



Enaminones-Assisted Synthesis of Disperse Dyes. Part 3: Dyebath Reuse and Biological Activities



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It is worth noting that industrial waste resulting from dyeing processes in the textile industry has a harmful impact on the environment. The majority of the industry uses disperse dyes and these colors produce effluent subsequent to coloring. In this study the dyebath of disperse dyeing at 100 or 130 °C utilized again without depleting them. Finally the biological activities of the synthesized disperse dyes like antimicrobial, antitoxicity and antioxidants were investigated.

Introduction

The utilization of disperse dyes has been constantly expanded in the industrial textile since the manufactured textures have been found. Disperse dyes can be applied to about all the manufactured textures utilizing straightforward weariness procedures, especially. Disperse azo dyes cause environmental concern because of their far reaching use. Water reuse in textile procedures has been a subject of most as of late years investigates and advancement work (1-3). The incentives for reuse of water are great, since there is a potential for reduction of both water requirements and the costs of wastewater treatment. The idea of dyebath renovation and reuse has started in the middle 1970s when energy costs became a critical factor in overall manufacturing costs. Dyebath renovation and reuse has been shown to be an effective method of cost reduction. One of the approaches to reuse the dyebath is to reconstitute the dye bath by adding the required amount of dyes and chemicals after analyzing the dye liquor. The above method is applicable only if the dyeing process does not change the characters of the residual dye in the bath such as disperse dyes (3-7). Dyebath reuse has long been recognized as a strategy to prevent pollution. In this study, polyester fabrics were

dyeing with disperse dyes in a dyebath reuse system after the original dyeing process. The target of the examination was to save chemicals and water and to reduce the quantities of effluent discharged during the dyeing of polyester fabrics. Rather than releasing the dyebath after every coloring cycle

Materials and Methods

General Procedure for the Synthesis of Disperse Dyes 1-6

The disperse dyes were prepared according to the method that we published in our previous research [8].

Dyeing procedure

The disperse dyes 1-6, a dispersion of the dyes were produced by dissolution of the appropriate amount of dyes (1% shades) in 2 ml DMF and then added dropwise with stirring to the dye bath (liquor ration 1:30) containing (1.5%) of leveal MDL as anionic dispersing agent (TANATEX chemicals) and TANAVOL EP 2007 (1%) as anionic eco friendly carrier (TANATEX chemical) in case of dyeing at 100 °C or just use dispersing agent in case of dyeing at 130°C. The pH of the dye bath was adjusted to 5.5 with aqueous acetic acid, and the wetted-out polyester fabrics (3 gm) were added. We performed dyeing by raising the

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dye bath temperature to 100 or 130°C at a rate of 3°C/min and holding it at this temperature for 60 min. After they were cooled to 50°C, the dyed fibers were rinsed with cold water and reduction-cleared (1 g/L sodium hydroxide, 1 g/L sodium hydrosulfite, 10 min, 80°C). The samples were rinsed with hot and cold water and, finally, air-dried.

Dyebath reuse procedure

After dyeing, the dyebath was analyzed and reconstituted with the necessary amount of fresh water to maintain a constant liquor ratio of the original volume.

Residual dyebath pH was measured in order to keep pH at 5.5. The Second dyeing was being carried out by the same procedures of the previously two techniques.

Finally areduction-cleared using sodium hydroxide (3 g/L) and sodium hydrosulphite (2 g/L) and soaped with 2% nonionic detergent (pH 8) at 50°C for 15 minutes to improve washing fastness.

Color Measurements

The colorimetric parameters of the dyed polyester fabrics were determined on a reflectance spectrophotometer. The color yields of the dyed samples were determined by using the light reflectance technique performed on an UltraScan PRO D65 UV/VIS Spectrophotometer. The color strengths, expressed as K/S values, were determined by applying the Kubelka-Mink equation.

Evaluation of Cytotoxicity, Antioxidant and Antimicrobial Activities

The cytotoxicity, antioxidant, and antimicrobial activities were determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University, Cairo, Egypt.

Evaluation of Cytotoxic Effects of Certain Chemical Compound Mammalian cell lines: HepG-2 cells (human Hepatocellular carcinoma), was obtained from the VACSERA Tissue Culture Unit. Chemicals used: dimethyl sulfoxide (DMSO), crystal violet, and trypan blue dye were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDTA were purchased from Lonza (Morrison, NJ, USA). The published method was followed when evaluating anticancer activities [15].

DPPH Radical Scavenging Activity The methanol solution of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10 °C. A methanol solution of the test disperse dyes were prepared. A 40 µL aliquot of the methanol solution was added to 3mL of DPPH solution. The decrease in absorbance at 515 nm was determined continuously, with data recorded at (1 min) intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical in the absence of the antioxidant (control) and the reference compound ascorbic acid was also conducted. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = \left[\frac{(AC - AT)}{AC} \right] \times 100 \quad (2)$$

where AC represents the absorbance of the control at t = 0 min and AT represents the absorbance of the sample + DPPH at t = 16 min [16].

The IC₅₀ (50% inhibitory concentration), the concentration required to inhibit DPPH radical by 50%, was calculated from graphic plots of the dose response curve.

Antimicrobial Activity Test

The antimicrobial activities of dyed fabrics with disperse dyes 1-6 were tested using the agar-well diffusion technique against ten different microbial cultures. Pure cultures of Streptococcus mutants RCMB 017 (1) ATCC 25175, Micrococcus sp. RCMB 028(1), and Enterococcus faecalis (ATCC 29212), (Gram-positive bacterium), Escherichia coli (RCMB 010052) ATCC 25955, Enterobacter cloacae RCMB 001 (1) ATCC 23355 and Proteus vulgaris RCMB 004 (1) ATCC 13315 (Gram-negative bacterium), and Candida albicans RCMB 005003 (1) ATCC 10231 (fungi) were used in the test, and the published method was followed when evaluating antimicrobial activities [17].

Results and Discussion

In continuation of our previous work, which was published in our strategy in constructing some of the diffuse dyes, in this study six dyestuffs were prepared, through a reaction of substituted acetophenones with dimethylformamide dimethylacetal (DMFDMA) that gave enamines which coupled with aryldiazonium salts to afford disperse dyes 1-6 (Figure 1) [8].

