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Analysis and Phytochemical Screening of the Polyphenolic Antioxidant Activity of the White Dragon Fruit (*Hylocereus undatus*)

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ABSTRACT

Background and Objective: Dragon fruit is a well-known and much-used herbal remedy, particularly in Asia, with various intriguing bioactive ingredients and health-promoting qualities. The purpose of this study was to examine the different extracts of white dragon fruit for bioactive components and assess its total phenolic content and antioxidant potential. **Materials and Methods:** Using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging activity assay, the antioxidant activity was ascertained. With the Folin-Ciocalteu technique, the total phenolic content was ascertained. Triterpenoid, alkaloid, flavonoid and saponin were detected by phytochemical screening of the white dragon fruit. **Results:** The study found the highest total polyphenols recorded in methanolic extracts are higher (246.2±3.32 mg/L) as compared to aqueous extract (197.09±2.41 mg/L), whereas in the methanolic extract, the flavonoid content was also higher (196.7±2.01 mg/L) compared to aqueous extract (168.32±2.56 mg/L). Antioxidant activity measured in terms of DPPH free radical scavenging assay showed an IC_{50} of 210 µg/mL, the extract demonstrated robust antioxidant activity in methanolic extract. **Conclusion:** The study findings provide new insight into the stable intermediates and phytochemicals related to dragon fruit that are present in different solvent extracts of the fruit. Thus, there is the potential to use dragon fruit, which contains numerous phytochemicals, as a natural active pharmaceutical ingredient.

KEYWORDS

Dragon fruit, flavonoids, tannins, alkaloids, total phenolic, antioxidant activity

INTRODUCTION

Fruits and vegetables are a rich source of flavonoids, which are polyphenolic compounds. They can be divided into flavones, isoflavones, flavonones, flavonols, anthocyanidins and chalcones based on their chemical structure. Flavonoids have the significant property of being antioxidants[1]. Reactive oxygen species, including singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite, can cause damage to cells. Antioxidants may shield cells from these harmful effects. Oxidative stress, which results in cellular damage, is caused by an imbalance between reactive oxygen species and antioxidants. Cells produce ROS as a controlled physiological process, but increasing ROS becomes pathological and leads to oxidative stress and disease[2]. Fruits are a vital portion of an adequate diet and serve as food supplements and appetizers[3]. Antioxidants are substances that have the ability to stop if not completely

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reverse, the damage that normal physiological oxidation causes to human tissue[4]. Numerous biological actions of flavonoids, including anti-inflammatory, antibacterial, antiviral, antiallergic and cytotoxic antitumor effects, have been documented[5]. Antioxidants like flavonoids may aid in preventing certain illnesses. The majority of the antioxidants utilized for this are currently produced synthetically.

A wonderful new edible fruit plant brought from Thailand is the white dragon fruit, which has a golden exterior and a white interior. The terrestrial, vining dragon fruit has meaty stems. The intricate flowers only open at night. Dragon fruit is high in vitamins, contains fiber that aids in digestion, guards against diabetes and colon cancer, neutralizes toxins like heavy metals and lowers blood pressure and cholesterol. Lycopene, a naturally occurring antioxidant that has been shown to prevent heart disease, fight cancer and decrease blood pressure, is present in the red-fleshed variety. White dragon fruit is a kind of cactus plant for which there is currently insufficient reference data regarding phytochemistry and pharmacology to allow for the best possible usage as an alternative medicine.

The usage of these plants in traditional medicine is supported by empirical data, hence it is necessary to establish a scientific foundation using contemporary biology and chemistry research techniques regarding the uses and varieties of bioactive chemicals found in dragon fruit. The current study aims to investigate the polyphenolic antioxidant activity of the different extracts of white dragon fruit as well as phytochemical screening. The current study's objectives were to assess the antioxidant and total phenolic content as well as the role that white dragon fruit can play in public health campaigns encouraging the fruit's daily consumption through phytochemical screening.

MATERIALS AND METHODS

The dragon fruits were collected from a local farmer of Vita-Mayanai Village from Sangli District of Maharashtra State during October and November, 2023. The analysis of the fruit was carried out at Department of Botany Laboratory, Sadguru Gadge Maharaj College, Karad (Maharashtra).

