

Evaluation of antioxidant activity and phytochemicals of *Ginkgo Biloba* leaf extract using ferric reducing antioxidant power (FRAP) method

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Abstract

Introduction: *Ginkgo biloba*, widely recognized as the maidenhair tree, is one of the most ancient and pharmaceutically valuable plants, known for its high content of phytochemicals such as flavonoids and terpenoids. These bioactive compounds are well-documented for their potent antioxidant activity, which plays a critical role in mitigating oxidative stress, a condition characterized by an imbalance between reactive oxygen species (ROS) and the body's natural antioxidant defenses. Oxidative stress is implicated in the pathogenesis of various chronic and degenerative diseases, including cardiovascular conditions, neurodegenerative disorders like Alzheimer's and Parkinson's disease, and certain types of cancer.

Objectives: The objective of this study was to evaluate the antioxidant potential of *Ginkgo biloba* leaf extract using the Ferric Reducing Antioxidant Power (FRAP) assay and to analyze its phytochemical constituents, particularly focusing on the presence of flavonoids and terpenoids.

Methods: The leaves were subjected to extraction using the maceration method with ethanol as the solvent. The antioxidant activity was assessed by FRAP, which measures the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), indicated by the formation of a blue complex measured spectrophotometrically. The antioxidant capacity of the extract was compared with ascorbic acid as a reference standard. In addition, phytochemical screening was conducted to detect the presence of flavonoids and terpenoids, and FTIR (Fourier-Transform Infrared) spectroscopy was employed to identify functional groups that contribute to the antioxidant properties.

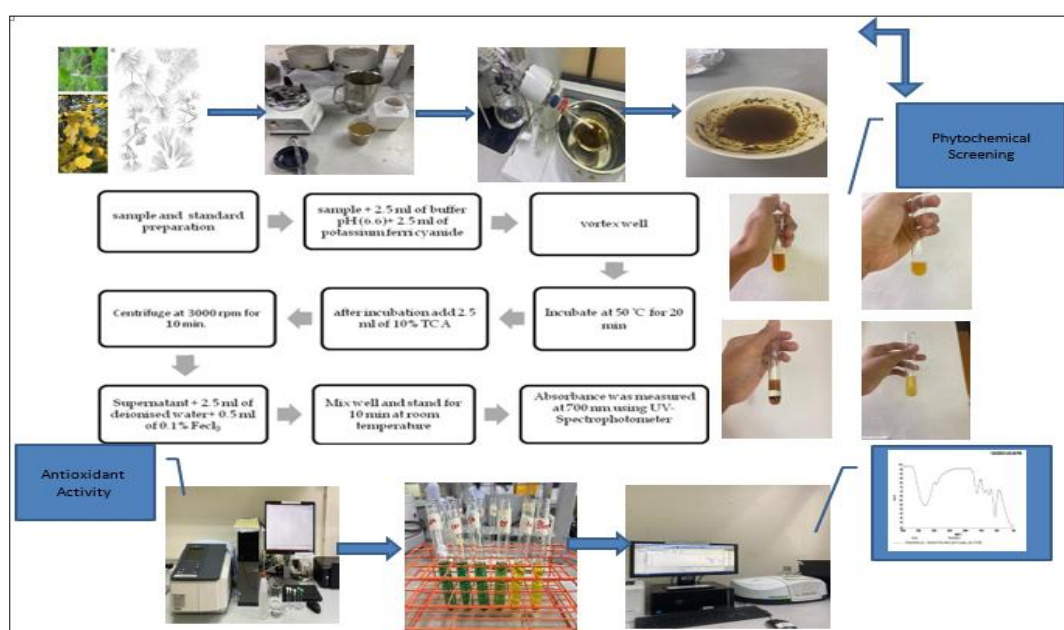
Results: The results revealed a concentration-dependent increase in antioxidant activity, with FRAP values significantly rising from 10 to 200 mg/mL. At higher concentrations, the antioxidant effect of the extract was found to be comparable to that of ascorbic acid, indicating substantial ferric-reducing power. Phytochemical tests confirmed the presence of flavonoids and terpenoids, while FTIR spectra exhibited characteristic absorption bands of hydroxyl (-OH), carbonyl (C=O), and aromatic ring structures, all of which are known to be involved in antioxidant mechanisms.

Conclusion: The study concludes that *Ginkgo biloba* leaf extract possesses significant antioxidant potential and could be utilized as a natural antioxidant in pharmaceutical, nutraceutical, and cosmetic applications. Moreover, its combination of bioactivity and historical use in traditional medicine underscores its relevance in modern therapeutic contexts. Future research should include *in vivo* studies, toxicological assessments, and advanced chromatographic analyses to quantify individual active compounds and validate their specific health benefits in biological systems.

Keywords: *Ginkgo biloba*, antioxidant, FRAP assay, flavonoids, terpenoids, phytochemical screening, FTIR spectroscopy

Introduction

Graphical Abstract



Ginkgo biloba is a well-known plant, commonly referred to as the maidenhair tree. The lineage of this remarkable plant dates back over 250 million years, making it as a living fossil (Boateng & Yang, 2022) [4]. This plant is the sole surviving member of the Ginkgoaceae family as most of its relative has extinct after several centuries. In fact, the Ginkgoatae stem group is estimated to be around 325 million years old, although the radiation of extant Ginkgo species is much more recent, tracing back to about 390,000 years ago (Zhang *et al.*, 2024) [17]. According to Boateng and Yang (2022) [4], this plant was rediscovered in 1670 by Kaempfer in the temple gardens of Asia. It's a native plant in China, Japan and Korea but also could be found in regions such as the United States, Europe, South America, India, and New Zealand due to global cultivation. Celebrated for its perseverance, the Ginkgo tree is not only a symbol of longevity but also of great cultural and therapeutic value. Traditional Chinese medicine has long prized its unusual fan-shaped leaves and seeds for its capacity to heal a range of conditions, including lung and kidney diseases, bronchitis, and stomach pain (Boateng & Yang, 2022) [4]. The medical use of Ginkgo seeds is recorded in ancient sources as the Herbals from 1350 CE, therefore stressing its historical relevance. Along with vitamins A, B, D, and E, ginkgo seeds are high in starch (60–70%), proteins (9–13%), and lipids (3.69–5.6%), as well as trace minerals like calcium, magnesium, iron, phosphorus, and selenium (Zhang *et al.*, 2024) [17]. For its numerous therapeutic properties, *Ginkgo biloba* has evolved into one of the most investigated plants in the current field of modern medicine (Boateng & Yang, 2022) [4]. Especially using standardized extracts like GBE 761, its leaves are used widely in the treatment of numerous known disorders including cognitive decline, vascular dementia, Alzheimer's disease, and cardiovascular diseases. The great diversity of bioactive compounds in its leaves, terpenoids such ginkgolides and bilobalide, and flavonoids such quercetin, kaempferol, and isorhamnetin has been found to be responsible for these medicinal qualities (Zhang *et al.*, 2024) [17]. Since these compounds demonstrate a broad spectrum of therapeutic actions from antioxidant and neuroprotective effects to anti-inflammatory and anticancer capabilities, the *Ginkgo biloba* extract is a valuable weapon in treating over age-related and chronic diseases (Zhang *et al.*, 2024) [17]. The plant's capacity to boost blood circulation and control neurotransmitter and receptor activity in the brain underlines its possibility to support cognitive health and control diseases including Alzheimer's disease and vascular dementia (Boateng & Yang, 2022) [4]. Furthermore, the bioactive elements in it shield cellular structures from oxidative stress, which is a main factor causing degenerative disorders including Parkinson's and cardiovascular diseases (Zhang *et al.*, 2024) [17]. Its anti-inflammatory action emphasizes even more its worth in controlling inflammatory diseases including arthritis and other autoimmune disorders (Zhang *et al.*, 2024) [17]. Apart from its therapeutic uses, *Ginkgo biloba* is quite important in the worldwide herbal supplement market since annual sales of it surpass USD 1.26 billion (Boateng & Yang, 2022) [4]. Its extracts are included in a range of health supplements, drinks, and beauty products because of their multifarious uses that satisfy customer needs for natural and environmentally friendly treatments.

