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Determination of Vitamins, Trace Elements, and Phytochemical Compounds in Ginkgo Biloba Leaves Extracts

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Abstract:

The present study was conducted to extract the vitamins, trace elements and phytochemical constituents of Ginkgo biloba leaves extracts using de-ionized water and ethanol (99%) as solvents, by Soxhlet extractor. The yield of the extract by the two solvents was (12.355gm/100gm) by deionized water and was (20.525 gm/100gm) by ethyl alcohol solvent. Different types of vitamins were characterized with the above extracts using High Performance Liquid Chromatography (HPLC). Vitamins A, K, E, D3, C, B1, and B2showed interesting results, for example, vitamin K is the most abundant in ethanol, whereas vitamin C is the most abundant in aqueous extract. These findings are motivated for deep research about the vitamins in Ginkgo biloba and their antioxidant relevance for therapeutic herbal medicine. Different metal ions, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn, were measured using Atomic Absorption Spectrometer (AAS). The results of the qualitative detection of the two extracts indicated the existence of Alkaloids, Steroids, Terpenes, Phenols, Carbohydrates, Glycosides, Proteins, Saponins, Tannins, and Flavonoids. Phenols and flavonoids are the major compounds exist in the plant, which are thought to be responsible about the observed antioxidant activity.

Keywords: Ginkgo Biloba; Soxhlet; Vitamins; HPLC;AAS; Phytochemical.

1. Introduction:

Medical plants are characterized by physiological active principles that have been utilized in traditional medicine years ago in the treatment of different diseases and disorders[1].One of these medicinal plants is Ginkgo biloba, which is also one of the most studied medicinal plants[2].Ginkgo Biloba leaves are rich in a variety of natural active ingredients and have a wide range of pharmacological activities[3], which play an important role in food[4], health care[5],medicine[6],supplements[7],anti-inflammatory,anti-cancer,antioxidant properties[8],and other fields. Ginkgo leaves are used for different purposes such as practiced antioxidant mechanisms by suppressing free radicals and reactive oxygen produced during oxidative stress[9].The traditional methods of extracting and the determination of active substances from Ginkgo biloba include solvent extraction[10], cloud point extraction[11], accelerated solvent extraction[12], ultrasound-assisted extraction[13], supercritical fluid extraction and hydro distillation (HD)[14]. Vitamins are a disparate group of compounds; they have little in common either chemically or in their metabolic functions. Nutritionally, they form a cohesive group of organic compounds that are required in the diet in small amounts (micrograms or milligrams per day) for the maintenance of normal health and metabolic integrity. They are thus differentiated from the essential minerals and trace elements (which are inorganic), and from essential amino and fatty acids, which are required in larger amounts[15]. Generally, the human organism cannot synthesize vitamins, and for this reason, they must be included in the human diet. The determination of vitamins plays an important role in nutritional and biochemical studies, and analytical methods are availably capable of determining these vitamins in different samples that are extracted from any part of the plant [16].Many analytical methods have been used for the analysis of vitamins: chromatographic methods[17], electromigration methods[18], microbiological assays[19], and several other methods[20].HPLC has preferred for vitamin quantifications due to its giving more decisions and more specificity than other methods[21].Minerals are inorganic substances that are found in all body tissues and fluids. Their presence is required for the preservation of certain

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physicochemical processes that are necessary for life, and they are chemical constituents that the body uses in a variety of ways. Even though the fact that they produce no energy, they play a crucial role in a variety of bodily functions[22].The analysis of micronutrients in food samples is of great interest both regarding nutrition and commercial aspects. This kind of analysis is so important to know the contents of foods and environmental samples given their toxicity and essential properties[23]. The study aimed is to verify the concentrations of vitamins, metal ions, and phytochemical compounds in both two extracts, and compare the presence of these species between both, then show the role of the Ginkgo plant in the treatment of several diseases.

2. Experimental:

2.1. Collecting ginkgo Biloba leaves:

The leaves of Ginkgo biloba were collected from Sulaymaniyah north of Baghdad, Iraq, in September 2021, then washed with deionized water, dried in shade for several days at room temperature, and ground to powder.

2.2. Sample Extraction:

2.2.1. Preparation of aqueous extract:

The samples were extracted using the traditional Soxhlet procedure. A 20 g dried plant sample was refluxed in 300 mL de-ionized water for 10-12 hours. Using the Soxhlet Apparatus at a boiling water temperature. Using a rotary evaporator, the extracts were filtered and concentrated till dry. Store in a dark place for use in the next step[23].

2.2.2. Preparation of alcohol extract:

Alcohol extract was prepared in the same way as the aqueous extract separation, but using ethanol instead of water.

