https://ijrps.com

ISSN: 0975-7538
Research Article

# Evaluation of *in vitro* antioxidant activity of ethanolic extract of leaves of *Rhynchosia beddomei Baker*

**Jaya Sankar Reddy V*[1] and Deval Rao G[2]**

[1]Department of Pharmacy, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India
[2]K. V. S. R Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India

**ABSTRACT**

To evaluate the antioxidant capability of the ethanolic extract of the leaves of *Rhynchosia beddomei*. *In vitro* antioxidant activity was evaluated by studying total phenol content, flavonoid content, DPPH radical scavenging activity, nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power, super ion scavenging activity and metal chelating activity. Gallic acid, ascorbic acid, Butylated hydroxyl toluene and quercetin were used as standards for all the experiments. The total phenolic content, total flavonoid content, and IC 50 values were observed for DPPH radical scavenging activity, nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power, super ion scavenging activity and metal chelating activities were determined to be 52.59, 48.6, 209, 191.18, 192.14, 218.4, 190.97 and 220.73. Each experiment was carried out in triplicate and results were expressed as mean ± SD. The results clearly indicated that ethanolic extract of leaves of *Rhynchosia beddomei* of the study species is effective in scavenging free radicals and has the potential to be a powerful *in vitro* antioxidant.

**Keywords:** *Rhynchosia beddomei;* antioxidant; Fabaceae; free radicals.

## Introduction

Free radicals have been implicated in the causation of several problems like asthma, cancer, cardiovascular disease, cataract, diabetes, gastrointestinal inflammatory disease, liver disease, muscular degeneration and other inflammatory process (Sen Set al., 2010). Reactive oxygen species (ROS) are continuously produced during cell metabolism and under normal conditions, they are scavenged and converted to nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system (Shao HB et al., 2008). Over production or an ineffective elimination of ROS may induce oxidative stress and cause damage to all types of biomolecules such as proteins, lipids and nucleic acids (Droge W, 2002). Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activators of antioxidative defense enzyme system to suppress the radical damages in biological systems (Murphy MP, 2011; Venkatesh S et al., 2009). Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species (Peng KT et al., 2011; Ling LU et al., 2011). Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of these diseases. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance (Battu GR et al., 2011; Lawrence R et al., 2011).

***Rhynchosia beddomei Baker*** India has rich and varied heritage of biodiversity covering ten bio-geographical zones. The Indian subcontinent is blessed with a wide variety of aromatic and medicinal plants. India nurtures enormous plant diversity and as many as 140 genera out of 5285 angiosperm species are endemic to the country (Annual Report, 2001). *Rhynchosia beddomei* is commonly called as Adavi Kandi and Vendaku occurring in dry deciduous forests. It is endemic to Southern India, found in Andhra Pradesh and Karnataka states. In Andhra Pradesh it is distributed in parts of Kadapa, Chittoor and Anantapur districts. In Chittoor district, it is common in Talakona forest and Japaliteertham, Gogarbham area, Sandralamitta and near deer park of Tirumala hills (Pullaiah T, 2006). *Rhynchosia beddomei* Baker belongs to Fabaceae family and is a rare and endemic medicinal plant distributed in Seshachalam hills of Eastern Ghats of Andhra Pradesh, India (Madhavachetty et al., 2008). Its present status is “Vulnerable” in the IUCN Red data book of Indian plants mainly because of its restricted distribution in Tirumala hills and small number of individuals left in the world (Sudhakar Reddy Ch et al., 2006). The genus Rhynchosia

* Corresponding Author
Email: shankarpharmacology@gmail.com
Contact: +91- 9959348676
Received on: 19-08-2013
Revised on: 22-11-2013
Accepted on: 25-11-2013

consists of approximately 200 species and occurs in both the eastern and western hemisphere in warm temperate and tropical regions (Grear J. W, 1978). Bakshu et al., (2001), studied the phytochemical properties of the leaves and reported the presence of alkaloids, indole alkaloids, anthracene glycosides, anthraquinones, carotenoids, coumarins, fatty acids, flavonoids, steroids and triterpenoids. . From the leaves of *Rhynchosia beddomei* Baker, a new flavonone- O- and C-glycoside were isolated and characterized (Bakshu LMD and Raju RRV, 2001). The plant material was also reported to contain flavonoid compounds viz., Flavones, flavonols and flavanones (Bakshu and Raju, 2009). Bakshu and Raju (2009) reported that the leaves of *Rhynchosia beddomei* have abortifacient, antibacterial, antifungal, antidiabetic and hepatoprotective properties. The leaves are also used for healing wounds, cuts, boils and rheumatic pains byadivasi tribes (Adinarayana D, 1980). The present investigation is focused on the antioxidant property of the *Rhynchosia beddomei* Baker is due to the presence of constituents like phenols, flavonoids, tannis and triterpenoids. The antioxidant activity on the plant not yet reported and therefore the present study was undertaken to evaluate the *in vitro* antioxidant studies for ethanolic extract of leaves of *Rhynchosia beddomei Baker*

**Figure 1: Ethanolic Extract of leaves**

**Chemicals**

DPPH (1, 1-diphenyl-2-picryl hydrazyl), Gallic acid, ascorbic acid, Follin-Ciocalteu's phenol reagent and BHT (Butylated hydroxy toluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (petroleum ether, chloroform, ethyl acetate, ethanol, ), sulphuric acid, sodium phosphate, ammonium molybdate, ferric chloride, , hydrochloric acid, copper sulphate, aluminium chloride, lead acetate, acetic acidand ammonia from Merck (Pvt.) Ltd. (Germany).