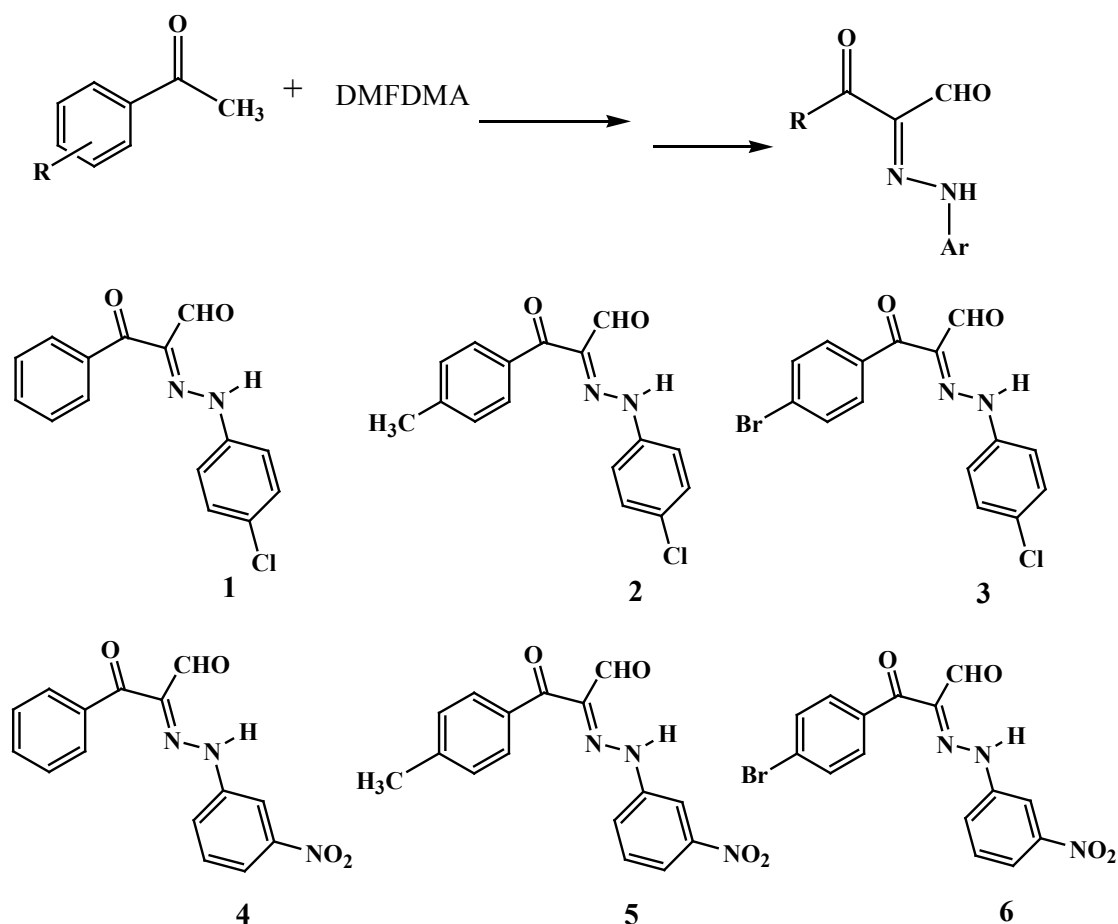


Fig. 1. Chemical structures of the disperse dyes.

Dyebath reuse

The prepared disperse dyes were used in dyeing polyester fabrics at 100 or 130 °C, and it is noticed that the dye effluents contains an amount of dye, which negatively affects the environment so our strategy was to use that dye effluents waste in new polyester fabric dyeing, for optimal use of the dye used and at the same time not to throw any colored waste that harms the environment.

From the data obtained in table (1) that represented in figure (2) we observed that color strength measurement K/S value of the dyebath reuse process in the dyeing process at 100 °C vary from 10, 20, 30, 60, 90% of its original value in the first dyeing process and this prove that dyeing reuse is an effective method of reduction costs, pollution prevention ,water ,energy and chemicals.

From the data obtained in table (2) that represented in figure (3) we observed that color strength measurement K/S value of the dyebath reuse process in the dyeing process at 130 °C

equal about 5-10% of its original value in the first dyeing process and this prove that HT pressure method is a favorite dyeing process as it give a good colorful shade and also prove that dyeing reuse is an effective method of reduction costs, pollution prevention ,water ,energy and chemicals.

Antimicrobial activities.

The antimicrobial activities of six dyes were tested against six different microbial cultures using the diffusion agar technique, well diameter, 6.0 mm (100µl was tested). Pure cultures of *Escherichia coli* and *poretus vulgaris* (Gram negative bacteria), *Bacillus subtilus* and *Staphylococcus auerus* (Gram positive bacteria) and *aspergillus flavus* (fungi), positive control for fungi (ketoconazole) and positive control for bacteria (gentamycin). The samples were tested at 10 mg/ml concentration, mean zone of inhibition in mm beyond well diameter produced on arrange of pathogenic microorganisms results are depicted in the table (3). The inhibition zones

given in that table reveal that all of the tested dyes showed positive antimicrobial activities against at least five tested microorganisms. Dye 1 show strong activities with significant inhibition zone > 9 mm against gram positive, negative bacteria and fungi. Dye 2 show strong activities with significant inhibition zone > 9 mm against gram positive, negative bacteria and *candida albicans* but no active against *aspergillus flavus*. Dye 3 show strong activities with significant inhibition zone > 9 mm against gram positive and negative bacteria, but no active against both *aspergillus flavus* and *candida albicans*. Dye 4 show strong activities with significant inhibition zone > 11

mm against all tested microorganisms. Dye 5 show strong activities with significant inhibition zone > 10 mm against all tested microorganisms. Dye 6 show strong activities with significant inhibition zone > 9 mm against gram positive, negative bacteria and *candida albicans* but no active against *aspergillus flavus*.

Cytotoxicity activities.

Cytotoxic effect was carried out at the regional center for mycology and biotechnology at Alazhar university against Hepatocellular carcinoma cells. (IC_{50}) is the concentration required to cause toxic effects in 50% of intact cells.

TABLE 1. Effect of the dyebath reuses dyeing process at 100 °C and K/S of dyed fabrics.

