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Research Article

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Use of *Beta vulgaris* as natural coloring agent for foods and cosmetics in Libya

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ABSTRACT

Color is the main feature of any food item as it enhances the appeal and acceptability of food. Currently, there is increasing awareness among people towards natural dyes and dye yielding plants due to severe health problems. Beet scientifically known as Beta vulgaris belongs to Chenopodiaceae family and locally known in Libyan markets as "Banjer". The present study aimed to study of beet from Libyan markets as coloring agent in food and cosmetic. Beet juice was prepared by hot extraction using methanol and then examined for its activity against bacteria by using the agar well diffusion method and also as antioxidant by using 2,2, Diphenyl-1-picryhydrazyl (DPPH$^{\bullet}$) assay. Beet juice also investigated phytochemically which indicated that beet is a rich source of flavonoids. Beet juice examined for the presence of aflatoxines (B1, B2, G1and G2), the beet juice was free from mentioned toxic substances. Therefore, the juice is safe. Finally Beet juice was evaluated as a food (Cakes) and cosmetic (Hair dye) coloring agents. The juice showed a weak antioxidant activity, good antimicrobial activity against gram positive bacteria and accepted to many volunteers in color and taste questionnaires when it's added to homemade cakes. The ability of juice to augment of Henna color; the best mixture with long lasting coloring was a 50:50 mixture.

Key words: Antimicrobial activity, Antioxidant activity, *Beta vulgaris*, *Chenopodiaceae*, Coloring agent.

INTRODUCTION

Coloring agents are one of the most important attributes of foods, have been determined as indicator for acceptability of foods and being considered as one of a quality indicators in foods industry [1]. In the past few years, consumer preference for natural colorants has greatly increased, this increase has been led to increase of availability and use, in addition to legislative action, which has continued the delisting of approved artificial dyes [2]. The secret behind consumer preference for naturally derived colorants is because they are not harmful and they have good quality comparing with synthetic colorants that tend to impart undesirable taste and are harmful to human beings [2]. Currently, in the European Union and United States a lot of colorants has authorized as food coloring agents. In both Europe and the US, most of the listed color agents are derived from natural sources [2].

Beta vulgaris (*B. vulgaris*), also known as red beet (Family: Chenopodiaceae), is a small sized plant, popularly known as 'Banjer', is an erect annual herb with tuberous root stocks. It is native of the coasts of Mediterranean, is extensively cultivated in Europe, America and many parts of Asia [3]. It has been used for centuries as a traditional natural coloring agent in dairy and meat products in many cuisines [4]. Medicinally, the roots and leaves of the *B. vulgaris* have been employed as a folk remedy to treat a wide variety of ailments including immune system stimulation, liver and kidney diseases. It is also useful to prevent of some kinds of cancers [5]. *B. vulgaris* seeds have a cooling and diaphoretic effects, while the roots have a good nutrient effect [5]. The present study was designed to evaluate The possibility of *B. vulgaris* use as coloring agent in Libyan food and in hair dyeing.

EXPERIMENTAL SECTION

Materials

The fresh roots of *B. vulgaris* and Henna powder were purchased from the local Tripoli markets, Libya. Strain of gram-positive bacteria *S. aureus* (ATCC 29213), gram-negative bacteria E. coli (ATCC.25922), and fungal strain *C. albicans* (ATCC 10231) were procured from the American type of cell culture collection. Nutrient broth, Mueller Hinton agar and Sabouraud Dextrose agar were procured from Liofilchem CO, Italy. Methanol, chloroform, DPPH (2,2, Diphenyl-1-picryhydrazyl) and standards including (Ascorbic Acid, Rutin, Ciprofloxacin, Amphotericin B and Aflatoxines B1, B2, G1, G2) were purchased from Sigma, UK.

Preparation of Extract

the roots were washed with fresh water to remove adhering dirt and foreign particles. air-dried, reduced to powder and kept in tightly closed dark glass container. One kg of powdered plant was extracted by hot extracted with 3 liters of 99% methanol in a Soxhlet apparatus for 72 h. The crude extracts were then dried using rotary evaporator at 40-50 °C and stored at -20 °C until further use [6].

Preliminary Phytochemical Screening

The phytochemical screening of the *B. vulgaris* juice was performed according to the standard procedures Mayer's and Dragendorff's tests for alkaloids and/or basic nitrogenous compound, Fehling's test for carbohydrates, Liebermann-Burchard's test for steroids, Frothy test for saponins, Shinoda's and sodium hydroxide tests for flavonoids and Fluorescence test for coumarins [7, 8].

Antioxidant Activity

Qualitative DPPH Antioxidant Assay

Qualitative antioxidant assay was performed by the standard TLC-DPPH method (16), Test samples were spotted on a TLC plate and air dried, then plates were sprayed with 0.2% methanolic DPPH solution using an atomizer. Ascorbic acid was used as the positive control [9].

Quantitative DPPH Antioxidant Assay

The free radical scavenging activity of *B. vulgaris* juice was evaluated by using DPPH˙ method [10], where the stock solution of *B. vulgaris* juice (10mg/ml) was prepared. 400 µl of 0.1µM of DPPH solution was added to 1ml cuvet. juice solution at different doses (10–1000µg) was added. A volume 600 µl of 99% ethanol was added and the mixture was shaken vigorously and allowed to stand in dark place at room temperature for 5 min. Then the absorbance was measured at 517 nm in UV-Visible-NIR spectrophotometer (Varian Cary 5000, USA). The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation:

Percent of DPPH inhibition= $[(A_A - A_B)/AB] \times 100$

Where A_A and A_B are the absorbance values of the test and the blank sample respectively, a percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value. Rutin was used as the positive control.