**Plant material**

The fresh leaves of *Rhynchosia beddomei Baker* were collected in December 2009 from sesachala hills of Tirupati, Andhra Pradesh (India) and authenticated by Dr. K. MadhavChetty, Assistant Professor, Taxonomist, Dept. of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India (Madhavachetty et al., 2008). A voucher specimen has been kept in our laboratory for future reference

**Preparation of extract**

The whole plant was cleaned, air dried and grounded into powdered. The dried powdered plant material was passed throμgh sieve 40 and stored in air tight container. The powdered material was subjected to successive solvent extraction using petroleum ether, chloroform, ethyl acetate, ethanol and water, based on the increasing order of polarity of solvent. Initially 100gm of crude powder was taken and packed in a packing paper. This pack was placed in a Soxhlet extractor for extraction with different solvents i. e. (Petroleum ether, chloroform, ethyl acetate, 70% ethanol, water) and the temperature was adjusted as per the solvent been used in the extraction. The extract is then concentrated and dried under reduced pressure. The percentage yield obtained was calculated and reported (Harborne JB., 1998; Khandelwal. K. R., 2005; Kokate. CK., 2009).

$$\text{Percentage yield} = \frac{\text{weight of the extract}}{\text{weight of plant material}} x\ 100$$

**Preliminary phytochemical analysis**

The preliminary phytochemical studies were conducted on the active extracts using standard procedures adopted by Gibbs R. D., 1974, Kokate C. K., 1994 and Harborne JB., 1998. Preliminary phytochemical analysis on plant extracts was performed using the following chemicals and reagents: Flavonoids (Mg metal and HCl), phenolics (FeCl3), protein and amino acid (Million's and Ninhydrin reagent), alkaloids (Mayer and Dragendorff's reagent), saponins (Foam test), phytosterols and triterpenoids (Liebermann- Burchard Test) and carbohydrates. (Fehling's solution A and B) (Gibbs R. D., 1974; Kokate C. K., 1994; Harborne JB., 1998)

**Determination of *in vitro* antioxidant activity**

The antioxidant activity was evaluated by eight ways which are as follows;

**Total polyphenol content determination**

Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method (*Demiray et al*., 2009). 1 ml of the plant extracts/standard of different concentration solution was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (7. 5% sodium carbonate) of sodium carbonate. The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for color development. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. Absorbance of samples and standard were measured at 765 nm using spectrophotometer against blank. A typical blank solu-

tion contained the solvent used to dissolve the plant extract. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg gallic acid equivalent of dry extract. All determination was performed in triplicate.

**Determination of total flavonoids content**

Aluminum chloride colorimetric method was used for flavonoids determination (Wang and Jiao, 2000). 1 ml of the plant extracts/standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. Quarcetin was used as standard for the calibration curve. Total flavonoid content of the extracts and fractions were expressed as mg quarcetin equivalents (QE) per gram of sample (mg/g).

**Free radical scavenging activity (DPPH method)**

The scavenging activity for DPPH free radicals was measured according to the procedure (*Blios et al., 1958).* Ethanolic solution of the sample extract at various concentrations (50, 100, 150, 200 and 250 µg/mL) was added separately to each 5 ml of 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27c. After incubation, the absorbance of each solution was determined at 517 nm using spectrophotometer. Ascorbic acid and BHT (butylatedhydroxy toluene) was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavengening activity percentage} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} x100$$

IC50 value is the concentration of the sample required to scavenge the 50% DPPH free radical.

**Nitric oxide scavenging capacity assay**

Nitric oxide scavenging assay was carried out using sodium nitroprusside (Sreejayan and Rao, 1997). This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 min. The absorbance was measured at 546 nm. A typical blank/control solution contained the same solution mixture without plant extract or standard. The absorbance of the blank/control solution was measured at 546 nm. The percentage inhibition was calculated according to the following equation:

$$\text{Percentage of inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} x100$$

**Scavenging of hydrogen peroxide**

Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide (Jayaprakasha et al., 2004). 1 ml of various concentrations of the extract, sub-fractions and standards in ethanol was added to 2 ml of hydrogen peroxide solution in phosphate buffered saline (PBS, pH 7.4). Then finally the absorbance was measured at 230 nm after 10 min. Ascorbic acid and BHT were used as standard. Control sample was prepared containing the same volume without any extract; standard and the absorbance were read at 230 nm using a spectrophotometer. The percentage inhibition was calculated according to the following equation:

Hydrogen peroxide scavenging activity (%) was calculated as:

$$\text{Percentage of inhibition} = \frac{\text{A0} - \text{AS}}{\text{AO}} x100$$

Where AO= absorbance of Control; AS = absorbance of sample

**Reducing power capacity assessment**

Assay of reducing power was carried out by potassium ferricyanide method (Yildirim et al., 2001). 1 ml of extract and its sub-fractions (final concentration 5 to 200 g/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. To this mixture, 2.5 ml of trichloroacetic acid was added, which was then centrifµged at 3000 rpm for 30 min. Finally, 2.5 ml of the supernatant solution was collected and mixed with 2.5 ml of distilled water and 0.5 ml ferric chloride and absorbance was measured at 700 nm. Ascorbic acid and butylated hydroxy toluene (BHT) were used as standard and phosphate buffer as blank solution.

The reducing power capacity assessment was calculated as:

$$\text{Reducing power capacity} = \frac{\text{AO} - \text{AS}}{\text{AO}} x100$$

**Superoxide Anion Radical Scavenging Activity**

Superoxide Anion Radical Scavenging Activity was measured with some modifications (Duan et al., 2007). The various fractions of plant extracts were mixed with 3 ml of reaction buffer solution (pH, 7.4) containing 1. 3 µM riboflavin, 0.02 M methionine and 5.1 µM NBT. The reaction solution was illuminated by exposure to 30 W fluorescent lamps for 20 minutes and the absorbance was measured at 360 nm using a spectrophotometer. Ascorbic acid and BHT was used as positive con-

trol and the reaction mixture without any sample was used as negative control.

