RESEARCH ARTICLE

Comprehensive Pharmacognostic Analysis and Evaluation of Antioxidant Properties in *Ginkgo biloba* Leaf Extracts: Implications for Therapeutic Applications in Traditional and Modern Medicine

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ABSTRACT

Ginkgo biloba leaves contain flavonoids, terpenoids, bioflavonoids, organic acids, polyprenols, ginkgolides, and bilobalides, among other active chemical constituents. The plant's many therapeutic qualities, such as its antioxidative, anticancer, antibacterial, antiviral, anti-inflammatory, and neuroprotective qualities, are attributed to these constituents. The objective of this study is to conduct a comprehensive pharmacognosy analysis, including the classification of primary and secondary metabolites, as well as HPTLC screening of Ginkgo biloba leaf extracts. Pharmacognosy analysis was perform according to methodology mention drafted by, CCRAS, Ministry of AYUSH, Govt. of India, New Delhi. Upon microscopic examination, the structural makeup of the *Ginkgo biloba* leaf was found to include calcium oxalate, starch grains, sclereids, long fibers, and spiral tracheids. Physiochemical analyses revealed that the sample contained earthy materials and inorganic salts, with a moisture content of 11.29% and water and alcoholsoluble extract was 18.53% and 16.52% was found. The *Ginkgo biloba* leaf contained primary metabolites (carbohydrates and proteins) as well as secondary metabolites (alkaloids, tannins, glycosides, and phenolic substances), according to qualitative phytochemical testing. In HPTLC screening maximum five chemical constituents are separated multiple wavelengths. The phenolic content of the *Ginkgo biloba* leaf was determined to be 789.94±61.07 (mg GAE per hundred 100 gram dry extract) and the flavonoid content was 6.59±0.91 (mg of quercetin equivalent per hundred gram dry extract). These results highlight the *Ginkgo biloba* leaf's complex composition and the existence of beneficial bioactive substances including flavonoids and phenols, which may support plant defense mechanism and have potential health benefits.

Keywords: *Ginkgo biloba*; total phenol content; total antioxidant capacity; DPPH assay; total flavonoid content; HPTLC.

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INTRODUCTION

The fan-shaped leaves of the *Ginkgo biloba*, sometimes called the maidenhair tree, turn golden-yellow in the fall before dropping off.[1] The leaves have a dichotomous venation pattern and are typically 5–10 cm long.[2] Due to its dioecious nature, the tree has distinct male and female branches. Male trees are favored for cultivation because female trees have a bad-smelling fruit.[3] Typically growing to a height of 20–35 meters with an uneven, spreading crown, *Ginkgo biloba* trees are enormous. The tree's gray, rough bark has shallow to moderately deep furrows that run along its length. The ginkgo tree is native to America, Asia, and Europe, where it prefers a temperate environment.[4] Numerous active chemical components found in *Ginkgo biloba* leaves contribute to the plant's medical capabilities, according to a wealth of study on the subject.[5,6] These comprise, among other things, ginkgolides, bilobalides, organic acids, flavonoids, terpenoids, and bioflavonoids. 10% organic acids, 5%–24% flavonoid glycosides, 6% terpenoids, and other bioactive substances are present in the standardized leaf extract of *Ginkgo biloba*.[7,8] Quercetin, kaempferol, isorhamnetin, rutin, luteolin, delphidenon, and myricetin are the main bioactive substances present in *Ginkgo biloba* leaves.[9] These flavonoids have antioxidative, anticancer, antibacterial, antiviral, anti-inflammatory, and neuroprotective properties. Polyprenols: These are resistant to Aβ 25–35 attacks and possess antibacterial qualities. Organic acids with anticancer activity and xanthine oxidase (XOD) inhibition include ginkgolic acid, a derivative of benzoic acid, and acids containing N. Moreover, several pharmacological characteristics of Ginkgo biloba, including antibacterial, antioxidant, anti-inflammatory, antiallergic, and cytotoxic anticancer effects, have been shown.[10,11] The plant's wide range of medical uses, which include the management of ailments including cancer, cardiovascular disease, and neurological diseases, are made possible by its complex chemical composition.[12,13] Objective of this study is to conduct a comprehensive pharmacognosy analysis, primary,

