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Evaluation *in-vitro* the antioxidant activity of some dithiolethiones

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ABSTRACT

The dithiolethiones are classified as an antioxidant compounds where it was noted that eating foods containing these compounds has led to a significant decline in cancer patients. The aim of the present study is to determine the antioxidant activity of the two derivatives of dithiolethiones were evaluated by phosphomolybdenum assays. The results showed that all dithiolethiones to be good reducers with AEAC values ranged between 1.73 and 3.097M.

Keywords: Dithiolethiones, Antioxidant activity, AEAC, phosphomolybdenum assays.

INTRODUCTION

Free Radicals are molecules with an unpaired electron and are important intermediates in natural processes involving cytotoxicity, control of vascular tone, and neurotransmission. Free radicals are very unstable and react quickly with other compounds, and try to capture the needed electron to gain stability. A chain reaction thus gets started. Once the process is started, it can cascade, and finally results in the disruption of a living cell.The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including cancer [1], cardiovascular disease [2], neural disorders[3], Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis [4].

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged [5]. Research on relationships between antioxidants and prevention of non-communicable disease such as cardiovascular disease has been increasing sharply in recent years.

Epidemiological studies suggest that high dietary intake of fruit and vegetables protects against tumorigenesis in many tissues, including the colon, but little is known about the mechanisms by which these types of food serve as cancer chemopreventive agents in humans.

Among vegetables with anticarcinogenic properties, members of the Cruciferae family, particularly those of the *Brassica* genus, such as broccoli, Brussels sprouts, cabbage, and cauliflower, appear to be most effective at reducing the risk of colorectal cancer [6].

Crucifers that are widely consumed are especially rich in several organosulfur compounds including isothiocyanates and dithiolethiones[7].

A number of isothiocyanates and a limited number of glucosinolates that were examined effectively block chemical carcinogenesis in animal models. Many isothiocyanates are also potent inducers of phase 2 proteins. Substantial

evidence supports the view that phase 2 enzyme induction is a highly effective strategy for reducing susceptibility to carcinogens [8].

Dithiolethioneshave been established as anticarcinogens, and protect against radiation injury [9] and hepatotoxicity induced by carbon tetrachloride and acetaminophen [10]. All of these pathologies are suspected to have an oxidative stress-based etiology. This suggests that the protective effect of sulferlem under those conditions may be related to an antioxidant function of the drug. Our studies show that a protective effect of sulferlem against oxidative lipid damage has been evident.

In previous article[11], we reported that dithiolethiones have a higher chelation activity to chelate ferrous ions which the metal chelation capacity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation.

The other aim of our work was to determine the antioxidant activity of some dithiolethiones with the phosphomolybdenum method that was proposed by P. Prieto et al.[12].

MATERIALS AND METHODS

Phosphomolybdenum Assay (PM)

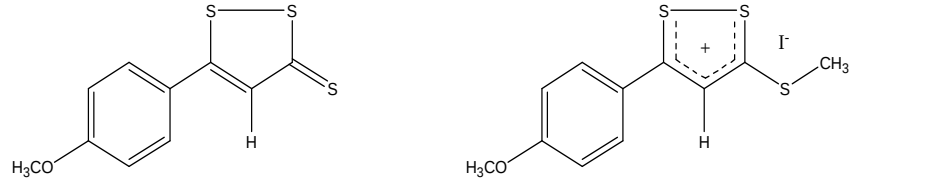
An aliquot of 0.1 ml of sample solution containing a different concentration of dithiolethiones dissolved in ethanol was combinedin tube with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the ethanolic solution of each was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid according to a new term called AEAC (Ascorbic Acid Equivalent Antioxidant Capacity)

We define the AEAC as the molar concentration of the ascorbic acid has a reducing power equivalent to concentration of 1Mofdithiolethiones.

RESULTS AND DISCUSSION

The antioxidant activity of 5-p-methoxyphenyl-1,2-dithiol-3-thione (sulferlem) and3-methylthio-5- methoxyphenyl-1,2-dithiol-3-thionecontre ion (I^-) was evaluated by the phosphomolybdenum method according to the procedure describe by Prieto et al.[12]. The assay was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green phosphate/Mo (V) complex at acidic pH with the maximal absorption at 695 nm. Higher absorbance indicates a higher total antioxidant capacity. Total antioxidant capacity of dithiolethiones are summarized in Table 1.



5-p-methoxyphenyl-1,2-dithiol-3-thione 3-methylthio-5- methoxyphenyl-1,2-dithiol-3-thionecontre ion (I^-)

Figure 1: The structure and name of dithiolethiones compounds

The 3-methylthio-5-methoxyphenyl-1,2-dithiol-3-thionecontre ion (I^-) showed higher phosphomolybdenum reduction (3.097 M) while the 5-methoxyphenyl-1,2-dithiol-3-thionegive a low capacity antioxidant (1.73M) .

Table 1: reducing power of dithiolethiones

Dithiolethiones	AEAC (M)
5-methoxyphenyl-1,2-dithiol-3-thione	1.73
3-methylthio-5-methoxyphenyl-1,2-dithiol-3-thionecontre ion (I^-)	3.097

According to the results of the antioxidant capacities, the salt of sulferlem3-methylthio-5-methoxyphenyl-1,2-dithiol-3-thionecontre ion (I^-) is able to reduce the Mo(VI) to Mo(V), this may be explained by the fact that the transfer of electrons from antioxidants depends on the structure of the antioxidants.

Ionization potential (IP) is one of the major factors that determine the mechanism and the efficacy of antioxidants, The estimated IP (Ionization potential) could provide an understanding of the initial energy required to release an electron from the compounds [14].In previous article[11], we found the IP of 3-methylthio-5-methoxyphenyl-1,2-dithiol-3-thione contre ion (I^-) is 190.064 Kcal/mol and for 5-methoxyphenyl-1,2-dithiol-3-thione is 2014.223 Kcal/mol. The lower of the ionization potential (IP), the easier is the electron abstraction[13].This is what leads to the compound, which has the lowest ionization potential shows the highest anti-oxidant capacity.

CONCLUSION

The antioxidant activities were evaluated by phosphomolybdenum assays, and the results indicated that these compounds have good scavenging activities. The increased concentration of dietary foods containing antioxidative nutraceuticals can help humans reduce the deleterious reactive oxygen species and free radicals.

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