The superoxide anion radical scavenging activity (%) was calculated as:

$$\text{Radical scavengening activity percentage} = \frac{AO - AS}{AO} \times 100$$

**Metal chelating activity**

The chelating of ferrous ions by the extract of leaves of *Rhynchosia beddomei* was estimated by the method described *by Dinis et al., 1994*. Various concentrations of the extracts viz., 50, 100, 150, 200, 250 and 300 µg/ml of sample was added with 1 ml of 2mM FeCl2 separately. The reaction was initiated by the addition of 5mM ferrozine (1ml). Absorbance was measured at 562nm after 10min. Ascorbic acid and BHT was used as standards

$$\text{Chelating activity percentage} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} x100$$

**Result**

**Successive Solvent Extraction**

The powdered material was subjected to soxhlation using petroleum ether, chloroform, ethyl acetate, 70% ethanol and water by successive solvent extraction method based on the increasing order of polarity of solvent. The following chemical constituents are reported in each extract that given in below table.

**Phytochemical screening**

The preliminary phytochemical studies were performed on the extracts by using standard procedures and result were given in table no 2.

The 70% ethanolic extract of leaves of *Rhynchosia beddomei* contains alkaloids, glycosides, phenols, tannins, flavonoids, steroids and amino acids while carbohydrates and resins are absent

**Determination of total phenolic contents**

Graph 1 shows the total phenolic content of ethanolic extract of leaves of *Rhynchosia beddomei* was compared to the calibration curve of Gallic acid. The total antioxidant capacity was found remarkable in ethanolic soluble fraction to be 52.59 mg Gallic acid equivalent/g extract. Phenolics or polyphenols are secondary plant metabolites that are present in plants and plant products. Many of the phenolics have been shown to contain high levels of antioxidant activities. Phenolic compounds contribute to the overall antioxidant activities of plants mainly due to their redox properties. Generally, the mechanisms of phenolic compounds for antioxidant activity are neutralizing lipid free radicals and preventing decomposition of hydro peroxides into free radicals. TPC of extracts was determined by Folin-Ciocalteau (F-C) assay using Gallic acid as a standard phenolic compound. The F-C assay for total phenolics contents is a fast and simple method and can be useful in characterizing and the total phenolic content expressed in terms of GAE and yield (%) of extract was found to be 52.59mg of GAE/g. The total phenolic contents were calculated using the following linear equation based on the calibration curve of gallic acid standard curve. F-C method is based on oxidation of phenolics in F-C reagent to yield a colored product with λmax 765. A linear calibration curve of Gallic acid, in the range of 20 – 80 µg/ml with coefficient of determination ($r^2$) value of 0.9998 was obtained. F-C assay gives a crude estimate of the total phenolic compounds present in an extract/fraction.

**Total flavonoid content**

Graph 2 shows the total flavonoid content of ethanolic

**Table 1: % of yield of different extracts of *Rhynchosia beddomei***

| S NO | Parameters | Pet ether | Chloroform | Ethyl acetate | Ethanol | water |
|---|---|---|---|---|---|---|
| 1 | Consistency | Waxy | Oily | Oily | Viscous | Viscous |
| 2 | Color | Greenish black | Light green | Brownish green | Reddish black | Cream |
| 3 | % of yield | 4.704% | 3.36% | 5.96% | 12.5% | 4.24% |

**Table 2: Phytochemical screening of different extracts of *Rhynchosia beddomei***

| S NO | Phyto constituents | *Phytochemical screening of Rhynchosia beddomei* | | | | |
|---|---|---|---|---|---|---|
| | | Pet ether | Chloroform | Ethyl acetate | Ethanol | water |
| 1 | Alkaloids | - | - | + | + | + |
| 2 | Glycosides | + | + | + | + | - |
| 3 | Carbohydrates | - | - | + | - | - |
| 4 | Amino acids | + | + | + | + | + |
| 5 | Flavonoids | + | + | + | + | + |
| 6 | Steroids | + | - | + | + | - |
| 7 | Saponins | - | - | - | + | - |
| 8 | Phenol's | + | + | + | + | + |
| 9 | Tannins | + | + | + | + | + |
| 10 | Resins | + | + | - | - | - |

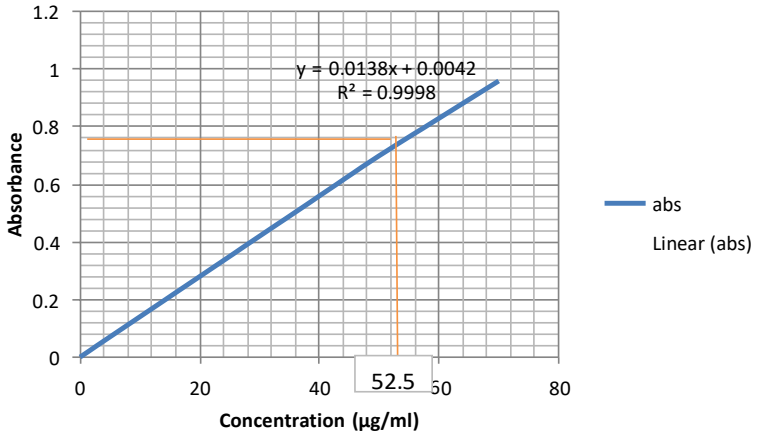


**Figure 2: Total phenolic content of ethanolic extract of *Rhynchosia beddomei***

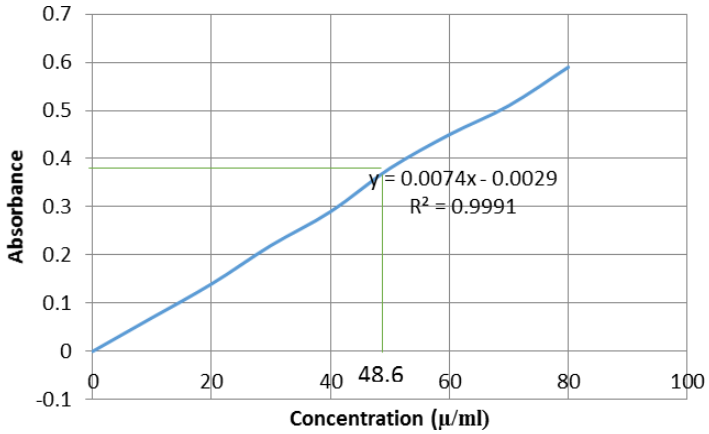


**Figure 3: Total flavonoid content of ethanolic extract of leaves of *Rhynchosia beddomei***

extract of leaves of *Rhynchosia beddomei* was compared to the calibration curve of quarcetin. The total antioxidant capacity was found remarkable in ethanolic soluble fraction to be 48. 6 mg quarcetin equivalent/g extract.

