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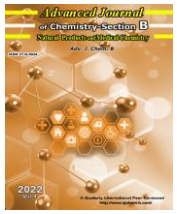

Original Research Article

Evaluation of some Chemical and Biochemical Constituents in Ocimum Basilicum Available in Msallata City- Libya

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ABSTRACT

The total phenolic, total antioxidant, ash, moisture, and metal contents of basil leaves collected within Msallata, Libya, were evaluated. To investigate the bioactive compounds, the basils were extracted using four solvents: water, ethanol, chloroform, and ethyl acetate. Phytochemical screening experiments revealed the presence of a variety of bioactive compounds in ethanolic and aqueous extracts, including coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, and proteins. Folin-Ciocalteu method was used to determine the total phenolic content of basil ethanolic extract. Furthermore, antioxidant activities were measured using phosphomolybdenum method. The total antioxidant capacity of basil was determined to be (49.8 14.7 mg ascorbic acid equivalent/g dry weight), while the total phenols were 39.75 (mg gallic acid equivalent/g dry weight). Likewise after dry digestion, the macro- and micro-metals; i.e. potassium, sodium, calcium, magnesium, and phosphorus, iron, copper, and zinc, in basil leaves were determined using Flame Photometry and Atomic Absorption Spectrophotometry. The mineral composition revealed that potassium (58288.33 370.32 mg/kg) had the highest concentration, while zinc had the lowest (18.39 0.37 mg/kg). The results showed that basil aqueous extract had the highest yield (14.80%) and ethyl acetate extract had the lowest (2.41%). However, the moisture and ash contents were determined to be 16.00% and 15.31%, respectively. Finally, the basil extracts demonstrated the potential for use as health-promoting food ingredients.

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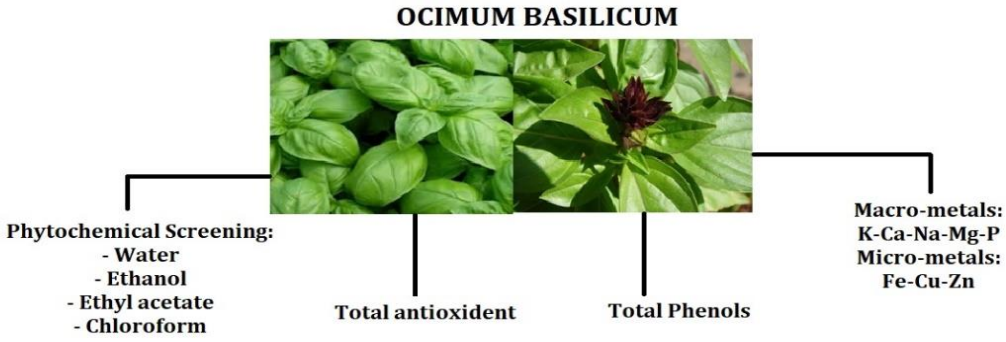
GRAPHICAL ABSTRACT



1. Introduction

Medical plants are useful in disease prevention and treatment, and they can even prevent and reduce the side-effects of conventional treatments. They may contain chemical compounds that are biologically and pharmacologically significant. Medical plants have historically been a source of successful drugs and will continue to play an important role in the screening of new lead compounds [1]. Herb combinations are recommended by traditional medicine for the treatment of common diseases such as fever, joint calcification, and other diseases. Individual and community health benefit greatly from medicinal plants. Approximately, 3.4 billion people in the developing world use plant-based traditional medicines. This represents approximately 88% of the world's population, with traditional medicine serving as the primary source of primary health care. The World Health Organization (WHO) supports traditional medicine as long as it has been proven to be effective and safe [2].

