Synthesis and Antioxidant Activity Evaluation of New Organic Reagent 2-\[2^−-(5-Nitro thiazolyl)azo]-4,6-dimethoxy benzoic acid (NTADMBA).

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Abstract. This study concerned synthesis and characterized new organic reagent 2-[2^−(5-nitro thiazolyl)azo]-4,6-dimethoxy benzoic acid (NTADMBA) and also its antioxidant, biomolecule protective. The organic reagent was characterized by FTIR, NMR and mass spectroscopy. The antioxidant tests showed the enhanced potency of E. coli bacteria. The study suggests that the organic reagent (NTADMBA) herb unequivocally is a potential source of antioxidants and could aid in alleviating oxidative stress-mediated disorders.

1. Introduction:
Thiazolylazo dyes are sensitive and selective chromogenic reagents. On the other hand to being interesting complexing agents, and have been comprise the largest group of it used as organic reagent in spectrophotometric analysis, solid phase extraction [1,2], and liquid chromatography [3]. These uses are due to that its distinguished by chromophoric azo group (-N=N-) offering a wide range spectrum of colors. The application in spectrophotometry is depends on the colored compounds resulting from their reaction with most metals, particularly some transition metals, frequently stable chelate complexes are produced[4]. They have been used in separation procedures, because of their low solubility in aqueous solution but higher in organic solvent. Some of them have also demonstrated to be particularly useful as indicators in complex metric titrations. They are found in a various groups of industrial applications because of their color fastness. Its used for coloring consumer goods such as leather, clothes, food, toys, plastic and cosmetics[5]. In this study we applied this reagent as antioxidant. Where the reactive oxygen species (ROS) comprise free radicals, such as superoxide anion (O^2−) and hydroxyl (HO ·) radicals, and non-free radical species, such as H2O2 and singlet oxygen (‘O2), and these comprise Various formatsof activated oxygen [6]. ROS are known to damage cellular membranes by Stimulating lipid peroxidation, damage DNA, proteins and lipids [7]. Thus, over production of free radicals/ROS cause to oxidation of biomolecules, and this has been implicated in the etiology of several human diseases, such as atherosclerosis, ischemia reperfusion injury, ageing and liver-related diseases [8]. To fortify the cells and organ systems of the body from free radicals/ROS, the human body has developed a highly sophisticated and complex antioxidant
protection system. Antioxidants are capable of stabilizing, or deactivating, free radicals before they destruction cells. The body’s antioxidant vindication system comprises endogenous antioxidant enzymes, viz. superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, etc., that catalyze free radical quenching reactions. In addition, dietary antioxidants, like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids and other low molecular weight compounds, such as glutathione and lipoic acid, also aid in stabilizing free radicals [9,10]. Plant-derived substances, combined called phytoneutrients or phytochemicals, are known for their antioxidant activity [10]. Attrition of natural antioxidants is erlated with miniured risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing [11].

2. Methods and Materials

2.1. Materials

2-Amino-5-nitrothiazole(C₃H₃N₃O₂S) from Cheng Du Micxy Chemical, 2,4-Dimethoxy benzoic acid (C₁₂H₁₀SN₄O₆) from Bidepharmtech, sodium nitrate (NaNO₂), nitric acid(HNO₃), Formic acid(CH₂O₂), Sulfuric acid(H₂SO₄) from B.D.H, Ferric chloride (FeCl₃), Ascorbic acid(C₆H₅O₆), Pyrdine(C₅H₅N), Methanol(CH₃OH), Hydrochloric acid(HCl) from Fluka, Sodium hydroxide (NaOH), Hydrogen Peroxide(H₂O₂), Ethanol Absolute (CH₃CH₂OH) from Scharlau, Agarose, Etidium bromide(C₂₁H₂₀BrN₃) from Bio Basic Canada Inc., TBE buffer from Bioneer.Korea. All the reagents used were analytical grade pure with no further purification, and all the solutions were prepared with deionized water.

2.2. Synthesis of 2-[2-(5-Nitro thiazolyl)azo]-4,6-dimethoxy benzoic acid (NTADMBA)

The azo reagent NTADMBA was Synthesis by dissolving 2-Amino-5-nitrothiazole(3.38 gm, 0.01 mol) in a mixture consisting of (6) ml of concentrated sulfuric acid (8) ml of formic acid, and its was cooled to (3) oC. Added this mixture to solution of (0.69gm,0.01mol) of sodium nitrate in 30 ml of distilled water was added dropwise at 0-5 oC and the mixture was stirred for 30 min. This diazonium chloride solution was added dropwise in 500 ml beaker containing (1.822 gm,0.01mol) of 2,4 dimethoxy benzoic acid) with stirring dissolved in a mixture of (15) milliliters of pyridine and (20) milliliters of methanol and cooled to 0-5 oC. The mixture was stirred for an additional 2 hours, in ice-bath allowed to stand over night and acidified with dilute hydrochloric acid to pH=6.5-7. The precipitate crude dyes were collected by filtration and recrystallization from hot ethanol and dried in a vacuum desiccator over anhydrous CaCl₂. [12]
2.3. Antioxidants section, procedure of antioxidants[13,14]:

1- Extract the DNA plasmids from the E. coli bacteria by extraction cut G-spin ™ Total plasmid DNA Extraction Kit from intron / Korea
2- Two samples were removed from the DNA plasmid extracted by the electrophoresis apparatus using 1% and 50 volts acroz gel for a period of 45 minutes.
3- Fenton's reagent has been prepared and is made up of 30 mM H₂O₂, 50 µM ascorbic acid, and 80 MM FeCl₃)
4- Attended two test tubes placed in each tube ((0.5µg of plasmid with fenton reagent and placed in the first compound tube (NTADMBA)
5- The two samples were removed after the interaction between the plasmid and the Fenton reagent

3. Result and discussion

3.1. Physical and chemical properties of azo dye (NTADMBA)

The thiazolylazo dye ligand is a dark red crystals is sparingly soluble in water. It is soluble in methanol, acetone and chloroform, but easily soluble in ethanol, DMF, DMSO and alkaline aqueous solutions and strongly acidic solutions. as shown in table (1)

| Table (1). Physical properties and elemental analysis for reagent (NTADMBA) |
3.2. Fourier transform infrared spectroscopy (FT-IR) of the reagent.