Acetone, butanol, chloroform, ethyl acetate, ethanol, methanolic acid, n-hexane, hydrochloric acid, sulfuric acid, phenolic acid, anhydrous acetic acid, silica gel GF254 plates and magnesium were among the chemicals and solvents that were purchased from E-Merck. Gallic acid, Folin-Ciocalteu reagent and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) were acquired from Sigma. Analytical grade reagents were obtained from local sources, including sodium carbonate, acetic acid, ascorbic acid, Dragendorff reagent and Lieberman-Burchard reagent.

Extraction and isolation of compounds: The white dragon fruit pulp was washed, dried, roughly chopped and then macerated in ethanol for 24 hrs at room temperature. Whatman No. 42 (125 mm) filter paper was used to filter the extracts. Using a rotary evaporator (BUCHI Rotavapor R200) and a water bath heated to 50°C, the extract was evaporated and concentrated under reduced pressure. The subsequent investigation also made use of the crude extracts.

Phytochemical evaluation: Standard analytical protocols were used to analyze the crude extract for the presence of terpenoids, flavonoids, alkaloids, tannins and steroids[6].

Qualitative analysis of phytochemicals[7-12]: To identify the presence of various phytochemicals such as alkaloids, flavonoids, tannins, steroids, glycosides, phenols, terpenoids, saponins, coumarins, anthraquinones and quinines, the preliminary phytochemical screening of the dragon fruit extract was conducted using recommended laboratory procedures.

Phytochemical screening for flavonoids (alkaline reagent test): A few drops of 20% NaOH were combined with each 2 mL of the filtered sample. It was seen that a bright yellow tint was forming. The yellow color vanished after the addition of a few drops of hydrochloric acid that had been diluted by 70%. The appearance of flavonoids is indicated by the creation and disappearance of the yellow color.

Phytochemical screening for phenols (ferric chloride test): Each 2 mL of filtered sample was mixed with 2 mL of 5% aqueous $FeCl_3$. The formation of the blue color points out the occurrence of phenols.

Phytochemical screening for tannins (ferric chloride test): In 2 mL of the fruit sample, 10% of alcoholic $FeCl_3$ was added. The formation of black/brownish blue confirms the presence of tannins.

Phytochemical screening for alkaloids (Dragendroff's test): While 2 mL filtered sample was taken, added diluted hydrochloric acid and filtered. Into the filtrate Dragendroff's reagent was added and the formation of a red precipitate indicates the presence of alkaloids.

Phytochemical screening for terpenoids (chloroform test): In a test tube, 2 mL of filtered sample, 0.5 mL chloroform and 0.5 mL of acetic anhydride were taken and a few drops of concentrated sulfuric acid were added. The formation of reddish-brown precipitate indicates the presence of terpenoids.

Phytochemical screening for anthraquinones: For potassium hydroxide solution, each was added to 2 mL of filtered sample. The blood-red colour shows the presence of anthraquinones.

Phytochemical screening for saponin (foam test/frothing test): While 2 mL of filtered sample was added with 4 mL of distilled water, mixed well and shaken vigorously. The formation of foam designates the presence of saponins.

Phytochemical screening for quinones: About 1 mL of sodium hydroxide was added to 1 mL of fruit sample. The formation of blue, green or red colors shows the presence of quinones.

Phytochemical screening for coumarins: In 1 mL of fruit sample 3-4 drops of 1% KOH in absolute ethanol was added. The formation of yellow color directs the occurrence of coumarins.

Phytochemical screening for glycosides (Keller-Kiliani test): In a filtered sample (2 mL) 0.5 mL glacial acetic, three drops of 1% aqueous $FeCl_3$ solution and 0.5 mL concentrated H_2SO_4 were added. A brown ring formed between the layers, which showed the entity of cardiac steroidal glycosides.

Phytochemical screening for steroids: A 2 mL fruit sample and a 2 mL chloroform mixture were mixed. Next, it was mixed with 2 mL of concentrated H_2SO_4. The chloroform layer will turn red and the acid layer will glow a greenish yellow colour if steroids are present.

Determination of antioxidant activity: The DPPH radical-scavenging assay was used to measure antioxidant activity. The method described, with minor adjustments, was used to test the dragon fruit extract's capacity to scavenge free radicals (DPPH)[13]. A portion of 0.5 mL of dragon fruit extract methanol at varying concentrations (10, 30, 50 and 70 ppm) was combined with 500 µL of 1 mM DPPH that had been dissolved in ethanol till 5 mL. After giving the mixture a good shake, it was allowed to stand at room temperature in a dark room for half an hour.

