Research Article

Screening of the Antioxidant Potential of the Leaves and Flowers Extract of *Erythrina variegata* L. : A Comparative Study

[1]S. Hemmalakshmi, [1]S. Priyanga, [1]B. Vidya, [1,2]V.K. Gopalakrishnan, [1]K. Devaki*
[1]Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, India.
[2]Department of Bioinformatics, Karpagam University, Coimbatore, Tamil Nadu, India.
*Corresponding author's E-mail: dr.devaki.bc@gmail.com

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ABSTRACT

Oxidative stress results from the accumulation of reactive oxygen species and has been connected with all the diseases. Generally, there is a balance between the free radicals produced in the body and the antioxidant mechanisms. It quenches these free radicals and prevent them from triggering harmful effects in the whole body. The current study was to examine the *in vitro* free radical scavenging ability of the ethanolic extract of *Erythrina variegata* L. leaves and flowers. Radical scavenging assays like DPPH, $ABTS^+$, OH, H_2O_2, NO, super oxide radical inhibition assay, FRAP assay and reducing power activity were carried out using the recognized protocols. The results were compared with the *Erythrina variegata* L. radical scavenging activity of leaves and flowers. Its exhibited good radical scavenging activity against the above mentioned radicals. The antioxidant capability of the ethanolic extract of the *Erythrina variegata* L. leaves and flowers were compared with the ascorbic acid and a standard drug. Based on the findings, it is suggested that *Erythrina variegata* L. flowers possesses more scavenging power than that of the leaves extract. In the conclusion, *Erythrina variegata* L. flowers have more antioxidant property and it may be used in all medicinal preparations to overcome diseases that are associated with the oxidative stress including diabetes and related diseases.

Keywords: *Erythrina variegata,* Free radical, Medicinal plant, DPPH.

INTRODUCTION

Fundamentally free radicals are the main cause for several ailments in the humans. They are generated as unevenness between the formation and neutralization of prooxidants resulting in the oxidative stress[1]. Reactive oxygen or nitrogen species (ROS, RNS) are important free radicals which complicates the human functions. ROS such as the superoxide anion radical (O_2^-) and hydroxyl radicals (OH^-) are physiological metabolites. They are produced as an outcome of the respiration in the aerobic organisms but their excessive levels have been linked to the onset of diseases such as cancer, stroke and diabetes[2].They play a vital physiological tasks such as immuno-competence, apoptosis, vascular tone, hormonal regulation, signal transduction, transcription factors, defense genes and adaptive reaction to the enzymes[3]. Recently, much awareness has been directed towards the improvement of the ethno medicines with strong antioxidant property and low cytotoxicity[4].

Many plant products have been recognized as a good protector in opposition to the free radicals by trigger antioxidant gene expression. For that account, natural antioxidants from the plant sources have been viewed as a secure medicinal drugs against many diseases like diabetes, cancer and some other disorders[5].

Erythrina variegata species (Family: Fabaceae) or Indian coral tree, is in an average size and grows rapidly in the deciduous forests all over India[6]. The studies on phytochemical of *Erythrina variegata* have demonstrated alkaloids and flavonoids as major constituents[7,8]. *E. variegata* L. parts (leaves, flowers, barks and roots) have been used in the natural medicines as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. Traditionally, it has potential effects to heal some of the diseases like convulsion, fever, alzheimer, inflammation, bacterial infection, cough, ulcer, cuts and wounds[9].

The Juice of the leaves is mixed with honey and ingested to kill tapeworm, roundworm and threadworm. It is also used to give relief from ear ache and toothache[10].

The previous studies on the preliminary phytochemical screening of the leaves, flowers, and bark with different extracts suggested that the ethanolic extract of the flowers and leaves have more phytoconsituent than that of the bark. So, the present investigation is designed to evaluate the free radical scavenging effect of the ethanolic extract of the *Erythrina variegata* L. leaves and flowers.

MATERIALS AND METHODS

Plant Collection and Authentication

The fresh plant samples were collected in the month of August from Kodaikannal and its surroundings, Dindigul District, Tamil Nadu, India. They were botanically authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus, Coimbatore. The specimen was deposited in the Herbarium for future reference (voucher number: BSI/SRC/5/23/2013-14/Tech/1500). The plant samples were thoroughly washed in the running tap water to remove adhering dust particles and dried under the shades for about two weeks. It was grounded into a

coarse powder and stored in an airtight container, which can be use for the further investigations.

