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Phytochemical Screening and Anti–Oxidant Profiling of Dragon Fruit

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Abstract:
The present investigation was carried out to evaluate the phytochemical constituents and antioxidant potential of *Hylocereus undatus* (dragon fruit) fruit pulp extracts using different solvents such as aqueous, chloroform, and hexane. Initially, the yield of solvent extracts was determined. Among the different solvents used, the aqueous extract showed the highest yield (5 g) from 50 g of dried fruit pulp powder, followed by chloroform extract (2.5g) and hexane extract (1.5g). The aqueous and chloroform extracts appeared as dark brown coloured paste, whereas the hexane extract showed a light brown coloured paste. Preliminary phytochemical screening revealed the presence of various secondary metabolites in the fruit pulp extracts. The aqueous extract showed the presence of carbohydrates, tannins, saponins, anthocyanins, quinones, cardiac glycosides, terpenoids, triterpenoids, phenols, acids, and steroids. The chloroform extract contained carbohydrates, saponins, alkaloids, cardiac glycosides, triterpenoids, phenols, and coumarins, while the hexane extract exhibited carbohydrates, saponins, anthocyanins, quinones, phenols, and acids. These phytochemicals are known to possess significant biological and antioxidant properties. The antioxidant potential of *H. undatus* fruit pulp extracts was evaluated using the DPPH radical scavenging assay. The results demonstrated that the percentage inhibition of DPPH increased with increasing concentration of extracts. Among the extracts, the aqueous extract exhibited stronger antioxidant activity compared to chloroform and hexane extracts, although the activity was lower than that of the standard L-ascorbic acid. At the highest concentration (200 µg/ml), the percentage inhibition observed was 63.49% for aqueous extract, 43.01% for chloroform extract, and 23.34% for hexane extract, whereas the standard L-ascorbic acid showed 77.45% inhibition.
Keywords: *Hylocereus undatus*, dragon fruit, chloroform extract, carbohydrates, saponins, alkaloids

Introduction

Dragon fruit *Hylocereus undatus* commonly called as pitaya is a climbing vine cactus species, which has received worldwide recognition first as an ornamental plant and then as a fruit crop. Its fruit is the most beautiful in the family *Cactaceae* with bright red skin studded with green scales and white or red flesh with green scales and or red flesh with tiny black seeds. Dragon fruit has high economic value and is useful for treating various types of diseases. Dragon fruit is believed to lower cholesterol concentration, to balance blood sugar concentration, to prevent colon cancer, to strengthen kidney function and bone, to strengthen the brain working capacity and increase the sharpness of eyes as well as it is used in cosmetic ingredients (Suryono, 2006).

Materials and Methods

Collection and Authentication of Dragon Fruit

Dragon fruit, Hylocereus undatus were collected from local fruit market, Nellore, Andhra Pradesh, India, it was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S V University, Tirupati.

Taxonomical Classification

Kingdom : Plantae (Plants)
Order : Caryophyllales
Family : Cactaceae (Cactus family)
Genus : Hylocereus
Species : undatus

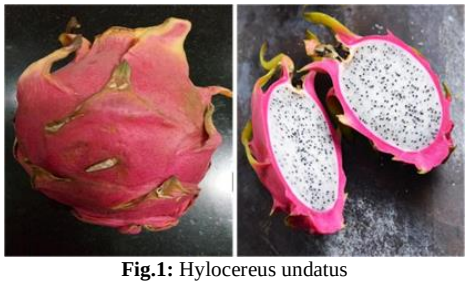

Fig.1: Hylocereus undatus

Preparation of Fruit Extracts Aqueous Extraction

After collecting dragon fruits from market, the outer epicarp of the fruit was removed and the endocarp was dried at room temperature. In a container, a 5% (w/v) suspension was prepared by mixing hot, boiled, distilled water to 50 g of dried fruit endocarp and held at 37°C and 200 rpm for 4 h in a shaker. The suspension was shaken, then cooled to room temperature before being run through four layers of No. 1 Whatman filter paper. The strained aqueous extract was dehydrated, stored at -20°C, and then subjected to various biological processes (Rajesh et al., 2016b).

