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Mixtures of Natural Functional Dyes in Finishing and Coloration of Natural Fabrics

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ABSTRACT

As an environmentally friendly method of producing textiles, this article addresses the use of natural functional dye mixtures, specifically"turmeric (natural yellow 3), madder (nature red 8) and Cochineal (natural red4)" in finishing and dyeing of natural fabrics "Cotton, Wool, and Silk" as an eco-friendly approach to textile production. advantages and disadvantages of utilizing combinations of natural dyes are examined, including improved color fastness and dye absorption,using self-dyes and mixing dyes for each of the three fabrics. Furthermore, for the dyed fabrics, measurements were made of the color strength values of the samples as well as the variables "concentration, temperature, and time" that affect the dye extraction process.Color strength (K/S) and color data (L*, a*, b*, ΔE) are measured. Also, fastness properties, light, washing, perspiration and rubbing, for the dyed fabrics are tested and evaluated. The antimicrobial effect of positive and negative bacteria, fungi also detected. In addition to discussing additives to improve mixtures' dyeing qualities, natural and chemical mordents were highlighted. The advantages of employing natural dyes from an economic and cultural standpoint are also emphasized. The article concludes that the use of mixtures of natural functional dyes in textile production has significant potential for creating innovative and environmentally friendly products.

1. Introduction

Colors are amazing, in which add beauty to the world and are vital to human life [1,2]. They can be applied to textiles in two ways: either by dyeing or printing. The art of coloring with natural dyes has been mastered since the ancient civilizations of Egypt, Sumerian, Greece, Mexico, and Rome [1]. Natural colorants can be extracted from leaves, fruits, seeds, flowers, bark, plant roots, insects, fungi, bacteria, and some microorganisms [1, 2]. There has long been knowledge about the use of natural dyes to color textiles; in fact, the earliest documented use of these dyes dates back to 2600 BC in China [3]. Considered important artifacts that portray society and culture from antiquity to the present, historical textiles act as a repository for the development of practical materials [4].

Natural dyes are defined as colorants derived from natural sources whether plant, animal, or mineral [3, 5]. According to some definitions, dyeing is the act of directly applying colorant to fibers, yarn, or fabrics without the use of additives. From ancient times until the nineteenth century, natural dyes were the only ones used to color textiles [6]. natural sources of color include cutch, turmeric, abol ,madder and purple cabbage. local plants appear to have been used in Nubia:dyes containing pixin and a red dye that is similar to madder but has a higher protein content have been discovered [1]. Awareness of environmental concerns and the health effects of synthetic dyes have spurred resurgence in the use of natural dyes[2]. Many natural materials, including plant and animal sources are being identified as potential colorants, such as tea leaves. All over the world, tea is mostly consumed in huge quantities as a beverage tea dust was utilised as a colourant and its fastness property of silk fabric under various mordanting methods and mordants was evaluated. It was noted that the tea leaves used contained colourants, but more effort was involved in the extraction of to achieve the desired shade [2]. The post mordanting method was found to have better color fastness and visual properties. Potassium dichromate and alum. significantly improved the fastness properties of the dyed samples [2].

Natural dyes have been used for dyeing fabric, leather, body, hair, for cosmetic purposes, crafts, food coloring, dye sensitive solar cells, and functional textile finishing [1, 7, 8]. In Europe, dying was a traditional craft that dates back to the Bronze Age. Primitive dyeing techniques included pasting plants onto cloth or rubbing powdered dyes onto the cloth [8].Until the latter half of the nineteenth century almost all dyes were vegetable or animal in origin [1,8].

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Natural dyes from crushed fruits,berries and other plants were boiled into the fabric to give it lightness and water fastness (resistance) through the development of techniques [8]. The majority of nations import natural dyes, especially, Indigo and Lac from India [1]. The trade in natural dyes began to decline [9], particularly in 1856 with the discovery of the first industrial dye [3]. As a result, markets closed not only in Europe but also in India, Japan, Egypt, and other countries, which were dependent on India for natural dye resources [1].

It has been stated that, dying textiles with natural dyes requires asignificant amount of chemicals; mainly, metal salt mordants, which pose serious environmental challenges. To reduce this pollution, the dyeing industry should increasingly adopt environmentally friendly, biodegradable, biocompatible, and renewable products [10]. Scientists' current concerns about natural dyes for textile coloring and the safe synthesis of valuable fabric components led to the development and use of traditional natural colorants due to the potential environmental risks [4].Special emphasis has been paid to the use of natural dyes for fabric functional finishing, such as antimicrobial finishing and UV protection properties [9]. The bioactivity of natural dyes has been demonstrated and a great deal of research has been done on how to incorporate them into textiles. According to certain studies, The application of natural dyes on textile substrates will be advantageous due to their innatet functional properties along with their non-toxicity and eco-friendly nature. They discuss the effect of natural dyes on surface modification of most used natural and synthetic fibers and its subsequent effects on their anti-microbial, UV protection and insect repellent properties with natural dyes. Natural dyes have demonstrated their environmental friendliness in an attempt to improve bioactive functions in textile materials [11].

Recently, natural dyes are considered safe alternatives to industrial dyes because they have been proven to be carcinogenic, and many of the chemicals used in them are toxic and polluting the environment. Thus, the drainage resulting from the use of natural dyes is less problematic and can be eliminated more easily; therefore there is a great interest in the application of natural dyes in the coloring of textiles worldwide [1-3, 5, 8, 12,13]. These dyes can be obtained with or without a simple chemical process [14], and must be used in conjunction with binding substances, usually natural or mineral salts [1].

Fabrics dyed with natural dyes often have poor fastness properties particularly when applied to fabricthat contain minerals. these can be improved by applying mineral salts, metal ions combine with the dye molecule to form a compound that is water insoluble, thereby enhancing color stability [12]. The functional benefits of natural dyes, including make their use very important in various fields [7]. There are currently several studies available on the multifunctional properties of natural dyes such as antimicrobials effect [5].using alum, copper, and ferrous sulfate as resources, the rich tannin extract of the Kirkos in Victoria plant was used to study cotton fabric antimicrobial activity was then tested against both Gram-positive and Gram-negative bacteria [12]. a study was taken up as an exploratory study to test if some natural dyes have inherent antimicrobial activity with a view to develop protective clothing from these. Four natural dyes Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia and Rumex maritimus - were tested against common pathogens: Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa. Quercus infectoria dye was most effective and showed maximum zone of inhibition thereby indicating best antimicrobial activity against all the microbes tested. Minimum inhibitory concentration was found to be varying from 5 to 40 μg. The textile material impregnated with these natural dyes, however, showed less antimicrobial activity, as uptake of these dyes in textile material is below MIC (Minimum inhibition concentration) [15].