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Apart from its therapeutic uses, *Ginkgo biloba* is quite important in the worldwide herbal supplement market since annual sales of it surpass USD 1.26 billion (Boateng & Yang, 2022) [4]. Its extracts are included in a range of health supplements, drinks, and beauty products because of their multifarious uses that satisfy customer needs for natural and environmentally friendly treatments. In cultural and decorative settings, the tree is also quite prized; its adaptability and symbolic links with lifespan and adaptation make it a common option for public areas and metropolitan settings (Boateng & Yang, 2022) [4].

Ginkgo biloba's unusual mix of ancient pedigree, adaptable medicinal uses, and cultural relevance makes it appealing. With continuous investigations trying to find fresh possible uses and benefits of its bioactive chemicals, this "living fossil" remains a topic of great scientific interest (Zhang *et al.*, 2024) [17]. Its flexibility and high bioactive content confirm its rank as one of the most remarkable plants in both traditional and modern settings, therefore bridging the gap between historical medical practices and modern healthcare advancements (Zhang *et al.*, 2024) [17].

Therapeutic uses

For generations, humans have valued the traditional medicinal herb *Ginkgo biloba* for its extensive therapeutic range. Chinese traditional medicine has traditionally used this plant's leaves and nuts to treat many medical issues. While nuts have been valued for treating respiratory problems including asthma, chest pain, cough, bladder irritation and even alcoholism, ginkgo leaves were historically used to cure heart disorders and skin infections (Tabassum *et al.*, 2022) [14]. Many of these traditional uses are supported by modern studies; *Ginkgo biloba* extract (GBE) is now often used to reduce bronchitis and asthma symptoms.

Ginkgo biloba offers therapeutic possibilities for neurological and cardiovascular conditions as well. Researchers have found that it can help people with Alzheimer's disease, epilepsy cerebrovascular diseases, stroke, and peripheral vascular diseases. Pharmacological studies also show that GBE has a few positive effects, including lowering the risk of cardiovascular disease, increasing cerebral blood flow, decreasing oxidative damage induced by ischemia, and providing hepatoprotective properties. Moreover, its capacity to antagonize platelet aggregation emphasizes its part in enhancing circulatory condition (Tabassum *et al.*, 2022) [14].

Though poorly investigated, GBE shows anticancer activity and suggests use in oncology. Especially entire extracts taken from *Ginkgo biloba* fruit seem to offer more complete therapeutic advantages than isolated molecules (Tabassum

et al., 2022) [14]. This overall effectiveness supports the plant's importance as a versatile medical agent by bridging traditional knowledge with contemporary pharmacological discoveries.

Phytoconstituents of *Ginkgo biloba*

Among the phytochemicals found in *Ginkgo biloba* are 110 known flavonoids, including kaempferol 3-O- α -[6000-p-coumaroyl(β -D)-glucopyranosyl(1,2)-rhamnopyranoside]. Strong antioxidant effects abound within these seven classes of flavonoids: flavanones, isoflavones, flavones, BI flavones, flavan-3-ols, flavanols, and ginkgolides (Tabassum *et al.*, 2022) [14]. Notable substances are glycosides of quercetin, kaempferol, and isorhamnetin, which predominate in

young leaves and have varied biological actions including antioxidant, anti-cancer, anti-bacterial, and neuroprotective properties (Patrycja Biernacka *et al.*, 2023) [3, 20]. Along with recently identified bilobalide isomers and nor-sesquiterpenoids, the terpenoid profile comprises ten diterpenoid lactones—ginkgolides A, B, C, J, K, and M—as well as sesquiterpene bilobalide (Tabassum *et al.*, 2022) [14]. With ginkgolide B showing antioxidant, anti-inflammatory, and anti-apoptotic properties, these terpenoids greatly help to maintain cardiovascular and cerebrovascular health (Patrycja Biernacka *et al.*, 2023) [3, 20].

Examples of phytoconstituent structures found in *Ginkgo biloba* is depicted in Figure 1.

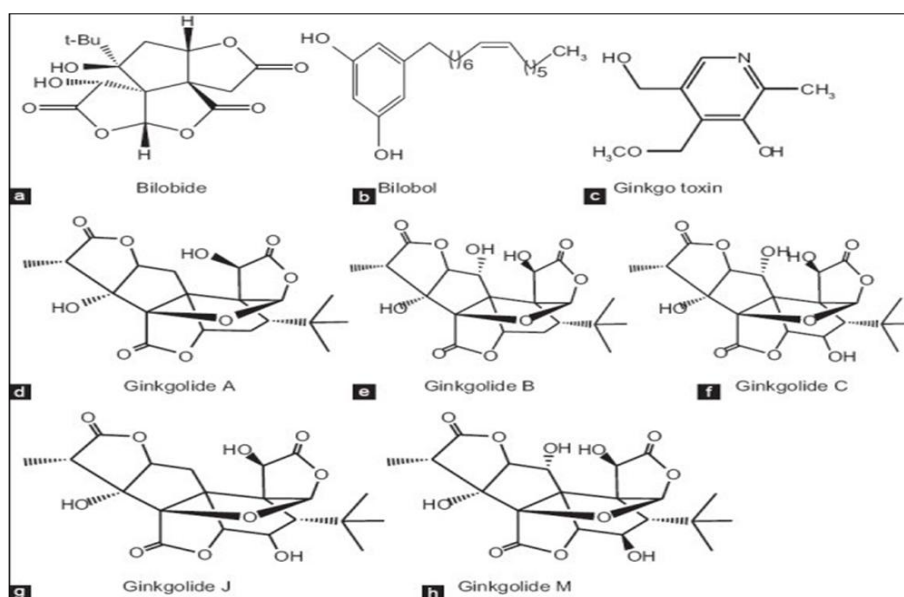


Fig 1: *Ginkgo biloba* phytoconstituents. Image adapted from (Isah, 2015).

Ginkgo biloba also has alkylphenols in it, such as ginkgolic acids, cardol's, cardanols, and urushiols. These chemicals are harmful and can cause mutations, but they have sometimes been shown to have therapeutic effects, like fighting tumours (Patrycja Biernacka *et al.*, 2023) [3, 20]. Strong antioxidant and free radical-scavenging properties abound in carboxylic acids including gallic acid, ferulic acid, and p-coumaric acid as well as quinic and shikimic acids (Patrycja Biernacka *et al.*, 2023) [3, 20]. Present in many plant sections, lignans include pinoresinol, lariciresinol, ginkgool, and sesamin support antioxidant capabilities and structural integrity in cell walls.