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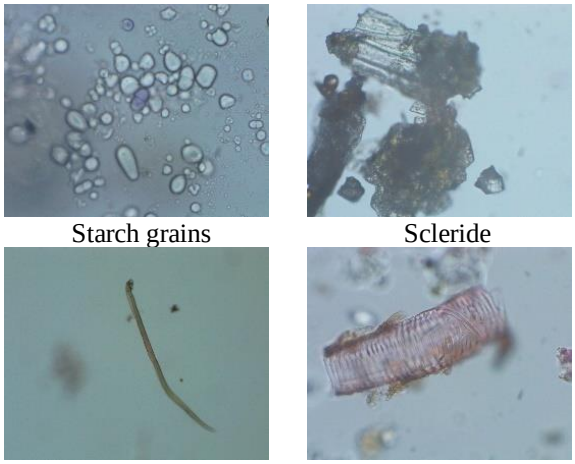
Figure 1: Leaf of *Ginkgo biloba*



Starch grains Scleride

Long Fiber Spiral tracheid

Figure 2. Powder Microscopy Leaf of *Ginkgo biloba*

secondary metabolites and HPTLC screening, determine the total phenol content and total antioxidant capacity and evaluate the potential health benefits and medicinal properties of Ginkgo biloba based on its chemical composition.

MATERIAL AND METHOD

The current investigation of the pharmacognosy profile and antioxidant capability of Ginkgo leaf used as in vitro methodology.

Reagents and chemicals

All required chemicals, including Mayer's reagent, Millon reagent, Seliwanoff's reagent, Molisch's reagent, CH_3OH, HCl, NaOH, $C_6FeK_4N_6$, CH_3COOH, $Na_2[Fe(CN)_5NO]$, H_2SO_4, K_2CrO_4, $C_6H_5CH_3$, CCl_4, KSCN, KI, H_3PO_2, HNO_3, NH_4OH, $PbS0_4$, $BaCl_2$, $FeCl_3$, C_6H_6, $HClO_4$, Na_2S, CH_3COCH_3, BiI_7K_4, $KNaC_4H_4O_6{\cdot}4H_2O$, $C_8H_8O_2$, NH_4Cl, C_2H_6O, $CHCl_3$, I_2 Solution, C_5H_5N, $C_9H_6O_4$, , $CuSO_4$, KOH, Dragandrof reagent, Na_2SO_4, Na_2CO_3, Folin-Ciocalteu reagent, $AlCl_3$, Tris, DPPH, etc. were purchased from Avantor, Merck, and HiMedia companies.

Plant Material

The GMP-certified Ayurvedic manufacturer BMRL, Rajasthan, is the source of *Ginkgo biloba* and has verified its authenticity.

Powder Microscopy

By taking pictures of various tissue segments and using an image analyzer to gather observations, the powder microscopy of leaves was investigated in accordance with conventional protocols.[14,15]

Loss on drying

The test sample that had not been dried before was weighed at around 10 g and put in a tared evaporating dish. I used a hot air oven set to 105 degree Celsius for five hr. to dry it. Drying and weighing should be done every hour until the discrepancy between two successive weight readings is less than 0.25%. The weight was deemed constant if there was a variation of no more than 0.01 g between two successive weigh-ins after a 30-minute drying and 30-minute cooling interval in a desiccator. Ascertain the percentage of loss concerning 10 grams of the specimen under examination.

Alcoholic Extractive Value

For 24 hours, macerate 5 g of the coarsely ground, air-dried test sample in a closed flask with 100 ml of alcohol. Shake the mixture regularly for the first 6 hr., and then leave it alone for the left behind 18 hours. Filter as soon as possible, taking care to avoid solvent loss. Next, evaporate twenty-five milliliters of the filtrate in a shallow dish with a flat bottom until it achieves a constant weight, and then weigh it. Dried at a temperature of 105 degree Celsius. Calculate the extractive % of alcohol in respect to the test sample that has been dried. found the methanol-soluble extractive by substituting methanol for alcohol.

Aqueous Extractive Value

For 24 hours, macerate 5 g of the coarsely ground, air-dried test sample in a closed flask with 100 ml of distilled water. Shake the mixture regularly for the first 6 hr., and then leave it alone for the left behind 18 hours. Filter as soon as possible, taking care to avoid solvent loss. Next, evaporate twenty-five milliliters of the filtrate in a shallow dish with a flat bottom until it achieves a constant weight, and then weigh it. Dried at a temperature of 105 degree Celsius. Calculate the extractive percentage that is soluble in water relative to the test sample that is dried.