Natural dyes are divided according to their source into three sections, as shown in Fig. 1A. The first section is the plant sources. The coloring matter from these plant dyes is derived from seeds, leaves, roots, bark, stem, fruits, and flowers. Over 500 species of plants have been identified as dye sources. These dyes have been extracted from different plant species such as pomegranate, turmeric, indigo, red sandal, peelings, Henna, pomegranate, madder, onion, turmeric, and tea ….etc. as shown in Fig. 1B. The secound section is the nimal sources. Colorants are obtained from the secretions of insects such as the Abyssinian worm, and from substances used by herbalists and perfumers, such as cochineal kermis, and lac, as well as common shellfish, as shown in Fig. 1C. The scarlet-colored Tyrian dye is one of the most expensive ancient dyes, as it is prepared from a special type of shellfish found in the Mediterranean Sea. The third section is the mineral sources. Thsese species is represented by many inorganic minerals and metal oxides. There was once a species known as Iron buff. They are extracted by soaking thin strips of iron in a barrel with a mixture of water and wine for several days. chrome yellow, iron buff and Prussian blue are the three most significant mineral dyes [1, 3] as shown in Fig. 1D.

Figure 1. Sources of natural dyes. (A) Natural food coloring. (B) Natural plant dyes. (C) Natural animal Source" Cochineal . (D) Natural Mineral Sources.

It's important to note that efforts are continuously made to discover new sources of dye extraction,like those found in coton flowers, coffee seeds,or apple tree bark, as well as renewable sources, such as dyes extracted from the bark of mango trees or mushrooms [3].

There are various kinds of natural dyes such as, Madder, Henna, Indigo, Tyrian purple, Annatto, Saffron, Red Sandalwood, Eucalyptus hybrid seed, Seed of Cassia Tora, Pomegranate rind, Deodar leaf, Eucalyptus leaf, Wattle bark, Chrysanthemum flower, Eucalyptus bark, Teak leaves, Grape skin waste, Euphorbia leaves, Areca Catechu nut, Beet sugar, Tulsa leaves, Tea, Turmeric, Cochineal, Onion Skin, Lac, Persian berries, Logwood, Juglone, Remex maritimus, Gallnut fruit, Wood, Sumac, Oak ,Coreopsis, Caesalpinia, Bougainvillea, Goldenrod, Sunberry, Pivot, Alder, Berries, Canna, Lily, Nettles, organisms like cassia, Ganoderma lucidum, Curious versicolor, and Amanita muscaria [8].The following dyes are the ones that will be used in the current research Experimental part:

Cochineal Dyes is red dye used in Mesoamerica before the Spanish conquest. During the 16th century, they were imported to Europe. These insects are still collected in Peru and India to present a permanent bright scarlet [16, 17]. Botanical name is Dactylopius Coccus and its chemical class is anthraquinone, C.I. Name is natural Red 4 and the whole insect is used partis. The Composition of cochineal dye as follows: Insects must be dried to about 30% of their original weight before being stored without decomposing. Crushed Scarlet female insects give a deep scarlet natural color that can be used to produce a range of shades [9] as shown in Figure 2 (A,B).

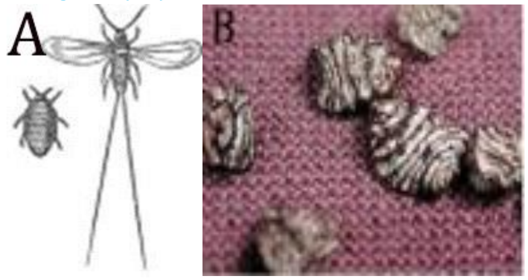

Figure (2) Cochineal Dye Composition. (A) Cochineals' male right and female left.. (B) Dry Cochineal.

Extraction of Cochineal dye is done by immersion in hot water, exposure to sunlight, Steam, or heating in an oven, where insect bodies are boiled in a solution of sodium carbonate. Each method produces a different color that results in a varied appearance of commercial Cochineal, to produce a range of scarlet, red, pink, and orange [9, 17].

Chemical structure of carminic acid, as shown in Fig. 3, is the main dye of cochineal, and responsible for the purple color of this dye. Carminic acid consists of large glucose units connected by an anthraquinone anion and max in water is 530 nm [16, 17,18].

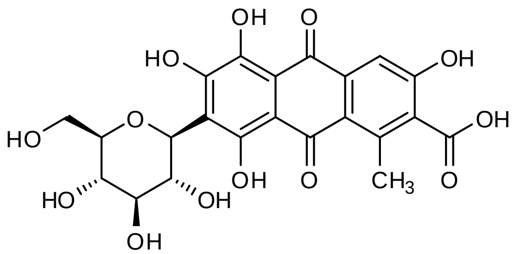

Fig. 3 Chemical structure of carminic acid (Cochineal Dye)
3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-7-((2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6 (hydroxymethyl)tetrahydro-2H-pyran-2-yl)-9,10-dihydroanthracene-2-carboxylic acid.

Turmeric or curcumin Dyes is historically one of the most famous and brightest of naturally occurring yellow dyes [17, 18] (Fig. 4A).

Fig. 4. Turmeric or curcumin Dyes.(A) Turmeric dye (B) Turmeric plant.

Turmeric dyes is extracted from the fresh or dried rhizomes (botanical name is *Curcuma tinctoria*, chemical class is carotenoid, C.I. name is natural yellow 3, and the used part is rhizome) as shown in Fig. 4B. It is a substantive dye capable of directly dyeing silk, wool, and cotton. Turmeric has a deep yellow natural color that can be used to produce a range of shades. On mixing with different mordant, a variety of colors are obtained, i.e.., lively yellow with alum, orange with thin, brown with iron, and olive with chromium [16-18]. The chemical structure of turmeric dyes as shown in Fig. 5, is the only natural yellow dye belonging to diferuloyl methane group. It is a diferuloyl-methane [18, 19].

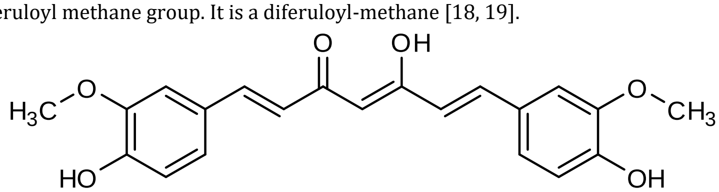

Fig. 5. Chemical structure of curcumin or turmeric -Enol form (Turmeric dye).
(1E,4Z,6E)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (or) (1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione.

Madder dye has been used as a dye for thousands of years, it was first recording in Egypt in the fourteenth century BC, its original origin is currently India, but its cultivation has spread to Europe and the Middle East, there are about 35 species of madder and the colored substance is concentrated in the rhizome of the plant at a concentration of up to 4% (Fig. 6) [20].