Dye No	% shade	L*	a*	b*	C*	h*	K/S	
							Dye reused	First dyeing
1	1%	91.30	-5.52	23.47	24.11	103.23	0.69	9.95
	2%	91.17	-8.10	46.30	47.00	99.93	2.54	21.91
	3%	90.03	-8.22	50.88	51.54	99.17	3.33	16.30
2	1%	91.95	-9.97	46.55	47.60	102.09	2.86	7.94
	2%	88.36	-11.27	52.64	53.84	102.09	6.28	15.08
	3%	87.06	-10.15	63.06	63.88	99.14	11.15	11.68
3	1%	89.03	-9.89	38.23	39.49	104.50	2.07	12.64
	2%	88.09	-10.54	50.11	51.20	101.88	4.08	17.43
	3%	87.38	-10.85	58.48	59.47	100.51	8.12	14.39
4	1%	89.38	-4.94	20.16	20.67	103.77	1.09	5.58
	2%	89.69	-3.87	35.79	36.00	96.18	2.59	11.35
	3%	86.90	-4.80	35.11	35.43	97.78	3.28	13.75
5	1%	92.15	-4.07	19.65	20.07	101.69	0.82	6.76
	2%	88.75	-5.27	24.02	24.59	102.38	1.58	19.37
	3%	84.50	-4.33	48.04	48.24	95.16	1.13	11.37
6	1%	89.65	-6.24	23.03	23.86	105.16	1.58	1.44
	2%	88.72	-5.96	26.84	27.50	102.52	1.93	3.40
	3%	87.49	-3.18	22.64	22.86	98.00	1.12	3.01

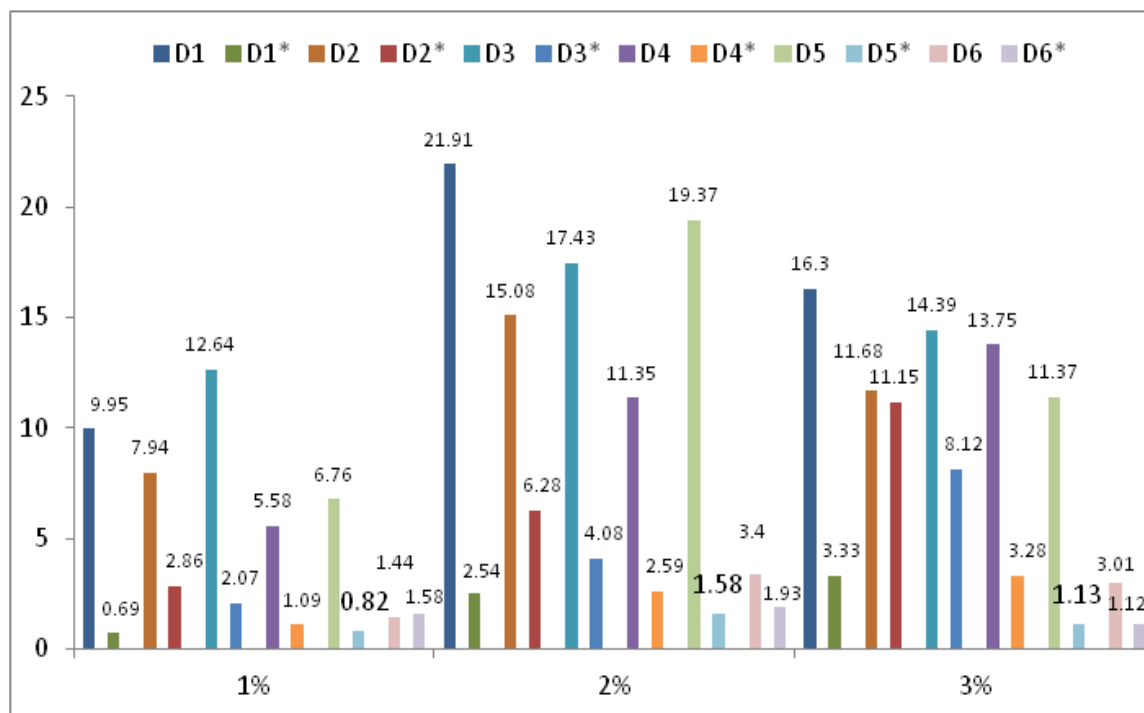


Fig. 2. Effect of the dye bath reuses dyeing process at 100 °C and K/S of dyed fabrics.

TABLE 2. Effect of the dye bath reuses dyeing process at 130 °C and K/S of dyed fabrics.

Dye No	% shade	L*	a*	b*	C*	h*	K/S	
							Dye reused	First dyeing
1	1%	90.43	-5.32	17.41	18.21	107.00	0.46	9.70
	2%	89.92	-7.67	29.48	30.46	104.58	1.05	16.04
	3%	88.97	-7.80	38.29	39.08	101.51	1.62	17.14
2	1%	90.64	-5.86	17.77	18.71	108.25	0.59	13.92
	2%	90.61	-6.58	20.43	21.47	107.84	0.69	13.70
	3%	90.40	-9.42	32.72	34.05	106.06	1.64	15.54
3	1%	91.41	-4.19	11.12	11.88	110.64	0.28	11.49
	2%	90.80	-6.72	19.80	20.91	108.75	0.62	13.51
	3%	90.44	-7.87	24.61	25.84	107.73	0.88	13.76
4	1%	90.59	-3.81	12.04	12.63	107.54	0.57	7.94
	2%	92.27	-4.38	22.65	23.07	100.94	1.19	15.60
	3%	89.03	-6.21	29.39	30.04	101.93	2.98	19.93
5	1%	90.79	-5.00	15.66	16.44	107.71	0.86	11.90
	2%	90.44	-5.51	18.42	19.23	106.64	1.12	15.18
	3%	98.94	-5.91	24.01	24.73	103.84	1.77	16.47
6	1%	91.51	-4.67	12.28	13.14	110.82	0.64	7.19
	2%	89.65	-8.34	31.64	32.72	104.77	4.05	11.19
	3%	89.98	-6.32	37.63	38.16	99.54	4.35	13.95

