

Antioxidant and Immunomodulatory Activity of Hydroalcoholic Extract and its Fractions of Leaves of *Ficus benghalensis* Linn.

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ABSTRACT

Background: *Ficus benghalensis* is a folk medicine indigenous plant of India. Several studies on this plant reported and focused on the biological profile of the plant. **Objectives:** This study is aimed to evaluate the antioxidant and immunomodulatory activity of *F. benghalensis* leaf extract using various *in vitro* screening methods of both parameters. **Materials and Methods:** Hydroalcoholic (FB1) extract and its four fractions viz. n-hexane (FB2), n-butanol (FB3), chloroform (FB4), and water (FB5) of leaves of *F. benghalensis* investigated for their free radical scavenging activity using 1-1-diphenyl-2-picrylhydrazyl and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radicals. A dose-response curve was plotted and IC_{50} values were determined to assess antioxidant activity. Nitroblue tetrazolium test, phagocytosis of killed *Candida albicans* and candidacidal assay were carried out to assess the immunomodulatory activity. Positive non-lymphoid cell number, mean particle number of killed *C. albicans*, percent value of killed *C. albicans* by neutrophils were calculated and presented. **Results:** All extracts showed antioxidant and prominent immunomodulatory activity with compared to standard. **Conclusions:** Hydroalcoholic (FB1) extract and its four fractions viz. n-hexane (FB2), n-butanol (FB3), chloroform (FB4), and water (FB5) showed promising antioxidant and immunomodulatory activity.

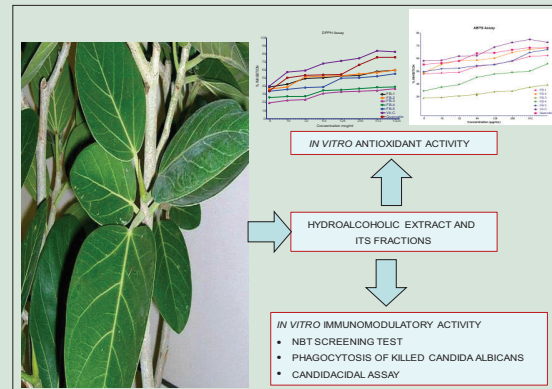
Key words: Antioxidant, *Candida albicans*, *Ficus benghalensis*, Free radicals, Immunomodulatory

SUMMARY

- Hydroalcoholic extract and its fractions of *F. benghalensis* Linn exhibited different DPPH and ABTS scavenging activity in concentration dependent manner.
- The extract, fractions and reference antioxidants showed DPPH scavenging effect in the order of Vit-C > Quercetin > FB2 > FB1 > FB5 > FB4 > FB3 and

ABTS scavenging effect in the order of Vit-C > Quercetin > FB1 > FB2 > FB5 > FB3 > FB4.

- FB2 and FB3 showed promising immunomodulatory activity at all concentrations.



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INTRODUCTION

Immune system dysfunction is responsible for various diseases such as arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer, and infectious diseases.^[1] The degree to which the patient becomes abnormally susceptible to infections by the microbial environment depends on the extent of immunosuppression. The suppression of the immune system is characterized by a reduction in the number and phagocytic function of the neutrophils and macrophages, as well as an impairment of the intracellular bactericidal capacity of these cells. This immunosuppression allows opportunistic pathogens to overwhelm the host to cause secondary infections.^[2] This problem can be overcome by boosting the immune system by the use of immunomodulatory drugs.^[3] Immunomodulators improve the host defense mechanism.

According to Ayurveda, rasayana, is the drug used for enhancement of body resistance against infection acts as an adaptogen, immunomodulatory, and antimutagenic.^[4,5] Chemotherapeutic agents available today have mainly immunosuppressive activity. Most of them are cytotoxic and exert a variety of side effects. This has given rise to stimulation in the search for investigating natural resources showing immunomodulatory activity. Many plants used in traditional medicine were found to have immunomodulatory properties it could be because of effects of phytoconstituents such as phenolics, terpenoids,

steroids, flavonoids, etc. The endogenous antioxidant system prevents the deleterious influence of the free radical on the immune cells and preserves their normal function. Impairment in the immune system leads to overutilization of endogenous antioxidant.^[4,5]

Many *Ficus* species have long been used in folk medicine as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics, and anti-dysentery drugs.^[6] It is believed that some *Ficus* species can be used as a remedy for visceral obstructive disorders, diabetes, leprosy, respiratory disorders and certain skin diseases^[7] and as an absorbent for inflammatory swellings and burns.^[8] *Ficus benghalensis* belongs to the family *Moraceae*, which is commonly known as Banyan tree. In earlier study glucoside, 20-tetratriacontene-2-one,

