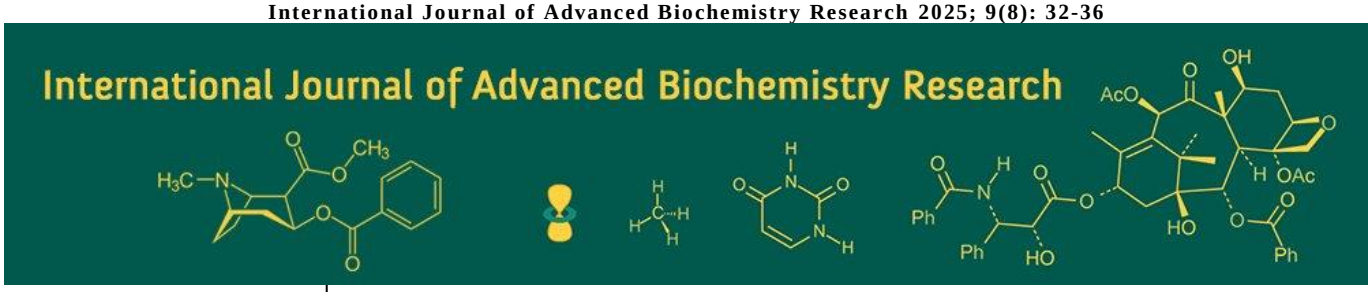

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Phytobioactive and *in vitro* antioxidant analysis in leaf extract of *Ficus carica* L.

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Abstract
The current research was conducted to determine the antioxidant capacity of *Ficus carica* L. leaves ethanol extract and its phytochemical analysis for its total phenolic, polyphenols, and total flavonoids. Using herbal leaf extracts as analytes, the nutraceutical effects of phytochemicals were investigated in defense against oxidative stress, inflammation, and immune-related diseases. The metabolic effects of antioxidant activities were biochemically altered through *in vitro* assays to compare the antioxidant properties, which included quantification of leaf extracts by FRAP (Ferric Reducing Antioxidant Power) assay, ABTS scavenging activity against ascorbic acid and DPPH assay. Phytochemicals were revealed to significantly increase phenols, polyphenols, and flavonoid compounds, which possess an essential role in chemoprevention of aging. Phenolic compounds extensively possess metabolic effects in scavenging free radicals, antioxidant properties and the reducing capability of *Ficus carica* L. leaf extract. *in vitro* antioxidant profiling showed a vital character in neutralizing the scavenging of free radicals present in the body, which protects tissues and cells from the oxidative damage. Metabolic profiling of DPPH, FRAP, and vitamin C assays showed an increase in oxidative stress of cellular health and diagnosed or reduced the risk of anti-inflammatory, antibacterial, antidiabetic, antifungal, and neurodegenerative aging.

Keywords: *Ficus carica* L, phytochemicals, phenols, flavonoids, aging

1. Introduction
Worldwide, human beings use diverse forms of conventional medicinal plants to predict phytochemically bioactive substances and therapeutic effects of aging, which can be metabolizing the cellular antioxidants. The chemo preventive measure of *in vitro* antioxidants is to detect the neurodegeneration volatility during the period of aging, which leads to delineating cognitive decline (Kuriyama *et al.*, 2006) [11]. A medicinal plant such as *Ficus carica* L. (Moraceae: Family) possesses foremost metabolic components such as vitamins A, E, ascorbic acid, flavonoids, polyphenols, which are phenolic compounds and found to be rich in antioxidant potentials. The antioxidant substrates help to mitigate the oxidative stress and protect against diseases like gastric ulceration, diabetes (T2D), and neurodegenerative diseases. The antioxidant compounds source are phytochemicals such as the phenolics, ascorbic acid, flavonoids, coumarins, carotenoids, amino acids, saponins, and polyphenols present in different herbal plant species related to promoting antioxidant capacities (Abdulhafiz *et al.*, 2020) [1]. Up-to-date medicine, phytochemicals, and *in vitro* antioxidant profiling are essentially reliable for the significant or biological effect on food supplements. Traditional complementary alternative medicines are primarily obtained from plants (Swilam and Nematallah, 2020) [18].
Ficus carica (L.) comes under Moraceae family, commonly known as figs, local regionally in Tamil it's called as **அத்திப்பழம்** its origin of phenolic compounds; proanthocyanidins, however, phenolic chemicals found in red wine and tea have lower phenol content than figs. (Li *et al.*, 2007) [12]. Its root, leaves and fruit stand used in historic medicine to treat gastrointestinal (gastritis), respiratory (bronchitis), and atherosclerosis and as antispasmodic and anti-inflammatory activity. Fig leaf extracts from various plants show significant affluence in phytochemicals like flavonoids, phenolic acids, saponins, coumarins,

