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Eco-Friendly Dyeing of Cotton Fabrics Using a Blend of *Olea europaea* L. and *Ziziphus spina-christi* L. Leaf Extracts: Chemical Profiling and Color Fastness Properties

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Abstract

Natural dyes are gaining great attention as eco-friendly alternatives to synthetic dyes for textile applications. In this study, methanolic extracts of olive (*Olea europaea* L.) and ziziphus (*Ziziphus spina-christi* L.) leaves were mixed in a 75–25 ratio and used as natural dyes for cotton fabrics for the first time. To identify the chemical constituents present in the major extract, we performed ultra-performance liquid chromatography-mass spectrometry (UPLC–MS/MS). The color fastness and characteristics of the dyed fabrics were evaluated by spectrophotometry and standard methods. The results showed that the olive-ziziphus leaf extract mixture produced a yellowish-brown colour on cotton with good light, washing, and sweat fastness. This study demonstrated the potential of olive and ziziphus leaves as eco-friendly sources of natural dyes for cotton dyeing.

Keywords: Color fastness properties; Olive leaves; UPLCMS analysis; Cotton fabrics; Oleuropein

1. Introduction

Natural dyes have been reported as colorants derived from diverse sources (e.g. plants, animals, or minerals) and served for textile dyeing since ancient times [1-3]. They have several advantages over synthetic dyes, such as being eco-friendly, biodegradable, nontoxic, revealing medicinal and health benefits, along with unique washing and sunlight fade-resistant properties [4-6]. However, they also possess some drawbacks, such as low color fastness, poor shade reproducibility, and high cost [4, 7]. Therefore, there is a need to improve the quality and performance of natural dyes by optimizing their extraction techniques and application methods.

The plant kingdom is well known as a great source of natural dyes that are found in whole plant parts [8]. Olive (*Olea europaea* L.) and ziziphus (*Ziziphus spina-christi* L.) leaves are two potential sources of natural dyes that have been reported to have high phenolic content and antioxidant activity [9, 10]. Olive leaves are mainly composed of polyphenols, while ziziphus leaves contain flavonoids, tannins, saponins, and alkaloids [11-13]. These compounds can produce yellowish-brown to reddish-brown colors on cotton fabrics depending on the extraction and dyeing conditions.

In this context, several studies have been conducted to explore the potential of plant-based natural dyes. For instance, a study by Özlenen Erdem İşmal experimented with the by-product of olive oil as a source of natural dye. The study utilized prina, a by-product of olive oil production, in wool dyeing [14]. Another study discussed the environmental and functional properties of plant-based natural dyes, their extraction methodology, and their applications [15]. On the other hand, the use of ziziphus extract as a natural dye has been explored in various studies due to

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its rich colorant properties [16, 17].

The concept of using mixed plant extracts as natural dyes is not new and has been explored in various studies [18, 19]. The idea behind this approach is to combine the unique properties of different plant extracts to create a more versatile and effective natural dye.

In the present investigation, we aimed to explore the feasibility of using a mixture of methanolic extracts of olive and ziziphus leaves (75–25) as an eco-friendly dye on cotton. As olive extract was the major component, the study encompasses an advanced dimension through ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis. This analytical technique enables an intricate dissection of the constituents within olive leaves, shedding light on their complex chemical composition [20]. UPLC–MS/MS analysis revealed the detection of 12 metabolites, including oleuropein as the major metabolite. Furthermore, we evaluated the color properties and fastness of the dyed fabrics by spectrophotometry and standard techniques.

2. Experimental Section

Plant material

The leaves of olive (*Olea europaea* L.) and ziziphus (*Ziziphus spina-christi* L.) Figures S1&S2 were collected from a private garden at An Nawahid, Belad Al Mal Al Bahri, Abu Tesht, (26°06'52.6"N 32°00'24.4"E), Qena Governorate, Upper Egypt, in 2022. The plants were identified by Prof. Ahmed M. Moharram and stored at the chemistry department of the Faculty of Science, Al-Azhar University (with serial numbers CHM201 and CHM202).