Chloroform, Ethyl acetate, Hexane, Methanol Extraction

For aqueous, chloroform and hexane extracts are prepared by taking each 50 g of dried fruit endocarp were extracted with aqueous, chloroform and hexane respectively. Separate conical flasks containing dried fruit endocarp were immersed in 1:10 w/v solutions of chloroform, ethyl acetate, hexane and methanol for 72 h at room temperature. The filterates were concentrated at 45-55ºC under lowering pressure using a vacuum rotary evaporator; after the extracts being filtered using Whatman No. 1 filter paper (ROLEX). The extracts were measured and studies were carried out on the concentrated extracts (Rajesh et al., 2016b).

Preliminary Phytochemical Screening

Qualitative tests for the screening of phyto-constituents like acids, alkaloids, anthocyanin and betacyanin, carbohydrates, cardiac glycosides, coumarins, flavonoids, glucosides, phenols, proteins, quinones, saponins, starch, sterols, tannins, terpenoids and triterpenoids were done by method of Kokate (1994) in all the five extracts of H. undatus. Extracts were dissolved in their respective solvents and used for phytochemical studies.

Tests for carbohydrates

Molisch test: To 1ml of test + 2-3 drops of α- naphthol + Conc. sulphuric acid= appearance of purple ring

Fehling's test: To 1 ml of test sample+ Fehling's solution A and B + heat = brick red precipitate

Benedict's test: To 1 ml of test sample, equal quantity of Benedict's reagent was added and boiled. Red colour precipitate confirmed the presence of carbohydrates.

Test for alkaloids

All the extracts were first treated with dil. hydrochloric acid separately and then filtered. The filtrate of all the extracts was subjected to following tests:

Mayer's test: 1 ml of the filtrate + 1ml of Mayer's reagent = cream precipitate

Hager's test: 1 ml of the filtrate + 1mL of Hager's reagent = yellow precipitate

Wagner's test: 1ml of the filtrate + 1ml of Wagner's reagent = reddish brown precipitate

Tests for terpenoids

Salkowski test: Chloroform solution of test sample was treated with equal amount of conc. sulphuric acid. The presence of steroid components in the test sample was observed by the appearance of red color in chloroform layer and green fluorescence in acid layer.

Libermann - burchard test: To 2ml of test sample, chloroform was added beforethe addition of 2-3 drops of acetic anhydride and conc. sulphuric acid. The test solution was observed for colour change from red to blue and then finally to bluish green which confirmed the presence of steroids in the test extracts.

Tests for flavonoids

Lead acetate test: Lead acetate solution was added to the extract. Flavonoid was confirmed on the basis of formation of yellow precipitate.

Alkaline reagent test: 1mL of test sample was dissolved in dilute sodium hydroxide solution that resulted in formation of yellow colour precipitate.

Tests for tannins and phenolic compounds

5% $FeCl_3$ solution: Few drops of 5% FeCl3 solution was added to the small amount of extract that leads to the formation of deep blue-black colour complex.

10% lead acetate solution: To 2 ml of extract, few drops of 10% lead acetate solution were added, white precipitate was formed.

Gelatin test: After dissolving some quantity of extract in distilled water, 2 ml of 1% gelatin solution containing 10% NaCl was added which led to the formation of white precipitate indicating the presence of phenolic compounds.

Tests for saponins

A. Froth test: 1 ml of test sample + 20 ml of distilled water + shacked for 15min=Formation of persistent foam

Test for proteins and amino acids

Ninhydrin test: 3 ml of the test solution + 3 drops of 5% ninhydrin + heat for 10 min + observe colour change

Biuret test: Test sample was treated with same volume of 1% copper sulphate and 4% sodium hydroxide solution and appearance of violet or pink colour was observed.

