORAC. The TRAP assay involves the initiation of lipid peroxidation by generating water-soluble peroxyl radicals and is sensitive to all known chain breaking antioxidants, but it is relatively complex and time-consuming to perform, requiring a high degree of expertise and experience. Both ORAC and TRAP assays are time consuming

and use fluorescence detector. Moreover, they are temperature sensitive (Ronald *et al*., 2005). Even, small temperature differences can decrease the reproducibility of the assay (Lussignoli, *et al*., 1999).

1.7.5 DPPH radical scavenging assay

The 2,2,diphenyl-1-picrylhydrazyl (DPPH•) assay (Deby and Magottease, 1970) was applied successfully to polyphenolic compounds (Brand-Williams *et al*., 1995) and phenolic acids and derivatives (Silva *et al*., 2000). It is a kind of nitrogen centered radical assay. When this radical reacts with polyphenols, dehydrogenation occurs on polyphenol molecules and DPPH• changes into DPPHH, the structure of which is also shown in Figure 1.5. The absorbance of DPPH• is decreased at 515 nm by the addition of the antioxidant into the solution (Brand-Williams *et al*., 1995; Ancerewick *et al*., 1998).

$$ArOH + DPPH\bullet \rightarrow ArO\bullet + DPPHH \qquad \text{Eqn 1. 17}$$

where ArO• represents the antioxidant radical. From the methodological point of view, DPPH• method is recommended as highly reproducible and comparable to other free radical scavenging methods such $ABTS\bullet^{+}$ (Gil *et al.,* 2000*)*.

DPPH **DPPHH**

Figure 1.6: Structures of DPPH• and DPPHH

Researchers agree that polyphenols inhibits propagation by "trapping" and stabilizing free radical species, such as lipid peroxyl radicals, and that this is done through donation of a hydrogen atom. Wright *et al*. (2001) found that for a large number of phenolic antioxidants, HAT is expected to be the dominant mechanism of reaction. Also, under neutral to acidic conditions and in non-protic solvents, HAT was found to be the preferred antioxidant mechanism of curcumin, a polyphenol used in Asian cuisines which bears the methoxyphenol moiety within its structure (Jovanovic *et al.*, 1999). Masuda *et al*. proposed that the H-atom donation occurred primarily from the phenolic group. Bors *et al*., (1990) and Zhang, *et al*., (2000) also indicated that HAT dominated in phenols. Huang *et al*. (2005) suggested that study on the HAT based assay is the more relevant for explaining the antioxidant activity as the HAT is the key step in the radical chain reaction.

DPPH • assay method has been proven to be adequate for measuring the HAT type antioxidant reactions in effective way (Sanchez *et al*., 1998; Goupy *et al*., 1999 & 2003). DPPH • stimulates reactive oxygen and nitrogen species affecting biological systems (Arnao, 2000; Cevallos-Casals, 2000). Ability of polyphenols to act as radical scavengers

should be discussed from the point that the scavenging (quenching) reaction rate of radicals by the antioxidant. Regardless of antioxidant mechanism, the end result is the same *i.e* hydrogen atom transfer, but kinetics differs. The antioxidant ability of antioxidants is discussed based on the rate at which the radical is scavenged. It is important that assay should not require any additional reagent to measure the chemical parameter to measure the radical scavenging ability without any interference. DPPH• is a readily available radical, which does not have to be generated as in other radical scavenging assays.

1.8 Kinetic study of antioxidant reaction

Without the reaction rate constant, it is difficult, if not impossible, to compare the antioxidant properties with other well established antioxidants. Kinetic information also can be used in food systems to design strategies to inhibit lipid, flavor, and color oxidation and preserve the quality of foods. It can also be used to design strategies to reduce oxidative stress *in vivo*, where antioxidants will scavenge, quench, or interact with superoxide, hydroxyl, and peroxyl radicals, and nitric oxide produced from cell or biochemical reaction systems. The function of antioxidants is to intercept and react with free radicals at a rate faster than the substrate. Reaction kinetics indicates how fast an antioxidant reduces the rate of oxidation. The generally accepted way of their grouping is according to their reaction rate constants toward a chosen radical (Atkinson, 1986; Christopher Evans & Ingold, 1992). The hydrogen atom donating ability generally characterizes antioxidant activity of polyphenols (Pokorny, 1987).

The rate of hydrogen atom transfer is governed by the kinetics of the reaction and also reaction medium in which the reaction occurs (Foti *et al*., 2001; Snelgrove *et al*., 2001; Valgimigli *et al*., 1999; Barclay e al., 1999; Howard *et al*., 1964). Solvent effects on the H-atom transfer between phenols and various radicals were studied in the 90's (Foti *et. al.*, 1994, Barclay *et. al.*, 1999, MacFaul *et. al.*, 1996, Avila *et. al.*, 1995) and are of great importance because the reactions of phenolic antioxidants are relevant to biological systems where reactions take place in aqueous media or in lipid membranes. Also, polyphenols are found to be localized on the polar surfaces of phospholipid bilayers (Ratty, 1988). Hence, the chance of solvents effect in the reaction between antioxidants and radicals should also be taken into account when trying to understand the effectiveness of antioxidants.

The H atom donating capacity of polyphenols is an important biologically significant property**,** in line with the ability of these plant antioxidants to convert potentially damaging reactive oxygen species (oxyl and peroxyl radicals) into non-toxic species. The effectiveness of an antioxidant is determined by several factors, among which the rate measurement of hydrogen atom transfer reactions from the antioxidant molecules to the reactive radicals formed are the most important. Pascale *et al.*, (2002) characterized a series of dietary polyphenols belonging to the most representative families (flavanols, caffeic acid), not only by their total stoichiometries but also by their kinetic parameter (second order rate constant, *k*) of H atom abstraction by DPPH•. Senba *et al.* (1999) analysed the radical scavenging ability of tea catechins using the second order rate constants and activation parameters.

Different approaches can be used to find kinetic parameter (rate constant, *k*) from the spectrometer response, but the most important (Casado *et al.,* 1986) are:

(1) the initial rate method (differential);

(2) the integral method;

All of them have advantages and disadvantages; for instances, the initial rate method takes a short time, but the experimental signal must be known during the early stages of the reaction; the integral method can be very precise, but most of the kinetic curve is needed and so it takes a longer time; the fixed-and the variable time methods do not need data handling and can easily be automated, but their precision is very dependent on, among others, the capacity to reproduce the value of the initial experimental signal. The measurement of the rate constants for radical-molecule reactions by direct time-resolved monitoring of the decay of the radical, generally using UV-visible absorption spectroscopy was first exploited by radiation chemists. The reaction rate can be measured by mixing the free radical solution with antioxidant manually if the reaction takes more than about 20 s (Pedulli *et al*., 2001). Stopped-flow machine (SFM) provides a powerful means of studying rapid reactions involving chemical steps that cannot be monitored by conventional UV-visible spectrophotometer.

One must decide upon a single method or use multiple methods to be more confident on the results obtained in order to explain antioxidant activity. Some polyphenols that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH• due to steric inaccessibility. To avoid this discrepancy, it is decided to include another method,

which could explore the antioxidant reactivity in conjunction with their structural arrangements has been proposed. Theoretical computational methods have been growing in investigating the structure-activity relationships of antioxidants for designing novel and non-toxic antioxidants. Accordingly, some rational-design strategies for antioxidants were proposed and applied in practice (Zhang, 2005).

There is confusion in the literatures that which part of polyphenols (CH or OH) donates its hydrogen atom. Jovanovic *et al*., (1999) proposed that the preferred antioxidant mechanism is that of H-atom transfer from the CH_2 group, especially at pH <7 (Jovanovic *et al*., 1999). In acidic solutions, he argued, the keto form of the molecule dominates, and in this form the C-H bonds are very weak, so the molecule lends itself to the mechanism of donating the H-atom from the central CH_2 group. Based on later research, Jovanovic *et al*. (2001) stated that both the β-diketone moiety and the phenol group are responsible for the exceptional antioxidant properties of curcumin, a polyphenol. Researchers maintain that the antioxidant capability of curcumin does not come from the central CH_2 group, but from the phenolic groups on either side of the molecule. Barclay *et al*. (2000) concluded that the phenolic groups of curcumin, rather than the central CH_2 group, were responsible for its antioxidant capacity. Masuda *et al*. (1993) also believe that it is largely the phenolic groups that contribute to the antioxidant effect of curcumin, with a minimal contribution from the β- diketone group. Sun *et al*. (2002) looked at bond dissociation enthalpies (BDEs) of the OH and CH bonds in curcumin to try to determine the antioxidant mechanism.

Hence theoretical computational studies play an important role in revealing the nature of hydrogen-bonding interactions because direct information, such as the geometry of hydrogen-bonded complexes and the strength of specific binding interactions, can be more readily obtained from calculations compared with experimentation (Wright *et al.*, 2001).

Researchers report that hydrogen atom donation of polyphenols is governed by the OH bond dissociation enthalpy (OH BDE) (Denisov *et al*., 1987, Tanaka *et al*., 1991; Tomiyama *et al*., 1993; Zhu *et al*., 1997; Eugenia *et al*., 1997) because rate of antioxidant-free radical reaction depend on the intrinsic reactivity of the two reactants. These intrinsic reactivities are largely determined by the BDEs (Denisov *et al*., 1987). But the characteristic of polyphenols is that they have many phenolic hydroxyl groups and, as a result, have many active points in radical scavenging. Also it is not currently not clear in the literature which OH (or combination of OH components) in polyphenols are effective in scavenging the free radicals, and what their mechanism of action is. Hence, the present work aimed to investigate BDEs of all OHs in their phenolic structures using theoretical approach (and as an additional method) to explain the antioxidant activity of phenolic compounds.

Numerous theoretical studies addressed thermo chemistry of the related bond breaking of phenol (Lloyd, 1974; Chipman, 1994; Qin, 1995; Nwobi, 1997; Wight, 1997; DeTuri, 1998; Dilabio, 1999; Cabral, 2002; Guedes, 2003). The determination of OH BDE and the correlation of OH BDE with antioxidant activity have received much attention lately

(Zhang, 1999, Wright *et al.*, 2001, Bakalbassis *et al.*, 2003). BDE is not only a physicochemical parameter, but also an important theoretical parameter to characterize the radical scavenging activity. Zhang *et al*., (2002) correlated the OH BDE with free radical scavenging activities. The hydrogen atom transfer (HAT) reactions of phenol with different free radicals have been studied recently by numerous authors (Victor and Siegbahn, 1998; Lundqvist, 2000; Luzhkov, 2001; Mayer, 2002; Wu 2005) using theoretical method especially density functional theory (DFT) techniques.

1.9 Computational chemistry

Due to the rapid advancement in computer technology, theoretical calculations gained popularity amongst the scientific community. Theoretical calculations are broadly classified into the following two categories; Molecular mechanics and Quantum mechanics. Molecular mechanics calculations are based on the laws of classical physics whereas Quantum mechanics calculations are based on the laws of quantum mechanics. Molecular mechanics calculations do not explicitly include the electrons for any molecular system. Since molecular mechanics methods do not treat the electrons explicitly, they cannot be used to deal with problems where electronic effects predominate as in bond breaking and/or bond making, *i.e.* chemical reactions.

1.9.1 Quantum mechanics calculations

Electrons are considered explicitly in quantum mechanical calculations and the potential energy of a molecule is given by the sum of nuclear and electronic energies obtained by

solving the Schrödinger equation. Every quantum chemical calculation aims at obtaining an exact solution to the Schrödinger equation:

$$H\psi = E\psi \qquad \text{Eqn 1.18}$$

Although it is impossible to obtain an *exact* solution to the Shrödinger equation for any system except for the hydrogenic atoms, it is possible to obtain a fairly good approximate solution for a large variety of systems through the use of high performance computers. Quantum mechanical methods can therefore be categorized in the following manner, based on the different of approximations used to solve the Schrödinger equation:

1. Semi-empirical methods.
2. *ab initio* methods.
3. Density functional theory methods.

1.9.2 Semi-empirical methods

Semi-empirical calculations use parameters derived from experimental data to simplify the computation. They are relatively inexpensive and provide reasonable qualitative description of molecular systems and fairly accurate quantitative predictions of energies and structure for systems when good parameter sets exist.

1.9.3 *Ab initio* methods

Ab initio methods do not use any experimental parameters except physical constants such as the speed of light, masses and charges of electrons and nuclei and Planck's constant in their computation. These calculations provide high quality quantitative results for a broad range of systems and can handle molecules in the ground state or excited state and in the

neutral, ionic and radical forms. However, all *ab initio* methods are computationally very demanding.

1.9.4 Density functional theory (DFT)

The basic principle behind the DFT is that the energy of a molecule can be determined from the electron density instead of a wave function. These calculations are based on the Hohenberg-Kohn theorem, according to which, the electron density can be used to determine all properties of a system under consideration (Kohn *et al*., 1996). While *ab initio* and semi-empirical calculations use the wave function, DFT calculations are based upon a strategy of modeling electron correlation via general functions of electron density. Electron correlation takes into account how electrons in a molecular system interact with one another and consequently affect molecular properties. DFT methods are attractive because they include the effects of electron correlation. Thus DFT methods can provide the benefits of some more expensive *ab initio* methods at essentially Hartree-Fock (HF) cost. The DFT functional partitions the electronic energy into several components and computes them separately. The components which arise from the DFT functionals are (i) the kinetic energy, (ii) the electron-nuclear interaction; the coulomb repulsion and (iii) an exchange correlation term. The exchange correlation term plays a key role in DFT, because this term accounts for electron-electron interaction and is divided into separate exchange and correlation components in DFT formulations. However, the efficiency of computational approach depends on the level of theory.

1.9.5 Level of theory

The level of theory plays a kernel role in computational calculations. Regardless of whether the process of optimization is efficient or not, the final geometry obtained will be what is predicted by the level of theory, being used to compute the energy. Each level of theory has its own significance and accuracy.

1.9.6 Basis sets

In general, a basis set is an assortment of mathematical functions used to solve differential equation. In quantum chemical calculations, the term "basis set" is applied to a collection of contracted Gaussians representing atomic orbitals, which are optimized to reproduce the desired chemical properties of a system. Standard *ab initio* software packages generally provide a choice of basis sets that vary both in size and in their description of the electrons in different atomic orbitals. Larger basis sets include more and greater range of basis functions to improve the accuracy. Therefore, a larger basis function can better refine the approximation to the true molecular wave function (De Paz *et al*., 1993), but requires correspondingly more computer resources. Alternatively, accurate wave functions may be obtained from different treatments of electrons in atoms. To improve the molecular integral calculation, Levine, 1999 introduced the usage of Gaussian type functions (GTF) instead of Slater type orbitals (STO) for the atomic orbital in a linear combination of atomic orbitals (LCAO) wave function. Standard basis sets for electron structure calculations use linear combination of Gaussian functions to form the orbitals.

1.9.7 Minimal basis set

Minimal basis set contains the minimum number of atomic orbital (AO) basis functions needed to describe each atom (*e.g.*, 1s for H and He). A typical example of a minimal basis set is STO-3G, which uses three Gaussian type functions (primitives) (3G) per basis function to approximate the atomic Slater type orbital (STO). Although minimal basis set not recommended for consistent and accurate prediction of molecular geometries, their simple structure provides a good tool for visualizing qualitative aspects of chemical bonding.

1.9.8 Split-valence basis set

In split valence basis sets, additional basis functions (one contracted Gaussian plus some primitive Gaussians) are allocated to each valence atomic orbital. The resultant linear combination allows the atomic orbitals to adjust independently for a given molecular environment. Split valence basis sets are characterized by the number of functions assigned to valence orbitals. Contrary to the minimal basis set, split valence basis set uses two or more STO for each valence AO, but only one STO for each inner shell (core) AO. Split valence basis sets such as 3-21G (Hehre, 1969) and 6-31G have two or more sizes of basis functions for each valence orbital. For example, H_2 and C are represented as follows;

H: 1s, 1s

C: 1s, 2s, 2s, 2px, 2py, 2pz, 2px, 2py, 2pz

where the paired and unpaired orbitals are different in size. The number of functions assigned to valence orbital characterizes split valences basis-set. Basis sets developed by

Pople and coworkers (Frisch, *et al*., 1998) are denoted by the number of Gaussian function used to describe inner shell and outer shell electrons. Thus the split-valence basis set 6-31G (Szabo, 1982) describes an inner shell atomic orbital with a contracted Gaussian composed of six primitives and six Gaussians, an inner valence shell with a contracted Gaussian composed of two primitives and outer valence shell with one primitive. Other split-valence shells include 3-21G (Szabo, 1982).

1.9.9 Polarization basis set

Polarization functions can be added to basis sets to allow for non-uniform displacement of charge away from atomic nuclei, thereby improving descriptions of chemical bonding. In order to allow for smaller displacements, the center of electronic charge placed away from the nuclear positions, it is necessary to include functions of higher quantum number (d-type functions on heavy atoms and p-type functions on hydrogen and f functions to transition metals) in the basis set and these are termed as polarization functions. The 6-31G(d) polarized basis set is constructed by the addition of a set of six second-order (d-type) Gaussian primitives to the split 6-31G basis set for each heavy atom. This basis set is also known as 6-31G*. Another popular basis set known as 6-31G(d, p) it is also known as 6-31G** which adds p orbitals to hydrogen atom, in addition, d orbital to the heavy atoms. The addition of p orbitals to hydrogen is particularly important in systems having hydrogen bridging atom. The primary purpose of polarization function is to give additional angular flexibility to the LCAO-MO process in forming the valence molecular orbitals (Jack, 1991). Polarization functions are essential in strained ring compounds because they provide the angular flexibility needed to direct the electron density into regions between bonded atoms.

1.9.10 Diffuse basis set

Species with significant electron density far removed from the nuclear centers (*e.g.* anions, lone pairs, and excited states) require diffuse functions to account for the outer most weakly bound electrons. They allow orbitals to occupy a larger region of space. Diffuse basis sets are recommended for calculations of electron affinities, proton affinities, inversion barriers and bond angles in anions. The addition of diffuse s- and p-type Gaussian functions to non-hydrogen atoms is denoted by a plus sign + as in 6-31+G. Further addition of diffuse functions to both hydrogen and larger atoms is indicated by double plus 6-31++G(d). Diffuse functions on hydrogen atoms seldom make a significant difference in accuracy. The valence and polarization functions described above do not provide enough radial flexibility to adequately describe either of these cases.

1.9.11 High angular momentum basis sets

High angular momentum basis sets consists of split valence basis-set plus polarization and diffuse functions. Larger basis sets add multiple polarization function per atom to the triple zeta basis set. Multiple polarizations are now practical for many systems and although not generally required for a HF calculation, multiple polarization functions are useful for describing the interactions between electrons in electron correlation methods. The examples of high angular momentum basis set are as follows:

* 6-31G (2d) – In this basis set two d functions are added to heavy atoms.

*6-311G (2df, pd) – Besides the (311) valence functions two d functions and one f functions are added to heavy atoms, p and d function are added to the hydrogen atom.

* 6-311G (3df, 2df, p) – three d functions and one f function are added to atoms with Z >11, two d, functions and one f function to first-row atoms (Li to Ne) and one p function to hydrogen. High angular momentum basis sets augmented with diffuse functions represent the most sophisticated basis sets available in the Gaussian program. Most widely used high accurate ab initio calculation would be produced by reasonably sophisticated polarized split-valence basis sets augmented with high angular momentum and diffuse atomic orbitals.

All these methods are available in commercially available software packages such as Gaussian, Hyper Chem and Chem3D. The relative evaluation of antioxidants through molecular descriptors such as OH BDE (Zhang, 1999, Wright *et al.*, 2001, Bakalbassis *et al.*, 2003), ionization potentials (Wright *et al.*, 2001), spin delocalization of phenoxyl radicals (Cheng *et al.*, 2003), enthalpy of electron transfer or chemical hardness (highest occupied molecular orbital, HOMO- lowest unoccupied molecular orbital, LUMO) (Zhang, 1999, Cheng *et al.*, 2003) successfully measured theoretically using the B3LYP density functional theory (DFT) method in Gaussian package.

1.10 Objectives of the study

Consumers have increased their demand for functional foods and nutraceuticals. It seems intuitively unwise to produce therapeutic drugs, supplementation or food fortification until more information is known about the structural activity by which polyphenolic antioxidants differ, and the effects of scavenging on free radicals. It is time-consuming to evaluate the structural effectiveness for antioxidant compound individually as researchers have so far identified over 8000 polyphenols (Harborne, 1994). It is well known that

experimental analysis is costly and eventually out of reach for many research laboratories. On the other hand, high-level *ab initio* calculations in conjunction with BDEs calculation may also be significant. Furthermore, a single antioxidant is usually not present alone in biological or food systems but acts in combination with other antioxidants. It conveys the importance of studying the model phenols, which can represent a majority family of polyphenols structure to interpret the structural activity. To elucidate the structural requisites for activity, it is decided to explore key portions of the structure of polyphenols: the OH group model compounds, catechol moiety (and pyrogallol moiety) compounds, and finally tea catechins.

As the effectiveness of antioxidants (both synthetic and natural) depends on the rate of the reaction, the reaction rate must be sufficiently fast enough that the reaction can be completed in a short time. Stopped-flow spectroscopy technique is adapted in this study for the analysis of polyphenols. **Chapter 2** describes the method used for the kinetic and theoretical analysis in this study.

Chapter 3 describes the selection of phenolic model compounds and kinetic studies aimed at looking into the number and position of hydroxyl group for the effective radical scavenging activity. Efforts were also made to correlate the theoretical parameter BDE with the experimental parameters activation energy (E_a) through comparisons. **Chapter 4** discusses the systematic computational analysis on phenols model aimed at clarifying the active hydroxyl (OH) sites using the calculated OH BDEs. Synthetic phenolic antioxidants such as gallic acid and alkyl esters of gallate are widely used as food additives for scavenging reactive oxygen species that are responsible for the rancidity of

different foodstuffs (Nakagawa *et al*., 1997 & Halliwell, B 1995). Study on the gallate derivatives will provide a useful clue to the design of new and effective antioxidants. **Chapter 5** describes the kinetic and computational studies on catechol gallate moiety. **Chapter 6** describes the kinetic and computational studies on pyrogallol moiety. **Chapter 7** explains the radical scavenging ability of tea catechins. In **Chapter 8**, all the important results obtained in this study are summarized for future research in the antioxidant analysis are emphasized.

2. METHODS USED FOR STUDY

2.1 Rapid kinetic study

To date, very few researchers have applied rapid mixing technologies to the investigation of antioxidant reaction with free radicals (Squadrito, 1995; Allegra, 2003). Monitoring the rate of decomposition of reactant under a variety of conditions is used by the majority of researchers as the sole means of inferring information about an antioxidant mechanism. The advantage of rapid reaction studies is that they allow the researcher to observe an individual antioxidant reaction as it happens. The stopped-flow technique is used to study reactions which occur between 0.001 to 10s.

2.2 Instrumentation

A schematic diagram of the stopped-flow spectrometer system is given in Figure 2.1. The stopped-flow spectrometer was purchased from BioLogic, France. It consists of a regulated light source (150W Xenon lamp), stopped-flow module (SFM 300), and the photomultiplier module. Stopped-flow spectrophotometer is a three–syringe stop flow kinetics system capable of mixing rapidly three solutions at a time (Figure 2.1). The instrument is controlled by Bio-Kine software (version 4.0). The reaction cuvette cell is made up of quartz and has 1.5 mm path length. All SFM syringes, valves and cuvette are enclosed in a water jacket to allow temperature regulations of the reactants and permit kinetic studies between 2°C and 80°C. The temperature of the solution in the stopped-

flow cell can be monitored with a thermistor probe. The syringe plungers are driven by stepping motors.

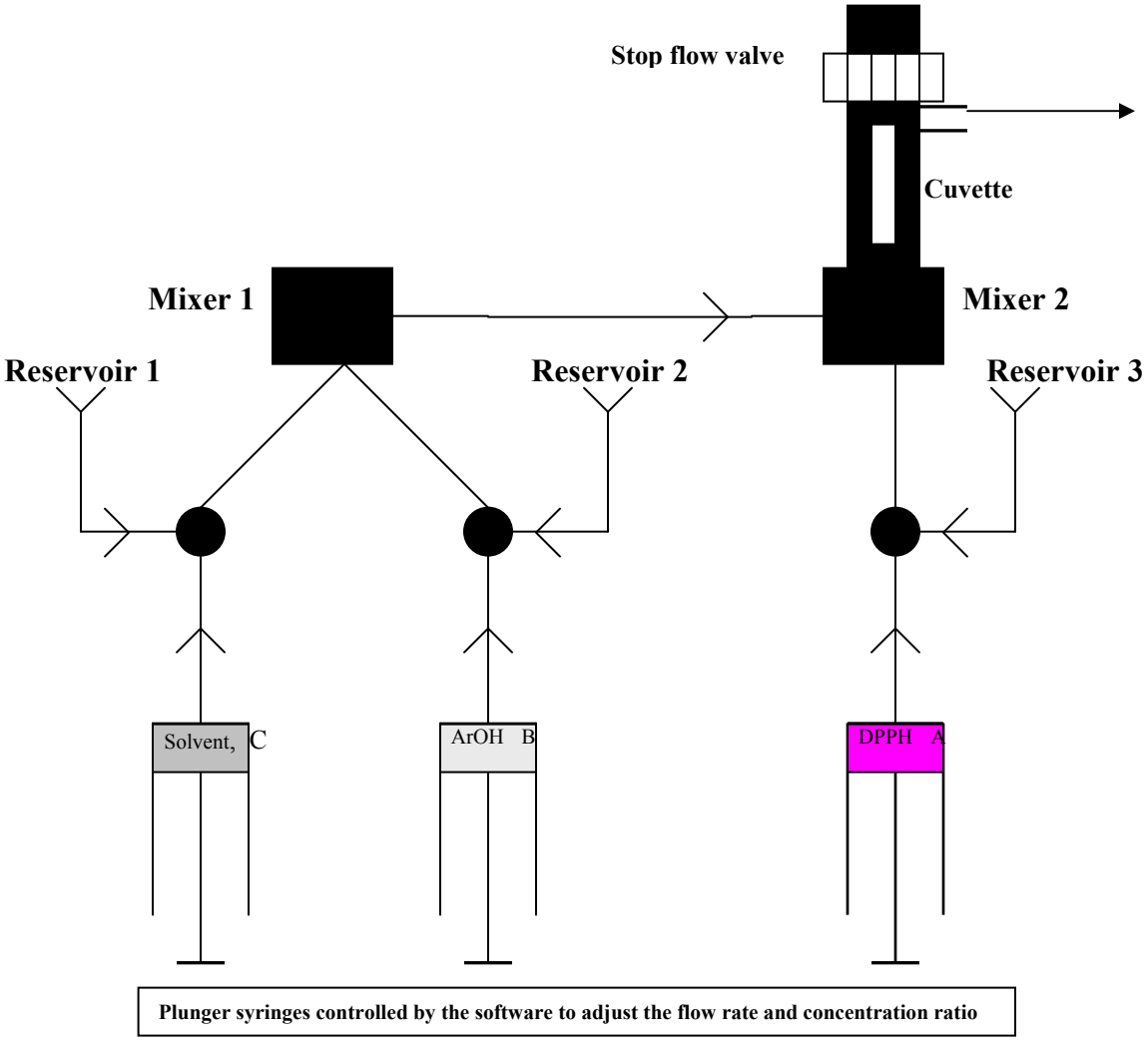


Figure 2.1: Schematic diagram of stopped –flow instrument

2.3 General principle of experiments with the stopped-flow spectrometer technique

The stopped-flow experiment occurs in two stages as shown in Figure 2.2. In the first stage, the flow is initiated by the two syringe plungers. The plungers force reactants A and B through a mixer and along a flow path into an observation cuvette. The resulting mixture of reactants A and B initiates the reaction to form C as it travels along the flow path and into the cuvette. This continues until a steady state condition arises in which the reaction of the reactant mixture is completely linear with respect to the distance along the flow path. Dead time is the time at which the mixed solution (A+B) goes from the centre of the mixer to the observation point. The dead time depends only on the flow rate of the mixture. Thus, as the flow rate is increased, the dead time will be decreased. The second stage of the experiment begins when the flow is stopped. At this point, the reactant mixture A and B in the cuvette becomes stationary and continues to react. Observation of the reactant mixture in the cuvette after the stop therefore represents a time course of the reaction. In our experiments, reactant A (DPPH$\bullet$) has strong absorbance, while reagent B (antioxidant) and C (solvent) do not. Therefore, as the reaction proceeds, the absorbance of a mixture B and C should decrease as A is diminished.

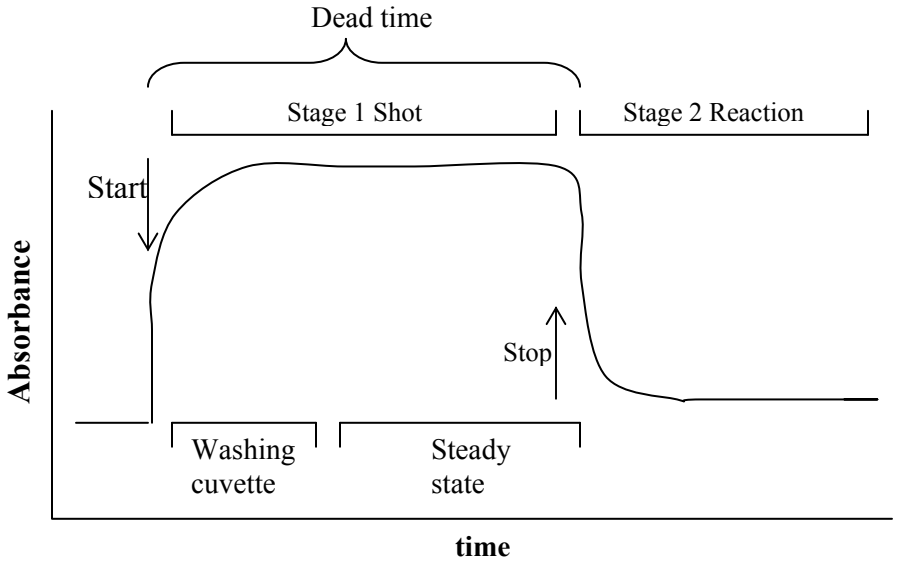


Figure 2.2: Stopped flow experiment time course profile

2.4 Reagents

Catechol, resorcinol, hydroquinone, 1,2,4-benzenetriol, pyrogallol, phloroglucinol, catechol derivatives, gallate esters, tea catechins were purchased from Sigma-Aldrich, Singapore, and were of 95% purity. All solvents used were of HPLC grade obtained from Fisher Scientific, Singapore. 2,2-diphenyl-1-picrylhydrazyl (DPPH•) was also purchased from Sigma-Aldrich, Singapore.