Preparation of Ethanolic Extracts of Leaves and Flowers

The powdered plant samples of leaves and flowers (100g) were weighed and mixed with 500 ml of ethanol. Then it is kept in an orbital shaker at 190-220 rpm for 48 hours. The supernatant was collected, filtered through Whatman No.1 filter paper and then concentrated by evaporating to dryness which gave a solid amorphous residue and it was dried thoroughly to remove the solvent used. The obtained dried extract was then accurately weighed, stored in a small vials at -20°C and used for the subsequent studies.

Free Radical Scavenging Assays

Free radical scavenging activity was analyzed by using the following assays. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) assay, was adapted from Blois[11] and the scavenging activity against ABTS cation radical was measured according to the method Re[12], Hydroxyl radical scavenging assay was performed by Klein[13], H_2O_2 radical scavenging assay was done by the standard method of Ruch[14], Nitric oxide radical scavenging assay was analysed by Green[15], Superoxide anion scavenging assay was estimated by the method Liu[16], FRAP assay was carried out as described by Benzie and Strain[17] and Reducing power assay was determined according to the method of Oyaizu[18].

Statistical Analysis

The data obtained were statistically analyzed and recorded by using of triplicate (n=3) values of the Mean ± Standard deviation of the results.

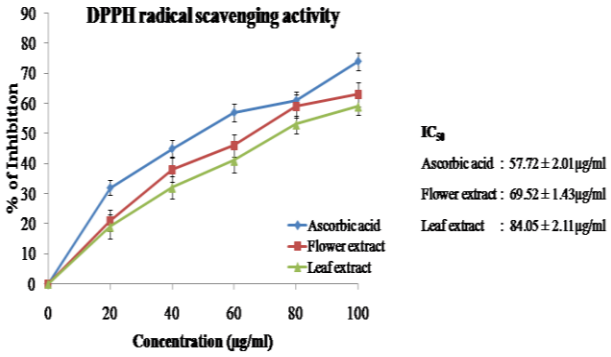
RESULTS AND DISCUSSION

Phytochemicals have received much interest owing to their molecular structure which consists of hydroxyl groups of aromatic rings and it has been associated with their function as oxidant scavengers[19]. Multiple assays were conducted since the antioxidant capacity could not be evaluated from a single method. Based on the fact that the oxidative stress and damage can result from the different mechanism. The antioxidative effectiveness of the herbal formulation was determined using the DPPH scavenging, FRAP, ABTS scavenging and reducing power assay. Radical scavenging activities play an imperative role to prevent the damaging functions of the free radicals in different disorders, including diabetes[20,21].

DPPH Radical Scavenging Activity

DPPH free radical scavenging is a conventional mechanism used for screening the antioxidant activity of the plant extracts. The absorbance decreases as a result of a colour change from purple to yellow hence the radical is scavenged by the antioxidants.

All the samples were able to reduce the stable free radical DPPH to the yellow-colour 1,1-diphenyl-2-picrylhydrazyl[22]. The ability of antioxidants for DPPH radical scavenging is supposed to be due to their hydrogen donating property[23]. When DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up[24].



Values are expressed as Mean ± S.D

Figure 1: DPPH Radical Scavenging Assay

Figure 1 shows the DPPH radical scavenging activity of the ethanolic extracts of *Erythrina variegata* L. leaves and flower, Ascorbic acid was used as a reference standard. The scavenging effects of plant extracts on DPPH were examined at the different concentrations (20, 40, 60, 80, 100µg/ml).

Among the extracts, *Erythrina variegata* L. flowers possessed the highest activity when compared to the leaves. Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated. A lower IC_{50} value denoted a higher antioxidant activity. IC_{50} values of ethanolic extracts of *Erythrina variegata* L. leaves and flower, and ascorbic acid were 84.05 ± 2.11 µg/ml 69.52 ± 1.43 µg/ml, and 57.72 ± 2 µg/ml, respectively.

$ABTS^+$ Radical Scavenging Activity

The $ABTS^+$ chemistry implicates nonstop production of $ABTS^+$ radical mono cation by means of no contribution of any mediator radical[25]. It is converted to its radical cation by the addition of potassium persulfate. This radical cation is blue in color and absorbs the light at 734 nm[26].