In an 85:15 ratio, proanthocyanidins—including prodelfinidin and procyanidin—have cardiovascular advantages with anti-hypertensive and anti-inflammatory properties (Patrycja Biernacka *et al.*, 2023) [3, 20]. Further improving the plant's medicinal profile are polyphenols with immunomodulating effects and polysaccharides from seeds, leaves, and sarcotesta, which show anti-cancer and anti-inflammatory action (Patrycja Biernacka *et al.*, 2023) [3, 20]. Other elements include essential oils high in sesquiterpenes and 4'-O-methylpyridoxine, a poisonous chemical in seeds (Tabassum *et al.*, 2022) [14]. Furthermore, providing essential macronutrients, vitamins, and minerals, *G. biloba* has leaves high in carbohydrates and seeds have good nutritional value.

Antioxidant activity

Long used for medicine, *Ginkgo biloba* is known for its strong antioxidant qualities, which help to explain its therapeutic effectiveness in many different health disorders. Terpenes, flavonoids, and bioflavonoids abound in the plant and are all very important in neutralizing destructive free radicals and lowering oxidative stress. Byproducts of ambient elements and metabolic activity, free radicals are unstable chemicals that can seriously damage DNA, proteins, and lipids among other components of cells. Along with cardiovascular conditions, this oxidative damage is associated to the development of many degenerative diseases including neurological conditions like Alzheimer's disease and Parkinson's. The antioxidant-rich makeup of *Ginkgo biloba* helps offset these negative effects, therefore supporting cellular health and avoiding the damage caused over time by ageing or disease.

One of the main chemicals in *Ginkgo biloba*, ginkgolide B, has been especially found to be able to lower the generation of reactive oxygen and nitrogen species (ROS/RNS) in human cells (Tabassum *et al.*, 2022) [14]. Various tissues have proven that this decrease in ROS/RNS generation reduces oxidative stress, therefore shielding against ischemic damage and stopping more oxidative damage. The brain and cardiovascular system especially benefit from such antioxidant properties since oxidative stress is usually

a main cause of ageing process and the beginning of degenerative disorders. *Ginkgo biloba* is a crucial herb for enhancing general health and longevity since it can prevent ischemic damage as well as treat disorders connected to inadequate blood flow, such stroke and peripheral vascular disease (Tabassum *et al.*, 2022) ^[14].

Apart from its internal health advantages, *Ginkgo biloba* is also attracting interest for its antioxidant properties in cosmetics. One of the main outside variables causing skin ageing, UV light causes ROS to develop in the skin. By breaking down collagen, elastin, and other structural proteins, these ROS induce oxidative damage to cellular components like lipids, proteins, and DNA, therefore hastening the ageing process on skin. The release of matrix metalloproteinases (MMPs) aggravates this damage especially MMP-1, which breaks down dermal collagen and causes wrinkles and drooping skin (Wang *et al.*, 2020) ^[16]. With its great concentration of flavonoids, *Ginkgo biloba*'s leaf extract has been demonstrated to efficiently scavenge ROS, hence lowering oxidative stress and stopping MMP activity. *Ginkgo biloba* is a great weapon in anti-aging skincare treatments since it helps shield the skin from the obvious symptoms of UV exposure, like small lines, wrinkles, and loss of suppleness.

Furthermore, the antioxidant properties of *Ginkgo biloba* go beyond their benefits on ageing skin to include maintaining collagen integrity and boosting circulation, so enhancing the general health of the skin. It is a great addition to cosmetic products meant to improve skin texture and stop early ageing brought on by environmental damage since it can boost microcirculation and shield against oxidative stress (Wang *et al.*, 2020) ^[16]. *Ginkgo biloba* is a very sought-after component in skincare products aiming at ageing and oxidative damage caused by UV exposure because of its antioxidant, anti-inflammatory, and skin protective qualities. Ultimately, *Ginkgo biloba*'s broad medicinal uses depend critically on its antioxidant properties. *Ginkgo biloba* protects against oxidative damage, so preserving good cellular function and preventing the start of age-related degenerative diseases whether used to counteract the effects of ageing on the brain, cardiovascular system, or skin. Both for internal health and outside beauty treatments, this herb is quite helpful since it helps the body defend itself and neutralizes free radicals (Wang *et al.*, 2020) ^[16]. *Ginkgo biloba* is probably going to stay a major participant in both traditional and modern medicine as studies reveal several advantages.

Need of natural antioxidant

Among *Ginkgo biloba* most important features is its vast abundance in natural antioxidants, which are necessary in lowering oxidative stress arising from an imbalance between free radicals and antioxidants in the body (Tabassum *et al.*, 2022) ^[14]. This imbalance destroys cells that hastens ageing and is connected to chronic disorders such diabetes, neurological diseases including Alzheimer's disease, heart disease, even some types of cancer. Among other bioactive substances in *Ginkgo biloba*, terpenoids and flavonoids show great antioxidant action. Taken together, these substances neutralize free radicals, preserve cellular structures, and enable the body to be generally in physiological homeostasis. *Ginkgo biloba* lowers oxidative damage, thereby improving health results and lowering the chance of the development of chronic diseases. The natural

supply of antioxidants of *Ginkgo biloba* explains one of its most important effects: a safer and more biocompatible substitute for manufactured antioxidants. Strong synthetic antioxidants have generated questions regarding probable toxicity and harmful consequences when used in high concentrations over a long period of time. Conversely, *Ginkgo biloba* has long been used in traditional medicine and taken at the right dosage, there is no evidence of any negative consequences. *Ginkgo biloba* leaves and seeds were used in traditional Chinese medicine to treat a variety of conditions, including cognitive impairment, circulatory difficulties, and pulmonary congestion (Barbalho *et al.*, 2022) ^[2]. Modern studies underline the antioxidant properties of the plant for their power to protect nerve cells, improve vascular health, and boost memory and cognitive performance especially in populations of ageing people, so supporting these benefits. These features of *Ginkgo biloba* offer promise as a treatment for age-related disorders and aid to retain energy.

Moreover, quite helpful in reducing oxidative stress-related damage to the cardiovascular systems and brain are the antioxidants of *Ginkgo biloba* (Barbalho *et al.*, 2022) ^[2]. Important in reducing heart attacks and strokes, the flavonoids in its leaves have been found to increase blood flow, strengthen capillaries, and lower the chance of blood clot formation. Its terpenoids, ginkgolides and bilobalide, lower inflammation, boost neurotransmitter function, and insulate neurons from oxidative damage, thereby having neuroprotective properties. *Ginkgo biloba* is a full natural remedy for ageing symptoms since twin uses in brain and cardiovascular health. Furthermore, its ability to lower oxidative stress in cellular structures could have protective consequences against the growth of certain malignancies, therefore strengthening its overall spectrum of advantages (Barbalho *et al.*, 2022) ^[2].