**DPPH radical scavenging activity**

To evaluate the antioxidant activity of the ethanolic leaf extract, the radical scavenging capacity based on DPPH assay was determined and the results are shown in Table 3 for the species of *Rhynchosia beddomei*. The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentrations of the extract from 50 -250 µg/mL. The percentage of inhibition of the DPPH radical was varying from 18. 33% (in 50 µg/mL of the extract to 62.25% (in 250 µg/mL of extract). The IC50 value of the ethanolic leaf extract of this species was determined to be 209 µg/mL. The extracts in all concentrations showed the percentage of inhibition of free radicals.

**NO Scavenging activity**

The different concentrations of the extract of leaves of *Rhynchosia beddomei* were from 50 -300µg/ml produces the percentage of inhibition varying from 22.12% (in 50 µg/ml of the extract to 70.01% (in 300 µg/ml of extract). The IC50 value of the ethanolic leaf extract of this species was determined to be 191. 18 µg/ml. The percentage of inhibition of free radicals and IC 50 value are given in table no -4

**$H_2O_2$ Scavenging activity**

Hydrogen peroxide, althoµgh not a radical species play a role to contribute oxidative stress. The generation of even low levels of $H_2O_2$ in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with $H_2O_2$ in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects (Miller et al., 2000). Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Halliwell et al., 1987). Thus, removal of $H_2O_2$ is very important for protection of food systems. $H_2O_2$ Scavenging activity of extracts may be attributed to their phenolics, which can donate electrons to $H_2O_2$, thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. The percentage of inhibition of the $H_2O_2$ was varying from 26.13% (in 50µg/ml of extract) to 72.08% (in 300 µg/mL extract). The IC50 value of the ethanolic leaf extract of the study species was 192.14µg/ml. All the concentrations of sample extracts showed higher percentage of inhibition of $H_2O_2$ Scavenging power to the respective concentration of standard drµg, BHT and ascorbic acid.

