Advances in Bioresearch
Adv. Biores., Vol 15 (4) July 2024:131-137

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.4.131137

Advances in Bioresearch

ORIGINAL ARTICLE

Exploring The Antioxidant Properties of Methanolic Extract from *Euphorbia neriifolia* Linn. through *In Vitro* Free Radical Scavenging Analysis

Susmita Dhar*, Akash Yadav, Naveen Gupta, Dharmendra Singh Rajput
Patel College of Pharmacy, Madhyanchal Professional University, Ratibad, Bhopal, Madhya Pradesh, India, 462044.
***Corresponding author: Susmita Dhar**
Correspondence Email: susmitadhar620@gmail.com

ABSTRACT
The relevance of natural antioxidants for human health is underscored by the widely recognized function of reactive oxygen species (ROS) in inducing cellular damage linked to numerous illnesses. Hence, safeguarding human health is greatly assisted by natural antioxidants. We conducted in vitro studies to determine if extracts from the stems of Euphorbia neriifolia (EN) have antioxidant characteristics and could scavenge reactive oxygen species. Antioxidant activity, phenolic content, and reducing power were assessed by testing methanol extracts. On top of that, we checked the extracts' ability to neutralize DPPH, hydroxyl, superoxide, nitric oxide, and hydrogen peroxide radicals to see how efficient they were. The EN extract included cardiac glycosides, alkaloids, tannins, saponins, and flavonoids, according to the analysis. In addition, we used conventional assay techniques to determine the total phenolic content. The results showed that the extract had a dose-dependent, strong antioxidant effect. The following concentrations were used to obtain the IC50 values: 85.11 ± 0.53 µg/ml for DPPH, 144.22 ± 2.1 µg/ml for nitric oxide, 58.10 ± 2.10 µg/ml for superoxide, and 25.12 ± 2.71 µg/ml for hydroxyl radicals. The extract also showed reductive potential that was dosage dependent. The stem extract's antioxidant and free radical scavenging properties were enhanced by the study, which showed that 10 mg of the extract contained 675.6 µg of phenolic components. Finally, our work emphasizes that methanol extracts from EN stems have antioxidant capabilities, which might make them useful as free radical inhibitors or natural antioxidants.
Keywords: *Euphorbia neriifolia, reactive oxygen species, antioxidant, reactive nitrogen species and total phenolic content*

Received 26.03.2024 Revised 18.04.2024 Accepted 19.06.2024

How to cite this article:
Susmita D, Akash Y, Naveen G, Dharmendra S R. Exploring The Antioxidant Properties of Methanolic Extract from *Euphorbia neriifolia* Linn. through *In Vitro* Free Radical Scavenging Analysis. Adv. Biores. Vol 15 [4] July 2024. 131-137

INTRODUCTION

It is believed that oxidative stress brought free radicals and reactive oxygen species (ROS) speeds up a number of physiological problems, such as cancer, ageing, Alzheimer's disease, Parkinson's disease, arthritis, coronary heart disease, emphysema, gastric ulcers, diabetes mellitus, cirrhosis, inflammation, and a number of other diseases. In a general sense, reactive oxygen species (ROS) consist of a variety of different molecules. In most cases, the production of the most potent of these radicals, the superoxide anion radical (O_2^-), occurs first when cells take in oxygen. [1]

Antioxidants are compounds that, in a nutshell, prevent or at least greatly lessen the amount of oxidative damage that is caused to certain molecules from occurring. What distinguishes antioxidants from other substances is their capacity to take in and eliminate free radicals. [2] Flavonoids, phenolic acids, and polyphenols are all examples of antioxidant chemicals. These compounds work by scavenging free radicals like peroxide, hydroperoxide, and lipid peroxyl, which allow them to inhibit the oxidative processes that are associated with degenerative diseases. [3]

