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Article

Synthesis and Determination of Antioxidant Activity of Alizarin Derivatives

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ABSTRACT

In the present work, we have synthesized nitro, phenyl, nitrophenyl, and methyl phenylhydrazone derivatives of alizarin and compared their antioxidant activity. The antioxidant activities of each derivative were compared using electrochemical assay with glassy carbon electrodes. The X-ray diffraction studies of compounds revealed that prominent peaks at 43 2q and 49 2q confirm the formation alizarin, whereas the intensity varies accordingly with the functional groups attached to it. This was further confirmed by H^1 nuclear magnetic resonance. The antioxidant activities were interpreted by cyclic voltammetry that depends on the parameters such as anodic peak current, anodic peak potential, and area swept by the curve. The maximum antioxidant activity was observed in phenylhydrazone derivative of alizarin. The area of the peak and peak potential was observed at 108.017 µc and 347.498 mv, respectively. Hence, the synthesis of the above derivatives of alizarin clearly explains the influence of functional groups on antioxidant activity.

Key words: Antioxidant Activity, Alizarin, Phenylhydrazone.

1. INTRODUCTION

Alizarin or 1,2-dihydroxyanthraquinone is an organic compound with anthraquinone as a parent moiety having molecular formula $C_{14}H_{18}O_4$. It is widely used in pharmacological activities in studies involving bone growth, osteoporosis, bone marrow, calcium deposits in the vascular system, cellular signaling, gene expression, tissue engineering, and mesenchymal stem cells. It is also used as an effective antioxidant to overcome oxidative stress caused by reactive oxygen species and used in textile fabrics as a dyeing agent [1-3]. Antioxidants are the substances that may protect cells from the damage caused by unstable molecules known as free radicals. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. In the present work, we have synthesized alizarin and the same was derivatized to get products of alizarin with unique functionalities, namely nitro, phenyl, nitrophenyl, and methyl phenylhydrazone derivatives in a conventional method. The participation of functional groups of these derivatives in exhibiting antioxidant activity was studied.

2. EXPERIMENTAL

2.1. Chemicals Used

Phthalic anhydride, concentrated sulfuric acid, boric acid, phenol, 10% KOH, conc. HCl, 1,2-dihydroxy-9,10-anthraquinone, hydrazine hydride, glacial acetic acid, phenylhydrazine, 4-nitrophenylhydrazine, 4-methylphenylhydrazine sulfate ethanol, silica gel, hexane: ethyl acetate mixture, and pyrocatechol were used.

2.2. Synthesis of 1,2-Dihydroxy-9,10-Anthraquinone (Alizarin)

About 9 g of phthalic anhydride was dissolved in 25 ml of concentrated sulfuric acid with continuous stirring. Catalytic amount of boric acid was added and transferred the reaction mixture into round-bottomed flask and was refluxed. Once the reaction attained the temperature of 80°C, 18 g of pyrocatechol was added and continued the reflux for about 6–8 h at 150°C. The progress of the reaction was checked using thin-layer chromatography method using hexane: ethyl acetate as solvent. After the completion of reaction, the reaction mixture was poured into 500 ml beaker containing ice. The reaction mixture was allowed to settle, and then, it was filtered and washed with hot water for 3–4 times [4]. The filtrate was collected and 10% KOH was added and it was neutralized with concentrated HCl, after 20 min product was filtered. Specific reaction conditions of various alizarin derivatives are shown in Table 1.

2.3. Synthesis of Derivatives

2.3.1. Antioxidant assay

Antioxidant activity of alizarin and its derivative was studied electrochemically in a three-electrode cell with glassy carbon electrode, saturated calomel as reference electrode, and platinum wire as auxiliary electrode. The cleaned glassy carbon electrode was transferred into electrochemical cell containing 10 ml 0.1 M PBS buffer. The potent were swept from +0.1 V to +0.8 V and then reversed with a scan rate of 250 mVs^{-1}. A blank reading was taken for PBS; then, PBS was replaced by alizarin solution, readings were recorded. To the same electrochemical cell, 50 µl of 0.1 M p-benzoquinone was added and CV was recorded. The experiment was repeated for derivatives of alizarins [5].

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Table 1: Reaction conditions of various alizarin derivatives.