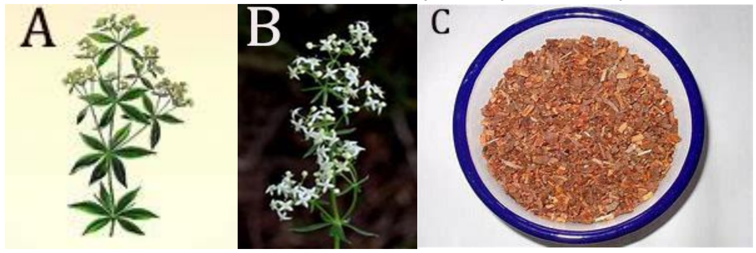

Figure 6. Madder dye. (A) Madder plant. (B) Wild madder. (C)Madder plant natural dye

The structure of Madder dye is a perennial plant in the Rubiaceae family that grows 50–150 cm tall and has a yellow bloom, a red rhizome, and a climbing red-brown stem (botanical name is *Rubia tinctorium,* chemical class is anthraquinone, C.I. name is natural red 4, and the used part is rhizome, park and stems) as shown is Fig. 6A-B. Madder in particularly important among red pigments. Although it has a lower coloring power compared to pigments extracted from insects, it has the advantage of producing different shades of pink to Black, Scarlet and red with different mordents. Madder on cotton produces a red shade with alum., and lemon, a pink tint with tin, and a brown tint with chrome [21, 22]. The extraction of Madder dye is peformed by Stirring dry madder roots in water at room temperature for 90 minutes, resulting in a suspension containing Pseudopurpurin, munjistin, alizarin and nordamncanthal as shown is Fig. 6C.The chemical structure of madder dye is depicted in Fig. 7. The most significant pigments in the madder plant's rhizome include anthraquinone, alizarin, rubiadin, purpurin, pseudopurpurin, and mungistin. Madder depends in its chemical composition on the compound anthraquinone [20, 21].

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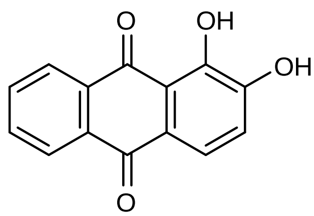

Fig. 7 Alizarin Crimson chemical structure (Madder dye) (1,2-dihydroxyanthracene-9,10-dione).

In this study, the fabrics are dyed with self -produced natural dyes shade or, the dyes were mixed before dyeing the fabrics, color strength (K/S) and color data (L*, a*, b*, ΔE) are measured. Also, fastness properties, light, washing, perspiration and rubbing, the dyed fabrics are tested and evaluated. The antimicrobial effect of positive and negative bacteria, fungi also detected. This article will provide a review of chitosan uses in textile finishing for cotton fabric.

2. Materials and methods

2.1. Materials

٢.1.1. Fabrics

Three fabrics - wool 100%, woven1/1 weight 221 gm2 , cotton 100%,woven1/1 and weight 144gm2 silk 100%,woven1/1 and weight 68gm2- were used in this studywool was obtained from Miser for Spinning and weaving Company, Mahalla El-Kobra, Egypt while the silk was purchased from local market.

2.1.2. Dyes

Three dyes were used in this study turmeric and madder supplied from the local market in Egypt they were in powder form, Cochineal natural dye were purchased from company Maiwa, Canada.

2.1.3. Mordants

Tannic acid and ferrous sulphate ($FeSO_4$) were the two mordant used in this study tannic acid was added at liquor ratio 1:40 during the dyeing process using 10% of the fabric weight as mordant .

2.2. Methods

2.2.1. Fabric Scouring

Before using the fabric (5 g / 1) is treated with a solution of non-ionic detergents (hostabal cv, Clariant) at 50°C for 30 minutes to eliminate impurities, then the fabric is thoroughly washed with water and dried at room temperature.

2.2.2. Extraction

Conventional extraction was carried out in 1000 ml of water using a different amount of dye powder (10-40 g) for turmeric and madder, concentration (1-5 g) for Cochineal, and this for different periods of time (20-100 min) and at a temperature (50° C-90° C), then filtered using nylon fabric.

2.2.3. Cotton Treatment with Chitosan

Samples fabrics of cotton were treated with freshly prepared aqueous solutions containing concentration (3% W.O.F) of chitosan high molecular weight and obtained in liquid form by dissolving in distilled water with 2% acetic acid using liquor ratio (1:20) for 60 min at 60°C using conventional heating the chemical structure of Chitosan is shown in Figure (8).

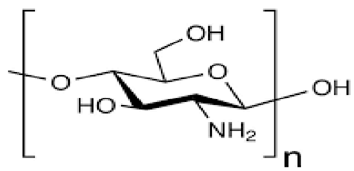

Figure (8) Chitosan structure.

Using 20 ml of dye for 1g of fabric, the dye extract of madder, turmeric, and cochineal was dyed at 90° C for 60 minutes. the pH values of turmeric, madder was 5 and cochineal 4.

2.2.4. Mixing of dyes

The fabrics are dyed according to the extent of mixing the dyes on each fabric used and the extent of the resulting color tone, so mixing was done in proportions of 1:1, 1:2, 1:3, 2:1, and 3:1 using each two dyes separately in the same pure dyeing method.

2.2.5. Washing

The dyed fabrics were soap using 3 gm/L of nonionic detergent (hostabal, CV Clariant) at 60°C for cotton and at 50°C for wool and silk this for 30 min. then rinsed with water and air dried.

2.2.6. Antimicrobial Effect of the Dyed Fabrics

Fabrics were tested against two types of bacteria and one type of fungus using agar propa Tests gation method. Agar was made with a size of 6 mm in Petri dishes containing bacterial inoculum and textiles samples dyed with natural dyes. fabrics dye samples were dissolved in 100 mg of water in 500 ml for 10 minutes. The prepared dishes were kept at room temperature to enable the extract to spread into the agar. After incubation for 24 hours at 37 °C, antibacterial activity was observed, indicated by A zone of inhibition surrounds the sample that contains the natural dye. The area of inhibition was measured and expressed in millimeters [7].

2.3. Measurements

The extracted dyes solution was measured Ultraviolet-Visible (UV/Vis) Spectrophotometer (Model, Lambda 3B). After series of dilution and at the detected weave length for each dye. The colorimetric analysis of the colored fabrics was recorded using a spectrophotometer with pulsed xenon lamps as light source (Ultra Scan Pro, Hunter Lab, and USA) 10°C observer with D65 illuminant, d/2 viewing geometry and measurement area of 2 mm. All measurements were occurred at different λ .The color strength (K/S) in visible region of the spectrum (350-700) nm was calculated based on Kubelkae – Munk equation [21].

$$\mathrm{k/S} = \frac{(2-R)}{2R} - \frac{(1-R_\circ)}{2R_\circ}$$

R is the dyed fabric reflectance expressed as decimal fraction. R_0 is the undyed fabric ‘s reflectance expressed as decimal fraction. K is the absorption coefficient. S stand for Scattering coefficient

The colorimetric properties of dyed fabrics were obtained with Hunter Lab DP-9000 Color- Spectrophotometer. The total difference CIE (L*, a*, b*) was measured using the Hunter-Lab spectrophotometer (model: Hunter Lab DP-9000). CIE (L*, a*, b*) between two colors each given in terms of L*, a*, b* is calculated from.

$$\Delta E = L^2 + (a^2 + b^2)^{1/2}$$

Where ΔE is the total difference between the sample and the standard, L the lightness from black (0) to white (100), a* is a red (+)/green (−) ratio and b* is yellow (+)/blue (−) ratio. 3. L* value: indicates lightness, (+) if sample is lighter than standard, (-) if darker. A* & b* values: indicate the sample and standard's relative locations in CIE Lab space, from which can be used to determine the kind of difference that exists [22].