The dry, hilly, and rocky *Euphorbia neriifolia* Linn. (Euphorbiaceae) plant belongs to the family Euphorbiaceae and is known as "thohar" or "Sehund" in Hindi. It is endemic to the Deccan Peninsula in India. The northern, middle, and southern regions of the country are all possible locations for its presence. Ayurveda provides a description of the bitter and pungent properties that are associated with *Euphorbia neriifolia*. [4] Fever, enlarged spleen, inflammation, leucoderma, piles, delirium, bronchitis, tumours, loss of consciousness, anaemia, piles, inflammation, spleen enlargement, ulcers, and bronchitis are just some of the many conditions that this plant can alleviate. The plant is known for its laxative, carminative, and appetite-improving properties, as well as its medicinal applications. [5, 6]

Despite the abundance of research that has been conducted on the medicinal advantages of *Euphorbia neriifolia*, the antioxidant capabilities of stem extracts from the plant have not been given much attention in vitro. As a result, the purpose of this research is to evaluate the antioxidant properties of methanolic extracts derived from *Euphorbia neriifolia* in a laboratory environment that is under controlled conditions. [7]

MATERIAL AND METHODS

Plant Material

Birbhum, a rural location in West Bengal, India, was the source of the *Euphorbia neriifolia* stems. The plant was authenticated at Shibpur, West Bengal, India, at the Botanical Survey of India (BSI) to make sure it was real.

Afterwards, a mechanical grinder was used to finely powder 500 g of entire stems that had been air-dried. Using a Soxhlet apparatus, the material was reduced to a fine powder before being extracted with methanol. After the solvent was extracted, it was completely evaporated at reduced pressure using a rotating vacuum evaporator.

Vacuum desiccators were used to meticulously preserve the concentrated extract that was produced, which had a yield of 35.42 percent.

Chemicals

Sigma Chemicals in the US supplied 1,1-diphenyl-2-picrylhydrazyl. Ascorbic acid, trichloroacetic acid (TCA), ethylene diamine tetra acetic acid (EDTA), thiobarbituric acid (TBA), sodium hydroxide (NaOH), butylated hydroxyanisole (BHA), hydrogen peroxide (H2O2), and nitroblue tetrazolium (NBT) were also purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

DPPH radical scavenging activity

Some tweaks were made to an earlier reported approach in order to test its DPPH radical scavenging activity. [8] At different concentrations, 0.2 ml of DPPH solution (100 μM in methanol) was mixed with 2.8 ml of either the test solution or standard ascorbic acid (dissolved in methanol), resulting in a total amount of 2.8 ml. At 517 nm, the absorbance of the solution that was produced was measured with a spectrophotometer after it had been incubated for thirty minutes at a temperature of 37 degrees Celsius.

We compared the test findings of an extract-treated group to those of a control group that received no treatment in order to calculate the percentage of DPPH radical inhibition. Additionally, we used the following formula to do this comparison. [9]

$$\text{Percentage inhibition} = [(C-T)/C] \times 100$$

Nitric oxide

The generation of nitrite ions is the process that leads to the production of nitric oxide. This process takes place in an aqueous solution with a physiological pH, and it involves the combination of sodium nitroprusside and oxygen. Quantification of these nitrite ions is possible through the use of the Griess method. [10]

Before starting the experiment, 0.1 ml of 10 mM sodium nitroprusside was mixed with 1 ml of either the test solution or standard ascorbic acid at several dosages. After that, the mixture was put into a pH 7.4 phosphate buffer that contained 100 mM of phosphate. Thereafter, the mixture was left to incubate for 150 minutes at a temperature of 25 degrees Celsius. [11]

Immediately following the completion of the incubation period, 1 millilitre of the mixture was removed and combined with 1 millilitre of Griess reagent. Sulfanilamide and orthophosphoric acid make up the Griess reagent. The finishing touch is a mixture of water and 0.1% naphthyl ethylene diamine dihydrochloride. These two components are in a 1:1 ratio with one another. The next step was to let the combination cool to ambient temperature for ten minutes without lighting it, after which it was moved to the refrigerator. [12]

Following this, the chromophore's absorbance was assessed at 546 nm. Combining naphthyl ethylene diamine dihydrochloride with nitrite that had been diazotized with sulfanilamide yielded the

chromophore. A calculation was made using the absorbance data in order to determine the proportion of nitric oxide generation that was suppressed.

