Free Radicals and Antioxidants, 2017; 7(1): 8-12
A multifaceted peer reviewed journal in the field of Free Radicals and Antioxidants
www.antiox.org | www.phcog.net

Original Article

In vitro Antioxidant and Cytotoxicity Analysis of Leaves of *Ficus racemosa*

Aslam Khan[1], Vijaya Anand[2], Varalakshmi Badrinarayanan[3], Karpagam Thirunethiran[3] and Pushpa Natarajan[4]

[1]Department of Biochemistry, Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli–612 001, Tamil Nadu, INDIA.
[2]Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore–641 046, Tamil Nadu, INDIA.
[3]Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli–620 002, Tamil Nadu, INDIA.
[4]Department of Microbiology, Cauvery College for Women, Tiruchirappalli–620 018, Tamil Nadu, INDIA.

ABSTRACT

Objectives: The present study assessed the phytochemical components, *In vitro* antioxidant ability and cytotoxicity of leaf extract of *Ficus racemosa*. **Methods:** Preliminary phytochemical analysis in aqueous and ethanol was carried out for the presence of phytochemical components. Of the two extracts used ethanolic extract possessed the highest phytochemical constituents compared to aqueous extract. Hence the antioxidant activity of ethanolic extract of *F. racemosa* was performed by several antioxidant assays including 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay, nitric oxide (NO) scavenging assay, reducing power and superoxide radical (SO) scavenging assay. **Results:** From the results, *F. racemosa* has been found to have the significant antioxidant activity in a dose-dependent manner and IC_{50} value was 150 µg/ml for DPPH and 100 µg/ml for both NO and SO scavenging assays. Further, the cytotoxicity analysis was determined against Dalton Lymphoma Ascites (DLA) cell line and the IC_{50} value was found to be 175 µg/ml for ethanolic leaf extract of *F. racemosa*. **Conclusion:** Hence, the current study attests that *F. racemosa* is enriched in phytochemicals and a fine source of natural antioxidants with anticancer agents and can be used in pharmaceutical preparations for the treatment of diseases induced by oxidative stress.

Key words: *Ficus racemosa*, DPPH assay, Dalton Lymphoma Ascites, MTT assay.

Correspondence :

B. Aslam Khan,
Research Scholar, Department of Biochemistry,
Manonmaniam Sundaranar University,
Abishekapatti, Tirunelveli – 612 001,
Tamil Nadu, INDIA.
Mobile no: +91 99621 51454

E-mail: avamiet@yahoo.com
DOI: 10.5530/fra.2017.1.2

INTRODUCTION

Oxidative stress can damage cells as well as tissues, which therefore leads to various dreadful degenerative diseases like cancer.[1] 127 lakhs of new cancer cases was estimated according to the International Agency for Research on Cancer in 2008. The global burden is may rise to 21.4 million by 2030.[2] Hence, supplementation of herbal antioxidants is necessary to suppress the oxidative stress in a healthier way. Use of man-made antioxidants like butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are restricted due to their side effects.[3] Recently, various antioxidants are acquired from naturally available plants that have the capacity to scavenge free radical or active oxygen.[4]

Ficus racemosa Linn. (Family; Moraceae) is used in traditional system of medicine for the treatment of various diseases. Medicinal uses of the *F. racemosa* are motioned in the ancient scriptures of Ayurveda, Siddha, Unani and Homeopathy. *F. racemosa* is a huge tropical, deciduous, evergreen tree with greater than 800 species. Aerial parts and root are often used for the management of ailments. It gives a unique fruit which is in fact an inverted flower. Antioxidant compounds such as polyphenols and flavanoids are present in *F. racemosa*, which are used for the treatment of various diseases associated oxidative stress.[5] Therefore, the present study is aimed to examine the *In vitro* antioxidant potential and cytotoxicity effect of *F. racemosa* leaves by several methods.

