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ORIGINAL ARTICLE

Antibacterial and Antioxidant Properties of Colorant Extracted from Red Onion Skin

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KEYWORDS

Red onion Skin;
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ABSTRACT: Due to the abundance and cheapness of red onion skin scrubs and its high consumption in Iran, we decided to evaluate its antioxidant and antimicrobial properties as a natural source of anthocyanin. The onion skin was collected from the Qazvin local market. Extraction was carried out using water and glycerol. The total anthocyanin concentration was determined by pH-differential method. MIC and MBC were determined using microdilution method and diameter of the bacteria inhibition zone by disc diffusion method on extracted color from Onion skin against *Staphylococcus aureus* and *Escherichia coli* strains. The antioxidant activity was determined by measuring the 2,2-diphenyl-1-picrylhydrazyl(DPPH) and total phenol content by the Folin Ciocalteu. The mean total anthocyanin concentration at 40°C was (60.67, 8.4) mg/g. The highest and the lowest mean diameter of the non-growth zones of the extracted colorant in *Staphylococcus aureus* was 0/83±0/14 and 0/4±0/17 and in the *E. coli* 0/9±0/22 and 0/5±0/20 respectively. Inhibitory concentration of 50% (IC50) in the extracted colorant was obtained at 14/718±0/20 mg / ml. The total phenolic content was obtained as an average of 114.326±2/36 mg/g of gallic acid per gram of onion powder. According to the results of the study and the high consumption of onions in various types of household foods and as a result of increasing their waste, antioxidant and antimicrobial properties, in addition to coloring, can be used as a cheap dye source in various food industries.

INTRODUCTION

Colors are important factors in human life and psychologically are very effective in emotional feelings and nerve stimulation. Man chooses many colors in an unconscious way, but the color of food is one of the first features to be taken into consideration and it has an intuitive aspect. It is an important factor in accepting food. Color of food can also be associated with the quality and freshness of food.

Adding color to food is long lasting that was made by Egyptians in 1500 BC. The colors are added to the food, for

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the following reasons: 1) Reduced color reduction during processing 2) Creation Color in foods that are naturally colorless 3) Reduce color variations during processing or variation based on climate or season 4) Increase the color of food and ensure the uniformity resulting from the heterogeneity of the components of natural colors 5) Coordinate with the customer's taste and appealing food show.

The pigments in the foodstuff are divided into three categories: 1) natural 2) Pseudo-natural 3) synthetic. Synthetic colors are used more than natural colors because they are cheaper, more abundant and more stable. According to the cultural and health considerations of each country, the using and the type of color in food are different [1, 2].

According to the FDA, the amount of artificial dye intake in foods between 1950 and 2012 has risen 5 times and has risen from 12 mg per person to 68 mg per day. In recent years, based on studies, negative effects of consuming artificial colors have been proven, which include hyperactivity and IQ reduction in children that were performed through different tests of the IQ and evaluated various dietary, Autism, leukemia and abortion, weakened immune system, decreased WBC, lymphocyte count and vitamin B6, sleep disturbances, and asthma [3-5].

Of course, all of these items alone do not relate to the use of color additives, and there are other confusing factors, such as other additives.

Natural pigments are very effective in preventing many diseases, such as Alzheimer's disease, heart disease and cancer. One of the most widely used natural pigments is anthocyanins. At the beginning, anthocyanins were known only for redness, but in recent years, due to the antioxidant properties that are effective in preventing neurological, cardiac, cancer, inflammation and diabetes, the color range they are between orange and blue and are stored in plant vacuoles. The sources of anthocyanin in adult diet include berries (20%), wine (16%), grapes (11%), purple or red vegetables (8%), juice (6%), yogurt (6%), and resources Another food (33%) [6,7].

Onion is one of the most important vegetables belonging to the Alliaceae family, a two-year-old herb. Its further growth is in the bulb area, and this part of the plant is a reserve organ, thus making the onions more suitable for storage than other vegetables. It can be planted with rain and irrigated, and has high adaptability to soil and climate types. Onions can be used in different forms, including raw and fried [8]. The use of onions in traditional and herbal treatments has long been traditionally used, but its use in the laboratory environment was 20 years ago, due to the extraction of flavonoids from this plant. Sugars, volatile sulfur compounds, Vitamin C, and mineral compounds, various enzymes, glycosides in onions, have been used in people's diets [9, 10].

