

Comprehensive Phytochemical Profiling and Comparative Assessment of Antioxidant Capacities of Sequential Leaf Extracts from *Ficus callosa* Roxb.: Implications for Therapeutic and Nutraceutical Applications

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ABSTRACT

Ficus callosa is a tropical fig tree that belongs to the Moraceae family and has numerous biological applications and ecological significance. From southern China and India to portions of Southeast Asia, including Malaysia, it is indigenous to these regions. There has been no previous study to prove that *Ficus callosa* crude leaf extract has antioxidant activity. Therefore, this study was conducted to evaluate the antioxidant effects of various leaf extracts of *Ficus callosa*. Two techniques were used to assess the antioxidant activity of *F. callosa* leaf extracts: DPPH and ABTS radical scavenging assays. The Petroleum ether extract had IC₅₀ values of 100 and 63 µg/ml, and the chloroform extract had IC₅₀ values of 87 and 53 µg/ml. The ethanolic extract showed IC₅₀ values of 96 and 60 µg/mL, respectively, and the IC₅₀ value of standard ascorbic acid for both methods was 40 µg. Therefore, *Ficus callosa* leaves have a dose-dependent antioxidant activity. Because of that, it contains various medicinal properties such as cancer, inflammatory diseases, neurodegenerative disorders, cardiovascular problems, kidney disease, and diabetes.

Keywords: *Ficus callosa*, Moraceae, Antioxidant, DPPH, ABTS, Cancer, leaf extract, pharmacology.

INTRODUCTION

Ficus callosa, a species belonging to the Moraceae family, is a tropical fig tree known for its ecological importance and diverse biological applications. It is native to regions extending from southern China and India to parts of Southeast Asia, including Malesia. This species typically grows in tropical and subtropical forests, often reaching heights of 30–35 meters. The tree's foliage is broad and elliptical, with a glossy green upper surface and a lighter underside. [1-2] The reproductive biology of *Ficus callosa* follows the classic fig-wasp mutualism, where specialized wasps pollinate the fig while using it as a reproductive site. This mutualistic relationship plays a crucial role in maintaining forest biodiversity, as the figs serve as a food source for various frugivorous animals. Due to its ecological significance, *Ficus callosa* has been the

subject of extensive botanical and ecological research. [3-7] Beyond its ecological role, *Ficus callosa* has garnered attention for its phytochemical properties. Studies have identified bioactive compounds, including flavonoids and terpenoids, with potential antioxidant and antimicrobial properties. These compounds have been explored for their medicinal applications, particularly in traditional medicine. Current research aims to understand further this species' chemical composition and potential therapeutic uses. [8-9] With its diverse ecological and pharmacological relevance, *Ficus callosa* remains an important subject of study in botany, conservation, and natural product chemistry. Future research may focus on its conservation status, given habitat fragmentation and environmental changes affecting its distribution. [10-11]

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Free radicals and reactive oxygen species (ROS), which are produced under normal physiological settings but turn harmful when not removed by the body's own processes, are a manifestation of oxidative stress. Actually, oxidative stress is caused by an imbalance between the body's natural antioxidant mechanisms and the production of reactive oxygen species. Both in vivo and in vitro, ROS are important sources of primary catalysts that start oxidation and produce oxidative stress, which leads to a variety of illnesses and conditions like cancer, heart disease, neurological conditions, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol-induced liver disease, ulcerative colitis, aging, and atherosclerosis. Overproduction of ROS is detrimental because it triggers bimolecular oxidation, which results in oxidative stress and cell death. Furthermore, oxidative stress results in unintentional enzyme activation and oxidative system damage in cells. [12] Antioxidants can be found in medicinal plants. Natural antioxidants lower the risk of several illnesses, including cancer, heart disease, and stroke, and boost the plasma's antioxidant capacity. [13-14] Plant secondary metabolites such as flavonoids and phenolics are effective scavengers of free radicals. They can be found in every part of plants, including the bark, roots, leaves, fruits, and seeds. [15]

MATERIALS AND METHODS

Collection of plant material and authentication:

The leaves of *Ficus callosa* were collected from Nithiravilai, Kanyakumari district in February and authenticated by Dr. S. Jaya kumar, associate professor in department of botany N. M. Christian College, Marthandam, Tamil Nadu, India.

