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Original scientific paper

IN VITRO ANTIOXIDANT ACTIVITY OF COTTON FABRIC TREATED WITH ETHANOL AND WATER *THYMUS SERPYLLUM L.* (WILD THYME) EXTRACTS

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Abstract

The plants contain a large number of various compounds that exhibit significant antioxidant activity. Among them, polyphenols and flavonoids are the most studied compounds with different biological properties, including antioxidant activity. The functionalization of cotton fabric with natural compounds from plants results in the material with improved antioxidative properties. In this study, cotton fabrics treated with ethanol and water Thymus serpyllum L. (wild thyme) extracts were investigated in terms of antioxidant properties and chemical composition. In vitro antioxidant capacity of ethanol and water extracts, and the cotton fabric before and after functionalization was evaluated using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radicals scavenging capacity assays. FTIR and UV–Vis spectroscopy elucidated the structure of the cotton fabric, extracts, and functionalized cotton fabric. FTIR spectroscopy demonstrated that the structure of cellulose was dominated in cotton, and also proved the presence of a low percentage of protein, pectin, oil, fat, and wax, as well as, binding of compounds from the extract by cross-linking with long chains of structure cotton. In dry extracts, the presence of various chemical functional groups was confirmed. UV-Vis analysis identified the active components in T. serpyllum extracts. Namely, absorption values for both spectra (water and ethanol samples) at 215-253 nm, 263-384 nm, are specific for phenolic acids, and flavonoids. Also, one of the aims of the study was to investigate the influence of various extracts (water and ethanol samples) on the antioxidant activity of cotton fabric. All extracts demonstrated excellent antioxidant properties, more potent than the reference antioxidant (ascorbic acid) in both antioxidant assays (between 85-86% in DPPH and 94-96% in ABTS assays). Pure T. serpyllum water extract was the most potent in the DPPH assay (86%), while pure ethanolic extract was the most potent antioxidant in the ABTS assay (96%). The antioxidant capacity of raw cotton fabric was insufficient (4%). On the other hand, in the DPPH assay, samples of cotton fabric with extract showed moderate activity with scavenging activity of 48% and 45% for ethanol and water extracts, respectively. Additionally, in the ABTS assay, the antioxidant activity of treated cotton fabric was 51% and 46% for ethanol and water extracts, respectively.

***Key words**: Thymus serpyllum extract, cotton knitted fabric, antioxidant activity*

Introduction

Thymus serpyllum L. (wild thyme, Lamiaceae) grows in almost all the countries bordering the Mediterranean, in Asia, and in parts of Central Europe (Farooqi et al., 2005). The plant belongs to the group of aromatic plants, which have a high level of essential oils and polyphenols. *T. serpyllum* possesses antiseptic, anthelmintic, diaphoretic, antispasmodic, antioxidative, expectorant, carminative, and diuretic activities (Hussain et al., 2013; Kulišić et al., 2006; Rasooli et al., 2002). *T. serpyllum* extracts have been traditionally used in the treatment of respiratory, digestive, and genitourinary diseases (Jarić et al., 2015). According to Kulišić et al. (2007) and Mihailović-Stanojević et al. (2013), water *T. serpyllum* extract showed nitric oxide-scavenging ability and inhibition of copper-induced oxidation. Polyphenol compounds are a large group of secondary metabolites of plants that are often employed as natural food preservatives, antioxidants, and additives (Casiglia et al., 2019). Flavonoids, as an important part of polyphenols, as well as natural antioxidants, can reduce oxidative stress in cardiovascular and neurodegenerative diseases, diabetes mellitus, asthma, and eye disorders (Andreescu et al., 2011). There is a growing interest to extract polyphenols from herbal sources, in order to obtain a safe, natural, and low-cost alternative to synthetic antioxidant components, out of which some possess toxic and mutagenic effects (Čadanović-Brunet et al., 2006). The chemical composition and biological potential of *T. serpyllum* extracts are the subjects of interest in the medicine, pharmaceutical, and food industries (Hussain et al., 2013). Furthermore, different procedures for the extraction of polyphenol compounds were established. In recent time, the application of modern extraction methods, such as heat-assisted extraction, have been evaluated (Jovanović et al., 2017a). The mentioned technique provides various benefits, including the saving of extraction medium, shorter extraction time, and higher extraction yield (Jovanović et al., 2017b). Additionally, it supports the concept of "green" solvent, i.e. minimizes the negative environmental effects from the consumption of large amounts of the extraction solvents. According to Mustafa and Turner (2011), simple alcohols and alcohol-water mixtures are more environmentally favorable solvents. From a toxicological point of view, water, and ethanol are safer and more suitable than other solvents used in food, pharmaceutical, cosmetic and other industries (Tauchen et al., 2015).

