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Assessment of Antioxidant Activity in Fruit Flesh Extract of *Hylocereus undatus* (Dragon Fruits): An *In Vitro* Study

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Abstract: Plants have been the mainstay of many traditional cultures for their low cost and wide accessibility for preventive and therapeutic uses. Antioxidant can be defined as any substances in low concentrations, derived from natural or synthetic, capable of delaying or inhibiting oxidation reaction significantly. Based on reaction mechanism, antioxidant assays can be grouped into five categories, namely radical scavenging assay, reducing power, chelating agent, lipid peroxidation reaction and synergist. These mechanisms are commonly exploited for assessment of antioxidant from natural sources. The present study was conducted to evaluate the antioxidant activity of *Hylocereus undatusd* fruit flesh. Antioxidant activity of *Hylocereus undatus* was determined by using different methods namely DPPH, hydroxyl radical, nitric oxide, superoxide anion, iron chelating activity and its IC_{50} values were found. The results indicated that the ethanol extract has potent antioxidant. This holds great promise for the use of *Hylocereus undatus* fruit flesh as a source of strong antioxidant compounds.

Keywords: Antioxidant activity, *Hylocereus undatus*, DPPH, Hydroxyl radical, Nitric oxide, Superoxide anion, Iron chelating activity

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Introduction

Plants and their products have been used since time immemorial as principal ingredients of various traditional medicines and have played a key role in global health improvement. Traditional medical practice which involves the use of herbs is viewed as an integral part of the culture in those communities (Mukherjee, 2002; Bodeker *et al.,* 2005). Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Reactive oxygen species including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reaction or from exogenous factors (Mishra *et al.,* 2006). Antioxidants are substances which are able to

prevent and protect against diseases related to oxidative stress through preventing the free radical formation, scavenging and neutralizing reactive oxygen species, and inhibiting oxidative reactions (Houston, 2013). Therefore, widespread interest has been recently concerned on the evaluation of antioxidant plants and phytochemicals for reducing the risk of various diseases and improving the quality of life (Yegdaneh *et al.,* 2016; Mesripour *et al.,* 2016).

Synthetic antioxidants have been shown to possess carcinogenic activity, which leads to a need for the replacement of synthetic antioxidant with naturally occurring ones. Therefore, there is a necessity for development of safer antioxidants particularly from natural sources. In recent days, researchers mainly focused on natural oxidants and their health benefits. Plants generate a variety of antioxidant compounds as protection to reactive oxygen species and free radicals (Vigneswaran *et al.,* 2018).

Medicinal plants have strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free radicals (Anitha and Nazeema, 2020). The present investigation was aimed at evaluating the *in vitro* antioxidant activity of extract of the fruit flesh of *Hylocereus undatus* by using DPPH radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, superoxide anion scavenging and iron chelating assay.

Materials and Methods

Collection of plant:

The fruits of *Hylocereus undatus* were collected from fruit shop, Thanjavur, Tamil Nadu, India. The collected fruits were washed in water, cleaned well to remove all traces of insects, dust and other kinds of impurities. The fruits were peeled, cut into pieces and collected the flesh and dried under shade for two weeks. The dried flesh was ground into powdered form and properly stored in sealed sterilized container for extraction.

Preparation of extract:

The dried flesh powder of dragon fruits (*Hylocereus undatus*) were extracted with ethanol using maceration technique for 24 h at room temperature. The extract was filtered by Whatman No. 42 (125 mm) filter paper. The solvent was evaporated and concentrated under reduced pressure using rotary evaporator with the water bath at 45°C and filtrate was used for *in vitro* antioxidant assays.

In vitro antioxidant activity:

DPPH radical-scavenging activity was determined by the method of Shimada *et al.* (1992). The superoxide anion radicals scavenging activity was measured by the method of Liu *et al.* (1997). The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al*. (2004). The metal chelating activity of the extract was assayed by Dinis *et al.* (1994) Nitric oxide radical scavenging activity was determined according to the method of Garrat (1964).