2.3.1. Fastness Properties

The fastness properties of the dyed samples were tested according to ISO standard methods. The specific tests were ISO 105-X12 (1987) for color fastness to rubbing, ISO 105-C02 (1989) for color fastness to washing, ISO 105-E04 (1989) color fastness to perspiration, ISO 105-B02 (1988) for color fastness to light (Xenon lamp) [17].

2.3.2. Microbiological analysis

Minimum inhibition concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. (MIC) is important to monitor the activity of new antimicrobials [23]. In order to determine the minimum inhibition concentration (MIC) for fibers, E. coli, *Staphylococcus*, aureus, and *Aspergellus* were used for the study [24] in which a standard protocol was followed for both tests. First, test samples were prepared and then three serial dilutions of each sample were made, at a final concentration of 50 mg/mL using sterile deionized water. Then 100 µL of each prepared concentrate and 95 µL of nutrient broth were added into each Petri dish. Negative and positive controls were also performed for each strain. Negative controls were performed by serial dilutions of samples in sterile distilled water (50%). The petri dishes of the test samples were incubated at the proper temperature required by each bacteria for 24 hours and growth kinetics assays for each strain were carried out by the bacterial cell counting device. MIC results for extracts are reported as mg/mL. These MIC spectral measurements were performed using a standardized Varioskan multilayer reader protocol after incubation for 24 h.

3. Results and Discussions

3.1. Effect of dye concentration in the extraction method

Measurement of more than one dye weight concentration in the dyeing bath with constant time 60 minutes and temperature at 90 °C. The concentration of both turmeric and madder dyes was measured by 4 different weights, (10, 20, 30, 40) g\L and measuring the concentration of Cochineal dye with different weights (1, 2, 3, 4, 5) g\L. The conclusion from Table 1 was that the greater the weight of the dye, the higher its concentration in the solution, so 40 g/L of turmeric and madder dyes and 5 g / L of Cochineal dyes, Fig. 9A, B, C.

Table 1: Measurement of dye weight concentration in extraction.

Concentration Dye absorbance				
Dye weight	Turmeric	Madder	Dye weight	Cochineal
40g	2.52	1.11	5g	1.61
30g	2.03	0.98	4g	1.13
20g	1.54	0.7	3g	0.85
10g	0.87	0.43	2g	0.64
--	--	--	1g	0.4

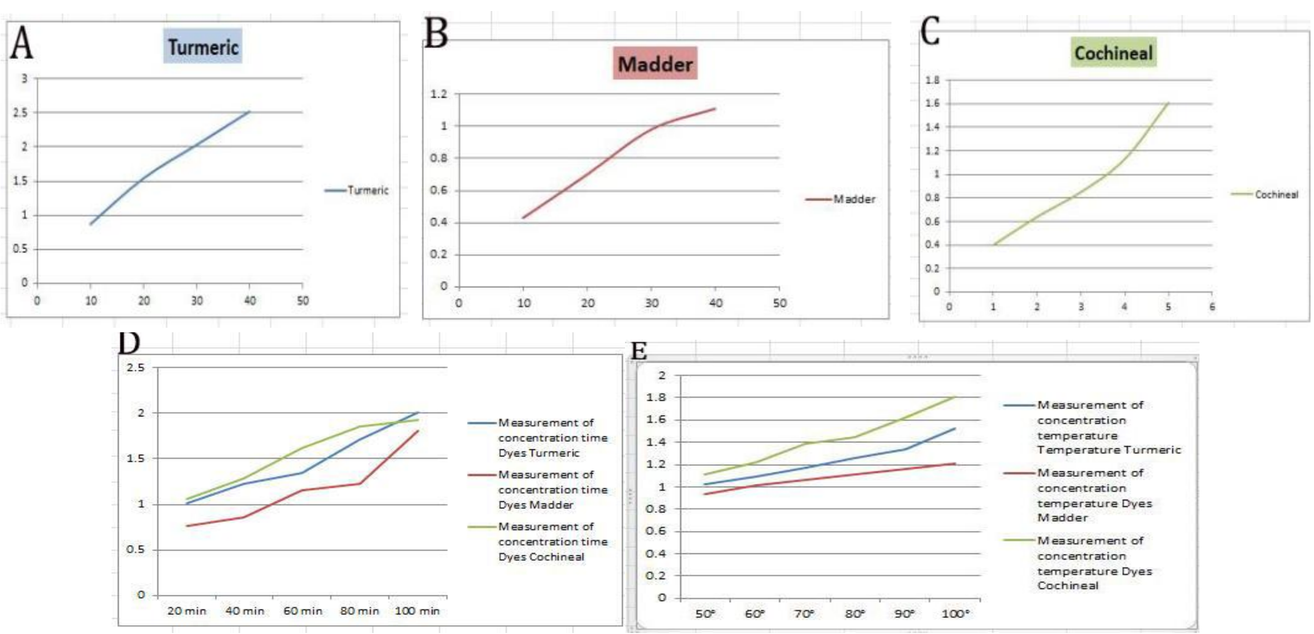

Fig. 9. Effect of Dye Concentration and time factor in the Extraction Method. (A) Turmeric tincture concentrate. (B) Madder tincture concentrate. (C) Cochineal tincture concentrate. (D) Measuring the time factor change. € Measurement of temperature factor change.

3.2. Effect of the extraction time

Measurement of the time factor in the process of dye preparation "dye bath" with the stability of dye weight 35 g/L for turmeric, madder dyes, and 5 g/L for Cochineal dye with temperature 90°C.The concentration of the time factor of dye bath for both turmeric, madder and Cochineal dyes was measured at different times to find out their best concentration for dye, dyeing bath and times were (20, 40, 60, 80, 100) minutes Fig. 9D,E. The conclusion from Table (2) was that the greater the time, the higher the dye concentration, but due to the time saving factor, the concentration of time factor, which represents 60 minutes, was used in the process of making the dye bath and dyeing.

Table 2**:** Measuring the time factor change in extraction

Time		Dyes Absorbance	
	Turmeric	Madder	Cochineal
20 min	1.01	0.76	1.06
40 min	1.22	0.86	1.28
60 min	1.34	1.16	1.62
80 min	1.71	1.23	1.86
100 min	2.01	1.81	1.92

3.3. Effect of temperature

Measurement of temperature factor change in the extraction process "dyeing bath" with stabilization of the dye weight concentration of 40 g/L for turmeric, madder, and 5g/L for Cochineal dye with time 60 minutes. Both turmeric dyes, madder, and Cochineal were measured at different temperatures to determine the best concentration of the dye, dyeing bath and the temperatures were(50°C, 60°C, 70C°, 80°C, 90°C, 100°C) degrees.

