Estimation the Scavenging Activities of Ascorbic Acid, Uric Acid, Gallic Acid and GSH to DPPH Radical

Ali M.A. Al-Kufaishi

Al-Furat Al-Awsat Technical University College of Health and Medical Techniques, Department of Medical Laboratory Techniques, Kufa, Iraq

Abstract

The "α,α-diphenyl-β-picrylhydrazyl radical **(**DPPH)" method from the estimation methods to determine the antioxidants activity in vitro, through the lowering the absorbance of DPPH with color changing from violet into yellow with increasing the strength of antioxidants activity. So, the results of this study show the uric acid is the strongest scavenging, then ascorbic acid when compared with other compounds used. But the reduced glutathione is the weakest although has acidic hydrogen.

Keywords: *DPPH, scavenging activity, antioxidants, Ascorbic Acid, Uric Acid, Gallic Acid.*

Introduction

The efficiency of antioxidants (scavenging activity) depends on the ability of free radicals scavengers (FRS) to given hydrogen to the free radicals [1]. The increase in rate and energy promotion for hydrogen ion transition from FRS to free radicals depend on the lowering in energy level for hydrogen bond with FRS. Efficient FRS results free radicals after donating hydrogen but not react rapidly with oxygen to produce superoxide. The efficiency of FRS as well depends on other factors as volatility, pH sensitivity and polarity [2].

In the present study, the antioxidants involve such as vitamin C, uric acid, gallic acid, and reduced glutathione.

Ascorbic acid (AA):

One from most powerful FRS [3,4]. It is found in several plants cells types. AA present in the reduced form under physiological conditions (90% of the ascorbate pool) in plants leaves and chloroplasts [5], were the concentration of AA (20 mM) in the cytosol, and (20-200 mM) in the chloroplast stroma [6]. The ability of AA to give the electron by many enzymatic and nonenzymatic reactions, therefore act as main detoxification antioxidants against ROS in the aqueous phase. So, the AA can directly be scavenging for singlet oxygen, superoxide radical, hydroxyl radical and reduced hydrogen peroxide to water by ascorbate peroxidase reaction [7].

AA capable to reduce tocopheroxyl radical into tocopherol that responsible about cell membrane protection. AA performs a number of non-antioxidant roles in the cells. It has been involved in the regulation of cell cycle progression from G1 to S phase, cell division, and cell elongation [8,9].

Glutathione (GSH):

"Tripeptide glutathione (□-glutamylcysteinylglycine)" is a plentiful compound in the tissues of plants. It is found in all compartments of the cell such as cytosol, mitochondria, and endoplasmic reticulum [10], were GSH has many roles in organisms such as storage for the sulphur, serve as precursors for phytochelatins, act as a detoxifier for xenobiotics via GSH-conjugation [11,12], and maintenance about redox form of the cellular membrane through (GSH-GSSG) system. Also, GSH has the ability to regulate gene expression. And regulation of cell cycle by GSH/GSSG system through -SH group [13,14].

GSH act as antioxidants through the nucleophilic center of –SH residue that responsible for higher reductive potential. So, its scavenger for singlet oxygen, superoxide radical and hydroxyl radical by non-enzymatic reactions, and with hydrogen peroxide and reducing to water by an enzymatic reaction [15]. GSH

has the ability to regenerate others antioxidants such ascorbic acid through the ascorbate-glutathione cycle [16,17].

Uric Acid (UA):

The final product of purine degradation in human and "Great Apes". It is a powerful scavenger for free radical such as singlet oxygen, hydroxyl radicals (.OH), and peroxyl radicals (RO. 2) [18,19]. In blood, stream urate considers from the major antioxidants against oxidative damage, so it is protected RBCs membrane from lipids peroxidation by scavenging oxygen radicals in aqueous media [20]. Uric acid some of the deleterious reactions, such as peroxide production by macrophages or autoxidation of haemoglobin [21].

In contrast, elevated the UA causes a lot of number from the epidemiology of hypertension [22], cardiovascular disease [23], visceral obesity [24], dyslipidemia [25], insulin resistance [26], kidney disease [19].

Gallic Acid (GA):

"GA is a 3,4,5-trihydroxybenzoic acid and its derivatives are widely spread in the plant kingdom and is a large family from secondary polyphenolic metabolites" in plants so it is from natural antioxidants [27]. GA present either methylated gallic acid form such as syringic acid, or in the orgalloyl conjugated form with catechin derivatives as "flanvan-3-ols, or in polygalloyl esters" form with glycerol, quinic acid or glucose [28].

The last two groups from "polyphenols are known as vegetable tannins, which their names derived from its ability to transform animal skins into leather" through the with collagen [29]. GA from the components of tea and some types of GA used as food additives to prevent peroxidation. Also, it is used in many phytomedicines due to its biological and pharmacological activities through the ability to free radicals scavenger [30], inducing apoptosis for the cancer cells [31,32], and inhibiting squalene epoxidase interfering the signal pathways involving calcium [33,34].