2.3. Determination of Vitamins concentration:

Identification of vitamins was done at the department of environment and water/Ministry of Science and Technology, using HPLC technique, (SykamS3210, Germany), with C18 column (4.6mm × 150 mm, 5µm).Mobile phase (Methanol: water) (35:65), flowrate(1mL/min),detection fluorescence system (254 nm).All the standard materials used for vitamins were pure, 99%, from Samarra/Iraq/Pharmaceuticals factory, a mixture of water: acetonitrile: glacial acetic acid was selected as a solvent for samples studied and by [94:5:1] respectively, using the mobile phase of the following mixture (Methanol: water) and by[25:75] respectively, the solution has been nominated with candidates with a diameter of 0.45µm,this combination has been chosen as a mobile phase for its suitability for the analysis of these vitamins. In the second stage, 200 mg of Ginkgo leaf extracts (aqueous and alcohol) were weighted and dissolved with a 5mLof standard solution, and then the mixture was transferred to a 25 ml volumetric flask, placed in a water bath at 0°C (65-75) °C for 10 minutes, stirring continuously until dissolved and then complete the volume with de-ionized water to the mark. Then (5 ml) from the previous extract solution was placed in a 50 mL volumetric flask, the solution was completed to the mark by deionized water, then the mixture was filtered, and the solution has been nominated with candidates with a diameter of 0.45µmbefore injection into the HPLC system[21][23].

2.4. Determination of trace element concentrations:

Flame Atomic Absorption Spectrophotometer (FAAS), Model AA646, Shimadzu Corporation, Kyoto/Japan, and Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS)were used to determine trace element concentrations at Ghazi Hariri Hospital in Baghdad. Herbal infusions and dilutions were made with deionized water [Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se and Zn], using a microwave digestion system before metal analysis. The microwave parameters were given in Table (1).

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Table (1) Microwave procedure for digestion procedure amountof the sample [24].

Amount of sample	1 g of plant sample
Digestion reagent	digested in HNO_3 + $HClO_4$ (3:1) (20) mL
Steps	leave for one night
	heated in a water bath
	cool at room temperature
	+ 70% $HClO_4$ (5 mL) heated first in a water bath for 30 minutes
	heated for 15 min until reduced the volume of the solution to 10 mL
	Diluted with deionized water to 30 mL
	filtered
	diluted with deionized water to 50 mL

A standard AAS reference stock solutions of the studied metals containing 1000 μgmL^{-1}were diluted with (3:1) ratio of HCl: HNO_3 to prepare different concentrations of solutions (0.5, 1, 2, 5, 10 and 20) $\mu g.mL^{-1}$[24].The standard solutions were used to generate calibration curves for the metals. Linear curves have been obtained after plotting the results. Operating conditions of spectrophotometer technique were shown in Table (2)

2.5. Phytochemical compounds:

Qualitative detection of Ginkgo extracts have been carried out to make sure there is presence of active compounds using standard procedures: alkaloids (Mayer's Test), steroids (chloroform + concentrated H_2SO_4), terpenoids (Salkowski Test),carbohydrates (Benedict's reagent), glycosides (Keller Kilianin Test), proteins (NaOH+ copper sulfate), Saponins (Foam Test), phenols, tannins (lead acetate solution),and flavonoids (Alkaline reagent Test)[25].

3. Results and Discussion:

3.1 Extraction yield:

a metric for how effective a solvent is at extracting specific components from the material, and has been defined as the number of extracts recovered by mass compared to the original amount of the sample,It is determined as follows [26]:

Mass extraction yield (g/100g %) = (weight of extract/weight of Ginkgo leaves) ×100

The aqueous extract yield was found to be(12.355gm/100gm), while alcoholic extract yield was found to be (20.525gm/100gm).

3.2 Vitamins:

The results were as follows Table (3) by the measurements of the HPLC system, Column C18, and wavelength (254nm) have been used with standard substances for each type of vitamin measured.The concentration per vitamin was calculated by comparing the area of the pick of the standard substance with the area of the pick for the desired vitamin and according to the following equation:

C (sample) = [A (sample)/A (standard)] ×C (standard)

In the aqueous extract, the presence of dissolved vitamins in water (C, B2, and B1) was confirmed and the concentration of vitamin C was the highest and had the best separation of the peak at retention time (4.628 min). The result showed the presence of water-soluble vitamins at a range retention time (4-7min) at a wavelength of 254 nm Figure (1) and Table (3).

