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Research Article

ANTIOXIDANT ACTIVITY OF FEW PHYTOESTOGENS BY DPPH ASSAY

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Abstract:

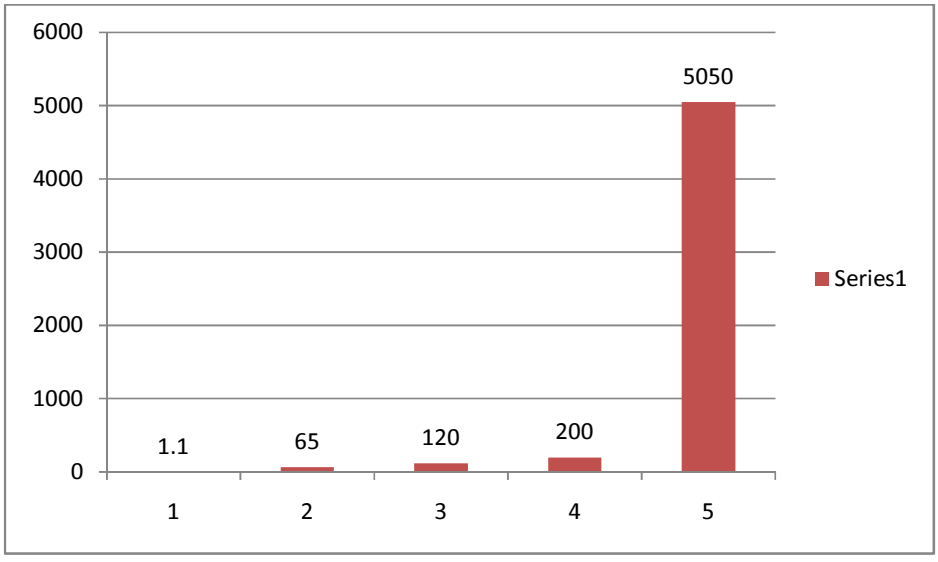
Introduction: Phytoestrogens are the compounds derived from the plant sources which produce responses similar to the natural estrogens due to the structural and functional similarity with the natural estrogens.

Mthodology: In the present study the antioxidant activity of *Asparagus racemosus, Saraca asoka, Diascorea bulbifera and Ipomoea digitata* are studied using DPPH Assay by using ascorbic acid as standard at 517nm.

Results: The IC_{50} values of slected plants was *Ascorbic acid* 1.1µg, *Saraca asoka* 65 µg, *Diascorea bulbifera* 120 µg, *Asparagus racemosus* 200 µg and I*pomoea digitata* 5050 µg in ascending order of IC_{50} Values with the significance *P value* is <0.001.

Conclusion: The free radical scavenging potential varies in comparison to standard Ascorbic acid The IC_{50} values of selected plants was *Ascorbic acid*, *Saraca asoka*, *Diascorea bulbifera*, *Asparagus racemosus* and I*pomoea digitata* in ascending order of IC_{50}Values.

Graphycal Abstract:



Key Words: DPPH Assay, Asparagus racemosus, Saraca asoka, Diascorea bulbifera, Ipomoea digitata, Phytoestrogens.

Introduction:

Oxidative stress plays a key role in the prognosis of the various diseases. An imbalance between the free radicals and antioxidant leads to oxidative stress. DPPH Assay is a very reliable and effective method to know the in-vitro Antioxidant activity of the plant extracts. DPPH Assay is based on the free radical scavenging effect of the extracts was assessed by the discolorations of the alcohol solution of DPPH which is measured at 517nm spectrophotometrically (A. M. Prokhorov 1936). Phytoestrogens are the compounds derived from the plant sources which produce responses similar to the natural estrogens due to the structural and functional similarity with the natural estrogens.

Materials:

Ascorbic acid (Sigma-Aldrich.), DPPH (Sigma-Aldrich.), Conc.HCl (Qualigen), Tris Buffer (SD.Fines.), Absolute alcohol (SD.Fines) Spectrophotometer (Lab India U.V. 3000+).

Methodology:

Asparagus racemosus, Saraca asoka, Diascorea bulbifera, Ipomoea digitata are selected based on the phytoestrogenic properties. Selected plants are procured and authenticated by botanist, dried material is the subjected for the size reduction and extracted with absolute alcohol using rota evaporator. The extracts are subjected to the Free radical scavenging activity by DPPH Assay.

DPPH: 0.5mM of DPPH is prepared by dissolving 4.8mg of the DPPH in 25ml of absolute alcohol. DPPH solution should be made freshly and should be kept in dark (Blois 1958). TRIS BUFFER: Dissolve 1.21gm of Tris Hcl in distilled water about 75ml by adjusting pH 7.4 with dilute sodium hydroxide make up the volume 100ml with distilled water.

The potential antioxidant activity of the above extracts has been determined in comparison with the standard preparation ascorbic acid by calculating IC_{50} values. 1mg/ml stock solution is prepared by dissolving 100mg of ascorbic acid in demineralised water. Aliquot samples are taken from the stock solution to plot the standard graph ranging from the 1µg to 10µg solutions. The volume is made to 1ml with Tris Hcl buffer and 1ml of the DPPH solution. The above solutions are allowd to stand for 20min in the dark. After 20min the absorbance of the solutions is measured at 517nm. Similarly trail (n=6) is conducted for the test samples.