Ginkgo biloba has become somewhat popular in the modern setting as a dietary supplement for those trying to improve their general well-being and reduce the consequences of oxidative stress produced by surroundings and way of life. Sometimes pollution, poor diets, and stress overwhelm the body's natural antioxidant defenses; so, adding natural antioxidants such as those included in *Ginkgo biloba* becomes quite crucial. *Ginkgo biloba* not only solves the basic causes of many chronic diseases but also increases lifespan and better quality of life by helping the body's defensive systems to reduce oxidative damage. Still a pillar of natural medicine, *Ginkgo biloba* illustrates the range of health advantages by crossing conventional wisdom with recent scientific evidence.

Reason and Method of Assay of *Ginkgo biloba*

An evaluation of the antioxidant action of *Ginkgo biloba* will help one to decide its therapeutic value and guarantee the potency of its extracts. The capacity of the plant to neutralize harmful reactive oxygen species (ROS) and reactive nitrogen species (RNS) is basic to its power to reduce oxidative stress-related ailments including cardiovascular diseases, neurological diseases, and ageing-related skin damage (Tabassum *et al.*, 2022) ^[14]. Standardizing Ginkgo-based supplements and identifying important bioactive ingredients like terpenoids and flavonoids supporting their favorable effects depends also on quantitative evaluation of antioxidant activity. Depending on its antioxidant characteristics, this work is

essential for the development of ideal medical and cosmetic compositions.

The antioxidant power of *Ginkgo biloba* is extensively studied by several techniques. Inspired by a purple to yellow color change, the DPPH radical scavenging assay investigates if antioxidants may provide hydrogen atoms to stabilize DPPH radicals (Schaich *et al.*, 2015) [13]. Though basic and often used, it examines only one type of radical and has little physiological value. Although the ABTS radical cation scavenging assay measures the potential of hydrophilic and lipophilic antioxidants to neutralize ABTS radicals, its non-specificity can magnify antioxidant capacity (Schaich *et al.*, 2015) [13]. Though it takes time and requires equipment, the ORAC (Oxygen Radical Absorbance Capacity) assay measures the capacity of antioxidants to scavenge peroxy radicals using fluorescent probes. The Total Phenolic Content (TPC) assay counts phenolic substances to indirectly assess antioxidant capacity but unable of measuring direct antioxidant activity (Schaich *et al.*, 2015) [13].

Among these techniques, the Ferric Reducing Antioxidant Power (FRAP) assay is thought best appropriate for evaluating *Ginkgo biloba*'s antioxidant capacity. Measuring the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), the FRAP assay generates a blue complex readily visible spectrophotometrically (Munteanu & Apetrei, 2021) [10]. This low-cost, basic, dependable approach produces consistent outcomes even without specific gear. Significantly, the FRAP test measures the overall reduction capability of antioxidants, providing a more complete view of antioxidant capacity than methods as DPPH and ABTS that concentrate on radical scavenging. Its biological relevance also arises from its capacity to replicate deteriorating conditions in biological systems, thereby enabling a more accurate portrayal of the antioxidant activity of *Ginkgo biloba in vivo*. These properties characterize FRAP as the recommended technique for thorough and consistent assessment of antioxidants.

Methodology

Dried *Ginkgo biloba* leaves are first cleaned and powdered into a fine powder to prepare the sample. Since ethanol is extremely effective at extracting phenolics and flavonoids, usually at a concentration of 70%,

it will be used as the extraction solvent. A specified quantity of the powdered leaves, 50 g, is placed into a container for the maceration process and 500 mL of ethanol is added to completely submerge the sample (Abubakar & Haque, 2020) [1]. The mixture is left at room temperature for 24 to 72 hours and the container is tightly closed to stop evaporation (Abubakar & Haque, 2020) [1]. To improve the extraction efficiency, the solution is stirred intermittently, that is, every eight hours during this duration. The mixture is filtered through Whatman filter paper following the maceration phase to separate the liquid extract from the plant residue. To eliminate ethanol, the filtrate is next concentrated under low temperature (about 40°C) using a rotary evaporator, hence producing the crude extract. Until further research is conducted, this extract is kept at 4°C in an airtight amber container.

Ferric Reducing Antioxidant Power (FRAP) assay

Ferric Reducing Antioxidant Power (FRAP) assay helps one evaluate the extract's antioxidant activity. Several reagents are made for this assay: as a standard, phosphate buffer (0.2M, pH 6.6), potassium ferricyanide (1%), trichloroacetic acid (10%), and ferric chloride (0.1%). 2.5 mL 0.2M phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide are combined with various concentrations of the *Ginkgo biloba* extract (e.g., 10-200µg/mL) starting the test (Vijayalakshmi & Ruckmani, 2016) [15]. The mixture is vortexed, then incubated in a water bath at 50°C for twenty minutes. 2.5 mL of 10% trichloroacetic acid is added to stop the reaction; the solution is centrifuged for 10 minutes at 3,000 rpm (Vijayalakshmi & Ruckmani, 2016) [15]. The supernatant (2.5 mL) then is combined with 0.5 mL of 0.1% ferric chloride and 2.5 mL of deionized water. Finally, a UV-spectrophotometer's absorbance of the mixture is recorded at 700 nm against a blank.

The results are interpreted by constructing a calibration curve with ascorbic acid as the standard.

The ferric-reducing potential of the *Ginkgo biloba* extract is then ascertained by a comparison with the standard. Every reagent should be made fresh, and solutions should be kept from direct sunlight both during preparation and analysis. This approach lets us methodically assess *Ginkgo biloba* extract's antioxidant qualities. The steps of FRAP assay are shown in Figure 2.

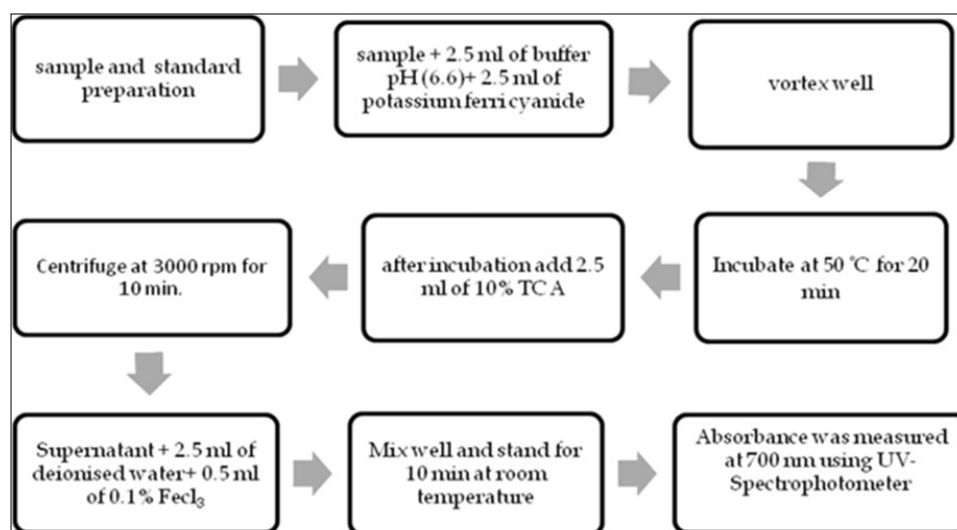


Fig 2: Steps of FRAP assay

Test for flavonoids and terpenoids content

Qualitative phytochemical studies were conducted to validate the presence of flavonoids and terpenoids in the

maceration-prepared *Ginkgo biloba* extract. For flavonoids, the Alkaline Reagent Test was employed to detect the presence of phenolic hydroxyl groups. In this procedure, 2 mL of 2% sodium hydroxide (NaOH) was added to the extract, resulting in the formation of an intense yellow color. Upon the addition of diluted hydrochloric acid (HCl), the disappearance of the yellow color confirmed the presence of flavonoids. For terpenoids, the Salkowski test was used, in which 5 mL of extract was combined with 2 mL of chloroform and 3 mL of concentrated sulphuric acid was carefully poured along the walls of the test tube to generate a separate layer. A red-brown color at the interface indicated the presence of terpenoids (Das *et al.*, 2014) [24]. These findings demonstrated that the maceration process worked well for extracting flavonoids and terpenoids.

