SYNTHESIS AND CHARACTERIZATION OF SEMICARBAZIDE AND THIOSEMICARBAZIDE BEARING BUTYLATED HYDROXYTOLUENE MOIETY FOR ANTIOXIDANT APPLICATION

SYABILAH BINTI SAZELI

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITI MALAYA
KUALA LUMPUR

2021

SYNTHESIS AND CHARACTERIZATION OF SEMICARBAZIDE AND THIOSEMICARBAZIDE BEARING BUTYLATED HYDROXYTOLUENE MOIETY FOR ANTIOXIDANT APPLICATION

SYABILAH BINTI SAZELI

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY

INSTITUTE FOR ADVANCED STUDIES
UNIVERSITI MALAYA
KUALA LUMPUR

2021

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Syabilah binti Sazeli

Matric No: HGA150001 / 17005215/1

Name of Degree: Master of Philosophy

Title of Project Dissertation: Synthesis and Characterization of Semicarbazide and Thiosemicarbazide Bearing Butylated Hydroxytoluene Moiety for Antioxidant Application

Field of Study: Chemistry

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

SYNTHESIS AND CHARACTERIZATION OF SEMICARBAZIDE AND THIOSEMICARBAZIDE BEARING BUTYLATED HYDROXYTOLUENE MOIETY FOR ANTIOXIDANT APPLICATION

ABSTRACT

New multipotent antioxidants (MPAOs) semicarbazides and thiosemicarbazides bearing thiolated butylated hydroxytoluene (BHT) moiety were synthesized by a simple reaction between acid hydrazide, aryl isocyanates, and isothiocyanates. The antioxidant activity of these compounds was evaluated by using *in vitro* α, α-diphenyl-β-picrylhydrazyl (DPPH) assay. Thiosemicarbazides **5a'-h'** were found more active in free radical scavenging activity than semicarbazides **5a-h**. Compound 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(naphthalen-2-yl)hydrazine-1-carbothioamide **5f'** showed the best antioxidant activity against DPPH radical (IC_{50} of 25.47 ± 0.42 μM) compared to the standard BHT. Compound **5f'** and 2-(2-((3,5-di-tert-butyl-4-hydroxybenzyl)thio)acetyl)-N-(naphthalen-2-yl)hydrazine-1-carboxamide **5f** were blended into trimethylolpropane trioleate (TMPTO) synthetic lube oil subjected for isothermal differential scanning calorimetric (DSC) measurement at 125 ºC and 150 ºC to investigate their oxidative stability. At 125 ºC, TMPTO blended with 0.25 wt.% of **5f'** showed 2 and 1.5 times higher oxidative stability than that of BHT and **5f**, respectively. It was postulated that the promising oxidative stability of **5f'** was due to strong auto-synergistic effect.

Keywords : antioxidants, BHT, semicarbazide, thiosemicarbazide, lubricant additives

SINTESIS DAN CIRI-CIRI SEMIKARBAZIDA DAN TIOSEMIKARBAZIDA GALAS BAHAGIAN TERBUTIL HIDROKSITOLUENA UNTUK APLIKASI ANTIOKSIDAN

ABSTRAK

Semikarbazida dan tiosemikarbazida yang mengandungi hidroksitoluena butilasi (BHT) tertiola telah disintesis melalui tindak balas mudah antara asid hidrazida dan aril isosianat dan isotiosianat. Aktiviti antioksidan sebatian ini dinilai dengan menggunakan ujian *in vitro* α, α-difenil-β-pikrilhidrazil (DPPH). Tiosemikarbazida (**5a'-h'**) didapati lebih aktif dalam aktiviti pemulung radikal bebas daripada semikarbazida (**5a-h**). Sebatian 2-(2-(3,5-di-tert-Butil-4-hidroksibenziltio)asetil)-N-1-naftilhidrazinakarbotio amida **5f'** menunjukkan aktiviti antioksidan terbaik terhadap radikal DPPH (IC_{50} 25.47 ± 0.42 μM) berbanding piawai BHT. Sebatian **5f'** dan 2-(2-(3,5-di-tert-Butil-4-hidroksibenziltio)asetil)-N-(1-naftil)hidrazinakarboamida **5f** telah diadun menjadi bersama minyak pelincir sintetik trimetilolpropana trioliat (TMPTO) tertakluk untuk pengukuran kalorimetrik pengimbasan perbezaan isoterma (DSC) pada 125 ºC dan 150 ºC untuk penyiasatan kestabilan oksidatif. Pada 125 ºC, TMPTO diadun dengan 0.25 % berat **5f'** masing-masing menunjukkan kestabilan pengoksidaan 2 dan 1.5 kali lebih tinggi daripada BHT dan **5f**. Telah didalilkan bahawa kestabilan oksidatif **5f'** yang menjanjikan adalah disebabkan oleh kesan auto-sinergistik yang kuat.

Katakunci : antioksida, BHT, semikarbazida, tiosemikarbazida, aditif minyak pelincir sintetik.

ACKNOWLEDGEMENTS

With the name of Allah, the Most Gracious The Most Merciful, All Praise is due to Allah, Lord of the worlds. I would not have finished this study without the permission of Allah. Highest gratitude to Allah for giving me the strength and capability to complete successfully. This master journey has lead my life getting to know You deeper to realize how great You are. Ya Rabb, may this research benefit to the ummah one day.

During the course of this work, I was very fortunate to have special guidance from organic expertise advisor, Dr Wageeh Abdulhadi Yehya. His fatherly support, patience, motivation, enthusiasm and immense knowledge helped me in all the time of research and writing of this thesis. My journey is completed when my supportive co-supervisor, Dr Lee Hwei Voon gave her encouragement and faith in me. Many thanks to Ministry of Higher Education Malaysia (MOHE) through MyBrain15, University of Malaya through (GC001C-14AET), PPP Grant PG272- 2015B, and FP127-2019A and all the staff in Nanocat Department, Institute for Advanced studies.

Also, I thank my fellow lab mates for the stimulating discussion, for the sleepless we were working together before deadlines and the fun we have had together. For all the struggles we've been through, may Allah shower us with His blessings.

Finally, words do not suffice to express my gratitude to my beloved family. Especially, my beloved parents, Adida Ibrahim and Sazeli Sani, without their prayer, blessing and financial support, I might not be able to wind up my master.

Back then during my high school, my chemistry teacher, Teacher Saadah had planted a seed deep in my heart to pursue study in chemistry to the highest level. This master is the stem from the seed of chemistry that she planted in my heart. She owned my forever humble respect.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEME

LIST OF SYMBOLS AND ABBREVIATIONS

ADPA	:	Alkylated diphenyl amine
ASTM	:	American Society for Testing and Materials
BDE	:	Bond dissociation energy (enthalpy change)
BHA	:	Butylated hydroxyanisole
BHT	:	Butylated hydroxytoluene
DMSO	:	Dimethyl sulfoxide
DPPH	:	Radical 2,2-diphenyl-1-picrylhydrazyl
DCM	:	Dichloromethane
DSC	:	Differential Scanning Calorimetry
FTIR	:	Fourier-transform infrared spectroscopy
HREIMS	:	High resolution electron ionization mass spectral
IC_{50}	:	Half maximal inhibitory concentration
LCMS	:	Liquid chromatography with mass spectrometry
MPAO	:	Multipotent antioxidants
NMR	:	Nuclear magnetic resonance
OIT	:	Oxidative induction time
PTSA	:	*p*-toluenesulfonic acid
RNS	:	Reactive nitrogen species
ROS	:	Reactive oxygen species
RT	:	Room temperature
SAR	:	Structure-activity relationship
TLC	:	Thin layer chromatography
TMPTO	:	Trimethylolpropane trioleate
ZDDP	:	Zinc dialkyldithiophosphates

CHAPTER 1: INTRODUCTION

1.1 Research Background

Over the past decades, there has been a dramatic increase in the development of environmental friendly lubricants from renewable resources to reduce dependency on under earth fossil-based resources due to the rising ecological concerns (Nath, Yehye, Zulkifli, & Johan, 2018). The use of fossil-based resources is no longer reasonable in view of the depletion of fossil fuels and its detrimental impact on the environment which has led to the critical need to seek alternative sources (Zainal, Zulkifli, Gulzar, & Masjuki, 2018). Hence, providing green lubricant and replacing mineral lubricant oil from renewable resources have been extensively explored recently. Synthetic polyol ester-based lubricant which derived from naturally occurred oil or fats has excellent lubricity, high viscosity index and biodegradable. However, the polyol ester-based lubricant has its limitation - poor oxidative and thermal stability. The presence of unsaturated bond in the oleic acid part of the molecule is sensitive to chemical oxidation in the presence of oxygen, water, and metals under elevated temperature (Alias, Yunus, Idris, & Omar, 2009; He, Shi, Wang, & Gao, 2018). As a result, free radicals are produced in this lubricant oil causing a shorter lubricant life cycle and may damage the machinery (Nath et al., 2018; S. Yu, Feng, Cai, & Liu, 2017). Besides manufactured goods, the free radicals are also harmful to human. Oxidative imbalance in the human body leads to many chronic diseases. Thus, our interest was concentrated on the development of multipotent antioxidants that scavenge free radicals and enhance the oxidative stability of synthetic ester-based lubricant oil.

1.2 Problem Statement and Research Scope

Pre-mature oxidation is one of the major hindrances in the development of a good synthetic lubricant. The growing concerns of ecological and environmental sustainability encourage the use of environmental-friendly lubricants, i.e., synthetic ester-based

lubricant. Trimetylolpropane trioleate is the most widely used ester-based lubricant that derived from oleic acid (Alias et al., 2009; Kiriliauskaitė, Bendikienė, & Juodka, 2011; Nath et al., 2018). It has an outstanding lubricity, low-temperature property and high viscosity index. However, TMPTO suffers from pre-mature oxidative degradation due to its poor oxidative stability (Nath et al., 2018). Therefore, research has been directed towards the development of antioxidants with better oxidative stability properties and less toxic.

BHT is one of the most common primary synthetic antioxidants which has been used in a wide range of application. Studies have shown that combination of BHT and secondary antioxidant was able to give a better oxidative stability in synthetic ester-based lubricant (Duangkaewmanee & Petsom, 2011; Mousavi, Wang, Grant, Oxenham, & Hauser, 2006). Antioxidant synergism is the subsequent impact of utilizing at least two antioxidants together conveys more noteworthy oxidation soundness than any individual antioxidant functions (Rawat, Joshi, Lamba, Tiwari, & Kumar, 2015). Synergistic antioxidants frameworks compromise convincing answers for issues where a single antioxidant is deficient to offer satisfactory outcomes (Rudnick, 2017). The mixture of various antioxidants in definite proportion could perform in a decent synergistic impact where oxidation stability is superior to the exclusively utilized antioxidants (Duangkaewmanee & Petsom, 2011; Fox & Stachowiak, 2007).

Organosulfur compounds are frequently used in lubricant compositions with BHT to enhance synergistic effect because they are essential additives for antioxidant, antiwear and extreme pressure properties. Though the least amount of sulfur-containing additive should be applied in advanced engine oil formulation as set forth to the current environmental and economic constraint. In fact, the use of several antioxidants in lubricant formulation may rise the environmental issue and increase the cost. Therefore,

multi-functional antioxidant has become more popular to cut down the number of several antioxidants in lubricant composition. Since multifunctional antioxidants can possess several antioxidant functional groups in their structure, which can provide auto-synergistic antioxidant system by exhibiting radical scavenging and peroxide decomposing properties (Rudnick, 2017).

So, we envisioned to assemble several antioxidant functions in one structure by incorporating BHT moiety into semicarbazide and thiosemicarbazide compounds to obtain better antioxidant auto-synergistic effect. This research deals with the preparation and evaluation of new series of semicarbazide and its corresponding thiosemicarbazide derivatives bearing BHT moiety for their antioxidant activities using DPPH assays and DSC. BHT moiety has been incorporated into semicarbazide and thiosemicarbazide through thioether bridge to obtain multifunctional antioxidant which can offer better auto-synergistic antioxidant system. The chemical behaviour of thiosemicarbazide is similar to that of its counterpart semicarbazide, however the thione group has greater chemical fliexibility than the keto group, resulting in more diversified behaviour of thiosemicarbazide (Keri et al., 2017). Thus, this study would help researchers to have deep understanding on potential and comparison between multifunctional semicarbazide and thiosemicarbazide derivatives as antioxidant inhibitor for future global lubricant oxidative stability industry perspective.

1.3 Objectives

The objectives of this research are:

1- To synthesize a series of novel semicarbazides and thiosemicarbazides bearing butylated hydroxytoluene (BHT) moiety.
2- To evaluate the antioxidant activity of the semicarbazides and thiosemicarbazides using DPPH assay.

3- To measure the oxidative stability of selected semicarbazide and thiosemicarbazide in base oil trimethylolpropane trioleate (TMPTO).

1.4 Organization of Thesis

This thesis is structured into five respective chapters.

Chapter 1:

Present a general review on oxidative stress and its causes, antioxidants, classification of antioxidant and its functions as scavenging oxidations. The need of antioxidants as synthetic lubricant additives has been clearly described along with the objectives of work.

Chapter 2:

Highlight on the review of free radical, classification of antioxidants with their types and examples, BHT, application of semicarbazides and thiosemicarbazides, and recent study on antioxidant additives for lubricant.

Chapter 3:

Describe the methodology of this study such as spectroscopic techniques, synthesis and characterization of semicarbazides and thisemicarbazides derivatives and antioxidant activities of the target compounds using DPPH assay and DSC.

Chapter 4:

Discuss specifically about the unique characterization of 5g and 5g' and results obtained from the experiment on antioxidant assays.

Chapter 5:

A closure by answering the objectives and recommendations for future work improvement related to this research.

CHAPTER 2: LITERATURE REVIEW

2.1 Free Radicals

The existence of free radicals in our surrounding was discovered approximately 70 years ago (Commoner, Townsend, & Pake, 1954). Free radicals are known as independent atoms or molecules containing one or more unpaired electrons in an atomic orbital executing them to be unstable, short lived and extremely reactive. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are classified as free radical existed and derived from both endogenous and exogenous sources (Phaniendra, Jestadi, & Periyasamy, 2015). Endogenous free radicals are produced from mitochondria, peroxisomes, immune cell activation, phagocytic cells, inflammation, aging, mental stress, exercise, and infection. Whereas, exogenous ROS/RNS are resulted from environmental factors for instance air and water pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs like halothane, paracetamol, and radiation, that could penetrate into the body by different routes. The radicals could be found in different form such as hydroxyls (HO^-), superoxide (O_2^-), peroxyl (ROO^-), alkoxyl (RO^-), nitric oxides (NO^-) and nitrogen dioxide (NO_2^-). The presence of one unpaired electron of free radicals tends to donate it or to obtain another electron from other molecules to attain their stability. These molecules are responsible for oxidation in biological and several industrial products such as pharmaceutical, rubber, foodstuffs, plastics and oil (Aguilar, Mazzamaro, & Rasberger, 2010; Hovorka & Schöneich, 2001; Shelton, 1959).

Free radicals might be either beneficial or harmful to human body depends on their delicate balance production. At moderate level of concentration, ROS/RNS provide biological advantages and important involvement in various physiological functions such as in redox regulation, mitogenic response, cellular signaling pathways and immune function (defense against pathogenic microorganisms) (Nordberg & Arnér, 2001;

Phaniendra et al., 2015; Valko et al., 2007). The production of free radicals in natural immune system of human body is important to prevent from granulomatous disease. The patients with this disease would unable to release the superoxide anion radical ($O^{2-\cdot}$) due to abnormal membrane-bound NADPH oxidase system, thereby the patients suffer from multiple and persistent infections. Certainly, as part of the body's defense mechanism against disease, it is vital for phagocytes (neutrophils, macrophages, monocytes) to release free radicals to destroy invading pathogenic microbes (Dröge, 2002; Pham-Huy, He, & Pham-Huy, 2008; Young & Woodside, 2001).

Through lipid peroxidation process, the excess free radical can extremely damage the cell membrane and lipoprotein. When an overproduction of free radicals cannot be gradually being eradicated, their accumulation in the body generates a phenomenon called oxidative stress (Pham-Huy et al., 2008). The free radicals can change the structure of proteins causes the loss of enzyme activities. However, human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ*, or externally supplied through foods and supplements. Unfortunately, if the antioxidant system is unable to scavenge the excessive free radical, the body starts to develop chronic and deteriorating diseases as well as aging process, severe pathologies and degenerative illness. Many researchers have reported that cancer, autoimmune disorders, neurodegenerative disfunction, and other related chronic diseases are the results of oxidative stress (Pham-Huy et al., 2008; Phaniendra et al., 2015; Sangeetha, Das, Koratkar, & Suryaprabha, 1990). Most of the diseases caused by excessive free radicals were outlined in Figure 2.1.

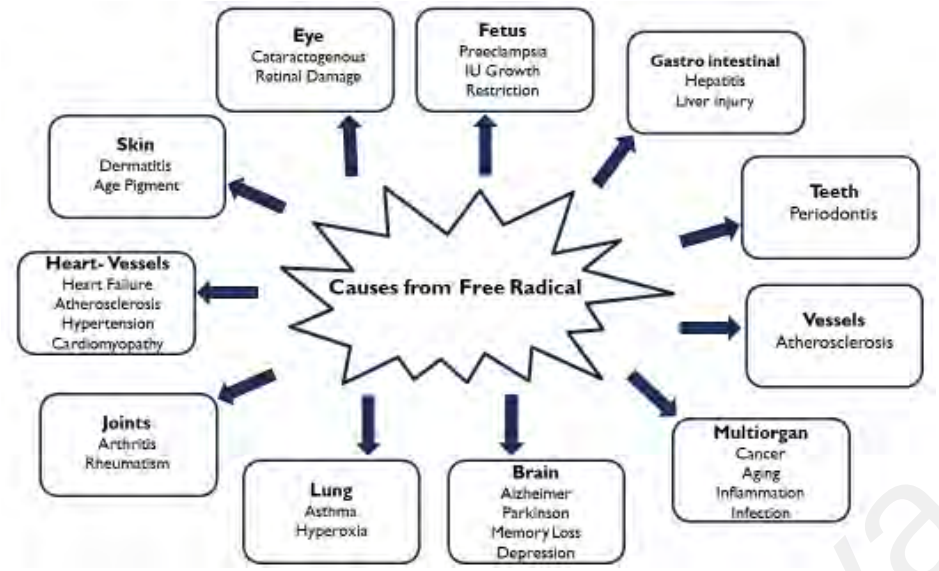


Figure 2.1: Several diseases in the human body caused by oxidative stress.

2.2 Antioxidants and Its Classification

Antioxidants are the main body's defense mechanism to combat ROS/RNS. The natural antioxidant defense system has been classified into endogenous and exogenous antioxidants to inhibit free radicals' formation. Alternatively, certain antioxidants, known as synthetic antioxidants, may be synthesized. Figure 2.2 shows the classification of antioxidants in a simplified chart.

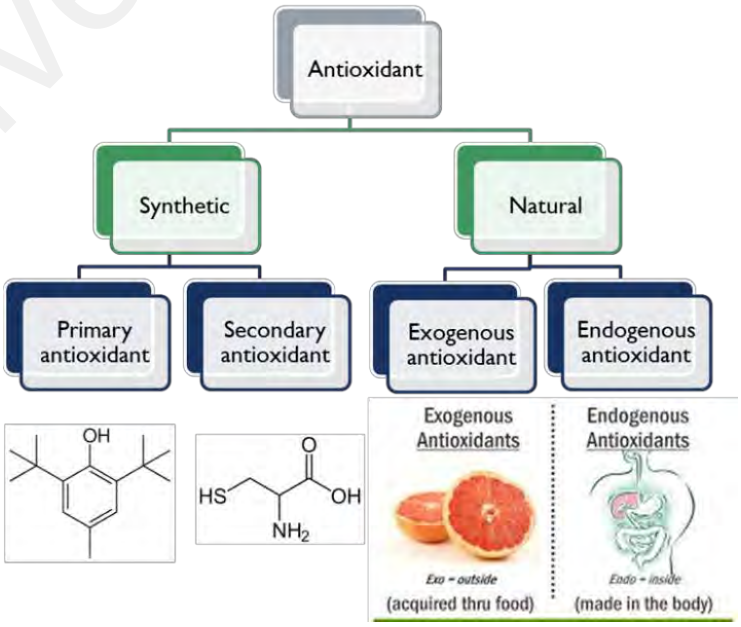


Figure 2.2: Classification of antioxidants

Endogenous antioxidants as shown in Figure 2.3 are naturally produced inside the human body and can be categorized into two groups: enzymatic and non-enzymatic antioxidant, which are depending on their activity (Nimse & Pal, 2015). Enzymatic antioxidants break down and remove free radicals by converting dangerous oxidative products to hydrogen peroxide (H_2O_2) and then water in cofactors' presence through a multi-step process. Their mechanism was mentioned in several review articles (Aziz, Diab, & Mohammed, 2019; Lobo, Patil, Phatak, & Chandra, 2010; Nimse & Pal, 2015). Non-enzymatic antioxidants are antioxidants generated by metabolism in the human body and are working by interrupting the reaction chain of free radical. Glutathione, vitamin C, vitamin E, plant polyphenol and carotenoids are the examples of non-enzymatic antioxidants (Nimse & Pal, 2015).

Exogenous antioxidants are antioxidants that cannot be generated in the body. Figure 2.3 display some examples of exogenous antioxidants found in fruits and vegetables. These antioxidants support the activity of endogenous anti-oxidative defense. It has to be consumed through supplements or diet intake to maintain sufficient capacity in the body (Pisoschi & Pop, 2015). These antioxidants are classified into two divisions: natural and synthetic antioxidants, depending on the sources of antioxidants. When present at low concentrations, these substances can be hydrogen donors or an electron donor (usually containing phenolic compounds) (Atta, Mohamed, & Abdelgawad, 2017; Wageeh A Yehye, 2012).