Statistical analysis:

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals' concentration by 50%, IC_{50}, was graphically determined by a linear regression method using MS- Windows based GraphPad Instat (version 3) software. Results were expressed as graphically/ mean ± standard deviation.

Results and Discussion

In vitro antioxidant activity of the fruit flesh extract of *Hylocereus undatus* was investigated in the present study by DPPH, hydroxyl radical, nitric oxide, superoxide anion, iron chelating assays. In this study, it is evident that the *Hylocereus undatus* possess effective antioxidant activity (Tables 1-5; Figs. 1-5). The free radical scavenging capacity of the plant can be linked to its flavonoid and phenolic content, which are compounds responsible for antioxidant activity (Cheynier, 2012; Kaneria and Chanda, 2013).

Table 1: DPPH radical scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid

Concentrations (µg/ml)	% of inhibition	
	Hylocereus undatus	**Std. (Ascorbic acid)**
20	16.61±0.05	20.17±0.06
40	26.70±0.07	36.20±0.09
60	51.63±0.10	58.16±0.13
80	68.84±0.15	75.66±0.20
100	87.83±0.23	92.58±0.24
IC_{50} (µg/ml)	59.65	52.88

Values are expressed as mean ± Standard deviation for triplicates; IC: Inhibition concentration

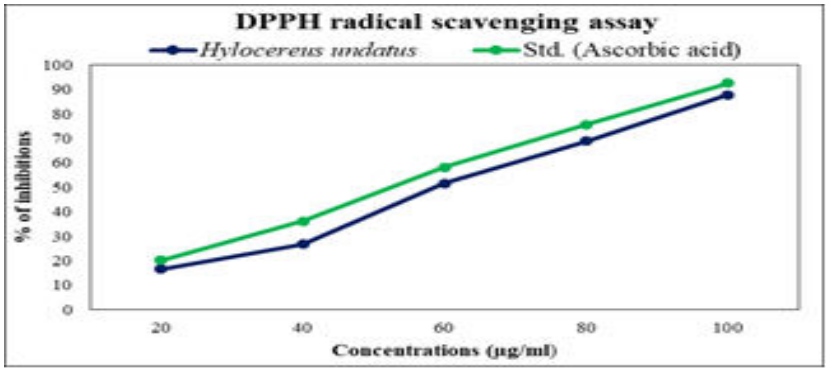

Fig. 1: DPPH radical scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid.

DPPH radical scavenging activity:

The result of reduction of DPPH radicals causes discoloration from purple color to yellow pale color which demonstrates the scavenging activity (Akar *et al.,* 2017). DPPH radical scavenging activity of *Hylocereus undatus* fruit flesh extract was determined and compared with ascorbic acid.

The fruit flesh extract and ascorbic acid showed the minimum DPPH inhibitory activity at 20 µg/ml concentration range (16.61% and 20.17%) while maximum inhibitory activity was noticed at 100 µg/ml concentration range (87.83% and 92.58%) (Table 1; Fig. 1). The half inhibition concentration (IC_{50}) of fruit flesh extract and ascorbic acid were 59.65 µg/ml and 52.88 µg/ml, respectively. *Hylocereus undatus* fruit flesh extract has potential DPPH activity and near to standard. This is comparable to the findings of Narendhirakannan and Rajeswari (2010) who discovered that the ability of garlic extracts to scavenge DPPH radicals increased with extract concentration. It is also in agreement with Tariq *et al.* (2022) who found that the DPPH radical scavenging potentials of *Moringa Oleifera* followed a similar pattern.