Extraction of colorant

The collected fresh plant leaves (1000 gm of each plant) Figure S3, were applied to cleaning, air drying and grinding to afford a fine powder (250 gm for each plant), and then kept away from the light until performing the proposed study. The leaf powder was subjected to extraction using methanol (3x 2L) for 60 minutes at an ambient temperature using an ultrasonic bath. Then, the afforded extract was concentrated in vacuo until dryness to afford 50.4 and 28.6 gm of crude extract, respectively, Figures S4&S5.

Substrates

Scoured and bleached cotton fabric was obtained from the local market.

Microwave dyeing process

A mixture comprising olive and ziziphus leaf extracts was combined in a proportion of 75% olive and 25% ziziphus. This blend was subsequently applied to cotton fabrics using a material-to-liquor ratio of 1:100. The dyeing process was executed at varying pH levels within the dye bath. Employing a Samsung oven model MS404MADXXBB, the dyeing was conducted over one hour.

Color measurements

The color intensity and light reflection of the cotton fabrics dyed with natural dyes were measured by a Reflectance Spectrophotometer. The extent of dye uptake on the dyed textile was determined through spectroscopic measurements. To ascertain the color intensity of the dye, expressed as K/S, Kubelka-Munk's equation was applied according to Yang and Kruse (2004) [21].

Fastness properties

Colorfastness to washing

The evaluation of color stability during the washing process was conducted following the ISO 105-C02:1989 standard [22]. A representative piece of the dyed fabric was positioned through two pieces of bleached material, which were from cotton and wool fabrics, respectively. These two pieces were meticulously joined through manual stitching. Subsequently, the composite was immersed in an aqueous solution containing nonionic detergents at a concentration of 5 g/L, maintaining a liquor ratio of 1:50. This immersion took place for 30 min at 60°C. The sample was then thoroughly rinsed through manual pressure and left to be dried. A grayscale was used to evaluate the color fastness to wash.

Color fastness to perspiration

The artificial sweat liquid was obtained by mixing L-histidine monohydrochloride monohydrate ($C_6H_9N_3O_2 \cdot HCl \cdot H_2O$) (0.5 g), NaCl (5 g), and sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) (2.2 g) and adding to 1L of distilled H_2O. The pH of the solution was adjusted to 5.5 using 0.1 N NaOH to make it acidic. Similarly, the basic sweat solution was afforded by adjusting the pH to 8.0 [23].

The fastness assessment was performed as follows: A piece of dyed fabric (5×4 cm) was stitched between two distinct uncoloured pattern segments. These fabric assemblies were immersed in both sweat solutions, ensuring thorough wetting by agitation, and pressing for 15-30 minutes. The samples underwent a load of 4-5 kg while positioned between plates made from plastic or glass. Subsequently, the plates were vertically kept at 37°C for 4 hours. The visualization of color fastness to perspiration was conducted by applying a grayscale change technique [24].

Color fastness to light

To evaluate the color fastness stability of the natural dye against light, the ISO 105-B02:1988 methodology was followed. The dyed samples were exposed to a carbon arc lamp for 35 hours. The color changing of the examined samples was then assessed using the blue color scale [23].

Phytochemical analysis of the major extract (olive)
UPLC–MS/MS analysis

UPLC is a modern and powerful liquid chromatography technique that can separate complex mixtures of compounds with similar polarity. It offers better resolution, faster analysis, and lower solvent consumption than HPLC. This is achieved by using a smaller column packed with particles usually smaller than 2 micrometres. To quickly identify the compounds, an MS for each compound was assigned as it was received from the LC column. The ESI-MS and LC instruments are usually connected to get *in situ* effective online LC/MS with high accuracy. The defatted extract of olive (*Olea europaea* L.) was dissolved in H_2O/MeOH (10:90) mixtures and injected into the UPLC/ESI-MS. UPLC analysis was performed using a Dionex Ultimate 3000 UPLC system (Sunnyvale, CA, USA: Thermo Scientific™Dionex™)., and a reverse-phased Hypersil Gold UPLC C18 column (2.1150 mm, 1.9 m) was used, according to the previously reported method by Asmaey et al. [20, 25].