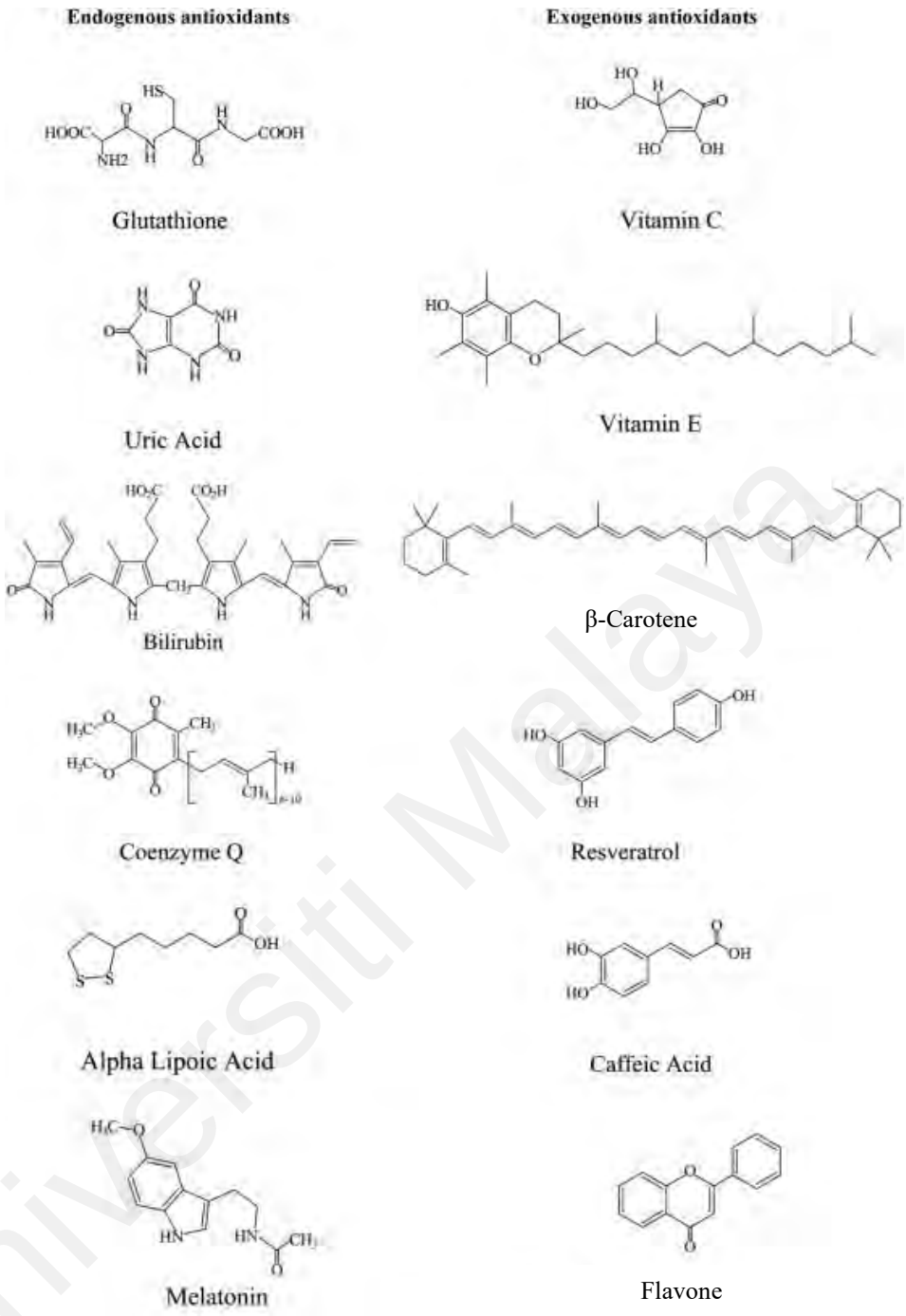


Figure 2.3: Structures of major endogenous and exogenous antioxidants (Pisoschi & Pop, 2015).

2.3 Synthetic Antioxidant

Synthetic antioxidants do not occur in nature. They are radical scavenger that chemically synthesized and added to food or industrial goods in order to protect from oxidation damages (Atta et al., 2017). Limited sources of natural antioxidants and their instability activity has brought synthetic antioxidants to an extensive application for

various industrial purposes. In addition, synthetic antioxidants are easily prepared in laboratory and effective even at low concentrations (Makahleh, Saad, & Bari, 2015). In lubricant industry, the most effective way to stabilize oxidants is to introduce synthetic antioxidants into lubricating oil formulations (He et al., 2018). However, give due attention to selecting and adding antioxidants because large doses of synthetic antioxidants have been reported to impart safety problems, leading to a pro-oxidant effect and showing potential adverse health effects (Atta et al., 2017). For this reason, synthetic antioxidants are tested for safety and must be permitted for use in food. Permissible limits for the use of antioxidants vary hugely from country to country, depending on the food product under consideration (Wageeh A Yehye, 2012).

Synthetic antioxidant can be classified based on their respective mechanism of action. The main classification of synthetic antioxidants as oxidation inhibitors are primary antioxidants and secondary antioxidants. The primary antioxidant function is scavenging free radicals, thus breaking the chain. In contrast, the secondary antioxidant is used to convert hydroperoxides into non-radical products as a preventive action (Santos-Sánchez, Salas-Coronado, Villanueva-Cañongo, & Hernández-Carlos, 2019; Wu, Li, Zhang, & Wang, 2013).

2.4 Primary Antioxidant

Hindered phenol and arylamine antioxidants are two common types of primary antioxidants. Both antioxidants function as free radical scavengers by donating hydrogen atoms to terminate alkoxy and alkyl peroxy radicals to disrupt the radical chain mechanism of the auto-oxidation process. The difference between these antioxidants is their source of hydrogen atoms (Soleimani, Dehabadi, Wilson, & Tabil, 2018).

Antioxidant activities of hindered phenols have been carried out to study the effect of substitution on the phenolic ring. It has been reported extensively that electron-donating

substituents, such as methyl and *tert*-butyl on 2,4 and 6-positions, increase phenols' antioxidant activity (Kajiyama & Ohkatsu, 2001). This is due to the lowering of the phenolic O-H bond dissociation energy (BDE) (Giovanni Brigati, 2002; Lucarini, Pedulli, & Cipollone, 1994) and the stabilization of the phenoxyl radical by inductive and hyperconjugative effects (X. W. Yu, Li, & Wu, 2004). Figure 2.4 shows the substituents at ortho-position would cause steric hindrance to minimize undesirable reactions such as pro-oxidation (Ariffin, Rahman, Yehye, Alhadi, & Kadir, 2014).

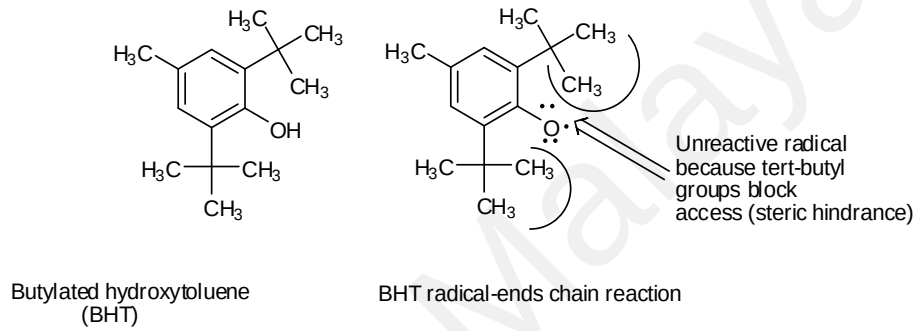


Figure 2.4: Steric hindrance effects on stabilization of phenolic antioxidants (Ariffin et al., 2014)

Butylated hydroxytoluene (BHT) and butylated hydroxyl anisole (BHA) represent the core skeleton of phenolic compound and widely used worldwide for increasing shelf life and protecting from autoxidation of solvents, oil, lubricants and many other handwork materials. To be specific, phenolic antioxidant eradicates free radical by donating hydrogen atom, from its hydroxyl group derivatives (Alisi, Uzairu, & Abechi, 2020). The proposed mechanism of BHT is shown in Figure 2.5.

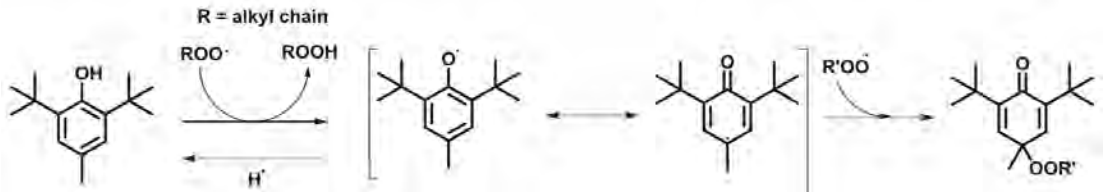


Figure 2.5: Proposed mechanism of radical scavenging of BHT (Higgins, Filip, Afsar, & Hayes, 2019a)

BHT can react with free radicals via donating a hydrogen atom to produce a stable product and the corresponding antioxidant free radical. The antioxidant free radical is itself adequately stable and does not appear to initiate or propagate a chain reaction, stopping the autoxidation chain reaction (Liu & Mabury, 2020).

Arylamine or aromatic amine is the other primary antioxidant class that can easily donate hydrogen atom on nitrogen to peroxy radicals (Soleimani et al., 2018). Alkylated diphenyl amine (ADPA) is one of typical groups of substituted amine antioxidant that are synthesized by the reaction between alkylated agents and diphenylamine. This range of antioxidant is used in lubricants as well as rubber vulcanite and synthetic polymer. The action mechanism of arylamines can be simplified as presented in Figure 2.6.

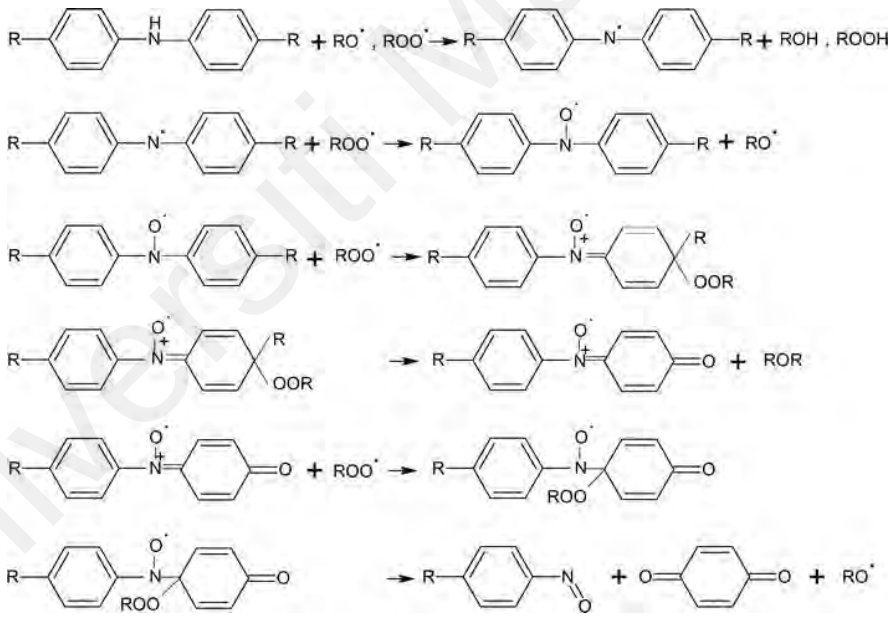


Figure 2.6: Mechanism of sequential reaction of alkylated diphenyl amine with peroxy radical (Soleimani et al., 2018)

Past studies have reported that the performance of diphenylamine's antioxidant activity depends on the substituents in the para position. Diphenylamine molecules tend to have four peroxy radicals scavenging potential to be compared to sterically hindered phenol antioxidants which only have two. In lubricant application, amine antioxidant has higher antioxidant activity than phenolic antioxidant. This could be its catalytic manner of

reaction and regeneration over several cycles of scavenging and breaking of chain reactions of oxidation (Soleimani et al., 2018).

The general antioxidant of H-atom transfer mechanism is when free radical (R·**)** has been initiated to form a chain reaction, yielding many lipid molecules (R-H) which are converted into lipid hydroperoxide (ROOH). The role of antioxidant (ArOH) is to interrupt the chain reaction by giving its hydrogen. To be effective, the ArO· must be a relatively stable free radical, so that it reacts slowly with the substrate, RH but rapidly with ROO·, hence the term "chain-breaking antioxidant" (Wageeh A Yehye, 2012).

2.5 Secondary Antioxidant

Sulfur- or phosphorous–based compounds are the typical secondary antioxidants that can decompose hydroperoxides (Luo, You, Zhang, Zheng, & Wu, 2020). Hydroperoxides could form hydroxyl and alkoxyl radicals when react with metals. It is significant to remove hydroperoxides at first because those hydroxyl and alkoxyl radicals could abstract hydrogen from lipids, generating carbon-centered radicals that further the propagation cycle (Berdahl, Nahas, & Barren, 2010). So, basically the main function of secondary antioxidant is to restrain the autocatalytic oxidation process. Organosulfur compounds like thiols, thioesters, thioamides, thioether and related compounds are the common example for organic compound containing sulfur that can work as hydroperoxide decomposers. The action of oxidation inhibitor starts with the reduction of an alkyl hydroperoxides to a less reactive alcohol, with the sulfide being oxidized to a sulfoxide intermediate (Rudnick, 2017). A general reaction between dialkyl sulfides with hydroperoxide molecules by converting them to sulfoxides are shown in Figure 2.7.

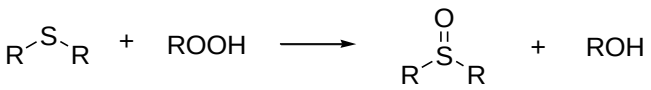


Figure 2.7: General reaction between dialkyl sulfides with hydroperoxide molecules by converting them to sulfoxides (Soleimani et al., 2018)

Extensive research shows that rapid development of sulfur-containing compound in various application mostly in food, medicinal and lubricant industries (Divar et al., 2017; Waheed, Jaafar, & Ahmed, 2019). Similarly, synthesis of phosphorus-containing derivatives likewise had become an important auxiliary antioxidant predominantly in polymeric processing. Organophosphorus like phosphites possessed high antioxidative activity under high temperatures (150-200 ^{o}C) which can adapt to the trend of high temperature processing of plastics nowadays. The first production of novel phosphorus containing compound as antioxidant begins in the past few decades and it is still under inventions till today (Rizvi, 2003; Takahashi, Yachigo, & Ishii, 1985; Zhang et al., 2020).

2.6 Multipotent Antioxidant

After nearly a century of great research and development, the assortment of antioxidants has changed from simple to complex and their proficiency has changed from low to high. Modern research has demonstrated that antioxidant configurations have a synergistic effect on industrial demand. Synergistic effect in antioxidant activity is always describe as the better performance of two or more combined antioxidants that can be obtained by the equivalent amount of any of the components alone (Rawat et al., 2015). This combination could be from the same or different types of antioxidants.

The typical example of the same antioxidant form is the mixture of primary antioxidants, such as diphenylamine and phenolic antioxidants, which function concurrently. This type of combination is called binary antioxidants (de Guzman, Tang, Salley, & Ng, 2009; J. Yao, Gaston, & Steven, 2009). However, these antioxidants'

synergistic effect is limited because they are homosynergism, which only showed the same action mechanism (He et al., 2018; R. Kumar, Yang, Kumar, & Cholli, 2011; S. Yu et al., 2017).

On the other hand, heterosynergism, which addresses dual-functional antioxidants with different action mechanisms, exhibits more competent antioxidant activity. As studied by Wang and the team, one approach to incorporate the assembly is combining two types of antioxidants. It was an example of a combination of primary phenolic antioxidants and secondary sulfur-bearing antioxidants. (Wang et al., 2013).

The mixture of two different antioxidants was not a favor since the effectiveness of multipotent antioxidants by integrating primary and secondary into one hybrid molecule was superior to antioxidant performance. He et al. reported that multipotent antioxidants could form intramolecular synergism. Figure 2.8 shows the possible mechanism of intramolecular synergism of sulfur-containing diphenylamines (He et al., 2018). As proclaimed from previous studies, their high molecular weight property was advantageous. Compared with butylated hydroxyl toluene, the antioxidant additive of synthetic lubricating oil with higher molecular weight reduces the volatility. Low volatility compound is vital for the product to stand under high-temperature conditions (R. K. Singh et al., 2017). Not just that, it also carries strong thermal stabilities, antioxygenic properties, tolerance to extraction and polyolefin compatibility (Zhang et al., 2020).

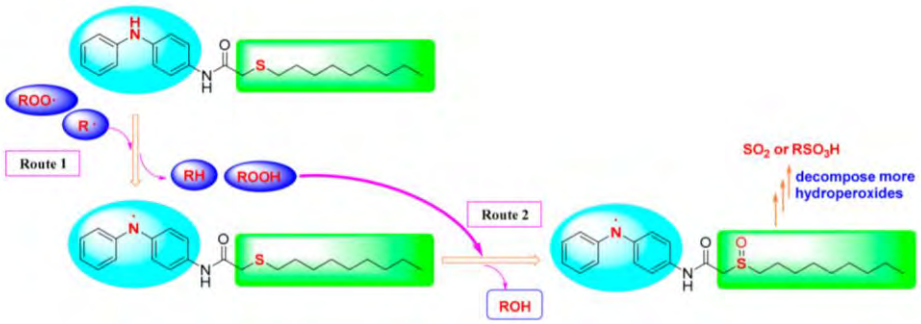


Figure 2.8: Possible mechanism of the dual-functional antioxidant. (He et al., 2018)

2.7 BHT Application

BHT is the most common primary synthetic antioxidant used in various applications such as food packaging products, cosmetics, pharmaceutical, petroleum products, and rubber, as shown in Figure 2.9. It was used by the combination with secondary antioxidant to obtain better oxidative stability of synthetic ester based lubricant (Duangkaewmanee & Petsom, 2011; Mousavi et al., 2006). The impact of utilizing at least two antioxidants brings more noteworthy oxidation soundness than that of any individual antioxidant function known as antioxidant synergism (Rawat et al., 2015). Synergistic antioxidant frameworks offer convincing answers for issues where an individual antioxidant is deficient in providing satisfactory outcomes (Rudnick, 2017). The mixture of various antioxidants in a particular proportion can result in a decent synergistic impact where oxidation stability is superior to the exclusively utilized antioxidants (Duangkaewmanee & Petsom, 2011; Fox & Stachowiak, 2007). BHT is frequently used with organosulfur compounds to provide a better synergistic effect. Organosulfur compounds are essential additives in the lubricant compositions because of their antioxidant, antiwear, and extreme pressure properties. According to the modern environmental and economic requirement, the least amount of sulfur-containing additive should be used in the advanced engine oil formulations. So, several antioxidants in lubricant formulation may raise the environmental issue and increase the cost. Multifunctional antioxidants have become more influential than several antioxidants in lubricant formulation.

Multifunctional antioxidants may have some functional antioxidants in their structure, which provide an auto-synergistic antioxidant system including radical scavenging and peroxidant decomposing properties (Rudnick, 2017).

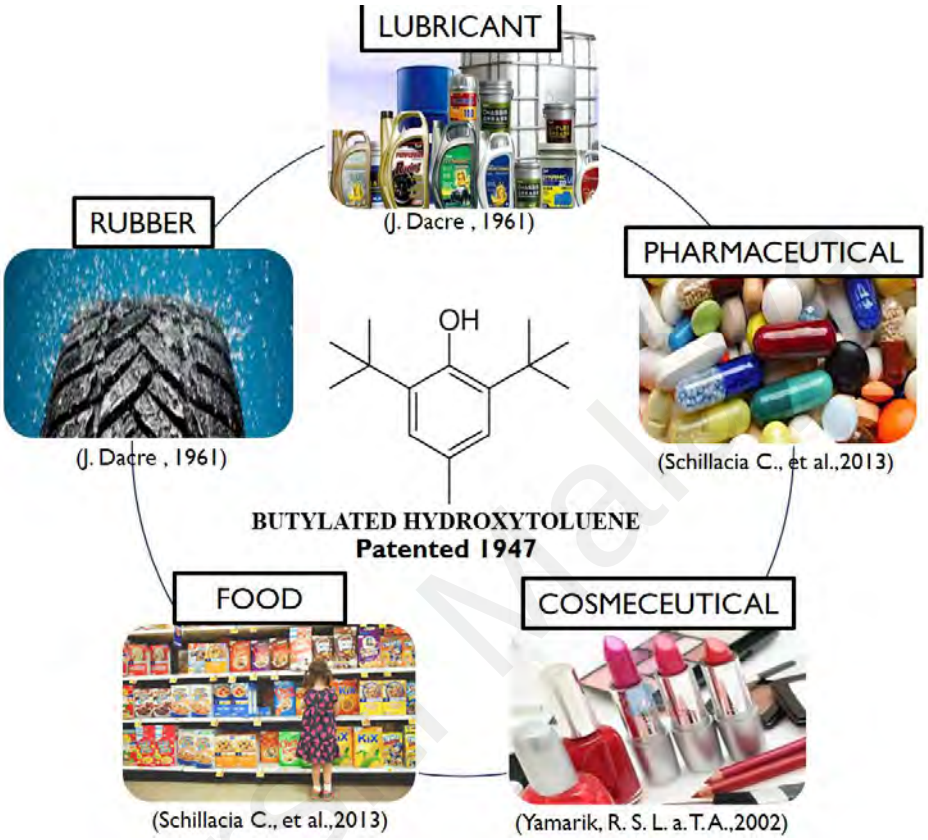


Figure 2.9: Application of BHT in various industries.