Total ash

2 to 3 g of the powdered sample should be burned in a silica crucible at a temperature no elevated than 600 degree celsius until the sample is carbon-free. After cooling, weigh it. In the event that carbon-free ash could not be produced using this procedure, the burned mass was put out, the remains was assemble on ashless filter paper, the remains and filter paper were burned together, the filtrate was added, the evaporated material was dried, and the mixture was ignited at a temperature not to exceed 600 degree celsius. Find the proportion of ash in the test sample that has been dried.

Acid-insoluble ash

I filled the crucible with all of the ash and then added 25 milliliters of diluted hydrochloric acid. Whatman 41 ashless filter paper was used to collect the insoluble particles, and the filtrate washed in hot water until it reached a neutral pH. I burned it until it achieved a steady weight after placing the filter paper with the insoluble material back into the original crucible and letting it dry

Table 1. Physiochemical Tests result of *Ginkgo biloba (Leaf)*

S. No	Name of Tests	Value
1.	Loss on Drying (% w/v)	11.29
2.	Aqueous Extractive Value (w/w)	18.53
3.	Alcoholic Extract Value (w/w)	16.52
4.	Total Ash (w/w)	5.45
5.	Acid Insoluble Ash (w/w)	0.74
6.	Water Soluble Ash (w/w)	3.85

on a hot plate. I immediately weighed the residue after allowing it to cool for thirty minutes in the proper desiccator. Ascertain the reference dry test sample's acid-insoluble ash %.

Water-Soluble Ash

I filled the crucible with all of the ash and added 25 milliliters of distilled water. Whatman 41 ashless filter paper was used to collect the insoluble particles, and the filtrate washed in hot water until it reached a neutral pH. I burned it until it achieved a steady weight after placing the filter paper with the insoluble material back into the original crucible and letting it dry on a hot plate. I immediately weighed the residue after allowing it to cool for thirty minutes in the proper desiccator. Ascertain the reference dried test sample's water-soluble ash percentage.[16]

Analysis of Primary and Secondary Metabolite Qualitatively

Phytochemical testing is used to identify the primary (carbohydrate, protein, and amino acid) and secondary (alkaloids, glycosides, tannin, saponin, and phenolic compounds) metabolites. The alcoholic and aqueous extracts were used to look for primary and secondary metabolites in test samples.

Molisch's Test: After adding two milliliters of test solution and two milliliters of Molisch's reagent, and giving it a gentle shake, one milliliter of concentrated H2SO4 was poured out the test tube's surface and permitted to stand for a minute. A purple ring at the link of the two layers indicated the existence of carbohydrate.

Benedict's test

Sugars were reduced using this technique, which mainly used copper sulfate and sodium hydroxide. Four milliliters of the drug's aqueous solution were combined with one milliliter of Benedict's solution, and the mixture was heated to almost boiling. As the concentration of simple sugar rose, colors such as green, yellow, orange, red, or brown were generated in the test solution as a result of the production of cuprous oxide.

Fehling solution test

Usually used to lower sugar levels, this test consists of two solutions blended in-situ. Fehling solution A includes 0.5% $CuS0_4$, and Fehling solution B is composed of $C_4H_4O_6KNa\cdot_4H_2O$. Two milliliters of the drug's aqueous solution were added after one milliliter each of Fehling A and B solutions were blended. The mixture was next brought to a boil in a water bath for five to ten minutes.

Dragondroff's Reagent

Table 2. Phytochemical Tests of *Ginkgo biloba (Leaf)*

S. No	Tests	Aqueous Extract	Ethanolic Extract
A.	Carbohydrate		
1.	Molish test	+	-
2.	Benedict test	-	-
3.	Fehling test	-	+
B.	Alkaloids		
1.	Dragendorff test	+	-
2.	Wagner's test	-	-
3.	Hager's test	-	+
C.	Amino Acid		
1.	Ninhydrine test	+	+
D.	Protein		
1.	Biuret test	+	-
2.	Xenthoprotic test	-	+
3.	Millon test	+	-
E.	Saponin		
1.	Foam test	+	-
F.	Glycosides		
1.	Borntrager's test	+	-
G.	Phenolic compound		
1.	Phenolic test	+	+
H.	Steroids		
1.	Salkowaski	-	-
I.	Tannins		
1.	$FeCl_3$	+	+
2.	Lead acetate	+	+
3.	Pot. Dichromate	-	-

Two milliliters of the test solution and two milliliters of bismuth subnitrate and potassium iodide solution, or Dragondroff's reagent, were added to a test tube. The presence of alkaloids was demonstrated by the materials' capacity to produce an orange precipitate.

