Antioxidant and Free-Radical-Scavenging Activity of *Sarcostemma Brevistigma*, Wight & Arn. - An Ethnomedicinal Plant

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Abstract: ***The plant Sarcostemma brevistigma (family-Asclepiadaceae) commonly known as "Kodikali" is used in many ayurvedic formulations. The present study was designed to evaluate the in vitro antioxidant potential of an ethanolic extract of Sarcostemma brevistigma. In vitro antioxidant activity was carried out by the reducing power assay, DPPH radical scavenging activity, Hydroxyl radical scavenging activity, Nitric oxide radical inhibition assay, Superoxide radical scavenging activity, Phosphomolybdenum assay and Determination of total phenolic content. The antioxidant activities of the study plant were found to increase with an increase in concentration. The extract exhibited the maximum reductive capacity, which was more or less equivalent to standards. The ethanolic extract (10, 20, 40, 60, 80 & 100 µg/ml) was compared with Ascorbic acid and Quercetin as a standard. The results of the present study clearly indicated that an ethanolic extract of Sarcostemma brevistigma possesses powerful in vitro antioxidant activity.***

Keywords: *Sarcostemma brevistigma*, free radicals, reducing power assay, phospohomolybdenum assay and Quercetin

1. Introduction

The use of plant-based drugs and chemicals for curing various ailments and personal adornment is as old as human cultivation. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments (Yadav and Khan, 2012). The history of the discovery and use of different medicinal plants is as old as the history of the discovery and use of plants for food (Ibrar, 2002). In the past, almost all the medicines used were from plants, with the plant being man's only chemist for ages. A study by the WHO depicts that over 80% of the world's population directly depends on the natural diversity and its associated traditional system of medicine for their primary healthcare demands (WHO, 2000). Today, a vast store of knowledge concerning the therapeutic properties of different plants has been accumulated (Evans *et al*., 2002).

Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health (Azaizeh *et al*., 2003; Gurib-Fakim, 2006). Bitters for example, are known to stimulate digestion, while phenolic compounds could be responsible for the anti-inflammatory and anti-oxidative activity of plant extracts. (Torras *et al*., 2005; Afolayan *et al*., 2007; Diouf *et al*., 2009). The use of herbal remedies as alternative medicine plays a significant role in the cultures and beliefs of the indigenous populations of South Africa (Hutchings *et al*., 1996). However, there is a dearth of information on the efficacy and more importantly, the toxicity of these remedies (Steenkamp *et al*., 2005). There is therefore a need for more scientific validation of these claims.

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes (Desai *et al*., 2008). The condition is brought by excessive generation of free oxygen and nitrogen species or their inefficient quenching in the cell. Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body (Craig, 1999). Exogenous sources of free radicals include cigarette smoke, exposure to ozone, ionizing radiation such as X-rays, and drugs, among others. On the other hand, endogenous sources of free radicals include the electron transfer chain reactions in the mitochondria, xanthine oxidase pathway, during disease states such as inflammation, ischemia, and reperfusion injury (Manda Spring, 2010).

With the advancement of science and a great understanding of chemistry, man was able to extract a great variety of pharmaceuticals that were previously unknown to the scientific world or unidentified. The science of pharmaceutical chemistry blossomed at the beginning of the 20th century with the synthesis of scores of complex organic compounds and the production of chemotherapeutics. Nevertheless, medicinal plants and herbal drugs have advantages over synthetic chemical compounds. Active constituents in plants are always biologically balanced, do not usually accumulate in the body and are capable of even neutralizing the harmful effects of other chemical compounds. As such, medicinal plants and their bioactive ingredients are once again attracting the attention of physicians, pharmacists and researchers.

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The majority of diseases / disorders are mainly linked to oxidative stress due to free radical formation. Free radical generating substances can be found in the food we eat, the drugs and medicines we take in, the air we breathe and the water we drink. They include a number of external and internal factors. These free radicals are implicated in more than 80 harmful diseases, including diabetes, cancer and ageing (Sangameswaran *et al*., 2009).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals, these radicals can start chain reactions that can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions. Plants are known to have potent antioxidant properties. Phenolic compounds and flavonoids are widely distributed in plants and have been reported to exert multiple biological effects, including antioxidant and free radical scavenging abilities (Buyukokuroglu *et al*., 2001). Current research is directed towards the discovery of naturally occurring antioxidant substances of plant origin.

2. Materials and Methods

Collection and identification of plant materials

The plant *Sarcostemma brevistigma* was collected from Pillur Beat (Pillur slope RF and Nellithurai RF), Karamadai Range, Western Ghats, Tamil Nadu, and India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, and Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2015/Tech./2334. The aerial parts of this species was washed under running tap water, shade dried at room temperature and powdered.

***In-Vitro* antioxidant studies**

Reducing power assay (Oyaizu, 1986)

The reducing power was determined according to the method of Oyaizu (1986). Various concentrations of ethanolic extract (2.5 ml) were mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 ºC for 20 min. Then 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionised water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as standard.

DPPH radical scavenging activity (Rajesh and Natvar, 2011)

Reagents

- Diphenyl-2-picrylhydrazyl (DPPH)
- Methanol

Principle:

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH*with an odd electron gives a maximum absorption at 517 nm (purple colour). When antioxidants react with DPPH*, which is a stable free radical; it becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPHH and as a result, the absorbance is decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, it gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Procedure

The hydrogen donating ability of extracts was examined in the presence of DPPH stable radical. One millilitre of 0.3 mm DPPHethanol solution was added to 1 ml of MEAL (1000 µg/ml) at different concentration and allowed to react at room temperature. After 30minutes the absorbance values were measured at 517 nm. Ethanol solution was used as a blank and DPPH solution (1.0 ml, 0.3 mm) with 1 ml ethanol served as negative control. Ascorbic acid (1000 µg/ml) was taken as the positive control. The capability to scavenge the DPPH radical was calculated using the following equation

$$\%\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}\ X\ 100} \qquad \text{------ (1)}$$

Where ‘A $_{control}$’ was the absorbance of the control reaction and ‘A $_{test}$’ was the absorbance in the presence of the extract/standard. The mean values were obtained from triplicate analysis. The antioxidant activity of the extract was expressed as IC_{50}.

Hydroxyl radical scavenging activity (Kumar *et al*., 2008)

Solutions of 1 mM Deoxy Ribose and 0.2 mM phenyl hydrazine hydrochloride were prepared in 50 mM phosphate buffer (pH 7.4). Deoxy ribose 0.6 ml (1 mM) and 0.4 ml of extract solution (Different concentrations) were mixed and the volume was made upto 1.6 ml using phosphate buffer. The tubes were then incubated for 10 min. then 0.4 ml of 0.2 mM phenyl hydrazine hydrochloride was added and incubated for 1 h and 1 ml each of 2.8% TCA and 1% TBA were added. The mixture was then heated on a boiling water bath for 10 min and cooled and then the absorbance was measured at 532 nm. Ascorbic acid was taken as a standard. The percentage inhibition was calculated and compared with the standard.

Nitric oxide radical inhibition assay (Chakraborthy, 2009)

Nitric oxide radical inhibition was estimated by the use of Griess Illosvoy reaction. In this investigation, Griess Illosvovy reagent was generally modified by using naphthyl ethylene diamine dihydrochloride (0.1 % w/v) instead of the use of 1- naphthylamine (5 %). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and the extract (10-50 µg/ml) standard solution (rutin, 0.5 ml) were incubated at 25ºC for 150 min.

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A control test compound equivalent amount of ethanol was taken. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml sulfanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotization.

Further, 1 ml of the naphthyl ethylene diamine dihydrochoride was added, mixed and was allowed to stand for 30 min at 25^{0}C. The concentration of nitrite was assayed at 540 nm and was calculated with reference to the absorbance of the standard nitrite solutions. Ascorbic acid was taken as a standard. The percent inhibition was calculated using the formula:

% inhibition = [(A cont - A test) / A cont] X 100

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of samples with the extracts.

Superoxide radical scavenging activity (Liu *et al.*, 1997)
Superoxide radical scavenging activity is generally based on the anion radical which is associated with PMSNADH system. The measurement of superoxide scavenging activity is based on method as described by Liu *et al.* (1997) with slight modifications. They are generated within PMSNADH systems by the oxidation of NADH and are assayed by the reduction of nitroblue tetrazolium (NBT). Po4 buffer (100 μM, pH 7.4) containing 1 ml NBT (156 μ M) solution, 1 ml NADH (468 μ M) solution on and a sample solution of extract (20-100 μg/ml) in methanol were mixed. The reaction was started when 0.1 ml of phenazine methosulfate (PMS) solution (60 μM) was added to the mixture. The reaction mixture was incubated at 25ºC for 5 min, and the absorbance was read at 560 nm against the corresponding blank samples. Ascorbic acid was used as a reference drug. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity.

Phosphomolybdenum assay (Prieto *et al.,* 1999)
The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex. An aliquot of 0.2 ml of sample solution (20-100μg/ml) was combined in a vial with 2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95ºC for 30 min. After incubation the samples were cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

Determination of total phenolic content (Singleton and Rossi, 1965)
Phenolic compounds concentration in the ethanolic extract was estimated by a colorimetric assay, based on procedures described by Singleton and Rossi with some modifications (Singleton & Rossi, 1965). Briefly, 1 ml of sample was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (Shimadzu UV - 1700 UV-Visbile Spectrophotometer). Quercetin was used to calculate the standard curve (0.01– 0.4 mM). Estimation of the phenolic compounds was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg of quercetin equivalents/g of extract.