Antimicrobial Activity

The antimicrobial activity of *B. vulgaris* juice was examined using the agar well diffusion bioassay method [11] against different microorganisms. microorganisms were identified and obtained from the American type of cell culture collection (ATCC), including *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231). Mueller Hinton agar plates were seeded with 0.1 ml bacterial suspension (equivalent to 10 7 –10 8 CFU/ml) and Sabouraud dextrose agar plates seeded with fungal strain. The seeded plates were incubated for12 h at 37°C, allowed to set and on each plate wells were made by sterile cork borer. Each well was filled with 50µl (10 mg/ml) of the juice and the plates were then re-incubated for further 24 h at 37°C. The diameters of zones of inhibition were measured and Ciprofloxacin and Amphotericin B were used as reference compounds for antibacterial and antifungal activities. The results have been recorded in the form of inhibition zones (diameter, millimeter) with a transparent scale and activity values are shown in table 3. The experiment was carried out in triplicate and the mean value was taken.

Aflatoxins Detection

The detection of Aflatoxins was by using TLC plate method [12]. detection was done by visual of *B. vulgaris* juice spots under ultraviolet lamp at wavelength 366nm. The mobile phases used to develop of the plate are methanol - chloroform mixture at percent 10: 90. Aflatoxines B1, B2, G1 and G2 were used as positive standards.

Color and Taste Sensory Test

The *B. vulgaris* juice was added to homemade cakes to evaluate the acceptance of its color and its taste between Libyan volunteers as, Figure 1, 2. Questionnaire was carried out with 200 volunteers.

Figure 1: Questionnaire represents the opinion of voluntaries regarding to the use of *B. vulgaris* juice as a coloring material to homemade cake

Volunteer NO.	Taste			Color		
	Accepted	Rejected	Comment	Accepted	Rejected	Comment
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Hair Dye Augmentation Test

This test was done by mixing of *B. vulgaris* juice with Henna in different percent 20:80, 40:60 and 50:50, Figure 3.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

In our preliminary phytochemical tests of *B. vulgaris* juice revealed that, the main active constituents of *B. vulgaris* roots are carbohydrates, flavonoids, steroids, alkaloids and/or basic nitrogenous compound, while saponin and coumarins were absent.

Antioxidant Activity

a lot of phytochemical studies have been conducted Previously and shown the presence of flavonoids, phenolics and betalains in ***B. vulgaris*** and the presence of such active constituents have been confirmed by preliminary phytochemical and chromatographic screening studies, and These constituents have been reported to be a potent antioxidants [3].

Qualitative DPPH Antioxidant Assay

Result of assay showed that *B. vulgaris* juice possesses antioxidant activity, this indicated by a yellow spot on a reddish purple back ground of the TLC plate, this due to reduced of the DPHH (purple) to diphenylpicryl hydrazine (yellow).

Quantitative DPPH Antioxidant Assay

B. vulgaris juice showed a weak antioxidant activity, where IC_{50} of the methanolic extract was (836 µg/ml) in comparing with Rutin, Ascorbic acid as standard compounds (IC_{50} 57.2 and 15 µg/ml) , respectively.

Antimicrobial Activity

Phenolic compounds have high levels of antimicrobial activity, this activity related to their effects on cell membranes, such as altering function and in some cases altering structure, which lead increasing permeability, cell swelling and ending by cell death [13]. *B. vulgaris* juice showed a good antimicrobial activity against *S. aureus* which is one of the most common gram-positive bacterium causing food poisoning with zone of inhibition 17 mm,, while not having any effect on other organisms, Table 1.

Table 1: Antimicrobial activity of *B. vulgaris*

Name	Zone of inhibition (mm)		
	S .aurous	***E.Coli***	***C.albicans***
***B. vulgaris* juice**	17	-	-
Ciprofloxacin	30	32	-
Amphotericin B	-	-	23

The results summarized are the mean values of n=3, (-) = No activity

Aflatoxins Detection

TLC plate of *B. vulgaris* juice showed freedom from any aflatoxins products in extract.

Color and Taste Sensory Test

The *B. vulgaris* juice was added to homemade cakes to see its effect on color and taste. In the questionnaire, the taste was very Delicious to 43 of the volunteers, accepted to 129 and 28 of them didn't have comments. The cake color was accepted to 139 volunteers, While other volunteers are not accepted.

Figure 2: Homemade cakes with *B. valgaris* juice

Hair Dye Augmentation Test

The mixture Henna-*B. valgaris* juice at 50:50 was given a good dye for hair.

Figure 3: The result of mixing of *B. vulgaris* with Henna in different Percentages

Figure 2a: color of 100% Henna as hair dye

Figure 2b: color of 80%of Henna +20% of *B. vulgaris* juice as hair dye mixture

Figure 2c: color of 50% of Henna +50% of *B. vulgaris* juice as hair dye mixture

Figure 2d: color of 60% of Henna +40 % of *B. vulgaris* juice as hair dye mixture

CONCLUSION

Natural dyes are not only having dyeing property but also having the wide range of medicinal properties. Currently, there is increasing awareness among people towards natural dyes and dye yielding plants. Due to their non-toxic properties, less side effects, more medicinal values, natural dyes are used in day-to-day food products and in pharmaceutical industry. In this study *Beta vulgaris* juice was investigated to evaluate the antioxidant, antimicrobial activities and as source of food and cosmetic coloring agents. The juice showed a good antioxidant and anti microbial activity and when it's added to homemade cakes was accepted to many Libyan volunteers in color and taste questionnaires. The juice was mixed with Henna to evaluate its ability to augment Henna color, the best mixture was 50:50. To conclude, there is need for proper methods, documentation and characterization of dye yielding plants for further development of pharmaceutical industry to formulate the natural plant pigments into therapeutically beneficial pharmaceutical formulations/dosage forms for safe use [14].

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