EFFECT OF SOME FREE RADICALS ON BIOMOLECULES, AND PROTECTIVE ROLE OF SOME ANTIOXIDANT

By

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RESULTS AND DISCUSSION

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ABSTRACT

Eslam Sabry Ahmed Bendary: Effect of some Free Radicals on Biomolecules, and the Protective Role of some Antioxidants. Unpublished Ph.D. Thesis, Department of Agricultural Biochemistry, Faculty of Agriculture, Ain Shams University, 2014.

The scavenging behavior of a series of 36 phenolic, 17 anilines and 11 heterocyclic compounds toward H_2O_2 and DPPH was examined. The efficient concentration (EC_{50}) was calculated for all compounds under investigation by using H_2O_2 scavenging activity assay. The antiradical efficiency (AE), EC_{50} and the antioxidant activity index (AAI) were calculated for all investigated compounds by applying DPPH scavenging activity assay. Wide differences among compounds in each series and among the three series were observed. Where, the most active phenolic compound is 2- naphthol; 2,3-diaminophthaline comes in the first order of anilines compounds; and L-ascorbic acid is the most active heterocyclic compound in H_2O_2 scavenging activity. In H_2O_2 scavenging activity assay, the aniline series are more active than the phenolic series due to the reduction properties of the aniline compounds. While, in the DPPH scavenging activity, the phenolic compounds are more active than the aniline compounds due to the lower bond dissociation energies (BDE) of O-H than that of N-H. Caffeic acid comes in the first order of phenolic compounds in DPPH

scavenging activity, 2,3-diaminophthaline of aniline compounds and α-tocopherol of heterocyclic compounds. The antioxidant activity related to the compound structure was found to be dependable on the number of the included active group (-OH or -NH_2).The more active compound is the more included active groups. The position of the active groups also plays an important role of structure-antioxidant relationship activity. The *ortho* position was found to be the more active one, due to its ability to form intra-molecular hydrogen bonding (iHB), followed by *para* position and then *meta* position of compounds. Also, the presence of double aromatic ring in the chemical structure of phenolic and anilines compounds has a positive effect on the scavenging activity in both assays. In contrast, electron withdrawing group has a positive effect on the activity in H_2O_2 scavenging assay, but it has a negative effect on the activity in DPPH scavenging assay. The correlation between H_2O_2 scavenging assay and DPPH scavenging assay is very weak and reversed in phenolic compounds due to the different reaction mechanisms. The protective role of the most active phenolic and aniline compounds in linoleic acid model was also examined. Wide differences among compounds in each series and between the two series are observed. 1-naphthol and 2-aminophenol come in the first order of phenolic compounds in linoleic acid model and 2,3-diaminophthaline of aniline compounds. While, α-tocopherol is the most active compound in linoleic acid. The hydrophobic compound has more protective role than hydrophilic compound. Also, hydrogen atom transfer (HAT) mechanism is more favorable than singlet electron transfer (SET) mechanism.

Key Words:

Antioxidants, free radical, phenolic compounds, aniline compounds, heterocyclic compounds, H_2O_2 Scavenging, DPPH scavenging, H_2O_2 scavenging and structure-activity relationships (SARs), DPPH, antioxidant reaction mechanism, The efficient concentration (EC_{50}), The antiradical efficiency (AE), the antioxidant activity index

(AAI), Lenoleic acid model.

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INTRODUCTION

Oxidation and production of free radicals and reactive oxygen-containing species (ROS) are an integral part of life and metabolism of our body. Actually, free radicals and ROS may be produced in our body in deliberately use to kill some strains of invading bacteria and fungi. Superoxide plays a useful role in the regulation of cell growth and intercellular signaling. Free radicals and ROS are useful, however, only when they are produced at the right amount, place and time. Otherwise, they can be very damaging because they are extremely reactive and almost instantly attack molecules, which are very close to them. Therefore, free radicals and ROS react with non-radicals and can initiate adverse chain reactions such as lipid peroxidation. They also damage other important molecules including proteins, carbohydrates and DNA.