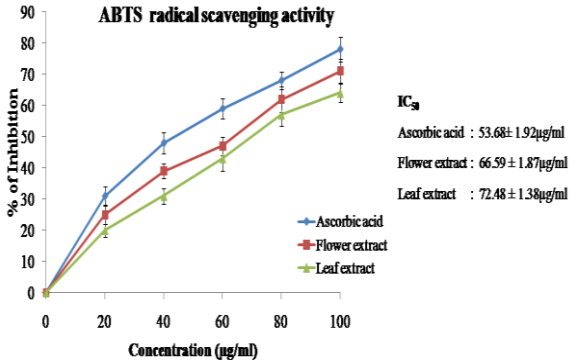


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Figure 2: $ABTS^+$ Radical Scavenging Assay

The samples of *E. variegata* L. leaves and flowers exhibited a significant antioxidant capacity.

The highest $ABTS^+$ scavenging activity was observed in *Erythrina variegata* L. flowers (71%) followed by leaves (64%).

The IC_{50} of ethanolic extracts of *Erythrina variegata* L. leaves and flower, and ascorbic acid were 72.48 ± 1.38, 66.59 ± 1.87, and 53.68 ± 1.92 µg/ml, respectively (Figure 2).

Hydroxyl Radical Scavenging Activity

The mutagenic capability of free radicals is due to the straight relations of hydroxyl radicals with DNA, resulting in DNA breakdown and therefore playing a significant work in a cancer development[21].

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals such as Fe^{2+} and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate the cell damage *in vivo*[27,28].

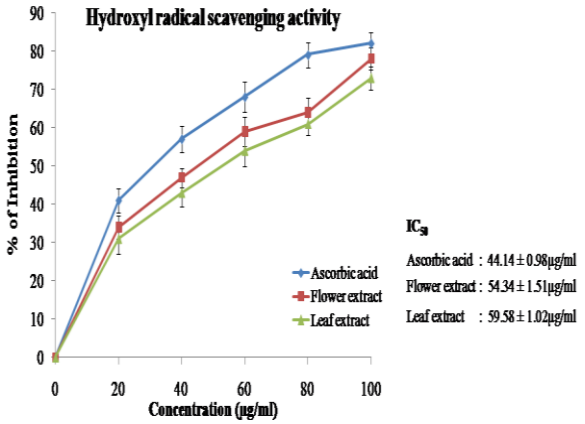

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Figure 3: Hydroxyl Radical Scavenging Assay

The radical was also effectively scavenged by *Erythrina variegata* L. leaves and flowers compared to the standard, ascorbic acid.

The hydroxyl radical scavenging activity of the *Erythrina variegata* L. flowers (78% at 100µg/ml) and ascorbic acid (82% at 100µg/ml).

The IC_{50} of the ethanolic extracts of the *Erythrina variegata* L. leaves and flower, and ascorbic acid were 59.58 ± 1.02 µg/ml, 54.34 ± 1.51 µg/ml, and 44.14 ± 0.98µg/ml, respectively (Figure 3).

The aqueous extract demonstrated high radical scavenging potential, and it is reported that the experimental evidence is similar to those of Devaki[7].

H_2O_2 Radical Scavenging Activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of the essential thiol (-SH) groups. It is an important reactive oxygen species because of its capability to penetrate biological membranes[29].

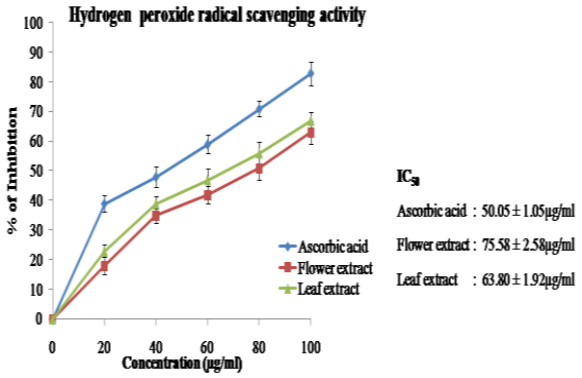

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Figure 4: Hydrogen Peroxide Radical Scavenging Assay

Once within the cell, hydrogen peroxide can possibly react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical and it may be the foundation for many of its toxic effects[30]. The ethanolic extract of the *Erythrina variegata* L. leaves and flowers demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner with an IC_{50} and the value of leaves were 63.80 ± 1.92 µg/ml, while the IC_{50} of the standard ascorbic acid was 50.05 ± 1.05µg/ml (Figure 4). However, the *Erythrina variegata* flowers extract gave the highest IC_{50} 75.58 ± 2.58 µg/ml. The extracts with lower IC_{50} shows more potential of free radical scavenging ability than the extracts with higher IC_{50}. Thus implying the ethanolic extract had a powerful radical scavenging activity.