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6-heptatriacontene-10-one, pentatriacontan-5-one, beta-sitostirrolalpha-D-glucose, and meso-inositol have been isolated from the bark of *F. benghalensis*.^[8] Leaves contain crude protein 9.63%, crude fibres-26.84%, CaO-2.53%, and phosphorus-0.4%. It yields latex containing caoytchoue (2.4%), resin, albumin, cerin, sugar, and malic acid. It is used in Ayurveda for the treatment of diarrhea, dysentery and piles,^[9,10] teeth disorders,^[11] rheumatism, skin disorders such as sores,^[12] to boost immune system,^[13] and as a hypoglycemic.^[14-17] The extracts of *F. benghalensis* were also reported to inhibit insulinase activity from liver and kidney,^[18] fruit extracts exhibited anti-tumor activity in the potato disc bioassay.^[19] Two flavonoid compounds, viz. 5, 7-dimethyl ether of leucopelargonidin 3-0-alpha-Lrhamnoside and 5, 3'-dimethyl ether of leucocyanidin 3-0-alpha-D galactosyl cellobioside were obtained from the bark of *F. benghalensis* and were evaluated for anti-oxidant activity in hyperlipidemic rats.^[20] It was also found to inhibit the lipid peroxidation.^[21] Various extracts of *F. benghalensis* was screened for its anti-allergic and anti-stress potential in asthma by milk induced leukocytosis and milk induced eosinophilia.^[22,23] In Ayurveda, *F. benghalensis* has been reported for their skin disorder, rhumatits, diabetes, antiseptic, aphrodisiac, hemostatic, nerve disorder, and menorrhagia. This plant is used throughout India for the purpose of complication related to blood, bone, endocrine system, toothache, gastric disorder, reproductive disorder, urinary disorder, and fever.

F. benghalensis is a holy plant largely found in the region of the forest of Deccan, and Southern India. It is also known for the tribal area of Maharashtra for their holiness and medical uses. This is evergreen plant having large source of leaves; Nalpamaram is an important group of Ayurvedic formulation that constitutes bark of *F. benghalensis* used in the treatment of skin disease and other ailments. In view of the medical importance and literature, the present study is aimed to evaluate *F. benghalensis* leaves for antioxidant and immunomodulatory activity.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *F. benghalensis* Linn. were collected from the Western Ghat of Belgaum region in Karnataka. The plant was identified and authenticated by Regional Medical Research Center (RMRC), Belgaum. The herbarium was prepared and deposited at RMRC, Belgaum, Karnataka with voucher specimen No. RMRC-918.

Preparation of extracts

Hydroalcoholic extract of leaves of *F. benghalensis* Linn. was prepared from 500 g of leaf powder by maceration at room temperature using 2000 mL 50% v/v ethanol for 7 days with occasionally shaking. The extract was evaporated and concentrated by using rotary vacuum evaporator (IKA'RV 10 digital) to get semisolid mass further drying was done by vacuum oven.

Semisolid extract was further successively extracted with n-hexane, n-butanol, chloroform and water. All extracts were stored in desiccator until further use.

Chemicals and reagents

All the chemicals and solvents are of analytical grade and obtained from Qualigens and Sigma-Aldrich. 1-1-diphneyl-2-picrylhydrazyl (DPPH) radical, 2, 2'azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical, potassium persulfate, nitroblue tetrazolium (NBT), *Candida albicans* suspension, hanks balanced salt solution, sodium deoxy cholate, methylene blue solution, supplemented minimum essential media, and wright stain.

Antioxidant activity

Antioxidant property of the extracts and fractions of leaves of *F. benghalensis* Linn. was investigated by using DPPH and ABTS radical scavenging activity.

1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity

A solution of DPPH was prepared by dissolving 25 mg of DPPH in 10 mL of methanol and the solution kept in the dark at 4°C. Stock solution of extract and fractions of concentration 1,000 µg/mL were prepared. A volume of 100 µL of stock solution (1,000 µg/mL) was added to the well and it was further down diluted to the lowest concentration (7.8 µg/mL) by performing a serial 2-fold dilution in a 96-well microtiter plate. A row of negative DPPH control was developed in the same 96-well microtiter plates by adding 100 µL of methanol into each well. Quercetin and Vitamin C were used as positive controls. Five microliters of methanolic DPPH were added into each well and reaction was allowed to proceed for 30 min in the dark. The absorbance was measured at 492 nm by a microplate reader.^[24] The DPPH radical scavenging effect (%) was calculated using the formula:

$$\text{DPPH radical Scavenging effect (\%)} = \frac{\text{OD (DPPH)} - \text{OD (DPPH + Sample)}}{\text{OD (DPPH)}} \times 100$$