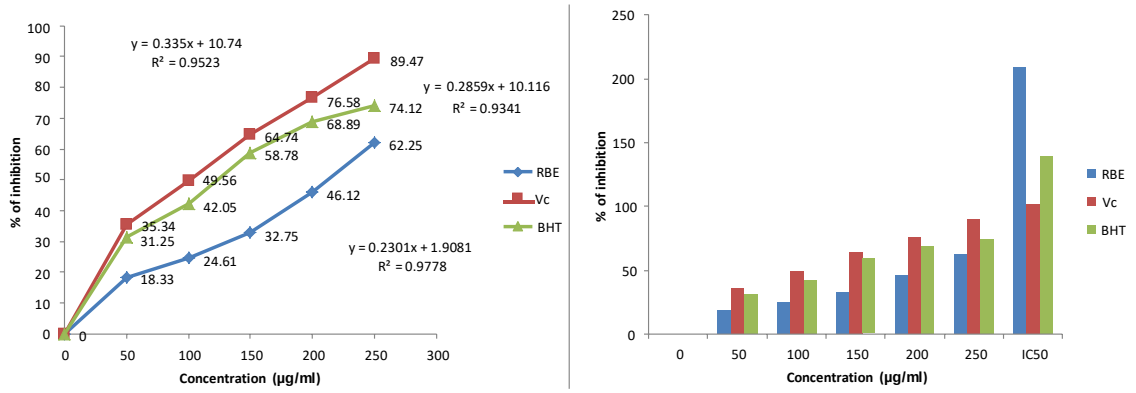


**Figure 4: DPPH radical scavenging activities of ethanolic extract of *Rhynchosia beddomei***

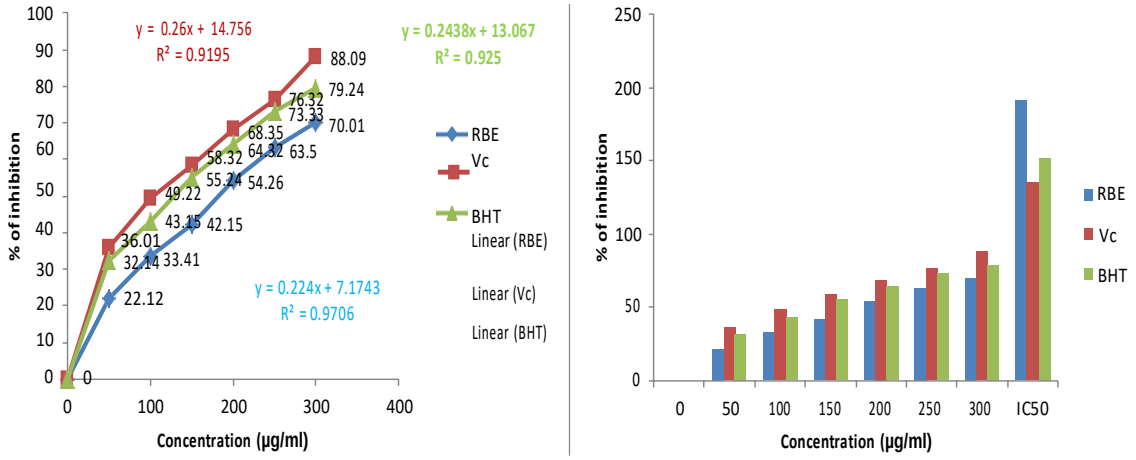


**Figure 5: NO Scavenging activity of ethanolic extract of *Rhynchosia beddomei***

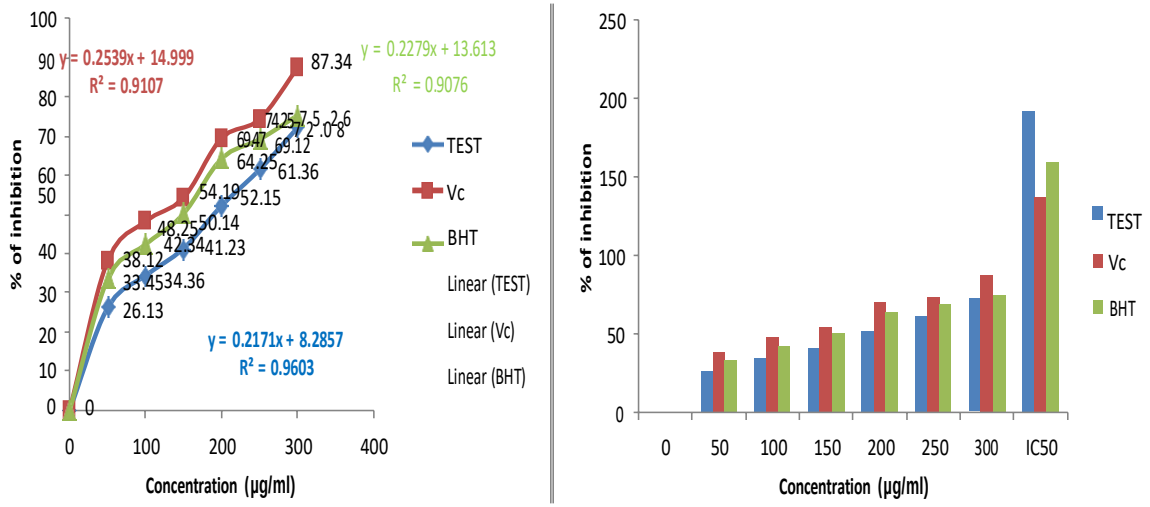


**Figure 6: $H_2O_2$ scavenging activities of ethanolic extract of *Rhynchosia beddomei***

**Reducing power assay**

The results of antioxidant activity of the leaf extract of *Rhynchosia beddomei* based on reducing power were presented in Table 4. The scavenging activity was determined to be increased with the increase in the concentration of extract from 50 to 300 µg/mi. The percentage of inhibition of the reducing power was varying from 18.21% (in 50µg/mL of extract) to 69.41% (in 300µg/mL extract). The IC50 value of the ethanolic leaf extract of the study species was 218.40 µg/ml. All the concentrations of sample extracts showed higher percentage of inhibition of reducing power to the respective concentration of standard drug, BHT and ascorbic acid.

**Superoxide anion radical scavenging activity**

Percentage inhibition of superoxide radical generation was determined and compared with same doses of BHT and ascorbic acid in table No -5. The IC 50 value of *Rhynchosia beddomei* was found to be 190.97% whereas the standards BHT and ascorbic acid were found to be 168. 13 and 147. 85% respectively.

