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Antioxidant activity analysis of *Ficus racemosa* leaf extract

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Abstract
In this era significant volume of research has been dedicated towards a new hype i.e. natural antioxidants, dietary compounds of many plants be a plant part or its isolated phyto-compounds, have shown more effective antioxidant activities than synthetic antioxidants like vitamin C or E, which can be beneficially utilized for ameliorating diseases. The present study encompasses the ascertainment of *in vitro* antioxidant capacity of the ethanolic extract of *Ficus racemosa* leaves and its phytochemical analysis for the estimation of its polyphenols and flavonoids. The antioxidant activities were determined by in vitro assays to compare the antioxidant effects which includes quantification of extracts by FRAP (Ferric Reducing Antioxidant Power) assay, TBARS(Thiobarbituric Acid Reactive Species) assay and scavenging activity against H_2O2. High amount of polyphenolic and flavonoid compounds were found. Phenolic compounds significantly contributed the antioxidant property i.e. free radical scavenging activity and reducing ability of the *Ficus racemosa* leaf extract**.**

Keywords: *Ficus racemosa*, antioxidant, phenolis, flavonoids, *in vitro*, etc.

Introduction
Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics or medicinal materials to replace synthetic antioxidants, which are being restricted due to their potential risk, carcinogenicity [1] and harmful effects. Natural antioxidants are gaining popularity due to their fewer side effects and more benefits. The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received very generous response and increasing attention for their elevated role in the prevention of human diseases [2]

Ficus racemosa Linn. (family: Moraceae) commonly known as gular is a widely cultivated plant all over India. It is one of the oldest medicinal herb mentioned in all ancient scriptures of Ayurveda and different parts like root, bark, and stem have been used to isolate various chemical constituents like tannins, phenols, flavonoids, alkaloids, etc. to cure a vast range of diseases. The leaf of *F. racemosa* contains tetra-triterpene, glauanol acetate, and racemosic acid. Among various pharmacological properties, *Ficus Racemosa Linn* employs an important role as an anti-oxidant, with wound healing properties, antibacterial and antioxidant activities [3]. Also used in dysentery, diarrhea and as a probable radio protector. Role as a radio protector mainly is based on its action as free radical scavenger because radiation induced cytotoxicity is mediated via generation of free radicals in the biological system. Many diseases are due an imbalance between formation and detoxification of pro-oxidants i.e. "oxidative stress".

Oxidative stress is the increase in the number of free radicals (that are normally produced by the metabolism of the oxygen in the body and generate (ROS) reactive oxygen species) more than their scavenging by the antioxidant system working in the body. Antioxidant compounds such as polyphenols and flavanoids present in *F. racemosa*, are known to be used for the treatment of various ailments associated with oxidative stress.

The aim of the present study was to investigate and evaluate the *in vitro* antioxidant potential of *Ficus racemosa* used in ethnoveterinary via studying the phytochemical properties of the selected herbal extract like polyphenols, flavonoids etc, which are responsible for its antioxidant activity.

2. Materials & Methods

2.1 Collection and Preparation of extracts

Collection of herbs was done on the basis of indigenous technical knowledge (ITK).

In the present study, *F. racemosa* was collected from the Dehradun district, India, and identified by the experts at Department of Botany at Bareilly College, Uttar Pradesh, India. Leaves of the selected plant were collected after proper identification and subjected to dry in the shade. Dried leaves were grinded to make the powder. The powder was subjected to ethanolic extraction in Soxhlet apparatus and was concentrated under reduced pressure. The extract was filtered using Whatmann filter paper no.1 and evaporated to dryness over rotary evaporator under room temperature. Finally, the extract was stored in refrigerator until the use.

