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FREE RADICAL SCAVENGING EFFICACY OF VARIOUS EXTRACTS OF *Ficus racemosa* BARK

A. Poongothai[1*] and S. Annapoorani[2]

[1]*Assistant Professor, Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur – 635 601, Tamil Nadu, India.*

[2]*Professor, Department of Biochemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India.*

Abstract

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infections and degenerative diseases. Modern research is now directed towards natural antioxidants originated from plants due to safe therapeutics. Antioxidant activity of different organic extracts of *Ficus racemosa* (FRE) bark was investigated for its free radical scavenging activity by adopting various *in vitro* models such as 1, 1-diphenyl, 2- picryl hydrazyl (DPPH), nitric oxide, hydroxyl and hydrogen peroxide scavenging activity was found to be more effective in the ethanolic extract than that of petroleum ether and ethyl acetate extracts. The ethanolic extract showed maximum scavenging activity for DPPH followed by nitric oxide, hydrogen peroxide and hydroxyl radical. Due to its safety and potent activity, it is worth in incorporating this extract in herbal formulations for hepatoprotective and antioxidant properties. This potent antioxidative role might be due the active secondary metabolites of ethanolic extract of *Ficus racemosa* bark.

Key words: Antioxidants, Free radicals, Scavenging activity and *Ficus racemosa*

1. Introduction

Oxidative Stress (OS) is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species (ROS) as a result of one of three factors: a) an increase in oxidant generation, b) a decrease in antioxidant protection, or c) a failure to repair oxidative damage. ROS are either free radicals, reactive anions containing oxygen atoms, or molecules containing oxygen atoms that can either produce free radicals or are chemically activated by them (Allen, 2003). Examples are hydroxyl radical, superoxide, hydrogen peroxide and peroxynitrite. The main damage to cells results from the ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Additionally, oxidative stress and ROS have been implicated in disease states, such as Alzheimer's disease, Parkinson's disease, Cancer, and Aging (Allen, 2003; Bheemachari *et al*., 2007). The effects of free radicals are expressed by the accumulation of oxidative damage to biomolecules: nucleic acids, lipids and proteins (Bheemachari *et al*., 2007).

Antioxidants synthesized within the body or taken in the diet that form a natural defense against free radical induced damage (Cesquini *et al*., 2003). The oxidative stress in animals or cell cultures has been successfully induced by hydrogen peroxide (Conforti *et al*., 2008), and was chosen for induction of oxidative stress on isolated

Corresponding author*: **Dr. A. Poongothai
Tel.: *Mobile:* +918940673409
E-mail: kumuthaannadurai@gmail.com
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frog heart. *Ficus racemosa,* a moderate to large sized tree belonging to the family Moraceae. It is a very common plant distributed throughout the India. The different parts of the plant are employed in native medicine for treating diarrhea, dysentery and diabetes, hypercholesterolemia and spermatozoic. Unripe fruits and bark of the same plant in treating inflammation, root bark as anti-oxidant. *Ficus racemosa* also been reported to produce anti filarial, antibacterial activities, in treating wounds and ulcers. Various literature surveys showed that no study has been done on antioxidative stress activity of *Ficus racemosa.* Therefore, we undertook the present investigation to examine the antioxidant activities of various extracts of *Ficus racemosa* bark through different *in vitro* models.

2. Materials and Methods

Plant materials

Ficus racemosa bark was collected from Coimbatore district, Tamil Nadu, India.

Extraction

The shade dried and powdered bark of *Ficus racemosa* were extracted with different organic solvents such as ethanol, ethyl acetate and petroleum ether using Soxhlet apparatus respectively at 79 ^{o}C, 77 ^{o}C and 55 ^{o}C. These extracts were concentrated using flash evaporator and dissolved in known volume of respective solvents and used for further experiments.

Evaluation of Antioxidant activity by *in vitro* Techniques

DPPH radical scavenging assay

DPPH radical assayed as described by Elizabeth and Rao (1990). The reaction mixture contained Methanol - 50 ml. DPPH (Diphenyl-2-picryl hydrazyl radical) – 1 mM 3 ml of 1 mM DPPH in methanol was added to 100 µl of plant extract with concentrations ranging from 10 µg to 100 µg. DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. When DPPH reacts with antioxidant in the sample, it was reduced and the color changed from deep violet to light yellow. This was measured at 518 nm. The percentage scavenging activity was calculated by the following formula.