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity

The ABTS radical was prepared by reacting equal volumes of 1.1 mg/mL aqueous ABTS and 0.68 mg/mL potassium per sulfate ($\text{K}_2\text{S}_2\text{O}_8$) and stored in dark for 6 h at room temperature. A stock solution of extracts and fractions at a concentration of 1,000 µg/mL was prepared. A volume of 100 µL of stock solution (1,000 µg/mL) was added to the well and it was further down diluted to the lowest concentration (7.8 µg/mL) by performing a serial 2-fold dilution in a 96-well microtiter plate. A row of negative ABTS control was developed in the same 96-well microtiter plates by adding 100 µL of methanol into each well. Quercetin and Vitamin C were used as positive controls. Twenty-five microliters of ABTS radical were added into each well and reaction was allowed to proceed for 30 min in the dark. The absorbance was measured at 734 nm by a microplate reader.^[24] The ABTS radical scavenging effect (%) was calculated using the formula:

$$\text{ABTS radical Scavenging effect (\%)} = \frac{\text{OD (ABTS)} - \text{OD (ABTS + Sample)}}{\text{OD (ABTS)}} \times 100$$

In vitro immunomodulatory activity

NBT screening test, phagocytosis of killed *C. albicans* and candidacidal assay were carried out at KLE University's Dr. Prabhakar Kore Basic Science Research Center, Belgaum to assess *in vitro* immunomodulatory activity. Ethical clearance was obtained from the Research and Ethical Committee, KLE University's V.K. Institute of Dental Sciences, Belgaum prior to study.

Nitroblue tetrazolium screening test

One part of 0.3% NBT solution was prepared in 0.35% sucrose solution and was used fresh. Hundred microliters of whole blood and 100 µL of Hank's Balanced Salt Solution (HBSS) were added to each Eppendorf tube and mixed well. The stock solution of hydroalcoholic extract and its fractions of leaves of *F. benghalensis* Linn. in different concentrations of 1,000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL were added individually to tubes containing suspension of blood cells and 50 µL of NBT solution. In another tube 50 µL of *Escherichia coli* endotoxin

was added to 50 μL NBT solution and suspension of blood cells, which served as positive control (standard). A normal control was maintained in another tube with only suspension of blood cells and NBT solution. All tubes were incubated at 37°C for 20 min and were gently shaken at room temperature for 20 min. A smear of fluid from each tube was taken on microscope slide, air dried, fixed in methanol for 2 min, stained with Giemsa stain for 15 min, rinsed with distilled water, dried, and observed under microscope. Hundred neutrophils were counted and percentage of NBT-positive cells containing blue deposits was determined.^[25]

Phagocytosis of killed *Candida albicans*

Preparation of *Candida albicans* suspension

The *C. albicans* culture was incubated in Sabouraud broth overnight and then centrifuged to form a cell button at the bottom and supernatant was discarded. The cell button was washed with sterile HBSS and centrifuged again. This step was repeated 3–4 times. The final cell button was mixed with a mixture of sterile HBSS and human serum in the proportion of 4:1. The cell suspension of concentration $1 \times 10^5/\text{mL}$ was used for the experiment.

Phagocytosis evaluation

Fifty microliters of concentrations of the hydroalcoholic extract and its fractions of leaves of *F. benghalensis* Linn. (1,000 $\mu\text{g}/\text{mL}$, 500 $\mu\text{g}/\text{mL}$, 250 $\mu\text{g}/\text{mL}$, 125 $\mu\text{g}/\text{mL}$, 62.5 $\mu\text{g}/\text{mL}$) were taken in separate Eppendorf tubes and 100 μL of HBSS, 100 μL of *C. albicans* suspension and 100 μL of leucocytes suspension ($2 \times 10^6/\text{mL}$) were added to each tube. A positive control (standard) and a normal control were maintained. All the tubes were shaken gently, incubated at 37°C for 30 min and centrifuged at 200 g for 5 min. The supernatant was removed leaving a small droplet into which sediment was resuspended. Smears were made, air-dried and stained with Giemsa stain. Two hundred neutrophils were observed under a microscope, a number of ingested *C. albicans* associated with each cell was counted and mean particle number (MPN) associated with each neutrophils was calculated.^[26]