According to FAO, Iran is the fourth largest onion producer in the world in 2012, and consumes 22 kilograms of onion in Iran, which is 18.2 times the global average of 1.10 kilograms per year, Specifies the importance of this product in the country's food basket, Which is as high as the production of onion skin lesions [11].

The use of waste from the food industry is one of the most important challenges around the world. Many large amounts of solid and liquid waste are produced annually in the food industry, whose disposal has many environmental problems, while the waste contains bio-degradable materials, the cost of treatment of waste from the food industry and Agriculture is very high. Therefore, reuse of waste can be applied, including the production of edible gelatin from fish waste, citric acid from palm waste and etc.

Based on research on red onion skin, it has been shown to have various anthocyanin species including acylated and non acylated anthocyanins, such as cyanidine 3 mono and di-glucosid, 4 cyanidine 3, 5 cyanidine 3-galactose, 6 Peonidin mono and di-glucosid 7-petonidin glucosid 8 and 5 carboxypyrano - 3 cyanidine glucoside 9 The main accumulation of flavonoids in onions in the outer layer and outer layer is that they are destroyed by peeling onions and disposing of the skin. The pigments and antioxidant properties of onions are related to These are the components [12-14].

Due to the abundance and cheapness of red onion skin scrubs and its high consumption in Iran, we decided to evaluate its antioxidant and antimicrobial properties as a natural source of anthocyanin.

MATERIALS AND METHODS

Plant material

In this experimental study, which was carried out in the laboratory of the Faculty Health of Qazvin University of Medical Sciences in winter 2018, red onion skin was collected from the local market of Qazvin. The spoiled parts were separated and washed with distilled water. Then dried in the darkness.

Extraction

After drying, powdered it with mixer and mix some of the powder with solvent (liquid to solid ratio 50ml / g) with a mixture of water (70% w/v) and glycerol (30% w/v) and put it in a glass container. The container was then placed in a shaker incubator at 200 rpm for 4 hours at 40 and 60°C. The sample was then centrifuged with (Froilabo) at 5000 rpm for ten minutes. The transparent sample was kept at a temperature of 20°C for subsequent use [15]. After determining the total anthocyanin level at 40 and 60°C, the sample with the highest anthocyanin content was used to continue the experiments.

Bacteria

The culture of Lyophilization of *S.aureus* (ATCC 25923) and *E.coli* (ATCC 25922) were prepared, from the Microbiology Department of Faculty of Medicine of Qazvin University of Medical Sciences.

Preparation of chemicals

All chemicals and solvents used in this study were purchased from Merck (Germany) and DPPH Free Radical from sigma aldrich, (Germany). Antibiotic disks were purchased from the Padtan Teb co. and the cultivating media from Charles Co.

Determination of total anthocyanin content

We took 10 ml of the solution to pH 1 and 4.5 with hydrochloric acid or sodium hydroxide 1 M. The absorption rate was determined by UV-Vis spectrophotometer at 520 and 700 nm. The total anthocyanin mononomic concentration was calculated based on cyanidin-3-o-glucoside using formulas 1 and 2. Total anthocyanin content (mg/g):

$$TMA = A \times Mw \times DF \times 1000 / \varepsilon \times L \quad (1)$$

$$A = (Abs_{520} - Abs_{700})_{pH=1} - (Abs_{520} - Abs_{700})_{pH=4.5} \quad (2)$$

Where Mw= The molecular weight of cyanidine 3 glucoside, DF= dilution factor, L= cell length per cm, ε= molar absorption, MW and ε in the formula are respectively 449/2 and 26900, respectively, of cyanidine 3 glucoside [13, 16].