Calculation of 50% inhibitory concentration (IC_{50})
The concentration (mg/ml) of the ethanol extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at six different concentration of the extract. Percentage inhibition was calculated using the formula:

Percentage inhibition = (Ac-As/Ac) × 100

Where,
Ac – absorbance of the control
As – absorbance of the sample

3. Results

***In vitro* Antioxidant Activity**
In this study, the various antioxidant assays such as, reducing power assay, DPPH radical scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging, superoxide radical scavenging, phosphomolybdenum and total phenolic content on the radical scavenging assay were evaluated *in vitro* in an ethanolic extract of *S. brevistigma.*

Reducing power assay
Reducing power assay is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe+3) to form potassium ferrocyanide (Fe+2), which then reacts with ferric chloride to form ferrous complex, that has an absorption maximum at 700nm. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table – 1 and Fig.- 1 showed the reducing power potential of the test plant in comparison with the standard drug ascorbic acid. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The reductive ability will be indicated by the increase in absorbance. The IC_{50} value of the test plant in ethanol extract was 10.99μg/ml. This value is comparable with the IC_{50}value of the standard (31.85μg/ml) ascorbic acid. The reducing power of the study plant was found to be dose dependent and the increase in the percentage of absorbance was also dose dependent. The results have proved the effectiveness of the ethanol extract as compared to the standard ascorbic acid.

DPPH$^•$ (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity
The DPPH is a stable organic free radical which has been extensively used for evaluating the free radical scavenging potential of natural antioxidants. Table – 2 indicates the DPPH$^•$ scavenging activity of ethanolic extract of *S. brevistigma* at six different concentrations in the reaction mixture. The extract exhibited dose dependant increase in activity. The percentage of DPPH$^•$ radical scavenging activity of the test plant ranged between 18% and 87%. As shown in Fig.- 2,the IC_{50} values of the plant (Ethanol) extract and standard (Ascorbic acid) on DPPH$^•$radical scavenging

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activity were 64.21µg/ml and 33.27µg/ml respectively. The highest DPPH radical scavenging potential as indicated by the lowest IC_{50} concentration was recorded for ethanol extract. The extract had a enhanced hydrogen donating ability with an IC_{50} value of 64.21µg/ml and the value was found to be higher than the standard (IC_{50} value of 33.27µg/ml), ascorbic acid.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was assessed by generating the hydroxyl radicals using ascorbic acid as standard. Table-3 and Fig.- 3 showed the hydroxyl radical scavenging ability of ethanolic extract of *S. brevistigma*. The hydroxyl radical scavenging activity (10, 20, 40, 60, 80 and 100µg/ml) was analyzed and the results were reported in terms of IC_{50} values. The extracts exhibited concentration dependent scavenging activities against the hydroxyl radicals generated during the reaction system using phenyl hydrazine hydrochloride. The IC_{50} values of the extract (Ethanol) and standard (Ascorbic acid) were 52.27µg/ml and 11.37µg/ml, respectively. The lowest IC_{50} value of the plant extract showed the potential of the extract for hydroxyl radical scavenging. The percentage inhibition of ethanolic extract of *S. brevistigma* on hydroxyl radical scavenging activity was 53%, 56%, 58%, 60%, 63% and 64% at the concentrations of 10, 20, 40, 60, 80 and 100µg/ml respectively.

Nitric oxide radical scavenging activity

The nitric oxide radicals are generated from sodium nitroprusside and found to be inhibited by ethanolic extract of *S. brevistigma*. The nitric oxide radical scavenging activity of ethanolic extract at different concentrations (10, 20, 40, 60, 80 and 100µg /ml) were analyzed in terms of IC_{50} value. The extracts moderately inhibited nitric oxide in a dose dependent manner (Table- 4 & Fig.- 4). The IC_{50} values of the test plant extract and the standard were respectively 69.34µg/ml and 57.61µg/ml. The extract exhibited radical scavenging activity between 16% and 78%. The ethanol extract appears to be more potent oxide scavenger, exhibiting the lowest IC_{50} value. This value is comparable with the IC_{50}value of the standard (57.61µg/ml). The percentage inhibition of the extract was compared with Ascorbic acid and found that the percentage inhibition was more than the standard. The above results revealed that ethanolic extract of *S. brevistigma* showed a significant antioxidant activity.

Superoxide radical scavenging activity

Superoxide anion radical ($O_2\cdot^-$) is generated by four-electron reduction of molecular oxygen into water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide radicals ($O_2\cdot^-$) are also formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils and the production of $O_2\cdot^-$ is an important factor in the killing of bacteria by phagocytes. The ethanol extract of S. *brevistigma* was a potent scavenger of superoxide anion and the results are presented in the Table- 5 & Fig.- 5. The IC_{50} value of ethanol extract of *S. brevistigma* is 31.43µg/ml. The standard ascorbic acid showed higher superoxide scavenging activity with an IC_{50} value of 14.81µg/ml. The plant extract had more scavenging potential when compared to the standard drug ascorbic acid. It was evident from the lowest IC 50 value of the test plant.

Phosphomolybdenum assay

Phosphomolybdenum assay is based on the reduction of Mo (VI) – Mo (V) and forms green colour Phosphomolybdenum (V) complex, which showed maximum absorbance at 695nm. Table - 6 revealed the antioxidant capacity of ethanolic extract of *S. brevistigma* and standard ascorbic acid. From Fig. - 6, it was found that the absorbance of the extract increased with an increase in concentrations. Total antioxidant capacity was reported as ascorbic acid equivalents. The total antioxidant capacity of the ethanolic extract was 59.62µg/ml. The ethanolic extract exhibited the highest antioxidant capacity for phosphomolybdate reduction when compared with standard drug.

Total phenolic radical scavenging activity

Phenolics are the most wide spread secondary metabolites in the plant kingdom. Total phenolic radical scavenging activity of the study plant was increased markedly with the increase in concentration (Table 7). The percentage inhibition of the plant extract and the standard ranged from 21% and 88%. As shown in Fig.-7, the IC_{50} values of the plant extract on total phenolic radical scavenging activity was39.09 µg/ml (ethanol extract). This value is comparable with the IC_{50} value of the standard Quercetin (16.57µg/ml).

Table 1: Reducing power assay of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (ascorbic acid)		Ethanol Extract	Ethanol Extract
		O.D Value	Percentage inhibition	O.D Value	Percentage inhibition
1	10	0.66 ± 0.002	26 %	0.46 ± 0.001	48 %
2	20	0.72 ± 0.002	18 %	0.57 ± 0.001	35 %
3	40	0.80 ± 0.002	9 %	0.75 ± 0.001	16 %
4	60	1.00 ± 0.002	13 %	0.97 ± 0.001	10 %
5	80	1.21 ± 0.002	36 %	1.17 ± 0.001	32 %
6	100	1.44 ± 0.032	62 %	2.00 ± 0.001	12 %
	Ic_{50} values		Ic_{50}= 31.85µg/ml		Ic_{50}= 10.99µg/ml

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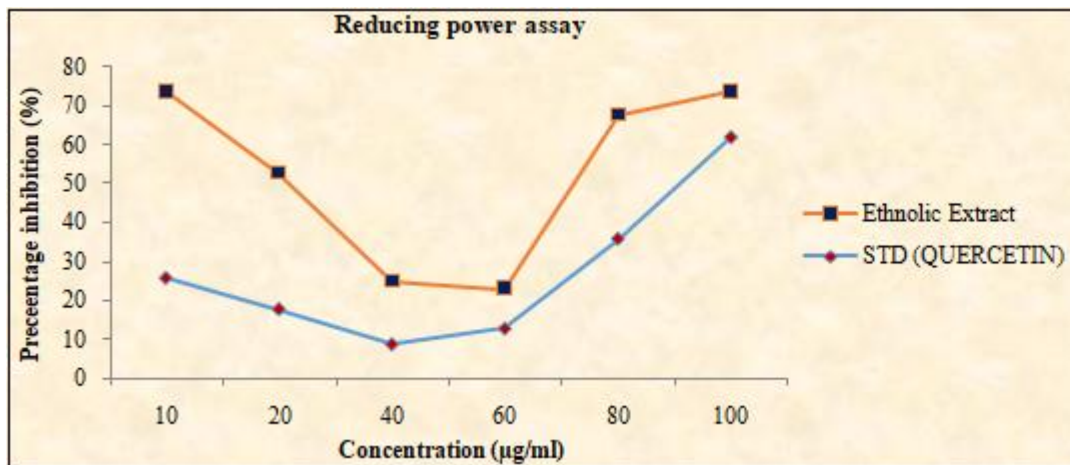