Nitric Oxide Radical Scavenging Activity

At the physiological pH, nitric oxide generated from the aqueous sodium nitroprusside (SNP) solution interacts with the oxygen to produce nitrite ions, which may be quantified by the Griess Illosvoy reaction[31]. Nitric oxide is a very unstable species under aerobic condition. It reacts with O_2 to produce stable products, nitrate and nitrite through intermediates. It also plays an important role in the pathogenesis of pain, inflammation, neural signal transmission, immune response, control vasodialation and blood pressuren[32].

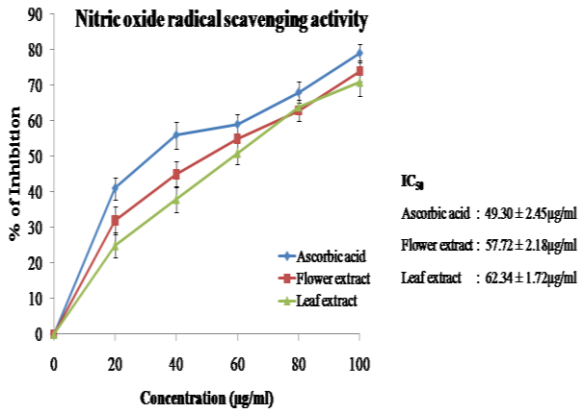

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Figure 5: Nitric Oxide Radical Scavenging Assay

Among the extracts, the highest inhibition was noted in the ethanolic extract of the *Erythrina variegata* L. flowers compared with the leaves extract (Figure 5).

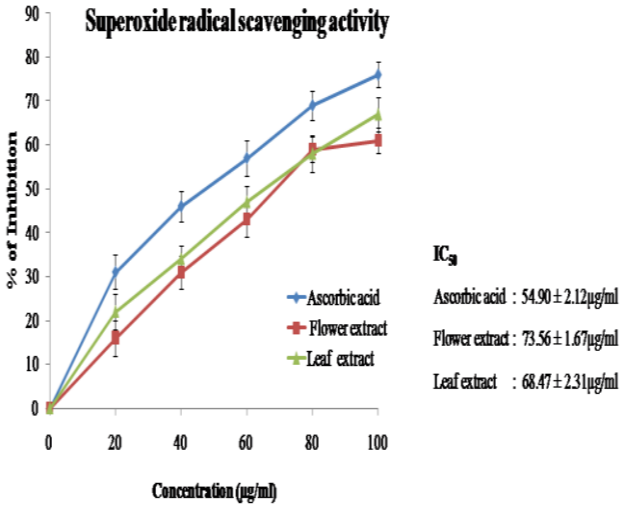
It exhibits a high scavenging capacity of 75% at 100µg/ml with an IC_{50} value of 57.72 ± 2.18 µg/ml which was approximately equal to that of the standard ascorbic acid that exhibits a scavenging capacity of 79% at 100µg/ml with an IC_{50} value of 49.30 ± 2.45 µg/ml and leaf extract was not having scavenging capacity as that flowers (IC_{50} value of 62.34 ± 1.72µg/ml).

Superoxide Anion Scavenging Activity

Super oxides are formed from oxygen by a single electron transfer during the process of auto oxidation.

The superoxide anions are scavenged by antioxidants and consequently, decrease the rate of pyrogallol auto oxidation or even inhibit it[33].

Superoxide anion radical is one of the most strongest reactive oxygen species among the free radicals and also very harmful to the cellular components[31].