Preparation of Extract:

After being allowed to dry in the shade at room temperature, the plant root was ground into a coarse powder using a dry grinder. 20 grams of this coarse powder was placed into a Soxhlet apparatus and subjected to sequential extraction with 150ml of petroleum ether, chloroform, and ethanol. The extraction process was repeated until the solvent's hue in the siphon tube turned colorless. Using a rotary evaporator petroleum ether, chloroform, and ethanol extracts were evaporated at 60°C.

Percentage Yield:

After being allowed to dry at room temperature, the % yield of petroleum ether, chloroform, and ethanol extract was calculated.

Qualitative phytochemical analysis:

Several active phytoconstituents, including steroids, alkaloids, tannins, phenolic compounds, flavonoids, diterpenes, triterpenes, and saponins, were investigated in petroleum ether, chloroform, and ethanol.

EVALUATION OF ANTIOXIDANT ACTIVITY:

DPPH scavenging activity:

The DPPH scavenging activity of the sample was evaluated using the 1,1-diphenyl,2-picrylhydrazyl (DPPH) method. To summarize, a 0.4 mM DPPH solution was prepared in methanol, and 2 ml of this solution was mixed with petroleum ether, chloroform and ethanol extracts at varying concentrations of 20, 40, 60, 80, and 100 µg/ml. The mixture was allowed to sit at room temperature for 20 minutes before measuring the absorbance at 517 nm against blank samples. Ascorbic acid was used as a reference compound, and the experiment was repeated three times. The control cuvette included all reagents except the test sample extract.

$$\text{Inhibition (\%)} = (\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100$$

ABTS radical scavenging activity:

ABTS radical scavenging activity of the samples was evaluated using the 2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay. In summary, an ABTS solution was prepared by combining an ABTS stock solution (7mM) with a potassium persulfate solution (100mM) in methanol. Two milliliters of this solution was then added to various concentrations (20, 40, 60, 80, and 100 µg/ml) of petroleum ether, chloroform and ethanol extracts. The solution was allowed to sit at room temperature for 20 min before the absorbance was measured at 734 nm against blank samples. Ascorbic acid was used as the reference compound and the experiment was performed in triplicate. The control cuvette contained all reagents, except for the test sample extract.



Inhibition (%) = (Abs control - Abs sample / Abs control) x 100 ^[16]

RESULTS

Percentage yield:

The percentage yield of extract of *Ficus callosa* was shown in Table No.1, All the extracts were stored in a well-closed container at 4°C.

Table 1: Percentage yield of various extracts of *Ficus callosa*

Extract	Percentage yield (%w/w)
petroleum ether	2.8
chloroform	2.0
ethanol	1.6

Table 2: Result of Phytochemical analysis of *Ficus callosa* extract of petroleum ether, chloroform and ethanol

Constituents	petroleum ether extract	Chloroform extract	Ethanol extract
Alkaloids	+++	++	+++
Carbohydrates	++	+	++
Glycosides	-	-	-
Saponins	+	-	+
Proteins	-	-	+
Amino acid	-	-	-
Phenols	+	++	++
Flavonoids	-	+	-
Terpenoids	+	++	-
Steroids	+	+++	+

+++ = strong reaction, ++ = moderate reaction, + = mild reaction, - = absence.

DPPH radical scavenging assay

The free radical scavenging activity of the *F. callosa* leaf extracts was determined using the DPPH method. The petroleum ether, ethanolic, and chloroform extracts were evaluated and compared with standard

Qualitative phytochemical analysis:

Petroleum ether, chloroform, and ethanol extracts of leaves of *Ficus callosa* were analyzed phytochemically, and the results are shown in Table No.2.