Scavenging activity (%) = A518 (control)-A518 (sample)/A518 (Control) × 100

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964). The reaction mixture (3 ml) containing 2 ml of sodium nitroprusside (10 mM), 0.5 ml of phosphate buffer saline (1 M) were incubated at 25 ^{0}C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33 %) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1 % NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess illosvery reaction at 540 nm.

Hydrogen peroxide scavenging assay

Hydrogen peroxide assayed as described by Ruch *et al.* (1989) who proposed an assay for the determination of antioxidant activity of compounds by their ability to scavenge the oxidant hydrogen peroxide. The reaction mixture contained Phosphate buffer (pH - 7.4) hydrogen peroxide in phosphate buffer (40 mM). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer. Plant extracts at the concentration of 10 mg/10 µl was added to a hydrogen peroxide solution (0.6 ml, 40 mM). The total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm. The blank solution contained phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the plant extract was calculated as follows:

Percentage of scavenged H_2O_2 = A0 - A1/A0 × 100

Where,

A0 - Absorbance of control

A1 - Absorbance in the presence of plant extract

Hydroxyl radical scavenging activity

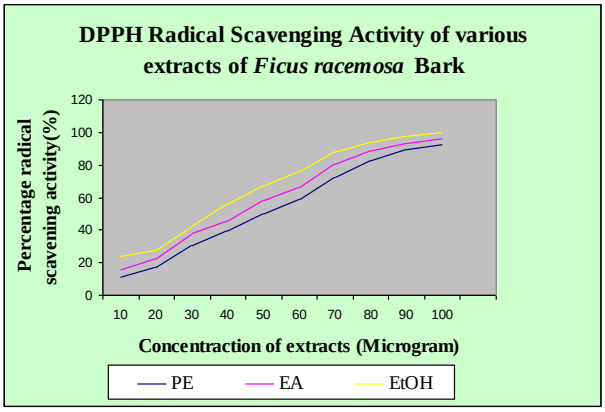
Hydroxyl radical assayed as described by Elizabeth and Rao (1990). The assay was based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate – EDTA – H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8 mM), 0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1 mM), 0.1 ml Ascorbate (0.1 mM), 0.1 ml KH_2PO_4 - KOH buffer, pH 7.4 (20 mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37 ^{0}C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

3. Results and Discussion

Free radicals are known to have an important role in stimulation of phagocytosis, induction of drug detoxification pathways and stimulation of signal transduction pathways. However, the same radicals can be implicated in the pathogenesis of various diseases such as atherosclerosis, aging, ischemia, and reperfusion injury of many tissues, central nervous system injury, gastritis, including cancer (Costa *et al*., 2009). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of these diseases. Phytocompounds like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Conforti *et al*., 2008; Kalaivani and Mathew, 2009). Therefore, medicinal plants can be a potential source of natural antioxidants (Cesquini *et al*., 2003).

DPPH radical scavenging activity

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1- picrylhydrazyl DPPH. The DPPH Scavenging activity of ethanol, ethyl acetate and petroleum ether extracts of *Ficus racemosa* bark was illustrated in Figure I. The percentage of DPPH radical Scavenging activity of ethanolic extract of *Ficus racemosa* bark was exhibited a maximum DPPH radical activity of 95.70 % at 100 µg/ml whereas ethyl acetate and petroleum ether extracts were found to be 98.23 % and 95.46 %. The, IC_{50} values of these extracts found to be 39 µg/ ml, 47 µg/ ml and 54 µg/ ml were respectively.



*All values are expressed as mean ± SEM for three determinations

Figure – I

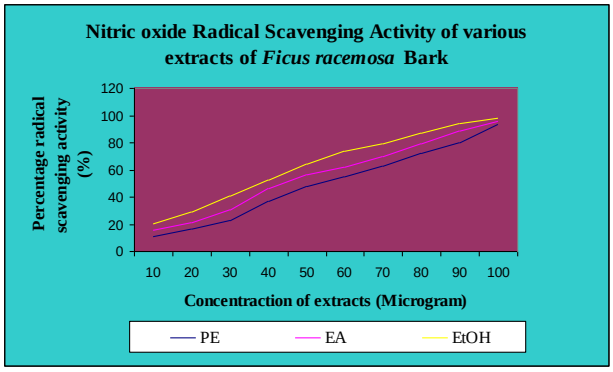
Nitric oxide radical scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems. So, it was worthful to investigate the NO scavenging potential of the plant extract. The reduction of nitric oxide radical by the ethanol, ethyl acetate and petroleum ether extracts of *Ficus racemesa* bark was illustrated in Figure II. The maximum NO radical scavenging activity of 97.33 % at 100 µg/ml whereas ethyl acetate and petroleum ether extracts were found to be 94.86 % and 95.67 %. The, IC_{50} values of these extracts found to be 42 µg/ ml, 45 µg/ ml and 54 µg/ ml were respectively.