In the alcoholic extract, the presence of dissolved vitamins in fat (E, D_3, A,and K) was confirmed and the concentration of vitamin E was the highest and had the best separation of the peak at retention time (6.480 min) according to the peak of standard substances. The result showed the presence of fat-soluble vitamins at a range retention time (5-12min) at a wavelength of 254nm.Figure (2) and Table (3)**.**

Table (2) the standard operating conditions of the Spectrophotometer

Traceelement	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
The wavelengthOfabsorption (nm)	228.0	242.5	357.9	324.8	248.3	279.0	232.0	217.0	196.0	307.6
Lamp Current (mA)	9	10	5	5	10	4	10	9	8	7
Carrier Gas Flow (ml/min)	250	300	300	300	300	250	250	300	400	300

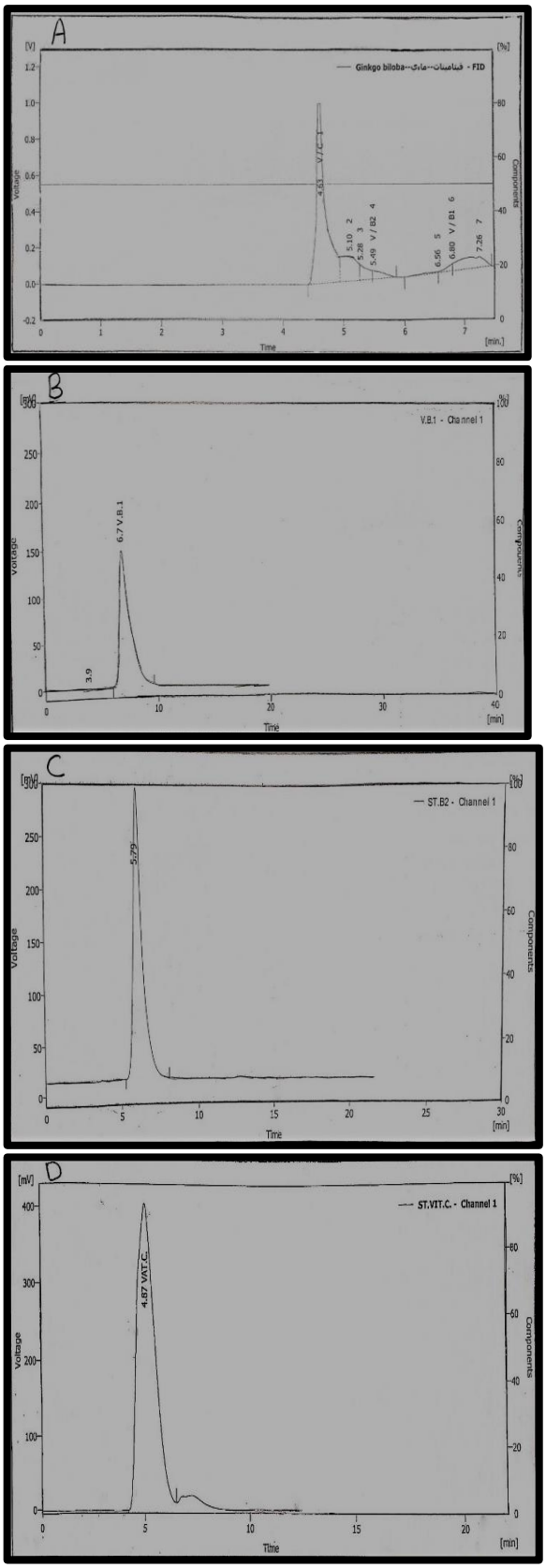

Figure (1):- HPLC analysis for (A) Vitamins water-soluble.(B) Vitamin B1 standard, (C) Vitamin B2 standard, (D) Vitamin C standard.

