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An Automated Study of Antioxidant Potentials of Polar Extract of Turmeric as Influenced by Ultraviolet Radiation

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By

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Turmeric polar extract (TPE) was obtained by dielectric-precipitation of turmeric slurry and found to contain three proteins with two in the 10-11 KDa range being dominant. Antioxidative activity and persistence (AP) of TPE (5%, w/v) respectively showed 87% and 85% greater generation of alkoxy- and peroxyl radicals compared the non-redox-active buffer alone showing significant ($p<0.05$) pro-oxidative behavior. Conversely, purified curcumin (CU) (0.1% w/v) was dramatically antioxidative with AA and AP values of 2,828 and 1,129%, respectively, compared to the blank. However, a combination of the two at the same concentration dropped these values to 590 and 389%, respectively, reflecting dramatic dampening of the efficacy of CU. Ultraviolet radiation significantly modulated the efficacy of CU where UVB (300 nm) exposure gave the highest enhancement when limited to five min. Data showed that turmeric contains highly pro-oxidant polar proteins that significantly dramatically diminishes the beneficial antioxidative efficacy of its principal phytochemical, CU.

Keywords: Turmeric polar extract, curcumin, ultraviolet radiation, antioxidative activity, pro-oxidan.

DEDICATION

My thesis is dedicated to God (Allah) the most merciful, everyone who loves me, and to the spirit of my father whom I thank for being an inspiration to my life.

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Now, that I am ending this chapter of my life, I feel like I have gained new knowledge and skills, which did not come easy, but today I can say I did it! I have deep gratitude to those who helped me not only to learn but who also stood by me and taught me to overcome the obstacles that I faced during this time.

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LIST OF ABBREVIATIONS

AA	Antioxidant activity
ABAP	2,2’-azo-bis (2-methylpropionamidine) dihydrochloride
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AC	Antioxidant Capacity
AD	Alzheimer’s disease
AP	Antioxidant persistence
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxyl- toluene
CU	Curcumin Concentrations
CU 0.1	Curcumin 10^{-1} %, (w/v)
CU 0.01	Curcumin 10^{-2} %, (w/v)
CU 0.001	Curcumin 10^{-3} %, (w/v)
CU 0.0001	Curcumin 10^{-4} %, (w/v)
DE	Diethyl ether $C_4H_{10}O$
HAT	Hydrogen atom transfer
LSD	Least Significant Difference
PP	Phosphorous pentoxide P_4O_{10}
RFU	Relative fluorescence unit
RLU	Relative light unit
RNS	Reactive nitrogen species

ROS	Reactive oxygen species
RSS	Reactive sulfur species
SAS	Statistical Analysis Software
SET	Single electron transfer
SDS-PAGE	Sodium dodecyl Sulfate-Polyacrylamide Gel Electrophoresis.
TBHQ	Tertiarybutylhydroquinone
TPE	Turmeric Polar Extract
TRAP	Total radical trapping antioxidant potential
UV	Ultraviolet
UVA	Ultraviolet Radiation A
UVB	Ultraviolet Radiation B

CHAPTER I

INTRODUCTION

It is normal for the body to deal with free radicals of intrinsic and extrinsic origin through multicomponent antioxidative systems but cell damage leading to health deterioration occurs if these occur in excess (Albano, 2006). In addition to environmental radical producing chemicals and pollutants, our bodies generate free radicals every day mostly during the process of oxidative phosphorylation and digestion (Vinogradov & Grivennikova, 2005). The human body has a defense system of antioxidants (enzyme systems). However, this system is sometimes lost or reduced, so the body needs to compensate for the decrease. It can do this by taking the antioxidant from the different sources such as synthetic and natural sources to protect the body from many diseases such as aging, degeneration and cancer (Ringman et al., 2005). Reactive oxygen species play an important role in oxidative stress which has related with many diseases such as ulcerative colitis and neurological degenerative diseases (Khanduja & Bhardwaj, 2003).

Currently, research has shown that oxygen and free radicals participate in aging and diseases processes such as cancer, diabetes, cardiovascular diseases. Other reactive oxygen species cause excessive production of free radicals which can cause oxidative damage to functional molecular such as proteins, lipids, and DNA. Natural antioxidants are considered more acceptable than synthetic antioxidants (Huang et al., 2010).

Free radicals that occur in the environment can lead to an interaction series that causes oxidative damage to biological cells and later results in cancer, heart disease, and autoimmune diseases (Mitscher et al., 1997). The term reactive oxygen species (ROS) is not only oxygen- centered radical such as $\dot{O}_2$ and $OH^{\bullet}$ but also non-radical derivatives of oxygen, for example, single oxygen O2, hydrogen peroxide $WATER_2$, and O_3 (Poljšak & Dahmane, 2012). A free radical is an atom which is unstable. So, to achieve a more stable state, free radicals can "steal" a hydrogen atom from another molecule or interact in other ways with free radicals (Albano, 2006). Therefore, there is interest considerable interest in the study of compounds naturally occurring in herbs and spices, such as phenols and peptides that can counter the harmful effects if these molecules. They have been shown to prevent detrimental harmful health complications such as those due to aging, tumors and certain types of cancers (Scapagnini et al., 2011).

Fruits and vegetables are a primary source of natural antioxidants in the diet. Other sources of natural antioxidants include spices, herbs, and teas; all of which have a great variety of phenolic compounds (Yanishlieva-Maslarova, 2001). In the food industry, synthetic antioxidants are inexpensive though very effective. However, they have some defects that include harmful toxicological effects (Kiritsakis et al., 2010). Synthetic and natural phenolic antioxidants have been shown to a counter the damaging effects of ultraviolet radiation-induced free radical that cause oxidative damage (Brewer, 2011).Ultraviolet can generate reactive species such as free radicals. Because that, UV causes mutations in genome like hydrogen peroxide and superoxide (D'Orazio et al., 2013). However, this generation of free radicals also occurs as part of physiological

processes in the body such as mitochondria. So there is antioxidant enzymes subjectivity (Chen et al., 2012).

It is a well-known fact that UV radiation is harmful to human health. So it is important to provide protection from UV, especially the skin. Ultraviolet can cause many side effects as sunburns, aging, and cancer (Korać & Khambholja, 2011). Other results have shown that the products which contain natural extracts can protect the body, especially the skin from UV damage. Curcumin is extracted from (Curcuma longa), which can prevent the damaging effects of ROS generated from radiation UV (Chan et al., 2003; Garcia-Bores & Avila, 2008). Many studies were conducted on the research of curcumin and curcuminoids that were obtained from turmeric.

Antioxidants from natural sources have acquired increasing attention (Moure et al., 2001) towards plant materials that are rich in a phenolic such as fruits, vegetables, and herbs. Recently, antioxidants have become an important topic of growing interest in the field of medicine. Antioxidants are the most common substances that play active roles in protecting the body from the destroying effects of free radicals and preventing oxidation or delaying the oxidative process (Hurtado-Fernández et al., 2010). It has become a worldwide trend to use natural additives in food and cosmetic products (Yanishlieva et al., 2006), Natural compounds have also become popular as researchers seek efficient and safe natural sources of antioxidants. There are many food stuffs and beverages from their antioxidant activity such as teas, herbs, spices, and coffee, in addition to fruits and vegetables (Devi et al., 2011). These plants may have the ability to delay oxidative deterioration of lipids and improve the quality of food (Kahkonem et al., 1999).

Using natural products as therapeutic materials to treat diseases caused by free radicals has been the focus of many studies over the years. The outcomes of these studies have led to an increased interest in the antioxidant activities of these compounds, particularly those of herbaceous plant origin (Mancuso, 2015).

Plant derived spices have been coveted around the world for many centuries not only for their flavor and aroma but also for their ability to enhance the shelf life of foods and for various medical treatments (Elżbieta et al., 2008; Yanishlieva-Maslarova, 2001). Spice such as turmeric may provide health benefits and have been shown to counteract oxidative stress (Modak et al., 2007).

Turmeric is belonging of the ginger family, (Zingiberaceae) and spice that comes from the root of plants Curcuma longa. It is considered a root crop, an herbaceous. It is one of the most valuable and sacred spices that contains amounts of 69.4% carbohydrates, 5.1% lipids, 2.6% fiber, and 6.3% proteins (Kamal & Yousuf, 2012). Turmeric is rich in minerals such as calcium, iron, phosphorus, and vitamin A. It has been shown to be important not as a cosmetic and spice but also as a medicinal plant and a coloring material in the textile industry (Hossain & Ishimine, 2005). Curcuminoids are the components of turmeric which include mainly curcumin, desmethoxycurcumin, and bisdemethoxycurcumin (Chainani-Wu, 2003).

Curcumin is the biological activity of turmeric, soluble in ethanol and acetone but difficult in water(Joe et al., 2004a). Curcumin 95% is the most bioactive and soothing portion of turmeric and has many properties such as antioxidant, antiplatelet, and anti-inflammatory (Naz et al., 2010). In current years, a substantial number of empirical studies have been conducted regarding the beneficial effects of various phenolic

compounds in herbs and spices known to prevent or reduce several age-related diseases, such as cancer, Alzheimer's disease, and neurological diseases (Scapagnini et al., 2011). The potent health effects of curcumin, in addition to its applications in culinary and industrial purposes, have made it a focus of considerable scientific interest (Lee et al., 2013)

Certain spices have been recognized to have beneficial physiological and medicinal properties; among these are their uses as effective anti-carcinogenic and anti-inflammatory compounds as well as their ability to stimulate digestion (Hossain et al., 2011). Natural antioxidants are compounds which may prevent the oxidation of materials by scavenging free radicals and reducing the catalytic metals which reduce stress oxidation. In this study, turmeric rhizomes were used for the extraction of turmeric polar extract (TPE). This protein extract is considered a natural source of antioxidants and has medicinal and antimicrobial properties (Kulkarni et al., 2012).

A specific point of interest was focused on determining the effect of radiation on the structure of curcumin (CU), especially in enol because the enol form has a more stabilized structure than the keto form (Priyadarsini, 2014). These forms of the compound have a significant effect on the antioxidant capabilities of the curcumin compound. Turmeric polar extract was obtained by using cold acetone solvent and a vacuum to get a slurry containing a mixture of proteins, fiber, sugars, and metals.

The methods of proximate analysis were used to estimate the total content of chemical compounds for TPE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to determine the molecular weight of turmeric polar extract. Total radical antioxidant potential by using ABAP to generate alkoxyl and peroxyl

radicals source and evaluate antioxidants used radical cation $ABTS^{\bullet+}$. The results of this study may contribute information about the free radical defenses of curcumin at different concentrations with exposure to UVA and UVB at various times 5, 10, 15, and 20 min with and without TPE.

1.1 Objectives

The overall objective was to extract and study the polar proteins in turmeric. The specific objectives were to:

- Extract polar proteins TPE from turmeric (C. longa) rhizome using dielectric extraction (cold acetone at -20˚C).
- Investigate antioxidative efficacy of TPE and purified curcumin (CU) using real-time chemiluminescence methods.
- Determine the effect of combining TPE and CU (as in typical turmeric powder) on antioxidative efficacy.
- Characterize the effect of ultraviolet radiation (UVA and UVB) on the antioxidative efficacy of CU and TPE and their combinations.

CHAPTER II

LITERATURE REVIEW

2.1 Antioxidant

The antioxidant is defined, as a material or substance that can delay, remove, or inhibit oxidation (Wang et al., 2000). Antioxidants can deactivate or reduces free radical, non-free radical, reactive oxygen species (ROS), and reactive nitrogen species (RNS) before they attack cells which can lead to stabilization of biological targets (Atoui et al., 2005). Antioxidants can delay the progression of many diseases. So, there is a need to determine safe sources of antioxidants as a natural alternative, especially from plant origin (Gülçin et al., 2012). Many studies have been conducted in various fields, including pharmacology and food processing that shows the importance of antioxidants in protecting tissues or organisms (Magalhaes et al., 2009).

Antioxidants have become an important topic of growing interest in the field of application to different medical treatments. An antioxidant is the most common active substance of nutrition that plays an active role in protecting the body against cellular damage. The body's defense system of antioxidants prevents free radical damage and can also prevent oxidation or delay the oxidative process (Hurtado-Fernández et al., 2010).

2.1.1 Synthetic antioxidant

There is synthetic antioxidant that have been used in food industry as food additives to increase shelf life as well as pharmaceutical products supplements such as,

butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). Those are the most common synthetic antioxidant in addition to natural antioxidants. Furthermore, BHA and BHT are synthetic phenolic compounds that have been used since the 1950s and are widely applied synthetic antioxidant (Botterweck et al., 2000)

Currently, there are many studies try to reduce the use of synthetic antioxidants in the food industry as a food additive and reduce intake amounts on the human body because of toxic and carcinogenic and /or mutagenic effects, many countries have identified or even prohibited the use these additives in food processing (Capitani et al., 2013). Some studies suggest that synthetic antioxidants may stimulate the growth of cancer cells in the bodies of mice (Rahman et al., 2014). Also, they may cause genotoxicity at high concentrations (Gutteridge & Halliwell, 2010; Shebis et al., 2013). Their potential toxicity and health risks (Dudonné et al., 2009). Also have effects on enzyme systems (Inatani et al., 1983). Although, synthetic antioxidants have been used to maintain the quality of food products for eating and has become common (such as butylated hydroxytoluene BHT and butylated hydroxyanisole BHA) for a long time. However, the consumer concern about their safety has stimulated the food industry and researchers to find and seek natural alternatives. These natural alternatives may be used for many purposes, not only for food preservation but also for therapeutic, cosmetics and medical purposes. Thus, many studies make use of natural or alternative antioxidants sources (Inatani et al., 1983). Moreover, the shelf-life of food and food preservation via antioxidants may begin to derive primarily from many natural sources (Devi et al., 2011).

2.1.2 Natural source of antioxidant compounds

Free radicals are produced extensively and naturally in all cells of the body. There are many types of natural defenses, like natural antioxidants, that can prevent the formation of free radicals (Knight, 1998). A brief summary of the types of natural antioxidants are listed below:

A) The antioxidant enzymes such as; catalase, superoxide dismutase, and glutathione peroxides work together and can be synthesized (in vivo) to protect the human cells from toxic ROS. Therefore, these enzymatic antioxidants can defend the human body from ROS accumulation, which is the cause of oxidation and body damage (Matés et al., 1999).

B) Non-enzymatic antioxidants are either lipid soluble (vitamin E and carotenoids) or water-soluble (vitamin C and phenolic compounds) (Podsędek, 2007). Some non-enzymatic antioxidants (e.g., b-carotene or a-tocopherol) can be obtained by ingesting from the diet (Sies, 1986).

Nevertheless, internal anti-oxidation systems are not enough. Over the decades, many of researches have been carried out to understand further the different kinds of antioxidants which are present in the diet and various natural sources to reduce the free radical concentrations and keep it at a low level (Pietta, 2000).

The naturally occurring antioxidants are found more in edible plants, especially spices and herbs. Natural antioxidants can help to protect the human body from chronic diseases including cancer, cardiovascular, and aging diseases. The antioxidant properties of plant extracts have been recognized to contain polyphenol (Göktürk Baydar et al., 2007). However, herbs and spices are a primary source of phenolic compounds such as

flavonoids, phenolic acid, and tocopherols (Maizura et al., 2010). On the other hand, some of the spices and herbs such as (oregano, rosemary, and thyme) have limited application, although they have antioxidant activity, as they impart a special herbal flavor to the food (Moure et al., 2001). However, many experiments have shown that these products may have a toxic or carcinogenic effect on humans (Göktürk Baydar et al., 2007).

2.2 Free radical and oxidative stress

2.2.1 Definition of oxidative stress

Oxygen is the final electron acceptor in the system of electron flow which produces energy in the form ATP. Oxidation was defined as transferring the electrons from one atom to another which is an essential part of our metabolism and aerobic life (Burton & Jauniaux, 2011; Pietta, 2000). Organisms produce energy from food, and this causes loss of electrons through a process called "oxidative stress". Thus, Oxidative stress is a fundamental and critical reaction in the human body that can damage many biological particles such as proteins and DNA. It has been shown in previous studies (Gülçin et al., 2010) that an oxidative stress has defined as the imbalance between the antioxidant defense and the production of ROS which leads to a disorder of antioxidant reactions and pro-oxidant reaction.

However, (Rodríguez-Mañas et al., 2009) study that oxidative stress is either a growing formation of ROS and/or a weakly protected system of cellular antioxidant with later consumption of antioxidants. Excessive production of ROS prevents the natural functions of cellular proteins, lipids, and DNA. On the other hand, it has also been suggested that oxidative stress unbalancing produce the ROS/NOS and antioxidant

defense (López-Alarcón & Denicola, 2013). Because food tissues are efficient and living, they are exposed to oxidative stress from free radicals, ROS, and pro-oxidants which were created both internally (H_2O and transition metals) and externally (heat and light) (Brewer, 2011; López-Alarcón & Denicola, 2013).

On the other hand, (2014) a study by Ristow, defines oxidative stress as an excessive burden of reactive oxygen species ROS, which causes permanent damage at the cellular level (Ristow, 2014). For example, the body oxidizes food to get energy and produces oxygen with products that are harmful in oxidative stress, called free radicals. These free radicals can divide cell molecules and cause destruction, making the human body susceptible to numerous inflammatory diseases, viruses, and cancers (Magder, 2006). Oxygen major role for all aerobic organisms is labor as fertile land for electrons. If electrons cannot flow through the chain; the proton driving force has to go to waste and cannot continue to produce ATP. However, the mitochondria are considered the main place for the production of energy in the cells (Cadenas & Davies, 2000).

2.2.2 Definition of free radicals

Free radical has been defined as is as any "species able to independent existence which contains one or two unpaired electrons, an unpaired electron being one that is alone in an orbital". Moreover, they are very unstable and react quickly with other compounds to gain stability (Halliwell, 1993). In 1900, Gomberg of the University of Michigan was the first person to write a description of free radical as atoms, or ions with unpaired electrons in their outer shell making them interactive significantly in biological systems (Chen et al., 2012).

Free radical promote oxidative damage to biomolecules such as proteins, carbohydrates, and nucleic acids these may cause many diseases and can damage proteins resulting is a loss of activity by the enzyme (Devasagayam et al., 2004). Therefore, free radicals are the main cause of many diseases in the human body including cancer's diseases (Kinnula & Crapo, 2004), Alzheimer's diseases (AD)(Smith et al., 2000) and aging (Hyun et al., 2006).

Furthermore, environmental factors such as pollution, radiation, cigarette smoke, drugs, pesticides, and herbicide can also produce free radical which produce similar toxic effects on human health (Bagchi et al., 2000). In fact, some of these elements are considered essential to life (especially oxygen). These reactive species have adverse effects on the human body (Lobo et al., 2010). A decrease in ROS was achieved by using protective agents contain natural antioxidants; such as fruit, roots, and leaves. Although, the effectiveness of the antioxidant endogenous system is not enough. Therefore, humans need different types of antioxidant to exist in the diet. This source can help to maintain the free radical concentration in low level (Pietta, 2000).

2.2.3 Mechanisms of free radicals

To avoid diseases and improve the quality of life, an understanding of the interactions that occur in the human body that reduce or prevent many diseases from occurring is required. Currently, there are three own areas which are supposed to help in delaying the aging process; free radicals, antioxidant, and co-factors (Bagchi et al., 2000; Rahman, 2007).

