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Phytochemical Investigation and Evaluation of *In Vitro* Antioxidant and Anti-Inflammatory Activity of *Ficus Recemosa* Fruit Extracts Using Different Solvents

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ABSTRACT

Ficus recemosa is an underutilized vegetable; however, it has many folk medicinal uses. Still there is a lack of systematic report on the phytochemicals present in this underutilized vegetable. The objectives of this study were to investigate the phytochemicals and to evaluate the *in vitro* antioxidant as well as anti-inflammatory activity of *Ficus recemosa* fruit extracts using five different solvents. Five different extracts (in methanol, ethanol, chloroform, n-hexane and petroleum ether) of mature *Ficus racemosa* fruits were prepared by employing maceration process. Both qualitative and quantitative analyses of phytochemicals in the extracts were performed. Several *in vitro* assay methods were used to investigate the antioxidant and anti-inflammatory activity. Qualitative test detected the presence of alkaloids, flavonoids, flavonols, resins, saponins, proanthocyanidins, coumarins, steroids and tannins in the extracts. Quantitative analysis demonstrated the highest content of polyphenols such as phenolics (56.90±1.37 mg gallic acid/ g dry weight), flavonoids (32.33±2.60 mg of catechin/ g dry weight), flavonols (4.21±.19 mg of quercetin/ g dry weight) and proanthocyanidins (12.59±.39 mg catechin/ g dry weight) in ethanolic extract. DPPH and Superoxide radical scavenging assays of methanolic extract showed the strongest antioxidant activity (IC_{50} values are 42.80±1.78 and 72.95±4.10 µg/ml, respectively) than those of other extracts, whereas, ABTS and nitric oxide scavenging assays of ethanolic extract showed the highest activity with the IC_{50} value of 65.15±3.4 and 140.3±2.35 µg/ml, respectively. In egg albumin and BSA denaturation assay ethanolic extract was found to be the most effective. Extracts of *Ficus racemosa* fruits possesses a wide range of pharmacologically important phytochemicals which exhibited strong antioxidant and anti-inflammatory activity.

Keywords: Antioxidant, Anti-inflammatory activity, *Ficus racemosa* fruits extract, Phytochemicals.

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INTRODUCTION

Over production of reactive oxygen species (ROS) leads to damage of proteins, DNA and lipid, and is associated with the chronic degenerative diseases including coronary artery disease, hypertension, cancer and diabetes etc. [1, 2, 3]. Antioxidant compounds have the ability to scavenge free radicals as well as to delay or prevent oxidative stress [1, 4]. Enzymes such as catalase, superoxide dismutase, peroxidase glutathione etc. of endogenous defense system are not enough to scavenge reactive oxygen species; hence exogenous anti-oxidants are required [5]. Food industries use several synthetic antioxidants which may be responsible for liver damage and carcinogenesis [6, 7]. So interest in the use of natural antioxidants has increased. Medicinal plants play important role in disease prevention or control due to the presence of antioxidant rich constituents such as phenolic acids, flavonoids, vitamins, terpenoids, alkaloids, stilbenes, tannins, quinones, coumarins etc. [8].

Ficus racemosa, popularly known as the cluster fig or goolar fig, is a plant species in the *Moraceae* family which is native to India, Australia, and Southeast Asia [9]. Many studies have reported that *Ficus racemosa* fruits possesses antiulcer, hypoglycemic and antioxidant activities and are a good antidote for visceral obstruction and also useful in regulating diarrhea and constipation [10, 11]. It was also found that a uterine tonic prepared using the aqueous extract of fruits shows effect similar to oxytocin [12]. Anti-inflammatory activity of stem bark and leaf extracts of *Ficus racemosa* has been found in some studies but a very few study was done with fruit extracts [13, 14]. Moreover, antioxidant and anti- inflammatory activity of *Ficus racemosa* fruit extracts in different solvents is yet to be investigated. Hence, the intent of the present study was to investigate the phytochemical components of *Ficus recemosa* fruit extracts using five different solvent systems (i.e., ethanol, methanol, chloroform, n-hexane and petroleum ether) and to evaluate their antioxidant and anti-inflammatory activity.