Table 3: Temperature factor in extraction

Temperature	Dyes Absorbance		
	Turmeric	Madder	Cochineal
50°C	1.02	0.94	1.11
60°C	1.09	1.01	1.22
70°C	1.17	1.06	1.39
80°C	1.26	1.11	1.45
90°C	1.34	1.16	1.62
100°C	1.52	1.21	1.81

The conclusion from Table 3 was that the higher the temperature, the higher the dye concentration, However, to save the energy , the solution was prepared at a temperature of 90°C for both the extraction bath and dyeing.

3.4. The effect of pure-dyeing on fabrics

Turmeric dye is excellent with both wool and silk, while it is good for cotton. The color strength (K/S) is measured at 390 nm. Madder dye is excellent with wool, silk, and cotton when using chitosan. The color strength (K/S) is measured at 500 nm. Cochineal dye is excellent with wool but weak with cotton and silk. The color strength (K/S) is measured at 530 nm, as shown in Table 4.

Table 4: The effect of pure dyeing on fabrics.

Turmeric	K/S	λ_{max}	Madder	K/S	λ_{max}	Cochineal	K/S	λ_{max}
Cotton	2.64	390	Cotton	1.53	500	Cotton	0.50	530
Wool	11.22	390	Wool	3.61	500	Wool	3.06	530
Silk	12.44	390	Silk	1.04	500	Silk	0.41	530

3.5. The effect of mixing dyes on fabrics

The mixing of turmeric and madder in different proportions (1:1), (1:2), (1:3), (2:1),and (3:1) was performed on wool, cotton, and silk fabrics when turmeric dye and madder were mixed on cotton, wool, and silk fabrics in equal proportions ,they produced different color tones, increasing the percentage of madder and reducing turmeric resulted in an orange color tone, while increasing the percentage of turmeric and reducing madder led to a yellow color tone. The color value ranged between 1.58 to .8.63 as shown in Table 5 .

The mixing of turmeric and cochineal dyes in different proportions (1:1), (1:2), (1:3), (2:1), and (3:1) was performed on silk, the color strength ranged from (1.03 to1.85), for wool, the mixing ratios were (1:1), (1:2), and(2:1) ,and the color strength ranged from (7.68:19.78), Additionally, the mixing of madder and cochineal dyes in three proportions was conducted on cotton fabric, with the mixing ratios of (1:1), (1:2),and (2:1) the color strength measurement was (3.27:5.84) as indicated in Table 6.

Table 5: Mixing Dye Turmeric & Madder.

Cotton	K/S	Wool	K/S	Silk	K/S
Mix(1:1)	2.23	Mix(1:1)	8.63	Mix(1:1)	2.12
Mix(1:2)	2.74	Mix(1:2)	5.52	Mix(1:2)	1.58
Mix(1:3)	2.54	Mix(1:3)	5.13	Mix(1:3)	1.72
Mix(2:1)	2.05	Mix(2:1)	7.90	Mix(2:1)	2.19
Mix(3:1)	2.28	Mix(3:1)	7.65	Mix(3:1)	2.11

Table 6: Mixing Dye Turmeric & Cochineal, Madder & Cochineal

Turmeric & Cochineal		Turmeric & Cochineal		(Madder & Cochineal	
Silk	K/S	Wool	K/S	Cotton	K/S
Mix(1:1)	1.65	Mix(1:1)	10.45	Mix(1:1)	5.84
Mix(1:2)	1.24	Mix(1:2)	7.68	Mix(1:2)	3.27
Mix(1:3)	1.03	Mix(2:1)	19.78	Mix(2:1)	5.63
Mix(2:1)	1.85	--	--	--	--

3.6. The effect of mordents on fabrics during dyeing

The addition of tannic acid as a mordant during the dyeing process was done when dyeing wool, silk and cotton with turmeric. The color value ranged from (5.78to 22.48), Additionally, when dyeing the three fabrics with madder, the color strength ranged from (3.75 to 13.26). Wool and cotton were also dyed with cochineal resulting in a color strength of 3.06 to 10.01,However, it did not provide any color to the silk fabric (0.01) as displayed in Table (7).

As shown in table (8), The addition of mordant ($FeSO_4$) during the dyeing process resulted in color values ranging from (3.20to20.87) when dyeing wool, silk and cotton with turmeric. Similarly, when dyeing the three fabrics with madder the color strength ranged from (4.42 to 8.83). Wool and cotton were dyed with cochineal resulting in a color strength range of (3.47 to 8.01) ,However, no color was obtained on silk fabric (0.02). During the dyeing process, the color value when dyeing wool, silk and cotton with turmeric, was in the range of (380 to 390 nm), similarly, when dyeing the three fabrics with madder, the color strength was observed within the (380:390 nm)range. Wool and cotton were dyed with cochineal and yielding the color strength (530 nm).

Table 7: The effect of Tannic acid Mordant

	Mordant ($FeSO_4$)		
Fabric	Turmeric K/S	Madder K/S	Cochineal K/S
Silk	10.32	4.73	0.02
Cotton	3.20	8.83	3.47
Wool	20.87	4.42	8.01

Table 8: The effect of $FeSO_4$ Mordant

	Mordant (tannic acid)		
Fabric	Turmeric K/S	Madder K/S	Cochineal K/S
Silk	14.26	3.75	0.01
Cotton	5.78	3.84	3.06
Wool	22.48	13.26	10.01

3.7. The effect of color strength (K/S) and color data (L*, a*, b*, ΔE).

In Tables 9-11, the effect of color strength (K/S) and color data (L*, a*, b*, ΔE) is measured with and without the effect of mordants.

Table 9: Color measurements without mordant effect.

Dye	Fabric	k/s	L	A	B	ΔE
Cotton	Turmeric	4.10	76.93	0.55	60.47	64.15
Silk	Turmeric	8.47	61.06	61.06	-1.35	47.48
Cotton	Turmeric & Madder	4.59	64.87	11.38	44.60	53.59
Cotton	Madder	3.20	66.41	16.65	11.83	30.25
Silk	Madder	3.84	57.95	57.5	14.89	13.10
Silk	Turmeric & Madder	7.88	60.10	60.10	6.77	45.21
Wool	Turmeric	18.83	63.50	6.54	58.04	53.74
Wool	Madder	16.48	44.66	33.23	25.16	53.65
Wool	Cochineal	10.64	43.13	28.57	-0.08	49.77
Wool	Cochineal & Madder	7.03	47.52	26.95	15.23	45.17
Wool	Cochineal & Turmeric	12.30	50.90	19.51	23.36	40.42
Wool	Turmeric & Madder	11.98	52.72	17.68	34.36	43.65

Table 10: The effect of mordant Tannic acid on color measurements.