Candidacidal assay

Fifty microliters each of different concentration of hydroalcoholic extract and its fractions of leaves of *F. benghalensis* were taken in separate Eppendorf tubes and 100 μL of HBSS, 50 μL of *C. albicans* suspension ($1 \times 10^5/\text{mL}$) and 100 μL of leucocytes suspension ($7 \times 10^6/\text{mL}$) were added to each tube. In another tube, 50 μL of pooled serum was added to 100 μL of HBSS, 50 μL of *C. albicans* suspension and 100 μL of leucocytes suspension which served as positive control (standard). A normal control was maintained in another tube containing leucocytes suspension, HBSS and *C. albicans* suspension. Each tube was incubated at 37°C for 60 min with shaking every 15 min. After 30 min, 100 μL solutions of 0.5% sodium deoxycholate were added to each tube and mixed causing lyses of leucocytes without damage to *Candida* cells. One milliliter of 0.01% methylene blue was added to each tube, mixed and centrifuged at 1,500 g for 5 min. The supernatant containing methylene blue was carefully removed with Pasteur pipette leaving behind droplet to resuspend the organism. The suspension was put in ice bath until ready for counting. The suspension was observed on Neubauer counting chamber under a microscope. The proportion of dead cells (stained blue) was determined.^[26]

Statistical analysis

The values were expressed as mean \pm standard error of the mean ($n = 3$). The results were analyzed using one-way analysis of variance followed by Dunnet's *t*-test to determine the statistical significance.^[27]

RESULTS

Extraction of leaves of *F. benghalensis* with cold maceration gives hydroalcoholic extract 25% w/w, and successive fractions of n-hexane,

n-butanol, chloroform and water 7% w/w, 2.8% w/w, 1.5% w/w and 14% w/w, respectively.

1-1-diphenyl-2-picrylhydrazyl radical scavenging activity

The free radical scavenging activity of hydroalcoholic extract and its different successive fractions of a leaf of *F. benghalensis* was determined according to the DPPH radical scavenging method and is shown in Figure 1. According to this method, a compound with high antioxidant activity effectively bind with the radical hence prevent its propagation and the resultant chain reaction. Different concentrations of five different samples, that is, hydroalcoholic extract (FB1), n-hexane fraction (FB2), n-butanol fraction (FB3), chloroform fraction (FB4) and water fraction (FB5) were analyzed. The results were expressed as IC_{50} values and calculated from regression lines as presented in Table 1.

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity

The free radical scavenging activity of hydroalcoholic extract and its different successive fractions of a leaf of *F. benghalensis* was determined according to the ABTS radical scavenging method and is shown in Figure 2. According to this method, a compound with high antioxidant activity effectively bind with the radical hence prevent its propagation and the resultant chain reaction. Different concentrations of five different extracts, that is, hydroalcoholic (FB1), n-hexane (FB2), n-butanol (FB3), chloroform (FB4) and water (FB5) extracts were analyzed. The results

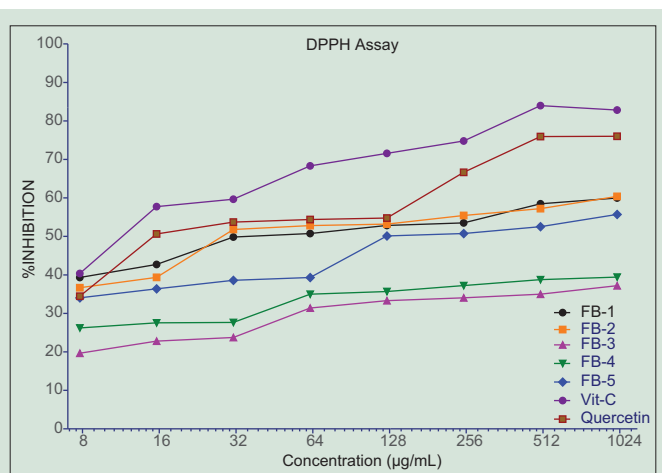


Figure 1: Free radical scavenging activity of hydroalcoholic extract and its fractions of *Ficus benghalensis* leaves measured using the 1-1-diphenyl-2-picrylhydrazyl-assay. Results are mean \pm standard error of the mean ($n = 3$)

Table 1: IC_{50} values of hydroalcoholic extract and its fractions of FB leaves and reference compounds for scavenging of DPPH free radicals

Sample	IC_{50} value ($\mu\text{g}/\text{ml}$)
Vitamin-C	11.5 \pm 0.052
Quercetin	15.4 \pm 0.120
Hydroalcoholic (FB 1)	32.3 \pm 1.320
n-Hexane (FB 2)	28.2 \pm 0.993
n-Butanol (FB 3)	>1000
Chloroform (FB 4)	>1000
Water (FB 5)	125.0 \pm 0.025

DPPH: 1-1-diphenyl-2-picrylhydrazyl; FB: *Ficus benghalensis*

were expressed as IC_{50} values and calculated from regression lines as presented in Table 2.

