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IN-VITRO ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF BARK OF *FICUS RACEMOSA* LINN. (MORACEAE)

Saurabh Rajvaidhya [*1] and V. V. Byahatti [2]

Moulana Azad Institute of Pharmacy, Jodhpur - 342008, Rajasthan, India.
Department of Pharmacognosy, K.L.E's College of Pharmacy, Hubli - 580031, Karnataka, India.

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Correspondence to Author:
Saurabh Rajvaidhya

Moulana Azad Institute of Pharmacy, Jodhpur - 342008, Rajasthan, India.

E-mail: srajvaidhya@gmail.com

ABSTRACT: The plant *Ficus racemosa* L., is a woody large deciduous tree distributed all over India, and grows in the evergreen forest, moist localities, along with the sides of ravines and banks of streams belonging to the family Moraceae, locally know as Attimara and Hindi known as Gular. Ethnobotanically the bark used for anti-diabetic, wounds, useful in asthma and piles recommended in uropathy and treatment of menorrhagia. Biological activities like anti-inflammatory, anti-diarrhea, anti-diuretic, antibacterial, hepatoprotective, were reported. The present study finds outs the scientifical evidence of bark of *Ficus racemosa* L. for its antioxidant property using different screening models. The different extract of bark was obtained by successive extraction with petroleum ether (40-60 ºC), butanol, ethyl acetate, alcohol by Soxhlet method. These extracts were taken for an *in-vitro* antioxidant study, were carried out by using *in-vitro* antioxidant screening models like DPPH radical scavenging activity and total phenolic contents (TPC). The few successive plant extracts showed significant dose-dependent activity by using various *in-vitro* antioxidant models. Antioxidant activity of bark showed a greater free radical sequestering activity. In the present study, ethanol extract showed a greater antioxidant activity was found to be 100 µg/mL expressed as significant antioxidant activity of *Ficus racemosa* L. This might be due to the presence of phyto-compounds flavonoids, phenols, saponins, steroids, tannins and terpenoids present in the preliminary phytochemical screening.

INTRODUCTION: In general, the effect of anti-oxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule [1].

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Anti-oxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease [2].

It has also been proposed that antioxidant activity of plant origin components can be mainly ascribed to the presence of phenolic compounds [3]. Phenolic compounds are not evenly distributed in plant parts; they are present at elevated amounts in the outer parts of the fruits, leaves, and barks [4]. Tree bark is usually rich in compounds with medicinal properties, and many cultures use it for centuries. Asians, Polynesian, and American indigenous

people used bark components to treat heart failure among other medicinal applications [5]. Following the great success of Taxol (a chemotherapic anticancer drug), there has been a massive search by ethnobotanists and biochemists for bark components, leading to the discovery of some useful products [6].

Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, cancer, wound healing diabetes asthma and, aging [1]. Free radicals induced by per-oxidation have gained much importance because of their involvement in several pathological conditions such as atherosclerosis, ischemia, liver disorder, neural disorder, metal toxicity, drugs toxicity and pesticide toxicity [7]. Together with other derivatives of oxygen, they are inevitable by-products of biological redox reactions of biological cycles8. Anti-oxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition of oxidants.

In general, the effect of antioxidants is to break up the chains formed during the propagation process by providing a hydrogen atom or an electron to the free radical and receiving the excess energy possessed by the activated molecule to reduce oxidation[1]. Anti-oxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease and increase healing [2]. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of pro-oxidant metals, quenches of singlet oxygen or hydrogen providers, *etc*. [9] The antioxidants can interfere with the oxidation process by reacting with free radicals by reducing those [10].

