

Coloristic and antimicrobial behaviour of polymeric substrates using bioactive substances

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Abstract. A major concern in reducing microbial contamination of healthcare and hygiene products motivated us to seek viable alternatives in order to create such barriers. The antimicrobial and anti-oxidant effects of natural extracts are well-known, their application onto polymeric supports is still challenging in terms of investigation. To our knowledge, the method of natural dyeing of different polymeric substrates using bioactive substances derived from black currant and green walnut shells, in conjunction with biomordants, and their long term effects have not been very consistently reported. The main objective of the study is based on the comparative study of different polymeric fibrous substrates dyed by means of laboratory scaled classic methodology with extracts from black currant fruits and green walnut shells, with the assistance of conventional and biomordants (copper sulphate, citric and tannic acids). The assistance of biomordant in the dyeing process seems to conduct to improved synergetic colouring and antibacterial performances. The main results demonstrated that the extract of green walnut shells reinforced by the biomordants solutions expressed the best antimicrobial behaviour. The present research is a milestone in the identification of potential technological alternatives applied in dyeing of synthetic and natural textile supports, quantified and controlled by antimicrobial response correlated with colorimetric features.

1. Introduction

There is an intense research dedicated to the "greening" of textile polymeric supports through the utilization of various bioactive extracts, as well as establishment of antimicrobial potential, such as natural pigments or antimicrobial products, process referred as functionalization [1,2]. In order to enhance the products competitiveness, and at the same time based on environmental protection reasons, lot of researches have been developed regarding the use of antimicrobial active agents, as well as natural colorants, biopolymers or other medical vegetal resourced components with potential application on fibrous polymeric supports. Thus, active substances, like chitosan and Aloe Vera extracts, Eucalyptus essential oils have been used onto cellulosic and chemical supports [3,4].

Other previous studies and researches belonging to the authors support the alternative of natural dyes onto different fibrous polymeric supports [5-7]. It is well-known that dyeing with natural extracts is a sustainable alternative to the synthetic dyes. Chinese researchers obtained some microcapsules comprising of herbs introduced in chitosan-sodium alginate (CSA) blend matrix using emulsion-



chemical cross-linking method. The study proved an efficient grafting onto the surface of cotton fabrics. In addition the microcapsules did not affect the cells, in terms of toxicity, being used in clinical treatment of atopic dermatitis (AD) [8].

The present paper deals with the potential application of natural extracts on different fibrous polymeric supports. On the other side the present study is a synthetic analysis of the most relevant coloristic/chromatic attributes by a comparative utilization of some biomordants and a classical one for ecologic dyeing of textiles, as well as highlighting of the antimicrobial activity aiming at the development of clothing garments designed to human health. The novelty of the present study stresses the bactericidal potential of bioactive components from natural extracts (black currant and green walnut shell). In fact the study is an extension of ecologic dyeing with complementarity on the antioxidant activity/anti-inflammatory response/behavior of the studied extracts. The study reflects the constant concerns of authors regarding the relevant biocidal properties of bioactive components from extracts with the synergic effects of dyeing.

2. Experimental protocol

Textile supports and chemical reagents

The methodology implied the use of bamboo, polyamide and woolen textile supports with the following features: relon 100%, raw, washed-degreased, with a weight of 67g/m², 11 type woolen fibers based support, washed and white bamboo knitting, with yarns fineness of 34/1 Nm. The dyeing methodology assumes the use of black currant fruits and green walnut shells extracts, as well as biomordants such as citric acid (CA) and tannic acid (TA), in comparison with a standard mordant copper sulphate CuSO₄. The dyeing bath contains the alcoholic extracts of black currant and nut shells, and 3 and 5 % mordant.

The study used the exhaustion dyeing method, the dyeing bath consisting of black currant and walnut shell extracts, adding the mordant, in order to stabilize the natural pigment, all the quantities being established to the studied polymeric support (woolen, polyamide, or bamboo).

Antimicrobial and colouring testing

The testing was performed on two standardized bacterial strains: *Staphylococcus aureus* ATCC 25923 (Gramm positive) and *Pseudomonas aeruginosa* ATCC27853 (Gramm negative).

