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IN VITRO ANTI-OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF FLOWERS *HYMENOCALLIS LITTORALIS*

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ABSTRACT

Hymenocallis littoralis is commonly referred as Beach spider Lilly, is a medicinal plant and ornamental plant. It is used as emetic, and has shown wound healing, anti-viral, antineoplastic and cytotoxic properties. *Hymenocallis littoralis* Flowers were extracted using ethanol as solvent. *Hymenocallis littoralis* have been evaluated for its anti-oxidant activity of ethanolic extract of flowers of *Hymenocallis littoralis* on male Wistar albino rats .The aim of this research is to explore the anti-oxidant potential of this selected plant material, by using antioxidant activity of *Hymenocallis littoralis* with standard drug such as Ascorbic acid is used by using three methods they are 2,2-Diphenyl-1-picrylhydrazyl(DPPH) , Reducing power scavenging activity. Hydrogen peroxide radical. This result shows that the ethanolic extract of *Hymenocallis littoralis* have effective oxidative properties that closely resembles the standard anti-oxidant drug like Ascorbic acid.

INTRODUCTION

Hymenocallis littoralis is a bulbous perennial herb. It has 60 to 70cm in height and 7-10cm in diameter of bulb. It is mostly found in Southern eastern united states, Mexico, Central America, Northern states of America. The parts used are leaves, bulbs, flowers, roots and stem. It often grows as an ornamental plant. The part we used is flower to show its anti-oxidant activity. The *H. littoralis* have medicinal uses like wound healing, emetic, anti-neoplastic, cytotoxic properties and anti-viral. The phytochemical screening of flowers yielded four alkaloids are lycorine, hippeastrine, 11 hydroxyvittatine and (+)-8-O-demethylmaritidine, and two flavonoids like quercetin,3-O-glucoside and rutin and pancreastatin are present. The volatile constituent of the plant flowers was analysed by GC/MS, which lead to identification of 26 known compounds.

Finally, the antimicrobial activity of the petroleum ether extract of the flowers of *H. littoralis* was investigated. In this study we know about the *H. littoralis* showing the anti-Oxidant property comparing with standard drug Ascorbic acid.

2. MATERIALS AND METHODS:

2.1. Materials used: Ethanol

2.2. Preparation of extract: Fresh *Hymenocallis littoralis* flowers were collected

(400g) from grounds of Vignan institute of pharmaceutical technology, Duvvada Visakhapatnam. About 400g of fresh flowers were minced and were extracted using Ethanol as solvent at room temperature until the colour becomes pale. The extract obtained were filtered separately using Whatman No.1 filter paper, this was repeated for 2-3 times (subsequent maceration) and similarly pooled together and the filtrate was collected and concentrated at low temperatures on Rota vapour or Heating mantle. This concentrated ethanol extract is used for further studies. The residual extracts were weighed and stored in desiccator in china dish. The percent extractive values were calculated by the following formula.

Percentage extract = weight of dried extract /weight of flower material**.**

3. *In Vitro* ANTIOXIDANT STUDIES

3.1. DPPH radical scavenging Activity:

PRINCIPLE:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH is methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the nonradical from DPPH-H.

This transformation results in a colour change from purple to yellow, which is measured spectrophotometrically. This disappearance of the purple colour is monitored at 517nm. The free radical scavenging activity can be measured by using 2,2-diphenyl-1-picryl-hydrazyl by the method of Mccune and johns (2002). Ascorbic acid, Gallic acid and, BHA, quercetin, rutin can be used as positive controls. This assay is useful for comparing the reduction potential of unknown materials like plant extracts.

Preparation of stock solutions of extract and Ascorbic acid:

0.1gm each of plant extract and ascorbic acid were taken and dissolved in 100ml of water to give a standard stock solution of plant extract and ascorbic acid respectively to provide 1mg/ml solution. From this, various dilutions of extract such as 0.1, 02, 0.3, 0.4, 0.5 micrograms/ml were made with water.