2.5 Kinetic method

2.5.1 Measurement of kinetic rate constants for the reaction of phenols with DPPH•

Huang *et al*. (2005) and Ronald *et al*., (2005) stressed that a standardized assay should meet the following "ideal" requirements: (1) measures chemistry actually occurring in potential application(s); (2) simple; (3) readily available; (4) good within-run and between-day reproducibility. Diphenyl picryl-hydrazyl (DPPH•), a coloured radical assay was proven to meet all the above requirements and successfully used to compare the radical scavenging activity of antioxidants (Blois, 1958; Nanjo *et al*., 1996; Soares *et al*., 1997; Sanchez-Moreno *et al*., 1998; Valgimigli, 1995; Potier, 1999; Dangles, 2000 and Shi, 2000).

Biologic stopped-flow spectrophotometer was employed to measure the decay in absorbance of DPPH• at 515 nm. For our study, one syringe was filled with phenols/solvent and the other syringe was with DPPH•/solvent. Pure solvent was filled up in the last syringe for washing the cuvette after each run. The dead-time for stop-flow mixing was kept 4.6 ms for all run. The total flow rate of DPPH• and ArOH per run was set to 8 mL s^{-1}. The stock concentration 2.5 x 10^{-5}M of DPPH• was prepared for all the phenolic reaction. The concentrations used for the phenols-DPPH• reaction were given in Table 2.1. Phenols were always used in large excess over [DPPH•] to maintain the pseudo order reaction condition. Generally, a minimum of a 20-fold excess is necessary

but 50-fold or 100-fold excess is preferable.* In this study, the pseudo first order rate constants k' were obtained for phenols, in each solvent, with the ratio of excess antioxidant to DPPH• varied in the range of 25-135 times depending on the reactivity of the phenols. The SFM programming allows to obtain the reactants mixing concentration ratios in the cuvette by varying the volume of the reactant solution in the syringes S2 and S3 (see Figure 2.3). The temperature of the solution was controlled by the microprocessor-based digital controller (Polyscience, USA) using ethylene glycol as the circulant. All kinetic measurements were carried out at six temperatures (15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC) with the accuracy of ± 0.2 ºC. All tests were run in triplicate (n=3) and rate constants were averaged.

Table 2.1: Phenol-radical concentration ratio for the kinetic study

Concentration in the Cuvette		**[ArOH]/[DPPH•]**
[DPPH•] 10^{-5} M	**[ArOH]** 10^{-4} M	
1.3	3.23	25
0.8	4.30	54
0.6	4.84	80
0.5	5.16	103
0.4	5.38	135

* http://www.chm.davidson.edu/ChemistryApplets/kinetics/IsolationMethod.html.(Last accessed, 24 January, 2007).

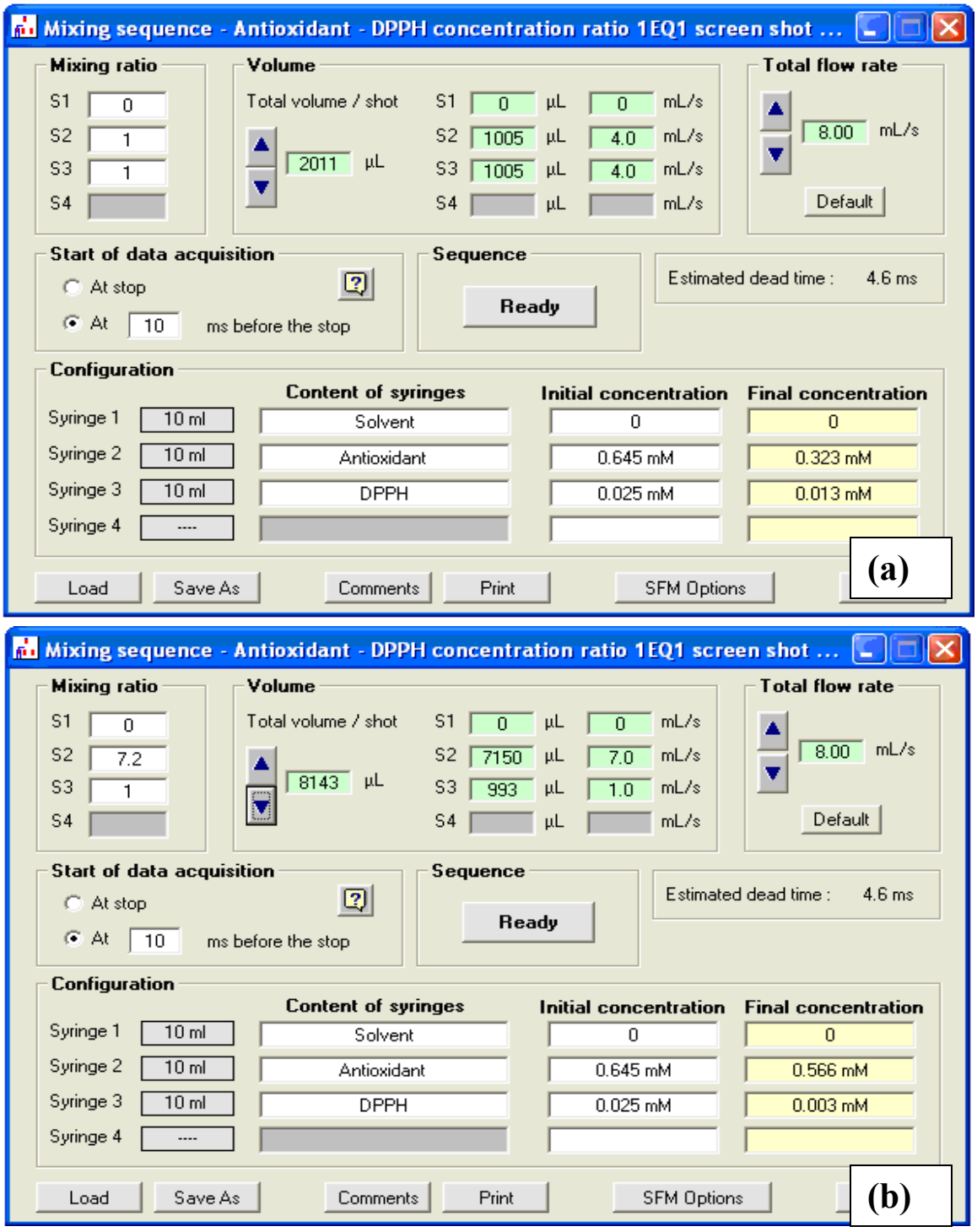


Figure 2.3: Screen shot showing the set up of method for (a) [ArOH]/[DPPH•] ratio of 25 and (b) [ArOH]/[DPPH•] ratio of 188 for phenol-radical reaction

The decay in radical absorbance is observed when hydrogen atom is transferred from the OH of phenol (ArOH) to the radical DPPH•, given in Eqn 2.1.

$$ArOH + DPPH\bullet \rightarrow ArO\bullet + DPPHH \qquad \text{Eqn 2. 1}$$

The rate of the decay of DPPH• can be written as follows;

$$r = -\frac{d[DPPH\bullet]}{dt} = k[ArOH][DPPH\bullet] \qquad \text{Eqn 2. 2}$$

Since the concentration of the phenolic antioxidants (ArOH) are kept constant by having its concentration in excess to the concentration of DPPH•, the equation 2.2 can be rewritten as follows;

$$r = -\frac{d[DPPH\bullet]}{dt} = k'[DPPH\bullet]_0 \qquad \text{Eqn 2. 3}$$

where $k' = k[ArOH]$ since $[ArOH] >> [DPPH\bullet]$

Pseudo first order rate constant k' of phenols obtained after integrating the rate equation 2.3 with the condition [DPPH•] = $[DPPH\bullet]_0$ at t=0;

$$[DPPH\bullet] = [DPPH\bullet]_0 e^{-k't} \qquad \text{Eqn 2. 4}$$

Second order rate constants, k were calculated from the slope by the least squares fitting of plots k' against [ArOH].

2.5.2 Effect of temperature on phenols

2.5.2.1 Measurements of activation parameters

Study of the effect of temperature on the polyphenols is unpopular in an attempt to explain the radical scavenging ability. Comparisons of rate constants at a given temperature may sometimes provide misinformation on reactivity of a compound. Karlin *et al*. (1997) indicated that discussion of activation or thermodynamic parameters should be preferred wherever possible because reaction rate is possibly controlled by the activation parameter (E_a). Arrhenius equation has been successfully used to quantify the effect of temperature:

$$\ln k = \ln A - \frac{E_a}{RT} \qquad \text{Eqn 2. 5}$$

where, k is the second order rate constant ($M^{-1}s^{-1}$), A is pre-exponential factor, R is the gas constant (8.314 J mol^{-1} K^{-1}), E_a is the activation energy (kJ mol^{-1}) and T is the temperature (K). Plot of ln k vs. $1/T$ is linear with slope ($-E_a/R$). The change in enthalpy occurs generally due to the breaking and forming of bonds that accompanies the chemical reaction and hence, the kinetics and thermodynamics for chemical reaction are intimately related (Pilling, 1992). Such a relationship for phenols can be examined using the Eyring's transition state theory (Eyring, 1935) as follows;

$$ArO-H+DPPH\bullet \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} [ArO-H\cdots HPPD]^{\#} \xrightarrow{k_{second}} ArO\bullet + DPPH-H \qquad \text{Eqn 2. 6}$$

$$\ln\frac{k_{second}}{T} = -\frac{\Delta H^{\#}}{R}\frac{1}{T}. + \ln\frac{k_B}{h} + \frac{\Delta S^{\#}}{R} \qquad \text{Eqn 2. 7}$$

The rate constants of phenols increased upon increasing the temperature in all solvents.

$$\ln\frac{k}{T} = -\frac{\Delta H^{\#}}{R}\frac{1}{T} + \ln\frac{k_B}{h} + \frac{\Delta S^{\#}}{R} \qquad \text{Eqn 2. 8}$$

where k is the second order rate constant ($M^{-1}s^{-1}$), T is the temperature (K), $\Delta H^{\#}$ is the activation enthalpy (kJ mol^{-1}), $\Delta S^{\#}$ is the activation entropy (J mol^{-1} K^{-1}), k_B is the Boltzmann constant (1.3807 x 10^{-23} J K^{-1}) and R is the gas constant (8.314 J mol^{-1} K^{-1}). A plot of *ln* (*k/T*) vs *1/T* produces a straight line with slope ($-\frac{\Delta H^{\#}}{R}$) and intercept ($\ln\frac{k_B}{T} + \frac{\Delta S^{\#}}{R}$) to obtain the activation enthalpy ($\Delta H^{\#}$) and activation entropy ($\Delta S^{\#}$) respectively

2.6 Computational method

Density functional theory (DFT) is amenable to large sized molecules with accuracy comparable to traditional *ab initio* methods at a lower computational cost . Since phenolic compounds larger in size, we used DFT approach in this project for our theoretical analysis.

2.6.1 Hardware details

Five different computing systems were used for running the computational calculations. Three systems were low-end Windows XP operating system using Pentium IV 3.2 GHz processors and a fast ethernet-based network. The other systems were the high-end Linux cluster Compaq; HP; SGI; Sun machines using multi processors. Due to the limited access to this parallel computer, only frequency calculations were run on the large number of processors. Figure 2.4 explains the flow chart of steps involved in computational method. Computational methods used are described in detail in the following section.

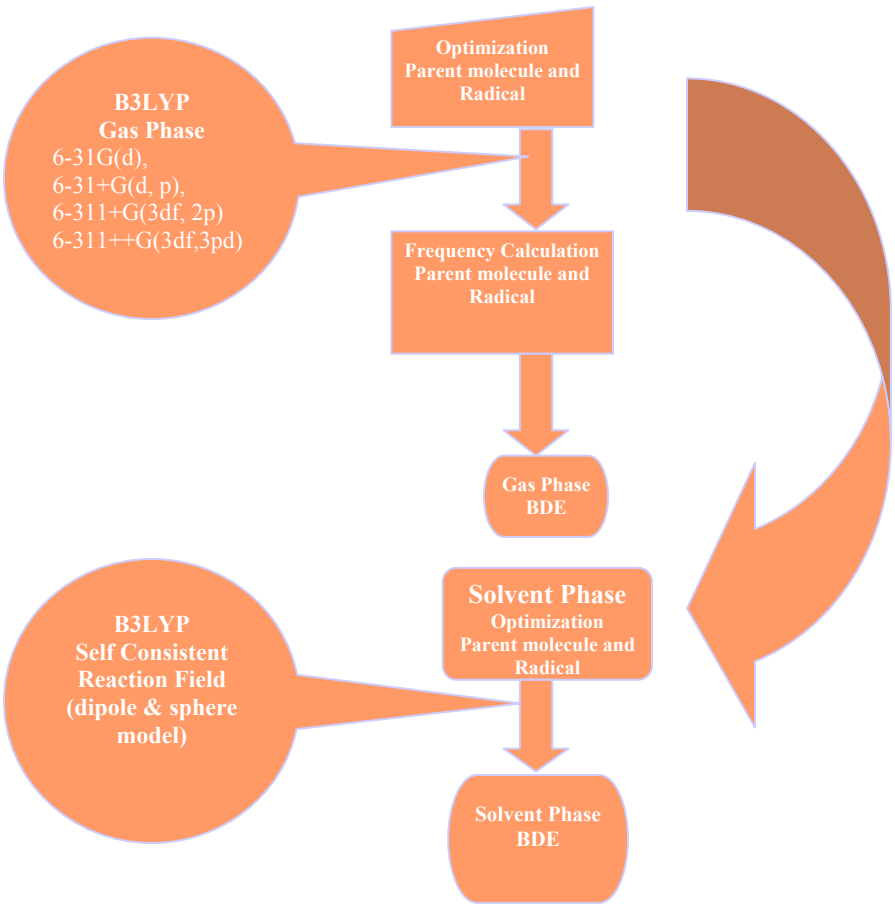


Figure 2.4: Flow chart on the steps involved in the computational method

2.6.2 Theoretical measurement of OH BDE in gas phase

As shown in Equation 2.1, free radical scavenging activity of polyphenols is characterized by its hydrogen atom donating ability to scavenge the radicals such as diphenyl picryl-hydrazyl (DPPH•). Ability to donate hydrogen atom is governed by the OH bond dissociation enthalpy (OH BDE) (Denisov and Khudyakow 1987; Tanaka, *et al*., 1991; Tomiyama *et al*., 1993; Zhu *et al*., 1997; Eugenia *et al*., 1997). In order to

efficiently quench all of the destructive radical intermediates formed during reaction, it was suggested (Denisov and Khudyakow, 1987) that the BDE of an antioxidant should be considerably lower than 368–376 kJ mol^{-1} *i.e*. about 40 kJ mol^{-1} lower than those of peroxides and hydroperoxides. The antioxidant radicals are about 1 x 10^7 times more stable than the peroxyl and hydroperoxyl radicals (Zhu *et al.*, 1997). Accurate estimation of BDE from theoretical calculations is also a challenging task, since high levels of calculation are necessary for taking into account the effect of both dynamical and non-dynamical part of electron correlation. B3LYP method is known to present little spin contamination in the treatment of free radicals with calculated <S2> values being very close to the expected value of 0.75 (Baker *et al.*, 1993).

All gas phase calculations were carried out using the B3LYP method and basis sets 6-31G(d), 6-31+G(d, p), 6-311+G (3df, 2p) and 6-311++G (3df, 3pd), as implemented in the Gaussian 98 program package (Frisch *et al*., 1998). This function was shown to provide accurate geometries for the phenolic systems (Wright *et al*., 1997). Each phenolic compound under investigation can have several possible stable conformations and each stable conformation has an antioxidant radical conformation which is most likely to be produced. Full geometry optimizations and frequency calculations were performed using restricted B3LYP (R-B3LYP) method for the parent molecule and unrestricted (U-B3LYP) for the radical. The OH BDEs at 298.15K were calculated using Equation 2.9, for the most stable ArOH conformer and the weakest ArO-H bond.

$$BDE\ (OH) = H(Ar\text{-}O^{\bullet}) + H(^{\bullet}H) - H(ArO\text{-}H) \qquad \text{Eqn 2. 9}$$

H (*j*) in Equation (2.9) is the enthalpy of chemical *j* at 298.15K and 1 atm. An 0 K enthalpy, H (H) of the hydrogen atom (-0.5 a.u) was used for the BDE calculations (DiLabio *et al*., 1999).

The most stable ArOH compound was calculated to be that conformer which, if possible, could form one or more intramolecular hydrogen bonds (IHB). Furthermore, the weaker the OH bond, the smaller the BDE and the greater the free radical scavenging ability of the antioxidant. The weakest ArO-H bond was determined by first calculating BDE on all the OH sites and equating to the lowest BDE value at the B3LYP/6-31G(d) level. After identifying the weakest OH site in the structure, a bench work was carried out at the four different basis sets {6-31G(d), 6-31+G(d, p), 6-311+G(3df, 2p) and 6-311++G(3df, 3pd)} to optimize the basis set influence on BDEs of all compounds.

The antioxidant reactions occur in condensed phase. Hence, OH BDE calculations were carried out in solution phases as well. The self-consistent reaction field model (SCRF) (Onsager, 1936; Wong *et al*., 1991) was used for the BDE calculation.

2.6.3 Theoretical measurement of OH BDE in solution

The SCRF Onsager reaction field (dipole and sphere) model can simply and substantially describe the interaction between a molecule and its reaction medium (Wong *et al*., 1991). The basic treatment of the solute-solvent interaction is performed by placing the solute in a spherical cavity within a homogeneous solvent reaction field. Hence, a solute radius is required and was computed for all compounds by a gas-phase molecular volume calculation (Using the volume = tight option in the Gaussian program suite). The volume

was then used to calculate solvent effect on the BDEs of all phenols. The optimized gas phase structure of phenols was used as the input for the solution phase optimization step. Frequency calculations were performed after obtaining the solution phase optimized structure at the restricted R-B3LYP and unrestricted (U-B3LYP) for phenols and the radicals respectively. Since SCRF model did not predict any significant difference in the enthalpy value of the hydrogen atom, H_f (H) in solution, the value of -0.5 a.u was used for calculating BDEs in solution.

3. KINETIC STUDY ON PHENOLS

3.1 Introduction

Recently, natural plant polyphenols have been the source for the search of new potent antioxidants against free radicals. More than 8000 compounds were markedly identified as polyphenols (ArOHs), which differ in structures depending on the multiple combinations of hydroxyl, oxygen and methyl groups attached on the two benzene rings of the basic structure of flavonoids (Harborne, 1994) (Figure 3.1). These differences in chemical structure determine their differences in potency (Butkovic *et al*., 2004).

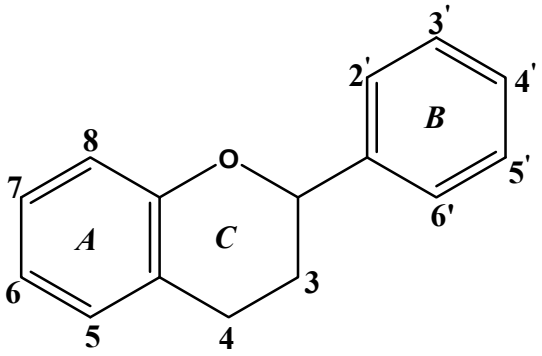


Figure 3.1: Basic structure of flavonoids

More details about the structural activity by which polyphenols differ, and their effects on scavenging the free radicals, would also be useful for producing therapeutic drugs, supplements or food additives. A single antioxidant may not be present alone in biological systems, but acts in combination with other antioxidants. It conveys the importance of studying model compounds, which can be commonly seen in or represent

the majority family of phenolic antioxidants in its structures. Cao *et al*. (1997) reported that the more OH substitutions, the stronger the free radical scavenging activity.
Rice-Evans *et al.* (1996) noticed that quercetin (3,3',4',5,7-pentahydroxyflavone), which has the same number of OHs as morin, but exists in different position (2',3,4',5,7-pentahydroxyflavone), showed better radical scavenging activity than morin.

In this work, it was decided to carry out the study on radical scavenging activity of phenols (catechol, resorcinol, hydroquinone, pyrogallol, phloroglucinol, and 1,2,4-benzenetriol as shown in Figure 3.2) mainly because they are observed as a core in most of the flavonoids structure (Rice-Evans, 2001). Study on solvent effects on the kinetics of the antioxidant reactions should be explored since the solvent is the active medium in facilitating the H- atom transfer from the phenolic antioxidant to the free radical. So the solvent plays a major role in the radical scavenging activity of phenols. Hence, the work is also preceded by investigating the solvent effect on the rates of hydrogen atom abstraction. To our knowledge, no comprehensive study has been carried out for kinetic and thermodynamic analysis of phenols (ArOHs). In the present work, we carried out the kinetic study on radical scavenging activity of phenols was carried out.

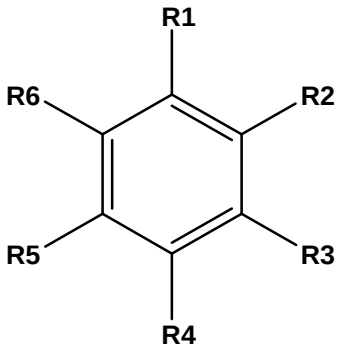


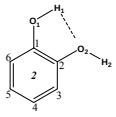
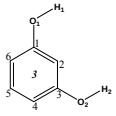
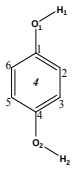
Figure 3.2: Chemical structure of different phenols on the basis of number and position of OHs

Catechol, (Cat)	R1, R2 = OH;	R3, R4, R5, R6 = H
Resorcinol, (Res)	R1, R3 = OH;	R2, R4, R5, R6 = H
Hydroquinone, (HQ)	R1, R4 = OH;	R2, R3, R5, R6 = H
Phloroglucinol, (Phl)	R1, R3, R5 = OH;	R2, R4, R6 = H
Pyrogallol, (Pyr)	R1, R2, R3 = OH;	R4, R5, R6 = H
1,2,4-benzenetriol, (Benz)	R1, R2, R4 = OH;	R3, R5, R6 = H

3.1 Results and discussion

Solvents of different polarity such as polar protic methanol (ε = 33), aprotic acetone (ε = 7.52), aprotic acetonitrile (ε = 36.6) and apolar tetra hydrofuran (THF) (ε = 7.52) were used as the medium for the kinetic study of phenols and at temperatures 15ºC – 40ºC. Calculated rate constants and activation parameters (Arrhenius and Eyring) for both 2-OHs phenols and 3-OHs phenols in all solvents are presented in Tables 3.1 - 3.4. The plot between ln$k$ and activation energy E_a was drawn and shown in Figures 3.3 and 3.4.

Table 3.1: Rate constants (*k*), activation Energy (E_a) of phenolics with 2-OHs in different solvents

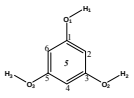
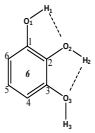
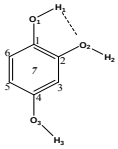
phenols	solvent	Second order rate constant, *k* ($M^{-1}s^{-1}$)						Arrhenius parameters	
		15°C	20°C	25°C	30°C	35°C	40°C	E_a^m kJ mol^{-1}	lnA^m $M^{-1}s^{-1}$
Cat	MeOH[h]	66	98	204	300	455	800	74.8 ±3.1	35.4 ±1.3
	AcN[j]	16	30	45	90	126	300	84.1 ±5.0	37.9 ±2.0
	AcE[k]	10	21	45	78	105	200	87.4 ±4.9	38.9 ±2.0
	THF[l]	0.7	1.8	4.0	7.0	9.5	18	93.6 ±7.0	38.9 ±2.8
Res	MeOH[h]	0.25	0.60	1.50	2.70	5.0	9.0	106.7 ±4.3	43.3 ±1.7
	AcN[j]	0.042	0.13	0.21	0.70	0.92	2.0	113.2 ±7.8	44.3 ±3.1
	AcE[k]	0.03	0.10	0.2	0.475	0.7	1.8	116.6 ±6.8	45.4 ±2.7
	THF[l]	0.004	0.01	0.03	0.06	0.13	0.8	150 ±12.1	56.9 ±4.9
HQ	MeOH[h]	26	50	80	137	238	430	82.4 ±1.9	37.7 ±0.7
	AcN[j]	5.0	8.0	16	31	54	100	91.5 ±2.5	39.7 ±1.0
	AcE[k]	3.0	5.0	9.0	18	40	58	93.1 ±4.2	39.9 ±1.7
	THF[l]	0.4	1.1	3.2	5.0	7.0	13	100.7 ±9.0	41.4 ±4.0

Abbreviations: [h]methanol, [j]acetonitrile, [k]acetone, [l]tetrahydrofuran.

Data are presented as the mean ± standard error.

n=3

Table 3.2: Rate constants (k), activation Energy (E_a) of phenolics with 3-OHs in different solvents

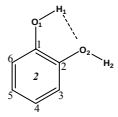
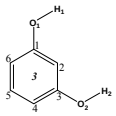
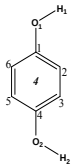
Phenols	solvent	Second order rate constant, k ($M^{-1}s^{-1}$)						Arrhenius parameters	
		15°C	20°C	25°C	30°C	35°C	40°C	E_a^m kJ mol^{-1}	$\ln A^m$ $M^{-1}s^{-1}$
Phl	MeOH[h]	0.2	0.5	1.9	3.0	4.2	7.8	108.3 ±11.6	43.9 ±4.6
	AcN[j]	0.009	0.02	0.05	0.15	0.4	1.0	143.9 ±6.0	55.3 ±2.4
	AcE[k]	0.008	0.014	0.044	0.15	0.3	0.9	145.7 ±7.3	55.8 ±2.9
	THF[l]	0.001	0.011	0.023	0.06	0.15	0.26	157.5 ±18.3	59.6 ±7.3
Pyr	MeOH[h]	620	990	1700	2185	3600	6000	66.2 ±2.8	34.0 ±1.1
	AcN[j]	291	450	740	1250	1900	2700	68.5 ±1.6	34.3 ±0.6
	AcE[k]	202	336	560	892	1400	2200	71.5 ±0.3	35.2 ±0.1
	THF[l]	16	51	63	145	190	250	79.7 ±9.6	36.4 ±3.9
Benz	MeOH[h]	364	560	998	1444	2325	3427	67.9 ±1.5	34.3 ±0.6
	AcN[j]	191	360	522	928	1434	2100	71.6 ±2.2	35.2 ±0.9
	AcE[k]	223	340	565	919	1478	2338	71.3 ±1.2	35.1 ±0.5
	THF[l]	12	32	54	105	145	202	83.2 ±7.4	37.4 ±3.0

Abbreviations: [h]methanol, [j]acetonitrile, [k]acetone, [l]tetrahydrofuran.

Data are presented as the mean ± standard error.

n=3

Table 3.3: Activation enthalpy ($\Delta H^{\#}$), and entropy ($\Delta S^{\#}$), free energies of activation ($\Delta G^{\#}$) of phenolics with 2-OHs in different solvents

phenols	Solvent	Eyring Parameters		
		$\Delta H^{\#}$ kJ mol^{-1}	$\Delta S^{\#}$ J mol^{-1} K^{-1}	$^{p}\Delta G^{\#}$ 25°C kJ mol^{-1}
Cat	MeOH[h]	72.3 ±3.1	41.13 ±1.9	60.07
	AcN[j]	81.6 ±4.9	61.09 ±1.8	63.36
	AcE[k]	84.9 ±6.9	70.26 ±1.7	63.95
	THF[l]	91.1 ±4.9	70.54 ±1.8	70.09
Res	MeOH[h]	104.4 ±4.2	106.72 ±1.8	72.62
	AcN[j]	110.7 ±7.8	114.66 ±1.7	76.53
	AcE[k]	114.1 ±6.8	124.03 ±1.8	77.13
	THF[l]	147.5 ±12.1	220.11 ±1.6	81.89
HQ	MeOH[h]	79.9 ±1.8	59.98 ±1.9	62.04
	AcN[j]	88.9 ±2.5	76.99 ±1.9	66.03
	AcE[k]	90.6 ±4.2	78.21 ±1.8	67.27
	THF[l]	98.2 ±9.9	90.96 ±1.6	71.07

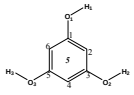
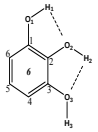
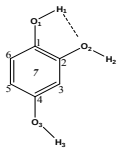
Data are presented as the mean ± standard error.