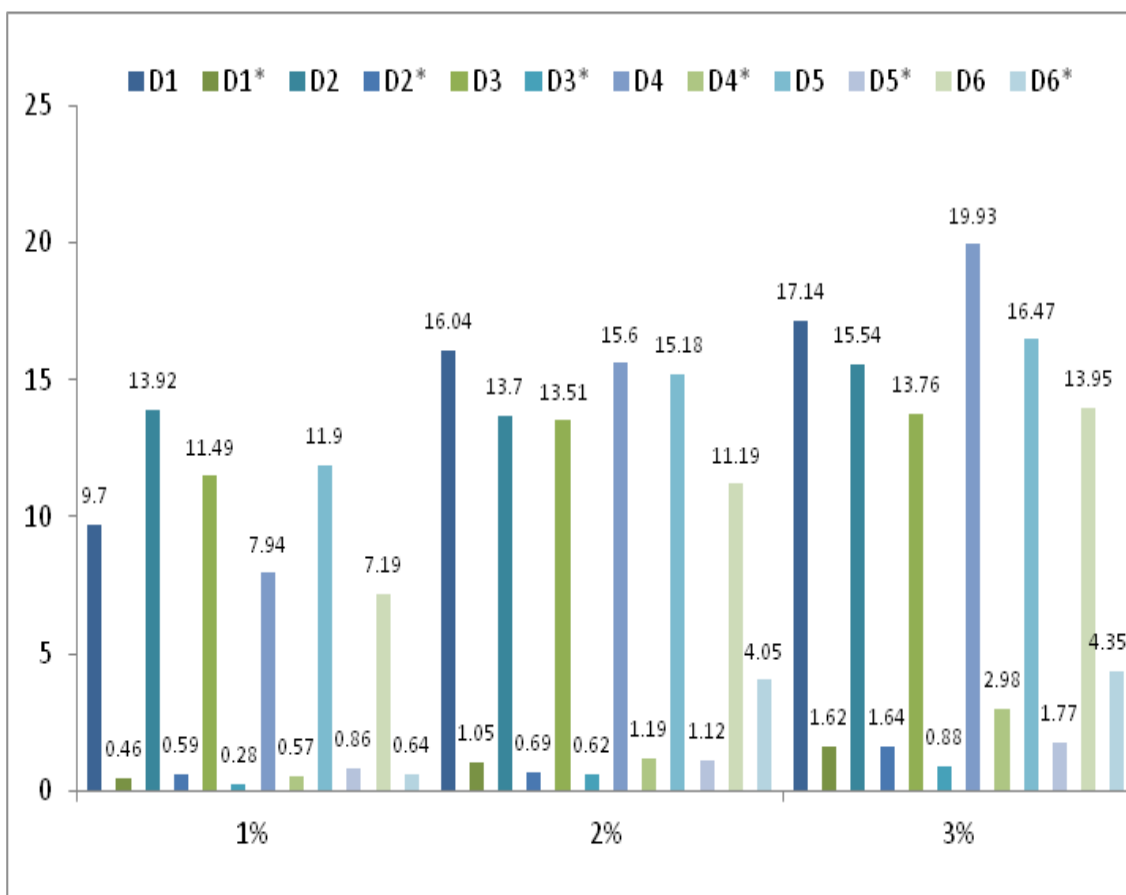


Fig. 3. Effect of the dyebath reuses dyeing process at 100 °C and K/S of dyed fabrics.

TABLE 3. Antimicrobial results of the synthetic disperse dyes.

Microorganisms	Dye Numbers						Control
	1	2	3	4	5	6	
<u>FUNGI</u>							<i>Ketoconazole</i>
<i>Aspergillus flavous</i> (RCMB 002002)	9	NA	NA	12	11	NA	16
<i>Candida albicans</i> RCMB 005003 (1) ATCC 10231	13	11	NA	14	12	10	20
<u>Gram Positive Bacteria</u>							<i>Gentamycin</i>
<i>Staphylococcus aureus</i> (RCMB 010010)	14	10	9	13	10	11	24
<i>Bacillus subtilis</i> RCMB 015 (1) NRRL B-543	15	11	12	13	12	13	26
<u>Gram Negative Bacteria</u>							<i>Gentamycin</i>
<i>Escherichia coli</i> RCMB 010052 ATCC 25955	12	9	10	12	10	11	30
<i>Proteus vulgaris</i> RCMB 004 (1) ATCC 13315	15	10	12	13	12	13	25