Hydroxyl radical scavenging activity:

The *in vitro* hydrogen peroxide (H_2O_2) free radical scavenging method is built on the concept that when H_2O_2 radicals are reduced to two molecules of water (H_2O) by an antioxidant substance, absorbance of H_2O_2 decreases (Arika *et al.,* 2019). In this study the fruit flesh extract and ascorbic acid showed the minimum hydroxyl radical scavenging inhibitory activity at 20 µg/ml concentration range (19.78% and 21.22%) while maximum inhibitory activity was shown at100 µg/ml concentration range (81.65% and 90.28%) (Table 2; Fig. 2). The half inhibition concentration (IC_{50}) of fruit flesh extract and ascorbic acid were 60.54 µg/ml and 53.27 µg/ml, respectively. *Hylocereus undatus* fruit flesh extract has

Table 2: Hydroxyl radical scavenging activity of Hylocereus undatus fruit flesh extract and ascorbic acid

Concentrations (µg/ml)	% of inhibition	
	Hylocereus undatus	**Std. (Ascorbic acid)**
20	19.78±0.09	21.22±0.12
40	30.57±0.14	38.12±0.17
60	48.92±0.18	56.47±0.23
80	66.90±0.25	73.02±0.28
100	81.65±0.32	90.28±0.37
IC_{50} (µg/ml)	60.54	53.27

Values are expressed as mean ± Standard deviation for triplicates; IC: Inhibition concentration

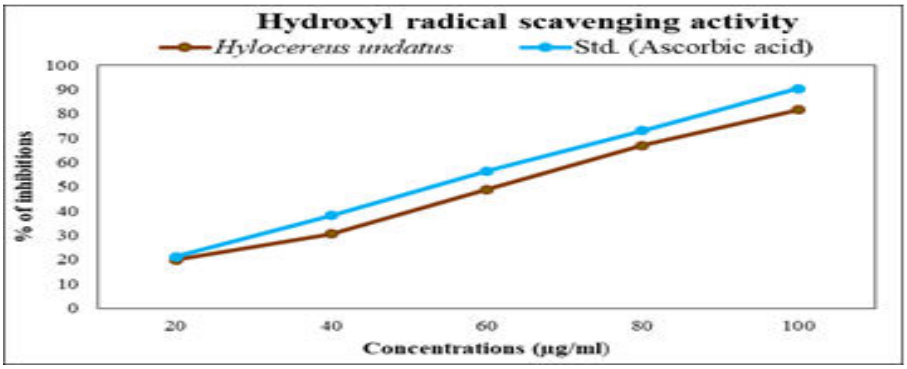

Fig. 2: Hydroxyl radical scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid.

potential hydroxyl radical scavenging activity which is near to standard. The present study also agree with those of Roghini and Vijayalakshmi (2018) who found that *Citrus paradisi* extracts had potent hydrogen peroxide scavenging activity though their activities were significantly lesser than those of the reference.

Nitric oxide scavenging activity:

Nitric oxide plays a vital physiological role in respiratory, immune, and neuromuscular systems. Nitric oxide (NO), which is an important bioactive molecule, possesses several physiological functions (Marcocci *et al.,* 1994). The ability to scavenge NO- radicals is evaluated by using sodium nitroprusside, as it generates NO- at physiological pH, which then will interact with oxygen to produce stable nitrite and nitrate ions (Azizan *et al.,* 2020). Nitric oxide is a potent pleiotropic mediator of various physiological processes. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological systems (Miller *et al.,* 1993). In this study we determined nitric oxide scavenging activity of *Hylocereus undatus* fruit flesh extract and compared with ascorbic acid. The fruit flesh extract and ascorbic acid showed the minimum nitric oxide scavenging inhibitory activity at 20 µg/ml concentration range (17.16% and 20.60%) while maximum inhibitory activity was recorded at 100 µg/ml concentration range (78.96% and 89.27%) (Table 3; Fig. 3). The half inhibition concentration (IC_{50}) of fruit flesh extract and ascorbic acid were 65.20 µg/ml and 56.06 µg/ml, respectively. *Hylocereus undatus* fruit flesh extract has potential nitric oxide scavenging activity which is near to standard.