Fabric	Dye	k/s	L	a	B	ΔE
Cotton	Turmeric	1.73	81.61	.-2.20	37.35	40.79
Silk	Turmeric	1.56	71.37	-1.33	18.20	18.05
Cotton	Turmeric & Madder	2.99	70.32	14.35	39.07	47.55
Cotton	Madder	2.74	66.06	18.57	12.27	31.91
Silk	Madder	1.24	66.88	9.53	8.54	18.15
Silk	Turmeric & Madder	1.94	68.64	3.74	21.56	22.63
Wool	Turmeric	5.97	71.54	3.42	41.82	35.35
Wool	Madder	4.76	55.35	22.12	24.64	39.03
Wool	Cochineal	2.47	56.40	19.19	-4.30	35.16
Wool	Cochineal & Madder	3.46	54.32	20.13	10.45	35.07
Wool	Cochineal & Turmeric	5.61	63.16	8.24	30.13	30.32
Wool	Turmeric & Madder	5.71	59.76	16.99	33.42	38.16

Table 11: The effect mordant $FeSO_4$ color measurements

Fabric	Dye	k/s	L	a	B	ΔE
Cotton	Turmeric	3.20	74.97	4.06	25.58	30.96
Silk	Turmeric	5.60	65.89	4.97	36.97	37.77
Cotton	Turmeric & Madder	3.88	64.42	9.47	21.39	34.27
Cotton	Madder	3.60	65.27	10.66	12.61	28.54
Silk	Madder	2.83	64.51	4.34	14.84	21.22
Silk	Turmeric & Madder	3.97	64.51	2.21	26.30	28.97
Wool	Turmeric	6.90	56.68	9.81	34.37	38.04
Wool	Madder	7.04	50.60	9.01	19.63	35.09
Wool	Cochineal	5.29	58.15	2.19	13.76	24.91
Wool	Cochineal & Madder	5.68	53.74	6.29	16.24	30.50
Wool	Cochineal & Turmeric	13.66	48.28	8.68	28.47	40.65
Wool	Turmeric & Madder	8.76	49.28	10.93	27.96	40.19

3.8. The effect of Fastness properties, such as light, washing, perspiration and rubbing, for the dyed fabrics.

Table 12: Fastness Properties of fabrics without mordants

	Fabric & dye	Washing			Rubbing		Perspiration						Light
							Acidic			Alkaline			
		St.*	St.**	Alt.	Dry	Wet	St.*	St.* *	Alt.	St.*	St.**	Alt.	
1	Cotton : Turmeric	2-3	3	3-4	3-4	3-4	3	3-4	3-4	3	4	4	3
2	Silk : Turmeric	3-4	3-4	3-4	4	4	3-4	3-4	4	3-4	4	4	3-4
3	Cotton: Turmeric & Madder	3-4	3	3-4	3	2	3-4	3-4	4	3-4	3-4	4	3-4
4	Cotton : Madder	3-4	3	3-4	3-4	3-4	3-4	3-4	4	3-4	4	4	3
5	Silk : Madder	4	4	4	3-4	4	4	4	4	3-4	4	4	5-6
6	Silk : Turmeric & Madder	3-4	3-4	3-4	4	4	3-4	3-4	3-4	4	4	4	4-5
7	Wool : Turmeric	3-4	4	3-4	3-4	2-3	3-4	4	4	4	4	4	4
8	Wool : Madder	3-4	3-4	3-4	3-4	2-3	3-4	4	4	4	4	4	4-5
9	Wool : Cochineal	4	4	4	4	4	4	4	4	4	4	4	6
10	Wool : Cochineal & Madder	3-4	3-4	4	4	3	4	4	4	3-4	4	4	5-6
11	Wool : Cochineal & Turmeric	4	4	4	3-4	3-4	4	4	4	4	4	4	4-5
12	Wool : Turmeric & Madder	3-4	3-4	4	4	3-4	4	4	4	3-4	4	4	4

Table 13: Fastness properties of fabrics with mordant tannic acid

	Fabric & dye	Washing			Rubbing		Perspiration						Light
							Acidic			Alkaline			
		St.*	St.**	Alt.	Dry	Wet	St.*	St.* *	Alt.	St.*	St.**	Alt.	
1	Cotton : Turmeric	3-4	3-4	3-4	4	3-4	3-4	4	4	3-4	3-4	4	5
2	Silk : Turmeric	3-4	3-4	3-4	4	4	4	4	4	4	4	4	5
3	Cotton: Turmeric & Madder	4	4	4	4	3-4	4	4	4	4	4	4	4-5
4	Cotton : Madder	4	4	4	4	3	4	4	4	4	4	4	3-4
5	Silk : Madder	4	3-4	4	4	4	4	4	4	4	4	4	5-6
6	Silk : Turmeric & Madder	3-4	3-4	3-4	3-4	4	4	4	4	4	4	4	5-6
7	Wool : Turmeric	4	4	3-4	3-4	3-4	4	4	4	4	4	4	4
8	Wool : Madder	4	4	4	2-3	2	4	4	4	4	4	4	4-5
9	Wool : Cochineal	4	4	4	2-3	2	4	4	4	4	4	4	6
10	Wool : Cochineal & Madder	4	4	4	3	2	4	4	4	4	4	4	6
11	Wool : Cochineal & Turmeric	3-4	4	4	1-2	1-2	4	4	4	4	4	4	4-5
12	Wool : Turmeric & Madder	4	4	4	3-4	3-4	4	4	4	4	4	4	5

Table 14: Fastness properties of fabrics with mordant $FeSO_4$

	Fabric & dye	Washing			Rubbing		Perspiration						Light
							Acidic			Alkaline			
		St.*	St.**	Alt.	Dry	Wet	St.*	St.* *	Alt.	St.*	St.**	Alt.	
1	Cotton : Turmeric	4	4	4	4-5	4	4	4	4	4	4	4	2-3
2	Silk : Turmeric	4	4	4	4	4	4	4	4	4	4	4	5
3	Cotton: Turmeric & Madder	3-4	4	4	3-4	3-4	3-4	4	4	3-4	4	4	2-3
4	Cotton : Madder	4	4	4	3	3	3-4	4	4	3-4	4	4	2-3
5	Silk : Madder	4	4	4	3-4	3-4	4	4	4	4	4	4	5
6	Silk : Turmeric & Madder	4	4	4	3-4	3-4	4	4	4	4	3-4	4	5
7	Wool : Turmeric	4	4	4	3-4	3	4	4	4	3-4	4	4	3-4
8	Wool : Madder	4	4	4	2-3	2-3	4	4	4	3-4	4	4	4-5
9	Wool : Cochineal	4	4	4	4	4	4	4	4	4	4	4	6
10	Wool : Cochineal & Madder	4	4	4	2-3	3-4	4	4	4	4	4	4	6
11	Wool : Cochineal & Turmeric	4	3-4	4	3-4	3-4	4	4	4	4	4	4	3-4
12	Wool : Turmeric & Madder	4	4	4	3	3	4	4	4	4	4	4	4-5

St.* = Staining on cotton; St. ** = Staining on wool; Alt. = alteration in color.

In tables 12-14, the effect of fastness properties such as light, washing, perspiration, and rubbing on the dyed fabrics is tested and evaluated.

2.1. Microbiological analysis

The data was analyzed using one-way ANOVA and means. Minimum inhibition concentration assays. In order to obtain MIC values of samples against different pathogens and microorganisms, the MIC values for *E. coli*, *Staphylococcus aurous*, and *Aspergillus flavus* are shown in the following tables 1٥, ١٦ :Cotton and turmeric, Cotton and madder, Silk and turmeric, Silk and madder, Wool and madder, Wool and Cochineal and Wool and turmeric .