Abbreviations: [h]methanol, [j]acetonitrile, [k]acetone, [l]tetrahydrofuran

$^{p}\Delta G^{\#}_{25^{\circ}C} = \Delta H^{\#} - T\Delta S^{\#}$

n=3

Table 3.4: Activation enthalpy ($\Delta H^{\#}$), and entropy ($\Delta S^{\#}$), free energies of activation ($\Delta G^{\#}$) of phenolics with 3-OHs in different solvents

phenols	Solvent	Eyring Parameters		
		$\Delta H^{\#}$ kJ mol^{-1}	$\Delta S^{\#}$ J mol^{-1} K^{-1}	$^{p}\Delta G^{\#}$ 25°C kJ mol^{-1}
Phl	MeOH[h]	105.75 ±11.6	111.28 ±1.6	72.59±5.8
	AcN[j]	141.45 ±4.23	206.11 ±1.8	80.02±2.0
	AcE[k]	143.14 ±7.3	210.37 ±1.7	80.45±5.0
	THF[l]	155.03 ±18.3	241.90 ±1.4	82.94±9.8
Pyr	MeOH[h]	63.75 ±2.7	30.17 ±1.9	54.76±2.3
	AcN[j]	66.01 ±1.6	31.63 ±1.9	56.58±1.8
	AcE[k]	68.99 ±0.3	39.13 ±1.9	57.34±1.1
	THF[l]	77.21 ±9.6	49.04 ±1.7	62.59±5.0
Benz	MeOH[h]	65.43 ±1.5	31.55 ±1.9	56.03±1.6
	AcN[j]	69.14 ±2.2	39.54 ±1.9	57.36±2.0
	AcE[k]	68.76 ±1.2	38.70 ±1.9	57.23±1.5
	THF[l]	80.59 ±7.3	57.89 ±1.8	63.34±4.5

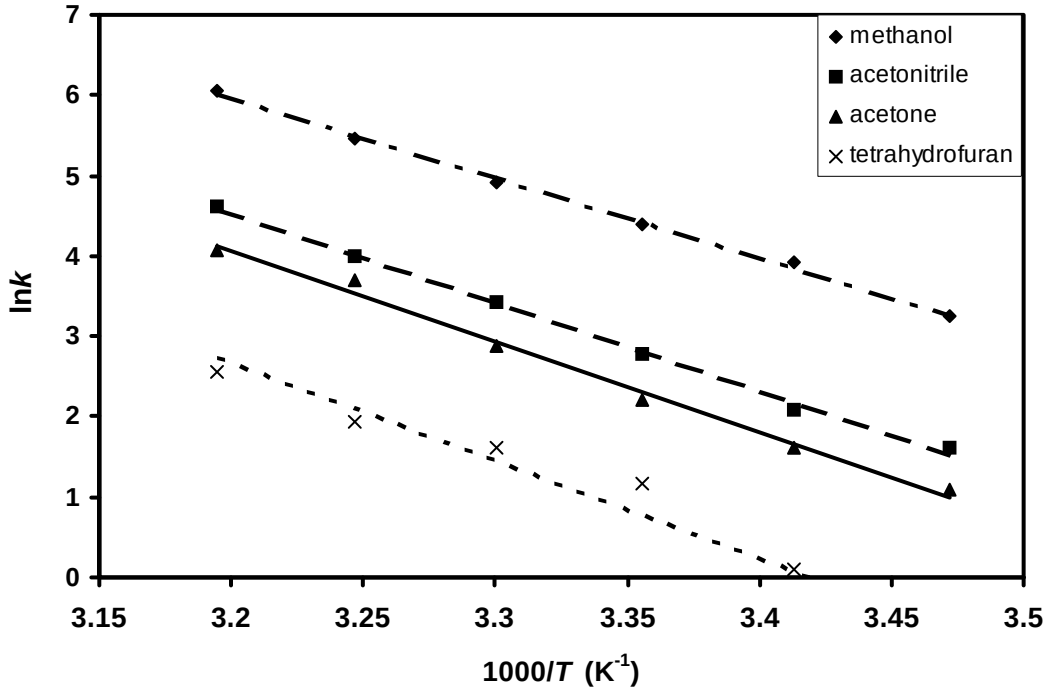
Abbreviations: [h]methanol, [j]acetonitrile, [k]acetone, [l]tetrahydrofuran

$^{p}\Delta G^{\#}_{25^{\circ}C} = \Delta H^{\#} - T\Delta S^{\#}$

Data are presented as the mean ± standard error.

n=3

Figure 3.3: Arrhenius plot for catechol (2-OHs *ortho* phenol) in different solvents



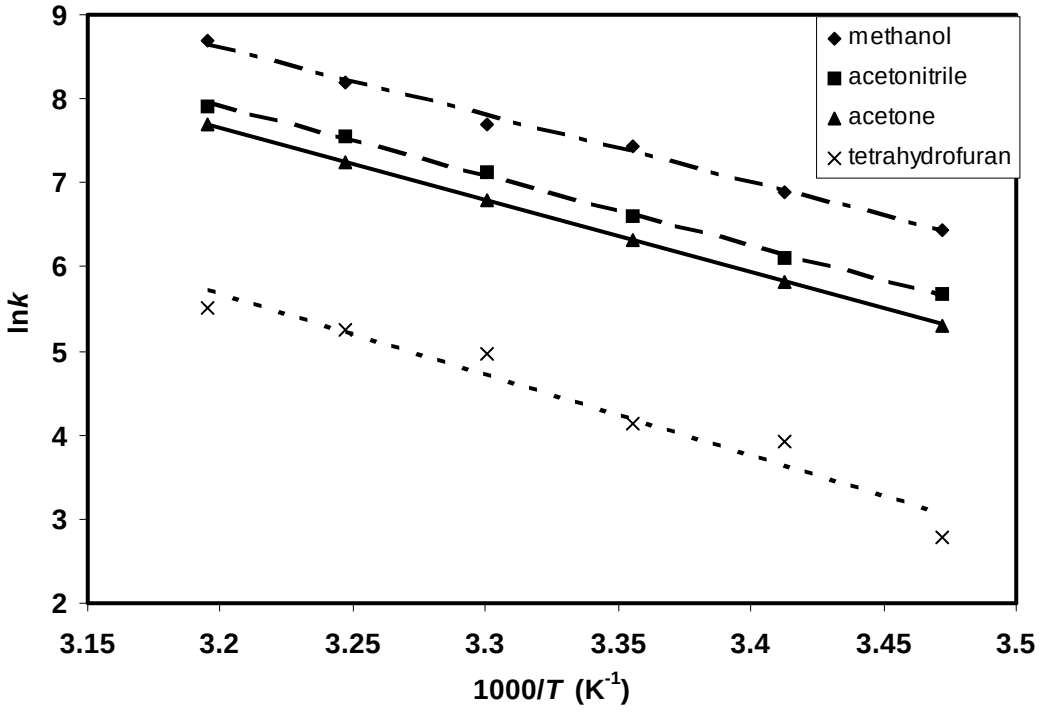


Figure 3.4: Arrhenius plot for pyrogallol (3-OHs *ortho* phenol) in different solvents

3.1.1 Effect of 2-OH phenols

Among the 2-OHs phenols, the rate constants *k* of catechol was found to be highest at all temperatures and in all solvents, followed by hydroquinone and resorcinol. Arrhenius plot of *ln k vs. 1/T* was plotted for all 2-OHs phenols. Only the plot for catechol in solvents is shown in Figure 3.3 and the rest were given in the Appendix I. The calculated activation barriers (both activation energy E_a and enthalpy $\Delta H^{\#}$) were found to be lowest for catechol (*ortho* OH) followed by hydroquinone (*para* OH), whereas for resorcinol (*meta* OH), it was the largest. Whereas *ortho* OH phenol has the fastest radical scavenging activity against DPPH• *meta* OH phenol has the slowest. The *ortho* OH phenol showed an E_a value by *ca*. 30-50 kJ mol^{-1} lower than that of *meta* OH phenol, and 8-12 kJ mol^{-1} lower than that of *para* OH phenol. This indicates that the *ortho* OHs play dominant role in the rate of radical scavenging reaction. On the other hand, comparing the kinetic data of resorcinol and hydroquinone (Table 3.1), it can be observed that the *para* OH showed lower E_a than that of *meta* by about 25-50 kJ mol^{-1}. Hence, the importance of *para* position in 2-OH phenols can also be inferred. The study on 2-OH phenols revealed that the reactivity of phenols against the free radical follows the order: *ortho* > *para* >> *meta*.

3.1.2 Effect of 3-OH phenols

It can be seen in Table 3.2 that the pyrogallol (3-OHs in the vicinal position) showed the largest rate constants of all the 3-OHs phenols studied in this work and in all solvents. Only the plot for pyrogallol in all solvents is shown in Figure 3.4 and the rest are given in

the appendix I. The activation barrier (E_a and $\Delta H^{\#}$) was found to be the lowest for pyrogallol. Pyrogallol, which possesses double-*ortho* OH arrangement in its structure, showed the E_a *ca*. 2 – 3 kJ mol^{-1} lower than that of 1,2,4-benzenetriol which has one *ortho* and one *para* OH arrangement. Hence, on analyzing the structure and kinetic results of pyrogallol and 1,2,4-benzenetriol (Tables 3.2 and 3.5), the more dominant role of *ortho* position can be inferred.

3.1.3 Comparison of 2 and 3-OH phenols

Phloroglucinol, which possesses 3-OHs positioned *meta* to each other, showed smallest rate constants and highest activation energy E_a in all solvents, which is quite similar to the results obtained for 2 *meta* OH phenol (resorcinol). This result indicates that the arrangement of OH in *meta* position do not contribute to radical scavenging activity of phenols irrespective of the number of OHs. The E_a of pyrogallol (3-OHs in two-*ortho* OH arrangement) is calculated to be lower (*ca*.10 kJ mol^{-1}) than that of catechol (2-OHs in one-*ortho* OH arrangement) in all solvents. It indicates that the more number of *ortho* OHs in phenols, the lower the E_a and hence the faster the rate of radical scavenging reaction. Ideally, both catechol and hydroquinone could display the equal number of resonance structures in their radical form, however, catechol showed the larger rate constants *k*. Similarly, pyrogallol and 1,2,4-benzenetriol could also exhibit same number of resonance structures in their radical form due to the electron delocalization, but the pyrogallol showed lowest activation barrier E_a. In catechol, pyrogallol and 1,2,4-benzenetriol, the intramolecular hydrogen bond (IHB) is expected to arise due to the

presence of *ortho* OH (see Figure 3.5). Ingold and co-workers (1981) reported that the rate constant for H-atom abstraction depends on the degree of stabilization of the aroxyl radical. In our case, the intramolecular bond is developed due to the *ortho* arrangement of OHs of phenols and that could bring the more stability of the aroxyl radical, and thus render faster radical scavenging activity. A greater number of *ortho* OHs could bring more stability via more IHBs to radical and this could account for the fastest radical scavenging activity of pyrogallol (see Figure 3.5).

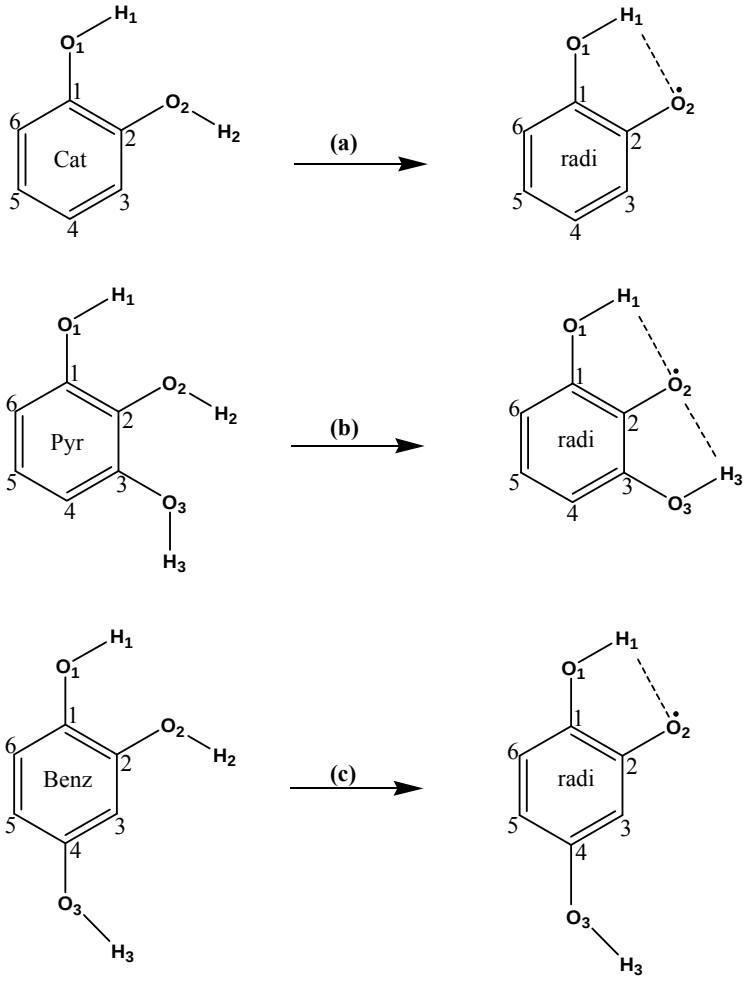


Figure 3.5: Intramolecular hydrogen bond (IHB) exerted stability of aroxyl radical derived from (a) catechol, (b) pyrogallol and (c) 1,2,4-benzenetriol.

In all phenols, lower $\Delta G^{\#}$ was found to be associated inversely with larger rate constants. The activation enthalpy, $\Delta H^{\#}$ followed the same trend with calculated activation energy, E_a. Positive values of activation entropy ($\Delta S^{\#}$) were obtained for all the phenol-radical reaction in this study, which indicated that the higher rigidity of the reactant state could be obtained compared with the transition state. Linear dependences between enthalpy and entropy were observed for the phenols-radical reaction (Figure 3.6) within temperatures 15°C-40°C in all solvents, which suggest that a single mechanism operates along the solvents. From the kinetic study of both the 2 and 3-OHs phenols, it is clear that the rate of radical scavenging reaction is sensitive to the position of OHs of phenols, and not to the number of OHs.

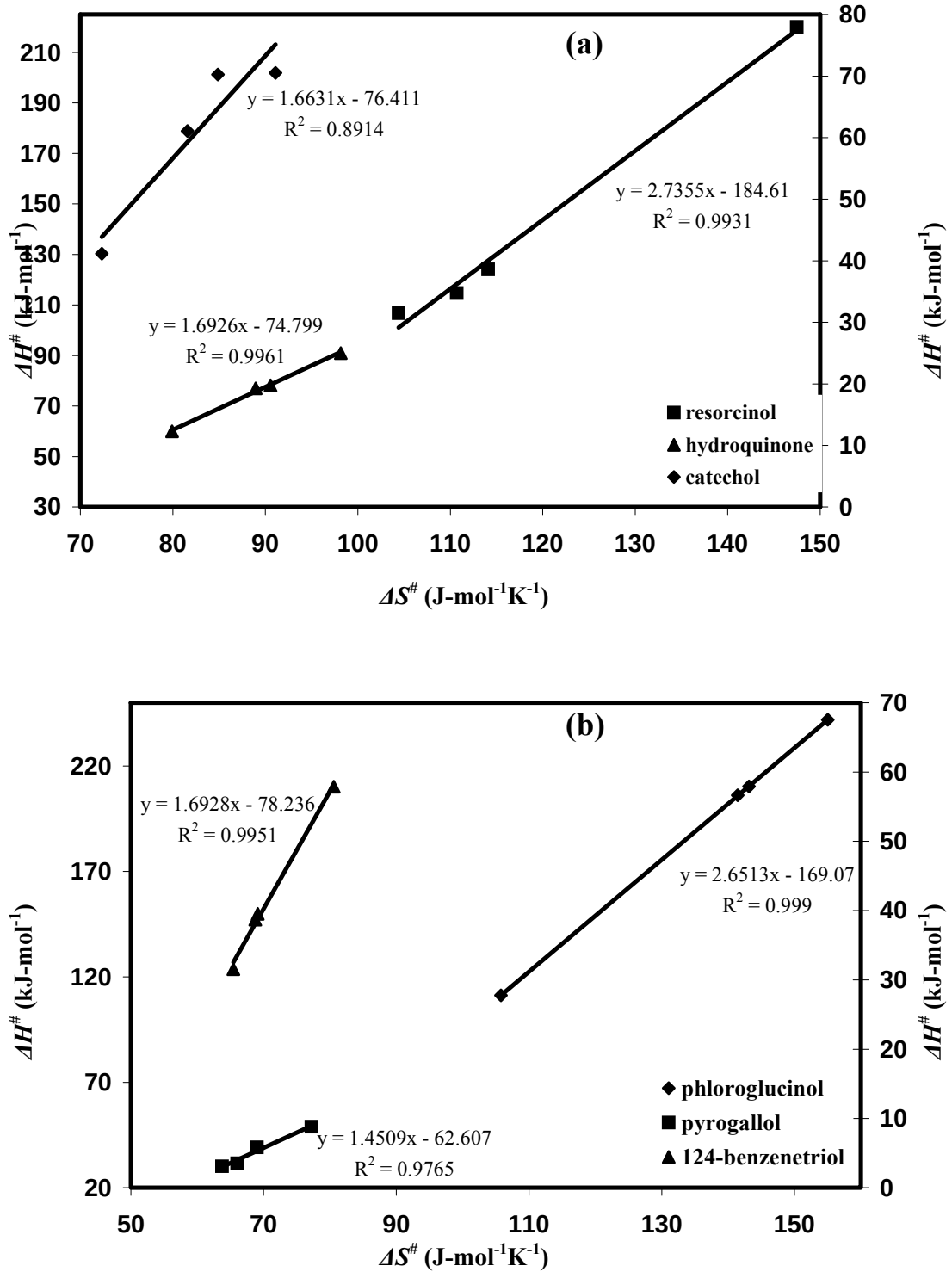


Figure 3. 6: Activation enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) compensation for (a) phenolics with 2-OHs and (b) 3-OHs.

3.1.4 Effect of solvation

Activation energy of all phenols was plotted against the reaction medium and shown in Figure 3.7. Activation energy of all phenols was found to be the lowest in methanol, whereas it was the highest in THF.

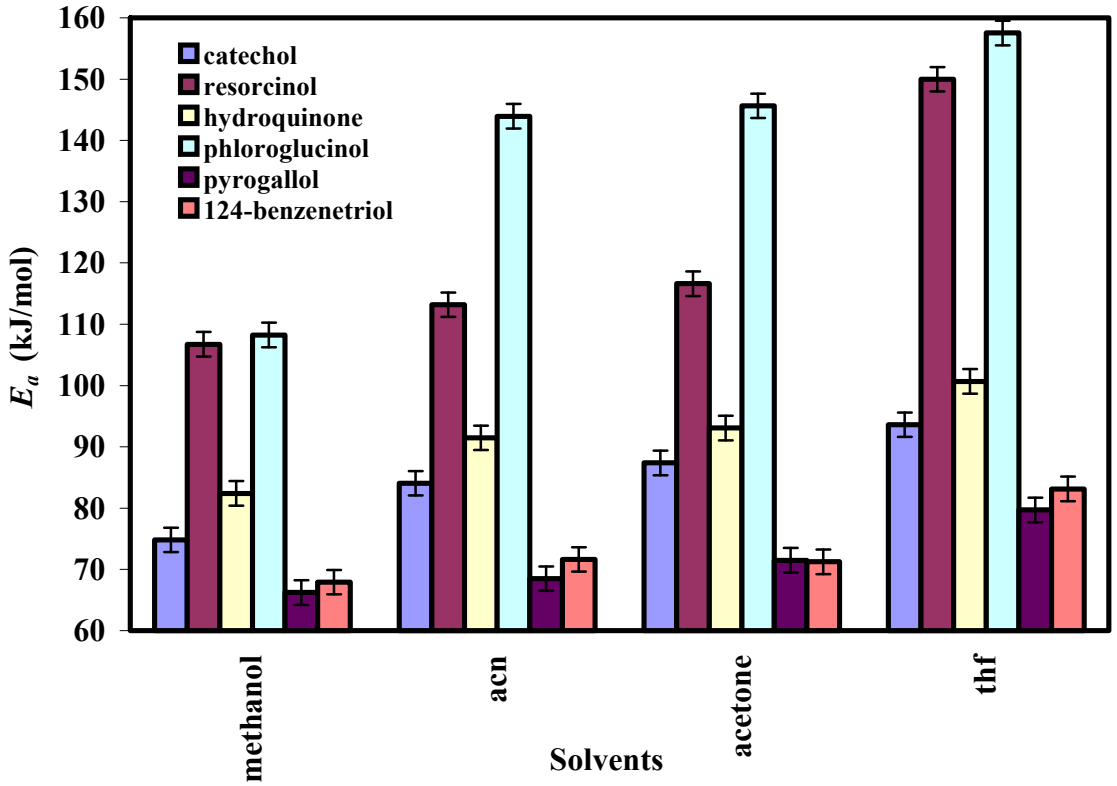


Figure 3.7: Plot of experimental activation energy E_a with respect to different solvents

Rate constants of catechol were found to be highest in polar protic methanol compared to polar aprotic acetonitrile (3.5 times) and acetone (4-6 times). As seen in Tables 3.1 and 3.2, highest activation energy was noticed for pyrogallol and 1,2,4-benzenetriol in polar

protic, methanol; and lower in non-polar solvent, THF. Acetonitrile is more polar than methanol, so the reaction was expected to be faster in methanol. However, it was noticed in this study, reactivity in methanol (ε = 33) was the fastest, followed by acetonitrile which has a dielectric constant (ε = 36.6) higher than methanol. These differences in the rate constants could result from the increased radical scavenging ability of the phenols in the presence of a polar protic medium. Methanol, a polar protic solvent, favors free radical scavenging because the nucleophile and proticity of the solvent helps to release the hydrogen from the OH of phenolic antioxidant. As shown in Figure 3.8, the possibility of continuous regeneration of phenols from its aroxyl radical by the abstraction of methanolic hydrogen could provide more phenolic hydrogen available to be donated to the radical, which may account for the fastest kinetics in protic medium. Hence, protic solvents could accelerate the overall process of H atom transfer.

Of all the phenols, pyrogallol was found to have the lowest activation parameters for the reaction with free radical in methanol. In pyrogallol (Figure 3.9), the hydrogen, H_2 could suffer strong pull effect due to the presence of intramolecular and intermolecular hydrogen bonds with methanol. Hence, H_2 is expected to be dissociated first in the hydrogen atom transfer process. Our recently published work on computation study also suggested that H_2 could be the possible hydrogen atom to be donated to the free radical first based on their OH BDEs (Thavasi *et al*., 2006). On comparing the kinetics of pyrogallol with 1,2,4-benzenetriol on the basis of all possible interaction, pyrogallol has additional intramolecular hydrogen bonding (IHB) attraction at H_2 from O_3 due to the presence of a second *ortho* OH in its structure. This could cause the donation of hydrogen

atom more efficiently and the reason for their faster radical scavenging ability. The effect of solvents on the thermodynamic and kinetic behavior of catechol and 1,2,4-benzenetriol was also significant (Tables 3.1-3.4). Catechol and 1,2,4,-benzenetriol like pyrogallol could also experience the pull effect at the region H2 as shown in Figures 3.10 and 3.11 due to the protic nature of solvent and IHB developed *ortho* OH. Hence, both *ortho* and polar protic nature of solvent could play an important role in increasing the kinetic reactivity of phenols against the free radical.

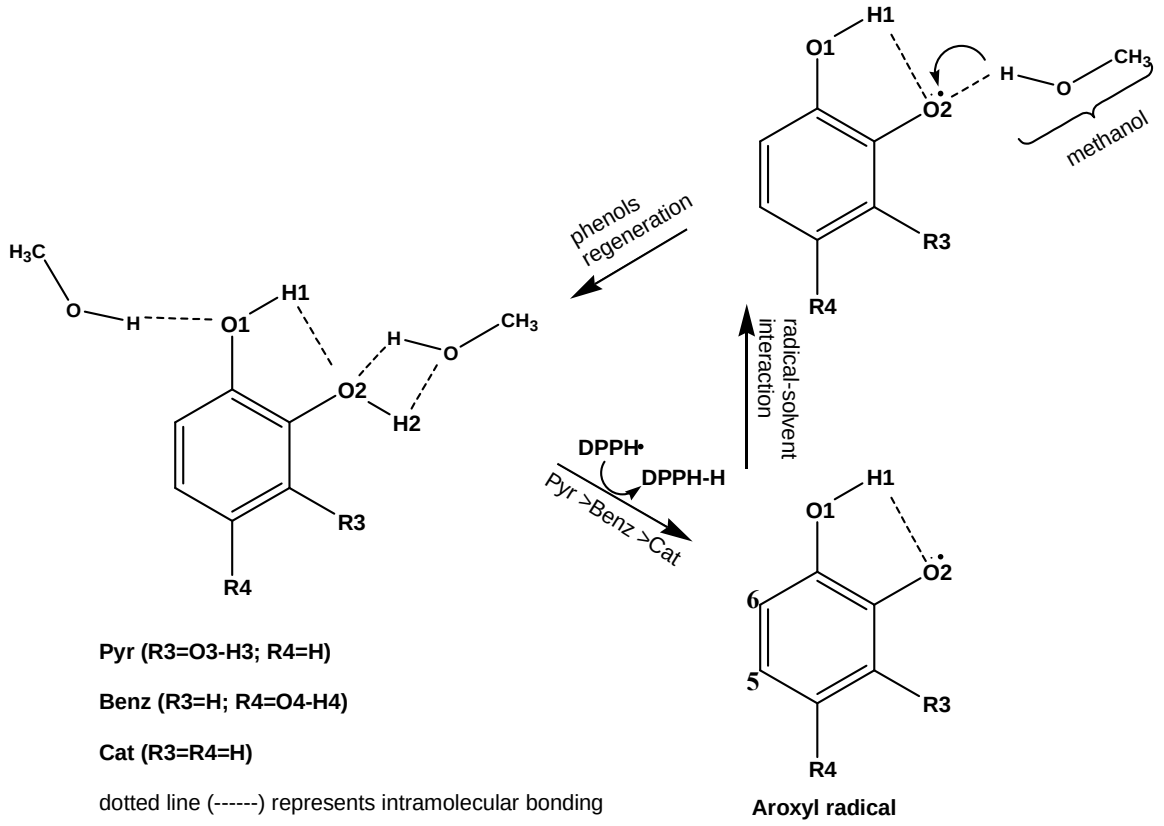


Figure 3.8: Polar protic solvent effects on both parent phenols and radical

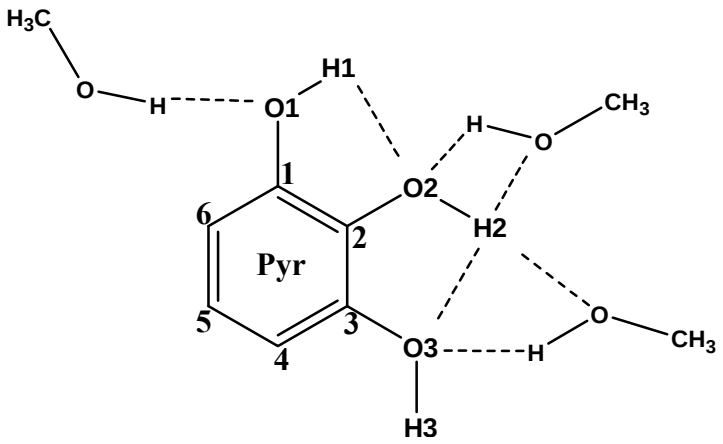


Figure 3.9: Possible *ortho* and polar protic solvent (methanol) interactions on pyrogallol

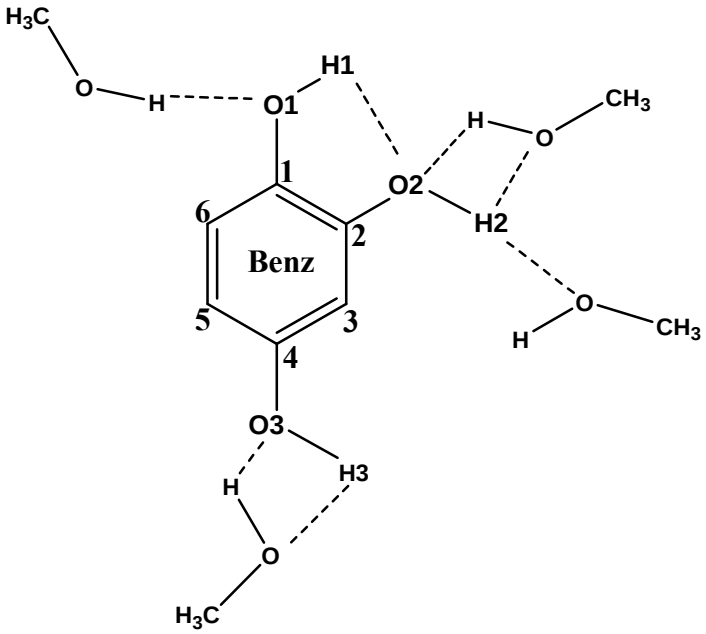


Figure 3. 10: Possible polar protic solvent interactions on 1,2,4-benzenetriol

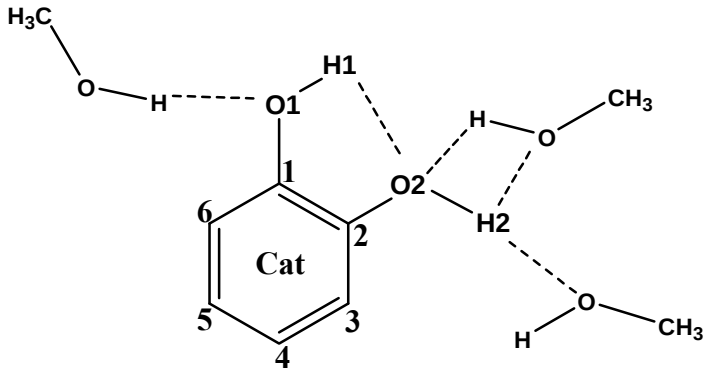


Figure 3.11: Possible polar protic solvent interactions on catechol

Radical scavenging activity was found to be higher in acetonitrile than that of acetone, which are in good agreement with the results reported in the literatures on reactivity of diols in acetonitrile and acetone (Foti *et al*., 2001). The difference in the kinetic activity of phenols within the polar aprotic solvents acetonitrile and acetone could be due to the differences in their ability to engage through intermolecular interaction with the OH of phenols. Figure 3.12 shows that interaction between phenols and acetonitrile is weaker due to the lower electronegative nature of N atom. This may enable the phenols to release their H atom considerably faster in acetonitrile compared to acetone and THF. Higher electronegative nature of O atom in acetone and THF could exert stronger interaction with the OH of the phenols (Figure 3.12). As a result, the hydrogen donation is restricted, which could be the reason for their slower rate of reactants.

And from polar methanol to apolar THF, the thermodynamic and kinetic behaviors of all phenols were observed to be very slow in THF. Decrease in rate constants are accompanied by the increase in activation enthalpy. A similar decrease in the reactivity of the phenolic reaction with DPPH• was measured by Litwinienko and Ingold (2003) on

changing from acetonitrile to THF. These differences in the rate constants could have resulted from the decreased ability of the phenols to react with the free radical because of the significant contribution of the solvent medium. A very strong intermolecular attraction between the OH of phenols and lone pair oxygen atom in THF was expected due to the low dipole moment and strongest electronegative nature of O atom, which could limit the number of 'free' phenol available for hydrogen atom abstraction (Figure 3.12). Since hydrogen abstraction is prevented, the reactivity is reduced in THF, which reflected in reaction rate constants shown in Tables 3.1-2.