Free radicals are ions or molecules with unpaired electrons which are highly active towards chemical interactions with other molecules. Free radicals are derived from

three elements: first the oxygen, second the nitrogen, and finally the sulfur (Poljšak & Dahmane, 2012). Thereby, forming a ROS, RNS , and reactive sulfur species (RSS) respectively (Sen et al., 2010). ROS are constantly produced by using the body's oxygen. Such as breathing. There are three major types of reactive oxygen species (ROS) which are included free radicals such as the superoxide anion ($O_2^{\bullet}$) substantially, present in cells because of infusion from the respiratory chain in mitochondria; hydroperoxyl radical ($HOO^{\bullet}$), and peroxyl radical ($ROO^{\bullet}$) (Matés et al., 2010). Also, the non-free radicals are there such as hydrogen peroxide (H_2O_2), resulting directly from the action of oxidase enzymes, hypochlorous acid (HOCl), nitric oxide (NO), and Lipid hydroperoxide (LOOH).

These enzymes can lead to the deterioration of the food (Gülçin et al., 2012). The reactions of products of ROS are displayed in the (figure 2. 1) by (Carocho & Ferreira, 2013; Flora, 2009). RSS is the reaction of ROS with thiols. RNS are formed by reacting (NO) with ($O2^{-\cdot}$) and forming peroxynitrite ($ONOO^{-}$) (Lü et al., 2010). All these molecules increase the factors that affect the injury of cellular and aging (Gulcin et al., 2002).

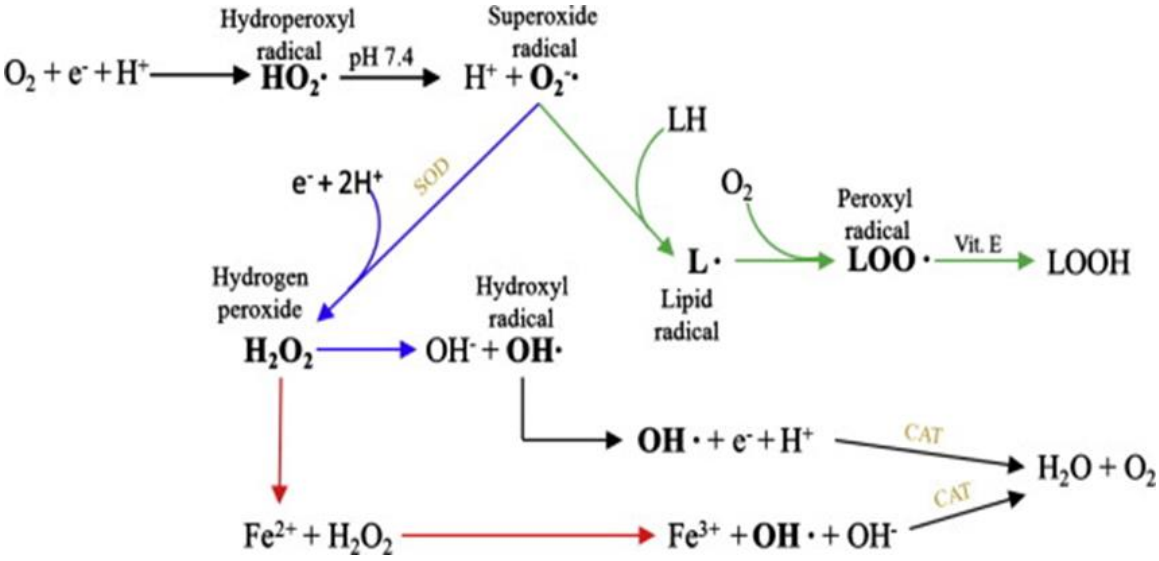


Figure 2.1 The reactions lead to the production of reactive oxygen species (ROS).

The green arrow represents lipid peroxidation; blue arrow represents the Haber–Weiss reaction and the red arrow represent the Fenton reactions. The big letters represent radicals or molecules with the same behavior. $WATER_2$, SOD and CAT are respective abbreviations for hydrogen peroxide, and the enzymes superoxide dismutase and catalase (Flora, 2009; Lü et al., 2010).

2.3 Ultraviolet radiation (UVA, UVB)

Ultraviolet radiation is defined as a portion of the electromagnetic waves between visible light and X-rays (Webber et al., 1997). There are three kinds of ultraviolet rays: UVC (200-290 nm), UVB (290-320 nm) and UVA (320-400 nm). The amount of ultraviolet rays which reaches to the ground up to about 6% approximately of the sunlight (Garcia-Bores & Avila, 2008). Recently, human activities have increased and emission of chlorofluorocarbon compounds (CFCs) have led to the destruction of the ozone layer. This has led to an increase in the amount of solar radiation that reaches the Earth.

UVC is not of biological importance for human beings because ozone filters it. UVB radiation can cause redness of the skin and cause sunburn. The UVA and UVB, we can see the wavelength of UVA is longer than UVB which can break through deeper into

the skin (Kullavanijaya & Lim, 2005). However, UVA has negative effects like skin cancer and ocular damage (Young 2003), but UVA-ray is useful because it increases of production the vitamin D3 (Azevedo et al., 1999). Mishra and others, have reported in 2011 that UV radiations produce compounds that are ROS or free radicals that are harmful compounds and caused aging and skin cancer (Mishra et al., 2011). It has been indicated in previous studies (Zhang & Björn, 2009) that the radiation of wavelength 280–315 nm UVB, considered one important factor to the production of secondary metabolites by using plants of useful as medicines.

It has been shown in previous studies (Hollósy, 2002) that UV cause the destruction of amino acid and disable activity of enzymes and proteins. (Takahashi et al., 2010) that the environmental stress factors play the primary role in the influence on the nutritional value and chemical composition in plants such as UV considered one of this factors, which has the wavelengths (280–400 nm), are more efficient photo inhibitors than the photosynthetically active radiation which has wavelengths from (400-700 nm).

2.4 An overview of Turmeric

2.4.1 History of turmeric

Turmeric of the wild turmeric has been classified as Curcuma aromatic, and the cultivated domestic species is known as C. longa (Chattopadhyay et al., 2004). It is the rhizomatous herbaceous annual plant belonging to the Zingiberaceae family. Typically, it is grown in tropical South Asia and to a lesser extent in Africa. But today, it is also cultivated in subtropical regions and around the world. India is the largest country that produces turmeric, which is used as a remedy in the home for several diseases

(Priyadarsini, 2014). Iran is considered the main importer of turmeric in the Middle East as well as the main consumer of turmeric (Asghari et al., 2009).

In Bangladesh, turmeric production annually is about 41.50 thousand tons, and it is cultivated in about 16.06 thousand hectares of land (Kamal & Yousuf, 2012). The profound orange-yellow powder known as turmeric is prepared from boiled and dried rhizomes of the plant. The yellow pigment is due to curcuminoids (diferuloylmethane) (3–4%) that comprised of curcumin I also knew simply as curcumin (main curcuminoids) (94%), curcumin II knew simply as demethoxycucumin (6%) and curcumin III or bisdemethoxycurcumin (0.3%) (Ruby et al., 1995). The protein content of turmeric can vary. In semitropical Okinawa island of Japan, turmeric was cultivated in pots with dark red soil, gray soil, and red soil and the protein content of powder rhizome grow in the dark red soil was 5.2% higher than that of other soil types (Hossain & Ishimine, 2005).

The powdered rhizome has usually been used as a spice for culinary as well as medicinal purposes in traditional medicine for conditions including inflammation due to traumatic injuries such as sprains and swelling. Also, it has been used as a stimulant, aspirant, carminative, cordial, emmenagogue, astringent, detergent, and diuretic, preservative and coloring agent (Subash C. Gupta et al., 2013)

There are many studies that show the effect of turmeric in protecting protect the body from oxidative stress. (Lebda, 2014) reported that turmeric decreased iron overload caused by oxidative stress and had the protective effect on liver and kidney function in rats (Nada et al., 2012) showed that the aqueous extract of turmeric was better for protection from radiation due to its non-toxic properties if taken at a high dosage. Moreover, it can be used in aqueous preparations.

In this study, crude turmeric extract containing the polar components rich in proteins and peptides was prepared by organic solvent induced precipitation using solvents with a low dielectric constant (Smallwood, 2012) diethyl ether (ε=4.3) and cold acetone (ε=20.7) (-20˚C) (details given in methods section).

2.4.2 Antioxidant preparation of Turmeric

Turmeric plays a role as a free radical scavenger. In the study done by Qader and others in (2011), it has been shown that ethanol extracts of turmeric had high antioxidant activity more than aqueous extracts (Qader et al., 2011). Several studies have been conducted f on antioxidative compounds and it has been found that these compounds do not carry any harmful side effects when used in food industry and preventive medicine. Therefore, they have been effective as drugs in the growing interest in the useful effects of some phenolic and peptide materials such as those used in a spice or seasoning and herbs in the prevention age-related morbidities such as cancer, aging, and neurodegenerative disease.

However, these effects remain to be clarified (Scapagnini et al., 2011). (Ramadas & Srinivas, 2011) reported that (BGS- Haridra), a glycoprotein, is one of the proteins that is isolated and filtered from turmeric extract after boiling in water. Its molecular weight is (~28 KDa). This study showed that protein with antioxidative properties prevents reactive oxygen species (ROS) induced lipid peroxidation.

The homogeneity of β-Turmeric was assured by its movement as a single band both in SDS-PAGE as well as in native PAGE. Also, the antioxidant properties of B-turmeric were studied and comparison with standard antioxidants such as BHA and a-tocopherol. Ningappa and others in (2008) have shown that the antioxidant protein of

curry leaves powder had scavenged about 85% hydroxyl and DPPH radicals (Ningappa & Srinivas, 2008).

2.4.3 Hydrophobic protein in Turmeric

In the last century, there has been considerable attention to the study of protein and the extent of its importance in the living cell configuration. There are three major classes of protein: Hydrophobic proteins, which have a low propensity to be in contact with water and have the inability of water to form hydrogen bonds with certain side chains, hydrophilic proteins, which are water-friendly; and charged residues separately. Most protein molecules have a hydrophobic basis. A protein molecule is a polymer of amino acids which is joined by peptide bonds (Aftabuddin & Kundu, 2007). There are 20 types of amino acids that occur in nature, and each of them has specific features, of the side chain, which give it its unique role in a protein structure. Amino acids such as alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, cysteine, and methionine, are considered hydrophobicity. The basic driving force that causes the process of the folding in protein was reduced the number of hydrophobic side chains of exposed to water (Giovambattista et al., 2008; Rose et al., 2006).

The studies of electrophoresis of hydrophobic proteins have made considerable developments in different fields to overcoming the difficulty of assembling hydrophobic proteins (Lin et al., 1999). There are a variety of proteinaceous inhibitors in plants which work to protect itself from enzymes. According to a study by (Lekshmi et al., 2012), turmeric is one of these plants which have an antioxidant capacity against glucosidase.

(Chethankumar, 2010) has reported a study that turmeric has a molecular weight of ~34 KDa, has the ability to protect living organs from snake poison that caused

oxidative damage. On the other hand, curcumin is an active compound and a hydrophobic polyphenol derived from the rhizome of the herb turmeric (Curcuma longa). Turmerin, which has ~5 KDa peptide, water soluble, was found to be protectant the DNA and an efficient antioxidant due to it contains three residues of methionine that are responsible for the antioxidant activity. Turmerin is the noncyclic peptide which containing 40 amino acid residues. This protein "Turmerin" was non-cytotoxic up to milligram concentrations in human lymphocytes. This protein is insensitive to UV radiation at 345 nm, and pepsin (Shalini & Srinivas, 1987; Srinivas et al., 1992).

It has been shown in previous studies (Cohly et al., 1998) that turmeric and its derivatives, a water soluble extract and lipid soluble (curcumin) which has antioxidant properties that provide protection against oxidative stress in a renal cell line. Furthermore, other studies have indicated (Cohly et al., 2003) that the same compounds have potent effectiveness as an anti- HIV. Furthermore, it has been shown in previous studies in experimental animal (Chethankumar, 2010) that turmerin has a statistically significant inhibition of NV-PLA2 enzyme activity. It is about a 34% increase in the percent inhibition of the enzyme activity when compared to the aqueous crude extract.

According to (Smitha et al., 2009) showed that obtained a β- turmerin from turmeric (Curcuma longa) waste grits and the molecular mass is ~34KDa by SDS-PAGE. β- turmerin showed effective in preventing lipid peroxidation at low concentration. Another study by (Dinesha & Srinivas, 2010) showed that turmeric protein which has named as BGS-haridrin a ~28 KDa glycol protein isolated from boiling water extract of turmeric (Angel et al., 2013), used eight curcumin specie had molecular weight had 66 KDa which solved by SDS-PAGE as 12 and 14 KDa proteins and these proteins showed

effectiveness as antioxidant activity. These proteins 12 KDa and 14 KDa were seen in SDS-PAGE and found in all these curcuma species. (Srinivas et al., 1992) studied a water-soluble 5 KDa peptide called turmerin from turmeric (curcumin longa) which isolated from aqueous turmeric extract was decolorized and fractionation or a sephadex G-10 Column.

2.4.4 Chemical composition of Turmeric

Turmeric is a rich rhizome of phenolic compounds and curcuminoids which contain protein 6.3%, fat 5.1%, mineral 3.5%, carbohydrates 69.4%, and moisture 13.1% (Table 2.1) (Kamal & Yousuf, 2012).The essential oil (5.8%) obtained by steam distillation of rhizomes has a-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberene (25%) and Sesquiterpenes (53%). Curcumin (diferuloylmethane) (3–4%) is responsible for the yellow color and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%). Demethoxy and bisdimethoxy derivatives of curcumin. Also were isolated. Curcumin chemical structure was determined by Roughly and Whiting 1973 (Bhat et al., 2015; Chattopadhyay et al., 2004). It forms a reddish-brown salt in contact with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform (Kumar et al., 2011). The melting point of curcumin, C2H20O6, is 184 ºC. Curcumin compound is soluble in ethanol and acetone, but insoluble in water (Joe et al., 2004b).

2.4.5 Curcuminoids; phenolic compounds

Phenolic compounds phenolic compounds constitute a group of substances that are abundant in the plant kingdom, where more than 8000 are known, with different

chemical structures and activities. Flavonoids, especially flavones, flavanones, anthocyanins, and isoflavones, are constituents of fruits, vegetables, nuts, and plants such as tea, herbals, and Eastern traditional medicine (Hsu & Yen, 2008). The phenolic compounds are naturally present in the plant materials, and they are an output of the plant metabolites (Gulcin, 2006).

The polyphenols of turmeric were named (curcuminoids), which include: Curcumin diferuloylmethane, demethoxycurcumin, and bisdemethoxycurcumin. Both of demethoxycurcumin and bisdemethoxycurcumin have antioxidant properties while curcumin has antimicrobial properties and antioxidant properties. Curcuminoids are defined as the most bioactive and it has been diagnosed as a group of bis-α, β-unsaturated β-diketone polyphenols; namely curcumin (72%), desmethoxycurcumin (DMC) (12%), and bisdemethoxycurcumin (BDMC) (3%) (Krishnakumar et al., 2015).

Table 2.1 Chemical composition of turmeric

Parameter	Percentage
carbohydrates	69.4
Moisture	13.1
Protein	6.3
Fat	5.1
Minerals	3.5

Source: (Bhat et al., 2015)

2.4.6 Curcumin: Derivative, structure, and stability

Curcumin was the original name for saffron which is Latin name derived from the Arabic word, “Kourkouma” (Scartezzini & Speroni, 2000). It is a phytochemical compound found in turmeric and is responsible for the yellow color of turmeric. It has the ability to color, so it used as a preservative in food and textile industries (Cohly et al., 2003).

Several studies and investigations have shown that curcumin has the characteristics of a safe, and useful pharmacological agent. It is a nutritionally acceptable component, and is also therapeutic to prevent or/and treat a variety of inflammatory diseases such as cancer, Alzheimer’s disease (AD, and inflammatory (Bansal et al., 2011; Begum et al., 2008; Shehzad et al., 2010).

Curcumin is a principal bioactive component that is extracted from turmeric (Curcuma longa). Chemically, it has been known as (diferuloylmethane) ($C_{21}H_{20}O_6$) (3-4%) which is of the compounds of the curcuminoid family. It is responsible for the yellow pigment and has a polyphenol structure, is used as a common coloring, and flavoring agent in food. It may also have chemotherapeutic properties, and has been studied for antioxidant, anti-inflammatory, anti-toxin, and cancer chemopreventive properties as well (Kim & Lee, 2010; Rahman et al., 2006)

The first isolation of curcumin was by Vogel and Pelletier in 1815, and the chemical structure was determined by J. Milobedzka and V. Lampe in 1910. However, it can be isolated as a diferuloylmethane, consisted of two aryl buten-2-one (feruloyl) chromophores joined by a methylene group and identified in 1913 (Priyadarsini, 2009). In 1972, it was documented that curcumin has the ability to decrease blood sugar levels in

human (Aggarwal & Sung, 2009). In a previous study (Aditya et al., 2013) has shown that curcumin gets easily decayed in high temperatures, in alkaline pH (>7), and light. Furthermore, it is a hydrophobic component. It can be removed or eliminated quickly from the body, but with little absorption in the gastrointestinal tract. So this bioactive component cannot be inserted into food products because of it natural instability.

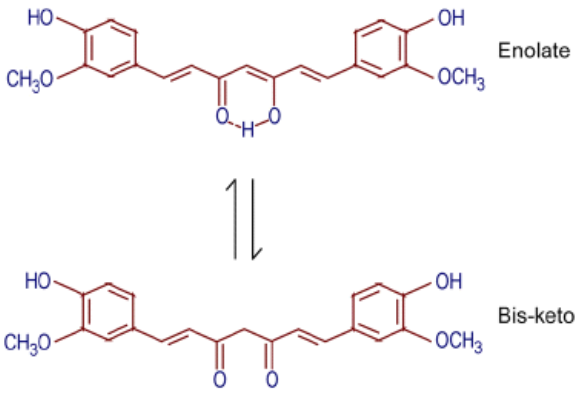
Another study (Aditya et al., 2015) has shown a synergistic action increase in the biological activity of curcumin when used in conjunction with some compounds. Curcumin and catechin enriched food products can be used to maintain general health and can be used as a nutraceutical to treat diseases.

In this study by (Aditya et al., 2013) has indicated that curcumin and genistein in their formations have a therapeutic effect against prostate cancer by increasing the bio-accessibility of these nutraceuticals during digestion in the small intestine. So both of these studies have shown the effect of curcumin, in conjunction with other compounds, have a better result. Curcumin antioxidant properties can be possible because of its unique structure that includes an enol form of a β-diketone and two methoxylated phenols (Rahman et al., 2006).