For the *in vitro* testing, the Mueller Hinton agar culture medium was used, 24 hours bacterial cultures with a 0,5 Mac Farland Standard microbial density equivalent (1,5 x10⁸ cells). The samples were examined after 24 hours incubation at 37°C.

The tested samples were equally dimensioned, having a surface of 1 cm². The fabrics made of woollen and knitting samples were embodied in the structure of culture medium, in order to avoid the contact errors with microorganisms from test culture. The samples were placed, so that their centres to be distanced one from the other at least 24 mm. After the incubation under aerobiosis conditions at 37°C, for 24 hours, the samples were evaluated and interpreted in terms of diameter of inhibition area developed around the tested sample area. The determination of inhibition area is performed macroscopically, including the measurement of the diameter of tested sample. To set a limit, we will consider the area on which a relevant increase is not visible.

The absence of inhibition area means the absence of antimicrobial activity.

The results were compared with gentamicin, in terms of diameter of inhibition area of a micro-tablet having a diameter of 10 mm and a gentamicin concentration of 10 µg. According to the standard CLSI [9,10], the best antimicrobial action is transposed through inhibition area of 19-27 mm belonging to gentamicin onto *Staphylococcus aureus* strain (ATCC 29213) and of 16-21 mm onto *Pseudomonas aeruginosa* strain (ATCC 27853).

The experimental dyeing protocol and the colour differences measured by reflexion spectrophotometry can be associated with the green walnut shell quantity, as well as the textile substrate the extracts were applied on. The colour modifications were performed by using the dyeing with natural extract, without mordant, as reference.

The experimental data were acquired with Datacolor 110 LAV reflection spectrophotometer in the CIELab system for parameters, as: ΔL^* , Δa^* , Δb^* , ΔC^* , Δh^* and colour differences ΔE^* .

3. Results and discussions

Table 1 reveals the colour coordinates, in terms of luminosity and colour differences for the polyamide (PA1 and PA2) samples dyed with extracts A and B, woollen and bamboo samples. The values of luminosity and colour differences changes in relation to the mordant type used in dyeing process (copper sulphate, citric and tannic acids).

Table 1. The values of luminosity and colour differences for the wool, polyamide and bamboo samples dyed with extracts A and B, derived from green walnut shells and black currant fruits

Woollen, polyamide, bamboo sample/mordant	CIE DL*	CIE Da*	CIE Db*	CIE DC*	CIE DH*	CIE DE*
3% copper sulphate	-6.11/3.09	-1.07/-	4.39/-1.31	2.95/-5.19	3.42/4.33	7.60/7.43
WO	-6.47/2.60	6.63	2.89/-8.61	6.48/-12.93	-10.82/1.46	18.83/13.27
PA	7.72	12.28/-	4.35	-15.35	5.56	18.06
BAMBOO		-15.73				
5% copper sulphate	-6.55/-0.82	-2.01/-				
WO	-6.00/0.92	6.87	3.92/-6.10	2.07/-9.13	3.89/1.09	7.89/9.23
PA		14.85/-	14.85/-9.91	7.60/-13.01	-13.00/1.61	21.27/13.14
		9.91				
BAMBOO	6.70	-11.42	7.91	-9.78	-9.86	15.42
3% acid citric						
WO	-4.14/0.92	3.68/2.05	12.83/6.39	12.63/6.19	4.31/2.59	13.98/6.77
PA	-6.30/-0.09	8.45/0.89	8.30/0.11	9.79/0.69	-6.66/-0.57	13.41/0.90
BAMBOO	-2.16	2.20	2.03	2.04	2.18	3.69
5% acid citric						
WO	-11.15/2.88	5.03/2.76	12.13/7.26	12.75/7.30	3.14/2.62	17.23/8.28
PA	-6.36/1.93	8.38/3.03	9.37/3.02	10.78/4.28	-6.46/-0.15	14.08/4.69
BAMBOO	0.71	3.05	3.55	2.92	3.66	4.73
3% acid tannic						
WO	-1.28/0.97	0.61/3.20	7.56/8.12	6.54/8.26	3.85/2.83	7.70/8.79
PA	-4.23/3.78	6.89/2.52	7.9/3.06	8.04/3.96	-4.38/0.23	9.33/5.48
BAMBOO	6.26	-1.53	2.17	-1.68	2.06	6.80
5% acid tannic						
WO	-1.56/1.40	2.95/4.07	9.11/9.34	9.09/9.76	3.03/2.94	9.70/10.28
PA	-5.46/7.44	7.65/3.50	8.05/6.98	9.76/7.53	-5.11/2.06	16.43/10.78
BAMBOO	7.42	-3.46	1.81	-3.60	1.50	8.38