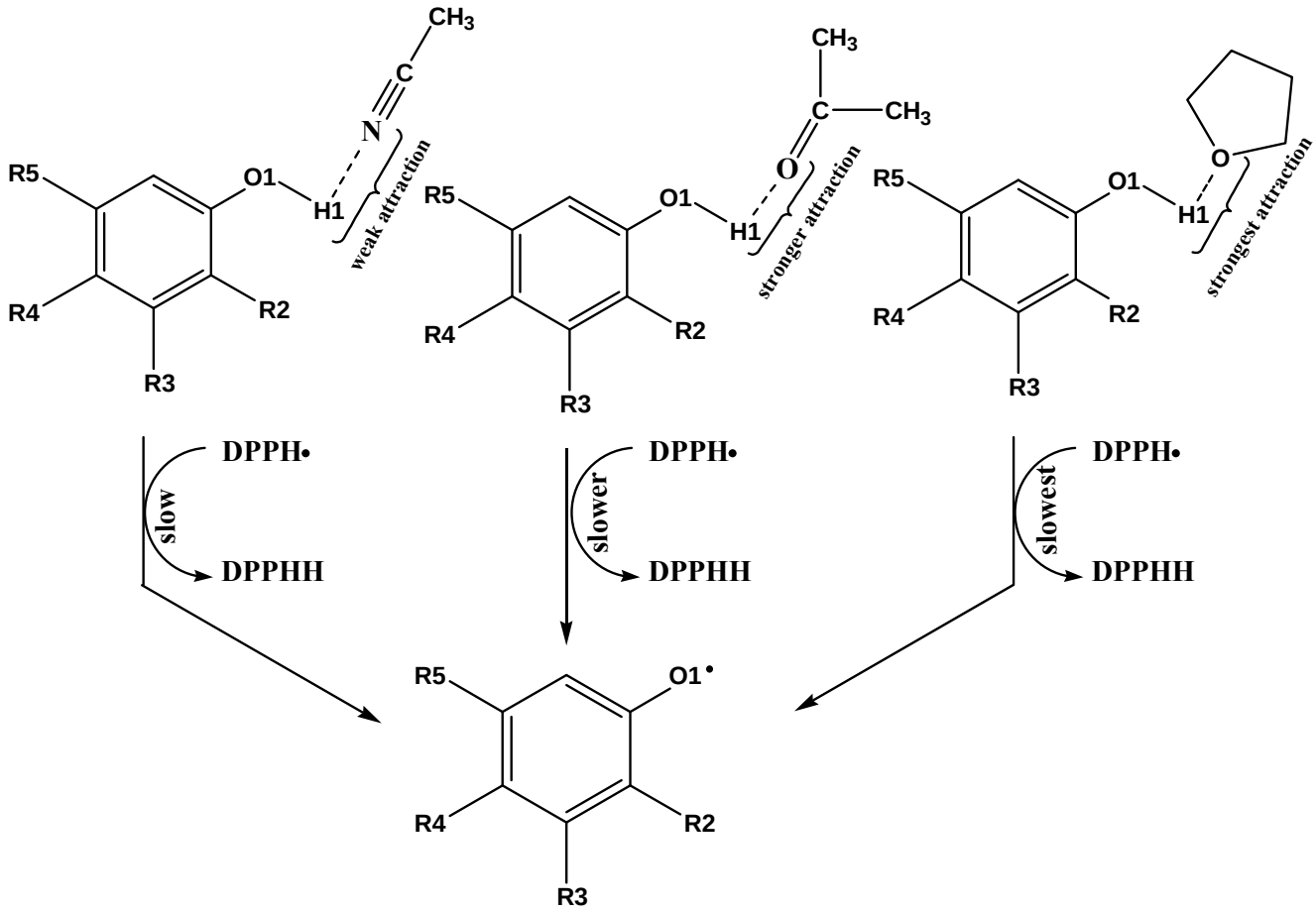


Figure 3.12: Radical scavenging ability of phenols under aprotic acetonitrile, acetone and apolar THF

3.2 **Conclusion**

Reaction rate constant increases as the activation barriers (E_a, $\Delta H^{\#}$) decrease as less energy is required for phenols to scavenge the free radical. In all solvents, pyrogallol was found to be the most effective radical scavenger, followed by 1,2,4-benzenetriol. In *ortho* OHs phenols, lower activation parameters resulted due to the presence of the dominating intramolecular H bonding and stability. Kinetic results on resorcinol and phloroglucinol indicate that OHs in *meta* position do not influence the radical scavenging ability at all and moreover, independent on both temperature and solvents. Results signify that the kinetics of radical scavenging is mainly contributed by the *ortho* arrangement of OH in phenols. Study on the effect of solvent reveals that polar protic solvent facilitates the radical scavenging activity due to its role in regenerating the availability of phenolic OHs.

The following conclusions can be made: (i) 3 OH in the vicinal provide the largest radical scavenging activity (ii) activation parameters seem to be important in explaining free radical scavenging ability of phenols. (iii) Polarity and protic nature of solvent play a vital role in the radical scavenging activity. In short, this study concludes that position of OHs and nature of reaction medium are important in defining the kinetics of radical scavenging activity of phenols, with the number of OH in the *ortho* position being the most effective. The observed results can be summarized as follows:

Reactive OH position: *ortho* > *para* > *meta*

Kinetic rate of phenols: pyrogallol > 12,4-benzenetriol > catechol > hydroquinone >> resorcinol ~ phloroglucinol;

Solvent reactivity: methanol > acetonitrile > acetone > THF.

Consequently, we believe that this experimental approach will ultimately provide the possibility of not only explaining the radical scavenging activity of existing antioxidants but also be of value for the design of new synthetic antioxidants.

4. COMPUTATIONAL STUDY ON PHENOLS

Polyphenolic antioxidants are the subject of intense scientific research because of the way they work to prevent or lower the risk of various cancers (Pace-Asciak *et al*., 1995; Bravo, 1998; Halliwell, 1999; Cox *et al*., 2000). Cancer caused or induced by free radicals can be effectively scavenged by polyphenols (Periera *et al*., 2000; Czinner *et al*., 2001; Lodovici *et al*., 2001). Excellent scavenging property of polyphenols is attributed to the phenolic OHs present in the ring structures (Rice-Evans, 1995; Rice-Evans *et al*., 1996; Wang *et al*., 1997). Flavonoids are the most common group of polyphenols. As shown in Figure 3.1, flavonoids share the common structure of two benzene rings, A and B, on either side of a carbon ring, C, but they are classified differently according to the various combination of the substitution groups such as OH attached to these structures. The radical scavenging ability of polyphenols depends on its individual structure. It is time-consuming to evaluate the structural effectiveness of antioxidants individually as researchers identified over 8000 as polyphenols (Periera *et al*., 2000; Czinner *et al*., 2001; Lodovici *et al*., 2001). Instead, model compounds that contribute to most of the polyphenolic structures can be chosen to study and interpret the structural activity. The potential importance of the number and arrangement of OH groups in polyphenols in drug absorption study across bio-membranes is well known (Potts *et al*., 1995; Pugh *et al*., 2000; DuPlessis *et al*., 2001).

Hence, in this study, we decided to elucidate the effect of OH with respect to the position and number towards radical scavenging ability. Therefore, on the basis of the number and

position of OH groups in the benzene ring, phenol, catechol, resorcinol, hydroquinone, pyrogallol, phloroglucinol, 1,2,4-benzenetriol and 5-hydroxypyrogallol were selected. Importantly, they are also observed as a nucleus in most of the flavonoids (Rice-Evans, 1996).

4.1 Theoretical measurement of BDE in solution

The SCRF Onsager reaction field model can simply and substantially describe the interaction between a molecule and its reaction medium (Onsager, 1936; Wong *et al*., 1991). The basic treatment of the solute-solvent interaction is performed by placing the solute in a spherical cavity within a homogeneous solvent reaction field. Hence, a solute radius is required and was computed for all compounds by a gas-phase molecular volume calculation[†]. The volume was then used to calculate solvent effect on the BDEs of all phenols. Optimization and frequency calculations were performed at the RB3LYP and UB3LYP for phenols and the radicals respectively. Since SCRF model did not predict any significant difference in the enthalpy value of the hydrogen atom, H_f (H) in solution, the value of -0.5 a.u was used for calculating BDEs in solution.

4.2 Results and discussion

4.2.1 Identification of active OH site in phenols

The weaker the OH bond, the smaller the BDE and the greater is the free radical scavenging ability of the antioxidant. Since polyphenols have a number of OH groups, it is necessary to locate the weakest OH bond site. The BDEs of all the possible conformers

[†] Using the volume = tight option in the *GAUSSIAN* program suite.

for all compounds were first calculated at the B3LYP/6-31G(d) level but only most stable conformer of the parent and the radical are reported here. The resulting BDEs were used to identify the active OH group need for further higher level calculations.

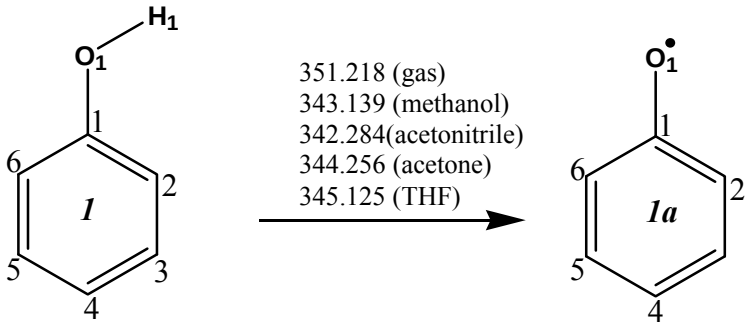


Figure 4.1: BDEs (kJ mol^{-1}) using B3LYP/6-31 G(d) for phenol and radical

Phenol, ***1*** (Figure 4.1) is observed in B ring of polyphenols such as apigenin, naringeniene kaempferol, and pelargonidin. BDE calculations at the B3LYP/6-31G(d) level using the SCRF model revealed that there was a difference between the gas and liquid phases of 6 - 9 kJ mol^{-1} (Figure 4.1).

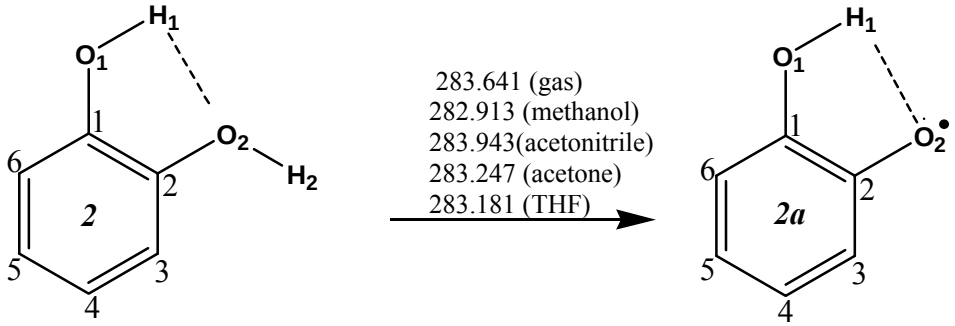


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Figure 4.2: BDEs (kJ mol^{-1}) using B3LYP/6-31 G(d) for catechol and radical

Catechol, ***2*** has two OHs *ortho* to each other C(1,2) in the benzene ring as shown in Figure 4.2. Most of the flavonoids such as quercetin, rutin, taxifolin, catechin, epicatechingallate, cynaidin have a catechol moiety in the B ring of their structure. Gas phase and solution phase calculations at 6-31G(d) showed that BDE for the route catechol to the radical ***2a*** was the smaller. Hence, for catechol compound **2**, the possible radical is ***2a.***

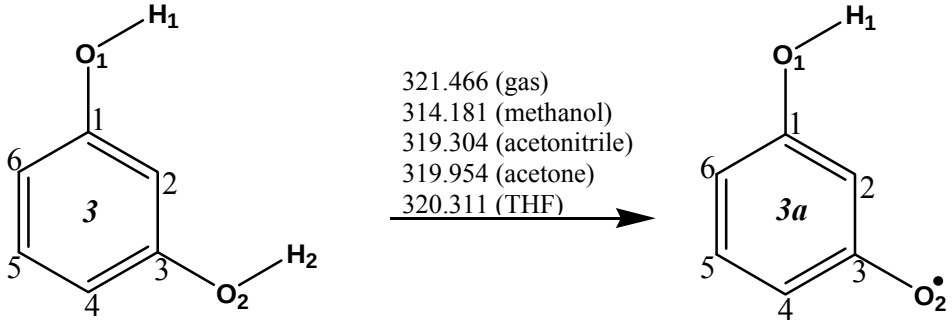


Figure 4.3: BDEs (kJ mol^{-1}) using B3LYP/6-31 G(d) for resorcinol and radical

Resorcinol ***3*** has two OHs positioned *meta* to each other C(1,3) in the benzene ring as shown in Figure 4.3. Most of the hydroxylation pattern in the A ring of flavonoids is of resorcinol type. There are a few studies available on the molecular and electronic structures of resorcinol in the gaseous phase and in hydroxylic solvents (Bouchoux, 2002). Calculations using the 6-31G(d) basis set for resorcinol, ***3*** to the radical ***3a*** showed BDEs (321.466 kJ mol^{-1}) in the gas phase. In methanol, resorcinol showed the lower BDE than that of the gas phase by about 7 kJ mol^{-1}.

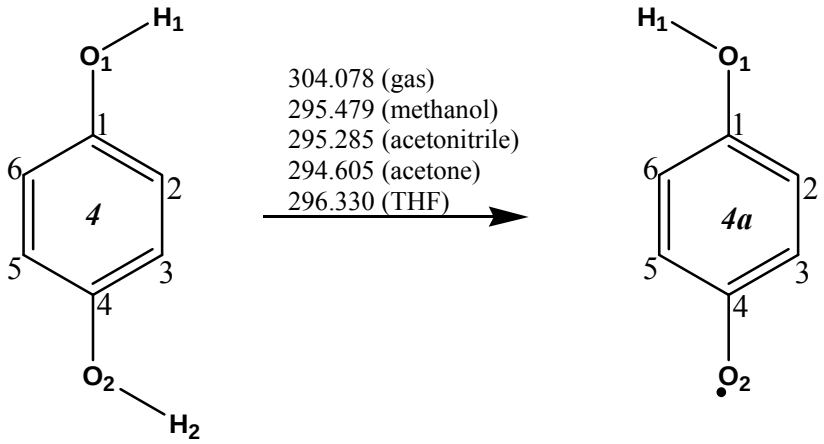


Figure 4.4: BDEs (kJ mol^{-1}) using B3LYP/6-31 G(d) for hydroquinone and radical

Hydroquinone, **4** has two OH in *para* position C (1,4) as shown in Figure 4.4. Hydroquinone ***4*** is found to have protective role against coronary heart disease (Pace-Asciak *et al*., 1995). B3LYP/6-31G(d) level calculations on the route for the hydroquinone ***4*** to the radical ***4a*** showed BDEs as 304.078 kJ mol^{-1} in gas phase. SCRF solvent calculations at the same level showed quite closer BDEs for the route hydroquinone ***4*** to the radical ***4a*** and but smaller by about 9 kJ mol^{-1} from their gas phase BDE.

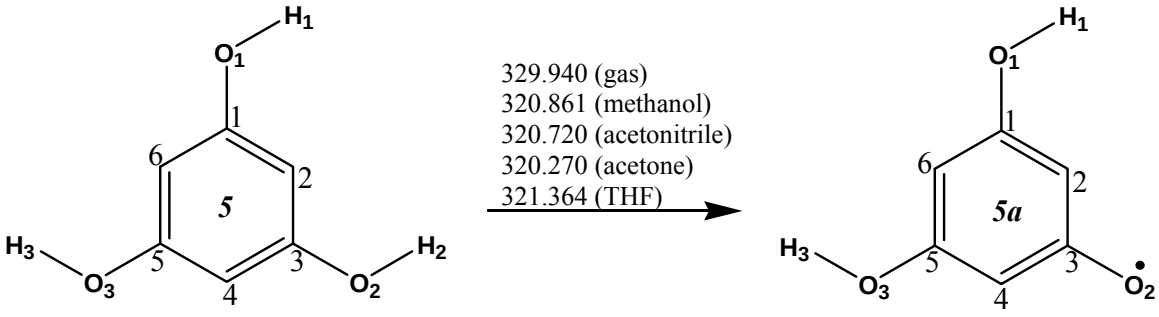


Figure 4.5: BDEs (kJ mol^{-1}) using B3LYP/6-31G(d) for phloroglucinol and radical.

Phloroglucinol, ***5*** is widely distributed in plants (Ghisalberti, 1996; Matsuhisa, 2002) and is of great interest in medicinal chemistry due to its antimicrobial and antiallergy activity. Phloroglucinol ***5*** possesses three OHs attached in *meta* to each other C(1,3,5) in the benzene ring. Among three- OH compounds, phloroglucinol (Figure 4.5) showed the gas phase BDE of 329.940 kJ mol^{-1} for the radical ***5a***. BDEs of ***5*** in each solvent were similar for the route to the radical ***5a*** at 6-31G(d) level. Null effect of solvent on the stability and molecular geometry of phloroglucinol ***5*** was noticed at 6-31G(d).

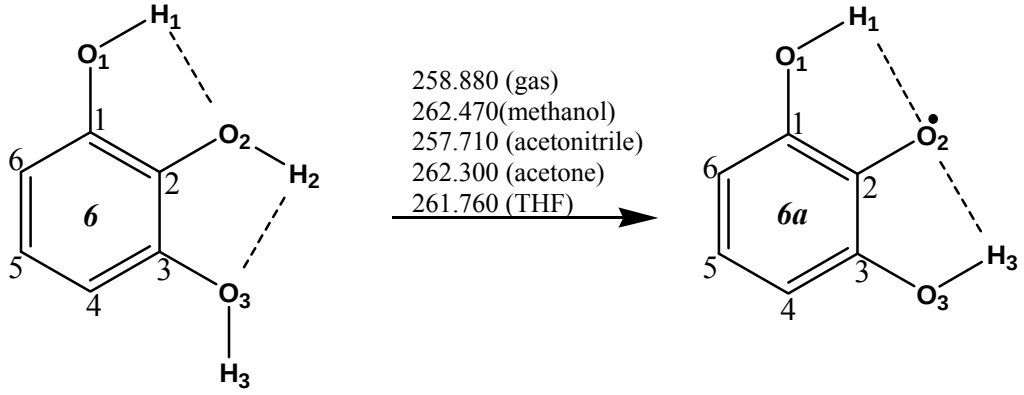


Figure 4.6: BDEs (kJ mol^{-1}) using B3LYP/6-31 G(d) for pyrogallol and radical.

As shown in Figure 4.6, pyrogallol, *6* has the structure of three OH attached to the benzene in the vicinal C (1,2,3) position and can be seen as B ring in tea catechins, myricetin, and delphinidin. Biochemical studies (Saeki *et al*., 2000; Benelli *et al*., 2002; Wang *et al*., 2003) predicted that a pyrogallol-type B ring structure is the minimal requirement for induction of apoptosis by catechins. BDE calculations at 6-31G(d) for the route pyrogallol ***6*** to radical ***6a*** provided the value 258.880 kJ mol^{-1}, which is lowest. The radical form ***6a*** found to be most preferable in all phases. Recent EPR studies on

pyrogallol derivative also confirmed this radical center for pyrogallol (Lucarini *et al*., 2002).

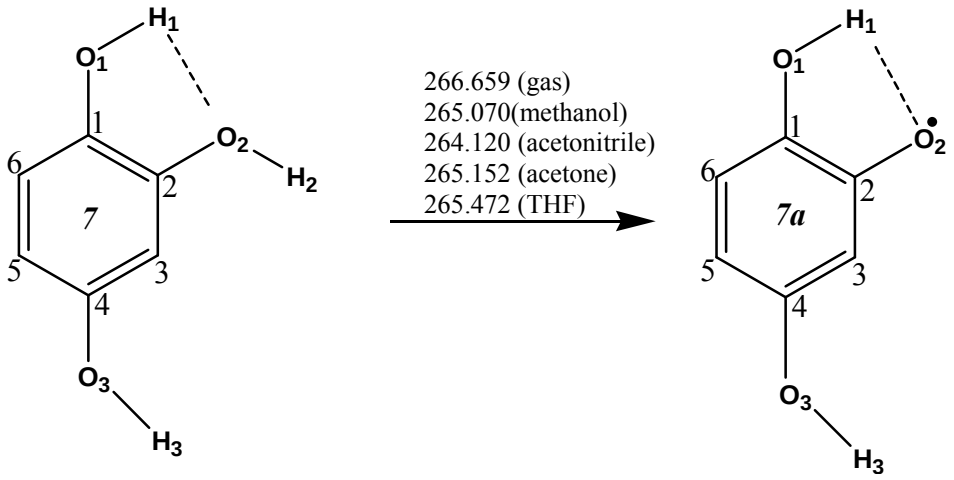


Figure 4.7: BDEs (kJ mol^{-1}) using B3LYP/6-31 G(d) for 1,2,4-benzenetriol and radical

1,2,4-benzenetriol, *7* has two OHs in C(1) and C (2) positions but the third OH in the C(4) position of benzene ring (Figure 4.7). As seen in Figure 4.7, both gas phase and solution phase BDE calculations of 1,2,4-benzenetriol *7* predicted that ***7a*** was the most possible radical**.**

From the BDE calculations of all phenols at the B3LYP/6-31G(d) level, it can be suggested that for the compounds, which do not have *ortho* arrangement of OHs in the benzene ring (resorcinol, hydroquinone and phloroglucinol), all the OH sites may have the equal possibility in donating its hydrogen atom. The active site in that case may be decided by the orientation of the molecule at the particular reaction condition. Overall, the BDE calculations provided an estimate of the possible active OH site for all phenols.

Hence only the stable radical forms ***1a, 2a, 3a, 4a, 5a, 6a*** and ***7a*** for the compounds phenol 1, catechol ***2***, resorcinol ***3***, hydroquinone ***4***, phloroglucinol ***5***, pyrogallol ***6***, 1,2,4-benzenetriol ***7*** respectively were considered for the high basis set gas and solution BDE calculations.

4.3 Gas phase calculations

4.3.1 Basis set effects on BDE calculations

BDE of phenol ***1*** was used as the reference compound for these basis set calculations. The influence of the basis set on the performance of the B3LYP was investigated. BDEs of all compounds and their respective radicals using different four different basis set were calculated and given in Table 4.1.

Table 4.1: B3LYP gas-phase OH BDEs (kJ mol^{-1}) as a function of basis sets

Compounds	6-31G(d)	6-31+G (d, p)	6-311+G (3df, 2p)	6-311++G (3df, 3pd)		
				BDE	ΔBDE^1	ΔBDE^2
Phe[a],	325.494	342.762	349.394	351.218	NA	NA
Cat[b],	283.554	306.317	311.334	312.849	-38.37	NA
Res[c],	321.454	338.270	344.424	346.450	-4.78	-33.59
HQ[d],	304.033	321.428	327.676	329.228	-21.99	-16.38
Phl[e],	329.920	347.054	352.242	353.996	-2.78	-41.15
Pyr[f],	258.880	287.697	287.000	289.385	-61.89	23.52
Benz[g],	266.659	291.588	293.234	294.849	-56.37	18.00

The enthalpy of hydrogen radical was used for all basis set = 0.5 hartree.

[a]phenol, [b]catechol, [c]resorcinol, [d]hydroquinone, [e]phloroglucinol, [f]pyrogallol, [g]1,2,4-benzenetriol.

[i]BDE in kJ mol^{-1} calculated from Eqn 2.9

$\Delta BDE^1 = BDE_{phenol} - BDE_{ArOH,}$ at B3LYP/6-311++G (3df, 3pd)

$\Delta BDE^2 = BDE_{catechol} - BDE_{ArOH}$ at B3LYP/6-311++G (3df, 3pd)

NA = Not applicable

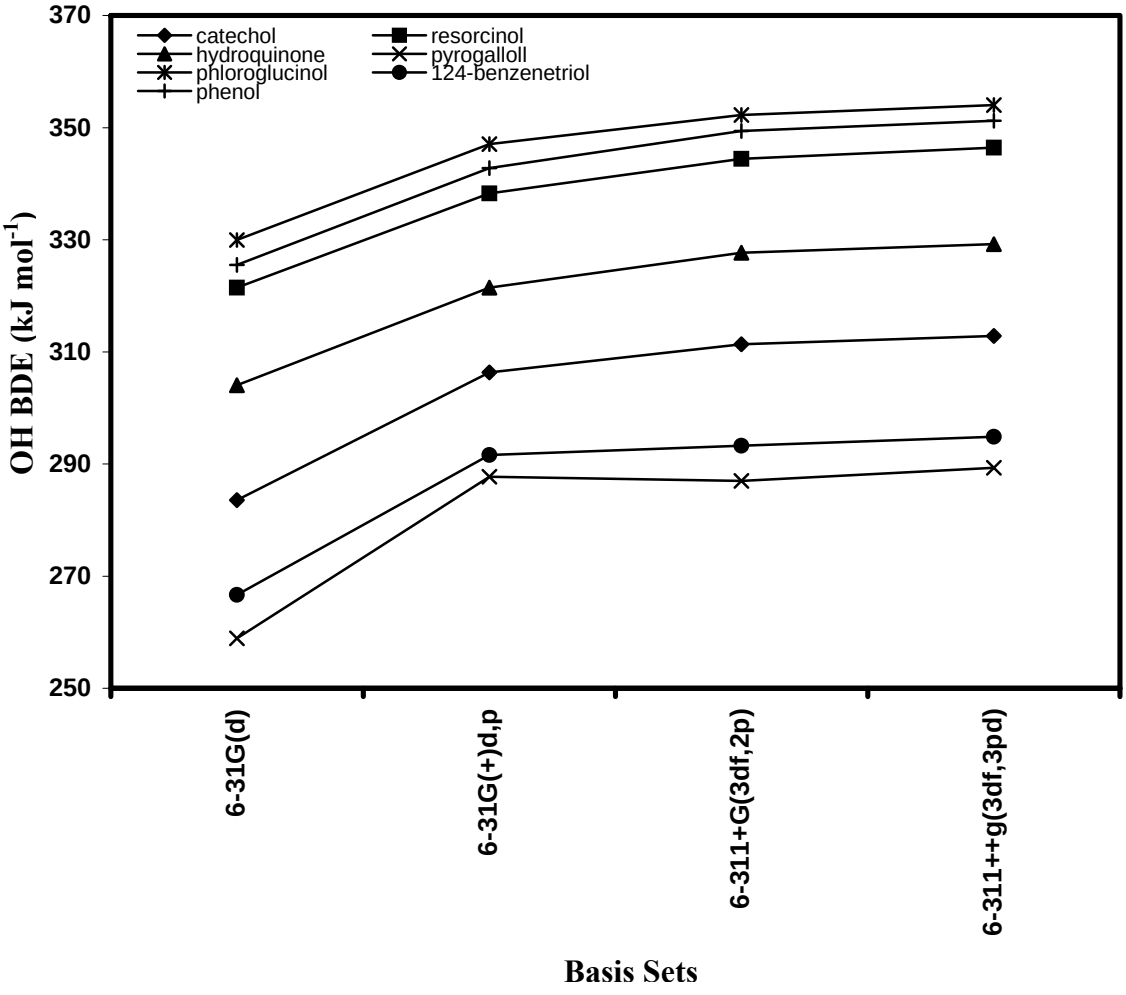


Figure 4.8: Plot of computed gas phase OH BDE with respect to basis sets

According to Figure 4.8, it is very clear that BDEs calculated at different levels of theory are very different from each other and shows that BDE of the phenolic compounds depend very much on the basis sets up to 6-31G+ (d, p). At 6-31+G (d, p) basis set, the BDE of phenols increased sharply to 17 - 28 kJ mol^{-1} compared to that of values obtained at 6-31G(d). Especially BDEs of *ortho* OH compounds catechol ***2***, pyrogallol ***6***, and 1,2,4-benzenetriol ***7*** increased by 23, 28.8 and 25 kJ mol^{-1} respectively. It shows that

inclusion of one diffuse functions and polarization functions on H has a considerable effect in evaluating the BDEs. Incorporating *3d* and one *f*' functions on C and O atoms in addition to the *2p*' and one primitive function on H atoms [6-311+G (3df, 2p)] slightly increased the BDEs for the phenols. The addition of '*3pd*' polarization functions on the H-atom is common for organic systems using large basis sets (Dust *et al*., 1983). Basis set 6-311++G basis set does not show much of a change in the distances or angles. Addition to *3p* and one set of '*d*' functions together with a set of diffusion functions on the heavy atoms [6-311+G (3df, 2p)] series provided an increase of about 2 kJ mol^{-1} in the BDEs for all compounds (Table 4.1).

The CPU time increased dramatically for this basis set, compared to 6-311+G (3df, 2p). Basis set 6-311++G (3df, 3pd) is believed to have an advantage over 6-311+G (3df, 2p) since computational efficiency is a function of the number of basis functions. A BDE value of 351.22 kJ mol^{-1} (84 kcal mol^{-1}) was obtained for phenol at 6-311++G (3df, 3pd) level, which is closer to the reported experimental value 359.82 kJ mol^{-1} (86.7 ± 0.7 kcal mol^{-1}) (Mulder *et al*., 2005). This BDE is also in agreement with the recommended range (85.1- 88.3 kcal mol^{-1}) based on measurements in the gas phase. In addition, the geometry of *1* (Table 4.2) using 6-311++G (3df, 3pd) was found to be close to the experimental values (Larsen , 1979). No experimental structure available for the phenoxyl radical *1a* and hence we compared with other theoretical studies (Table 4.3) and learnt that intramolecular distances in phenoxyl radical ***1a*** calculated from our calculation were very close to the literature values (Chipman, 1994). The expectation value of the spin squared operator <S2> for all our radicals were found between 0.78 to 0.79, close to the expected value of pure doublet wave function 0.75 (Brinck *et al*., 1997; Quin *et al*., 1994).

Hence, this chapter focuses on the results for all phenolic compounds that are obtained using B3LYP 6-311G++(3df, 3pd) in order to determine accurate OH BDEs irrespective of the computation time.