3. Results and Discussion

Factors affecting the dyeing process:
Effect of natural dye concentration

We applied different concentrations (2, 4, 6, 8 and 10 g/L) of the new natural dye to cotton fabric and measured the color strength by the K/S value. As illustrated in Table 1, the data recognized that the optimal K/S value was verified at a concentration of 6 g/L, which had the same K/S value as the concentration of 10 g/L. This revealed that increasing the concentration beyond 6 g/L did not improve the color quality of the dyed fabric.

Influence of pH of the dyeing bath

The influence of varying the pH levels of the dyeing bath on the color strength measurements of the natural dye applied to cotton fabrics is comprehensively displayed in

Table **2**. The maximum K/S value (i.e., the most vivid color), was achieved at pH 2, Figure S7, while the minimal K/S value (i.e., the most faded color), was obtained at alkaline pH. This could be explained by the fact that some polyphenols suffer from degradation when the pH value increases [26, 27]. Additionally, it is consistent with the fact that natural dyes work better in acidic conditions, as they form stronger bonds with the fibres.

Fastness properties

Table **3** displayed the results of the color fastness assessments for the cotton fabrics dyed with natural dye. The color fastness tests evaluated how well the color of the dyed fabrics can withstand washing, sweating, and sunlight. The results show that the dyed cotton fabrics have a low lightfastness rating of 5 on the blue scale, which means that the color fades easily when exposed to light Figure S7. This is a common drawback of natural dyes, as they are sensitive to UV rays and oxidation [5]. The results showed as well that the stained cotton fabrics have a high wash fastness and fluid fastness rating of 5 on the grayscale, which means that the color does not change much when washed or sweated on. This is a great benefit of natural dyes, as they are eco-friendly and biodegradable [4].

Identification of chemical components of olive extract using UPLC/MS-MS

In the pursuit of chemical characterization, a meticulous technique was performed to ascertain both the accurate molecular mass and formula. This was undertaken through LC–ESI/TOF, with further insights garnered from the fragmentation pattern extracted through LC–ESI/MS/MS analyses. These findings were cross-referenced with authentic standards and established literature data for validation. Employing the negative ion mode for ESI was selected because it proved instrumental, contributed to enhanced sensitivity, and facilitated a straightforward interpretation of the acquired spectra [28]. This can be ascribed to the intrinsic existence of the phenolate analogues reported in this study.

The methanolic extract of olive leaves presented 12 diverse compounds, (based on UPLC-MS/MS), which were classified into 4 secoiridoids, 1 phenolic compound, 1 phytotoxin, 1 triterpenic acid, 1 chalcone and 4 unknown compounds. A comprehensive tabulation of these compounds is meticulously presented in Table **4** and Fig. 1, while Fig. 2 shows their chemical structures.

Table 1. Influence of dye source concentration and extraction time on K/S.

Sample	Conc. g/L	Dyeing time	K/S	L	A	b
A1	2	1 hour on microwave	0.83	77.59	-2.78	13.84
A2	4		1.47	70.98	-0.82	15.07
A3	6		2.22	69.72	-2.39	20.97
A4	8		1.52	74.80	-3.14	17.76
A5	10		2.23	71.50	-3.06	21.92

K/S = color strength, L= perceptual lightness, a = green color, b = yellow color

Table 2. Influence of dyeing bath pH on K/S of extracted dye at 6 g/L.

Sample	pH	Dyeing time	K/S	L	a	b
A1	2	1 hour on microwave	5.11	61.05	-2.06	16.47
A2	4		1.55	71.04	-2.80	16.24
A3	8		1.46	73.24	-3.30	16.12
A4	12		1.02	75.80	-2.77	14.52

Table 3. Fastness characteristics of dyed cotton textiles.

