Egypt. J. Food. Sci. **Vol. 49,** No.2, pp. 297-304 (2021)

Egyptian Journal of Food Science
http://ejfs.journals.ekb.eg/

Characterization of Dragon Fruit (*Hylocereus undatus*) Antioxidative Components to Explore Their Utility as a Natural Food Additive

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HYLOCEREUS belongs to the Cactaceae and has recently gained attention because of its nutraceutical value. The study emphasizesto explore the use of *Hylocereus* peel as a natural food additive because of its color and food preservative properties due to the presence of betacyanin, flavonoid, polyphenol content, and antimicrobial activity. The peel containsa high concentration of these antioxidative compounds.The extract of the fruit is rich in antioxidants and the color of the betalain pigments increases the aesthetic appeal of the extract thus allowing us to use the extract in the form of natural food color. It is not only safe to consume but it also helps in improving the quality of food. Besides being a powerful antioxidant, it has antimicrobial activity as well. The antimicrobial studies are also promising as the test performed indicates no increase in microbial count in presence of peelextract and for this, it's utility as food preservative was studied. Due to its desirable properties of antioxidant and antimicrobial activities, the fruit peel can be exploited for its commercialprospects as anatural food color and a food preservative.

Keywords: Antioxidants, Antimicrobial, Natural food preservative, Betacyanin, Flavonoids, Polyphenols

Introduction

Hylocereus undatus or dragon fruit is a trending fruit in India. It may be consumed raw as a fresh fruit or can be made into juices, wines, flavorings, etc. It is getting popularity among cultivators because of its attractive fruit color and mouth-watering pulp with edible black seeds embedded inside the pulp. It has agre at nutraceutical value, excellent export potential, and thus a highly remunerative fruit (Mitcham et al., 2013).

Food Preservative is a substance, a pigment, or a chemical that is added to food for preventingits decomposition from unwanted microbial growth or any chemical changes (Lück et al., 2002). Food preservative reduces the microbial count thus reducing the risk of microbial spoilage which subsequently reduces foodborne infectious diseases and preserve fresh attributes and nutritional quality. The increasing demand for ready to eat food has shifted the consumer's focus on increasing the shelf life of the food and these challenges are met by the use of artificial preservatives. However, the artificial preservatives may have negative concomitant effects (Theron et al., 2007).

Hylocereus Undatus peel is almost 30-35% of its dry weight and its extract contains pigments like betalains (mostly beta-cyanine) (Woo et al., 2011) and besides pigments, polyphenols (mainly flavonoid) (Kim et al., 2011). The attractive and appealing color is the added attribute and also safe to consume as they are natural and free from chemicals, thus reducing the risk of adverse effects caused by artificial food colors. Above all, these compounds are very good antioxidants

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Received: 22/2/2021; accepted: 30/8/2021
DOI: 10.21608/ejfs.2021.61698.1098

and are very beneficial in lowering the LDL (Low density lipids) composition of the body and reduce the risk for several major maladies like cancer, diabetes, etc (Tesoriere et al., 2004).

Betalains

Betalains are a class of indole-derived pigments ranging from yellow to shades of red colors. Betalains are further classified into 2 categories: beta-cyanins and beta-xanthin. The molecular mass of betalains is 550.5 g/mole and its molecular formula is $C_{24}H_{26}N_2O_{13}$ (Castellar et al., 2003). Betalains show a maximum absorption between 506-610 nm and betacyanin has a maximum wavelength at 538 nm which is used for its estimation (Gandía-Herrero et al., 2005).

Betacyanin is a natural anti-oxidant as it has a good tendency to lose electrons and get oxidized. Betalamic acid, the core structure of betacyanin reduces Fe^{+3} ions to Fe^{+2} ions as it easily donates electrons (Gandía-Herrero et al., 2012). The antioxidant activity of betacyanin varies according to its structure.

Flavonoids

Flavonoids are a kind of polyphenols or polyhydroxy phenols which are natural organic chemicals found in fruits and vegetables. These compounds have many health-promoting effects due to their rich anti-oxidant effect as they have high free radical scavenging properties (Cushnie et al., 2005). Flavonoids are ismetabolite of a plant that play a key role in a variety of biological activities in animals and plants and is not just a color pigment. They are detoxifying agents, anti-microbial defensive compounds, and are assigned to have positive effects on chemoprevention(Dixon et al., 2010). Flavonoids are the pigments that provide coloration from yellow to red/blue range. Chemically flavonoids are a 15-carbon skeleton, which consists of two phenyl rings (A and B) and a heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6 (McNaught et al., 2019).