Reagents:

1, 1-diphenyl-2-picrylhydrazyl (DPPH, 0.004%) solution: 4mg of DPPH was dissolved in 100 ml of ethanol and kept it overnight in dark place for the generation of DPPHradical.

PROCEDURE:

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the difference in optical density of DPPH radical is monitored. According to Manzocco et al., 1998 firstly the sample extract (0.2 mL) is diluted with methanol and then 2mL of DPPH solution (0.5 mM) is added. The mixture was shaken and left to stand overnight .Finally the absorbance is measured at 517 nm by visible spectrophotometer.

The percentage of the DPPH radical scavenging is calculated using the equation as given below:

%Inhibition of DPPH free radical= A0-A1/A0*100

The molecule 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as it would be the case with most other free radicals. Due to the delocalization of electron it gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centred at 517nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour.

3.2. Reducing power scavenging activity:

PRINCIPLE:

Reducing power method is based on the principle that substances, which have reduced potential activity, that react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then react with the ferric chloride to form ferric-ferrous complex that has absorption at 700nm. Graph 2 shows that how the APPD increases with the increase with the amount of sample. Percentage inhibition of APPD is linear with that of standard ascorbic acid. The IC-50 of APPD is and ascorbic acid is. It represents that APPD is having more antioxidant activity than ascorbic acid.The ability of extract to reduce to reduce Fe3+ to Fe2+ was determined according to the

method described by the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Dietary antioxidant such as ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that can act as primary and secondary antioxidants.

REAGENTS: Potassium ferric cyanide, trichloro acetic acid, ferric chloride, sodium hydroxide, potassium phosphate

PROCEDURE:

3.3. Hydrogen peroxide radical scavenging activity:

PRINCIPLE: Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, micro- organisms, food and beverages. Human beings exposed to H2O2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure.H2O2 enters the human body through inhalation of vapour and through eye or skin contact .In the body,H2O2 is rapidly decomposed into oxygen and water, and this may produce hydroxyl radicals that can initiate lipid peroxidation and cause DNA damage .The ability of test sample and ascorbic acid.

Preparation of stock solutions of extract and Ascorbic acid: 0.1gm each of plant extract and ascorbic acid were taken and dissolved in 100ml of water to give a standard stock solution of plant extract and ascorbic acid respectively to provide 1mg/ml solution. From this, various dilutions of extract such as 0.1, 0.2, 0.3, 0.4, 0.5 µg/ml were made with phosphate buffer.

Reagents: Hydrogen peroxide (43Mm) Solution: 0.45ml of H2O2 was dissolved in 100 ml of phosphate buffer (0.1M) solution (7.4Ph)

PROCEDURE: The ability of plant extract to scavenge H2O2 was determined .A solution of H2O2(43Mm) was prepared in phosphate buffer and concentration was determined spectrophotometrically at 230nm (UV-Vis).Plant extract (10-50 µg/ml) in distilled water was added to a hydrogen peroxide solution (0.6ml,43mM) and the absorbance of H2O2 at230nm was determined after 10 min against a blank solution in phosphate buffer without hydrogen peroxide .The percentage of scavenging of hydrogen peroxide of plant extract and standard compounds was calculated using the following equation :

4. RESULT: Antioxidant from natural source can improve the antioxidant system in body for scavenging free radicals. It says that antioxidant from natural sources are increasing faster than synthetic sources. Ethanolic compounds which naturally present in *Hymenocallis littoralis* plant can reduce the risk of many diseases and its effects which correlated with the antioxidant compounds. Recently there are some reports about *H. littoralis* flowers which are rich in ethanolic compounds like flavonoids, gallic acid, rutin and quercetin as antioxidant activity. *H. littoralis* is one the Mexico traditional plant that has multipurpose biological activities.

