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PHYTOCHEMICAL ANALYSIS, PHYSICOCHEMICAL INVESTIGATION AND FLUORESCENCE CHARACTERIZATION OF ETHANOLIC EXTRACT OF *GINKGO BILOBA* LEAVES

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ABSTRACT
Indigenous medicine is outlined as "the expertise, abilities, and protocols based on indigenous theories, beliefs, and experiences used to maintain wellness and prevent, diagnose, improve or treat physical and mental illness. The most common reasons for using traditional medicine are that it is less expensive; more closely corresponds to the patient's ideology, alleviates concerns about the side effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and allows greater public access to health information. Herbal medications are mostly used for health promotion and therapy for chronic, rather than life-threatening, illnesses. *Ginkgo biloba* leaves were analyzed physicochemically and phytochemically. The purpose of this study was to determine the phytochemical ingredients and physicochemical parameters of *Ginkgo biloba* using the conventional pharmacopoeial approach. The presence of various metabolites such as alkaloids, steroids, flavonoids, tannins and amino acids. Carbohydrates glycosides, saponins, fix oil and fat was not found in *Ginkgo biloba* leaves, when quantified using the literature method. Total Ash (27%), Water Insoluble Ash (1.28gm), Acid Insoluble Ash (1.19 gm) is all included in the physicochemical analysis. TLC and Floroscence analyses, as well as established procedures, determine the loss on drying (11.33%) and Rf value (0.64 (UV).

KEYWORDS: *Ginkgo biloba* L. Physicochemical analysis, phytochemical Investigation, Floroscence analysis, TLC.

1. INTRODUCTION
Ginkgo biloba L. is a member of the Ginkgoaceae family and the Ginkgoales order. The tree which can live for up to 1000 years and can reach a height of 30 meters, is native to East Asia.[1] Yinxing (Chinese) is the oldest known gymnosperm species alive, having persisted for millions of years and is thus regarded a 'living fossil'.[2] The discovery of *G.biloba* fossil records suggests the survival of the wild species in the Dalou Mountains in south western China.[3] The ultrastructure of stomatal development in this species has been researched[4], and there is a remarkable relationship between modern-day *Ginkgo biloba* and Triassic-era French ginkgophyte seedlings. This species' products are currently available in a variety of forms, including tea, bars, tablets, capsules, and extracts.[5]

This extract is now one among the world's best-selling and most popular botanical medicine formulations. The suggested dosage is 40-80 mg three times a day. *G. Biloba* seeds have yielded important proteins with functional characteristics For thousands of years, the Chinese have utilized these seeds to cure and manage a variety of diseases, including ischemia, lung congestion, and asthma.[6]

This phytomedicine is now used to treat sexual dysfunction, premenstrual syndrome, dementia, and cognitive impairment in various regions of the world, particularly China, Germany, and France. *Ginkgo biloba*, one of the world's oldest living deciduous trees contains a wide spectrum of pharmacologically active chemicals, making it an essential traditional medicine therapeutic agent for thousands of years.[7,8]

The plant is a gymnosperm that originated in eastern China and is now found all over the world, mainly in northern temperate climates. The tree grew largely in China and other parts of Asia until the early 1700s; thus, Chinese medical traditions have made substantial use of ginkgo since 2800 BCE.[9,10]

Its leaves, fruits, and roots have medicinal properties *Ginkgo* preparations based on the leaves and fruits are the most often used in Chinese herbal medicine. Treatment of asthma and other respiratory difficulties;

infectious diseases such as tuberculosis and gonorrhea; circulatory problems; and a variety of other disorders are among the therapeutic applications. *Ginkgo biloba* has been used medicinally in China and other Asian civilizations, including Ayurvedic medicine in India, since ancient times as a mainstay of acknowledged medical therapy.[13]

In contrast, ginkgo-based products have been used in the United States and other Western countries since the 1960s, following the invention of a process for extracting and commercializing the plant's subcomponents.[14] The usage of Ginkgo as a health supplement and alternative to prescription formulations has expanded significantly in the last decade.[15] It is commonly supplied as a standardized extract of dried leaves (EGb761), which contains 24% ginkgo flavonol glycosides and 6% terpene lactones in edible caplet form. The reasons for self-administration of this herb vary greatly, depending on the desired health objectives. Ginkgo is said to promote three primary areas of health: (i) neurological effects, (ii) circulatory effects, and (iii) sexual effects.[16,17]