In addition, these extracts can be used in textile materials dyeing because they are environment-friendly, and non-toxic to humans (Vankar and Shukla, 2019; Madhukar Thakker and Sun, 2022). Cotton seeds contain antioxidant flavonoids, with various pharmacological properties (Dong et al., 2022). Cotton fibers are widely used in everyday life and industry, while in recent decades, biological textiles with antimicrobial, antioxidant and cosmetic properties have been developed. According to Shahmoradi-Ghaheh et al., (2017) due to the synergistic effect can be transmission occurs the high amount of active component transfer with functionalized materials on the skin. This could have vital benefits such as skin protection, product, or skin moisturizer (Shahmoradi-Ghaheh et al., 2017).

The aim of the present study was the investigation of antioxidant properties and chemical composition of cotton fabrics treated with ethanol and water *T. serpyllum* extracts.

Materials and Methods

Materials and reagents

The dried *T. serpyllum* was from the Institute for Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia. The following reagents were used: ethanol (Merck, Germany), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) - ABTS, and 2,2-diphenyl-1-picrylhydrazyl - DPPH (Sigma-Aldrich, USA), and ultrapure water (Simplicity UV® water purification system, Merck Millipore, Merck KGaA, Germany). The single 100% cotton knitted fabric with mass per unit weight of 111.89 g/m^2 and density of 17cm^{-1} was used in this study.

Extraction procedure

T. serpyllum extracts were prepared employing water and 50% ethanol, as extraction mediums, using heat-assisted extraction (incubator shaker at 200 rpm and 80ºC, KS 4000i control, IKA, Germany). The solid-to-solvent ratio was 1:30 g/mL and the extraction time was 60 min. The samples were filtered through filter paper after the extraction and the extracts were stored at 4°C until further analyses.

Functionalization of cotton knitted fabric

The preparation cotton fabric sample was performed according to the literature (Koh and Hwa Hong, 2016) with small changes. Briefly, cotton knitted fabric (20 x 20 cm) was immersed in the 20 ml of ethanol or water *T. serpyllum* extracts solution for 2h at room temperature. Subsequently, the fabrics were dried on air.

Characterization methods

Fourier-transform infrared (FTIR) analysis

FTIR spectra of pure cotton and ethanol and water *T. serpyllum* extracts, as well as the cotton fabric functionalized by extracts, were recorded in the transmission mode between 400 and 4000 cm^{-1} using Nicolet iS10 spectrometer (Thermo Scientific, Sweden). Before the analysis, the solvent (water or 50% ethanol) from the extracts was evaporated.

UV–Vis spectroscopy

UV–Vis spectroscopy was used with the aim to identify the active compounds in ethanol and water *T. serpyllum* extracts. UV–Vis spectra of properly diluted extracts were recorded using UV-1800 spectrophotometer (Shimadzu, Japan).

Determination of antioxidant capacity (DPPH and ABTS assays)

In vitro antioxidant capacity of ethanol and water *T. serpyllum* extracts, and the cotton fabric before and after functionalization by using ethanol and water extract was evaluated using DPPH and ABTS radicals scavenging capacity assays.

The DPPH assay was based on the procedure described by Horžić et al. (2012) and Koh and Hwa Hong (2016) with a slight modification and the absorbance was measured at 517 nm. Ascorbic acid was used as the standard and the results were expressed as a percentage (%).

