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COLORIMETRIC CHARACTERISTICS OF ULTRASOUND DYED TEXTILES WITH EXTRACTS OF *Reynoutria japonica* AND COPPER-BASED MORDANTS

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Abstract: In this study, the influence of the type of extract (leaf and rhizome of *R. japonica*) and the type of copper-based mordant (copper (I) oxide - Cu_2O and copper (II) sulfate - $CuSO_4$) on the spectroscopic and antimicrobial properties of dyed wool knitwear was investigated. The antimicrobial activity of all samples was tested against the bacteria *Staphylococcus aureus* and *Escherichia coli* and the yeast *Candida albicans*. It was observed that knitted fabrics dyed with *R. Japonica* leaf extract and Cu_2O show a better antimicrobial effect on *S. aureus* bacteria compared to knitted fabrics dyed with the addition of $CuSO_4$. However, in the case of knitwear dyed with *R. Japonica* rhizome extract, we have the opposite case; $CuSO_4$ proved to be better as a mordant. Conductance was measured using the dielectric spectroscopy method in the frequency range from 20 Hz to 100 kHz. The highest increase in conductivity was observed in the sample dyed with the rhizome extract of *R. Japonica* and Cu_2O , where the increase at the frequency of 24 kHz was 20 times higher compared to the initial sample. The obtained results of the coloration spectrophotometric analysis of the samples show that the highest colour strength (K/S) was achieved with the sample dyed with *R. Japonica* leaf extract and $CuSO_4$. The obtained results indicate the possibility of using the tested knitwear for antimicrobial as well as electro protection.

Keywords: Colorimetric characteristics, Antibacterial properties, Dielectric properties, Wool knitwear, *Reynoutria japonica*, Copper (I) oxide, Copper (II) sulfate.

1. INTRODUCTION

The growth of microorganisms on textiles causes a series of unfavorable impacts, both on the clothes and on the people wearing the clothes. These effects include the creation of unpleasant odors, staining and discoloration of clothing, a reduction in the mechanical strength of the fabric, and increased contamination by microorganisms [1-3]. The goals of using antimicrobial finishes are to significantly limit the frequency of bacterial growth, reduce the formation of odors as a result of the microbiological degradation of sweat, and avoid the transmission and spread of pathogenic microorganisms [4]. During the regulation of body temperature, sweat is secreted, and its amount depends on body weight, physical activity, propensity to sweat, climatic conditions, thermal and sorption properties of the clothes. A thin layer of liquid on the skin is formed when the evaporation of sweat is blocked, which is an undesirable, because then the best conditions for the formation of fungi and bacteria are created.

The effectiveness of textiles with active antimicrobial effects is based on the principle of diffusion, that is, bioactive substances are dispersed at a variable rate from the surface or fibers during ion exchange, replacing cations with anions. For the antibacterial treatment of textiles, mainly ions, silver, zeolites, chitosan, plant extracts are used, depending on the desired effect [5].

Plant extracts in the textile processing, first dye the textile, and then give to it some other properties, which improve its quality and provide a wider practical application. One of the properties that can be assigned to textiles is antimicrobial [6].

Due to the fact that biologically active substances from plants can slow down or prevent the growth of microorganisms, there is a growing interest in studying their application in textile processing [7, 8]. In the last few years, the reasons for the increased interest in the antimicrobial finishing treatment of textiles are: promotion of health and a physically active lifestyle; increasing awareness of the harmful impact of microorganisms on textiles, human health and hygiene; greater use of materials from synthetic fibers and their mixtures for the manufacture of products, such as shirts, socks, blouses and underwear, which tend to increase the moisture of the skin due to poorer moisture transport compared to natural fibers due to sweating.

Due to strict environmental standards, which are introduced by many countries in response to toxic and allergic reactions associated with the use of synthetic dyes, there is a need to find new ways to use plant extracts for textile dyeing. Natural dyes for dyeing textiles, which are usually aqueous or alcoholic extracts of various plants, are more environmentally friendly than their synthetic counterparts, have better biodegradability and, in principle, have greater compatibility with the environment. When it comes to textile dyeability, i.e. visual experience of the surface, the raw untreated sample has the highest reflectance values, which indicates its lighter shade. As a rule, with an increase in the concentration of colour on the textile substrate, as well as with darker shades, the reflection decreases, i.e. remission value of light [6, 9].