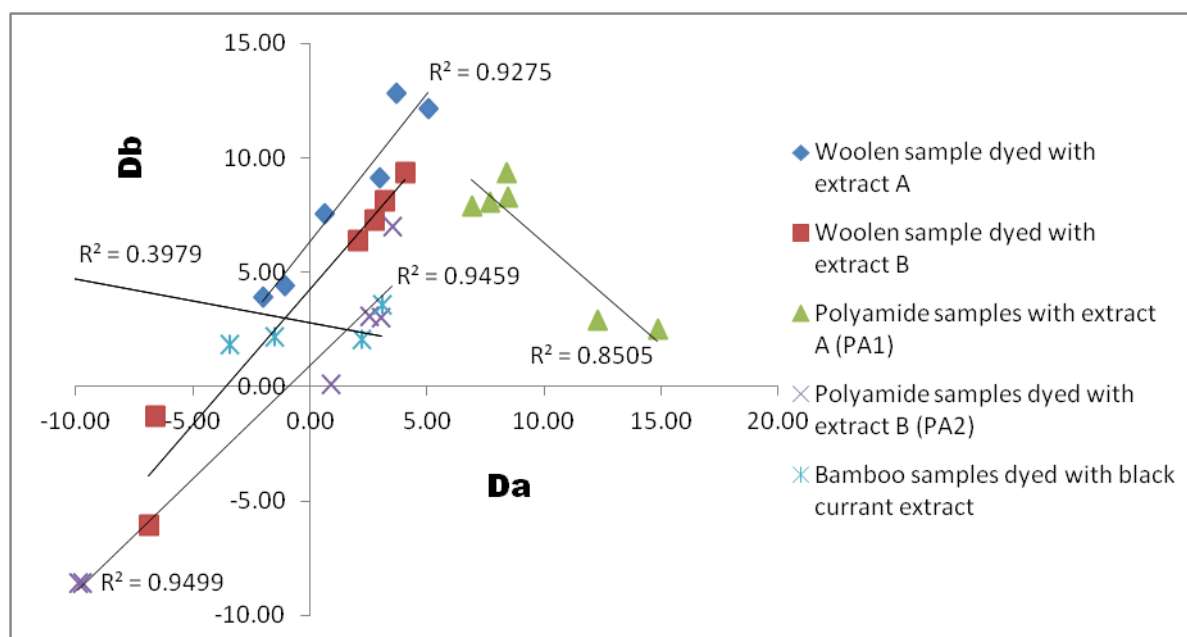


Figure 1. The graphic representations of colour attributes onto the red-green and yellow-blue axis, for the woollen, polyamide (PA1, PA2) samples dyed with green walnut shells extract (A/B (water:ethanol 1:1/water:ethanol 3:1) and bamboo samples dyed with black currant extract

