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Antioxidant Activity of Lumichrome and Its Reduced Form, 5,10-Dihydro-7,8-dimethyl Alloxazine

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Abstract*: The antioxidant activity of lumichrome and its derivative (its reduced form) namely 5,10- dihydro-7,8-dimethyl alloxazine which was synthesized from lumichrome were investigated. Both compounds were found to be active but the IC_{50} value of lumichrome was found to be even better than that of the standard ascorbic acid. This finding aptly reflects that further research could be carried out on lumichrome (7,8-dimethyl alloxazine) for development as a new potential antioxidant drug for treating the pathological states caused by oxidative stress.*

keywords: *Antioxidant, Free Radical, Lumichrome, DPPH Assay.*

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I. Introduction

Many of the human diseases/disorders are mainly connected to oxidative stress because of free radicals. These free radicals also known as reactive oxygen species (ROS) are capable of leading to oxidative changes within the cells. In oxidative stress, cellular antioxidant defenses are insufficient to keep the levels of ROS below a toxic threshold value (1). ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to overproduction of reactive species, induced by exposure to external oxidants or a failure in the defense mechanisms, damage to cell structures, DNA, lipids and proteins occur which increases risk of more than 30 different disease processes, some of which are ischemia, anemia, arteriosclerosis, atherosclerosis, heart disease, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, liver disease, ageing process, Alzheimer's disease and dementia (2-4).

Antioxidants, which scavenge free radicals, are known to possess an important role in preventing these free radical-induced diseases. Besides these, various synthetic antioxidants such as tert-butyl-1-hydroxytoluene (BHT), butylated hydroxytoluene (BHA), propyl gallate (PG) and tert-butyl hydroxyquinone (TBHQ) used as food additives to increase shelf-life are known to have not only toxic and carcinogenic effects on humans but also abnormal effects on enzyme systems. Therefore, the interest in natural antioxidants as well as synthesis of new antioxidant compound has greatly increased in recent years (5).

Lumichrome, also known as 7,8-dimethyl alloxazine is formed by photolysis of riboflavin in acid or neutral aqueous solution(6). Only three biological activities of lumichrome have so far been reported. It has been found that lumichrome has potential as a UVA/blue light photosensitizer in antibacterial photodynamic therapy (7). Lumichrome was also found to reduce bone loss in ovariectomized mice by inhibiting osteoclastogenesis(8). It also inhibited the growth of lung cancer cells (9-11). Besides these, literature survey also indicates that lumichrome's reduced form, 5,10- dihydro-7,8-dimethyl alloxazine has not been studied for any biological activity. This prompted us to investigate various biological activities of these two compounds and their antioxidant activity had been found promising which is presented in this paper.

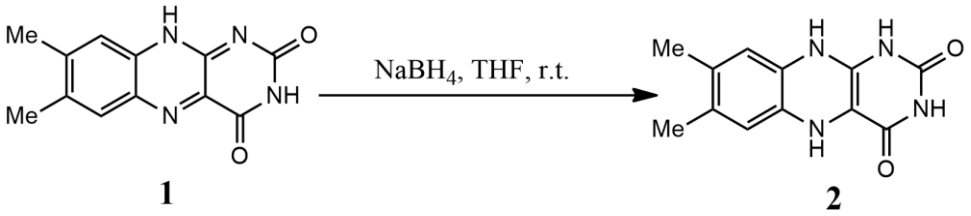
II. Materials and Methods

Chemicals:

Lumichrome (**1**) and 1,1-Diphenyl- 2- picrylhydrazyl (DPPH) was purchased from commercial sourceSigma Aldrich. All other chemicals used in the research including methanol, sodium borohydride ($NaBH_4$), Tetrahydrofuran (THF)are of analytical grade procured from Merck. Distilled water was used in the entire study.