Table 4.2: Comparison of bond length (Å) of optimized phenol in gas phase with experimental and other theoretical methods

Phenol *1*			
Bond	Bond length		
	B3LYP / 6-311G++(3df, 3pd)	Experimental	B3LYP / 6-31+G(, 3pd)
R(C1-C2)	1.390	1.391	1.400
R(C2-C3)	1.390	1.392	1.402
R(C3-C4)	1.387	1.395	1.401
R(C4-C5)	1.390	1.395	1.404
R(C5-C6)	1.390	1.394	1.399
R(C1-C6)	1.390	1.391	1.399
R(C1-O)	1.360	1.375	1.083
R(O-H)	0.960	0.957	1.081
R(C2-H)	1.080	1.081	1.081
R(C3-H)	1.080	1.084	1.081
R(C4-H)	1.080	1.080	1.080
R(C5-H)	1.080	1.084	1.403
R(C6-H)	1.080	1.086	0.966

Table 4.3: Comparison of bond length (Å) of optimized phenoxide radical in gas phase with experimental and other theoretical methods

phenoxide radical ***1a***			
bond	bond length		
	B3LYP 6-311G++ (3df, 3pd)	CAS-SCF 6-311G(2d, p)	B3LYP 6-31+G(, 3pd)
R(C1-C2)	1.450	1.454	1.443
R(C2-C3)	1.370	1.370	1.386
R(C3-C4)	1.440	1.411	1.413
R(C1- O●)	1.240	1.228	1.298
R(C2-H)	1.080	1.073	1.081
R(C3-H)	1.080	1.074	1.081
R(C4-H)	1.080	1.073	1.081

Table 4.4: B3LYP/ /6-311++G(3df, 3pd) gas phase BDEs (in kJ mol^{-1}) for phenols

Effect of position of OHs	Phenols	BDE[a]	ΔBDE[1]
Meta effects	Res[c], ***3***	346.450	-4.78
	Phl[e], ***5***	353.996	-2.78
Ortho (IHB)	Cat[b], ***2***	312.849	-38.37
	Pyr[f], ***6***	289.385	-61.89
Para effect	HQ[d], ***4***	329.228	-21.99
Combined (*ortho* and *para*)	Benz[g], *7*	294.849	-56.37
	HP[h], ***8***	250.280	-100.9

[a]BDE of phenol ***1*** was calculated as 351.2 kJ mol^{-1}

[b]catechol, [c]resorcinol, [d]hydroquinone, [e]phloroglucinol, [f]pyrogallol, [g]1,2,4-benzenetriol, [h]5-hydroxypyrogallol

$\Delta BDE^{1} = BDE_{phenol} - BDE_{ArOH}$

Results on BDEs of all phenols at B3LYP/6-311++G(3df, 3pd) level are presented in Table 4.4. From the calculations, it was noted that BDEs were mainly influenced by the presence of both *ortho* position and *para* position of OH. It is discussed in the following section:

4.3.2 *Ortho* (IHB) effect

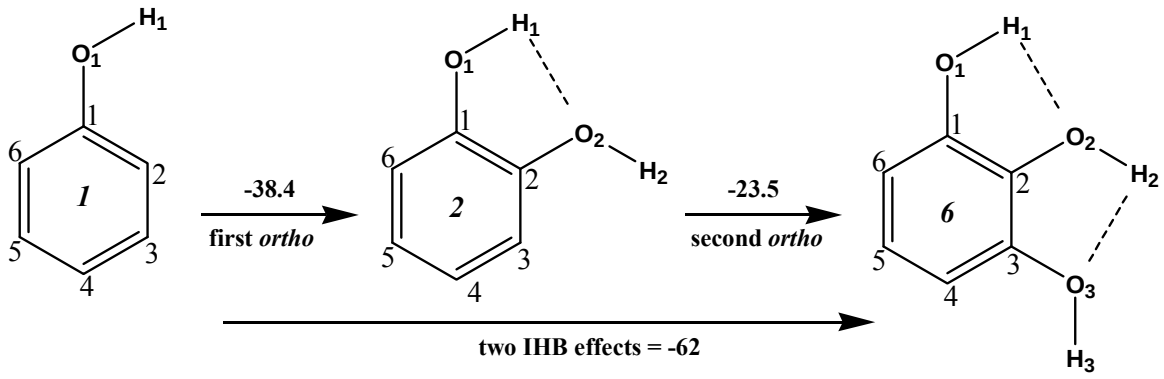


Figure 4.9: IHB effects on phenol and catechol (values above and below arrows are changes in BDE in kJ mol^{-1})

As shown in Table 4.4, BDE of catechol, ***2*** (*ortho*- 2 hydroxyl benzene) was calculated as 312.8 kJ mol^{-1}. In catechol, *ortho* arrangement exerted due to the presence of second OH in C(2) position could develop an intramolecular H-bond (IHB) between the two neighboring OH groups. Hence the radical ***2a*** from catechol is more stable than the phenoxide radical ***1a***. This could be accounted for the lower BDE of catechol as compared to that of phenol. As clearly shown in Figure 4.9, an *ortho* OH in phenol could reduce the BDE of about 38.4 kJ mol^{-1}. This relative BDE is also found to be closer to the experimental (30.1 kJ mol^{-1}) (Hong-Yu, 2003) and other theoretical (34.5 kJ mol^{-1}) (Sun, 2004) values. The *ortho* arrangement of OHs could make catechol a better radical scavenger than phenol.

Among the two and three OH compounds studied, the lowest BDE (289.4 kJ mol^{-1}) was observed for pyrogallol *6*, which has OHs in C(1,2,3) position of the benzene ring. This is

close to the literature value (304.2 kJ mol^{-1}) reported by Wright *et al. (*2001*)*, lower than the values (323.8 kJ mol^{-1}) reported by Bakalbassis *et al*., (2003) at 6-31+G(, 3pd) and by Hong *et al*., (2005) (322.7 kJ mol^{-1}) at CCSD/6-31+G(d).

In our study, for both pyrogallol ***6*** and 5-hydroxypyrogallol ***8***, the middle OH was considered broken to donate H atom, as it is believed that both sides of OH could provide more stability to the radical in the center. Our bench work-study on BDE calculation on all the OHs also indicated that middle OH was the weakest one. A NMR study (Sawai *et al*., 2005) also indicated that the peak intensity for C = O was observed in the center OH site of pyrogallol after it was reacted with the free radical. Hence, the equal stability exerted by both side OHs could cause the radicals *6a* and *8a* to be the most stable ones.

In Figure 4.10, it can be also seen that the presence of OH in the C(3) position in pyrogallol ***6*** introduces the second possibility of IHB formation. The two OHs located at the C(2) and C(3) positions exert two IHBs, which could lower the BDE significantly. Thus, the more IHBs in the structure, the more stable the radical, and thus the lower the BDE. As expected, the lower the BDE, the higher the free radical scavenging ability.

Figure 4.10: Two IHB effects on resorcinol (the value below the arrow is the change in BDE in kJ mol^{-1})

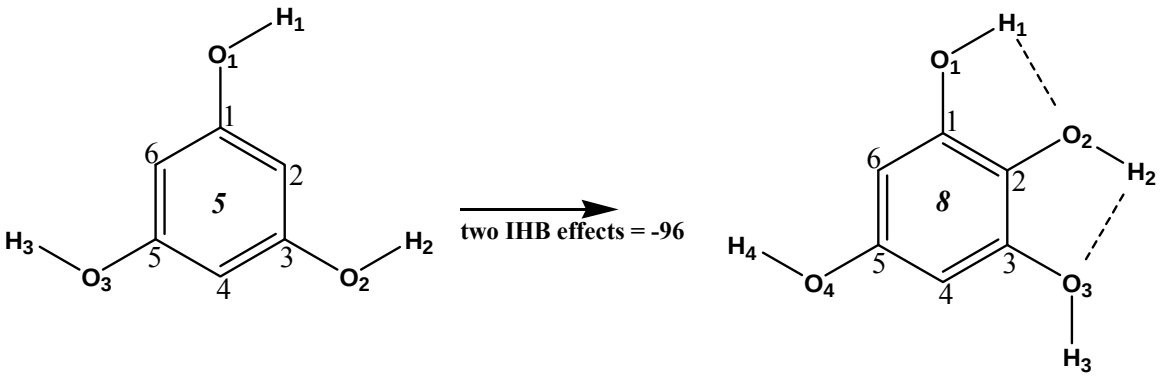


Figure 4.11: Two IHB effects on phloroglucinol (the value below the arrow is the change in BDE in kJ mol^{-1})

Figures 4.11 and 4.12 clearly indicate that the introduction of OH in the C(2) of resorcinol, ***3*** as well as phloroglucinol ***5*** could provide two possible IHBs in their structure *i.e* called pyrogallol ***6*** and 5-hydroxypyrogallol ***8*** respectively. The relative BDE of pyrogallol to resorcinol is estimated as 64.6 kJ mol^{-1} and 5-hydroxypyrogallol to

resorcinol is about 96 kJ mol^{-1}. This results confirm that the *ortho* bridge (IHB) has a great influence on BDEs and hence the free radical scavenging ability.

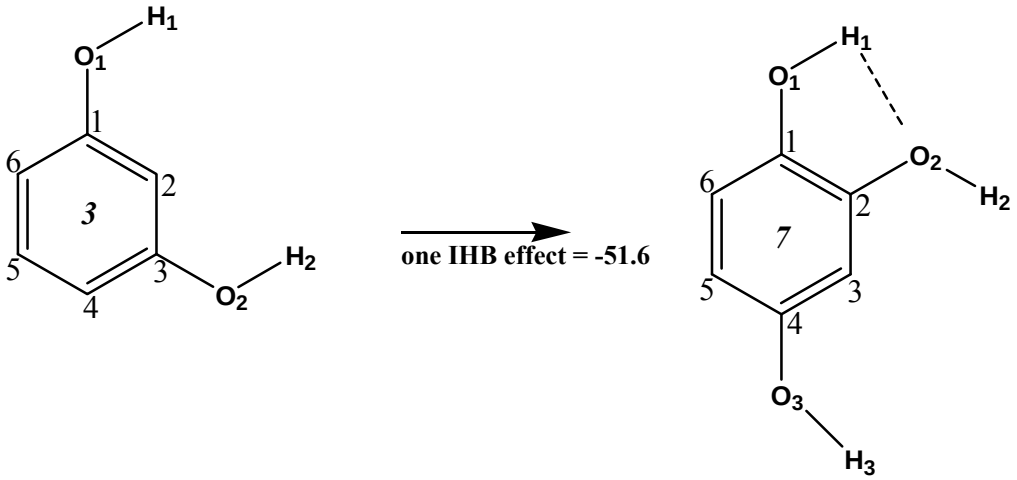


Figure 4.12: One IHB effect on resorcinol (the value below the arrow is the change in BDE in kJ mol^{-1})

As shown in Figure 4.12, resorcinol *3* can be modelled to 1,2,4-benzenetriol *7* by bringing out an OH in either C(6) or C(4) position in resorcinol. Our calculations show that the BDE of 1,2,4-benzenetriol is lower by 51.6 kJ mol^{-1} than that of resorcinol.

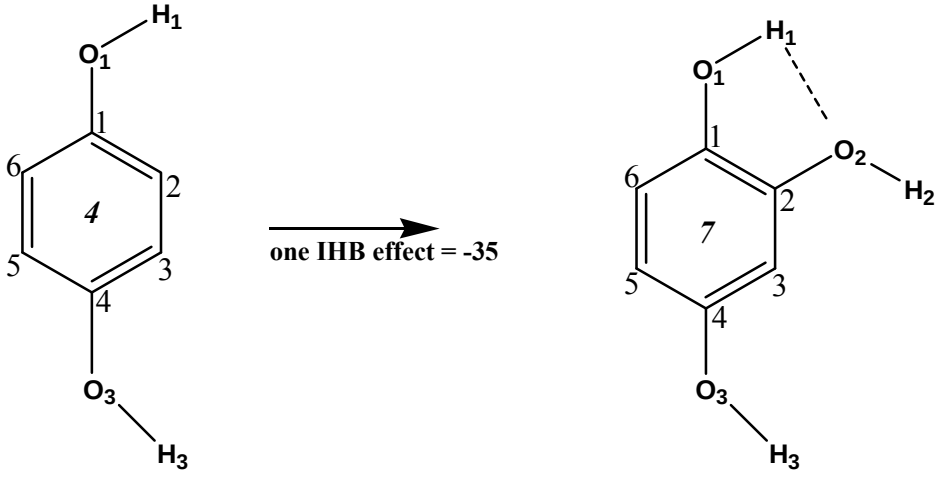


Figure 4.13: One IHB effect on hydroquinone (the value below the arrow is the change in BDE in kJ mol^{-1})

Similarly, introducing the IHB effect by placing an OH in the C(2) position of hydroquinone ***4*** may lead to the compound called 1,2,4-benzenetriol ***7***, whose BDE is lower than that of hydroquinone by 35 kJ mol^{-1}. This is shown in Figure 4.13.

Over all, it can be stated from the gas phase studies that the *ortho* caused IHB in the structure. This plays a vital role in the BDEs and thus free radical scavenging ability. This supports the statements made by Barclay *et al*. (1999) and Burton *et al.* (1981 and 1985), that the main factor controlling BDEs of most of the flavonoids is the stabilization by the IHB.

4.3.3 *Para* effect

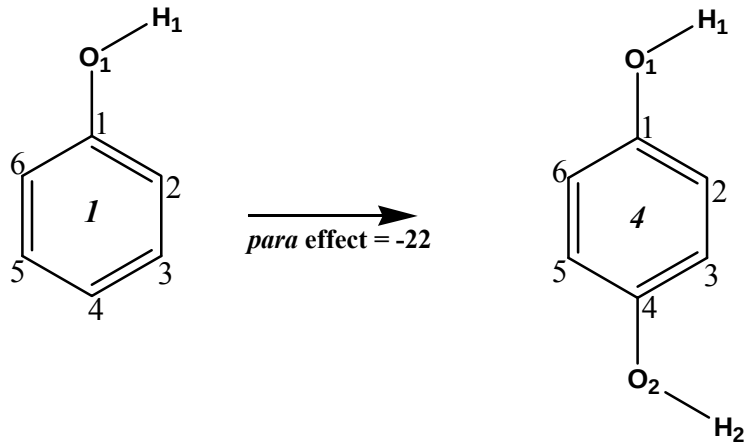


Figure 4.14: *Para* effect on phenol (the value below the arrow is the change in BDE in kJ mol^{-1})

BDE of hydroquinone ***4*** (*para*- 2 hydroxyl benzene) was calculated as 329.2 kJ mol^{-1}, which is lower by 6 kJ mol^{-1} as compared to the experimental value (Lind *et al*., 1990). Even though IHB is absent in both resorcinol *3* and hydroquinone *4*, the BDE of hydroquinone is found to be lower than that of resorcinol and phenol by 17.2 kJ mol^{-1} and 22 kJ mol^{-1} respectively. This implies that the OH at the *para* position reduces the BDE of phenol considerably, whereas the OH at the *meta* position of phenol does not have any strong effect on the BDE. This findings support the argument (Hansch *et al*., 1991) that EDG (here as OH) at the *meta* position does not have any significant effect on the bond strength in comparison to the unsubstituted phenol, whereas the same at the *para* position reduces the OH bond strength significantly. The BDE of hydroquinone was also found to be higher than that of catechol by 16.4 kJ mol^{-1}. Results show that the second OH in the *para* position has more radical scavenging activity than that in the *meta* position of phenol, but certainly lesser than that of the *ortho* effect exerted due to the IHB.

4.3.4 Combined effects of *ortho* (IHB) and *para*

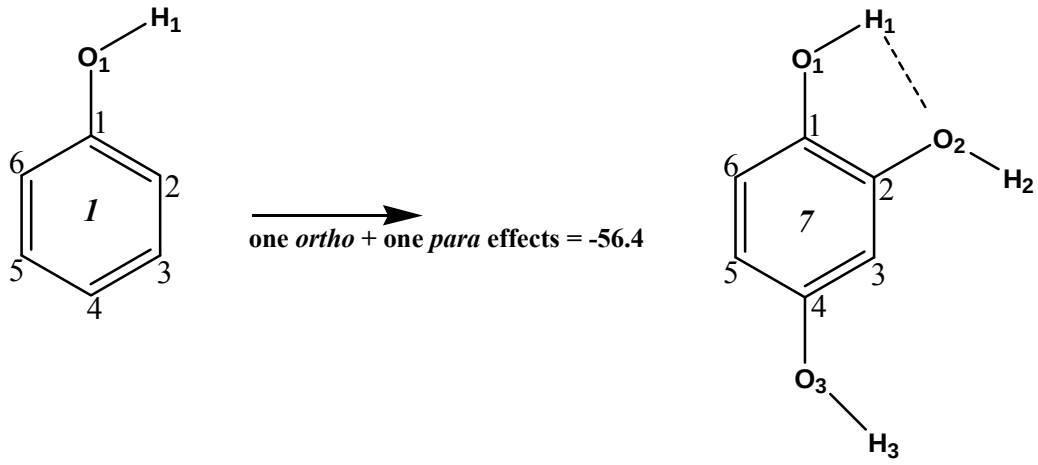


Figure 4.15: Combined effects of phenol to 1,2,4-benzenetriol (the value below the arrow is the change in BDE in kJ mol^{-1})

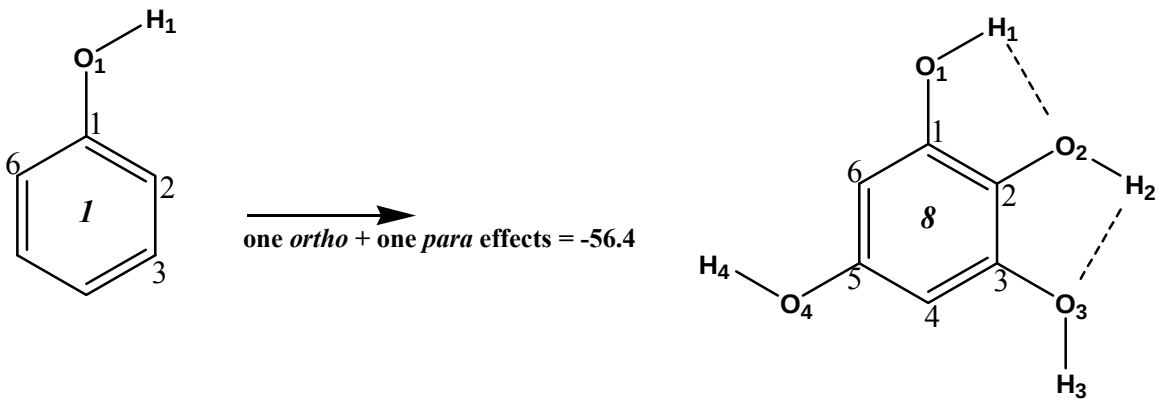


Figure 4.16: Combined effects of phenol to 5-hydroxypyrogallol (the value below the arrow is the change in BDE in kJ mol^{-1})

Positioning of one *ortho* and *para* OH in phenol simulates the structure of 1,2,4-benzenetriol. Calculations show that the relative BDE of 1,2,4-benzenetriol to phenol is about 56.4 kJ mol^{-1}. Comparing this value with the relative BDE of catechol to phenol (38.4 kJ mol^{-1}) and hydroquinone to phenol (22 kJ mol^{-1}), it can be understood that combined effect of both *ortho* and *para* may reduce the BDE significantly. 5-hydroxypyrogallol has two IHBs and one *para* with respect to C(2) position. Examining the relative BDE of 5-hydroxypyrogallol to phenol (100.9 kJ mol^{-1}) also confirms the importance of the combined effect. On comparing the BDE of phenol to the one *ortho* and one *para* OH structured compound (1,2,4-benzenetriol) and to the two *ortho* and one *para* OH structured compound (5-hydroxypyrogallol), it can be said that *ortho* plays a dominating role in reducing the BDE. This is clearly shown in Figures 4.16.

Table 4.5: Calculated OH BDEs (kJ mol^{-1}) of phenol, catechol, resorcinol, hydroquinone using SCRF/ B3LYP/6-311++G (3dp, 3df)

Compounds	Solvent	BDE$^{m}_{solv}$ (kJ mol^{-1})	BDE$^{n}_{Diff}$ (kJ mol^{-1})
Phe[a], ***1***	MeOH[h]	343.140	8.08
	EtoH[i]	343.397	7.82
	AcN[j]	342.276	8.94
	AcE[k]	344.256	6.96
	THF[l]	345.127	3.61
Cat[b], ***2***	MeOH[h]	310.933	1.92
	EtoH[i]	311.410	1.44
	AcN[j]	312.075	0.77
	AcE[k]	311.363	1.49
	THF[l]	308.948	3.90
Res[c], ***3***	MeOH[h]	343.612	2.84
	EtoH[i]	342.407	4.04
	AcN[j]	342.998	3.45
	AcE[k]	343.392	3.06
	THF[l]	344.038	2.41
HQ[d], ***4***	MeOH[h]	318.536	1.24
	EtoH[i]	318.460	1.32
	AcN[j]	318.447	1.33
	AcE[k]	317.690	2.08
	THF[l]	319.392	0.38

[a]phenol, [b]catechol, [c]resorcinol, [d]hydroquinone, [h]methanol, [i]ethanol, [j]acetonitrile, [k]acetone, [l]tetrahydrofuran

Table 4. 6: Calculated solvent-Phase OH BDEs (kJ mol^{-1}) of phloroglucinol, pyrogallol and 1,2,4-benzenetriol using SCRF/ B3LYP/6-311++G (3dp, 3df)

Compounds	Solvent	BDE$^{m}_{solv}$ (kJ mol^{-1})	BDE$^{n}_{Diff}$ (kJ mol^{-1})
Phl[e], ***5***	MeOH[h]	343.728	10.09
	EtoH[i]	342.565	11.26
	AcN[j]	343.623	10.20
	AcE[k]	342.987	10.83
	THF[l]	344.298	9.52
Pyr[f], ***6***	MeOH[h]	291.830	-2.44
	EtoH[i]	291.609	-2.22
	AcN[j]	291.829	-2.44
	AcE[k]	291.667	-2.28
	THF[l]	291.365	-1.98
Benz[g], *7*	MeOH[h]	292.008	2.84
	EtoH[i]	290.958	3.89
	AcN[j]	289.503	5.35
	AcE[k]	292.139	2.71
	THF[l]	292.678	2.17

[e]phloroglucinol, [f]pyrogallol, [g]1,2,4-benzenetriol, [h]methanol, [i]ethanol, [j]acetonitrile, [k]acetone, [l]tetrahydrofuran

The BDE difference between non-polar solvents and gas phase was considerably higher for all the compounds. It can be seen that the difference between liquid phase and gas phase BDE values are higher for phloroglucinol ***5*** and smallest for pyrogallol, ***6***. Hence, both gas and solution DFT study on OH BDE provides more evidence for the already known importance of the catechol moiety (pyrogallol, 1,2,4-benzenetriol) among all other substitution group on the B and C ring of flavonoids (Bors *et al*., 1990; Haenen *et al*., 1997; Zhang *et al*., 2003)

4.3.5 *Meta* effect

BDE of resorcinol ***3***(*meta*- 2 hydroxyl benzene) was calculated as 346.5 kJ mol^{-1}, which is higher than that of catechol by 33.6 kJ mol^{-1} but closer to that of phenol by only 4.8 kJ mol^{-1}. This result shows that the second OH in the C(3) position of the phenol (two OHs in *meta* position*)* does not have much effect on the BDE of phenols and is almost equal to the one hydroxyl compound (phenol). Table 4.6 shows that BDE of phloroglucinol, which has OHs in C(1,3,5) position is estimated to be 353.9 kJ mol^{-1}. Interestingly, the relative BDE for phloroglucinol to phenol (2.8 kJ mol^{-1}) indicates that it is very closer to that of phenol and higher than that of the *meta* 2-OH compound by only 7.5 kJ mol^{-1}.

Of all the compounds studied, BDE is the highest for phloroglucinol, which has 3-OHs in number. This result emphasize that radical scavenging ability of polyphenols depends mainly on the positioning of OHs and certainly not on its number. Among the two, three and four OH groups compounds studied, catechol, pyrogallol and 5-hydroxypyrogallol

have the lowest BDEs. This result confirms that only the position of OH group is important for potent free radical scavenging ability. The BDE of phenols decreases in the order; 5-hydroxypyrogallol > pyrogallol > 1,2,4- benzenetriol > catechol > hydroquinone >> resorcinol ~ phloroglucinol ~ phenol. Our findings on the order of radical-scavenging ability are also in good agreement with the NMR conformational studies on the polyphenols. Hence, our DFT study on gas phase BDE provides more evidence for the already known importance of the catechol moiety (pyrogallol, 1,2,4-benzenetriol) among all other substitution group on the B and C ring of flavonoids (Bors *et al*., 1990; Zhang *et al*, 2003).

4.4 Conclusion

We presented computational results on the phenols to provide a deeper understanding of the effect of OHs with respect to position and numbers in BDE calculations. We conclude based on our BDE that the relative activity position of OH in the benzene ring is:

C(1,2,3,5) > C(1,2,3) > C(1,2,4) > C(1,2) > C(1,4) > C(1) ~ C(1,3) ~ C(1,3,5).

This study also concludes that the vicinal trihydroxy moiety (5-hydroxypyrogallol and pyrogallol) is superior to that of the *ortho*-dihydroxy moiety (1,2,4-benzenetriol and catechol). Hence there is every reason to believe that the *ortho* OH moiety can play a significant role in radical-trapping ability.

Overall, two points seem clear: (i) the position of OHs is very important for lower BDEs but not the number of OHs (ii) increasing the number of OH in the vicinal position *i.e* more the IHBs decrease the BDEs. Conversely, increasing the number of OHs in the

alternative position increases the BDEs; OH in the *para* position also lowers the BDEs and hence the largest radical scavenging activity is expected for 5-hydroxypyrogallol.

(OH) BDE of all phenols were calculated in polar protic solvents; methanol and ethanol, polar aprotic solvents; acetonitrile and acetone, and apolar solvent; tetrahydrofuran (THF) using SCRF/B3LYP/6-311++G (3df, 3pd) model and reported in the Tables 4.7 and 4.8. Geometry optimization using SCRF model on phenol *1* shows that geometry was unchanged in solvents and almost identical each other in each solvent. Unlike polar continuum model (PCM) solvent model studies (Bakalbasis, 2003; Alexandra *et al*., 2005; Wayner *et al*., 1995 and Leopoldini, 2004), self consistent reaction field (SCRF) solution BDEs of phenols were found to be lower than the gas phase BDEs. This observed trend is similar to the findings by Shukla *et al* (2003), on benzothiazoline derivatives.

5. SUBSTITUENTS EFFECT ON RADICAL SCAVENGING ABILITY OF CATECHOL

Results from the chapter 4 showed that the *ortho* bridge catechol and pyrogallol increased activities against the free radical. As such, catechol and pyrogallol moiety are the crucial moiety for the radical scavenging activity of polyphenols. Flavanoids, a major polyphenol can be found with variety of functional groups on the parts of the catechol especially on the C(4) side of catechol (see Figure 5.1). Therefore, it was of our interest to explore and compare the effect of different functional groups as substitutents at the C(4) position of catechol. In consideration of substitution groups for catechol, the substituents CN, COOH, CHO were chosen as electron withdrawing groups (EWGs), while the alkyl groups methyl (CH_3), ethyl (C_2H_4) and butyl (t-C_4H_9) as electron donating groups (EDGs) (Figure 5.2 and Table 5.1).

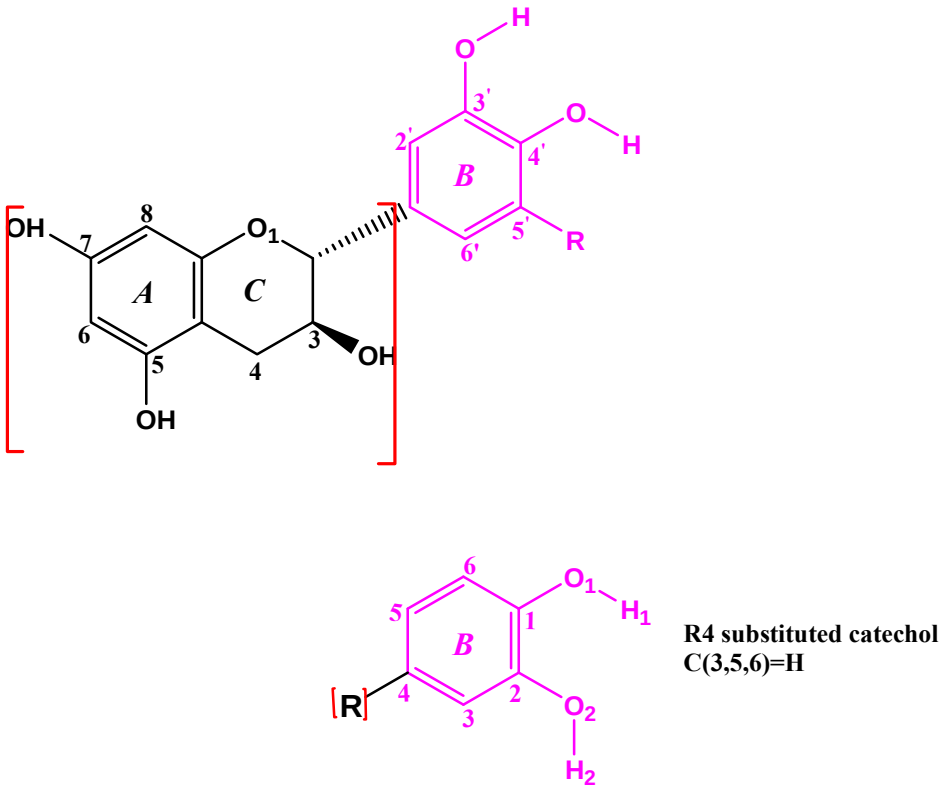


Figure 5.1: Identification of catechol compound in the structure of flavonoids.

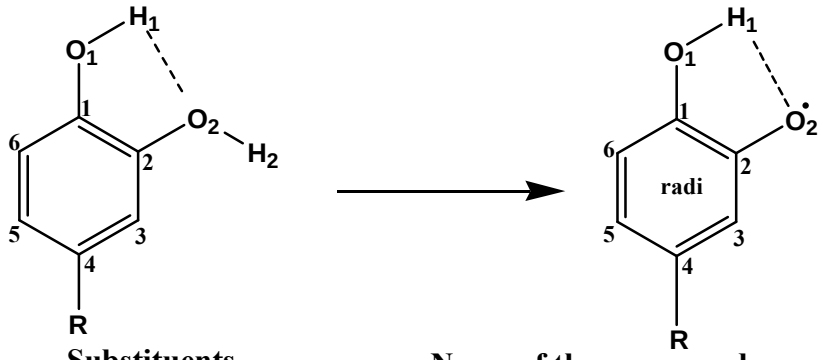


Figure 5.2: Substituents in the catechol moiety

5.1 Kinetics results and Discussion on substituted catechol

The kinetic study of selected *para* substituted catechols with DPPH• in methanol was carried out using stopped flow spectrometer at temperatures (15°C - 40°C) and kinetic parameters were tabulated in Table 5.2. Figures (5.3 & 5.4) show the Arrhenius plots of ln$k$ vs. 1/T observed for substituted catechols reaction and Table 5.2 shows the corresponding activation parameters (E_a and ln$A$) for the substituted catechols.