Curcumin, one of the active ingredients in turmerin which can exist in at least two tautomeric forms, Keto and Enol. Curcumin is the active ingredient of turmeric rhizome. The tautomeric of curcumin was demonstrated under different physiological conditions (Sa & Das, 2008). As well as under acidic and neutral conditions, the Bis-keto form (bottom) is mostly prevailing than the Enolate form (top) (Figure 2.2). Commercial curcumin contains three main types of curcuminoids shown in (Figure 2.3), which are the dietary phytochemicals of turmeric plant such as curcumin (diferuloylmethane) or

curcumin one about 77%), (desmethoxycurcumin) or curcumin II ~ 17%) and (bisdemethoxycurcumin) or curcumin III ~ 3%) (Chattopadhyay et al., 2004; Kulkarni et al., 2012) and contains about 2-5% curcumin alone.

Turmeric Plant with Rhizome



Curcumin

Figure 2.2 Curcuma Longa plant and chemical structure of curcumin (Sa & Das, 2008).

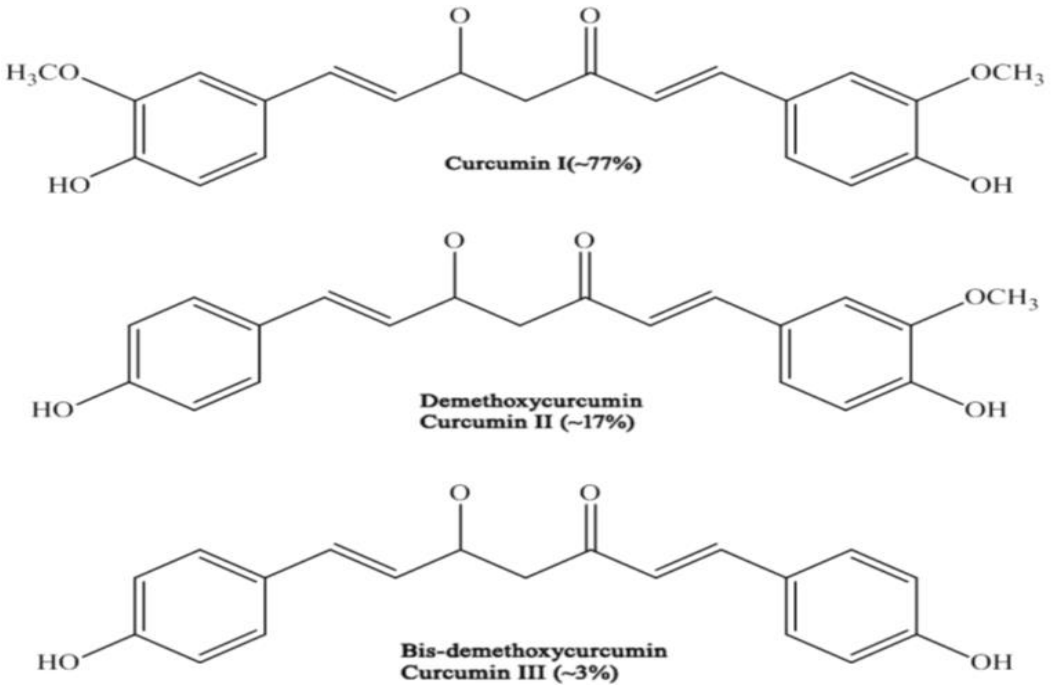


Figure 2.3 Chemical structure of curcumin, demethoxycurcumin and bisdemethoxycurcumin

(Chattopadhyay et al., 2004).

2.5 Medicals properties

Medical plants are considered the most important sources of new therapeutic substances. It is still in the process of chemical and biological research. Today, increasing knowledge on the cell cycle editing in cancer has reinforced the introduction of phytochemicals, which can directly alter cell cycle regulatory molecules (Pârvu & Pârvu, 2011).

Several plants are still not evaluated biologically or chemically, but these plants may be an excellent source of new therapeutic factors. Although, chemicals compounds have used to produce drugs, recent research has found some chemicals don't have much effectiveness as an antimicrobial. Since then, studies have shown that plant extracts have been effective against fungi and microbial diseases that can harm plants. Thus, it provides

high efficacy, low cost, and safety for all organisms (Abubakar, 2009; Carrillo-Muñoz et al., 2006).

2.5.1 Cancer and Turmeric

According to some reports, curcumin has shown an anticancer effect on numerous cancer cell types (Abe et al., 1999). Moreover, clinical trials have proved that curcumin took orally (12 g/day) was safe for human consumption and conferred chemopreventive benefits in patients with high-risk and scars cancer (Dhillon et al., 2008).There are many studies widely addressing the chemical treatment possibility of curcumin. It is considered non-toxic plant derived polyphenol and has shown significant promise against cancer and other inflammatory diseases (Sa & Das, 2008). It also suppresses invasion, angiogenesis, and metastasis of cancer cells (Lin et al., 2009) as well as the effect of curcumin on cell growth and telomerase activity in human cancer cell lines. It has been shown in previous studies that preserving telomere's protected cells from apoptosis and the inhibition of telomerase (Cui et al., 2006). Telomerase is an enzyme participating in telomeric DNA elongation, elicits an apoptotic response in cancer cells (Chiodi & Mondello, 2012).

Curcumin, the active substance in the spice turmeric, it has therapeutic potential for cancer prevention. Many of evidence have shown that curcumin can inhibit the forming of tumors in animal models. A study that was done in Taiwan referred to one of the leading causes of cancer-related death is lung cancer, and about 32.8 persons per 100 thousand died annually lung. So, curcumin has anti-metastatic potential by decreasing invasiveness of cancer cells (Abe et al., 1999; Lin et al., 2009).

2.5.2 Traditional medicine and Turmeric

It is interesting; curcumin was used for thousands of years as a folk medicine to treat diverse diseases in China and India long before the recent intensive studies revealed various biological activities of curcumin. Ayurveda, the oldest medical system in the world, which provides potential to find active and useful compounds for therapy by plants. (Ali et al., 2008). Ayurveda (Indian traditional medicine) and Chinese medicine has shown turmeric powder use for therapy of anorexia, inflammation, biliary disorders, and hepatic disorders (Shishodia et al., 2005), and also skin eye infections, stomach upset, and acnes. Also, used topically for the prevention of skin diseases and internally to purify blood and stimulate the stomach and as a disinfectant (Mahajan, 2011).

2.5.3 Alzheimer's disease and Turmeric

Alzheimer's disease is the common form of dementia and progressive neurodegenerative disease that is related to age which weakens memory, speech loss, and personality changes as well as cognitive functions (Essa et al., 2012; Mancuso et al., 2012). Oxidative stress is the main key of the AD. One of the reasons for oxidative stress is mitochondrial function and inflammatory effect has a major role in the generation and aggregation free radical ROS (Butterfield et al., 2007; Dumont & Beal, 2011).

In addition, there is 40-42 amino acid peptide found in human brain and plays a significant role in the development of AD. The amyloid precursor protein exists everywhere in the membrane protein (Butterfield et al., 2007; Pithadia & Lim, 2012).

The studies discovered that the therapeutic potential of herbs using antioxidant and flavonoid-rich natural products contributes to the preventative agents of AD as well as fruits, vegetables and nuts are of paramount importance. Curcumin has shown various

health beneficial effects. One of these benefits of curcumin its reported efficacy against AD. Alina and others in 2012 revealed that curcumin is insoluble in water and its hydrophobic nature (Anand et al., 2007). However, it can be overcome this problem by the synthesis and created of curcumin nanoparticles laminated with PLGA (Poly lactic-co-glycolic acid) coating; it was easy to use as a drug in treating AD that has convergence to the neurons and destroys amyloid aggregates (Mathew et al., 2012).

2.6 Laboratory evaluation methods

2.6.1 Proximate analysis

There are different important analyses performed on food products such as protein, fat, and ash. There are several methods used to determine proximate analysis recommended by AOAC 2002. Moisture content is considered one of the important analytical procedures that can be implemented on various food such as plants and herbs. Water may occur in food in three forms: free water, absorbed water, and water of hydration. Depending on that, there are three categories of moisture; high, medium, and low moisture in food. So, the method that is used to measure the moisture may show low or high in moisture present (Nielsen, 2010).

A study by Marshall in (2010), has shown that ash is the inorganic residue remaining after either complete oxidation of organic matter or ignition. There are two main types of ash; dry ash and wet ash. Wet ash is used to prepare samples for certain types of a proximate analysis of minerals (Nielsen, 2010). Protein is as also known as having great importance in the cell structure and function. In addition, it is one of the most elements and compounds abundant in cells. There are many techniques used to measure the protein content in various types of food, but the basic principle of these

techniques depends on the presence of nitrogen, peptide bonds, and amino acids in proteins. Kjeldahl method is the best ways to estimate the nitrogen in the food and determine the amount of protein present in food. If proteins contain the high amino acids ratio that it means contain more nitrogen (López et al., 2010).

Turmeric (Curcuma longa) considers one of the important plant food which has therapeutic potential against diseases that is because to the bioactive components. (Nisar et al., 2015)was showed that proximate composition of turmeric contained moisture 13.02%, crude protein 6.47%, crude fat 5.33%, crude fiber 4.80%, and ash 3.49%. It has been shown in previous studies (Ikpeama et al., 2014) that the turmeric contains 9.40 % crude protein and 67.38 % carbohydrate which make it a good source of protein and carbohydrate. Whereas, (Lim et al., 2011) indicated that turmeric powder contains high levels of minerals (K, P and Ca).

2.7 Antioxidant measurement methods

There are many different methods used to measure the total antioxidant activity of a compound (Magalhães et al., 2008; Thaipong et al., 2006). For measuring free radical scavenging ability. The method can be divided according to the mechanisms of interaction and the basic kinetic models of preventing autoxidation of antioxidant capacity assays into two main groups: HAT hydrogen atom transfer (HAT) and single electron transfer (SET). The HAT reaction methods that the antioxidant is able to quench free radical through donating of hydrogen. SET methods depend on the transfer of the electron to reduce any substance such as metal ions, carbonyls, and radicals to quench and detect the ability of antioxidant (Huang et al., 2005; Prior et al., 2005). Both types of reactions can occur at the same time. These methods are considered common because of

their sensitivity and ease. The methods are dependent on chromogen compounds radical nature such as (2, 2′-azinobis (3 ethyl benzothiazoline 6-sulfonate) ABTS and (2, 2-diphenyl-1-picrylhydrazyl) (DPPH), as well as oxygen radical absorbance capacity (ORAC), total radical absorption potentials TRAP assay (Dudonné et al., 2009; Sonkawade & Naik, 2015).

2.7.1 Total Radical Antioxidant Potential (TRAP) assay

Total radical trapping antioxidant parameter was based on the protection offered by the antioxidants on the fluorescence decay through controls of the peroxidation reaction. In the TRAP assay, the azo compound 2, 2'-Azo-bis (2- amidino propane) (ABAP) was used a radical source; evaluation of the antioxidative potential was done through decoloration by the measured decay of fluorescence. TRAP values are also calculated from the length of the lag phase of the sample compared with standard (Alam et al., 2013). The TRAP assay was initially used to measure the antioxidant status of human plasma which depends on the ability of plasma to trap a flow of peroxyl radical at a steady rate. The peroxyl radical was created at a controlled rate by the thermal decomposition of $AAPH^{\bullet}$ or $ABAP^{\bullet}$ in aqueous buffer at 37°C (Sánchez-Moreno, 2002). The TRAP assay uses R-phycoerythrin (R-PE) as a fluorescent probe, the intensity of fluorescence of R-PE decreases with time under the flux of the peroxyl radicals. In the presence of the tested sample containing antioxidants, the decay of R-PE fluorescence was retarded, and the reaction progress was controlled fluorometrically (Sánchez-Moreno, 2002). So, TRAP assays can be applied to hydrophilic chain breaking antioxidant activity against peroxyl radicals.

According to a study by (Wayner et al., 1985), TRAP is some moles of ROO•, which was stuck in a liter of biological fluid, and furthermore, is the most used method for measuring antioxidant capacity in fluids or human plasma. In a previous study of (López-Alarcón & Denicola, 2013), have shown that the TRAP is dependent on the absorption of oxygen in the peroxidation process of human plasma. Luminol (o- amino phthaloyl hydrazide) or pyranine (8-hydroxy-1, 3,6-pyrene tri-sulfonic acid) is used as probes to determine the TRAP index of the natural product. Furthermore, a study by Lissi and other in (1995), shown that the evaluation of the additives capacity to reduce the concentration of free radicals may be done by use of luminol chemiluminescence (Lissi et al., 1995). The mechanism of luminol chemiluminescence has been discussed by (Lissi et al., 1992) study, which shows the hydroxyl radical generated in vitro by pyrolysis ABAP 2, 2'-Azo-bis (2-amidinopropane) and unquenched radicals detected by chemiluminescence of luminol to determine the TRAP (Lissi et al., 1992).

$$ABAP \rightarrow 2\ R^{\bullet}$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + LH_2 \rightarrow ROOH + LH^{\bullet}$$

In the last step of the reaction, the luminol derived radicals (LH) will come to balance with the concentration of radical anions (L^-). The capacity to "trap" luminol derived and ABAP radicals is the ability of a component to quench the luminol derived luminescence (Lissi et al., 1995).

2.7.2 2, 2'-azinobis (3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical assay

This test has used the composite 2, 2'-a zinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as a source of radical cations $ABTS^{\bullet+}$ by oxidation ABTS with potassium persulfate which leads to formation of the blue-green color in the reaction (figure 2.4) (Magalhaes et al., 2009; Prior et al., 2005). There are many studies which examine and study the kinetics of the reaction of $ABTS^{\bullet+}$ with antioxidant substances based on the ability of antioxidants to remove the color the (2, 2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation. This radical has an absorbancy maximum of 734 nm and has blue in solution. Comparing with a standard amount of Trolox (6-hydroxy-2, 5, 7, 8- tetramethylchroman-2-carboxylic acid), a water-soluble analog of vitamin E (Ozgen et al., 2006; Sekher Pannala et al., 2001).

Furthermore, it has been used in many modern studies related to a reveal of antioxidant properties of the plants (Srinivasan et al., 2007). This method of colored free radicals has been employed to determine antioxidant capacity. This method reported by (Miller et al., 1996) and modified by (Re et al., 1999). This test was useful for a variety of analytical purposes. however, the ABTS•+ radical is not generated naturally in the biological systems (Huang et al., 2005; Prior et al., 2005).

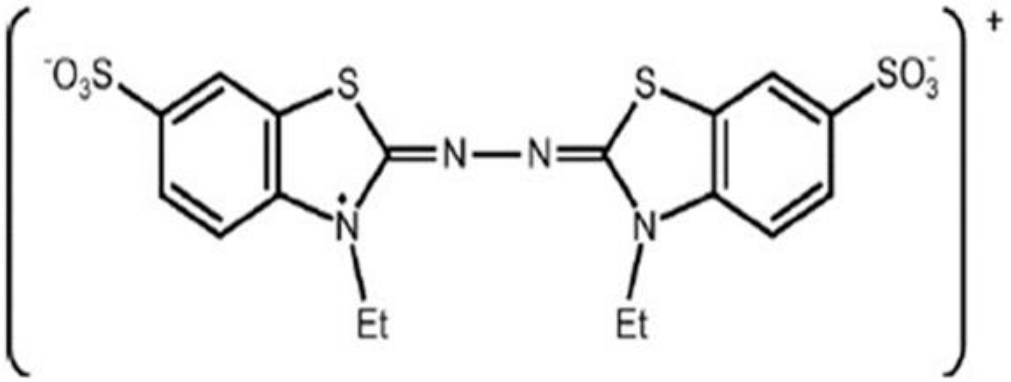


Figure 2.4 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$) radical cation

(Magalhães et al., 2008).

CHAPTER III

MATERIAL AND METHODS

3.1 Materials

Fresh turmeric (Curcuma longa) rhizomes were purchased (~7lbs) from eBay. It was not possible to obtain information about the history or storage of the turmeric root source. Once the rhizomes were delivered, they were stored in airtight Ziploc bags and stored at -20ºC until needed.

Diethyl ether $C_4H_{10}O$ (99.0%), acetone C_3H_6O (99.9%), phosphorous pentoxide (PP) O_5P_2 (99%) were purchased from Sigma- Aldrich (Milwaukee, WI, US.). Curcumin standard, Net weight 5g, part #: ASB-00003927-005, Lot #: 00003927-103 were purchased from ChromaDex company (Irvine, US.), The standard that was using as molecular weight markers were Precision Plus protein ^{TM} Dual Xtra standards 500 µL, cat. # 161-0377 got from (Bio-RAD, US.). Whatman 1 and 2 filter paper, peptide molecular weight standards, 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (ABAP), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and potassium persulfate (di-potassium peroxidisulfate) were obtained from (Sigma-Aldrich chemical Co., St. Louis, MO.). Trolox (6-hydroxy- 2, 5, 7, 8-tetramethylchroman-2-carboxylic acid was purchased from (Sigma-Aldrich, Milwaukee, WI, US..). Food grade absolute ethanol was purchased from Department of Chemistry (Mississippi State University, MS, U.S.). white, clear bottom 96 well plates (Costar®, Cole-Parmer, IL,

US.), nitrile exam gloves were purchased from (fisher scientific, Malaysia). Test samples were placed in 96 well plates placed within a Flex Station®3 Luminometer (Molecular Devices, LLC, CA, USA) for all data acquisition. Luminol ($C_8H7N_3O_2$) (Sigma-Aldrich Co., St. Louis, MO, US.

3.2 Methods

3.2.1 Preparation of turmeric polar extract (TPE)

The flowchart of the extraction process is shown in (figure 3.1). The turmeric rhizomes were cleaned with water to remove the soil and extraneous matter, dried with a clean cloth to removed excess water, cut into small pieces and pulverized using a coffee grinder. The pulverized paste 100g was placed in a beaker mixed with 300 ml cold -20ºC DE to produce a slurry with constant stirring using a magnetic stirrer for 30 minutes following removal of the DE by filtering through a Whatman 2 filter. The slurry thus collected on the Whatman paper was washed three times with 100 ml of DE and the organic extract containing a polar component was set aside and stored at -20ºC. The collected slurry was dispersed in 300 ml deionized water, chilled to 4˚C, gradually poured with constant whisking into 600 ml of cold acetone at -20˚C that was prepositioned in a salt/ice bath, to produce a precipitate that was left undisturbed for 45 min and collected on Whatman # 2 filter paper placed on a Buchner funnel connected to a side-arm flask by means of a neoprene adapter. The collected acetone powder was washed twice with cold -20˚C acetone, dried by aspiration by connecting a vacuum source to the air-tight side-arm flask, transferred to an air-tight desiccator, kept overnight over phosphorus pentoxide until dry, and stored in an airtight container at -20˚C until needed. According to the studies by (Devahastin & Niamnuy, 2010) showed that

nutritional changes and physical changes occur during the use of temperature for drying fresh turmeric roots. The total yield of TPE was 8.0055 g ~ 8% (w/w).

