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Effects on Antibacterial and DNA Protection of Organic Dyestuff Extracts Obtained from Hazelnut Nuthusk

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Abstract: Hazelnut is of great important in agricultural export of our country. It has an annual export of one and a half billion dollars. Every new product that will increase the value of the nut is very important. In recent years, natural dye materials used especially in textile materials are important and those of plant origin are preferred. For this purpose, we have made a natural dye extracts from hazelnut nuthusk after collected, dried and grinded of samples. Some biological activities have been carried out showing the suitability for industrial use of the dye extracts obtained from hazelnut nuthusk. Within the scope of our study, the effect of extracts obtained from the hazelnut nuthusk of the Düzce region was evaluated on anti-bacterial and DNA protection. From the extracts obtained, antibacterial activity tests were analyzed by disk diffusion method using 3 different standard bacterial strains as S. aureus, *E. coli* and *P. aeruginosa*. pBR322 plasmid DNA and UV-C method was used for DNA protective activity. Four different extracts of dyestuff has showed antibacterial activities to S. Aureus and two different of them *P. aeruginosa* but none of the samples antibacterial properties to E. coli bacterial strains. It has been determined that the all of dyestuffs extracts obtained from the hazelnut nuthusk have potential for protection against the effects of UV-C and H_2O_2 on DNA.

Keywords: UV-C, reflux, H_2O_2, DNA

1. INTRODUCTION

Before the discovery of the synthetic dyestuff agents in 1876, all the dyestuff processes were made with natural materials [1]. Nowadays, it has become clear that the synthetic dyestuff agents have harmful effects both on the people as well as on the environment since a majority of them are carcinogenic or create carcinogenic intermediates with fragmentation [2]. For this reason, people are turning to the natural dyestuff agents like in the past years which can be divided into two groups including artificial and natural. The main materials used for the synthesis of the artificial dyestuff agents and pigments are coal tar compounds known as aromatic hydrocarbons. The petrochemical industry is gaining importance in providing these materials, while the natural dyestuff agents are obtained from natural materials found in the nature. It is known that the herbal dyestuff agents are obtained from plants which are found in the nature and have dyestuff features. While the whole of some plants are used for coloring,

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there are also plants whose certain parts (flowers, leaves, roots and nark etc.) are used only. The disadvantage of the natural dyes is that they can be produced in a low amount and that it is difficult the catch the same color tone [3]. The advantage of the natural dyestuff agents is that they do not pose a risk to health and do not create environmental pollution as the synthetic dyestuff agents. Textile industry is a sector that uses excess amount of water therefore it generates excessed amount of wastewater too. The content of these wastewaters consists of fiber impurities, used dyestuff agents and various chemicals. The dyestuff agents that are included within the wastewater give color to this water which destroys the aesthetic appearance of the water and also reduces the amount of the dissolved oxygen which is the life source of the living creatures within the aquatic environment by reducing the light permeability and causes the death of these living creatures. Considering that the share of the textile industry in Turkey is 20 % in the industrial production, the necessity of the treatment of the textile wastewater becomes more important. The direct disposal of such wastes into the water is dangerous and may cause infection [4].

As a result of chemical and biological changes the organic materials such as dyes and detergents which are included within the wastes cause the dissolution of the dissolved oxygen in the water and cause the destruction of the fishes' life environment. However, naturel dyestuff agents are biodegradated quickly in the naturel cycle [5]. This demand will be ensured when the textile materials are colored with natural dyestuff agents. Among the natural dyestuff agents, the plant coloring agents are the most widely used. These can be obtained from plants called dyestuff plants or from plant wastes. Turkey which is one of the countries having the most favorable conditions for planting and growing hazelnuts grows hazelnuts on about 600 thousand hectares and gives 65 % of the hazelnuts produced throughout the world. Approximately 550 thousand tons of hazelnut are grown in Turkey each year and from these approximately 275 thousand tons of hard shell and about 500 tons of hazelnut nut husk waste emerge [6]. Therefore this study intended to examine the effects of the herbal dyestuff agents obtained from the hazelnut nuthusk through appropriate optimization and extraction methods on antimicrobial, antibacterial and DNA protection. Bacteria can grow very quickly on the textile surface when the appropriate temperature, humidity and other conditions are provided. It is possible to encounter two different hazards on the textile surface as a result of bacterial growth. The first of them, as a result of the uncontrolled bacterial growth is the emergence of bad odor, loss of comfort and the potential risk to harm the human health. Secondly, there may occur stains, discoloration and performance losses on the fabric surface [7,8]. Therefore, it is very important to use antibacterial dyestuff agents for the coloring of the textile products. It is known that the various solvents obtained from the pomegranate extracts [9,10], aloe Vera [11], green tea [12], chilly seed oil [13], peppermint oil [14] and rosemary oil have antibacterial activities and that these are applied to the textile surface directly or through microencapsulation. However, the recovery and antibacterial properties of the dyestuff agent obtained from the hazelnut shells have not yet been reported.