Table 5.2: Hammet constant for the *para* substitution groups

Group	Substitution at C(4)	Hammet constant [a]σ_p
EDG	OH	-0.37
	CH_3	-0.17
	t-C_4H_9	-0.15
EWG	CN	+0.66
	COOH	+0.45
	CHO	+0.42
[b]Cat	H	0

[a] Data obtained from the reference:- Hansch, *et al*., 1991.
[b]catechol

Table 5.3: Rate constants (k), activation energy (E_a) of substituted catechols in methanol

Nature of Group	Substitutions at C(4)	Second order rate constant, k ($M^{-1}s^{-1}$)						Arrhenius parameters		ΔE_a [o]
		15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	E_a kJ mol^{-1}	lnA $M^{-1}s^{-1}$	kJ mol^{-1}
EDG	OH	364	560	998	1444	2325	3427	67.9 ±1.5	34.3 ±0.6	-6.9
	CH_3	124	233	368	610	1000	1400	72.89±2.1	35.32±0.7	-1.9
	t-C_4H_9	139	260	440	645	1050	1508	70.74±2.4	34.56±0.9	-4.1
EWG	CN	29	52	93	161	275	440	82.03±0.6	37.63±0.2	+7.2
	COOH	3	7	10	27	41	65	91.94±5.6	39.63±2.2	+17.1
	CHO	2	6	13	24	39	60	99.91±7.8	42.68±3.1	+25.1
[b]Cat	H	66	98	204	300	455	800	74.8 ±3.1	35.4 ±1.3	0

[o]$\Delta E_a = E_a$ of given compound - E_a of catechol (74.8 kJ mol^{-1})

[b]catechol

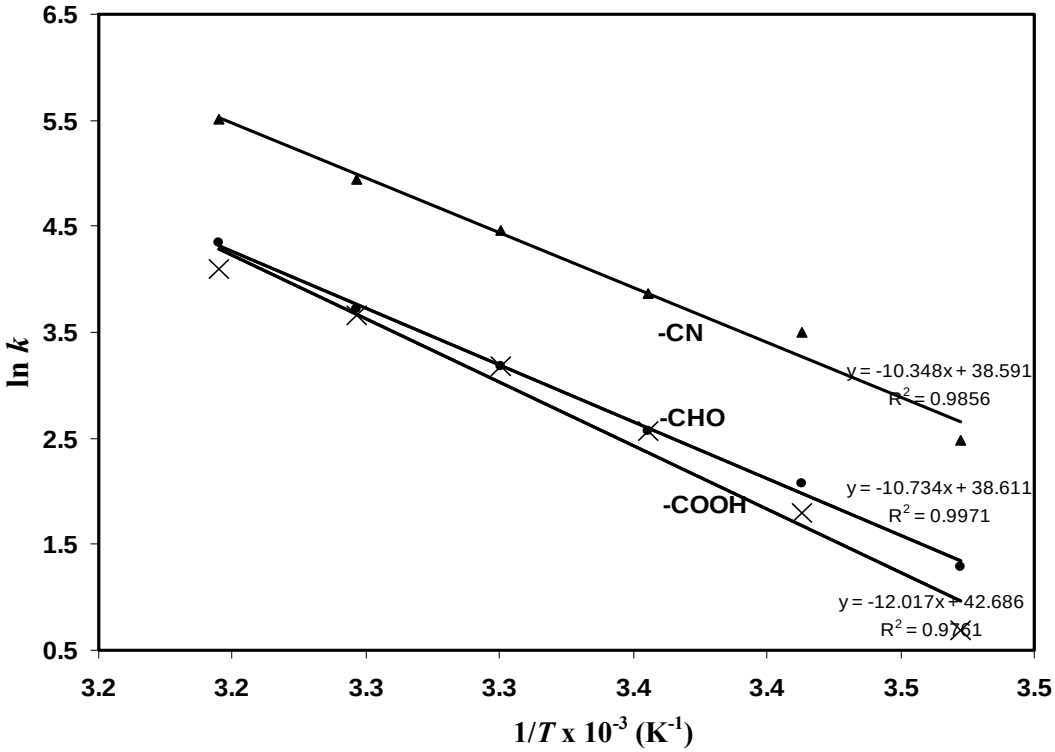


Figure 5.3: Arrhenius plot for EWG substituted catechols with DPPH•

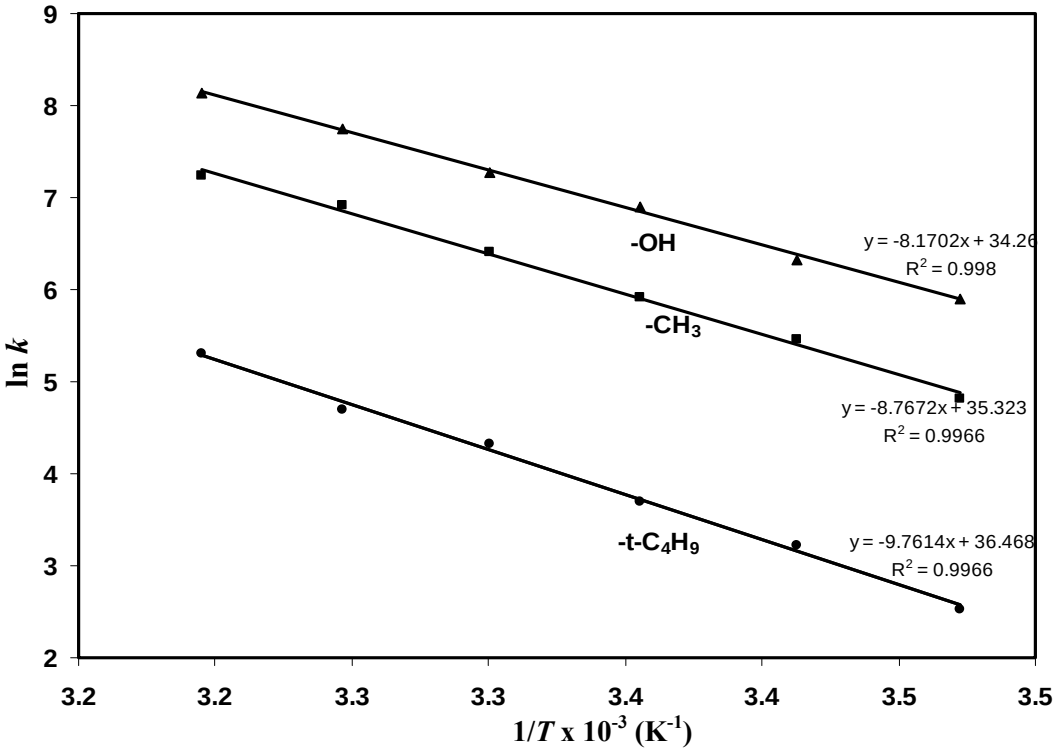


Figure 5.4: Arrhenius plot for EDG substituted catechols with DPPH•

The result obtained for the substituted catechols from the kinetic experiments were analyzed on the basis of the nature of substitutents as follows;

5.1.1 Effect of EDGs on the kinetics of catechol

Larger rate constants (*k*) and lower E_a were observed for EDGs substituted catechols compared to the EWGs substituted catechols. Using OH as the substitutent at the para position, the E_a of catechol was found to be reduced by about 7 kJ mol^{-1}, which is largest effect observed for EDGs. Alkyl substitutions were found to increase the rate of radical scavenging ability of catechol. This is reflected in the larger rate constants of 4-methyl and 4-butyl catechols. Larger rate constants (*k*) were obtained for 4- butyl catechol compared to the 4-methyl catechol. It can be seen that the E_a of catechol was reduced by *ca*. 4 kJ mol^{-1} for t-C_4H_9 as para substitutent compared to 2 kJ mol^{-1} by CH_3 group (see Table 5.2). It indicates that the presence of a longer alkyl chain at the C(4) position of catechol enables the catechol a faster radical scavenger.

5.1.2 Effect of EWGs on the kinetics of catechol

Each of the EWG substituents causes a bigger increase in activation energy of catechol. Smaller rate constants (*k*) and larger E_a were observed for 2,3-dihydroxy benzoic acid and they indicate that the presence of a non conjugated carboxylic group (–COOH) at C(4) position of catechol increases the E_a of catechol by 17 kJ mol^{-1} and thus reduce radical scavenging ability of catechol. Replacing the carboxylic group with an aldehyde (CHO) again resulted in a substantial reduction in the rate of radical scavenging reaction.

2,3-dihydroxybenzaldehyde showed E_a of 100 kJ mol^{-1} and it is noticed larger than that of catechol by 25 kJ mol^{-1}, which is the largest value seen among the studied EWGs. The 4- cyano catechol showed relatively faster rate of reaction compared to the other EWGs studied in this study. However, on comparing the kinetic parameters of 4-methyl catechol with that of EWGs, one can realize that the impact of EWGs is considerably bigger in reducing the rate of radical scavenging reaction of catechol.

5.1.3 Significance of Hammet relation

One of the ways to explain the effect of EWGs and EDGs is to use Hammet constant (Hammet, 1937). Hammet constant (σ) based structure-reactivity relationship has been remarkably successful for chemists for the past half-century. Hammet equation (Eqn 5.1) is generally used to study the influence of *meta*- or *para*-substituents (X) on the reactivity of the functional group (Y) in the benzene derivatives XC6H4Y.

$$\ln(k / k_0) = \rho\sigma_x \qquad \text{Eqn 5.1}$$

where k is the rate constant for the given reaction of *m*- or *p*-substituted benzene derivatives (X-C6H4-Y); k_0 refers to the reaction of C_6H_5-Y, i.e. X = H; σ_x, is the electronic effect of substituent x relative to hydrogen. σ_x is determined based on the influence of a substitutent on the ionization of benzoic acid. σ_x values were reported in literatures (Hansch, *et al*., 1991; Hammett, 1937) for a wide number of substituents, including the substituents at the *para*, *meta* and *ortho* positions, based on the ionization of benzoic acids. If $\sigma < 1$, it means that rate decreases with EWG but increases with

EDG. As seen in Table 5.1, σ_p is slightly negative for EDGs used in this study, indicating that electron-donating substituents on the phenolic ring increase the rate of radical scavenging reaction. Attempt was made to plot between Hammet constants for *para* substitutents and the measured activation energy (E_a) (Figure 5.5). Linear fit indicates that the radical scavenging property of catechols depend on the electronic property of the substituents. However, fit with with $r^2 = 0.7$ indicates that these corraltion may not be very mch reliable.

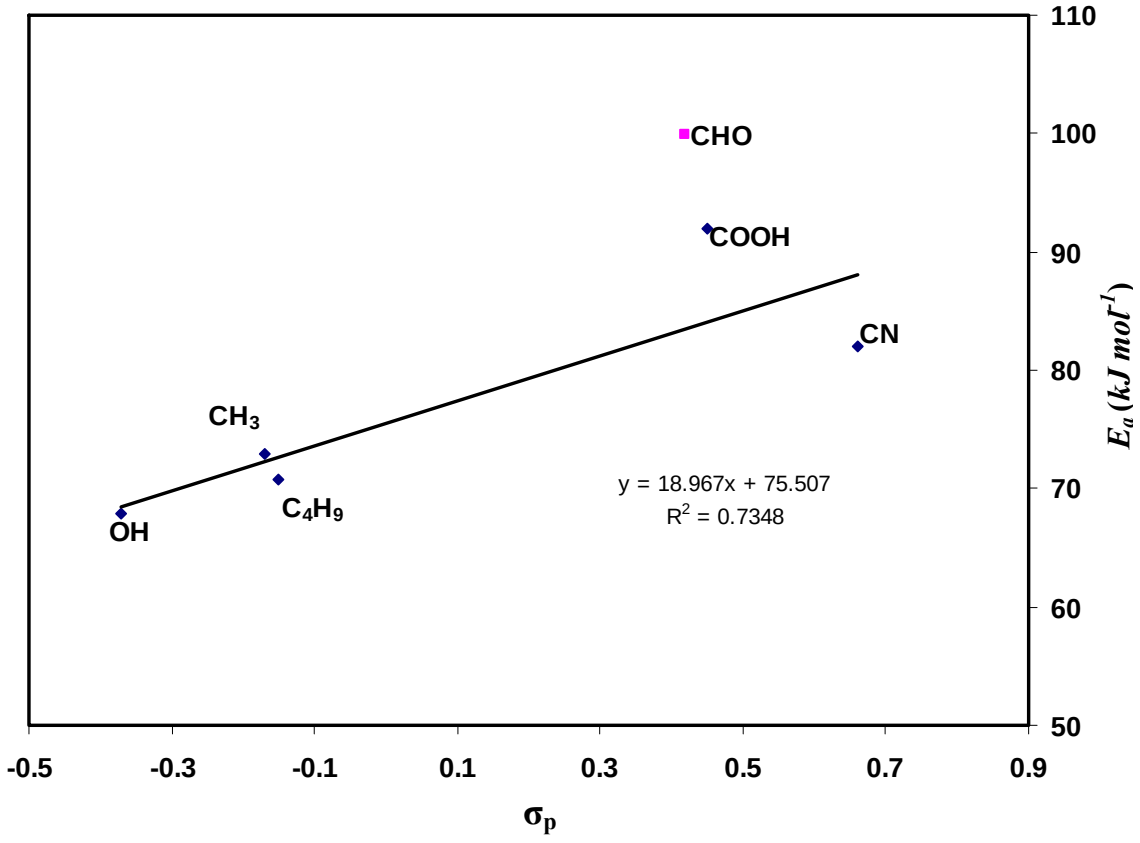


Figure 5.5: Correlation between the E_a of substituted catechols and Hammett parameter σ_p

5.2 Conclusion on catechol kinetics

The influence of nature of substituents on the kinetics of the *para* substituted catechols against free radical reaction was discussed. Phenols possessing strong electron-donating substituents showed faster reactivity against free radical, but depending on the type of these substituents, the different reactive order of activity was observed. However, EDGs attached to the 4-catechol moiety are preferred for faster radical scavenging activity.

5.3 Computational study of substituted catechols

As discussed earlier in Chapter 4, in catechol, after the O_2-H_2 bond is broken, the aroxyl radical is able to rearrange itself by forming intramolecular H bonding with $O_2\bullet$ to attain the most stable conformation (Figure 5.6). When the hydrogen atom leaves the phenolic group, the nature of substitution affect the stability of both the ground state and the radical. Hence this computational study was mainly focused to explore the effect of substituents on the (O_2-H_2) BDE of catechol.

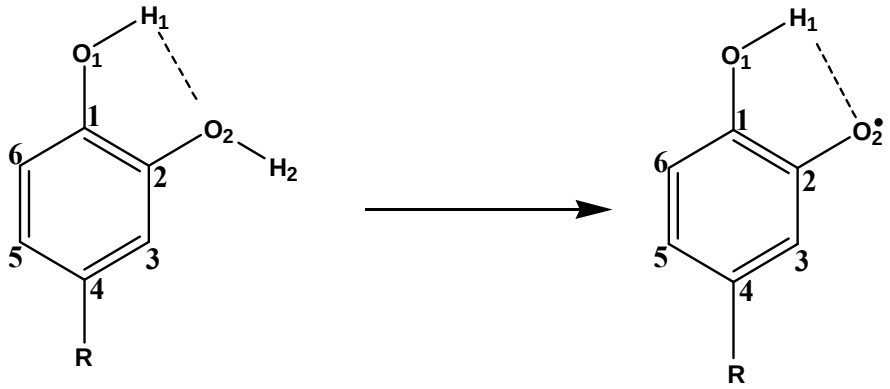


Figure 5. 6: Stable arrangement of radical from substituted catechol

5.4 Computational results and discussion on substituted catechols

The OH BDE of selected substituted catechols was calculated using B3LYP/ 6-311 G++(3df, 3pd) in methanol and the values are reported in Table 5.3.

Table 5.4: Effect of *para* substitutions on the (O_2-H_2) BDE of catechols

Nature of substitution group	Substituents	C(4)	(O_2-H_2) BDE (kJ mol^{-1})	ΔBDE_1[a] (kJ mol^{-1})
EDG	Methyl[c],	CH_3	306.101	-6.7
	Ethyl[d],	C_2H_4	305.658	-7.8
	Butyl[e],	t -C_4H_9	305.010	-7.4
EWG	DBAcid[h],	COOH	319.949	+7.1
	Cyano[i],	CN	320.479	+7.6
	DBAlde[j],	CHO	329.427	+16.6
	[b]Cat	H	312.850	0

[a] ΔBDE_1 = BDE of given compound – $BDE_{catechol}$

[b]catechol, [c]4-methyl catechol, [d]4-ethyl catechol, [e]4-t-butyl catechol, [h]2,3-dihydroxy benzoic acid, [i]4-cyano catechol, [j]2,3-dihydroxy benzaldehyde,

The results obtained from the computational calculations on the *para* substituted catechols were analyzed on the basis of the nature of substitutents as follows;

5.4.1 Effect of EDGs on OH BDE of catechol

The result show that electron donating groups (-CH_3, -C_2H_4 and –t-C_4H_9) reduced the OH BDE of catechol and supports the initial hydrogen atom abstraction. OH BDE calculations show that the presence of alkyl groups reduces the BDE of catechol by ca. 8 kJ mol^{-1}. This could be due to the reason that EDGs such as alkyl chains induce weakening of the OH bond by the combination of effects *i.e* i) the destabilization of the parent catechol and ii) stabilization of the aroxyl radical by delocalization of the unpaired electron (Pedulli *et al*., 1997). Substituents that stabilize the ground state usually increase the OH BDE while stabilization of the radical results in lower BDE.

Radical delocalization is represented by the canonical forms. Four possible canonical forms are shown for phenoxyl radical from catechol in Figure 5.7. EDG in the benzene ring could enrich the electron density at *ortho* and *para* position (C3, C5 and C1) by providing electron to the benzene ring. Hence the existence of all the four canonical forms (I, II, III and IV) are possible for the phenoxyl radical obtained from EDG substituted catechols. Greater the number of canonical forms, the greater the electron delocalization. This results in better radical stability, and hence lowers the OH BDE.

5.4.2 Effect of EWGs on OH BDE of catechol

Electron-withdrawing substituents (-CN, CHO, COOH) showed negative effects toward initial hydrogen atom abstraction from O_2-H_2 of catechol. Unlike EDGs, EWGs on the catechol destabilize the radical but stabilize the ground state. This increases the energy

gap between the ground state and the radical, and so the BDE increases. Presence of EWG in the benzene ring pulls an electron from the benzene ring and thus weakens the electron density at *ortho* and *para* position (C3, C5 and C1). Hence the existence of canonical forms; II, III and IV could be diminished for the phenoxyl radical obtained from the EWG substituted catechols. This destabilization could be accounted for the larger OH BDEs of EWGs substituted catechols.

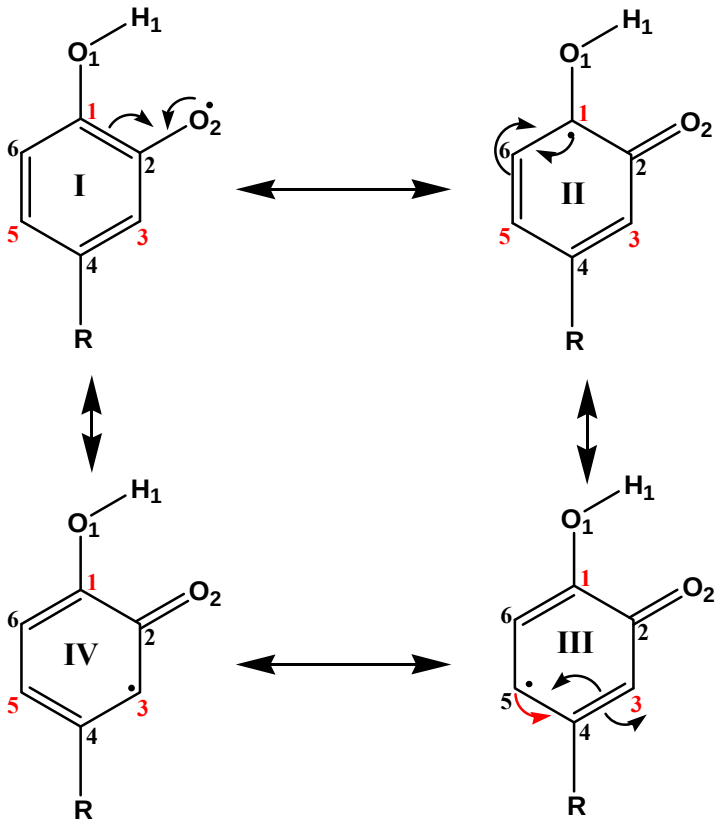


Figure 5.7: Canonical forms (I, II, III and IV) of phenoxyl radical

As discussed earlier in this chapter, Hammett's substituent parameter (σ) reflects the electron withdrawing and electron donating properties of a substituent. Hence, the electronic effect (σ_p) of substitutent was also correlated with the O-H BDE and shown in Figure 5.8.

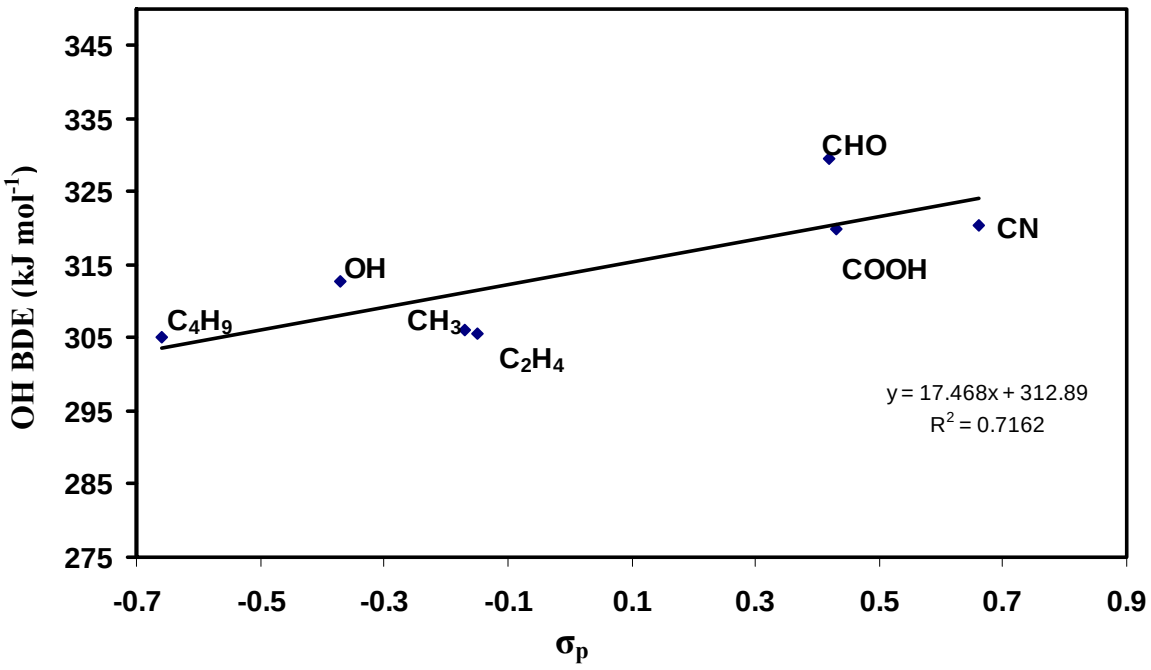


Figure 5.8: Correlation between the O-H BDE of substituted catechols and Hammett parameter σ_p

The present computational results on OH BDEs of substituted catechols are in fair agreement with our kinetic reactivity order of substituted catechols against DPPH•. It indicates that the OH BDE and rate parameters are the valid tool for measuring the free radical scavenging ability of phenols.

One of the ways to check their validity is to correlate the rate parameters with their OH BDE values (Lucarini *et al*., 1996; Burton *et al*., 1985; Valgimigli *et al*., 1999). According to Donkers and Workentin (2001), the rate constant of a biological or chemical process can be related to the activation free energy ($\Delta G^{\#}$) as given below;

$$k \ \alpha \ \exp(\Delta G^{\#}/kT) \qquad \text{Eqn 5.1}$$

Saveant's model relates the activation free energy ($\Delta G^{\#}$) to the reaction free energy (ΔG^{0}) using the equation 5.2, which contains contributions from the bond dissociation enthalpy (BDE) in addition to the reorganization energy (λ). (Saveant, 1993; Andrieux, 1986):

$$\Delta G^{\#} = \frac{\lambda + BDE}{4}(1 + \frac{\Delta G^{0}}{\lambda + BDE})^{2} \qquad \text{Eqn 5.2}$$

As the numeric value of BDE is greater than the free energy of reaction, ΔG^{0} the numerical value of term $(1+\frac{\Delta G^{0}}{\lambda + BDE})^{2}$ may be neglected. Hence the equation 5.2 can be rewritten as:

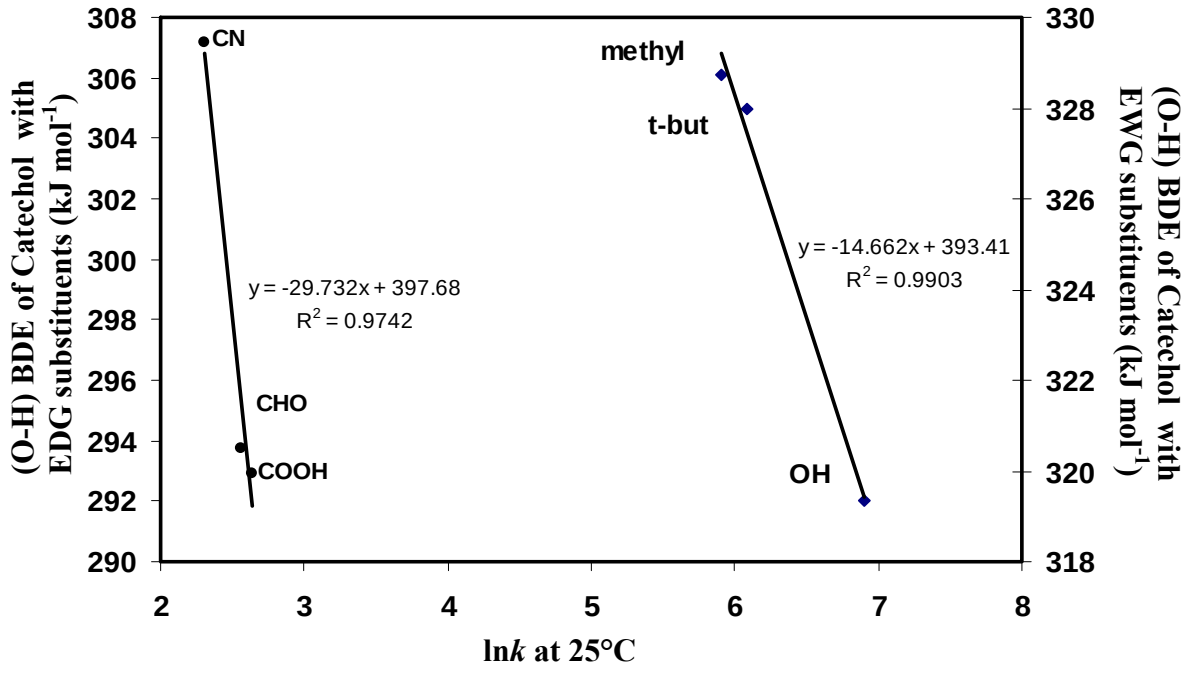
$$\Delta G^{\#} = \frac{\lambda + BDE}{4} \qquad \text{Eqn 5.3}$$

On substituting the Eqn 5.3 into Eqn 5.1, we obtain:

$$k\ \alpha\left(\exp\frac{\lambda + BDE}{4}/kT\right) \qquad \text{Eqn 5.4}$$

It can be seen from the equation 5.4 that the logarithm of rate constant is proportional to the bond dissociation enthalpy. It shows that the rate constants for the hydrogen atom abstraction reactions are dependent upon BDEs. Hence, plot was made between ln*k* and OH BDE of substituted catechols and shown in Figure 5.9. Another correlation was made between the activation energy of the reaction (E_a) and the corresponding O_2-H_2 BDE (see Figure 5.10). Good correlation obtained between experimental rate parameters and computed OH BDEs emphasizes that E_a, *k* and OH BDE are the suitable parameters to explain the radical scavenging ability of phenols.

Figure 5.9: Correlation between rate constant at 25 °C and OH BDE of substituted catechols.



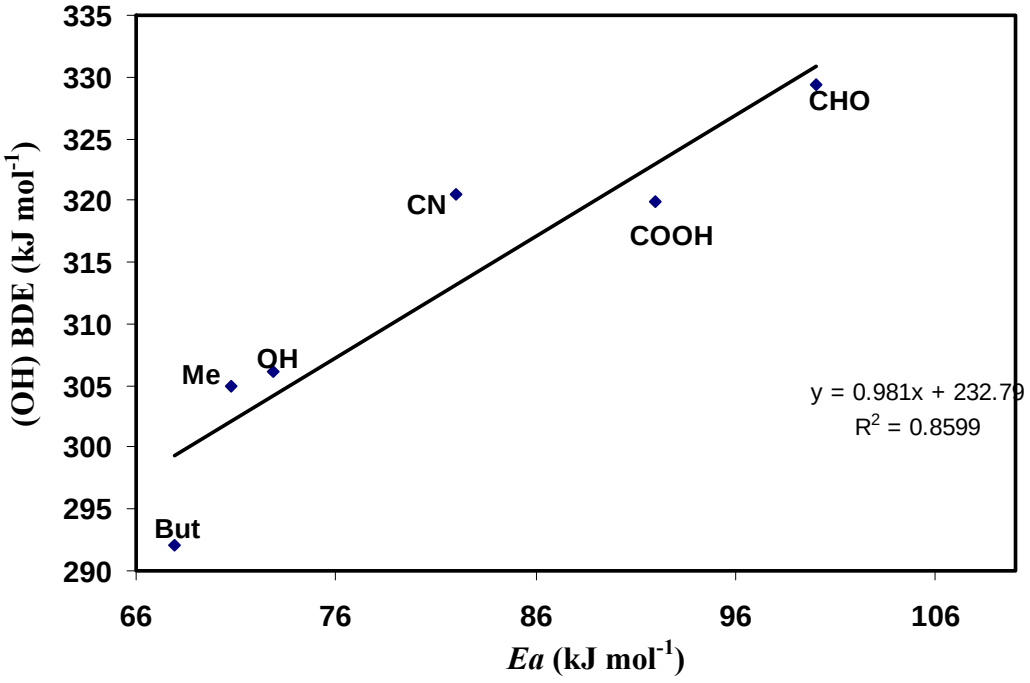


Figure 5.10: Correlation between activation Energy E_a and OH BDE of substituted catechols.