Colored solutions are usually used for dyeing textiles, which give the textile product a certain degree of coloring, as well as antimicrobial and deodorizing properties, UV protection, etc. New procedures for processing textile materials with plants with medicinal ingredients have enabled natural, unique and stable processing of textile products with an additional medicine effect in the treatment and prevention of several diseases or protection of human skin [10]. Plant extracts are preferred during processing for the following reasons: the processing is environmentally friendly; no occurrence of toxic products; treated textiles are skin-friendly; the use of processed textiles is safe; antimicrobial treatment prevents further development of microbes [11].

The final antimicrobial treatment must achieve the following: effective control of the growth and reproduction of bacteria, molds and fungi; selective activity towards unwanted microorganisms; absence of toxic effects for producers and consumers; permanent resistance to washing, dry cleaning and rinsing; application without harmful effects on the fabric; acceptable moisture transfer properties; compatibility with other finishing agents; simple application and compatibility with standard textile processing [12, 13]. Clothing items made of fabrics treated in this way, in contact with human skin, protect against many diseases, transferring the healing properties of plants to the skin and body. The colour of plant extracts and their antimicrobial effect come from substances of different chemical compositions. These can be anthocyanins, anthraquinones, indicans, flavonoids, etc. [14].

Reynoutria japonica Houtt. is a perennial herb that has a strong and hollow knee-shaped stem with alternately arranged lanceolate leaves. It blooms in autumn with fragrant, honey-like, whitish flowers. The above-ground part of the plant dies in the winter, and a strong rhizome remains underground, which gives a new stem in the spring. The plant grows in dense assemblages and monodominant communities where there are almost no other plants [11]. The biologically active substances found in the rhizome are various phenolic compounds: flavanols, flavones, stilbenes, hydroanthraquinones, and phenolic acids [15, 16]. The content of individual phenolic compounds varies depending on the parts of the plant as well as the habitat where the plants grow [17, 16]. There are many different biologically active substances in the leaves, but the presence of stilbene and some flavanols in the rhizome is many times higher [16]. The rhizome extract of R. japonica showed good dyeing abilities of different types of fabrics, which derives from the content of different flavonoids [18]. The degree of coloring and antimicrobial activity of treated fabrics depends on the previous treatment as well as the type of wetting agents. Thus, the dyeing of knitted fabrics made of cotton, bamboo, polyester, polyester/cotton (50/50%) and poly-

ester/bamboo (50/50%) with alcoholic rhizome extract of R. japonica, in the presence of molasses (3% KNaC₄O₆·H₂O), significantly affected on staining intensity compared to control samples without wetting agents [19]. Knitwear dyed with an extract wetting agent also showed better colour fastness after washing. In these experiments, dyed knitted fabrics showed antimicrobial activity against Staphylococcus aureus, but not against Escherichia coli and Candida albicans. On the other hand, pre-treatment plasma water vapor of knitwear made of cotton, bamboo and cotton/bamboo (50/50%) and dyeing with alcohol extract had the effect of reducing the intensity of the coloring while increasing the antimicrobial effect on the growth of the S. aureus culture [20, 9]. The use of Al, Cu and Fe as mordants in silk dyeing with R. japonica extract affected the speed and intensity of silk dyeing, as well as the antimicrobial properties exhibited against S. aureus and Klebsiella pneumoniae [18]. Dyeing cotton with R. japonica leaf extract, with pretreatment with cations, gave a light brown colour that was not reduced by washing.

The dielectric properties of polymer materials provide information on the size, shape and distribution of fillers within the material [21-23]. Dielectric spectroscopy is widely used for textile material characterization [24-27], also the knowledge of the dielectric properties enables designing of textile-based devices, such as various kinds of sensors, antennas, energy harvesting devices or wearable electronic textiles [28-34]. The dependence of the dielectric properties of textile materials on the crystallinites, chain orientations, relative humidity, temperature, fillers, yarn structure, fabric construction and fiber orientation provides a new possibility in textile materials characterization [21, 35]. The use of textile materials for antistatic and electromagnetic interference shielding aplication is one of the most important applications of materials that can be easily managed by knowing the dielectric properties of the material [28, 36, 37]. It has been shown in many scientific papers that the addition of copper to the polymer matrix increases the electrical conductivity [21, 35]. Textile materials modified by copper have been widely used for electromagnetic interference shielding aplication due to an increase in the conductivity by adding copper [38].

