



Received on 05 September 2019; received in revised form, 23 September 2019; accepted, 27 September 2019; published 30 September 2019

IN-VITRO ANTI-OXIDANT STUDIES OF MACERATED ETHANOLIC AND AQUEOUS EXTRACT OF *HYLOCEREUS UNDATUS* FRUITS

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Keywords:

Cactaceae,
Hylocereus undatus,
Anti-oxidants, Ascorbic acid,
Ethyl acetate

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ABSTRACT: *Hylocereus undatus* is an important medicinal plant belonging to the family Cactaceae and widely distributed in tropical forest areas. Fruits are used for anti-bacterial, anti-oxidant, antipyretic, antidiabetic and hepatoprotective activities of the plants are scientifically proved. The aim of the present study is to evaluate *Hylocereus undatus* pharmacologically for the anti-oxidant study. The study also includes the Pharmacognostical and phytochemical evaluation of the plant. The pharmacognostic evaluation revealed authentication and identification of the plant and extraction of plant material using ethanol by Soxhlet extraction method. Preliminary phytochemical studies were carried out using various chemical tests. Phytochemical studies showed the presence of glycoside, phenolic, flavonoids, triterpenoids, tannins and reducing sugar. The phenolic content of the ethyl acetate extract of the roots separated by chromatographic technique and the total phenolic content was calculated. Anti-oxidant activity studies using nitric oxide and DPPH scavenging assay showed that the ethyl acetate and aqueous extracts could be promising sources of natural anti-oxidants.

INTRODUCTION: Now, there is a new trend in drugs of natural origin because they are considered as herbal medicine or green medicine. Herbal medicine is always safe compared with the synthetic drug which is harmful to humans and environment¹. The human race is constantly being challenged by many dreadful diseases and it is an uphill task to combat them in the present scenario. Leading healthy life is a challenge in a highly competitive world, where people hardly get a chance to care about their diet or assure the quality of the food they eat. Oxidation reaction can form free radical and these start chain reactions that damage cells.

Anti-oxidants are a molecule capable of inhibiting the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reaction². Different types of fruits in India have antioxidants property documents in standard literature. Green leafy vegetables and fruits are good sources and vitamins. Ethano-botanical properties of green leafy vegetables and fruits like anti-diabetic, anti-carcinogenic, hypolipidemic and Anti-bacterial activity.

The phytochemicals present in fruits and vegetables have overlapping and complementary mechanism including stimulation of the immune system scavenging of oxidative agent, hormone metabolism and antibacterial and antiviral effects³. *Hylocereus undatus* (white dragon fruit) are fruits belonging to Cactaceae with a bright red skin studded with green scales and white with tiny black seeds.

	<p>QUICK RESPONSE CODE</p>
	<p>DOI: 10.13040/IJPSR.0975-8232.IJP.6(9).319-22</p>
<p>The article can be accessed online on www.ijjournal.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.6(9).319-22</p>	

The flower is so beautiful that it is nicknamed as Noblewomen or Queen of the night. Dragon fruits contain rich amount of vitamins, minerals, fat, carbohydrate, flavonoid, Phenolic, carotene, iron and phytoalbumin⁴. The present study has been undertaken to evaluate the phytochemical and antioxidant properties of *Hylocereus undatus*.

MATERIALS AND METHOD:

Collection of Sample: Dragon fruits were purchased from LULU Mall Supermarket in Edapally, Ernakulam District, Kerala.

Sample Preparation and Extraction:⁵ Dragon fruits were washed and peeled in order to separate the peels from the pulp. To collect 10 - 30 g of the edible portion was crushed using a blender to a paste-like state.

Alcohol Maceration: The homogenized samples 20 g were placed with whole of the 360 ml alcohol in close vessels for seven days. During this period shaking was done occasionally after seven days liquid was strained and vacuum dried.

Aqueous Maceration: The homogenized samples 20 g was placed with the whole of the 360 ml chloroform water in close vessels for seven days. During this period shaking was done occasionally after seven days liquid was strained and vacuum dried.

Preliminary Phytochemical Screening: Preliminary phytochemical analysis was carried out on the macerated ethanolic and water extracts using the standard procedure to identify the constituents present by characteristics color changes⁶.

Nitric Oxide Scavenging Assay: Sodium nitroprusside (5 µm) in standard phosphate buffer solution was incubated with different concentrations of the test extracts dissolved in standard phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25 °C for 5 h. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by

mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and the activity was compared with ascorbic acid, which was used as a standard antioxidant. Nitric oxide scavenging that is calculated by the formula⁷.