Figure 1: Representation Photograph of plant and leaves of *Ginkgo biloba.*

Ginkgo biloba is a one-of-a-kind tree with no living relatives. The ginkgo tree is a species that flourished during the Mesozoic era 150 million years ago, peaking during the Jurassic and Cretaceous periods.[18]

As a result, it is one of the most well-known examples of a living fossil. Because ginkgo is a gymnosperm, its seeds are not protected by an ovarywall. Ginkgo trees are currently widely cultivated in Asia, Europe, North America, New Zealand, and Argentina.[19] Since the early Chinese herbals, ginkgo seed has been listed as a source of medicine. The leaf was advised for medical purposes as early as 1509 and is still used in teas today. Ginkgo leaf extracts are now available in the form of capsules.[20]

2. MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant leaves of *Ginkgo biloba* were collected from Indore and authenticated by Dr. S.N. Dwivedi, Professor & Head, department of Botany, Janta PG college, APS university, Rewa, M.P. The Voucher Specimen was deposited for future reference. The seeds and leaves were shade dried and stems and roots were cut into small fragments and then shade dried. Then dried plant material of seeds, stems and roots was powdered individually by using mixture grinder except leaves which were manually grinded and stored at room temperature for further analysis.

Extraction of Plant

The dried plant material was crushed into the fine particle (powder), the powdered plant material (100gm) was extracted in 250 ml of ethanol by using Soxhlet apparatus for 24 hours at a temperature of 50^{o} C. After 24 hours the obtained extracts were filtered by whatman filter paper and the eathanol was evaporated at 55^{o}C with the help of heating mantle and the samples were further dried in vacuum desiccators. After complete dryness of extracts, the yield value was calculated and kept in the refrigerator at 4^{o}C until further study.

The leaves of *Ginkgo biloba* plants were analyzed for the Alkaloids, Steroids, flavonoids, tannins and carbohydrates glycosides, saponins, fix oil and fat, protein and amino acids (Table 1) using standard methods.

PHYSICO-CHEMICAL ANALYSIS[21-23]

Determination of loss on drying

Two grams of crude powder were placed in an evaporating dish and dried in a 105°C oven until a steady weight was attained. The weight after drying was recorded, and the drying loss was determined. The percentage was derived using the initial sample size.

Determination of total ash

Two grams of dry powder were placed in a silica crucible and gradually heated to 500°C until white, demonstrating the absence of carbon.

Ash was quickly cooled in a desiccator and weighed. The total ash value was estimated in milligrams per gram of air-dried material.

Determination of water soluble ash

25 cc of water was added to the crucible containing the complete ash and heated for 5 minutes. The insoluble materials were collected using ash-free filter paper. It was cleaned with hot water and cooked for 15 minutes in a crucible. The weight of insoluble materials was removed from the overall ash weight. The water soluble ash concentration was determined in mg g-1 of air dried material.

Determination of acid insoluble ash

To the crucible holding complete ash, 25 ml of hydrochloric acid (70 g/l) was added. It was gradually heated for 5 minutes to boil, covered with a watch-glass. The watch glass was rinsed with 5 ml of hot water before being placed in the crucible. The ash-free filter paper was used to capture the insoluble materials, which was then washed with hot water until the filter was neutral. The insoluble substance was transferred to the original crucible via filter paper, which was dried on a hot plate and heated until a steady weight was reached. The residue was allowed to cool in desiccators for 30 minutes before being weighed. The acid insoluble ash was estimated in milligrams per gram of air.

Thin Layer Chromatography (TLC)[22,23]

The Rf values of the various extracts were evaluated using thin layer chromatography in various solvents with Silica gel G as an adsorbent.

Qualitative phytochemical analysis of plant extract

Khandelwal and Kokate performed a preliminary phytochemical investigation on the *Ginkgo biloba* obtained using normal procedures. The extract was tested for the presence or absence of phenolic compounds, carbohydrates, flavonoids, also glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acids, and tannins.