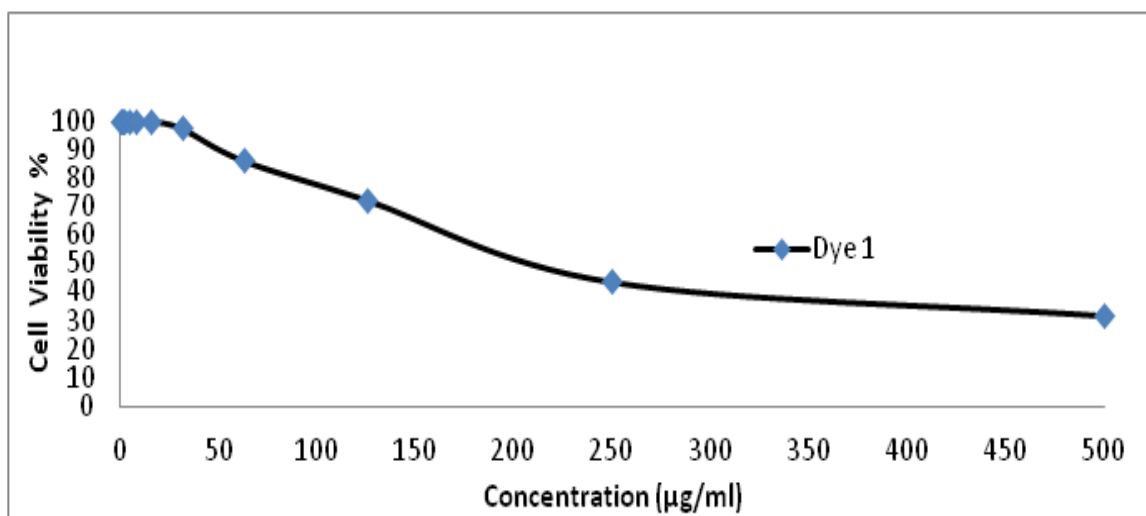
NA: No activity

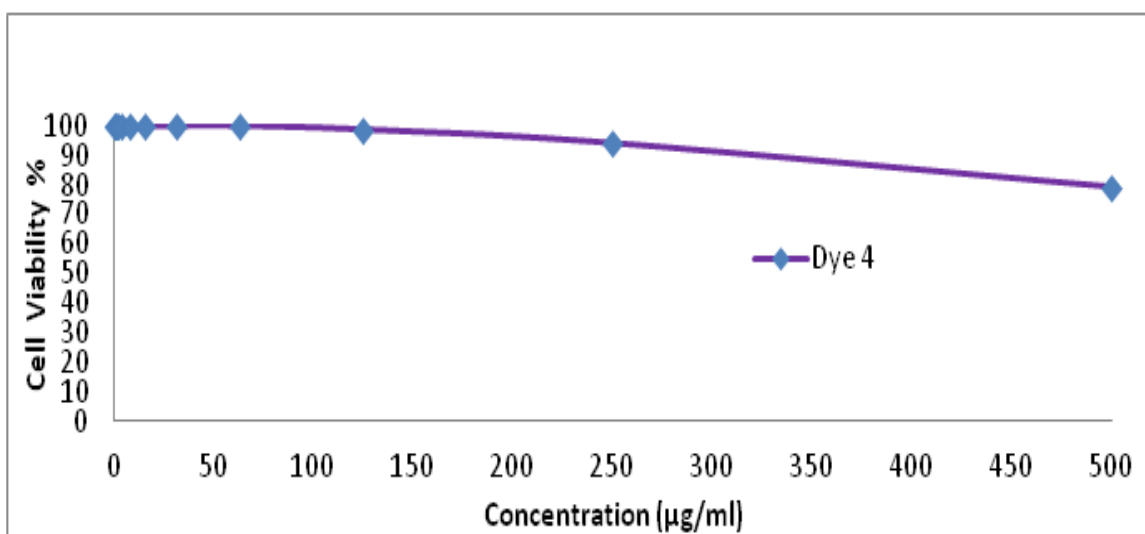
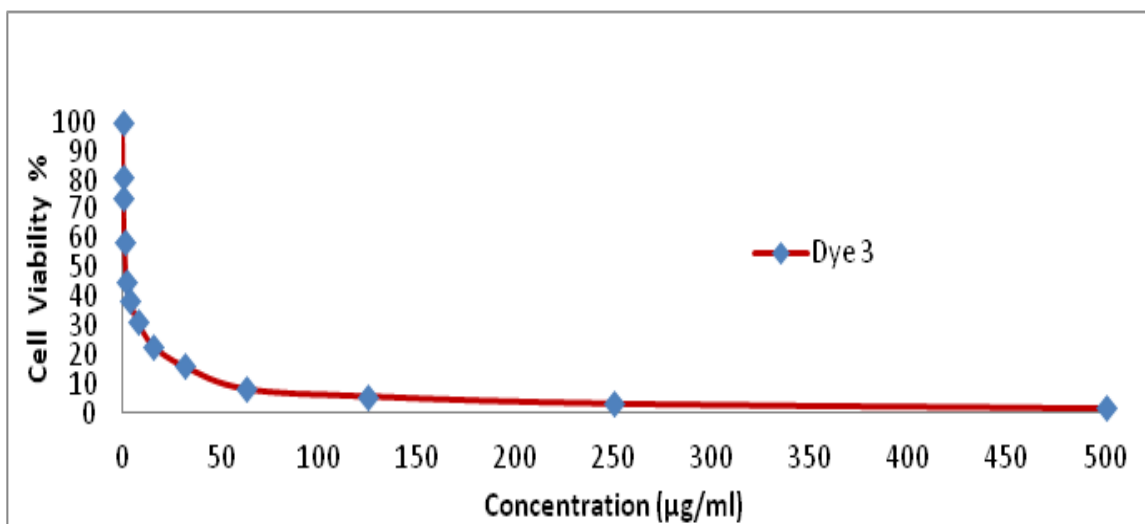
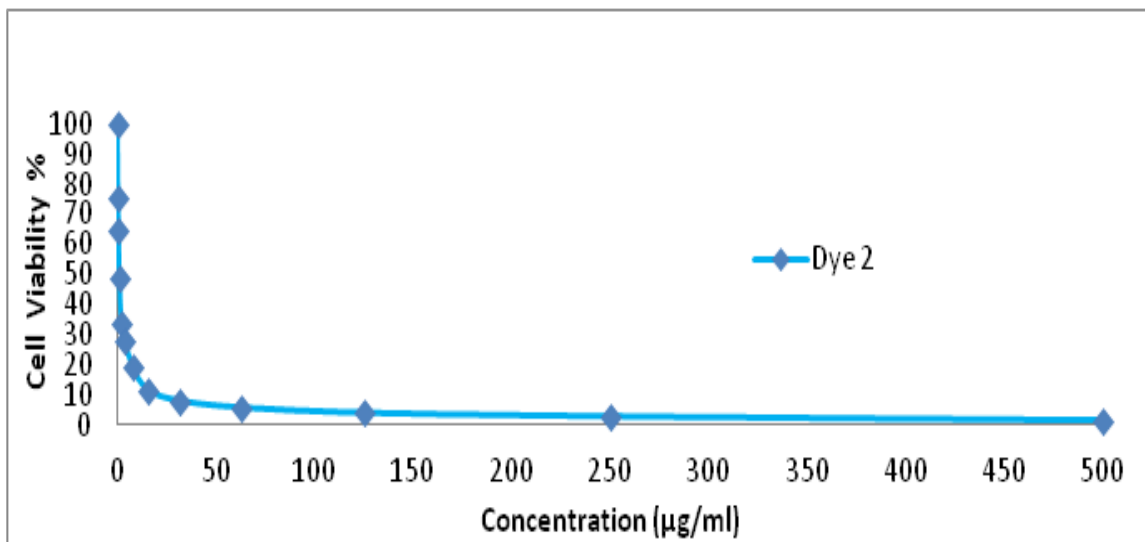
TABLE 4. Antitumor and antioxidant activities of the disperse dyes 1-6.