Table 15: Antimicrobial activity of samples, solid diffusion test: total inhibition (clear zone, mm)*

Microorganism	Samples							
	Control	1	2	3	4	5	6	7
E.coli	0^{a}	1^{a}	2^{b}	3^{c}	2^{a}	2^{b}	3^{c}	3^{c}
Staphylococcus aureus	0^{a}	2^{b}	3^{c}	3^{c}	2^{a}	2^{b}	3^{c}	3^{c}
Aspergellus	0^{a}	1^{a}	2^{b}	2^{b}	3 c	3^{c}	3^{c}	3^{c}

* Total inhibition or clear zone means no growth of microorganism. An inhibition of 90 mm represents total inhibition (Petri dish diameter). Inhibition diameter is the mean of three different observations taken from three different experiments. * Data followed by different letters in the row are statistical significantly different.

Total inhibition or clear zone means no growth of microorganism. An inhibition of 90 mm represents total inhibition (Petri dish diameter). Inhibition diameter is the mean of three different observations taken from three different experiments.

Table 16: The total bacterial numbers of samples dyed with one type of dye were examined after their preparation*

Microorganism	Samples							
	Control	1	2	3	4	5	6	7
E.coli	0	$12x10^{3d}$	$10x10^{3c}$	$6x10^{3b}$	$4x10^{3b}$	$6x10^{3b}$	$6x10^{3b}$	$10x10^{3c}$
Staphylococcus aureus	0	$10x10^{3c}$	$9x10^{3c}$	$6x10^{3b}$	$3x10^{3a}$	$6x10^{3b}$	$5x10^{3b}$	$9x10^{3c}$
Aspergellus flavus	0	$12x10^{3d}$	$10x10^{3c}$	$6x10^{3b}$	$4x10^{3b}$	$6x10^{3b}$	$6x10^{3b}$	$10x10^{3c}$

*Minimum inhibition concentration(MIC) effect of finishing on antimicrobial activities of fabric *for E.coli, Cocci*, and *Aspergellus flavus*

In the current study it was observed that fibers silk dyed with the encapsulated madder has antibacterial activity compared to cotton and wool. It gives higher resistance results than turmeric and cochineal the madder dyed silk was able to inhibit the growth of all the bacteria tested. Solid diffusion test: total inhibition (clear area, mm) log read 2^{a}, 2^{a}, 3^{c} (*E.coli, S. aureus, Aspergellus flavus*)respectively, and recorded $4x10^{3b}$, $3x10^{3a}$, $4x10^{3b}$ (*E.coli, S. aureus, Aspergellus flavus*)respectively. The study proved the antimicrobial activity of madder that it was more Efficacy against (*E.coli, S. aureus, Aspergellus*), cochineal, and then turmeric. The activity is due to the presence of the substance Alizarin It has a higher antimicrobial property than turmeric.

Tables 17 and 18 show the antimicrobial activity of turmeric, madder and cochineal for cotton, wool and silk dyed fabric. With the combination of two types of natural herbs on one sample (Turmeric with madder cotton, Turmeric with madder silk, Cochineal with madder wool, with Turmeric wool, and Turmeric with madder wool).Results are visible on fabrics dyed with these mixed proportions of natural dyes Antimicrobial activity.

Table 17: Antimicrobial activity of samples dyed with two types of natural dyes per sample, solid diffusion test: total inhibition (clear area, mm)*

Microorganism	Samples					
	control	1	2	3	4	5
E.coli	0	2^{b}	2^{b}	3^{b}	3^{b}	2^{b}
Staphylococcus aureus	0	3^{b}	3^{b}	3^{b}	3^{b}	2^{b}
Aspergellus flavus	0	2^{b}	2^{b}	3^{b}	3^{b}	3^{b}

* Total inhibition or clear zone means no growth of microorganism. An inhibition of 90 mm represents total inhibition (Petri dish diameter). Inhibition diameter is the mean of three different observations taken from three different experiments. * Data followed by different letters in the row are statistical significantly different.

Table 18: The total bacterial numbers of samples dyed with two types of natural herbs per sample were examined after their preparation*

Microorganism	Samples					
	Control	1	2	3	4	5
E.coli	0	$9x10^{3c}$	$4x10^{3b}$	$5x10^{3b}$	$6x10^{3b}$	$5x10^{3b}$
Staphylococcus aureus	0	$8x10^{3c}$	$3x10^{3a}$	$5x10^{3b}$	$5x10^{3b}$	$5x10^{3b}$
Aspergellus flavus	0	$10x10^{3c}$	$4x10^{3b}$	$5x10^{3b}$	$6x10^{3b}$	$6x10^{3b}$

*Minimum inhibition concentration (MIC) Effect of finishing on antimicrobial activities of fabric for *E.coli ,Cocci, and Aspergellus flavus*

Table 17,18 the decrease in bacteria concentration is observed in treated wool samples When comparing wool dyed with Cochineal and madder, better results are recorded compared to wool dyed with madder and turmeric, followed by wool dyed with Cochineal and turmeric. It exhibits a very high resistance activity against bacteria *Escherichia coli*.

Table 19: Antimicrobial activity of samples, solid diffusion test: total inhibition (clear zone, mm)*. ($FeSO_4$)

Microorganism	Samples							
	Control	1	2	3	4	5	6	7
E.coli	0^{a}	3^{b}	3^{b}	4^{a}	4^{a}	3^{b}	4^{a}	4^{a}
Staphylococcus aureus	0^{a}	2^{b}	4^{a}	4^{a}	3^{b}	3^{b}	4^{a}	4^{a}
Aspergellus flavus	0^{a}	3^{b}	3^{b}	3^{b}	4^{a}	4^{a}	4^{a}	4^{a}

* Total inhibition or clear zone means no growth of microorganism. An inhibition of 90 mm represents total inhibition (Petri dish diameter). Inhibition diameter is the mean of three different observations taken from three different experiments. * Data followed by different letters in the row are statistical significantly different.

Table 20: The total bacterial numbers of samples dyed with one type of dye were examined after their preparation**

Microorganism	Samples							
	Control	1	2	3	4	5	6	7
E.coli	0	$9x10^{3d}$	$9x10^{3c}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$9x10^{3d}$
Staphylococcus aureus	0	$10x10^{3c}$	$8x10^{3c}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$3x10^{3b}$	$10x10^{3c}$
Aspergellus	0	$9x10^{3d}$	$9x10^{3c}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$9x10^{3d}$

*Minimum inhibition concentration (MIC) Effect of finishing on antimicrobial activities of fabric for *E.coli; ,Cocci, and Aspergellus flavus*

In tables 19 and 20 Silk, cotton, and wool samples were treated with $FeSO_4$ dyed and then dyed with three different natural dyes(Madder, Turmeric and Cochineal) The results showed the antibacterial activity of the dyed samples against *E. coli*, which was compared with the treated samples containing silver nanoparticles. The results indicated that the treated fabrics containing these natural dyes exhibited excellent resistance to bacteria compared to the samples in Table 15, 16. As well as those treated with Ag nanoparticles. Turmeric-dyed fabric had a lesser effect on reducing the concentration of bacteria compared to the madder-dyed fabric.