α,α-diphenyl-β-picrylhydrazyl radical (DPPH):

DPPH is described as relatively stable free radical due to delocalization phenomenon for the unshared electron overall whole molecule, so this radical do not suffer from dimerization as other radicals. The delocalization gives this radical the deep violet color when dissolved in absolute ethanol and absorbed at 517 nm. DPPH used to estimation the antioxidants activity for any sample or free radicals scavenging material [35].

Material and Methods

Prepared Serial Solutions:

Prepared Ascorbic acid solutions:

This is done by dissolving ascorbic acid in distilled water (D.W.) as the following concentrations: 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 mg/dl.

Prepared GSH solutions:

This is done by dissolving GSH in distilled water (D.W.) as the following concentrations: 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 mg/dl.

Prepared UA solutions:

This is done by dissolving UA in distilled water (D.W.) (pH 8.5) as the following concentrations: 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 mg/dl.

Prepared GA solutions:

This is done by dissolving GA in absolute ethanol as the following concentrations: 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 mg/dl.

DPPH procedure:

DPPH preparation:

Prepared 0.1 mM from DPPH, by dissolving appropriate weight in absolute ethanol.

Principle of method:

The principle of this method depends on the reduce the absorbance values of DPPH after addition the sample due to conversion the violet colour into yellow colour depending on the antioxidants activity of the sample. The colour intensity measured at 517 nm (36) (figure 1).

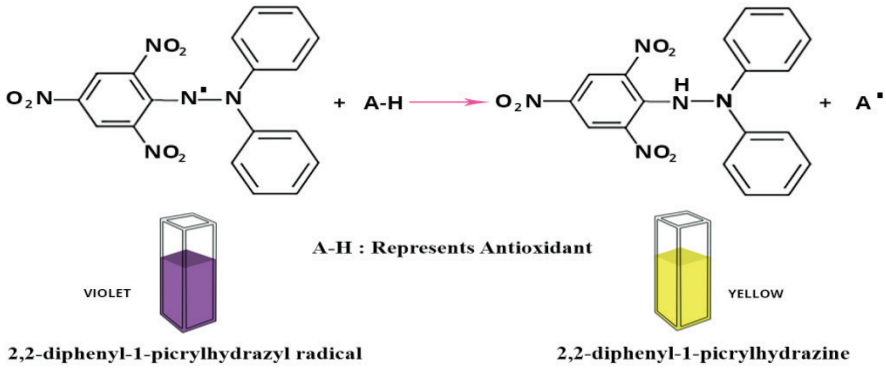

Fig. 1: Reduction effect of AH to DPPH.

Procedure: The procedure of addition as following:

Reagent	Sample/µl	Blank/µl
DPPH	1000	1000
Sample	100	-------
D.W.	-------	100

Incubate for 20 min at 25ºC. In the dark condition, then read the absorbance at 517 nm.

Results and Discussion:

The following results (Table 1) and (Figure 2) the inhibition values of DPPH by the antioxidants compounds. In other words, the absorption values for DPPH after scavenging by ascorbic acids, GSH, UA, and GA.

Table 1: Absorption values for DPPH after scavenging by UA, GA, GSH, and ascorbic acid.

Concentration (mg/dl) of U.A, Gallic acid, GSH, Ascorbic acid	UA	Gallic acid	GSH	Ascorbic acid
2.5	0.302	0.323	0.352	0.314
5	0.289	0.321	0.352	0.292
10	0.280	0.320	0.351	0.288
15	0.275	0.315	0.344	0.286
20	0.269	0.311	0.344	0.280
25	0.259	0.310	0.344	0.279
30	0.251	0.303	0.343	0.274
35	0.247	0.291	0.343	0.268
40	0.243	0.290	0.343	0.261
45	0.238	0.289	0.332	0.254
50	0.211	0.262	0.332	0.238
55	0.193	0.249	0.332	0.226
60	0.169	0.232	0.332	0.212
65	0.145	0.221	0.332	0.195
70	0.123	0.214	0.332	0.185