carotenoids and polyphenols. These compounds have been quantified and used for their antioxidants. Antioxidants neutralize free radicals according to (Re *et al.*, 1999) [15], which can cause cellular damage and contribute to chronic diseases. Medicinal benefits Fig are antispasmodic, metabolic, respiratory and cardiovascular, it has various phenolic compounds, which enhance the anti-inflammatory properties and also improve the neuroinflammation a putative marker for neurodegeneration. Phytochemicals retain a extensive assortment of biological functions, which includes antioxidant, antiapoptosis, anticarcinogen, anti-atherosclerosis, antiinflammation properties, endothelial capacity improvement, including angiogenesis inhibition. Phenolic compounds confirm the antioxidant capacity, which is essential in reducing the effect of free radicals and neutralizing oxidative stress. It is a substantial source of neurodegenerative diseases in pathogenesis for dementia like Alzheimer's, Parkinson's, schizophrenia, and psychosomatic disease Prior *et al.*, 2005 [14].

Recently, more attention has been persistently focused on determining the phytochemicals and their metabolic effects, showing more antioxidants like polyphenols, flavonoids, and phenolic substances, particularly in crude extracts from traditional medicinal herbs (Christova-Bagdassarian *et al.*, 2014) [5]. Most antioxidant phytochemicals have been found to have high neuroinflammatory and antimicrobial properties (Alabri *et al.*, 2014) [3]. The present study analyses the scavenging of free radicals and antioxidants, which are DPPH, ABTS, FRAP & total antioxidant capacity, and phenolic metabolites such as flavonoids and polyphenols. Phenolic content can facilitate and protect the neuronal cells from aging and longevity, which also leads to supporting neurodegeneration.

2. Materials and Methods

The plant material was collected from the farm of Dhanalakshmi Srinivasan Agriculture Farm, Perambalur, during February 2025. The leaves of *F. carica* L. were dried instantly subsequent to harvest in the shade and well aired in the Biochemistry laboratory, DSAC, Perambalur in two weeks. Next, they were packed in plastic stacks and kept in a dark room overnight. After that the leaves were managed using a Soxhlet extractor using dissimilar solvents subjected to ethanol, ethyl acetate, and methanol. 50 g of powdered sample of fig leaves were weighed and soaked in 50 ml of three different solvents. Then the mixture was incubated in the orbital shaker at 40°C with 140 rpm for 48 hours. Now, the mixture was filtered through Whatman filter paper, and the filtrates were evaporated, concentrated in room temperature and stored at 4°C until further use.

2.1 Phytochemical analysis

a) Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent technique was used to determine the total phenolic content (Azhagu Madhavan *et al.*, 2024) [16]. Its reagent 250 µl was combined with 100 µl of leaf extract. Let it sit at room temperature for five minutes. After adding 1.5 cc of 20% sodium bicarbonate, incubate for two hours. The UV spectrophotometer was used to detect the maximum absorption at 765 nm. µg of gallic acid equivalents (GAE)/mg of dry extract was used to express the results.