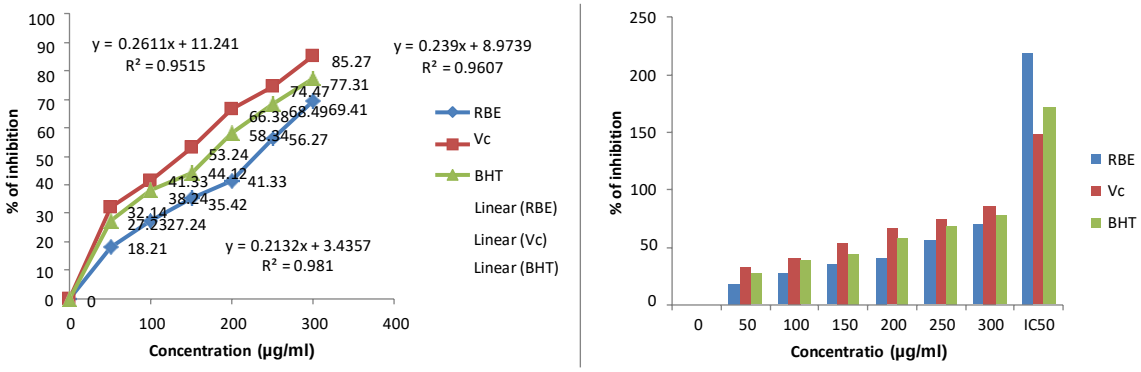


**Figure 7: Reducing power assay of ethanolic extract of *Rhynchosia beddomei***

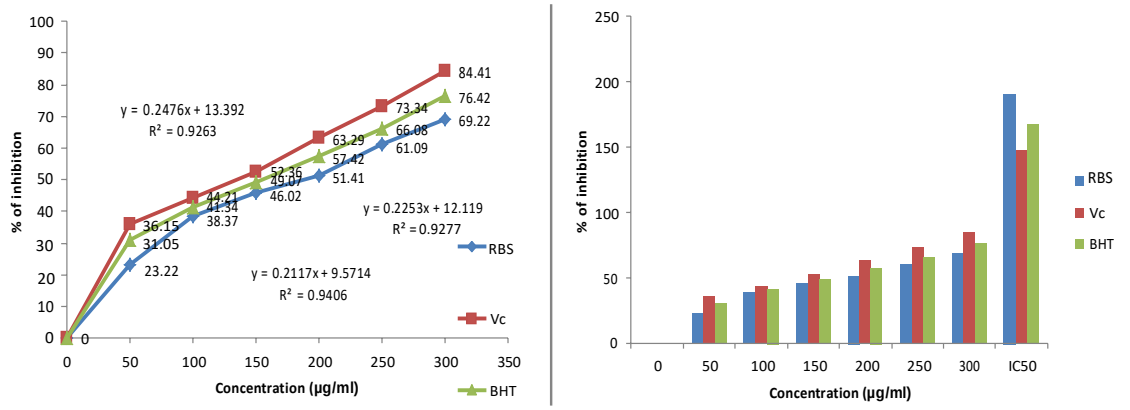


**Figure 8: Superoxide anion radical scavenging activity of ethanolic extract of *Rhynchosia beddomei***

### Metal chelation activity

The results of antioxidant activity of the leaf extract of *Rhynchosia beddomei* based on metal chelating activity are given in Table No -6. As observed in DPPH and NO radical scavenging assays, the percentage of metal chelating activity was determined to be sample concentration dependent and it was increasing with the increase in the concentration of extract from 50 to 300 µg/mL. The percentage of inhibition of the metal chelation was varying from 16.02 % (in 50 µg/ml of extract) to 66. 18% (in 300 µg/ml extract). The IC50 value of the ethanolic leaf extract of the study species was 220.73 µg/mL. The percentage of inhibition of free radicals by various concentrations of sample were less than the respective concentration of standard drµg and BHT

### Discussion

The consumption of vegetables, fruits and some part of the plant can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames B.M et al., 1993). Oxidants, commonly known as "free radicals" is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals, which are the harmful by-products generated during normal cell aerobic respiration in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology (Gutteridge, J. M. C. and Halliwell, B., 2000). An over production of these reactive species can occur, due to oxidative stress broµght about by the imbalance of the bodily antioxidant defense system and free radical formation (Lekameera R., 2008). Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. They are the first line of defense against free radical damage and are critical for maintaining optimum health (Cheeseman K. H. and Slater T. F, 994). Therefore, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and/or the propagation of oxidative diseases (Willet W. C., 1994). The most common antioxidants present in vegetables and part of the plants are vitamins C, vitamin E, Carotenoids, phenols and flavonoids.

*In vitro* antioxidant activity of the ethanolic extract leaves of *Rhynchosia beddomei* was investigated in the present study by total phenol content, flavonoid content, DPPH, nitric oxide scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, super oxide ion scavenging activity and metal chelating assays. The antioxidative effect is mainly due to phenolic components, such as phenolic acids, phenolic diterpenes and the presence of hydroxyl groups (Shahidi F et al., 1992; Shah R et al., 2010). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Shah R, 1994). Phenolic compounds and terpenoids have been shown to be responsible for the antioxidant activity of plant extracts. (Rice-Evans et al., 1996). A number of studies have focused on the biological activities of phenolic compounds, which are

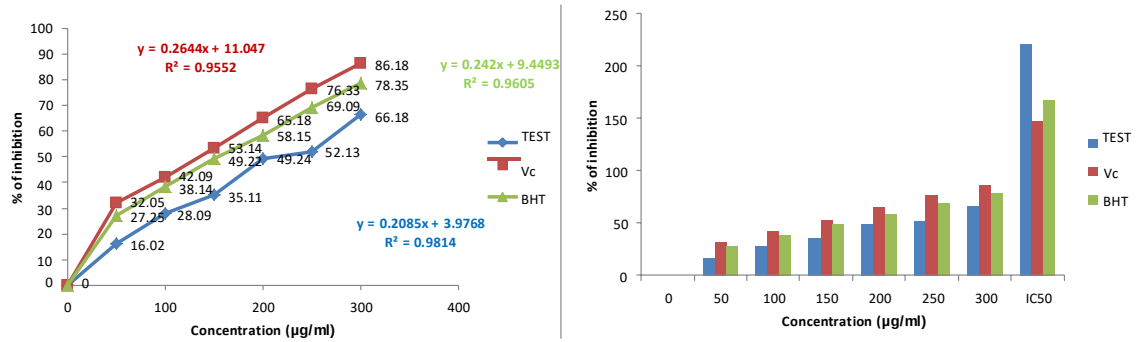


**Figure 9: Metal chelation activity of ethanolic extract of *Rhynchosia beddomei***

potential antioxidants and free radical scavengers (Ara N and Nur H. 2009). Therefore, the content of total phenols in the extract was investigated by the Folin-Ciocalteu method. The content of total phenols is expressed as gallic acid equivalents (mg gallic acid/g extract). It is suggested that polyphenol compounds have inhibitory effects on mutagenesis and carcinogenesis in humans (Sawant O et al., 2009). The total phenol content of the ethanolic extract of *Rhynchosia beddomei* was given in Graph no 1. The amount of total phenol was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 0.013x + 0.0042, R2=0.9998, Where y is absorbance at 760 nm and x is totalphenolic content in the extracts of *Rhynchosia beddomei* expressed in mg/gm. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidant on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1 - 1 diphenyl - 2 - picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug (Sochor J et al., 2010). The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant activity (Jun M et al., 2004). In the present study, the ethanolic extract of leaves of *Rhynchosia beddomei* had significant scavenging effect on the DPPH radical which was increasing with the increase in the concentration of the sample from 50 - 250 µg/mL. Similar trend of DPPH free radical scavenging activity was reported for the species, Lippia Alba (Shah R et al., 2010).

Nitric oxide (NO) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, smooth muscle relaxation, inhibition of platelet aggregation and regulation of cell mediated toxicity and antitumor activities (Hagerman et al., 1998). Despite the possible beneficial effects of NO., its contribution to oxidative damage is also reported. This is due to the fact that NO. Can react with superoxide to form the peroxy nitrite anion, which is a potential oxidant that can decompose to produce OH and NO. The procedure is based on the principle that, sodium nitro-prusside in aqueous solution at physiological pH (7.2) spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO. May lead to tissue damage. Suppression of released NO may be partially attributed to direct NO scavenging activity, as the extracts of *Rhynchosia beddomei* decreased the amount of nitrite generated from the decomposition of SNP in vitro. The scavenging of NO by the ethanolic extract of leaves of *Rhynchosia beddomei* was increased in dose dependent manner from 50 to 300 µg/ml under aerobic conditions. H2O2 is highly important because of its ability to penetrate biological membranes. $H_2O_2$ itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells (Sakat S. S et al., 2010). The results showed that extracts and fractions of *Rhynchosia beddomei* had an effective $H_2O_2$ scavenging activity.