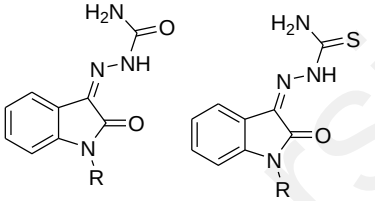
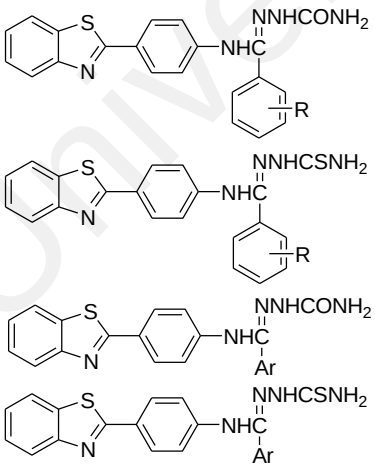
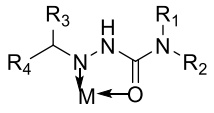
2.8 Application of Semicarbazide and Thiosemicarbazide

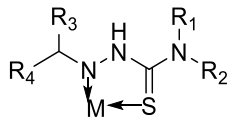
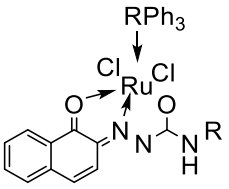
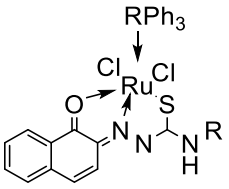
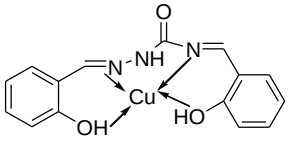
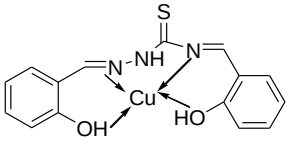
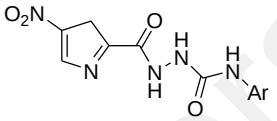
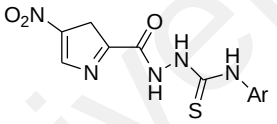
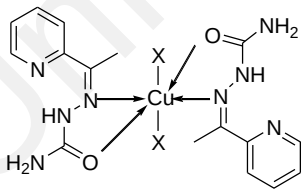
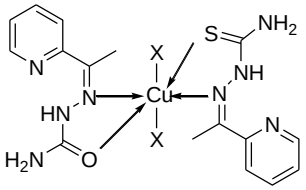
In the history of development of antioxidants, many scientists have proven semicarbazide and thiosemicarbazide derivatives as a superior oxidation inhibitors. In 2013, Perkovic and the team pointed out that hydroxyl derivative of semicarbazide displayed the highest antioxidant activity (Perković et al., 2013). Eventually, in 2016, Huda and her colleagues reported that thiosemicarbazide derivatives showed highly active antioxidant assays with the lowest IC_{50} value for DPPH radical scavenging. (Kareem, Nordin, Heidelberg, Abdul-Aziz, & Ariffin, 2016). Research continued with Molnar and the team in 2017 when they found that thiosemicarbazide derivatives showed

an excellent radical scavenger in comparison with standard ascorbic acid. Thus, it is clear that semicarbazide and thiosemicarbazide derivatives exhibit potential antioxidant activity *in vivo* and *in vitro*.

This type of compound has rising interests in the research group because of its multi-donor ligands. They had a wide range of pharmacological activities not only as antioxidant, but also as antifungal, antibacterial, antitumor, antimalarial, antiviral, antimicrobial, anti-tubecular agents, which made it biologically active and nontoxic compounds (Ali et al., 2018; Huda, 2016; V. A. Kumar et al., 2018; Nguyen & Bui, 2013; Salah BA, 2018). Table 2.1 shows carbazone derivatives of semicarbazide and thiosemicarbazide from previous studies in various applications.

Table 2.1: Carbazone derivatives from previous studies in various applications.

Structures	Applications	References
	Cytotoxic activity against breast cancer cell lines.	(Divar et al., 2017)
	Antimicrobial activities against Gram-positive and Gram-negative bacteria	(M. Singh, Singh, Gangwar, Nath, & Singh, 2016)
	Anti-fungal ,anti-bacterial , anti- cancer ,anti-human immunodeficiency virus, anti-inflammation, anti-neoplastic ,inhibition corrosion, antioxidant ,antiradical.	(Waheed et al., 2019)

 M = metal		
	The antioxidant activity against the DPPH radical and OH radical	(Anitha, Viswanathamurthi, Misini, & Linert, 2013)
		
	Antibacterial activity	(Md. Saddam Hossain, 2017)
		
	Antimicrobial and anti-tubercular activity	(Rane et al., 2014)
		
	Antibacterial Screening	(Chandra, Raizada, & Verma, 2012)
		

2.9 Synthesis of Semicarbazides

Semicarbazide is an important class from acid hydrazide with functional group of $NH_2NHC{=}ONH_2$. Substituted semicarbazide had been an excellent intermediate compound to prepare semicarbazone. Amit and Wageeh did a successful high yield reaction study to synthesis semicarbazides utilizing 3 different solvents: toluene, dichloromethane (DCM) and ethanol. Among the solvents, the reactions' products started to form almost instantly in toluene whereas in DCM and ethanol required around 10 and 20 minutes, respectively, for the solid product to be visible. Hence, most reactions were performed in toluene except a few aryl isocyanates which insoluble in the media. In this case, the reaction was performed in DCM. Besides solvents, the nature of the semicarbazides substituents on benzene ring of the aryl isocyanates determines their reactivity as well. It is observed that the presence of a weakly electron-withdraw body like phenyl isocyanate, 4-fluorophenyl isocyanate, 2,4-dichlorophenyl isocyanate, m-tolyl isocyanate, and naphthyl isocyanate, had a very short completion time, but in the case of moderate deactivate like 4-acetylphenyl isocyanate and 3-(ethoxycarbonyl) phenyl isocyanate, the required reaction time was noticeably longer (5 hours) (Nath & Yehye, 2018). The structure of the semicarbazides is shown in the Figure 2.10.

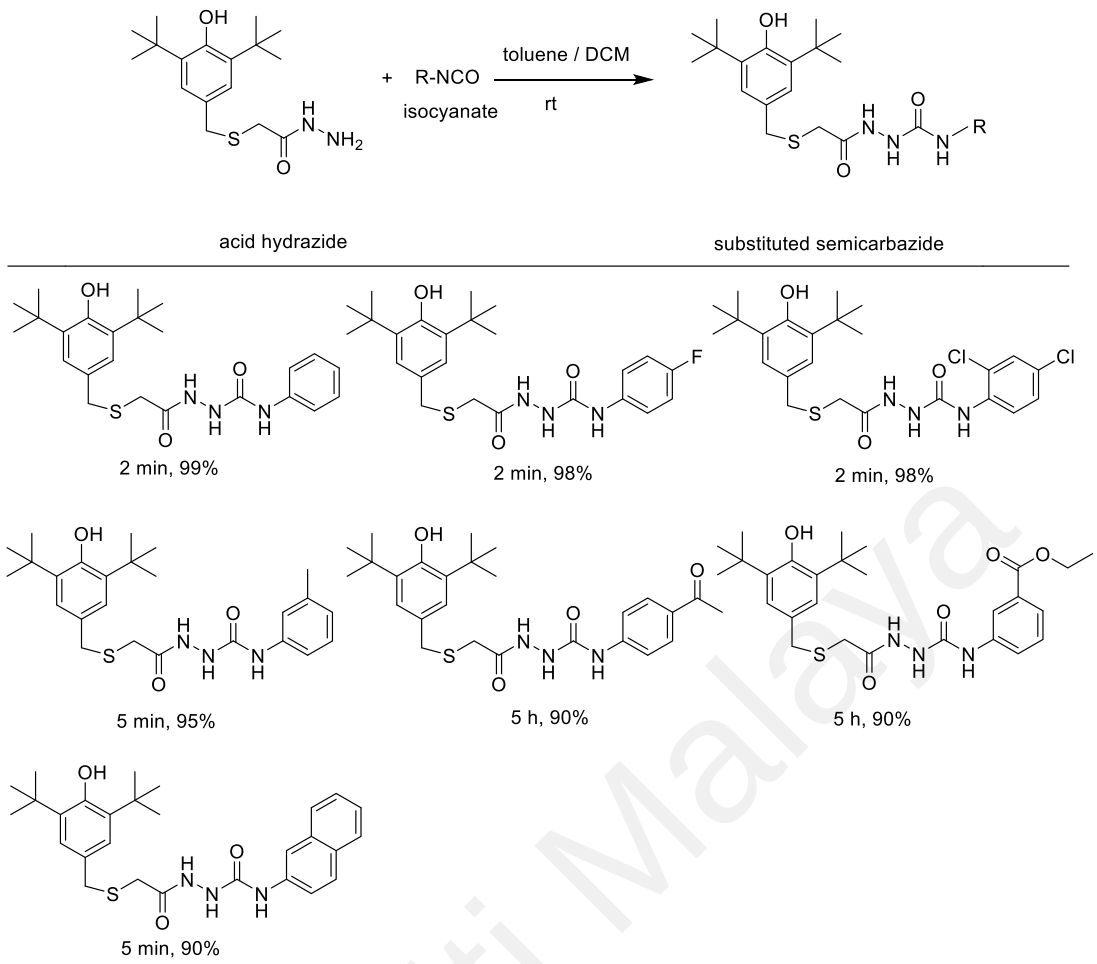


Figure 2.10: Synthetic scheme and structure of synthesized semicarbazides (Nath & Yehye, 2018)

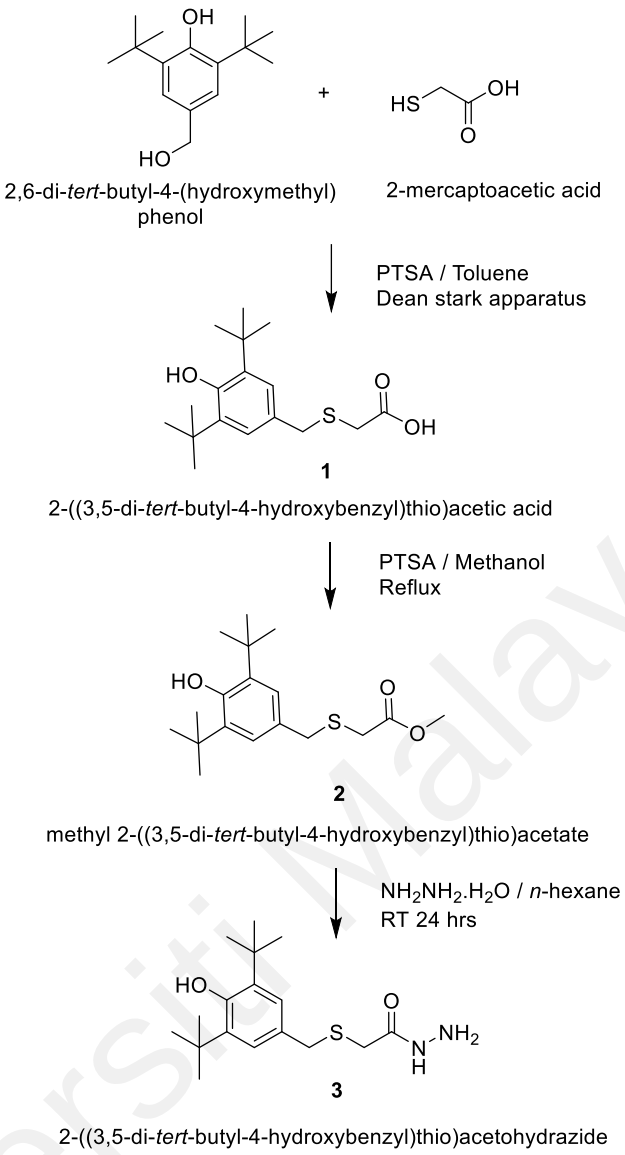
2.10 Synthesis of Thiosemicarbazides

Thiosemicarbazide is the basic hydrazine derivative of thiocarbamic acid (Acharya, Bhavsar, Jethava, Patel, & Patel, 2021). This heterocyclic molecules of sulfur and nitrogen-containing compounds is known in the literature as important synthetic and analytical applications.

Consequently, Wageeh et al reported a higher yield method in preparation of acid hydrazide. The preparation of the precursor compound started with 2,6-di-tert-butyl-4-(hydroxymethyl)phenol in dry toluene refluxed with thioglycolic acid using dean stark apparatus. The optimized condition for the reaction was using PTSA as a catalyst to give

a very good yield (93%) of carboxylic acid compared to other conventional method (35%). The reaction then continued to one of the most common esterification method, fischer esterification using a large excess of methanol to shift the equilibrium towards the product formation. The desired ester was obtained in 90% yield (Wageeh A Yehye, 2012).

An alternative method was used to prepare hydrazide instead of using common synthetic approach, direct hydrazination. It was found that the reaction in alcoholic solvent at room temperature (RT) undergo hydrolysis, giving many products. It could be attributed to the polar reaction condition where the polar solvent used dissolved the nucleophile which enhanced nucleophilic reaction. The indirect hydrazination of esters with hydrazine hydrate involve in the presence of *n*-hexane enabled the conversion to desired product, acid hydrazide to occur with a very good yield (87%) within 24 hours (Scheme 2.1) (Ariffin et al., 2014; Wageeh A Yehye, 2012).



Scheme 2.1: Synthetic scheme of acid hydrazide (Ariffin et al., 2014)

At the final stage, the reaction was carried out by a straightforward reaction between acid hydrazide and isothiocyanates at room temperature using toluene as solvent for 6 hours. (Figure 2.11) (Ariffin et al., 2014).

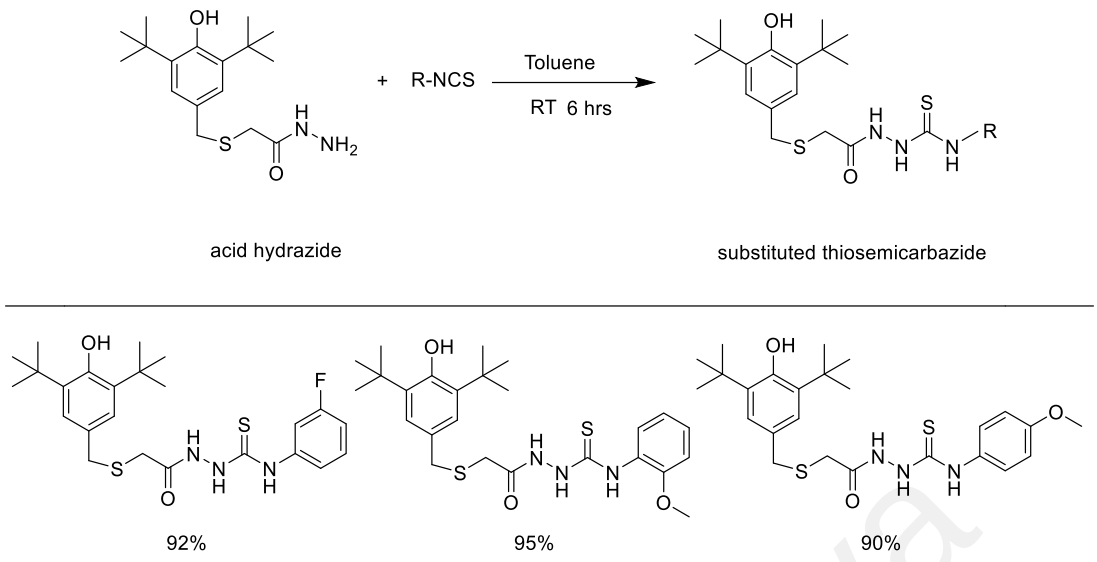


Figure 2.11: Synthetic scheme and structures of thiosemicarbazides (Ariffin et al., 2014)

2.11 Lubricating Oil

Modern lubricants are made from a variety of chemical additives and base fluids. Base fluids are the base oil which are derived from petroleum-based, synthetic or bio-based compound. According to American Petroleum Institute (API), petroleum-based crude oil can be categorized into three groups according to its sulfur content. Group IV base oils are fully synthetic (polyalphaolefin) oils, while group V is not included in Classes I – IV for all other base oils (Sharul Hafiq, 2017).

The addition of different chemical additives to the base fluid improves and sustains lubricant properties. Additives are added to optimize properties such as antifriction and anti-wear, anticorrosion, viscosity booster, detergents and dispersants, antioxidant stability, and pour-point depressant (Roy M Mortier, 2010). Certain additives improve lubricant efficiency under severe conditions such as extreme pressures and temperatures and heavy pollution. Through these additives, the base fluid serves as the carrier and must also retain them in solution under all regular operating environment. Recently, most of lubricating oil contained at least one additive, and some oils include many different types

of additives. The proportion of additive used in a wide range from a few hundredths of a percent to 30% or more (Nasser, 2015). Figure 2.12 illustrates the mixture of base oil and the lubricant additives.

Figure 2.12: Base oil and the additives for lubricant (Ahmed & Nassar, 2011)

2.11.1 Oxidation in Combustion Engine

Engine oils function under severe oxidative conditions. The engine oil's oxidative breakdown creates sludge and deposits, deteriorates the oil's viscosity characteristics, and produces acidic bodies that cause engine parts' corrosion (Tynik, Donnelly, & Aguilar, 2011). The most critical aspect of lubricating oils is to maximize oxidative stability. Exposure of hydrocarbons to oxygen and heat will accelerate the oxidation process. The internal combustion engine is an excellent chemical reactor for catalyzing the process of oxidation as well as the engine's metal parts, such as copper and iron, act as effective oxidation catalysts. Thus, engine oils are probably more susceptible to oxidation than any other lubricant application. Figure 2.13 shows the effects of oxidation process of the oil.

Figure 2.13: Sludge in engine

Lubricants are subject to oxidation during processes involving exposure to heating, shear, oxygen and metals. Lubricating oil will undergo oxidative deterioration in the following sequence step 1 to 3 shown in Scheme 2.2. In step 1, lubricating oil will decompose into alkyl radicals. An alkyl radical will react with one oxygen molecule in step 2. Thereby a peroxide and alkyl radical will start generated. The peroxide generated will react with two normal lubricating oil molecules, producing another alkyl radical.

Step 1: $RH \rightarrow R\cdot$

Step 2: $R\cdot + O_2 \rightarrow ROO\cdot$

Step 3: $ROO\cdot + RH \rightarrow ROOH + R\cdot$

$ROOH + 2RH \rightarrow ROH + H_2O + 2R\cdot$

RH: hydrocarbon (lubricating oil)
R・: alkyl radical
ROO・: peroxy radical
ROOH : peroxide

Scheme 2.2: Oxidative deterioration steps in lubricating oil.

Figure 2.14 shows the step of oil oxidation in the engine. Lubricating oil may further decompose into alkyl radicals due to oxygen and high temperature causes (Ahmed & Nassar, 2011).

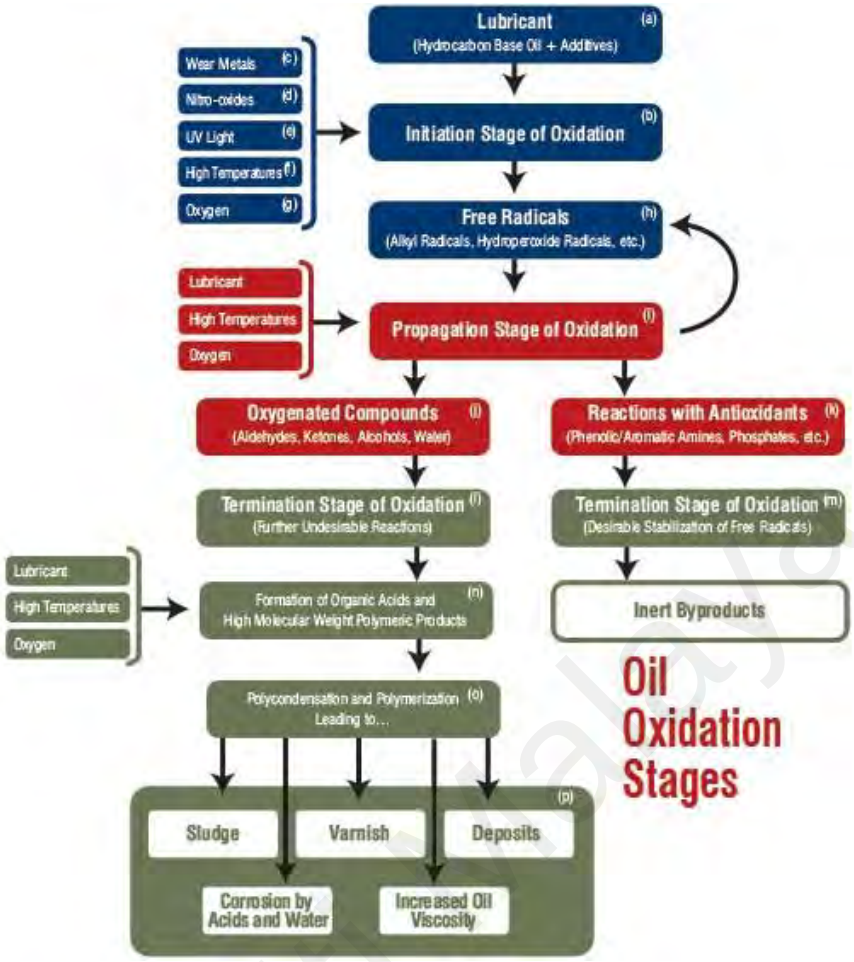


Figure 2.14: Oil oxidation stages in engine
(https://www.machinerylubrication.com/Read/30165/oil-oxidation-stages)

The types of lubricants now on market were made possible through antioxidants' development and use. Without them, the lubricants we rely on would not have the properties necessary to fill the many applications we now take for granted. Just as with human body, antioxidants of various kinds and functions protect lubricants during processing and long-term use.

