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Evaluation Of Antioxidant Activity Of *Hemidesmus Indicus* By Three Different Invitro Methods

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Abstract : Free radicals are one electron short atoms, to quench their thirst of ion/ electron they attack neighboring molecule and makes it unstable, the chain continues to damage the cell, tissue, organ and leads to pathological condition. Hence there is the need for the antioxidants which breaks the chain of free radicals and also prevents the generation of free radicals. The herb Sariva(*Hemidesmus indicus*) is attributed with Rakta prasadana and Rasayana karma is screened for the antioxidant property by three different invitro methods namely, DPPH, Total Phenolic Content, Phosphomolybdenum Assay. The test drug Sariva (*Hemidesmus indicus*), both aqueous and alcoholic extracts were Shown positive result in all the three methods. Hence Sariva (*Hemidesmus indicus*) can be utilized as a one of the best sources of antioxidant as it can be used as beverage and also as a medicine.

***IndexTerms* -. Antioxidants, Free Radicals, Sariva, *Hemidesmus indicus*, In vitro study, Rakta Prasadana**

I. INTRODUCTION

Antioxidants are nature's way of protecting the body & cells from damaging free radicals. Antioxidants are compounds that inhibit the process of oxidation. Oxidation increases due to stress, anxiety and fast pace of life. In recent times, due to modernized, sedentary lifestyle, the society is suffering from many health hazards and one of the causative factors is free radical, which is the byproduct of the oxidation process. For that, everyone is looking for the antioxidants, which are very much costly and not abundantly available. So the paper is aimed to provide evidence of antioxidant properties of drugs by in vitro methods.

In this research work, the drug Sariva is subjected for the screening of antioxidant activity by in vitro method. Sariva having multifaceted activity & attributed with Rasayana & Rakta prasadana activity as per classics. It is also included under Varnya gana of Charaka Samhita[1,2]. With the evidence of references available in classical texts, Sariva can be a good source of Antioxidants.

Sariva, botanically identified as Hemidesmus indicus belongs to the Asclepidaceae family. It is a slender, twining, semi erect shrub. The parts used are the roots. Roots are woody and aromatic[3]. The roots are subjected for antioxidant activity by three different Invitro methods, namely DPPH, Total Phenolic Content and Phosphomolbdenum assay.

Materials & Methods

Invitro methods

1. DPPH Assay [4]

Procedure : This essay was carried out based on the method of Costa et al. (2012) and Plank et al. (2012) with slight modification.

0.1mM of methanolic DPPH stock solution was prepared freshly using 10 mg DPPH dissolved in 125 ml methanol in a 250 ml volumetric flask. A 0.4ml diluted sample or standard solution was added into test tube containing 5.6 ml Methanolic DPPH. Test tubes sealed with parafilm were incubated in water bath(Memmert, Germany) at 370 C for 30 min. The absorbance was measured against methanol(Blank) at 517 mm UV- Visual spectrophotometer (secomam Prim, France). Trolox calibration solutions of 50- 500 um concentration were used to generate the standard curve. The results were expressed as umol TE/ 100 ml.

2. Total Phenolic Content[5]

Procedure : The phenolic content of samples was determined spectrophotometrically according to Folin–Ciocalteu method with slight modification by Mahadavi et al. (2010) and singleton & Rossi (1965). An amount of 0.4 ml sample or standard solution was added into 10 ml volumetric flask, containing 3.6 ml of distilled water. Folin- Ciocalteu reagent(0.4) was added into the mixture. About 4ml of 7% sodium carbonate was also added following 5 min. The solution was made up to 10 ml with distilled water, mixed thoroughly and allowed to stand at room temperature for 90 min. The absorbance was measured at 765nm using UV-Visual spectrophotometer(Secomam Prim, France) against distilled water as blank. Calibration curve was plotted using Gallic acid standard solution of 0- 250 mg/L. The result was expressed gallic acid equivalent(mg GAE/ 100ml).

3. Phosphomolybdenum assay [6]

Procedure : The total antioxidant capacity of te fractions was determine by phosphomolybdenate method using ascorbic acid as standard. An aliquot of 0.1ul of sample solution was mixed with 1 ml of reagent solution. The tubes were capped & incubated in a water bath at 95^0 C for 90 min. After the samples had cooled at room temperature, the absorbance of the mixture was measured at 765nm against blank. A typical blank obtained 1 ml of reagent solution & the appropriate volume of the solvent * incubated under the same conditions. Ascorbic acid was used as standard. The antioxidant capacity was estimated using formula,

$$\% = \frac{\text{(Control absorbance - Sample absorbance)} \times 100}{\text{Control absorbance}}$$