Hydrogen peroxide scavenging assay

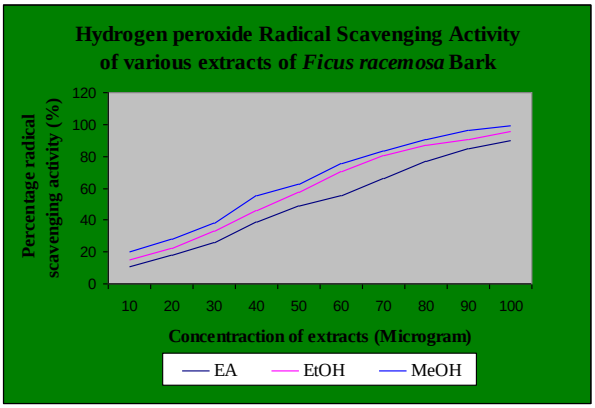
Hydrogen peroxide (H_2O_2) may contribute to the pathogenesis of ischemic-

reperfusion injury in the heart. The hydrogen peroxide radical by the ethanol, ethyl acetate and petroleum ether extracts of *Ficus racemesa* bark was illustrated in Figure III. The ethanolic extract of *Ficus racemosa* bark was exhibited a maximum H_2O_2 radical activity of 97.49 % at 100 µg/ ml. whereas ethyl acetate and petroleum ether extracts were found to be 98.29 % and 95.07 %. Then, IC_{50} values of these extracts found to be 35 µg/ml, 40 µg/ml and 48 µg/ml were respectively.



*All values are expressed as mean ± SEM for three determinations

Figure – II

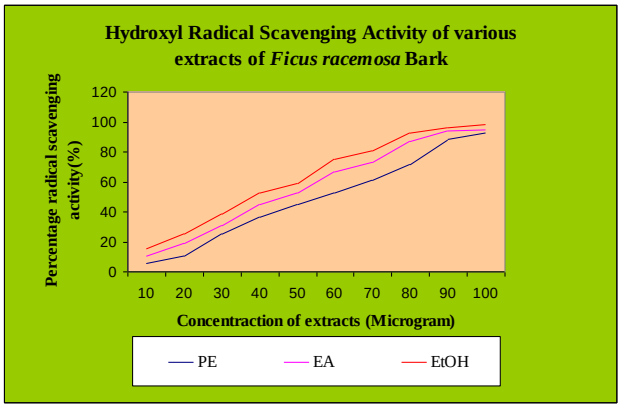


*All values are expressed as mean ± SEM for three determinations

Figure – III

Hydroxyl radical scavenging activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. Hydroxyl radicals are the major Reactive Oxygen Species causing lipid peroxidation and enormous biological damage. The reduction of hydroxyl radical by the ethanol, ethyl acetate and petroleum ether extracts of *Ficus racemesa* bark was illustrated in Figure IV. The maximum hydroxyl radical scavenging activity of ethanolic extract of *Ficus racemesa* bark 98.22 % at 100 µg/ml. whereas ethyl acetate and petroleum ether extracts were found to be 98.47 % and 97.54 %. The, IC_{50} values of these extracts found to be 30 µg/ml, 39 µg/ml and 42 µg/ml were respectively.



*All values are expressed as mean ± SEM for three determinations

Figure – IV

4. Conclusion

From the above results, it can be concluded that, among the three extracts the ethanolic extract was found to be more efficient antioxidant than that of ethyl acetate and petroleum ether extracts. These ethanolic extract of *Ficus racemosa* bark is a potent source of natural antioxidants, which might be helpful in preventing the progress of various oxidative stresses in diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders and neurological disorders. Further investigations need to be carried out to isolate and identify the active compounds present in the above plant extracts and the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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