Fourier-transform infrared analysis

Fourier-transform infrared (FTIR) analysis was performed to characterize the functional groups present in the *Ginkgo biloba* extract. Using a FTIR spectrometer with a resolution of 4 cm⁻¹, spectra were recorded from 4000 to 400 cm⁻¹, with an average of 4 scans per sample to increase the signal-to-noise ratio. Background correction was conducted, and all spectral data were baseline-corrected and normalized prior to analysis.

Characteristic absorption bands were investigated to identify significant chemical components. The broad –OH stretching vibration (~3360 cm⁻¹), C–H stretching (~2930 cm⁻¹), and carbonyl C=O stretching (~1690 cm⁻¹) were attributed to flavonoid glycosides and phenolic acids typically present in *G. biloba* preparations (Rimkiene *et al.*, 2021) [44]. To confirm the identification of important functional groups, the spectra were compared with published FTIR profiles *Ginkgo biloba* leaf constituents.

Result

Extraction Yield and Physical Characteristics

The maceration of 50 g of powdered *Ginkgo biloba* leaves in 70% ethanol yielded approximately 7.8 g of crude extract after rotary evaporation at 40°C. The extract appeared as a brownish, viscous semi-solid with a distinctive herbal odour. It was stored in refrigerator in an evaporating dish covered with aluminium foil until further analysis.

Qualitative Phytochemical Screening

The alkaline reagent test for flavonoids showed a positive result. The addition of 2% sodium hydroxide to the extract produced an intense yellow coloration, which disappeared upon subsequent addition of dilute hydrochloric acid—indicating the presence of phenolic hydroxyl groups characteristic of flavonoids.

For terpenoids, the Salkowski test also yielded a positive result. A reddish-brown ring formed at the interface between chloroform and concentrated sulphuric acid confirmed the presence of terpenoid compounds in the *Ginkgo biloba* extract. These results demonstrate that the maceration method effectively preserved key phytoconstituents.

Ferric Reducing Antioxidant Power (FRAP) assay

The antioxidant activity of *Ginkgo biloba* extract was tested using the Ferric Reducing Antioxidant Power (FRAP) assay, a well-established method for measuring the reducing

capacity of phytochemical-rich materials. This test works by using antioxidants in the sample to change ferric (Fe³⁺) ions into ferrous (Fe²⁺) ions. This creates a blue-colored ferrous complex that can be evaluated by looking at the absorbance at 700 nm. Various concentrations ranging from 10 to 200 µg/mL of the *Ginkgo biloba* extract were examined. The absorbance values obtained for each concentration are reported in Table 1. A progressive and constant increase in absorbance was seen with increasing doses of the extract, indicating a dose-dependent antioxidant response as we can see in Figures 3,4,5. The greatest absorbance was 200 µg/mL, which shows that this is the highest level of ferric reduction capacity in the measured range. Absorbance verses ascorbic acid is shown in Figure 6.

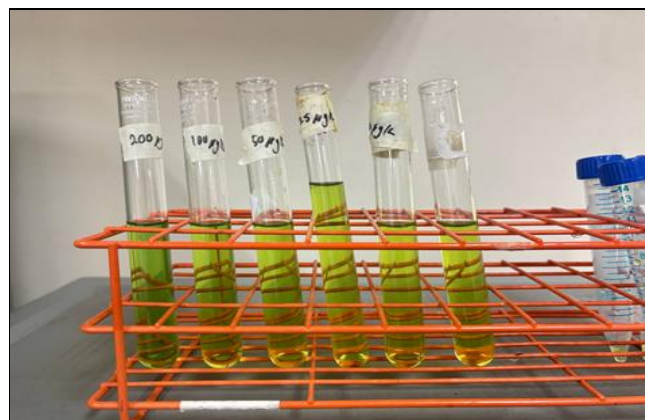


Fig 3: Gradual increase in blue coloured complex with increasing ginkgo concentration

Table 1: Result of *Ginkgo biloba* extract FRAP assay

Sample Concentration (µg/mL)	Absorbance at 700nm wavelength
10	0.007
25	0.016
50	0.048
100	0.083
200	0.211

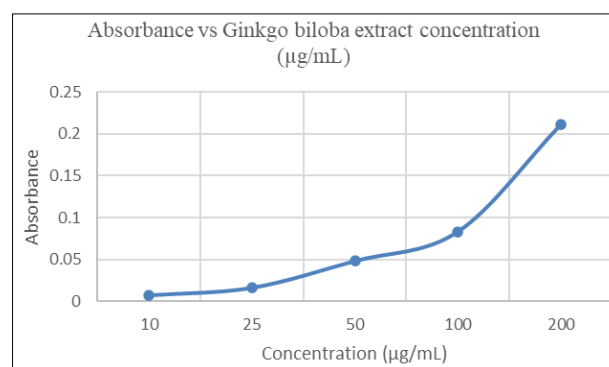


Fig 4: Absorbance vs *Ginkgo biloba* extract concentration (µg/mL)

A calibration curve was made with ascorbic acid as the standard antioxidant to measure the antioxidant activity of the extract. Ascorbic acid was evaluated at concentrations ranging from 10 µg/mL to 200 µg/mL, and the resulting absorbance values are reported in Table 2. A linear regression model was created using these data. The model has a high correlation coefficient ($R^2 = 0.9451$), which shows that the assay was accurate.

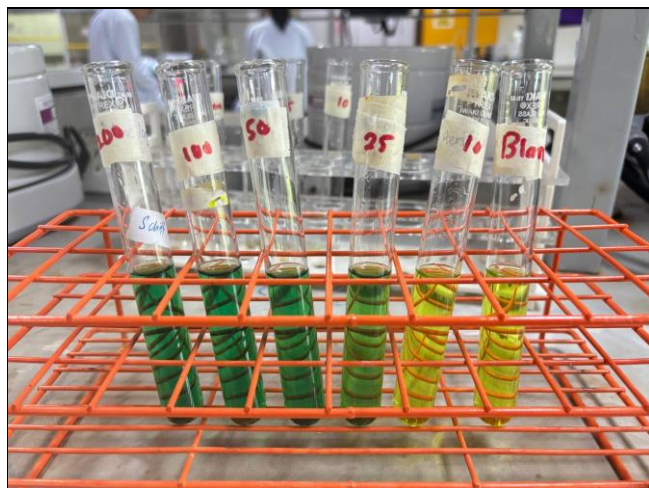


Figure 5: Gradual increase in blue coloured complex with increasing ascorbic acid concentration

Table 2: Result of Ascorbic acid FRAP assay

Sample Concentration (µg/mL)	Absorbance at 700nm wavelength
10	0.003
25	0.442
50	0.942
100	1.249
200	1.326

An ascorbic acid standard's absorbance data was used to create a calibration curve (Figure 17). The regression equation from the curve ($y = 0.3453x - 0.2435$) made it

possible to figure out the ascorbic acid equivalent (AAE) values for the *Ginkgo biloba* extract.