FT-IR spectrometric (Shimadzu 8400S, Japan) analysis was used to characterize the chemical structure of (NTADMB) reagent. The sample was prepared as KBr Pellet and spectra in the frequency range (4000-400) cm⁻¹.

The IR spectra of the thiazolyl azo reagent with was listed in Table (2) and Figure (1).

| COMPOUND       | COLOR  | M.P | M.F  | Found (Calc.)% |
|----------------|--------|-----|------|----------------|
| C₁₂H₁₀SN₄O₆   | DARK RED | 183 | 338.308 | 41.725, 2.84, 15.633, 9.421 |
|                |        |     |      | (42.604, 2.984, 16.561, 9.477) |

Table (2). The IR (in cm⁻¹) data of thiazolylazo dye the (NTADMB) reagent Group.

| Group | Reagent(NTADMB) |
|-------|-----------------|
| ν (O-H) | 3178(s) Aro, 2841(s) |
| ν (C-H) | 3047(w) |
| ν (C=C) | 1537(sh) |
| ν (C=N) | 1699(s) |
| ν (N=N) | 1465(sh) |
| ν (C-S) | 1211, 1165(sh) |

S = strong , m= medium , w = weak , br=broud.
3.3. 1H-NMR Spectra for reagent

The 1H-NMR spectra of thiazolylazo dye ligand (NTADMBA) showed in figures 1. It was measured in DMSO-d6 as solvent with TMS as an internal reference (300MHz). The thiazolylazo dye ligand (NTADMBA) has been studied and listed in Table 3.

| Reagent LH, δ, ppm, (H atoms, peak, assignment) | J-J-Coupling |
|-----------------------------------------------|--------------|
| 11.59(S,1H,OH)                                | 0.95         |
| 0.99(S,6H,OCH₃)                               | 1.03         |
| 6.32-7.42(M,4H,Ar-H)                          | 17.26-14.73  |

3.4. Mass spectra for reagent

The mass spectra of the thiazolylazo dye ligand (NTADMBA) gave a range of peaks Figs 3. The results of the expected mass fragment ion are shown in scheme2 [11].
Figure (3). Mass spectrum of thiazolylazo reagent (NTADMBA)

Scheme(2). Mass spectrum fragmentation of thiazolylazo reagent(NTADMBA)

3.5. Antioxidants study:-
To demonstrate whether the compound (NTADMBA) had the ability to work as antioxidants, the plasmid was extracted from E. coli bacteria and according to the method of extraction of the acids, then the plasmid samples were carried over to demonstrate the validity of the extraction by using the electrophoresis apparatus and as shown in Figure 4 that the extraction was good and gave clear packages. The way to know the susceptibility of the compound to work as anti-oxidants is to display the plasmid to vehicles that work on its oxidation, where the fenton reagent was used, and it contains hydrogen peroxide, which is a strong oxidizing agent with auxiliary factors, ferric chloride and ascorbic acid, as these materials work to oxidize the plasmid and destroy it and thus when the plasmid is transferred After smashing, it does not give one packet, but it gives two or more packets, or the packets disappear completely, according to the degree of smashing. In our experiment, two packages appeared, and this indicates that the plasmid was split into two parts due to the oxidizing factors in the Fenton reagent, as shown in Figure 5 in sites 1 and 2. Whereas, when we added the compound (NTADMBA) to Tube 1 and 2, it appeared that the Fenton reagent did not affect the plasmid, as the two compounds acted as antioxidants, dealt with the Fenton reagent and canceled its susceptibility to oxidation, so single packages appeared in sites 1 and 2 indicating that the plasmid was not affected by the oxidizing agent due to the presence of the compounds that She worked as an antioxidant. These results are consistent with many studies such as Sakina et al [13] and Amina et al [14].

![Image](image_url)

**Figure 4.** The figure shows the migration process of the plasmid extracted from E. coli bacteria. Where the electrophoresis device was used. 1% acros at 50 volts. Using 0.5 X of TBE BFR for 45 minutes. Ethidium bromide was used with acrylic gel and the image appeared with ultraviolet light.
Figure 5. The migration image of the plasmid after DNA plasma (0.5µg) was used with the fenton reagent (30 mM H$_2$O$_2$, 50 µM ascorbic acid, and 80 µM FeCl$_3$. (Where NTADMBA added to Model No. 1 and NTADMBA for Compound.

4. Conclusions

1- The new organic reagent (NTADMBA) was synthesized.

2- The synthesis of the new organic reagent was demonstrated and validated using techniques.

3- Apply the new organic reagent, using it as an antioxidant.

4- The antioxidant assays showed the enhanced potency of E. coli bacteria. the study suggests that the organig reagent (NTADMBA) herb unequivocally is a potential source of antioxidants and could aid in alleviating oxidative stress-mediated disorders.

5. References

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