The absorbance was measured with a UV-vis spectrophotometer at 515 nm. The ascorbic acid was used as standard:

$$I(\%) = \frac{A(\text{blank}) - A(\text{sample})}{A(\text{blank})} \times 100$$

The percentage (I%) of inhibition of DPPH radical scavenging activity was calculated, where sample represents the absorbance of the sample and blank represents the absorbance of the blank solution (which contains all of the reagents except for the test sample). The graph of scavenging activity against the concentrations of the dragon fruit samples was used to calculate the IC_{50} value.

Determination of total phenolic content: The Folin-Ciocalteu method, as reported by Lamien-Meda *et al.*[14], was utilized to ascertain the total phenolic content (TPC). Folin-Ciocalteu phenolic reagents (0.5 mL) and deionized water (7.5 mL) were combined with 0.5 mL of the extract. Five minutes later, 1.5 mL of 20% sodium carbonate was added to the mixture. Using a spectrophotometer, the absorbance was measured at 760 nm against a water blank after being maintained at 40°C for 20 min. Using gallic acid (0, 40, 80, 120, 160 and 200 mg/L), a typical calibration curve was drawn.

The gallic acid calibration curve was used to determine the TPC amounts for the sample. The findings were presented as gallic acid equivalents (GAE) in gram per gram of dry plant materials. Every measurement was carried out three times.

RESULTS AND DISCUSSION

Extraction and phytochemical evaluation: One kilogram of dragon fruit was extracted and dried in two liters of methanol for 24 hrs, producing one liter of filtrate and, after evaporating, 320 g of crude extract. Four chromatogram spots with Rf 0.48, 0.62, 0.72 and 0.73 were found using TLC scanning analysis with mobile phase butanol: acetic acid: water (4:1:5). The spectrophotometer investigation results indicated that the first spot tested positive for flavonoids. The phytochemical screening validated this analysis as well. Triterpenoid, flavonoid, alkaloid and saponin were detected during screening; sterol and tannins were not detected.

Qualitative analysis of phytochemicals: Plants create secondary metabolites when the environment is unfavorable to them. Alkaloids, flavonoids, tannins, saponins, phenols, steroids, quinones and other secondary metabolites are formed in response to numerous harmful environmental circumstances[12-14]. The current work examined dragon fruit extracts for their phytochemical components in five different solvents. Ten phytochemicals produced strong favorable results in the current studies of phytochemical screening of dragon fruit extract. The phytochemical screening of the various extracts obtained from the fruits of dragon fruit (*Hylocereus* spp.) revealed the presence of alkaloids, phenols, saponins, steroids, tannins and terpenoids, while glycosides and anthocyanin were found to be absent (Table 1).

The main goal was to do a thorough phytochemical screening of several extracts made from the dragon fruit. Identifying and characterizing the bioactive compounds found in these extracts was the aim. Alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids were all found using a variety of specific tests. Notably, alkaloids, phenols, saponins, steroids, tannins and terpenoids were found in dragon fruit. These results highlight the pharmacological characteristics of dragon fruits exhibit naturally, indicating their potential as important sources of therapeutic compounds. It's vital to remember that these fruits' secondary metabolites are known to provide a range of medicinal uses that can improve human health.

Table 1: Phytochemical test for white dragon fruits in different solvent extracts

Phytochemical test	Ethanol	Methanol	Chloroform	Petroleum ether	Water
Alkaloids	-	++	+	+	-
Flavonoids	+	+	+	-	-
Carbohydrates	-	+	++	+	++
Glycosides	-	-	-	-	-
Phenols	++	+	+	-	-
Anthocyanins	-	-	-	-	-
Saponins	+	-	-	+	-
Steroids	+	+	+	+	+
Tannins	+	++	++	-	-
Terpenoides	++	-	-	-	-

+: Presence of bioactive compound and -: Absence of bioactive compound

Table 2: Quantitative analysis of phytochemicals (mg/L) of dragon fruit

	White dragon fruit	
Phytochemicals	Water extract	Methanol extract
Total polyphenols	197.09±2.41	246.2±3.32
Total flavonoids	168.32±2.56	196.7±2.01

Determination of antioxidant activity: The measurement of antioxidant activity in dragon fruit is crucial because the fruit's quality and level of antioxidant activity can be utilized as a benchmark when it comes to using it as an herbal remedy for health. Since the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical is used to test antioxidant capacity, the rapid, easy and affordable DPPH approach was used. The ability of substances to function as hydrogen donors or free radical scavengers, as well as the antioxidant activity of food, are frequently tested using DPPH. Recently, it has also been applied to the quantification of antioxidants in intricate biological systems.