In this paper, colorimetric characteristics, as well as the antimicrobial and dielectric properties

of ultrasonically dyed knitwear with extracts of the leaves and rhizomes of the plant *R. Japonica* with the addition of copper (I) oxide and copper (II) sulfate, were investigated.

2. EXPERIMENTAL SECTION

2.1. Materials

The military winter socks made of green wool were selected for the study since they are an item of gear that comes into direct contact with the skin. As a result, testing them is vital because they must have specific qualities that would make them comfortable to wear. To keep the feet warm and comfortable in cold weather, the socks are constructed of wool knit with a big surface mass. The reason the socks are green is because they camouflage with their surroundings, thus it's interesting to see how the plant *Reynoutria japonica* L. affects the socks' color.

2.2. Experimental methods

2.2.1. Dyeing with extracts of medicinal plants in an ultrasonic bath

The concentration of the alcoholic (methanolic) extract of the plant *Reynoutria japonica* L. is 50 mg/mL. Before processing, the mass of the sample of textile material is measured, and based on this, the amount of extract required for antimicrobial treatment is determined (fleet ratio 1:20). An alcoholic extract with a working concentration of 50 mg/mL is poured into a laboratory beaker. Then Citric acid (8 g/L), Tannin (2 g/L), Cu₂O (2 g/L) or CuSO₄ (2 g/L) were added and as the last sample of textile material. The ultrasonic bath is heated to the appropriate temperature (45 °C) and the sample is processed for 30 minutes. After finished processing, the samples are dried in a dryer at a temperature of 40 °C.

The procedure of antimicrobial dyeing of wool knitwear and labeling of the samples are schematically shown in Figure 1.

The characterization of dyed woolen knitwear consisted of the following tests:

- colorimetric characteristics determined by a spectrophotometer,
- antimicrobial action of knitwear by the method of diffusion in agar and
- dielectric properties by dielectric spectroscopy.

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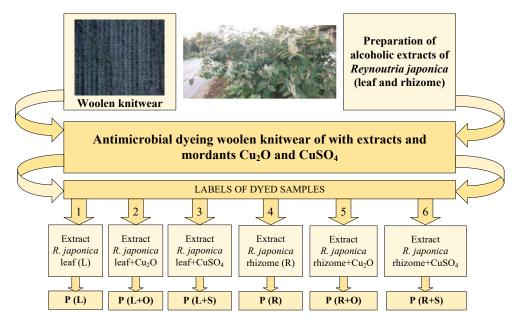


Figure 1. Antimicrobial dyeing of wool knitwear

2.2.2. Spectrophotometric colour analysis of textile

The colour of (coated) objects is visualized and quantified by using the CIELab colour space. The 3-dimensional colour space is built-up from three axes that are perpendicular to one another [39, 40]. Coordinates (L* - the lightness, a* - green-red axis, b* - blue-yellow axis) were determined using the Konica Minolta CM-2600d (Measurement light source D65, observation angle 10°, illumination geometry d:8°, measuring caliber - \emptyset 8 mm). Measurment were conducted five times. Colour difference (Δ E) is calculated with the equation (1):

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \tag{1}$$

where:

 ΔE - colour difference,

 ΔL – lightness difference,

 Δa - green-red axis value difference,

 Δb - blue-yellow axis value difference, as it is shown in Figure 2.

Table 1. Contain description of colour difference between two colours [41].

When it comes to the colorability of textiles, i.e. visual experience of the surface, the results of reflection spectroscopy can give certain interpretations and explanations based on the dependence of reflection (R) and parameters K/S on the wavelength in the visible light region. The degree of coloring of fabric samples is analyzed by spectrophotometric measurements before and after treatment with plant extracts, and based on the data for reflection (R) the parameter K/S (colour strenght) is calculated, with the equation (2):

$$K/S = (1 - R)^2/2R$$
 (2)

where:

R – reflection, K – absorbance and S – scattering.