Figure 1: Reducing power assay of ethanolic extract of *S. brevistigma*

Table 2: DPPH˙ radial scavenging activity of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (ascorbic acid)		Ethanol Extract	Ethanol Extract
		O.D Value	Percentage inhibition	O.D Value	Percentage inhibition
1	10	1.288 ± 0.002	16 %	1.527 ± 0.002	37 %
2	20	0.782 ± 0.003***	30 %	1.195 ± 0.002***	74 %
3	40	0.306 ± 0.002***	72 %	1.985 ± 0.002***	78 %
4	60	0.075 ± 0.001***	93 %	1.727 ± 0.002***	55 %
5	80	0.047 ± 0.003***	96 %	0.456 ± 0.002***	59 %
6	100	0.032 ± 0.001***	97 %	0.347 ± 0.002***	68 %
	Ic_{50} values		Ic_{50}=33.27 µg/ml		Ic_{50}= 64.21µg/ml

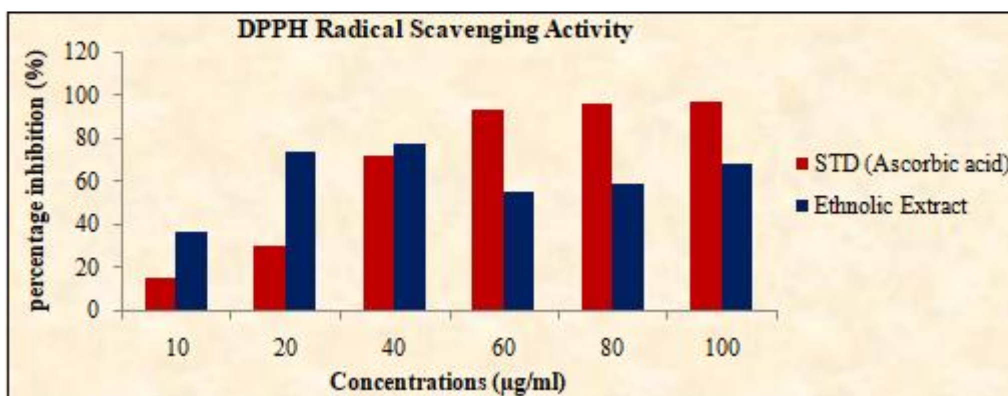

Figure 2: DPPH radial scavenging activity of ethanolic extract of *S. brevistigma*

Table 3: Hydroxy radial scavenging activity of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (ascorbic acid)		Ethanol Extract	
		Absorbance Value	Percentage inhibition	Absorbance Value	Percentage inhibition
1	10	0.162 ± 0.002	64 %	0.216 ±0.002	53 %
2	20	0.156 ± 0.001	66 %	0.203 ±0.002	56 %
3	40	0.147 ± 0.002	68 %	0.194 ±0.002	58 %
4	60	0.140 ± 0.001	70 %	0.186 ±0.002	60 %
5	80	0.132 ± 0.002	71 %	0.172 ±0.002	63 %
6	100	0.121 ± 0.001	74 %	0.166 ±0.001	64 %
	Ic_{50} values		Ic_{50}=52.27µg/ml		Ic_{50}=11.37 µg/ml

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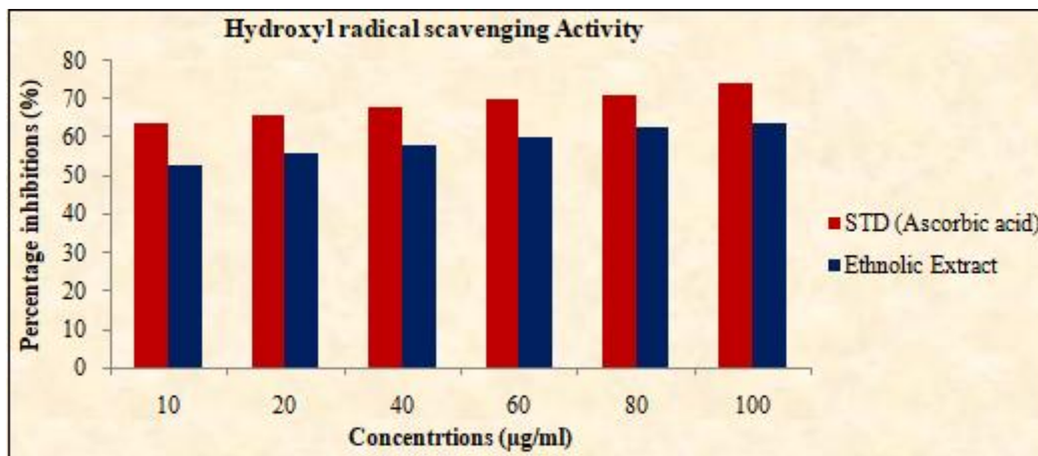

Figure 3: Hydroxy radial scavenging activity of ethanolic extract of *S. brevistigma*

Table 4: Nitric oxide radial scavenging activity of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (ascorbic acid)		Ethanol Extract	Ethanol Extract
		O.D Value	Percentage inhibition	O.D Value	Percentage inhibition
1	10	0.562 ± 0.002	28 %	0.657 ±0.002	16 %
2	20	0.482 ± 0.002	38 %	0.602 ±0.002	23 %
3	40	0.436 ± 0.002	44 %	0.453 ±0.001	42 %
4	60	0.416 ± 0.002	47 %	0.437 ±0.002	44 %
5	80	0.384 ± 0.003	51 %	0.215 ±0.002	72 %
6	100	0.312 ± 0.002	60 %	0.176 ±0.002	78 %
	Ic_{50} values		Ic_{50}= 57.61 µg/ml		Ic_{50}=69.34 µg/ml

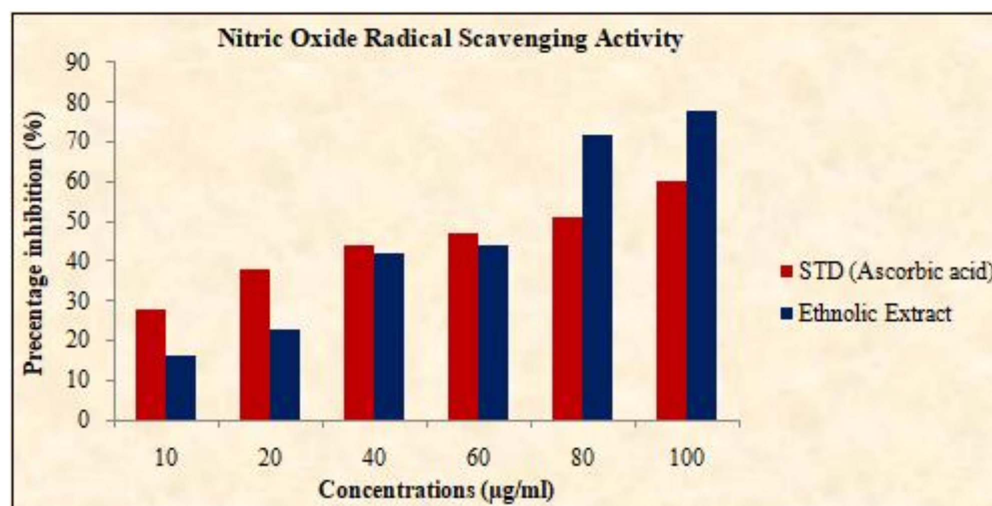

Figure 4: Nitric oxide radial scavenging activity of ethanolic extract of *S. brevistigma*

Table 5: Superoxide radical scavenging activity of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (ascorbic acid)		Ethanol Extract	
		Absorbance Value	Percentage inhibition	Absorbance Value	Percentage inhibition
1	10	0.145 ± 0.002	22 %	0.243 ± 0.002	31 %
2	20	0.152 ± 0.002*	18 %	0.258 ± 0.005***	39 %
3	40	0.157 ± 0.002***	15 %	0.315 ± 0.001***	40%
4	60	0.166 ±0.002***	10 %	0.343 ± 0.002***	85 %
5	80	0.167 ± 0.002***	9 %	0.420± 0.001***	126 %
6	100	0.177 ± 0.002***	4 %	0.435 ± 0.002***	135 %
	Ic_{50} values		Ic_{50} = 14.81µg/ml		Ic_{50} = 31.43µg/ml

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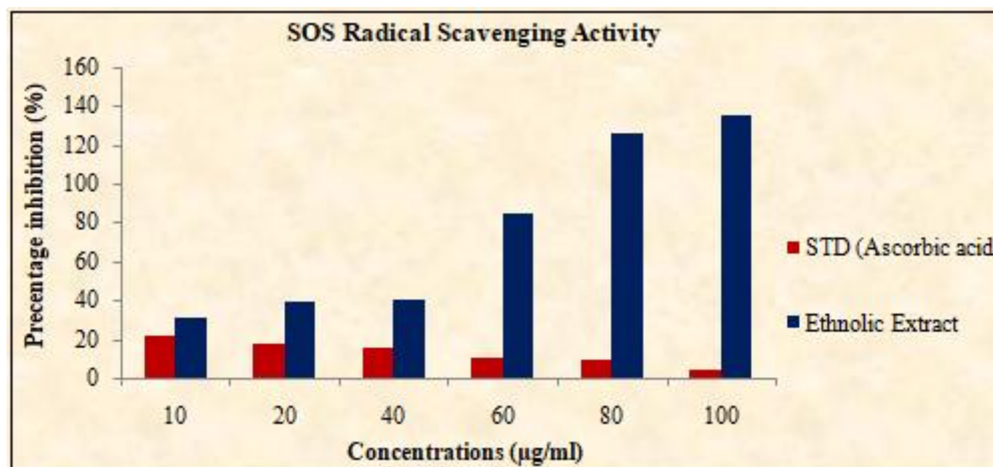