Values are expressed as Mean ± S.D

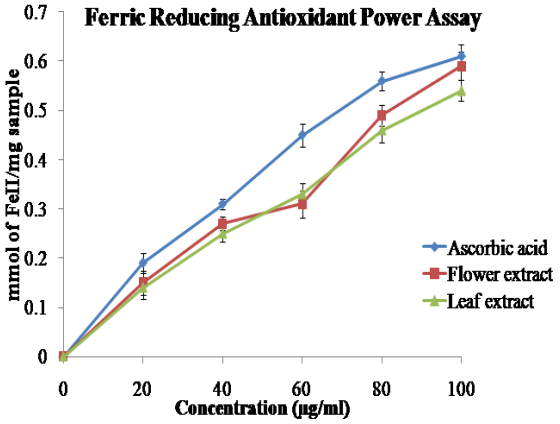
Figure 6: Superoxide Anion Scavenging Assay

Figure 6 revealed the scavenging activity of ethanolic extracts of *Erythrina variegata* L. and implicate that the leaves have more anti radical scavenging activity, while comparing with the flower extract.

Antioxidant activity of the *Erythrina variegata* L. extracts were shown in the following order: Ascorbic acid (54.90 ± 2.12µg/ml) > *Erythrina variegata* L. leaves (68.47 ± 2.31µg/ml) > *Erythrina variegata* L. flowers (73.56 ± 1.67 µg/ml).

FRAP Activity

Ferric Reducing Antioxidant Power (FRAP) assay is clearly reproducible and linearly interconnected to the molar concentration of the antioxidant present and it was described that its work as a free radical scavenger, proficient of transforming reactive free radical species into conventional non radical products[34].



Values are expressed in Mean ± S.D

Figure 7: Ferric Reducing Antioxidant Power Assay

This assay was used to assess the ability of the herbal formulations to reduce the $Fe^{3+}$to Fe^{2+}. It measures the changes in the absorbance at 593 nm owing to the formation of blue colored Fe - tripyridyltriiazine compound from the colourless oxidized and the Fe form the action of electron donating antioxidants[35]. The present study showed the highest ferric reducing potency. The FRAP ability of ethanolic extract of *Erythrina variegata* L. flower, leaves and standard were depicted in the Figure 7. Different concentrations of the plant extracts were used (20–100µg/ml) and the ethanolic extract of the flower showed the strong reducing power at the concentration of 100µg/ml than the leaves and its activity increases in a dose dependent manner when compared to the standard ascorbic acid at the same concentration.

Reducing Power Activity

The reducing power of a complex acts as a marker of its potential antioxidant activity. The reducing properties are generally connected with the presence of reductones which show the evidence of antioxidant activity by breaking the chain reactions by donating the hydrogen atoms[36].

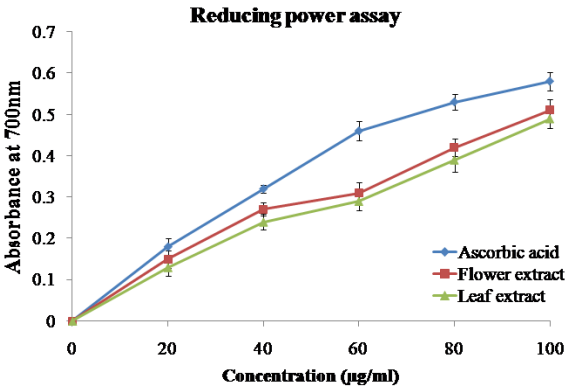


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Figure 8: Reducing Power Assay

The reduction potential of ethanolic extracts of *Erythrina variegata* L. leaves and flowers at various concentrations

is illustrated in the Figure 8. Reducing capacity of both the kinds of extracts are compared with ascorbic acid for the reduction of Fe^{3+} - Fe^{2+}.

On the basis of this investigation it is reported that there is a concentration dependent increase in reducing the power of the extracts.

It is found that the ethanolic extract of *Erythrina variegata* L. flowers have a higher percentage of reduction potential as compared to leaf extract. *Erythrina variegata* L. flowers, leaves and reference standard are found to be 0.59±0.04, 0.54±0.021 and 0.61±0.0 respectively, and it is evident that the absorbances of the both extract and standard are almost the same.

In this assay increase absorbance of the reaction mixture indicates its reductive ability.

Reducing the capacity of a compound may serve as a significant indicator of its potent antioxidant activity.

However, the activities of antioxidants have been attributed to various mechanisms such as prevention of chain initiation, decomposition of peroxides, reducing the capacity and radical scavenging ability[37,38].

Our previous studies demonstrated that flavonoids were the important constituent in *Erythrina variegata* L. and abundantly present in the flowers than the leaves[7,8].