Plants contain various phytochemicals, which are also known as secondary metabolites. Individual, additive, or synergistic actions of phytochemicals to improve health make them useful in the treatment of certain complaints [3]. In the pharmaceutical industry, phytochemicals are essential for the development of new drugs and the preparation of therapeutic agents [4]. The discovery of active constituents in natural sources is the initial step in the development of new drugs. Screening plant extracts is a novel method for identifying therapeutically active compounds in various plant species [5]. Flavonoids, tannins, saponins, alkaloids, and terpenoids have a variety of biological properties, including antioxidant, anti-inflammatory, anti-diarrheal, anti-ulcer, and anticancer properties [6]. Ocimum Basilicum L. is a member of the Lamiaceae plant family, and it has traditionally been used as a medicinal herb to treat headaches, diarrhoea, warts, coughs, constipation, and kidney malfunction [3,7] as an antispasmodic, carminative, aromatic, and stomachic tonic [4,8].

When using herbs to treat specific conditions, it should be noted that, in addition to their pharmacological effect, they can be toxic if they contain toxic contaminants such as pesticides or heavy metals [9]. Prolonged exposure to plants containing heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), and nickel (Ni) above the maximum allowable limit is thought to cause severe health problems such as dermatitis, poisoning, organ dysfunctions, cancer, mental retardation, nervous system damage, anaemia, and so on [10]. Heavy metals can be essential or non-essential in the body, depending on their

role. Essential metals or micronutrients like Cr, Co, Cu, Mn, Mo, Ni, Fe, Se, and Zn are required for the proper operation of biological and biochemical processes, including humans. As, Cd, Hg, and Pb are non-essential metals with no known biological function. Heavy metals are not biodegradable, so even at low concentrations they can be toxic, posing a serious threat to both the environment and human health [11]. Minerals are necessary for proper body function. Many micronutrients are active centres for enzymes and vitamins. Minerals such as potassium help to maintain healthy blood pressure, and zinc helps to lower blood sugar and improve cholesterol levels. Iron is a protein component that is essential for the transport of oxygen from the lungs to all body cells. Manganese is an important component of many physiological processes and an enzyme activator [12]. Because medicinal plants are widely used as dietary supplements and in folk medicine, extensive research on these plants was required. The importance of the present study was established by determining the bioactive active components of medical plants, studying their content of total phenolic compounds and total antioxidants, and estimating their content of vital minerals for the body. Therefore, the goal of this study was to identify bioactive compounds in four extracts (water, ethanol, ethyl acetate, and chloroform) of the ocimum basilicum plant through qualitative phytochemical screening and to determine moisture, ash, total phenols, and total antioxidants contents of the studied plant. In addition, the levels of potassium, sodium, calcium, magnesium, phosphorus, copper, iron, and zinc in the herbal plant sample were determined using flame photometry and flame atomic absorption spectrometry.

2. Experimental

Sample Plant Collections

Fresh basil leaves were purchased from local markets within Msallata. The plant under study was collected in January, February, and March, 2021.

Plant Sample Preparation

The plant samples were washed several times with tap water, and then with distilled water to remove the dirt and dust that had accumulated on them. The plant samples were then air-dried at room temperature for 25 days before being ground using an electric grinder, sieved, and stored in sealed glass bottles.

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade and used without further purification. These Reagents, solvents, and chemicals are listed in Table (1).

Phytochemical Screening

Carbohydrates, proteins, phenols, alkaloids, flavonoids, tannins, saponins, steroids, glycosides, Coumarins, and terpenes were identified in four plant extracts (aqueous, ethanolic, ethyl acetate, and chloroform) using procedures described in the literature [13-18].

Yield

The extraction yield (g of dry extract per 100 g of fresh plant sample) was calculated. A solvent extracted plant was evaporated in a water bath at 40 °C and then dried for 24 hours in an air oven at 40 °C. Extraction yield was calculated using the final dry weight. The yield of the plant extract was calculated using the following equation:

$$\% Y = \frac{\text{Wt of dry extract (g)}}{\text{Wt of fresh sample (g)}} X\ 100 \qquad (1)$$

Where, % Y is Yield [14].