Figure 5: Superoxide radical scavenging activity of ethanolic extract of *S. brevistigma*

Table 6: Total antioxidant activity (Phosphomolybdenum assay) activity of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (QUERCETIN)		Ethanol Extract	
		O.D Value	Percentage inhibition	O.D Value	Percentage inhibition
1	10	0.673 ± 0.002	40 %	0.683 ± 0.002	42 %
2	20	0.432 ± 0.002***	10 %	0.593 ± 0.002^{ns}	23 %
3	40	0.327 ± 0.002***	32 %	0.433 ± 0.002***	10 %
4	60	0.284 ± 0.002***	41 %	0.338 ± 0.028***	24 %
5	80	0.256 ± 0.002***	47 %	0.282 ± 0.002***	42 %
6	100	0.183 ± 0.002***	62 %	0.143 ± 0.002***	70 %
	Ic_{50} values		Ic_{50} = 52.25µg/ml		Ic_{50} = 59.62µg/ml

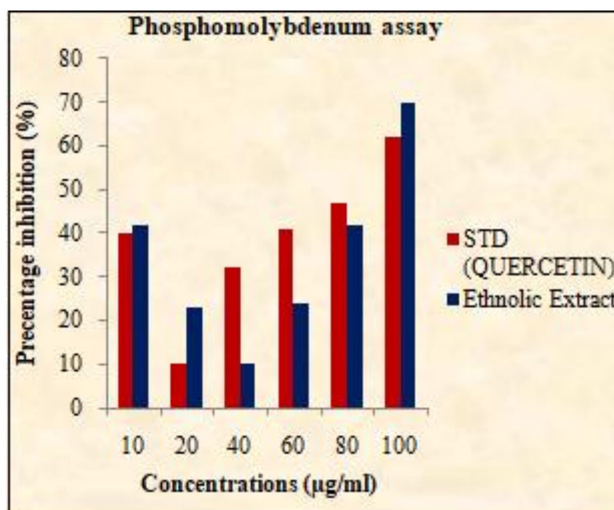

Figure 6: Total antioxidant activity (Phosphomolybdenum assay) activity of ethanolic extract of *S. brevistigma*

Table 7: Total phenolic content radical scavenging activity of ethanolic extract of *S. brevistigma*

S. No.	Concentration	Standard (Quercetin)		Ethanol Extract	Ethanol Extract
		O.D Value	Percentage inhibition	O.D Value	Percentage inhibition
1	10	0.415 ± 0.002	62 %	0.134 ± 0.002	88 %
2	20	0.640 ± 0.001	42 %	0.173 ± 0.002	84 %
3	40	0.871 ± 0.001	22 %	0.22 ± 0.001	80 %
4	60	1.236 ± 0.002	11 %	0.492 ± 0.002	56 %
5	80	1.338 ± 0.002	20 %	0.737± 0.002	34 %
6	100	1.417 ± 0.002	27 %	0.882 ± 0.002	21 %
	Ic_{50} values		Ic_{50}= 16.57µg/ml		Ic_{50}=64.85 µg/ml

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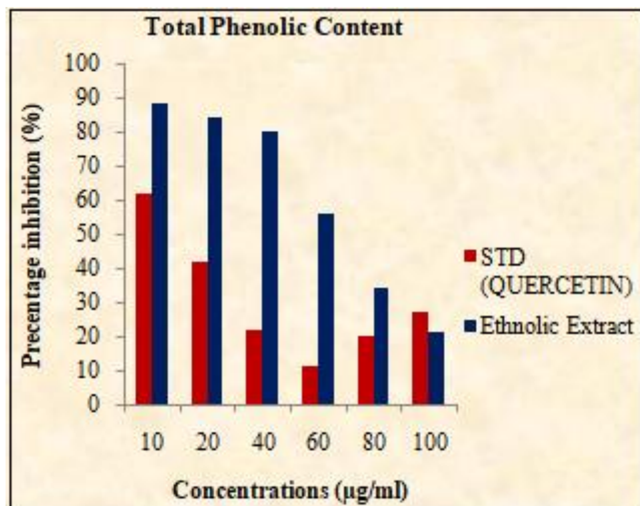

Figure 7: Total phenolic content radical scavenging activity of ethanolic extract of *S. brevistigma*

4. Discussion

In vitro antioxidant studies

Reactive oxygen species are ubiquitous and occur naturally in all aerobic organisms arising from both endogenous and exogenous sources (Shackelford *et al*., 2000). Normally cellular metabolism produced these Reactive oxygen species as by products and are capable of damaging biomolecules, provoking immune response, activating oncogenes and enhancing aging process (Devasakayam and Kamat, 2000). Reactive oxygen species metabolites can be generated by the stepwise reduction of oxygen leads to the production of series of oxidant molecules such as super oxide and nitric oxides (Jenner, 1999). Hence, compounds especially from natural sources capable of protecting against the reactive oxygen species mediated damage may have potential applications in the prevention of chronic diseases like cancer, diabetes and ulcer (Rao and Agarwal, 2000; Cuzzocrea *et al*., 2001).Thus there has been an increasing demand to evaluate the antioxidant properties of plant extracts or isolated products of plant origin, rather than looking for synthetic ones (Mc Clements and Decker, 2000). Antioxidant activity of the multiple plant extracts cannot be evaluated by a unidimensional method, due to complex nature of the phytochemicals present in them (Frankel and Meyer, 2000).Therefore the antioxidant activity is generally evaluated by a number of methods to explain the different mechanism of antioxidant function. In view of this, the antioxidant potential of ethanol extract of *S. brevistigma* was evaluated by employing different chemical assays and *in vitro* methods.

Free radicals

Free radicals are chemical species, which contain one or more unpaired electrons, due to which they are highly unstable, in order to attain stability, they extract electrons from other molecules and cause damage to them (Leong *et al*., 2008). Free radicals and other reactive oxygen species (ROS) are produced as by products in human body during physiological and biochemical processes. To eliminate these free radicals, human body has developed many mechanisms but this is not sufficient in severe oxidative stress conditions. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA) leading to many chronic diseases, such as cancer, diabetes, atherosclerosis, aging and other degenerative diseases in humans (Uttara *et al*., 2009). Use of natural antioxidants, for inactivating free radicals is receiving a lot of attention, because they are natural and non-synthetic products. These natural product drugs play a leading role in pharmaceutical business as they are the most productive leads for development of drugs particularly anticancer agents and anti infective agents. Almost half of the drugs approved, since 1994, are based on natural products (Newman and Cragg, 2007; Butler, 2008) due to which, there is a growing interest in developing the products, that contain mixtures of natural compounds from traditionally used medicines (Charlish, 2008).

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolics, tannins, flavonoids, quinones, alkaloids, betalins and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001;Cai *et al*., 2003). Antioxidant plays a major role in protecting our body from disease by reducing the oxidative damage to cellular component caused by free radicals and ROS (Huda *et al*., 2009). There is increasing evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in medicinal plants (Osawa *et al.*, 1994; Noda *et al*., 1997). Recent investigations suggest that the antioxidants of plant origin with free radical scavenging properties may have great therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, gastrointestinal diseases, and other diseases associated with ageing (Ashokkumar *et al*., 2008; Veerapur *et al*., 2009). In the present study ethanolic extract of *S. brevistigma* showed high free radical scavenging properties.

Reducing power potentials

Reducing power can be attributed to the anti-ROS activity, as oxidation is the predominant mechanism behind free radical medicated damage, even though a potent antioxidant should possess individual free radical scavenging properties, besides potentials to chelate metals and inhibit lipid peroxidation (Pokorny, 2007). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging activity (Yilidirim *et al*., 2000; Selvamani *et al*., 2004; Yu *et al*., 2004). The reducing power potential of ethanol extract of the present study sample was comparable with standard antioxidant ascorbic acid.

The reducing properties of the plant extracts are generally associated with the presence of reductants, which have been shown to exert antioxidant action, by breaking the free radical chain through donating a hydrogen atom (Gordon, 1990). Reductants are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The data obtained in the present study suggested that it was likely to contribute significantly towards the observed

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antioxidant effects. However, the antioxidant activity has been attributed by various mechanisms, like prevention of chain initiation, binding of transition metal ion catalysts, prevention of continued hydrogen abstraction, reductive capacity, radical scavenging activity and decomposition of peroxides. In the present study the reducing power of the extract was significant and it was increased with increasing concentration (Kavimani *et al*., 2014).

The presence of reductants (antioxidants) in *S. brevistigma* ethanolic extract exhibited the maximum reductive capacity. Extract causes the reduction of the Fe^{3+}/ferric cyanide complex to the ferrous form (Naznin and Hasan, 2009) who present study has the similarity with previous investigation of Vadivelan *et al*. (2009). They reported that the antioxidant activity may be due to the presence of phytochemicals in the titled plant (Ganesh *et al*., 2010). According to Jayanthi and Lalitha (2011), the leaf extract of *Eichhornia crassipes*, the fruit extract of *Calotropis procera* (Yazna srividya *et al*., 2013) and the leaf and bark extract of *Dolichandrone atrovirens* (Kavimani *et al.,* 2014)had higher reducing power potential.