The phytoconstituents present in the petroleum ether extract are Alkaloids, Carbohydrates, Saponins, Proteins, Phenolic compounds, and Steroids. Chloroform extracts showed the presence of Alkaloids, Carbohydrates, Phenolic compounds, Flavonoids, Steroids, and Terpenoids. Ethanolic extract showed the presence of Alkaloids, Carbohydrates, Saponins, Proteins, Phenolic compounds, and Steroids.

ascorbic acid. The petroleum ether extract showed an IC₅₀ value of 100µg/mL. The chloroform extract showed an IC₅₀ value of 87µg/mL and the ethanolic extract showed an IC₅₀ value of 96 (µg/mL) and the IC₅₀ value of standard ascorbic acid is 40 µg/ml.

Table.no 3: IC₅₀ concentration of petroleum ether, chloroform and ethanolic extract of *Ficus callosa* and compare with standard in DPPH method

Extracts	Concentration(µg/ml)	Percentage inhibition	IC ₅₀ Value
petroleum ether extract	Control	0	100(µg/mL)
	20	14.85±0.67	
	40	16.73±0.34	
	60	21.23±0.11	
	80	35.10±0.48	
	100	50.77±0.22	
Chloroform extract	Control	0	87(µg/mL)
	20	12.02±0.34	
	40	30.16± 0.17	



	60	42.46± 0.11	
	80	44.97± 0.25	
	100	66.10± 0.27	
Ethanol extract	control	0	96(µg/mL)
	20	12.13±0.20	
	40	24.16±0.27	
	60	32.32±0.07	
	80	43.82±0.208	
	100	52.40±0.41	
Standard (Ascorbic acid)	control	0	40 (µg/mL)
	20	35.84±0.11	
	40	50.10±0.39	
	60	64.67±0.33	
	80	78.42±0.12	
	100	87.83±0.22	

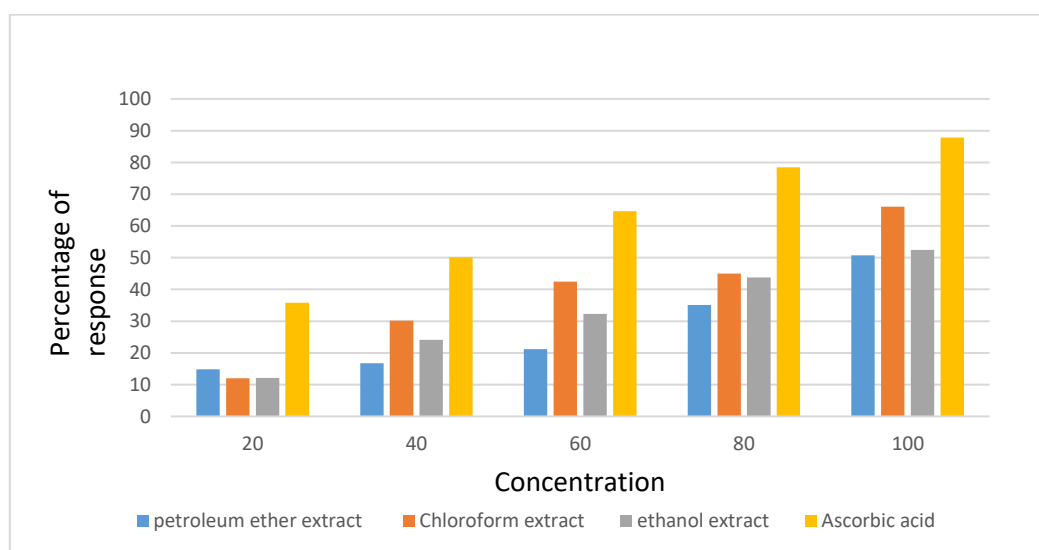


Fig.no 1: Graphical representation of DPPH method of extracts and standard

ABTS scavenging assay

The free radical scavenging activity of the *F. callosa* leaf extracts was determined using the ABTS method. The petroleum ether, ethanolic, and chloroform extracts were evaluated and compared with standard