MATERIALS AND METHOD

Collection of plant material and authentication

Fruits of *Ficus recemosa* were collected from local area near the University of Rajshahi (north-western part of Bangladesh) and authenticated by the Department of Botany, University of Rajshahi, Bangladesh. The fruits were first washed with tap water to remove adhering filth and then chopped into small pieces and shed dried. After complete drying, the entire portions were grinded into a coarse powder by a grinding machine and stored in an airtight container for further use.

Chemicals

Folin–Ciocalteu, gallic acid, quercetin, butylhydroxytoluene (BHT), 2,2´-diphenyl-1-picrylhydrazyl (DPPH), ABTS [2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)], potassium persulfate were purchased from Sigma–Aldrich (USA). Methanol, ethanol, chloroform, n-hexane, petroleum ether and hydrochloric acids, sodium carbonate, NBT (Nitro-blue tetrazolium), hydroxylamine hydrochloride, EDTA, ferric chloride, ascorbic acid, Potassium ferricyanide, aluminum trichloride, dimethyl sulfoxide (DMSO) were obtained from Merck (Germany) and Trichloro acetic acid (TCA) from Loba chemicals (India).

Preparation of plant extracts

Powdered plant material (25 g) was taken in a conical flask and extracted with five different organic solvents (250 mL) named ethanol, methanol, chloroform, n-hexane and petroleum ether in a mechanical shaker with temperature control (Room temperature) at constant stirring at 200 rpm. It was left for 48 hours and solids were filtered using Whatman No. 1 filter paper. The whole process was repeated three times for complete extraction. Afterwards, the solvents were evaporated under reduced pressure at 40 ºC using rotary evaporator and the residues stored in small sterile vials under refrigerated conditions until used.

Phytochemical analysis

Qualitative analysis

Qualitative tests for phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resins, saponins, steroids and tannins were carried out by dissolving samples in specific reagents using standard methods [15, 16].

Determination of total phenolics content

Total phenolic contents in each extract were determined by the Folin-Ciocalteu method [17], where gallic acid was used as a standard. Briefly, 300 µl of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent, diluted (1:10) in distilled water and allowed to stand for 5 min at room temperature; and then 2.25 ml of sodium carbonate (60 g/l) solution was added to the mixture. After 90 min incubation at room temperature, the absorbance was measured at 725 nm using a spectrophotometer. The total phenolic content of the extracts was calculated and expressed as gallic acid equivalents per gram of sample dry weight (mg GAE/g DW).

Determination of total flavonoids content

Total flavonoid contents were determined using the method described by Abu Bakar et al. [17] and Catechin was used as a standard. Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15 ml of 5% $NaNO_2$ solution and kept at room temperature for 6 min. After that, 0.3 ml of a 10% AlCl3.6H2O solution was added and allowed to stand for another 5 min before the addition of 1.0 ml of 1 M NaOH. The mixture was then mixed well using a vortex. The spectrophotometric reading was measured

immediately at 510 nm using a spectrophotometer. Results were calculated and expressed as catechin equivalents per gram of dried sample (mg CAE/g DW).

Determination of total flavonols content

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran [18]. An aliquote of 2.0 ml of extract/standard was taken in test tube to which 2.0 ml of 2% $AlCl_3$ (dissolved in ethanol) and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorbance at 440 nm was measured after 2.5 h at 20 °C. Total content of flavonols was expressed in terms of quercetin equivalent, mg of QUE/g of sample dry extract.

Determination of total proanthocyanidins

Estimation of proanthocyanidins content was carried out based on the method as described by Sun et al. [19]. A volume of 0.5 ml of 0.1 mg/mL of extracts/standard solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml of hydrochloric acid; the mixture was allowed to stand for 15 minutes. The absorbance was recorded at 500 nm and the result was expressed in terms of catechin equivalent, (mg of CAE/g of dry extract).