Dye no	**Washing fastness**			**Perspiration fastness**						**Light fastness**
				Acidic			**Alkaline**			
	Alt	SC	SW	Alt	SC	SW	Alt	SC	SW	
1	5	5	5	5	5	5	4-5	4-5	4-5	2 - 3

Alt = Alterations, CS = Cotton staining, WS = Wool staining

The quasi molecular ion peak shown at *m/z* 153 $[M\text{-}H]^-$ along with a subsequent fragment ion peak at *m/z*123 correspond to the loss of a CH_2OH group Figure S8, agreeing with hydroxytyrosol (**1**), representing one of the common compounds obtained from olive leaves [29].

Compound **2** appeared at R*t* 2.33, displaying a notable molecular ion peak at *m/z* 389 $[M\text{-}H]^-$, being attributed to oleoside, from which fragment ion peak at *m/z* 345 indicative for the expulsion of a carboxylic acid group (CO_2, 44 Da) was discerned. A further fragment ion peak at *m/z* 183 was displayed due to a subsequent elimination of additional CO_2, Figure S9 [24].

Compound (**3**) (R*t* 2.39 min) exhibited a molecular ion peak at *m/z* 403 ($[M\text{-}H]^-$), Figure S10, from which the MS/MS produced product ions *m/z* 371 ((arising from the loss of CH_3OH $[M\text{-}H\text{-}CH_3OH]^-$) and 223 (being attributed to the formation of a dehydrated elenolic acid as a result of H_2O molecule elimination. This pattern of destruction is matching well elenolic acid hexoside profile (a degradation product of oleuropein) [25].

A prominent peak observed at R*t* 2.97 min was distinctly evident, showing a precursor ion peak at *m/z* 539.18 $[M-H]^-$, Figure S11. Specifically, the MS^2 exhibited a base peak at *m/z* 377 aligned with the distinct expulsion of hexose moiety $[(M-H)\text{-}162]^-$. This was followed by the presence of two further peaks (*m/z* 307 and *m/z* 275) corresponding to subsequent expulsion of C_4H_6O $[M-H-162\text{-}70]^-$ and CH_3OH groups $[(M-H)-162-70\text{-}32]^-$, respectively. Surprisingly, the observed data match well with the fragmentation pattern of oleuropein (**5**). Oleuropein is often reported as the leading phenolic metabolite produced by olive leaves, [30] constituting as much as 9% of the leaves [31]. Similarly, the remaining compounds (**6**, **8**, **10**, and **12**) were tentatively ascertained and classified as well.