ascorbic acid. The petroleum ether extract showed an IC₅₀ value of 63 µg/mL. The chloroform extract showed an IC₅₀ value of 53 µg/mL and the ethanolic extract showed an IC₅₀ value of 60 (µg/mL) and the IC₅₀ value of standard ascorbic acid is 40 µg/mL.

Table.no 4: IC₅₀ concentration of petroleum ether, chloroform and ethanolic extract of *Ficus callosa* and compare with standard in ABTS method

Extracts	Concentration(µg/mL)	Percentage inhibition	IC ₅₀ Value
petroleum ether extract	Control	0	63(µg/mL)
	20	39.62±0.57	
	40	41.98±0.74	
	60	48.11±0.56	
	80	54.12±0.16	
	100	58.01±0.32	



Chloroform extract	control	0	53($\mu\text{g/mL}$)
	20	34.92 $\pm$ 0.28	
	40	40.78 $\pm$ 0.06	
	60	56.33 $\pm$ 0.27	
	80	67.34 $\pm$ 0.06	
	100	74.64 $\pm$ 0.39	
Ethanol extract	control	0	60($\mu\text{g/mL}$)
	20	30.71 $\pm$ 0.22	
	40	43.25 $\pm$ 0.29	
	60	50.97 $\pm$ 0.16	
	80	65.21 $\pm$ 0.11	
	100	71.10 $\pm$ 0.06	
Standard (Ascorbic acid)	control	0	40($\mu\text{g/mL}$)
	20	36.61 $\pm$ 0.38	
	40	50.14 $\pm$ 0.42	
	60	64.96 $\pm$ 0.22	
	80	80.70 $\pm$ 0.29	
	100	89.05 $\pm$ 0.68	

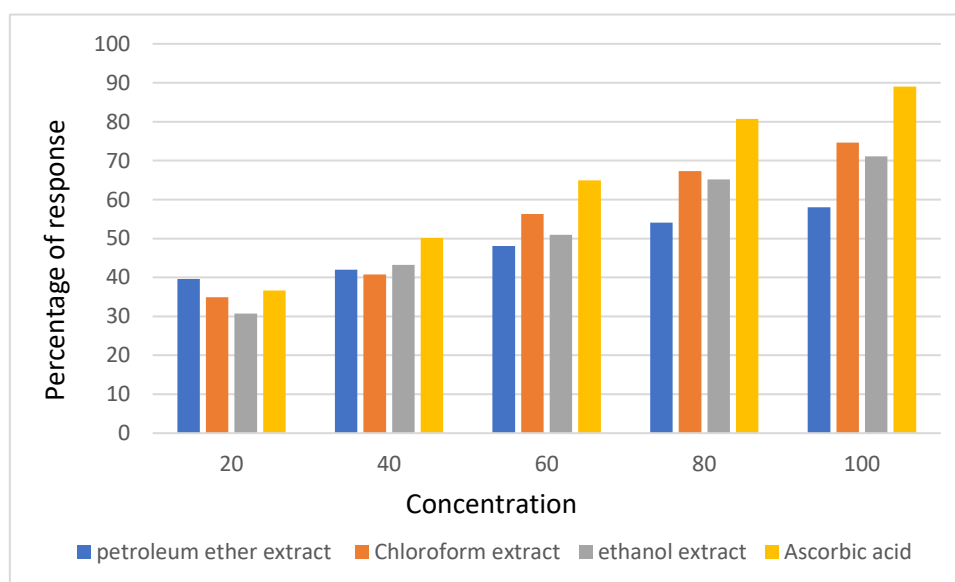


Fig.no 2: Graphical representation of DPPH method of extracts and standard

DISCUSSION

An imbalance between free radicals and antioxidants leads to oxidative stress. This imbalance is connected to a variety of health conditions, including cancer, inflammatory diseases, neurodegenerative disorders, cardiovascular problems, kidney disease, and diabetes with its associated complications. Free radicals, typically encompassing reactive oxygen species and reactive nitrogen species, are characterized by their reactivity and instability, functioning as both oxidizing and reducing agents.[17] Growing interest