Synthesis of 5,10-dihydro-7,8-dimethyl alloxazine (2):

The synthesis of 5,10-dihydro-7,8-dimethyl alloxazine (**2**) was based on reduction of lumichrome with $NaBH_4$ in THF at room temperature(**Scheme 1**). (12)



1 **2**

Scheme 1

Scheme1: Synthesis of a lumichrome (1) derivative- 5,10- dihydro-7,8-dimethyl alloxazine (2)

Method:

In order to synthesize 5,10-dihydro-7,8-dimethyl alloxazine (**2**), one mole of Sodium borohydride ($NaBH_4$) for one mole of lumichrome (**1**) was used for the reaction.

Powdered crystals of sodium borohydride (0.05 g, 1.24 mmol) was slowly added in batches to the mixture of Lumichrome (0.3g, 1.24 mmol) and 15ml of THF in a round bottom flask. Using a magnetic stirrer the reaction mixture was then stirred overnight. The THF wasevaporated off under reduced pressure using a rotary evaporator. The crude residue, thus obtained, were then washed with little distilled water and filtered. 0.22 g of crystals of the product were obtained in 72.5% yield.

Antioxidant activity test by radical scavenging assay:

In vitro antioxidant activity of Lumichrome and the synthesized compound 5,10-dihydro-7,8-dimethyl alloxazine (**2**)was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method of Brand-Williams, *et al* with some modification (15).

0.1 mM DPPH solution in methanol was prepared and 2 ml of this solution was added to 1 ml of the sample solution in methanol at different concentrations (500 to .977µg/ml). The mixture was then incubated in the dark for 30min at room temperature. The change in color (from deep violet to light yellow) was measured as absorbance (Abs) at 517nm using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA).

Ascorbic acid was used as a standard compound. The DPPH radical scavenging activity percentages (RSA%) of lumichrome (7,8-dimethyl alloxazine) and 5,10-dihydro-7,8-dimethyl alloxazine were determined as follows:

$$\text{DPPH RSA}(\%) = (1 - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \times 100$$

Where$\text{Abs}_{\text{control}}$ represents the absorbance of the control (without sample) and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample. The concentration required to scavenge 50% of the DPPH radical (IC_{50}) was used to evaluate the antioxidant capacity and estimated by logarithmic regression. All measurements were performed in triplicates.

III. Results

Characterization data of synthesized compound:

5,10-dihydro-7,8-dimethyl alloxazine (**2**). Pale orange crystal powder. Yield: 70%. IR (KBr) cm^{-1}: 3772.76, 3693.68, 3433.29, 1710.86, 1620.21. ^1H NMR (CD_3OD, 400 MHz): δ2.464 (s, 3H, -CH_3), 2.484(s, 3H, -CH_3), 7.629 (s, 1H, aromatic H), 7.856 (s, 1H, aromatic H).

Its NMR spectrum is consistent with its structure. The two methyl groups give two singlets at 2.464 and 2.484 ppm respectively. The aromatic two protons impart also two singlets at 7.629 and 7.856 ppm. Since the NMR spectrum was taken in CD_3OD, NH peaks were not observed in the spectrum.

Antioxidant activity:

Following graph represents the DPPH radical scavenging activity percentages (RSA%) of lumichrome and synthesized derivative 5,10-dihydro-7,8-dimethyl alloxazine compared to standard ascorbic acid at different concentrations ranging from 0.977 to 500µg/ml.