Determine the inhibitory effect of onion waste colorant against S.aureus (25923) and E.coli (25922) and compare it with different antibiotics using disk diffusion method

For this experiment, 24-hour culture of the bacteria containing 10^8 bacteria per ml was used. To perform disc diffusion test, discs with a diameter of 6.4 mm were used which were saturated with color concentration of 50 mg / ml. Then we placed the discs on a sterile plate for 1-hour full color absorption. To compare non-growth zone, standard antibiotics tetracycline, amoxicillin, ampicillin and chloramphenicol were used. In these experiments, the Muller Hinton Agar culture medium was used. After pouring the culture medium into the plate and closing it, 100 µl of the microbial suspension containing 10^8 cfu / ml was poured onto the medium and spread with pasteurized glass pipette in all media. Then the discs were prepared from different colors at different concentrations at right intervals. The culture media was kept in a 37 ° C cabinet for 24 hours. Then the diameter of the non-growth holes was measured with the ruler and the results were reported as average [17].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Using microdilution method, (MIC) and (MBC) were determined for the target microorganisms. In summary, 96 wells were used in this test with a volume of 200 µl, extracted from the colorant Concentrations of 100-50-25-12.5-6.25-3.125-1.56-0.78 mg/ml were prepared. Due to the solubility of color in water, water was used to prepare different concentrations. To determine the minimum inhibitory concentration of colorant of the bacterium, 80 µl of each well was added at a desired concentration and 100 µl of BHI was added. Then, 20 µl of bacterial suspension was added to each well. The well was considered as a positive control containing 180 µl BHI medium plus 20 µl bacteria. A well to control possible contamination including 80 µl of color and 120 µl of BHI was prepared. Microplate contents were mixed with a shaker for 2 minutes. The Microplate was incubated at 37 ° C for 24 hours and after incubation, the turbidity or lack of turbidity in the wells was observed as an ophthalmic concentration. The first well was obtained without turbidity the growth of bacteria was considered as (MIC). To determine the minimum bacterial concentration (MBC), the color of the well in which the bacterial growth was inhibited was used. The sterile anise was impregnated with the contents of the well and cultivated in a superficial manner on the surface of the medium. The plate was placed at 37° C for 24h and then examined for bacterial growth. Minimum concentration that inhibited the growth of 99.9% of bacteria was considered (MBC) [18, 19].

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The ability to color hydrogenation was measured by colorless purple methanol solution (DPPH) staining. In this spectroscopic evaluation, the stable radical in phenylpicrolyl hydrazil DPPH was used as a reactive agent. 0.5 ml of a methanol solution containing concentrations of 1-5-10-20-30mg/ml to 2.5 ml of methanol solution of 0.004% DPPH was added. The solution was stored at room temperature and darkness for 30 minutes after being stylized and then absorbed at 515 nm as compared to control. Free radical inhibitory DPPH was calculated based on percentages. For each solution, the percentage of DPPH free radical containment was calculated using the following formula [20].

$$\%\ DPPH_{\text{radical scavenging}}=[(A_o-A_s) \div A_o]\times 100$$

Where A0 is the absorbance of the control and as is the absorbance of the sample.

Total phenolic content

The phenol assay was performed using a Folin Ciocalteu method. And Gallic acid reaction as standard. We mixed 200 µl of the extracted colorant with 1000 µl of Folin diluted Ciocalteu Reagent to a ratio of 1:10. It was kept at room temperature for 5 minutes. Then, 800 µl of sodium carbonate solution was added to 7.5% and then placed again at 50°C for 5 minutes. After absorbance at 765 nm, the spectrophotometer was read. The steps mentioned for standard gallic acid solutions were also performed and a standard curve was obtained. The total amount of phenolic compounds was expressed using the equation of the line drawn for gallic acid, based on mg gallic acid per gram of dry onion powder [20].

Statistical analysis

The data are expressed as mean±SD. In this Study one-way analysis of variance (ANOVA) were applied to determine the statistical significance for non-growth zones between the subjects as classified by antibiotic (amoxicillin vs. ampicillin vs. Tetracyclin vs. Choloramphenicol) concentration (100 vs. 50 vs .25 vs. 12.5 vs. 6.25 vs. 3.125 vs. 1.56 vs. 0.78) Statistical analyses were performed using the statistical package SPSS for Windows, PC software, version 11.5.1 (SPSS Inc., Chicago, IL, USA). The statistical significance was defined as $p < 0.05$.