Derivative	Reactants	Reaction duration (h)
Alizarin hydrazone	2 g of alizarin was dissolved in 25 ml of ethanol to this 5 ml of acetic acid and 30 ml of hydrazine hydride was added dropwise with stirring.	24
Alizarin phenylhydrazone	2 g of alizarin was dissolved in 25 ml of ethanol to this 10 ml of acetic acid and 17 ml of phenylhydrazine was added with stirring.	20
4-methyl phenylhydrazone	2 g of alizarin was dissolved in 25 ml of ethanol to this 5 g of 4-methyl phenylhydrazine sulfate was added with stirring.	8
4-nitrophenylhydrazone	2 g of alizarin was dissolved in 25 ml of ethanol to this 10 ml of acetic acid and 5 g of 4-nitrophenylhydrazine sulfate was added with stirring.	8

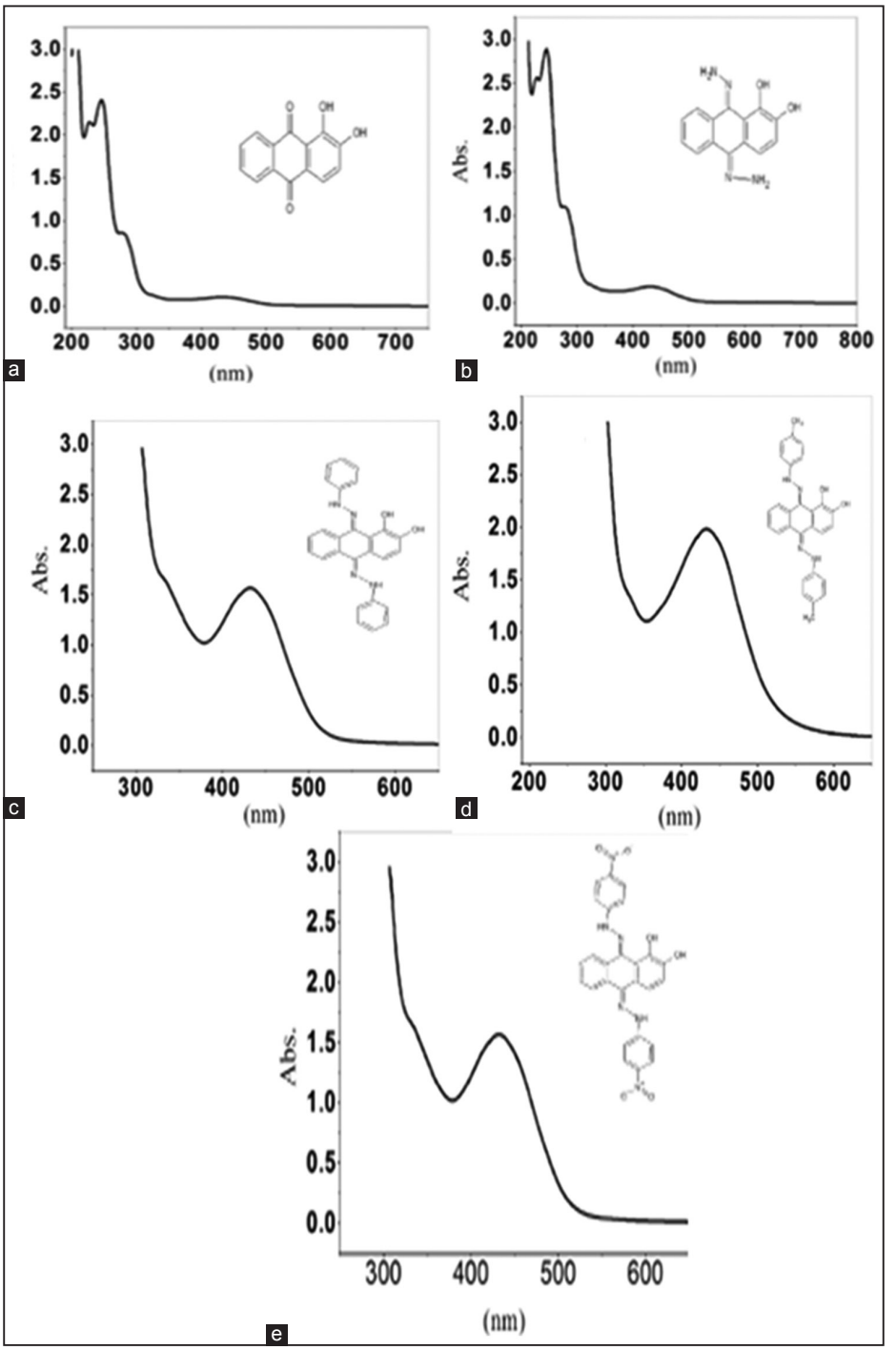

Figure 1: UV-visible spectrum of (a) alizarin, (b) alizarin hydrazone, (c) alizarin phenylhydrazone, (d) alizarin methyl phenylhydrazone, (e) alizarin-4-nitrophenylhydrazone