Nitroblue tetrazolium test

The hydroalcoholic (FB1) extract of leaves of *F. benghalensis* has stimulated the neutrophils to phagocytic activity to the extent of 45% at concentration of 1,000 $\mu\text{g}/\text{mL}$ and 41.66% at concentration of 500 $\mu\text{g}/\text{mL}$ very significantly. n-Hexane (FB2) fraction showed 80.33% at concentration of 1000 $\mu\text{g}/\text{mL}$, 78.00% at concentration of 500 $\mu\text{g}/\text{mL}$, 76.66% at concentration of 250 $\mu\text{g}/\text{mL}$, 44.00% at concentration of 125 $\mu\text{g}/\text{mL}$. n-Butanol (FB3) fraction showed very highly significant result at all concentrations. Chloroform (FB4) fraction has stimulated the neutrophils to phagocytic activity to the extent of 55% at concentration

of 1,000 $\mu\text{g}/\text{mL}$, 54.00% at concentration of 500 $\mu\text{g}/\text{mL}$ and 36% at concentration of 250 $\mu\text{g}/\text{mL}$, while water (FB5) fraction does not show significant activity. The results are presented in Table 3.

Phagocytosis of killed *Candida albicans*

The hydroalcoholic (FB1) extract and its four fractions n-hexane (FB2), n-butanol (FB3), chloroform (FB4) and water (FB5) of leaves of *F. benghalensis* stimulated the phagocytosis of killed *C. albicans*. The MPN was found to be 5 at concentration of 1,000 $\mu\text{g}/\text{mL}$ for hydroalcoholic (FB1) extract; 5, 5, 4, 3-4, 2-3 for n-hexane (FB2) fraction; 5-6, 5, 5, 4-5, 3 for n-butanol (FB3) fraction at down diluted concentrations from 1,000 $\mu\text{g}/\text{mL}$ to 62.5 $\mu\text{g}/\text{mL}$; 4, 3-4 for Chloroform (FB4) fraction at concentration of 1,000 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$, respectively. Results are compared with positive control (standard) that is pooled serum (6, 5-6, 5, 5, and 4 at down diluted concentrations from 1,000 $\mu\text{g}/\text{mL}$ to 62.5 $\mu\text{g}/\text{mL}$). Water (FB5) fraction does not show significant stimulation of phagocytic activity. The results are represented in Table 4.

Candidacidal assay

The hydroalcoholic (FB1) extract of leaves of *F. benghalensis* has shown significant candidacidal activity to the extent of 29% at concentration of 1,000 $\mu\text{g}/\text{mL}$; n-hexane (FB2) fraction has shown 36.33% and 32.66% at concentrations of 1,000 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ respectively. n-Butanol (FB3) fraction has shown 35.33% and 33% at concentration of 1,000 $\mu\text{g}/\text{mL}$ and 500 $\mu\text{g}/\text{mL}$ respectively when compared to standard, that is pooled serum (40% and 37.33%) and to normal control (21.66% and 19.66%) at the same concentrations. Chloroform (FB4) fraction and water (FB5) fraction does not showed significant stimulation of candidacidal activity. The results are represented in Table 5.

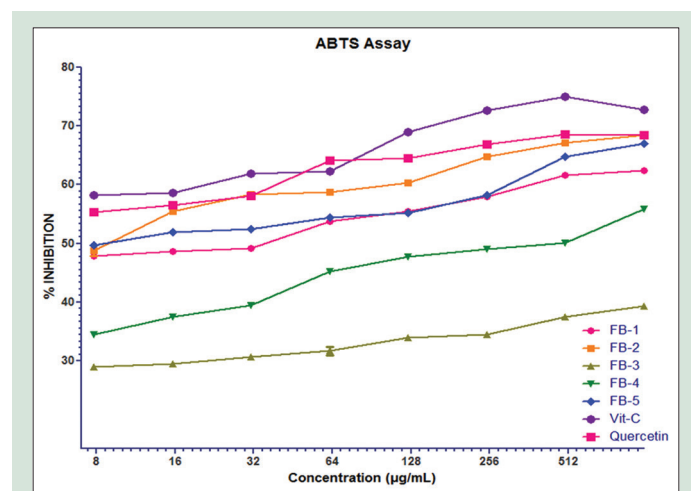


Figure 2: Free radical scavenging activity of hydroalcoholic extract and its fractions of *Ficus benghalensis* leaves measured using the 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)-assay. Results are mean \pm standard error of the mean ($n = 3$)

