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Investigation of Antioxidant Activity in Thiocarbamides using the DPPH Free Radical Scavenging Assay

P.L. Harale[1], M.E. Shelke[2], D.T. Tayade[3]

[1]Research Centre and Department of Chemistry, Padmashri Vikhe Patil College, Pravaranagar.

[2]Department of Chemistry, Govt. Vidarbha Institute of Science and Humanity, Amaravati.

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Abstract: The DPPH free radical scavenging method is a well-known and trustworthy technique for assessing chemical compounds' antioxidant potential. The color change of thiocarbamate compounds using DPPH (2,2-Diphenyl-2-Picrylhydrazyl) and their percentage scavenging activity with strong absorption at 517 nm were used to assess their antioxidant capacity. Compared to ascorbic acid, ultimate thiocarbamate compounds showed remarkable antioxidant activity and unmatched percentage scavenging activity, as well as reduced IC50 values (effective concentration for scavenging 50% of the starting DPPH value for µM).

Keywords: *Antioxidant activity, Thiocarbamides, DPPH method, IC50 value*

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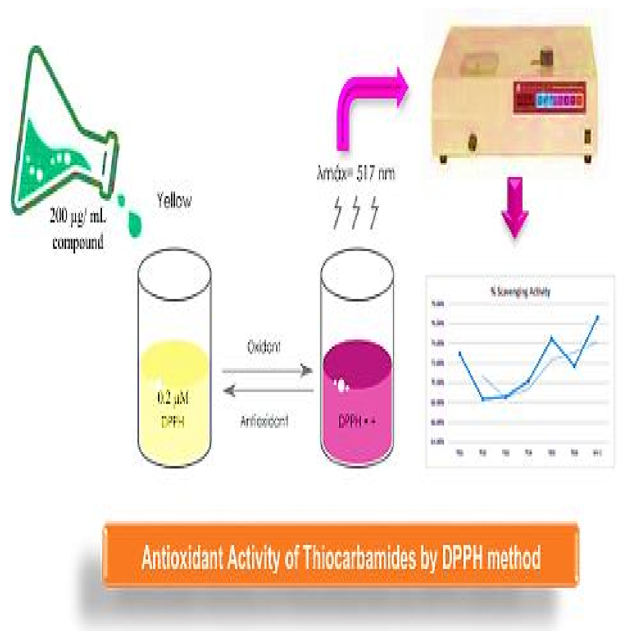
I.INTRODUCTION:

Oxidative stress is widely understood for its role in aging and the development of human diseases, particularly cancer, cardiovascular problems, metabolic disorders, diabetes, autoimmune diseases, and neurological disorders. An imbalance between the generation of reactive oxygen species and the natural antioxidant defense system's ability to defend against free radicals and prevent the oxidation of lipids and proteins is known as oxidative stress. Every part of a plant's and animal's life depends on oxygen. The majority of the oxygen combines with hydrogen to generate water throughout the metabolic process, while 4-5% of the oxygen forms superoxide anions. The metabolism of water can produce hydrogen peroxide, which is made up of free radicals. Since oxygen is the most accessible source of electrons in a living organism, oxygen radicals will be produced. Free radicals will target Cell membranes and exhibit significant oxidative activity and vivacious activity due to their instability. When they interact with unsaturated fatty acids found in the cell membrane, lipid peroxidation rises and free radical chain reaction takes place. Reactive oxygen species can be produced by aerobic organisms, which also have an active oxygen scavenging mechanism (antioxidant enzymes and antioxidants) that may transform active oxygen into less active compounds. There is protection for the basal body. Several kinds of cell destruction are associated with reactive oxygen species, among which are superoxide anions (O2-), hydrogen peroxide (H2O2), hydroxyl radicals (OH), and lipid peroxidation, which requires a sequence of chain reactions initiated by free radicals[1-4].Thiocarbamide compounds have wide spectrum applications in various fields such as medicinal, pharmaceutical, agriculture, and synthetic chemistry [5-9]. An antioxidant prevents the oxidation of molecules inside a cell. Free radicals produced during the biological oxidation chain reaction can lead to damage to a cell. Antioxidants act as reducing agents by terminating a biological chain reaction and eliminating free radical mediates, also preventing oxidative damage and protecting cells from severe body complaints [10-13]. Researchers are constantly working on synthesizing compounds with highly potent antioxidant activity [14-19]. The DPPH test is one of the very stable and reliable free radical scavenging methods to measure the antioxidant activity of organic compounds [20-25]. DPPH is reduced by accepting hydrogen from hydrogen donor compounds and free radical scavenging compounds reduction shows a colour change from violet to yellow. A study of antioxidant activity synthesized compounds was done by calculating its % scavenging activity values and half-maximal inhibitory concentration values or effective concentration for scavenging 50% of the initial DPPH value (IC_{50} value) [26-28].