3. RESULTS AND DISCUSSION

3.1. Characterization of Purified Compounds

3.1.1. UV absorption spectroscopy

The purified compounds were scanned for their absorption properties, from 200 nm to 900 nm in a Shimadzu, UV-visible spectrometer. In Figure 1a and b represents for alizarin and alizarin hydrazone shows prominent peak at λ_{max} 279 nm which corresponds to the literature [6], and in Figure1 c-e, alizarin methyl hydrazone, alizarin phenylhydrazone, alizarin methyl phenylhydrazone, and alizarin-4-nitrophenylhydrazone show the λ_{max} at 450 nm. A bathochromic shift was observed due to the presence of the substituents such as methyl, phenyl, and nitro groups.

3.2. Characterization of Synthesized Compounds by P-X-ray Diffraction (XRD)

p-XRD profile of all alizarin derivatives shows two prominent peaks around 40 and 50 two theta values which indicate a monoclinic crystal lattice [7]. Figure 2 hydrazone derivative of alizarin shows same pattern as alizarin, but due to hydrazone formation, the peak intensity has been reduced. In the case of Figure 1c and d, due to electron donating groups as functionality shows high peaks intensity and reveals the bulkiness of the molecule. Figure 1e reveals that additional peaks with high intensity due to strong π-π stacking effect due to the presence of electron withdrawing nitro group as functionality.

3.3. Characterization of Purified Compounds using H^1-Nuclear Magnetic Resonance

Alizarin shows peaks at δ=12.80 (Ar-OH), δ=7.28 (1H,d), δ=7.82 (1H,m), δ=8.32 (1H,t), alizarin hydrozone at δ=2.34 (NH), δ=7.28 (1H,d), δ=7.82 (1H,m), δ=8.32 (1H,t), δ=12.80 (Ar-OH), alizarin phenylhydrazone at δ=2.21 (NH), δ=12.80 (Ar-OH), δ=8.32 (1H,t), δ=7.28 (1H,d), δ=7.82 (1H,m), alizarin methyl phenylhydrazone at δ=12.80 (ArOH), δ=2.17 (NH), δ=7.82 (1H,m), δ=7.28 (1H,d), δ=1.58 (3H,S), alizarin-4-nitrophenylhydrazone shows its peak at δ=6.63 (1H,d), δ=7.28 (1H,d), δ=7.82 (1H,M), δ=8.08 (1H,d), δ=8.32 (1H,t), δ=12.80 (Ar-OH), δ=2.17 (NH) (Figure 3).