Determination of antioxidant activity

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) was determined by the method of Prieto et al. [20] with some modifications. Briefly, 0.5 ml of extract at different concentrations was mixed with reaction mixture (3 ml) containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate, and incubated at 95 °C for 10 min to complete the reaction. The spectrophotometric reading was taken at 695 nm against blank after cooling at room temperature. A standard graph of Catechin at various concentrations was also constructed in a similar manner for comparison.

Ferric reducing antioxidant power (FRAP) assay

The reducing power was measured following the method of Oyaizu (1986) with some modification [21, 22]. Briefly, an aliquot of 250 µL of extract with different concentrations were mixed with 1.75 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of potassium ferricyanide (1%). The mixture was then incubated at 50 °C for 20 min followed by the addition of 1 ml of TCA (10%). An aliquot (1 ml) from the incubation mixture was mixed in a test tube with 1 ml of distilled water and 0.2 ml of ferric chloride (0.1%). The absorbance of the resulting solution was measured at 700 nm after 10 min. Increased absorbance of the reaction mixture is the indication of increased reducing power [23]. A standard with ascorbic acid at various concentrations was also made in a similar manner for comparison.

DPPH free radical scavenging assay

The free radical scavenging activity of various extracts was measured *in vitro* by 2,2´-diphenyl-1-picrylhydrazyl (DPPH) assay according to the established method described earlier [24, 25]. Different concentrations of extracts were added at an equal volume (2 ml) to a 0.1 mM methanolic solution of DPPH. The mixture was kept at 25 ºC for half an h and then absorbance was measured at 517 nm. Ascorbic acid and BHT was used as positive control. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Scavenging Activity} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Where, $A_{control}$ = Absorbance of control, A_{sample} = Absorbance of sample.

Then percentage DPPH radical scavenging activity was plotted against concentration, and from the graph IC_{50} was calculated.

ABTS radical scavenging activity

The antioxidant capacity was determined in terms of ABTS radical scavenging activity following the method previously described [26]. ABTS radical was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12–16 h before use. ABTS radical solution (stable for 2 days) was diluted with water to obtain an absorbance of 0.70±0.02 at 734 nm. ABTS radical solution (3 ml) was added to 1 mL of test sample with various concentrations and mixed vigorously. The absorbance was measured at 734 nm after 6 min. Ascorbic acid and BHT was used as positive control.

The ABTS radical scavenging activity of the samples was expressed as

$$\% \text{ Scavenging Activity} = [(A_{control} - A_{sample}) / A_{control})] \times 100$$

Where, $A_{control}$ is the absorbance of the blank control (ABTS radical solution without test sample) and A_{sample} is the absorbance of the test sample.

Scavenging activity of Superoxide

This activity was measured using NBT (nitrobluetetrazolium reagent) method as described by Sabu and Ramadasan [27] with some modification. Test solutions of extract (20–300 µg/mL) were taken in a test tube. Then 1 mL of 5 mM sodium carbonate, 0.4 mL of 0.24 mM NBT and 0.2 mL of 0.1 mM EDTA solutions were added to the test tube and immediate reading was taken at 560 nm. About 0.4 mL of 1 mM of hydroxylamine hydrochloride was added to initiate the reaction then reaction mixture was incubated at 25 ^{0}C for 15 min and reduction of NBT was measured at 560 nm. Ascorbic acid and BHT was used as standard. Absorbance was recorded and the percentage of inhibition was calculated according to the following equation**:**

$$\% \text{ Scavenging Activity} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the initial reading of sample/standard and A_1 is the absorbance of final reading.