Table 21: Antimicrobial activity of samples, solid diffusion test: total inhibition (clear zone, mm)*. (Tannic acid)

Microorganism	Samples							
	Control	1	2	3	4	5	6	7
E. coli	0^{a}	4^{a}	3^{b}	5^{a}	4^{a}	5^{a}	5^{a}	5^{a}
Staphylococcus aureus	0^{a}	4^{a}	4^{a}	5^{a}	3^{b}	5^{a}	6^{a}	6^{a}
Aspergellus flavus	0^{a}	3^{b}	3^{b}	4^{a}	4^{a}	4^{a}	5^{a}	4^{a}

* There is no microbial growth when there is total inhibition or a clear zone. total inhibition (Petri dish diameter).is represented an Inhibition of 90 mm as indicated in table 21, inhibition diameter is the mean of three different observations taken from three different experiments * Data followed by different letters in the row are statistical significantly different.

Table 22: The total bacterial numbers of samples dyed with one type of dye were examined after their preparation*

Microorganism	Samples							
	Control	1	2	3	4	5	6	7
E.coli	0	$9x10^{3d}$	$9x10^{3c}$	$3x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$
Staphylococcus aureus	0	$8x10^{3c}$	$8x10^{3c}$	$3x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$3x10^{3b}$	$3x10^{3b}$
Aspergellus	0	$9x10^{3d}$	$9x10^{3c}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$	$4x10^{3b}$

*Minimum inhibition concentration (MIC) Effect of finishing on antimicrobial activities of fabric for *E.coli; ,Cocci*, and *Aspergellus flavus*

Various studies have reported MIC values for samples were treated by Tannic acid) turmeric record. MIC values are ($6x10^{3b}$) µg/mL for S. aurous and ($6x10^{3b}$) µg/mL for *Escherichia coli* and recorded solid diffusion test: total inhibition (clear zone, mm) (3^{c}, 3^{c}) *S. aurous*, *Escherichia coli* respectively While better results were recorded when treated with compared to samples treated with or dye samples without turmeric treatment, $3x10^{3b}$ µg / ml for *S. aureus* $3x10^{3b}$ µg/mL for Escherichia coli as shown in Table 22. Compare microbiological data obtained using Madder against microorganisms, better results were obtained with silk samples to which madder dye was added and treated tannic acid compared to other samples.

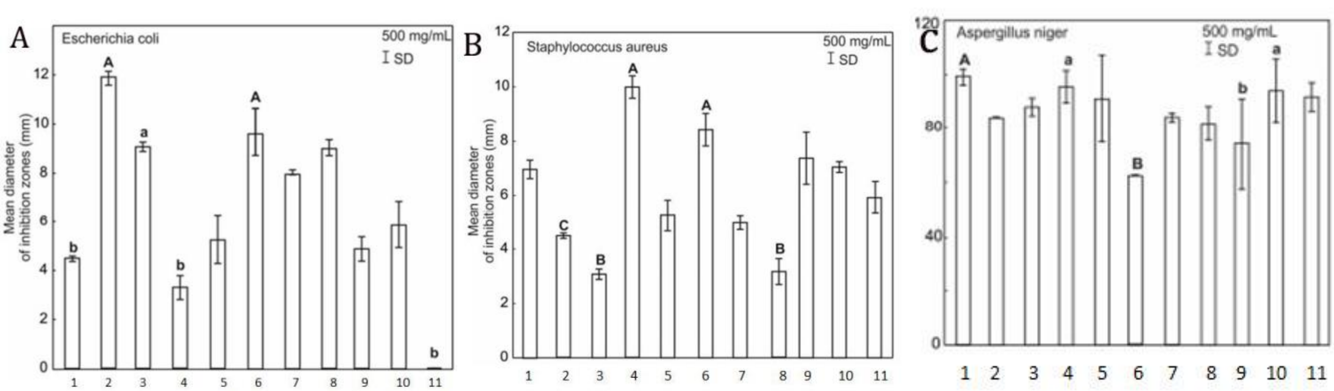

Figure 10.(A) Results of the ANOVA test. A. Results of ANOVA test for the mean inhibitory activity of all tested against *E. coli*. Statistical differences are indicated by different letters and their significance at p ≤ 0.001 with capital letters (A), $p \le 0.05$ with small letters (a). Upper and lower case letters above. (B) Results of the ANOVA test for the mean inhibitory activity of all tested against *S. aureus*. Statistical differences are indicated by different letters and their significance at $p \le 0.001$. (C) Results of the ANOVA test for the mean inhibitory activity of all tested against *A. niger.* Statistical differences are indicated by different letters and their significance at $p \le 0.001$.

4. Conclusions

The study aimed to identify the extent of the use of natural dyes on cotton, wool and silk fabrics and determine the best dye for natural fabrics. The dyeing process was carried out at a temperature of 90°C for 60 minutes. The concentration of cochineal was 5 g/L, and the concentration of turmeric and madder dye was 40 g/L. , the dyes were mixed and confused on natural fabrics, it was concluded that cochineal is poorly pigmented on both Cotton and silk fabrics, but it is good on wool fabrics, unlike the effect of turmeric and madder dyes, their effect is good on the three natural fabrics. Two types of mordant were used during the dyeing process, tannic acid and ferrous sulphate ($FeSO_4$), and each had a distinct impact on fabrics. Color strength (K/S) and color data (L*, a*, b*, ΔE) are measured. The fastness properties, light, washing, perspiration and rubbing the dyed fabrics are tested and evaluated. The antimicrobial effect of positive and negative bacteria, fungi also detected. The use of natural functional dyes in the finishing and coloration of natural fabrics is a promising approach for achieving eco-friendly textile production. Mixtures of natural functional dyes can offer a wider range of colors and properties than single dyes, while also providing enhanced stability and fastness properties. Research has indicated that the combination of different natural dyes can yield significant synergistic effects, resulting in improved dye absorption, color fastness, and light fastness. The use of natural mordants and additives can further enhance the properties of the mixtures. Additionally, the use of natural dyes can provide economic benefits to local communities and promote the preservation of traditional dyeing techniques and cultural heritage. In conclusion, the use of natural functional dyes mixtures in finishing and coloration of natural fabrics is a promising and sustainable approach that can offer a range of benefits to both the environment and society. Further research and development in this area can lead to the creation of innovative and eco-friendly textile products.

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Author Contributions

All authors contributed to this work. Prepared H. Mashaly and N. Kamal the samples and completed the experimental measurements. Both M. Eladawy shared writing and followed the performance of the experiments. N. Kamal helped the first author complete the sample preparation. N. Galal with N. Kamel completed the paper writing, analyzing the data, and validation. M. Eladawy with N. Galal followed the revision and submission of the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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