Wagner Test

The presence of alkaloids was indicated by the production of a reddish-brown precipitate after a few drops of Wagner's reagent (diluted iodine solution) were added to two milliliters of test solution.

Hager test

Picric acid was dissolved in a saturated aqueous solution. After the test filtrate was treated with this reagent, an orange-yellow precipitate was formed, indicating the presence of alkaloids.

Ninhydrin test

This test was used to identify proteins with free amino groups and alpha-amino acids. The characteristic deep blue or light blue color is caused by the synthesis of a mixture between two ninhydrin molecules and the nitrogen of free amino acids.

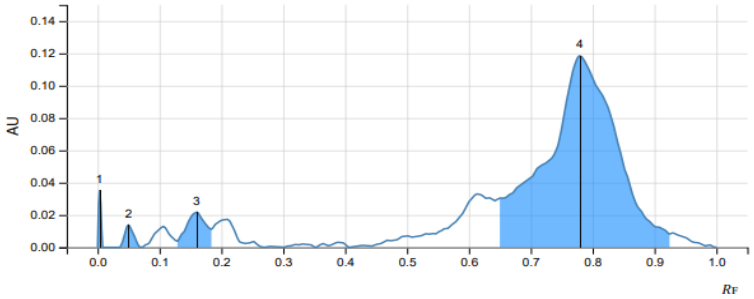
Biuret test

The mixture was mixed with one milliliter of 4% sodium hydroxide solution, five milligrams of residue, and a drop of 1% $CuSO_4$copper sulfate solution. The proteins were indicated by the emergence of a violet or pink tint.

Xanthoprotic test

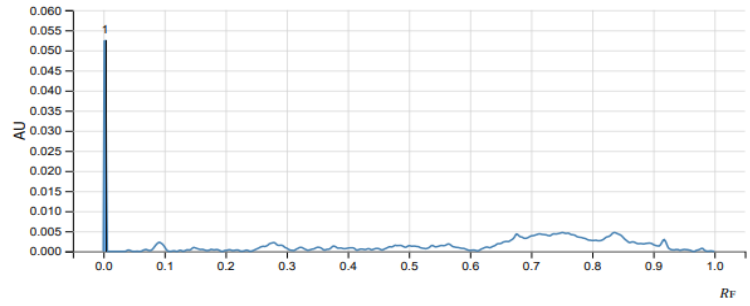
A small portion of the test material was treated with two milliliters of water and five milliliters of strong HNO_3.

Table 3. Thin Layer Chromatography screening at 254 nm wavelength of *Ginkgo biloba (Leaf).*



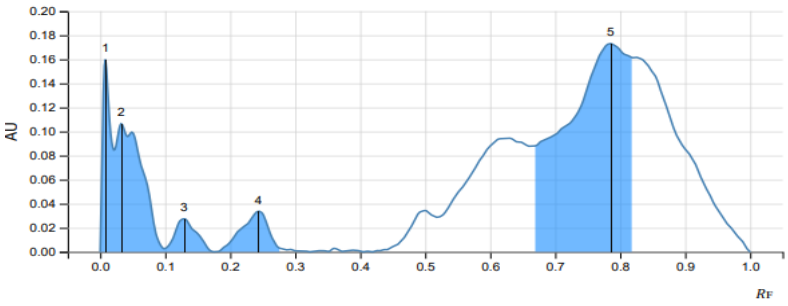
Peak	Start		Max			End		Area	
	R_f	Height	R_f	Height	%	R_f	Height	A	%
1	0.000	0.0000	0.003	0.0355	18.73	0.008	0.0000	0.00016	0.97
2	0.035	0.0000	0.050	0.0138	7.29	0.069	0.0000	0.00023	1.35
3	0.129	0.0038	0.160	0.0217	11.46	0.184	0.0113	0.00081	4.82
4	0.648	0.0301	0.779	0.1187	62.52	0.924	0.0082	0.01558	92.86

Table 4. TLC screening at 366 nm wavelength of *Ginkgo biloba (Leaf).*



Peak	Start		Max			End		Area	
	Height	R_f	Height	%	Height	R_f	Height	%	Height
1	0.000	0.0000	0.003	0.0524	100	0.006	0.0000	0.00020	100