As recommended by Azhagu Madhavan (et al., 2024) [16], the Folin-Ciocalteu reagent was used to measure the total phenolic content [16]. Its reagent 250 µl was was mixed with an aliquot of 100 µl of the leaf extract. For five minutes, the mixture was left to stand at room temperature. Following the addition of 1.5 ml of a 20% sodium bicarbonate solution, the mixture was incubated for two hours. The UV spectrophotometer was used to record the highest absorption at 765 nm, for the dry extract, the results were reported as µg of gallic acid equivalents (GAE)/mg.

b) Total flavonoid contents (TFCs)

The total flavonoid content (TFCs) was measured based to aluminium chloride colorimetric technique Azhagu Madhavan (*et al.*, 2024) [7] and catechin equivalents expressed as mg (CE)/g of the dried extract. The standard of catechin was used for the determination of total flavonoids and analysed at 510 nm, total TFCs were declared in terms of catechin equivalents (CE) in µg/mg.

c) Total Polyphenols

The Li *et al.* (2007) [12] technique was used to calculate the total amount of polyphenols, the 100 µL of each of the extract was taken and mixed Folin-Ciocalteu reagent 500 µL (10%). Afterward, four min, the 7.5% sodium carbonate (Na_2CO_3) solution 400 µL was added. Prior to the estimation of polyphenol content, the chemicals was adjusted through responding at room temperature (25 + 1 °C) for 2 hours to estimate the absorbance at 760 nm, total polyphenols was stated in terms of mg of gallic acid equivalents (mg GAE/g extract).

2.2 *In vitro* Antioxidant Assay

a) ABTS radical scavenging assay

The total capacity of antioxidant of the extracts was analyzed according to Sumathy *et al.*, 2024 [6]. ABTS [2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid] radicals were generated through an oxidation reaction with potassium persulfate. The mixing of the ABTS + radical cation with potassium persulfate was kept for 16 hrs. under incubation at room temperature until further use. The ABTS assay was performed with leaf extract in addition to the reagent, which was diluted with 95% ethanol until the absorption measured reached maximum at 734 nm. The ABTS radical scavenging activity of the extract, compared to ascorbic acid, was used as standard and calculated.

b). Ferric reducing antioxidant power (FRAP)

According to Sumathy *et al.*, 2023 [4], plant leaf extract is assayed with FRAP reagent, which consists of TPTZ 10 mM in HCl 40 mM, $FeCl_3$20 mM, and sodium acetate buffer (pH 3.6) 250 mM. FRAP reagent was newly prepared through TPTZ solution mixing. A solution of $FeCl_3$, with acetate buffer, was prepared in a volumetric 1:1:10 a ratio, an aliquot of 100 µl of the extract solution was varied with FRAP reagent 900 µl, following a the incubation period at 37°C of 4 min in room temperature, the absorbance measurement was carried out at a wavelength of 593 nm, with the results compared to a blank control. The standard Butylated hydroxytoluene (BHT) was used as reference solution for FRAP assay, the results existed quantified and expressed as µg of BHT corresponding per mg of sample.

c). DPPH Radical Scavenging Assay

The potential antioxidant of leaf extracts of *Ficus carica* L. was evaluated in relation to hydrogen donation or free

radical scavenging capacity, using stable radical DPPH (2,2'-diphenyl-1-picrylhydrazyl) as a reagent at 4 mg/100 mL concentration, the interaction with a proton donor, such as an antioxidant, this radical exhibits a colour change from purple to yellow, accompanied by a reduction in absorbance (Vinothini *et al.*, 2017) [13].

The samples 50 µL in different concentrations obtained from DPPH solution 850 µL were added, in ten different test tubes. The readings at 517 nm were recorded after 20 minutes of incubation in dark, at room temperature of (25±1°C). Quercetin solution was used as positive control, the inhibition percentage of DPPH radical was determined using the following formula:

Inhibition% = (AB—AE/AB) × 100

Where,

AB denotes the absorbance of the control (-), and AE signifies the absorbance of the extract.

2.4 Data and statistical analysis

The plant extracts of *Ficus carica* L. were analysed in triplicate for phytochemicals, *in vitro* antioxidants, phenolic, flavonoid, and polyphenol content. The results of antioxidant activity and phenolic-related content are shown as mean±standard error (SE). The mean (n = 3) of phenolic compounds are shown with Duncan Multiple Range Test (DMRT) result for the probability value of less than 0.05. Using SPSS Statistics 21.0 software (SPSS Inc., Chicago, USA), data from phenolic content and *in vitro* antioxidant analysis were subjected to multiple range test (Tukey's test) and analysis of variance (ANOVA) (Daoud *et al.*, 2021) [17].