Hydroxyl radical scavenging activity

In order to evaluate the capability of scavenging hydroxyl radicals, an alternate approach was developed and utilised. In distilled deionized water, deoxyribose (10 mM), EDTA (1 mM), FeCl3 (10 mM), ascorbic acid (1 mM), and hydrogen peroxide (10 mM) were all broken down into their respective dissolved forms. [13]

There was a specific order in which the succeeding volumes were inserted: The test methodology consisted of several different components, including the following: (in water at different concentrations) 0.1 millilitres of EDTA, 0.01 millilitres of FeCl3, 0.1 millilitres of hydrogen peroxide, 0.36 millilitres of deoxyribose, and 1.0 millilitres of either the extract or the normal ascorbic acid. Following that, 0.1 millilitres of ascorbic acid and 0.3 millilitres of phosphate buffer with a concentration of 50 millimolar and a pH of 7.4 were added. After that, the mixture had to be incubated for one hour at a temperature of 37 degrees Celsius. [14]

After allowing the combination to incubate for a certain amount of time, one millilitre of each of the following substances was added to it: 0.025 M sodium hydroxide, 0.5% thiobarbituric acid (TBA), and 0.025% butylated hydroxyanisole (BHA). It was at a wavelength of 532 nm that the pink chromogen was produced by the combination of these components.

In order to report the extract's hydroxyl radical scavenging activity, the percentage suppression of deoxyribose degradation that was previously reported was utilised.

Reductive activity

Trichloroacetic acid (TCA), Potassium ferricyanide, and ferric chloride ($FeCl_3$) were mixed to generate a colourful complex, which was used to quantify the reducing power of the test materials. [15]

Approximate 1 milliliter each of the extract and standard ascorbic acid solutions were combined with 2.5 milliliters each of potassium ferricyanide solution (1% concentration) and phosphate buffer (pH 6.6) in order to carry out the procedure. The next step was to incubate the mixture for 20 minutes at 50°C.

After 10 minutes of incubation, the mixture was centrifuged at 3000 rpm before 2.5 ml of a 10% TCA solution was added. Accumulated 2.5 ml of water, 0.5 ml of a 0.1% $FeCl_3$ solution, and 2.5 ml of the supernatant were combined, and the absorbance was assessed at 700 nm.

Analyzed products exhibited lowering power as indicated by greater absorbance values of the reaction mixture.

Analysis of total phenolic concentration

The Folin-Ciocalteu (FC) reagent, with minor adjustments to a conventional technique, was used to quantify the total phenolic content. Then, in a conical flask, 1 mL of FC reagent, 45 mL of distilled water, and 1 mL of the extract were combined. After then, it was shaken for half an hour. [16]

The mixture was then subjected to further shaking for a further two hours at room temperature after adding three millilitres of a sodium carbonate solution with a concentration of 2%. At 760 nm, the absorbance of the finished solution was compared to that of distilled water, which was used as a control. [17]

For this purpose, we calculated the total phenolic content using the following formula:

Total phenolic content (μg)=Absorbance at 760 nm−0.00330.001×Pyrocatechol (μg)Total phenolic conten t (μg)=0.001Absorbance at 760 nm−0.0033×Pyrocatechol (μg)

In this case, the concentration of a recognized phenolic chemical utilized as a benchmark is Pyrocatechol (μg).

Data analysis

Standard error of the mean (SEM) is computed from three independent measurements and displayed as the average plus or minus it.

Plotting concentration against percentage inhibition data allowed us to determine the 50% inhibitory concentrations (IC_{50}) using Graph Pad Prism, version 4.03. It was from these numbers that the IC_{50} values were calculated.

RESULTS

In all three studies, the IC_{50} values (mean ± SEM) were consistently determined at the μg/ml level, indicating that the *E. neriifolia* extract has an impressive ability to scavenge reactive species.

While peroxynitrite and NO are more harmful reactive nitrogen species in the body, DPPH is a good chemical to employ for evaluating free radical scavenging capability due to its potency and stability. In particular, the extract shows strong scavenging action against every reactive nitrogen species.