Results:

The results of the DPPH Assay are represented with reference to Ascorbic acid as the standard. Table 1: shows the results of standard ascorbic acid and %inhibition respectively. Table 2, 3, 4& 5: show the results of test samples of Saraca Asoka, Diascorea bulbifera, Asparagus racemosus and Ipomoea digitata and % inhibition where as Table 6 shows the IC_{50} of the standard and the test samples. Figure 1 show the comparative bar diagram of the test sample with reference to the standard ascorbic acids IC_{50} value.

Table 1: Standardisation and % Inhibition of Ascorbic Acid.

S.NO	CONC µg	MEAN ± S.D	%INHIBITION
1	1	1.226 ±0.173	42.87
2	2	1.247 ±0.178	41.89
3	4	1.638 ±0.101	23.67
4	6	1.259 ±0.196	41.33
5	8	1.191 ±0.251	44.5

Table 2: Standardisation and % Inhibition of Saraca Asoka.

S.NO	CONC µg	MEAN ± S.D	%INHIBITION
1	10	2.094 ±0.030	42.87
2	20	2.154 ±0.020	41.89
3	40	2.073 ±0.044	23.67
4	60	1.966 ±0.060	41.33
5	80	1.822 ±0.054	44.5
6	100	1.393 ±0.055	35.08

Table 3: Standardisation and % Inhibition of Diascorea Bulbifera.

S.NO	CONC µg	MEAN ± S.D	%INHIBITION
1	100	1.05 ± 0.102	51.07
2	200	1 ±0.097	53.4
3	400	1.084 ±0.218	49.48
4	600	1.475 ±0.719	32.028
5	800	0.77 ±0.067	64.119
6	1000	0.794 ±0.030	63.187

Table 4: Standardisation and % Inhibition of Asparagus Racemosus

S.NO	CONC µg	MEAN ± S.D	%INHIBITION
1	100	1.388 ±0.052	35.32
2	200	1.271 ±0.226	40.77
3	400	1.305 ±0.041	39.189
4	600	1.429 ±0.342	33.41
5	800	1.23 ±0.641	42.68
6	1000	1.303 ±0.086	39.28

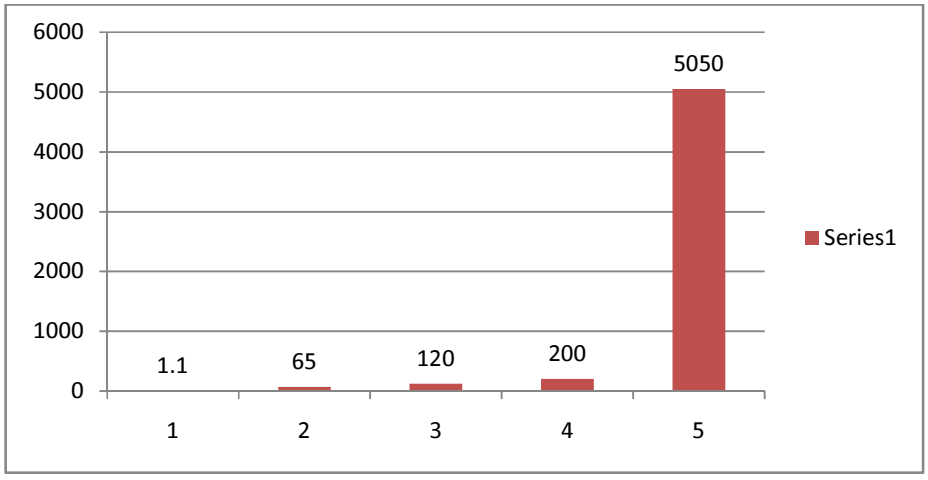
Table 5: Standardisation and % Inhibition of Ipomoea Digitata

S.NO	CONC mg	MEAN ± S.D	%INHIBITION
1	2	2.063 ± 0.002	3.86
2	4	2.021 ±0.001	5.82
3	8	1.921 ±0.001	10.48
4	12	1.505 ±0.003	29.86
5	16	1.633 ±0.002	23.9
6	20	1.514 ±0.002	29.45

Table 6: Comparative IC_{50} Value of Ascorbic Acid and the Test Samples.

S.NO	SAMPLE	IC50 VALUE µg
1	ASCORBIC ACID	1.1
2	SARACA ASOKA	65
3	DIASCOREA BULBIFERA	120
4	ASPARAGUS RACEMOSUS	200
5	IPOMOEA DIGITATA	5050

Figure 1: Comparative IC_{50} Value of Ascorbic Acid and the Test Samples



1. *Ascorbic acid (Standard), 2. Saraca asoka, 3. Diascorea bulbifera, 4. Asparagus racemosus and 5. Ipomoea digitata* and their respective IC_{50} value with the significance P value is <0.001.

Discussion:

DPPH Assay is the invitro antioxidant study for the estimation of the free radical scavenging capacity of any compound that has a potential to act as the antioxidant. The compounds under study have shown that they have a capacity exhibit free radical scavenging activity. However, there is a variation on the IC_{50} value which is the

differentiating factor and tells about the varying potential between the selected agents. The descending order of the potential is Saraca Asoka, Diascorea bulbifera, Asparagus racemosus and Ipomoea digitata respectively.

Saraca Asoka showed the free radical capacity close to the standard ascorbic acid. While Diascorea bulbifera, Asparagus racemosus and Ipomoea digitata showed a greater IC_{50} far from the standard. However, it is found that the antioxidant capacity of the test compounds is less that the standard.

This study can substantiate the role of these compounds as antioxidants and also can be the basis for their mechanism of action in the models that has a significant role of the oxidative stress in their pathological prognosis.

References:

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