3. Results and Discussion

3.1 Phytochemical Analysis

Fig. 1 shows phytochemical analysis using different solvent methods evaluated based on the different assays against *in vitro* antioxidants, which are found to be more in ethanol extract (90%) compared to other solvents. Macerated or powdered leaves were treated with ethanol in herbal plants of *Ficus carica* L. Different concentrations were found to facilitate those with the maximum antioxidant activity, such as polyphenols, and also the DPPH and FRAP assay concentrations reveal the strongest anti-inflammatory and neurodegeneration effects in relation to brain health. Glutamine, polyphenols, total phenolic and flavonoid content are compounds that are more beneficial from the perspective of neuroinflammation due to their anti-inflammatory and antioxidant properties (Fig. 2). DPPH activity is found to be as 98.8% and ABTS activity is 94.7% in fig leaf extracts, different analyte concentrations *in vitro* antioxidants showed higher concentration (Fig. 3).

The restorative efficiency of these cellular oxidative stress molecules targets scavenging the free radicals and depends on their bio accessibility. Fig leaf extracts might possess a pivotal role in nerve cell changes, and their metabolic effects show high antioxidant content, predominantly ABTS, which inhibits neuroprotective properties, and they are also rich in antioxidant activities, which show improved cognitive function. Furthermore, fig (*Ficus carica* L.) plant leaf extract possesses helpful insight into phytobioactive compounds such as vitamin C, glutamine, polyphenol, alkaloids, flavonoid, and phenolic content (Li *et al.*, 2007) [12], supporting the immune response, which scavenges the free radicals and helps in improving the neuroinflammatory, antidiabetic, antiarthritic, and antimicrobial properties.

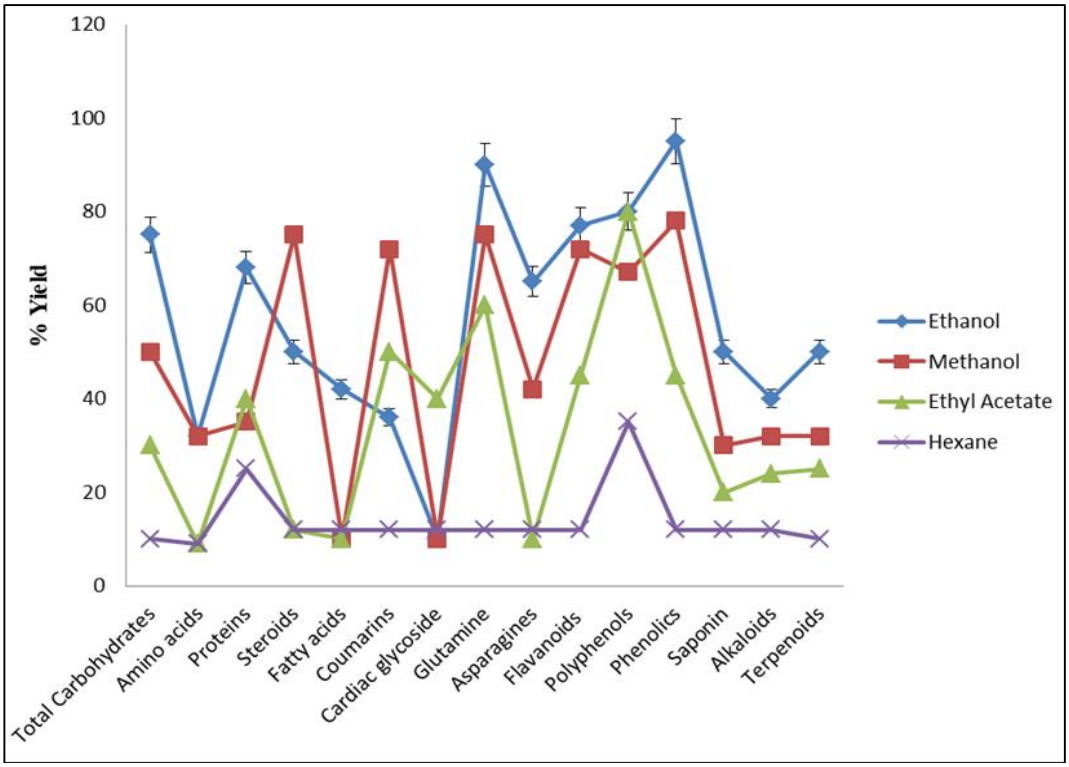

Fig 1: Phytochemical analysis using different solvents in leaf extract-*Ficus carica* L.