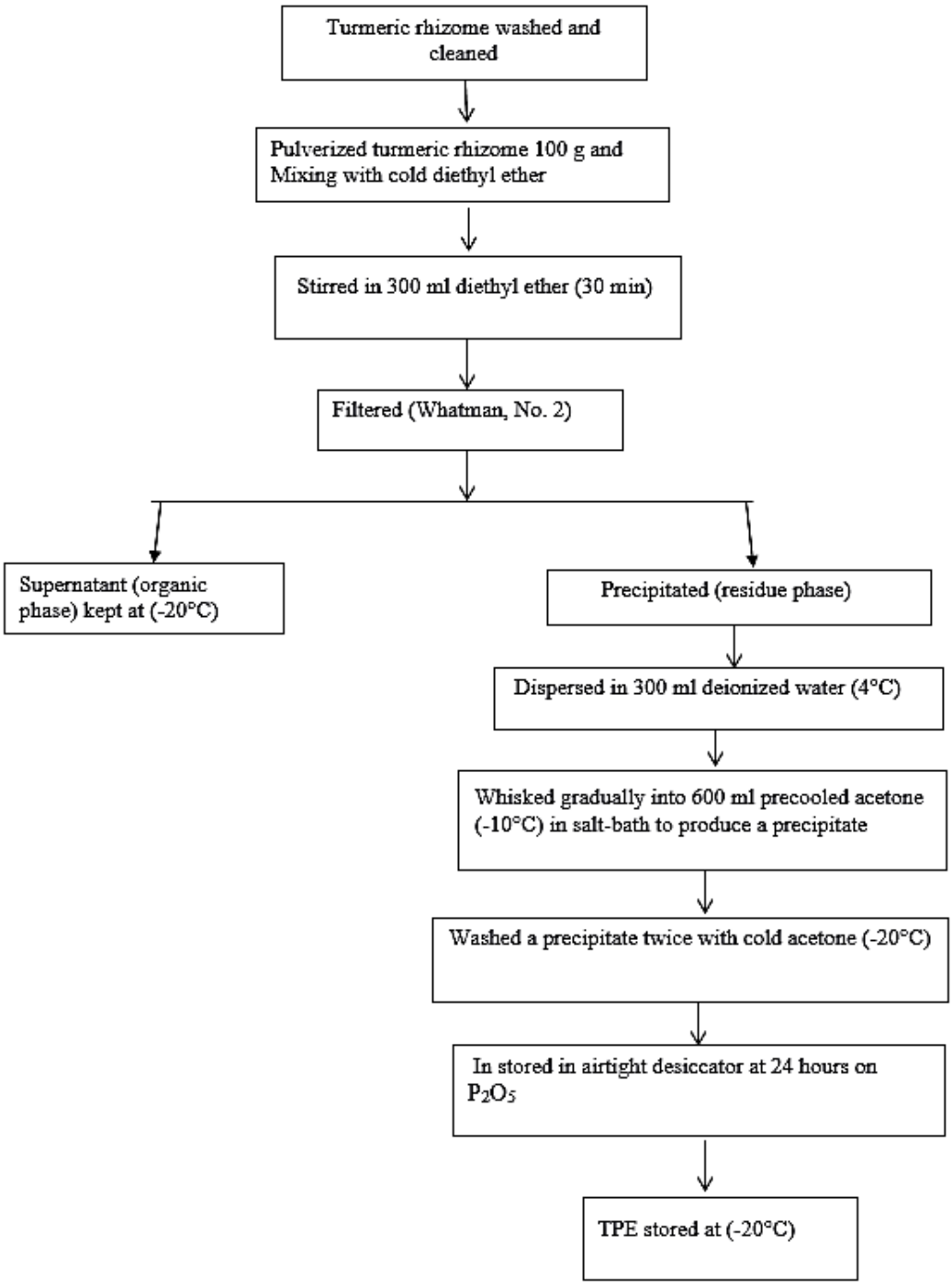


Figure 3.1 Extraction procedure for turmeric rhizome

3.2.2 Preparation of buffer and Samples

There are two main steps to prepare MacIlvaine's iso-ionic buffer (pH 7.0) was used in the experiments related to the assessment of antioxidative efficacy. The steps involved in buffer preparation were as follows:

Step -1-: Dissolved 53.614 g of sodium phosphate dibasic heptahydrate (HNa_2O_4 P).$7H_2O$ at MW: 268.07g/ mol (Sigma-Aldrich Co., St. Louis, MO) into 1000 mL distilled water in a beaker to get 0.2 M of HNa_2O_4 P $7H_2O$ dissolved 21.01 g citric acid monohydrate (citric acid) ($C_6H_8O_7$.) at MW:210.1 g/ mol (Sigma- Aldrich Co., St. Louis, MO) into 1000 mL distilled water in another beaker and thus get 0.1M citric acid.

Step-2-: For 1L (1000 ml) of buffer solution, mix 177 ml of above prepared citric acid solution with 823 ml of above prepared HNa_2O_4 P.7 H_2O.

Luminol $C_8H_7N_3O_2$ having an MW: 177.16 and at a concentration of 10 mM was prepared by adding 1.77 g of luminol to 100 mL of the Sodium Hydroxide 1N solution (1.005-0.995N) in a glass beaker. The prepared buffer and luminol were stored in the refrigerator at 4°C until used for each test. McIlvaine's buffer and luminol were prepared by following the method of preparation as described by (Haque et al., 2014; Mukherjee, 2015). This experiment was conducted to study the antioxidant activity of four concentrations of curcumin exposed to UVA and UVB radiation for different of time 5, 10, 15, 20 minutes, respectively, with or without the addition of TPE.

The first step was to prepare 1% (w/v) of curcumin solution (stock) by dissolving 1 g curcumin powder in 100 ml food grade absolute ethanol. The mixture was then stirred with a stir bar until the solution became homogeneous. The second step was to prepare a series of concentrations of curcumin by using ethanol and make a series of dilutions.

Curcumin stock 1% was diluted to make stock solutions of 0.1, 0.01, 0.001, and 0.0001%, respectively of curcumin and stored in tubes 1, 2, 3 and 4 respectively. Another sample of TPE was prepared from 5g TPE mixed with 100 mL McIlvaine's buffer (w/v) in a centrifuge tube and spun at 6000 RPM for 20 minutes at 25°C. Lastly, the solution was filtered by use of Whatman #1 filter paper.

3.2.3 Subjecting curcumin concentrations to the ultraviolet; UVA and UVB

In the present work four concentrations of curcumin 0.1, 0.01, 0.001, 0.0001%, respectively, were exposed to ultraviolet at wavelength 380 nm and 300 nm, respectively, at the different time 5,10, 15, 20 min, respectively. The aim was to study and determine the effect of this ultraviolet on the structure of the active substance of curcumin and TPE regarding its efficiency as antioxidant. This method is summarized as follows:

Two 96 well plate (figure 3.3) were prepared to use the first plate with UVA and the second plate with UVB. The same way and design were used to all ultraviolet at various time 5, 10,15, 20 minute and the same amount of concentrations of curcumin 50 µL of each concentration and mixed with ethanol 50 µL (Food grade absolute ethanol) to avoid the evaporate of concentration. In 1-2 column was added CU0.1%, in the 3-4 column added CU0.01%, in the 5-6 column added CU0.001%, and the 7-8 column added CU0.0001%, respectively. After that, the 96 well plate in was placed in the FlexStation®3 machines. The machine setting was previously set to obtain the experimental reading by opening the machine for 3 minutes using the SoftMax. The setting option "Read Mode" was selected and the option of (Fluorescence) "RFUs", under the "Wavelength" 380 nm for UVA, and under "kinetic" option the "Timing" was set up 5 minutes. Afterward, the exposure step of UV used the same methods as above with 10,

15, and 20 minutes and with wavelength 300 nm UVB. Once the exposure experiments were finished, the antioxidant activity assays were immediately conducted.

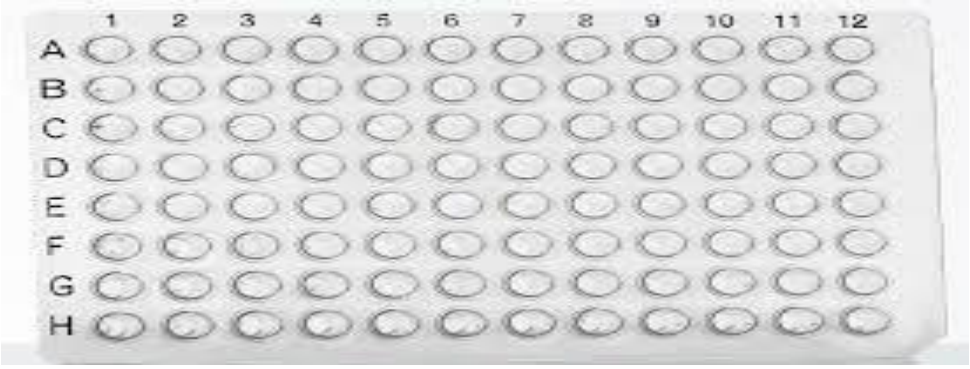


Figure 3.2 96 Well Plate Image

3.2.4 TRAP assay

All the reagents and protocols adopted to carry out the experiment in this study of TRAP was conducted by using the method of (Lissi et al., 1995; Wayner et al., 1987) and modified by (Mukherjee, 2015), but changed and designed for this study.

The composition of reaction mixture of different test samples of curcumin concentrations 0.1, 0.01, 0.001, and 0.0001% were stored in the refrigerator -20 °C until time of use and TPE 5% solution was stored in the refrigerator at 4°C, Oxygenated McIlvaine's iso-ionic buffer (PH=7.0) stored at 4°C, ABAP to be like a rapid, potent peroxyl free radical generator for the test, was stored in the refrigerator 4°C, and when it needed was dissolved mixture of ABAP 12.5 mM (0.0846g / 25 ml Mcllvaine's buffer (PH=7.0) into clean Petri plate and pipetting 50µL, and 50µL of Luminal 100 mM (disolvid1.77g in 100 ml sodium hydroxide 1N solution) also stored in refrigerator at

4°C. Test samples were placed in 96 well plates placed within a Flex Station®3 Luminometer (Molecular Devices, LLC, CA, USA) for all data acquisition.

TRAP method was the introduction of ABAP into the reaction mixture with or without the test samples in the 96 well plates generates free radicals due to pyrolysis. Luminol induced of chemiluminescence, caused by a form of presence of unquenched radicals and was recorded as RLU every 1:30 interval at 37°C in a Flex Station®3 Luminometer for one hour.

The chemiluminescence thus detected was directly proportional to the accumulation of the generated radicals. Therefore, lower the chemiluminescence, greater the antioxidative activity. by a number of free radicals generated. The TRAP is calculated from the length of the Lag Phase due to the samples compared with the standard. Getting the data for the first and second hours indicated the antioxidant activity (AA) and persistence (AP) of the samples, respectively by Mukherjee & Haque, (2015).

3.2.4.1 Procedures of antioxidant activity and persistence to curcumin concentrations and TPE

TRAP assay was conducted in two steps; the first step was conducted on four concentration of curcumin 0.1, 0.01, 0.001, 0.0001% (w/v), respectively with presence or absence of TPE 5% (w/v) without any exposure to UV radiation. The second step was conducted on four concentration of curcumin after exposed them to UV irradiation UVA and UVB at wavelength 380 nm and 300 nm, respectively at various times 5,10,15, and 20 min with the presence or absence of TPE.

Initially, the 96 well plate (Figure 3.3) was prepared and divided from 1 to 12 columns and from A to H shelf according to the treatments. In the first column was added

50 µL the McIlvaine's iso-ionic buffer (pH=7.0) without sample served as blank, in the second column was added 50 µL the TPE 5%, 3 to 4 columns were added 50 µL CU0.1, 25 µL of CU0.1 mixed with 25 µL TPE, respectively, 5 to 6 columns were added 50 µL CU0.01, 25 µL of CU0.01 mix with 25 µL TPE, respectively, 7 to 8 columns were added 50 µL CU0.001, 25 µL of CU0.001 mix with 25 µL TPE, respectively, 9 to 10 columns added 50 µL CU0.0001, 25 µL of CU0.0001 mix with 25 µL TPE, respectively, and 11 to 12 was added Trolox (0.5%, w/v) dissolved in McIlvaine's iso-ionic buffer (pH=7.0) buffer was the standard. Each reaction mixture contained 50 µL ABAP 12.5 mM, 50 µL McIlvaine's iso-ionic buffer (pH=7.0), and 50 µL luminol (the inducer of chemiluminescence). The "initial volume" was 200 µL of the composition of the reaction mixture and immediately placed into the FlexStation®3 machine which was set to 37°C.

The 96 well plate was then immediately placed into the imaging system which was used to analyze the reaction in the 96 well plate. The system then measured the range luminescence signal given off from each of the 96 well plates by a number of free radicals generated. The FlexStation®3 was prepped by turning it on it for 3 minutes at 37ºC and opening the software, SoftMax. In the software, the “Read Mode" option was selected as well as "Luminescence" "RLUs" around 500 nm, under the 'Kinetic" option the "Timing" was set to “1:00:00" and "interval" option was set to "00:01:30" Implying data capture at every 1:30 seconds during one hour. The "Compound Transfer before Read" option was set with an "initial volume" at 200 µl. Once the 96 well plate containing the reaction mixture was placed into the machine and, the “Read” option was selected in the software, and the reaction was allowed to run on the machine for one hour. Once the machine had ended its run, the data was saved on the computer and a graph of

the readings was created. The five surrounding readings of the point that the control showed the highest luminescence (which indicates to a maximum amount of free radical generation) was copied beside the five surrounding reading for all four curcumin concentrations with all treatments at the same time point and each of five reading was averaged and called "Lum Max C". The first hour of luminescence was "Lum Max C1" Antioxidant Activity (AA) and the second hour called "Lum Max C2" Antioxidant persistent (AP). The second hour was conducted using the same 96 well plate which was taken from the FlexStation®3 and another dose of 50 µL ABAP was added immediately at the same concentration in all columns. All "settings" were kept the same except "compound Transfer before Read "option was changed to "final volume" of 250 ml. The "Read" option was selected in the software and the reaction was allowed to run in the FlexStation®3 for another hour while recording the luminescence. Once the run had ended, the data was saved on the computer as an AP. The percent increase in AA or AP of concentrations compared to blank:

The inverse of Lum Max C was obtained through the calculation using the expression;

(AA or AP Blank - AA or AP samples) / AA or AP samples * 100) (3.1)

where, AA and AP were the highest luminescence of blank, AA and AP were the highest luminescence of each concentration with treatments. This value reflected the AA and AP of that concentration and was represented as a bar graph.

The second step was conducted on the 96 well plates that were prepared previously of exposure to ultraviolet rays either UVA or UVB at various times 5, 10, 15, and 20 minutes. This step was summarized as follows: A 96 well plate, which was

exposed to UVA at five minutes. It was brought and put on the table and started added 25 ml of the TPE in each of 2,4,6, and eight columns included curcumin concentrations exposed to UV, respectively, and was left each of 1,3,5, and seven columns as curcumin concentrations exposed to UVA without any addition of TPE, respectively. Each reaction mixture contained 50 µL ABAP, 50 µL McIlvaine's iso-ionic buffer (pH 7.0), and 50 µL luminol. The "initial volume" was 200 µL of the composition of the reaction mixture and immediately placed into the FlexStation®3 machine. All settings of FlexStation®3 was the same steps above. Initiation of reaction by pyrolysis of ABAP was determined as the first hour or Lum Max C1". Another dose of 50 µL ABAP was added immediately to all columns and change the volume to 250 µL and returned the plate to FlexStation. The AP was obtained after the second hour or "Lum Max C2". It was repeated in the same ways with all treatments that exposed to UVA and UVB at a different time.

3.2.5 Radical scavenging potential of curcumin concentrations and TPE by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS assay

The antioxidant capacity assay was measured as described by (Re et al. 1999) with modification. This assay is based on the inhibition by antioxidant of absorbance of radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) which has absorption spectrum showing at 734 nm.

The experiment was carried out to measure the antioxidant capacity of CU with or without the addition of TPE, which was exposed to UVA and UVB at different times 5, 10, 15, and 20 minutes. The loss of color when antioxidant samples were added to the blue-green $ABTS^{\bullet+}$ meant that this assay depends on decolonizing technique in which the $ABTS^{\bullet+}$ radical cation is produced directly in stable conditions in the presence of

antioxidants. The technique involves the production of blue-green $ABTS^{\bullet+}$ chromophore through the reaction between $ABTS^{\bullet+}$ and potassium persulfate. The addition of antioxidants to pre-formed radical cations reduce it to ABTS on time- scale depending on antioxidant activity. The extent of decolonization as percent inhibition of $ABTS^{\bullet+}$ radical cation is determined as a function of contentment and time, which involved the generation of ABTS•+ chromophore by the oxidation of ABTS with potassium persulfate. $ABTS^{\bullet+}$ radical cation was prepared by dissolving 0.076 g ABTS 2,2-azino-bis(3-ethylbenz- thiazoline-6-sulfonic acid), molecular weight: 548.68 g/mol in 20 ml of the Mcillvane's iso-ionic buffer (pH=7.0) in a dark bottle covered with aluminum wrap. A concentration of 7 mM.0.013 g of potassium persulfate $K_2O_8S_2$ (molecular weight =270.32) was added in the ABTS solution to prepare the $ABTS^{\bullet+}$ radical cation solution, which yielded a final concentration of 2.45 mM.

The mixture of $ABTS^{\bullet+}$ solution was kept at room temperature, in the dark, and stood for 12-16 hours before use. Trolox (6-hydroxy- 2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), water -soluble analog of vitamin E, was used as an antioxidant standard (Srinivasan et al., 2007).Trolox (2.5 mM), 0.5%, (w/v) was prepared daily as a stock standard by a dilution of 0.13 g Trolox mixed with 25 mL McIllvaine’s buffer.

3.2.5.1 Procedure of $ABTS^{\bullet+}$ radical cation

This experiment was based on testing the ability of antioxidant of TPE 5% in different concentrations of curcumin 0.1, 0.01, 0.001, 0.0001%, respectively, without any exposure to ultraviolet as well as with exposed to UV radiation at various times 5, 10, 15, 20 minutes. Initially, it used the same previous steps method of exposure to ultraviolet (UVA and UVB) and the ways and amounts of concentrations above in TRAP assay with

a difference, which is used free radical cation $ABTS^{\bullet+}$ and absorbance at 734 nm. This process is summarized as follows:

The first 96 well plate was prepared to add the concentrations. In the first column was added 50 µL the Mcillvaine's iso-ionic buffer (pH=7.0) without sample served as blank, in the second column was added 50 µL the TPE 5 g/ml, 3-4 column was added 50 µL CU0.1, 25 µL CU0.1+ 25 µL 25 TPE, respectively, 5-6 column CU0.01, CU0.01+TPE, respectively, 7-8 column CU0.001, CU0.001+ TPE, respectively, 9-10 column CU0.0001, CU0.0001+ TPE, respectively, as above in TRAP assay and 11-12 was added Trolox 0.5% as a standard. Each reaction mixture contained 100 µL ABTS 2.45 mM and100 µL Mcillvaine's iso-ionic buffer (pH 7.0). The "initial volume" was 250 µL of the composition of the reaction mixture and immediately placed into the FlexStation machine. All settings were similar to the TRAP assay except the "Read Mode" option was selected as "absorbance" around 734 nm (ʎ irradiation= 734 nm) for one hour, and the "initial volume" at 250 µL. Once the machine had ended its run, the data was saved on the computer in the folder as antioxidant capacity (AC) without exposure to UV.