In this part of the study, we used the high level theoretical method (B3LYP/ 6-311 G++(3df, 3pd) to examine the OH BDEs of substituted catechols. The results obtained in this work clearly show that the nature of the substituents and the radical scavenging ability of phenols are related. BDEs of catechols with EDGs in the *para* position decrease the BDEs while EWGs increase the BDEs. Study on substituent effects also showed that the presence of EDGs at the C(4) position of catechol play a key role. The presence of EDGs at the C(4) position contribute to lowering of the energetic expense for breaking the OH bond and favor the H-atom transfer to free radicals.

6. SUBSTITUENTS EFFECT ON THE RADICAL SCAVENGING ABILITY OF PYROGALLOL

Gallate esters (gallic acid, methyl gallate and ethyl gallate) are widespread in plant foods and beverages such as tea and wine. These compounds are reported to have anticarcinogenic effect (Polewski *et al*., 2002). Gallic acid and alkyl esters of gallate, especially propyl-, octyl-, and lauryl gallate are also used as food additives for scavenging the radicals (ROS) that are responsible for the rancidity of different foodstuffs (Sherwin *et al*., 1990; Nakagawa *et al*., 1997). Alkyl gallates were identified to have a high affinity factor for the cell membrane, and thus they could efficiently enter the cell membrane (Nakayama *et al*., 1998; Nakayama *et al*., 2000). The presumptive explanation was that the lipophilic alkyl side chain makes it easier for the benzene ring with three hydroxyl groups (pyrogallol moiety) to permeate the cell membrane and interact with the enzymes in the cells. This makes gallate esters more useful antioxidants than other polyphenolics in cells (Nakayama *et al*., 2000).

Not only in gallate esters and alkyl gallates but also in major polyphenols such as flavanoids, pyrogallol is the crucial moiety observed as shown in Figure 6.1. Hence, for this study, three widely used gallate esters namely gallic acid (COOH), methyl gallate ($OOCH_3$) and ethyl gallate (OOC_2H_4) were chosen in order to investigate the presence of substitution in the pyrogallol moiety. Selected substituents groups on the C(1) position of pyrogallol as shown in Figure 6.2.

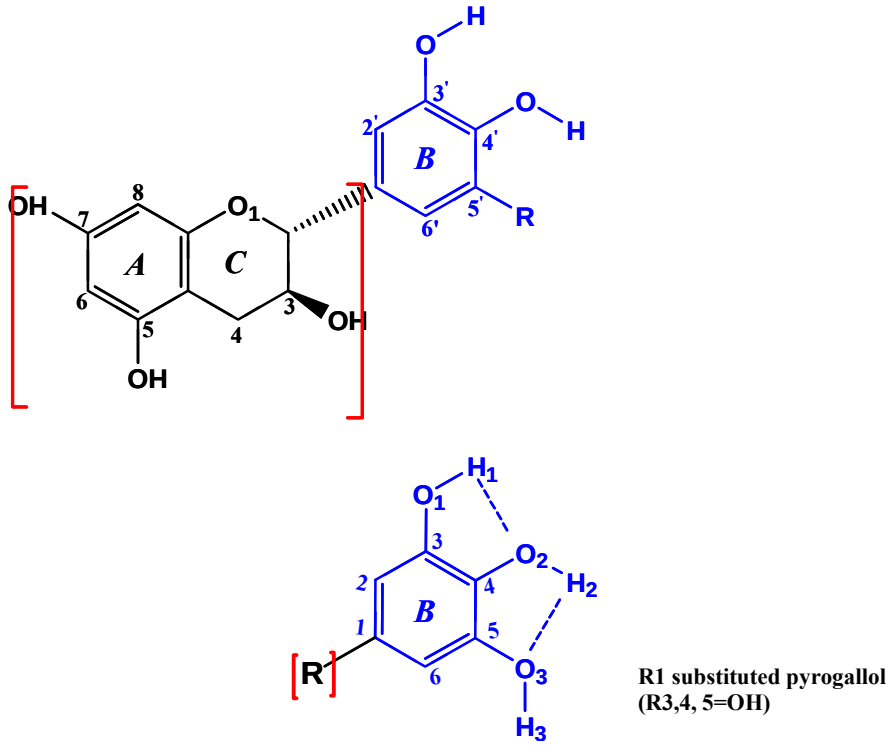
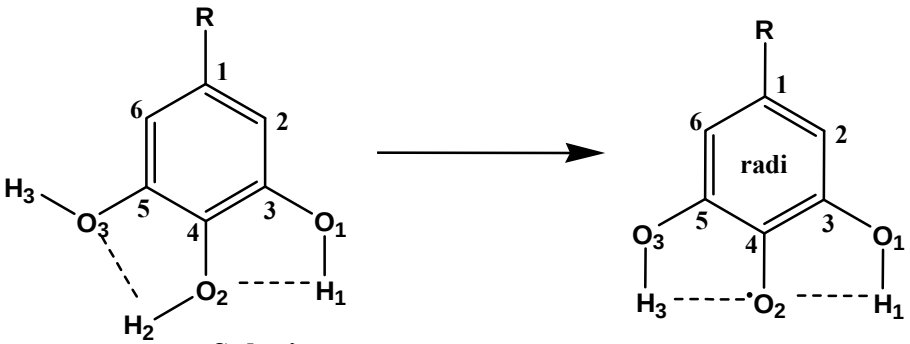


Figure 6.1: Importance of pyrogallol model compound in the structure of flavonoids.



Substituents		Name of the compound
R1 = H	→	Pyrogallol
R1 = $OOCH_3$	→	Methyl Gallate
R1 = OOC_2H_5	→	Ethyl Gallate
R1 = COOH	→	Gallic Acid

Figure 6.2: Substituents in pyrogallol moiety

6.1 Results and discussion on kinetics of substituted pyrogallols

The kinetic experiments were carried out for the reaction of substituted pyrogallols (COOH, $OOCH_3$ and OOC_2H_4) with DPPH• in methanol and rate parameters were measured. It is tabulated in Table 6.1. Arrhenius plot for substituted pyrogallols were drawn and shown in Figure 6.3.

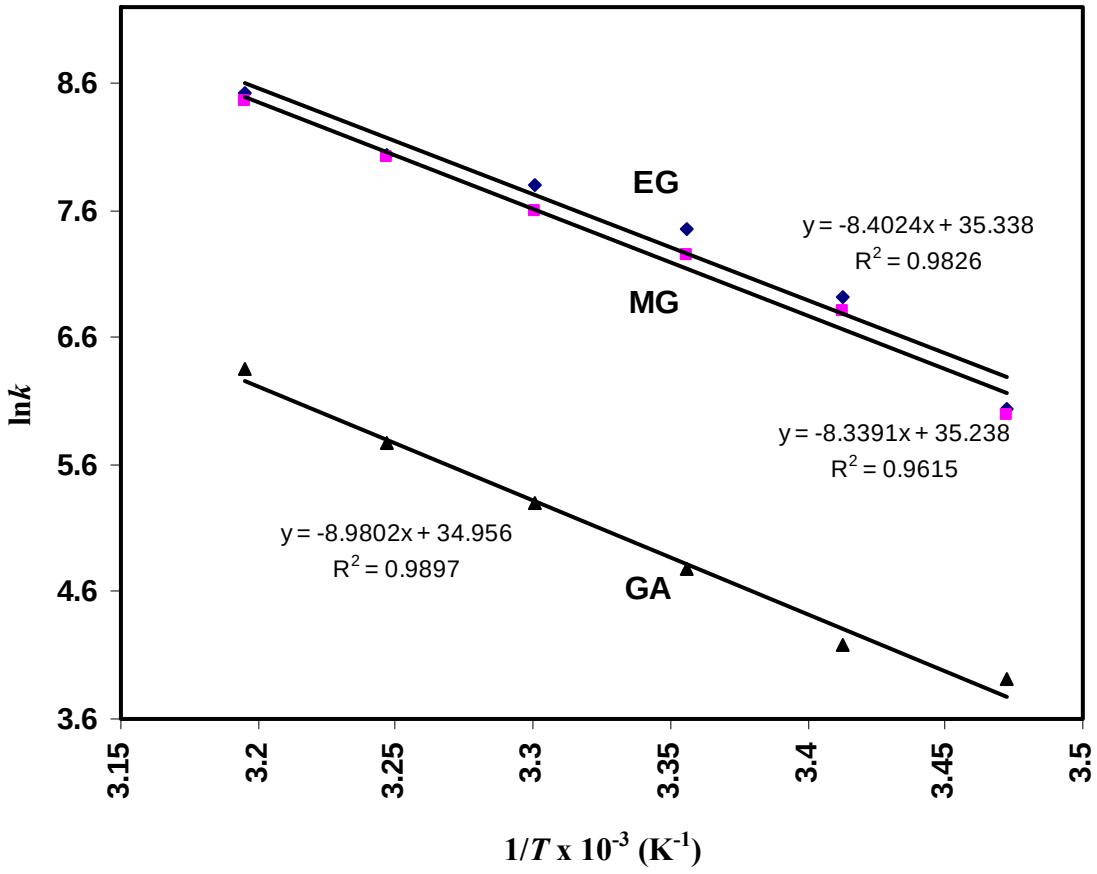


Figure 6.3: Arrhenius plot for gallic acid, methyl gallate and ethyl gallate against DPPH• in methanol

Table 6.1: Rate constants (k), activation energy (E_a) of substituted pyrogallols in methanol

Substitution group	Nature of group	Second order rate constant, k ($M^{-1}s^{-1}$) at different temperatures						Arrhenius parameters	
		15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	E_a kJ mol^{-1}	ln$A$ $M^{-1}s^{-1}$
[a]COOH	EWG	50	65	120	200	320	570	74.7±3.8	34.9±1.5
[b]$OOCH_3$		400	910	1405	2000	3020	4700	69.9±4.6	35.3±1.8
[c]OOC_2H_5		420	1010	1740	2450	3100	5030	69.3±6.9	35.2±2.7
[d]H		620	990	1700	2185	3600	6000	66.2 ±2.8	34.0 ±1.1

[a]gallic acid; [b]methyl gallate; [c]ethyl gallate; [d]pyrogallol

Table 6.2: Comparison of substituted pyrogallols activation energy with the pyrogallol

Nature of group	Compound	C(4)	E_a, kJ mol^{-1}	[d]ΔE_a, kJ mol^{-1}
EWG	GA[a]	COOH	74.7	+8.5
	MG[b]	$OOCH_3$	69.9	-3.7
	EG[c]	OC_2H_4	69.3	-3.1

[a]gallic acid; [b]methyl gallate; [c]ethyl gallate,
[d]ΔE_a = E_a of pyrogallol (66.2 kJ mol^{-1}) – E_a of compound

Substituting longer ester chain group in the pyrogallol moiety significantly affects rate constant with the trend $OOC_2H_4 > OOCH_3$. On comparing the ester substituted pyrogallol with the pyrogallol, it can be seen that the presence of ester groups $–OOCH_3$ and $-OOC_2H_4$ at C(1) did not increase the radicals scavenging ability of pyrogallol (Table 6.1). This is due to the electron withdrawing nature of the ester substituents. However, unlike EWG COOH, esters did not decrease the rate of radical scavenging reaction to a larger extent. This is reflected in the larger k of esters especially OOC_2H_4 (Table 6.1) and it could be due to weaker electron withdrawing nature of esters. On the other hand, COOH substituent showed smaller rate constants and lower E_a compared to the esters and this is due to its stronger electron withdrawing property of COOH substituent.

6.2 Computational study for substituted pyrogallols

As discussed in the Chapter 4, pyrogallol has three OHs attached in the vicinal C(1,2,3) position and OH BDE calculations showed the lowest BDE observed at O_2-H_2 of all OH BDEs. This could be due to the fact that the radical always tend to stay in the lower energy state, which can be attained in this case by forming two intramolecular bonding ($O_2 \bullet$ ---H_1 and $O_2 \bullet$ ---H_3) (Figure 6.2). A recently reported study on pyrogallol derivatives using electron paramagnetic resonance (EPR) also confirmed that $O_2\bullet$ is the radical center for pyrogallol (Lucarini *et al*., 2002). Hence in this work, computational study was mainly focused to investigate the effect of C(1) substitutents on the hydrogen atom donating ability of the O_2-H_2 of pyrogallol.

6.3 Computational results and discussion for substituted pyrogallols

The computational calculation of gas phase O_2-H_2 BDE using B3LYP/ 6-311G++(3df, 3pd) on COOH substituted pyrogallol (gallic acid) and esters substituted pyrogallol (gallate esters) were carried out and reported in Table 6.3.

Table 6.3: (O_2-H_2) BDE of substituted pyrogallols

Compound	Substitution position C(1)	Nature of group	Gas phase 6-311++G (3df, 3pd)			
			[d]H_p [h]hartree	[e]H_r [h]hartree	O_2-H_2 BDE kJ mol^{-1}	[f]ΔBDE kJ mol^{-1}
GA[a]	COOH		-646.6	-645.9	305.1	+15.7
MG[b]	$OOCH_3$	EWG	-685.8	-685.2	303.4	+14
EG[c]	OOC_2H_4		-725.1	-724.5	295.1	+5.7
Pyr[g]	H		-457.9	-457.4	289.4	0

[a]gallic acid; [b]methyl gallate; [c]ethyl gallate
[d]H_p is enthalpy of parent molecule
[e]H_r is enthalpy of radical obtained from parent molecule
[f]ΔBDE = BDE of pyrogallol– BDE of given compound
[g]pyrogallol
[h]1 hartree = 2625.5 kJ mol^{-1}

All the calculated OH BDE of the compounds were found to be greater than that of pyrogallol. The presence of $OOCH_3$ in the C(1) position of pyrogallol increased the OH BDE of pyrogallol by 14 kJ mol^{-1}. Changing to OOC_2H_4 group increased OH BDE of pyrogallol only by *ca*. 6 kJ mol^{-1}. On comparison, the $OOCH_3$ group displayed higher OH BDE for pyrogallol than the OOC_2H_4 substituent. These results indicate that the length of alkyl chain present in the esters could be associated with the radical scavenging activity as such longer the chain the lower OH BDE. The substitutent -COOH showed largest increase in O_2-H_2 BDE of pyrogallol (*ca.* 16 kJ mol^{-1}). This is be due to the stronger

electron donating nature of COOH. This supports our earlier results that stronger EWGs (CHO and COOH) decrease the radical scavenging activity of phenols to a larger extent.

The correlation was made between theoretical OH BDE and experimentally calculated activation energy (E_a) of substituted pyrogallols and obtained the straight line with the regression coefficient r^2=0.67 (see Figure 6.4).

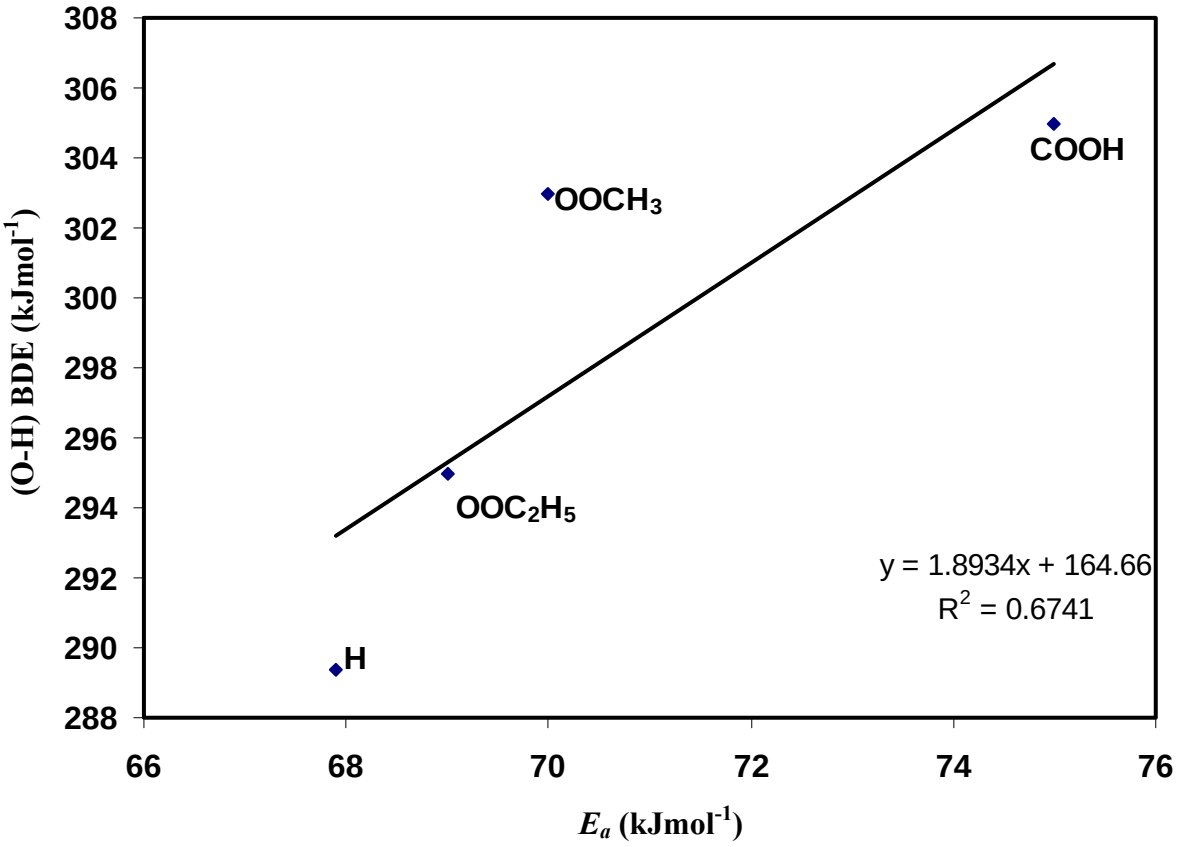


Figure 6.4: Correlation between E_a and OH BDE of substituted pyrogallols

6.4 Conclusion for substituted pyrogallols

The results from both computational and experimental kinetic measurements on substituted pyrogallols conveyed that the stronger EWG in the polyphenols larger the OH BDEs and could slow down the radical scavenging activity. From the studies on gallate esters, the longer the alkyl chain presents in the ester group, the better the radical scavenging activity.

7. STUDY ON RADICAL SCAVENGING ABILITY OF TEA POLYPHENOLS

Green tea is widely consumed and its primary nutritious constituents are the polyphenols (Lambert, 2003; Lakenbrink, 2003). The chief polyphenols in green tea are flavonoids such as catechin and proanthocyanidins, with the four major polyphenols being (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epicatechin (EC) (Junji *et al*., 1994). Green tea polyphenols possess anticarcinogenic and antibiotic properties. Epidemiologic studies suggested that the consumption of green tea is effective for cancer prevention (Oguni *et al*., 1989; Ines and Federico, 2000). Green tea's active components include catechins were found to be effective for treating and reducing gastrointestinal cancers (Hasan and Nihal, 2000). Green tea was found to reduce blood sugar (Leitzmann, 2003). Their antioxidative effect seems to be most important as lipid oxidation is involved in the human cancer (Negre-Salvayre *et al*., 1991; Decharneux *et al*., 1992). Recent studies also showed that the flavanol absorption in plasma was enhanced when tea polyphenols were taken as a capsule compared to that of raw food and beverages (Susanne *et al*., 2004). They suggested that the supplements retain the beneficial effects of green tea and may be used in future chemoprevention studies.

However, reports on pro-oxidative effects of flavonols (Yen *et al*., 1997; Cao *et al*., 1997; Miura *et al*., 1998; Ohshima *et al*., 1998; Moetodiewa *et al*., 1999) triggered us to evaluate the contribution of the individual antioxidant compounds to the overall

antioxidative effects of tea polyphenols. Until more is known about the chemical property (kinetic data) and structural activity by which tea polyphenols differ in their radical scavenging ability, it seems intuitively inadequate information to proceed for food fortification and supplementation with tea polyphenols. In this work, kinetic experiment was carried out for EC, EGC and EGCG to determine the kinetic parameters and computational study on EC and EGC for OH BDE calculations.

7.1 Kinetic study on radical scavenging ability of tea catechins

In this study, the radical scavenging activity of tea catechins was carried out against DPPH• using stopped-flow kinetic spectrometer at different [ArOH]/[DPPH•] as shown in Chapter 2. The rate constants obtained for five different temperatures (15°C-40°C) were tabulated in Table 7.1. Arrhenius plot of ln k vs. 1/T was drawn for tea catechins to obtain the activation energy E_a. The measured E_a of tea catechins were also given in Table 7.1.

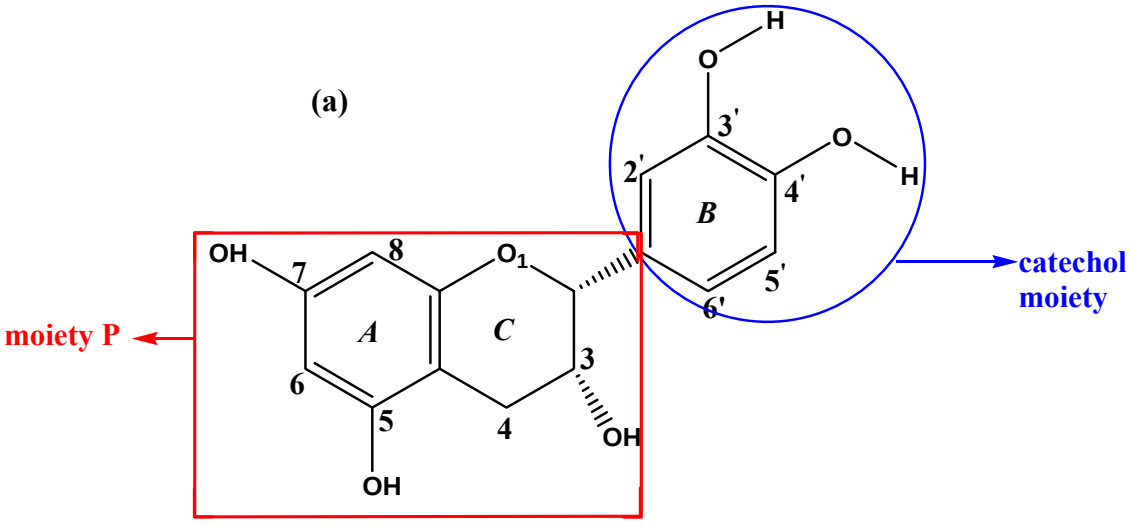
Table 7.1: Rate constants (k), activation Energy (E_a) of tea polyphenols against DPPH• in methanol

Compound	Second order rate constant, k ($M^{-1}s^{-1}$) at different temperatures						Arrhenius parameters	
	15 °C	20 °C	25 °C	30 °C	35 °C	40 °C	E_a kJ mol^{-1}	ln$A$ $M^{-1}s^{-1}$
EC[a]	260	492	688	1300	1736	2648	68.7±3.2	34.33±1.3
EGC[b]	1200	2850	3890	5000	7020	13000	63.7±6.4	33.90±2.5
EGCG[c]	1900	3030	4604	6901	9300	15400	60.9±1.7	33.00±0.7

[a] -(-) epicatechin; [b] -(-) epigallocatechin; [c] -(-) epigallo catechin gallate

From the Table 7.1, it can be seen that the EGCG was found to have larger k for all temperatures studied and the lower E_a compared to EC and EGC. This can be explained from their structural point of view.

Figure 7.1: Segmented structure of EC (a), EGC (b) and EGCG (c)



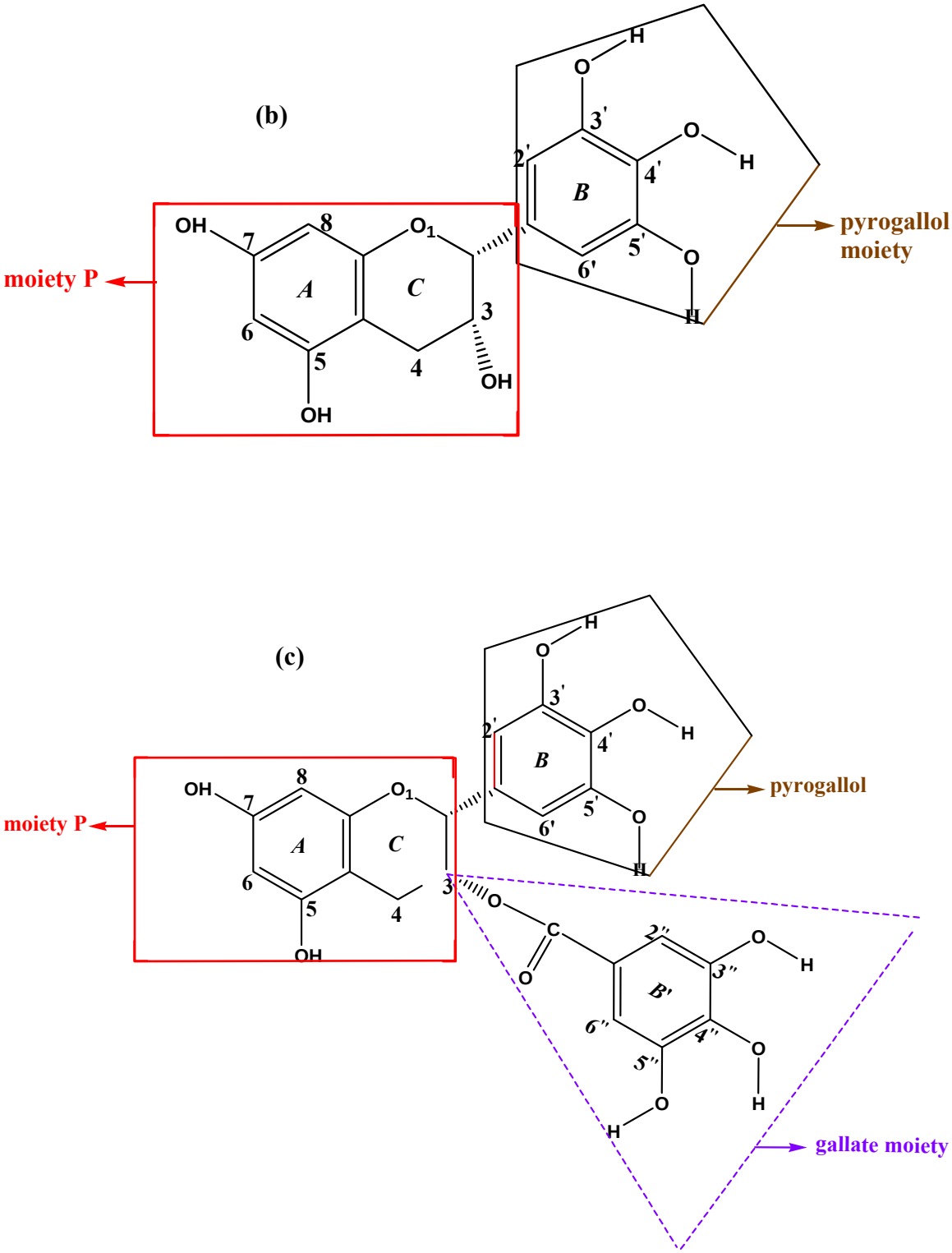


Figure 7.2: Segmented structure of EC (a), EGC (b) and EGCG (c)

As shown in Figure 7.1a, the structure of EC can be considered to have major two moieties (P and B), in which the moiety B represents the catecholic structure i.e two OHs in the *ortho* position. The structure of EGC has an additional OH in the B ring (Figure 7.1b**)** and the presence of third OH could be associated with the larger k of EGC compared to that of EC. The structure of EGCG has gallate moiety in addition to moiety B and P, and this could be responsible for the larger rate constants (k) and lower E_a compared to EGC and EC. From the kinetic results obtained on EC, EGC and EGCG, it can be suggested that the gallate and pyrogallol moiety contribute considerably to the radical scavenging activity of polyphenols.

In addition, we also decided to evaluate the effect of moiety P in the radical scavenging ability of tea catechins. On comparing E_a of catechol to EC and E_a of pyrogallol to EGC, it is possible to demonstrate the effect of moiety P in EC and EGC. As shown in Table 7.2, the presence of moiety P in catecholic structure decreased the E_a of catechol around 6 kJ mol^{-1} but only 2.5 kJ mol^{-1} for pyrogallol. It means that the moiety P in pyrogallol contribution is only 2.5 kJ mol^{-1}, which is lower than that of moiety P contribution in pyrogallol structure i.e EGC. This indicates that the double *ortho* structure in pyrogallol moiety should play dominating role and increases the radical scavenging ability of tea catechins. It hence strengthened our argument as suggested in the earlier chapters that greater the number of *ortho*, the faster the radical scavenging activity.

Table 7.2: Effect of moiety P present in the tea catechins

Tea catechins	E_a (kJ mol^{-1}) *(1)*	Model Compounds	E_a (kJ mol^{-1}) *(2)*	*(1-2)* $^{d}\Delta E_a$ (kJ mol^{-1})
EC[a]	68.7	Catechol	74.8	-6.1
EGC[b]	63.7	Pyrogallol	66.2	-2.5

[a] -(-) epicatechin; [b] -(-) epigallocatechin;
[d] $\Delta E_a = E_a$ of tea catechins - E_a of model active compounds.

In epigallocatechin gallate (EGCG), not only the presence of third OH in the B moiety and but also the gallate moiety B' enhance the radical scavenging activity by increasing the more hydrogen atoms available to be donated to the radical. This is reflected as the larger rate constant values for EGCG as shown in the Table 7.1. The kinetic study using the stopped flow spectrometer on tea catechins showed that the order of reactivity against the DPPH• was found as follows: EGCG > EGC > EC. The literature reported findings on tea catechins against OH and N_3 radicals is also found similar in the reactivity order (Wolf *et al*. 1999).