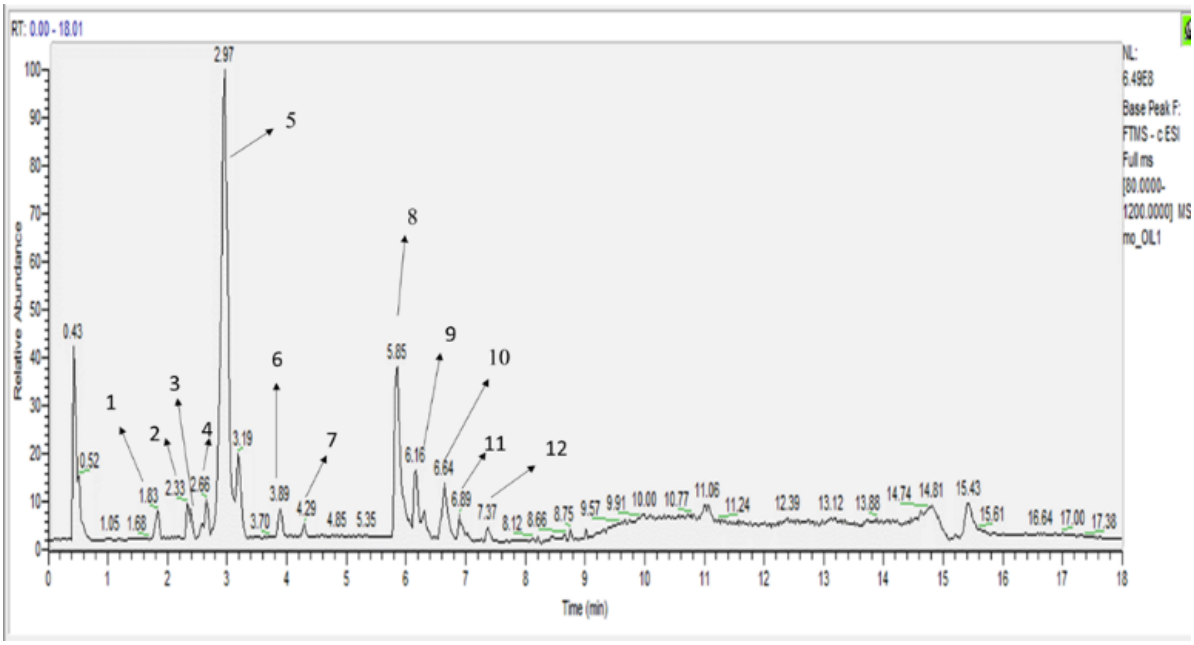

Fig. 1. UPLC–MS chromatogram of *Olea europaea* L. methanolic extract.

Table 4. UPLC–MS data of *Olea europaea* L. leaf extract.

No.	t_R (min.)	Class	Tentative Identification	Molecular Formula	[M-H]$^-$ *m/z*	*m/z* fragments	Ref.
1	1.83	Phenolic alcohol	Hydroxytyrosol	$C_8H_{10}O_3$	153.0546	123	[32]
2	2.33	Secoiridoid	Oleoside	$C_{16}H_{22}O_{11}$	389.1089	345, 209, 121, 69	[33]
3	2.39	Secoiridoid glycoside	Elenolic acid glucoside	$C_{17}H_{24}O_{11}$	403.1243	371, 223, 179, 89	[34]
4	2.66		Unknown		463.16	403, 174, 112	
5	2.97	Secoiridoid monoterpenoids	Oleuropein	$C_{25}H_{32}O_{13}$	539.1766	377, 307, 275, 223, 179, 129, 101, 89	[35]
6	3.19	Secoiridoid monoterpenoids	Oleuropein Isomer	$C_{25}H_{32}O_{13}$	539.1767		[34, 35]
7	3.89, 4.29		Unknown		391.1397	255, 149	
8	5.88	Phytotoxin	Zinniol	$C_{15}H_{22}O_4$	265.1477	97	[36]
9	6.16		Unknown		309.1740		
10	6.64	Chalcones	(1*E*,4*E*)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one	$C_{19}H_{18}O_3$	293.1791	265, 174, 112	
11	6.89		Unknown		555.2839	225, 81	
12	7.37	Triterpenic acid (oleananes)	Maslinic acid	$C_{30}H_{48}O_4$	471.3475	235, 175, 113	[37]

