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COMPARATIVE EVALUATION FOR IN VITRO ANTIOXIDANT ACTIVITY OF *GYMNOSPORA MONTANA* LEAVES AND STEM

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ABSTRACT

Gymnospora Montana occurring throughout the arid, dry areas of India and is traditionally claimed to be useful in various ailments. A detailed review of literature afforded no more information on the in vitro antioxidant activity of the same plant. The main objective of this study was to evaluate the free radical scavenging activity of *G.Montana*leaves and stem by using DPPH radical scavenging method. For the present study ascorbic acid was used as reference standard and it shows IC_{50} value25 μg/ml. The extracts were prepared only in ethanol. The ethanol extracts of leaves and stem exhibited DPPH scavenging activity with IC_{50} value 34 μg/ml and 36 μg/ml respectively. Result suggests a close relations in between the ethanol extracts of leaves and stem of *G.Montana* antioxidant activity. Therefore it was concluded that ethanol extract of leaves and stem of *G.Montana* showed potent antioxidant activity. It can be used as antioxidant for the treatment of various diseases.

Key Words : - *Gymnospora Montana*, free radical, DPPH, antioxidant, ascorbic acid.

1. INTRODUCTION :-

Antioxidant are the compounds that neutralize free radicals which otherwise damages the body. Free radicals are chemically active product of metabolism andinclude reactive oxygen species or reactive nitrogen species. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and otherdisorders [1]. Various pathological conditionssuch as cancer, cataracts, chronic inflammation as well as diabetes mellitus, cardiovascular and neurodegenerative diseases believed to the associated with oxidative stress [2,3,4].

Synthetic antioxidants has been widely used. The incorporation of these synthetic antioxidants in food preparations have been questioned due to potential health risks, toxicity and

carcinogenicity [5,6]. It is increasingly being realized that a majority of the disease of today are due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant [7]. Plant material have evolved a wide range of mechanisms to contend with this problem, with variety of antioxidant molecules and enzymes.

In recent years much attention has been devoted to natural antioxidant and their association with health benefits and many plants exhaustively studied in the last few years for their antioxidant and radical scavenging activities [8]. *G.Montana*plant has been used traditionally and useful in treating ulcer, gastrointestinal disorders, toothache, dysentery [9], antispasmodic [10] and hepatoprotective [11] effects were also reported. A detailed review of literature afforded no information on the in-vitro antioxidant potential of the plant were investigated by using DPPH scavenging method.

Therefore, taking into consideration the vast potentiality of plants as sources for antioxidants a systematic investigation was undertaken to screen the local flora for radical scavenging activity. The leaves and stem of *G.Montana*were extracted in ethanol and tested free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ascorbic acid was used as antioxidant reference compound.

2. MATERIAL AND METHODS :-

2.1 Plant material: The leaves and stem of *G.Montana*were collected from Balaghat region of Beed district of Maharashtra.

2.2 Extraction : The leaves and stem of *G.Montana*were dried under shade and then powdered. The dried powdered material was subjected to extraction with ethanol. 10 gm of powdered plant material was dissolved in 100 ml of ethanol and kept on a magnetic stirrer for 24 hrs. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated out to dryness. The obtained material was stored at 4^0c in airtight bottles for further studies.

2.3 Evaluation of antioxidant activity :-

DPPH Method

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of DPPH (1.5 mg/ml in methanol) was prepared such that 75 μl of it in 3 ml of methanol. Decrease in the absorbance in the presence of sample extract at different concentration (10-125μgm/ml) was noted after 15 min. IC_{50} value was calculated from % inhibition.

Preparation of stock solution of the sample :-

10 mg of extract was dissolved in 10 ml of methanol to get 1000μg/ml solution. Then were prepared diluted test solution of different concentration as 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml from stock solution. 15 mg for DPPH solution was dissolved in 10 ml of methanol. The resulting solution was covered and protected from light.

75 μl of DPPH solution was taken and the final volume was adjusted to 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 75 μl of DPPH and 100 μl of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Absorbance at zero time was taken in UV-visible spectrophotometer at 517 nm for each concentration. Final decrease in absorbance of DPPH with sample of different concentration was measured after 15 minute at 517 nm.

Percentage inhibitions of DPPH radical by test compound were determined by the following formula.

$$\% \text{ Inhibitions} = \left(\frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \right) \times 100$$

IC_{50} value was calculated by using graphical method.

3. OBSERVATION :-

Antioxidant activity testing

Table.1. DPPH Free Radical Scavenging Activity of Ascorbic Acid.

Absorbance of the sample at 517nm Absorbance of Control = 0.490

Sr. No.	Concentration In (µg/ml)	Absorbance of Ascorbic acid	% Inhibition	IC_{50} Value (µg/ml)
1	10	0.295	39.79	25
2	20	0.270	44.89	
3	30	0.246	49.79	
4	40	0.225	54.08	
5	50	0.203	58.57	
6	60	0.184	62.44	
7	70	0.161	67.14	
8	80	0.142	71.02	
9	90	0.120	75.57	
10	100	0.087	82.24	

Table.2. Antioxidant Activity of Ethanol Extract of *G.Montana*(leaves)