Recently interest has increased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity and drug interactions [11]. The food industry uses natural anti-oxidants as a replacement of conventional synthetic anti-oxidants, to avoid their side effects [12]. The plant *Ficus racemosa* L., is a woody evergreen belonging to the family Moraceae, locally knows as Attimara and in Hindi known as Gular. Ethnobotanically, root, and sap of root, bark, leaves, and fruits, latex are used for various medicinal uses. Roots used in dysentery, diarrhea hydrophobia and fluid obtained from it by incision is administered as a powerful tonic. Leaves are used in bronchitis, antihypertensive and inflammation, lymphadenitis, in sprains and fibrositis. Ripe fruits used to check leprosy, menorrhagia or excessive bleeding during menstruation, nose bleeding and for expelling intestinal worms. Biological activities such as antimicrobial studies and preliminary phytochemical studies reported that the presence of various classes of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids [13], this report confirmed antioxidant potential of bark of *Ficus racemosa* L. After the scrutiny of literature, so far no work has been carried out regarding anti-oxidant activity of fruits of selected plant. Hence, in the present study, the antioxidant activity of bark of *Ficus racemosa* L. was done [14, 15, 16, 17, 18, 19].

MATERIALS AND METHODS:

Collection of Plant Material: The stem bark of *Ficus racemosa* Linn. was collected from the Botanical Garden, Karnataka University, Dharwad (Karnataka State) and authenticated by Dr. G.R Hegde, Professor, and Head, P.G. Department of Botany, Karnataka University, Dharwad. A voucher specimen (no. 04PG0356, Saurabh Rajvaidhya) has been deposited in the PG Pharmacognosy laboratory of the college for future reference.

Preparation of the Plant Extracts: The collected bark materials were shade-dried and coarsely powdered using a pulverizer. The coarse powders were subjected to successive extraction with organic solvents of increasing polarity such as petroleum ether (40-60 ºC), butanol, ethyl acetate, alcohol by Soxhlet method, and water extract by maceration of the mark of successive extract. The extracts were collected and distilled off on a water bath at atmospheric pressure, and the last trace of the solvents was removed in vacuum and stored at 4 ºC. The resulted extracts were used for the *in-vitro* antioxidant activity.

Inhibitory Effects on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical Assay: DPPH is a free radical which when dissolved in ethanol has a blue-violet color. When it reacts with the reducing agent, the solution loses color indicates radical scavenging activity of test material [20]. 3 ml of 60 µM DPPH in ethanol was added to different concentrations of extracts (10-1000 µg/mL) and then incubated at room temperature for 15 min. Absorbance was read at 517 nm using a spectrophotometer (Simtronics, India). The percentage of DPPH radical scavenging activity was calculated by comparing the absorbance values of control not treated with the extract. Ascorbic acid used as a positive control. All determinations were performed three times, and the results were expressed as a mean ± S.E.M.

Total Phenolic Assay: The amount of total phenolic was measured using the Folin-Ciocalteu reagent method [21]. One milliliter of extracts was taken into test tubes and mixed with 1 ml 95% ethanol, 5 ml distilled water and 0.5 ml 1N Folin-Ciocalteu reagent. After 5 min, 1 ml of 5% Na_2CO_3 was added, and the reaction mixture was allowed to stand for 60 min before the absorbance at 725 nm was measured.

A standard curve was established for each assay using 50-500 µg of gallic acid in 95% ethanol and expressed as gallic acid equivalent (GAE) (milligram of a gallic acid equivalent/gram of various extracts). All determinations were performed three times, and the results were expressed as a mean ± S.E.M.

RESULTS: All the extract showed concentration-dependent activity in various extracts. The results of anti-oxidant activity were given here as follows in **Table 1**.

Inhibitory Effects on 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) Radical Assay: S. petroleum ether extract showed maximum activity was observed as 61.54 ± 0.72 at 1000 µg/mL followed by 22.65 ± 0.61 at 100 µg/mL and 14.37 ± 1.21 at 10 µg/mL concentrations respectively. S. butanol extract showed maximum activity as 69.36 ± 0.80 at 1000 µg/mL followed by 28.28 ± 0.78 at 100 µg/mL & 18.60 ± 0.74 at 10 µg/mL concentrations respectively.

S. ethyl acetate extract showed maximum activity as 82.31 ± 0.80 at 1000 µg/mL followed by 38.18 ± 0.70 at 100 µg/mL and 26.50 ± 0.42 at 10 µg/mL concentrations respectively. S. ethanol extract showed maximum activity as 91.68 ± 0.95 at 1000 µg/mL and followed by 52.26 ± 1.03 at 100 µg/mL and 28.82 ± 2.24 at 10 µg/mL concentrations respectively. All the values were compared with the control ascorbic acid was observed as 97.22 ± 1.54 at 1000 µg/mL and followed by 69.43 ± 2.80 at 100 µg/mL & 36.46 ± 1.43 at 10 µg/mL concentrations respectively **Fig. 1**.