Table 5. Thin Layer Chromatography screening at 540 nm wavelength of *Ginkgo biloba (Leaf).*



Peak	Start		Max			End		Area	
	Height	R_f	Height	%	Height	R_f	Height	%	Height
1	0.000	0.000	0.008	0.1599	31.95	0.021	0.0847	0.00229	7.50
2	0.021	0.0847	0.032	0.1065	21.27	0.098	0.0026	0.00513	16.79
3	0.098	0.0026	0.129	0.0274	5.47	0.176	0.0000	0.00105	3.44
4	0.176	0.0000	0.244	0.0337	6.72	0.287	0.0016	0.00164	5.37
5	0.661	0.0877	0.785	0.1731	34.58	0.819	0.1613	0.02046	66.91

The presence of proteins was shown by the emergence of a yellow hue.

Foam test

A tiny amount of the sample was added to a test tube together with a small amount of water and sodium bicarbonate, and the mixture was shaken vigorously. Saponins were identified by a continuous, identifiable froth that looked like honeycomb.

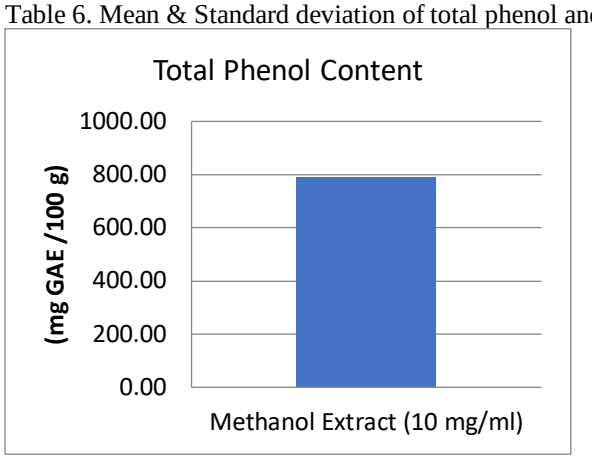
Borntragor's Test

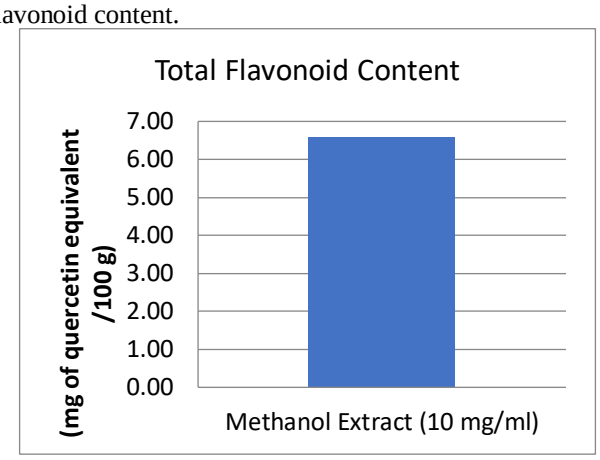
The ethanol extract was mixed with 1 milliliter of C_6H_6 and 0.5 milliliter of diluted NH_4OH solution. This produced a reddish-pink hue, which indicated the presence of glycoside.

Phenolic compound test

Two milliliters of $FeCl_3$ solution were added to the sample extract after it had been heated in water. After that, the mixture was left to be observed to see if any green or blue

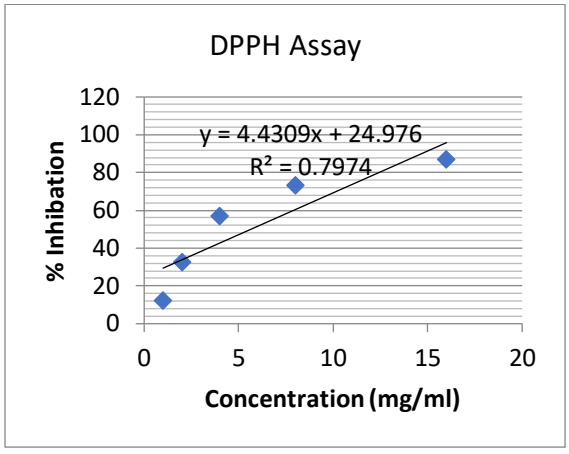
Table 6. Mean & Standard deviation of total phenol and flavonoid content.