Due to their reducing nature, they are oxidized by reactive free radical oxygen species to form a stable intermediate which makes them less reactive or inactive (Korkina et al., 1997). Some of the flavonoids are such reducing agents that they can directly scavenge superoxide whereas some can scavenge the highly reactive oxygen-scavenging due derived radical called peroxynitrite. Researchers have reported that a few flavonoids such as epicatechin and rutin show powerful radical scavenging due to its inhibitory activity on the enzyme Xanthine oxidase (XO). Some researchers proved that by scavenging radicals, flavonoids can inhibit LDL Oxidation in-vitro (Kerry et al., 1997)

Antimicrobial Activity of Hylocereus undatus

One of the major food-related concerns in our society is food nutrition, food hygiene, and spoilage. *Hylocereus undatus* or Pitahaya fruit contains 30-35% of the peel that is usually considered as waste and will be cast aside during processing. The uses of different parts of *H. undatus* inpromoting wound healing in diabetic rats have been reported, and it also shows high anti- microbial activity (Perez et al., 2005).

In the past decade, interest on the topic of antibacterial property of plant extracts has been growing (Lee et al., 2007). Scientists are switching back towards traditional folk medicines or natural products to uncover the scientific basis of remedial effects such as antibacterial agents (Haslam et al., 1996). Many food companies and industries are facing issues regarding the shelf life of the food with ready to eat packaged foods, drinks, and canned edibles. The major concern is with the high growing number of food borne outbreaks and illnesses associated with microorganisms. Many organisms also have become far more resistant to many antibacterial agents. 70% of the cases involved the strains that are resistant to at least one antibacterial agent (Cushnie et al., 2005). Natural compounds in fruits and vegetables such as polyphenols like flavonoids and tannins have shown very promising results in combating bacteria, fungus, and viral (Ahmad et al., 2001).

The present study aimed to evaluate the antibacterial properties of methanol and hexane extracts from *H. undatus* peel using disc diffusion and broth microdilution methods in determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Materials and Methods

Equipment

Cooling centrifuge (REMI CM-12 Plus), UV-Visible spectrophotometer (Shimadzu UV 1800), incubator (Remi), Micropipette (Eppendorf), Petri plates (Borosil), Autoclave (Scientech SE-101), conical flask, gel puncture.

Chemicals

80% Methanol, 10% $AlCl_3$, 5% $NaNO_2$, 1

M NaOH, Quercetin Standards, Gallic Acid Standards, Folin-Ciocalteau reagent, sodium carbonate, Methanol, n-hexane, Luria Bertani broth and agar, Muller Hinton agar.

Cultures

Bacillus cereus (Isolated from garden soil), *Staphylococcus aureus* (Isolated from skin swab),and *Escherichia coli* (Isolated from waste water).

Sample preparation

White Dragon Fruit (*H. Undatus*) was bought from the market. The fruit was washed with tap water to remove impurities such as dirt, grit, and dust. Then the fresh peel was separated. 50g of it was weighed and cut into small pieces to increase the surface area that will increase the extraction efficiency.

Color extraction

For the extraction 50 g of peel was taken, 200 mL of 80% methanol was added to the sample and 1:4 W/V extract was prepared. The extracts were prepared in triplicates (n=3). Then the extract was kept in a refrigerator for the maceration process for 24 hr. From the extract, the solid material was separated by the use of a funnel through What man No. 42 filter paper to obtain a colored solution. The solid residue was re-extracted twice to increase yielding efficiency. The filtrate was then centrifuged for 15 min at 8000 rpm using REMI CM-12 plus centrifuge model and the supernatant was collected. The obtained filtrate was used for the determination of total betacyanin content, total phenolic content, and total flavonoid content. The filtrate was then concentrated by evaporation and dried to obtain a powder.

Determination of Betacyanin concentration

Betacyanin was quantified by measuring its absorbance at 538nm with a UV-Vis spectrophotometer. The quantification of betacyanin was done using the formula described by Priatni et al (Priatni et al., 2015). The concentration of betacyanin (mg/100gof fresh weight) was estimated by the following formula: -

$$\text{Concentration of betacyanin (mg/100gm of fresh weight)} = \frac{A538 \times M.W \times V \times D.F \times 100}{\epsilon \times l \times w}$$

Where, A_{538}= absorbance at 538 nm (λ max), **l** (path length) =1.0cm,DF dilution factor, V volume of extract= 250 mL, w fresh weight of extracting material=50g. For betacyanin, ϵ (mean molar absorptivity or molar extinction co-efficient) =6.5 x 10^4 l/mol cm and MW(molecular weight) = 550 g/mol.