2.11.2 Current Antioxidant for Lubricant.

Each lubricant is designed with an oxidation controlling method. The formulation of each lubricant, therefore, contains antioxidants. These antioxidants are designed to be sacrificial, meaning they react or oxidize to protect the lubricant's remainder (the base oil). This protection is the only mechanism saving the lubricating oil from pre-mature

failure. The addition of antioxidant additives to lubricating oils prevents all resins, lacquers and acidic compounds (Ahmed & Nassar, 2011). However, to combat the effects of oxidation, engine oils are formulated with an array of antioxidants including, zinc dialkyl dithiophosphate (ZDDP), ADPAs and hindered phenols compounds. The use of ZDDP in engine oils is declining due to phosphorus's poisoning effect on exhaust after-treatment catalyst (Tynik et al., 2011). In addition, sulfur levels in engine oils are also in decline due to sulfated ash exhaust after-treatments. Thus, a need exists for effective antioxidant chemistry to reduce or eliminate the need for phosphorus and sulfur-containing antioxidants. Additionally, organomolybdenum compounds have been used as a component in engine oils as an antioxidant (Ellington, Loper, & Mathur, 2011). However, the industry has an interest in minimizing the reliance on molybdenum-based antioxidants due to the high costs.

Due to the environmental requirements in recent years, there has been a general trend in the industry toward machinery being built smaller yet operating at higher speeds and higher operating temperatures. However, under such operating conditions, the lubricants' thermal and oxidative stress have become severe, that conventional ADPA antioxidants insufficiently stabilize the oils (Dong, 2009). Hindered phenols are the most widely used antioxidants. They are highly effective in an extensive range of applications includes cosmetic (Lanigan & Yamarik, 2002), food, pharmaceutical (Carlo Schillacia, 2013), rubber and petroleum industries (Abraham et al., 2003; Dacre, 1961). They are beneficial antioxidants for lubricating oils, specialty oils, synthetic lubricants, motor gasoline, aviation turbine fuels, transformer oils, feed and forage products, industrial fats, fatty acids, paraffin waxes, etc. They function as free radical scavengers and protect high-temperature processing operations and end-use at elevated temperatures. Butylated hydroxytoluene (BHT) is one of the most commonly used phenolic antioxidants in petroleum products. In fact, BHT was first synthesized in the 1940s to protect petroleum

from oxidation (Liu & Mabury, 2020). Unfortunately, the use and exposure to BHT indicated that it might contribute to the health problems. Among synthetic phenolic antioxidants, BHT was the dominant congener detected causing environmental pollution (Liu & Mabury, 2020).

2.12 Antioxidant Activity (DPPH Method)

It has been a century since a violet-coloured radical, called 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical, was first introduced in 1922 by Goldschmidt and Renn (Goldschmidt & Renn, 1922). Blois then suggested practical approaches that this artificial free radical could be a method used for measuring the antioxidant activity of chemicals (Blois, 1958). Subsequently, in 1995, Brand-Williams and the team had developed the assay in the form adopted by the vast majority of researchers. It has widespread use because this method is fast, inexpensive and only require simple equipment to measure antioxidant capacity as a screening experiment (Santos-Sánchez et al., 2019; Wageeh A Yehye, 2012). This test is based on the ability of DPPH to abstract H-atoms from R-H bonds. DPPH is synthetic organic nitrogen radicals of deep purple colour, which can be reduced in the antioxidant presence with the consequent decolourization. The antioxidant potential can be measured by the reduced adsorption at particular wavelengths of 517nm. The colour of the solution changes from deep purple to a light yellow. Strong antioxidant compounds will result in a rapid decline in the absorbance of the DPPH. The reaction has been simply illustrated by Becker and the team in the Figure 2.15 (Becker et al., 2019).

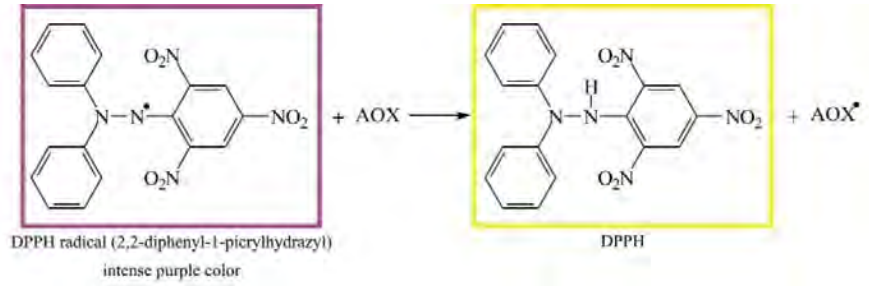


Figure 2.15: Chemical reaction involved in the DPPH with antioxidant (Becker et al., 2019).

Different antioxidant concentrations are used in DPPH assay to determine the antioxidant concentration that quenches 50% of the initial DPPH• radical in a specific time interval. This concentration is called inhibitory concentration, IC_{50}. The lower the value of IC_{50} shows the higher the antioxidant capability (Sazeli et al., 2021). However, there are many issues regarding the IC_{50} value and its differences in reaction conditions. The concentration of DPPH (22.5-250 uM), incubation time (5min-60min), reaction solvent and pH of the reaction mixture have been exerted widely different protocols from various research groups. As a result, IC_{50} values for even the standard antioxidants such as butylated hydroxytoluene and ascorbic acid vary greatly. Therefore, the legal assay requirement is crucial to compare the results from different laboratories (Sharma & Bhat, 2009). Thus, for the sake of uniformity, Sharma and Bhat in 2009 have recommended that the antioxidant assay based on scavenging of DPPH radical is at 50 uM in methanol. The use of methanol as a solvent is also known as the most utilized solvents for determining the antioxidant activity (Marinova & Batchvarov, 2011). Figure 2.16 shows the best examples of miniaturized DPPH assay scheme.

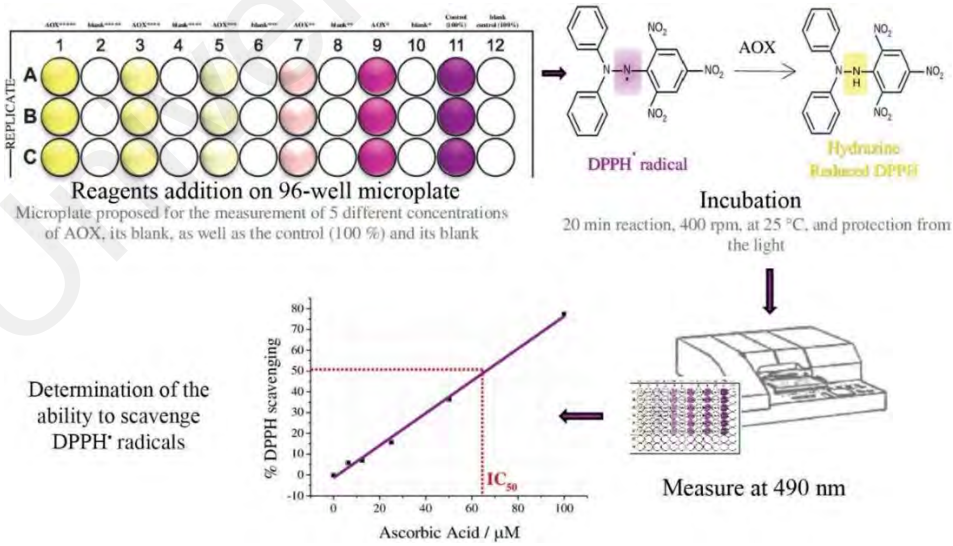


Figure 2.16: Miniaturized DPPH assay scheme (Becker et al., 2019).

Despite all, this artificial radical's long scientific life has continued to assist in justifying a significant theme of free radical chemistry: the search for perfect antioxidant (Brand-Williams, Cuvelier, & Berset, 1995; Foti, 2015).

2.13 Antioxidant Activity (DSC Method)

Differential scanning calorimetry has diverse applications as an analytical instrument. Its possibility to classify oils has been realized for more than 70 years. The recent developments on the use of DSC reflect the DSC technique's versatility to evaluate the oxidative deterioration of oils (C. Tan & Man, 2002). DSC's basic principle is to monitor the oxidation process in phase transition or change in energy based on thermal release (Shahidi & Zhong, 2015). The DSC technique can establish the oxidative resistance study of oils because the transfer of oxygen molecule to an unsaturated fatty acid requires energy (exothermic process). DSC is a technique used in which the time to oxidation is measured when the sample is maintained at a constant temperature in a high-pressure atmosphere of oxygen (Rose, 1991). Through research and developments, American Society for Testing and Materials (ASTM) standard methods for oxidative induction time have been established in Table 2.2.

Table 2.2: ASTM standard methods for oxidative induction time (Blaine & Harris, 1997).

Method	Title
D 3350	Specification for Polyethylene Plastics Pipe and Fittings Materials
D 3895	Test Method for Oxidative Induction Time of Polyolefins by Differential Scanning Calorimetry
D 4565	Test Methods for Physical and Environmental Performance Properties of Insulations and Jackets for Telecommunications Wire and Cable
D 5483	Test Method for Oxidation Induction Time of Lubricating Greases by Pressure Differential Scanning Calorimetry

D 5885	Test Method for Oxidative Induction Time of Polyolefin Geosynthetics by High Pressure Differential Scanning Calorimetry
E 1858	Test Method for Oxidative Induction Time of Hydrocarbons by Differential Scanning Calorimetry

Among phase transition techniques, DSC is non-chemical, simple operation, rapid, low instrumentation cost, and shows excellent correlations with other accelerated methods and chemical analyses (Shahidi & Zhong, 2005; Shahidi & Zhong, 2015). Previous researchers have conducted many comparative studies to determine the oils oxidative stability using DSC isothermal modes with a purge purified oxygen (C. P. Tan, Che Man, Selamat, & Yusoff, 2002). Table 2.3 shows some substantial studies on oxidation application using DSC.

Table 2.3: Studies on oxidation application using DSC.

Studies on oxidation application using DSC	References
Screening test to the analysis of the antioxidants, dioctyldiphenyl amine (DODPA) and Topanol '0' in three base oils.	(Rose, 1991)
A polyethylene film sample, inhibited with a hindered phenol antioxidant, appears to be the best currently available candidate and is offered for consideration as an OIT Reference Material.	(Blaine & Harris, 1997)
The high correlations found between DSC T_0 values and OSI measurements imply that DSC can be recommended as an appropriate objective method for assessing the oxidative stability of various edible oils.	(C. P. Tan et al., 2002)
A comparative study on antioxidant behavior in polypropylene	(Wang et al., 2013)
Oxidation Induction Time (OIT_{time}) and Oxidation Induction Temperature (OIT_{temp}) on six different grades of polyethylene were measured by DSC.	(Schmid, Ritter, & Affolter, 2006)
The antioxidant behavior of four compounds 2-(alkylthio)-N-(4-(phenylamino)phenyl)acetamides as additives of base oil triisodecyl trimellitate	(He et al., 2018)

(TIDTM) is evaluated by non-isothermal and isothermal DSC analyses.	
The antioxidant ability of the diphenylamine-phenols was evaluated using Differential Scanning Calorimetry (DSC).	(Higgins, Filip, Afsar, & Hayes, 2019b)
Antioxidant ability of a series of novel dendrons featuring 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionic ester chain ends was evaluated using pressurized differential scanning calorimetry and when blended with a lubricant base oil, at 0.5% w/w.	(Higgins et al., 2019a)
Thermal oxidation behaviour of extra virgin olive oil samples was studied using DSC as an indicator of their quality and stability during thermal processing.	(Malvis et al., 2019)

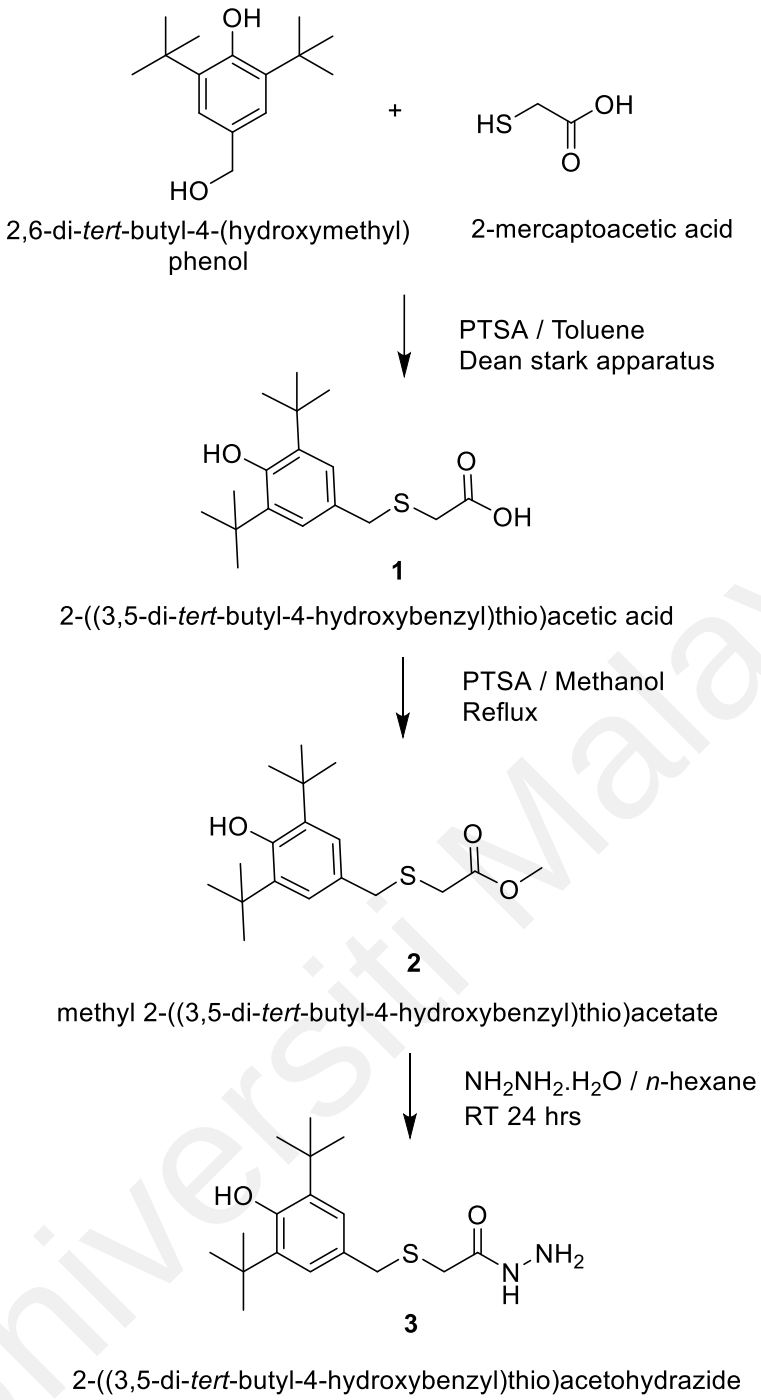
CHAPTER 3: EXPERIMENTAL

3.1 General Instrumentation

All materials and solvents were purchased in analytical grade from commercial suppliers and used without further purification. Toluene was dried over 4Å molecular sieves (Sigma-Aldrich) prior to use. BHT and DPPH were purchased from Sigma-Aldrich. Merck TLC aluminium sheets (Silica gel 60 F_{254}) were used for thin layer chromatography to monitor the reaction by ultra-violet UV light. All synthesized target compounds were characterized using fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectrometer, liquid chromatography with mass spectrometer (LCMS), and melting point instrument. Infrared spectra were recorded on FTIR-Spectrum 400 spectrometer Perkin Elmer by attenuated total reflectance (ATR) technique at wave number δ from 4000-400 cm^{-1}. Proton (1D) and carbon-13 (2D) NMR experiments were obtained at 600 and 150 MHz respectively on a Bruker Advance III 600 ultra-shield spectrometer. Chemical shifts were reported in ppm relative to deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide-d_6 (DMSO-d_6) or tetramethylsilane (TMS). The coupling constants are given in Hz. High resolution-mass spectra (ESI) were obtained using an agilent 6550 i-Funnel Q-TOF spectrometer 70 eV with Agilent ZORBAX Eclipse Plus C18 column. Melting points were determined with Mel-Temp II melting point apparatus.

3.2 Preparation of Precursor Compounds

The preparation of the starting material is outlined in Scheme 3.1. 2-((3,5-di-tert-butyl-4 hydroxybenzyl)thio)acetohydrazide, **3**, was synthesized according to previously described method (Ariffin et al., 2014). Compound was solid and stored at ambient temperature.



Scheme 3.1: Synthetic scheme for the preparation of 2-((3,5-di-tert-butyl-4-hydroxybenzyl)thio)acetohydrazide, **3**

3.2.1 *S*-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolic acid, 1

Thioglycolic acid (4.8 g, 52 mmol) was added to a stirred solution of 3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol (6 g, 25 mmol) in dry toluene (52 ml). *p*-toluenesulfonic acid (PTSA) (0.02 g) was added, and the solution was refluxed for 6 hours by using a dean

stark apparatus. The mixture was cooled and filtered. The resulting residue was washed with distilled water to remove PTSA and unreacted thioglycolic acid. The crude product was 7.21 g of white solid product (93%). Mp 97-99 ˚C.

3.2.2 Methyl-*S*-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolate, 2

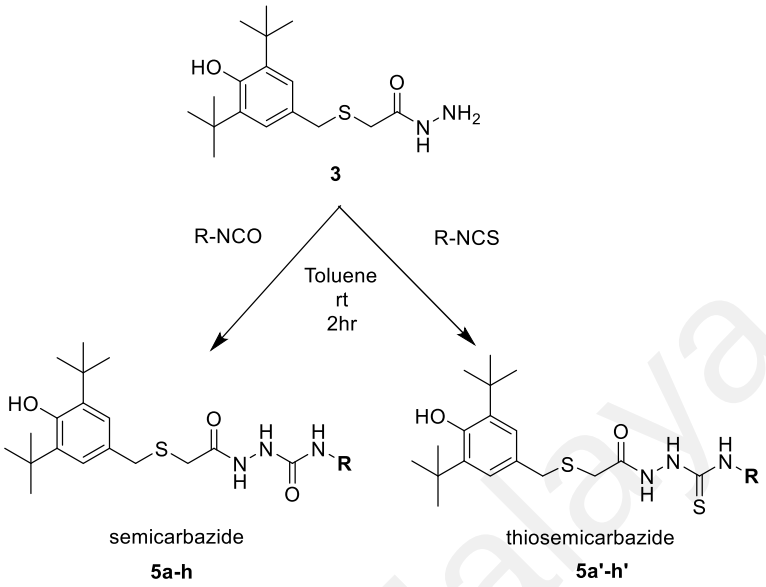
S-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolic acid (6 g, 19.3 mmol) and PTSA (0.06 g) in 200mL of anhydrous methanol were refluxed for 24 hours. After cooling to room temperature, 100 ml of $NaHCO_3$ (3%) was added. The aqueous solution was extracted with ethyl acetate, (3x50 mL). The organic layers were combined, washed with brine, dried over anhydrous sodium sulfpreate, and evaporated. The crude product was purified by column chromatography (*n*-hexane-ethyl acetate, 9:1) to give 6.01 g of brown oil (96 %). Bp 260-262˚C. IR (Nujol), cm^{-1}: 3647 (free OH, wide), 1738 (C=O, asymmetry), 1434 (C-H methylene bending), 1030 (C-O stretching). ^1H NMR ($CDCl_3$, ppm) δ 1.43 (s, 18H, 2 × *t*-Bu), 3.12 (s, 2H, H-8), 3.72 (s, 3H, -OCH3), 3.76 (s, 2H, H-7), 5.19 (s, 1H, OH), 7.11 (s, 2H, H-3 and H-5). ^{13}C NMR ($CDCl_3$, 100 MHz), δ, ppm : 30.34 (6C, 2 × $–C(CH_3)_3$), 32.55 (1C, C-8), 34.39 (2C, 2 × $–C(CH_3)_3$), 36.93 (1C, C-7), 52.44 (1C, OCH_3), 125.96 (2C, C-3, C-5), 127.48 (1C, C-4), 136.02 (2C, C-2, C-6), 153.10 (1C, C-1), 171.13 (1C, C-9). HRMS(ESI): m/z $[M]^+$ (calcd for $C_{18}H_{28}O_3N_2S$ 324.1759; found: 324.1763).

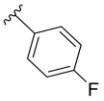
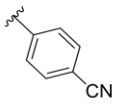
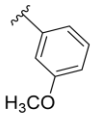
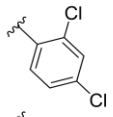
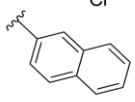
3.2.3 *S*-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolic acid hydrazide, 3

Methyl-*S*-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolate (5 g, 15.40 mmol) was stirred with hydrazine hydrate 85% (2 ml) for 15 minutes. *n*-hexane (150 ml) was added after 1 hour, followed by 6 ml of hydrazine hydrate and stirred at room temperature for 24 hours. The crude product was filtered, washed with distilled water (3x25ml) to remove unreacted hydrazine. Dried, and recrystallized from *n*-hexane to give white solid

4.34 g (87%). Mp 108-110˚C. IR (KBr pellet), cm^{-1}: 3634 (free OH), 3275-3324 (-NH-NH_2), 2872-2962 (C-H of *t*-Bu), 1637 (C=O), 1433 (CH methylene bending). ^{1}H NMR (DMSO-d_6, 400 MHz), δ, ppm: 1.31 (s, 18H, 2 × *t*-Bu), 2.95 (s, 2H, H-8), 3.67 (s, 2H, H-7), 4.21 (bs, 2H, NH2), 6.85 (s, 1H, OH), 6.99 (s, 2H, H-3, H-5), 9.10 (s, 1H, NH). 13C NMR (DMSO-d_6, 100 MHz), δ, ppm : 30.86 (6C, 2 × –C(CH3)3), 32.92 (1C, C-8), 34.99 (2C, 2 × –C(CH3)3), 36.79 1C, C-7), 125.73 (2C, C-3, C-5), 128.88 (1C, C-4), 139.70 (2C, C-2, C-6), 153.09 (1C, C-1), 169.00 (1C, C-9). HRMS(ESI): m/z $[M]^+$ (calcd for $C_{17}H_{28}O_2N_2S$ 324.1872; found: 324.1881).

