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Determination of Vitamins, Trace Elements, and Phytochemical Compounds in Boswellia Carterii Leaves Extracts

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ABSTRACT: ***Background:*** *The assessment of the extraction yield and nutritional content of Boswellia carterii extracts, including vitamins and trace elements, is significant.* ***Objective:*** *The study aims to identify phytochemical compounds present, and concluding on the plant's potential health benefits and dietary contributions.* ***Methods:*** *The present study was conducted to extract the vitamins, trace elements and phytochemical constituents of Boswellia carterii leaves extracts using distilled water, ETOH (99%), and Ethylacetate by soxhlet. The yield of extracts was (18.361g/100g distilled water) while (29.322g/100g ETOH) and the Ethylacetate was found to be (27. 312g/100g). Different types of vitamins were estimated in all extracts utilizing HPLC.* ***Results:*** *Vitamins showed interesting results by revealing (A, B6, and B12). Vita. B12 is the most abundant in Alcohol extract, whereas vita. B12 is the most abundant in aqueous extract, and vita. B12 is the most abundant in ethyl acetate compared with vitamin A, and B6. These findings call for more research into the vitamins in Boswellia carterii and their antioxidant relevance in therapeutic herbal medicine. Different metal ions were measured using FAAS (Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, and Zn). The results of the qualitative detection of all extracts indicated the existence of Alkaloids, Steroids, Terpenes, Phenols, Carbohydrates, Glycosides, Proteins, Saponins, Tannins, and Flavonoids.* ***Conclusions:*** *The results of the qualitative detection of all extracts indicated the existence of Alkaloids, Steroids, Terpenes, Phenols, Carbohydrates, Glycosides, Proteins, Saponins, Tannins, and Flavonoids.*

KEYWORDS: *Boswellia carterii; Vitamins; HPLC; AAS; Phytochemical*

INTRODUCTION

Medicinal plant is used to treat or prevent disease or to relieve pain by altering the physiological or pathological process. Medicinal plants contain active physiological features that have been utilized in conventional medical practice for years to deal with a wide range of disorders [1]. One of these medicinal plants is Boswellia carterii, which is also one of the most researched medicinal plants [2]. Boswellia carterii leaves are abundant in a number of natural active ingredients and have a wide range of pharmacological properties [3] which play a significant role in food [4], health care [5], drugs[6], dietary supplements [7] anti-inflammatory, anti-cancer, antioxidant properties[8], and other fields. Boswllia leaves practiced antioxidant mechanisms by stifling free radicals and reactive oxygen produced during oxidative stress [9]. The traditional techniques of removing and determining ingredients substances from Boswellia carteii include Solvent extraction [10]. Extraction of cloud points [11] accelerated solvent extraction [12] extraction with the aid of ultrasound [13], Supercritical fluid extraction and Hydrodistillation (HD) [14]. The vitamins are a diverse set of components they have few in common either chemically or in their metabolic effects. Nutritionally, they share a cohesive collection of organic components that are needed in the diet in minute quantities (micrograms or milligrams daily) for the maintenance of normal health and metabolic integrity. They are thus

distinguished by the necessary minerals and trace elements (which are inorganic) and by necessary amino and fatty acids, which are needed in bigger quantities [15]. Generally the human body is unable to produce vitamins, and due to this, they must be consumed through food. The identification of vitamins plays a crucial role in nutritional and biochemical investigations, and analytical techniques are availably possible to identify these vitamins in various collection that are extracted from any component of the plant [16]. Many analytical methods have been used for the examination of vitamins: chromatography approaches [17]. electromigration methods [18] microbiological assays [19] and several other methods [20] HPLC has to its giving more decisions and more specificity than other procedures [21]. Minerals are inorganic compounds that are in all physiological fluids and tissues. Their found is needed for the preservation of several physicochemical activities 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 essential role in a variety of bodily functions [22]. The analysis of micronutrients in nutritional collections is very interesting from a dietary and business perspective. This kind of research is essential for comprehending the contents of foods and environmental samples given their harmfulness and crucial characteristics [23].