Table 1. Chemicals, solvents, and reagents used in the study

Reagent	Chemical Formula	Purity percentage	Company
1-Naphthol	$C_{10}H_8O$	≥99%	Riedel-DeHaen
Sodium Potassium Tartarate	$NaKC_4H_4O_6.4H_2O$	99%	Cosmic chemicals
Iron (III)Chloride	$FeCl_3$	96%	Winlab
Lead Acetate	$(CH_3COO)_2Pb.3H_2O$	99.5%	T-Baker Lab Chemicals
Ammonium Molybdate	$(NH_4)_6MO_7O_{24}.4H_2O$	99%	Winlab
ChloroForm	CH_3Cl	99%	Winlab
Folin-Ciocalteu's reagent	-	-	Sigma-Aldrich
Gallic acid	$C_7H_6O_5$	97.5-100%	Sigma-Aldrich
Ascorbic acid	$C_6H_8O_6$	≥99%	Merck
Ethyl acetate AR	$CH_3COOC_2H_5$	99.5%	Pspark
Chloroform	CH_3Cl	99%	Winlab
Ethanol	C_2H_5OH	96%	Sigma-Aldrich
Copper(II) sulfate	$CuSO_4.5H_2O$	99%	Riedel-deHaen
Tri-sodium citrate	$C_6H_5Na_3O_7.2H_2O$	99%	Scharlau
Sodium bicarbonate	$NaHCO_3$	99.7%	Riedel-De Haen
Sodium hydroxide	NaOH	≥98%	Carlo Erba
Sulphuric acid	H_2SO_4	-	Carlo Erba
Hydrochloric acid	HCl	-	Riedel-deHaen
Potassium iodide	KI	99.5%	Riedel-deHaen
Iodine Resublimed	I_2	99.8%	Winlab
Nitric acid	HNO_3	65%	Riedel-deHaen
Acetic acid	$C_2H_4O_2$	99-100%	Riedel-deHaen
Potassium Ortho Phosphate	K_3PO_4	95%	Riedel-deHaen
Sodium carbonate	Na_2CO_3	99.5%	Riedel-deHaen

Moisture and Ash

Plant samples were chemically analysed for moisture and ash using the Association of Official Analytical Chemists' official methods of analysis [19].

These methods rely on determining the mass of water in a known mass of sample before and after evaporation. The following equation can be used to calculate the moisture content:

$$\% \text{ Moisture} = \frac{wt_1 - wt_2}{wt_1} \text{ X } 100 \quad (2)$$

Where, wt_1 is the weight (g) of plant sample before drying and wt_2 is the weight (g) of plant sample after drying. 1.0 g of plant sample was dried in an oven at 60 °C for 1 hour. The sample is removed from the oven, allowed to cool in a desiccator, and weighed in grams. The process is repeated several times till constant weight is obtained. To obtain an accurate measurement of the moisture content of plant using evaporation methods, it is necessary to remove all of the water molecules that were originally present in the plant, without changing the mass of the plant matrix.

The ash content was determined using the method described in the literature [19]. A gram of plant material was dried in an oven at 100 °C to 105 °C. The dried sample was then ashified in a muffle furnace for one and a half hours by gradually increasing the temperature from 100 °C to 600 °C. It was then placed in a desiccator. After cooling, the ash was weighed, and the ash content was calculated using Equation (3):

$$\% \text{ Ash} = \frac{\text{Wt of ash (g)}}{\text{Wt of fresh sample (g)}} \text{ X } 100 \quad (3)$$

Estimation of total phenolic contents

The total phenolic content was determined using the Folin-Ciocalteu reagent method [20], which was slightly modified. It is based on the conversion of phenols to phosphomolybdate-phosphotungstic acid in an alkaline medium, where the solution is coloured blue and absorption at 760 nm is measured [20]. Ethanolic extract (0.25 ml) was placed in a separate test tube and mixed with dilute (1:10) Folin-Ciocalteu reagent (1.0 ml), which was then diluted to 10 ml with distilled water. After 5 minutes in the dark, (0.8 ml) Na_2CO_3 Solution (7.5%) was added to each test tube. The solution was thoroughly mixed by hand, and a similar procedure was used for gallic acid standards. All the test tubes were kept in a dark place for 60 min. The absorbance of the tested samples was measured by the UV spectrophotometer at the fixed wavelength 760 nm.