MATERIALS AND METHODS

Preparation of extracts and preliminary phytochemical analysis

The leaves of *F. racemosa* were collected from Trichy, Tamil Nadu, India. The collected leaves were washed thoroughly in running tap water, distilled water, shade dried and ground into fine powder. Ethanolic extract of the plant material was prepared using a soxhlet extractor. Aqueous extract was prepared freshly. The extract was evaporated and the powder was stored in a sterile container until use. Standard methods,[6] were used for the preliminary phytochemical screening of phytocomponents present in the aqueous and ethanolic leaf extracts of *F. racemosa*.

In vitro antioxidant analysis

DPPH radical scavenging assay

The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay was done by the method of Brand-Williams *et al.*[7] About 1.0 ml of the 0.004% methanol solution of DPPH was added to 1 ml of ethanolic leaf extract of *F. racemosa* at various concentrations (25, 50, 100, 150 and 200 µg/ml). Ascorbic acid was used as standard. The reactants were vortexed and incubated in dark at room temperature for half an hour. The absorbance was measured at 517 nm in a spectrophotometer. The assay was carried out in triplicates. DPPH radical scavenging activity was calculated as a percentage using the formula:

$$\% \text{ Scavenged} = \frac{[\text{Abs control} - \text{Abs sample}]}{[\text{Abs control}]} \times 100$$

The IC_{50} value was calculated, which is the effective concentration at which the antioxidant activity is 50%.

Nitric oxide scavenging activity

NO scavenging activity of *F. racemosa* was assayed by the method of Fan *et al.*[8] 2 ml sodium nitroprusside (10 mM in 0.5 mM phosphate buffered saline at pH 7.4) was mixed with 0.5 ml of ethanolic leaf extract of *F. racemosa* at various concentrations (25, 50, 100, 150 and 200 µg/ml).

Ascorbic acid was used as standard. The mixture was incubated at 25°C for 2.30 hours. After incubation, 0.5 ml of the reactants was removed; 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand at room temperature for 5 minutes for a complete diazotization reaction. 1 ml of naphthyl ethylene diamine dichloride (0.1%*w/v)* was added and the mixture was allowed to stand for 30 minutes at room temperature. Absorbance was measured at 540 nm in a spectrophotometer. The assay was carried out in triplicates. NO percentage was calculated using the formula:

$$\text{No inhibition percentage} = \frac{[\text{Abs control} - \text{Abs sample}]}{[\text{Abs control}]} \times 100$$

The IC_{50} value was calculated, which is the effective concentration at which the antioxidant activity is 50%.

Ferric reducing power assay

The reducing power of ethanolic leaf extract of *F. racemosa* was determined by the method of Oyaizu.[9] Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

The ethanolic leaf extract of *F. racemosa* at various concentrations (25, 50, 100, 150 and 200 µg/ml) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared 0.1% ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in a similar manner excluding samples. Ascorbic acid was used as standard. The assay was carried out in triplicates. Increase in absorbance of the reaction mixture indicates stronger reducing power.

Superoxide radical scavenging assay

The superoxide radical generated from the photo reduction of riboflavin was detected by NBT (Nitro blue tetrazolium) reduction.[10] The reaction mixture contained EDTA (0.1 M), 0.0015% NaCN, riboflavin (0.12 mM), NBT (1.5 mM) and ethanolic leaf extract of *F. racemosa* at various concentrations (25, 50, 100, 150 and 200 µg/ml) and phosphate buffer (67 mM, pH 7.8) in a total volume of 3 ml. The tubes were uniformly illuminated for 15 minutes and the optical density was measured at 530 nm before and after the illumination. The percent scavenged was calculated by the following equation.

$$\text{Percent scavenged} = \frac{[\text{Abs control} - \text{Abs sample}]}{[\text{Abs control}]} \times 100$$

The IC_{50} value was calculated, which is the effective concentration at which the antioxidant activity is 50%.