The ABTS assay was based on the procedure described by Re et al. (1999) and Koh and Hwa Hong (2016) with a slight modification and the absorbance was measured at 734 nm. Ascorbic acid was used as the standard and the antioxidant activity was expressed as a percentage (%).

All spectrophotometric measurements were performed in an UV-1800 spectrophotometer (Shimadzu, Japan).

Statistical analysis

The statistical analysis of the antioxidant potential of pure cotton and ethanol and water *T. serpyllum* extracts, as well as the cotton fabric functionalized by extracts was performed by using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p<0.05$.

Results and Discussion

The impact of ethanol and water *T. serpyllum* extracts on the antioxidant potential of cotton, as well as the chemical composition of pure cotton, ethanol and water *T. serpyllum* extracts, and the cotton functionalized by the extracts were investigated. The results are presented in Figure 1 (antioxidant potential), Figure 2 (FTIR spectra), and Figure 3 (UV-Vis spectra).

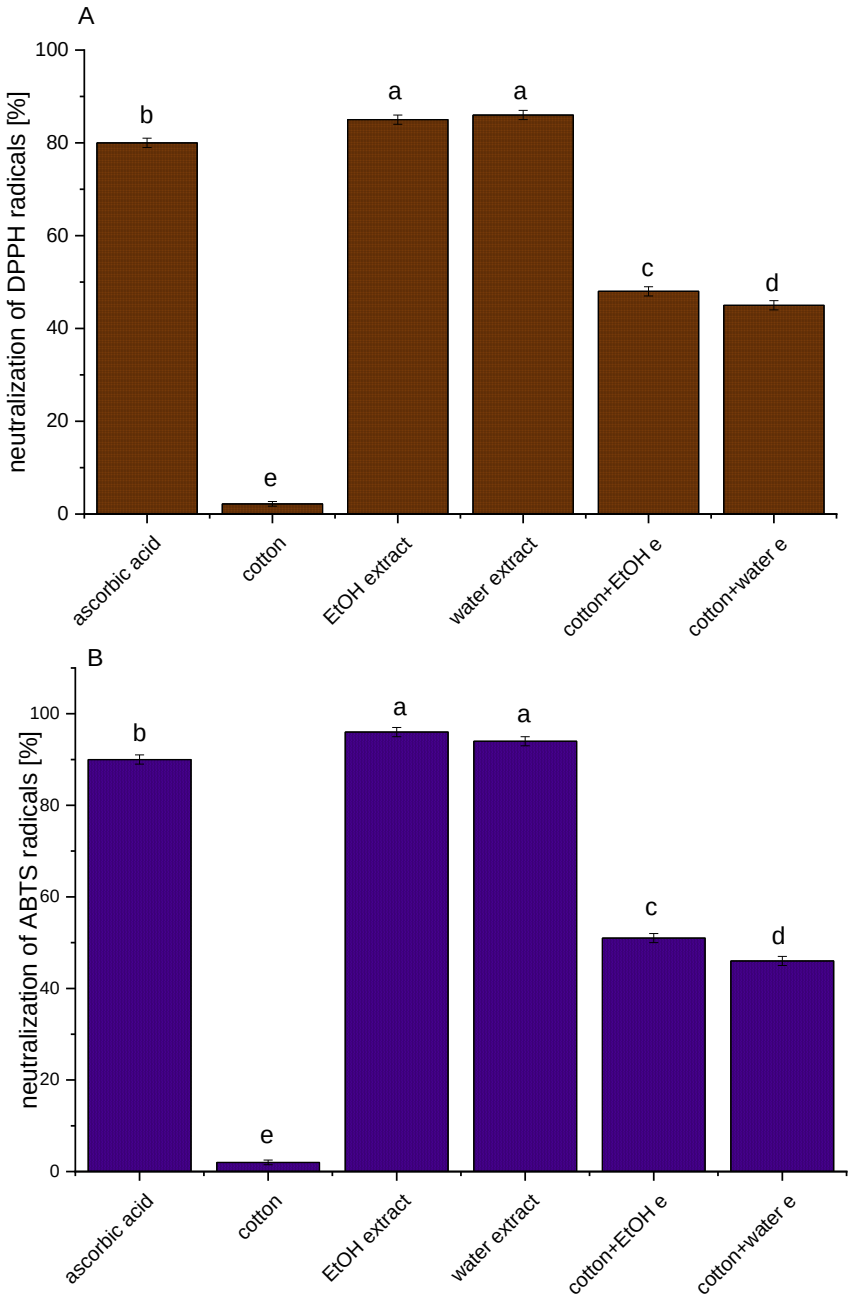

Figure 1. (A) DPPH and (B) ABTS radical scavenging potential of pure cotton, ethanol and water Thymus serpyllum extracts and the cotton functionalized by the extracts; the letters (a-e) above bars showed statistically significant differences ($p<0.05$; $n=3$; analysis of variance, Duncan's post-hoc test).