Percentage of nitric oxide scavenging = (control OD-sample OD)/ Control OD × 100

DPPH Radical Scavenging Study:^{8,9} Free radical scavenging activity of aqueous and ethanolic macerated extracts of fruits of *Hylocereus undatus* were measured by DPPH. In brief 0.1 mm solution of DPPH in ethanol was prepared from the above solution 1 ml was added to 3 ml of different extract in ethanol at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature 30 min. Absorbance was measured at 517 nm by using UV-visible spectroscopy. The reference standard compound being used was ascorbic acid and experiment was done in triplicate IC₅₀ value of sample was calculated.

RESULTS: Preliminary phytochemical analysis of macerated ethanolic and aqueous extracts of *Hylocereus undatus* revealed the presence of some secondary metabolites in **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF HYLOCEREUS UNDATUS FRUITS EXTRACT

S. no.	Phytochemical constituents	Ethanolic extract	Aqueous extract
1	Carbohydrate	+	+
2	Saponins	-	+
3	Tannins	+	+
4	Flavonoids	+	+
5	Steroid and triterpenoids	+	-
6	Phenols	+	+
7	Alkaloids	+	+
8	Glycoside	+	+

TABLE 2: NITRIC OXIDE SCAVENGING ASSAY OF HYLOCEREUS UNDATUS FRUITS EXTRACT

S. no	Extract	Concentration	Absorbance 546nm	Percentage of Inhibition
1	Control	-	1.666 ± 0.303	-
2	Standard drug (Ascorbic acid)	50	1.303 ± 0.152	21.78
		100	0.933 ± 0.057	43.64
		150	0.573 ± 0.057	65.60
		200	0.216 ± 0.057	87.03

3	Alcoholic extract	50	1.321 ± 0.001	20.70
		100	1.031 ± 0.001	38.11
		150	0.677 ± 0.112	59.36
		200	0.269 ± 0.001	83.85
4	Aqueous extract	50	1.540 ± 0.001	7.56
		100	1.249 ± 0.001	25.03
		150	0.961 ± 0.001	42.31
		200	0.739 ± 0.001	55.64

N = 3 absorbance expressed as Mean ± SD

TABLE 3: DPPH RADICAL SCAVENGING ASSAY OF DIFFERENT EXTRACT OF *HYLOCEREUS UNDATUS*

S. no.	Extract	Concentration	Absorbance 546nm	Percentage of Inhibition
1	Control	-	0.253 ± 0.0186	-
2	Standard drug (Ascorbic acid)	50	0.133 ± 0.002	47.43
		100	0.113 ± 0.001	55.33
		150	0.086 ± 0.002	66
		200	0.061 ± 0.001	75.89
3	Alcoholic extract	50	0.143 ± 0.001	43.48
		100	0.121 ± 0.001	52.17
		150	0.103 ± 0.001	59.28
		200	0.078 ± 0.002	69.16
4	Aqueous extract	50	0.156 ± 0.005	38.33
		100	0.138 ± 0.001	45.45
		150	0.116 ± 0.001	54.15
		200	0.097 ± 0.001	61.66

N = 3 absorbance expressed as Mean ± SD

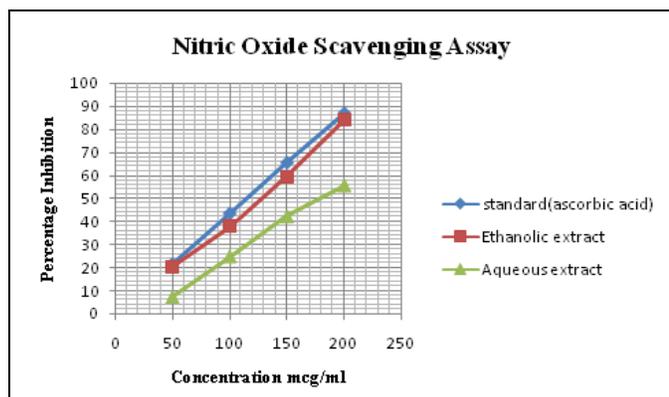


FIG. 1: PERCENTAGE INHIBITION OF NITRIC OXIDE BY DIFFERENT EXTRACT OF *HYLOCEREUS UNDATUS*

Both nitric oxide scavenging and DPPH radical scavenging assay shows the beneficial anti-oxidant study was shown in Fig. 1 and 2. In nitric oxide scavenging assay of alcoholic extract shows high antioxidant activity compared with aqueous extract. DPPH radical scavenging assay of alcoholic extract shows high antioxidant activity compared with aqueous extract.