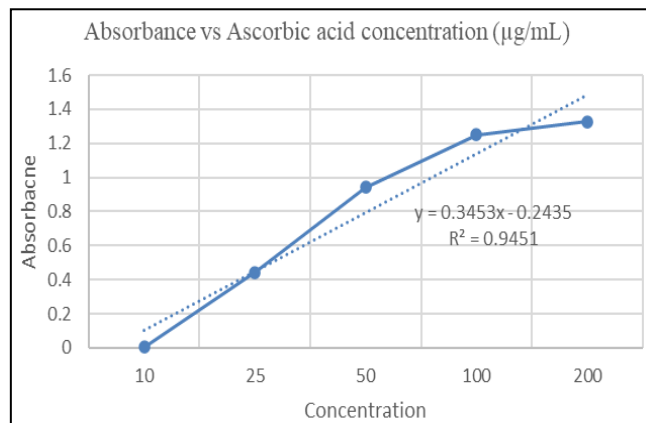


Fig 6: Absorbance vs Ascorbic acid concentration (µg/mL)

Ascorbic Acid Equivalent (AAE) quantifies the antioxidant capacity of a sample (e.g., *Ginkgo biloba* extract) by comparing it to a known antioxidant standard — ascorbic acid. AAE is usually expressed in µg of ascorbic acid equivalent per µg of extract or per volume (e.g., µg AAE/mL). In this equation, y is the concentration and x is the AAE.

$$y = 0.3453x - 0.2435 \rightarrow x = \frac{y + 0.2435}{0.3453}$$

Table 3: Ascorbic Acid Equivalent of different *Ginkgo biloba* extract concentration

<i>Ginkgo biloba</i> Extract Concentration (µg/mL)	Ascorbic Acid Equivalent (µg/mL)
10	$x = \frac{0.007 + 0.2435}{0.3453} = 0.725$
25	$x = \frac{0.016 + 0.2435}{0.3453} = 0.751$
50	$x = \frac{0.048 + 0.2435}{0.3453} = 0.844$
100	$x = \frac{0.083 + 0.2435}{0.3453} = 0.946$
200	$x = \frac{0.211 + 0.2435}{0.3453} = 1.316$

The antioxidant activity of *Ginkgo biloba* extract at each concentration was measured and reported as Ascorbic Acid Equivalent (AAE) using this calibration curve. At 10µg/mL, the *Ginkgo biloba* extract displayed an AAE of 0.725µg/mL, slightly greater than that of ascorbic acid at the same concentration, although this may be within the margin of experimental error. As the concentration went up, the antioxidant activity went up as well. At 25, 50, 100, and 200µg/mL of extract, the AAE values were 0.751, 0.844, 0.946, and 1.316 µg/mL, respectively.

The gradual rise in AAE shows that the antioxidant capacity of *Ginkgo biloba* depends on the dose, although its ability to

reduce is still much lower than that of ascorbic acid at the same doses. For instance, at 100µg/mL, *Ginkgo biloba* achieved an AAE of just 0.946µg/mL, demonstrating that a much higher quantity of extract is necessary to match the reducing activity of a relatively small amount of ascorbic acid. These findings represent the moderate antioxidant capacity of *Ginkgo biloba* as determined by its ferric reducing activity and demonstrate that while it contains bioactive components capable of electron donation, it does not rival the potency of ascorbic acid in this assay.

FTIR analysis

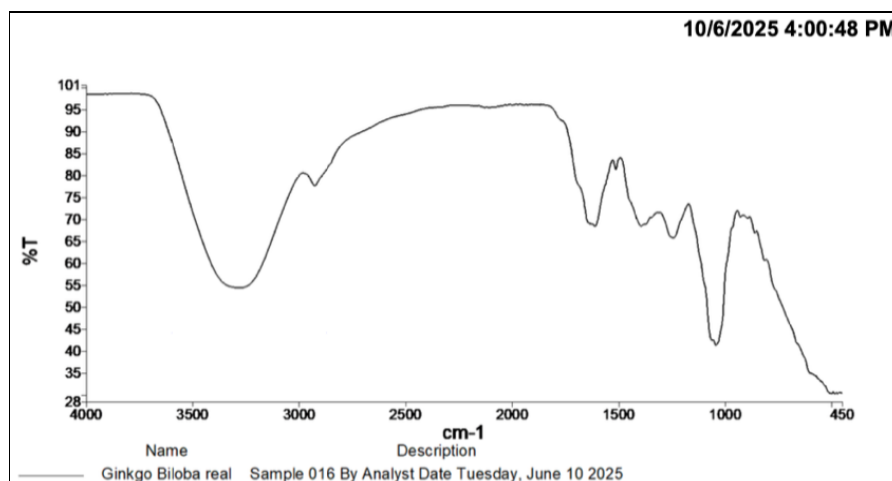


Fig 7: Result of FTIR spectrum of *Ginkgo biloba* sample

The FTIR spectrum of *Ginkgo biloba* extract exhibited multiple different absorption bands, revealing its phytochemical composition. One of the most important peaks was at about 3271 cm^{-1} , which is where the O–H stretching vibration of hydroxyl groups happens. This is common in phenolic compounds like flavonoids and tannins. It is known that these chemicals help *Ginkgo biloba* work as an antioxidant. There was also a noticeable band about 2927 cm^{-1} , which is due to C–H stretching vibrations of aliphatic groups. This means that some phytoconstituents have hydrocarbon chains or methyl groups in their structure. A sharp absorption peak at 1612 cm^{-1} was detected, which is a sign of C=C stretching in aromatic rings or alkenes. This shows that there are aromatic or unsaturated molecules, such as flavanols, which have conjugated double bonds. This band is more likely to be aromatic or alkene functional groups than carbonyl groups, which usually absorb about 1700 cm^{-1} . This supports the idea that the extract contains flavonoids and maybe terpenoids. These kinds of chemicals commonly take part in redox processes and are recognized to be very important for antioxidant systems. Extra bands in the $1200\text{--}1000\text{ cm}^{-1}$ range are C–O stretching vibrations, which are usually caused by alcohols, ethers, or glycosidic bonds. These are common for glycosylated flavonoids, which are found in large amounts in *Ginkgo biloba* leaves. Examples include quercetin and kaempferol derivatives (Zhao *et al.*, 2021). Peaks in this area help prove that sugar units and polyphenolic glycosides are there. The FTIR spectrum generally supports the idea that there are many functional groups compatible with what we know about the phytochemical profile of *Ginkgo biloba*. These include phenolic hydroxyls, aromatic alkenes, and ether or alcohol linkages, which together suggest a rich presence of bioactive flavonoids and terpenoids.