3.3 General Procedure for The Synthesis of Semicarbazides (5a – 5h) and Thiosemicarbazide (5a' – 5h')



Compound	R
5a, 5a'	
5b, 5b'	
5c, 5c'	
5d, 5d'	
5e, 5e'	
5f, 5f'	
5g, 5g'	
5h, 5h'	

Scheme 3.2: Synthetic scheme for the preparation of **5a** – **5h** and **5a'** – **5h'**.

The preparation of the target compounds is outlined in Scheme 3.2.

General procedure for the preparation of semicarbazides and thiosemicarbazide:

To a solution of *S*-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolic acid hydrazide (**3**, 0.3241 g, 1 mmol) in dry toluene (5 mL) was added isocyanate (1 mmol). The reaction mixture was stirred at room temperature for 2 hours until all reactants were consumed when monitored on TLC. The white precipitate was collected by filtration, washed with water and hot *n*-hexane, and dried at 50 ºC. The same procedure is applied to respective isothiocyanate group of reactants. Compound structure was confirmed by IR, ^{13}C NMR, ^{1}H NMR and HRMS analysis.

3.3.1 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-phenylhydrazine-1-carboxamide, 5a

Yield: 0.42 g (95%); white solid; mp 151 ºC; FTIR (ATR): 3619, 3252, 2960, 1605 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.83 (s, 1H), 8.72 (s, 1H), 8.13 (s, 1H), 7.46 (d, J = 6 Hz, 2H), 7.26 (t, J = 6 Hz, 2H), 7.08 (s,2H), 6.96 (t, J = 6Hz, 1H), 6.89 (s, 1H), 3.79 (s, 2H), 3.13 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.49, 155.68, 153.32, 140.06, 139.63, 129.12, 128.92, 125.73, 122.38, 118.90, 36.56, 34.95, 32.92, 30.83; HRMS (ESI): m/z $[M+Na]^+$ (calcd for $C_{24}H_{33}NaN_3O_3S^+$ 466.2135; found: 466.2161)

3.3.2 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(4-fluorophenyl)hydrazine-1-carboxamide, 5b

Yield: 0.44 g (96%); white solid; mp 160-162 ºC; FTIR (ATR): 3634, 3292-3344, 2871-2955, 1656 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.81 (s,1H), 8.76 (s, 1H), 8.15 (s, 1H), 7.47 (m, 2H), 7.10 (m, 2H), 7.07 (s, 2H), 6.89 (s, 1H), 3.78 (s, 2H), 3.12 (s, 2H), 1.37 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.49, 158.64, 157.06, 153.31, 139.64,

136.40, 128.92, 120.70, 115.66, 115.52, 36.55, 34.94, 32.93, 30.82; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{24}H_{33}O_3N_3SF^+$ 462.2221; found: 462.2225)

3.3.3 *N*-(4-cyanophenyl)-2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)hydrazine-1-carboxamide, 5c

Yield: 0.42 g (91%); white solid; mp 141-148 ºC; FTIR (ATR): 3589-3649, 3337, 2956-3025, 2234, 1601 cm^{-1} ; ^1H NMR (600 MHz, DMSO-d_6): δ 9.89 (s, 1H), 9.27 (b, 1H), 8.43 (s, 1H), 7.71 (d, J = 6 Hz, 2H), 7.65 (d, J = 6 Hz, 2H), 6.88 (s, 1H), 3.78 (s, 2H), 3.14 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.51, 155.24, 153.33, 144.66, 139.64, 133.64, 128.89, 125.73, 119.75, 118.74, 103.86, 36.53, 34.95, 32.87, 30.82; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{25}H_{33}O_3N_4S^+$ 469.2268; found: 469.2265)

3.3.4 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(3-methoxyphenyl) hydrazine-1-carboxamide, 5d

Yield: 0.44 g (93%); white solid; mp 134-136 ºC; FTIR (ATR): 3624, 3288, 2961, 1683 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 9.82 (b, 1H), 8.72 (b, 1H), 8.12 (b, 1H), 7.16 (m, 1H), 7.08 (s, 2H), 6.98 (d, J = 6 Hz, 1H), 6.88 (s, 1H), 6.54 (dd, J = 6, J = 6 Hz, 1H), 3.79 (s, 2H), 3.71 (s, 3H), 3.13 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.49, 158.64, 157.06, 153.31, 139.64, 136.40, 128.92, 120.70, 115.59, 36.55, 34.94, 32.93, 30.82; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{25}H_{37}O_4N_3S^+$ 475.2442; found: 475.2470)

3.3.5 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(2,4-dichlorophenyl)hydrazine-1-carboxamide, 5e

Yield: 0.46 g (90%); white solid; mp 104-106 ºC; FTIR (ATR): 3644, 3184-3332, 2862-2956, 1591 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.00 (s, 1H), 8.91 (s, 1H), 8.33 (b, 1H), 8.11 (d, ^{3}J = 6 Hz, 1H), 7.62 (d, ^{4}J = 0 Hz, 1H), 7.37 (dd, ^{3}J = 6, ^{4}J = 0 Hz, 1H), 7.07 (s, 2H), 6.89 (s, 1H), 3.78 (s, 2H), 3.13 (s, 2H), 1.37 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.40, 155.03, 153.33, 139.63, 135.50, 129.06, 128.84, 128.14, 126.80, 125.74, 123.28, 122.57, 36.52, 34.94, 32.65, 30.81; HRMS (ESI): m/z $[M+Na]^+$ (calcd for $C_{22}H_{31}O_3NaN_3SCl_2^+$ 510.1336; found: 510.1373)

3.3.6 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(naphthalen-2-yl)hydrazine-1-carboxamide, 5f

Yield: 0.44 g (89%); white solid; mp 121-123 ºC; FTIR (ATR): 3639, 3200-3302, 2873-2961, 1610 cm^{-1} ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.96 (s, 1H), 8.79 (s, 1H), 8.43 (s, 1H), 8.06 (d, ^{4}J = 12 Hz, 1H), 7.92 (m, 1H), 7.80 (b, 1H), 7.67 (d, ^{3}J = 6 Hz, 1H), 7.53 (m, 2H), 7.46 (t, ^{3}J = 6, ^{3}J = 6 Hz, 1H), 7.08 (s, 2H), 6.90 (s, 1H), 3.80 (s, 2H), 3.16 (s, 2H), 1.36 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.60, 156.42, 153.32, 139.69, 134.58, 134.19, 128.90, 128.74, 126.41, 126.21, 126.16, 125.74, 124.25, 122.38, 119.42, 36.59, 34.92, 32.91, 30.81; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{28}H_{36}O_3N_3S^+$ 494.2472; found: 494.2473)

3.3.7 *N*-(but-3-en-1-yl)-2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)hydrazine-1-carboxamide, 5g

Yield: 0.37 g (90%); white solid; mp 127-129 ºC; FTIR (ATR): 3619, 3436.3-3515, 3139, 2867-2956, 1675 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.63 (s, 1H), 7.86 (s, 1H), 7.06 (s, 2H), 6.86 (s, 1H), 6.47 (t, ^{3}J = 6, ^{3}J = 6 Hz, 1H), 5.80 (m, 1H), 5.15 (dd, ^{3}J = 18, ^{2}J = 0 Hz, 1H), 5.03 (dd, ^{3}J = 12, ^{2}J = 0 Hz, 1H), 3.75 (s, 2H), 3.66 (t, ^{3}J = 6, ^{3}J = 6 Hz, 2H), 3.08 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.39, 158.27, 153.30, 139.62, 136.75, 128.96, 125.68, 114.94, 41.94, 36.62, 34.94, 32.99, 30.84; HRMS (ESI): m/z $[M+Na]^+$ (calcd for $C_{21}H_{33}O_3NaN_3S^+$ 430.2135; found: 430.2154)

3.3.8 *N*-butyl-2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)hydrazine-1-carboxamide, 5h

Yield: 0.37 g (87%); white solid; mp 82-84 ºC; FTIR (ATR): 3375 , 3132, 2849-2917, 1635 cm-1; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.60 (s, 1H), 7.75 (s, 1H), 7.06 (s, 2H), 6.87 (s, 1H), 6.26 (t, ^{3}J = 6, ^{3}J = 6 Hz, 1H), 3.75 (s, 2H), 3.07 (s, 2H), 3.01 (m, 2H), 1.37 (s, 18H), 1.26 (m, 2H), 0.87 (t, ^{3}J = 6, ^{3}J = 6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.35), 158.41, 153.30, 139.61, 128.93, 125.69, 39.30, 36.59, 34.93, 32.92, 32.42, 30.82, 19.88, 14.17; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{22}H_{38}O_3N_3S^+$ 424.2628; found: 424.2637)

3.3.9 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-phenylhydrazine-1-carbothioamide, 5a'

Yield: 0.43 g (94%); white solid; mp 126-128 ºC; FTIR (ATR): 3634, 3144, 2956, 1670, 1225 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.10 (s, 1H), 9.67 (s, 1H), 9.60 (b, 1H), 7.45 (s, 2H), 7.34 (t, ^{3}J = 6, ^{3}J = 6 Hz, 1H), 7.17 (t, ^{3}J = 6, ^{3}J = 6 Hz, 1H), 7.08 (s, 2H), 6.90 (s, 1H), 3.79 (s, 2H), 3.17 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 181.39, 169.38, 153.34, 139.66, 139.55, 128.87, 128.59, 126.09, 125.70, 125.51, 36.67, 34.95, 33.35, 30.84; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{24}H_{34}O_3N_3S_2^+$ 460.2087; found: 460.2096)

3.3.10 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(4-fluorophenyl)hydrazine-1-carbothioamide, 5b'

Yield: 0.45 g (95 %); white solid; mp 109-111 ºC; FTIR (ATR): 3624, 3139-3283, 2956, 1670, 1220 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.47 (b, 1H), 8.54 (b, 1H), 8.11 (s, 1H), 7.39 (m, 2H), 7.12 (s, 2H), 7.11 (d, J = 6 Hz, 2H), 5.24 (s, 1H), 3.85 (s, 2H), 3.27 (s, 2H), 1.44 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 166.22, 162.09, 160.46, 153.29, 136.47, 132.47, 127.07, 126.92, 125.86, 116.64, 116.49, 37.75, 34.35, 33.76, 30.23; HRMS (ESI): m/z $[M+Na]^+$ (calcd for $C_{24}H_{32}O_2NaN_3S_2F^+$ 500.1812; found: 500.1827)

3.3.11 *N*-(4-cyanophenyl)-2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)hydrazine-1-carbothioamide, 5c'

Yield: 0.43 g (90%); white solid; mp 152-154 ºC; FTIR (ATR): 3634, 3127-3205, 2956, 2229, 1600, 1240 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.81 (b, 2H), 8.93 (b, 1H), 7.66 (d, J = 6 Hz, 2H), 7.53 (d, J = 6 Hz, 2H), 7.03 (s, 2H), 5.17 (s, 1H), 3.76 (s, 2H), 3.25 (s, 2H), 1.34 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 179.79, 167.41, 153.42, 142.01, 136.63, 133.03, 126.59, 125.78, 122.55, 118.55, 107.53, 37.78, 34.36, 33.97, 30.22; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{25}H_{33}O_2N_4S_2^+$ 485.2039; found: 485.2043)

3.3.12 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(3-methoxyphenyl)hydrazine-1-carbothioamide, 5d'

Yield: 0.46 g (94%); white solid; mp 109-111 ºC; FTIR (ATR): 3634, 3283-3332, 2834-2956, 1674, 1162 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.09 (b, 1H), 9.68 (s, 1H), 9.54 (b, 1H), 7.24 (t, ^{3}J = 6, ^{3}J = 6 Hz, 1H), 7.17 (s, 1H), 7.08 (s, 2H), 7.04 (dd, ^{3}J = 6, ^{4}J = 6 Hz, 1H), 6.89 (s, 1H), 6.74 (d, ^{3}J = 6 Hz, 1H), 3.79 (s, 2H), 3.74 (s, 1H), 3.17 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 181.20, 169.37, 159.50, 153.34, 140.67, 139.66, 129.37, 128.86, 125.71, 110.93, 55.57, 36.68, 34.95, 33.33, 30.83; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{25}H_{36}O_3N_3S_2^+$ 491.2293; found: 491.2245)

3.3.13 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-(2,4-dichlorophenyl)hydrazine-1-carbothioamide, 5e'

Yield: 0.51 g (96%); white solid; mp 131-136 °C; FTIR (ATR): 3634, 3174-3278, 2965, 1651, 1275 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.19 (b, 1H), 9.90 (b, 1H), 9.48 (b, 1H), 7.67 (s, 1H), 7.43 (d, ^{3}J = 6 Hz, 2H), 7.07 (s, 2H), 6.89 (s, 1H), 3.78 (s, 2H), 3.16 (s, 2H), 1.37 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 182.34, 169.41, 153.34, 139.66, 136.35, 132.86, 132.49, 131.94, 129.28, 128.85, 127.78, 125.69, 36.69, 34.94, 33.24, 30.83; HRMS (ESI): m/z $[M+Na]^+$ (calcd for $C_{24}H_{32}O_2NaN_3S_2Cl_2^+$ 526.1138; found: 526.1147)

3.3.14 2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)-*N*-1-(naphthalen-2-yl)hydrazine-1-carbothioamide, 5f'

Yield: 0.47 g (92%); white solid; mp 119-121 °C; FTIR (ATR): 3644, 3166-3258, 2956, 1698 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.79 (b, 1H), 9.04 (b, 1H), 8.54 (s, 1H), 7.92 (m, 1H), 7.82 (m, 1H), 7.76 (d, ^{3}J = 12 Hz, 1H), 7.58 (d, ^{3}J = 12 Hz, 1H), 7.46 (m, 1H), 7.45 (s, 1H), 7.42 (m, 1H), 7.00 (s, 2H), 5.08 (s, 1H), 3.72 (s, 2H), 3.07 (s, 2H), 1.31 (s, 18H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 179.16, 165.29, 153.24, 136.29, 134.51, 132.15, 129.58, 128.57; 128.53, 127.27, 126.87, 126.83, 125.87, 125.63, 124.92, 122.10, 37.62, 34.31, 33.69, 30.24; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{28}H_{36}O_2N_3S_2^+$ 510.2243; found: 510.2241)

3.3.15 *N*-(but-3-en-1-yl)-2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)hydrazine-1-carbothioamide, 5g'

Yield: 0.47 g (85%); white solid; mp 113-124 °C; FTIR (ATR): 3619, 3439-3512, 3141, 2868-2956, 1674, 1230 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.88 (s, 1H), 9.32 (s, 1H), 8.05 (s, 1H), 7.07 (s, 2H), 6.88 (s, 1H), 5.82 (m, 1H), 5.13 (dd, ^{3}J = 18, ^{2}J = 6 Hz, 1H), 5.04 (dd, ^{3}J = 12, ^{2}J = 0 Hz, 1H), 4.10 (s, 2H), 3.75 (s, 2H), 3.11 (s, 2H), 1.38 (s, 18H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 182.35, 169.39, 153.33, 139.64, 135.31, 128.87, 125.68, 115.76, 46.31, 36.66, 34.94, 33.27, 30.84; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{21}H_{34}O_2N_3S_2^+$ 424.2117; found: 424.2110)

3.3.16 *N*-butyl-2-(2-((3,5-di-*tert*-butyl-4-hydroxybenzyl)thio)acetyl)hydrazine-1-carbothioamide, 5h'

Yield: 0.41 g (93%); white solid; mp 122-127 °C; FTIR (ATR): 3619-3639, 3346, 3215-3273, 2868-2956, 1664, 1225 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.83 (s, 1H), 9.20 (s, 1H), 7.82 (s, 1H), 7.06 (s, 2H), 6.89 (s, 1H), 3.75 (s, 2H), 3.42 (d, ^{3}J = 6 Hz, 2H), 3.10 (s, 2H), 1.46 (m, 2H), 1.38 (s, 18H), 1.25 (m, 2H), 0.88 (t, ^{3}J = 6, ^{3}J = 6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 192.18, 169.49, 158.64, 157.06, 153.31, 139.65, 128.86, 125.69, 43.80, 36.63, 34.94, 33.19, 31.32, 30.83, 19.88, 14.25; HRMS (ESI): m/z $[M+H]^+$ (calcd for $C_{22}H_{38}O_2N_3S_2^+$ 440.2400; found: 440.2424)

3.4 Antioxidant Assay (DPPH scavenging assay)

DPPH radical scavenging assay was carried out according to the protocol reported by Ahmad and co-workers (Ahmad et al., 2019). Briefly, 0.2 mM DPPH solution in 25 mL methanol was added to various concentrations of antioxidant samples 10, 20, 40 and 60 μM. Each assay was carried out in triplicates. The mixtures were vortexed and incubated in the dark for 60 min at room temperature. Absorbance at 517 nm for each sample was measured. BHT and DPPH solution served as the as positive and negative controls. The free radical scavenging activity of the compounds was calculated as a percentage of radical inhibition by using the formula:

Percentage of inhibition (%) = [(Ac − As)/Ac] × 100,

where

As = Absorbance of the compounds/ positive control, and

Ac = Absorbance of control (DPPH solution and methanol).

Percentage of DPPH inhibition of each compounds were plotted against extract concentration in order to determine the concentration required to achieve 50 % inhibition (IC_{50}) of DPPH radical.

3.5 Differential Scanning Calorimetry

The antioxidant effect of hindered phenols and their synergistic effect with secondary antioxidant was demonstrated in thermo-oxidative stability test by differential scanning calorimetry according to ASTM D3895 with some modification (Riga & Patterson, 1997). Free radical contents of the oxidized oils were determined to explain the antioxidation synergistic effect of hindered phenols with secondary antioxidant compounds.

The DSC experiment involves heating a thin film of oil sample on an aluminium pan in an oxygen atmosphere and detecting the exotherm corresponding to the onset of rapid and accelerating oxidation. This was carried out in isothermal temperature programmed mode where the temperature was held constant and the elapsed time to the onset of oxidation (oxidative induction time) was measured. A longer oxidative induction time indicate an improvement of oxidation stability. The DSC test conditions were as follow:

Sample weight = 10 mg

Gas flow rate = 20 ml/min

Gas pressure = 2 bar

Temperature programmed = 10 ºC/min

Isothermal temperature = 125 ºC

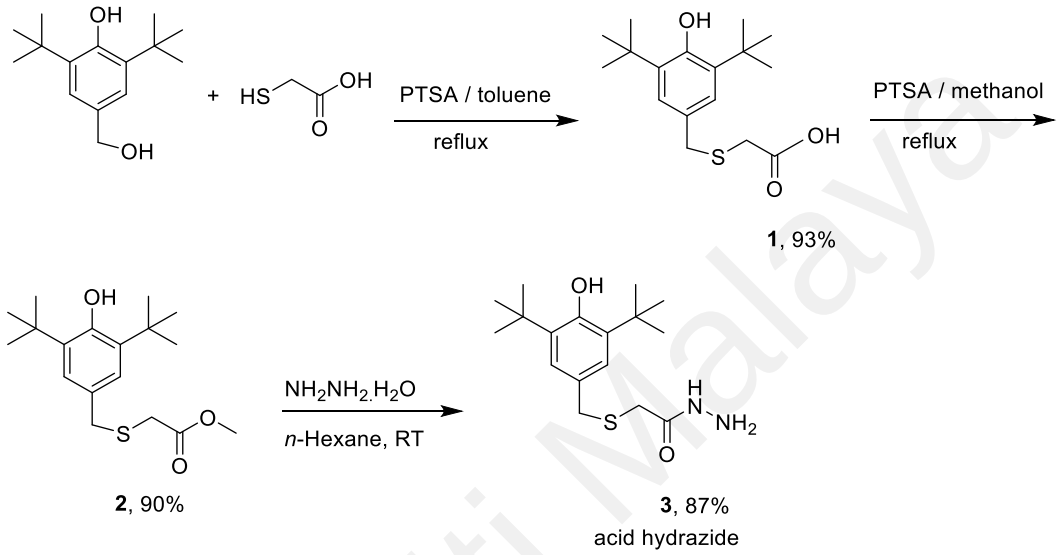
Antioxidant composition range = 0.25 wt %

The induction period from the isothermal experiments were obtained by extrapolation of the front edge of the exothermic peak to the baseline.