Five dilutions along with pure methanol extract of betacyanin were prepared and their absorbance was taken at 538nm using a UV-vis spectrophotometer and their concentration were estimated using the above formula. All tests were performed in triplicates (n=3)

Determination of total flavonoid content

The total flavonoid content of peel was determined with the use of aluminum chloride ($AlCl_3$) method using quercetin as a standard described by Shoib et al. (2015) and Stanojevi et al. (2009) The calibration curve was plotted (absorbance v/s concentration) by preparing 1 mL aliquots of 50, 100, 200, 400, 600, 800, 1000 µg/mL.4 mL of distilled water was added followed by 0.3 mL of 5% $NaNo_2$, 0.3 mL of 10% $AlCl_3$, 1M NaOH and 1.4 mL, 1.9 mL of distilled water to make up the volume to 3.4 mL. The absorbance was read at 510 nm using a UV-Visible spectrophotometer. The experiment was performed in triplicates (n=3).

Quantification of antimicrobial activity

The methanolic extract prepared above was tested as aninhibitin gagent against the inhibiting effect of chloramphenicol (concentration10µg/mL). Three bacterial species *Bacillus cereus, Staphylococcus aureus, and Escherichia coli* were selected as test organisms for the study of inhibiting effect through the disc- diffusion method. Four or five well-isolated colonies (depending on the size of the colonies) were picked from an overnight plate culture and inoculated into saline suspension at room temperature. The turbidity was then adjusted to 0.4 McFarland standards (10^9CFU/mL) and streaked onto Mueller-Hinton agar plates using a sterile nichrome wire loop. What man No. 1 filter paper discs of 6mm diameter were impregnated with 50 µL of the extracts.

Chloramphenicol disc was used as a positive control. The filtrate was used as a negative control. The plates were incubated at 37°C for 24 h. The screening of antibacterial activity was assessed based on the diameter of the clear zone surrounding the paper disc (including the disc diameter) in millimeter (mm). The tests were conducted in triplicates and repeated three times. The microdilution method was also used to find out the proliferation of the cells. In this method, the primary culture of every organism was taken

and 80µL of each culture was inoculated in 50 mL of LB broth. Along with the culture, 2 mL extract of different concentration diluted with distilled water were also inoculated and incubated for 24 hr. One standard tube of each culture was placed without inoculation of the extract and the absorbance of all cultures was measured at 600 nm and the percent proliferation of bacterial cells was calculated.

Results and Discussion

The concentration of both betalains and flavonoids was found significantly higher than all present natural food colors. Besides that, it's inhibition against micro-organisms has given us a scope to use it as a food preservative as well. Betacyanin stability was found to be only affected by light intensity and the temperature

TABLE 1. Concentration of betacyanin from various dilutions.

Dilutions	Concentration (mg/100 g)
PURE	2.172 ± 0.0199
1:1	2.144 ± 0.0399
1:2	2.073 ± 0.0598
1:3	2.313 ± 0.0798
1:4	2.256 ± 0.0998
1:5	2.115 ± 0.1196

does not have a major effect on its stability. It is also stable in a wide range of pH that is 3-7. The

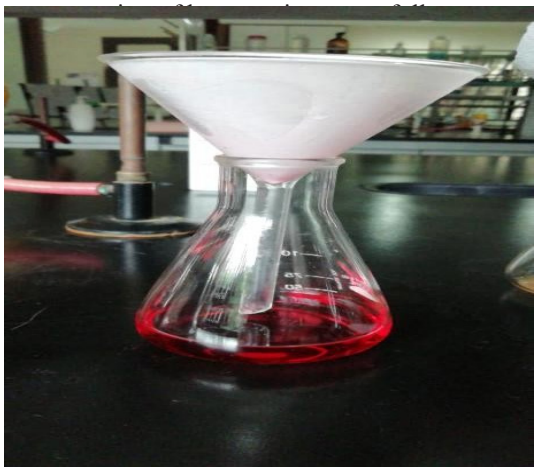

Fig. 1. Methanol extract of *H. undatus* peel containing beta-cyanin.

