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"Invitro Studies Of Antioxidant Activity From The Leaf Extract Of Ficus Bengalensis"

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ABSTRACT

The present study investigates the in-vitro antioxidant potential of the leaf extract of Ficus benghalensis, commonly known as the Banyan tree. The plant is traditionally used in Ayurveda for its medicinal properties, including anti-inflammatory and wound-healing effects. In this study, methanolic and aqueous extracts of Ficus benghalensis leaves were evaluated for their free radical scavenging activity using standard antioxidant assays, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, and ferric reducing antioxidant power (FRAP) assay. Phytochemical screening confirmed the presence of flavonoids, tannins, and phenolic compounds, which are known to contribute to antioxidant activity. The results indicated that the methanolic extract exhibited higher radical scavenging activity compared to the aqueous extract, suggesting a higher concentration of bioactive compounds in organic solvents. The IC50 values obtained for the DPPH and ABTS assays were comparable to those of standard antioxidants like ascorbic acid and quercetin. These findings highlight the potential of Ficus benghalensis leaf extract as a natural antioxidant source, which could be utilized in pharmaceutical and nutraceutical formulations. Further research is recommended to isolate and characterize the active compounds responsible for the observed antioxidant activity.

Keywords: Ficus benghalensis, antioxidant activity, DPPH, ABTS, FRAP, phytochemicals

INTRODUCTION

Plants are the oldest and the most important source of medicines. At the end nine after the synthesis of aspirin, research on herbal products was halted and researchers started synthetic and semi-synthetic drugs. But in the last few decades, there is an upsurge in research on the natural origin, especially botanical drugs. About 25% of the prescribed drugs are higher plants and this value is increased to 50%, if animal and microbial products are also Ficus bengalensis L., belonging to the family Moraceae, which is commonly known as Banyan Darakht-e-Reesh and Bargad (Unani medicines) and Bohar (Urdu). . Different parts of the banyan tree are used as a traditional medicine to get remedies for several ailments. The bark is useful for burning sensations, hemorrhages, diarrhea, dysentery, diabetes, ulcer, and skin diseases. The leaves are good for ulcers, leprosy, skin allergies, etc. The buds are used in diarrhea and dysentery. Latex is useful in rheumatism, hemorrhoids, inflammation, and skin diseases.