Table 2: IC_{50} values of hydroalcoholic extract and its fractions of FB leaves and reference compounds for scavenging of ABTS free radicals

Sample	IC_{50} value ($\mu\text{g}/\text{mL}$)
Vitamin-C	6.40 \pm 0.015
Quercetin	7.05 \pm 0.115
Hydroalcoholic (FB 1)	52 \pm 0.722
n-Hexane (FB 2)	58.2 \pm 0.714
n-Butanol (FB 3)	491 \pm 0.555
Chloroform (FB 4)	>1000
Water (FB 5)	20.3 \pm 0.133

ABTS: 3-ethylbenzthiazoline-6-sulphonic acid; FB: *Ficus benghalensis*

Table 3: Percentage of reduced neutrophils after treatment with extract and fractions of FB by NBT test

Sample	Concentration ($\mu\text{g}/\text{ml}$)				
	1000	500	250	125	62.5
Blood/without endotoxin	20.66 \pm 1.20	21.33 \pm 0.57	21 \pm 1.15	20 \pm 0.57	22 \pm 0.57
Blood with endotoxin	92.33 \pm 1.45	91.66 \pm 1.20	88.66 \pm 0.88	88 \pm 1.15	87.66 \pm 0.33
Hydroalcoholic (FB 1)	45 \pm 0.57**	41.66 \pm 0.88**	25.0 \pm 2.30 ^{NS}	21 \pm 0.57 ^{NS}	20.33 \pm 0.66
n-Hexane (FB 2)	80.33 \pm 0.66***	78 \pm 0.57***	76.66 \pm 0.33***	44 \pm 0.57**	26.66 \pm 2.84*
n-Butanol (FB 3)	89.66 \pm 0.33***	83.5 \pm 2.85***	82.66 \pm 0.88***	61 \pm 1.00***	32.66 \pm 0.88**
Chloroform (FB 4)	55 \pm 1.00***	54 \pm 0.57***	36 \pm 0.57**	26 \pm 1.52 ^{NS}	21.33 \pm 0.88
Water (FB 5)	20.33 \pm 0.33 ^{NS}	20 \pm 0.57 ^{NS}	18.33 \pm 0.66 ^{NS}	18.0 \pm 0.00 ^{NS}	16.66 \pm 0.33

The data are expressed as mean percentage reduced neutrophils \pm SEM. Significant difference from positive control (standard) by one-way ANOVA followed by Dunnett's *t*-test. ($n=3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Blood without endotoxin (normal control), blood with endotoxin (positive control). ^{NS}Not significant, NBT: Nitroblue tetrazolium; SE: Standard error of mean; FB: *Ficus benghalensis*; ANOVA: Analysis of variance

Table 4: MPN of killed *Candida albicans* phagocytosed

Sample	Concentration (µg/ml)				
	1000	500	250	125	62.5
Pooled serum	6	5-6	5	5	4
Normal control	2	2	2	2	2
Hydroalcoholic (FB 1)	5	3-4	3	2	2-3
n-Hexane (FB 2)	5	5	4	3-4	2-3
n-Butanol (FB 3)	5-6	5	5	4-5	3
Chloroform (FB 4)	4	3-4	3	3	2-3
Water (FB 5)	3	2-3	2	2	2

The data are expressed as mean particle number phagocytosed. Pooled serum (positive control). MPN: Mean particle number; FB: *Ficus benghalensis*

Table 5: Percentage of killed *Candida albicans* by candidacidal assay

Sample	Concentration (µg/ml)				
	1000	500	250	125	62.5
Pooled serum	40±0.57	37.33±0.88	32.66±0.66	26.66±1.45	24±0.57
Normal control	21.66±1.20	19.66±0.66	16.66±0.33	15±0.57	13.66±0.33
Hydroalcoholic (FB 1)	29±0.57**	20.33±0.33*	19.33±0.88	17±0.57	17.66±0.33
n-Hexane (FB 2)	36.33±1.45***	32.66±0.88**	19.33±0.66	17±0.57	17±0.57
n-Butanol (FB 3)	35.33±0.66***	33±0.57***	22±1.00	18.66±0.33	17±0.57
Chloroform (FB 4)	20±0.33	17.66±0.88	21±0.57	17±0.57	13.66±0.33
Water (FB 5)	21.66±0.66	20±0.57	21.33±0.88	17±0.57	18.66±0.33