The second 96 well plate, which was exposed to UVA at five minutes was brought and put on the table and started added 25 µL of the TPE in each of 2,4,6, and eight columns, respectively, and was left each of 1,3,5, and seven columns as curcumin concentrations exposed to UVA without any addition of TPE, respectively. Each reaction mixture contained 100 µL ABTS, 100 µL Mcillvaine's iso-ionic buffer (pH 7.0). The "initial volume" was 250 µL of the composition of the reaction mixture and immediately placed into the FlexStation machine. All settings were similar to the above, selected as

"absorbance" around 734 nm (ʎ irradiation= 734 nm) for one hour, and the "initial volume" at 250 µL. Once the machine had ended its run, the data was saved on the computer in the folder as AC after exposure to UV. Afterward, the data was saved on the computer as AC for CU with or without TPE, that was subjected to UVA and was put into Microsoft Excel to calculate (means ± SD) and the graph of reading was created. The radical scavenging activity was calculated using Eq. (3.1) and expressed as percent inhibition, according to (Hiller & Lorenzen, 2010).

The following equation is used to calculate the AC of all treatments including TPE and Trolox:

$$AC\ \% = [(Absorbance_{Blank} - Absorbance_{Sample})/Absorbance_{Blank}] \times 100 \qquad (3.2)$$

3.2.6 Sodium Dodecyl Sulfate-Polyacrylamide Gel electrophoresis (SDS-PAGE) of TPE

It is a common method for the analysis of proteins by an electrophoresis depend on molecular weight, the polyacrylamide gel- based separation method. Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide gels are composed of pores which are sized by the concentration of the page gel 12% and 10%. Smaller proteins migrate through the pore faster while large proteins migrate slower, which determines the location of the band on the gel. The internal structure of the protein must first be decomposed to be able to use this method. The protein was carried out in slab gels according to the method (Laemmli, 1970).

3.2.6.1 Preparation of TPE dispersions

The turmeric polar content was extracted according to the method described by (Smillie & Krotkov, 1960). Weigh out of 1 g of TPE powder in a 50 ml polyethylene tube

with 10 ml of 50 mM Tris-HCL (pH 7.5) buffer. The mixture was centrifuged at 2500 XG for ten min at 4Cº. Collect the supernatant (protein and peptide) in another clean centrifuge tube (if necessary, one more time centrifuge). Then, filtering by using filter paper Whatman two to remove any undissolved contaminations. The protein concentration was determined by using Bradford assay. The concentration was determined to be 560 µg/ml buffer Tris-HCL (pH7.5).

3.2.6.2 Preparation solutions for electrophoresis

All solutions were prepared as follows: 10% SDS in deionized water was adjusted to pH7.2 and heated at 68 Cº 15 min using HCL. The final volume was adjusted to 100 ml. 10% APS (Ammonium persulfate);1 g mixed with 10 ml water and set aside. A 1.5 M Tris-HCl (pH 8.8) solution was prepared using 181.7 g of Tris base 181.7 g and 800 ml water. The pH was adjusted to 8.8 by adding (~30 ml) HCl. Lower concentration of Tris-H Cl stock solution: 1.0 M Tris- HCL (pH 6.8) was prepared by dissolving 121g Tris in 800 ml water and adjusting pH to 6.8 by adding HCL (~50 ml). Final volume was adjusted to a1000 mL. Tris- Glycine buffer (Running buffer) 1 L: Tris base- 15.1g [0.125 M], Glycine 94 g [1.75 M], SDS 5.0g [0.5% w/v] were dissolve in 800 ml water. This was diluted fivefold prior to use and the pH was adjusted to 8. Dilute protein samples 1:1 in 2x sample buffer before loading on SDS-PAGE gels. Staining solution- for 1L solution:25% ethanol- 250 ml, 10% glacial acetic acid- 100ml, 0.1% Coomassie brilliant blue R- 250 1g. Added distilled WATER- 650 ml. Stirred well and filtered with Whatman 1 paper. Distaining solution was composed of 100 ml acetic acid, 50 ml ethanol, and 850 ml distilled water.The running gel was prepared by using 3.75 mL of 40% acrylamide.

3.2.6.3 Preparation of gels for SDS-PAGE electrophoresis

A-Running gel 12% (10ml) was prepared by following; 1.5 M Tris- HCl (PH8.8) 2.5 ml, 10% SDS 0.1 ml, 40% Acrylamide stock 3.75 ml, 10% APS 0.1 ml, N,N,N'N'-Tetra methylethylene diamine (TEMED) 0.004 ml, distilled water 3.55 ml.

B- Stacking gel (4 ml) was prepared by following; 1.0 M Tris- HCl (PH 6.8) 0.5 ml, 40% Acrylamide stock 0.5 ml, 10% SDS 0.04 ml, 10% APS 0.04 ml, TEMED 0.004 ml, distilled water 2.87 ml.

Sodium Dodecyl Sulfate- poly Acrylamide Gel Electrophoresis of protein out in slab gels according to the method of (Laemmli, 1970). It was taken 100 µl of protein solution of sample TPE with 100 µl Laemmli loading buffer in one tube. Heating the mixture at 95°C for five min (this will help to denaturation the protein) then cool it to room temperature. Loading 10 µl of sample in the gel 12%. The protein standard that we had used. Set voltage ~60V for 4% stacking gel and ~100V for 12% resolving gel and run the gel 2-3 hours until the tracking dye front reaches the bottom of the gel or until the blue line left and be under the green line. Then, it has taken the glass plates and open it carefully to avoid tearing the gelatin layer. Staining the gel with a staining solution for 1-2 hours (Coomassie Brilliant blue) under shaking. Recoloring the gel with distaining solution overnight but without the dye.

3.2.6.4 Running the gel

Pipetted the gel solution between the glass plate while leaving ¼ of space free for stacking gel. After that covering the top of the resolving gel with 70% ethanol and waited 20 min until the resolving gel polymerized. Preparing theThe stacking gel was then pipetted on top of the resolving gel and a comb was place into it to create the wells on

gelation (2-30 min). Lastly, removed the comb and the gel was placed in the electrophoresis tank and Tris- Glycine-SDS buffer was poured into tank up to the designated mark.

3.2.6.5 Analyze the gel

The image was taken and analyzed by using (Bio-Rad Chemi Docx^{TM} XRS)(Bradford. MA) system.

3.2.7 Proximate analysis of TPE

The approximate analysis for chemical composition of TPE was carried in nutrition Lab in the department of animal and dairy sciences. The TPE powder was characterized for their proximate composition including total ash, moisture content, protein (N* 6.25) and carbohydrate content by following the (AOAC 2002) method on triplicate samples.

The proximate analysis of raw material is an important tool to evaluate the quality of this material. For this purpose, Turmeric polar extract was investigated for different parameters to find out its composition.

3.2.7.1 Protein

The Kjeldahl method (AOAC 2001.11) (Feldsine et al., 2002) with the use of (Kjeltec Auto Sampler System, 1035 analyzer, Foss Tecator™, North America) was utilized for the determination of protein content of TEP. 250 ml Kjeldahl digestion tubes were used. The first tube served as the blank and the second tube acted as the standard with 0.10g (NH4) 2SO4.For the remaining seven test tubes, approximately ~ 0.9 g of the sample was weighed and placed into each assigned test tube. 14 ml of sulfuric acid were

added into each tube along with 3.5g K_2SO_4 + 0.4g $CuSO_4$ in the form of one Kjeldahl tab. Tubes were then placed in a digestion block under a fume hood with the vent turned on and the use of the manifold and then heated to 415 °C for 1-2 hours or until all samples were a clear green color indicating the reaction. After the reaction, the tubes were removed from the block and left to cool under the fume hood for 10-20 minutes with the exhaust on until all fuming ended. The digest was pre-diluted manually before distillation by adding a few milliliters of deionized water to each tube to avoid mixing concentrated alkali with concentrated acid and to prevent the digest from solidifying. Additional water was added to each tube to a total of 80ml.

To perform distillation was used. 50ml 40% NaOH was dispensed into the alkali tank of the distillation unit. Automatic dilution was used by placing entire test tube rack with samples into the unit and analysis was performed. Titration was performed automatically using H3BO3 with standard 0.1000M HCL to a violet color. The amount of nitrogen in the sample is determined by the reaction between the moles of boric acid and moles of nitrogen. Total protein content in the samples was calculated using the conversion factor F (6.25) by following:

$$\text{Kjeldahl nitrogen (N), \%} = (VS-VB) * M * 14.01 / W * 10, \quad (3.3)$$

Crude protein, % = Kjeldahl N * F. All these abbreviations mean: VS= volume (ml) of standardized acid used to titrate a test; VB= volume (ml) of standardized acid used to titrate reagent blank; M= molarity of standard;14.01= atomic weight of N; W= weight(g) of test portion or standard; 10= factor to convert mg/g to present; and F= factor to convert N to protein.; F factor= 6.25. Measurement of moisture and ash of TEP

The basic principle is to evaporate the moisture from the samples by oven drying. The moisture content of the TEP powder is determined by using the (AOAC 934.01) (Feldsine et al., 2002) official method as residue remaining after drying. Moisture content is determined in duplicate by drying a 2g TEP sample in an aluminum dish at 100ºC for 24 hours in a forced-air oven (Precision Scientific Oven, Model 124, US). Samples were then moved to a desiccator and allowed to cool down. Then pans were weighed, and moisture was determined by subtractions the weight of the pans without a sample.

The ash method (AOAC 942.05, 2002); (Fisher Scientific, Muffle Furnace, US.) is used for the determination of ash content of TEP. Samples used for dry matter determination are placed in a cool temperature controlled furnace; it was then set to 500°C and held at this temperature for 5 hours. Once muffle furnace cooled, aluminum pans were transferred directly to desiccator then weighed immediately. Reporting percent ash to the first decimal place.

The calculation of the percent Dry Matter [% Lab DM= wet weight - dry weight / wet weight * 100] and the calculation of the percent Ash [%(w/w) Ash= (weight of portion test g - weight loss on ash)/ weight of test portion g *100].

The total percentage carbohydrate content in the TPE was determined by the difference method. This method included adding the total values of crude protein, moisture and ash constituents of TPE and subtracting it from 100 (Das et al., 2014) the value obtained is the percentage carbohydrate constituent of the sample.

$$\text{Carbohydrate} = 100 - (\text{protein} + \text{ash} + \text{moisture}) \quad (3.4)$$

3.3 Statistical analysis

This experiment involves testing of four concentrations of curcumin 0.1, 0.01, 0.001, 0.0001, (w/v) with or without TPE (5g/100ml) which were subjected to two types of ultraviolet irradiation, on a different wavelength, UVA and UVB (380 nm and 300 nm), respectively, at various times (5, 10, 15, and 20 min), respectively. The interactions were evaluated ($p<0.05$) using SAS version 9.4. A three-way cross-classification fixed effects model with one response per treatment combination was used. Thus, the four-factors interaction is assumed to be negligible and it is used as the error term. Fisher's Least Significant Difference (LSD) test was conducted to determine the significance of interactions among means of the results with ($p<0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Proximate analysis

The proximate chemical composition of TPE which contained protein 6.68%, ash 5.15%, and carbohydrate 88.30% on dry weight basis (Table 4.1). The preparation of the TPE was by cold precipitation of aqueous dispersions of using solvents of low dielectric constants was based on the premise that the curcuminoids intended to extract only the constituents that polarize. The low dielectric constants of the solvents used being 4.33 for diethyl ether and 20.7 for acetone compared to 80.1 for pure water at 22˚ (Smallwood, 2012). At lower dielectric data obtained was comparable to the previous studies. According to (Nisar et al., 2015) the Curcuma longa (powder) contained protein, ash, moisture)6.47, 3.45%, 13.2%, respectively(. The protein and ash contents consistent with works from previous authors (Ikpeama et al., 2014) reported turmeric contains protein, ash, moisture, and carbohydrate contents in turmeric to be (9.40, 2.85, 8.92, and 67.35, respectively). The moisture and ash contents were consistent, while protein content was higher, and carbohydrate was lower than the findings from the current study. The ash content of TPE exhibited the presence of considerable amount of minerals. (Lim et al., 2011) reported ash content of TPE to be four times higher than that of wheat flour. This indicates the presence of considerably higher levels of minerals (K, P and Ca).

Table 4.1 Proximate composition of turmeric extract powder (dry weight basis)

TPE composition*	Mean content %	CV**
Crude protein	6.68	0.216
Ash content	5.15	1.050
Total carbohydrate	88.3	-

* TPE is abbreviation for turmeric polar extract. Values are per 100g of TPE powder.
**CV is coefficient of variation (SD/mean).

4.2 Sodium Dodecyl Sulfate-Polyacrylamide Gel electrophoresis of TPE

The active antioxidant component of TPE was isolated or separate the protein by electrophoresis which uses a broken polyacrylamide gel as a support medium and sodium dodecyl sulfate SDS to denature the proteins. The method is called SDS-PAG. The results indicated TPE to be mainly composed of three fractions with molecular weights of 25.8, 11.7, and 10.2KDa, respectively.

The sample constitutes about 90% of the TPE (Figure 4.1) About 10%, as determined by densitometry of the protein content, consisted of a peptide with a molecular mass of ~10 KDa based on molecular mass markers (standard).

In previous studies, β- turmerin with a molecular mass of ~34KDa by SDS-PAGE was isolated from turmeric (Curcuma Longa) waste grits (Smitha et al., 2009). Another study (Ramadas & Srinivas, 2011) showed that isolated a ~28 KDa glycoprotein from boiling water extract of turmeric and named it BGS-haridrin which has the highest inhibition of ghost lipid peroxidation 78% compared with in the present study, the ~10 KDa showed the lower effectiveness of antioxidant compared with the curcumin. While, this study (Srinivas et al., 1992) was used a different method to study a water -soluble ~5

KDa peptide called turmerin from turmeric (curcumin longa), a Sephadex G-10 Column to fractionation.

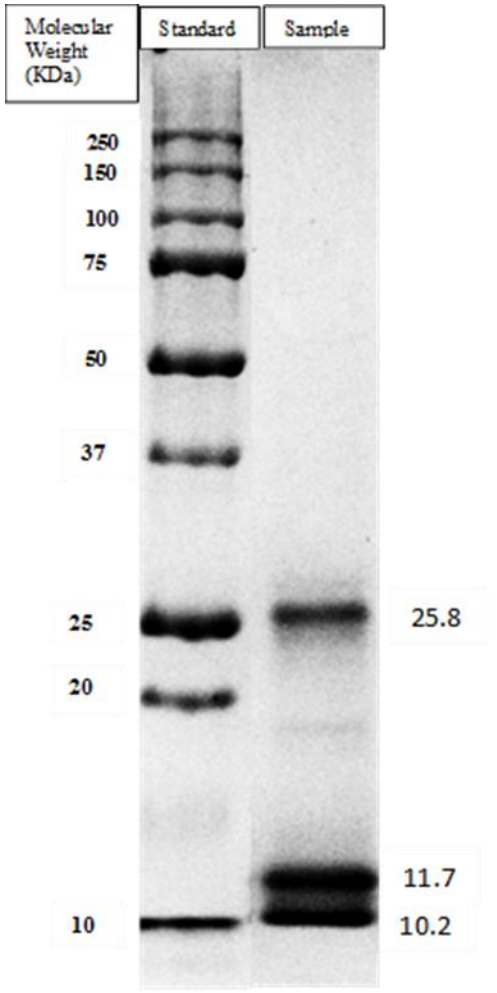


Figure 4.1 Sodium Dodecyl Sulfate-Polyacrylamide Gel electrophoresis (SDS-PAGE) of turmeric extract with the standard.

4.3 Total Radical Trapping Potential (TRAP) of curcumin and TPE

The present research work deals with the antioxidant evaluation of turmeric polar extract (TPE) and curcumin after treated with UV. In the beginning, the present study was performed using ultraviolet (UVA and UVB) in different times 5, 10, 15, 20 minutes

with various concentrations of curcumin 0.1, 0.01, 0.001, 0.0001. To know the impact of these rays in different wavelengths on the structure of curcumin before used the TRAP assay. This experiment was based on the principle of using turmeric polar extract (TPE) with and without the curcumin to know the antioxidant effect being able to quench peroxyl or alkoxyl radicals formed by the thermolysis of ABAP. All the results which will be discussed were obtained from SAS version 9.4.

4.3.1 Antioxidant Activity of different concentrations of curcumin with and without TPE, with and without exposure to ultraviolet at various

It was used 1 g/100 ml curcumin: food gread ethanol to prepared a series dilution by prepared four concentrations of curcumin as well as prepared TPE g/ml which was used mixed with curcumin after exposure to UV at different times before measured the antioxidant activity by TRAP assay. A similar study reported by (Mongkolsilp et al., 2004) which used DPPH assay of four different dilutions of Curcuma longa rhizome extract exhibited the highest radical scavenging activity.

The free radicals through using one concentration of ABAP dissolved in 2 mL McIlvaine's iso-ionic buffer (PH 7.0) according to previous studies (Wayne et al. 1985; Lissi et al. 1995).

The similar study was by (Sreenivasan & Haque, 2013) which involving TRAP, have indicated the use of different concentrations of ABAP including 12.5 mM to explore the maximum free radical available for studying the AA of four types of tea, while study by Lissi et al. (1995) conducted TRAP studies using 12.5 mM ABAP, which is the same used in this study.

The results of the first hour of pyrolysis of ABAP showed that the highest AA was in concentrations CU0.1, CU0.01, CU0.001, CU0.0001, respectively, the TPE absence and presence (Table 4.2, Table A1, and Table A2) and (Figure 4.2-4.3). However, there was no difference ($p<0.05$) RLU between CU0.001 and CU0.0001 at TPE absence. While the TPE presence in all curcumin concentrations showed (higher RLU) lower AA compared with the TPE absence (Table 4.2). The CU0.1 concentration was highest AA, lower RLU, within absent and present TPE, respectively, among all four concentrations which it showed 2828.5 and 590.3%, respectively. Whereas, CU0.01, CU0.001, and CU0.0001, within TPE absent respectively, showed 778.5, 275.2, and 247.8%, respectively compared with blank (Table 4.5). While, the presence of TPE within the curcumin concentrations, showed decreasing in AA between CU0.01, CU0.001, and CU0.0001, with 590.3, 7.4, -46.6, and -87%, respectively (Table 4.5).

Whereas, the turmeric polar extract showed (highest RLU) means lowest AA about -87% compared with a blank and with all four concentrations. It indicates that it tends to be pro-oxidant. Maybe the reasons either treatment conditions including the method of produce which is caused loss the antioxidant compounds (protein, peptide, aromatic compounds.... etc) and less stable or the curcumin concentrations were more stable.

There was a significant difference ($p<0.05$) between the RLU in all concentrations at the exposure of ultraviolet UVA and UVB or without exposure (Table 4.3), and it was noted that as CU0.1 concentration without UV the (lower RLU) highest AA compared with all other concentrations in the same treatment. However, there was no difference ($p<0.05$) between UVA and UVB at the same CU0.1 concentration, and it was no

difference between CU0.001 without UV, CU0.001 UVA, and CU0.0001 without UV. Where the AA of all concentration without UV exposure showed better than with UVA or UVB, although, the UVB was better than UVA and showed no great effect on structural of curcumin compared with UVA exposure (Table 4.4).