Superoxide anion scavenging activity:

Superoxide radical is considered a precursor of more reactive species, such as single oxygen and hydroxyl radicals, and is an initiator of lipid peroxidation. Superoxide anion can be generated in enzymatic systems by autoxidation reactions or by nonenzymatic electron transfer which reduces molecular oxygen (Wang *et al.,* 2007). In this study superoxide anion scavenging activity of *Hylocereus undatus* fruit flesh extract was

Table 3: Nitric oxide scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid

Concentrations (µg/ml)	% of inhibition	
	Hylocereus undatus	**Std. (Ascorbic acid)**
20	17.16±0.05	20.60±0.10
40	29.18±0.11	35.19±0.15
60	42.06±0.19	51.50±0.20
80	62.23±0.24	70.38±0.26
100	78.96±0.28	89.27±0.35
IC_{50} (µg/ml)	65.20	56.06

Values are expressed as mean ± Standard deviation for triplicates; IC: Inhibition concentration

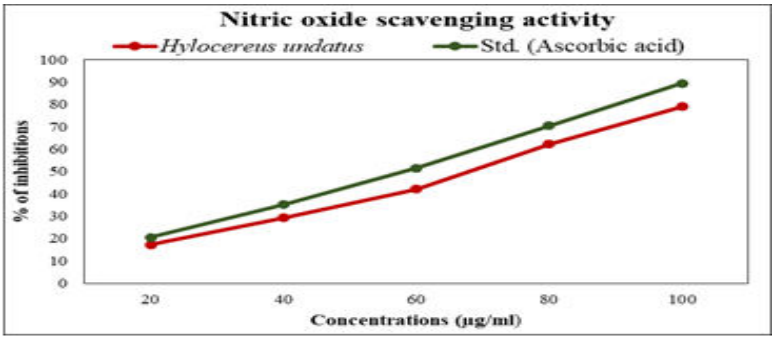

Fig. 3: Nitric oxide scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid.

Table 4: Superoxide anion scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid

Concentrations (µg/ml)	% of inhibition	
	Hylocereus undatus	**Std. (Ascorbic acid)**
20	20.43±0.11	22.93±0.13
40	34.05±0.17	39.42±0.20
60	48.74±0.19	56.63±0.24
80	69.53±0.22	75.62±0.27
100	88.17±0.31	91.75±0.39
IC_{50} (µg/ml)	57.44	51.63

Values are expressed as mean ± Standard deviation for triplicates; IC: Inhibition concentration

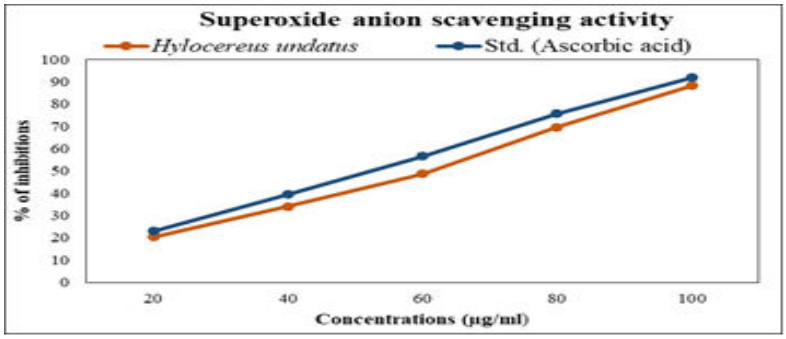

Fig. 4: Superoxide anion scavenging activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid.