In vitro cytotoxicity analysis

Cell line

The Dalton Lymphoma Ascites (DLA) cell line was obtained from the Amala Cancer Research Institute (Thrissur, India) and was propagated into transplantable tumors in the peritoneal cavity of female albino mice. The freshly aspirated cells from the mouse peritoneum were washed with phosphate-buffered saline (PBS) under sterile conditions and their concentration was determined using a hemocytometer before transplantation. The aseptically collected ascitic cells were washed with IMDM medium supplemented with 10% FBS, 100 IU/mL penicillin and 100 mg/mL streptomycin. The harvested DLA cells were incubated in a petri dish for one hour at 37°C in 5%, atmospheric CO_2 and non adherent cells were cultured and used for *In vitro* experiments.

MTT Assay

In vitro cytotoxic activity of ethanolic leaf extract of *F. racemosa* on DLA cells was assayed by the method described by Mosmann.[11] The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye reduction assay was performed to determine the cytotoxic effect of ethanolic leaf extract of *F. racemosa* at various concentrations (25, 50, 100, 150 and 200 µg/ml). The assay depends on the reduction of MTT by mitochondrial dehydrogenase, an enzyme present in the mitochondria of viable cells, to a blue formazan product. Briefly, the DLA cells at the concentration of 1×10^5cells/ml were plated onto 96-wells and treated with various concentrations of ethanolic leaf extract of *F. racemosa*. The wells were incubated for 24 hours (37°C, 5% CO_2 in a humidified incubator. After 24 hours of incubation, 10 µl of MTT (5 mg/ml in PBS) was added to each well, and the plate was incubated for a further four hours at 37°C. The resulting formazan was dissolved in 100 µl of dissolving buffer and absorbance of the solution was read at 595 nm using a scanning Multiwell spectrophotometer. All determinations were carried out in triplicates. Concentration of ethanolic leaf extract of *F. racemosa* showed 50% reduction in cell viability (IC_{50} value) was then calculated.

RESULTS AND DISCUSSION

Antioxidants have the capacity to protect the body from oxidative stress damage. Epidemiological studies indicate that intake of fruits; vegetables as well as indigenous herbal products have the capacity to prevent the free radicals in the human body. In this study, the phytochemical investigation and antioxidant properties of the ethanolic leaf extract of *F. racemosa* was assessed.

Preliminary phytochemical analysis

The results of preliminary phytochemical investigation exhibits that the leaf extract of *F. racemosa* have various classes of bioactive components (Table 1). Steroids, coumarin, tannins, saponin, flavonoids, quinines, anthraquinones, total carbohydrates, total proteins, total phenol and fixed oil were established to be present in both ethanolic and aqueous extracts. Additionally, ethanolic extract alone exhibit the presence of terpenoids and glycosides. Both extracts displayed negative results for alkaloids. Since the ethanolic extract showed the existence of more phytochemicals than aqueous extract it was used for further analysis.

Table 1: Preliminary phytochemical analysis of leaf extract of *F. racemosa*

Test name	Aqueous	Ethanol
Carbohydrates	+	+
Total proteins	+	+
Alkaloids	-	-
Total phenol	+	+
Tannins	+	+
Flavonoids	+	+
Saponin	+	+
Steroids	+	+
Fixed oil	+	+
Terpenoids and Glycosides	-	+
Anthroquinones	+	+
Coumarin	+	+

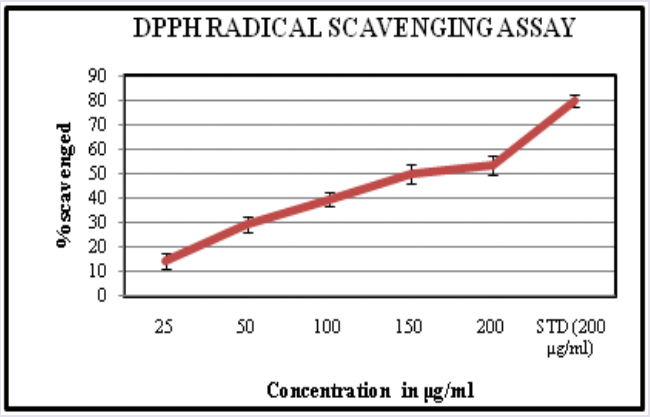


Figure 1: DPPH radical scavenging activity.