PLANT PROFILE

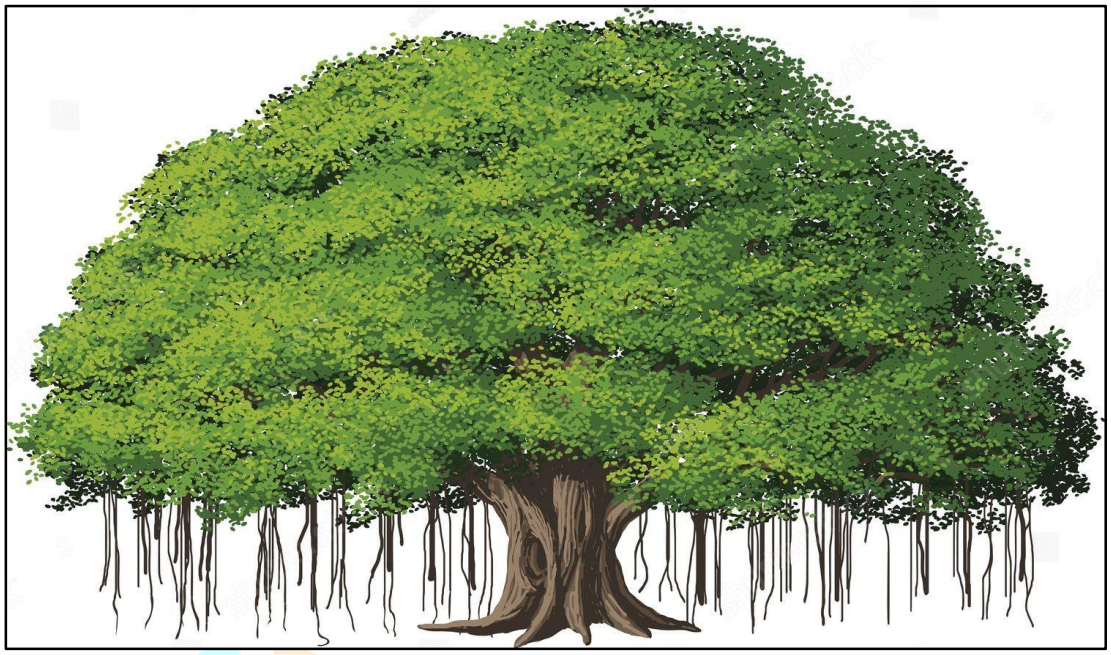


Figure 1: Whole plant of Banyan tree

Kingdom: Planate

Subkingdom: Tracheobinote

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Order: Urticales

Family: Moraceae

Genus: Ficus

Morphology:

- Plant F. bengalensis is a laticiferous tree, up to 30 m in height with widely spreading branches bearing many aerial prop roots.
- The bark is greenish white. Leaves are simple, alternate, and arranged often in clusters at the ends of branches.
- They are stipulate, 5-12 cm broad and 10-18 cm long, entire, broadly elliptic to ovate.

MATERIALS AND METHODS

Materials:

1. Plant material: Fresh or dried leaves of Ficus benghalensis.

2. Solvents: Methanol, ethanol, acetone, or other solvents for extraction.

3. Chemicals: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3- ethylbenzothiazoline-6-sulphonic acid)), FRAP (ferric reducing antioxidant power) reagents, and other chemicals for antioxidant assays

4. Equipment: Rotary evaporator, sonicator, centrifuge, spectrophotometer, and other laboratory equipment.

Methods:

Plant Material Preparation

1. Collect fresh or dried leaves of Ficus benghalensis.

2. Clean and dry the leaves to remove any dirt or moisture.

3. Grind the dried leaves into a fine powder.

Extraction:

1. Weigh 10g of powdered plant material.

2. Add 100ml of solvent (e.g., methanol, ethanol, or acetone) to the plant material.

3. Mix well and incubate for 24 hours at room temperature.

4. Filter the extract using filter paper or a membrane filter

Antioxidant Assay:

1. DPPH assay: Measure the ability of the extract to scavenge DPPH radicals.

2. ABTS assay: Measure the ability of the extract to scavenge ABTS radicals.

3. FRAP assay: Measure the ability of the extract to reduce ferric ions.

4. Other antioxidant assays: Measure the ability of the extract to scavenge other free radicals or reduce oxidative stress.

Phytochemical Analysis:

1. HPLC (High-Performance Liquid Chromatography): Analyze the phytochemical composition of the extract.

2. GC-MS (Gas Chromatography-Mass Spectrometry): Analyze the phytochemical composition of the extract.

3. Other phytochemical analysis methods: Analyze the phytochemical composition of the extract using other methods.

Statistical Analysis:

1. ANOVA (Analysis of Variance): Compare the antioxidant activity of different extracts.

2. Tukey's test: Compare the antioxidant activity of different extracts.

3. Other statistical analysis methods: Analyze the data using other statistical methods.

EXTRACTION METHODS:

Conventional Extraction Methods:

1. Solvent Extraction: Using solvents like methanol, ethanol, or acetone to extract bioactive compounds.
2. Maceration: Soaking the leaves in a solvent for an extended period to extract bioactive compounds.
3. Infusion: Steeping the leaves in hot water to extract bioactive compounds.
4. Decoction: Boiling the leaves in water to extract bioactive compounds.
5. Percolation: Passing a solvent through a bed of leaves to extract bioactive compounds.

Non-Conventional Extraction Methods:

1. Ultrasound-Assisted Extraction (UAE): Using high-frequency sound waves to enhance solvent extraction.
2. Microwave-Assisted Extraction (MAE): Using microwave energy to heat the solvent and enhance extraction.
3. Supercritical Fluid Extraction (SFE): Using high-pressure carbon dioxide to extract bioactive compounds.
4. Enzyme-Assisted Extraction (EAE): Using enzymes to break down cell walls and enhance solvent extraction.
5. High-Pressure Extraction (HPE): Using high pressure to enhance solvent extraction.
6. Pulsed Electric Field (PEF) Extraction: Using high-voltage pulses to enhance solvent extraction.
7. Subcritical Water Extraction (SWE): Using hot water under high pressure to extract bioactive compounds.