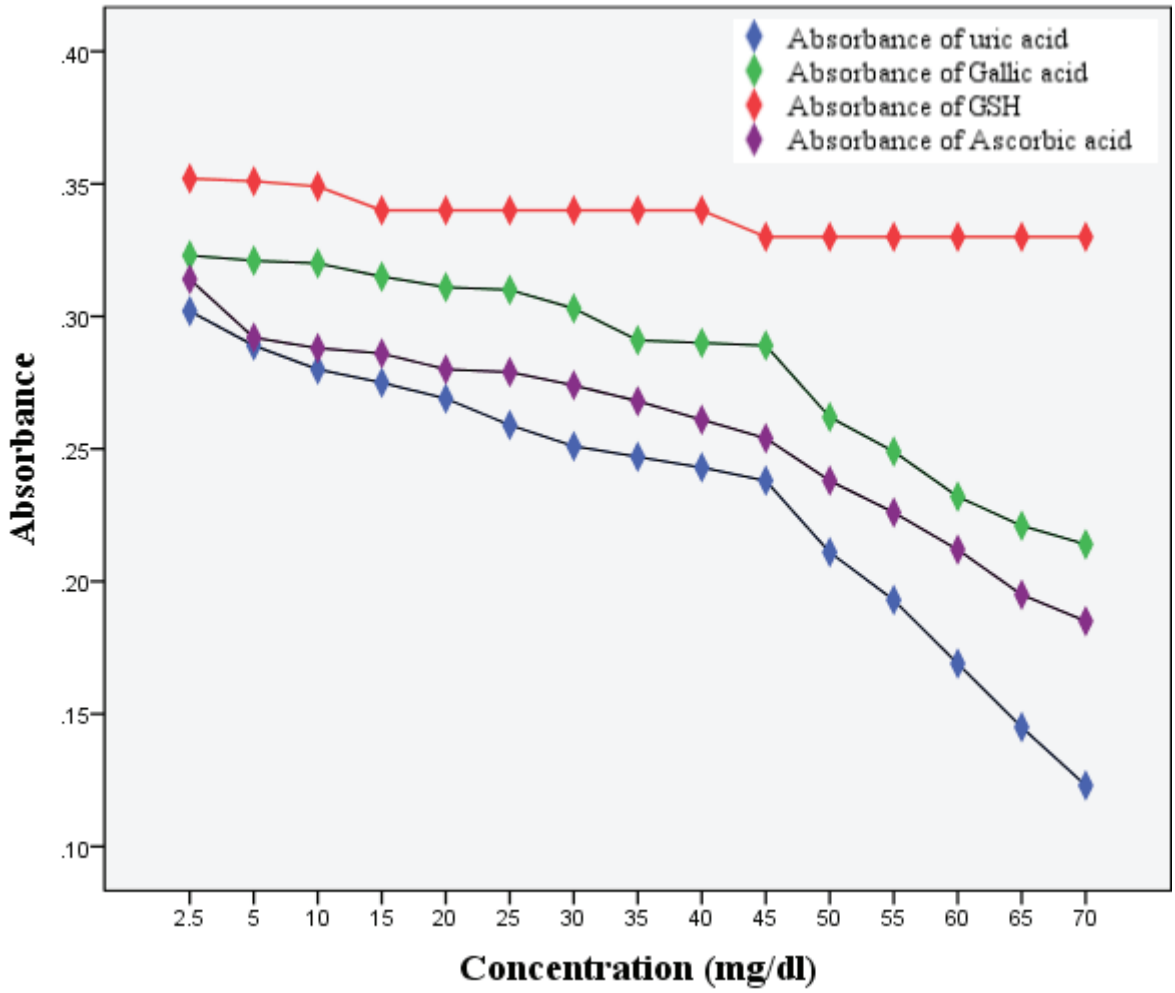

Fig. 2: Decline in absorption values for DPPH after scavenging by UA, GA, GSH, and ascorbic acid.

The scavenging effect for UA toward DPPH is more than other antioxidants were used in this study when compared in the same concentrations, may be due to the heterocyclic ring effect that responsible about delocalization and lowering energy of hydrogen bond with the compound (figure 3).

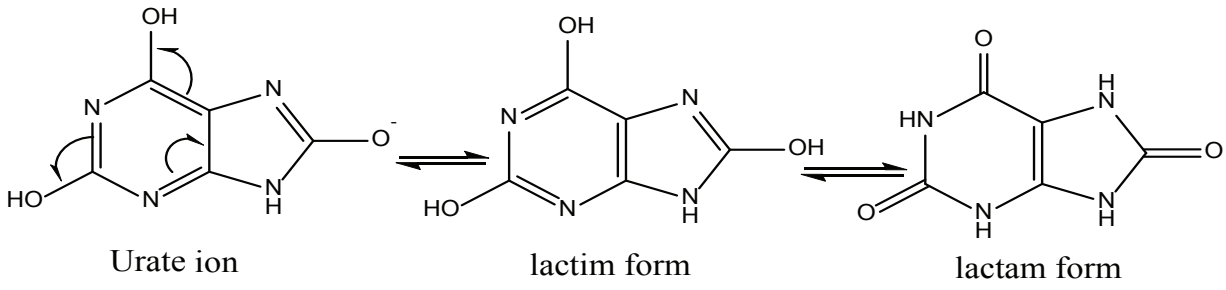

Fig. 3: Urate tautomerism

From the results show a slight decrease in the line chart (figure 2) for the GSH value, this means it is the weakest scavenging compared with other compounds used in this study may be due to the energy bond of hydrogen in the thiol group is relatively strongest than other compounds, therefore no change in the color of DPPH. However, the scavenging effect increased with an increase in concentrations of these antioxidants compounds.

Conclusion

In conclusion, the strongest scavenging in vitro the compounds that have the ability to reduce the DPPH and change its the color from violet into yellow, therefore the scavenging strength follow the sequence UA, AA, and GA, where the GSH is very weakest scavenging to DPPH at the same concentrations.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest

The authors declare that they have no conflict of interest.

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