The data are expressed as mean percentage reduced neutrophils±SEM. Significant difference from positive control (standard) by one-way ANOVA, followed by Dunnett's *t*-test. (*n*=3). **P*<0.05, ***P*<0.01, ****P*<0.001. Normal control, positive control (pooled serum). SE: Standard error of mean; FB: *Ficus benghalensis*; ANOVA: Analysis of variance

in the reaction. Figure 1 shows the DPPH scavenging activities of five different extracts, that is hydroalcoholic (FB1), n-hexane (FB2), n-butanol (FB3), chloroform (FB4), water (FB5) and reference antioxidants at down diluted concentrations from 1000 µg/mL to 7.8 µg/mL. The extracts exhibited different DPPH scavenging activities in a concentration-dependent manner. The scavenging effects of extracts and reference antioxidants on DPPH observed in the following order: Vitamin C > quercetin > FB2 > FB1 > FB5 > FB4 > FB3. The IC₅₀ of various fraction and reference antioxidants are listed in Table 1. FB1, FB2 exhibited effective radical scavenging activity while FB₅ showed less activity. FB3, FB4 showed very weak activity. The ABTS radical, which has a peak absorbance at 734 nm, should be performed by mixing ABTS and K₂S₂O₈. When antioxidants were added, the ABTS radical, which has a blue-green color, is reduced to ABTS (no color). Figure 1 shows the ABTS scavenging activities of five different extracts, that is hydroalcoholic (FB1), n-hexane (FB2), n-butanol (FB3), chloroform (FB4), water (FB5) extracts and reference antioxidants at different concentrations (7.8–1,000 µg/mL). The extracts exhibited different ABTS scavenging activities in a concentration-dependent manner. The scavenging effects of extracts and reference antioxidants on ABTS decreased in the following order: Vitamin C > Quercetin > FB1 > FB2 > FB5 > FB3 > FB4. The IC₅₀ of extract, fractions, and reference antioxidants are listed in Table 2. FB1, FB2 exhibited very strong radical scavenging activity while FB3, FB4 and FB5 showed weak activity.

Immunity plays an important role in the protection of the human body from foreign toxic matter. Many plants from Ayurveda have been used for the purpose of increase immunity. Immunomodulatory agents of plant and animal origin increase the immune responsiveness of the body against pathogens by activating the nonspecific immune system. However, there is a need to systemic studies on medicinal plants to substantiate the therapeutic claims made regarding their clinical utility. In this study, the hydroalcoholic (FB1) extract and its four fractions n-hexane (FB2), n-butanol (FB3), chloroform (FB4) and water (FB5) of leaves of *F. benghalensis* significantly increased the phagocytic function of human neutrophils, when compared with control indicating the

possible immunostimulating effect. The engulfment of microorganisms by leukocytes called phagocytosis, which is one of the main defense mechanisms of an organism. Hydroalcoholic extract and its fractions *F. benghalensis* have significantly increased the intracellular reduction of NBT dye to formazan (deep blue compound) by the neutrophils confirming the intracellular killing property and overall metabolic integrity of phagocytizing neutrophils.

Phagocytosis of killed *C. albicans* and candidacidal assay has supported the immunomodulatory activity of *F. benghalensis*, claimed as an enhancer of general immunity against various physical and mental disorders in the indigenous system of medicine.

CONCLUSIONS

The results clearly indicate that the leaves of *F. benghalensis* possess immune boosting properties and suggest usefulness in the disorder of immunological origin where the antioxidant system is adversely affected. Further study regarding the isolation of active chemical constituents responsible for immunomodulatory activity and its mode of action need to be determined.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA. Search of immunomodulatory agents. *Indian Drugs* 1990;28:56-63.
- Rao CS, Raju C, Gopumadhavan S, Chauhan BL, Kulkarni RD, Mitra SK. Immunotherapeutic modification by an ayurvedic formulation Septilin. *Indian J*