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT (Nitro-blue tetrazolium) resulting in the formation of blue formazan. In-vitro super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. The super oxide radical reduces NBT to a blue colored formazan that can be measured at 360 nm. From results, it was found that the ethanolic extract of leaves of *Rhynchosia beddomei* showed potent free radical scavenging activity compared to the ascorbic acid and BHT (standard) at low IC50. The ethanolic extract of

leaves of *Rhynchosia beddomei* is electron donordue to the presence of electron donating substituents groups like –OH, -CL and $–CH_3$. This compound donated their electrons to the superoxide and scavenges them to prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product (Wang W. K et al., 2005).

The presence of transition metal ions in a biological system could catalyze the Haber-Weiss and Fenton type reactions, resulting in the generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH generation and inhibition of peroxidation processes of biologicalmolecules (Chew YL, 2009). The metal ion scavenging effect was increasing with the increase in the concentration of the extracts from 50-300 µg/mL. The high metal ion scavenging activity of the ethanolic extract of leaves of *Rhynchosia beddomei* is probably due to the chelating agents, which form sigma bonds with the metal and effective as secondary antioxidants because they reduce the redox potential, thereby the oxidized form of the metal ion (Gulcin S et al., 2004)

In the present study, it is found that ethanolic extract of Rhynchosiabeddomei showed concentration dependent free radical scavenging activity and this antioxidant effect may be due to the higher content of alkaloids, flavonoids, steroids, glycosides, tannins and phenols (Gulcin S et al., 2004). The results of the present study indicate that the ethanolic extract leaves of *Rhynchosia beddomei* has significant antioxidant activities which is comparable to that of the standard drugs ascorbic acidand BHT. Thus, the ethanolic extract of leaves of *Rhynchosia beddomei* as promising natural sources of antioxidants can be used in nutritional or pharmaceutical fields for the prevention of free-radical-mediated diseases.

## CONCLUSION

The study was performed to evaluate the *in vitro* antioxidant activity of ethanolic extract of leaves of *Rhynchosia beddomei*. The results obtained from all the eight methods indicates the significant antioxidant activity through total phenol content, total flavonoid content, DPPH scavenging activity, NO Scavenging activity, $H_2O_2$ Scavenging activity, Reducing power assay, Superoxide anion radical scavenging activity and metal chelation activity. In the present study, it is found that ethanolic extract of *Rhynchosia beddomei* has showed concentration dependent free radical scavenging activity and this antioxidant effect may be due to the presence of higher content of alkaloids, flavonoids, steroids, glycosides, tannins and phenols. The results were compared with standard references Gallic acid, quercetin, ascorbic acid and BHT. Further research investigations may be carried out to isolate the actual phytoconstituents responsible for antioxidant activity.

## REFERENCES

Adinarayana D, Gunasekar D, Seligmann O. and Wagner H. Flavone o- and c-glycosides of *Rhynchosia beddomei*. *Photochemistry*; 1980; 19:480-481.

Ames, B. M., Shigena, M. K. andHagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. AcadSci., USA, 90: 7915 - 7922.

Ara N, Nur H. *In vitro* antioxidant activity of methanolic leaves and flowers extracts of Lippia alba. Research Journal of Medicine and Medical Sciences 2009; 4 (1): 107-110

Arulmozhi S., Mazumder P. M., Narayanan L. S. and Thakurdesai P. A., *In vitro* antioxidant and free radical scavenging activity of fractions from Alstoniascholaris Linn. R. Br., International Journal of Pharma Tech Research, 2010, 2, 18-25.

Bakshu LMD and Raju RRV. Antimicrobial activity of *Rhynchosia beddomei*. Fitoterapia; 2001. 72:579-582.

Battu GR, Ethadi SR, Veda Priya G, SwathiPriya K, Chandrika K, Venkateswara Rao A, et al. Evaluation of antioxidant and anti-inflammatory activity of Euphorbia heyneanaSpreng. Asian Pac J Trop Biomed 2011; 1 (2): 191-194.

Blios MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 26: 1199 - 1200.

Cheeseman, K. H. and Slater, T. F. (1994). Free radical in medicine, 49: 479724.

Chew YL, Goh JK, Lim YY. Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem 2009; 116: 13-18.

Demiray S, Pintado ME, Castro PML (2009). "Evaluation of Phenolic Profiles and Antioxidant Activities of Turkish Medicinal Plants: TiliaArgentea, Crataegi folium Leaves and Polygonumbistorta roots" World Acad. Sci., Eng. Technol. 54:312-317.

Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (Acetoaminophen, Salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315: 161-169.

Droge W. Free radicals in the physiological control of cell function. Physiology Rev 2002; 82: 47-95.

Duan, X., Wu, G., Jiang, Y., (2007). Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules, 12 (4), 759-771.

Fransworth, N. R. Biological and Phyto-chemical screening of medicinal plants. Journal of pharmaceutical Sciences. 1966: 55:225-276.

Gibbs, R. D. Chemotaxonomy of flowering plants. MC Gill Queens University press, Montreal and London. 1974.

Grear J. W, 1978. A revision of the New World species of Rhynchosia (Leguminosae-Harper, K. T. 1979. Some reproductive and life history characteristics of rare plants and implications for management. The endangered species: a symposium. Great Basin Naturalist Memoirs 3: 129–137.

Gulcin S, Beydemir H, Alici A, Elmasta M, Buyukokuroglu ME. *In vitro* antioxidant properties of morphine. Pharmacol Res 2004; 49: 59-66. 534.

Gutteridge, J. M. C. and Halliwell, B. (2000). Free radicals and antioxidants in the year 2000 Ahistorical look to the future, 899:136-147.

Harbone, J. B., Turner, B. L. Plant chemo-systematics. Academic press, London. 1984: P: 61-62.

Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis. London: Chapman and Hill; 1998.