Table no: 3.2.1 DPPH radical scavenging Activity

TEST SUBSTANCE		STANDARD	
Concentration	**Absorbance**	**Concentration**	**Absorbance**
50 µg	1.5089	500 µg	1.7404
100 µg	1.7349	400 µg	1.5346
200 µg	1.7214	300 µg	1.3517
300 µg	1.7169	200 µg	1.2420
400 µg	1.7448	100 µg	0.8549
500 µg	1.7602	50 µg	0.8135

Hydrogen peroxide radical scavenging activity

TEST SUBSTANCE		**STANDARD**	
Concentration	Absorbance	Concentration	Absorbance
10 µg	0.0537	500 µg	1.7404
20 µg	0.1145	400 µg	1.5346
30 µg	0.1782	300 µg	1.3517
40 µg	0.2325	200 µg	1.2420
50 µg	0.2984	100 µg	0.8549
		50 µg	0.8135

Reducing power scavenging activity

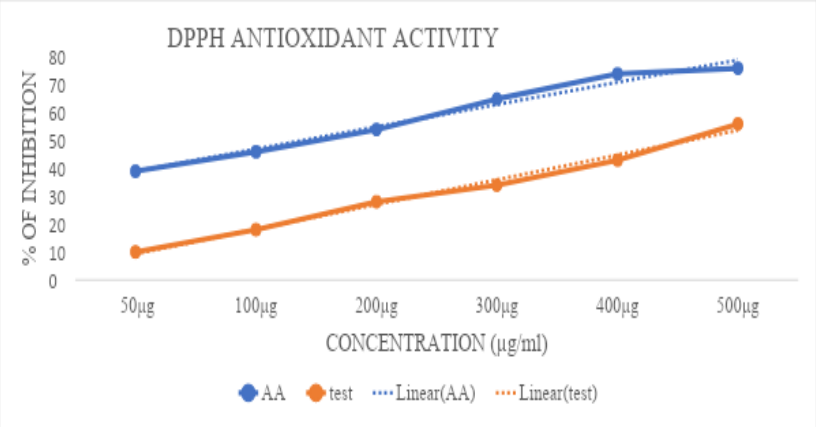
TEST SUBSTANCE			STANDARD	
Concentration	Absorbance	Average	Concentration	Absorbance
0.1conc			500 µg	1.7404
1.	1.1414		400 µg	1.5346
2.	1.1955	1.147	300 µg	1.3517
3.	1.1147		200 µg	1.2420
0.2conc			100 µg	0.8549
1.	0.9880		50 µg	0.8135
2.	1.1730	1.047		
3.	0.9989			
0.3conc				
1.	1.1535			
2.	1.2016	1.12		
3.	1.0226			
0.4conc				
1.	1.2169			
2.	1.1502	1.15		
3.	1.1115			
0.5conc				
1.	1.0549			
2.	1.0877	1.08		
3.	1.1111			

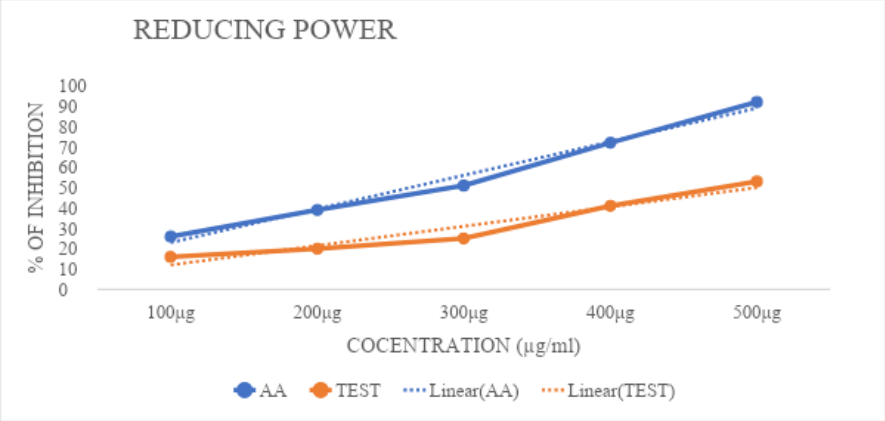
4.1. DPPH Antioxidant activity:

CONC	**AA**	**TEST**
50 µg	39	10
100 µg	46	18
200 µg	54	28
300 µg	65	34
400 µg	74	43
500 µg	76	56
IC50	2.38	5.59

4.2. Reducing power scavenging activity:

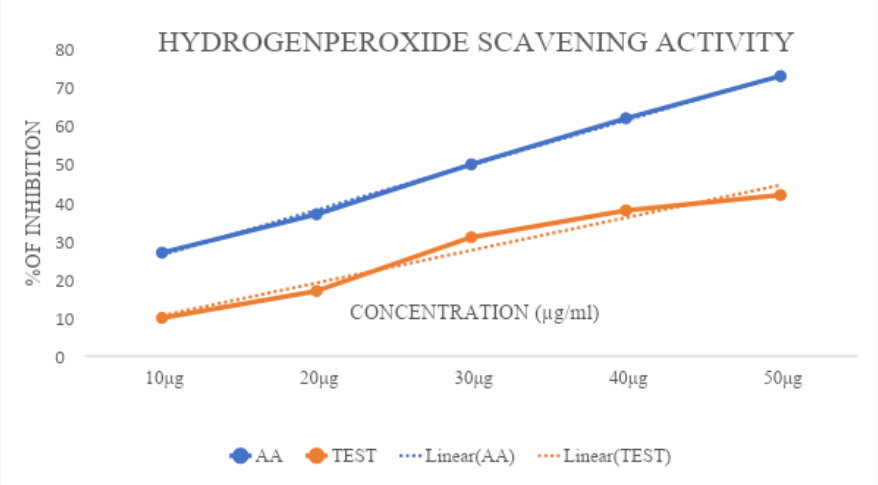
CONC	AA	TEST
100 µg	26	16
200 µg	39	20
300 µg	51	25
400 µg	72	41
500 µg	92	53
IC50	2.6	5.0





4.3. Hydrogen peroxide scavenging activity:

CONC	AA	TEST
10 µg	27	10
20 µg	37	17
30 µg	50	31
40 µg	62	38
50 µg	73	42
IC50	3.02	5.64



5. DISCUSSION:

The anti-oxidant property of plant *Hymenocallis littoralis* is determined by methods such as DPPH radical scavenging activity, Reducing power scavenging activity and the Phosphomolybdenum method, Hydrogen peroxide radical scavenging activity methods by comparing with that of the standard Anti-oxidant such as Ascorbic acid. Antioxidants from natural source can improve the antioxidant system in body for scavenging free radicals. An interest in antioxidant from natural sources increasing faster than synthetic sources. Phenolic compounds which naturally present in *H. littoralis* plant can reduce the risk of many diseases and its effects which co- related with antioxidant compounds. Recently there are some reports about *H. littoralis* which are rich in phenolic compounds such as flavonoids such as the rutin and quercetin. This study clearly shows that the antioxidant property of *H. littoralis* is more than that of the standard drug Ascorbic acid. It is proved by the values of the test and standards when compared for the IC50 values which are shown in results. Our study proves that ethanolic extracts of *Hymenocallis littoralis* has more potential as an antioxidant than that of the Ascorbic acid. *H. littoralis* is rich in anti-oxidant compounds like phenolic compounds as the major chemical constituents and hence showing the potent antioxidant activity. Still further work should be done to make of this plant for the production of safe and effective drug and make use of it effectively. Antioxidants neutralize the free radicals either by oxidation or reduction and make them harmless to the body. The various methods used for the determination of the antioxidant properties contains the compounds which form free radicals and our plant *H. littoralis* effectively inhibit the concentration of those free radicals more than that of the Ascorbic acid which is considered as the Standard in our study.

6. CONCLUSION:

The study on our plant clearly demonstrates that *Hymenocallis littoralis has* very effective Antioxidant property and reduce the free radicals and rescue the body system from damage. This helps us to get the effective treatment relating to this disease with fewer side effects but the further studies should be done on this plant to understand its mode of action. Our study helps to develop new effective and safe drugs upon further testing and identifying the other constituents present in the *H. littoralis*.

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