The antioxidant action of the extract was due to the presence of hydroxyl groups in the flavonoids which had the ability to transfer the proton and stabilize the radical intermediate because of the resonance delocalisation[21].

CONCLUSION

From the above study it can be concluded that the ethanolic extract of *Erythrina variegata* L. flowers demonstrated with the more powerful and promising antioxidant activity compared to the leaves and it also can be used as an effective protecting mediator against the oxidative stress and it helps to combat the diseases like cancer, diabetic mellitus and other diseases.

Further work is desired to isolate the secondary metabolites from the extract to deliberate its efficacy in various disorders.

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REFERENCES

1. Gangwar M, Gautam MK, Sharma AK, Tripathi YB, Goel RK, Nath G. Antioxidant Capacity and Radical Scavenging Effect of Polyphenol Rich *Mallotus philippenensis* Fruit Extract on Human Erythrocytes: An *in vitro* Study, Scientific World Journal, 2014, 1-12.
2. Kazeem MI, Ashafa AOT. *In-vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy 763-771. whole plant extracts. Journal of Herbal Medicine, 2015, 1-7, http://dx.doi.org/10.1016/j.hermed.2015.06.003.
3. Moukette BM, Pieme CA, Biapa PCN, Moor VJA, Berinyuy E, Ngogang JY. *Afrostyrax lepidophyllus extracts* exhibit *in vitro* free radical scavenging, antioxidant potential and protective properties against liver enzymes ion mediated oxidative damage, BMC Research Notes, 8, 2015, 344.
4. Ghosh S, Derle A, Ahire M, More P, Jagtap S, Phadatare SD, Patil AB, Jabgunde AM, Sharma GK, Shinde VS, Pardesi K, Dhavale DD, Chopade BA. Phytochemical Analysis and Free Radical Scavenging Activity of Medicinal Plants *Gnidia glauca* and *Dioscorea bulbifera*, PLoS ONE, 8, 2013, 1-18.
5. Zengin G, Cakmak YS, Guler GO, Aktumsek A. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei DC. Subsp.* hayekiana Wagenitz, Records of Natural Products, 5, 2011, 123–132.
6. Mangathayaru K, Sarah K and Balakrishna. Estrogenic effect of *Erythrina variegata* L. in prepubertal female rats. Indian Journal of Natural products and Resources, 5, 2014, 223-227.
7. Devaki K, Hemmalakshmi S, Priyanga S. HPTLC analysis and *in vitro* antioxidant activity of aqueous bark extract of *Erythrina variegata* L, Israel Journal of Plant Sciences, 2015, dx.doi.org/10.1080/07929978.2015.1096608.
8. Hemmalakshmi S, Priyanga S, Devaki K. Phytochemical screening and HPTLC fingerprinting analysis of ethanolic extract of *Erythrina variegata* L. Flowers, International Journal of Pharmacy and Pharmaceutical Science, 8, 2016, 210-217.
9. Cui L, Thuong PT, Fomum ZT, Oh WK. A new erythrinan alkaloid from the seed of *Erythrina addisoniae*, Archives of Pharmacal Research, 32, 2009, 325–328.
10. Nagar JC, Chauhan LS. Hypoglycemic and hypolipidemic activity of root extracts of *Erythrina variegata* in alloxan induced diabetic rats, Asian Journal of Biomedical and Pharmaceutical Sciences, 5, 2015, 30.
11. Blois MS. Antioxidant determinations by the use of stable free radical, Nature, 1, 1958, 1199-2000.
12. Re R, Pelligrini N, Proteggeenate M, Yang C, Rice Evans. Antioxidants activity of applying an improved ABTS radical cation decolorisation assay, Free Radical Biology and Medicine, 26, 1999, 1231-1237.
13. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system, Biochemistry, 20, 1991, 6006-6012.
14. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea, Carcinogenesis, 10, 1989, 1003-1008.
15. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and nitrate in biological fluids, Analytical Biochemistry, 126, 1982, 131–138.
16. Liu F, Ooi VEC, Chang ST. Free radical scavenging activity of mushroom polysaccarides extract, Life Science, 60, 1997,

17. Benzie IFF, Strain JJ. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay, Analytical Biochemistry, 239, 1996, 70-76.

18. Oyaizu M. Studies on product of browning reaction prepared from glucose amine, Japan Journal of Nutrition, 7, 1986, 307-315.