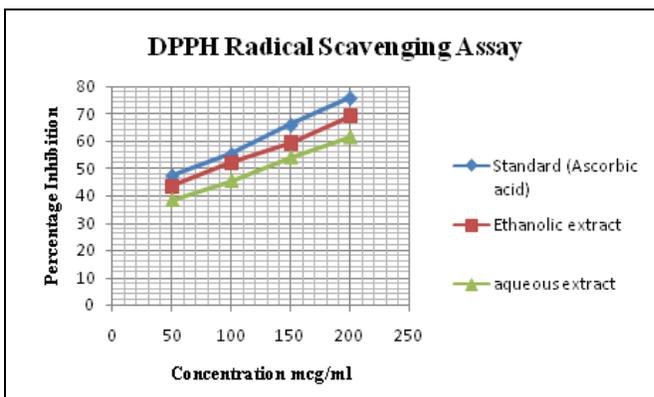


FIG. 2: PERCENTAGE INHIBITION OF DPPH RADICAL SCAVENGING ASSAY BY DIFFERENT EXTRACT OF *HYLOCEREUS UNDATUS* FRUIT

TABLE 4: IC₅₀ VALUE OF DIFFERENT EXTRACT

S. no.	Sample	IC ₅₀ Value(mcg/ml)
1	Standard (Ascorbic acid)	66.25
2	Alcoholic extract	87.5
3	Aqueous extract	126.25

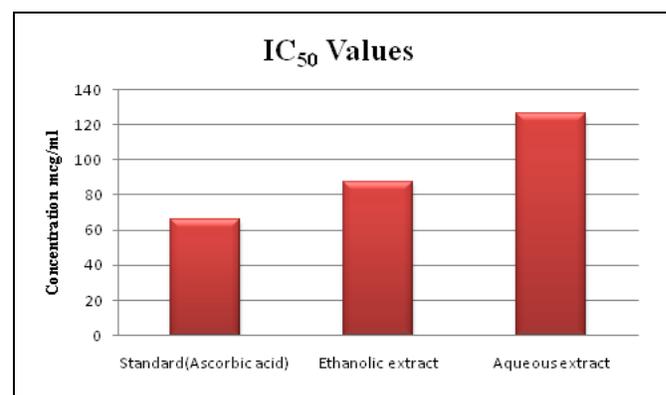


FIG. 3: IC₅₀ VALUE OF DIFFERENT SAMPLES

DISCUSSION: Both extracts of *Hylocereus Undatus* fruits show significant radical scavenging

activity. Extract contains high amount of radical scavenging compounds. Preliminary phytochemical analysis of ethanolic and aqueous extract showed the presence of alkaloids, tannins, flavonoids, phenols, glycosides. Plant phenolics constituents one of the major groups of compounds acting as primary anti-oxidant free radical terminator¹⁰. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophage involved in the regulation of different types of physiological activity. Excess concentration of nitric oxide to produced cytotoxic effect observed in disorders like AIDS, cancer and Alzheimer's. Oxygen reacts with nitric oxide to produce nitrites which act as free radicals¹¹.

Nitric oxide scavenging assay showed a significant dose-dependent antioxidant potential of alcoholic extract over aqueous extract. DPPH assay was carried out to measure the primary anti-oxidant activity of each sample in present study. The ability to remove scavenging free radicals is classified as primary anti-oxidant. Showing the presence of compounds responsible as free radical scavengers which reduced the initial DPPH concentration¹². DPPH radical scavenging assay showed a significant dose-dependent anti-oxidant potential of both alcoholic and aqueous extract. IC₅₀ value of different extract shows alcoholic extract has less IC₅₀ value compared with aqueous extract and higher with standard.

CONCLUSION: In this study, *Hylocereus Undatus* showed significant and dose-dependent free radical scavenging activity by nitric oxide scavenging assay and DPPH radical scavenging assay. The fruits and vegetables are the main sources of anti-oxidant in diet. *In-vitro* study proves that alcoholic extract has better antioxidant activity with IC₅₀ than aqueous extract. Further isolation and purification of *Hylocereus Undatus* have used as greater anti-oxidants.

ACKNOWLEDGEMENT: The authors wish to thank the Shijikumar P S, Principal, Jamia Salafiya

College of Pharmacy, Malappuram and Dr. Preeja G Pillai, Principal KVM College of Pharmacy, Cherthala, Kerala and for providing the necessary infrastructural facilities and Dr. Akhila S. Head of Department of Pharmacognosy, KVM College of pharmacy for valuable instruction and support on my study.

CONFLICT OF INTEREST: Nil.

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How to cite this article:

Anu V, Sarath D and Sneha PK: *In-vitro* anti-oxidant studies of macerated ethanolic and aqueous extract of *Hylocereus undatus* fruits. Int J Pharmacognosy 2019; 6(9): 319-22. doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.6\(9\).319-22](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.6(9).319-22).

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