Scavenging activity of nitric oxide

The method of Garrat et al. [30] was used to determine the nitric oxide radical scavenging activity of plant extracts with some modification. A volume of 2 mL of 10 mM sodium nitroprusside prepared in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of plant extract at various concentrations. The mixture was incubated for 150 min at room temperature. After incubation, the solution was mixed with 0.5 mL of Griess reagent [1.0 mL sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid)] and kept at room temperature for 5 min. After 1 mL of naphthylethylenediamine dichloride (0.1% w/v)] was added to the mixture. The mixture was incubated at room temperature for 30 min, followed by the measurement of absorbance at 546 nm. Ascorbic acid and BHT was used as positive control.

The NO radical-scavenging activity of the samples was expressed as

$$S\% = [(A_{control} - A_{sample})/A_{control})] \times 100$$

Where, $A_{control}$ is the absorbance of the blank control (NO radical solution without test sample) and A_{sample} is the absorbance of the test sample.

Determination of in-vitro anti-inflammatory activity

Egg albumin denaturation assay

The egg albumin denaturation assay was carried out according to the method previously described [28]. Briefly, the reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of extract so that final concentrations become 100, 200, 400, 600 and 1000 µg/mL. Double-distilled water was used as control. Then the mixtures were incubated at (37±2) ˚C in for 15 min and then heated at 70 ˚C for 5 min. After cooling, absorbance was measured at 660 nm by using vehicle as blank. Acetyl salicylic acid (ASA) was used as reference drug and treated similarly for determination of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage Inhibition (\%)} = [(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$$

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

BSA denaturation assay

The protein denaturation assay was determined using a modified method as described by Murugan and Parimelazhagan [29]. In short, 0.45 mL of bovine serum albumin (5% aqueous

solution) and 0.05 mL of distilled water were mixed to obtain 0.5 mL of reaction mixture. The pH was adjusted to 6.3 using a small amount of 1N HCL. An aliquot of 1 mL of extract with different concentrations was added to the reaction mixture followed by incubation at 37 ºC for 30 min. Then the mixture was heated at 57 ºC for 5 min. After cooling, 2.5 mL of phosphate buffer solution (pH 6.4) was added and absorbance was measured at 660 nm. A mixture of 0.05 mL of distilled water and 0.45 mL of bovine serum albumin was used as negative control. Acetyl salicylic acid was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition was determined by using the following formula:

Percentage Inhibition (%) = $[(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$

The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Statistical analysis

All experiments were done in triplicate and the results were represented as mean ± standard deviation (SD). SPSS software (version 16) was used to evaluate the significant relationships between experimental parameters and IC_{50} was calculated using Graph Pad Prism 6.

RESULTS

Phytochemical screening

Data of qualitative phytochemical investigation of five different extracts (namely EEFR, MEFR, CEFR, NEFR and PERF) of *F. racemosa* fruits are shown in the Table 1. The results demonstrated the presence of alkaloids (in MEFR and CEFR), carbohydrates (in EEFR, MEFR and PEFR), flavonoids, Glycosides (in EEFR, MEFR and PEFR), triterpenoids (in all except CEFR), resins (in all five extracts), saponins (in EEFR and NEFR), steroids (in all five extracts) and tannins (in EEFR,MEFR and PEFR).

Table 1: Qualitative test for phytochemicals of five different extracts of *F. racemosa*

Phytochemicals	Name of Test	Test sample				
		EEFR	MEFR	CEFR	NEFR	PEFR
	Dragendorff's test	-	+	-	-	-
Alkaloids	Hager's test	-	-	+	-	-
	Wagner's test	+	-	-	-	-
	Mayer's test	-	-	-	-	-
	Molisch's test	+	+	-	-	+
Carbohydrates	Benedict's test	+	+	-	-	-
	Fehling's test	+	+	-	-	-
	Anthrone test	+	+	-	-	+
Flavonoids	Shinoda's test	+	+	+	+	+
Glycosides	Molisch's test	+	+	-	-	+
Triterpenoids	Liebermann-Burchard's test	+	+	-	+	+
Resins		+	+	+	+	+

Saponins		+	-	-	+	-
Steroids	Liebermann-Burchard's test	+	+	+	+	+
	Salkowski reaction	+	+	+	+	+
Tannins		+	+	-	-	+

Note: '+' indicates the presence and '–' the absence of respective phytoconstituent.