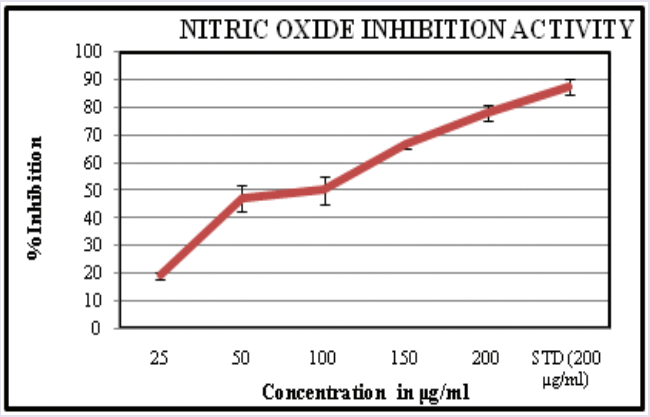


Figure 2: Nitric oxide scavenging activity.

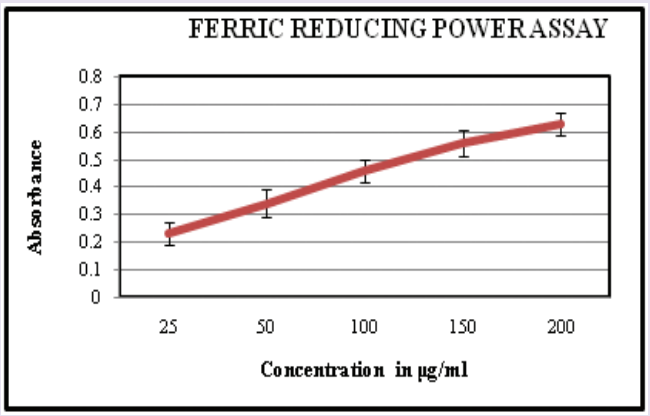


Figure 3: Ferric reducing power assay.

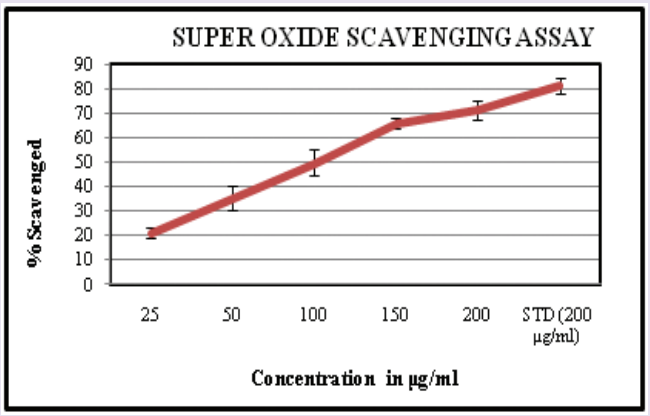


Figure 4: Super oxide scavenging assay.

DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron/hydrogen radical to become a stable diamagnetic molecule. Antioxidants on interaction with DPPH, transfer electron/hydrogen atom to DPPH and therefore reduce the free radical effect and convert it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration shows the scavenging activity of the drug.[12]

Figure 1 shows the DPPH radical scavenging activity of ethanolic leaf extract of *F. racemosa* and standard ascorbic acid. The extract showed significant DPPH scavenging activity and was comparable with ascorbic acid. Substrate concentration that affects 50% loss of the DPPH activity is called as IC_{50} value. In the present study the IC_{50} value for ethanolic leaf extract of *F. racemosa* was found to be 150 µg/ml.