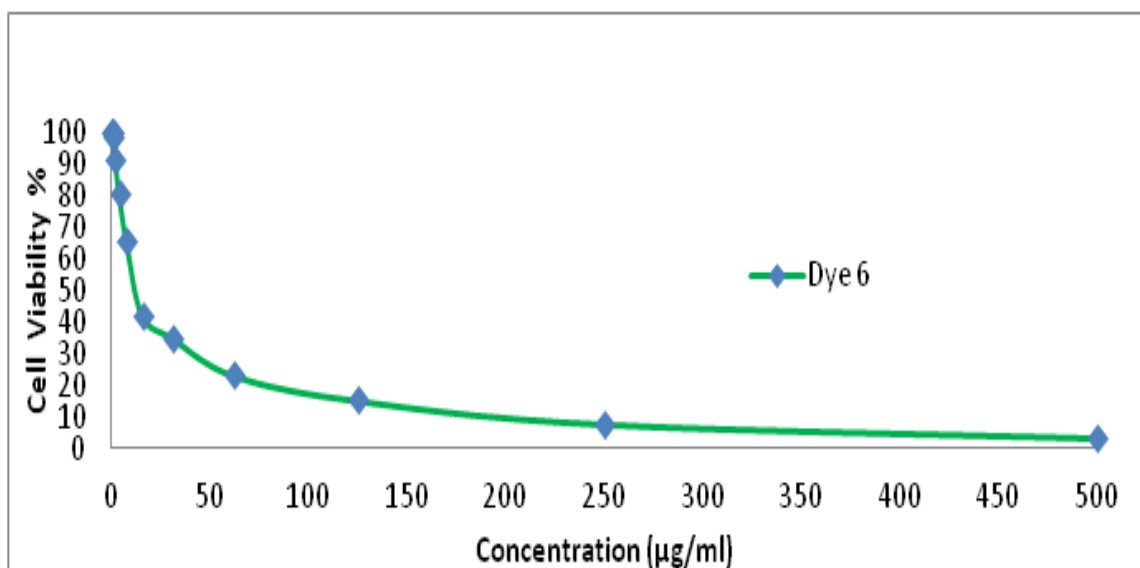
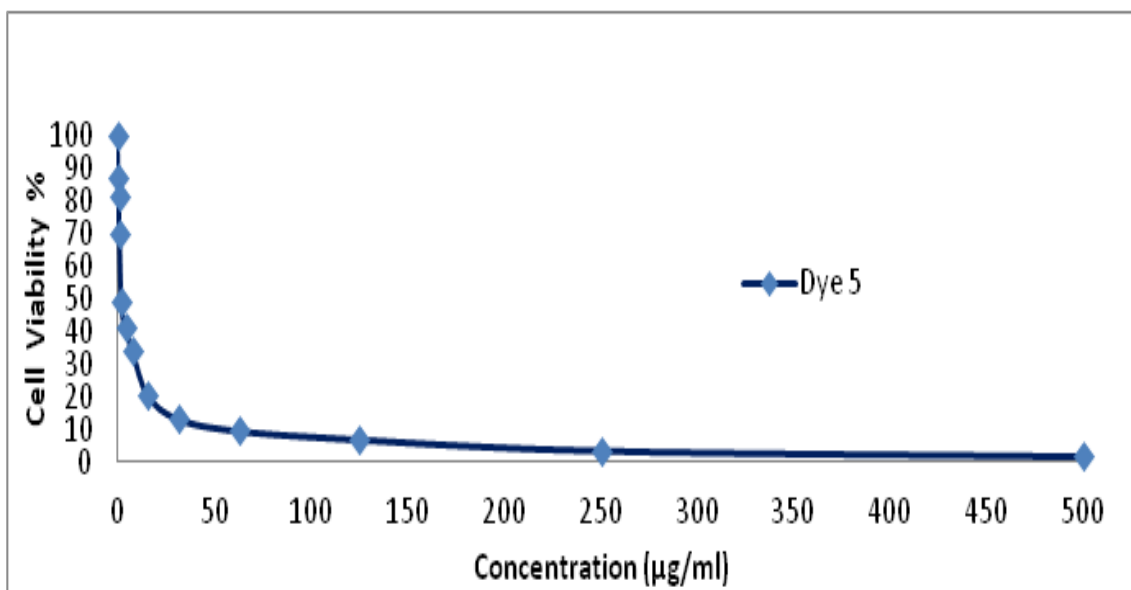
Dye Number	Cytotoxic activity (IC_{50} $\mu\text{g/ml}$)	Antioxidant activity
	HepG-2	(IC_{50} $\mu\text{g/ml}$)
Disperse dye 1	222 ± 6.20	63.80
Disperse dye 2	0.96 ± 0.08	111.0
Disperse dye 3	1.66 ± 0.12	74.90
Disperse dye 4	> 500	158.7
Disperse dye 5	1.96 ± 0.18	153.4
Disperse dye 6	13.0 ± 1.20	150.5
Doxorubicin	0.42	
Imatinib	18.9	
Ascorbic acid		14.20

In vitro cytotoxicity assays should have some of advantages, such as speed, reduced cost and potential for automation, and tests using human cells may be more relevant than some in vivo animal tests. However, they have some disadvantages because they are not technically advanced enough yet, to replace animal tests [9-13]. The in vitro growth inhibitory activity of the synthesized disperse dyes were investigated against human hepatocellular carcinoma (HepG-2), Data generated were used to plot a dose response curve of which the concentration of test compounds required to kill 50% of cell population (IC_{50}) was determined at the regional center for mycology and biotechnology Alazhar university. The cytotoxic effects observed for azo dyes might be

due to the action of dyes on the cells or, especially, to the formation of metabolites resulting from the azo bond reduction [16, 17]. Metabolites can react with the DNA molecule, damaging both its structure and function [18]. Because of the significant increase in chemical compounds being discharged into the environment, bioassays have been carried out using different organisms in order to identify and evaluate the harmful effects of various agents at their different concentrations and exposure periods [19]. The in vitro inhibitory activities of tested compounds against the hepatocellular carcinoma cell line (HEP G2) have the descending order as follow D2> D3> D5> D6> D1> D4 according to the data listed in table (4)