Observations & Results

1. Results of DPPH Assay

DPPH	**Vitamin C**	**Hemidesmus AQ**	**Hemidesmus Al**
1 µg	39.069	36.292	36.460
2 µg	40.970	37.566	37.285
4 µg	41.620	38.028	37.378
8 µg	42.121	38.726	37.740
10 µg	44.206	39.092	38.636
20 µg	46.320	39.237	39.234
40 µg	47.687	39.709	39.470
80 µg	48.078	40.288	40.194
100 µg	65.169	40.786	42.037
200 µg	80.320	40.970	43.717
400 µg	90.428	41.464	46.869
800 µg	95.096	42.260	48.233
1000 µg	95.154	43.045	57.058

2. Results of Total Phenolic Assay

Total phenolic content	**Gallic acid**	**Hemidesmus Aq**	**Hemidesmus Al**
1 µg	0.00825	1.700	3.425
2 µg	0.00865	2.025	3.625
4 µg	0.01365	2.225	4
8 µg	0.02525	2.525	4.375
10 µg	0.02685	2.950	4.65
20 µg	0.0492	3.150	5.65
40 µg	0.11615	3.350	11.4
80 µg	0.2077	4.325	17.8
100 µg	0.25615	6.325	43.425

3. Phosphomolybdenum Assay

Table of results

Conc. (µg/ml)	Hemidesmus indicus (AQ)	Hemidesmus indicus (AL)
1	0.035	13.339
2	1.972	14.357
4	2.806	15.393
8	3.597	17.000
10	4.125	24.214
20	5.028	25.464
40	8.486	29.500
80	10.806	31.196
100	12.653	39.464
200	28.056	51.625
400	44.958	96.964
800	75.694	146.821
1000	89.736	179.286

Discussion

In the present research work, the *Hemidemus indicus* is subjected for antioxidant activity by in vitro methods, DPPH Assay, Total Phenolic

Content & phosphomolybdenum Assay are the methods selected. Both aqueous & alcoholic extracts showed the presence of Antioxidant properties in all these methods.

DPPH Assay : In DPPH assay, gallic acid as benzoic derivatives with hydroxyl group is identified as potent radical scavengers, flavonoids, quercetin & Myrcitin which have additional hydroxyl group are identified as good scavengers and flavanones are poor in radical scavenging in DPPH assay. The results of *Hemidemus indicus* in DPPH assay showed the antioxidant property but poor compared to the standard Vitamin C. According to the chemotaxonomy of *Hemidemus indicus* , it is rich with flavanones, and flavanones are poor performers in DPPH assay, hence the results showed lesser compared to Standard Vitamin C. **Total Phenolic Content :** The assay was used to assess the presence of phenolic compounds in the test sample. Higher phenolic content is expected to exhibit good results in antioxidant activity, as the phenols are known for its free radical inhibition action. In the result *Hemidesmus indicus* shows 6.325 & 43 Gallic acid equivalent in 100 ug of both aqueous & alcoholic extraction. Phenols are both aqueous & alcoholic soluble compounds, in the present work the alcoholic extraction shows higher phenolic content than the aqueous extract.

Phosphomolybdenum Assay : This assay works on the principle of reduction of Mo VI to MO V. This reduction occurs by receiving ions from the sample. Both aqueous & alcoholic extraction showed good antioxidant activity. Amongst them alcohol showed good response compared to aqueous extract. This may be due to phytochemicals like Phenols, Saponins & flavonoids are known for the reduction of free radicals & the most of them are alcohol soluble.

Conclusion

Sariva, the drug mentioned in Ayurvedic classics as varnya & pittashamaka . It is attributed to Raktaprasadana karma. With these references the drug is been screened for antioxidant activity by these different in vitro methods. In all the methods the drug Hemidesmus indicus showed positive results, the extent of the activity compared to the standard in all three methods varies. This is due to the chemical constituents reacting in different invitro methods selected, as in DPPH Hemidesmus indicus shows less antioxidant activity compared to standard because in DPPH flavanones are poor performers, where as in Total Phenolic Content, it showed higher phenolic content hence proved to be good antioxidant drug. In phosphomolybdenum assay reduction of ion occurs because of phenols, saponins, hydroxyl compounds. Thus it can be concluded that Hemidesmus indicus can be a good source of antioxidants.

REFERENCES

1. Agnivesha, Charaka Samhita Vol- 1 (revised by charaka and Dridhabala) with commentary of Chakrapanidatta, Edited by Acharya Vidhydhara Shukla and Ravidatta Tripathi, Varanasi: Chaukhamba Sanskrit Pratishtan.
2. Sushrutha, Sushrutha Samhita Ayurveda tatva sandeepika, hindi commentary, Kaviraj Dr Ambika datta shastry, Edition 2007 published by Chaukhamba Sanskrit Pratishtan
3. J L N Shastry, Dravyaguna Vignana Part 2, Chaukhamba Orientalia. Reprint 2014, pp348
4. Prieto. (1999)
5. Sulaiman.CT, and Indira Balachandran. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J.pharm.Sci.2012: 74(3):258-260
6. Halliwell and Gutteridge, 1981