Scavenging activity of DPPH*

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants and it is used to determine the antioxidant activities of many plant extracts and compounds (Sancehz-Moreno, 2002) and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soare *et al*., 1997). DPPH assay is the most commonly used electron transfer methods for the assessment of antioxidant capacities of natural products. This method is based on a decrease in alcoholic DPPH solution in the presence of H binding antioxidant ($DPPH^{\bullet} + AH \rightarrow DPPH - H + A^{\bullet}$). The DPPH test provided information on the reactivity of test compounds with a stable free radical (Alluri *et al*., 2009).

In the present study, the percentage of $DPPH^{\bullet}$ radical scavenging activity of the test plant ranged between 18% and 87%. The IC_{50} value of the plant extract (Ethanol) on $DPPH^{\bullet}$ radical scavenging activity was 46.15µg/ml. The sample was compared with the standard Ascorbic acid (24.10µg/ml).The highest $DPPH^{*}$ radical scavenging potential as indicated by the lowest IC_{50} value. The ethanolic extract of *S. brevistigma* showed better percentage of inhibition of $DPPH^{\bullet}$ radicals more or less equal to that of standard ascorbic acid. Dey *et al*. (2015) have reported higher DPPH scavenging activities of methanol extract of *Streptocaulon sylvestre* indicated strong inhibition activities. DPPH a stable organic nitrogenous radical (Huang *et al*., 2005), was scavenged by *S. brevistigma* ethanolic extract at very low concentration.

Our findings were in agreement with the reports of Mary *et al.,* (2003) and Maryam *et al*. (2009). The results of the study carried out by Udhayasankar *et al.* (2012) in *Wattakaka volubilis* and Ahirwal *et al.* (2013) in *Gymnema sylvestre* revealed that the plants possess powerful DPPH activity. Oloyede *et al*. (2010) observed the antioxidant activity of different solvent extracts of *Adansonia digitata, Newbouldia laevis, Alchornea laxiflora* and *Cnidoscolus acontifolius* (Euphorbiaceae) and reported that the butanol and water extracts showed higher antioxidant activities in a concentration dependent manner. In the present study ethanol extract of test plant was found to possess the maximum ability to scavenge free radicals as $DPPH^{\bullet}$ in a concentration dependent manner. The antioxidant properties of plants have been linked to their therapeutic and protective effects in many diseases such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections and inflammation (Matook and Hashinaga, 2005; Tanko, 2008).

Hydroxyl radical scavenging activity

Hydroxyl radicals are generated in biological cells and are very active. Though short lived and low in concentration, hydroxyl radicals are the most reactive of all the reduced forms of dioxygen and are believed to be responsible for initial cell damage both *in vitro* and *in vivo* (Bailly *et al*., 2000). It is an extremely harmful ROS with tremendous potentiality to damage biomolecules (Huang *et al*., 2005). Super reactive hydroxyl radical may be formed in the presence of metal ions. For this reason, H_2O_2 inhibition activity is considered to be an important method for the determination of antioxidant characteristics.

The ethanolic extract of *S. brevistigma* showed potential hydroxyl radical scavenging activity and it was indicated by the lowest IC_{50} value the same was comparable with the standard ascorbic acid (IC_{50} 11.37µg/ml). The antioxidant properties exhibited by *S. brevistigma* were supported by the detection of plant phenols in the test plant. Polyphenols (electron-rich compounds) having the ability to go into electron-donation reactions with oxidizing agents and still from stable species and thus inhibit or delay the oxidation of different biomolecules (Khalaf *et al*., 2008). In the present study the ability of the extract to quench hydroxyl radicals seems to be directly related to the prevention of lipid peroxidation and scavenging of active oxygen species, thus reducing the rate of the chain reaction.

Ethanolic extract of the aerial parts of *S. brevistigma* was a potential source of natural antioxidants. This was in agreement with the findings of Thambiraj *et al.* (2012) who reported that the methanolic extract of *Acalypha fruticosa* was effective in scavenging hydroxyl radical. Few more reports, supports our present findings were Wettasinghe and Shahidi (2000) and Xing *et al.* (2005) in *Ecklonia cava*, Ahriwal *et al.* (2013) in *Gymnema sylvestre* and Dey *et al.* (2015) in *Streptocaulon sylvestre*.

Nitric Oxide radical scavenging activity

Nitric oxide is an essential bio-regulatory molecule, required for several physiological processes like neural signal transmission, immune response, control vasodilatation and control of blood pressure (Gold *et al*., 1990). Nitric oxide is reactive free radical, lipophilic in nature and diffuse easily between cells. Nitric oxide plays an important role in various types of inflammatory processes in the animal body, exhibits numerous physiological processes such as smooth muscle relaxation and regulation of cell mediated toxicity (Kumar *et al*., 2010). It is an important second messenger, acts as a neurotransmitter and plays an important role in the defence against pathogens as well as in the control of blood pressure reacts more rapidly with superoxide and is an important mediator of diverse physiologic and pathologic processes,

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including arthritis (Wallace, 2005). High levels of NO production results in direct tissue toxicity and contributes to various carcinomas and inflammatory conditions (Currás-Collazo, 2011). The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation.

In the present study, ethanol extract of *S. brevistigma* was found to be more potent scavenger of nitric oxide, exhibiting lowest IC $_{50}$ value. This was in a dose dependent manner. The concentration of nitrite after spontaneous decomposition of sodium nitro prusside indicating that ethanol extract may contain compounds able to scavenging NO (Prajakta *et al*., 2008). The present work complemented the earlier investigation of Vidyadhar *et al.* (2010) in *Securinega leucopyrus* and of Desai *et al.* (2008) in *Baliospermum montanum.* According to Ahirwal *et al.* (2013), all methanolic fractions of *Streptocaulon sylvestre* showed significantly higher nitric oxide radical scavenging activities than commercial antioxidants.

Superoxide radical scavenging activity

Superoxide anions are the most common free radicals *in vivo* and are generated in a variety of biological systems by either auto-oxidations processes or by enzymes. The concentration of superoxide anions increases under oxidative stress and related situations (Shyura *et al*., 2005). Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive species (Kavimani *et al*., 2014). Superoxide radicals are generated during the normal physiological process mainly in mitochondria (Suriyavathana *et al.,* 2010). These radicals produces tissue damage associated with inflammation in joints such as rheumatoid arthritis, osteoarthritis and crystal induced arthropathies (Afonso *et al*., 2007). Plants do not possess SOD enzyme, but they may contain SOD mimic activity with the help of which, superoxide dismutases converts superoxide to hydrogen peroxide, which is then removed by glutathione peroxidase or catalase (URL:http://www.thefreedictionary.com/superoxide+dismutase). In the present study it was noteworthy to mention that the ethanolic extract of *S. brevistigma* recorded the highest superoxide scavenging activity (10.78µg/ml) as reported in *W. volubilis* Udhayasankar *et al*. (2012).

The radical scavenging and antioxidant results obtained in ethanolic extract of *S. brevistigma* were not in agreement with the earlier literature of Gazzani et *al*. (1998). He reported that the alcoholic extract of *Euphorbia heyneana* was found to exhibit minimum radical scavenging activity. However these contradictory results are most likely due to differences in methodology and experimental conditions used in the different studies. Though, wide variety of potential antioxidant compounds such as vitamins, flavonoids, phenolic acids and sulphur compounds present in plants, differences in the method of sample extraction can results in a wide variation in the antioxidant activity of the extract (Nuutila *et al*., 2003). According to Kumaran and Karunakaran (2007) the methanolic extract of the genus *Phyllanthus debilis* possess highest superoxide anion scavenging activity. This was in agreement with the present investigation.

The higher superoxide anion scavenging activity was observed in aqueous fractions of *Ecklonia cava* (Senevirathne *et al*., 2006) it may be attributed to both neutralisation of O_2 radicals via hydrogen donation and inhibition of xanthine oxidase by various phenolics present in *Ecklonia cava* fractions. Kavimani *et al.* (2014) reported that the superoxide anion radical scavenging activity of methanolic extract of *Dolichandrone atrovirens* is more significant. According to Dey *et al*. (2015) the methanolic extract of *Streptocaulon sylvestre*, an endangered plant of sub-Himalayan plains of West Bengal and Sikkim displayed excellent membrane stabilizing capacity by inhibition of superoxide radical. These reports supported the present findings.

Phosphomolybdenum assay

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids, suggesting the presence of these compounds in the extracts. The different extracts were assayed for their antioxidant potency by the formation of green phosphomolybdenum complex. It is known that hydrogen and electron transfer from antioxidant analytes to DPPH and Mo(VI) complex occur in the DPPH and phosphomolybdenum assay methods. The transfers occur at different redox potentials in the two assays and also depend on the structure of the antioxidant (Renuka *et al*., 2012). DPPH scavenging assay detect antioxidants such as flavonoids and polyphenols, whereas, the phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherol, and carotenoids (Prieto *et al*., 1999). Ascorbic acid, glutathione, cysteine, tocopherols, polyphenols, and aromatic amines can be detected by the two assay models (Borges *et al*., 2005). Thus the antioxidant potential of the extracts differed in the foresaid type of assay.