RESULTS

At 60°C, the total anthocyanin concentration was 43.13±3.5 mg / g and at 40°C it was 60.67±8.4 mg / g.

The average antibacterial effect of the colorant by diffusion disc method is shown in Table 1. The results of analysis of variance showed that there was no significant difference between the diameter of the non-growth zone in the five different concentrations of the extracted colorant against the *S. aureus* (P= 0.070). According to the results of this test, the highest non-growth zone diameter was observed in the concentration of 1000 mg/ml (0.83±0.14) and the lowest non-growth zone diameter at 200 mg / ml (0.4±0.17). Also, the results of this test did not show a significant difference between the diameter of non-growth zones in five different color concentrations against *E. coli* (P=0.164). The highest non-growth zones diameter was related to the concentration of 1000 mg / ml (0.9±0.22) and the lowest non-growth zone diameter was 200 mg / ml (0.5±0.20) (Figure 1). Generally, in both bacteria, increasing the color concentration of the bacteria increased the diameter of the inhibition zone.

Table 1. Average diameter of non-growth zone of extracted colorant against the bacteria studied in different concentrations (cm)

Bacteria	Concentrations (mg/ml)					
	200	400	600	800	1000	P-value
S. *aureus*	0.4±0.17	0.5±0.17	0.6±0.17	0.7±0.17	0.83±0.14	0.070
E.*coli*	0.5±0.20	0.6±0.20	0.7±0.20	0.8±0.20	0.9±0.22	0.164

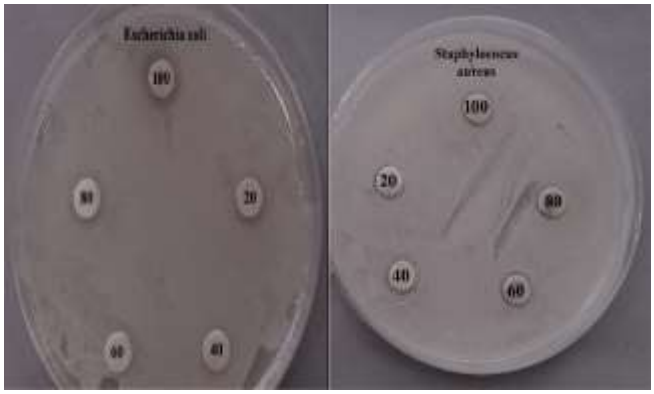

Figure 1. Disc diffusion method against the bacteria studied with different concentrations of extracted colorant

The average antibacterial effect of standard antibiotics against the bacteria studied is shown in Table 2. The results of analysis of variance showed that there was no significant difference between the diameter of the non-growth zones in the four antibiotics against the *S.aureus* bacteria or, in other words, the antibiotic type did not have a significant effect on the diameter of the inhibition zone (P= 0.134). The results showed that the diameter of the inhibition zone by antibiotics tetracycline and ampicillin was the highest (2.83±0.2) and by the antibiotic amoxicillin was the lowest (1.5±1.32) that This indicates that S aureus is more susceptible to ampicillin and tetracycline. Also, based on this test, there was a significant difference between the diameter of the non-growth zone in the four antibiotics studied against the *E. coli* bacteria, that is, the type of antibiotic had a significant effect on the diameter of the non-growth zones (P=0.005). The highest diameter of the non-growth zones was related antibiotic tetracycline (1.85±0.4) and the lowest non-growth zones diameter was related to ampicillin antibiotic (0.33±0.5). The antimicrobial effect of standard antibiotics was greater than the different concentrations of the color.