2.2. Antioxidant activity assessment.

Ferric-Reducing antioxidant Power Assay

In this assay, the antioxidant activity was determined on the basis of the ability of extract to reduce ferric (III) iron to ferrous (II) iron. The FRAP assay was carried out according to the procedure of Sahgal *et al.* [4]. FRAP reagent was prepared by mixing acetate buffer (25 ml, 300 mmo1/l, pH 3.6), 10 mmo1/1TPTZ solution (2.5 ml) in 40 mmol/1 HCl and 20 mmo1/1 $FeCl_3$ solution (2.5 ml) in proportions of 10:1: 1 (v/v), respectively. The FRAP reagent was freshly prepared and prewarmed to 37 °C in a water bath. 150µl of the sample was added to the FRAP reagent (4.5 ml) and absorbance of the reaction mixture was recorded at 593 nm after 4 min, the assay was carried out in triplicates. The standard curve was generated using $FeSO_4$ solution (0.5- 10 mg/ml). The results were expressed as µmol Fe (II)/g dry weight of plant extracts. L-ascorbic acid was used as a comparative model for this assay.

Assay of Ascorbate-Iron (III)-catalysed phospholipids peroxidation

The ability of the extract to scavenge hydroxyl radical was determined by the modified method of Aruoma *et al.* [5]. Here goat liver was mixed (1: 10) with mM phosphate-buffered saline (PBS, pH 7.4) and sonicated in an ice bath for the preparation of the homogenate liposomes. The liposomes (0.2 ml) were added with 0.5 ml of PBS buffer, 0.1 ml of 1 ml $FeCl_3$ and various volumes (100 µl and 200 µl) of herbal extract and then subsequently 0.1 ml of 1 mM ascorbic acid was added. After incubation at 37 °C for 60 min, 1 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 2000 rpm for 10 min at room temperature. Finally, one ml of 0.67% 2-thiobarbituric acid (TBA) in 0.05 M NaOH was added to the supernatant, vortexed and heated in a water bath at 100°C for 20 min. After cooling, 1 ml of distilled water was added and absorbance was recorded at 532 nm. Control containing all reagents except the extract was kept; the assay was carried out in triplicate. Comparative model for this assay was Vitamin E. The percentage inhibition activity was calculated as: [(Abs. of control - Abs. of sample)/ Abs. of control] × 100 %.

Determination of Scavenging Activity against Hydrogen Peroxide

The radical scavenging activity against hydrogen peroxide of herbal extract was determined using the method of Ruch *et al.* [6]. Samples with various concentrations were added to 0.1 M phosphate buffer solution (pH 7.4, 3.4 ml), respectively, and mixed with 43 mM hydrogen peroxide solution (0.6 mL). After 10 min, the absorbance of reaction mixture was determined at 230 nm. The reaction mixture without sample was used as the control. Ascorbic acid was used as a standard reference compound. The percentage inhibition activity was calculated as: [(Abs. of control -Abs. of sample)/Abs. of control] x 100%.

Determination of Phenolic content

The total phenolic content was measured using Folin-Ciocalteau method Biglari *et al.*[7]. The samples (0.4 ml) (1 mg/ml extracts) were transferred into test tubes and distilled water (1.0 ml) and Folin-Ciocalteu reagent (1.0 ml) were added to this solution, and the tubes were shaken thoroughly. Sodium carbonate solution (Na_2CO_3, 1.6 mL, 7.5%) was added after 1 min, and the mixture was allowed to stand for 30 minutes with intermittent shaking. Absorbance was taken at 765 nm using UV spectrophotometer. The content of phenolic compounds of the samples was expressed as gallic acid equivalents (GAE) in mg per gram dry weight. The content of phenolic compounds in the extract was calculated using this formula: C = A/B; where C is expressed as mg GAE / g dry weight of the extract; A is the equivalent concentration of Gallic acid established from calibration curve (mg); and B is the dry weight of the extract (g).

Determination of Flavonoids Content

The total flavonoid content of the extract was determined in accordance with the method ascribed by Nabavi *et al.* [8]. The sample solutions (0.5 ml) were mixed with distilled water (2 ml) then add 5% $NaNO_2$ solution (0.15 ml). After 6 min of incubation, 10% $A1C1_3$ solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 4% NaOH solution (2 ml) to the mixture. Subsequently, add water to the sample to bring the final volume to 5 ml and the mixture was shaken thoroughly and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm. Total flavonoid content was expressed in mg of catechin per gram of extract.