CHAPTER 4: RESULT AND DISCUSSION

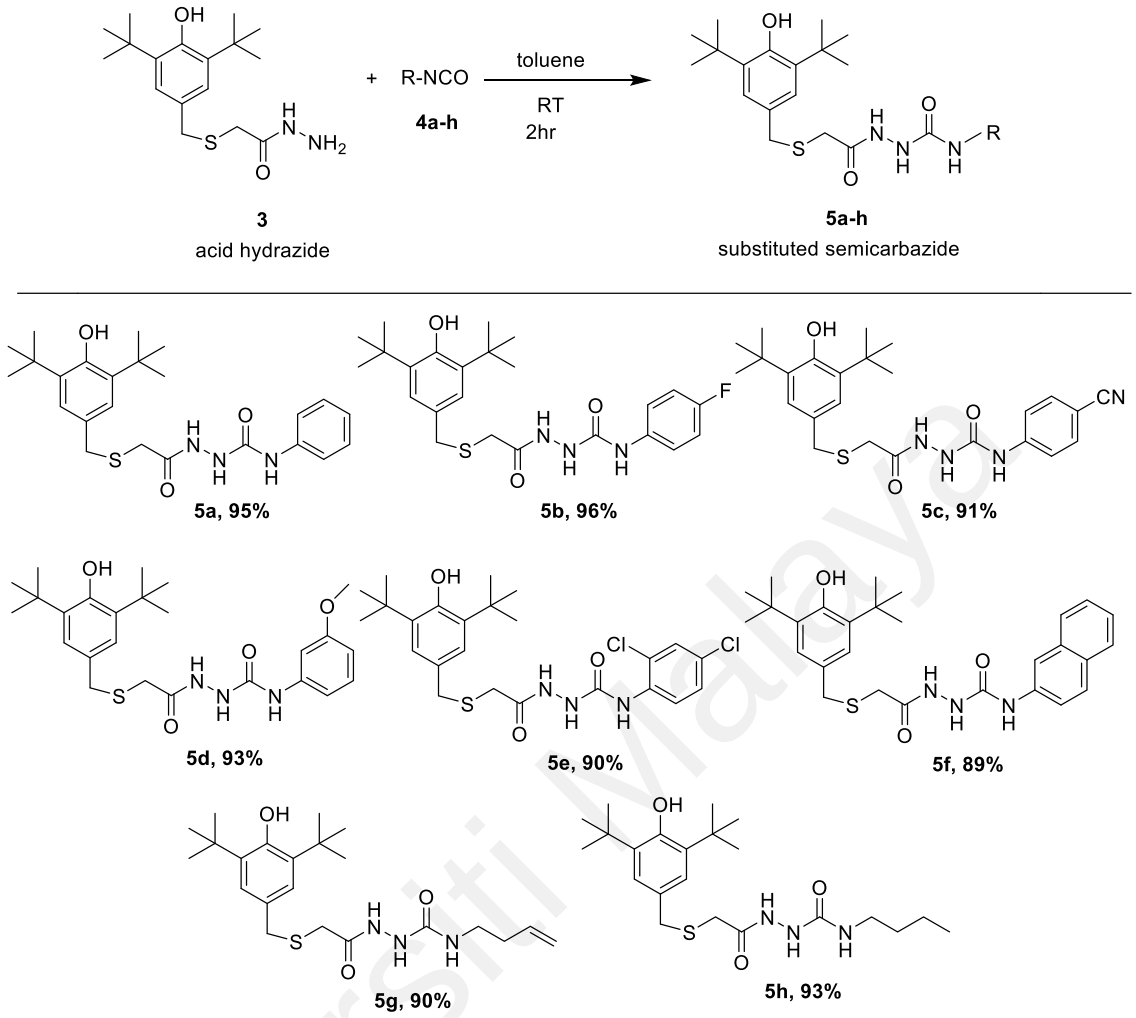
4.1 Chemistry of Multipotent Antioxidants

The preparation of precursor compound S-(3,5-di-*tert*-butyl-4-hydroxybenzyl)thioglycolic acid hydrazide, **3**, is outlined in Scheme 4.1 (Nath & Yehye, 2018; Wageeh A. Yehye et al., 2015).



Scheme 4.1: Preparation of acid hydrazide **3**

Subsequently, the reaction between acid hydrazide **3** and isocyanates **4a-h** at ambient temperature in dry toluene gave corresponding semicarbazides 5a-h. Visible product was formed upon mixing the reactants. The yields were excellent (89-96%). The results are summarized in Scheme 4.2. All synthesized products were confirmed by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry.



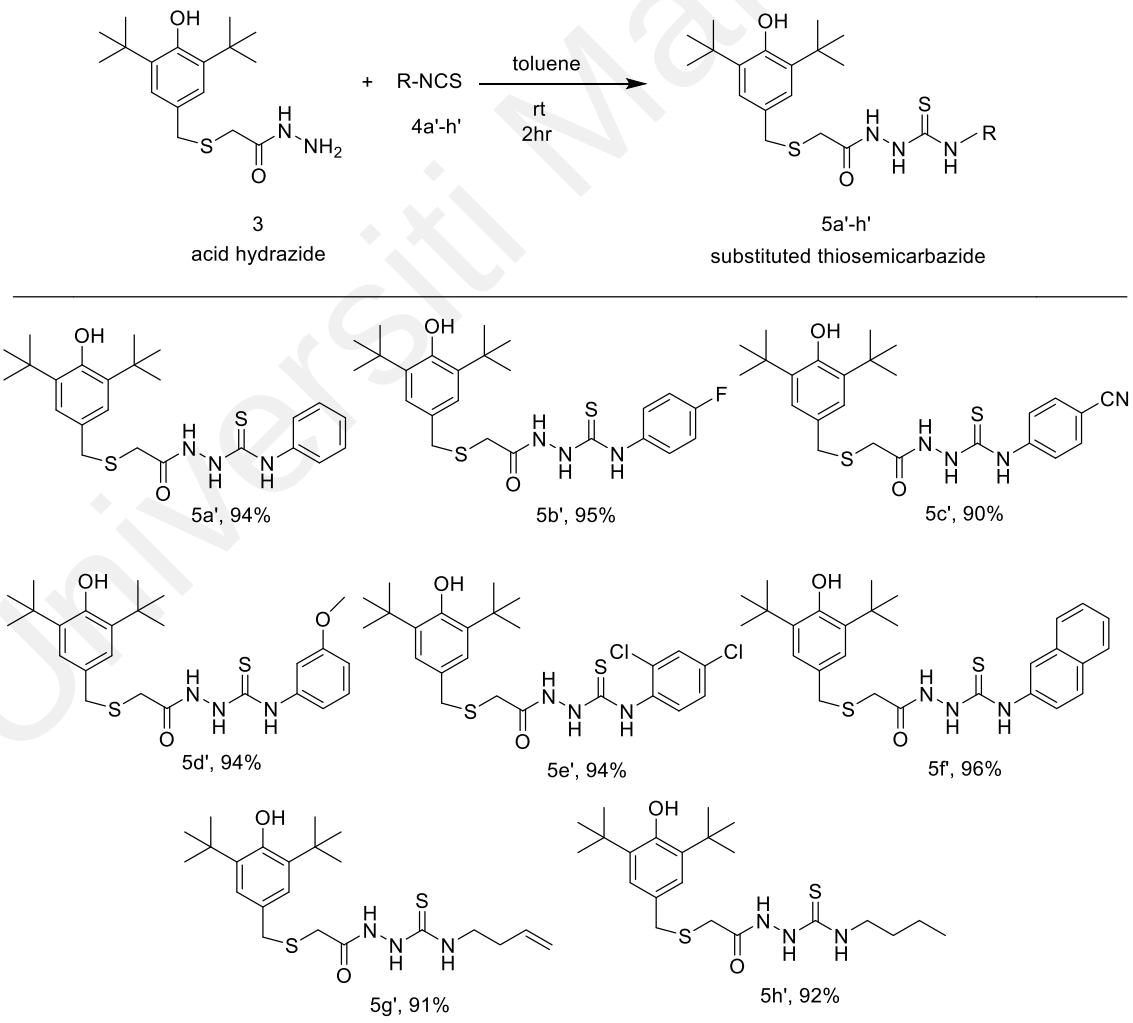
Scheme 4.2: Preparation of semicarbazides **5a-h**

The IR spectra of semicarbazides clearly showed a broad O-H stretching band at range 3619-3644 cm^{-1} and a C=O stretching band at 1703-1715 cm^{-1}. A strong band at 3242-3344cm^{-1} was assigned to the N-H stretching whereas C-H aliphatic bands appeared at 2955-2961 cm^{-1}. The mass spectrometric analysis confirmed the molecular weights of compounds **5a-h**.

The ^{1}H and ^{13}C NMR spectra of compounds **5a-h** showed different chemical shifts due to different substituents on the hydrazides. Generally, these semicarbazides showed a singlet peak at 1.36-1.38 ppm due to eighteen protons of *tert*-butyl groups. A singlet peak at the region between 6.87-6.90 ppm was attributed to free phenolic group. The H-7 and

H-8 of **5a-h** exhibited two singlet peaks in the region 3.75-3.80 ppm and 3.07-3.16 ppm, respectively. The aromatic H-3 and H-5 of the compounds are chemically equivalent and appeared as singlet at 7.06-7.08 ppm. All compounds exhibited broad signals at 9.60-10.00 ppm, 7.75-8.76 ppm and 6.26-9.27 ppm referring to NH-1, NH-2 and NH-3, respectively. ^{13}C NMR spectra of **5a-h** showed the presence of two carbonyls in the range of 155.03-158.41 ppm.

The thiosemicarbazides **5a'-h'** were also successfully prepared in good yield (90-96%) by treating acid hydrazide **3** with the corresponding thiocyanates **4a'-h'** in toluene at room temperature (Scheme 4.3).



Scheme 4.3: Preparation of substituted thiosemicarbazides **5a'-h'**

The spectroscopic features of **5a'-h'** were quite similar to **5a-h** corresponding compounds. In IR, a very strong band at the region around 1100 – 1200 cm^{-1} was assigned to the C=S stretching band that belongs to these thiosemicarbazides (Akinchan, West, Yang, Salberg, & Klein, 1995).

In ^{1}H NMR and ^{13}C NMR spectra, the major difference between **5a-h** and **5a'-h'** was the chemical shifts of N-H and C=S group. ^{1}H NMR spectra of **5a'-h'** showed three singlet peaks at 9.47 – 10.19, 8.11 – 9.68, and 7.82 – 9.90 ppm attributed to NH-1, NH-2, and NH-3, respectively. The chemical shifts of NH-2 and NH-3 were deshielded due to the adjacent C=S group. In ^{13}C spectra, the chemical shift of C-10 was also deshielded and appeared at 160.49 – 182.35 ppm. Thus, the NMR results confirmed the structures of thiosemicarbazides group.

The ^{1}H NMR spectrum of compound **5g** and **5g'**, recorded in DMSO-d_6, showed the presence of an allyl group as indicated by signals δ 5.80 (1H, ddd, *J*=24, 12, 6 Hz), 5.15 (1H, dd , *J*=18, 0 Hz) 5.04 (1H, dd, *J* =12, 0 Hz), 3.66 (2H, t, *J*=6, 6 Hz) (Pavia, Lampman, Kriz, & Vyvyan, 2008).

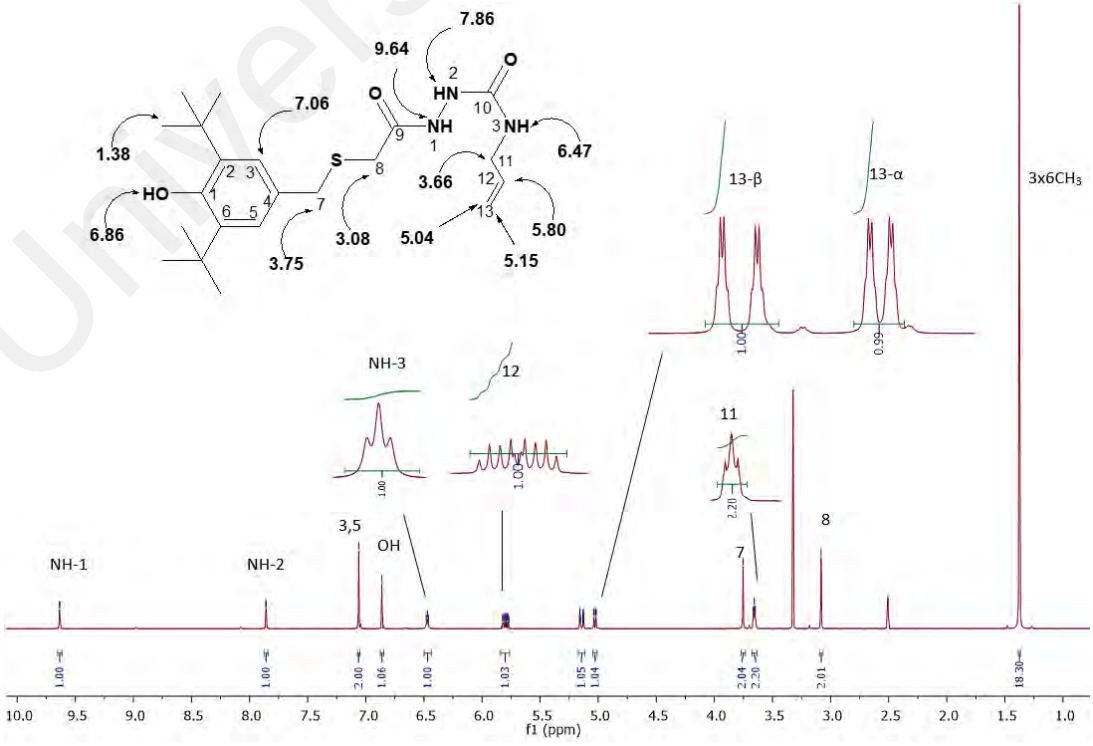
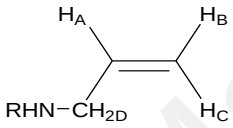


Figure 4.1: The 600-MHz ^{1}H NMR spectrum of allyl semicarbazide **5g.**

Those hydrogens as simplified in Figure 4.2, H_A = 5.80 ppm and H_B = 5.15 ppm showed germinal splitting with respect to each other. The third proton, H_C, appears at 5.04 ppm and was coupled differently to H_A (which is *trans*) than to H_B (which is *cis*). The hydrogen attached at C-12 (H_A) has the most complicated pattern *ddd*. This doublet of doublet of doublets pattern arises from coupling of HA with four protons (Hb, HC, and HD..HD…). H_C experienced a wider doublet of doublet pattern than H_B because ^3J *trans* is always larger than ^3J *cis* coupling for a vinyl system. Also, each of these multiplets were noticed to have 1H integration. Figure 4.2 showed the condition of H_A, H_B and H_C.



$d_B \neq d_C$
$J_{AC} \neq J_{AB}$
$J_{AB} \neq 0$

Figure 4.2: Condition of H_A, H_B and H_C.

Similar coupling patterns were also observed for 5g (Figure 4.3). However, comparing the chemical shift of the two results, C=S were found to cause deshielding effects to the proton neighbour NH-2 and NH-3 in compound **5g'** made them appeared at 9.33 and 8.05 ppm respectively. It is a significant different to be compared to its corresponding compound **5g** NH-2 and NH-3 which have 7.86 and 6.47 ppm respectively. The reason behind is because the properties of sulfur which more electronegative than oxygen thus decreases the electron density more in compound **5g'**. As a result, it leads to an increase in chemical shift value due to the shielding of the nucleus.

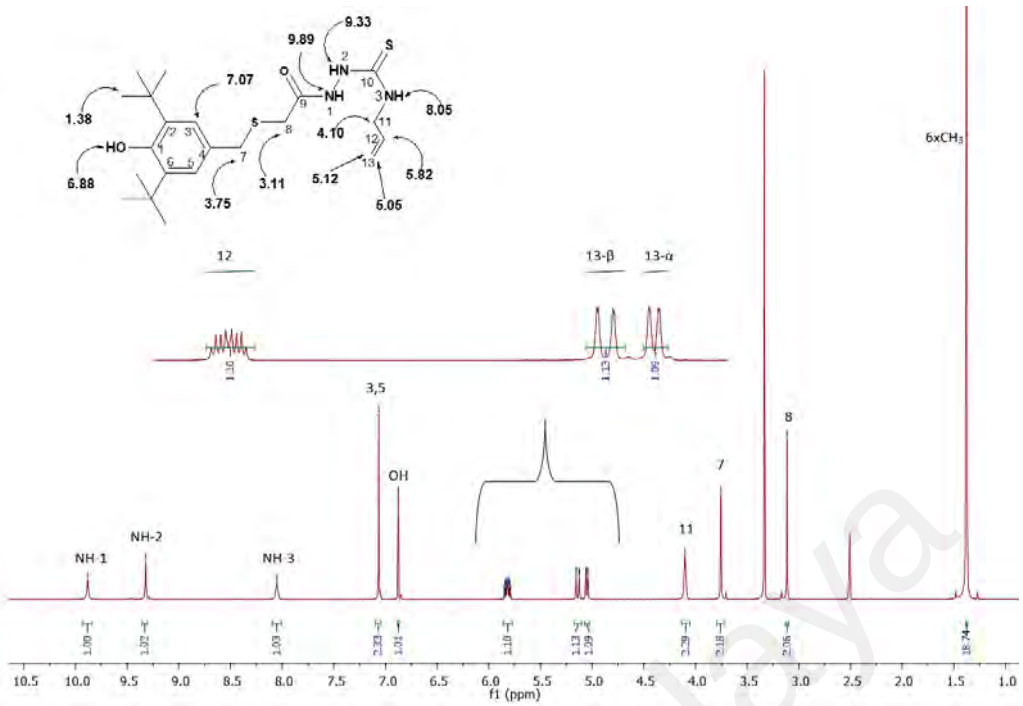


Figure 4.3: Experimental spectrum ^{1}H chemical shifts of allyl thiosemicarbazide 5g'.

4.2 Antioxidant Evaluation by DPPH

All the compounds were subjected for antioxidant evaluation using *in vitro* 1,1-diphenyl-2-picryl-hydrazine (DPPH) assay. DPPH assay is one of the most widely used methods to measure free radical scavenging ability of antioxidants due to its rapid, simple and inexpensive properties (Pyrzynska & Pękal, 2013). BHT was used as a standard in the DPPH assay. Delocalization of electron in DPPH gives a deep violet color at 517 nm absorption. However, the color turns into yellow when an antioxidant is added to the DPPH solution, resulting in the reduction of divalent nitrogen of DPPH (Abdulwahab et al., 2015). The radical scavenging activity is expressed by the half maximal inhibitory concentration (IC_{50}), which represents the concentration needed to inhibit 50% of DPPH. Thus, the compound which shows a lower IC_{50} would be considered a good antioxidant. The free radical scavenging properties of the synthesized semicarbazides (**5a-h**) and thiosemicarbazides (**5a'-h'**) were evaluated in 0.2 mM 1,1-diphenyl-2-picryl-hydrazine (DPPH) for 30 mins. The antioxidant activity of synthesized semicarbazides (**5a-h**) obtained from DPPH assay is summarized in Figure 4.4. All semicarbazides **5a-h** showed

good antioxidant activity in comparison with BHT. The decreasing order of IC_{50} values of **5a-h** was **5e** > **5h** > **5a** > **5g** > **5b** > **5f** > **5c** > **5d** > BHT.

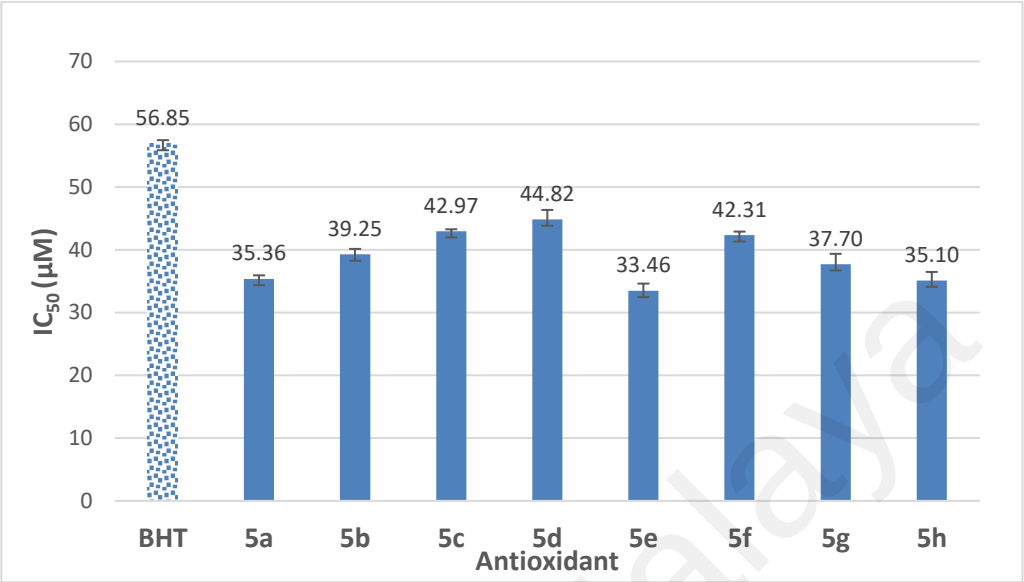


Figure 4.4: Antioxidant activity (IC_{50}) of synthesized compounds **5a-h** measured by DPPH assay.

The antioxidant activity of thiosemicarbazides (**5a'-h'**) is depicted in Figure 4.5. Good antioxidant activity was observed for all the compounds. Interestingly, thiosemicarbazides bearing naphthyl, 4-cyano, 2,4-di-chloro substituents showed promising free radical scavenging activity. Free radical scavenging properties of these compounds was in the descending order of **5f´** > **5c´** > **5e´** > **5a´** > **5d´** > **5g´** > **5b´** > **5h´** > BHT.

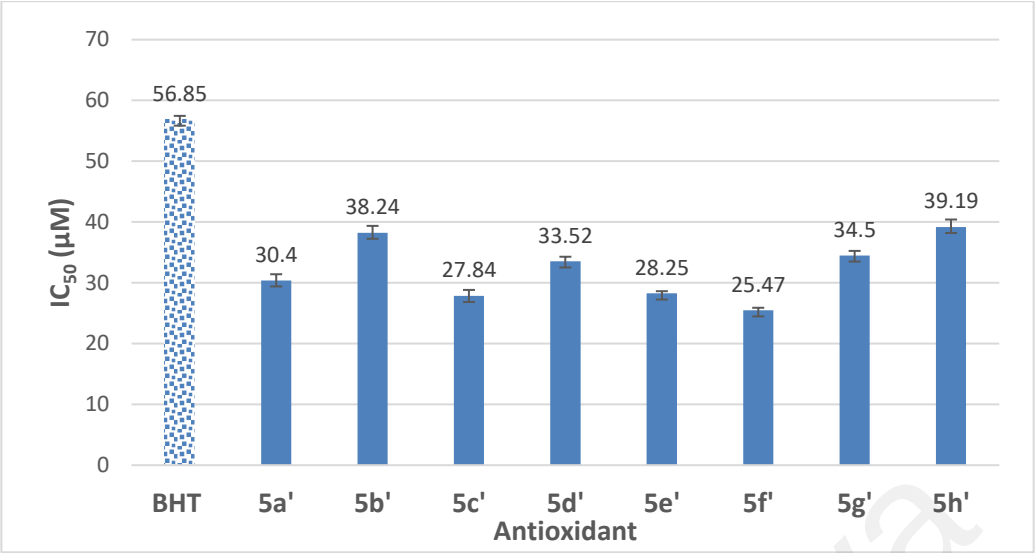


Figure 4.5: Antioxidant activity (IC_{50}) of synthesized compounds **5a'-h'** measured by DPPH assay.