Table 2. Average of the results of the antibiotic test for the non-growth zone of different antibiotic disks (cm**)**

Bacteria	Antibiotic				
	Amoxicillin	**Ampicillin**	**Chloramphenicol**	**Tetracycline**	**P-value**
S. *aureus*	1.5±1.32	2.83±0.2	2.5±0	2.83±0.2	0.134
E. *coli*	0.36±0.3	0.33±0.5	0.68±0.1	1.85±0.4	0.005

The results of the MIC and MBC measurements of the colorant against the bacteria are shown in Table 3. Based on independent t-test, the mean of MIC and MBC of the studied bacteria were significantly different (P= 0.020, 0.038). The amount of MIC and MBC against *S. aureus* was 41.66±14.43 and 83.33±28.86 mg/ml, respectively. In *E. coli* bacteria, MIC was 100 ± 0 and MBC was 0 ± 0. This indicates that the *E. coli* bacterium has a lower sensitivity to the extracted colorant than *S. aureus* (Figure 2).

Table 3. Minimum inhibitory concentration (MIC) and Bactericidal (MBC) of extracted colorant (mg / ml)

Bacteria	MIC(mg/ml)	MBC(mg/ml)
S.aureus	41.66±14.43	83.33±28.86
E.coli	100±0	0±0
p-value	0.020	0.038

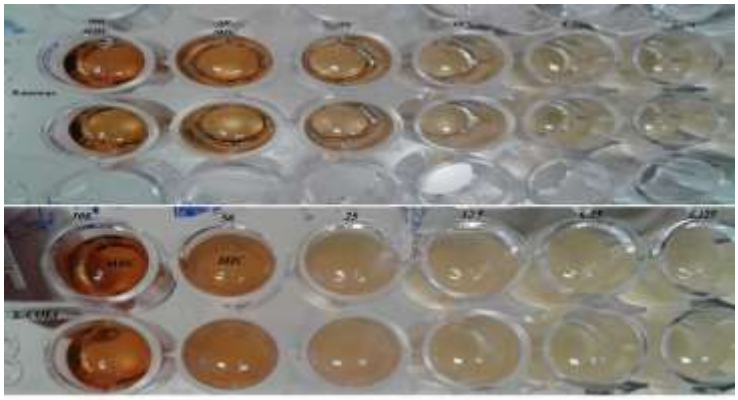

Figure 2. 96 home microplate (broth microdilution method)

The results of this study showed that the activity of DPPH radical scavenging in the extracted colorant increases with increasing concentrations. Inhibitory concentration of 50% (IC50) was obtained for extracted colorant, 14.718±0.20 mg/ml, and for gallic acid it was 2.512±0.048. The comparison of the mean results showed that there is a significant difference between the antioxidant properties of the extracted colorant with gallic acid (P<0.001), Indicating that the antioxidant power of the extracted colorant was lower than Stabilized antioxidant gallic acid.

The content of total phenol by the Folin Ciocalteu method was expressed as mg of gallic acid equivalents (GAE) per gram of powder on a dry weight (DW) basis, based on its comparison with standard Gallic acid solutions and according to the obtained equation (R2= 0.99882, y= 0.00348 x 0.02535) from the curve The Gallic acid standard (Gallic acid standard chart) was calculated (Figure 3). The total amount of phenol was obtained for the extracted color as an average of 114.33±2.36mg GAE/g dry onion skin.

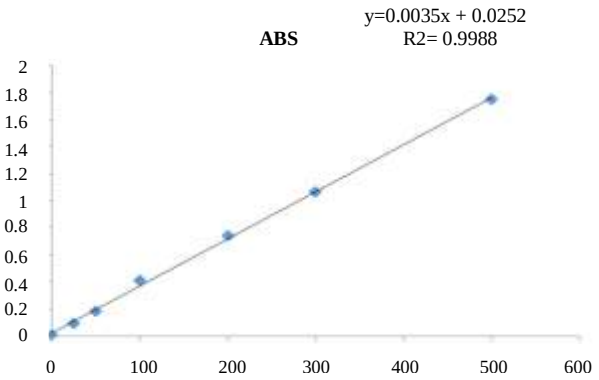

Figure 3. Standard Gallic Acid Absorption Scale Based on Folin Ciocaltue

DISCUSSION

In this study, color extraction was performed using two solvents water and glycerol and the total anthocyanin level was measured by differential pH method. Due to the higher total anthocyanin concentration at 40°C, all experiments were performed using the extracted colorant at this temperature. As the anthocyanin pigments are more sensitive to temperatures, when the temperature raises, the amount of anthocyanin decreases. In this study, the total anthocyanin level was decreased at a higher temperature.