As can be seen from Figure 1, all *T. serpyllum* extracts demonstrated excellent antioxidant potential, more potent than the antioxidant standard (ascorbic acid) in both antioxidant assays (between 85-86% in DPPH assay and 94-96% in ABTS assay). Pure *T. serpyllum* water extract was the most potent in the DPPH assay (86.5±1.1%), while pure ethanol

extract was the most potent antioxidant in the ABTS assay (96.3±1.3%). The antioxidant capacity of raw cotton fabric was insufficient in both assays (~2%, Figure 1). On the other hand, in the DPPH assay, samples of cotton fabric with extract showed moderate activity with the scavenging activity of 48.2±1.0% and 45.5±1.1% for ethanol and water extracts, respectively. Additionally, in the ABTS assay, the antioxidant activity of treated cotton fabric was 51.3±1.4% and 46.4±1.3% for ethanol and water extracts, respectively. Although there was no statistically significant difference between pure ethanol and water *T. serpyllum* extracts, cotton impregnated by ethanol extract has shown statistically significantly better antioxidant potential in both tests in comparison to water parallel. This suggests that the cotton fabric impregnated with ethanol extract showed the significance of the synergetic effect of the phenolic group from bonded active compounds in increasing the antioxidant activity.

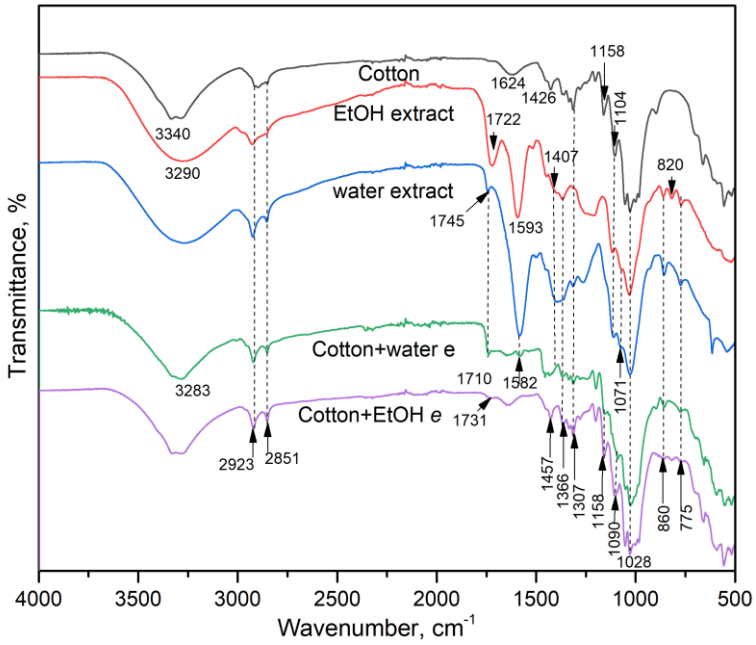

Figure 2. FTIR spectra of pure cotton, ethanol and water Thymus serpyllum extracts and the cotton functionalized by the extracts