Million's test: 3 ml of extract was mixed with 5 ml of Million's reagent. White precipitate formed which on heating turned to brick red, indicated the presence of proteins.

Tests for glycosides:

Borntrager's test: Dil. H2SO4was added to 3 ml of extract solution and boiled for 5 min. The solution was filtered and cooled. Same amount of chloroform was added while shaking the mixture. Ammonia was added to the organic solvent layer. Change in color of ammonical layer to pinkish red colour confirmed the presence of anthraquinone type glycosides.

Legal test: 1 ml of sodium nitroprusside was added in 1 ml of pyridine solution containing test sample and colour change was observed.

Keller killani test: To 2 ml of extract, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added. This solution was carefully transferred to the surface of 2 ml concentrated H2SO4 and the observation was noted down.

Tests for fats and oils

A. Spot test: One drop of extract was placed on filter paper and solvent was allowed to evaporate. An oily stain on filter paper indicated the presence of fixed oil.

Antioxidant Potential of H. undatus

DPPH radical scavenging assay:

The antioxidant potential of three different extracts of H. undatus fruit pulp was carried out by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay of Blois (1958).

Procedure

- The standard L-Ascorbic acid and all the three extracts of H. undatus fruit pulp were dissolved separately in methanol to achieve the desired concentrations.
- DPPH was made into a 0.1 mM solution by dissolving it in methanol.
- A precise 150 µl volume of the DPPH solution was pipetted into the microtitre wells, followed by addition of 50 µl of standard L-Ascorbic acid or H. undatus fruit pulp extracts at different concentrations (12.5-200 µg/ml).
- Methanol was added to DPPH solution to serve as a blank.
- The reaction mixture was thoroughly mixed with micropipette and incubated at room temperature in dark for 30 min.

The absorbance was measured at 520 nm using microplate reader and the percentage inhibition was calculated using the following formula:

$$\text{DPPH Inhibition (\%)} = \frac{\text{OD of Control} - \text{OD of Experiment}}{\text{OD of Control}} \times 100$$

The effective concentration of the reference standard and sample needed to scavenge the DPPH radical by 50% was determined using linear regression analysis (IC50 value).

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was performed based on reduction of ferric tripyridyltriazine complex to ferrous form at low pH. Absorbance was measured at 593 nm after incubation at 37°C for 10 minutes.

Results and Discussion

The dried fruit pulp of H. undatus is highly soluble in aqueous, chloroform and hexane. Aqueous and chloroform fruit pulp extracts of H. undatus resembled like a dark brown coloured paste, hexane resembled like a light brown coloured paste**.**

Preliminary Phytochemical Screening of H. undatus

In the current investigation, the analysis of phyto-constituents of H. undatus fruit pulp showed the presence of carbohydrate, tannins, saponins, anthocyanin, quinones, cardiac glycosides, terpenoids, triterpenoids, phenols, acids and steroids in aqueous extract, carbohydrate, saponins, alkaloids, cardiac glycosides, triterpenoids, phenols and coumarins in chloroform extract, carbohydrate, saponins, anthocyanin, quinones, phenols and acids in hexane extract.

Antioxidant Potential of H. undatus: The DPPH activity of aqueous, chloroform and hexane extracts of H. undatus fruit pulp were compared with L-Ascorbic acid (Standard). The DPPH activity inhibition was -15.55, -7.84,-3.49, -2.23 and -29.81 per cent at 12.5 µg/ml concentration of aqueous, chloroform and hexane extracts of H. undatus fruit pulp, respectively. As the concentration increased, DPPH inhibition also increased to -63.49, -43.01, -43.96, -23.34 and -79.79 per cent at 200 µg/ml concentration of aqueous, chloroform and hexane extracts of H. undatus fruit pulp, respectively.