Jayaprakasha GK, Rao LJ, Sakariah KK (2004). Antioxidant activities of flavidin in different *in vitro* model systems. Bioorg. Med. Chem., 12 (19):5141-5146.

Jun M, Tohru U, Jianzhang L, Takeshi F. Identification and evaluation of antioxidant activities of bamboo extracts. For Stud China 2004; 6: 1-5.

Khandelwal. K. R, Practical pharmacognosy, I edition, Nirali prakashan, Pune India Reprint, 2005, 27-35.

Kokate, C. K. Practical Pharmacognosy, 4th edn. Vallabh Prakan, New Delhi. 1994, 179-181.

Lawrence R, Kapil L. Antioxidant activity of garlic essential oil (Allium sativum) grown in north Indian plains. Asian Pac J Trop Biomed 2011; 1 (1): 1-3. Annual Report, 2001. Botanical Survey of India.

Lekameera, R., Vijayabaskar, P. and Somasundaram, S. T. (2008). Evaluating antioxidant property of brown ALGA (Derb. et sol), 2: 126-130.

Ling LU, Tan KB, Lin H, Chiu GNC. The role of reactive oxygen species and autophagy in safingol-induced cell death. Cell Death and Disease 2011; 2: 1-12.

Madhava chetty, K. Sivaji, K., Tulasi rao., mentioned in the Book of Flowering plants of chittoor district, Andhra Pradesh, II edition, India, 2008, 90 – 95

Madhava chetty, K. Sivaji, K., Tulasi rao., mentioned in the Book of Flowering plants of Chittoor district, Andhra Pradesh, II edition, India, 2008, 90 – 95

Murphy MP, Holmgren A, Goran Larsson N, Halliwell B. Unraveling the biological roles of reactive oxygen species. Cell Metab 2011; 13 (4): 361-366.

Pari L, Amudha K. Antioxidant effect of naringin on nickel-induced toxicity in rats: an in vivo and *in vitro* study. Int J Pharm Sci Res 2011; 2 (1): 151-158.

Peng KT, Hsu WH, Shih HN, Hsieh CW, Huang TW, Hsu RWW. The role of reactive oxygen species scavenging enzymes in the development of septic loosening after total hip replacement. J Bone Joint Surg 2011; 93-B: 1201-1209.

Pullaiah, T. 2006. Biodiversity and conservation with special reference to Eastern Ghats. In: Proc. Of National Conf. on Forest Biodiversity resources: Exploitation, Conservation and Management. CBFS, Madurai Kamraj University, Madurai

Rice-Evans C, Miller N, Paganga G, Structure-antioxidant activity relationships of flavonoids and phenolic acids, Free Radical Biology and Medicine 20, 933-956, 1996.

Sakat S. S., Juvekar A. R. and Gambhire M. N., *In vitro* antioxidant and anti-inflammatory Activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharmacy and Pharmaceutical Sciences, 2010, 2, 146-155

Sathishkumar P, Paulsamy S, nandakumar AM, Senthilkumar P. Effect of habitat variation on the content of certain secondary metabolites of medicinal importance in the leaves of the plant, Acacia aesia Willd. Journal of Advances in Plant Sciences 2009; 22 (11): 451-453.

Sawant O., Kadam J. and Ghosh R., *In vitro* free radical scavenging and antioxidant activity of Adiantum lunulactum, Journal of Herbal Medicine and Toxicology, 2009, 3, 39-44.

Sen S, Chakraborty R, Sridhar C, Reddy YSR. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharmaceutical Sci Rev Res 2010; 3 (1): 91-100.

Shah R Novel natural antioxidants for utilization in food and biological systems. In: Uritani I, Garcia VV, Mendoza EM (Eds) Post harvest biochemistry of plant food materials in the tropics. Japan Scientific Societies Press, Japan: 241251, (1994).

Shah R, Kathad H, Sheth R, Sheth N. *In vitro* antioxidant activity of roots of Tephosiapurpurea Linn. International Journal of Pharmaceutical Sciences 2010; 2 (3): 30-33.

Shahidi F, Janitha PK and Wanasundara PD, Phenolic antioxidants. CRC Critical Rev. Food Science and Nutrition. 32 (1): 67103, (1992).

Shao HB, Chu LY, Lu ZH, Kang CM. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int J Biolog Sci 2008; 4: 8-14.

Sochor J, Ryvolova M, Krystofova O, Salas P, Hubalek J, Adam V, Trnkova L, Havel L, Beklova M, Zehnalek J,

Provaznik I, Kizek R. Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages. Molecules 2010; 15: 8618-8640.

Sreejayan N, Rao MNA (1997). Nitric Oxide Scavenging by Curcuminoids. J. Pharm. Pharmacol. 49 (1):105-107.

SudhakarReddy Ch., Reddy, K. N., Pattanaik, C and Raju, V. S. 2006. Ethnobotanical Observations on some Endemic Plants of Eastern Ghats, India. Ethnobotanical Leaflets. 10:82-91

Venkatesh S, Deecaraman M, Kumar R, Shamsi MB, Dada R. Role of reactive oxygen species in the pathogenesis of mitochondrial DNA (mt DNA) mutations in male infertility. Indian J Med Res 2009; 129: 127-137.

Wang SY, Jiao H (2000). Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J. Agric. Food Chem. 48 (11):5672-5676.

Wang W. K., Park H. S., Ham I., Oh M., Namkoong H., Kim H. K., Hwang D. W., Hur, S. Y., Kim T. E., Park Y. G., Kim J. R., Kim J. W. Natural compounds, fraxin and chemically structurally related to fraxin protect cells from oxidative stress. Exp. Mol. Med. 2005; 37: 436–446.

Willet, W. C. (1994). Diet and health what should we eat? 264: 532 – 537

Yildirim A, Mavi A, Kara AA (2001). Determination of Antioxidant and Antimicrobial Activities of Rumex crispus L. Extracts. J. Agric. Food Chem. 49 (8):4083-4089.