ΔE between 0 and 1	Generally, difference cannot be noticed
ΔE between 1 and 2	Small colour difference, visible to "trained" eye
ΔE between 2 and 3.5	Medium colour difference, visible to "untrained" eye
ΔE between 3.5 and 5	Obvious colour difference
ΔE over 5	Massive colour difference

Table 1. The visual difference between two colours

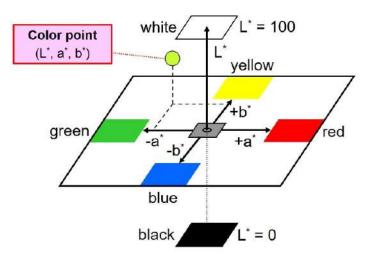


Figure 2. CIE Lab coordinate system

2.2.3. Examination of antimicrobial activity by agar diffusion method

The antibacterial activity of the modified fabrics, on the bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, was investigated by the method of diffusion in agar [42, 43]. For this investigation, the modified fabric samples were cut into patches of dimensions 2,5 x 2,5 cm.

The investigation procedure was performed as follows:

- 1. 100 μ L of each culture was added to the surface of a sterile Mueller-Hinton agar plate.
- 2. The culture suspension was carefully distributed on the surface of the whole petri dish with a sterile glass L-stick.
- 3. Fabric samples were placed on the surface of the seeded culture.
- 4. Petri dishes were incubated for 24–48 hours at 37 $^{\circ}$ C.

After incubation, zones of inhibition were measured from the following equation:

$$Z_i = \frac{(T-D)}{2} \ (mm) \tag{3}$$

where are:

 Z_i – width of zone of inhibition(mm),

T – width of sample + zone of inhibition (mm),

D – width of sample (mm).

If there is no zone of inhibition, and no growth below the sample, then it is defined as contact inhibition.

2.2.4. Investigation of dielectric properties of knitwear

Dielectric measurements were performed on a precision LCR Hameg 8118 instrument, in the frequency range from 20 Hz to 100 kHz, at a temperature of 20.5 °C. Knitted samples (85 mm in diameter) were placed in a capacitor cell, where dielectric measurements were performed using $U_0 = 1.0 \text{ V}$ [44, 45]. The conductance (G_m) and susceptance (B_m) of the samples, as well as the conductance (G_b) and susceptance (B_b) of the empty cell, were measured in the C_p mode of the instrument, while the specific conductance and susceptance were calculated using the following relations:

$$G_{spec} = G \frac{d}{s} \tag{4}$$

$$B_{spec} = B \frac{d}{s} \tag{5}$$

where *d* is the thickness, *S* is the surface area of the sample, $G = G_m - G_b$ and $B = B_m - B_b$.

3. RESULTS AND DISCUSSION

3.1. Colorimetric characteristics

Colour coordinates, calculated colour strength (K/S) and colour differences (ΔE) between knitted fabric samples dyed with *Reynoutria japonica* extract (leaf and rhizome) with the addition of Cu₂O and CuSO₄ and the knitted fabric (P) before dyeing are shown in Table 2. Graphic representa-

tion of spectral curves of knitted fabric samples dyed with *R. japonica* leaf and rhizome extract is given in Figure 3.

Sample label	L*	a*	b*	ΔΕ	K/S
Р	29.42	-0.23	7.90	-	13.51
P(L)	28.24	-0.06	10.13	2.53	24.14
P(L+O)	26.61	0.31	9.04	3.08	26.63
P (L+S)	28.74	-0.42	10.36	2.56	24.26
P (R)	28.24	0.56	13.65	5.91	24.26
P(R+O)	27.04	0.33	12.27	5.00	20.85
P(R+S)	28.70	0.11	13.54	5.70	19.34