The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare the calibration curve of gallic acid, the following concentrations were prepared: 10, 20, 40, 60, 80, and 100 mg/l. The amount of phenolic compounds in the various extracts was expressed as gallic acid equivalence (mg gallic/g of dry sample).

Determination of total antioxidants activity

The total antioxidant capacity (TAC) of basil ethanolic extract was determined spectrophotometrically using the phosphomolybdenum assay, as described in the literature [21]. In capped test tubes, 0.3 mL of each extract solution in ethanol was mixed with 3.0 mL of phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid). The samples were then incubated for 60 minutes in a 95 °C water bath. After cooling to room temperature, the absorbance of the solutions was measured against a blank using a UV-Vis spectrophotometer at 695 nm (0.3 mL ethanol without plant extract). TAC values were expressed in milligrams of ascorbic acid equivalents (mg ascorbic/g dry sample). The same procedure was used to create ascorbic acid standards. An ascorbic acid calibration curve was used to calculate the TAC of extracts which were obtained at the following ascorbic acid concentrations: 20, 40, 60, 80, 100, and 120 mg/l.

Metal Contents

The contents of minerals and heavy metals [sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphor (P), iron (Fe), and zinc (Zn)] in the powdered samples were determined using a Jenway flame photometer and a VARIAN 220 FS atomic absorption spectrometer.

Table 2. Instruments used in the study

Instrument	Model	Company
Atomic Absorption System (AAS)	Varian 220 FS	Varian
Flame Photometer	PFP7	Jenway
Drying Oven	EV-50	RAYPAnet
UV-Vis Spectrophotometer	6300	Jenway
pH meter	3540	Jenway
Analytical balance	ME54	Mettler Toledo

Dry digestion method [22] was used to prepare the samples for analysis. All glassware was cleaned by soaking it in a 10% nitric acid. solution overnight, and then rinsing it three times with distilled water. **Instruments** Various instruments were used in this study, as listed in the Table (2). **Statistical analysis**

All analyses were carried out three times and results were expressed as mean ± standard deviation.

3. Results and Discussion

Phytochemical Screening

The results of phytochemical screening experiments on basil plant extracts (ethanol, aqueous, chloroform, and ethyl acetate) revealed the presence of various bioactive compounds. The phytochemical compounds discovered are known to be medicinally significant. Table (3) presents the results of phytochemical screening.

Table 3. Phytochemical screening of four extracts of Ocimum Basilicum

Test		Solvents			
No.	Detection test	Ethanol	Water	Ethyl acetate	Chloroform
١	Steroids and triterpenes (Lieberman)	-	-	-	-
٢	Coumarins (NaOH)	+	+	-	-
٣	Flavonoids (NaOH)	+	+	-	-
٤	Alkaloids (Wagner)	+	+	-	-
٥	Alkaloids (Ferric chloride)	+	+	-	-
٦	Tannins (Ferric chloride)	+	+	-	-
٧	Tannins (Lead acetate)	+	+	-	-
٨	Phenols (Ferric chloride)	+	+	-	-
٩	Carbohydrates (Benedict)	-	-	-	-
١٠	carbohydrates (Fehling)	-	-	+	+
١١	Carbohydrates (Molisch)	+	+	-	-
١٢	Turbines	-	-	-	-
١٣	Saponins	-	+	-	-
١٤	Glycosides (Benedict)	-	-	-	-
١٥	Proteins	+	+	-	+