19. Zengin G, Guler GO, Aktumsek A, Ceylan R, Picot CMN, Mahomoodally MF. Enzyme Inhibitory Properties, Antioxidant Activities, and Phytochemical Profile of Three Medicinal Plants from Turkey, Advances in Pharmacological Sciences, 2015, 1-8.

20. Neergheen-Bhujuna VS, Munogeeb N, Coolenb V. Antioxidant and anti-inflammatory efficacies of polyherbal formulations and elixirs traditionally used in Mauritius for the treatment of rheumatoid arthritis, Journal of Herbal Medicine, 4, 2014, 1–9.

21. Rahman M, Islam B, Biswas M, Alam AHMK. *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Research Notes, 621, 2015, 1-10.

22. Rigano D, Conforti F, Formisano C, Menichini F, Senatore F. Comparative free radical scavenging potential and cytotoxicity of different extracts from Iris pseudopumila Tineo flowers and rhizomes, Natural Product Research, 23, 2009, 7-25.

23. Hatami *T*, Emami SA, Miraghaee SS, Mojarrab M. Total Phenolic Contents and Antioxidant Activities of Different Extracts and Fractions from the Aerial Parts of *Artemisia biennis* Willd, Iranian Journal of Pharmaceutical Research, 13, 2014, 551-558.

24. Sanchez-Moreno C. Methods used to evaluate the free radical scavenging activity in foods and biological systems, Food Science and Technology International, 8, 2002, 122–8.

25. Priyanga S, Hemmalakshmi S, Sowmya S, Vidya B, Chella Perumal P, Gopalakrishnan V K and Devaki K. *In vitro* Enzyme Inhibitory Evaluation and Free Radical Scavenging Potential of Ethanolic Leaf Extract of *Macrotyloma uniflorum* (L.), International Journal of Current Pharmaceutical Review and Research, 5, 2015, 169-17.

26. Raghavendra M, Reddy AM, Yadav PR, Raju AS, Kumar LS. Comparative studies on the in vitro antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of India, Asian Journal of Pharmaceutical and Clinical Research, 6, 2013, 96-9.

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

28. Sowndhararajan K, Kang SC. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn, Saudi Journal of Biological Sciences, 20, 2013, 319–325.

29. Bhatia L, Bishnoi H, Chauhan P, Kinja K, Shailesh S. *In-vitro* comparative antioxidant activity of ethanolic extracts of glycosmis pentaphylla and *Bauhinia variegata*, Recent Research in Science and Technology, 3, 2011, 1–3.

30. Gomathi D, Ravikumar G, Kalaiselvi M, Devaki K, Uma C. Antioxidant activity and functional group analysis of *Evolvulus alsinoides*, Chinese Journal of Natural Medicines, 12, 2014, 827-832.

31. Garratt DC. The quantitative analysis of Drugs. *Volume 3*. Chapman and Hall ltd, Japan, 1964, 456-458.

32. Divya BT and Mini S. *In vitro* radical scavenging activity of different extracts of *Butea Monosperma* bark. Int J Curr Pharm Res, 3, 2011, 114-116.

33. Benhammou N, Bekkara FA, Panovak TK. Antioxidant and antimicrobial activities of *Pistacia lentisus* and *Pistacia Atlanta* extracts, African Journal of Pharmacy and Pharmacology, 2, 2008, 22-28.

34. Priyanga S, Hemmalakshmi S, Sowmya S, Vidya B, Chella Perumal P, Gopalakrishnan VK, Devaki K. Quantitative evaluation and *in vitro* free radical scavenging ability of ethanolic stem extract of *Macrotyloma uniflorum* L. Der Pharmacia Lettre, 7, 2015, 225-233.

35. Gupta VK, Sharma SK. *In vitro* antioxidant activities of aqueous extract of *Ficus Bangalensis*, International Journal of Biological Chemistry, 4, 2010, 134-140.

36. Venkatachalam U, Muthukrishnan S. Free radical scavenging activity of ethanolic extract of *Desmodium gangeticum,* Journal of Acute Medicine, 2, 2012, 36-42.

37. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca Buxifolia* fruit, Osong Public Health and Research Perspectives, 4, 2013, 246-254.

38. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata,* BMC Complementary and Alternative Medicine, 8, 2008, 1-10.

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