The per cent inhibition of DPPH was directly proportional to the concentration. In the same way, DPPH activity of standard L-Ascorbic acid showed a gradual inhibition from -29.79 to -77.45 per cent, as the concentration increased from 12.5 µg/ml to 200 µg/ml, respectively (Table 3 and Fig. 1).

The per cent inhibition of DPPH in *H. undatus* methanol fruit pulp extract was lesser to L-Ascorbic acid (Standard). Statistical analysis as confirmed by two-way ANOVA revealed that the data were significant at P<0.001 level. The IC_{50} value of aqueous, chloroform, and hexane was found to be 124.188 µg/ml, 336.090µg/ml and 778.771 µg/ml respectively (Table 4). The results clearly indicate that *H. undatus* methanol fruit pulp extract showed much DPPH reducing activity in opposition to stable free radicals.

The FRAP assay measures ferric ion reducing antioxidant power of the extracts. The reducing ability increased with concentration in all extracts. L-ascorbic acid exhibited the highest reducing power, followed by aqueous, chloroform, and hexane extracts. The aqueous extract showed significantly higher ferric reducing activity compared to other solvent extracts, indicating a greater abundance of phenolic and antioxidant constituents. The results demonstrate that the antioxidant potential of H. undatus fruit pulp is concentration dependent and correlates with the presence of phytochemicals such as phenols and anthocyanins.

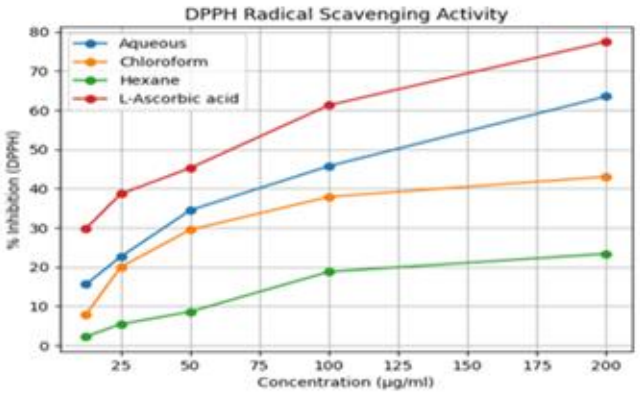

Fig.2: Line diagram showing DPPH activity of H. undatus fruit pulp extracts

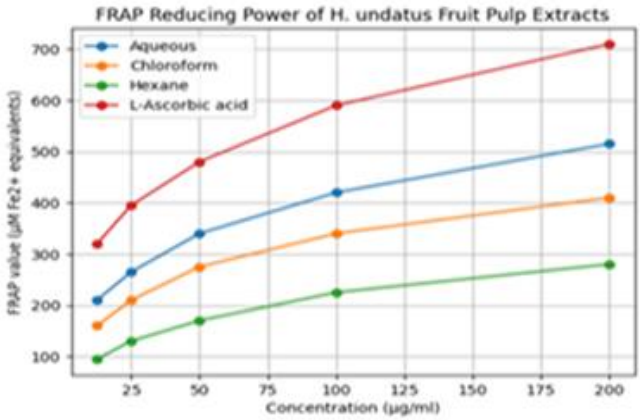

Fig. 3: Line diagram showing FRAP activity of H. undatus fruit pulp extracts

Table 1: Yield of *H. undatus* fruit pulp solvent extracts

S. No.	Solvents	Weight of dried powder (g)	Yield of Extract (g)	Colour	Consistency
1.	Aqueous	50	5	Dark brown	Paste
2.	Chloroform	50	2.5	Dark brown	Paste
3.	Hexane	50	1.5	Light brown	Paste