Absorbance of the sample at 517nm Absorbance of Control = 0.494

Sr. No.	Concentration In (µg/ml)	Absorbance of Ascorbic acid	% Inhibition	IC_{50} Value (µg/ml)
1	10	0.289	41.49	34
2	20	0.273	44.73	
3	30	0.261	47.16	
4	40	0.249	49.59	
5	50	0.232	53.03	
6	60	0.219	55.66	
7	70	0.196	60.32	
8	80	0.181	63.36	
9	90	0.165	66.59	
10	100	0.156	68.42	

Table.3. Antioxidant Activity of Ethanol Extract of *G.Montana*(stem)

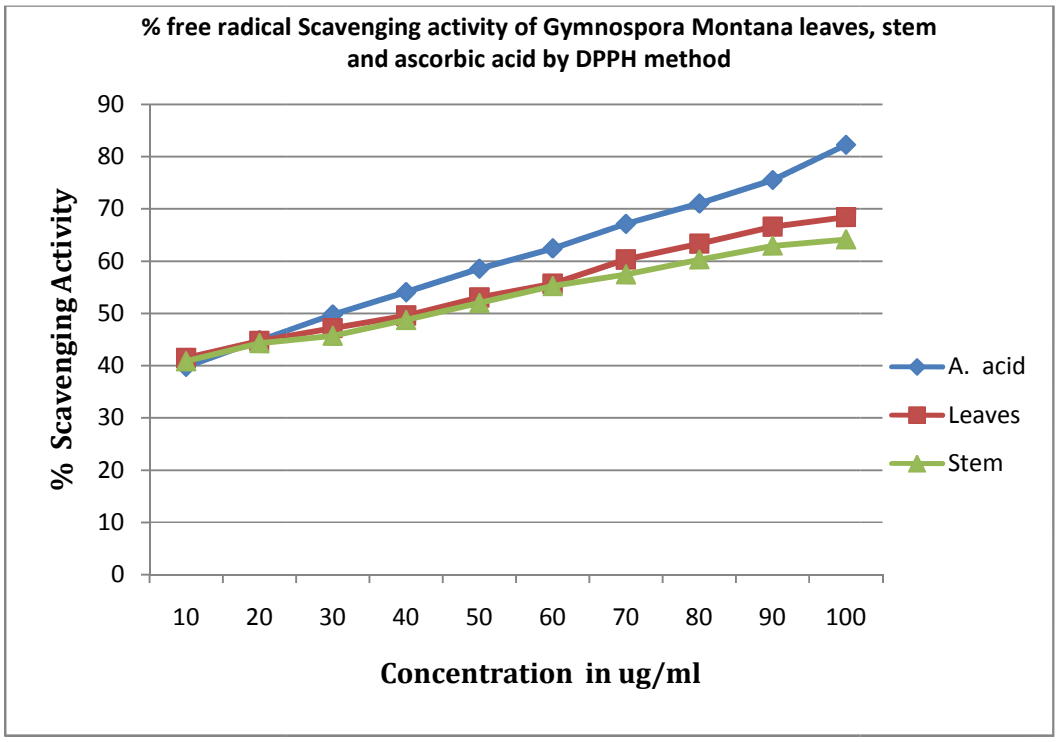
Absorbance of the sample at 517nm Absorbance of Control = 0.494

Sr. No.	Concentration In (µg/ml)	Absorbance of Ascorbic acid	% Inhibition	IC_{50} Value (µg/ml)
1	10	0.292	40.89	36
2	20	0.275	44.33	
3	30	0.268	45.74	
4	40	0.253	48.78	
5	50	0.237	52.02	
6	60	0.221	55.26	
7	70	0.210	57.48	
8	80	0.196	60.32	
9	90	0.183	62.95	
10	100	0.177	64.17	

4. RESULTS AND DISCUSSION :-

In vitro antioxidant activity of ethanol extracts of leaves and stem of *G.Montana*were tested for their antioxidant activity of DPPH scavenging method. Result shows that it has better antioxidant activity. It is observed that, phenolic compounds are responsible for antioxidant activity [12,13].

The phenolic compounds from *G.Montana* may be responsible for antioxidant activity. Phenolic compounds are effective hydrogen donors, which make them antioxidant [14]. The dose response curve of DPPH for extracts of *G.Montana* leaves and stem was compared with reference standard ascorbic acid as shown in figure.



DPPH scavenging activitywas found in ethanolicextracts of *G.Montana* leaves and stem. Compared to ascorbic acid the radical scavenging activities of both test samples has their IC_{50} values rangedfrom 34 and 36 µg/ml respectivelyin DPPH assay,(Table-2,3).

This indicates that ethanolicextracts of *G.Montana* leaves and stem hasgood potential as a source for natural antioxidants.Ascorbic acid was used as a standard. Which shows IC_{50} value of 25µg/mlin DPPH assay,(Table-1).

5. CONCLUSION :-

The results obtained in present study were indicated thatextracts of *G. montana* inhibits freeradical scavengingactivity. The overall antioxidant activity depends on itstriterpenoid and polyphenolic content and otherphytochemical constituents were present. It could be asource of natural antioxidant that could have greaterimportance as therapeutic agent in preventing or slowingoxidative stress related degenerative diseases.

It can be observed that ethanolic extract of leavesand stem of *G. montana* showed very closesignificant scavenging activity as compared to thereference standard ascorbic acid.

Therefore, it wasconcluded that ethanolic extract of *G. Montana* showed potent antioxidant activity.

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