- Exp Biol 1994;32:553-8.
3. Fulzele SV, Satturwar PM, Joshi SB, Dorle AK. Study of immunomodulatory activity of *Haridradi Ghrita* in rats. Indian J Pharmacol 2003;35:51-4.
 4. Thatte UM, Dahanurkar SA. Rasayana concept: Clues from immunomodulatory therapy. In: Upadhyay S, editor. Immunomodulation. New Delhi: Narosa Publishing House; 1997. p. 141-8.
 5. Ghaisas MM, Shaikh SA, Deshpande AD. Evaluation of immunomodulatory activity of ethanolic extract of the stem bark of *Bauhinia variegata* Linn. Int J Green Pharm 2009;3:70-4.
 6. Trivedi CP, Shinde S, Sharma RC. Preliminary phytochemical and pharmacological studies on *Ficus racemosa* (Gular). Indian J Med Res 1969;57:1070-4.
 7. Chopra RN, Mukerji B, Chopra IC. A Treatise on Tropical Therapeutics. Calcutta: UN Dhur and Sons; 1950.
 8. Bhattacharjee SK. Handbook of Medicinal Plants. Jaipur, India: Pointer Publishers; 1998. p. 118.
 9. Subramanian PM, Misra GS. Chemical constituents of *Ficus benghalensis* (part II). Pol J Pharmacol Pharm 1978;30:559-62.
 10. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP. Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. J Ethnopharmacol 1998;60:85-9.
 11. Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastav GN, et al. Dictionary of Indian Medicinal Plants. Lucknow, India: CIMAP; 1992. p. 546.
 12. Aiyer MN, Nambodiri AN, Kolammal M. Pharmacognosy of Ayurvedic Drugs. Trivandrum: The Central Research Institute, Trivandrum; 1957.
 13. Warriar PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants, (1-5). Madras: Orient Longman Ltd.; 1995.
 14. Gabhe SY, Tatke PA, Khan TA. Evaluation of the immunomodulatory activity of the methanol extract of *Ficus benghalensis* roots in rats. Indian J Pharmacol 2006;38:271-5.
 15. Shrotri DS, Aiman R. The relationship of the post absorptive state to the hypoglycemic action studies on *Ficus benghalensis* and *Ficus glomerata*. Indian J Med Res 1960;48:162-8.
 16. Deshmukh VK, Shrotri DS, Aiman R. Isolation of a hypoglycemic principle from the bark of *Ficus benghalensis* Linn. A preliminary note. Indian J Physiol Pharmacol 1960;4:182-5.
 17. Augusti KT. Hypoglycaemic action of bengalenoside, a glucoside isolated from *Ficus benghalensis* Linn, in normal and alloxan diabetic rabbits. Indian J Physiol Pharmacol 1975;19:218-20.
 18. Augusti KT, Daniel RS, Cherian S, Sheela CG, Nair CR. Effect of leucopelargonin derivative from *Ficus benghalensis* Linn. on diabetic dogs. Indian J Med Res 1994;99:82-6.
 19. Achrekar S, Kaklij GS, Pote MS, Kelkar SM. Hypoglycemic activity of *Eugenia jambolana* and *Ficus benghalensis*: Mechanism of action. In Vivo 1991;5:143-7.
 20. Mousa O, Vuorela P, Kiviranta J, Wahab SA, Hiltunen R, Vuorela H. Bioactivity of certain Egyptian *Ficus* species. J Ethnopharmacol 1994;41:71-6.
 21. Daniel RS, Mathew BC, Devi KS, Augusti KT. Antioxidant effect of two flavonoids from the bark of *Ficus benghalensis* Linn in hyperlipidemic rats. Indian J Exp Biol 1998;36:902-6.
 22. Shukla R, Gupta S, Gambhir JK, Prabhu KM, Murthy PS. Antioxidant effect of aqueous extract of the bark of *Ficus benghalensis* in hypercholesterolaemic rabbits. J Ethnopharmacol 2004;92:47-51.
 23. Taur DJ, Nirmal SA, Patil RY, Kharya MD. Antistress and antiallergic effects of *Ficus benghalensis* bark in asthma. Nat Prod Res 2007;21:1266-70.
 24. Muid S, Ali A, Yusoff K, Nawawi H. Optimal antioxidant activity with moderate concentrations of tocotrienol rich fraction (TRF) in *in vitro* assays. Int Food Res J 2013;20:687-94.
 25. Heroor S, Beknal AK, Mahurkar N. Immunomodulatory activity of methanolic extracts of fruits and bark of *Ficus glomerata* Roxb. in mice and on human neutrophils. Indian J Pharmacol 2013;45:130-5.
 26. Patil JK, Jalalpure SS, Hamid S, Ahirrao RA. *In vitro* immunomodulatory activity of extracts of *Bauhinia variegata* Linn stem bark on human neutrophils. Indian J Pharmacol Ther 2010;9:41-6.
 27. Kulkarni SK. Handbook of Experimental Pharmacology. Delhi: Vallabh Prakashan; 1999. p. 179-80.

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