In order to defend against damage from free radicals and ROS, humans and other living organisms develop powerful and complex antioxidant systems. Components of these systems are antioxidants, a diverse group of molecules that protect key biological sites from oxidative damage. They usually act by removing or inactivating chemical intermediates that produce free radicals. Antioxidants are either produced in the body (endogenous) or derived from the diet.

Clinical trials and epidemiological studies have established an inverse correlation between the intake of fruits, cereals and vegetables and the occurrence of diseases such as inflammation, cardiovascular disease, cancer, Alzheimer's, and aging-related disorders ***Halliwell,*** **(1992)** and ***Willet,*** **(2001**). Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases, **(*Ames et.al.,* 1995** and ***Kaur and Kapoor,*** **2001**).

There is increasingly growing market for nutraceruticals and functional food. Products containing nutraceuticals have reached a worldwide estimated value of

$65 billion. ***Lachance,*** **(2002**). Antioxidants have thus become a topic of increasing interest recently. A literature search revealed that the number of publications on antioxidants and oxidative stress have nearly quadrupled in the past decade (1684 in 1993; 6510 in 2003).

Antioxidants are compounds that, in low concentration, can prevent biomolecules (proteins, nucleic acids, polyunsaturated lipids, sugars) from undergoing oxidative damage through free radical mediated reactions. They can inhibit oxidizing chain reactions in several ways, including direct quenching of reactive oxygen species, inhibition of enzymes, and chelation of metal ions (Fe^{+3}, Cu^{+}). Their beneficial effects are related to diseases in which oxidative processes are remarkable, i.e., atherosclerosis, coronary heart disease, certain tumors, and aging itself. **(*Luximon-Ramna, et. al.,* 2003*, Toyokuni, et. al.,* 2003*, Caia, et. al.,* 2004 and *Romani, et. al.,* 2004).**

In fact, a diet rich in fruits, vegetables, cereals, and olive oil can prevent cardiovascular diseases and certain forms of cancer. The major antioxidant components of these common foods are the phenolic compounds. Their antioxidant activity seems to be related to their molecular structure, more precisely to the presence and number of hydroxyl groups, and to conjugation and resonance effects, (**Rice-Evans, *et. al.* 1996**). Recently, a quantum-mechanical investigation has shown that the antioxidant action of flavonols is related to radicals showing a planar conformation that allows extended electronic delocalization between adjacent rings, **(*Russo et. al.* 2000)**.

The major plant phenolics antioxidants can be divided into 4 general groups: phenolic acids (gallic, protochatechuic, caffeic, and rosmarinic acids), phenolic diterpenes (carnosol and carnosic acid), flavonoids (quercetin and catechin), and volatile oils (eugenol, carvacrol, thymol, and menthol). Phenolic acids generally act as antioxidants by trapping free radicals; flavonoids can scavenge free radicals and chelate metals as well, **(*Engeseth and Geldof,* 2002).**

The purpose of the present study is to evaluate the antioxidant activity of some

phenolic compounds, aniline compounds and heterocyclic compounds by two different methods. H_2O_2 scavenging activity and DPPH scavenging activity assays were used to determinate the antioxidant activity. The efficient concentration (EC_{50}) and Antiradical efficiency (AE) were calculated for all tested compounds. The comparison among EC_{50} values and among (AE) values was made to evaluate the most active group of compounds and the most active compound inside each group. The relationship between the chemical structure and the antioxidant activity was studied. Finally, the protective role of the most active tested groups using linoleic acid model was studied. The efficient concentration (EC_{50}) was calculated for all the tested compounds. The comparison among EC_{50} values was made. The relationship between the chemical structure and the protective role was studied. Correlation studies were made to determine the suitable antioxidant reaction mechanism for each tested compound.

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REVIEW OF LITERATURE

The two main parameters of this investigation are the free radicals and the antioxidants activity, so, the review of literature is presented under these two main topics as follows:

I- FREE RADICALS

I.1- Free Radicals and Reactive Oxygen Species (ROS) Nature

***Gutteridge* (1987)** reported that hydroxyl radical produced in living cells among ROS is the most active and strongest oxidizing agent and can react with almost substances at diffusion rate.

***Huang et al.* (2005)** reported that experimental evidence has directly or