 Table 2. Colour coordinates, colour difference and colour strength results

It can be noticed that the difference in brightness of all samples is barely noticeable to a human eye and shows darker samples, but device detected coordinates show that undyed sample is the lightest, and samples appear darker after dyeing. The addition of $CuSO_4$ gives the effect of a slightly darker colour, addition of Cu_2O makes samples appear darker. On the a* axis the changes are close to zero, and in combination with only 10% positive yellow content on b* axis, it can be said that the samples are dark gray. Regarding the change in colour and the difference between the samples, the change is noticeable even to the untrained eye compared to the initial sample. It increases with the addition of both plant extracts. With the addition of plant leaf extract, the difference is medium, visible to the untrained eye, while with the addition of rhizome extract, the difference in colour is massive. The strength of the colour almost doubles with the addition of the extract. Their values are approximately the same, however, adding mordants changes this value. With the leaf extract, with the addition of mordants, there is an increase in colour intensity, while in the rhizome extract, there is a decrease in colour intensity.

Spectral curves are shown on Figure 3. As it is shown there is not much variation between the samples until high wavelengths are reached. In the range from 660 nm to 740 nm there is slightly distinction between the samples, from the point of view of colour the difference is small considering that it is below 60% of the reflected value, so although this area belongs to the reflection of red color, the colour is quite dark to visually notice the difference. However, by reading the data from the device, it can be noticed that the samples for both extracts significantly lost their brightness when Cu₂O was added. Although the samples from the leaf extract, are slightly brighter than the rhizome ones.

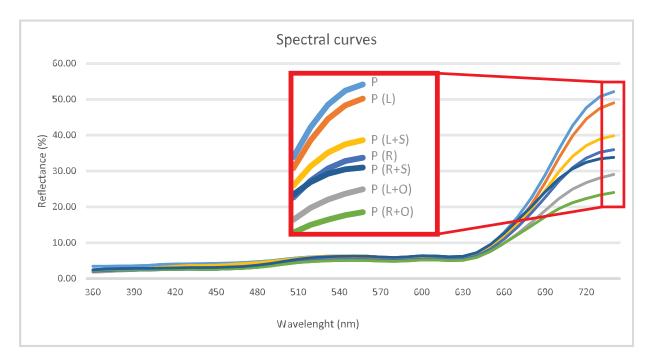


Figure 3. Graphic representation of the samples spectral curves

3.2. Antimicrobial examination

The results of testing the antimicrobial effect of knitwear dyed with *Reynoutria japonica* plant extracts (leaf and rhizome) with the addition of Copper (I) oxide (Cu₂O) and Copper (II) sulfate (CuSO₄) are shown in Table 3 and Figures 4 and 5.

Based on the results of testing the antimicrobial effect of knitted fabrics dyed with *Reynoutria japonica* leaf and rhizome extracts, it can be seen that the processed knitted fabrics show a significant antimicrobial effect on *Staphylococcus aureus* bacteria in the form of an inhibition zone. It can also be noticed that knitted fabrics dyed with *R. japonica* leaf extract with the addition of Cu₂O show a larger zone of inhibition compared to those dyed with the addition of CuSO₄. When dying with *R. japonica* rhizome extract, a larger zone of inhibition was noticed when CuSO₄ was added compared to Cu₂O. Knitted fabrics treated with *Escherichia coli* do not show any antimicrobial activity. Knitted fabrics dyed with extracts of the leaves and rhizomes of the plant *Rey*- *noutria japonica* with the addition of mordants Cu_2O and $CuSO_4$ showed a weaker antimicrobial activity (SKI) against the yeast *Candida albicans*.

		2	4			
	Antimicrobial activity on microorganisms					
Sample	Staphylococcus aureus	Escherichia coli	Candida albicans			
P (L)	DKI	NA	SKI			
P(L+O)	$Z_i = 0,920 \text{ mm}$	NA	SKI			
P(L+S)	$Z_i = 0,250 \text{ mm}$	NA	SKI			
P (R)	$Z_i = 0,750 \text{ mm}$	NA	NA			
P (R+O)	$Z_i = 1,125 \text{ mm}$	NA	SKI			
P(R+S)	$Z_i = 2,250 \text{ mm}$	NA	SKI			
Z _i – inhibition zone (mm) SKI – weak contact inhibition (25-50% reduction of						