Total flavonoid content of peel was found significantly higher than some other fruits; hence

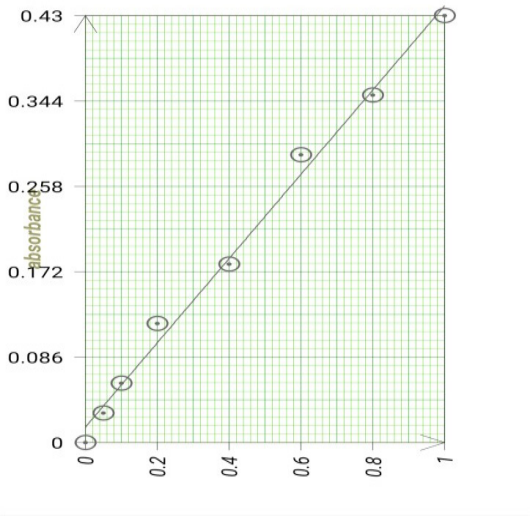

Fig. 2. Graph showing standard concentration of flavonoids.

f(x) = mx+c equation was:

f(x) = 0. 4244x + 0.01538

R^2 value = 0.9931

Linear line obtained when the regression line is plotted between concentration and absorbance. eobtainedregres sionequation for quercetinasthest and ardflavonoid solution was

y=0.4244x+0.01538.

The determination coefficient (R^2) obtained forst and ardflavonoid solution was 0.9931. Good correlation coefficient shows the correlation between concentration and absorbance. The higher the concentration of quercetin, the greater the absorbance was observed.

The concentration of flavonoid content of methanolic extract of dragon fruit from the above equation was 158.6 µg/mg quercetin equivalents.

The total betacyanin content was 2.2mg/100g of the peel.

Disc diffusion assay

The present study focuses on testing the sensitivity of 3 bacterial species viz., *Bacilluscereus, Staphylococcus aureus, and Escherichia coli* to the obtained extract of *H.undatus*. The diameters of inhibition zone of exhibited by each species are listed in the below table.

The zone of inhibition clearly indicates the effect of these pigments on the inhibition of bacterial species which gives us an edge over to use it as a food preservative.

Micro-dilution assay

TABLE 2. Calculation of mean diameter of the disk.

Bacteria Strains	Mean diameter of inhibition zone (in mm)	
	Extract (0.5µL/disc)	Chloramphenicol (10µg/disc)
Staphylococcus aureus	14.3±0.19	30±0.28
Escherichia coli	6.8±0.11	8.0±0.23
Bacillus cereus	1.2±0.05	29.0±0.34

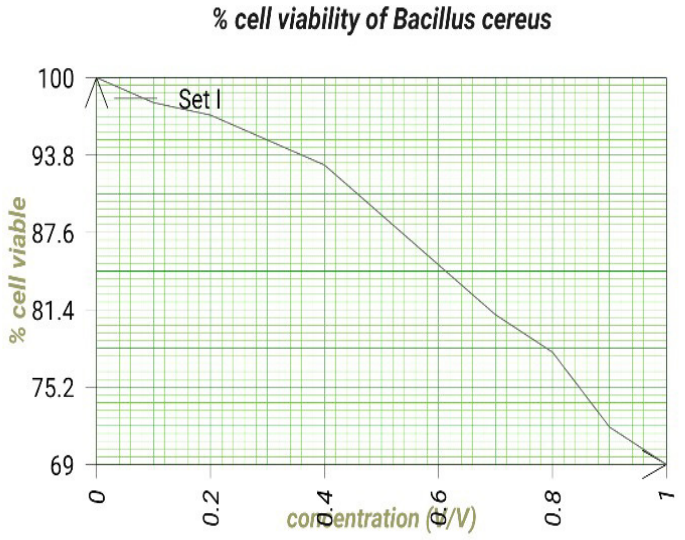

Fig. 3. Graph showing variation between concentration and cell viability of *Bacillus cereus.*