Total Phenolic, Flavonoid, Flavonol and Proanthocyanidin contents

Table 2 showed the contents of total phenolics, flavonoids, flavonols and proanthocyanidins in different extracts of *F. racemosa*. All the five extracts of *Ficus racemosa* were standardized for their phytochemical contents. Phenolic, flavonoids, flavonols and proanthocyanidins contents of extracts ranged from 10.75±1.24 to 56.90±1.37 (mg of GAE/g of dry extract), 6.35±0.97 to 32.33±2.60 (mg of CAE/g of dry extract), 1.786 ±.411 to 5.409±.237 (mg of QUE/g of dry extract) and 3.754±.568 to 12.595±.394 (mg of CAE/g of dry extract), respectively. The highest content of phenolic, flavonoids and proanthocyanidins was found in ethanolic extract (EEFR), whereas that of flavonols was in methanolic extract.

Table 2: Total Phenolics, Flavonoids, Flavonols and Proanthocyanidins contents of *F. racemosa*

Name of extract	Phenolics (mg of GAE/g of DW)	Flavonoids (mg of CAE/g of DW)	Flavonols (mg of QUE/g of DW)	Proanthocyanidines (mg of CAE/g of DW)
MEFR	46.21±1.73	26.41±1.57	5.409±.237	10.468±.465
EEFR	56.90±1.37	32.33±2.60	4.212±.196	12.595±.394
CEFR	16.41±1.22	9.87±2.25	2.952±.196	6±.375
NEFR	10.75±1.24	17.64±1.15	1.786±.411	3.754±.568
PEFR	19.67±0.98	6.35±0.97	4.338±.250	7.205±.394

Results are expressed as mean ± standard deviation. DW: Dry weight of extract.

Antioxidant activity

The total antioxidant capacity (TAC) of different extracts of *Ficus racemosa* and Catechin (standard) is shown in Figure 1. The extracts demonstrated increased total antioxidant capacity with the increasing concentration of the extracts. All the five extracts strongly reduced Mo (VI) to Mo (V) which is comparable to standard, Catechin. Among these five extracts, EEFR exhibited higher activity and NEFR showed the lowest activity.

The Ferric Reducing Antioxidant Power (FRAP) of different extracts of *Ficus racemosa* and Ascorbic acid (standard) is shown in Figure 2. Each extract was found to demonstrate higher reducing potential with the increasing concentration of the extracts among which methanol extract (MEFR) showed the highest activity.