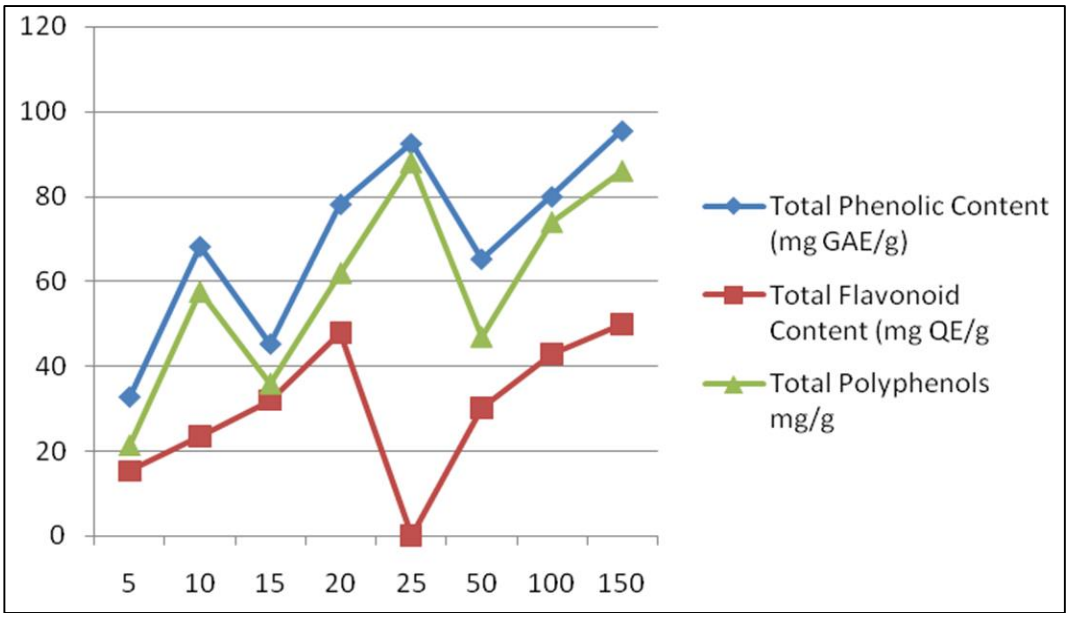

Fig 2: Physiochemical content in leaf extract-*Ficus carica* L.