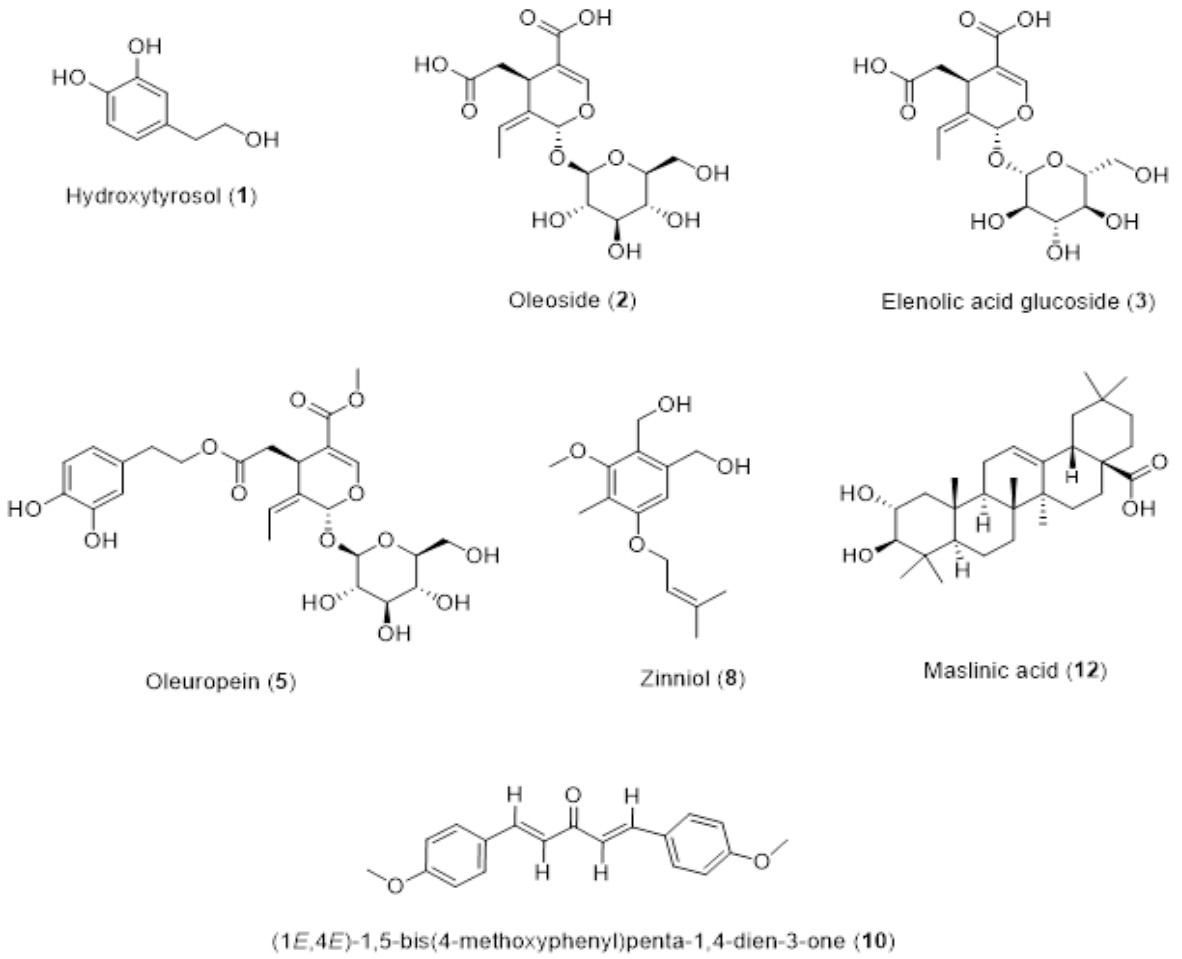

Fig. 2. Chemical structures of the identified metabolites from *Olea europaea* L. leaf extract

4. Conclusion

In conclusion, this work is considered the first investigation that demonstrated utilizing a blend of methanolic extracts from Egyptian olive and ziziphus leaves in a (75–25 ratio) as a natural dye for cotton fabrics. This innovative approach leverages the unique properties of these plant extracts, offering an eco-friendly alternative to synthetic dyes. A crucial dimension of this investigation was the integration of UPLC–MS/MS analysis, which offered a comprehensive view of the chemical composition of olive leaf extract. The analysis revealed the presence of 12 metabolites in the olive extract, with oleuropein as the major compound. Furthermore, the dyed fabrics displayed good color properties and fastness at pH 2, suggesting the suitability of the natural dye for textile applications. This study also provided detailed insight into the chemical composition of the olive extract, which could help to understand its dyeing mechanism and performance.

Conflicts of interest

"The authors declare there are no conflicts to declare".

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