Comparative antioxidant studies by Raghavendra *et al.*[13] reported the IC_{50} value of DPPH radical scavenging activity in selected plant extracts and standard ascorbic acid in following order: Ascorbic acid (6.8 µg/ml) > *Mentha arvensis* (25 µg/ml) > *Moringa oleifera* (185 µg/ml) > *Amaranthus viridis* (190 µg/ml) > *Tamarindus indica* (210 µg/ml) > *Spinacia oleracea* (475 µg/ml) > *Trigonella foenum-graecum* (620 µg/ml).

In another study by Charalampos Proestos *et al.*[14] reported the IC_{50} values of *Nepeta melissifolia*, *Mentha pulegium* and *Phlomis lanata* (5.1 ± 0.4, 13.5 ± 0.5, 23.9 ± 0.4 µg/ml) and were found to be similar to BHT and ascorbic acid (18.5 ± 0.4, 3.9 ± 0.3 µg/ml). Atoui *et al.*[15] also investigated the antioxidant activity and phenolic profile of Tea and herbal infusions and reported that they showed higher hydrogen-donating capacity for DPPH assay. The results of the present study also synchronized with the result of others.

Nitric oxide scavenging activity

NO is a free radical produced in mammalian cells, have a major role in the regulation of several physiological process, including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. However, excess production of NO is linked with several diseases. It would be interesting to increase potent and selective inhibitors of NO for possible therapeutic use.[16]

In the present study, ethanolic leaf extract of *F. racemosa* effectively scavenged NO radical, which competes with oxygen to react with NO and the formation of nitrite radical is inhibited. The antioxidant activity of plant is the most effective way of preventing tissue damages and undesired transformation, thus maintain health. The results of the ethanolic leaf extract of *F. racemosa* showed significant NO scavenging efficacy and the IC_{50} value was 100 µg/ml (Figure 2).

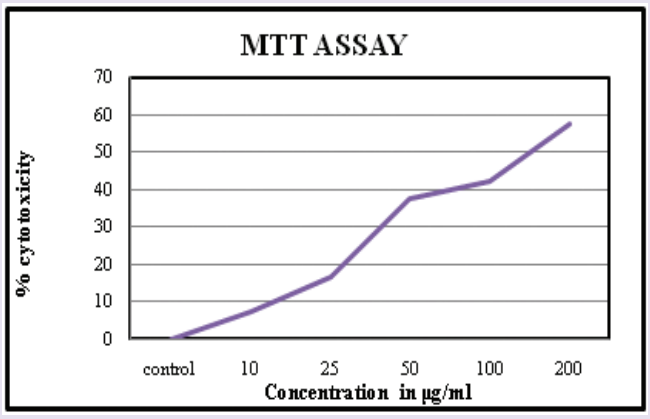


Figure 5: MTT Assay.

Ferric reducing power assay

Reducing power is a reflection of antioxidant activity.[17] Compounds with reducing power can donate electron donors and thus reduce the intermediates of lipid peroxidation and act as primary and secondary antioxidants.[18] The reducing power of ethanolic leaf extract of *F. racemosa* is shown in Figure 3. The reducing power of the standard ascorbic acid and the extract at various concentrations (25, 50, 100, 150 and 200 µg/ml) showed a significant increase in absorbance (0.23, 0.34, 0.46, 0.56 and 0.63) at 700 nm.

Earlier authors have found a direct relationship among reducing ability and antioxidant activity of some herbal extracts.[19] The reducing properties might be due to the presence of reducing compounds,[20] which can donate hydrogen atom and breaks the free radical chain reaction thus exerts antioxidant action.[21] Reductones react with precursors of peroxides and prevent peroxide radical formation.

Saha *et al.*[22] investigated the reducing ability of the leaf extract of *Mimusops elengi* compared to ascorbic acid and reported that the reducing power of extract of *Mimusops elengi* was found significant and the reducing power of the extract increased with the increasing concentration.

Pratap Chandran *et al.*[23] studied the *In vitro* free radical scavenging activity of aqueous and methanolic leaf extracts of *Aegle tamilnadensis* and reported that the methanolic extract showed higher activity in a dose dependant manner than aqueous extract which is due to the presence of reductones. The result of the current analysis is also concurrent with the results of others.