(+): Present and (−): Absent

According to Table 3, analysis of the aqueous and ethanolic extracts showed that the studied plant contained coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, and proteins, but no glycosides, steroids, or terpenes were detected. However, based on Table 3, the chloroform and ethyl acetate extracts of the studied plant contained only carbohydrates and proteins (in the case of the chloroform extract) and none of the glycosides, saponins, phenols, tannins, alkaloids, steroids, or terpenes were present. Compared the results of the basil ethanolic extract with previous studies, Al-Aubadi [23] discovered that ethanolic extract contains terpenes, tannins, flavonoids, saponins, alkaloids, and steroids, which are consistent with our findings except for glycosides. Adham [24] also found carbohydrates, tannins, flavonoids, phenols, and alkaloids in ethanolic extract, which agreed with our findings. Furthermore, in Al-Aubadi's study [23], the basil aqueous extract contained tannins, flavonoids, saponins, and alkaloids, which was consistent with our findings, but they differed from our findings for terpenes, glycosides, and steroids. Daniel *et al.* [25] study results agreed with our results for saponins, tannins, and steroids, but not for alkaloids and flavonoids. In addition, Azam and Irshad [26] found carbohydrates, tannins, coumarins, steroids, and flavonoids in the aqueous extract. These findings corroborated the findings of this study. However, it was in contrast to the results of proteins and saponins. In terms of tannins, steroids, terpenes, flavonoids, saponins, and glycosides, the results of Mousavi *et al.* [27] study on basil ethyl acetate extract differed from our results. Suriyavathana and Punithavathi's [13] study on basil extract found that chloroform extract contained alkaloids, carbohydrates, proteins, and glycosides, which agreed with our findings. They did, however, differ from our findings in terms of the presence of saponins, flavonoids, steroids, and terpenes. While the results of Mousavi *et al.* [27] differed from ours in terms of tannins, steroids, terpenes, flavonoids, saponins, and glycosides.

Yield

The yields were determined as a weight percentage of the extracts to the original weight of the dried sample used. According to Table (4), the percent yields of basil extracts were ranged from 2.40% to 14.80%. Still, the water extract provided the highest (14.80%), followed by ethanol (5.2%), and then chloroform (4.5%) and ethyl acetate extract yielded the lowest (2.4%). The percentage yield of basil was presented in Figure (1).

Table 4. Percentage yield of Ocimum Basilicum

Plants name	Yield (%Mass)			
	Ethanolic extract	Aqueous extract	ethyl acetate extract	chloroform extract
Ocimum Basilicum	5.23	14.80	2.41	4.50

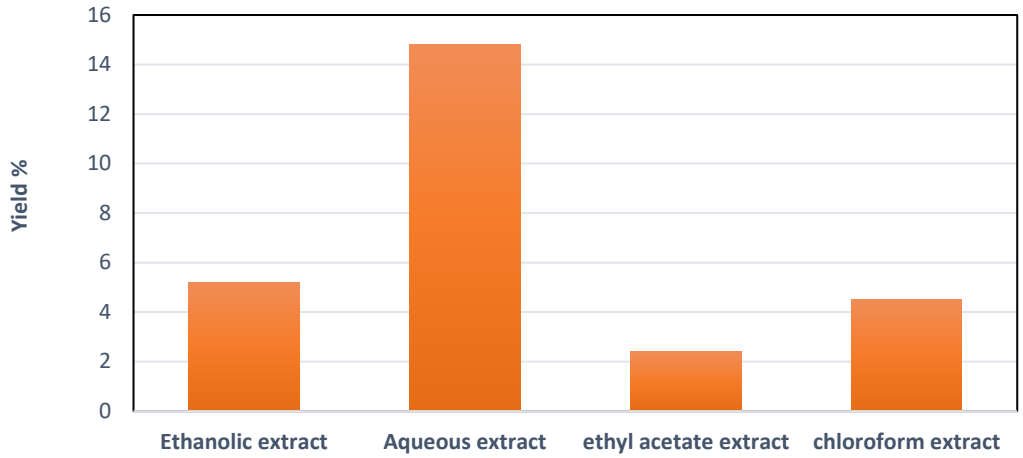


Fig. 1. Percentage yield of Basilicum

Moisture and Ash content

Table (5) shows the results of moisture measured and displays the ash content (as percentage) for the studied plant. It was observed that the ash content of the basil, which is 15.31%, while moisture was 16.00%. The moisture and ash contents of studied plant were presented in Figure (2).