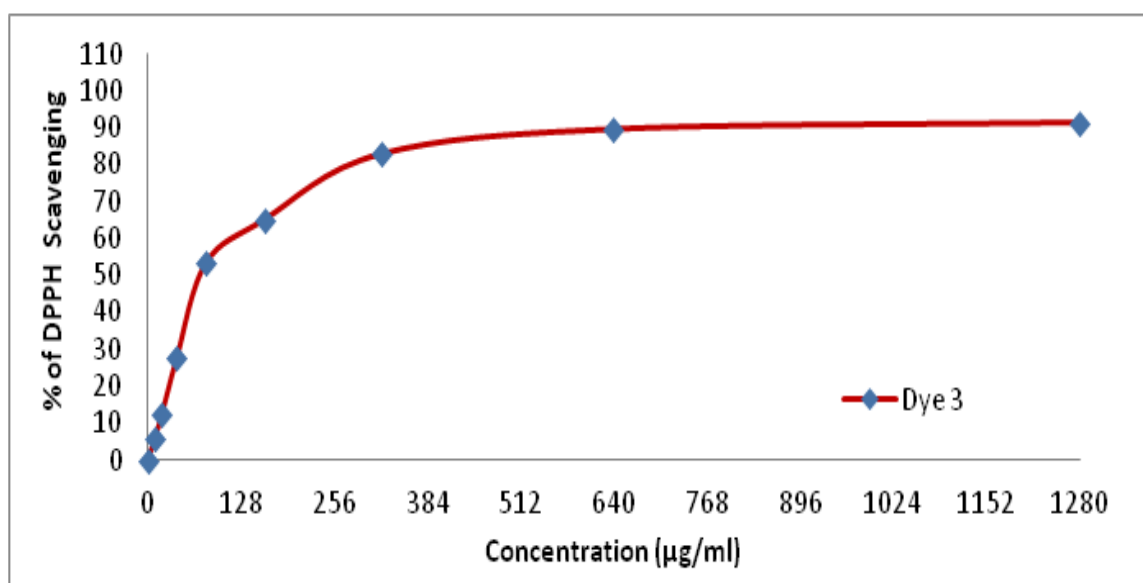
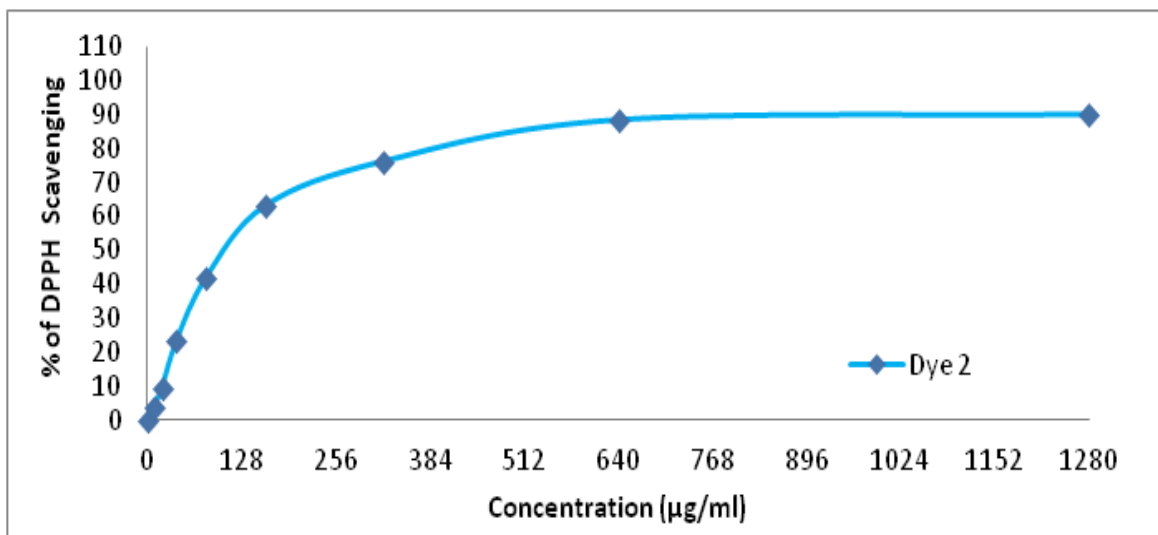
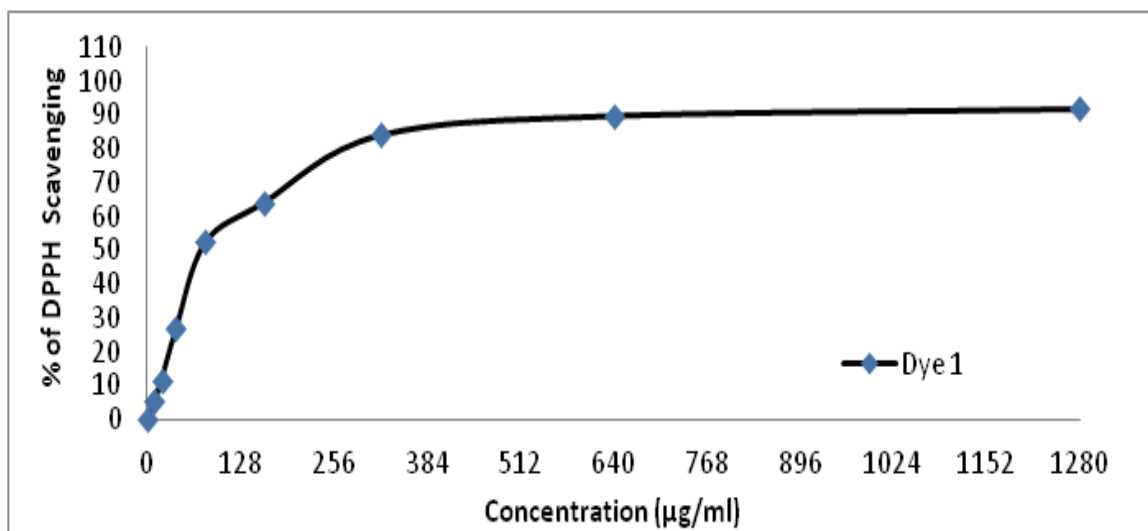