Super oxide scavenging assay

Superoxide anion is a radical that are generated in the living cells from oxygen. Superoxide anion changes to other damaging ROS and free radicals such as hydrogen peroxide and hydroxyl radical, which stimulates oxidative damage.[24]

In the present study, ethanolic leaf extract of *F. racemosa* exhibited IC_{50} value of 100 µg/ ml for superoxide radical scavenging activity (Figure 4).

Rajamanikandan *et al.*[25] investigated the free radicals scavenging ability of ethanolic extract of *Mollugo nudicaulis* (whole plant) by using (DPPH), reducing power, ferric reducing antioxidant power, hydroxyl radical scavenging assay, SO scavenging assay, hydrogen peroxide radical scavenging assay, NO scavenging assay and total antioxidant capacity assay. The results were compared with standard ascobic acid. The results obtained that the ethanolic extract of *Mollugo nudicaulis* had a significant antioxidant activity and is a better source of natural antioxidants. The radical scavenging activity of ethanolic extract of *Mollugo nudicaulis* at a concentration of 2.5 mg/ml was 74.96% for DPPH assay and 45% for reducing power when compared with standard BHT. 2.5 mg/ml ethanolic extract of *Mollugo nudicaulis* scavenged (58%) superoxide anion, whereas standard scavenged 60%. This was proved in *Psidium guajava* also.[26]

In vitro cytotoxic effect

In vitro cytotoxic effect of ethanolic leaf extract of *F. racemosa* versus DLA cell line determined by MTT assay is shown in Figure 5**.** The cytotoxicity improved with increase in concentration of the extract. At 10 µg/ml concentration 7.21% cell death was noticed, whereas at high concentration (200 µg/ml) 57.37% of cell death was observed and showed an IC_{50} value of 175 µg/ml.

Akhila Sravya Dantu *et al.*[27] studied hydroalcoholic extract of *Tabernaemontana divaricata* in anticancer activity against Hela cell lines of several concentrations and reported that as the concentration increases, there was an elevation in the cell growth inhibition and the IC_{50} value was more than 100 µg/ml. In another research anticancer activity of *Artocarpus heterophyllus* (methanolic extract) was tested by MTT assay on A549 cell line. The IC_{50} value was found to be 35.26 µgm/ml by MTT assay against A549.[28]

The conventional cancer treatments like chemotherapy and radiotherapy are costly and also cause many adverse effects such as vomiting, alopecia, diarrhoea, constipation and some severe effects like myelosuppression, neurological, cardiac, pulmonary and renal toxicity. All adverse effects can decrease the quality of life and discourage patients to observe medication procedures which then results in progression of cancer and associated complications. Further, many of these current treatments limited for anti-cancer activity.[29] Hence it is necessary to identify another anticancer drugs, which are more potent, selective and less toxic than conventional treatment. In previous eras, research on plant based compounds has been efficacious in the field of anticancer drugs.

Earlier examples of antileukemic alkaloids such as vinblastine and vincristine from the Madagascar periwinkle (*Catharanthus roseus*), paclitaxel isolated from *Taxus baccata* used in the treatment of lung, ovarian, breast, neck cancers and advanced forms of Kaposi's sarcoma.[30]

CONCLUSION

In the present study, ethanolic leaf extract of *F. racemosa* exhibited IC_{50} value of 150 µg/ ml for DPPH radical scavenging assay and 100 µg/ml for both NO and SO radical scavenging assays. Thus the results reveals that the ethanolic extract of *F. racemosa* is a better scavenger for NO and SO radicals than DPPH radical and a good source of natural antioxidants and also a promising source of anticancer agents.

CONFLICT OF INTEREST

No funding source and there is no conflict of interest.