Table 5. Moisture and Ash content of Ocimum Basilicum

%	Basil
Moisture	16.00
Ash	15.31

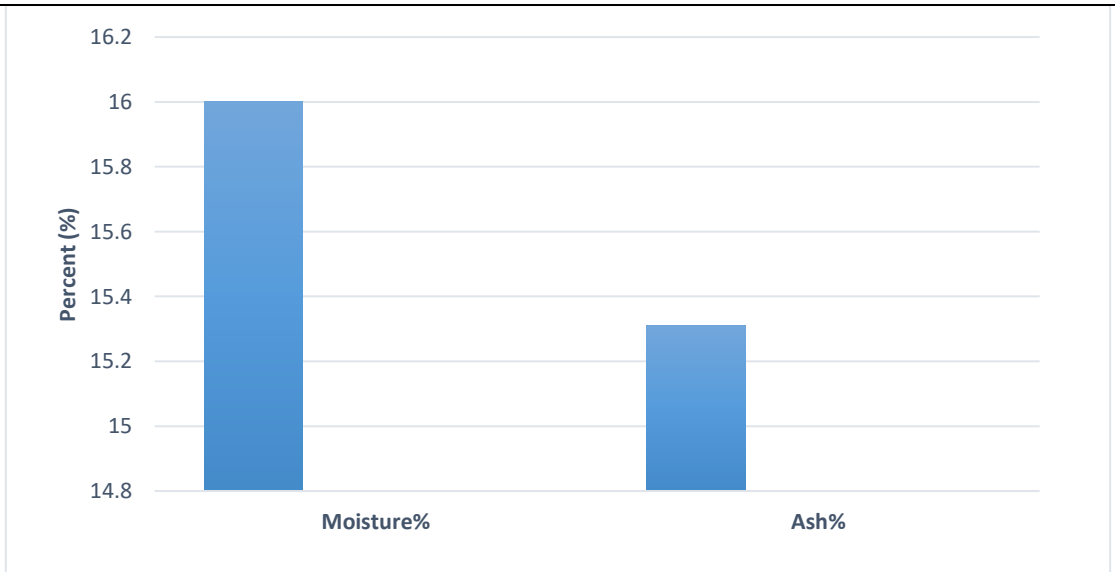


Fig. 2. The moisture and Ash content of Basilicum

Total Phenols

As a basis, phenolic content was measured using the Folin–Ciocalteu reagent in ethanolic extract. The results were derived from a calibration curve; Figure (3), (y = 0.0058 x, R^2 = 0.9704) of gallic acid (10–80 mg/L) and expressed in gallic acid equivalents (GAE) per gram dry extract weight (Table 6). The content of phenolic compounds in ethanolic extract was (39.75±14.84 mg GAE/g). It should be noted that the phenolic content and extract concentration have a linear relationship, indicating the suitability of the applied method, as depicted in Figure 5. Table 6 compares the results of the total phenolic content with some of the findings from earlier studies.