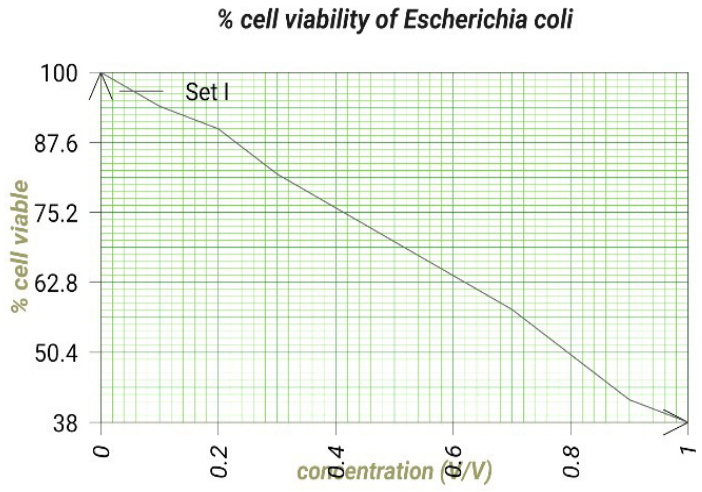

Fig. 4. Graph showing variation between concentration and cell viability of *Escherichia Coli.*

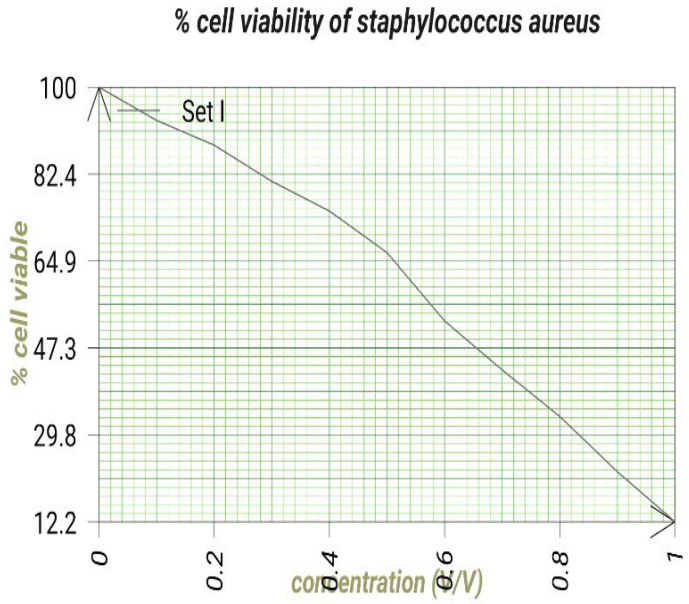

Fig. 5. Graph showing variation between concentration and cellviability of *Staphylococcus aureus.*

The below graph shows the % cell viability for every species which is found out by using absorbance of the media.

It is clear from the graphs that the extract has shown maximum inhibitory activity in the case of *S. aureus* and least in the case of *Bacillus cereus*. These results are significant to show the inhibitor activity of the extract against micro-organisms. Thus, it may be used as a preservative.

A Higher amount of betacyanins, flavonoids and phenolic acids was reported in flesh as well as peels of H. undatus. These data demonstrated that a more precise quantification of extract phytochemical constituents could be possible by using a sub-fractionation procedure. Betacyanins exhibited the highest antioxidant activities, and they were almost 10 times more abundant in peels than in flesh. Thus, it was highlighted that there are differences in composition of both edible and non- edible portions. Finally, polyphenolic fractions of flesh and peels were able to inhibit the food- borne pathogens tested. Our results indicated peel extracts as a good source of bioactive phytochemicals. The peels, an inedible waste product of fruit represents up to one third of the fruit weight, may be subjected to simple and economical purification methods to provide extracts and sub-fractions for the formulation of health-care products and food applications indicated for food coloring and food preservation, thus, contributing to a new natural food additive (Tenore et al., 2012).

Conclusion

Food colors and preservatives till now have just been used to improve the appearance of food, however they do not have any nutritional or health benefit effects. Moreover, some researchers believe that artificial food colors and preservatives may be harmful to human health. The present study effectively deals with the production of a food color from dragon fruit which may also act as a preservative and is completely natural. The color comprises various pigments like betalains and phenolics like flavonoids which provide an appealing appearance to the food and also these pigments are a major source of anti-oxidants which may act as a potent killer of reactive oxygen species thus reducing the risk of cancer and several cardiovascular diseases (Castellar et al., 2003; Gandía-Herrero et al., 2005). Till now it was unimaginable to produce a food color or preservative having any health benefit. The only focus of the research was on to finding the least toxic material as food additives. The pigment composition of dragon fruit not only allows us to produce a least toxic additive but also it has a definite health benefit. This food additive can be used as a food color as well as its important application as a food preservative can be explored.

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