In the present study, the antioxidant activity of novel substituted thiocarbamide compounds was investigated by using the DPPH free radical scavenging method and comparing its activity with ascorbic acid. The fields of organic transformations and antioxidant synthesis, which protect plants and animals from negative effects brought on by aging and other stresses, are dependent on the discovery of new synthetic antioxidants.

Graphical Abstract:



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2. Experimental Section:

2.1. General

All synthesized substituted thiocarbamide compounds were purified by recrystallization with ethanol. DPPH (2,2-Diphenyl-2-Picrylhydrazyl) and ascorbic acid of AR grade were used. Micropipettes are used for the preparation of the microliter solution. Absorption at 517nm was measured using a Systronic (2202 model) make Double-beam UV Spectrophotometer.

2.2. Antioxidant activity / DPPH free radical scavenging activity

Novel thiocarbamide compounds displayed antioxidant activity and were determined by using free radical scavenging against 2,2-Diphenyl-2-Picrylhydrazyl (DPPH). 0.2 µM DPPH solution was prepared in 95 % ethanol and used as the negative control. Then, different substituted novel thiocarbamide compounds 200 µg/ mL were mixed with DPPH solution which was then incubated in the dark for 30 min. at room temperature. Vitamin C is used as a standard for comparison because of its high inhibition ability with the presence of OH group stabilized free radical. The absorbance of the reaction mixtures was then measured at 517 nm. By using a spectrophotometer. Plotting the % scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

% scavenging activity = [(Abs. Blank−Abs. Sample) / Abs. Blank] ×100.

Thiocarbamides have significant antioxidant activity show low IC50 value (half-maximal inhibitory concentration) by the DPPH method.

Table 1: Antioxidant activity expressed as IC50 values of TD5 thiocarbamides

Concentration (µg/ml) of TD5	% Scavenging Activity	IC50 value
50	45.03	1.44
100	56.12	6.57
150	65.48	11.70
200	74.44	16.84
250	85.58	21.97
300	93.73	27.11

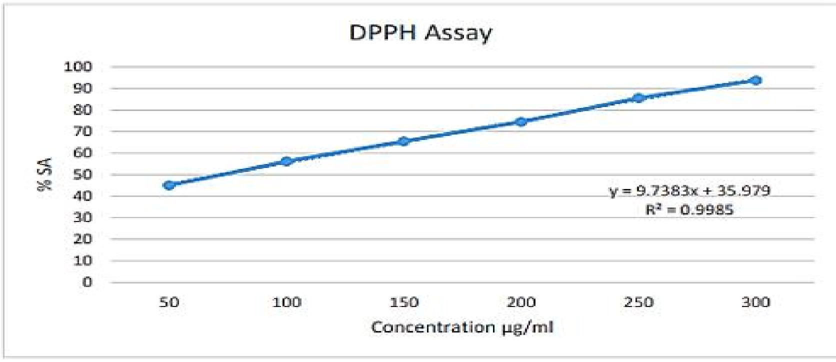
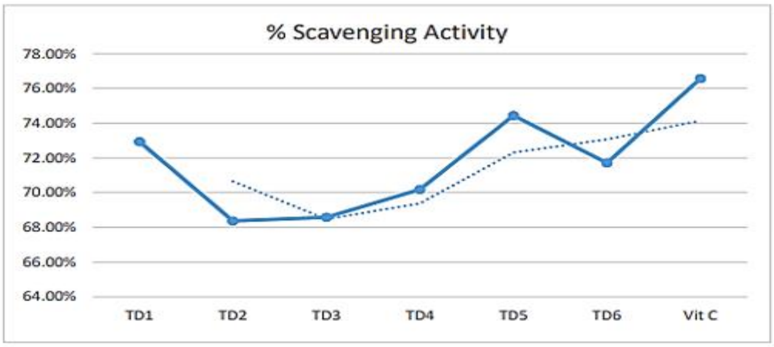


Table 2: Antioxidant activity of different thiocarbamides TD1-TD6

Thiocarbamide Derivatives 200 (µM) solution	Ph-R	Abs.	% Scavenging Activity at 200 (µM)
TD1	-H	0.254	72.94
TD2	-Me	0.297	68.37
TD3	-Allyl	0.295	68.58
TD4	-Ph	0.289	70.18
TD5	-PClPh	0.235	74.44
TD6	-mClPh	0.265	71.71
Vit.C		0.220	76.57



3. Result and Discussion:

Novel thiocarbamide compounds were investigated for their free radical scavenging ability in ethanol by using DPPH assay as DPPH radical changes from Purple to yellow color when quenched by antioxidants. DPPH radical scavenging was monitored by a Spectrophotometer at wavelength 517 nm. Most of the derivatives showed notable antioxidant properties as compared to Vitamin C. The Order of the activity depends on the free radical stability form in the derivatives.In the present research work, all the thiocarbamide compounds showed excellent antioxidant activity with minimum IC50 values and at the lower concentration due to the presence of the N-H group in its structure.

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Declaration:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: This has not been published elsewhere and is not currently under consideration for publication elsewhere

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