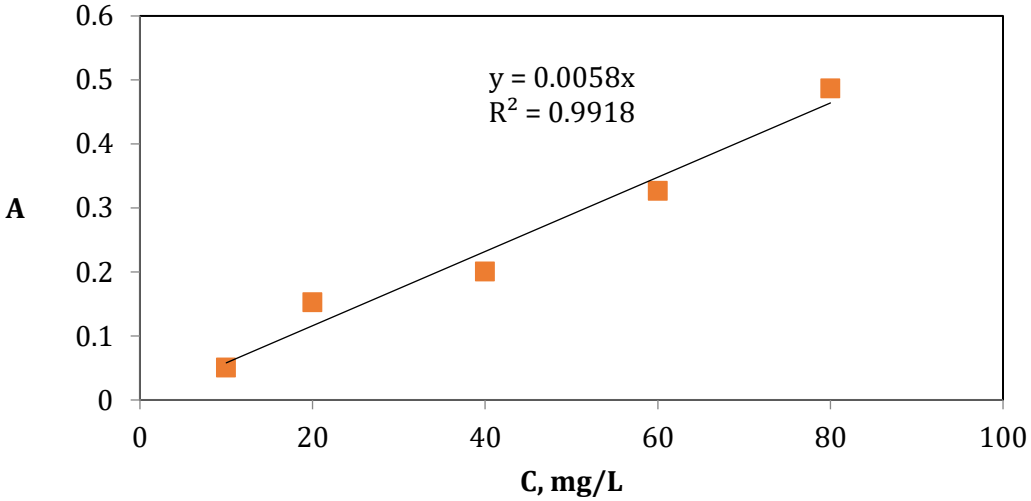


Fig. 3. Calibration curve of gallic acid

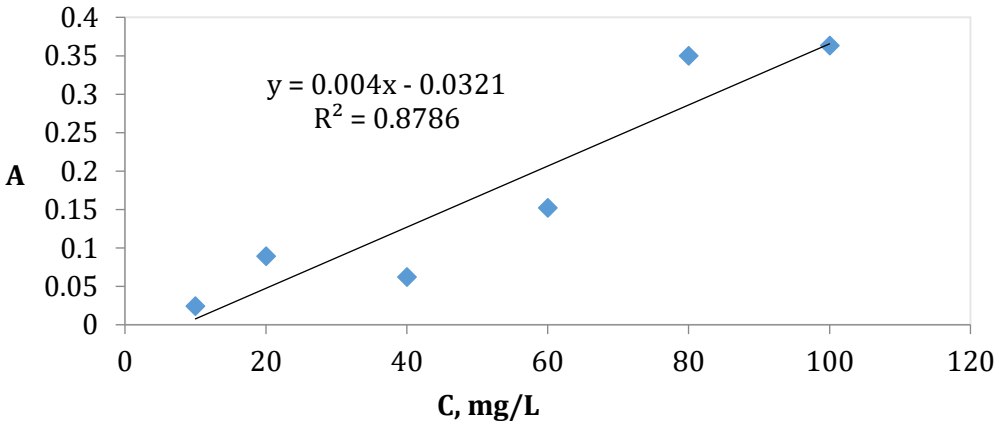


Fig. 4. Variation of phenolic content with extract concentration of Ocimum Basilicum

Phenolic compounds are important plant constituents with antioxidant activity due to their redox properties [28]. The hydroxyl groups in phenolic compounds play a role in free radical scavenging. Due to the presence of a hydroxyl group, phenolic compounds are more soluble in polar organic solvents, so ethanol was chosen as the extracting solvent [29]. Adebooye *et al.* [30] and Yen *et al.* [31], discovered lower total phenolic levels than the current study. Table 6 compares the current study's results to those of previous researches. The phenolic content values in this study differed slightly from those in the literature. This could be due to the presence of different amounts of sugars, carotenoids, or ascorbic acid, as well as the duration, geographical variation, or extraction methods, which could affect the amount of phenols [32].

Table 6. Total phenolic content and total antioxidant capacity of tested plant

Plant	Results	TPC (mg GAE/g DE)	Total Antioxidant (mg AAE/g DE)
Basil	Found	39.75 ± 14.84	49.8 ± 14.70
	Reported	20.68 – 255.93 [33]	69.33 – 89.22 [33]
		9.09 – 27.41 [34]	6.27 – 92.37 [34]
		55.60 – 57.10 [30]	1.29 – 11.23 [35]
		41.30 – 82.45 [35]	45.76 [12]
		52.61 [12]	