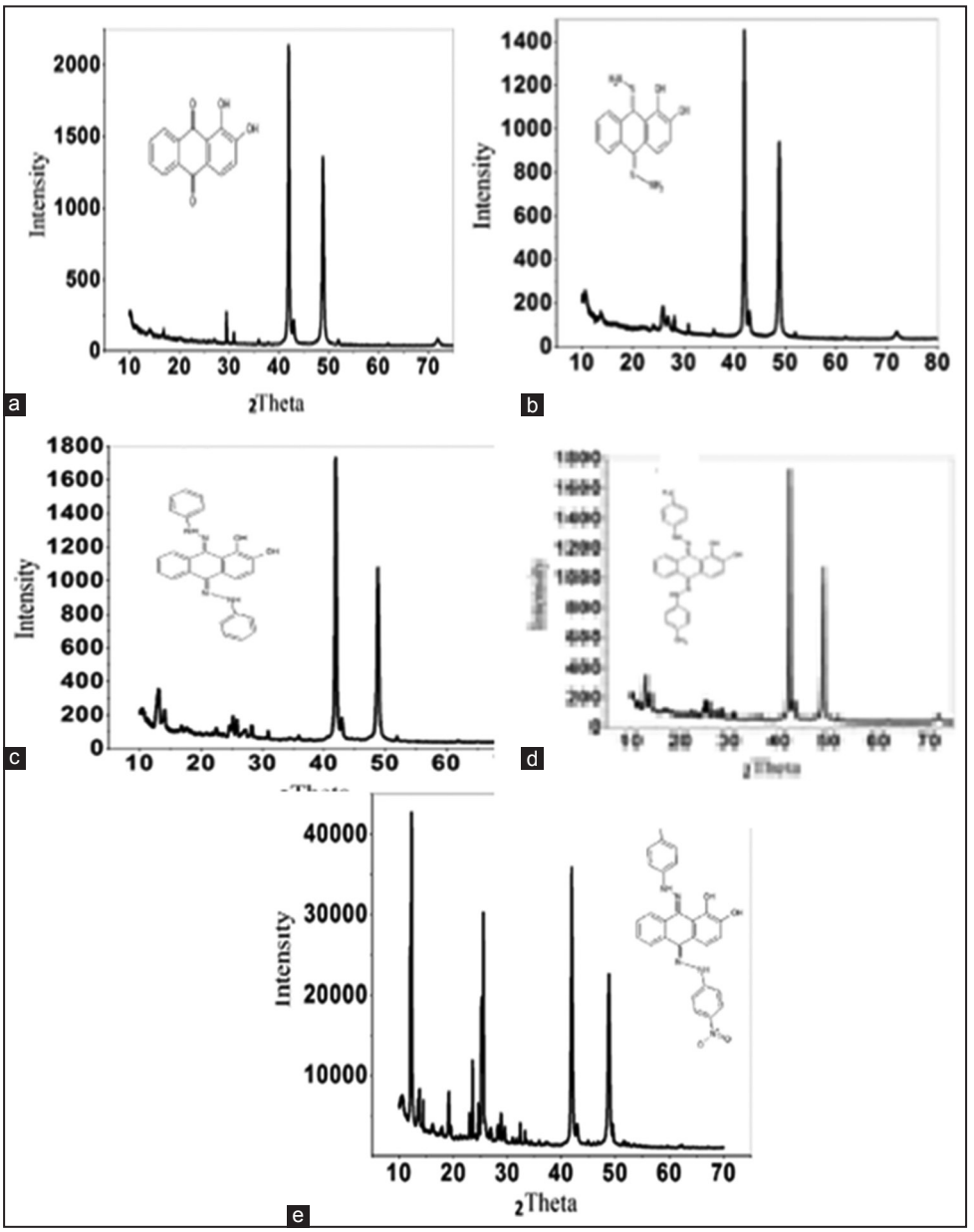

Figure 2: p X-ray diffraction of (a) alizarin (b) alizarin hydrazone (c) alizarin phenylhydrazone (d) alizarin methyl phenylhydrazone (e) and alizarin-4-nitrophenylhydrazone