in the possible health advantages of natural substances and plant extracts with antioxidant qualities has occurred in recent years. Phenolics, flavonoids, and carotenoids are among the compounds that have been shown to help lower the risk of chronic diseases and minimize oxidative damage. Investigating these endogenous antioxidants presents encouraging opportunities for both therapeutic and preventative approaches to oxidative stress-related illnesses. However, consuming high doses of antioxidants, even from natural sources, can be risky. In some instances, they may act as pro-oxidants, potentially worsening



the oxidative stress. Overconsumption of vitamins C or E, for instance, might cause redox imbalance, interfere with regular cellular signaling, or even encourage the formation of tumors. Additionally, such high doses may reduce the effectiveness of chemotherapy or radiotherapy by counteracting the oxidative stress required to target cancer cells. Further research is necessary to determine the safe and effective dosages for clinical use. Developing successful therapies requires an understanding of the dual function that free radicals play in health and illness. Even though the body has robust antioxidant defenses, the balance between the creation of free radicals and antioxidant capacity is greatly influenced by lifestyle, nutrition, and exposure to the environment. New treatments that use natural antioxidants to fight oxidative stress and enhance human health could result from developments in this area. [18–20] A phytochemical study has shown the presence of Alkaloids, Carbohydrates, Saponins, Phenolic compounds, proteins, flavonoids, terpenoids, glycosides, and steroids. There has been no previous study to prove that *Ficus callosa* crude leaf extract has antioxidant activity. Therefore, this study was conducted to evaluate the antioxidant effects of various leaf extracts of *Ficus callosa*. The antioxidant activity of *F. callosa* leaf extracts was determined by two methods: DPPH and ABTS radical scavenging assays. The Petroleum ether extract had IC₅₀ values of 100 and 63 µg/ml, and the chloroform extract had IC₅₀ values of 87 and 53 µg/ml. The ethanolic extract showed IC₅₀ values of 96 and 60 µg/mL, respectively, and the IC₅₀ value of standard ascorbic acid for both methods was 40 µg. Therefore, *Ficus callosa* leaves have a dose-dependent antioxidant activity. Because of that it contains various medicinal properties such as cancer, inflammatory diseases, neurodegenerative disorders, cardiovascular problems, kidney disease, and diabetes.

CONCLUSION

The anti-oxidant activity of *Ficus callosa* leaf has been screened using DPPH, ABTS methods, and compared with standard ascorbic acid, and all extracts showed dose-dependent activity. When compared with the three-extract chloroform extract has more anti-oxidant activity. There is no antioxidant activity

performed for a crude extract of *Ficus callosa*. But even with the promising in vitro results, more thorough full-scale studies are needed to confirm these preliminary results. The efficacy, safety, pharmacokinetics, and mechanism of action of such plant-derived compounds should be evaluated through comprehensive preclinical research employing suitable in vivo models, followed by designed clinical trials. Such evidence is required to support their therapeutic application in the prevention and treatment of cardiovascular disorders and a variety of malignancies.

ACKNOWLEDGEMENT

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Conflicts of Interest: None

Source of Funding: None

Conflict of Interest: The authors declare no competing interests.