Generally, the IC_{50} values of compounds **5a-h** and **5a'-h'** were better than that of BHT, indicating they were better antioxidants than BHT. The high antioxidant activity of semicarbazide was attributed to the synergistic effect of butylated hydroxytoluene (BHT) moiety and semicarbazide moiety. The NH group of semicarbazides can donate a hydrogen atom to the DPPH radical. After donating a hydrogen atom, semicarbazides **5a-h** exist in a radical form, and the radical can delocalize to the benzene ring of semicarbazides to produce a stable resonance system. The resulting resonance structure stabilizes the radical, preventing it from participating in a destructive chemical reaction. However, the synthesized substituted semicarbazides **5e**, **5h**, **5a** and **5g** showed IC_{50} value in the range of 33.46 – 37.70 µM. It is known that electron donating substituents enhance free radical scavenging properties on the benzene ring. The reason of this enhanced antioxidant properties might be attributed to the electron resonance effect of substituted benzene ring in **5e** making the radical more stable in the presence of electron-donating groups. In contrast, semicarbazides containing highly electron withdrawing group -CN (**5c**), -F (**5b**) showed higher IC_{50} value 39.26 µM and 42.97 µM respectively. Compound

5d which has an electron donating *O*Me group showed the highest IC_{50} value among all semicarbazides, likely due to a weak resonance effect (Wageeh A. Yehye et al., 2015).

On the other hand, thiosemicarbazides **5a'-h'** showed better free radical scavenging properties in comparison with the corresponding semicarbazides **5a-h**. Compound **5f'** which bears a naphthyl substituent exhibited the best antioxidant activity (25.47 ± 0.42 µM) among thiosemicarbazides, followed by **5c'** (CN group 27.84 ± 1.12), **5e'** (2,4-di-Cl 28.25 ± 0.37 µM) and **5a' (**30.40 ± 0.61). The antioxidant activity of **5f'** might be attributed to the delocalization of electron in naphthyl ring and steric hindrance. Similarly, compounds having naphthalene ring were found to exhibit good antioxidant activity (Foti et al., 2002; Ho & Ariffin, 2016; Ozen, Macit, & Toka, 2018). Interestingly, thiosemicarbazides with strong electron withdrawing substituent such as cyano (-CN) showed lower IC_{50} value compared with the corresponding semicarbazides. This phenomena might be attributed to the thioamide-thioimidic acid tautomerism of thiosemicarbazides Figure 4.6. Thiosemicarbazides might reduce the DPPH· radical in thioimidic acidic form as seen in Figure 4.6.

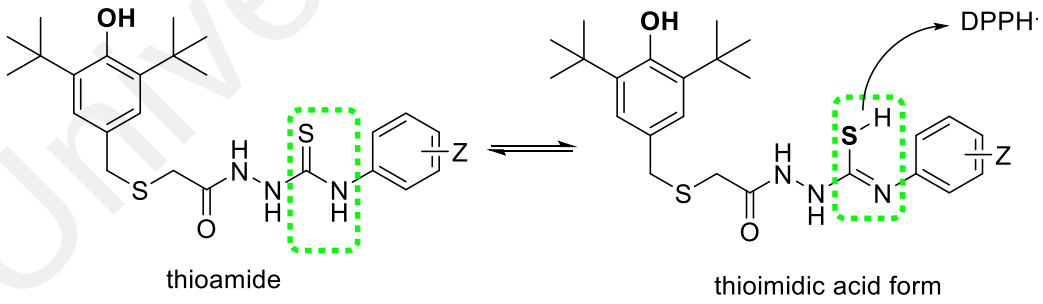
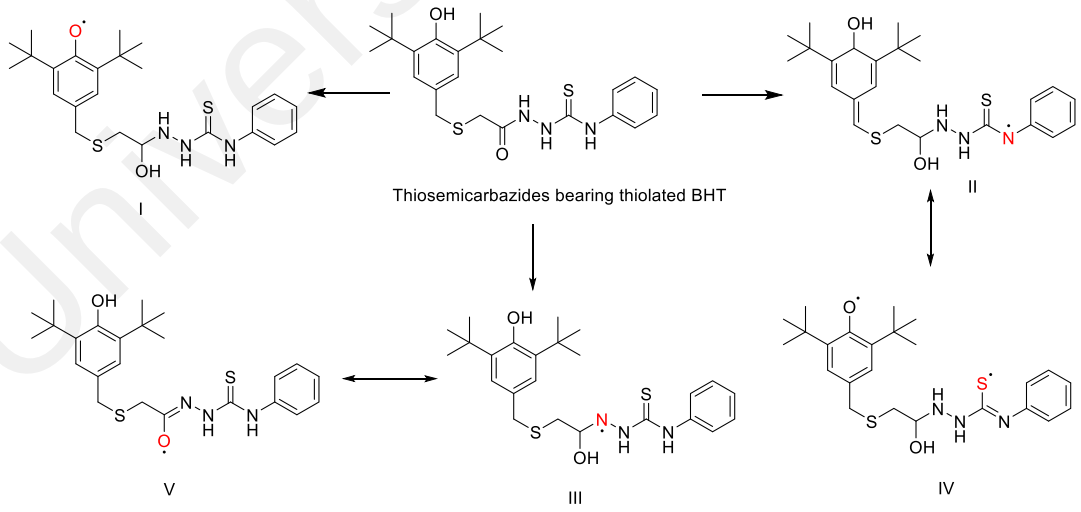


Figure 4.6: Possible thioamide-thioimidic acid tautomerism of thiosemicarbazides.

Similarly, thiosemicarbazide **5d'** showed a lower IC_{50} (33.52 ± 0.99) support the thioimidic acid form of thiosemicarbazide is lower than the corresponding semicarbazide **5d.** However, thiosemicarbazides with an electron donating substituent such as butyl

group (**5h´**) exhibited higher IC_{50} value (39.19 ± 0.75) than the corresponding semicarbazide **5h**.

It is obvious from DPPH result that thiosemicarbazides with thiolated BHA were found better antioxidant properties in comparison with semicarbazides. There may have two possible reasons for thiosemicarbazide to obtain a better antioxidant activity than semicarbazides: thioamide-thioimidic acid tautomerism and lower electronegativity of sulfur (2.58) than oxygen (3.44). Thiol can easily release proton to DPPH radical in comparison of hydroxyl group (Figure 4.6) (Abdulwahab et al., 2015; Ariffin et al., 2014; Kareem et al., 2016; Nguyen & Bui, 2013; Wageeh A Yehye, 2012). In addition, it was found that the degree of conjugation in thioamides is considerably higher than amides which made it a stronger π- electron attractor (Velkov, Velkov, Balabanova, & Tadjer, 2007). Thiosemicarbazides can convert into several radical forms by donating a proton to DPPH radical. A plausible radical formation of thiosemicarbazides is shown in Figure 4.4.



Scheme 4.4: Possible radical formation of thiosemicarbazides bearing thiolated BHT.

In summary, the DPPH results clearly showed the difference between semicarbazide and thiosemicarbazide and the substitution effect of different electron-donating groups and –withdrawing groups on the scavenging of free radicals. With the exception of **5h'**, the IC_{50} values of thiosemicarbazides **5a'-g'** were lower than that of semicarbazides **5a-g**. This study highlights a finding that the thiocarbonyl C=S of thiosemicarbazides played a better role in antioxidant activity than the carbonyl of semicarbazides. Further investigation on the structure-activity relationship is needed by computational and kinetic studies.

4.3 Evaluation of Antioxidants Stability by Thermal Analysis.

Based on the DPPH result, thiosemicarbazide **5f'** was the best antioxidant. Therefore, compound **5f'** and its corresponding semicarbazide compound **5f** were selected for oxidative stability evaluation using differential oxidation calorimetry (DSC). BHT was used as a standard antioxidant, and TMPTO was used as a synthetic ester-based lubricant. Compounds **5f´**, **5f** and BHT (0.25 wt%) were blended into TMPTO. Isothermal DSC was carried out with the blended samples to obtain oxidative induction time (OIT). The longer the OIT means the better oxidative stability of the oil (J. B. Yao, 1997). This experiment was conducted at two different isothermal temperature: 150 ºC and 125 ºC. At first, the isothermal DSC test was carried out at 150 ºC. Afterwards, the same test was conducted at 125 ºC. Both OIT results were outlined in Figure 4.7.

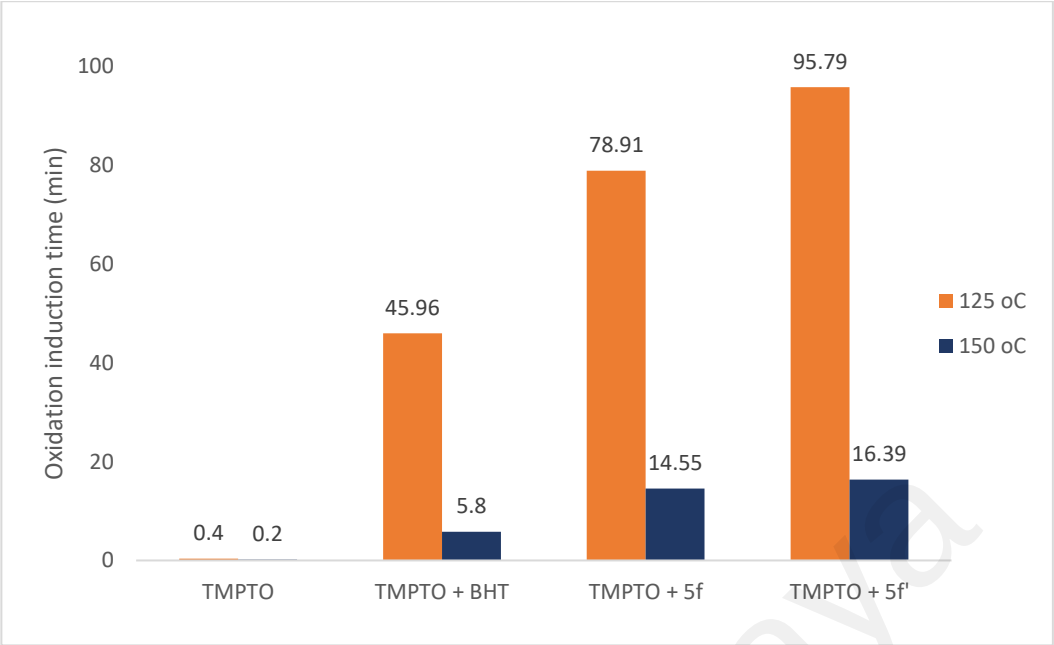


Figure 4.7: Oxidative induction time of TMPTO, TMPTO + BHT, TMPTO + **5f**, and TMPTO + **5f'** at 150 ºC and 125 ºC isothermal temperature.

According to Figure 4.7, TMPTO were stable only for 0.4 min at 150 ºC. Incorporation of 0.25 wt.% of **5f'** extended the OIT of TMPTO to 16.39 min. Incorporation the same amount of BHT into TMPTO resulted in the extension of OIT to 5.8 min. Therefore, TMPTO blended with **5f'** was 3 times more stable than TMPTO blended with the standard BHT.

A more significant extension of OIT was observed 5f at 125 ºC isothermal DSC. As seen in Figure 4.7, the OIT of TMPTO was only 0.4 min. However, addition of compounds **5f** and **5f'** increased the OIT of the base oil to 78.91 and 95.79 min, respectively. Both compounds showed better oxidative stability of TMPTO than BHT.

Taken together, these results showed that both 5f and 5f' have a great potential as antioxidant additives for synthetic lubricating oil i.e. TMPTO. Thiosemicarbazide **5f'** exhibited stronger oxidative stability than semicarbazides, **5f.** This observation was in agreement with that of DPPH result. The higher activity of **5f'** may be attributed to the presence of one primary antioxidant (BHT) and two secondary antioxidants (thioether

and thioamide) Figure 4.8, whereas **5f** possess two primary antioxidants (BHA, amide, and one secondary antioxidant (thioether). It was reported that antioxidants containing functional groups that can provide free radical scavenger activity moiety exhibit auto synergy (Rudnick, 2017; Sammaiah, Padmaja, Kaki, & Prasad, 2015). Thus, due to the auto-synergistic effect both the synthesized compound **5f** and **5f'** showed better oxidative stability than BHT.

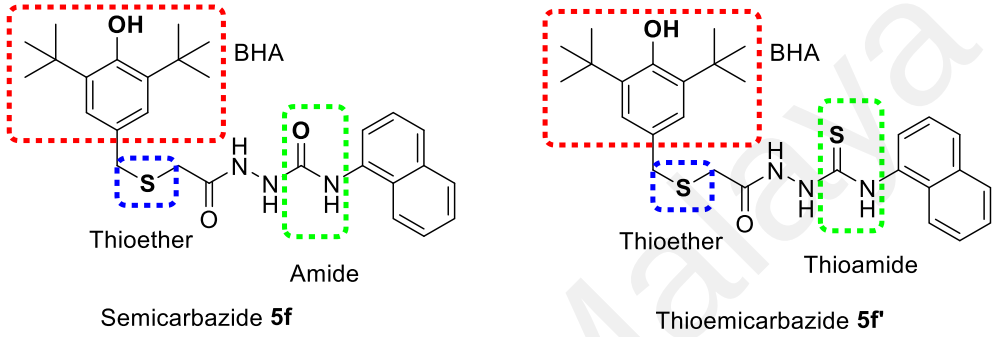


Figure 4.8: Important antioxidant functions in **5f** and **5f'** for potential oxidative stability of lubricant oil.

Organosulfurs are one of the important components in the lubricant compositions because of their antioxidant, antiwear and extreme pressure properties. They have been usually utilized together with hindered phenol or aromatic amine to get a better synergistic effect (Nath et al., 2018).

CHAPTER 5: CONCLUSION AND RECOMMENDATION

In conclusion, semicarbazides (**5a-h**) and thiosemicarbazides (**5a'-h'**) bearing thiolated BHA were synthesized in high yields. All the synthesized compounds were evaluated using DPPH assay for the antioxidant properties. The result showed that all the synthesized compounds were better antioxidant than BHT. Semicarbazides containing electron donating substituents showed enhanced free radical scavenging activity than that of semicarbazides with electro withdrawing substituents. Poor antioxidant activity was observed for semicarbazide containing electro donating methoxy group (**5d**) due to formation of intramolecular hydrogen bond. In comparison, thiosemicarbazides showed a better free radical scavenging activity than semicarbazides, likely due to the thioamide-thioimidic acid tautomerism. In addition, the thioamide of thiosemicarbazides may easily donate proton to DPPH radical due to a lower electronegativity of sulfur (2.58) compared to that of oxygen (3.44) from semicarbazide. Based on the DPPH result and solubility in TMPTO, compound **5f'** and **5f** have been chosen for isothermal DSC at two different isothermal temperatures to evaluate their oxidative stability in comparison with commercial antioxidant BHT. Compound **5f'** was found to exhibit a better oxidative stability in synthetic ester-based lubricant oil compared to BHT, attributing to its auto-synergism between two secondary antioxidant functions (thioether and thioamide) and a primary antioxidant function (BHA moiety). The DSC result of **5f'** is in agreement with its DPPH result.

Further investigations on structure-thermodynamic-antioxidant relationships employing double H^+/e^- process using density functional theory (DFT) calculations and quantitative structure-activity relationship (QSAR) modelling are required to identify the specific substituents effect and kinetic mechanisms.

In a future study, I would suggest to evaluate the toxicity of the compound. These compounds may be used as MPAO and various pharmacological applications, i.e. anti-inflammatory and anti-cancer. To be qualified as MPAO these compounds need further testings such as non-isothermal DSC analysis, four-ball tribology as well as anti-wear analyses.

REFERENCES

Abdulwahab, M. K., Ariffin, A., Yehye, W. A., Abdul-Aziz, A., Kareem, H. S., & Nordin, N. (2015). Synthesis of the Hydrazones of 2-((3, 5-Di-tert-butyl-4-hydroxybenzyl) thio) acetohydrazide and the Study of their Radical Scavenging Activity by the DPPH Assay and the Computational Method. *Bulletin of the Korean Chemical Society, 36*(11), 2716-2724. doi:https://doi.org/10.1002/bkcs.10553

Abraham, W. D., Adams, P. E., Lamb, G. D., Wolak, T. J., Denis, R. A., Kocsis, J. A., . . . Cowling, S. V. (2003). Lubricant compositions containing ester-substituted hindered phenol antioxidants. In: U.S. Patent No. 6,559,105.

Acharya, P. T., Bhavsar, Z. A., Jethava, D. J., Patel, D. B., & Patel, H. D. (2021). A review on development of bio-active thiosemicarbazide derivatives: Recent advances. *Journal of Molecular Structure, 1226*, 129268. doi:https://doi.org/10.1016/j.molstruc.2020.129268

Aguilar, G., Mazzamaro, G., & Rasberger, M. (2010). Oxidative degradation and stabilisation of mineral oil-based lubricants. In *Chemistry and Technology of Lubricants* (pp. 107-152): Springer. doi: https://doi.org/10.1023/b105569_4

Ahmad, M. H., Abd Rahman, N., Kadir, F. A., Al-Ani, L. A., Hashim, N. M., & Yehye, W. A. J. J. o. C. S. (2019). Design and synthesis of sulfur-containing butylated hydroxytoluene: antioxidant potency and selective anticancer agent. *131*(10), 1-11. doi:10.1007/s12039-019-1682-x

Ahmed, N. S., & Nassar, A. M. (2011). Lubricating Oil Additives. In D. C.-H. Kuo (Ed.), *Lubricating Oil Additives, Tribology - Lubricants and Lubrication* (pp. 320): InTech. doi: 10.5772/22923

Akinchan, N. T., West, D. X., Yang, Y. H., Salberg, M. M., & Klein, T. L. (1995). Magnetic and spectroscopic properties of copper (II) complexes with 1-salicoyl-4-phenylthiosemicarbazide. *Transition Metal Chemistry, 20*(5), 481-484. doi:https://doi.org/10.1007/BF00141522

Ali, B., Khan, K. M., Salar, U., Hussain, S., Ashraf, M., Riaz, M., . . . Perveen, S. (2018). 1-[(4′-Chlorophenyl) carbonyl-4-(aryl) thiosemicarbazide derivatives as potent urease inhibitors: Synthesis, in vitro and in silico studies. *Bioorganic chemistry, 79*, 363-371. doi:https://doi.org/10.1016/j.bioorg.2018.05.017

Alias, N. H., Yunus, R., Idris, A., & Omar, R. (2009). Effects of additives on oxidation characteristics of palm oil-based trimethylolpropane ester in hydraulics applications. *European journal of lipid science and technology, 111*(4), 368-375. doi:https://doi.org/10.1002/ejlt.200800136

Alisi, I. O., Uzairu, A., & Abechi, S. E. (2020). Free radical scavenging mechanism of 1,3,4-oxadiazole derivatives: thermodynamics of O–H and N–H bond cleavage. *Heliyon, 6*(3), e03683. doi:https://doi.org/10.1016/j.heliyon.2020.e03683

Anitha, P., Viswanathamurthi, P., Misini, B., & Linert, W. (2013). Synthesis, characterization, antioxidant, cytotoxic, and DNA cleavage studies of ruthenium (III) complexes containing N-substituted thiosemicarbazone/semicarbazone. *Monatshefte für Chemie-Chemical Monthly, 144*(12), 1787-1795. doi:https://doi.org/10.1007/s00706-013-1097-1

Ariffin, A., Rahman, N. A., Yehye, W. A., Alhadi, A. A., & Kadir, F. A. (2014). PASS-assisted design, synthesis and antioxidant evaluation of new butylated hydroxytoluene derivatives. *Eur J Med Chem, 87*, 564-577. doi:10.1016/j.ejmech.2014.10.001

Atta, E. M., Mohamed, N. H., & Abdelgawad, A. A. (2017). Antioxidants: An overview on the natural and synthetic types. *Eur. Chem. Bull, 6*, 365-375. doi:DOI: 10.17628/ecb.2017.6.365-375

Aziz, M. A., Diab, A. S., & Mohammed, A. A. (2019). *Antioxidant categories and mode of action*: IntechOpen.doi: 10.5772/intechopen.83544

Becker, M. M., Nunes, G. S., Ribeiro, D. B., Silva, F. E., Catanante, G., & Marty, J.-L. (2019). Determination of the antioxidant capacity of red fruits by miniaturized spectrophotometry assays. *Journal of the Brazilian Chemical Society, 30*(5), 1108-1114. doi:https://doi.org/10.21577/0103-5053.20190003

Berdahl, D., Nahas, R., & Barren, J. (2010). Synthetic and natural antioxidant additives in food stabilization: current applications and future research. In *Oxidation in foods and beverages and antioxidant applications* (pp. 272-320): Elsevier. doi: https://doi.org/10.1533/9780857090447.2.272

Blaine, R. L., & Harris, M. B. (1997). A proposed reference material for oxidative induction time by differential scanning calorimetry. In *Oxidative Behavior of Materials by Thermal Analytical Techniques*: ASTM International.doi: 10.1520/STP13823S

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*(4617), 1199-1200. doi:https://doi.org/10.1038/1811199a0

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology, 28*(1), 25-30. doi:https://doi.org/10.1016/S0023-6438(95)80008-5

Carlo Schillacia, R. N., Alessia Bellomariaa. (2013). Antioxidants in Food and Pharmaceutical Research. *Albanian Journal of Pharmaceutical Sciences, 1*, 9-15.

Chandra, S., Raizada, S., & Verma, R. (2012). Synthesis, characterisation and biological activities of copper (II) complexes with semicarbazones and thiosemicarbazones. *J. Chem. Pharm. Res, 4*(3), 1612-1618.