MATERIALS AND METHODS

Sample Collection

The study aimed is verify the number of vitamins, metal ions, and phytochemical compounds in the three extracts and compare the presence of these species between the three, and show the role of the boswellia plant in the treatment of several diseases such as myocardial disease, asthma, colitis, brain tumors, reduce skin damage due to radiotherapy in breast cancer patients [24].

Collecting Boswellia Carterii Leaves

The foliage of Boswellia cartetii was gathered at Sulaymaniyah north of Baghdad, Iraq, in September 2022, then washed with deionized water, dried in shade for several days at room temperature, and ground to powder.

Sample Extraction

Preparation of Aqueous Extract

Samples were extracted using the standard Soxhlet method. A 20 g dried plant sample was refluxed in 300 mL deionized water for 10-12 hours. Using the Soxhlet Apparatus at a boiling water temperature. Using a rotary evaporator, the extracts were concentrated and filtered till dry. Store in a dark area for use in the next step [25].

Preparation of Alcohol and Ethyl Acetate Extracts

The extract was prepared in the same manner as aqueous extract separation, but using ethyl alcohol and the ethyl acetate in place of water.

Determination of Vitamins Concentration

HPLC system (Sykam S3210, Germany) was used to identify vitamins in the Ministry of Science and Technology, Department of Environment and Water, employed C18 column (4.6mm × 150 mm, 5μm). Mobile phase (Methanol: water) (35:65), Flow Rate (1mL/min), detection fluorescence apparatus (254 nm). All the standard materials used for vitamins were exclusively 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 0.45 μm, this combination has been chosen as a mobile phase due to its compatibility for the analysis of these vitamins. In the second stage, 200 mg of boswellia leaf extracts (aqueous and alcohol) were weighed and dissolved with 5 mL of standard solution, and then the mixture was transferred to a 25 ml volume bottle, placed in a water bath at 0 °C (65-75) °C for 10 minutes, stirring continuously until dissolved and then complete the volume with deionized water to the mark. Then (5 ml) from the previous extract solution was added to a 50 mL volumetric flask, the solution

was completed to the mark by deionized water, then the combination was filtered, and the solution has been nominated with candidates with a diameter of 0.45μm before injection into the HPLC system [26], [27].

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 measure trace element concentrations at Ghazi Hariri Hospital in Baghdad. Herbal infusions and dilutions were prepared with deionized water [Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and Zn], using a microwave digestion system before metal analysis. A standard AAS standard stock solution of the studied metals having 1000 $\mu g.mL^{-1}$ was diluted serially with HCl: HNO_3 (3:1) ratio to achieve solutions of (0.5, 1, 2, 5, 10 and 20) $\mu g.mL^{-1}$ concentrations [28]. The standards were used to created calibration curves for the metals. Calibration curves of the metals demonstrated linearity results. The reference operating conditions of the spectrophotometer were provided for digestion procedure, the amount of sample was 1 g of plant sample, the digestion reagent was HNO_3 + $HClO_4$ (3:1) (20) mL leave for one night. The steps for the procedure were as following: sample heated in a water bath cool at room temperature, then + 70% $HClO_4$ (5 mL) heated first in a water bath for 30 minutes and heated for 15 min until reduced the volume of the solution was to 10 mL, after that diluted with deionized water to 30 mL filtered, and finally, diluted with deionized water to 50 mL.

Phytochemical Compounds

Qualitative detection of boswellia extracts has been applied to check certain there is the presence of active compounds using established techniques 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) [29].

RESULTS AND DISCUSSION

Extraction Yield

A metric for how effective a solvent is at extracting particular components from the material, and has been developed as the number of extracts recovered by mass compared to the initial amount of the sample, it is determined as follows [26]. Mass extraction yield (g/100g %) = (weight of extract/ weight of boswellia leaves) ×100. The aqueous extract yield was determined to be (18.361gm/100gm) while the Alcohol extract yield was determined to be (29.322gm/100gm) and the ethyl acetate extract yield was determined to be (27. 312gm/100gm).