The antioxidant potential of *S. brevistigma* ethanolic extract showed highest (59.62µg/ml) antioxidant potential in the phosphomolybdenum assay. Total antioxidant capacity was reported as ascorbic acid equivalents. This maximum antioxidant activity might be attributed to the presence of phytochemicals such as phenols, tannins and flavonoids in the test plant. The same has been reported in research article evaluation of antioxidant activity of mushroom (Sarikurkcu *et al*., 2008; Ferreira *et al*., 2009) and antioxidant properties and total flavonoid content of leaf extracts of three varieties of Carob tree (Hanane Hajaji *et al*., 2010) and the antioxidant activities of *Cinnamomum zeylanicum* and *Cinnamomum tamala* (Shahwar and Asamraza, 2010).

Recent studies have shown that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants (Khan *et al*., 2012). In case of *S. brevistigma* also, the results suggested that the strong antioxidant activity of extracts might be due to the presence of phenolics compounds present in the extract (Khan *et al*., 2012). Phenols also showed positive correlation with phosphomolybdenum assay. Such positive correlation between total phenolic content of plant extracts and related antioxidant activity has also been reported by different workers (Guleria *et al*., 2011; Kumar and Pandey, 2013; Yazna srividya *et al*., 2013).

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Similar to our results, literature survey revealed that some of the Indian medicinal plant extracts *viz. Koelreutaria paniculata, Sapindus trifoliatus, Alstonia scholaris* and *Ficus benjamina* provided good protection against the damage caused by free radicals (Bibi *et al*., 2011).

Percentage of free radical scavenging by Total phenolic content

The antioxidant activity of the plant extract is mainly due to presence of phenolic compounds, due to their redox properties, hydrogen donors and singlet oxygen quenchers (Hatano *et al.,* 1989; Osawa, 1994). Phenolics are the most wide spread secondary metabolites in plant kingdom and have multiple biological properties (Sharififar *et al*., 2009; Adsul *et al.,* 2012). These diverse groups of compounds have received much attention as potential natural antioxidant. Today, the inventory of antioxidative phytochemicals chiefly consists of phenolic and flavonoid compounds which have also demonstrated a vast range of bioactivities.

The present study displayed the percentage inhibitions of radical scavenging activity of total phenolic content of ethanolic extract of *S. brevistigma* in comparison to standard quercetin. The plant extract showed potent radical scavenging activity (0.492, 0.737 and 0.882µg/ml) at the concentration of 60, 80 and 100µg/ml respectively. The extract showed concentration dependent free radical scavenging activity, even compared to that of standard drug quercetin. Our findings are in agreement with the reports of Maryam *et al*. (2009) and Kedari and Malpathak (2014). Their observations clearly indicated a cross linkage between phenolics and antioxidant activity.

The methanolic leaf and bark extracts of *Dolichandrone atrovirens* showed strong antioxidant activity in various *in vitro* systems tested and the antioxidant effect of *Dolichandrone atrovirens* may be due to the phenolic compounds present in it (Kavimani *et al*., 2014). The strong antioxidant activity of *Chonemorpha fragrana* extracts might be due to the presence of phenolics compounds present in the extract (Kedari and Malpathak, 2014). Many authors have also correlated antioxidant activity with their polyphenolic or phenolic contents (Kaur *et al*., 2002; Ravishankara *et al*., 2002).

Antioxidants prevent the deleterious consequences of oxidative stress due to which, there is an increasing interest in exploring, protective biochemical function of natural antioxidants from medicinal plants. Antioxidant activity of *S. brevistigma* has pointed to interesting antioxidant prospect exhibited by its extract. It also reveals the ROS scavenging, thereby affirms its role as an antioxidant. Results supported the idea that this plant can be an effective source of natural antioxidants for medicinal applications. These antioxidant effects of *S. brevistigma* emphasises on the need of further investigation of their other beneficial biological properties, as such antioxidants are known to prevent cancer and cardiovascular diseases. Further research is also needed to investigate the type of chemical compounds that are present in this medicinal plant, especially in the extracts found with high antioxidant potential. This is the first report of antioxidant activity of *S. brevistigma.*

5. Conclusion

The present study was designed to evaluate the *In vitro* antioxidant activity of ethanolic Whole plant extract of *Sarcostemma brevistigma* as an antioxidant agent. The results of the present study clearly indicated that ethanolic extract of *Sarcostemma brevistigma* possesses powerful *in vitro* antioxidant activity. The encouraging results of the study plant with various *in vitro* antioxidant tests proved the plant as a reducing agent and effective scavenger of free radicals. In this study, significance was found between the antioxidant activity and total phenol and flavonoid contents, indicating that these compounds could be major contributors of antioxidant activity.

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References

[1] Adsul, V.B., Khatiwora, E. and Deshpande, N.R. 2012. Evaluation of antioxidant activity of *Ipomoea carnea* leaves. *J. Nat. Prod. Plant Reso.,* 2(5):584-588.

[2] Afolayan AJ, Jimoh FO, Sofidiya MO, Koduru S, Lewu FB. Medicinal potential of the root of *Arctotis arctotoides*. *Pharm Biol.* 2007;45:486–493.

[3] Afonso, V., Champy, R., Mitrovic, D., Collin, P., and Lomri, A. 2007. Reactive oxygen species and superoxide dismutases: role in joint diseases. *Joint Bone Spine*, 74(4): 324-9.

[4] Ahirwal, L., Singh, S., Hajra, S. and Mehta, A. 2013. Screening of methanolic extract of *Gymnema sylvestre* R. Br. leaves for antioxidant potential. *Int. J. Pharm. Sci. Rev. Res.,* 19(1): 87-91.

[5] Alluri, V.K., Chirravuri, V.R., Tayi, V.N.R., Reddy, K.N. and Trimurtulu, G. 2009. *In vitro* and *in vivo* antioxidant activity of *Aphanamixis polystachya* Bark. *Am. J. Infect. Dis.*, 5: 60-67.

[6] Ashokkumar, D., Mazumder, U.K., Gupta, M., Senthilkumar, G.P. and Selvan, V.T. 2008. Evaluation of antioxidant and free radical scavenging activities of *Oxystelma esculentum* in various *in vitro* Models. *J. Comp. Integ. Med*., 5(1): 9.

[7] Azaizeh H, Fulder S, Khalil K, Said O. Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. *Fitoterapia.* 2003;74:98–108.

[8] Bailly, F., Zoete, V., Vamecq, J., Catteu, J.P. and Bernier, J.L. 2000. Antioxidant actions of ovothiol-derived 4-mercaptoimidazoles: glutathione peroxidase activity and protection against peroxynitrite-induced damage. *FEBS Lett.*, 486:19–22.

[9] Bibi, G., Ihsan-ul-Haq, Ullah N, Mannan, A. and Mirza, B. 2011. Antitumor, cytotoxic and antioxidant potential of *Aster thomsonii* extracts. *Afr. J. Pharm. Pharmacol.,* 5(2): 252-258.