Table 4.2 Antioxidative activity of curcumin as reflected by a decrease in chemiluminescence maxima.

Concentrations (w/v)	TPE (w/v)	Chemiluminescence in relative light units
CU 0.1	Absent	23.0 [a]
CU 0.01	Absent	77.0 [b]
CU 0.001	Absent	180.4 [c]
CU 0.0001	Absent	194.6 [c]
CU 0.1	Present	98.0 [b]
CU 0.01	Present	630.1 [d]
CU 0.001	Present	1267.5 [e]
CU 0.0001	Present	1642.3 [f]

[a-f] Dissimilar letters indicate differences ($p<0.05$) in treatments mean between each concentration of curcumin with present or absent turmeric polar extract in column. CU and TPE are abbreviation for curcumin and turmeric polar extract, respectively.

Table 4.3 Effect of ultraviolet radiation on radical scavenging ability of the antioxidant activity of curcumin as a percentage compare to the blank (buffer alone).

Concentrations (w/v)	Ultraviolet (UV)	AA%
CU 0.1	Without UV	2,841 [a]
CU 0.1	UVB	2,048 [ab]
CU 0.1	UVA	974 [ab]
CU 0.01	Without UV	779 [b]
CU 0.01	UVB	138 [d]
CU 0.01	UVA	7.4 [e]
CU 0.001	Without UV	276 [c]
CU 0.001	UVB	-47 [f]
CU 0.001	UVA	-46 [f]
CU 0.0001	Without UV	248 [c]
CU 0.0001	UVB	-52 [g]
CU 0.0001	UVA	-59 [h]

[a-h] Dissimilar letters indicate differences ($p<0.05$) in treatments means between each of curcumin concentrations without and with exposure to ultraviolet in the same column. UVA and UVB are ultraviolet radiation at 380 and 300 nm, respectively. All other abbreviations are as given in the footnote of Table 4.2.

Table 4.4 Effect of time of exposure to UVB type of ultraviolet radiation on the antioxidative activity(AA) of curcumin as reflected by a decrease in chemiluminescence as a percentage compare to the blank (buffer alone).

Concentrations (w/v)	Time (min)	AA%
CU0.1	0	2,841 a
CU 0.1	5	4,128 a
CU 0.1	10	2,719 a
CU 0.1	15	2,233 a
CU 0.1	20	974 c
CU 0.01	0	779 c
CU 0.01	5	1,028 bc
CU 0.01	10	590 ce
CU 0.01	15	420 e
CU 0.01	20	138 g
CU 0.001	0	275 f
CU 0.001	5	254 f
CU 0.001	10	108 h
CU 0.001	15	86 i
CU 0.001	20	-47 k
CU 0.0001	0	247 f
CU 0.0001	5	218 f
CU 0.0001	10	71.7 ji
CU 0.0001	15	68 j
CU 0.0001	20	-51 l

[a-l] Dissimilar letters indicate differences ($p<0.05$) in treatments means between each of curcumin concentrations with exposure time to UV in the same column. All other abbreviations are as given in the footnote of Table 4.2.

Table 4.5 Antioxidative activity (AA) of curcumin without and with turmeric polar extract as reflected by decrease in chemiluminescence as a percentage compare to the blank (buffer alone).

Concentrations (w/v)	TPE (w/v)	AA %
CU0.1	Without TPE	2828.5
CU0.1	With TPE	590.3
CU0.01	Without TPE	778.5
CU0.01	With TPE	7.4
CU0.001	Without TPE	275.2
CU0.001	With TPE	-46.6
CU0.0001	Without TPE	247.8
CU0.0001	With TPE	-58.8

All other abbreviations are as given in the footnote of Table 4.2.

Table 4.6 Antioxidant activity (AA) of curcumin concentrations with and without turmeric polar extract, exposure to ultraviolet radiation as reflected by decrease in chemiluminescence maxima as a percentage compare to the blank (buffer alone).

Treatments	Concentration of CU (w/v)	Antioxidant activity (AA)%			
		Time of UV exposure (min)			
		5	10	15	20
CU-UVA	0.1	2,584.5	1,116.7	2,718.8	2,342.2
CU-UVB	0.1	6,282	4,264.5	4,410	3,556.8
CU-UVA+TPE	0.1	228.1	203.4	868.2	182.3
CU-UVB+TPE	0.1	745.6	826.7	699.6	653.3
CU-UVA	0.01	1,519.1	1,027.5	422.4	588.2
CU-UVB	0.01	3,153.7	356.2	896.3	804.4
CU-UVA+TPE	0.01	-55.2	26	73	-81.3
CU-UVB+TPE	0.01	33.7	-53	4.8	-44.4
CU-UVA	0.001	-47	254	92.8	83.7
CU-UVB	0.001	35	22.7	426	158.6
CU-UVA+TPE	0.001	-200	-58	-37.7	-89
CU-UVB+TPE	0.001	-47.7	-81	295.2	-88
CU-UVA	0.0001	-51.8	217.6	67.9	71.4
CU-UVB	0.0001	356.8	0.015	330.6	64.3
CU-UVA+TPE	0.0001	-84.4	-48.5	-53.5	-90
CU-UVB+TPE	0.0001	-53.9	-81.3	-66.5	-88.6

Abbreviations are as follows: CUVA, four curcumin concentrations exposure to ultraviolet (UVA at 380 nm); CUUVB, four curcumin concentrations exposure to ultraviolet (UVB at300 nm); CU-UVA+TPE are curcumin concentrations that exposed to ultraviolet at 380 nm and then mixed with turmeric polar extract; CU-UVB+TPE are curcumin concentrations which were exposed to ultraviolet (UVB at 300 nm) and then mixed with turmeric polar extract. At all concentrations, curcumin that was not subjected to UV treatment exhibited significant difference ($p<0.05$) antioxidant persistence AP relative to the blank (McIlvaine's iso-ionic buffer on its own).; 5, 10, 15, 20 min were the time of exposure to ultraviolet. The blank (McIlvaine's buffer), turmeric polar extract, and Trolox (a water-soluble vitamin E derivative used as the standard) exhibited chemiluminescence maxima of 1024.9 ± 10.97, 7926.2 ± 380.8 and 13.78±0.7 RLU, respectively.

From these findings, it was clear that UVB and without UV were significantly better compared to UVA at all concentrations (Table 4.2).

There was no ($p>0.05$) interaction between TPE*ultraviolet, but there was a significant difference ($p<0.05$) interaction between Time*ultraviolet for concentrations (Table 4.3).

It was noted that the CU0.1 concentration was (lowest RLU) highest AA than of all other concentrations in various times of exposure to UV. For CU0.1 concentration, there was no difference ($p<0.05$) between 0, 5,10, and 15 min exposure to UV, but there was a difference with 20 min UV exposure at CU0.1. However, the RLU at15 and 20 min were higher (lowest AA) than RLU at 0, 5,10, and15 min (highest AA) (Table 4.3). For the CU0.01 concentrations, there was the significant difference ($p<0.05$) between RLU at 0, 5, and ten min, respectively, which was higher than RLU at 15 and 20 min lowest in AA, and there was no difference between 15 and 10 min of CU0.01 concentrations, as well as, there was no difference between CU0.1 at 20 min and CU0.01 at ten min. For the CU0.001 concentrations, there was no difference between 0 and 5 min, and it showed the best AA (lower RLU) compared with other times, as well as, no difference between the above with CU0.0001 at 0 and 5 min. For the CU0.0001 concentrations, there was a difference ($p<0.05$) at all times except 0 min and 5 min and between 10 and 15 min, respectively, showed no difference (Table 4.3).

From these findings, it can be noticed that at zero min, the AA was the best in all concentrations and five min was markedly better than 10, 15, 20 min, respectively, with some differences depend on the condition of test.

In summary, at a CU0.1 concentration of curcumin without TPE showed increasing AA compared with other concentrations CU0.01, CU0.001, and CU0.0001 %, respectively, in the same situation. Whereas, when the TPE was added showed decreasing in AA among all concentrations. A minimum time of exposure to UV 5 and 10 min, showed the best AA compared with 15 and 20 min. The exposure of UVB was showed lower RLU compared with UVA among all four concentrations.

(Figure 4.2 and Figure 4.3) are a graphical representation of (Table A1 -Table A2) which shows the mean and standard deviation of all concentrations of curcumin with different treatments. Also, (Table 4.2, and Table 4.3) showed that when the RLU was lower that means to increase in AA with increasing the concentration. But, when adding the TPE, showed increasing the RLU level and thus reduce AA.

Most of the studies have shown that a solvent's types are important to an extractive capability of chemical compounds from plants and prove the antioxidant ability of these compounds. In the previous study by (Ghasemzadeh et al., 2011) has been used three kinds of solvents extracts (methanol, acetone and chloroform) from different parts of ginger which was showed that highest content of phenolic was found in methanol extract better than acetone and chloroform) in leaves, rhizomes, and stems, respectively. In this study, was used the acetone solvent to extract the polar compounds from turmeric rhizomes. Another studies by (Thongrakard et al., 2014) has been shown that the dichloromethane extract of turmeric has the highest antioxidant activity.

So, maybe this is one reason to make the TPE lower in AA compared with other samples.

At the highest concentration of CU0.1 we can see highest antioxidant activity AA. This indicates that curcumin at a higher level can protect against various times of UVA and UVB as compared to other concentrations without TPE. With the second highest concentration of CU0.01, we can see that it does not provide as much protection from UVA and UVB as compared to the highest concentration. However, it does provide more protection than the lowest concentrations. Another trend that can be noticed is that after 5 minutes of radiation in all sample concentration; UVA caused significantly lower antioxidant activity compared to UVB. This is consistent with previous studies by (Suva, 2014) showing that the produced compounds which used to protect the skin from harmful rays particularly UVB radiation; showed that the aqueous and ethanolic extract of rhizomes of Zingiber has active compounds which have protection against this UV

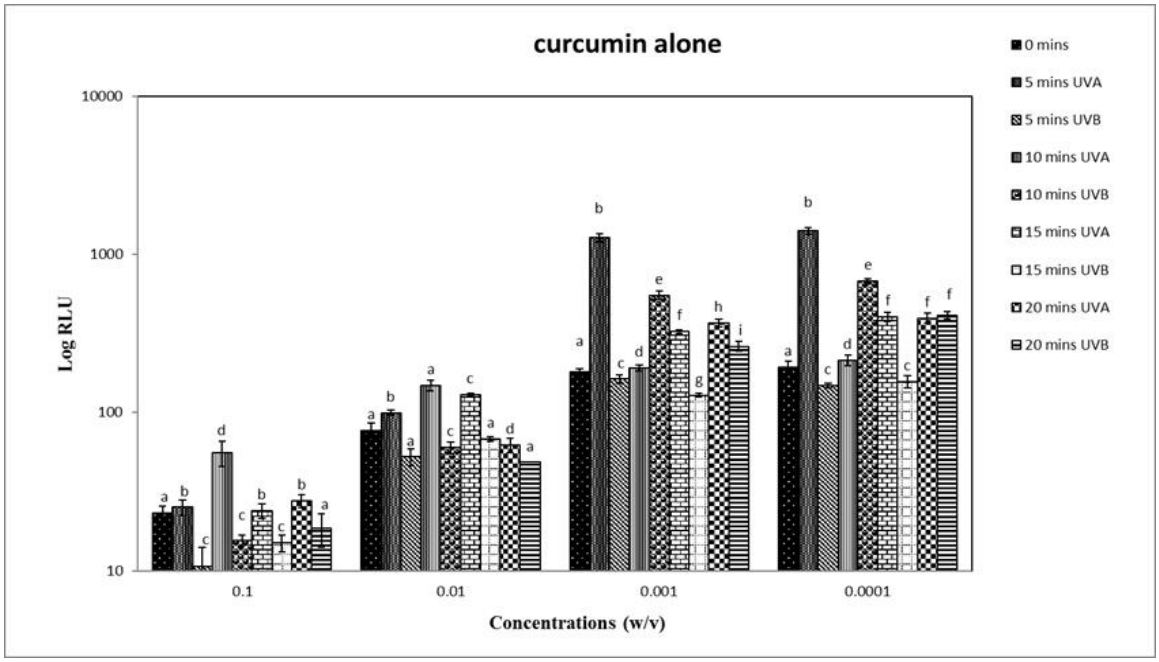


Figure 4.2 Effect of time of ultraviolet radiation on peroxyl and alkoxyl radical quenching efficacy of curcumin alone as reflected by decrease in chemiluminescence maxima at the point of maxima free radical proliferation.

Data are the mean of five chemiluminescence values recorded at 1:30 min intervals for a test sample following the first pyrolytic event of 2,2'-azobis (2 methylpropionamidine) dihydrochloride (referred to as the Lumax1).
[a-j] Dissimilar letters indicate differences ($p<0.05$) in exposure to UV at various times means between the curcumin concentrations.
The X and Y axes indicate CU 0.1-0.0001, (w/v) and chemiluminescence in the relative light unit (Log RLU), respectively.

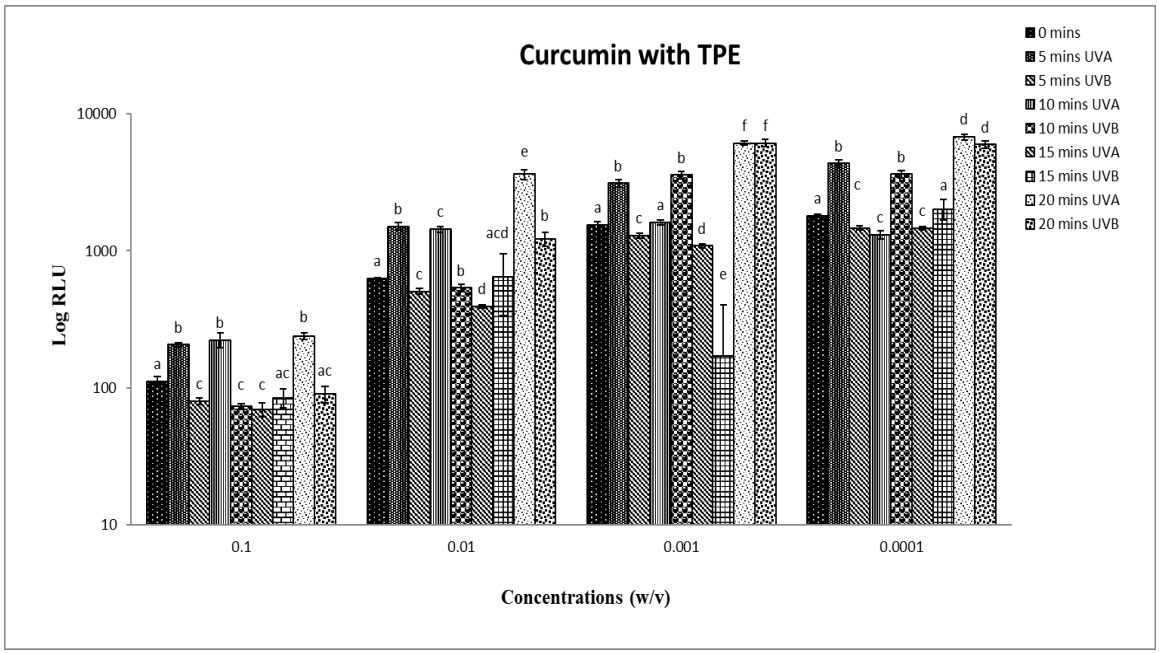


Figure 4.3 Effect of time of ultraviolet r on peroxyl and alkoxyl radical quenching efficacy of curcumin with turmeric polar extract (TPE) as reflected by chemiluminescence maxima at the point of maximal free radical proliferation.

Data are the mean of five chemiluminescence values recorded at 1:30 min intervals for a test sample following the first pyrolytic event of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (referred to as the Lumax1).
The statistical notation and X and Y axes are as explained in the legend of Figure 4.2.

4.3.2 Antioxidant Persistence AP of different concentration of curcumin with or without TPE, with and without exposure to ultraviolet at different

The second challenge of curcumin with and without TPE following the second pyrolysis of ABAP (LuMaxC2) that was calculated by averaging of data. All concentrations of curcumin with presence or absence TPE at various times of exposure to UV showed a significant difference ($p<0.05$) in antioxidant persistence AP compared with blank (Table 4.8, Table 4.9). However, the TPE alone showed (higher RLU) lower AP -85% which shows no difference within AA -87%. It indicates that it tends to be pro-oxidant. For CU0.1 concentration without TPE (absence) showed higher AP (lower RLU)

compared to all other concentrations, as well as showed highest AP with TPE (present) compared with the CU0.01, CU0.001, and CU0.0001, respectively. However, there was a difference between interaction concentration*TPE. There was no difference ($p<0.05$) between CU0.1 and CU0.01 of the TPE absent, as well as no difference between CU0.001 absence TPE and with CU0.1 concentration TPE presence (Table 4.7). Overall, AP of concentrations with TPE absent or present was exhibited results similar to those of AA. The AP showed the difference between concentrations of curcumin without TPE as CU0.1, CU0.01, CU0.001, and CU0.000, 1229.3, 1165.3, 177,8, and 62%, respectively, and the concentrations of curcumin with TPE as 389.4, -36.2, -65.5, -56.5%, respectively (Table 4.8). In this case, the results showed that the AP of CU0.1> the AP of CU0.01 and the AP of CU0.01> AP of CU0.0001. From ANOVA table that showed there was an interaction between concentration*time at the second hour of pyrolysis, there was a significant difference between each concentration with each time of exposure to UV. For CU0.1 concentration had lesser RLU; higher AP than CU0.1, CU0.001, and CU0.0001 concentrations at 0, 5, 10, and 20 min. However, there was no difference between CU0.1 and CU0.01 at 0, 5, 10, 15 min and with CU0.001 at 0, 5 min which was showed greater AP compared with other times. In addition, no difference between CU0.01 concentrations at 0 and 15 min with CU0.0001 concentrations at 15 min (Table 4.10). in summary, the findings showed that increase in AP regard to the increase the concentration and reduce the time of exposure to UV.

Table 4.7 Antioxidative persistence (AP) of curcumin with and without turmeric polar extract (TPE) as reflected by decrease in chemiluminescence maxima

Concentrations (w/v)	TPE (w/v)	Chemiluminescence in relative light units
0.1	absent	77.1 a
0.01	absent	81 a
0.001	absent	368.9 b
0.0001	absent	629.6 d
0.1	present	209.4 b
0.01	present	1605.9 e
0.001	present	2973.6 g
0.0001	present	2553.2 f

[a-f] Dissimilar letters indicate differences ($p<0.05$) in treatments means between each curcumin concentrations with present or absent turmeric polar extract in column.
All other abbreviations are as given in the footnote of Table 4.2.

Table 4.8 Antioxidative persistence (AP) of curcumin without with TPE as reflected by decrease in chemiluminescence maxima as a percentage of antioxidant persistence compared to the blank

Concentrations (w/v)	TPE (w/v)	AP %
CU 0.1	Without TPE	1129.3
CU 0.1	With TPE	389.4
CU 0.01	Without TPE	1165.3
CU 0.01	With TPE	-36.2
CU 0.001	Without TPE	177.8
CU 0.001	With TPE	-65.5
CU 0.0001	Without TPE	62.8
CU 0.0001	With TPE	-59.8

All abbreviations are as given in the footnote of Table 4.2.