Since the textile products are constantly in contact with the human body, the synthetic dyestuff agents used in the production can cause skin diseases moreover these materials may have allergic or carcinogenic effects [15].

The European Commission (EC) date of 2004/21 directive of the European Union limited the use of many azo dyes to 30 ppm. Some of the azo dyes cause the formation of free amino groups as a result of dyeing. The carcinogenic effects of these emerging free amino groups are known [16]. It is also known that the UV (Ultraviolet) rays that reach the Earth due to the destruction of the stratosphere layer have negative effects on the living creatures. The UV rays can cause serious diseases that may result in skin cancer or skin aging. The synthetic dyestuff agents used in textile products can cause irreversible damage to the skin when they got

combined with the effects of the UV rays. Therefore, in this study it tried to be detected that the obtained herbal dyestuff extracts had effects on DNA protection against the UV rays.

2. MATERIAL and METHODS

2.1. Plant Material

Hazelnut nuthusk samples from Düzce (district of Akcakoca) in Turkey were collected (August and September 2016). This samples were dried without the sun. The collected samples were ground to powder approximately 70-80 mesh and it was eliminated so as to be smaller than 80 mesh. Dried and sieved samples are wrapped with aluminum foil on the outside of the glass bottle (to be dark) and are stored in cold (+4℃).

2.2. Recovery of the Dyestuff Agents

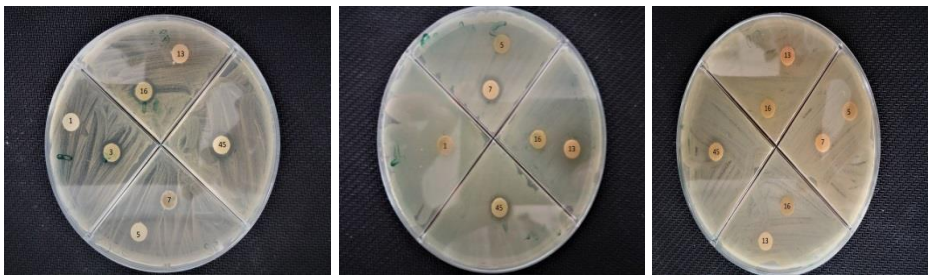
Organic dyestuff (H1, H2, H3, H4, H5, H6, H7) extraction was achieved from the hazelnut nuthusk according to the literature [17]. The obtained dry crude extracts were used for biological activity studies [18].

2.3. Determination of the Antibacterial Activity

In order to determine the antibacterial activity, the tests of the nuthusk dyestuff agents were carried out by considering the CLSI criteria of the disc diffusion test. 500 µl solvent was used for 50 mg DMSO (CH_3) 2SO of the obtained extracts. After the solutions were homogenized in the vortex device, then 20 µl was impregnated to sterile discs of 6 mm diameter. The extract was dried at room temperature for one day in order to remove the solvent from the impregnated discs. By taking an amount of the bacterial strains (S. aureus ATCC 6538, *E. coli* ATCC 25322, *P. aeruginosa* ATCC 27853) as shown that table 1. The extract was adjusted in a densitometer to 0.5 mcFarland standard (Barium Sulfate Turbidity Standard) in separate physiological saline. After vortexing the suspensions, they were spread with a sterile syringe bar by inoculating 100µl to MHA medium. They were left to dry for 10 minutes. The dried discs which were prepared one day before were placed on the medium with the help of a sterile forceps. The petri dishes were placed into an incubator and were left there for 24 hours at 35^0C. At the end of this period the diameters of the inhibition zones formed around the discs was measured according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) with a ruler as shown in Figure 1.