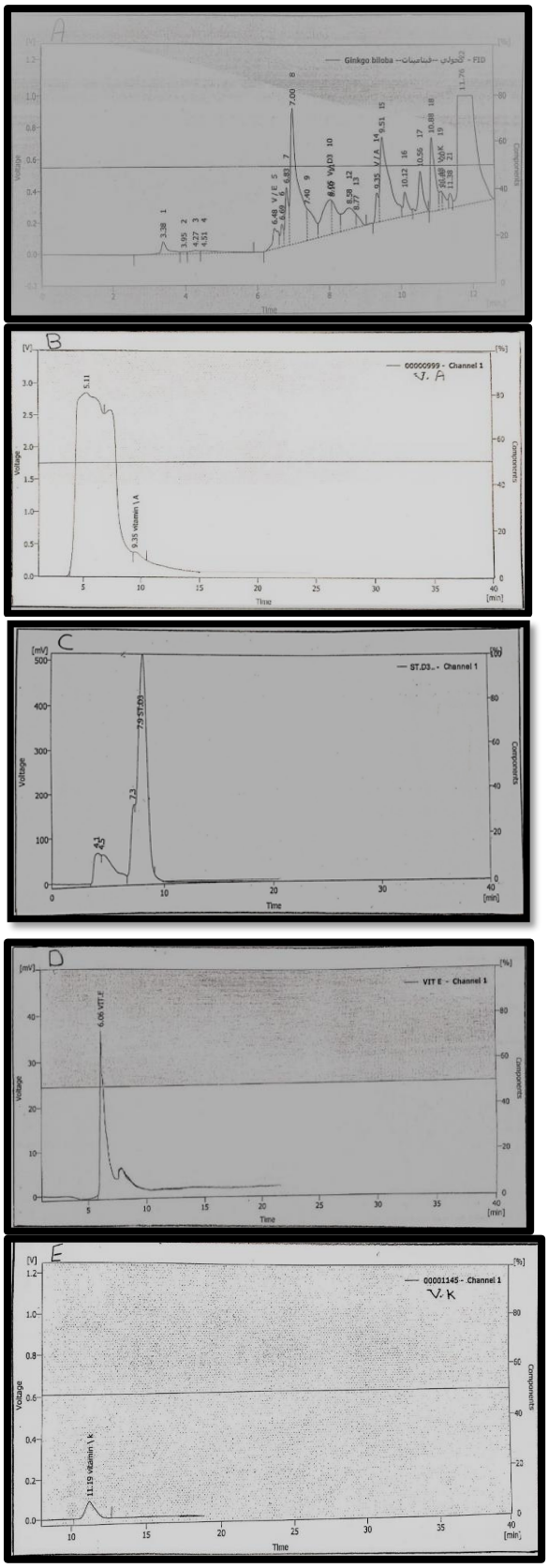

Figure (2):- HPLC analysis for (A) Vitamins fat-soluble. (B) Vitamin A standard, (C) Vitamin D3 standard, (D) Vitamin E standard, (E) Vitamin K standard

3.4. Trace elements: Plant samples of extracts were prepared using optimal digestion as described in the previous section? and analyzed according to the conditions are presented in Table)2 (.

The results of atomic absorption revealed that the presence of different amounts of trace elements necessary for human nutrition in leaves and water and alcoholic extracts Table (4).

3.5 Phytochemical compounds: Examining and studying the presence of plant chemical compounds in both extracts of Ginkgo Biloba leaves are shown in Table (5)

Table (3): Concentration of dissolved vitamins in the water and ethanol extracts of the Ginkgo biloba plant.

Vitamins	Resultsaqueousextract Areaconc. [mV.s]	[µg.mL^{-1}]	ResultsethanolicExtract Area [mV.s]	con. [µg.mL]	Retention Time [Min] Extracts	Retention Time [Min] Standard
A	_	_	1184.700	7.0782	9.352	9.345
E	_	_	1436.122	29.0106	6.480	6.058
D_3	_	_	3978.072	1.8614	8.048	7.890
K	_	_	671.179	0.8663	11.132	11.188
C	10787.128	2.3412	_	_	4.628	4.873
B_1	277.141	0.1467	_	_	6.800	6.700
.B_2	595.259	0.2741	_	_	5.492	5.793

Table (4) Contents of trace elements in the analyzed samples of Gingko Biloba (µg/g)

Element	Leaves (before the extraction) (µg/g)	Aqueous extract (µg/g)	Ethyl alcohol extract (µg/g)
Cd	0.163	0.067	0.022
Co	0.264	0.095	0.087
Cr	4.221	1.552	1.399
Cu	7.936	3.499	2.130
Fe	263.72	117.881	109.263
Mn	17.553	5.456	10. 535
Ni	3.836	1.572	1.233
Pb	6.092	3.216	1.671
Se	0.395	0.078	0.189
Zn	20.933	9.345	6.892

Table (5) Experiment reagents and chemical detection of Ginkgo biloba leaf extracts (water and ethanol) Active Compound

Active compounds	Experiments reagents	Indication	Water Extract	EthanolExtract
Alkaloids	Mayer's test	White precipitate	++	++
Steroids	chloroform+ concentrated H_2SO_4	Layer yellow+green fluorescence	–	++
Terpenes	SalkowskiTest	Reddish-brown layer	+	++
Phenols	Lead acetate	Whiteprecipitate	++	++
Carbohydrates	Benedict's test	Green solution	++	_
Glycosides	Keller KilianinTest	brown ring	++	++
Proteins	NaOH+ copperSulphate	color	++	+
Saponins	Foam Test	Foam white	++	–
Tannins	Lead acetate	yellowish precipitate	++	++
Flavonoids	Alkaline Reagent Test	colorless	++	++

Key: ++ = High concentration

+ = Presence of bioactive compound

– = Absence of bioactive compound

All the results obtained are in agreement with previous studies.

The Ginkgo plant if it is for the acomposition of vitamins, mineral, elements, and chemical compounds [26, 32], and the varying difference in the results of this study from previous studies isdue to the different habitats of plant growth used, from environmental and soil components and the quality of irrigation water, climate, temperature, and others that certainly have a significant impact on the plant.

4. Conclusions:

This study has revealed that a diet of Gingko Biloba can bring much health to people and further research should focus on the development of an appropriate form and route of administration of Ginkgo so that therapeutic effects are maximized.

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