Table 4.9 Antioxidative persistence (AP) of curcumin with and without turmeric polar extract (TPE), exposure to ultraviolet at various time as reflected by decrease in chemiluminescence maxima

Treatments	Concentrations of CU (w/v)	Antioxidant persistence (AP) %			
		Time of UV exposure (min)			
		5	10	15	20
CU-UVA	0.1	1,519	1,087.6	978.8	3,483.5
CU-UVB	0.1	3,153.6	6,04.1	8.735.3	4,434.5
CRUVA+TPE	0.1	312.6	782	392.7	83.2
CRUVB+TPE	0.1	741.5	112.8	78	872.8
CRUVA	0.01	260.4	519.6	360.6	942.6
CRUVB	0.01	1,277.5	2,090	1,566.5	1,270.1
CRUVA+TPE	0.01	-48	-53.8	81.5	-75
CRUVB+TPE	0.01	24.6	17.2	-67	-66
CRUVA	0.001	-38	175.5	69.2	37
CRUVB	0.001	379	0.19	12.3	177.8
CRUVA+TPE	0.001	-66	-60	232.7	-86.2
CRUVB+TPE	0.001	-67	-86.9	-85.2	-90.3
CRUVA	0.0001	-56	83	-42.5	-21.6
CRUVB	0.0001	247.3	-39	-41.4	14.1
CRUVA+TPE	0.0001	-73.4	-58.5	-64	-87.8
CRUVB+TPE	0.0001	-65.5	-83.8	-87	-87.8

Abbreviations are as follows: CUVA, four curcumin concentrations exposure to ultraviolet (UVA at 380 nm); CUUVB, four curcumin concentrations exposure to ultraviolet (UVB at300 nm); CU-UVA+TPE are curcumin concentrations that exposed to ultraviolet at 380 nm and then mixed with turmeric polar extract; CU-UVB+TPE are curcumin concentrations which were exposed to ultraviolet (UVB at 300 nm) and then mixed with turmeric polar extract. At all concentrations, curcumin that was not subjected to UV treatment exhibited significant difference ($p<0.05$) antioxidant persistence AP relative to the blank (McIlvaine's iso-ionic buffer on its own).; 5, 10, 15, 20 min were the time of exposure to ultraviolet. The blank (McIlvaine's buffer), turmeric polar extract, and Trolox (a water-soluble vitamin E derivative used as the standard) exhibited chemiluminescence maxima of 1024.9 ± 10.97, 7926.2 ± 380.8 and 13.78±0.7 RLU, respectively

Table 4.10 Effect of time of exposure to UVB type of ultraviolet radiation on the antioxidative persistence (AP) of curcumin as reflected by a decrease in chemiluminescence

Concentrations (w/v)	Time (min)	Chemiluminescence in relative light units
CU 0.1	0	1,231a
CU 0.1	5	740 a
CU 0.1	10	769 a
CU 0.1	15	393 a
CU 0.1	20	83 b
CU 0.01	0	1,165a
CU 0.01	5	925 a
CU 0.01	10	521 a
CU 0.01	15	362 a
CU 0.01	20	-66 f
CU 0.001	0	178 ab
CU 0.001	5	379 a
CU 0.001	10	0.20 c
CU 0.001	15	-67 f
CU 0.001	20	-86 h
CU 0.0001	0	63 b
CU 0.0001	5	-39 d
CU 0.0001	10	-55 e
CU 0.0001	15	-68 f
CU 0.0001	20	-88 h

[a-h] Dissimilar letters indicate differences ($p<0.05$) in treatments means between each of curcumin concentrations with exposure time to UV in the same column. All abbreviations are as given in the footnote of Table 4.2.

There was a difference ($p<0.05$) interaction between concentration * ultraviolet in AP at each treatment as UVA, UVB, and without UV. For CU0.1 concentration, there was no different between treatments on without UV, UVB, and with CU0.01 on without UV. However, the higher AP was with CU0.01 at UVB. There was no difference between CU 0.001 at treatment without UV and UVB. For CU0.001 showed a marked decrease in availability as antioxidant, indicated by a considerable decline in AP (Table 4.11). Overall, CU0.1 was the best AP compared with other concentrations. Also, there was no significant difference existed between UVB and without exposure to UV. There was no

difference between CU0.1 and CU0.01 at 0, 5, 10, and 15 min and trend to reduce the RLU, means higher AP.

Table 4.11 Effect of concentration of curcumin and time of exposure to ultraviolet radiation on the antioxidative persistence (AP) of curcumin as reflected by decrease in chemiluminescence

Concentrations (w/v)	Ultraviolet radiation (UV)	AP%
CU 0.1	Without UV	1,231 b
CU 0.1	UVB	1,087 b
CU 0.1	UVA	782 c
CU 0.01	Without UV	1,165
CU 0.01	UVB	1,859 a
CU 0.01	UVA	1,015 c
CU 0.001	Without UV	379 d
CU 0.001	UVB	177 e
CU 0.001	UVA	175 e
CU 0.0001	Without UV	63 f
CU 0.0001	UVB	-39 g
CU 0.0001	UVA	-56 h

[a-h] Dissimilar letters indicate differences ($p<0.05$) in treatments means between each of curcumin concentrations with or without exposure to ultraviolet in the same column. UVA is ultraviolet radiation at 380 nm, and UVB is ultraviolet radiation at 300 nm. All other abbreviations are as given in the footnote of Table 4.2.

The figure 4.4 and figure 4.5 are a graphical representation of (Table C1 and Table C2)) that shows the mean and standard deviation of all concentrations of curcumin with different treatments. Also, (Table 4.7, and Table 4.11) that showed that when the RLU was lower that means to increase in AP with increasing the concentration. But, when adding the TPE, showed increasing the RLU level and thus reduce AP. In summary, when using a higher concentration of curcumin without TPE (absence) consistently shows a greater degree of AP or AA in comparison to TPE (presence). Also, when to reduce the time of exposure to UV, the AP or AA was an increase. From another side, when treated with UVB shown better AA or AP compared with UVA.

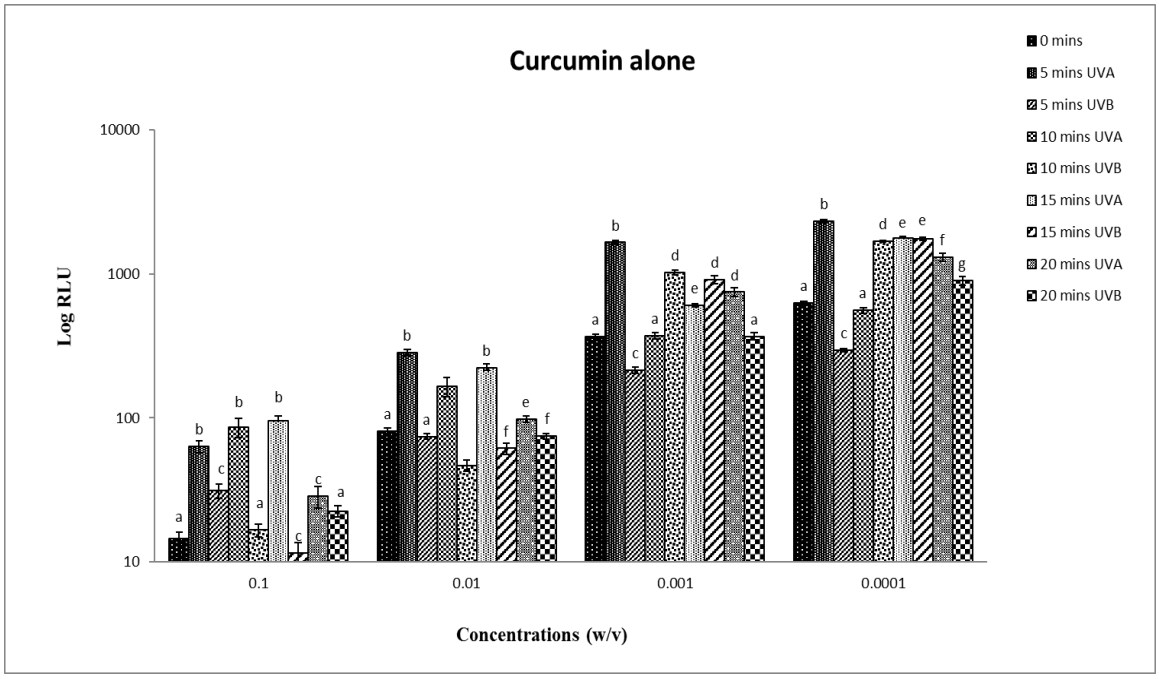


Figure 4.4 Effect of time of ultraviolet radiation on peroxyl and alkoxyl radical quenching efficacy of curcumin CU alone as reflected by chemiluminescence maxima at the point of maximal free radical proliferation.

The statistical notation and X and Y axes are as explained in the legend of Figure 4.2. Data are the mean of five chemiluminescence values recorded at 1:30 min intervals for a test sample following the second pyrolytic event of 2,2'-azobis (2 methylpropionamidine) dihydrochloride (referred to as the Lumax1

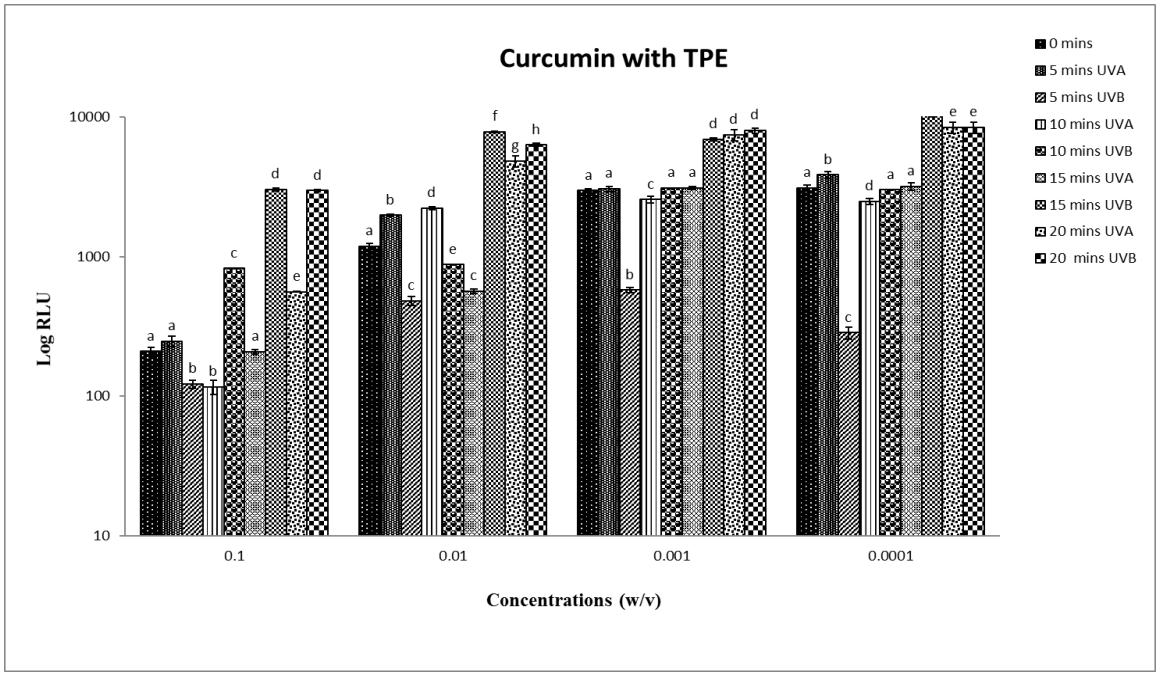


Figure 4.5 Effect of time of ultraviolet radiation on peroxyl and alkoxyl radical quenching efficacy of curcumin CU with TPE as reflected by chemiluminescence maxima at the point of maximal free radical proliferation.

The statistical notation and X and Y axes are as explained in the legend of Figure 4.2. Data are the mean of five chemiluminescence values recorded at 1:30 min intervals for a test sample following the second pyrolytic event of 2,2'-azobis(2-methylpropionamidine) dihydrochloride (referred to as the Lumax1); a-d Dissimilar letters indicate differences ($p<0.05$) in exposure to UV at various times means between the curcumin concentrations. All abbreviations and superscripts are the same as for Figure 4.4 and the X and Y axes are as explained in the legend for figure 4.4.

At all concentrations curcumin (CU) that was not subjected to UV treatment exhibited significantly greater ($p<0.05$) antioxidant persistence (AP) relative to the control (McIlvaine's iso-ionic buffer on its own). At concentrations of 0.1, 0.01, 0.001 and 0.0001% (w/v) CU showed 6968, 1165, 177 and 63% augmentations of AP compared to the blank. Thus, a concentration-dependent effect on augmentation of AP of CU was observed, depicting two, three and four-folds of enhancements, respectively, in AP with every ten folds of increase in the concentration of CU. However, turmeric TPE

imparted a significantly ($p<0.05$) adverse effect on the of the test samples. The AP of CU for concentrations of 0.1, 0.01, 0.001 and 0.0001% (w/v), exhibited increase AP of first concentration CU0.1 and third concentration CU0.001 after on hour (the second challenge), respectively. However, the second concentration CU 0.01 and fourth concentration CU 0.0001% showed a decrease of AP on second hours which was a decline of 1341, 110, 706 and 393 %, respectively following the addition of TPE. The effect of exposure to UV radiation varied markedly based on the type of UV radiation (UVA or UVB) and the duration of exposure.

At all concentrations, curcumin (CU) that was not subjected to UV treatment exhibited significantly greater ($p<0.05$) antioxidant persistence (AP) relative to the control (McIlvaine's iso-ionic buffer on its own). At concentrations of 0.1, 0.01, 0.001 and 0.0001% (w/v) CU showed 6968, 1165, 177 and 63% augmentations of AP compared to the blank. Thus, a concentration-dependent effect on augmentation of AP of CU was observed, depicting two, three and four-folds of enhancements, respectively, in AP with every ten folds of increase in the concentration of CU.

However, TPE imparted a significantly ($p<0.05$) adverse effect on the of the test samples.The AP of CU for concentrations of 0.1, 0.01, 0.001 and 0.0001% (w/v), exhibited increased AP of first concentration CU0.1 and third concentration CU0.001 after on hour (the second challenge), respectively. However, the second concentration CU 0.01 and fourth concentration CU 0.0001% showed a decrease of AP on second hours which was a decline of 1341, 110, 706 and 393 %, respectively following the addition of TPE. The effect of exposure to UV radiation varied markedly based on the type of UV light (UVA or UVB) and the duration of exposure.

4.4 Antioxidant capacity (AC) of curcumin concentrations with and without TPE by ABTS$^{\bullet+}$ radical cation assay

Turmeric polar extract was isolated from the fresh rhizome (g/ml) and curcumin was purchased from (ChromaDex® company, Irvine, CA 92618 USA) which was prepared four concentrations of curcumin (w/v). The method described gives a measure of the antioxidant activity of the range of concentrations of curcumin which exposure to ultraviolet at the different time and treated with or without turmeric extract and determined by the decolorization of the ABTS$^{\bullet+}$. The percentage of AC was expressed by calculating the suppression of the absorbance of the ABTS compared with a blank.

The results in (Table 4.12) showed that AC of CU 0.1 concentration was no significant difference ($p<0.05$) between TPE present and absent at the different time of exposure to UV. There was no difference ($p<0.05$) between TPE absent and present at five min exposure to UV and with 20 min TPE present, respectively, 54.6, 50, 46.4% AC, respectively. Also, there was no difference between ten min with TPE absent and present and with 15 min of the TPE present and absent in AC, 32.1, 32, 30.3, and 36.8% AC, respectively. However, the CU 0.1 without TPE at 15 min shown the higher AC compared to what mentioned above. For the TPE absent at 20 min had lesser ($p<0.05$) of AC compared with TPE presence with this concentration.

From these findings, it can be noticed that CU 0.1 concentration with TPE presence and absence TPE at five min of exposure to UV has the highest AC among all different times with or without TPE. Also, at ten min TPE absent and present was higher AC of all other concentrations.

For the concentration of 0.01 with or without TPE in different time of exposure to UV showed the significant difference ($p<0.05$). There was the difference at TPE

presence at ten min in this concentration and showed higher AC compared with all other times (Table 4.13). There was no difference ($p<0.05$) between TPE presence and TPE absent at five min exposure to UV. Whereas, without TPE at ten min showed lower AC compared with 10 minutes with TPE presence. There was no difference between TPE present and TPE absence at 15 min and 20 min of exposure to UV which was showed negative AC% (pro-oxidant properties) (Table 4.13).

(Table 4.15) was showed that CU 0.001 concentration has a difference ($p>0.05$) in AC between TPE present and TPE absence. The AC of TPE present at five min of exposure to UV was high (10.9%) compared with TPE absence at the same time of exposure to UV. However, there was no difference between TPE absence and present at ten min and between TPE absence at five min which was showed negative results. Whereas, there was no difference between TPE absence and TPE presence at 15 and 20 min which was showed negative AC.

From these findings, it can be noticed that with TPE presence at five min of exposure to UV is have the highest AC among all different times with or without TPE.

For a CU 0.0001 concentration with TPE absent or present at various times of exposure to UV, was showing different results. At the TPE presence had shown (22.5 %) higher ($p<0.05$) AC at five min exposure to UV compared with other times with or without TPE (Table 4.15). Whereas, when the TPE was absent at the same time (5 min) showed negative results. However, there was lower AC at ten min of exposure of UV TPE absence and presence compared with five min.,

Overall, there was a significant difference ($p<0.05$) in AC between all concentrations with TPE absence or present at the various times of exposure to UV. The

results of AC showed that CU0.1 concentration was markedly higher in AC during all times of exposure compared with other concentrations CU0.01, CU0.001, and CU0.0001, respectively.

(Figure 4.6) is a graphical representation of all tables of concentrations (Table 4.12-4.15), and it shows that the percentage of AC follows the downward trend; negative results (pro-oxidant) when increasing the time of exposure to UV with decreasing the curcumin concentrations. However, the percentage of AC with adding TPE in most cases both of five min and ten min with all concentrations of curcumin showed better antioxidant properties compared with other times as well as without adding TPE, reduce time showed better results.

This behavior of increase in AC of concentrations of curcumin with reducing the time of exposure to UV was also noticed through studies carried out by (Sreenivasan & Haque, 2013), but they used an increase in steeping temperature of different types of tea.

In summary, at a highest concentration of curcumin with TPE (presence), to obtain maximum AC at the least time of exposure to UV, in a minimum time of five min with TPE (presence) of curcumin concentration showed that CU0.1, CU0.0001, CU0.01, and CU0.001 respectively, were high in AC (54.6, 22.5, 31.9, and 10.9%), respectively. So, maybe to the short time of exposure to UV with a high concentration of curcumin with TPE will give better antioxidant properties.

Maybe the antioxidant activity of TPE cannot be attributed just to the presence of protein compounds. However, there are several compounds can be contributed to scavenge off the free radical such as phenolic, vitamins, and minerals such as (Se) (Gounder and Lingamallu, 2012).