Soxhlet Extraction

1. Extraction chamber: A cylindrical vessel made of glass or stainless steel
2. Condenser: A coil or tube that cools the solvent vapor.
3. Solvent reservoir: A container that holds the solvent.
4. Heating mantle: A device that heats the solvent reservoir.
5. Thimble: A porous container that holds the plant material.

Operation Method:

1. Prepare the plant material: Grind or chop the plant material into small pieces.
2. Assemble the apparatus: Connect the extraction chamber, condenser, solvent reservoir, and heating mantle.
3. Add solvent: Fill the solvent reservoir with the desired solvent.
4. Add plant material: Place the plant material in the thimble.
5. Heat the solvent: Heat the solvent reservoir using the heating mantle.
6. Extract the compounds: The solvent vapor rises into the extraction chamber, where it extracts the bioactive compounds from the plant material.

7. Condense the solvent: The solvent vapor is condensed in the condenser and returns to the solvent reservoir.

8. Repeat the cycle: The extraction cycle is repeated until the desired compounds are fully extracted.

9. Collect the extract: The extracted compounds are collected in the solvent reservoir.

Table 1: Phytochemical screening of Ficus Benghalensis plant leaves

S.No	Phytochemical group	Reagents	Aqueous phase(water)	Chloroform
01	Alkaloids test	Mayer's test	-	-
		Wagner's test	+	+
02	Tannins	Ferric chloride test	+	+
		Lead acetate test	+	+

03	Steroids test	Salkowski test	+	+
		Libermann test	-	+
04	Glycosides test	Keller-killani test	-	+
05	Protein test	Biuret test	+	+
06	Carbohydrates test	Bendict's test	+	+
		Molish's test	+	+

Result: (+) Present, (-) Absent

RESULT AND DISCUSSION

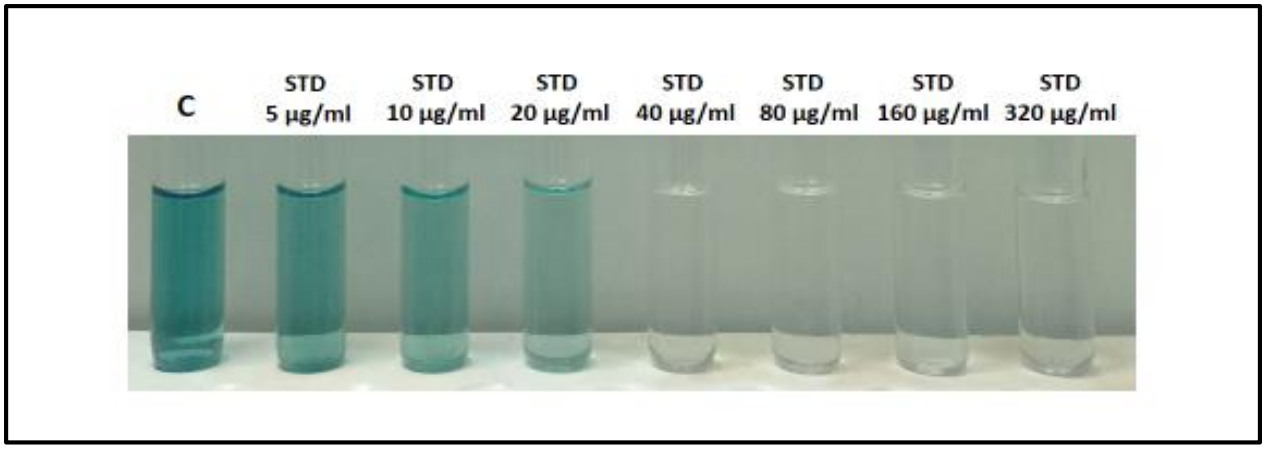
Study report;