Both the extract and standard ascorbic acid suppress DPPH in a way that is dosage dependent, as seen in **Figure 1**. In contrast to the standard ascorbic acid, which has an IC_{50} value of 8.54 ± 0.85 µg/ml, **Figure 6** demonstrates that the *E. neriifolia* extract possesses an exceptional DPPH scavenging effect, measuring 85.11 ± 0.53 µg/ml.

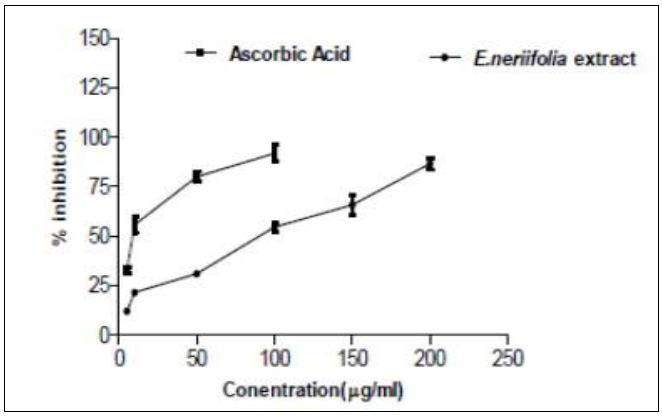

Fig 1: DPPH scavenging activity of Ascorbic acid, a standard, and *E. neriifolia* extract. This data shows the proportion of DPPH that was inhibited. The results shown by each point are the means ± SEMs of three separate experiments that were carried out in triplicate.

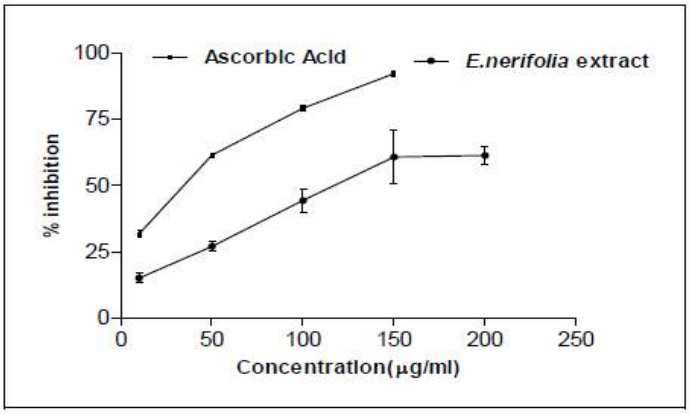

Fig 2: The ability of *E. neriifolia* extract and conventional ascorbic acid to scavenge radicals of nitric oxide. The numbers show what proportion of nitric oxide inhibition there is. The results shown by each point are the means ± SEMs of three separate experiments that were carried out in triplicate.

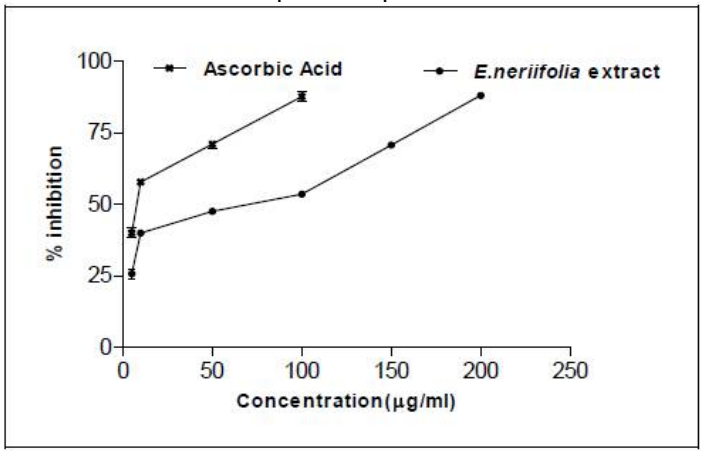

Fig 3: Comparison of *E. neriifolia* extract with the gold standard, ascorbic acid, in a superoxide radical scavenging test. These numbers show the extent to which superoxide inhibition was achieved. The results shown by each point are the means ± SEMs of three separate experiments that were carried out in triplicate.

