ISSN PRINT 2319 1775
Online 2320-7876

PHYTOCHEMICAL INVESTIGATION OF METHANOLIC EXTRACT *HYLOCEREUS UNDATUS* PEEL

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Submitted: 29-April-2022 Accepted: 10-July-2022 Published: 22-July-2022

ABSTRACT:

Hylocereus species or better known as dragon fruit or pitaya from the Cactaceae family had become interest subject to many researchers mainly due to its unique taste, shape and the flesh colour. The functionality of *H. polyrhizus* seed are including antioxidant property and the uses of different part of *H. undatus* in promoting wound healing in diabetic rats also have been reported. Therefore the present study was aimed to investigate the active phytochemical constituents of ethanol, chloroform and hexane extracts from *H. undatus* peel. The findings obtained in the study could support the potential application of pitaya peels as a natural source of various active chemical constituents with relation to its potential health benefit in providing perspectives of research and application.

Keywords: Hylocereus Undatus Peel, Phytochemical, antioxidants, Total phenolic content

INTRODUCTION:

Dragon fruit (*Hylocereus polyrhizus)* or red pitaya is one of the tropical fruits that belongs to the cactus family, Cactaceae 1. Dragon fruits are edible fruits with sweet taste and rich in bioactive compounds, helps in boosting the human immunity system, and improves physical and mental abilities of individuals 2. Dragon fruit species have been classified based on the color of flesh and the peel, *Hylocereus undatus* variety has white flesh with pink skin known as white dragon fruit, flesh of *Hylocereus polyrhizus* was red in color with pink skin know n as red pitaya and *Hylocereus megalanthus* with white pulp and yellow skin known as yellow pitaya 3,4.Dragon fruit peel (DFP), which accounts for more than 20% by weight of the whole fresh fruit, is usually discarded as waste during processing. Recent studies have indicated dragon fruit peel as a potential natural colourant, having good antioxidant activities 5. An antioxidant is a phytochemical compound commonly referred as a bioactive compound. There are many types of chemical structures and functions of phytochemicals in fruits and vegetables, and one of them is the phenolic compounds. The phenolic compounds play an important role in contributing to the overall antioxidant activity. These phenolic compounds have the potency to fight against reactive oxygen species (ROS) or better known as free radical species, by inhibiting the initiation of free radicals, breaking their chain reactions and suppressing the formation of free radicals

such as superoxide ion, hydroxyl radical, singlet oxygen and hydrogen peroxide. These reactive free radical species can damage the body of cells if they are present in high quantities 6.

Extensive researches have been conducted on the properties of red pitaya pigment and the antioxidant properties of flesh and peel of red pitaya. White dragon fruit is a type of cactus plants that still do not have complete reference information, both in terms of phytochemical and pharmacology in order to be optimally used as a form of alternative medicine. Utilization of these plants as traditional medicine is based on empirical evidence so there is a need to find a scientific basis about utilities and types of bioactive compounds in dragon fruit with the use of research approaches to biochemistry and modern biology. The objectives of the present study are phytochemical screening of methanolic extract of *Hylocereus undatus* (dragon fruit). The present study aimed to promote the contribution of dragon fruit in public health campaigns to encourage the daily consumption of white dragon fruit, through phytochemical screening and evaluation of functional properties.

Collection of Sample:

The fresh fruits of *Hylocereus undatus* were procured from Chikhali market, Dist Buldana, Maharashtra. The media, reagent, chemicals and solvents used in this study were obtained from Kamla Agencies Akola, Maharashtra, India.

Sample Preparation:

The dragon fruit peels were then dried using a cabinet drier set at 55°C for 11 hours or until the desired water activity was achieved. Next, the peels were ground using a blender, and then the powdered sample was weighed and placed in separate sealed containers and stored in the refrigerator until further use.