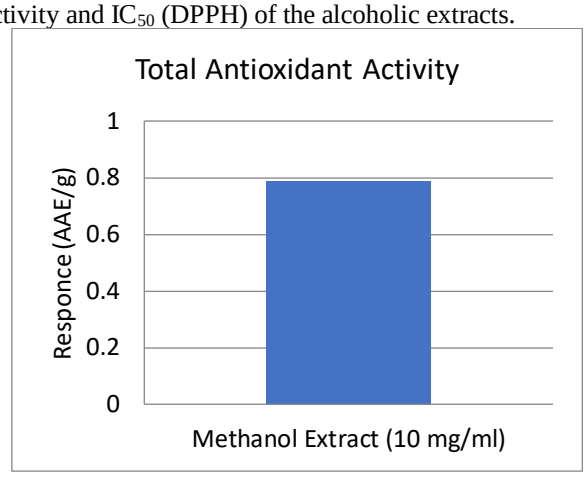




Total Phenol Content (mg GAE Per hundred gram dry extract)	Total Flavonoid Content (mg of quercetin equivalent Per hundred gram dry extract)
789.94±61.07	6.59±0.91

Table 7. Mean and Standard Deviation of total antioxidant Activity and IC_{50} (DPPH) of the alcoholic extracts.





DPPH Assay (IC_{50} Value)	Total Antioxidant Activity (AAE per gram dry extract)
5.6501 mg	0.7874±0.0710

hue developed, which would suggest the presence of phenolic chemicals.

Salkoweski reaction

5 mg of extract, 2 ml of $CHCl_3$, and 2 ml of concentrated H_2So_4 were introduced from the side of the test tube. The test tube was shaken for a few minutes. Redness was an indication that steroids were present.

Lead acetate: The test filtrate was mixed with a 10% w/v solution of basic $PbS0_4$ in distilled water. Precipitate formation suggested the presence of tannins.

Potassium dichromate

The filtrate was put to a solution of $K_2Cr_2O_7$ for this test. The presence of tannins was indicated by the black tint.

High-Performance Thin Layer Chromatography

One technique for determining and separating the various chemical components present in a test sample is chromatography. The stationary phase, mobile phase, and visualization phase of the chromatography process were all finished. Phase in station: 10 x 20 cm, silica gel 60 F_{254}. $C_6H_5CH_3$ (12 ml): $C_4H_8O_2$ (8 ml) is the mobile phase. R_f Value: The difference in the travel distances from the origin line of the solvent and solute. Visualized under 366, 540, and 254 nm. Derivatization Anisaldehyde sulphuric acid are used (17, 18, 19).

Total Phenol Content

Using the Folin-Ciocalteu technique, the total phenolic component concentration in the methanol extract was ascertained. A volume of 750 µL of the Folin-Ciocalteu reagent was added to each tube. Each tube was then filled with 100 µL of the extracts, vortexed, and the mixture was kept at 25°C for five minutes. After that, the tubes were filled with 750 µL of sodium carbonate, and the solutions were left at 25°C for 40 minutes. A standard curve was plotted after the absorbance at 725 nm was determined. Gallic acid equivalent milligrams per 100 grams (mg GAE per hundred grams) of dry extract were used to express the results.

Total Flavonoids Content

Through colorimetric examination with aluminum chloride, the total flavonoid concentration was ascertained. First, a 2% ethanol-based solution of aluminum chloride was made. The test sample's methanol extract (10 mg/ml) at different concentrations and 500 µL of the aluminum chloride solution (2%) were then added to separate tubes.

The samples were vortexed and then kept at 25 °C for an hour. At 420 nm in wavelength, absorbance measurements were made, and quercetin was used to create standard curves for flavonoids. The outcomes were reported as milligrams of quercetin equivalent (mg QE/100 g) of dry extract per 100 grams. Total Antioxidant Capacity: The phosphomolybdate technique was utilized to ascertain the total antioxidant capacity. One milliliter of the phosphomolybdate reagent and 0.1 milliliter of the extract were added to each tube. After being vortexed, the resultant solutions were heated to a boil for ninety minutes. At a wavelength of 695 nm, absorbance measurements were made, and ascorbic acid was used to create an antioxidant standard curve. The corresponding of ascorbic acid/gm. (AAE/g) of dry extract was used to express the results.