Total Phenol Assay: Petroleum ether extract showed maximum activity was observed as 72.51 ± 1.04 at 1000 µg/mL followed by 53.11 ± 0.65 at 100 µg/mL and 15.34 ± 0.41 at 10 µg/mL concentrations respectively. S. butanol extract showed maximum activity as 78.21 ± 1.36 at 1000 µg/mL followed by 60.12 ± 1.23 at 100 µg/mL and 21.24 ± 1.54 at 10 µg/mL concentrations respectively. S. ethyl acetate showed maximum activity as 82.57 ± 1.88 at 1000 µg/mL followed by 61.32 ± 1.23 at 100 µg/mL and 23.72 ± 1.34 at 10 µg/mL concentrations respectively.

S. ethanol extract showed maximum activity as 92.43 ± 1.60 at 1000 µg/mL and followed by 64.61 ± 1.89 at 100 µg/mL & 24.80 ± 2.00 at 10 µg/mL concentrations respectively. All the values were compared with the control gallic acid was observed as 98.44 ± 0.42 at 1000 µg/mL and followed by 71.35 ± 1.27 at 100 µg/mL & 32.13 ± 1.45 at 10 µg/mL concentrations respectively **Fig. 2**.

TABLE 1: DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF BARK OF *FICUS RACEMOSA* L.

Extracts	DPPH assay (Inhibition %)											
	S. Petroleum ether extracts (µg/mL)			S. Butanol extracts (µg/mL)			S. Ethyl acetate extracts (µg/mL)			S. Ethanol extracts (µg/mL)		
	10	100	1000	10	100	1000	10	100	1000	10	100	1000
Bark	14.3 ±1.21	22.65 ±0.61	61.54 ±0.72	18.60 ±0.74	28.28 ±0.78	69.36 ±0.80	26.50 ±0.42	38.18 ±0.70	82.31 ±0.80	28.82 ±2.24	52.26 ±1.03	91.68 ±0.95
Control	36.46 ±1.43	71.35 ±1.27	97.22 ±1.54	36.46 ±1.43	71.35 ±1.27	97.22 ±1.54	36.46 ±1.43	71.35 ±1.27	97.22 ±1.54	36.46 ±1.43	71.35 ±1.27	97.22 ±1.54

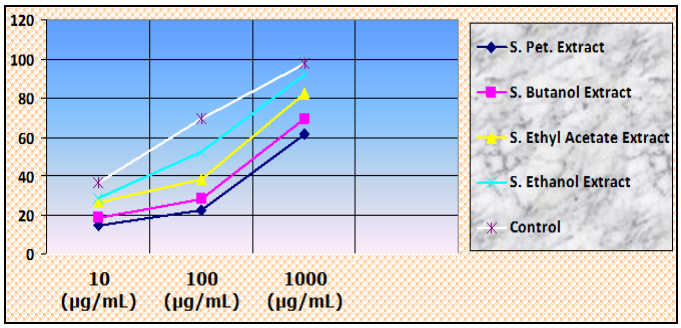

FIG. 1: DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF BARK OF *F. RACEMOSA* L.

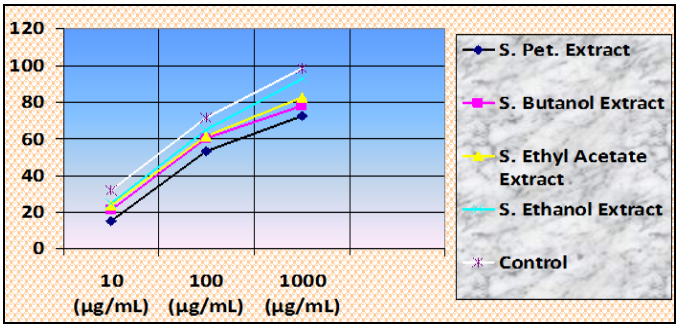

FIG. 2: TOTAL PHENOLIC CONTENT OF VARIOUS EXTRACTS OF BARK *OF FICUS RACEMOSA* L.