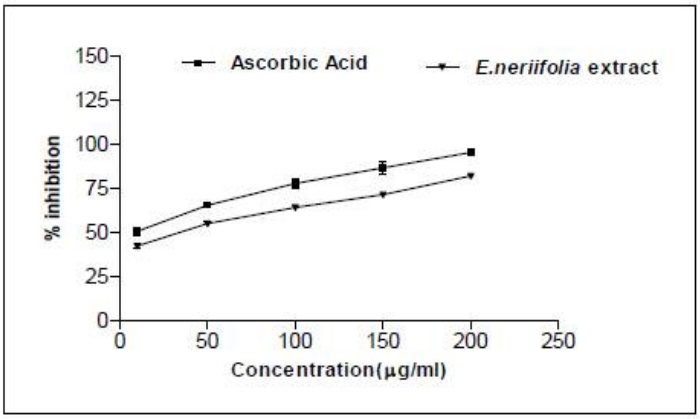

Fig 4: Comparing the hydroxyl radical scavenging capabilities of Ascorbic acid, a standard, and an extract from *E. neriifolia*. The results show the proportion of hydroxyl radicals that were inhibited. The results shown by each point are the means ± SEMs of three separate experiments that were carried out in triplicate.

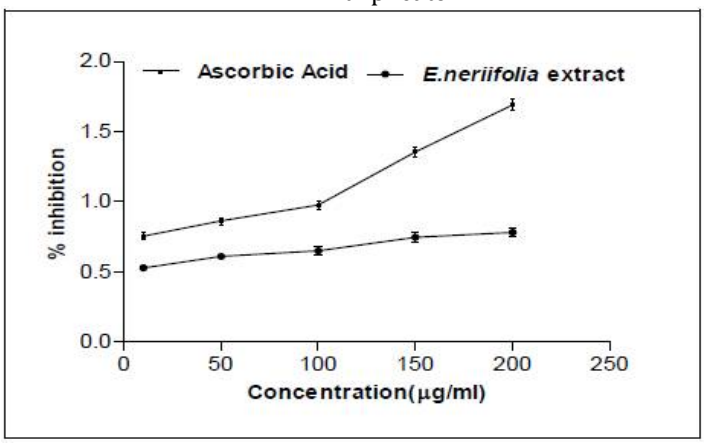

Fig 5: Comparison of *E. neriifolia* extract with conventional ascorbic acid: a reducing impact. The numbers show the proportion of reduction capacity. The results shown by each point are the means ± SEMs of three separate experiments that were carried out in triplicate.

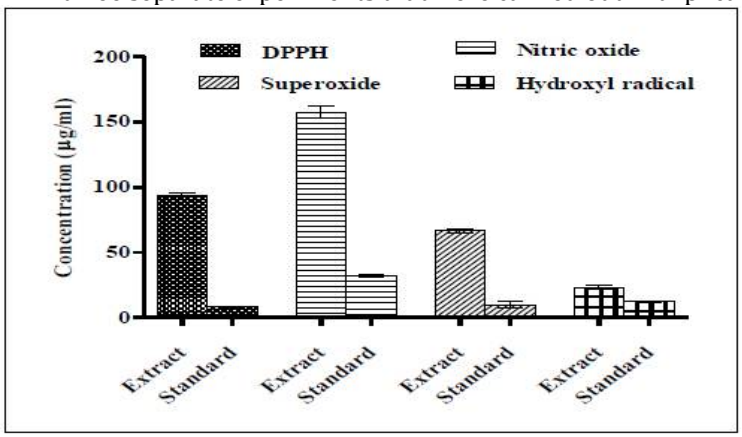

Fig. 6: The IC_{50} values of the following compounds were compared to the standard: DPPH, nitric oxide, superoxide, and hydroxyl radical. The data are presented as the mean ± SEM of three separate tests.