Total Antioxidants Activity

The phosphomolybdate technique was utilized to ascertain the overall antioxidant capability. One milliliter of the phosphomolybdate reagent and one milliliter of the methanol extract (10 mg/mL) were added to each tube. After being vortexed, the resultant solutions were heated to a boil for ninety minutes. At a wavelength of 695 nm, absorbance measurements were made, and ascorbic acid was used to create an antioxidant standard curve. The corresponding of ascorbic acid/gm. (AAE/g) of dry extract was used to express the results.

DPPH Assay

0.8 mL of buffer, 0.2 mL of extracts (10, 20, 40, 80, and 160 mg/mL solution) in methanol, and 1 mL of DPPH solution in methanol were added to each tube, and then the tubes were vortexed. After that, the solutions were incubated for 45 minutes at 25oC in the dark. The samples' absorbance was calculated at a wavelength of 517 nm after the allotted time. The subsequent formula was used to determine the absorbance values of the samples and the blank: To get the percentage inhibition, divide (A blank - A extract) by 100. The percentage of DPPH scavenging effect equals the percentage of inhibition. Absorbance in negative control for a blank. The absorbance of samples is called an extract. In order to compare the extracts' scavenging effects, the data were presented as IC_{50} values.[20,21,22]

RESULTS AND DISCUSSION

Ginkgo biloba leaves were examined under a microscope to detect calcium oxalate, starch grain, sclereids, long fiber, and spiral tracheids. These elements support the leaf's overall composition and structure (Fig. 1 & 2). The sample's ability to retain water was demonstrated by the loss-on-drying test, which revealed moisture content of 11.29%.

Important components were present, as evidenced by the extractive values of 18.53% for the water-soluble extract and 16.52% for the alcoholic-soluble extract, which are directly related to the drug's strength or potency. The amount of inorganic and earthy stuff measured by the ash value was 5.45%, with the water-soluble ash being 3.85% and the acid-insoluble substance being 0.74%. These numbers show that the sample contains earthy materials and inorganic salts (Table 1). Primary metabolites (carbohydrates, proteins, fats, etc.) and secondary metabolites (alkaloids, glycosides, tannins, etc.) can be distinguished using a qualitative phytochemical test. The Molisch test for the aqueous extract of *Ginkgo biloba* leaf is positive, indicating the presence of mono-, dis-, and polysaccharides. The sample's ethanolic extract yielded a positive test result, indicating the presence of reducing sugars. The aqueous extract was found to contain alkaloids as a consequence of both the Dragondroff and Wagner tests yielding positive results. Because of the positive Hager's test result, the ethanolic extract was also positive. Since both extracts include amino acids, the ninhydrine test yields a positive result. Additionally, proteins are present, as evidenced by positive results from the xenthoprotic test in the ethanolic extract and the Biuret and Millon assays in the aqueous extract. The aqueous extract of the material passed the foam test, indicating the presence of saponin. Both the sample's alcoholic and aqueous extracts passed the phenolic test, indicating the presence of phenolic chemicals. Tannin is present in the test sample, as indicated by the positive results of the lead acetate and $FeCl_3$ tests (Table 2). Four chemical constituents are visible in *Ginkgo biloba* leaf HPTLC at a wavelength of 254 nm; the corresponding relative frequencies (R_f values) are 0.003, 0.050, 0.160, and 0.779. One chemical component can be seen at a wavelength of 366 nm, and the R_f value was discovered to be 0.003. Five chemical constituents were observed after derivatization and visualization at 540 nm wavelength. The R_f values of these constituents were determined to be 0.008, 0.032, 0.129, 0.224, and 0.758 (Table 3-5). Both phenols and flavonoids are classes of organic compounds that are present in plants and are recognized for their potential health advantages, roles in the defense mechanisms of plants, and capacity to combat dangerous free radicals in the body, thereby lowering the risk of chronic illnesses like cancer and heart disease. The leaf of *Ginkgo biloba* is rich in flavonoids and phenols; we detected 6.59±0.91 mg of quercetin equivalent and 789.94±61.07 mg of GAE/100 g dry extract, respectively (Table 6,7).

CONCLUSION

The *Ginkgo biloba* leaf's complex composition and the occurrence of valuable bioactive compounds, such as phenols and flavonoids which are recognized for their prospective health benefits and contributions to plant defense mechanisms are demonstrated by a thorough microscopic, chemical, and qualitative phytochemical analysis.

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