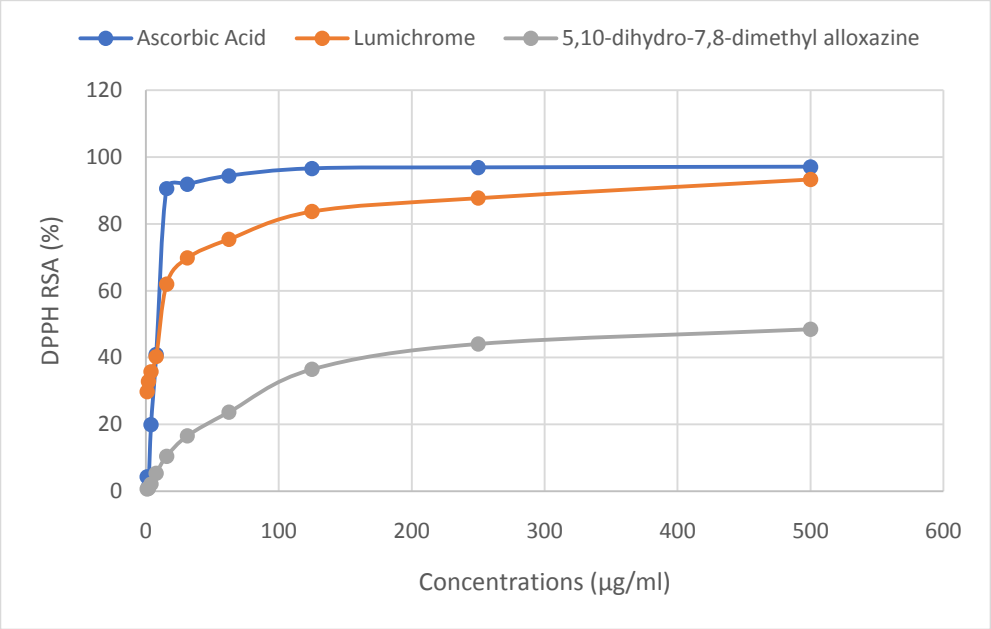


Figure 1 DPPH scavenging activity profile of lumichrome, 5,10-dihydro-7,8-dimethyl alloxazine and ascorbic acid (standard).

Table 1 represents the IC_{50} values of standard ascorbic acid, lumichrome and the synthesized compound 5,10-dihydro-7,8-dimethyl alloxazine which clearly indicates the potential antioxidant effect of lumichrome.

Table 1 DPPH radical scavenging activity profile of test compounds

No.	Sample	IC_{50} (µg/ml)
1	Ascorbic acid (standard)	10.46
2	lumichrome (7,8-dimethyl alloxazine) (**1**)	8.41
3	5,10-dihydro-7,8-dimethyl alloxazine (**2**)	879.63

IV. Discussion

The target compound 5,10-dihydro-7,8-dimethyl alloxazine (**2**) was anticipated to have important biological effects and thus was synthesized from lumichrome. The compound was successfully synthesized with 72.5% yield and characterized by spectrometric data.

DPPH free radical scavenging assay is one of the commonly used method for measuring the antioxidant property of a substance. Due to its delocalized electron, DPPH (2,2-diphenyl-1-picryl-hydrazyl radical) is a deep violet colored stable free radical which shows absorption band at 517nm in methanol solution. In DDPH assay hydrogen-donating ability of an antioxidant substance to convert stable DPPH free radical to 2,2-diphenyl-1-picrylhydrazine is measured. The radical scavenging effect changes the color of solution from deep violet to light yellow which can be quantitatively measured from the changes in absorbance using a UV-Visible Spectrophotometer(13-15).

From the graph (Figure 1) it can be postulated that both lumichrome and the synthesized compound have DPPH radical scavenging effect but the ability of lumichrome to reduce the stable free radical DPPH to the yellow colored diphenylpicrylhydrazine was comparatively more similar to standard ascorbic acid.The IC_{50} value ofascorbic acid was determined to be 10.46µg/ml whereas oflumichrome was 8.41µg/ml which is even better than standard. Though at 500µg/ml the scavenging activity of lumichrome was slightly less than ascorbic acid but the activity was increasing with the increase in concentration. Since lumichrome had marked antioxidant effect, it deserves to be carried out for further research on it. The IC_{50} value of 5,10-dihydro-7,8-dimethyl alloxazine was determined to be 879.63µg/ml (greater than 500µg/ml) which indicates its lower antioxidant effect (**Table 1**).

V. Conclusion

In this study a simple synthesis procedure of a derivative of lumichrome namely 5,10-dihydro-7,8-dimethyl alloxazine was described and the antioxidant profile of both of the compounds were investigated. The compound was successfully synthesized and characterized by IR, NMR spectrometric data. Following antioxidant study, the concentration of lumichrome required to scavenge 50% of free radical was found to be lower than standard ascorbic acid. We can conclude that lumichrome (7,8-dimethyl alloxazine) can be developed as a new potential antioxidant drug for treating the pathological states caused by oxidative stress.

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