ABBREVIATION USED

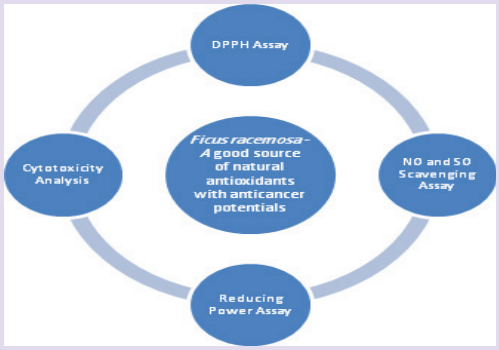
BHA: Butylated hydroxy anisole; **BHT:** Butylated Hydroxytoluene; **DLA:** Dalton Lymphoma Ascites; **DPPH:** 1,1-diphenyl-2-picryl-hydrazyl; **IC_{50}:** Half Maximal Inhibitory Concentration; **MTT:** 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; **NO:** Nitric Oxide; **SO:** Superoxide Radical.

REFERENCES

1. Wolf SP, Dean RT. Glucose auto-oxidation and protein modification. The potential role of autoxidative glycosylation in diabetes. Biochem J. 1987;245(1):243-50.
2. Jacques F, Hai-Rim S, Freddie B, David F, Colin M, Donald MP. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer. 2010; 127(12):2893-917.

3. Madhavi DL, Salunkhe DK. Toxicological aspects of food antioxidants. In: Madavi DL, Deshpande SS, Salunkhe DK, (Eds.). Food Antioxidants. Dekker, New York, 1995; pp. 267.
4. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: A cohort study. Brit Med J. 1996;312(7029):478-81.
5. Sirisha N, Sreenivasulu M, Sangeeta K, Madhusudhana Chetty C. Antioxidant properties of *Ficus* species-A review. International Journal of Pharm Tech Research. 2010; 2(4): 2174-82.
6. Trease GE, Evans WC. Pharmacognosy, 12th Edition ELBS Publication, Baillier Tindall, East Bourne, 1996, pp. 344-539.
7. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity, Lebensmittel-Wissenschaft und-Technologie/Food Science and Technology. 1995;28:25-30.
8. Fan YM, Xu LZ, Gao J, Wang Y, Tang XH, Zhao XN *et al.* Phytochemical and anti-inflammatory studies on *Terminalia catappa*. Fitoterapia. 2004;75(3):253-60.
9. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr. 1986;07:307-15.
10. McCord JM. Fridovich I Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244(22):6049-55.
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;16;65(1-2): 55-63.
12. Shazia Usmani. Screening for antioxidant and free radical scavenging potential of extracts of leaves and flowers of *Calotropis gigantea*. Asian J Pharm Clin Res. 2013;6:97-100.
13. Raghavendra M, Madhusudhana RA, Pulala RY, Sudharshan RA, Siva KL. Comparative studies on the *in vitro* antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of India. Asian J Pharm Clin Res. 2013;6(3):96-9.
14. Charalampos P, Konstantina L, Olga KM, Panagiotis Z, Vassileia J. Sinanoglou. Antioxidant capacity of selected plant extracts and their essential oils. Antioxidants. 2013;2(1):11-22.
15. Atoui AK, Mansouri A, Boskou G, Kefalas P. Tea and herbal infusions: Their antioxidant activity and phenolic profile. Food Chem. 2005;89(1):27-36.
16. Hepsibha BT, Sathiya S, Babu CS, Premalakshmi V, Sekar T. *In vitro* studies of antioxidant and free radical scavenging activities of *Azima tetracantha* Lam. leaf extract. Indian J Sci Technol. 2010;3(5):571-7.
17. Oktay M, Gulcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensum.Wiss. U. Technol. 2003; 36(2):263-71.
18. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research. 2009;3(13):981-96.
19. Tanaka M, Kuie CW, Nagashima Y, Taguchi T. Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi. 1988;54:1409-14.
20. Duh PD, Tu YY, Yen GC. Antioxidant activity of the aqueous extract of Harn jyur (*Chrysanthemum morifolium* Ramat). Lebensmittel-Wissenschaft and Technologie. 1999; 32(5):269-77.
21. Gordon MH. The mechanism of antioxidant action *in vitro*. In: BJF Hudson (Ed.), Food antioxidants Elsevier Applied Science, London, 1990; pp. 1-18.