3. Statistical analysis

The obtained results were expressed as Mean ± SE. Data were evaluated statistically with computerized SPSS package program (SPSS 9.00 software) using one-way analysis of variance (ANOVA). Significant differences among means were estimated at $P < 0.05$ according to Snedecor and Cochran [9].

4. Results and discussion

FRAP assay analyses the antioxidant activity of the extract by determining its ability to reduce ferric (III) iron to ferrous (II) iron. Reducing power is directly correlated with antioxidant activity and may serve as a significant reflection of the antioxidant capacity Oktay *et al.* [10]

The standard curve was generated the results were expressed as mmol ferrous ion equivalent per gram of extracts dry weight ($y = 0.646x + 0.042$, $R^2 = 0.942$). The FRAP shown by the ethanolic extract of *Ficus racemosa* was approximately 1.78-fold higher (0.739 ± 0.034 mmol Fe (II)/g) in Table.1 in comparison with ascorbic acid used as standard reference (0.413 + 0.019 mmol Fe (II)/g). Benzie and Szeto [11]; Gao *et al.* [12] and Zhu *et al.* [13] opinionated similar relations between iron (III) reducing activity and total phenolic content and FRAP assay in the literature. This is also in accordance with

the results produced by Zheng *et al.* [14] regarding the reducing potential of the antioxidant. Khan [15] studied the reducing ability of the leaf extract of *Ficus racemosa* and suggested the high reducing power of extract in a dose dependent manner. And the present analysis also revealed synchronized results with other authors.

Table 1: *In vitro* assay for antioxidant potential of *Ficus racemosa* leaves extracts (Mean ± S.E.)

Extracts	FRAP (mmol Fe II/ g)	H_2O_2 (%inhibition)	TBARS (%inhibition)	Total Phenol (GAE/ g)	Total Flavonoid(mg catechin/g)
Ficus racemosa	0.739[a]	51.28±0.91[a]	61.46±0.92[a]	39.03±0.92	5.126± 0.67
Standard (AsA)	0.413	49.2±0.95		-	-
Standard (Vit E)	-	-	40.64±0.57	-	-

a = statistically significant difference (P<0.05), when compared with the respective standards.

Property of bioactive compounds to inhibit the formation of 2- thiobarbituric acid reactive species (TBARS) by scavenging hydroxyl radicals generated by ascorbic-iron III at low concentration directly express their potency as an antioxidant. The current study indicates the protection of phospholipids by scavenging hydroxyl radicals before they reacted with the susceptible components within the lipid matrix, as the relative antioxidant ability of *F. racemosa* to scavenge the free radicals is more than the standard antioxidant i.e. Vitamin E. *Ficus* extract has shown high inhibition of TBARS (61.46±0.92 %) as compared to the comparative standard i.e. vitamin E (40.64±0.57%) which makes it a potent antioxidant containing plant. Similar observations were also reported in relation to the high inhibition of TBARS by Channabasavaraj *et al.* [16] in *Ficus glomerata* bark.

Manian *et al.* [17] revealed high free radical scavenging by the *Ficus racemosa* bark extract and *Camellia sinensis* tea. Sultana *et al*., 2013 also investigated the antioxidant activity of *F. racemosa* leaves by estimating the DPPH radical scavenging activity and reported higher hydrogen donating capacity for DPPH assay, these are in close agreement with our finding related to the hydrogen peroxide scavenging activity of the ethanolic extract of *Ficus racemosa* leaves (51.28±0.91) which was comparable with the standard ascorbic acid (49.2±0.95), as we are well versed that hydrogen peroxide is involved in the inactivation of different enzymes by the oxidation of essential thiol groups and our study is in a strong accord that *Ficus* leaf extract was able to actively quench H_2O_2 in a concentration-dependent manner.