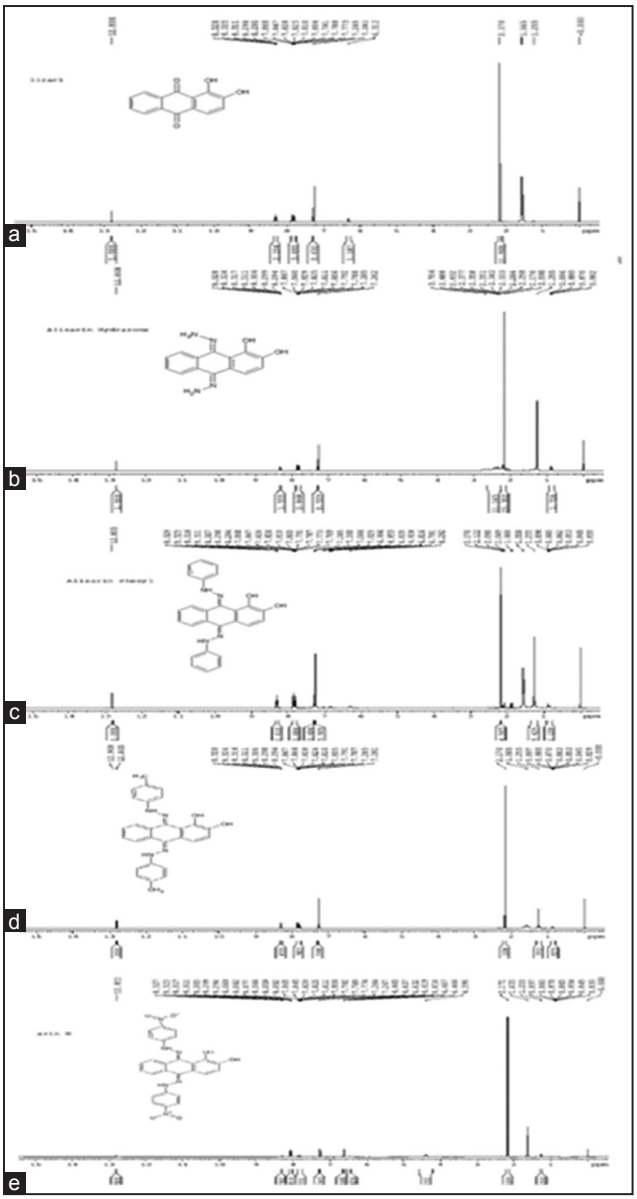

Figure 3: Nuclear magnetic resonance of (a) alizarin (b) alizarin hydrazone (c) alizarin phenylhydrazone (d) alizarin methyl phenylhydrazone (e) alizarin-4-nitrophenylhydrazone

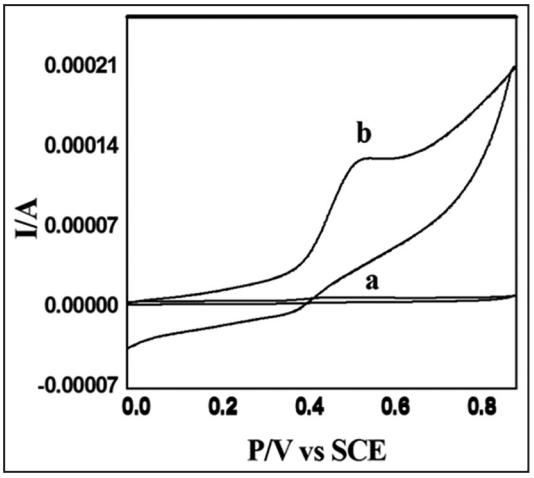

Figure 4: Cyclic voltammetry of 4-nitrophenyl alizarin (a) and alizarin phenylhydrazone, (b) in the presence of p-benzoquinone

Table 2: Electrode potential, peak current, and area of peaks covered by alizarin and its derivatives.

Compound name	E_{pa} (mv)	I_a (µA)	S (area) µc
Alizarin crude	422.641	14.766	12.112
Alizarin hydrazone	667.393	65.813	47.518
Alizarin phenylhydrazone	347.498	114.18	108.017
Alizarin nitrophenylhydrazone	473.861	4.758	3.055
Alizarin methyl phenylhydrazone	530.602	124.651	71.121

3.4. Antioxidant Assay

Cyclic voltammetry is shown to be effective tool to determine antioxidant capacity of anthraquinone compounds. Antioxidant capacity of anthraquinone compounds is based on the important cyclic voltammetric parameters such as anodic peak current, cathodic peak current, anodic peak potential, cathodic peak potential, and area under the anodic peak activity. The best antioxidants contain more polyphenolic compounds; they have a tendency to readily donate an electron at electrode surface. Hence, antioxidants when oxidize at lower potentials show higher antioxidant activity. To examine this, CV of alizarin and its derivatives was recorded in the presence and absence of p-benzoquinone. Hence, a good antioxidant readily gives an electron and converts p-benzoquinone to p-hydroquinone, which will be depicted in CV.

Figure 4 represents the difference in peak range of least antioxidant 4-nitrophenyl alizarin hydrazone and most active antioxidant of all derivatives being alizarin phenylhydrazone in the presence of p-benzoquinone. Furthermore, there were no reduction peaks observed at reverse scan which shows that electrochemical redox reactions of herb extracts exhibit irreversible redox process at GCE. This could be due to the inductive effects from ortho and/or a para hydroxyl group to the double bond, which is conjugated with the aromatic rings containing polyphenols. Hydrazone, methyl phenyl, and phenyl groups are electron donating groups showing slight similarities in area under the curve (S), phenyl group shows high area under the curve. Nitro-substituted compound shows least area under the curve due to electron withdrawing effect of nitro group. Antioxidant activity of alizarin and their derivatives in the presence and absence of hydroquinone is shown in Table 2. By this, we can conclude that order of antioxidant activity for the compounds follows the sequence alizarin phenylhydrazone > alizarin methyl phenylhydrazone > alizarin hydrazone > alizarin > 4-nitrophenyl alizarin hydrazone.

4. CONCLUSION

In this experiment, new and unique derivatives of alizarin were synthesized and their antioxidant activity revealed that phenyl-substituted derivative has the highest antioxidant property.

5. ACKNOWLEDGMENT

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***Bibliographical Sketch**

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