Vitamins

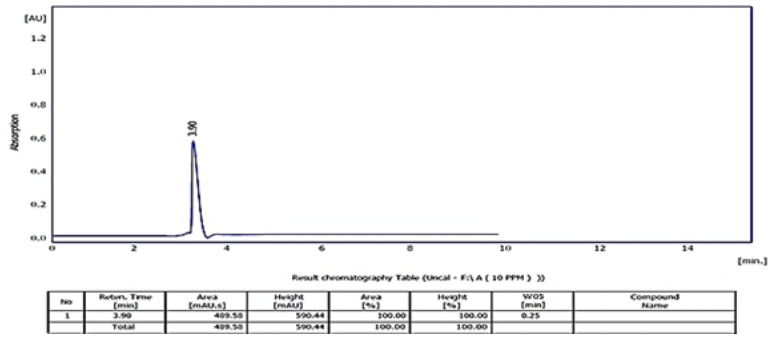
The results were as follows in Table 1 by the measurements of the HPLC system, Column C18, and wavelength (254nm) have been used with reference substances for every type of vitamin determined. The concentration per vitamin was calculated by comparing the area of the pick of the reference substance with the area of the pick for the desired vitamin according to the following equation:

$$C(Sample) = \frac{A(Sample)}{A(Standard)} \times C(Standard) \quad (1)$$

Table 1. The standard operating conditions of the spectrophotometer

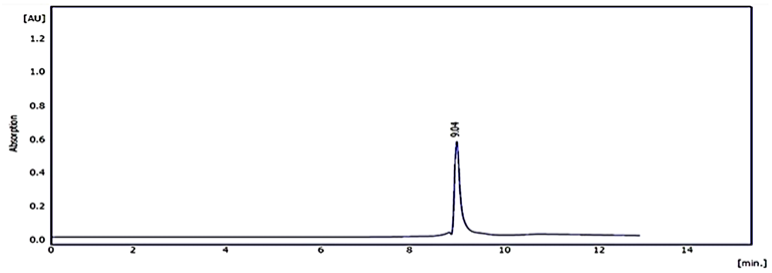
Trace elements	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn
The wavelength of absorption (nm)	288.0	242.5	357.9	324.8	248.3	279.0	232.0	217.0	196.0	307.6
Lamp current (mA)	9	10	5	5	10	4	10	9	8	7
Carrier Gas Flow (ml/min)	250	300	300	300	300	250	250	300	400	300

In the aqueous extract, the presence of vitamins (A, B6, and B12) was confirmed and the concentration of vitamin B12 was the highest while having the best separation of the peak at retention time (6.12 min). The result showed the presence of vitamins (A, B6, and B12) at a range retention time (3-9 min) at a wavelength of 254 nm Figures 1-4 and Table 2. In the alcohol extract, the presence of vitamins (A, B6, and B12) was confirmed and the concentration of vitamin (B12) was the highest while having the best separation of the peak at retention time (6.11 min) according to the peak of standard substances. The result showed the presence of vitamins at a range retention time (3-9 min) at a wavelength of 254nm as shown in Figure 5 and Table 2. In ethyl acetate extract., the presence of vitamins (B6 and B12) was confirmed and the concentration of vitamin (B12) was the highest and had the best separation of the peak at retention time (6.11 min) according to the peak of standard substances. The result showed the presence of vitamins at a range retention time (3-9min) at a wavelength of 254nm as shown in Figure 6 and Table 2.