Ficus racemosa has been konwn to contain tannins, kaempferol, rutin, arabinose, flavonoids, ficusin, coumarin and phenolic glycosides (Baruah and Gohain [18] Total phenol, as determined by the Folin Ciocalteu method, was expressed as gallic acid equivalents with respect to the reference gallic acid standard curve ($y = 0.150x – 0.005$ and $R^2 = 0.976$). In the present study the total phenolic content in the *Ficus racemosa* was 39.03±0.92 which is in accord to the similar study done by Manian *et al.* [17] who estimated the phenolic contents of ethanolic extract of *Ficus racemosa* bark and leaves but reported a high content of total phenols in extract of *Ficus racemosa* bark (51.5±0.2 mg GAE/ g). Likewise in our study there is a strong positive correlation R^2 =0.976, several other investigations established a linear correlation between the total phenolic content and the antioxidant capacity which means dominant the content of phenolics, higher the antioxidant capacity [2, 19] whereas, some studies attributed that there is no correlation between the total phenol content and antoxidant activity [20] which may be due to the presence of highly potent phytochemicals in some plants which in small quantity also can produce high antioxidative potential.

Phenols and polyphenolic compounds, such as flavonoids, tannins etc. are widely found in food products, derived from plant sources which showed significant antioxidant activities [21]. Flavonoids are also useful to prevent and treat cardiovascular disease, neurodegenerative problems, and cancer. Total flavonoid content was expressed as mg of catechin equivalent per gram with reference to standard curve ($y = 2.203x + 0.427$ and $R^2 = 0.987$). The value of total flavonoid (5.126± 0.67) in the present study was expressing significant efficacy and a positive correlation $R^2 = 0.987$ stipulated for an active antioxidant.

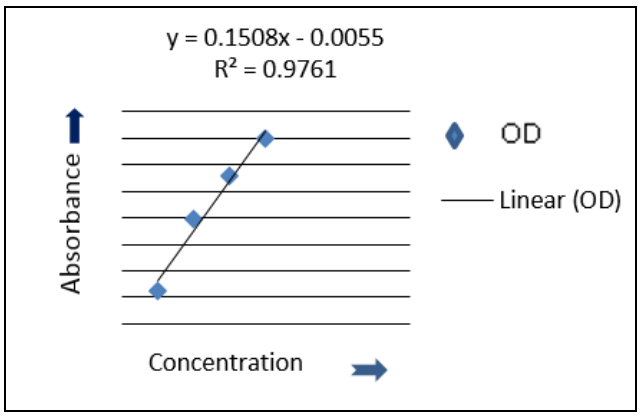

Fig 1: Standard curve for Total phenol *in vitro* antioxidant assay.

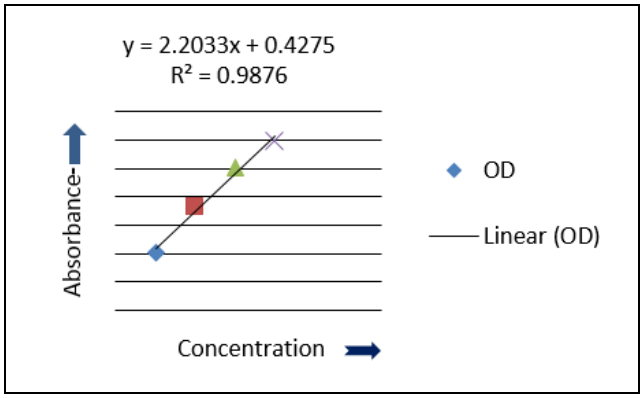

Fig 2: Standard curve for Flavonoids *in vitro* antioxidant assay.

Conclusion

The ethanolic extract of the leaves of *Ficus racemosa* revealed significant antioxidant activity with presence of elaborated amount of total phenol and flavonoids. A positive linear correlation between the phenolic content and the antioxidant activity is established. *Ficus racemosa* may be tested as an adjunctive therapy with other chemotherapeutic agents to extrapolate the additional beneficial effects. Futhermore, it should be kept in mind that there is an urgent need of validation for the exploration of ethno veterinary

practices as this traditional knowledge can deliver much to the frontier of veterinary medicine.

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