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[10] Borges, F., Roleira, F., Milhazes, N., Santana, L. and Uriarte, E. 2005. Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. *Curr. Med. Chem.*, 12(8): 887–916.
[11] Butler, M.S. 2008. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat. Prod. Rep.,* 25:475-516.
[12] Buyukokuroglu, M.E., Gulcin, I., Oktay, M. and Kufrevioglu, O. I., 2001.In vitro antioxidant properties of dantrolene sodium. *Pharmacological Res.,* 44: 491.
[13] Cai, Y.Z., Sun, M. and Corke, H. 2003. Antioxidant activity of betalains from plants of the Amaranthaceae.*J. Agric. Food Chem.*, 51(8): 2288–2294.
[14] Chakraborthy., G.S. 2009. Free radical scavenging activity of *Aesculus indica* leaves. *Int. J. Partech Res.,* 1 (3): 524-526.
[15] Charlish, P. 2008. Traditional remedies: latter day medicines. *Scrip World Pharmaceut.* 3351: 31-34.
[16] Craig WJ. Health-promoting properties of common herbs. Am J Clin Nutr 1999; 70:491- 9.
[17] Currás-Collazo, M.C. 2011. Nitric oxide signaling as a common target of organohalogens and other neuroendocrine disruptors. *J. Toxicol. Environ. Health. B. Crit. Rev*., 14(5-7): 495-536.
[18] Cuzzocrea, S., Riley, D.P., Caputi, A.P. and Salvemini, D. 2001. Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia reperfusion injury. *Pharmacol. Rev*., 53: 135-159.
[19] Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, *et al.* Medicinal plants and cancer chemoprevention. Curr Drug Metab, 2008;9:581-91.
[20] Desai, P.V., Wadekar, RR., Kedar, G.H. and Patil, K.S. 2008. Free radical scavenging activity of aqueous extract of roots of *Baliospermum montanum* Muell-Arg. *Int. J. Green Pharm*., 2: 31-3.
[21] Devasakayam, T.P.A. and Kamat, J.P. 2000. Free radicals and antioxidants in human health. *EMSI News letter*, 23: 3.
[22] Dey, P., Ray, S., Sarkar, M. P. and Chaudhuri, T. K. 2015. Chemical characterization and assessment of antioxidant potentiality of *Streptocaulon sylvestre* Wight, an endangered plant of sub-Himalayan plains of West Bengal and Sikkim. *BMC Complementary and Alternative Medi.,* 15:107.
[23] Diouf PN, Stevanovic T, Cloutier A. Study on chemical composition, antioxidant and anti-inflammatory activities of hot water extract from *Picea mariana* bark and its proanthocyanidin- rich fractions. *Food Chem.* 2009;113:897–902.
[24] Evans, C.W. Trease. and Evance. 2002. Pharmacognosy, 15thed. 3.
[25] Ferreira, I.C., Barros, L. and Abreu, R.M 2009. Antioxidants in wild mushrooms. Curr.*Medi. Chemi.,* 16: 1543-60.
[26] Frankel, E.N. and Meyer, A.S. 2000. The problems of using one-dimensional methods to evaluate multi functional food and biological antioxidants. *J. Sci. Food and Agri*., 80(13):1925-1941.
[27] Ganesh, T., Saikat, S. and Chakraborty, R. 2010. *In vitro* antioxidant activity of *Meyna laxiflora* seeds. *Int. J .Chem .Pharm. Sci.*, 1: 5–8.
[28] Gazzani, G., Papetti, A., Daglia, M., Berte, F. and Gregotti, C. 1998. Protective activity of water soluble components of some common diet vegetables on rat liver microsome and the effect of thermal treatment. *J. Agric. Food Chem*., 46: 4123-4127.
[29] Gold, M.E., Wood, K.S., Byrns, R.E. 1990. G-methyl-L-arginine causes endothelium-dependent contraction and inhibition of cyclic GMP formation in artery and vein. *Proc. Nat. Acad. Sci.,* USA, 87: 4430-4434.
[30] Gordon, M.H. 1990. The mechanisms of antioxidant action *in vitro*. In BJF Hudson (Ed.) Food antioxidants. Elsevier Applied Sceince, London. 1-18.
[31] Gulcin, I., Oktay, M., Kirecci, E. and Kufrevioglu, O.I. 2003. Screening of antioxidant and Antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem.,* 83: 371-382.
[32] Guleria, S., Tiku, A.K., Singh, G., Vyas, D. and Bhardwaj, A. 2011. Antioxidant activity and protective effect against plasmid DNA strand scission of leaf, bark, and heartwood extracts from *Acacia catechu*. *J. Food Sci.,* 76(7):959-64.
[33] Gurib-Fakim A. *Medicinal plants: traditions of yesterday and drugs tomorrow, Molecular Aspects of Medicine.* 2006;27:1–93.
[34] Hanane Hajaji, E.l., Nadya, L., Katim, A., Yahya, C., Abdellah, F., Abdesslam, E., Brahim, E.l., Bali and Mohammed, L. 2010. Antioxidant Properties and Total Phenolic Content of Three Varieties of Carob Tree Leaves from Morocco. *Rec. Nat. Prod*., 4(4): 193-204.
[35] Hatano, T., Edamatsu, R. and Mori, A. 1989. Effects of interaction of tannins with coexisting substances, *Chem. Pharm. Bull,* 37: 2016–2021.
[36] Huang, D, Ou, B. and Prio, R.L. 2005. The chemistry behind antioxidant capacity assays. *J. Agric. Food. Chem*., 53:1841–56.
[37] Huda, A.W., Munira, M.A., Fitrya, S.D. and Salmah, M. 2009. Antioxidant activity of *Aquilaria malaccensis* (Thymelaeaceae) leaves. *Pharm. Res.*, 1: 270-3.
[38] Hutchings A, Scoth AH, Lewis G, Cunningham AB. *Zulu medicinal plants, An inventory.* Pietermaritzburg: Natal University Press; 1996. pp. 195–196.
[39] Ibrar M. Responsibilities of Ethnobotanists in the Field of Medicinal Plants. In Proceeding of Workshop on Curriculum Development in Applied Ethnobotany. Published by the Ethnobotany Project, WWF Pakistan, 34-D/2, Sahibzada Abdul Qayuum Road Peshawar, Pakistan 2002; pp 16- 20
[40] Jayanthi, P. and Lalitha, P. 2011. Reducing power of the solvent extracts of *Eichhornia crassipes* (MART.) Solms. *Int. J. Pharm. Pharm Sci*., 3(3): 126-128.
[41] Jenner, P. 1999. Oxidative stress in Parkinson's disease and other neurodegenerative disorders. *Pathol. Biol*., 44: 57– 64.
[42] Kaur, C. and Kapoor, H.C. 2002. Antioxidant activity and phenolic content of some Asian vegetables. *Int. J. Food Sci. Techn.,* 37: 153-161.
[43] Kavimani, S., Saminathan, K. and Senthil Kumar, R. 2014. Antioxidant and free radical scavenging

Licensed Under Creative Commons Attribution CC BY

activities of *Dolichandrone Atrovirens* Using Various *In vitro* assay models. *Int.J. Phytopharm..*, 5(4): 293-300.

[44] Kedari, P. and Malpathak, N. 2014. Exploring antioxidant activity and dna protection potential of *Chonemorpha fragrans. Int. J. Pharma. Sci. Res.,* 5(12): 5452-5462.

[45] Khalaf, N.A., Shakya, A.K., Al-Othman, A., Agbar, Z.E. and Farah H. 2008. Antioxidant Activity of Some Common Plants. *Turk. J. Biol*., 32: 51-55.

[46] Khan, R.A., Khan, M.R. and Sahreen, S. 2012. Assessment of flavonoids contents and *In- vitro* antioxidant activity of *Launaea procumbens*. *Chem. Central. J*., 6: 43-47.

[47] Kumar, P., Deval Rao G., Lakshmayya and Ramachandra Setty S., 2008. Antioxidant and hepatoprotective activity of tubers of Momordica tuberosa Cogn., against CCl_4 induced liver injury in rats, *Indian J. Experimental Biol.,* 46: 510-513.

[48] Kumar, R., Phani Kumar, G. and Chaurasia, O.P. 2010. Invitro antioxidant activity of methanolic extract of *Rhodiola imbricate* Edgew. *Phcog. Net*., 2(7): 157-167.

[49] Kumar, S. and Pandey, A.K. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World J.,* 1-16.

[50] Kumaran, A. and Joel Karunakaran, R. 2007. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *Food Sci. Tech.,* 40(1): 344-352.

[51] Larson, R.A. 1998. The antioxidants of higher plants. *Phytochem*., 27: 969-78.

[52] Leong, C.N., Tako, M., Hanashiro, I. and Tamaki, H. 2008. Antioxidant flavonoids glycosides from the leaves of *Ficus pumila* L. *Food Chem.*, 109: 415–20.

[53] Lieu, R.H. 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr*., 78: 520.

[54] Liu, F., Ooi VEC., and Chang S. T., Free radical scavenging activity of mushroom polysaccharide extracts. Life Science, 1997, 60, 763-771.

[55] Manda Spring. Effects of Medicinal Plants on Cancer. Bright Hub, 2010

[56] Marcocci, L., Packer, L., Droy-Lefaix, M.T., Sekaki, A. and Gardes- Albert, M. 1994. Antioxidant actions of *Ginkgo biloba* extracts EGB 761, *Methods Enzymol,* 234: 462-475.

[57] Mary, N.K., Achuthan, C.R., Babu, B.H. and Padikkala, J. 2003. *In vitro* antioxidant and antithrombotic activity of *Hemidesmus indicus* (L) R.Br. *J. Ethnopharmac.* 87: 187-191.

[58] Maryam, Z., Aqil, F. and Iqbal, A. 2009. The in vitro antioxidant activity and total phenolic content of four indian medicinal plants. *Int. J. Pharma. Pharmac. Res*., 1(1): 88-94.

[59] Matook, S. and Hashinaga, M. 2005. Antioxidant activities of Banana (*Musa* AAA, *cv. Cavendish*) fruits peel. *Am. J. Biochem. Biotechnol*., 1(3): 126-132.

[60] Mc Clements, D.J. and Decer, E.A. 2000. Lipid oxidation in oil-in-water emulsion: Impact of molecular environment on chemical reactions in heterogeneous food systems. *J.Food Sci*., 65: 1270-1282.

[61] Nagai, T., Inoue, R., Inoue, H. and Suzuki, N. 2003. Preparation and antioxidant properties of water extracts of propolis. *Food Chemistry*, 80: 29-33.

[62] Naznin, A. and Hasan, N. 2009. *In Vitro* antioxidant activity of methanolic leaves and flowers extracts of *Lippia alba*. *Res. J. Med. Sci.,* 4: 107-110.

[63] Newman, D.J. and Cragg. G. 2007. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.,* 70:461-477.

[64] Noda, Y., Anzai-Kmori, A., Kohono, M., Shimnei, M. and Packer, L. 1997. Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectromoter system. *Biochem. Mol. Biol. Inter.* 42: 35-44.

[65] Nuutila, A.M., Puupponen-Pimia, R., Aarni, M. and Oksman Caldentey, K.M. 2003. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem*., 81: 485-493.

[66] Oloyede, G.K., Onocha, P., Julius, A., Oguntokun, S. and Emmanuel, O.T. 2010. Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. *Ann. Bio. Res*., 1 (2): 114-120.

[67] Osawa, T. 1994. Postharvest biochemistry. In: Uritani I, Garcia VV, Mendoza, EM, editors. Novel neutral antioxidant for utilization in food and biological systems. Japan: Japan Scientific Societies Press; 241-251.

[68] Owolabi, M.A., Coker, H.A.B. and Jaja, S.I. 2007. Flavonoid metabolites in urine after oral administration of the aqueous extract of *Persea americana* to rats. *J. Nat. Med*., 61: 200-204.