Table 3. Results of testing the antimicrobial effect of knitted fabrics dyed with plant *Reynoutria japonica* leaf and rhizome extracts with the addition Cu_2O and $CuSO_4$

 Z_i – minoriton zone (min) SKI – weak contact inhibition (25-50% reduction of microorganisms under the sample) NA – no activity

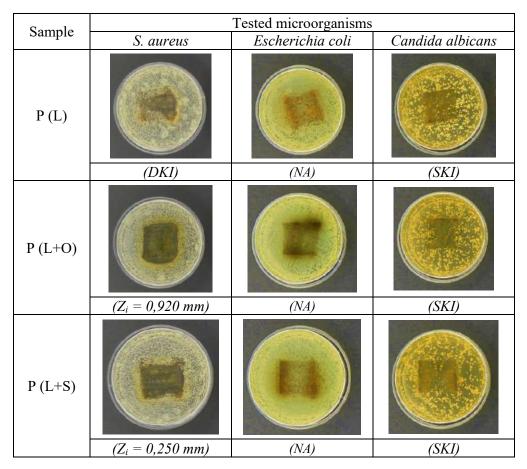


Figure 4. Procedure for testing antimicrobial properties of dyed woolen knitwear with R. japonica leaf extract

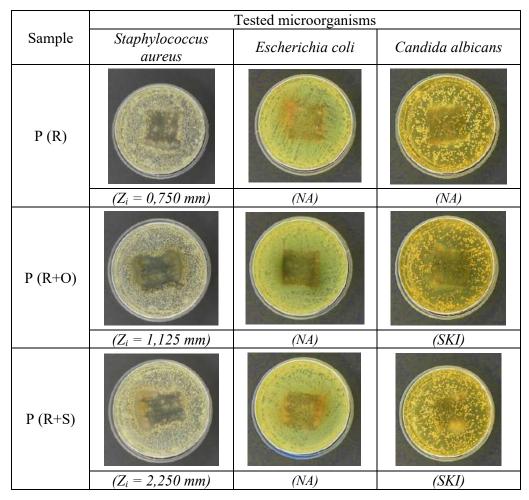


Figure 5. Procedure for testing antimicrobial properties of dyed woolen knitwear with R. japonica rhizome extract

3.3. Dielectric spectroscopy

Dielectric parameters, conductance and susceptance, were measured for all samples using the dielectric spectroscopy method in the frequency range from 20 Hz to 100 kHz, at a measuring voltage of 1 V. Based on the obtained results, specific values of conductance were calculated and the results were graphically processed and shown in Figures 6 and 7.

Figure 6 shows the change in specific conductance as a function of frequency for knitted fabric samples dyed with R. *japonica* extract (leaf), copper (I) oxide and copper (II) sulfate. All samples show an increase in conductance with frequency, which indicates the activation of different polarization mechanisms that are characteristic of dielectric materials. An increase in conductivity compared to the initial sample was noticed in all dyed samples, and the figure shows that the highest increase in conductivity was obtained in samples dyed with the addition of

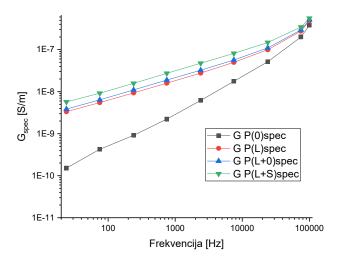


Figure 6. Specific conductance as a function of frequency for samples P(0), P(L), P(L+O) and P(L+S).

mordants Cu₂O and CuSO₄. The increase in conductivity at lower frequencies is the result of interfacial

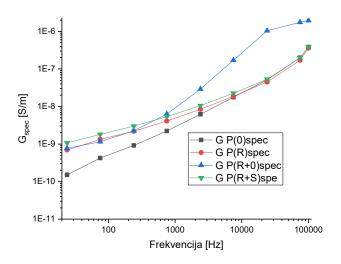


Figure 7. Specific conductance as a function of frequency for samples P(0), P(R), P(R+O) and P(R+S).

and space charge polarization caused by modifying the knitted fabric sample with copper oxide and copper sulfate, while the increase in conductivity at higher frequencies is caused by dipolar polarization. Specific conductances as a function of frequency for samples of knitted fabrics dyed with *R. japonica* (rhizome) extract, copper (I) oxide and copper (II) sulfate were shown at Fig. 7. The highest increase in conductivity was observed in the sample dyed with the addition of Cu₂O mordant, and this increase at the frequency of 24 kHz was 20 times higher than the initial sample.