Regarding the curcumin concentrations, maybe the wavelength of UV-influenced in the structure of curcumin which was negatively affected by antioxidant potential, as well as the long exposure time to UV plays a major role in this effect. Maybe, due to the interactive happened between the free radical and the UV-induced stress in the samples which caused to give pro-oxidant findings.

Maybe agree with a previous study (Ferreira et al., 2016) which has shown when exclusion the UV in Curcuma longa was not affected the curcumin content in the rhizome and also when the plant was grown in full sun or in shadings. As well as, when increasing the exposure time to UV and reducing the concentration level, resulted in negative results. when we add TPE, it can be noticed an increase in the number of significant difference within concentrations. The difference is especially noticeable during the highest exposure time of UV.

Overall, from this findings, indicated that using short time of exposure to UVB and UVA was giving maximum AC. In a low time of exposure to UVB and UVA was obtained a higher AC. Mostly, the UVB exposure showed a best result in AC compared with UVA.

The ABTS assay of radical scavenging activity of TPE from fresh rhizome is shown in (figure 4.6). A higher AC around 6.8% compared with the blank which is showed 3% AC. Whereas, the CU 0.1 concentration 1g/ml showed increased in AC% (20-60%) compared with TPE and blank and much lower AC when compared to that of standard Trolox who showed high percentage of AC of 90-100% at 0.05 g/ml level.

Table 4.12 Effect of time of exposure to ultraviolet radiation on antioxidant capacity of curcumin (0.1 w/v) with and without turmeric polar extract (TPE)

TPE (w/v)	Time (min)	Antioxidant capacity (%)
Present	5	54.6 a
Absent	5	50 a
Present	20	46.4 ab
Absent	15	36.8 bc
Present	10	32.1 c
Absent	10	32 c
Present	15	30.3 c
Absent	20	16 d

[a-d] Dissimilar letters indicate differences ($p<0.05$) in curcumin concentration CU0.1 means within the same column, with and without TPE at a different exposure times to ultraviolet radiation.

Table 4.13 Effect of time of exposure to ultraviolet radiation on antioxidant capacity of curcumin (0.01 w/v) with and without turmeric polar extract (TPE)

TPE (w/v)	Time (min)	Antioxidant capacity (%)
Present	10	31.9 a
Present	5	18.2 b
Absent	5	13.9 bc
Absent	10	2.9 cd
Absent	20	-1.5 d
Absent	15	-4.5 d
Present	20	-12.6 d
Present	15	-12.8 d

[a-d] Dissimilar letters indicate differences ($p<0.05$) in curcumin concentration CU0.01 means within the same column, with and without TPE at different exposure times to ultraviolet radiation.

Table 4.14 Effect of time of exposure to ultraviolet radiation on antioxidant capacity of curcumin (0.001 w/v) with and without turmeric polar extract (TPE)

TPE (w/v)	Time (min)	Antioxidant capacity (%)
Present	5	10.9 a
Absent	5	-1.3 b
Present	10	-1.8 b
Absent	10	-3.3 b
Absent	20	-10.9 c

[a-d] Dissimilar letters indicate differences ($p<0.05$) in curcumin concentration CU0.001 means within the same column, with and without TPE at a different exposure times to ultraviolet radiation

Table 4.15 Effect of time of exposure to ultraviolet radiation on antioxidant capacity of curcumin (0.0001 w/v) with and without turmeric polar extract (TPE)

TPE (w/v)	Time (min)	Antioxidant capacity (%)
Present	5	22.5 a
Absent	10	9.5 b
Present	10	2.8 bc
Absent	5	-4.6 cd
Absent	20	-6.5 cd
Absent	15	-6.8 cd

[a-d] Dissimilar letters indicate differences ($p<0.05$) in curcumin concentration CU0.0001 means within the same column, with and without at TPE different exposure times to ultraviolet radiation

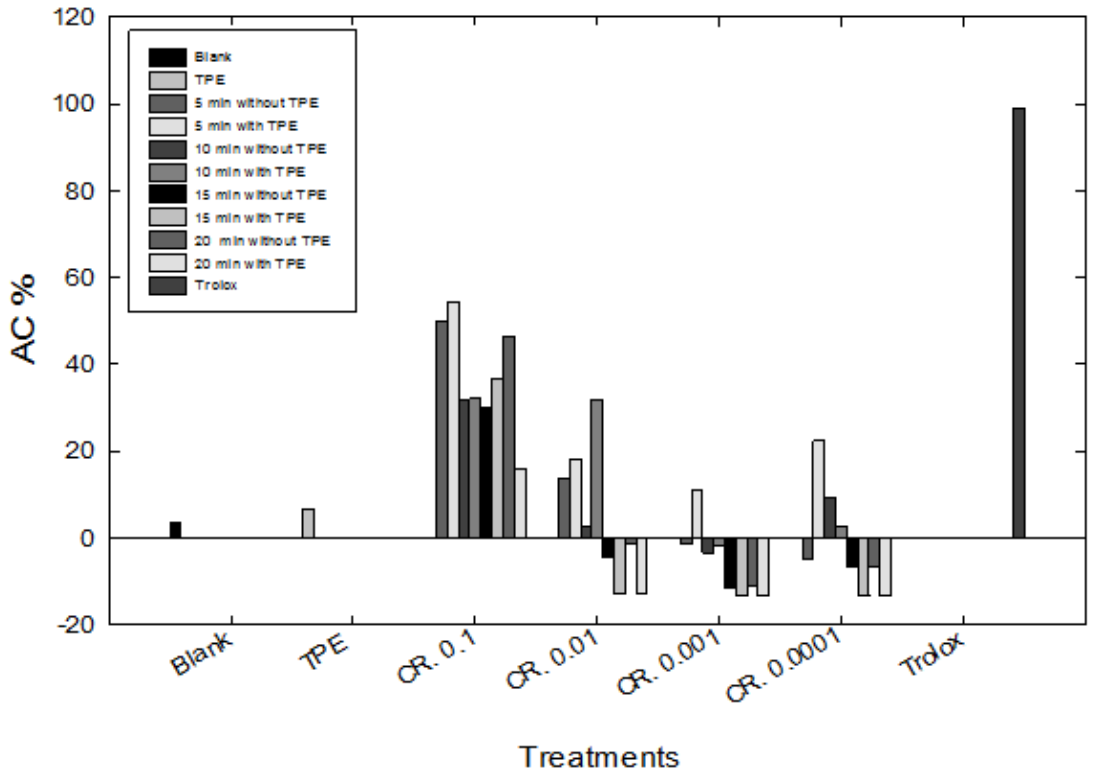


Figure 4.6 Antioxidant capacity (AC) of curcumin with and without turmeric polar extract following different periods of exposure to ultraviolet radiation, indicated by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) decolorization assay.

The x and y-axes represent the treatments of concentration of curcumin (w/v) with or without TPE (w/v) with the ultraviolet at various times and their antioxidant capacities, respectively. The X-axis represents different test samples where TPE is turmeric polar extract, CR is curcumin and the numbers that follow describe the concentration in weight by volume. The Y-axis represents the AC in % compared to Trolox.

CHAPTER V

CONCLUSION

The overall objective was to extract proteinaceous turmeric polar extract (TPE) from the rhizome of curcumin (C. longa) and investigate its antioxidative efficacy without and with purified curcumin (CU), the principal beneficial antioxidative phytochemical in turmeric. Analyses showed that TPE contained 6.68 % of proteins on a dry weight basis along with carbohydrates and ash which are known not to participate in electron/proton acceptance/donation related to anti- or pro-oxidative efficacy. SDS-PAGE showed that TPE contained three proteins with those in the ~10-11 KDa range being dominant. Proliferation of peroxyl and alkoxyl radical in the reaction mixture during the determination of antioxidative activity (AA) and persistence (AP) of TPE (5%, w/v) respectively increased by 87 and 85% compared to the non-redox-active buffer alone (blank). This demonstrated the intense pro-oxidative nature of the TPE proteins. Conversely, CU by itself (0.1% w/v) was dramatically antioxidative with AA and AP values of 2,828% and 1,129%, respectively, compared to the blank. However, combination CU with TPE at the same concentrations respectively reduced these values by 79 and 66% reflecting severe dampening effect of TPE proteins on the antioxidative efficacy of CU. Data clearly demonstrated the detrimental pro-oxidative effect of polar proteins in turmeric powder that must be considered for its therapeutic and nutraceutical applications.

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APPENDIX A

ANOVA TABLE FOR INTERACTIONS

Table A.1 ANOVA - table of interaction between factors after the first pyrolysis during determination of antioxidative activity

Source	DF	SS	MS	F Value	Pr > F
Con	3	139214093.9	46404698.0	3588.85	<.0001
Time	3	91745205.5	30581735.2	2365.14	<.0001
UV	1	1709914.1	1709914.1	132.24	<.0001
TPE	1	176531384.6	176531384.6	13652.6	<.0001
Con*Time	9	45475239.2	5052804.4	390.77	<.0001
Con*UV	3	1254641.6	418213.9	32.34	<.0001
Time*UV	3	36845258.7	12281752.9	949.85	<.0001
Con*TPE	3	80056038.6	26685346.2	2063.80	<.0001
Time*TPE	3	95494542.3	31831514.1	2461.79	<.0001
UV*TPE	1	661.0	661.0	0.05	0.8213
Con*Time*UV	9	20328849.9	2258761.1	174.69	<.0001
Con*Time*TPE	9	47259768.0	5251085.3	406.11	<.0001
Con*UV*TPE	3	2381487.1	793829.0	61.39	<.0001
Time*UV*TPE	3	13691279.2	4563759.7	352.95	<.0001
Con*Time*UV*TPE	9	6726781.8	747420.2	57.80	<.0001
Error	288	3723904.6	12930.2	-	-
Corrected Total	359	911955791.4	-	-	-

Abbreviations refer to: DF, a degree of freedom; SS, sum of squares; MS, mean square. Con, curcumin concentrations; UV, ultraviolet; TPE, turmeric polar extract, significant at ($p<0.05$).

Table A.2 ANOVA - table of interaction between factors after the second pyrolysis during determination of antioxidative persistence.

Source	DF	SS	MS	F Value	Pr > F
Con	3	472120111.1	157373370.4	4308.16	<.0001
Time	3	146527300.6	48842433.5	1337.08	<.0001
UV	1	38347605.9	38347605.9	1049.78	<.0001
TPE	1	539365734.6	539365734.6	14765.3	<.0001
Con*Time	9	69090030.2	7676670.0	210.15	<.0001
Con*UV	3	28766528.7	9588842.9	262.50	<.0001
Time*UV	3	71122700.8	23707566.9	649.00	<.0001
Con*TPE	3	209317402.6	69772467.5	1910.05	<.0001
Time*TPE	3	171813958.3	57271319.4	1567.82	<.0001
UV*TPE	1	52085116.2	52085116.2	1425.85	<.0001
Con*Time*UV	9	34245292.2	3805032.5	104.16	<.0001
Con*Time*TPE	9	80463981.7	8940442.4	244.75	<.0001
Con*UV*TPE	3	45607169.2	15202389.7	416.17	<.0001
Time*UV*TPE	3	17521537.4	5840512.5	159.89	<.0001
Con*Time*UV*TPE	9	12554177.5	1394908.6	38.19	<.0001
Error	288	10520397	36529	-	-
Corrected Total	359	2434824776	-	-	-

ANOVA - table for interaction between factors after the second pyrolysis (AP). All abbreviations are the same A1

APPENDIX B

ANTIOXIDANT ACTIVITY AA

Table B.1 Effect of combining turmeric polar extract with curcumin on antioxidative activity (AA) as reflected by decrease in chemiluminescence as a percentage compare to the blank (buffer alone).

Treatments	Concentration of CU (w/v)	Chemiluminescence maxima[1] in a RLU[2]
CU	0.1	23.2 ± 2.4
CU+TPE	0.1	98 ± 8.4
CU	0.01	77.2 ± 8.5
CU+TPE	0.01	630.1 ± 3.6
CU	0.001	180.4 ± 8.2
CU+TPE	0.001	1267.5 ± 76.8
CU	0.0001	194.6 ± 17.4
CU+TPE	0.0001	1642.3 ± 58.6

[1]Luminol emitted chemiluminescence maxima as a result of unquenched peroxyl and alkoxyl radical.
[2]Relative light units.
CU and TPE are abbreviations for curcumin and turmeric polar extract, respectively.

Table B.2 Effect of concentration of curcumin (CU) and time of exposure to ultraviolet radiation (UV) without and with turmeric polar extract (TPE) on the antioxidative activity as reflected by the chemiluminescence maxima

Treatments	Concentration of CU (w/v)	Chemiluminescence maxima[1] in a RLU[2] with UV Exposure (min)			
		Time of UV exposure (min)			
		5	10	15	20
CU-UVA	0.1	25.2±2.7	55.6 ± 9.8	24± 2.5	27.7±2.7
CU-UVB	0.1	10.5 ± 3.4	15.5±1.2	15 ± 1.8	18.5±4.5
CU-UVA+TPE	0.1	206.2±7.5	223 ± 27.5	69.6± 8.5	239.6 ± 13.1
CU-UVB+TPE	0.1	80±4.5	73 ± 3.7	84.6 ± 8.2	89.8 ± 11.9
CU-UVA	0.01	99.6±4.2	148.3±10.8	129.5 ± 2.7	62.3±6.4
CU-UVB	0.01	52.4±6.2	60 ± 5.2	67.9 ± 2.4	48.5±4.5
CU-UVA+TPE	0.01	1509 ± 97.3	1439.4 ± 71.2	391 ± 10.5	3622.1 ± 306.4
CU-UVB+TPE	0.01	506.1 ± 26	537±36.8	645.5 ± 53	1217 ± 141.5
CU-UVA	0.001	1276.2 ± 80	191.1 ± 8.8	325 ± 9	368.3 ± 18.5
CU-UVB	0.001	163 ± 9.2	551.2 ± 34.3	128.6 ± 3.7	261.6 ± 18.7
CU-UVA+TPE	0.001	3109 ± 175	1614.0 ± 69.4	1086.7 ± 39	6104.2 ± 231.6
CU-UVB+TPE	0.001	1293.2 ± 51.3	3571.8 ± 199.9	171.2 ± 94.8	6117 ± 404.1
CU-UVA	0.0001	1403.4 ± 63.3	213 ± 16.6	403. ± 28.4	394.8 ± 28.1
CU-UVB	0.0001	148.1 ± 5.6	676.4 ± 25.9	157.1 ± 14.2	411.7 ± 22.4
CU-UVA+TPE	0.0001	4342 ± 287.4	1313.2 ± 95.6	1455.5 ± 36.3	6774 ± 353.9
CU-UVB+TPE	0.0001	1468.2 ± 46.1	3625.2 ± 203.01	2020.12± 93.1	5960.3 ± 361.3

Abbreviations are as follows: CU-VA: Curcumin samples of different concentrations exposed to UVA radiation (380 nm) for various time periods. CU-UVB: Curcumin samples of different concentrations exposed to UVB radiation (300 nm) for various time periods. CU-UVA+TPE: Curcumin samples of different concentrations without and with TPE (5%, w/v) exposed to UVA radiation (380 nm) for various time periods. CU-UVB+TPE: Curcumin samples of different concentrations without and with TPE (5%, w/v) exposed to UVB radiation (300 nm) for various time periods.

All superscripts are as explained in the footnote of Table B1.

APPENDIX C

ANTIOXIDANT PERSISTENCE AP

Table C.1 Effect of combining turmeric polar extract with curcumin on antioxidative persistence (AP) as reflected by decrease in chemiluminescence as a percentage compare to the blank (buffer alone).

Treatments	Concentration of CU (w/v)	Chemiluminescence maxima[1] in a RLU[2]
CU	0.1	77.1 ± 1.5
CU+TPE	0.1	209.4 ± 14.4
CU	0.01	81 ± 3.6
CU+TPE	0.01	1189.8 ± 57.6
CU	0.001	368.9 ± 10.8
CU+TPE	0.001	2973.7 ± 70.1
CU	0.0001	629.6 ± 15.3
CU+TPE	0.0001	3106.6 ± 158.9

[1]Luminol emitted chemiluminescence maxima as a result of unquenched peroxyl and alkoxyl radical.
[2]Relative light units.
CU and TPE are abbreviations for curcumin and turmeric polar extract, respectively.

Table C.2 Effect of concentration of curcumin (CU) and time of exposure to ultraviolet radiation (UV) without and with turmeric polar extract (TPE) on the antioxidative persistence as reflected by the chemiluminescence maxima

Treatments	Concentration of CU (w/v)	Chemiluminescence maxima[1] in a RLU[2] with UV Exposure (min)			
		Time of UV exposure (min)			
		5	10	15	20
CU-UVA	0.1	63.3±6.1	86.3 ± 13.1	95 ± 7.6	28.6±4.9
CU-UVB	0.1	31.5±3.6	16.7±1.7	11.6±2	22.6±2.1
CU-UVA+TPE	0.1	248.4 ± 21.1	116.2 ± 13.8	208.1 ± 7.8	559.5 ± 5
CU-UVB+TPE	0.1	121.8 ± 8.9	481.7 ± 36.2	575.6 ± 26.6	284.8 ± 28.4
CU-UVA	0.01	284.4±15.5	165.4 ± 25.4	222.3 ± 16	98.3±5.1
CU-UVB	0.01	74.4±5.3	46.8±4.1	61.5±5.4	74.8±3.4
CU-UVA+TPE	0.01	1976.6 ± 42.5	874.6 ± 49.5	564.6 ± 19.7	4829.7 ± 463.3
CU-UVB+TPE	0.01	822.6 ± 40.8	2218.7 ± 45.1	3105.7 ± 138.5	3007.4 ± 244.8
CU-UVA	0.001	1654.6 ± 44.9	372 ± 18.3	605.6 ± 17.9	747.7 ± 51.5
CU-UVB	0.001	213.9 ± 10.1	1023.5 ± 43.8	912.7 ± 54.8	369 ± 20
CU-UVA+TPE	0.001	3048.8 ± 139.3	2561.5 ±141.6	3087.1 ± 67.7	7425.7 ± 666.3
CU-UVB+TPE	0.001	3014.1 ± 73	7813.8 ± 116	6925 ± 174	10581.1 ± 572.7
CU-UVA	0.0001	2322.2 ± 56.8	560 ± 25	1781.6 ± 31.3	1308.1 ± 85
CU-UVB	0.0001	295.1 ± 9	1682.5 ±20.5	1748.1 ± 36.6	898.6 ± 64
CU-UVA+TPE	0.0001	3850.4 ± 223.1	2475.6 ± 126.5	3191.7 ± 191.02	8385.1 ± 783.8
CU-UVB+TPE	0.0001	2973 ± 66	6309.3 ± 151.5	7989 ± 324.7	8385.1 ± 783.8

Abbreviations are as follows: CU-VA: Curcumin samples of different concentrations exposed to UVA radiation (380 nm) for various time periods. CU-UVB: Curcumin samples of different concentrations exposed to UVB radiation (300 nm) for various time periods. CU-UVA+TPE: Curcumin samples of different concentrations without and with TPE (5%, w/v) exposed to UVA radiation (380 nm) for various time periods. CU-UVB+TPE: Curcumin samples of different concentrations without and with TPE (5%, w/v) exposed to UVB radiation (300 nm) for various time periods.

All superscripts are as explained in the footnote of Table C1.