Commoner, B., Townsend, J., & Pake, G. E. (1954). Free radicals in biological materials. *Nature, 174*(4432), 689-691. doi:https://doi.org/10.1038/174689a0

Dacre, J. C. (1961). The Metabolism of 3:5-Di-tert.-butyl-4-hydroxytoluene and 3:5-Di-tert.-butyl-4-hydroxybenzoic Acid in the Rabbit. *Biochem. J., 78*, 758-766. doi:10.1042/bj0780758

de Guzman, R., Tang, H., Salley, S., & Ng, K. S. (2009). Synergistic effects of antioxidants on the oxidative stability of soybean oil-and poultry fat-based biodiesel. *Journal of the American Oil Chemists' Society, 86*(5), 459. doi:https://doi.org/10.1007/s11746-009-1373-8

Divar, M., Khalafi-Nezhad, A., Zomorodian, K., Sabet, R., Faghih, Z., Jamali, M., . . . Khabnadideh, S. (2017). Synthesis of some novel semicarbazone and thiosemicarbazone derivatives of isatin as possible biologically active agents. *Journal of Pharmaceutical Research International*, 1-13. doi:10.9734/JPRI/2017/35243

Dong, J. (2009). Liquid Additives for the Stabilization of Lubricant Compositions. In: U.S. Patent Application 12/476,423.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological reviews, 82*(1), 47-95. doi:https://doi.org/10.1152/physrev.00018.2001

Duangkaewmanee, S., & Petsom, A. J. T. I. (2011). Synergistic and antagonistic effects on oxidation stability of antioxidants in a synthetic ester based oil. *44*(3), 266-271. doi:https://doi.org/10.1016/j.triboint.2010.10.028

Ellington, J. R., Loper, J. T., & Mathur, N. C. (2011). Additives and lubricant formulations for improved antioxidant properties. In (Vol. No. 7,897,552.): U.S. Patent No. 7,897,552.

Foti, M. C. (2015). Use and Abuse of the DPPH• Radical. *Journal of agricultural and food chemistry, 63*(40), 8765-8776. doi:https://doi.org/10.1021/acs.jafc.5b03839

Foti, M. C., Johnson, E. R., Vinqvist, M. R., Wright, J. S., Barclay, L. R. C., & Ingold, K. (2002). Naphthalene diols: a new class of antioxidants intramolecular hydrogen bonding in catechols, naphthalene diols, and their aryloxyl radicals. *The Journal of Organic Chemistry, 67*(15), 5190-5196. doi:https://doi.org/10.1021/jo020184v

Fox, N., & Stachowiak, G. J. T. i. (2007). Vegetable oil-based lubricants—a review of oxidation. *40*(7), 1035-1046. doi:https://doi.org/10.1016/j.triboint.2006.10.001

Giovanni Brigati, M. L., * Veronica Mugnaini, and Gian Franco Pedulli. (2002). Determination of the Substituent Effect on the O-H Bond Dissociation Enthalpies of Phenolic Antioxidants by the EPR Radical Equilibration Technique. *J. Org. Chem., 67*, 4828-4832. doi:10.1021/jo025755y

Goldschmidt, S., & Renn, K. (1922). Zweiwertiger Stickstoff: Über das α, α-Diphenyl-β-trinitrophenyl-hydrazyl.(IV. Mitteilung über Amin-Oxydation). *Berichte der deutschen chemischen Gesellschaft (A and B Series), 55*(3), 628-643. doi:https://doi.org/10.1002/cber.19220550308

He, J.-B., Shi, H., Wang, Y., & Gao, X.-L. (2018). Synthesis, Characterization, and Performance Evaluation of Sulfur-Containing Diphenylamines Based on Intramolecular Synergism. *Molecules, 23*(2), 401. doi:https://doi.org/10.3390/molecules23020401

Higgins, C. L., Filip, S. V., Afsar, A., & Hayes, W. (2019a). Evaluation of thermal and oxidative stability of three generations of phenolic based novel dendritic fuel and lubricant additives. *Reactive and Functional Polymers, 142*, 119-127. doi:https://doi.org/10.1016/j.reactfunctpolym.2019.06.009

Higgins, C. L., Filip, S. V., Afsar, A., & Hayes, W. (2019b). Increasing the antioxidant capability via the synergistic effect of coupling diphenylamine with sterically hindered phenol. *Tetrahedron, 75*(51), 130759. doi:https://doi.org/10.1016/j.tet.2019.130759

Ho, K. W., & Ariffin, A. (2016). Synthesis, photophysical, and electrochemical properties of wide band gap tetraphenylsilane-carbazole derivatives: Effect of the substitution position and naphthalene side chain. *Russian Journal of Physical Chemistry A, 90*(13), 2590-2599. doi:https://doi.org/10.1134/S0036024416130124

Hovorka, S. W., & Schöneich, C. (2001). Oxidative degradation of pharmaceuticals: theory, mechanisms and inhibition. *Journal of pharmaceutical sciences, 90*(3), 253-269. doi:https://doi.org/10.1002/1520-6017(200103)90:3<253::AID-JPS1>3.0.CO;2-W

Huda, S. K. (2016). *Synthesis and antioxidant activity of conjugated oligo-aromatic compounds containing a 3, 4, 5-trimethoxybenzyl substituent/Huda Salah Kareem.* University of Malaya,

Kajiyama, T., & Ohkatsu, Y. (2001). Effect of para-substituents of phenolic antioxidants. *Polymer degradation and stability, 71*(3), 445-452. doi:10.1016/s0141-3910(00)00196-8

Kareem, H. S., Nordin, N., Heidelberg, T., Abdul-Aziz, A., & Ariffin, A. (2016). Conjugated oligo-aromatic compounds bearing a 3, 4, 5-trimethoxy moiety: investigation of their antioxidant activity correlated with a DFT study. *Molecules, 21*(2), 224. doi:https://doi.org/10.3390/molecules21020224

Keri, R. S., Chand, K., Budagumpi, S., Somappa, S. B., Patil, S. A., & Nagaraja, B. M. (2017). An overview of benzo [b] thiophene-based medicinal chemistry. *European journal of medicinal chemistry, 138*, 1002-1033. doi:https://doi.org/10.1016/j.ejmech.2017.07.038

Kiriliauskaitė, V., Bendikienė, V., & Juodka, B. (2011). Synthesis of trimethylolpropane esters of oleic acid by Lipoprime 50T. *Journal of industrial microbiology & biotechnology, 38*(9), 1561-1566. doi:https://doi.org/10.1007/s10295-011-0946-3

Kumar, R., Yang, S., Kumar, V., & Cholli, A. L. (2011). Nitrogen and hindered phenol containing dual functional macromolecular antioxidants: synthesis, performances and applications. In: Google Patents.

Kumar, V. A., Sarala, Y., Siddikha, A., Vanitha, S., Babu, S., & Reddy, A. V. (2018). Synthesis, Characterization Antimicrobial and Antioxidant Activities of 2, 4-dihydroxybenzaldehyde-4-phenyl-3-thiosemicarbazone (DHBPTSC) and its Pd (II), Ni (II) dppm Mixed Ligand and Cu (II) Complex having Heterocyclic Bases. *Journal of Applied Pharmaceutical Science Vol, 8*(04), 071-078. doi:10.7324/JAPS.2018.8410

Lanigan, R. S., & Yamarik, T. A. (2002). Final report on the safety assessment of BHT (1). *International Journal of Toxicology, 21*, 19-94. doi:10.1080/10915810290096513

Liu, R., & Mabury, S. A. (2020). Synthetic phenolic antioxidants: A review of environmental occurrence, fate, human exposure, and toxicity. *Environmental science & technology, 54*(19), 11706-11719. doi:https://doi.org/10.1021/acs.est.0c05077

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews, 4*(8), 118. doi:10.4103/0973-7847.70902

Lucarini, M., Pedulli, G. F., & Cipollone, M. (1994). Bond Dissociation Enthalpy of .alpha.-Tocopherol and Other Phenolic Antioxidants. *The Journal of Organic Chemistry, 59*(17), 5063-5070. doi:10.1021/jo00096a061

Luo, K., You, G., Zhang, S., Zheng, W., & Wu, S. (2020). Antioxidation behavior of bonded primary-secondary antioxidant/styrene-butadiene rubber composite: Experimental and molecular simulation investigations. *Polymer*, 122143. doi:https://doi.org/10.1016/j.polymer.2019.122143

Makahleh, A., Saad, B., & Bari, M. (2015). Synthetic phenolics as antioxidants for food preservation. In *Handbook of antioxidants for food preservation* (pp. 51-78): Elsevier.doi: https://doi.org/10.1016/B978-1-78242-089-7.00003-8

Malvis, A., Šimon, P., Dubaj, T., Sládková, A., Ház, A., Jablonsk , M., . . . Burčová, Z. (2019). Determination of the Thermal Oxidation Stability and the Kinetic Parameters of Commercial Extra Virgin Olive Oils from Different Varieties. *Journal of Chemistry, 2019*. doi:https://doi.org/10.1155/2019/4567973

Marinova, G., & Batchvarov, V. (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian Journal of Agricultural Science, 17*(1), 11-24.

Md. Saddam Hossain, C. M. Z., Md. Kudrat-E-Zahan* and B Zaman. (2017). Synthesis, Spectral and Thermal Characterization of Cu(II) Complexes withTwo New Schiff Base Ligand towards Potential Biological Application. *Pelagia Research Library, 8*(3), 380-392. doi:10.11648/j.ajmsp.20190401.16

Mousavi, P., Wang, D., Grant, C. S., Oxenham, W., & Hauser, P. J. (2006). Effects of Antioxidants on the Thermal Degradation of a Polyol Ester Lubricant Using GPC. *Industrial & Engineering Chemistry Research, 45*(1), 15-22. doi:10.1021/ie050539b

Nasser, R. M. (2015). *The Behavior of Some Acrylat Copolymers as Lubricating Oil Additives*: Lap Lambert Academic Publishing.doi: 10.11648/j.ajmsp.20190401.16

Nath, A. R., & Yehye, W. A. (2018). Acid Hydrazide: A Potential Reagent for the Synthesis of Semicarbazones. *Synthesis, 50*(21), 4301-4312. doi:10.1055/s-0037-1609557

Nath, A. R., Yehye, W. A., Zulkifli, N., & Johan, M. R. (2018). Ester of thiolated butylated hydroxytoluene: Potential antioxidant for synthetic lubricant oil. *Thermochimica Acta, 670*, 7-12. doi:https://doi.org/10.1016/j.tca.2018.09.021

Nguyen, D. T., & Bui, T. T. T. (2013). Antioxidant activities of thiosemicarbazones from substituted benzaldehydes and N-(tetra-O-acetyl-β-D-galactopyranosyl) thiosemicarbazide. *European journal of medicinal chemistry, 60*, 199-207. doi:https://doi.org/10.1016/j.ejmech.2012.10.004

Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *Rsc Advances, 5*(35), 27986-28006. doi:10.1039/C4RA13315C

Nordberg, J., & Arnér, E. S. (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free radical biology and medicine, 31*(11), 1287-1312. doi:https://doi.org/10.1016/S0891-5849(01)00724-9

Ozen, T., Macit, M., & Toka, M. (2018). Screening and Evaluation of Antioxidant Activities of Selected Naphthalene Compounds. *Jurnal Sains Kesihatan Malaysia (Malaysian Journal of Health Sciences), 16*(1). doi:http://dx.doi.org./10.17576/JSKM-2018-1601-06

Pavia, D. L., Lampman, G. M., Kriz, G. S., & Vyvyan, J. A. (2008). *Introduction to Spectroscopy*: Cengage Learning.

Perković, I., Tršinar, S., Žanetić, J., Kralj, M., Martin-Kleiner, I., Balzarini, J., . . . Zorc, B. (2013). Novel 1-acyl-4-substituted semicarbazide derivatives of primaquine—synthesis, cytostatic, antiviral and antioxidative studies. *Journal of enzyme inhibition and medicinal chemistry, 28*(3), 601-610. doi:https://doi.org/10.3109/14756366.2012.663366

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS, 4*(2), 89.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry, 30*(1), 11-26. doi:https://doi.org/10.1007/s12291-014-0446-0

Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry, 97*, 55-74. doi:https://doi.org/10.1016/j.ejmech.2015.04.040

Pyrzynska, K., & Pękal, A. (2013). Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Analytical Methods, 5*(17), 4288-4295. doi:https://doi.org/10.1039/C3AY40367J

Rane, R. A., Naphade, S. S., Bangalore, P. K., Palkar, M. B., Shaikh, M. S., & Karpoormath, R. (2014). Synthesis of novel 4-nitropyrrole-based semicarbazide and thiosemicarbazide hybrids with antimicrobial and anti-tubercular activity. *Bioorganic & medicinal chemistry letters, 24*(14), 3079-3083. doi:https://doi.org/10.1016/j.bmcl.2014.05.018

Rawat, D. S., Joshi, G., Lamba, B. Y., Tiwari, A. K., & Kumar, P. (2015). The effect of binary antioxidant proportions on antioxidant synergy and oxidation stability of Jatropha and Karanja biodiesels. *Energy, 84*, 643-655. doi:https://doi.org/10.1016/j.energy.2015.03.024

Riga, A. T., & Patterson, G. H. (1997). Development of a standard test method for determining oxidative induction time of hydrocarbons by differential scanning calorimetry and pressure differential scanning. In *Oxidative Behavior of Materials by Thermal Analytical Techniques*: ASTM International.doi: 10.1520/STP13819S

Rizvi, S. Q. (2003). Additives and additive chemistry. *In Fuels and Lubricants Handbook: Technology, Properties, Performance, and Testing*, 199-248.

Rose, D. J. (1991). *Analysis of antioxidant behaviour in lubricating oils.* University of Leeds,

Roy M Mortier, M. F. F., Stefan T Orszulik. (2010). *Chemistry And Technology Of Lubricants* (Third ed.): Springer.doi: https://doi.org/10.1007/978-1-4020-8662-5

Rudnick, L. R. (2017). *Lubricant Additives: Chemistry and Applications, Third Edition*: CRC Press.

Salah BA, K. A. a. A. E.-N. M. (2018). A Therapeutic Journey of Semicarbazide and Thio Semicarbazide Derivatives and their Transition Metals Complexes: Mini Review. *Research & Reviews: Journal of Chemistry, Volume 7*(Issue 1), 38-48.

Sammaiah, A., Padmaja, K. V., Kaki, S. S., & Prasad, R. B. (2015). Multifunctional lubricant additives derived from natural amino acids and methyl oleate. *Rsc Advances, 5*(95), 77538-77544. doi:https://doi.org/10.1039/C5RA15239A

Sangeetha, P., Das, U., Koratkar, R., & Suryaprabha, P. (1990). Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. *Free radical biology and medicine, 8*(1), 15-19. doi:https://doi.org/10.1016/0891-5849(90)90139-A

Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant compounds and their antioxidant mechanism. In *Antioxidants*: IntechOpen.doi: https://doi.org/10.1007/978-1-4020-8662-5

Sazeli, S., Nath, A. R., Ahmad, M. H., Zulkifli, N., Johan, M. R., Yehye, W. A., & Voon, L. H. (2021). Semicarbazide and thiosemicarbazide containing butylated

hydroxytoluene moiety: new potential antioxidant additives for synthetic lubricating oil. *Rsc Advances, 11*(13), 7138-7145. doi:10.1039/D0RA10626G

Schmid, M., Ritter, A., & Affolter, S. (2006). Determination of oxidation induction time and temperature by DSC: results of round robin tests. *Journal of thermal analysis and calorimetry, 83*(2), 367-371. doi:https://doi.org/10.1007/s10973-005-7142-5

Shahidi, F., & Zhong, H. J. (2005). Methods for Measuring Lipid Oxidation. *Bailey's Industrial Oil and Fat Products*, 1-27. doi:10.1002/047167849X.bio050.pub2

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods, 18*, 757-781. doi:https://doi.org/10.1016/j.jff.2015.01.047

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food chemistry, 113*(4), 1202-1205. doi:https://doi.org/10.1016/j.foodchem.2008.08.008

Sharul Hafiq, R. (2017). *Synthesis of surface capped molybdenum sulphide nanoparticles as an antiwear additives for bio-based lubricant oil/Sharul Hafiq Roslan.* University of Malaya,

Shelton, J. R. (1959). Mechanism of antioxidant action in the stabilization of hydrocarbon systems. *Journal of Applied Polymer Science, 2*(6), 345-350. doi:https://doi.org/10.1002/app.1959.070020616

Singh, M., Singh, S. K., Gangwar, M., Nath, G., & Singh, S. K. (2016). Design, synthesis and mode of action of novel 2-(4-aminophenyl) benzothiazole derivatives bearing semicarbazone and thiosemicarbazone moiety as potent antimicrobial agents. *Medicinal Chemistry Research, 25*(2), 263-282. doi:https://doi.org/10.1007/s00044-015-1479-5

Singh, R. K., Kukrety, A., Sharma, O. P., Poddar, M. K., Atray, N., & Ray, S. S. (2017). Synthesis of a novel efficient antioxidant for use in lubes and biodiesel. *Petroleum Chemistry, 57*(1), 100-105. doi:https://doi.org/10.1134/S096554411701011X

Soleimani, M., Dehabadi, L., Wilson, L. D., & Tabil, L. G. (2018). Antioxidants Classification and Applications in Lubricants. doi:10.5772/intechopen.72621

Takahashi, Y., Yachigo, S., & Ishii, T. (1985). Phosphorus-containing compounds, their production and their use as antioxidants. In: Google Patents.

Tan, C., & Man, Y. C. (2002). Recent developments in differential scanning calorimetry for assessing oxidative deterioration of vegetable oils. *Trends in Food Science & Technology, 13*(9-10), 312-318. doi:https://doi.org/10.1016/S0924-2244(02)00165-6

Tan, C. P., Che Man, Y. B., Selamat, J., & Yusoff, M. S. A. (2002). Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. *Food chemistry, 76*(3), 385-389. doi:https://doi.org/10.1016/S0308-8146(01)00272-2

Tynik, R. J., Donnelly, S. G., & Aguilar, G. A. (2011). Antioxidant additive for lubricant compositions, comprising organotungstate, diarylamine and organomolybdenum compounds. In: Google Patents.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry & cell biology, 39*(1), 44-84. doi:https://doi.org/10.1016/j.biocel.2006.07.001

Velkov, Z., Velkov, Y., Balabanova, E., & Tadjer, A. (2007). First principle study of the structure of conjugated amides and thioamides. *International Journal of Quantum Chemistry, 107*(8), 1765-1771. doi:https://doi.org/10.1002/qua.21234

Waheed, E. J., Jaafar, W. A., & Ahmed, A. A. (2019). Various applications of carbazone derivatives and their metal complexes. *Biochemical and Cellular Archives*.

Wang, X., Xing, W., Tang, G., Hong, N., Hu, W., Zhan, J., . . . Hu, Y. (2013). Synthesis of a novel sulfur-bearing secondary antioxidant with a high molecular weight and its comparative study on antioxidant behavior in polypropylene with two commercial sulfur-bearing secondary antioxidants having relatively low molecular weight. *Polymer degradation and stability, 98*(11), 2391-2398. doi:https://doi.org/10.1016/j.polymdegradstab.2013.08.030

Wu, Y., Li, W., Zhang, M., & Wang, X. (2013). Improvement of oxidative stability of trimethylolpropane trioleate lubricant. *Thermochimica Acta, 569*, 112-118. doi:https://doi.org/10.1016/j.tca.2013.05.033

Yao, J., Gaston, A., & Steven, G. (2009). Study on the synergistic effects of the antioxidants for lubricant. *Lubr. Oil, 24*, 38-44.

Yao, J. B. (1997). Evaluation of sodium acetylacetonate as a synergist for arylamine antioxidants in synthetic lubricants. *Tribology International, 30*(11), 795-799. doi:10.1016/s0301-679x(97)00046-7

Yehye, W. A. (2012). *Design, synthesis, multipotent antioxidant activity of new heterocycles and some reactions of acid hydrazide based on butylated hydroxytoluene (BHT) moiety*. University of Malaya,

Yehye, W. A., Rahman, N. A., Ariffin, A., Abd Hamid, S. B., Alhadi, A. A., Kadir, F. A., & Yaeghoobi, M. (2015). Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. *European journal of medicinal chemistry, 101*, 295-312. doi:http://dx.doi.org/10.1016/j.ejmech.2015.06.026

Young, I., & Woodside, J. (2001). Antioxidants in health and disease. *Journal of clinical pathology, 54*(3), 176-186. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1731363/pdf/v054p00176.pdf

Yu, S., Feng, J., Cai, T., & Liu, S. (2017). Schiff base bridged phenolic diphenylamines for highly efficient and superior thermostable lubricant antioxidants. *Industrial & Engineering Chemistry Research, 56*(14), 4196-4204. doi:https://doi.org/10.1021/acs.iecr.7b00313

Yu, X. W., Li, Y. Q., & Wu, D. (2004). Microencapsulation of tannase by chitosan-alginate complex coacervate membrane: synthesis of antioxidant propyl gallate in biphasic media. *Journal of Chemical Technology and Biotechnology, 79*(5), 475-479. doi:10.1002/jctb.1000

Zainal, N., Zulkifli, N., Gulzar, M., & Masjuki, H. (2018). A review on the chemistry, production, and technological potential of bio-based lubricants. *Renewable and Sustainable Energy Reviews, 82*, 80-102. doi:https://doi.org/10.1016/j.rser.2017.09.004

Zhang, Y., Li, H., Luo, Z., Li, M., Liu, T., Liu, W., . . . Hu, Y. (2020). Synthesis of Polyethylene-Grafted Phosphite Antioxidant and Its Antioxidant Behavior in Polypropylene. *Macromolecular Chemistry and Physics*, 1900540. doi:https://doi.org/10.1002/macp.201900540

https://www.machinerylubrication.com/Read/30165/oil-oxidation-stages)