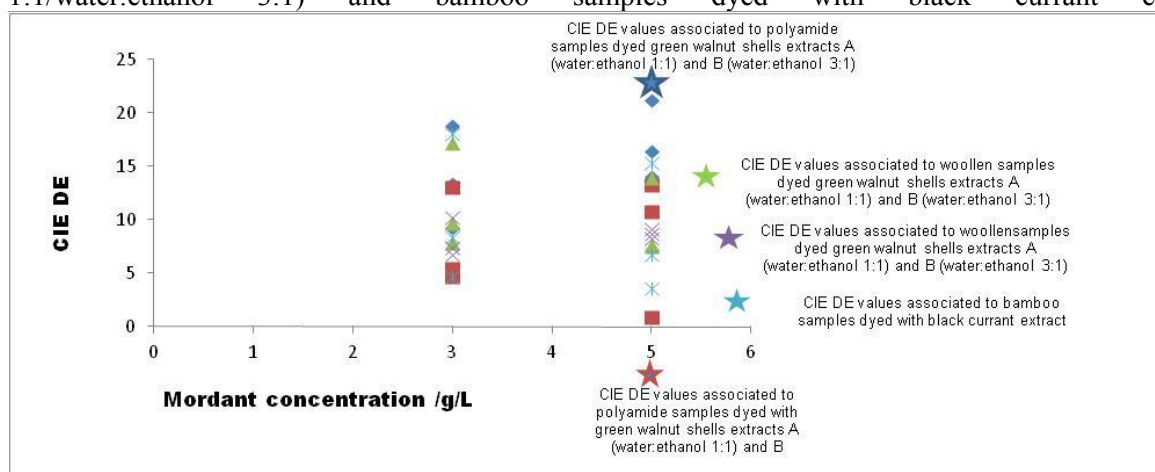


Figure 2. The graphic representations of colour difference – mordant concentration for: woollen samples dyed with extracts A and B; polyamide (PA1 and PA2) samples dyed with green walnut shells extracts A and B (water:ethanol 1:1 and water:ethanol 3:1); bamboo samples dyed with black currant extract

The highest colour changes are noticeable for bamboo samples treated with copper sulphate, while the lowest are occurred wherein the dyeing was assisted by CA and TA.

By comparison the three textile supports dyed with black currant and nut shells extracts, visualizing Fig. 1, it can assumed that major colour changes are remarkable when the dyeing is assisted by citric acid (CA) as biomordant, the classic one leading to irrelevant colour differences.

The lightness of bamboo samples reveals a decrease by using 3 and 5% concentrations of CA and a major augmentation when TA and copper sulphate assisted the dyeing. The plots recorded collinear points situated on the same line, in the colour diagram. Small differences in the red-green and yellow-blue areas are noticed, for the samples with biomordants. It is noteworthy to highlight the idea of linear correlation of Da (red-green) and Db (yellow-blue) components in case of woollen samples dyed

with extracts A and B, whilst the associated values of these chromatic parameters to polyamide and bamboo samples are not linear. This result reveals the fact that only woollen samples would have stable chromatic components (Da and Db), the other studied samples being with no consistency in this regard.

Fig 2 representing the colour difference versus mordant concentration for the woollen, polyamide and bamboo samples dyed with different natural extracts shows a coherent relation (CIE DE values for all samples are situated on the same line, in case of mordant concentration of 3 and 5 g/L, respectively) between colour differences for all samples, no matter the polymeric fibrous supports are.

For the woollen and polyamide samples, dyed with walnut shells, it can be noticed that the samples are brighter when the dyeing is assisted by the TA. Their modification which was quantified as colour difference has considerable values only by assistance of 5 % concentration of copper sulphate and TA, the other ones being in the acceptable ranges.

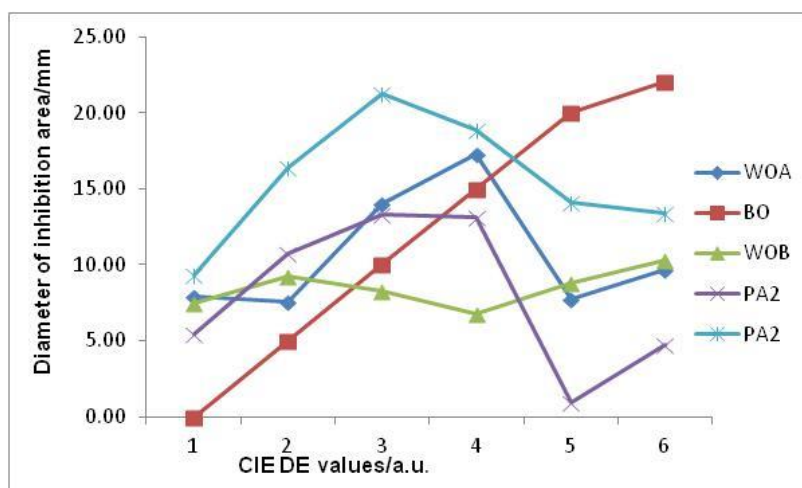


Figure 3. The correlation between colour difference and antimicrobial activity of woollen, polyamide and bamboo samples with green walnut shells extract B (b) and A (a) tested against *Pseudomonas aeruginosa* ATCC 27853