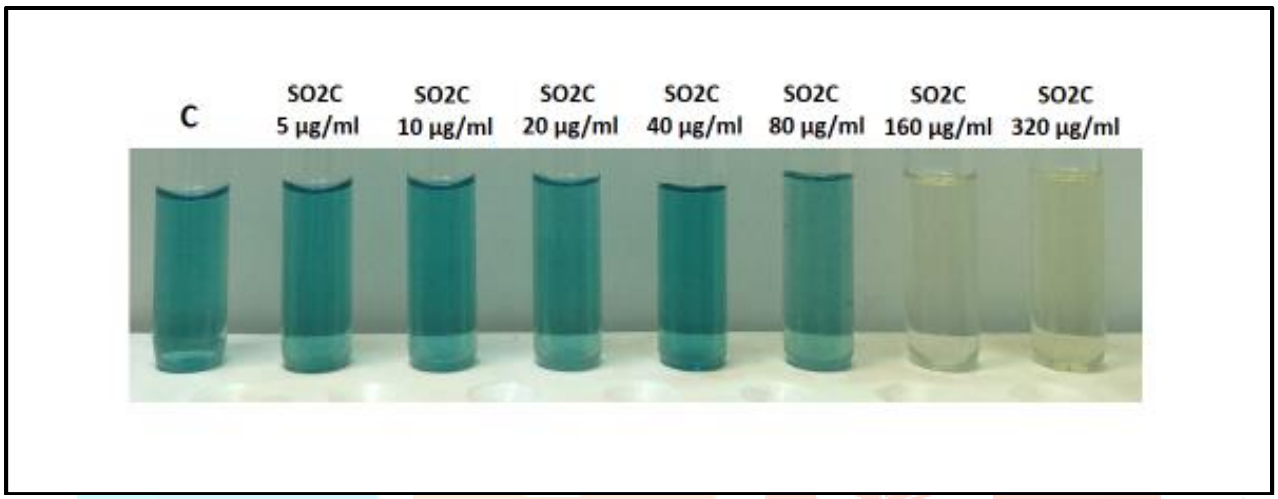
ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay:

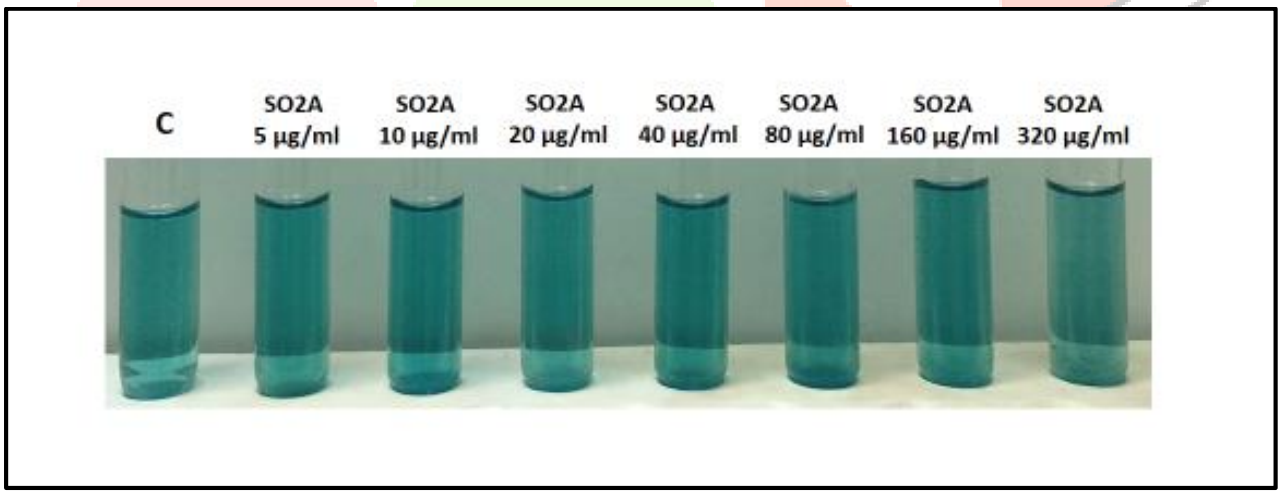
ABTS radical scavenging assay of test samples (SO2C, SO2A) was performed according to the modified method of Perumal et al., 2018. The ABTS (7 mM, 25 mL in deionized water) stock solution was prepared with potassium persulfate (K2S2O8) (140 mM, 440 µL). Different concentrations (5, 10, 20, 40, 80, 160 and 320 µg/mL) of test samples and standard (Ascorbic acid) were mixed with the ABTS working solution (2.0 mL) and the reaction mixture was allowed to stand at room temperature for 20 min; then, the Abs was measured using an ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity was given as ABTS radical scavenging effect was calculated by the equation: ABTS radical scavenging effect (%) = [(A0 - A1)/A0] ×100. Where, A0 is the control; A1 is the test