The DPPH method is used for both liquid and solid samples and it does not target any single antioxidant component; rather, it evaluates the sample's total antioxidant capacity. The red dragon fruit peel delivered on its promise to stop melanoma cells from growing. By employing the DPPH method to assess the antioxidant activity of red dragon fruit (Hylocereus polyrhizus), Rebecca *et al.*[15] demonstrated that the fruit's effective concentration (EC50) was 295 mM vitamin C equivalents/g of dry extract.

The IC_{50} value in this investigation was 203 µg/mL for the methanolic extract of white dragon fruit (*Hylocereus undatus*). This indicates that a concentration of 203 µg/mL of the white dragon fruit methanolic extract can inhibit free radical DPPH by 50%. These findings suggest that the white dragon fruit methanolic extract has the ability to inhibit the free radical DPPH, as it was able to block 50% of the radicals at doses below 210 µg/mL.

Due to the hydroxy group on the molecule where flavonoids have free hydroxy groups, flavonoids have the capacity to stop free radicals. The IC_{50} value of ascorbic acid, which is 40 g/mL, is significantly smaller than that of the methanolic extract of white dragon fruit.

Determination of total phenolic content: The activity of the extracts is probably caused by polyphenolic chemicals, which are known to have antioxidant activity. It's assumed that their redox qualities, which are crucial for absorbing and neutralizing free radicals, quenching singlet and triplet oxygen and breaking down peroxides, are primarily responsible for this action[16]. Since gallic acid is a standard for determining total polyphenolic content, the Folin-Ciocalteu method was employed, as it is a well-recommended analytical technique. In the present study, total polyphenols in methanol extract were 246.2±3.32 mg/L whereas in water extract it was 197.09±2.41 mg/L. The total flavonoids were also found higher in methanolic extract as compared to water extract (Table 2).

There is a notable total polyphenolic content in a few common fruits. There are various fruits that have high levels of antioxidants: Tomatoes have 350 µg/g, cherries have 670 µg/g, blueberries have 3180 µg/g, *Carica papaya* has 260 µg/g and *Musa* sp. has 110 µg/g. With a total phenolic content of 246 mg/L, 1 kg of dried white dragon fruit extract[15].

CONCLUSION AND RECOMMENDATION

The results of this study showed that white dragon fruit has a high flavonoid content and good antioxidant activity when tested using DPPH techniques. The amount of flavonoids in flavonoid compounds is positively connected with antioxidant activity. In summary, white dragon fruit, when eaten as a vegetable, can be utilized as a convenient way to receive organic antioxidants, which will have positive effects on health. The current study shows that the fruits of the dragon fruit are rich in bioactive chemicals that may be utilized as sources for therapeutic effects and medical applications. In light of these results, it is advised that future studies use cutting-edge chromatographic methods like High-Performance Liquid Chromatography (HPLC) to more thoroughly examine the components of the entire plant. This would advance our knowledge of the bioactive components of dragon fruit and how they affect human health on a broader scale. Moreover, bioactive substances with antioxidant qualities were found in dragon fruit (*Hylocereus* spp.) after preliminary phytochemical screening.

SIGNIFICANCE STATEMENT

Dragon fruits are a rich source of immune stimulating substances. The present study aimed to evaluate the concentrations of active phytochemicals in various solvent extracts of dragon fruit plants. The study found the highest total polyphenols recorded in methanolic extracts are higher as compared to aqueous extract, whereas in the methanolic extract, the flavonoid content was also higher as compared to aqueous extract. Antioxidant activity measured in terms of DPPH free radical scavenging assay showed an IC_{50} of 210 µg/mL. Understanding the health benefits nutritional significance, commercial value and potential uses of dragon fruit an easily available and affordable fruit for alternative medicine and prevention-requires an understanding of its phytochemical and antioxidant composition.

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