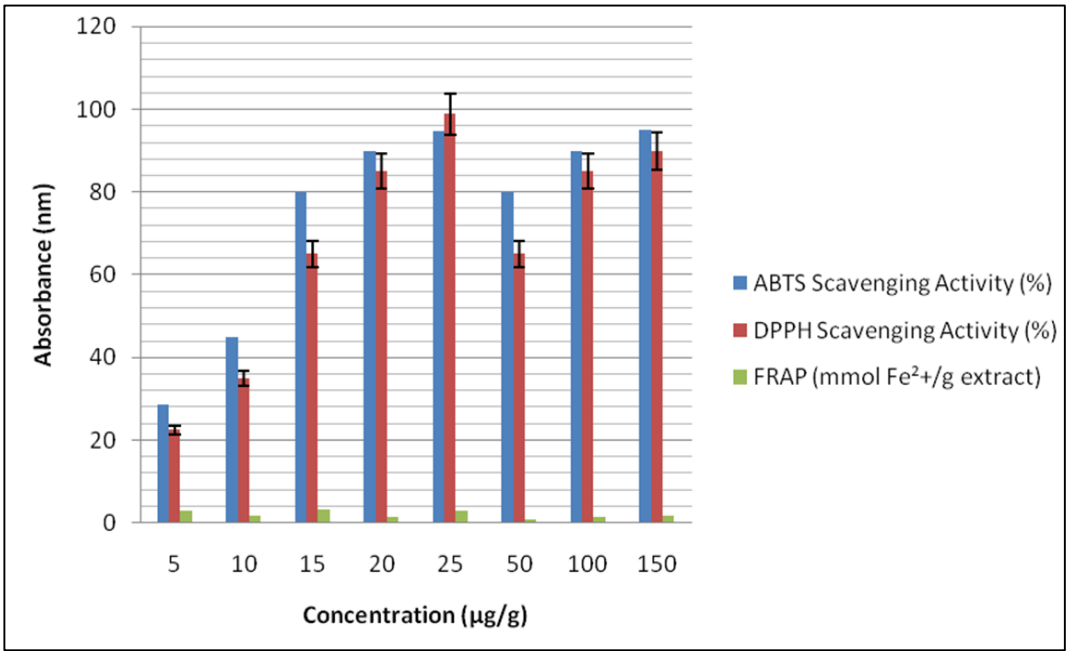

Fig 3: *In-vitro* antioxidant analysis of the leaf extract-*Ficus carica* L.

This phytochemical profile, potential neuroprotective effects and antioxidant properties of *Ficus carica* L was investigated during this study. The extract was found to be rich in bio-active compounds like flavonoids, phenolic acids, alkaloids and tannins (Christova-Bagdassarian *et al.*, 2014) [5]. These phytobioactive constituents are widely known for its antioxidant and neuroprotective properties that contribute towards cognitive health and mitigate neurodegenerative processes (Agati *et al.*, 2012) [2]. The antioxidant assays (e.g., DPPH, ABTS, FRAP) demonstrated significant free radical scavenging activity, suggesting that the extract can effectively neutralize oxidative stress—a key contributor to cognitive decline and neuronal damage according to (Prior *et al.*, 2005; Re *et al.*, 1999; Kahkonen *et al.*, 1999) [14, 15, 10]. High total polyphenolic, phenolic, and flavonoid contents were correlated positively with antioxidant activity, supporting the concept that polyphenolic compounds are important contributors for the pragmatic effects in phytoactive substances (Ivanov *et al.*, 2015) [8]. In the milieu of brain health, oxidative stress is ascertained with various neurological disorders, which include cognitive capability gradually impairing and having an affinity to turn down dementia-type Alzheimer's disease, schizophrenia, Parkinson's disease, and stroke. The bioactive and volatile compounds (Jun *et al.*, 2011) [9] in the plant extract might be helpful for protecting neuronal integrity in reducing free radicals, reactive oxygen species (ROS), improving inner mitochondrial function, and varying in cellular antioxidants. Likewise, a few phytochemicals recognized in the leaf extract (e.g., saponin, coumarin, glutamine, polyphenols, flavonoids, quercetin, kaempferol, or phytocatechins) are known to be involved along the blood-brain barrier and make use of an undeviating effect on nerve cells. Further, assays in nerve cells using plant-based treatments followed by *in vivo* or *in vitro* (e.g., neuronal cytotoxic cell activity, lipid peroxidation, or acetylcholinesterase inhibition) support its potential as a neuroprotective agent.

5. Conclusion

The leaves of *Ficus carica* L. are comprised of bioactive compounds like steroids, triterpenes, glycosides, saponins, tannins and flavonoids These secondary metabolites of the plant exhibit significant efficacy and are utilized in pharmacotherapy for a range of harmful diseases. The comprehensive analysis of phenolic and flavonoid concentrations demonstrates promising results in methanol, thereby facilitating drug discovery and enhancement. The phytochemical profile of *Ficus carica* L. elucidates the existence of multiple bio-active compounds; it has been found that figs are used in traditional medicine for their therapeutic properties in combating aging and neurodegenerative disorders. Antioxidants are important in mitigating oxidative stress, which is a major contributing factor in neurodegenerative diseases.

6. Conflicts of Interest

The authors declare that they have no conflict of interest**.**

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