Table 5: Iron chelating activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid

Concentrations (µg/ml)	% of inhibition	
	Hylocereus undatus	**Std. (Ascorbic acid)**
20	19.87±0.09	21.10±0.12
40	32.11±0.15	40.06±0.18
60	55.96±0.21	60.85±0.23
80	72.78±0.25	77.67±0.27
100	89.90±0.30	93.57±0.33
IC_{50} (µg/ml)	55.43	50.52

Values are expressed as mean ± Standard deviation for triplicates; IC: Inhibition concentration

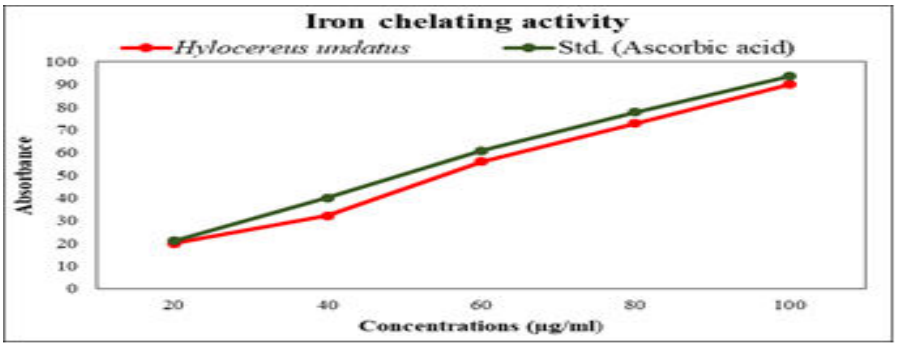

Fig. 5: Iron chelating activity of *Hylocereus undatus* fruit flesh extract and ascorbic acid.

determined and compared with ascorbic acid. The fruit flesh extract and ascorbic acid showed the minimum superoxide anion scavenging activity inhibitory activity at 20 µg/ml concentration range (20.43% and 22.93%) while maximum inhibitory activity was show3n at 100 µg/ml concentration range (88.17% and 91.75%) (Table 4; Fig. 4). The half inhibition concentration (IC_{50}) of fruit flesh extract and ascorbic acid were 57.44µg/ml and 51.63µg/ml, respectively. *Hylocereus undatus* fruit flesh extract has potential superoxide anion scavenging activity and near to standard. Similar results were reported regarding the scavenging effect of grape seeds (Parejo *et al.,* 2002).

Iron chelating activity:

Iron chelating activity of *Hylocereus undatus* fruit flesh extract and compared with ascorbic acid. The fruit flesh extract and ascorbic acid showed the minimum Iron chelating activity at 20 µg/ml concentration range (19.87 and 21.10) while maximum inhibitory activity was noticed at 100 µg/ml concentration range (89.90 and 93.57) (Table 5; Fig. 5). The half inhibition concentration (IC_{50}) of fruit flesh extract and ascorbic acid were 55.43 µg/ml and 50.52 µg/ml, respectively. *Hylocereus undatus* fruit flesh extract has potential iron chelating activity and near to standard. Iron chelating activities of organic extracts of *P. pygmaeus* were also shown to occur in a similar manner (Md Yusof *et al.,* 2013).

Antioxidants play major roles in providing cell's defense mechanisms where the molecular and enzymatic constituents of antioxidant compounds can act by scavenging chain-initiating radicals, quenching singlet oxygen, decomposing hydroperoxides, and chelating pro-oxidative metal ions (Martysiak-Zurowska and Wenta, 2012; Carocho and Ferreira, 2013). The actions of antioxidants in scavenging free radicals contribute to the prevention of chronic disease development caused by oxidative stress (Yu *et al.,* 2005; Rajendran *et al.*, 2014). Although the human body produces its antioxidants, the effectiveness of these antioxidants may not be

100 %, and it gradually decreases with age, denoting the need to gain antioxidants from other sources to maintain their optimum level in the body.

Conclusion

The results of this study showed that the alcoholic extract of *Hylocereus undatus* fruit flesh can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The antioxidant property of alcoholic extract of *Hylocereus undatus* fruit flesh due to the phytochemical components present in it. Therefore, further works are needed on the identification of the antioxidant components present in alcoholic extract of *Hylocereus undatus* fruit flesh.

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