Extraction:

The powdered peels were soaked in 95% Methanol for four days to solubilize the biologically active compounds present. The soaked samples were sonicated for 1 hour, then filtered. The residue was rinsed with fresh ethanol 6-7 times until the color of the liquid became clear. The combined filtrates were concentrated in vacuo using a rotary evaporator at 56°C water bath, 120 rpm, and 9-11.5°C condenser until the extract became 20-30 mL. The concentrated extract was placed in an Erlenmeyer flask, sealed, and kept refrigerated to prevent decomposition of the components that are sensitive to heat 7.

Preliminary Phytochemical Screening:

Pre-liminary phytochemical analysis was carried out on the macerated ethanolic extracts using the standard procedure to identify the constituents present by characteristics color changes 8.

Thin layer chromatography:

ISSN PRINT 2319 1775
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Thin layer chromatographic (TLC) analysis for dragon fruit extract was carried out to detect the secondary metabolites present. The five solvent systems were the following: SS1- toluene-chloroform (9:11); SS2 - toluene-acetone-chloroform (40:25:35); SS3 - n-butanol-acetic acid- water (4:1:5); SS4 - chloroform-acetic acid-water (50:45:5); and SS5 - chloroform-methanol (5:1) extract was spotted on the marked and labeled TLC plates (6 cm x 3 cm) and was developed in the different solvent systems in the developing chamber. The spots for a specific metabolite were visualized on the TLC plates and were exposed under UV light to check the separation of the different compounds. Then, different spray reagents were used to visualize the spots, and the distinct spots were traced with a pencil, and the Rf values were computed 9,10.

Determining total phenolic content (TPC)|:

In a 25 mL conical flask, 0.25 mL of extracted sample with 1.3 mL of 10-fold Folin-Ciocalteu reagent and 3.75 Ml of 7.5% sodium carbonate solutions were mixed. The mixture was then dilutedto the required volume with deionized water and inverted 20 times. Then, it was kept at room temperature for 30 minutes before being measured by a spectrophotometer 11.

Determining antioxidant activity:

The capability of DPPH free radical scavenging activity towards *Hylocereus undatus* foliage and peels extract was determined according to the method described with slight modifications 12 .In preparation of control sample (Acontrol), a 0.28 mL of DPPH solution (0.1 mM, in 95% ethanol)was added into a 10 mL of conical flask and it was then diluted to the required volume with ethanol.In preparing the test sample (Asample), 0.28 mL of DPPH solution and 0.28 mL of the sample were added into a 10 mL of conical flask and the mixture was then diluted to the required volume with ethanol. The mixture was then inverted several times and incubated in the dark room for 30 minutes at room temperature. The absorbance was measured against the control sample by using a spectrophotometer at 517 nm 13. The radical scavenging activity was measured as a decrease inthe absorbance of DPPH and was calculated by using the 14.

Following equation:

Scavenging effect (%) ¼ [1- (A sample e Acontrol)] _ 100%

Results:

Preliminary Phytochemical Screening:

Preliminary phytochemical analysis of macerated Methanolic extracts of *Hylocereus undatus* revealed the presence of some secondary metabolites in Table 1.

Sr.No	Phytochemical constituents	Methanol
1	Carbohydrate	+
2	Saponins	-

3	Tannins	+
4	Flavonoids	+
5	Steroid and triterpenoids	+
6	Phenols	+
7	Alkaloids	+
8	Glycoside	+

Table 1: Preliminary Phytochemical Analysis of *Hylocereus Undatus* Peel ExtractThin layer chromatography:

Thin-layer chromatography (TLC) confirmed the presence of phytochemical constituents observedin the preliminary test.