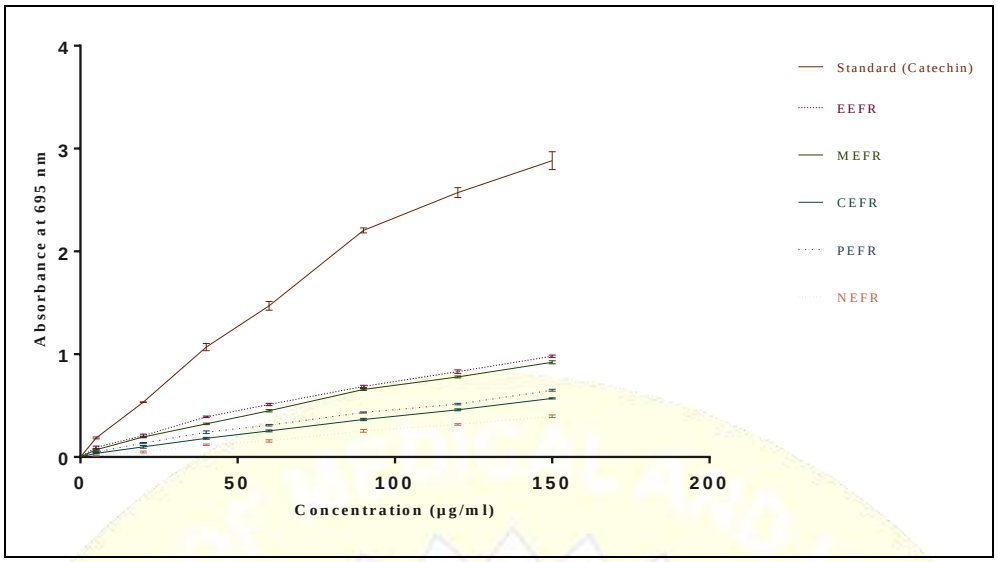

Figure 1: Total antioxidant capacity of different extracts of *Ficus racemosa*

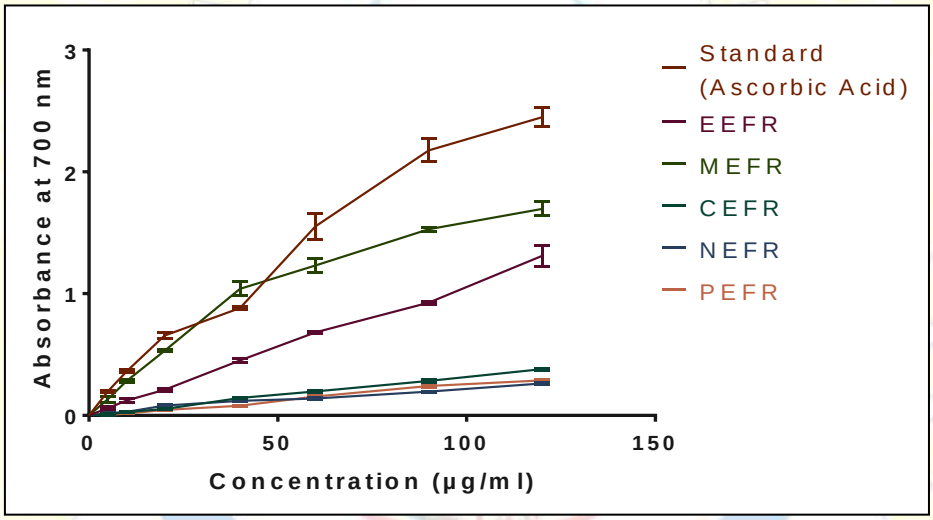

Figure 2: Ferric Reducing Antioxidant Power of different extracts of *Ficus racemosa*

In a concentration dependent manner, DPPH, ABTS, Superoxide and Nitric Oxide (NO) scavenging activity of five different solvent extracts of *F. racemosa* fruits and standards were determined and are presented in Table 3. It is evident from the Figure that the extracts reduced these radicals more with increasing concentrations. Lower values of IC_{50} indicated stronger scavenging capacity.

Table 3: IC_{50} values of *F. racemosa* extracts of different antioxidant assays

Extract Name	IC_{50} (µg/mL)			
	DPPH	**ABTS**	**Superoxide**	**Nitric Oxide**
MEFR	42.80±1.78	67.00±1.39	72.95±1.89	207.5±0.99
EEFR	47.08±1.57	65.15±1.41	99.04±1.64	140.3±2.35
CEFR	372±3.01	415.20±3.56	364.3±2.52	229.2±1.20
NEFR	318.3±2.85	264.5±2.14	ND	506.9±4.01
PEFR	216±2.46	539.3±3.97	93.38±2.23	283.6±1.94
Standard				
Ascorbic Acid	41.86±1.17	54.42±1.08	70.91±1.76	91.70±1.82
BHT	69.15±1.86	78.23±1.39	94.65±1.87	132.67±2.96

BHT: Butylhydroxytoluene. NA: Not tested.