Table 2: Phytochemical screening of *H. undatus* fruit pulp extracts

S. No.	Secondary metabolites	Aqueous	Chloroform	Hexane
1	Acids	+	-	+
2	Alkaloids	-	+	-
3	Anthocyanin	+	-	++
4	Carbohydrate	+++	+++	+++
5	Cardiac glycosides	++	+	-
6	Coumarins	-	+	-
7	Flavonoids	-	-	-
8	Glycosides	-	-	-
9	Phenols	+	+	+
10	Protein	-	-	-
11	Quinones	+	-	+
12	Saponins	+	++	+
13	Steroids	++	-	-
14	Tannins	+	-	-
15	Terpenoids	+	-	-
16	Triterpenoids	++	+	-

+++ Strongly present ++ Slightly present

Table 3: DPPH activity of *H. undatus* fruit pulp extracts

Conc.(µg/ml)	Aqueous	Chloroform	Hexane	L-Ascorbic acid
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0
12.5	84.45 ± 0.70*(-15.55)	92.16 ± 0.76*(-7.84)	97.77 ± 0.23*(-2.23)	70.21 ± 0.37*(-29.79)
25	77.43 ± 0.26*(-22.57)	80.06 ± 0.44*(-19.94)	94.62 ± 0.24*(-5.38	61.39 ± 0.18*(-38.61)
50	65.57 ± 0.37*(-34.43)	70.56 ± 0.45*(-29.44)	91.46 ± 0.14*(-8.54)	54.81 ± 0.27*(-45.19)
100	54.30 ± 0.29*(-45.69)	62.14 ± 0.46*(-37.86)	81.27 ± 0.31*(-18.73)	38.78 ± 0.23*(-61.22)
200	36.51 ± 0.50*(-63.49)	56.99 ± 0.50*(-43.01)	76.66 ± 0.42*(-23.34)	22.55 ± 0.17*(-77.45)

Table 4: The exact IC_{50} value of DPPH activity of *H. undatus* fruit pulp extracts

S. No.	Extracts	Concentration (µg/ml)
1.	Aqueous	124.188
2.	Chloroform	336.090
3.	Hexane	778.771
4.	L-Ascorbic acid	65.009

Table 5: FRAP Activity of *H. undatus* Fruit Pulp Extracts

Concentration (µg/ml)	Aqueous (µM Fe^{2+} equivalents)	Chloroform (µM Fe^{2+} equivalents)	Hexane (µM Fe^{2+} equivalents)	L-Ascorbic acid (µM Fe^{2+} equivalents)
Control	0	0	0	0
12.5	210 ± 4.1	160 ± 3.5	95 ± 2.4	320 ± 5.3
25	265 ± 4.6	210 ± 3.9	130 ± 2.8	395 ± 6.2
50	340 ± 5.2	275 ± 4.3	170 ± 3.1	480 ± 6.8
100	420 ± 5.8	340 ± 4.9	225 ± 3.7	590 ± 7.4
200	515 ± 6.5	410 ± 5.6	280 ± 4.2	710 ± 8.1

Conclusion

The present study demonstrated that Hylocereus undatus fruit pulp contains a wide range of bioactive phytochemicals, including phenols, tannins, saponins, anthocyanins, terpenoids, and triterpenoids. These compounds are known to contribute significantly to antioxidant activity. Among the different solvent extracts tested, the aqueous extract showed the highest yield and exhibited superior antioxidant activity compared to chloroform and hexane extracts. The results obtained from both DPPH radical scavenging assay and FRAP assay confirmed that the antioxidant activity increased with increasing concentration of the extracts. Although the antioxidant activity of the extracts was lower than the standard L-ascorbic acid, the results indicate that H. undatus fruit pulp possesses considerable natural antioxidant potential. The presence of phenolic compounds and anthocyanins may be responsible for the observed antioxidant effects. Therefore, H. undatus fruit pulp can be considered a valuable natural source of antioxidants and may have potential applications in pharmaceutical, nutraceutical, and functional food industries. Further studies involving isolation of active compounds and in vivo evaluation are recommended to explore its therapeutic potential.

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