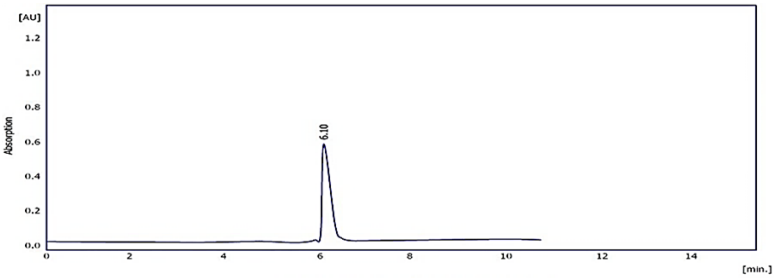
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.90	489.58	590.44	100.00	100.00	0.25	
	Total	489.58	590.44	100.00	100.00		

Figure 1. Chromatogram of vitamin A



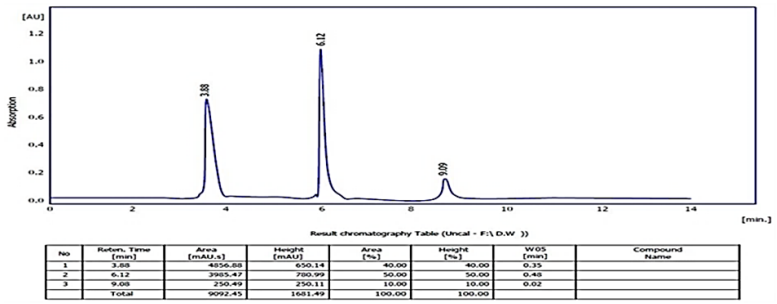
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9.04	690.48	599.05	100.00	100.00	0.25	
	Total	690.48	599.05	100.00	100.00		

Figure 2. Chromatogram of vitamin B6



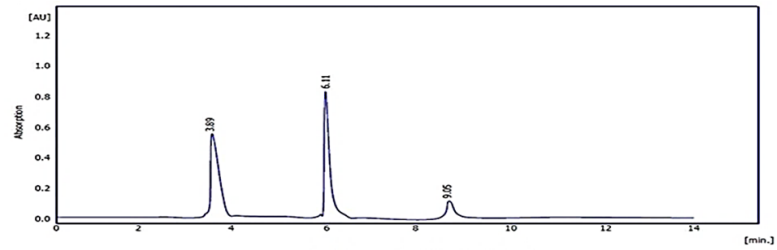
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.10	510.49	601.44	100.00	100.00	0.25	
	Total	510.49	601.44	100.00	100.00		

Figure 3. Chromatogram of vitamin B12



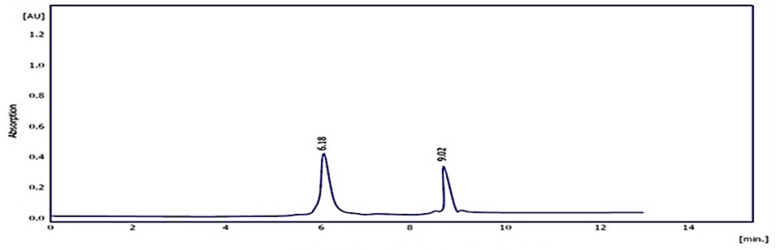
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.88	4856.88	650.14	40.00	40.00	0.35	
2	6.12	3985.47	700.99	50.00	50.00	0.48	
3	9.08	250.49	250.11	10.00	10.00	0.02	
	Total	9092.45	1681.49	100.00	100.00		

Figure 4. Chromatogram of aqueous extract



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.89	2562.15	590.15	40.00	40.00	0.35	
2	6.11	2894.15	745.89	50.00	50.00	0.48	
3	9.05	120.88	180.45	10.00	10.00	0.02	
	Total	5064.28	1895.98	100.00	100.00		

Figure 5. Chromatogram of alcoholic extract



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.18	1452.49	398.58	55.00	55.00	0.25	
2	9.02	954.88	370.11	45.00	45.00	0.20	
	Total	2407.15	768.69	100.00	100.00		

Figure 6. Chromatogram of ethyl acetate extract

Table 2. Concentration of dissolved vitamins in the water and alcohol extracts of the Boswellia carterii plant

Name	VIT (μg/g)	VIT B12 (μg/g)	VIT B6 (μg/g)
Ethyl acetate Ethanol	UDL	5.47	1.33
D.W	5.15	7.11	1.59
	8.15	11.25	3.65

Trace Elements

Plant samples of extracts were made utilizing optimum digestion as explained in the preceding section and analyzed according to the circumstances presented in Table 1. The results of atomic absorption revealed the found of different amounts of trace elements required for human nutrition in leaves and water and alcohol extracts Table 3.