4. CONCLUSION

Military uniforms are decorated with certain details, starting with the colour of the uniform, and ending with the material used to make those uniforms. All of these are very important segments that must be provided, because with their help, the lives of the soldiers who wear them are protected, and at the same time, their readiness to perform the assigned activity increases.

Based on the results of testing the antimicrobial effect and dielectric properties, as well as the spectrophotometric analysis of textile materials, intended for the production of military clothing, dyed with extracts of the leaves and rhizomes of the *Reynoutria japonica* plant, the following conclusions can be found:

-Knitted fabrics dyed with extracts of the leaves and rhizomes of the plant *Reynoutria japon*-

ica show, with the addition of Cu_2O and $CuSO_4$, a significant antimicrobial effect on the bacterium *Staphylococcus aureus* in the form of an inhibition zone, on the yeast *Candida albicans* a weak contact inhibition, while on the bacterium *Escherichia coli* they do not show any antimicrobial activity.

-All samples dyed show an increase in conductivity compared to the initial untreated sample. It is also evident that the highest increase in conductivity was obtained in samples treated with copper (I) oxide and copper (II) sulfate. A decrease in conductivity was observed in fabric samples that were dyed with extract of the leaf, together with additions of Cu_2O and $CuSO_4$, and in rhizome-dyed fabric and treated with Cu_2O , there was a decrease in conductivity by as much as 90%.

– Samples dyed with Reynutria japonica extracts gave a greenish color, with a much stronger effect achieved by rhizome dyeing. It is important to note that the addition of Cu_2O compared to the addition of $CuSO_4$ increases the intensity and durability of the coloring, which significantly improves the final effect on the material. It was found that the difference in colour (ΔE) between the initial sample and the samples after dyeing with *R. japonica* extract (leaf and rhizome) was medium, and with addition of mordants it was massive difference, and gave darker colour that is preferred by military equipment.

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КОЛОРИМЕТРИЈСКЕ КАРАКТЕРИСТИКЕ УЛТРАЗВУЧНО БОЈЕНОГ ТЕКСТИЛА ЕКСТРАКТИМА *Reynoutria japonica* И МОРДАНТИМА НА БАЗИ БАКРА

Сажетак: У овом раду истраживан је утицај врсте екстракта (лист и ризом *R. japonica*) и врсте морданата на бази бакра (бакар (I) оксид - Cu_2O и бакар (II) сулфат - $CuSO_4$) на спектроскопијске и антимикробне особине бојених вунених плетенина. Антимикробна активност свих узорака испитана је на бактерије *Staphylococcus aureus* и *Escherichia coli* и на квасницу *Candida albicans*. Уочено је да плетенине бојене екстрактом листа *R. Japonica* и Cu_2O показују боље антимикробно дејство на бактерију *S. aureus* у односу на плетенине бојене уз додатак $CuSO_4$. Међутим, код плетенина бојених екстрактом ризома *R. Japonica* имамо обрнут случај; као мордант бољи се показао $CuSO_4$. Методом диелектричне спектроскопије извршено је мерење кондуктансе у фреквентном опсегу од 20 Hz до 100 kHz. Највећи пораст проводности уочен је код узорка бојеног екстрактом ризома *R. Japonica* и Cu_2O , гдје је повећање на фреквенцији 24 kHz било 20 пута веће у односу на полазни узорак. Добијени резултати спектрофотометријске анализе показују да је највећа јачина боје (K/S) постигнута код узорка бојеног екстрактом листа *R. Japonica* и $CuSO_4$. Добијени резултати указују на могућност употребе испитиваних плетенина за антимикробну, као и електромагнетну заштиту.

Кључне ријечи: колориметријске карактеристике, антимикробне особине, диелектричне особине, вунена плетенина, *Reynoutria japonica*, бакар (I) оксид, бакар (II) сулфат.

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