TABLE 2: TOTAL PHENOLIC CONTENT OF VARIOUS EXTRACTS OF BARK OF *FICUS RACEMOSA* L.

Extracts	Total Phenol assay (Inhibition %)											
	S. Petroleum ether extracts (µg/mL)			S. Butanol extracts (µg/mL)			S. Ethyl acetate extracts (µg/mL)			S. Ethanol extracts (µg/mL)		
	10	100	1000	10	100	1000	10	100	1000	10	100	1000
Bark	15.34 ±0.41	53.11 ±0.65	72.51 ±1.04	21.24 ±1.54	60.12 ±1.23	78.21 ±1.36	23.72 ±1.34	61.32 ±1.23	82.57 ±1.88	24.80 ±2.00	64.61 ±1.89	92.43 ±1.60
Control	32.13 ±1.45	71.35 ±1.27	98.44 ±0.42	32.13 ±1.45	71.35 ±1.27	98.44 ±0.42	32.13 ±1.45	71.35 ±1.27	98.44 ±0.42	32.13 ±1.45	71.35 ±1.27	98.44 ±0.42

All the values are expressed as mean ± S.E.M.

DISCUSSION: Plants produce a significant amount of anti-oxidants to prevent the oxidative stress caused by photons, oxygen, and other biological reactions; they represent a potential source of new compounds with antioxidant activity [22]. High levels of free radicals or active oxygen species create oxidative stress, which leads to a variety of biochemical and physiological lesions and often results in a metabolic impairment, improper functioning and cell death [23]. There is continuing interest on the screening of medicinal plants with a view to determining new sources of natural anti-oxidants to avoid the side effect of synthetic one [24, 25]. Thus, continued research is being undertaken all over the world on different plant species and their therapeutic principles for the above mentioned purpose [26]. Several Indian medicinal plants have been extensively used slowing the process of aging and related disorders.

Several such plants have already been highlighted for their antioxidant activity and other related activity such as *Emblica officinalis*, *Curcuma longa*, *Mangifera indica*, *Sandalum album*, *Withania somnifera*, *etc*. [27] Active principles have been isolated from various plants, *e.g*. Mangiferin, from *Mangifera indica* L.; emblicanin A & B, two tannins from *Phyllanthus emblica* L. [28] and curcumin, and curcuminoids well-known compounds isolated from *C. longa* L. [29]. *Ficus racemosa* L., an important Indian medicinal plant, different extracts of bark was tested for the first time in the present study, for their free radical scavenging activity and total phenol content method *in-vitro*. The *Ficus racemosa* L., bark showed a greater free radical sequestering activity.

However, in general flavonoid and phenolic class of compound was observed in the extract which possesses greater anti-oxidant activity. In the present study, S. ethyl acetate and S. ethanol extract showed a greater antioxidant activity which may be attributed to the presence of flavonoid compound quercetin. The compound quercetin is well known antioxidant [30, 32]. Also, these results indicate that all the extracts have a noticeable effect on the scavenging of free radicals.

This activity also increases with increasing concentration. The extracts of this plant can be regarded as promising candidates for a plant-derived antioxidant compound. This study reveals that *Ficus racemosa* L., offer an interesting source of new anti-oxidative plant extracts being a potential for their use in different fields (foods, cosmetics, pharmaceuticals). Future studies will be aimed at investigating the effects of S. ethyl acetate and S. ethanol extract of bark on the regulation of cellular mechanisms and upon isolating and identifying the substances responsible for the antioxidant effects of the plant extracts.

CONCLUSION: From the above results, it can be concluded that S. ethyl acetate and S. ethanol extract extracts of the bark of *Ficus racemosa* L., showed the most potent *in-vitro* antioxidant activity with high percentage inhibition. This may be attributed due to the presence of secondary plant metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids which probably play a role as an effective free radical scavenger and effective antiasthmatic, hepatoprotective and antitumoral agent.

This study supports the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. Significant antioxidant activity showed by *Ficus racemosa* L., provides scientific validation for the traditional use of these plants. Further work on isolation and identification of active compounds and its efficacy needs to be done.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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