In the disk diffusion test, the antimicrobial effect of the extracted colorant against the gram positive bacteria *S.aureus* and gram negative bacteria *E.coli* were evaluated and compared with standard antibiotics: amoxicillin, ampicillin, tetracycline and chloramphenicol. Based on observations of *S.aureus* bacteria, it was more susceptible to ampicillin and tetracycline and *E. coli* was more susceptible to tetracycline. Different color concentrations had a greater inhibitory effect on *S.aureus* than *E.coli* on the other hand; the colorant had a greater inhibitory effect on gram positive bacteria. The antimicrobial effect of standard antibiotics was greater than the different concentrations of the colorant. In the antibiogram test showed that the *E.coli* bacterium was less sensitive to the colorant than *S.aureus*. The amount of MIC and MBC against *S.aureus* was 41.66±14.43 and 83.33±28.86 mg / ml, respectively. In *E. coli* bacterium, the MIC value was 100 ± 0 and MBC was 0 ± 0. This indicates that the *E. coli* bacterium has a lower sensitivity to the extracted colorant than *S.aureus*. According to the findings of this test, the inhibitory effect of the color on the gram-positive bacterium was higher.

The activity of DPPH radical scavenging in the extracted colorat increased with increasing concentrations. Inhibitory concentration of 50% (IC50) was obtained for extracted colorant, 14.718±0.20 mg / ml, and for gallic acid it was 2.512±0.048. The comparison of the mean results showed that there is a significant difference between the antioxidant properties of the extracted colorant with gallic acid ($P<0.001$), Indicating that the antioxidant power of the extracted colorant was lower than Stabilized antioxidant gallic acid.

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to the obtained equation (R2=0.99882, y=0.00348 x 0.02535) from the curve The Gallic acid standard (Gallic acid standard chart) was calculated. The total amount of phenol was obtained for the extracted color as an average of 114.33±2.3 g/g of gallic acid per gram of onion powder.

[15] conducted a study on the effect of colorant on rheological properties of yogurt using a green extraction method for color extraction. In addition to different glycerol and water percentages, they used cyclodextrin as a catalyst and according to the reported results; the highest total anthocyanin concentration was 3.13 mg/g at 80°C with 60% glycerol and 13% cyclodextrin and 27% water. The total amount of anthocyanin in this study was lower than the

total anthocyanin level in our study. Which can be attributed to the less use of water solvent and higher temperature? They stated that according to the results of this study, this color could be used as a substitute for artificial colors in yogurt. [20] by extraction with different percentages of alcoholic solvent and Different time extracted color of red onion skin, and examined the antioxidant, anti-microbial and total phenol content. The highest rate of this parameter was reported with 80 and 60% ethanol at 60 and 120 minutes, respectively, at 470 and 309 mg/g respectively. The amount of anthocyanin was higher in our study due to the solvent used. They also measured the effect of the extracted color on different bacteria such as Escherichia coli and Staphylococcus by similar method, but did not observe any inhibitory effect. The reason for this can be related to the type of microorganisms being tested and the method of extraction, but in our study, this effect was evident and significant results were obtained.

Studied the effects of yellow, red, and white onion on broiler portions on antioxidant properties, flavonoids, and anthocyanin levels. The colorant was extracted with ethanol and water[21]. The amount of total anthocyanin in red, yellow and white onions was (29.99±1.19), (9.64±1.30) and (0.75±0.40), respectively. The amount of anthocyanin in the red onion was less than our study, due to the use of onions in meat and domestic layers. But in the three species of onion, the test of red onions was higher in the anthocyanin level. in red onions The amount of antioxidants in this study was higher than in our study in antioxidants.

CONCLUSIONS

Based on the type of red onion, the type of layer used, the solvent type, and the type of bacteria, the antioxidant and antimicrobial potency of the extracted colorant can be different. Regarding the antimicrobial and antioxidant potential of red onion skin in this study, as well as the high consumption of onions in various types of household foodstuffs, resulting in an increase amount of its waste and its economic cost, it can be used as a cheap source of color in various food and pharmaceutical industries.

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