Discussion

This research examined the phytochemical profile and antioxidant capacity of *Ginkgo biloba* leaf extract acquired through maceration with 70% ethanol. The employed analytical techniques—specifically qualitative phytochemical screening, the Ferric Reducing Antioxidant Power (FRAP) assay, and Fourier-transform infrared spectroscopy (FTIR) provided extensive information regarding both the structural attributes and the functional antioxidant efficacy of the extract. The results align with the well-documented bioactivity of *Ginkgo biloba*, while also

elucidating extraction efficacy, ingredient classifications, and the correlation between phytochemicals and antioxidant activity.

Effectiveness of Maceration and Extraction Yield

The choice of 70% ethanol for maceration was based on its established efficacy in dissolving and retaining polar and semi-polar phytoconstituents, such as flavonoids and terpenoids. Ethanol–water combinations at this concentration are frequently appropriate for enhancing the solubility of phenolic hydroxyl groups, while also limiting microbial contamination during longer extraction periods (Abubakar & Haque, 2020) [1]. The yield of 7.8 g from 50 g of dried leaf powder (around 15.6%) is in the range of what was found in earlier *Ginkgo* extraction investigations, which usually produce between 6% and 15% depending on the quality of the solvent and the leaves (Kim *et al.*, 2014) [30]. The extract's thick, dark-brown color and strong herbal smell suggest that it has a lot of resins, polyphenols, and essential oils, which are all common in *Ginkgo* extracts. The relatively high yield also shows that the maceration procedure worked well. This was helped by stirring the mixture from time to time to help the solvent get into the mixture and the solute spread around. These mechanical and solvent-based elements all worked together to make sure that the target compounds were recovered in the best way possible.

Qualitative Screening and Phytochemical Profile

The qualitative phytochemical screening results validated the presence of notable antioxidant chemicals, notably flavonoids and terpenoids. The research clearly shows that these secondary metabolites are very important for the bioactivity of *Ginkgo biloba*. The unique short yellow color that showed up when NaOH was added and went away when HCl was added showed that phenolic hydroxyl groups were present. This was used to locate the flavonoids (Das *et al.*, 2014) [24]. The reddish-brown interface that was formed in the Salkowski test demonstrated that terpenoids were present. This was because triterpenoids or ginkgolides were present. These substances have qualities that preserve nerves and reduce inflammation.

These results support earlier studies that have consistently demonstrated the presence of diterpene lactones, including ginkgolides A, B, and C, as well as flavonoids such as quercetin, kaempferol, and isorhamnetin in *Ginkgo biloba*

leaves (Zhang *et al.*, 2015) ^[55]. These substances have bioactivities that assist them do their job of lowering oxidative stress. For example, they get rid of free radicals, stop lipid peroxidation, and make mitochondria work better (Chandimali *et al.*, 2025) ^[6]. This phytochemical information demonstrates that the extract might perform as an antioxidant. It also suggests that more studies should be done on the extract using structural analysis and quantitative tests.

FRAP Used to Measure Antioxidant Activity

The FRAP test was done to see how well the *Ginkgo biloba* extract could fight free radicals. This test looks at how efficiently antioxidants can change ferric (Fe^{3+}) ions into ferrous (Fe^{2+}) ions. The absorbance at 700 nm rose with the dose, much like the extract concentration rose from 10 $\mu\text{g/mL}$ to 200 $\mu\text{g/mL}$. This pattern clearly shows that the extract has redox-active chemicals that may give up electrons. This is an important part of decreasing oxidative stress (Jomová *et al.*, 2023) ^[27]. A standard curve of ascorbic acid was employed to assess the antioxidant activity of the extract. Ginkgo extract showed action at all concentrations, however it was always less effective than ascorbic acid, as shown by the Ascorbic Acid Equivalents (AAE) computations. The AAE values for the extract went from 0.725 $\mu\text{g/mL}$ at 10 $\mu\text{g/mL}$ to 1.316 $\mu\text{g/mL}$ at 200 $\mu\text{g/mL}$. This shows that *Ginkgo biloba* has antioxidant properties, but they are not as strong as those of the pure reference standard. These findings support earlier studies indicating that although *Ginkgo biloba* possesses antioxidant characteristics, the richness and diversity of its plant matrix may lead to reduced efficiency relative to pure synthetic antioxidants (Tabassum *et al.*, 2022) ^[14].

However, at 10 $\mu\text{g/mL}$ the *Ginkgo biloba* extract exhibited a somewhat elevated absorbance value compared to ascorbic acid, which may appear unexpected but can be elucidated by test sensitivity, extract complexity, and technical limitations. To begin with, instrumental noise and baseline changes can affect spectrophotometric tests like FRAP, especially when the analyte concentrations are low. Absorbance variations ranging from ± 0.005 to 0.01 frequently fall below the instrument's detection limit and may lack biological significance (Sooväli *et al.*, 2006) ^[48]. Secondly, plant extracts like *Ginkgo biloba* have a wide range of phenolic chemicals that may operate in a nonlinear or synergistic way at low concentrations. Calabrese (2021) ^[5] showed that some plant-based antioxidants work much better at lower doses because they include highly reactive minor components. This might help explain the first peak in lowering activity that was seen at 10 $\mu\text{g/mL}$. Finally, the FRAP test only looks at lowering power based on electron transfer and doesn't look at other way's antioxidants work, such chelating metal ions or scavenging radicals (Munteanu & Apetrei, 2021) ^[10]. Hence, a plant extract may exhibit anomalously high strength at specific concentrations, although having a moderate overall antioxidant capacity. These facts indicate that the elevated absorbance of *Ginkgo biloba* at 10 $\mu\text{g/mL}$ likely results from assay variability and the initial powerful activity of some phytochemicals, rather than a genuine advantage compared to ascorbic acid.

The findings indicate that FRAP is useful for assessing overall antioxidant capacity; nevertheless, it is restricted to the evaluation of reducing power and does not consider hydrogen atom donation or metal chelation, which may be significant *in vivo*.

Structural Insights from FTIR Analysis

The FTIR spectra give us a lot of information about the structural properties of the functional groups in the *Ginkgo biloba* extract, which confirms the findings of the initial phytochemicals test conducted. The stretching vibration of the O–H bond in hydroxyl groups caused a large absorption band to form at about 3271 cm^{-1} . This happens when the sample contains phenol or alcohol. These hydroxyl groups are particularly crucial for antioxidant processes because they may give up hydrogen atoms, which stops oxidative chain reactions and free radicals. Phenolic hydroxyls are particularly known for their redox properties, which underpin their ability to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

It can be hypothesized that the strong signal at 2927 cm^{-1} came from the stretching vibrations of the C–H bonds in the aliphatic $-\text{CH}_2$ and $-\text{CH}_3$ groups. These are typically associated with saturated hydrocarbon chains seen in terpenoids, which constitute a significant category of phytoconstituents in *Ginkgo biloba*. Ginkgolides and bilobalide are terpenoids that help the nervous system and lower inflammation. Long aliphatic chains are usually linked to their structural stability, which can be seen in FTIR spectra in this area.