Table 3. Contents of trace elements in the analyzed samples of Boswellia carterii (μg/g)

ELEMENT	Aqueous extract (mg/g)	Ethyl alcohol extract (mg/g)	Ethyl acetate (mg/g)
Cd	12.8	11.5	10.7
Co	0.084	0.076	0.086
Cr	4.2	8.1	3.8
Cu	7.1	7.5	3.6
Fe	-	-	-
Mn	5.6	4.216	4.112
Ni	3.2	4.2	3.9
Pb	5.8	3.7	3.5
Se	0.366	0.88	0.178
Zn	8.3	7.4	6.7

Phytochemical Compounds

Examining and researching the existence of plant chemical compounds in both extracts of Boswellia cartetii leaves are shown in Table 4.

Table 4. Experiment Reagents and Chemical Detection of Boswellia carterii leaf extracts (aqueous, alcohol and ethyl acetate) Active Compound

Active compounds	Experiments reagents	Indication	Water extract	Ethyl alcohol extract	Ethyl acetate
ALKALOIDS	Mayer'stest	Whiteprecipitate	++[a]	++	++
STEROIDS	chloroform+ concentrated H2SO4	Layer yellow+green fluorescence	++	++	++
TERPENES	SalkowskiTest	Reddish-brownlayer	+	++	+[b]
PHENOLS	Leadacetate	Whiteprecipitate	++	++	++
CARBOHYDRATES	Benedict'stest	Greensolution	++	++	++
GLYCOSIDES	KellerKilianin Test	brownring	++	++	++
PROTEINS	NaOH+ Copper Sulphate	Color	++	++	++
SAPONINS	FoamTest	Foamwhite	++	-[c]	-
TANNINS	Leadacetate	yellowishprecipitate	++	++	+
FLAVONOIDS	AlkalineReagent Test	colorless	++	++	++

[a] ++ = High concentration
[b] += Bioactive chemical present
[c] **-** = Bioactive compound absent

All the results obtained in this study matched significantly with many studies conducted in the past on the Boswellia plant if it is for the composition of vitamins, mineral elements, and chemical compounds[30], [31], and the varying difference in the results of this study from previous studies is caused to the different habitats of plant growth used, from environmental and soil factors and the quality of irrigation water, climate, temperature, and others that certainly have a significant impact on chemicals and their composition in the plant [32]–[34].

CONCLUSION

The study showed that Boswellia Carterii plant contains varying concentrations of vitamins, Phytochemical compounds and trace elements, which are comparable with other studies Plant were in the order Cd>Zn> Cu >pb> Mn>Ni>Se>Co. Also, the plant includes significant concentrations of vitamins A, B6, and B12, according to the results of the vitamin analysis. Therefore, the plant holds a terrific promise in providing a nutrient supply that could promote good health. It is possible to say that Boswellia Carterii contributes to the dietary intake of important elements and vitamins A, B6, and B12 when analysis results and suggested values are taken into account combined.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Mustafa T. Mohammed and Mohammed Z. Thani contributed to the study design; Heyam A. Hashim carried out the experimental work, data analysis, writing, drafting, and editing of the paper; Nisred K. Klichkhanov contributed to the discussion of the results.

FUNDING

None.

DATA AVAILABILITY STATEMENT

None.

ETHICAL APPROVAL

This study did not involve direct contact with humans, and all clinical isolates were obtained from hospital laboratories. Therefore, no ethical approval was needed.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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