Component	**Spray Reagent**	**Observation**	**Results**
Flavonoids, Steroids	Antimony (III) chloride	Fluorescing spots in long-wave UV light	+ +
Phenols, Tannin Flavonoids	Potassium-ferricyanide-ferric chloride	Blue spots	+ +
Alkaloids	Dragendorff's Reagent	Orange spots	+ +
Cardenolides	3,5 Dinitrobenzoic acid Kedde Reagent	Red-blue violet-colored zones	- -
Coumarins, Anthraquinones, Anthrones, Phenols	Methanolic potassium hydroxide (Bornträgerreagent)	Anthraquinones give orange coloration; Anthrones give yellow and Coumarins react to form blue (UV 365nm) colored zones	+ +
Anthraquinones	Magnesium acetate inmethanol	Orange-colored zones	+ +
Higher alcohols Phenols, Steroids, Essential oils	Vanillin-sulfuric Acid	Colorful zones	+ +

Table 2. List of plant constituents, their visualizing agents, the indication of a positive test, and the TLC confirmatory test results.:

The TLC results showing the best solvent system and the number of spots and Rf

ISSN PRINT 2319 1775
Online 2320-7876

values are presented in Table 3. Only the solvent system which gave the best separation of the spots. The spray reagents used to visualize the spots and observable colors are listed in Table 2. The results revealed samples was tested positive for flavonoids and steroids, consistent with the preliminary phytochemical analysis. Separation of components observed in the solvent system n-butanol-aceticacid-water (4:1:5).

Phytochemical	Solvent System	No. of Spots	Rf Value
Falvonoids and steroids	n-Butanol-Acetic Acid-Water	1	0.72
Phenols, Tannins and Flavonoids	Chloroform-Acetic Acid-Water	1	0.93
Alkaloids	Chloroform-Methanol	1	0.90
Coumarins, Anthraquinones, Anthrones and Phenols	n-Butanol-Acetic Acid-Water	1	0.60
Anthraquinones	Chloroform-Methanol	1	0.96
Higher Alcohols, Phenols, Steroids and Essential Oils	n-Butanol-Acetic Acid-Water	1	0.98

Table3. Rf values of the different spots developed in the solvent systems which gave the highest number of spots visualized using the appropriate spray reagents.:

The test for phenols, tannins, and flavonoids resulted in only one spot for the sample using the different solvent systems, but the highest Rf value was observed in chloroform-acetic acid-water (50:45:5). Likewise, the alkaloids and anthraquinones test resulted in only one spot in sample using chloroform-methanol (5:1). The test for coumarins, anthraquinones, anthrones, and phenolsshowed the separation of one spot in sample to be almost similar Rf values. Lastly, the test for higher alcohols, phenols, steroids, and essential oils yielded only one spot for the sample.

Determining total phenolic content (TPC):

TPC of dried peel dragon fruit extracted using maceration extraction methods. The results from this study found that TPC was significantly higher in peel extracted using maceration process. It is possible that the use of maceration extraction increased the release of bound phenolic compound from the peel of dragon fruit. Some authors have postulated that the loss of TPC in dried samples at high temperature could be resulted from

degradation of phenolic compounds due to thermal effect and/or reduced free phenolic compound. Besides, the reduction of TPC also contributed to the loss betacyanin as betacyanin also contained phenol structure in molecule. However, the present study found higher total phenolic compounds which may be resulted from increased betacyanin content when extracted using maceration process.

Determining antioxidant activity:

The antioxidant activity of Methanolic extracts of dragon fruit. From the data presented in the table, it was found that the functional properties of different concentrations of fruit samples differhighly ($P< 0.01$) significant. The antioxidant activity exhibited by fruits was dose dependent. The antioxidant activity increased as the concentration increased. The antioxidant activity of dragon fruit was observed by spectrophotometer using the DPPH method in the range of 18.500 to 30.000per cent.

Recommendation for future works:

It is recommended to have a further study on phytochemical screening and antioxidant activity of Hylocereus undatus foliage since there are many other specific antioxidant compounds present such as carotenoids, betalains and lutein. Further studies on these types of antioxidant activities could improve the nutritional values found in the Hylocereus undatus foliage. In addition, some further analyses are suggested and required so as to isolate and identify the main phenolic compounds present in each extract. Since this present study is very much preliminary in nature, further works need to be carried out in order to determine the total flavonoid content as well as phenolic and flavonoid acids.

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