The suppression of nitric oxide by the *E. neriifolia* extract and conventional ascorbic acid is shown in Figure 2, and it is dosage dependent. The IC_{50} values for the extract are 144.22 ± 2.1 µg/ml, whereas those for ascorbic acid are 30.05 ± 2.52 µg/ml, as shown in Figure 6.

Figure 3 shows that the extract effectively scavenges superoxide radicals, which are among the most significant reactive oxygen species (ROS) generated by inflammatory cells. According to Figure 6, the extract has an IC_{50} value of 58.10 ± 2.10 µg/ml, while ascorbic acid has an IC_{50} value of 7.52 ± 1.15 µg/ml.

Figure 4 shows that the extract is more effective than regular ascorbic acid at scavenging hydroxyl radicals. In this test, the extract had an IC_{50} value of 25.12 ± 2.7 µg/ml while the standard had an IC_{50} value of 10.85 ± 0.43 µg/ml (Figure 6).
As seen in Figure 5, the extract demonstrates a significant reduction in power compared to regular ascorbic acid, and this effect is dosage dependent.
The inclusion of 675.6 µg of phenolic compounds per 10 mg of plant extract enhances the extract's antioxidant and free radical scavenging activities.

DISCUSSION

Antioxidants are vital compounds that provide protection against oxidation to key biological macromolecules. It is well knowledge that antioxidants are substances that disrupt the chain reaction of oxidation. The focus of researchers has switched to natural antioxidants because, in comparison to many synthetic antioxidants, which have unfavourable side effects, natural antioxidants are far more effective in lowering oxidative stress and preventing the development of diseases such as inflammation.
Because it is able to remove an electron or hydrogen radical from appropriate reducing agents in an effective manner, DPPH has the potential to facilitate the formation of a diamagnetic molecular transition that is stable. In order to determine the radical scavenging capabilities of a sample, it is usual practice to test the decrease in DPPH absorbance. The results of our research indicate that the capacity of the *E. neriifolia* extract to donate hydrogen is the factor that is responsible for the dose-dependent stoichiometric suppression of the DPPH radical.
Alzheimer's disease and juvenile diabetes are two examples of the many inflammatory disorders that include nitric oxide radicals and peroxynitrite (ONOO-). All of these diseases are characterised by inflammation. Through the scavenging of peroxynitrite and the limitation of the generation of nitrite anions, which are a consequence of the interaction between oxygen and sodium nitroprusside, our findings suggest that the stem extract of *E. neriifolia* is capable of preventing the bleaching known as Evans blue.
Deactivating enzymes can be accomplished directly by oxidising essential thiol (-SH) groups, which hydrogen peroxide is capable of doing despite the fact that it has weak oxidising characteristics that it possesses. Because of its ability to traverse cell membranes and interact with Fe2+ and may be Cu2+ ions, the creation of the potentially damaging hydroxyl radical is made easier on account of this property. In the course of our research, we discovered that hydrogen peroxide had a dose-dependent scavenging effect, which indicates that it has the potential to prevent oxidative damage [7-14].
Myeloperoxidase, an enzyme found in neutrophils, is responsible for the production of HOCl, another hazardous byproduct, by the oxidation of calcium ions in inflammatory areas. During the reaction between this chemical and taurine, the TNB extract experiences a reduction in its colour [17]. It has been demonstrated through the findings that the extract of *E. neriifolia* suppresses HOCl in a dose-dependent way.
According to the results of the FC reagent test and the phytochemical analysis, the extract of *E. neriifolia* has a high phenolic content. This indicates that it is also abundant in triterpenes and flavonoids. Therefore, the effectiveness of the extract in reducing inflammation is most likely attributable to the presence of phenolic components inside it.

CONCLUSION

In this study, the concentration-dependent free radical scavenging effect of an alcoholic extract derived from the stem of *Euphorbia neriifolia* is studied and emphasised. It is because the extract has a high concentration of phenolic compounds, which include tannins, flavonoids, terpenoids, phenols, and saponins, that these effects are observed.
Further investigation is being conducted by researchers in order to determine which active compounds are responsible for these side effects. Research is also being conducted to determine the precise action mechanism that is responsible for the antioxidant activity that has been discovered. The purpose of this study is to get a deeper understanding of the potential applications of *Euphorbia neriifolia* and its constituents in the treatment of diseases that are brought on by oxidative stress.