REFERENCES

1. Corner EJH. *Ficus callosa* in The *Ficus* of Borneo. Royal Botanic Gardens, Kew; 1965:102.
2. Berg CC, Corner EJH. Moraceae (*Ficus*). Flora Malesiana. Vol 17. National Herbarium of the Netherlands; 2005:1-730.
3. Li H, Li J, Yang J, et al. Phylogeny Reconstruction of *Ficus* to Reveal the History of Fig-Wasp Mutualism. *Symbiosis*. 2007;45(1-3):45-56.
4. Rønsted N, Weiblen GD, Cook JM, et al. 60 Million Years of Co-Divergence in the Fig-Wasp Symbiosis. *Proc Biol Sci*. 2005;272(1581):2593-2599.
5. Harrison RD. The Role of Figs in Tropical Rainforest Diversity. *Bioscience*. 2005;55(12):1053-1064.
6. Shanahan M, So S, Compton SG, et al. Fig Consumption by Vertebrate Frugivores: A Global Review. *Biol Rev Camb Philos Soc*. 2001;76(4):529-572.
7. Harrison RD, Yamamura N. Evolutionary Hypotheses for Dioecy in *Ficus*. *Oikos*. 2003;100(3):628-635.



8. Phan VK, Cuong NX, Kim DC, et al. Chemical Constituents and Antioxidant Activity of *Ficus callosa*. *Nat Prod Commun*. 2011;6(2):207-210.
9. Phan VK, Cuong NX, Kim DC, et al. Flavonoid and Megastigman Constituents from *Ficus callosa*. *Chem Pharm Bull (Tokyo)*. 2011;59(5):605-607.
10. Hariri F, Rachman RY, Siregar UJ, et al. Molecular Identification and Morphological Characterization of *Ficus* Species from Bengkulu, Indonesia. *Biodiversitas*. 2021;22(6):36-44.
11. Zhou Y, Zhang Y, Wang X, et al. Predicting Seed Yield in *Ficus* Fruits Using Fruit Dimensions. *Plant Signal Behav*. 2019;14(8):1616653.
12. Chanda, S. and Dave, R., 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, 3(13), pp.981-996.
13. Rice-Evans C (2004). Flavonoids and isoflavones: absorption, metabolism and bioactivity. *Free Rad. Biol. Med*. 36: 827-828.
14. Prior RL, Cao G (2000). Antioxidant phytochemicals in fruits and vegetables. Diet and health implications. *Hortic. Sci*. 35: 588-592.
15. Mathew S, Abraham TE (2006). In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem. Toxicol*. 44: 198-206.
16. Vinothini, K.V. and Shervin Jose, S., 2024. Evaluation of anti-oxidant and anti-cancer activity of root extracts of *artocarpus hirsutus* by in-vitro methods. Vol 13, Issue 18, 615-636.
17. Sharma, N., Sharma, A. and Lee, H.J., 2025. The antioxidant properties of green carbon dots: a review. *Environmental Chemistry Letters*, pp.1-49.
18. Chandimali, N., Bak, S.G., Park, E.H., Lim, H.J., Won, Y.S., Kim, E.K., Park, S.I. and Lee, S.J., 2025. Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discovery*, 11(1), p.19.
19. Hrelia, S., Barbalace, M.C. and Angeloni, C., 2025. *Agri-Food Wastes as Natural Source of Bioactive Antioxidants—Third Edition*. *Antioxidants*, 14(2), p.198.
20. Mustafa, Y.F., 2025. Role of fruit-derived antioxidants in fighting cancer: a narrative review. *Indian Journal of Clinical Biochemistry*, pp.1-18.

HOW TO CITE: S. Shervin Jose*, R. Roshinee, G. M. Vishal Bharathi, J. Jenisha, S. Aishwarya Laxmi, V. Vinslet and M. J. Nishani, Comprehensive Phytochemical Profiling and Comparative Assessment of Antioxidant Capacities of Sequential Leaf Extracts from *Ficus callosa* Roxb.: Implications for Therapeutic and Nutraceutical Applications, *J. Pharm. Sci.*, 2025, 1 (11), 325-331. <https://doi.org/10.5281/zenodo.17551904>