Fluorescence analysis of leaf powder

An inconspicuous amount (1 gm) of dries and lightly ground A *Ginkgo biloba* Leaf leaf was treated with freshly made aqueous NaOH, 1N HCl, 5% FeCl3, 1M H2SO4, conc.HNO3, dil.HNO3, and 50% HNO3. They were tested for fluorescence in both daytime and UV-light (365 nm). The colors seen in various radiations were recorded.[24]

3. RESULTS AND DISCUSSION

The phytochemical quantitative compositions of *Ginkgo biloba* are shown in Table 1. Alkaloids, Steroids, flavonoids, tannins are positively present in leaves of *Ginkgo biloba* and carbohydrates glycosides, saponines, fixed oil and fat, protein and amino acids are not present in leaves of *Ginkgo biloba* L. also perform the fluorescence analysis where achieved observation and Observations Of Thin Layer Chromatographic Studies of *Ginkgo biloba* Leaves were achieved by Rf value 0.64.

The results of physico-chemical analysis of *Ginkgo biloba* L. are shown in Table 2. The Total amount of ash was 27%, Water soluble Ash was 1.28% and acid insoluble Ash were in range 1.19% and loss of drying was 11.33%, Rf value 0.64 (UV) and done Fluorescence Analysis of Powder of *Ginkgo biloba* L.

Table 1: Quantitative Phytochemical composition of plant samples on dry weight basis expressed as mg/100 gm dry weight of leaves of *Ginkgo biloba* plant.

Name of the Plant	Part Used	Chemical Constituents	Mark
Ginkgo biloba	Leaves	Alkaloids	+ve
		Carbohydrates	-ve
		Glycosides	-ve
		Steroids	+ve
		Flavonoids	+ve
		Saponins	-ve
		Fixed oils and fats	-ve
		Tannins Proteins and amino acids	+ve +ve

Table 2: Physicochemical Analysis of *Ginkgo biloba* L.

Sr. No.	Name of the Plant	Part Used	Physicochemical Test	Values
1	*Ginkgo biloba*	Leaves	Total Ash	27%
2			Water soluble Ash	1.28%
3			Acid insoluble Ash	1.19%
4			Loss of drying	11.33%

Table 3: Observations of Thin Layer Chromatographic Studies of *Ginkgo biloba* L.

Extracts	Mobile phase	No. of Spots	Rf values	Color	Intensity
Ethanol	Chloroform: ethanol	1	0.64(UV)	Y	+++ +++
	(8:2)				

Where +++ = Most intense, ++ = moderately intense, + = Least intense, Y=Yellow V= Visible (UV) = Ultraviolet 360nm

Table 4: Fluorescence Analysis of Powder of *Ginkgo biloba* L.

S. No.	Powdered drug + Reagent		Observation	
			Leaves	
		Visible/Day	UV (Long)	UV (Short)
		Light		
1	Powder + 1 M NaOH	Yellowish green	Brownish black	Blue at center and green at edges
2	Powder + 1 M HCl	Gray	Black at center and gray at edges	Light green
3	Powder + 5% $FeCl_3$	Yellowish brown	Black	Blue at center and green at edges
4	Powder + 1 M H_2SO_4	Light brown	Brownish black	Black at center and transparent at edges
				Black at center and
5	Powder + Conc. HNO_3	Dark brown	Black	greenish at edges
6	Powder + 1 N NaOH	Yellowish green	Brownish black	Blue at center and green at edges
7	Powder + 1 N HCl	Gray	Black at center and gray at edges	Light green
8	Powder + 1 N H_2SO_4	Light brown	Brownish black	Black at center and transparent at edges
9	Powder + dil. HNO_3	Dark brown	Black	Black at center and green at edges
10	Powder + 25% NH_3	Greenish yellow	Black	Brown at center and green at edges
11	Powder + dil. NH_3			
		Greenish yellow	Black	Greenish brown
12	Powder + 50% HNO_3	Dark brown	Black	Brown at center and green at edges
13	Powder + HNO_3+25% NH_3	Dark brown	Black	Brown at center and green at edges

4. CONCLUSIONS

This plant may be a good source of metabolites and steroids to treat number of diseases that are mainly caused due to the deficiency of those primary and secondary metabolites and can be utilized in Ayurvedic system to cure disease. This also aids in differentiating *Ginkgo biloba* plant material from that of related species. The phytochemical investigation provided valuable information about the various phytoconstituents present in the plant, which assists future investigators in the selection of the specific extract for further investigation of isolating the active principle and also provided an idea about different phytochemicals that have been found to possess a wide range of activities.

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