ACKNOWLEDGEMENT

Thanks to the kind support from Madhyanchal Professional University's Patel College of Pharmacy in Ratibad, Bhopal, Madhya Pradesh, India. Using this platform, the authors would want to express their deepest gratitude to the organization for granting them access to the resources needed to conduct this research.

DECLARATION OF INTEREST

When it comes to their work, the writers have not mentioned any potential conflicts of interest that may be present. They have total authority over the entirety of the document, including its composition and the content of the paper. In every respect, they are in total control of it.

REFERENCES

1. Singh L, Kaur N, Kumar P, (2009). "Reactive oxygen species, Oxidative damage and oxidative defence systems with emphasis on herbal antioxidants and human and cattle health", Biochem Cell Arch, 9(2), 135-144.
2. Pakutharivu T, Suriyavadhana M, (2010). "*In vitro* antioxidant activity of Entada pursaetha, Toddalia aculeata, and Ziziphus mauritiana", Phycog Journal, 2(2), 102-106.
3. Mohammad A M, Koji Y, Toshiki M, Yumi N, Katsumi S, Hiroaki S, Yoshifumi T, (2007). "Superoxide Anion Radical Scavenging Activities of Herbs and Pastures in Northern Japan Determined Using Electron Spin Resonance Spectrometry", Internal Journal of Biological Science, 3(6), 349-355.
4. Yamagishi S, Matsui T, *(2011). "Nitric oxide, a Janus-faced therapeutic target for diabetic microangiopathy-Friend or foe?", Pharmacol Res, 64, 187-194,
5. Wu Y Y, Li W, Xu Y, Jin E H, Tu Y Y, (2011). "Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses", J Zhejiang Univ Sci B, 12,744-751.
6. Hernández T, Canales M, Avila J G, Duran A, Caballero J, Vivar A R, (2003). "Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (México)", J Ethnopharmacol, 88, 181-188.
7. Chellaiah M, Muniappan A, Nagappan R, Savarimuthu I, (2006)."Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India", J Ethnobiol Ethnomedicine, 2, 43.
8. Cotelle A, Bernier J L, Catteau J P, Pommery J, Wallet J C, Gaydou E M,(2006). "Antioxidant properties of hydroxy-flavones", Free Radic Biol Med, 20, 35-43.
9. Sreejayan N, Rao M N A, (1997)."Nitric oxide scavenging by curcuminoids", J Pharm Pharmacol, 49,105–7.
10. Halliwell B, (1992)."Reactive oxygen species and the central nervous system", Journal of Neurochemistry, 59, 1609–23.
11. Jayprakash GK, Singh R P, Sakariah K K,(2001). "Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro, Food Chemistry, 73, 285-290.
12. Lin J Y, Tang C Y, (2007)."Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation", Food Chemistry, 101,140-147.
13. Kuo P C, Damu A G, Cherng C Y, Jeng J F, Teng C M, Lee E J,(2005). "Isolation of a natural antioxidant, dehydrozingerone from Zingiber officinale and synthesis of its analogues for recognition of effective antioxidant and antityrosinase agents", Arch Pharm Res, 28, 518-28.
14. Chanda S, Dave R, Kaneria M, (2011). "*In vitro* antioxidant property of some Indian medicinal plants", Research Journal of Medicinal Plant, 5 (2), 169-179.
15. Kundu S, Bala A, Ghosh P, Mukhopadhya D, Mitra A., Sarkar A.,(2011). "Attenuation of oxidative stress by Allylpyrocatechol in synovial cellular infiltrate of patients with Rheumatoid Arthritis", Free Radical Research, 45, 518–26.
16. Zhishen J, Mengcheng T, Jianming W, (1999). "The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals", Food Chemistry, 64, 555-559.
17. Govindappa M, Sravya S N, Poojashri M N, Sadananda T S, Chandrappa CP, (2011). "Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc", Journal of Pharmacognosy and Phytotherapy, 3(3), 43-51.