Regarding the antimicrobial potential, it can be stated that the more concentrated extract was used, the higher the antimicrobial activity and colour differences. The figure 3 reveals an increase of the inhibition diameter for polyamide samples dyed with the two extracts against CIE DE value (3 arbitrary units). It has been noticed an increase of the growth diameter with the CIE DE value, as approximately linear correlation.

The obtained results showed a favourable distributed antimicrobial action against Gram negative bacteria. The strain of *Pseudomonas aeruginosa* showed a moderate sensitivity in terms of woollen samples dyed with B extract (high concentration of green walnut shell extract) (Fig. 4) fixed with 3% CA (diameter of inhibition area of 14 mm), 5% AC acid citric (diameter of inhibition area of 16 mm), 3% TA (diameter of inhibition area is 13 mm), 5% TA (diameter of inhibition area of 15 mm), in comparison with Gentamicin, wherein the diameter of inhibition was 16-21 mm.

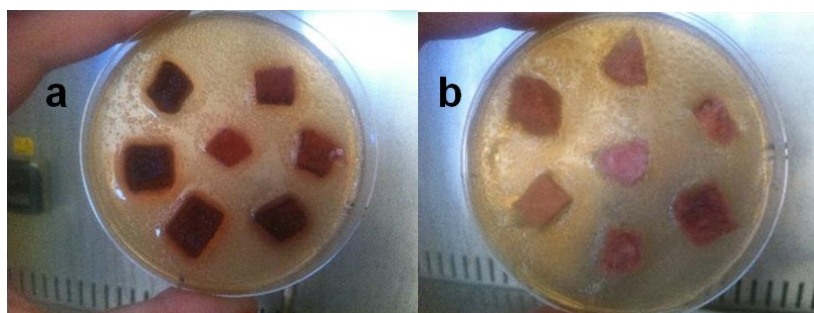


Figure 4. Woollen samples with green walnut shells extract B (b) and A (a) tested against *Pseudomonas aeruginosa* ATCC 27853.

These samples were dyed with the highest concentration of green walnut shell extract (more intense dyed samples – right side of the Fig. 4), with the assistance of CA and TA as biomordants.

The woollen dyeing with A extract (a small concentration of green walnut shell extract), by using the same mordants and tested against *Pseudomonas aeruginosa*, did not induced antimicrobial attributes to the tested samples. Nevertheless, this dyeing showed had a moderate antimicrobial activity against the strain of *Staphylococcus aureus*, by using biomordants. For instance, in case of using 3% copper sulphate the diameter of inhibition area was 12 mm, while for 5% copper sulphate, the diameter of inhibition area was 13 mm. With the assistance of 5 % TA, the diameter of inhibition area was 13 mm, in comparison with Gentamicin, which gave a diameter of inhibition area of 19-27 mm.



Figure 5. Woollen samples dyed with green walnut shell extract (A), tested onto *Staphylococcus aureus* ATCC 29213

The antimicrobial activity of woollen samples dyed with A extract, was relevant only for samples where TA and sulphate copper were used against Gram positive bacterial strains (*Staphylococcus aureus*). B extract showed a better antimicrobial activity for samples treated with CA and TA s biomordants against Gram negative bacterial strain (*Pseudomonas aeruginosa*).

This differentiated action might be related to the cell wall structure of Gram positive and Gram negative bacteria: Gram positive bacteria presents a thick cell wall (15-50 nm), relatively homogenous made of many layers of peptidoglycans, while the Gram negative bacteria posses a thinner cell wall (3-8 nm) with much more complex structure [11].