In DPPH and Superoxide scavenging assays, the methanol extract (MEFR) exhibited the highest scavenging activity with IC_{50} values of 42.80±1.78 (µg/mL) and 72.95±1.89 (µg/mL), respectively. In these assays, the order of scavenging activity were MEFR>EEFR>PEFR>NEFR>CEFR (in DPPH scavenging assay) and MEFR>PEFR>EEFR>CEFR (in superoxide scavenging assay). The methanol and ethanol extracts of *F. racemosa* were found as potent DPPH radical scavengers which were comparable to reference drugs (Ascorbic acid and BHT). On the other hand, methanol, ethanol and petroleum ether extracts of *F. racemosa* were found as potent superoxide radical scavengers as standard reference drugs. But, in ABTS and NO scavenging assays, ethanol extract (EEFR) showed the highest scavenging activity with IC_{50} values of 65.15±1.41 (µg/mL) and 140.3±2.35 (µg/mL), respectively. Strong correlation ($p \leq 0.05$) was found between the phenolic content and the radical scavenging activity which are shown in Table 4.

Table 4: Correlations between the antioxidative activities and total phenolic contents of the different extracts of *F. racemosa*

Assay	Correlation coefficient (R^2)				
	MEFR	**EEFR**	**CEFR**	**NEFR**	**PEFR**
DPPH	0.862*	0.860*	0.985**	0.933**	0.953**
ABTS	0.772*	0.827*	0.922**	0.929**	0.991**
Superoxide	0.893*	0.857*	0.882*	NA	0.852*
NO	0.925**	0.886*	0.871*	0.877*	0.870*

*Correlation is significant at the 0.05level, ** correlation is significant at the 0.01 level; NA-Not tested

Anti-inflammatory activity

The data on anti-inflammatory activity tests are presented in Table 5. In both egg albumin and BSA denaturation assay, each extract of *Ficus racemosa* demonstrated increased percentage of inhibition of denaturation with the increasing concentration of the extracts. EEFR exhibited the highest activity with the percentage inhibition of 22.98±1.61, 33.73±1.75, 42.20±1.09, 60.10±2.04 and 73.77±2.37 at concentrations of 100, 200, 400, 600 and 1000 µg/ml in egg albumin denaturation assay, whereas at the same concentrations the percentage inhibition in BSA denaturation assay were 26.54±1.24, 37.13±1.64, 46.73±1.87, 62.42±1.72 and 76.40±1.75.

Table 5: Anti-inflammatory activity of different extracts of *F. racemosa* and Acetyl salicylic acid

Concentration (µg/ml)	% inhibition of denaturation					
Egg Albumin denaturation method						
	MEFR	**EEFR**	**CEFR**	**NEFR**	**PEFR**	**ASA**
100	19.56±0.56	22.98±1.61	10.12±0.76	11.23±1.09	15.39±0.28	26.71±1.68
200	27.98±0.89	33.73±1.75	17.36±1.35	20.66±0.88	21.76±0.90	38.97±1.97
400	41.73±1.88	42.20±1.09	31.57±1.87	27.03±1.32	29.45±1.03	59.33±2.04
600	58.20±1.47	60.10±2.04	38.46±1.67	41.73±2.01	49.71±1.58	68.91±1.60
1000	68.43±2.08	73.77±2.37	43.05±1.88	53.21±1.64	60.43±1.85	79.87±2.34
BSA denaturation method						
100	24.51±1.23	26.54±1.24	11.56±0.73	13.61±0.64	18.56±1.03	28.69±1.32
200	33.47±1.57	37.13±1.64	19.32±0.91	21.98±1.35	25.56±1.25	39.15±1.58
400	45.72±1.64	46.73±1.87	32.65±1.32	30.25±2.02	35.44±1.74	61.07±2.14
600	60.42±1.98	62.42±1.72	43.47±1.21	44.88±1.63	52.64±1.72	72.64±1.73
1000	71.43±2.04	76.40±1.75	52.06±1.54	57.33±1.87	63.12±1.47	83.45±2.03

ASA: Acetyl salicylic acid;

DISCUSSION

Since ancient time, plants have been considered as an important source of biologically active substances. Phenolics, flavonoids, flavonols and proanthocyanidins are some of the important bioactive phytochemicals [31] found in plant. This study revealed the presence of different bioactive phytochemicals in *Ficus racemosa* (Fig).