There was a clear and strong absorption band at about 1612 cm^{-1} . This meant that alkenes that were conjugated or aromatic had C=C stretching vibrations. Many plants that have flavonoids and polyphenols shows these spectra. Aromatic conjugation makes the molecule more stable and allows electrons to move more freely. Resonance makes radicals more stable, which helps them fight off free radicals. This signal is a strong sign that the sample contains flavonoid aglycones such as quercetin and kaempferol. There are a lot of these chemicals in *Ginkgo biloba*, and they can get rid of free radicals (Zhao *et al.*, 2021).

There were also a lot of absorption peaks in the fingerprint region between 1000 and 1200 cm^{-1} that matched the vibrations of C–O and C–O–C stretching. When this signal is detected, they usually refer to ether and alcohol. This shows presence of glycosidic linkages in flavonoid glycosides. Ginkgo leaves have flavonoids that dissolve in water, such as quercetin-3-O-glucoside and kaempferol glycosides. These glycosides are significant at fighting free radicals. The presence of such compounds suggests a balance of both hydrophilic and lipophilic bio actives in the extract, enhancing its bio functional potential (Zhao *et al.*, 2021).

Moreover, minor peaks detected in the region of 1700–1750 cm^{-1} could be attributed to C=O stretching vibrations from ester or carboxylic acid groups, which are also common in secondary metabolites and may contribute to metal-chelating properties—another mode of antioxidant action not captured by FRAP but nonetheless relevant for biological applications.

The extract has a lot of various phytochemicals in it, like polyphenols, flavonoid glycosides, terpenoids, and maybe even acids or esters. All the spectrum properties make this evident. The way the hydroxyl, carbonyl, and aromatic groups are put together makes the extract more redox-active, which is in line with the FRAP values seen in the antioxidant test.

Implications and Limitations

This study points to *Ginkgo biloba* leaf extract as a potentially valuable natural antioxidant. That lines up well

with its long-standing use in traditional medicine and suggests it could also be useful in other areas as well. The presence of compounds like flavonoids and terpenoids, which are known for their effects on oxidative balance, makes it reasonable to consider adding *Ginkgo biloba* to products designed for health or wellness. In food, for instance, its antioxidant content might help slow down fat oxidation and extend shelf life naturally. It could also have benefits in skincare by helping protect against UV damage or harsh chemicals found in cosmetics. In medicine, it might play a role in managing health conditions linked to oxidative stress, such as diabetes, heart disease, or even neurodegenerative disorders (Olufunmilayo *et al.*, 2023) [40]. That said, while the results are promising, there are a few limitations which is worth discussing. To assess antioxidant activity, the study relied only on the FRAP assay, which mainly looks at how well the extract can reduce ferric ions (Fe^{3+}) to ferrous (Fe^{2+}). While this gives some insight, it doesn't tell the whole antioxidant process. Antioxidants also work by neutralizing free radicals, binding metal ions, and other mechanisms that FRAP doesn't cover. It would have been better to include a few more tests like DPPH, ABTS, or ORAC to get a clearer picture (Kotha *et al.*, 2022) [34].

Another point is that although phytochemical screening did show the presence of flavonoids and terpenoids, it was all qualitative. That means we know what's there, but not how much. It would've helped to run tests like the Folin-Ciocalteu method to measure total phenolics or use aluminum chloride to quantify flavonoids. More advanced methods like HPLC could also break things down further, identifying specific active ingredients like quercetin or ginkgolides (Luo *et al.*, 2013) [38]. This kind of information is especially useful if the extract is going to be used in products where consistency matters.

One of the bigger issues, though, is that study is only used *in Vitro* methods. Lab-based tests are useful for early findings, but they don't account for what happens in a living system. A compound might show strong antioxidant activity in a test tube but behave very differently in the body. For instance, compounds like flavonoid glycosides often need to be broken down by enzymes or gut bacteria before they can be absorbed properly (Murota *et al.*, 2018) [11]. To really understand *Ginkgo's* effects, future research needs to explore how it works in live animals or cell cultures.

It's also worth mentioning that the safety of the extract wasn't assessed here. *Ginkgo* has been used for centuries, yes, but that doesn't mean it's free of side effects. Some people have reported issues like nausea, headaches, allergies, or interactions with blood thinners (Mai *et al.*, 2025) [9]. Any plans to use it clinically or commercially should include proper toxicology studies.

All in all, the study backs up the idea that *Ginkgo biloba* has solid antioxidant potential, which could be useful in a range of products. Still, more in-depth research is needed—specially to address the limitations mentioned above. Doing so will help ensure the extract is not only effective but also safe and reliable for broader use.

Conclusion and Future Perspective

This study effectively illustrates the antioxidant capacity and phytochemical abundance of *Ginkgo biloba* leaf extract through a synergistic application of maceration extraction, Ferric Reducing Antioxidant Power (FRAP) assay, qualitative phytochemical screening, and Fourier-transform

infrared (FTIR) spectroscopy. The results confirm *Ginkgo biloba's* historical significance in herbal therapy and substantiate its utility as a source of bioactive chemicals for contemporary health applications.

The FRAP test showed that the extract has a moderate ferric reducing antioxidant capacity, which means it can give electrons and neutralize free radicals. This is in line with the existence of important phytochemical components, like flavonoids, terpenoids, and phenolic compounds, which were validated by early phytochemical assays. The FTIR spectral data confirmed the extract's chemical complexity by displaying functional groups like hydroxyl, carbonyl, and aromatic rings, which are linked to antioxidant action.

These results show that *Ginkgo biloba* has the potential to be a natural antioxidant that can be used in food, cosmetics, and pharmaceutical industries. Its diverse phytochemicals not only help control oxidative stress, but they also have other therapeutic effects, such as anti-inflammatory and neuroprotective effects.

Nevertheless, numerous domains persist for additional inquiry. The study utilized a singular *in Vitro* assay (FRAP), which, although beneficial, does not capture the complete range of antioxidant mechanisms. Subsequent investigations might include further assays, including DPPH, ABTS, and ORAC, to achieve a more thorough antioxidant profile. Furthermore, the shift from qualitative to quantitative phytochemical analysis employs modern methodologies like HPLC, LC-MS, or GC-MS which would facilitate accurate identification and standardization of bioactive components.

It is significant that *in Vitro* results do not directly correlate with biological efficacy in real cells. Consequently, subsequent investigations need to examine the extract's antioxidant activity *in vivo* models or cell-based systems to evaluate bioavailability, metabolism, safety, and therapeutic efficacy under physiological settings. Toxicological investigations are also very important for figuring out safe dosage limits, especially since the extract might have drug interactions and side effects.

Furthermore, improving extraction methods, such as ultrasound-assisted or microwave-assisted extraction, may increase yield and protect delicate phytochemicals. Comparative research investigating various solvents, extraction durations, and plant parts (e.g., seeds or roots) could enhance the production process and expand our comprehension of *Ginkgo biloba's* pharmacological versatility.

In conclusion, the present study validates the antioxidant potential and phytochemical abundance of *Ginkgo biloba* extract, while simultaneously facilitating further biochemical, pharmacological, and technological investigations. With ongoing study and innovation, *Ginkgo biloba* may become a fundamental element in the advancement of evidence-based, plant-derived antioxidant medicines in the future.

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