Result:

The IC50 values of the given test samples **(SO2C, SO2A)** and the standard drug **(Ascorbic acid)** were found to be **48.7 µg/ml, >320 µg/ml** and **11. µg/ml** 13 respectively.







***In vitro* Antioxidant activity - ABTS assay**

Table 2:

Sample	Conc.(µg/ml)	Singlet	Duplicate	Triplicate
Control	0	0.809	0.808	0.806

Table 3:

		OD at 734 nm		
Sample	**Conc.(µg/ml)**	**Singlet**	**Duplicate**	**Triplicate**
Ascorbic acid	5	0.656	0.654	0.657
	10	0.415	0.418	0.415
	20	0.227	0.225	0.222
	40	0.109	0.116	0.104
	80	0.056	0.057	0.052
	160	0.038	0.034	0.037
	320	0.016	0.013	0.018

Table 4:

		% inhibition					
Sample	**Conc. (µg/ml)**	**Singlet**	**Duplicate**	**Triplicate**	**Mean**	**SD**	**IC_{50}**
Ascorbic acid	5	18.9122373	19.0594059	18.48635236	18.8193319	0.297609089	
	10	48.7021014	48.2673267	48.51116625	48.4935314	0.217923114	
	20	71.9406675	72.1534653	72.45657568	72.1835695	0.259268222	**11.13**
	40	86.526576	85.6435644	87.09677419	86.4223049	0.732194699	
	80	93.0778739	92.9455446	93.54838719	93.1906019	0.316836667	
	160	95.302843	95.7920792	95.40942928	95.5014505	0.257272116	
	320	98.0222497	98.3910891	97.76674938	98.0600294	0.313879766	

Table 5:

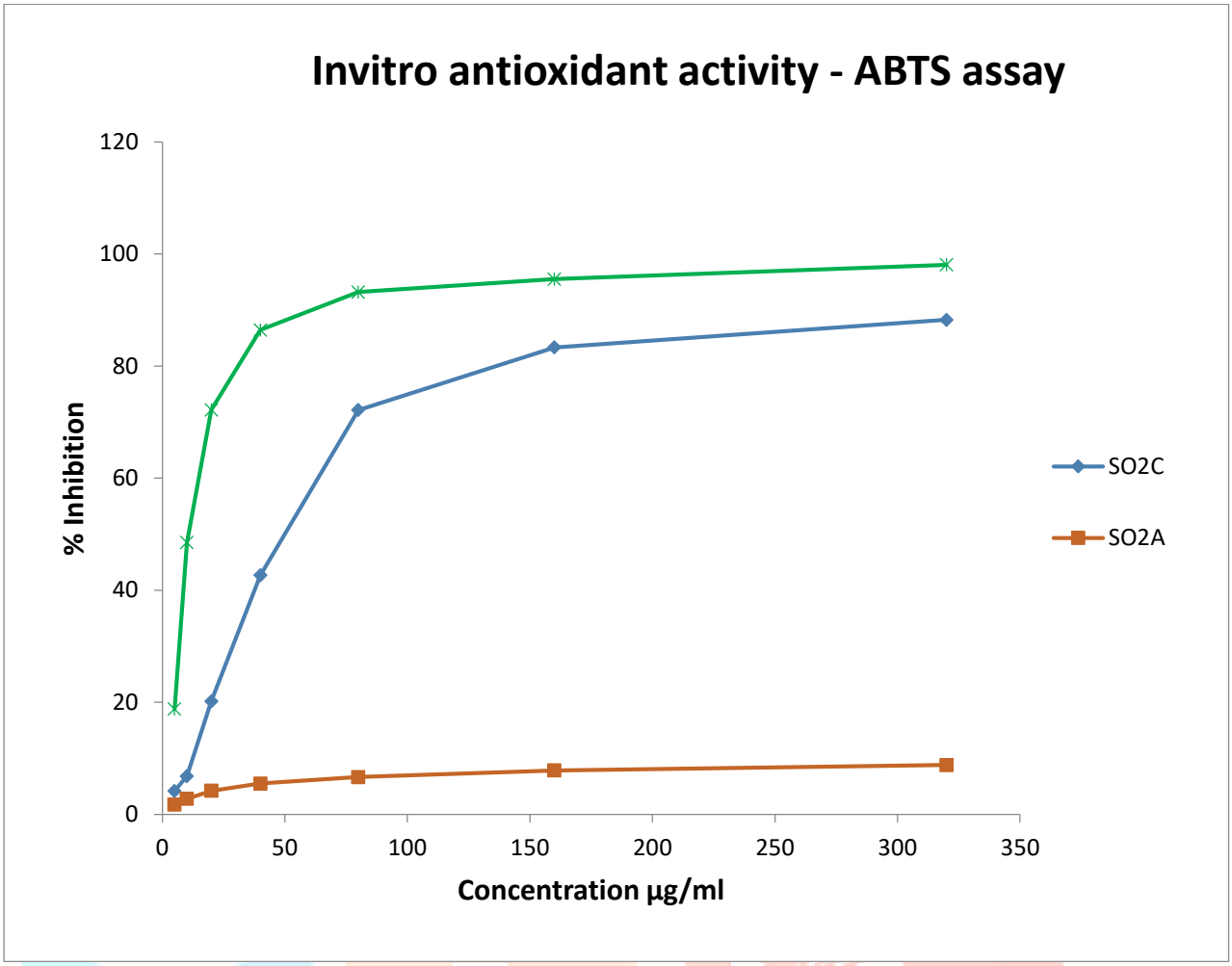
		OD at 734 nm		
Sample	**Conc.(mg/ml)**	**Singlet**	**Duplicate**	**Triplicate**
SO2A	5	0.793	0.796	0.792
	10	0.784	0.785	0.787
	20	0.771	0.776	0.774
	40	0.763	0.762	0.765
	80	0.752	0.757	0.753
	160	0.746	0.745	0.742
	320	0.735	0.741	0.734

Table 6:

		% inhibition					
Sample	**Conc.(mg/ml)**	**Singlet**	**Duplicate**	**Triplicate**	**Mean**	**SD**	
SO2A	5	1.97775031	1.48514851	1.736972705	1.73329051	0.24632154	
	10	3.09023486	2.84653465	2.357320099	2.76469654	0.373248066	
	20	4.69715698	3.96039604	3.970223325	4.20925878	0.422560806	
	40	5.68603214	5.69306931	5.086848635	5.48865003	0.347988001	**>320**
	80	7.046	6.31188119	6.575682382	6.64443302	0.3717264	
	160	7.78739184	7.7970297	7.94044665	7.84162273	0.085719585	
	320	9.14709518	8.29207921	8.933002481	8.79072562	0.444910223	

Table 7:

Conc.(µg/ml)	**SO2C**	**SO2A**	**Ascarbic acid**
5	4.168464742	1.73329051	18.81933188
10	6.809398523	2.764696537	48.49353145
20	20.18199894	4.209258783	72.18356951
40	42.67392711	5.488650027	86.42230486
80	72.14190667	6.644433015	93.19060186
160	83.32731845	7.841622732	95.5014505
320	88.23754003	8.790725623	98.06002939



Conclusion

The findings suggest that Ficus benghalensis leaves possess strong antioxidant properties, making them a potential natural source of bioactive compounds for pharmaceutical, nutraceutical, and cosmetic applications. The presence of polyphenols and flavonoids plays a crucial role in free radical scavenging, which may help in preventing oxidative stress-related diseases. Further research, including in-vivo studies and identification of specific bioactive compounds, is recommended to validate the therapeutic potential of Ficus benghalensis leaves.

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