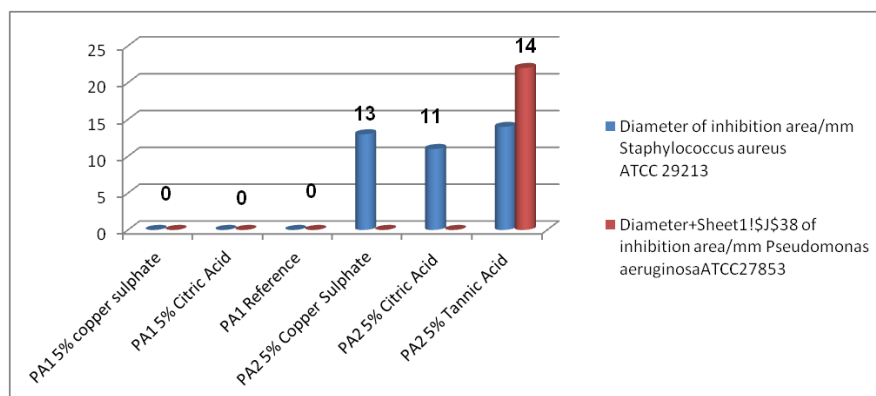


Figure 6. Antimicrobial activity of polyamide supports dyed with green walnut shells B extract

The dyeing with extract B (a high concentration of green walnut shell natural dye) onto polyamide support, with the assistance of 5% tannic acid (TA), as mordant, had a very good antimicrobial performance, the inhibition area diameter being 22 mm (Fig. 6).

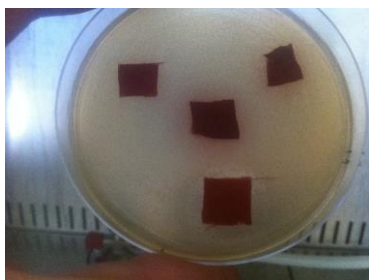


Figure 7. PA supports dyed with extract B (a high concentration of green walnut shell natural dye), with the assistance of 5% CuSO_4 , 5% CA and 5% TA, tested against *Pseudomonas aeruginosa* ATCC 27853

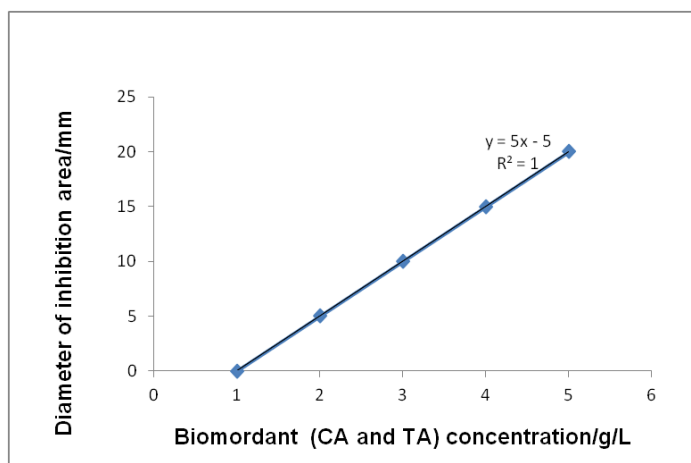


Figure 8. Correlation between biomordant concentrations and inhibition area diameters, of the studied samples, against *Pseudomonas aeruginosa* ATCC 27853

The figure 8 reveals a perfect linear correlation between biomordant concentrations and the bacteria growth inhibition diameters, in case of dyed woollen polymeric support with the assistance of 5 % CA and TA, against the strain *Pseudomonas aeruginosa* ATCC 27853.

4. Conclusions

The main results of this study revealed that the natural polymeric supports functionalized by the addition of bioactive compounds extracted from plant sources, under the conditions of an ecologic dyeing methodology, and assistance of biomordants showed good antimicrobial activity with minimal chromatic changes of the textile support.

A linear correlation between the fixation agent belonging to the natural dye and the inhibition diameter of treated samples was found with respect to the biomordant concentration and, consequently to the eco dye mass accelerating the dyeing.

The chromatic attributes of dyed samples are different in terms of the biomordant type, but undoubtedly it can be stated as alternative utilisation of CA and TA, within the ecologic dyeing, as well as the assignment of antimicrobial behaviour to the polymeric supports with the adding value in the area of hygiene and healthcare products.

Acknowledgements

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