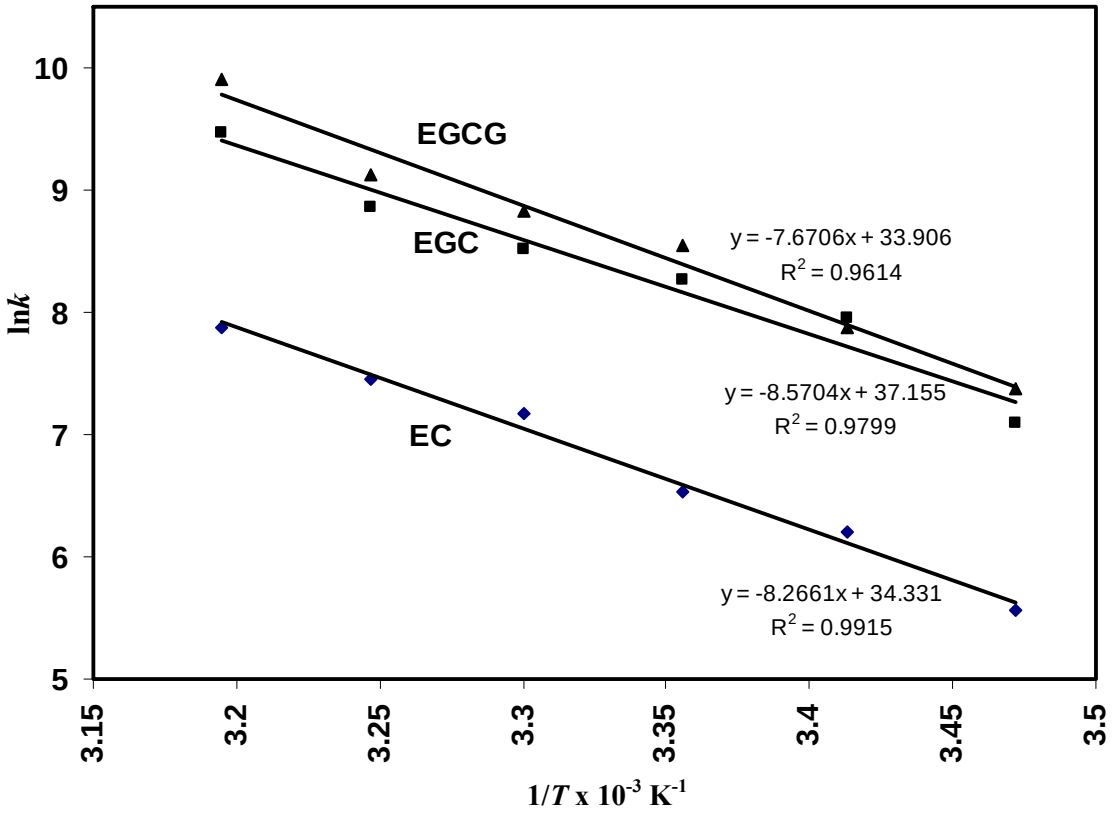


Figure 7.3: Arrhenius plot on EC, EGC and EGCG reaction with DPPH•

7.2 Computational study on tea catechins

As the tea polyphenol molecules modeled in this study are relatively larger and with polar functional groups, the computational analysis of EC, EGC was done at the smaller basis set (B3LYP/6-31G(d)) to reduce the expensive calculations. The OH BDEs for each hydroxyl group of EC and EGC were calculated at B3LYP/6-31G(d) and are listed in Table 7.3. As shown in Figures 7.3 and 7.5, the hydroxyl groups of ring B have the lowest BDEs in EC and EGC, which results from the stabilized radical by the *ortho* hydroxyl exerted intramolecular hydrogen bonds (IHB). The lowest OH BDE of epicatechin (EC) was observed at the region C(4')-OH and was 280 kJ mol^{-1}, lower than that of C(3')-OH in the B ring by 3 kJ mol^{-1} (see Figure 7.3 and Table 7.3). The next lowest OH BDE for EC was observed on ring C at C(3)-OH and was 322.6 kJ mol^{-1}.

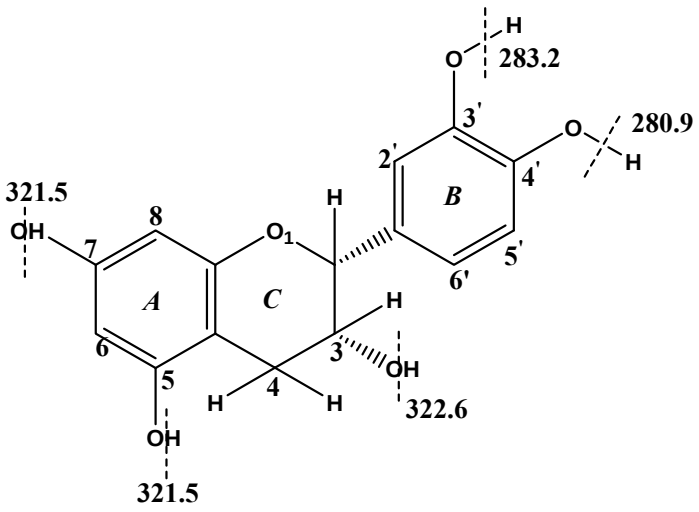


Figure 7.4: Calculated OH BDEs of epicatechin (EC) using B3LYP/6-31G(d)

Figure 7.5: Radical scavenging mechanism of epicatechin (EC)

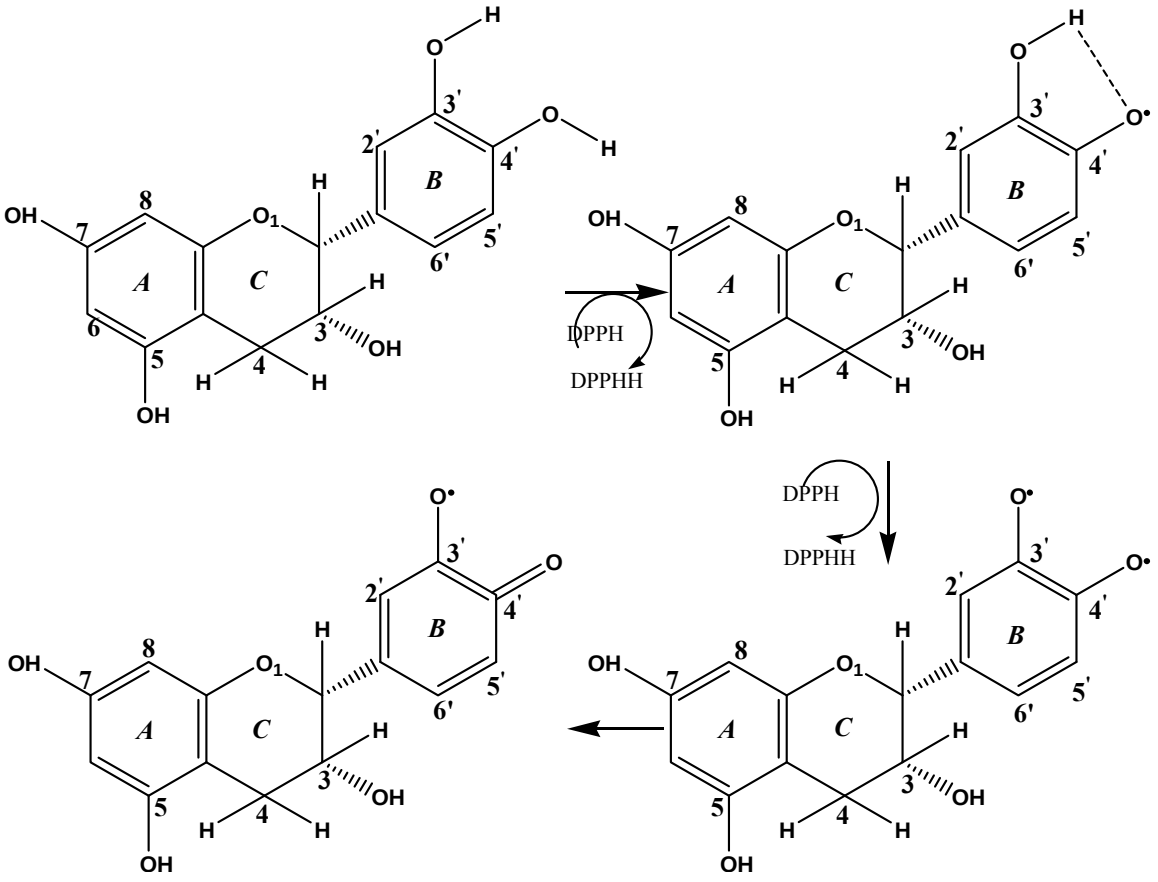


Figure 7.6: Radical scavenging mechanism of epicatechin (EC)

Computational study of EGC revealed that the lowest OH BDE was observed at the region C(4')-OH and was 259.5 kJ mol^{-1}, which is 30 kJ mol^{-1} lower than that of C(3')-OH and C(5')-OH (see Figure 7.5 and Table 7.3). The OH BDE at the region C(5')-OH was estimated as 293.7 kJ mol^{-1}, which is higher than that of OH BDE of EGC at C(4')-OH by 34.7 kJ mol^{-1}.

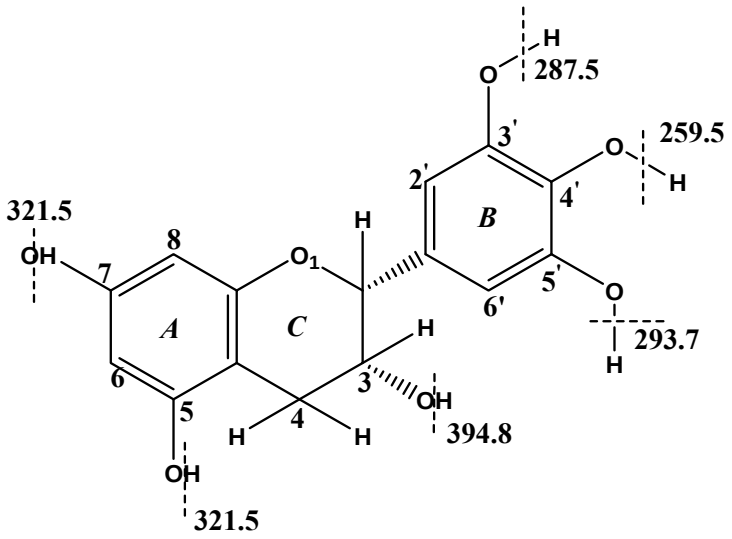


Figure 7.7: Calculated OH BDEs of epigallocatechin (EGC) using B3LYP/6-31G(d)

Table 7.3: OH BDEs of tea catechins using B3LYP/6-31G(d) in gas phase study

	Major model compounds observed in the tea catechins					
Tea catechins	**Ring B**			**Ring C**	**Ring A**	
	OH BDE[d] (3'-OH) (kJ mol^{-1})	**OH BDE[d] (4'-OH) (kJ mol^{-1})**	**OH BDE[d] (5'-OH) (kJ mol^{-1})**	**OH BDE[d] (3-OH) (kJ mol^{-1})**	**OH BDE[d] (5-OH) (kJ mol^{-1})**	**OH BDE[d] (7-OH) (kJ mol^{-1})**
EC[a]	283.2	280.9	NA	322.6	321.5	321.5
EGC[b]	287.5	259.5	293.7	394.8	321.5	321.5

[a](-)- epicatechin Catechin; [b](-)-epigallocatechin;
[d] calculated using the B3LYP/6-31G(d) using Gaussian 98
NA = Not applicable

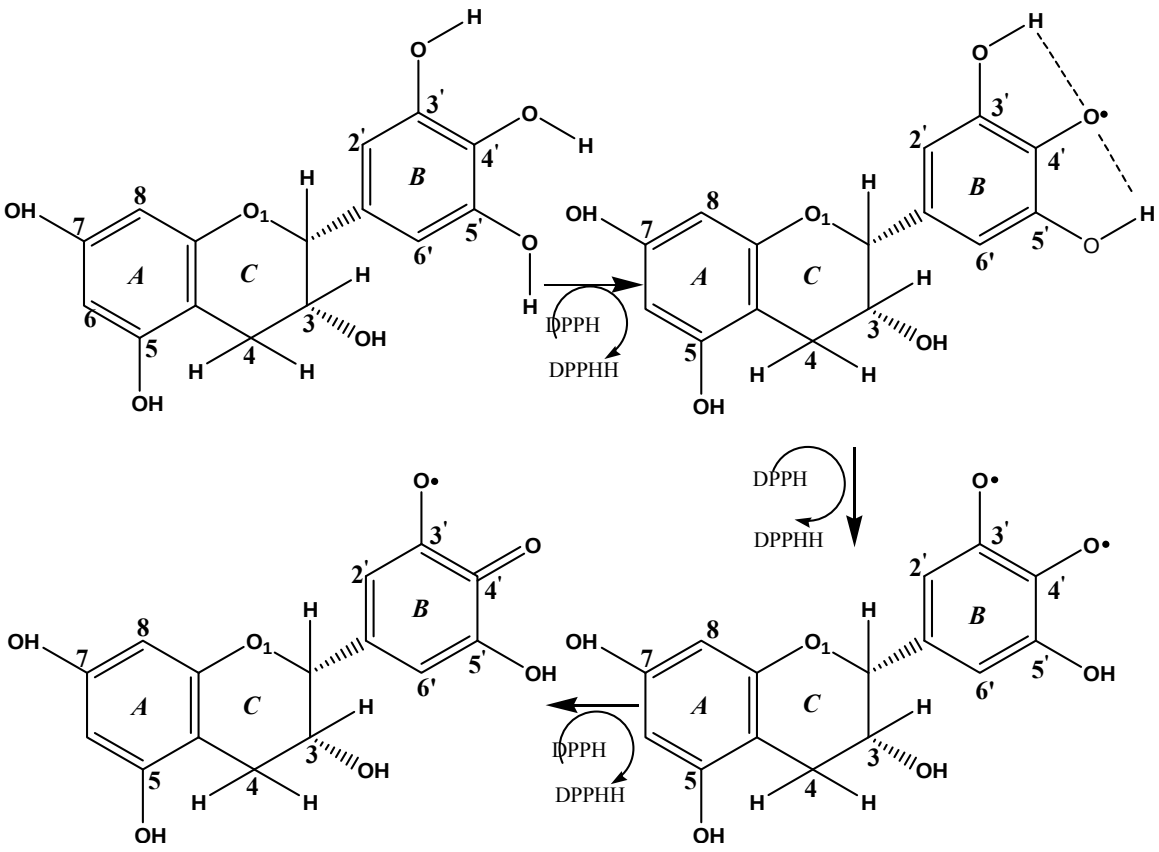


Figure 7.8: Radical scavenging mechanism of epigallocatechin (EGC)

The lowest OH BDE of EGC is 259.5 kJ mol^{-1}. This result suggests that H atom from the pyrogallol moiety B is more easily donated to the radical. This is due to the presence of additional OH in ring B of EGC which exerts two IHBs within the three OHs.

Now on comparing the obtained lowest OH BDEs on both EC and EGC, it can be seen that the presence of additional OH in the moiety B of EGC decreased the OH BDE by 21.4 kJ mol^{-1} than that of EC. This result also coincides with the results obtained from the

model compounds study with catechol and pyrogallol. Computational study on tea catechins showed that the lowest OH BDE of tea catechins is at the pyrogallol moiety, which coincides with our results obtained from the model phenols (chapter 4) that pyrogallol is the fastest radical scavenger. The present finding indicates that hydrogen transfer occurs from the C(4)-OH to scavenge the radicals. Based on these studies the mechanism for EC and EGC reaction towards DPPH• were proposed in Figures 7.4 and 7.6 respectively.

The OHs in the ring A of both EC and EGC likely represent the structure of resorcinol. In both EC and EGCs, OHs on the ring A are in the *meta* position showed the same OH BDEs and at the other hand, their values are much higher than that of ring B. It indicates that OHs on ring A do not really contribute to the radical scavenging ability of EC and EGC. These results support our earlier prediction from the model phenols that the OH in *meta* position will have higher BDEs and hence do not contribute for the radical scavenging activity. This implies that the sole sites of radical attack are certainly the catechol and/or pyrogallol groups in the B-ring of EC and EGC. These results indicate that giant structured polyphenolic molecules behave in the similar fashion as the smaller molecules. Hence, the computational studies on separate ring moiety of a large natural occurring polyphenols can be studied independently and correlated to understand the radical scavenging activities of the whole molecule.

8. OVERALL CONCLUSION

In the present study, radical scavenging ability of model polyphenols and tea catechins was evaluated using kinetic and computational approach.

8.1 Conclusion on kinetic results

Comparing the kinetics results obtained from three OHs model compounds pyrogallol and phloroglucinol, it can be concluded that the radical scavenging ability of phenols depend on the position of OHs in the ring and the greater the number of OH groups in the adjacent position of the benzene ring, the faster the radical scavenging ability. The reactivity towards free radical proceeds in the following order; double *ortho* (pyrogallol) > one *ortho* (catechol) > *para* (hydroquinone) >> *meta* (resorcinol and phloroglucinol).

The rate constants in polar protic medium were observed to be larger than that of non protic medium. This behavior is due to the interaction between the OHs of phenols and the hydrogen atom donor property of the solvent. Results from solvent based study also show that the polar protic solvent facilitates for the faster radical scavenging activity of phenols whereas apolar solvent does not. Hence, the kinetic studies on hydrogen atom abstractions from hydroxyl (OHs) are strongly dependent on the strength of the solvents. Hence, the nature of the solvent (protic or aprotic and polar or apolar) has a great influence on the free radical scavenging ability of phenolic antioxidants.

The kinetic experiments on the substituted catechols showed that the electron donating groups (EDGs) at the *para* position of catechol moiety increase the radical scavenging

activity, whereas EWGs decrease. Rate parameters correlated against Hammet constant indicated that the rate of radical scavenging reaction depends on the electronic property of substituents. The reactive order for EDGs in the catechol is as follows; OH > t-C_4H_9 > CH_3. Experimental results from the substituted pyrogallols concluded that the presence of stronger EWGs such as COOH, CHO decrease the rate of radical scavenging reaction to a larger extent, however, the weaker EWGs such as esters $OOCH_3$ and OOC_2H_4 showed smaller effect in rate of radical scavenging reaction of pyrogallol. Therefore, the radical scavenging ability of phenolic antioxidants could be further improved by altering both the number of OH groups, and the type and position in the unit. The experimental studies on tea polyphenols revealed that the number of OHs in the B ring play a vital role in increasing the rate of radical scavenging reaction. EGCG has the lowest activation energy E_a (60.9 kJ mol^{-1}) compared to EC and EGC and it is considered as the most effective polyphenol in scavenging DPPH•. The reactivity order of tea polyphenols was shown as EGCG > EGC > EC.

8.2 Conclusion on theoretical results

Theoretical methods based on the Density Functional Theory (DFT/B3LYP), which combines both speed and accuracy, was used to calculate the OH BDEs of phenolic antioxidants. The OH BDEs of polyphenols was found to be dependent on the stabilization of aroxyl radicals which could occur via; intramolecular hydrogen bonded phenols and resonance. Calculated computational data provide valuable insight into the effects of EDG and EWG substituents on the hydrolytic dissociation of polyphenols. It is concluded that the addition of electron-donating groups (EDGs) such as methyl, ethyl and

butyl to the aromatic ring of phenols lower the OH BDEs and hence increase the H-atom donating ability of catechol. Larger OH BDEs obtained for aldehyde and cyano substitutions on the *para* position of catechol revealed that the reactivity of phenols is reduced for electron-withdrawing substituents (EWGs). Based on the correlation obtained between computational and experimental results, it can be concluded that the BDE is a good primary indicator of radical scavenging ability of polyphenols on the theoretical side.

The following conclusions are drawn from the studies on the polyphenols and its ability as an efficient radical scavenger; (a) the presence of the *ortho* OH group is required for faster radical scavenging activity (RSA), (b) the pyrogallol moiety plays a dominant role in the tea catechins for the faster radical scavenging activity (c) EDG substitutions increase the free radical scavenging activity of phenols.

8.3 Future work

In attempting to design an optimum synthetic antioxidant using the results obtained in this work, one must also include the ionization potential (IP) of phenolic antioxidants which can be determined using the appropriate solvent model theory. Computational and kinetic studies were carried out in this work on phenols to understand their radical scavenging activity in different medium (gas and solvents), and hence the next probable study of phenolic antioxidants is to carry out the study in real matrix systems i.e food and biological systems. Extension of this study on this aspect can further be useful for the design, synthesis and application of novel synthetic antioxidants.

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COURSES, CONFERENCES AND PUBLICATIONS

INTERNATIONAL ADVANCED COURSE

1) International training course by the Product Design and Quality Management Group, Wageningen University, **Netherlands**, Dec 2004.

INTERNATIONAL CONFERENCES

1) T. Velmurugan, Lai Peng Leong, Ryan P A Bettens, Molecular Modelling Study of Antioxidant Molecules, **Singapore** International Chemical Conference 2005, Dec 2005.
2) T. Velmurugan, Lai Peng Leong, Ryan P A Bettens, Study on the Influence of Bioactive Functional Groups in tea catechins. The First Mathematics and Physical Science Graduate Congress in Bangkok, **Thailand**, Dec 2005.
3) T. Velmurugan, Lai Peng Leong, Ryan P A Bettens, Studies on Dietary Polyphenolic Antioxidant Molecules in Preventing Free Radicals Damage, Gene Regulation and Cell Function, **Kyoto-NUS** University International Symposium, **Indonesia**, Jan 2005.
4) T. Velmurugan, Lai Peng Leong, Ryan P A Bettens**. "**Structural, Temperature And Solvent Effects On Antioxidant Action Of Polyphenols: A Thermo kinetic Approach"**.** World nutra 2004. November 7-10, 2004, San Francisco, CA, **USA**.
5) T Velmurugan, Lai Peng Leong and Ryan P A Bettens Free Radical Scavenging Activity Of Polyphenols: A Thermodynamic Approach. **Singapore** International Chemical Conference 2003.
6) T Velmurugan, Lai Peng Leong and Ryan P A Bettens. Kinetic explanation for the role of antioxidant in scavenging the free radicals. Asia Pacific Conference and Exhibition on Anti- Ageing Medicine. **Singapore**, September 2003.
7) T Velmurugan, Lai Peng Leong and Ryan P A Bettens. Role of Quantum Computational study on antioxidant activity against free radicals. Asia Pacific

Conference and Exhibition on Anti- Ageing Medicine. **Singapore**, September 2004.

INTERNATIONAL JOURNALS:

1) Thavasi, Velmurugan.; Leong, Lai Peng.; Bettens, Ryan P. A. Investigation of the influence of hydroxy groups on the radical scavenging ability of polyphenols. Published in **J. Phy. Chem. A** 2006, 110, pp.4918.

2) Thavasi, Velmurugan.; Leong, Lai Peng.; Bettens, Ryan P. A. Temperature and solvent effects on radical scavenging ability of phenols- to be submitted soon to Journal of Physical Chemistry A

3) Thavasi, Velmurugan, Lai Peng Leong, Ryan P A Bettens. Stopped- flow kinetic experimental and Computational studies on Substitutional Effects on Catecholic moiety. - to be submitted soon to Journal of Physical Chemistry A

4) Thavasi, Velmurugan, Lai Peng Leong, Ryan P A Bettens. Studies on Substitutional Effects on gallate moiety - to be submitted soon to Journal of Physical Chemistry A.

APPENDIX I

Figure S3.1 Arrhenius plots for resorcinol in solvents.

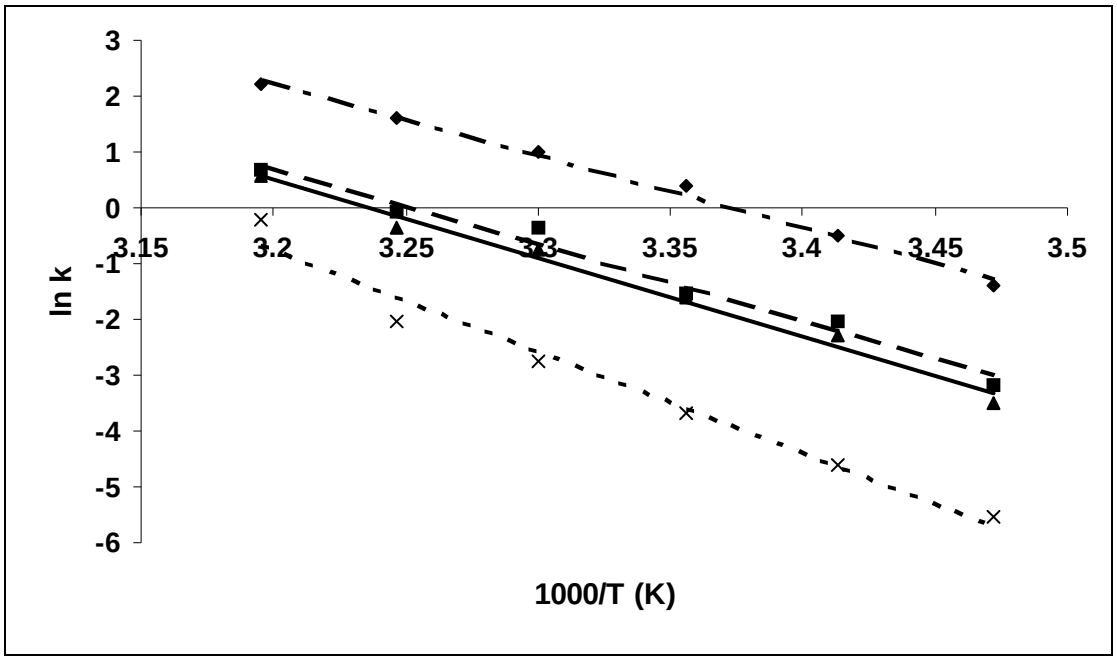

Figure S3.2 Arrhenius plots for hydroquinone in solvents

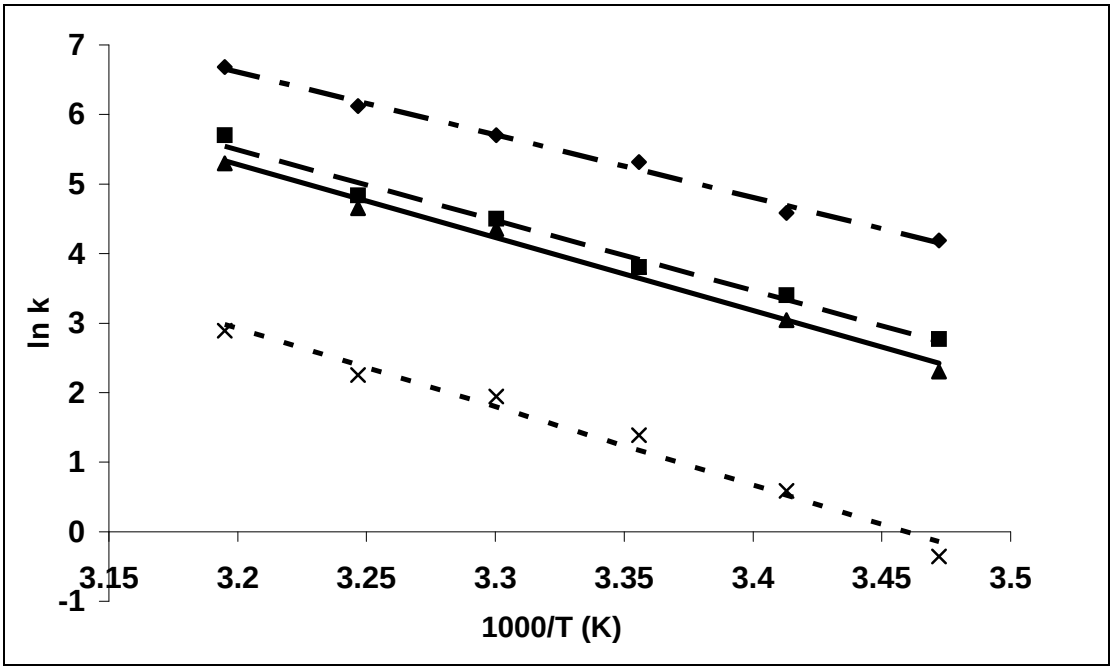

Figure S3.3 Arrhenius plots for phloroglucinol in solvents.

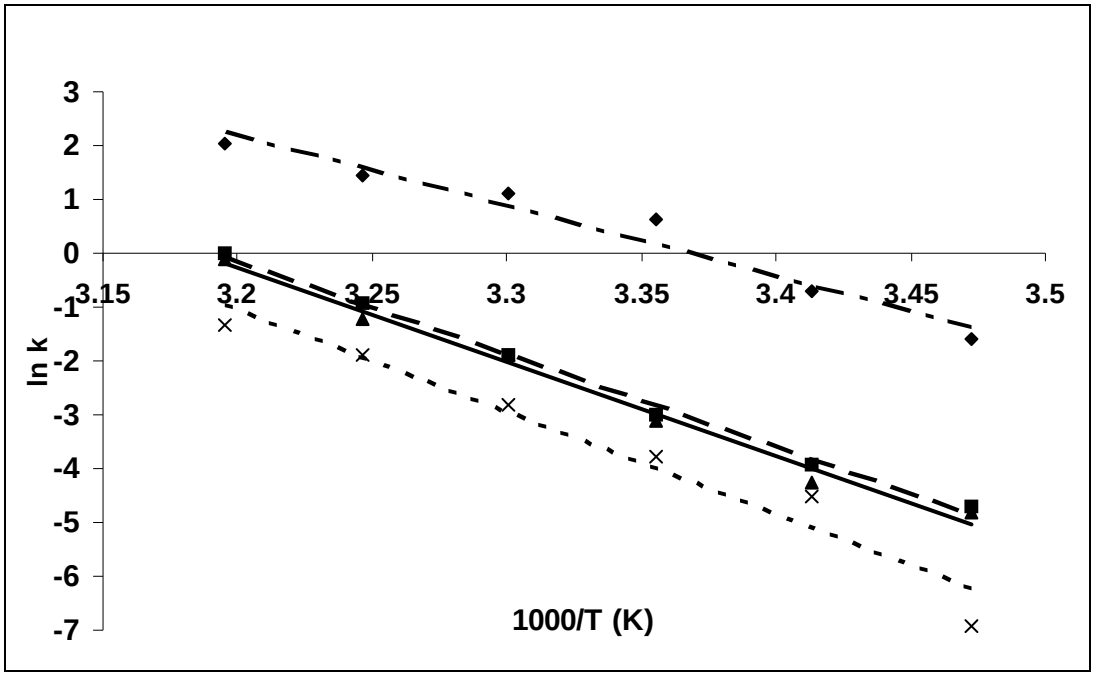

Figure S3.4 Arrhenius plots for 1,2,4-benzenetriol in solvents.