Phytochemicals are the chemical constituents in plants with diverse biological activities such as anti-inflammatory, antioxidant, anticancer, and antimicrobial properties. Phenolics and flavonoids are the most common antioxidants known in plants [32]. Moreover, Proanthocyanidins play preventive role in various diseases, like atherosclerosis, gastric ulcer, large bowel cancer, cataracts and diabetes. Flavonols also offer significant cardiovascular health benefits [31].

Previous studies have shown that fig is rich in polyphenol compounds. In this study, appreciable amount of total phenolic, flavonoid, flavonol and proanthocyanidin were detected. The results were supported by a previous study carried out with leaves and barks of *Ficus racemosa* plant [33].

A few studies reported that the differences in polyphenol content could be attributable to biological factors (genotype, cultivars), as well as environmental conditions (temperature, salinity, water stress and light intensity). Moreover, the extraction of phenolic compounds depends on the type of solvent used, the degree of polymerization of phenolics, and their interaction [34, 35, 36]. In this study, it was confirmed that among all the employed organic solvents, ethanol was the most effective solvent for the extraction of polyphenol from fig.

Recently, polyphenols have attracted particular attention due to their potentiality to reduce free radical induced tissue injury. Numerous studies have demonstrated that plant polyphenols are effective scavengers of free radicals and reactive oxygen species [31]. This study has followed several *in vitro* assay methods to evaluate the antioxidant activity of fig extracts. The total antioxidant capacity (TAC) assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acidic pH. Total antioxidant capacity was performed on the basis of the phosphomolybdenum method which evaluates both water-soluble and fat-soluble antioxidant capacity. In ferric reducing antioxidant power assay (FRAP), ferric-ferricyanide complex is reduced to the ferrous form due to the presence of antioxidants in the plant extracts [37]. In both TAC and FRAP assay, each extract showed increasing activity with the increase of concentration. In TAC assay ethanol extract of *Ficus racemosa* (EEFR) showed higher antioxidant activity than other extracts, whereas methanol extract (MEFR) represented higher activity in FRAP assay.

DPPH, a stable free radical, is widely used to study radical scavenging activities of extracts and pure compounds due to its property of delocalization of the spare electron on the whole molecule. When the odd electron becomes paired off in the presence of a free radical scavenger to form hydrazine, the absorption reduces and the DPPH solution is decolorized from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract [38]. The ABTS radical is also commonly used to measure the radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plants extracts [39.] Active free radicals such as hydrogen peroxide, hydroxyl radical and singlet oxygen can be generated from superoxide anions. Although hydrogen peroxide is not very toxic to cell but it may give rise to hydroxyl radical which have the ability to interact with DNA directly and therefore contribute to cancer development, ageing and cytotoxicity. Injurious NO is also generated in biological tissues by specific nitric oxide synthases [31, 40].

In this study, all of the extracts showed strong radical scavenging activity in which the methanol extract (MEFR) exhibited the higher scavenging activity in DPPH and Superoxide scavenging assays, whereas ethanol extract (EEFR) showed the highest scavenging activity in ABTS and NO scavenging assays. By scavenging free radical antioxidants play a preventive role in different diseases including cancer [31, 38, 40]. These results were found in agreement with those reported by previous studies [41, 42].

It has been reported that protein denaturation is one of the causes of rheumatic arthritis. Several anti-inflammatory drugs have been reported to inhibit thermally induced protein

denaturation at a dose dependent manner [43]. In this study, each extract showed the considerable anti-inflammatory activity which is comparable to standard ASA.

CONCLUSION

The study concluded that *F. racemosa* possesses strong antioxidant and anti-inflammatory activity, and can be a promising source to discover drug for the treatment of various human diseases. Further investigation is required to identify and characterize the active compounds present in the extract.

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