FTIR spectroscopy demonstrated that the structure of cellulose was dominated in cotton, and also proved the presence of a low percentage of protein, pectin, oil, fat, and wax, as well as binding of compounds from the extract by cross-linking with long chains of structure cotton (Figure 2). In cotton fabric the characteristic bands 3340 and 3283 cm^{-1} are assigned to -OH groups (Chung et al., 2004). The stretching and bending vibrations of $-CH_2$ and $-CH_3$ were observed in the region 2923-2851cm^{-1}, and region 1426-1307 cm^{-1}, respectively. The band at 1624 cm^{-1} and peaks at 1158-1028 cm^{-1} are attributed to bending O-H and stretching C-O groups in cellulose cotton, respectively, while the peak at 895 cm^{-1} ascribed to β-glycosidic linkages (Chung et al., 2004). In dry extracts, the presence of various chemical functional groups, including hydroxyl (O-H) at 3290 cm^{-1}, methyl and methylene groups at 2923-2851 cm^{-1}, carbonyl (C=O) at 1722 and 1745 cm^{-1}, phenyl ring (C=C) at 1593 cm^{-1}, bending of C-H and phenyl substituted ring at 860-775 cm^{-1}. After the functionalization of cotton knitted fabric was observed bands from natural compounds from plants. Observed new peaks at 1710 and 1582 cm^{-1} for cotton+water e and cotton+EtOH e, respectively. Due to, cross-linking binding other bands led to a change of intensity in spectra.

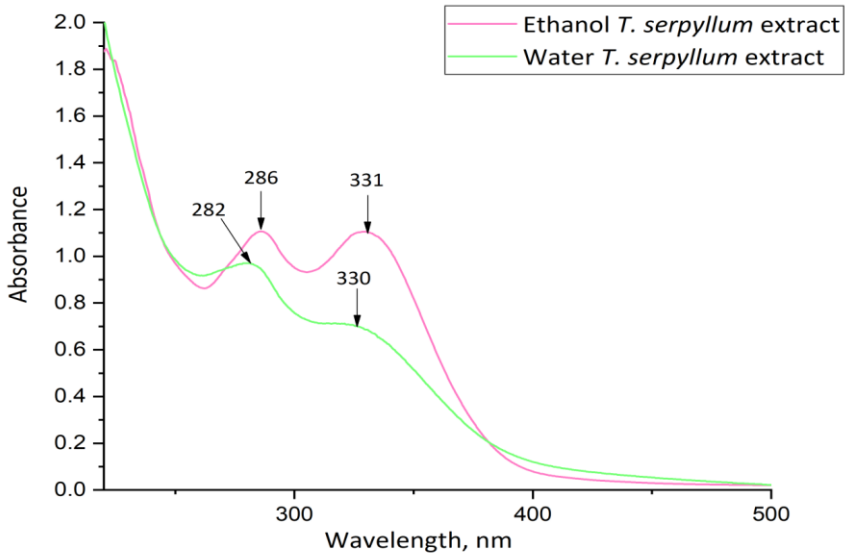

Figure 3. UV-Vis spectra of pure ethanol and water Thymus serpyllum extracts

UV-Vis analysis identified the active components in ethanol and water *T. serpyllum* extracts. Namely, absorption values for both spectra at 215-253 nm, 263-384 nm for ethanol and water extracts are specific for phenolic acids, flavonoids, and quinones. According to the literature data, the most dominant compounds in *T. serpyllum* were polyphenols, including phenolic acids and flavonoids (Jovanović et al., 2017a; Monographs 2014; PDR for Herbal Medicines, 2004).

Conclusions

The aim of the present study was the examination of antioxidant properties and chemical composition of ethanol and water *T. serpyllum* extracts, as well as cotton fabrics treated with the mentioned extracts. Although there was no statistically significant difference between pure ethanol and water *T. serpyllum* extracts, cotton impregnated by ethanol extract has shown statistically significantly better antioxidant potential in both tests in comparison to water parallel. UV-Vis analysis identified the active compounds in ethanol and water *T. serpyllum* extracts, including phenolic acids, flavonoids, and quinones, while FTIR spectra showed their binding for fabric.

Acknowledgments

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Conflict of interest

The authors declare that they have no financial and commercial conflicts of interest.

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