Table 1. Result of the antibacterial from nuthusk dysetuff

Indicator bacteries	H1	H2	H3	H4	H5	H6	H7
S. aurus	+	+	-	-	+	-	+
P. aeruginosa	+	-	-	-	+	-	-
E. coli	-	-	-	-	-	-	-



S. aureus *P. aeruginosa* *E. coli*

Figure 1. Inhibition zone diameters

As a result of these evaluations, as it was shown in Table 1, the 1st, 2nd, 5th and 7th samples of the nuthusk's dyestuff agents showed antibacterial activity against the strains of *S. aureus*. Antibacterial activities were not found in the other samples against these strains.

While antibacterial effects were found in the 1st and 7th samples against *P. aeruginosa*, none of the samples showed antibacterial effects against the *E. coli* strains

2.4. Determining the DNA Protector Activity of the NutHusk's Dyestuff Agent

The plasmid DNA of pBR322 (vivantis) was used to determine the DNA protection effect of the extracts obtained from the nuthusk raw materials against the UV and oxidative damages [19] Imaging was performed on 1.25% agarose gel by applying the method determined by Russo et. al (2000). Seven nuthusk extracts were used for the DNA protection activity test. 50 mg of the extracts were weighed and it was dissolved by adding 1000 µl of methanol in order to prepare a 5% stock concentration [20].

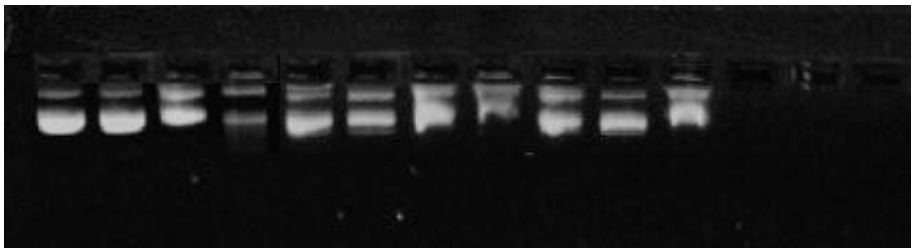


Figure 2. Dysetuff samples DNA protector activities band

Preparation Conditions of Control and Nuthusk samples (H):
C1: Control 1: Plasmid DNA (3µl) + dH_2O (6 µl)
C2: Control 2: Plasmid DNA (3µl) + dH_2O (6 µl) + UV
C3: Control 3: Plasmid DNA (3µl) + dH_2O (6 µl) + H_2O_2 (1 µl)
C4: Control 4: Plasmid DNA (3µl) + dH_2O (6 µl) + H_2O_2 (1 µl) + UV
H1: Plasmid DNA (3µl) +Nuthusk Extract (5 µl) + UV + H_2O_2 (1 µl)
H2: Plasmid DNA (3µl) + Nuthusk Extract (5 µl) + UV + H_2O_2 (1 µl)
H3: Plasmid DNA (3µl) + Nuthusk Extract (5 µl) + UV + H_2O_2 (1 µl)
H4: Plasmid DNA (3µl) + Nuthusk Extract (5 µl) + UV + H_2O_2 (1 µl)
H5: Plasmid DNA (3µl) + Nuthusk Extract (5 µl) + UV + H2O2 (1 µl)
H6: Plasmid DNA (3µl) + Nuthusk Extract (5 µl) + UV + H_2O_2 (1 µl)
H7: Plasmid DNA (3µl) + Nuthusk Extract (5 µl) + UV + H_2O_2 (1 µl)

As a result of examining the DNA protection activity of the nuthusk's dyestuff agents, it was determined that all the samples showed DNA protective activity as shown in Figure 2 but the H4 and H7 samples had lower activity than the other samples. H1, H2, H3, H5 samples had good protector activities than control groups, H4 and H7.