[69] Oyaizu,M. 1986. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. Japonesse. *J. Nutri*., 44: 307-315.

[70] Pokorny J. 2007. Are natural antioxidants better—and safer—than synthetic antioxidants. *Eur. J. Lipid Sci. Technol.,* 109: 629–42.

[71] Prajakta, V.D., Raju, R.W., Girish, H.K. and Kalpana, S.P. 2008. Free radical scavenging activity of aqueous extract of roots of *Baliospermum montanum* Muell-Arg.*Int. J.Green Pharm*., 2(1): 31-33.

[72] Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.,* 1999; 269:337-341

[73] Prieto, P., Pineda, M. and Aguilar, M. 1999.Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analy. Biochem*., 269(2): 337–341.

[74] Quideau, S., Deffieux, D., Douat-Casassus, C. and Pouységu, L. 2011. Plant polyphenols: chemical properties, biological activities, and synthesis angew. *Chem Int*., 50: 586–621.

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[75] Rajesh P.M, Natvar P.J. In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. *J. Advan. Pharma. Educ. Res.,* 2011; 1:52-68.

[76] Rao, A.V. and Agarwal, S. 2000. Role of antioxidant lycopene in cancer and heart disease. *J Am Coll Nutr.*, 19(5): 563-569.

[77] Ravishankara, M.N., Shrivastava, N., Padh, H. and Rajani, M. 2002. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R. Br. (Anantmul). *Phytomedi.,* 9: 153-160.

[78] Rees, D.D., Palmer, R.M. and Moncada, S. 1989. Role of endothelium derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci.,* USA, 86: 3375-3378.

[79] Renuka, D., Amit, S. and Nutan, M. 2012. Phytochemical Composition and Antioxidant Potential of *Ruta graveolens* L. *In Vitro* Culture Lines. *J. Bot*., 6.

[80] Sancehz-Moreno, C. 2002. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci Tech Intl.,* 8: 121-137.

[81] Sangameswaran B, Balakrishnan B.R., Deshraj, C. and Jayakar, B. 2009. In vitro antioxidant activity of roots of Thespesia lampas Dalz and Gibs. *Pak J Pharm Sci.,* 22(4): 368-72.

[82] Sarikurkcu, C., Tepe, B. and Yamac, M. 2008. Evaluation of the antioxidant activity of four edible mushrooms from the Central Anatolia, Eskisehir - Turkey: *Lactarius deterrimus, Suillus collitinus, Boletus edulis, Xerocomus chrysenteron*. *Bioresour. Technol.,* 99: 6651-655.

[83] Selvamani, P., Latha, S., Jyothi Sen, D. and Gupta, J.K. 2004. Dieuritc activity of isolated fractions of petroleum ether extracts from *Commiphora berryi* leaves. *Indian. J. Nat. Prod*. 20: 24-26.

[84] Senevirathne, M., Kim, S.H., Siriwardhana, N., Ha, J.H., Lee, K.W. and Jeon, Y.J. 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food. Sci. Tech. Int*., 12(1): 27-38.

[85] Shackelford, R.E., Kausmann,W.K. and Paules, R.S. 2000. Oxidative stress and cell cycle check point functions. *Free radical Biol.med*., 28: 1387-1404.

[86] Shahwar, D. and Asamraza, M. 2010. Comparative study on the antioxidant and antimicrobial activities of stem-bark extract of *Litchi chinensis* and its organic fractions. *J. Chem. Soci. Pakistan*, 32: 3.

[87] Sharififar, F., Dehghn-Nudeh, G., Mirtajaldini, M. 2009. Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chem.,* 112: 885-888.

[88] Shyura, L.F., Tsunga, J.H., Chenb, J.H., Chiua, C.Y. and Loa, C.P. 2005. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. *Int. J. Appli. Sci. Engi.,* 3(3): 195-202.

[89] Singleton, V.L. and Rossi, J. A. Jr. 1965. Colorimetric of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American J. Enology Viticulture.* 16, 144–158.

[90] Soare, J.R., Dinis, T.C.P., Cunha, A.P. and Almeida, L.M. 1997. Antioxidant activity of some extracts of *Thymus zygis*. *Free Radic Res.,* 26: 469-478.

[91] Sreejayan, N. and Rao, M.N.A. 1997. Nitric oxide scavenging by eucuminoids. *J. Pharmacy and Pharmacol*., 49: 105-107.

[92] Steenkamp V, Stewart MJ, Chimuka L, Cukrowska E. Uranium concentrations in South African herbal remedies. *Health Physiol.* 2005;89:79–83.

[93] Suriyavathana, M., Usha, V. and Shanthanayaki, M. 2010. Studies on phytochemical analysis and antioxidant activity of selected medicinal plants from kolli hills. *J. Pharma. Res.,* 3(2), 260-262.

[94] Tanko, Y., Yerima, M., Mahdi, M.A., Yaro, A.H., Musa, K.Y. and Mohammed, A. 2008. Hypoglycemic activity of methanolic stem bark of *Adansonnia digitata* extract on blood glucose levels of streptozocin-induced diabetic wistar rats. *Int. J. App. Res. Nat. prod.*, 1(2): 32.

[95] Thambiraj, J., Paulsamy, S. and Sevukaperumal, R. 2012. Evaluation of in vitro antioxidant activity in the traditional medicinal shrub of western districts of Tamilnadu, India, *Acalypha fruticosa* Forssk. (Euphorbiaceae) *Asian Pac. J. Trop. Biomed*., 127 - 130.

[96] Torras MAC, Faura CA, Schonlau F, Rohdewald P. Antimicrobial activity of Pycnogenol*. *Phytother Res.* 2005;19:647–648.

[97] Tripathi,Y.B., Singh,A.V. and Dubey,G.P. 2001. Antioxidant property of the bulb of *Scilla indica*. *Curr.Sci*., 80(10): 1267-1269.

[98] Udhayasankar, M. R., Danya, U. and K. Arumugasamy 2012. Phytochemistry and Free radical scavenging activity of *Wattakaka volubilis* (Linn. f.) Benth ex. Hook f. (Asclepiadaceae)- A rare and threatened medicinal plant. *Int. J. Pharm. Tech. Res.,* 4(3): 1025-1032.

[99] URL:http://www.thefreedictionary.com/superoxide+dismutase.

[100] Uttara, B., Singh, A.V., Zamboni, P. and Mahajan, R.T. 2009. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Current Neuropharma.,* 7(1): 65-74.

[101] Vadivelan, P., Rajesh, K.R., Bhadra, S., Shanish, A., Elango, K. and Suresh, B. 2009. Evaluation of antioxidant activity of root extracts of *Rubus ellipticus* Smith. *Hygeia.* 1: 7–10.

[102] Veerapur, V.P., Prabhakar, K.R., Parihar, V.P., Kandadi, M.R., Ramakrishana, S., Mishra, B., Satish Rao, B.S., Srinivasan, K.K., Priyadarsini, K.I. and Unnikrishnan, M.K. 2009. *Ficus racemosa* Stem Bark Extract: A Potent Antioxidant and a Probable Natural Radioprotector. *Evid. Based Comple. Alternat. Med.*, **6**(3)**:** 317-324.

[103] Vidhyadhar, C.H., Dayananda, S.B., Shashikala, G.L. and Kalagouda, B.G. 2010. Synthesis, characterization, anti-microbial study of 2-amino-N′-[(1E)-1-pyridine-2-ylethylidene] benzohydrazide and its Lanthanide (III) Complexes. *Der*`12345 *Pharma Chem.*, 2(5): 390-404.

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[104] Wallace, J.L. 2005. Nitric oxide as a regulator of inflammatory processes. *Mem. Inst. Oswaldo. Cruz.*, 100: 5-9.
[105] Wettasinghe, M. and Shahidi, F. 2000. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chem.*, 70: 17–26.
[106] Xing, R., Liu, S., Guo, Z., Yu, H., Wang, P., Li, C., Li, Z. and Li, P. 2005. Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities *in vitro*. *Bioorganic and Med.Chem.*, 13: 1573-1577.
[107] Yadav M, Khan KK. Some Ethno Medicinal Perceptions of Tribal Communities of Rewa District, Madhya Pradesh Indian J Sci Res. 2012;3(2):145-148, 2012 2.
[108] Yasui, K. and Baba, A. 2006. Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. *Inflamm. Res.*, 55(9): 359-63.
[109] Yazna srividya, B., Ravishankar, K. and Priya Bhandhavi, P. 2013. Evaluation of in vitro antioxidant activity of *Calotropis procera* fruit extract. *Int. J. Res. Pharma. Che.,* 3(3): 573-578.
[110] Yilidirim, A., Mavi, A., Oktay, M., Kara, A.A., Algur, O.F. and Bilaloglu, V. 2000. Comparison of antioxidant and antimicrobial activites of *Tilia, Sage* and black tea extracts. *J. Agric. Food Chem.*, 48: 5030-5034.
[111] Yu, W., Zhao, Y. and Shu, B. 2004. The radical scavenging activites of radix puerariae isoflavanoids. A chemiluminescence study. *Food Chem.*, 86: 525-529.
[112] Zheng, W. and Wang, S.Y. 2001.Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* 49(11): 5165–5170.
[113] WHO. 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, Geneva, Switzerland, pp. 1-80.

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