22. Saha MR, Hasan SMR, Akter R, Hossain MM, Alam MS, Alam MA, *et al. In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. Bangl J Vet Med. 2008;6(2):197-202.
23. Pratap CR, Vysakhi MV, Manju S, Kannan M, Abdul KS, Sreekumaran NA. *In vitro* free radical scavenging activity of aqueous and methanolic leaf extracts of *Aegle tamilnadensis* (Rutaceae). Int J Pharm Pharm Sci. 2013;5(3):819-23.
24. Al-Mamun M, Yamaki K, Masumizu T, Nakai Y, Saito K, Sano H. Superoxide anion radical scavenging activities of herbs and pastures in northern Japan determined using electron spin resonance spectrometry. Int J Biol Sci. 2007;3(6):349-55.
25. Rajamanikandan S, Sindhu T, Durgapriya D, Sophia D, Ragavendran P, Gopalakrishnan VK. Radical scavenging and antioxidant activity of ethanolic extract of *Mollugo nudicaulis* by *in vitro* assays. Indian Journal of Pharmaceutical Education and Research. 2011;45(4):310-6.
26. Manikandan R, Vijaya Anand A. Evaluation of antioxidant activity of *Psidium guajava* Linn. in streptozotocin–induced diabetic rats. Free Radicals and Antioxidants. 2016;6(1):72-6.
27. Akhila SD, Shankarguru P, Ramya DD, Vedha Hari BN. Evaluation of *in vitro* anticancer activity of hydroalcoholic extract of *Tabernaemontana divaricata*. Asian J Pharm Clin Res. 2012;5(3):59-61.
28. Rajesh PM, Sahil PK. Cytotoxic activity of methanolic extract of *Artocarpus heterophyllus* against A549, Hela and MCF-7 cell lines. Journal of Applied Pharmaceutical Science. 2011;1(7):167-71.
29. Mans DR, da Rocha AB, Schwartsmann G. Anti-cancer drug discovery and development in Brazil: targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. Oncologist. 2000; 5(3):185-98.
30. Takimoto CH, Calvo E. *Principles of Oncologic Pharmacotherapy.* In *Cancer Management: A Multidisciplinary Approach,* 11 Ed., edited by R. Pazdur, L.D. Wagman, K.A. Camphausen and W.J. Hoskins. New York: Cmp United Business Media 2008.

PICTORIAL ABSTRACT



SUMMARY

- *Ficus racemosa* consists of medicinally essential phytoconstituents.
- The ethanolic extract has a better scavenger for NO and SO radicals than DPPH radical.
- The IC_{50} value for cytotoxicity analysis against DLA cell line was found to be 175 µg/ml.
- It has a good source of natural antioxidants and also a promising source of anticancer agents.

ABOUT AUTHORS

Mr. B. Aslam Khan: Research Scholar, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu. He is doing Ph.D., in the Department of Biochemistry in phytopharmaclogy.

Dr. A. Vijaya Anand: Associate Professor and Head, Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore, Tamil Nadu, India. He is currently engaged in the field of phytopharmacology, neurogenetics, medical genetics and clinical biochemistry.

Dr. B. Varalakshmi: Assistant Professor, Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamil Nadu. She is engaged in the field of phytopharmacolocy and clinical biochemistry.

Dr. T. Karpagam: Assistant Professor, Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamil Nadu. She is engaged in the field of medicinal chemistry and clinical biochemistry.

Dr. N. Pushpa: Assistant Professor, Department of Microbiology, Cauvery College for Women, Tiruchirappalli, Tamil Nadu. She is engaged in the field of phytochemistry and clinical biochemistry.