3. DISCUSSIONS

Medical and aromatic plants are plants that are used as medicines to prevent diseases, maintain health or heal diseases. While the medical plants are used in fields such as nutrition, medicine, cosmetics, body care, incense or religious ceremonies, aromatic plants are used in the fields of fine food, aromatherapy and perfumery [16]. The secondary metabolites of the plants are in fact very important chemical compounds with complex mechanisms produced by the plants which have many functions used in various fields recently such as protection, defense, compliance to the environment, survival and the continuation of the generations. Secondary metabolites used as raw materials in many sectors nowadays, are chemical substances that are not directly related to the essential vital function of the plants instead they are as important as

the primary metabolites (protein, fat and carbohydrates) that are directly associated with the vital function of the plant [21]. Although the duties of the secondary products are different in the plants, those with cytotoxic effects against the microbial pathogens can be used as antibacterial agents in medicine. Also some secondary metabolites can be evaluated medically as anti-depressant, sedative and muscle relaxant or can be used as anesthetic drugs. There are various literatures on various chemical components in different mechanism about the use of the plant's secondary metabolites both in industrial as well as in the medical fields [22]. For this purpose, the antibacterial and DNA protective activity of the extracts of the husk was evaluated. The extracts of the husk which is the secondary metabolite of hazelnut were obtained through high temperature and suitable optimization in order to evaluate its usability as coloring material in the textile industry. Many of the dyestuff agents obtained from plants have antimicrobial activities besides of the fact that they do not have damaging effect on health. Many of the plants used for paint extraction are used medically and it have been discovered recently that some of them have significant antimicrobial activity [23].

Punica granatum (Pomegranate) and many commonly used natural dyestuff agents have been reported as antimicrobial agents due to the abundant presence of tannin (commonly referred to as tannic acid) Some other plant sources are rich in naphthoquinone. For example, the anti-bacterial and anti-fungal activity of lawsone in henna, juglone in walnuts and the lapachole in radix alkannae were reported [24]. In this study, in the solvent extracts of the dyestuff agent obtained from the hazelnut nuthusk wase valuated on antibacterial and DNA protection. The natural antimicrobial agents are used to inhibit the growth of mold and bacteria on fabric. A large class of the antimicrobial agents used in textile includes oregano-metallic, phenol, quaternary ammonium salts and oregano silicones [25]. The microbial agents should be safe and biodegradable and should not be toxic and it should be noted if the active agent used in the antimicrobial dyes is effective and can be used safely. Due to the fact that the natural dyestuff agents generally inhibit the growth of microorganisms without any toxic effects, it has become important to study and apply these dyestuff agents [26]. Recently, detailed studies have been carried out on the extracts obtained from medical and aromatic plants in order to investigate new compounds that can control the oxidative DNA damage causing cancer [27].The exposure to oxidative stress and the Ultraviolet rays (UV) can induce the skin damage and can trigger diseases such as aging, inflammation and cancer [28].. It is known that the UV rays reaching the Earth due to the destruction of the stratosphere layer have negative effects on the living creatures. UV rays may cause serious illnesses that can result in skin cancer of aging. In fact, the human skin has a number of mechanism that reduce the harmful effects of VIS (visible rays) and UV rays. However, the high exposure to UV rays can lead to the decrease in the amount of the cellular antioxidants and to UV-induced oxidative DNA damage caused by the reactive oxygen species [20]. There were some research about some additives as colorent of food in the literature. One of them red sorghum bran is known as a rich source for anthocyanins that used of food additives. To study the health benefits of anthocyanin from red sorghum bran, the total antioxidant activity was evaluated by biochemical and molecular methods as use of DNA nicking test. The antioxidant activity of the red sorghum bran was directly related to the total anthocyanin found in red sorghum bran [29].

The another report about a green food additive of chlorophyllin inhibitory effected on the genotoxicities of various carcinogens in Drosophila. The similar reported about purpurin, a component of a red food additive produced from mader root (*Rubia tinctorium*), inhibited the bacterial mutagenicity of hetero cyclicamines [30]. This reports were about food additives on genotoxicity and biochemical properties there were limited report about textile dyestuffs.

For this reason, in this study the DNA protective effect mechanism of the obtained dyestuff agents was evaluated against the UV rays. It was detected that all of the extract samples obtained in order to be used as dyestuff agents in the textile industry had DNA protective effects

against the UV rays. This result is significantly important since it was the first study in the literature that intended to evaluate the dyestuff agents in terms of their DNA protective activity. It is expected to increase the demand to apply this study to the extract of the dyestuff agents obtained from different plants.

Conflict of Interests

Authors declare that there is no conflict of interests.

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