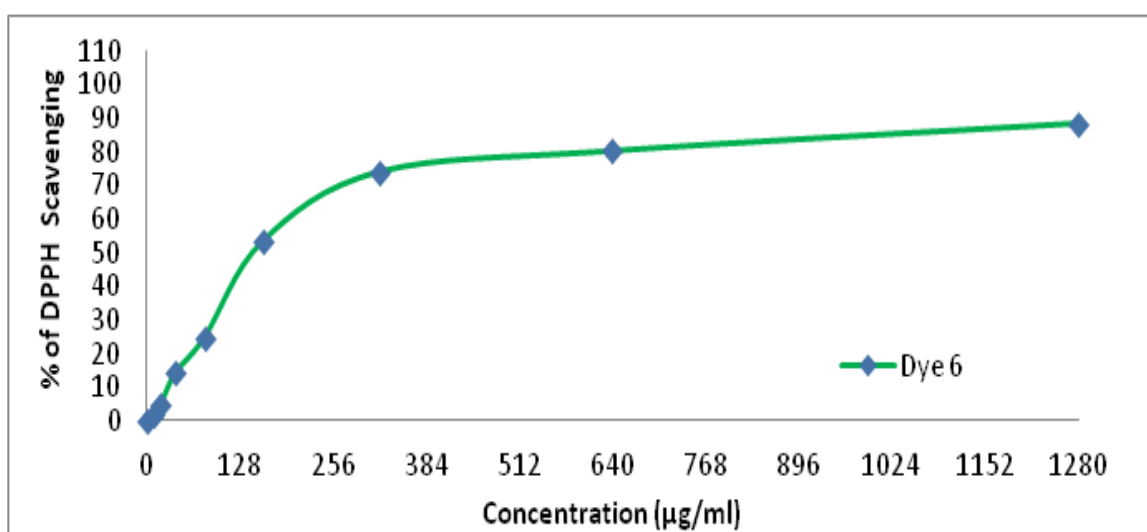
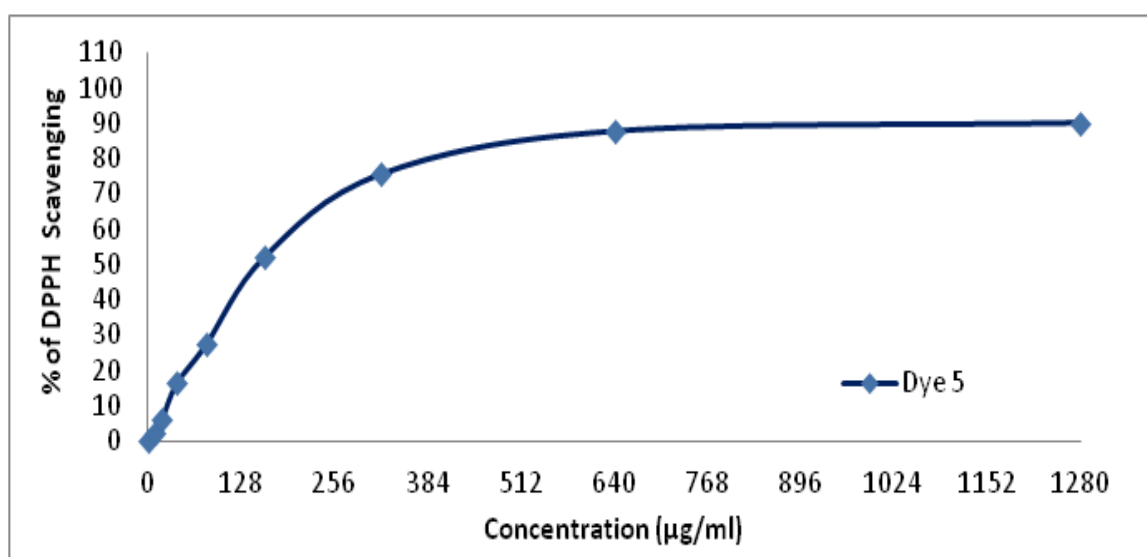
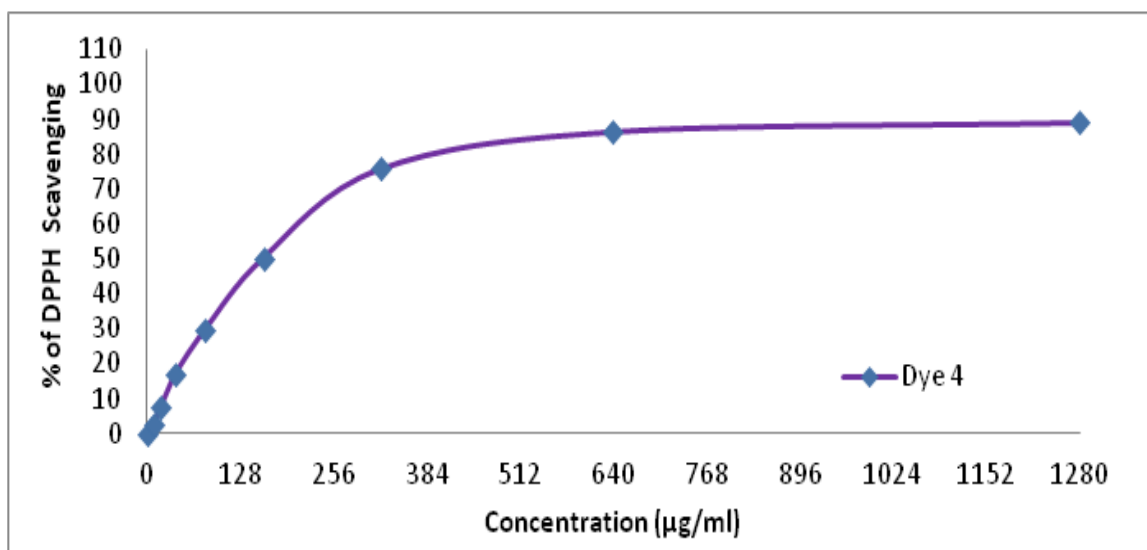


Antioxidant activities.

The 50% inhibitory concentration (IC_{50}), is the concentration required to inhibit DPPH radical by 50%. The ability to donate hydrogen or electron to the DPPH radicals is thought to be the reason for the antioxidant property of organic compounds. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The *in vitro* antioxidant activities of tested compounds have the descending order as follow D1> D3> D2> D6> D5> D4

according to the data obtained in table (4). IC_{50} value of ascorbic acid 14.2 µg/ml, which is a well known antioxidant scavenging of DPPH radical. Additionally, it seems to be that radicals scavenging activity of these Dyes is mainly due to azo groups that show strong antioxidant activity like phenolic compounds, as these compound characterized by its ability as a donating group beside that the position and the type of the substituent that bonded to the aromatic ring causing a slightly increasing or decreasing in the antioxidant activity.





Conclusions

In conclusion, a general method for the preparation of disperse dyes 1-6 was reported herein. The dyebath of disperse dyeing at 100 or 130 °C utilized again. The biological activities of the synthesized disperse dyes like antimicrobial, antitoxicity and antioxidants were investigated and some of the tested compounds showed moderate to weak effects

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تشبيد بعض الصبغات المنتشرة بمساعدة الإينامينونات. جزء ٣: إعادة استخدام حمام الصباغة و الانشطة البيولوجية

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تمت دراسة إعادة استخدام حمام الصباغة بعد الصباغة بهدف تعظيم العائد من الاستخدام الامثل للصبغات و كذلك الحد من التلوث البيئى. أيضا تمت دراسة الانشطة البيولوجية للصبغات المحضرة و التى أظهرت نتائج ممتازة تمتلكها تلك الصبغات.