Total Antioxidants

Total antioxidant capacity, expressed in terms of ascorbic acid equivalents (AAE) [36], is a better way of depicting the combined effect of phenolics, flavonoids, and other reducing compounds in plant extracts. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) via antioxidant action and the formation of a green phosphate-Mo (V) complex with a maximum absorption at 695 nm [37]. The results were derived from a calibration curve; Figure (5), (y = 0.025 x-0.004, R^2 = 0.9808) of ascorbic acid (2.0-20.0 mg/L) and expressed in ascorbic acid equivalents (AAE) per gram dry extract weight (Table 6). These values are much lower than what was found by Albayrak et al (143.19±0.1 mg AAE /g of Basil) in Methanolic extract [38], and Akoto *et al.* (374.8 ± 0.9 mg AAE/g of basil) [39]. The results of the current study compared to some previous researches are shown in Table 6. A significant linear correlation was found between the values for the concentration of extracts and the antioxidant activity of extracts (Figure 6).

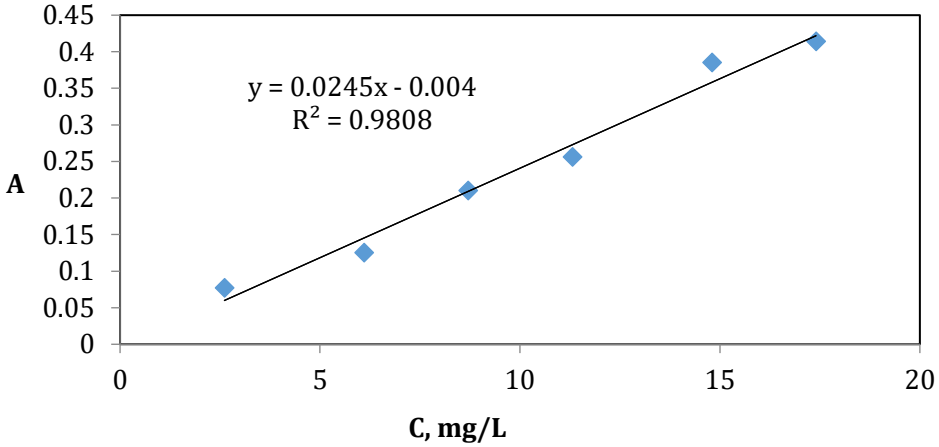


Fig. 5. Calibration curve for ascorbic acid for phosphate molybdate test

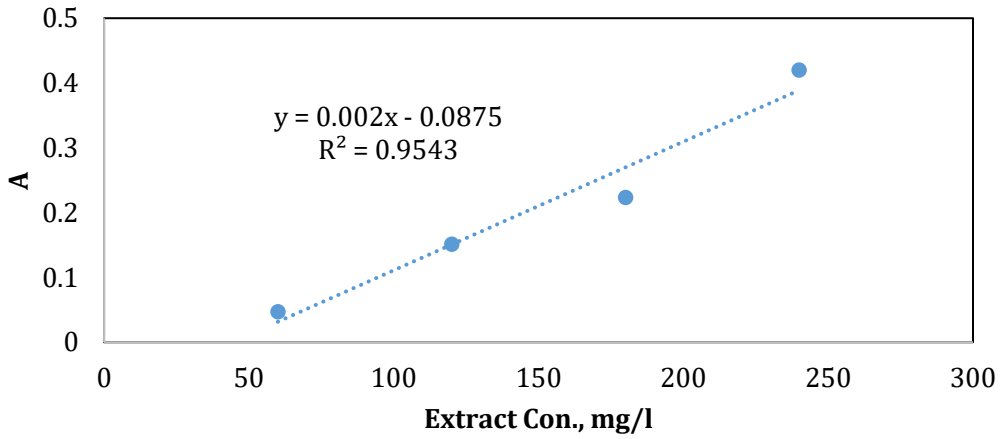


Fig. 6. Antioxidant activity contents of various extract concentrations of Ocimum Basilicum

Metal Contents

Table (7) indicates the data on total concentrations of the macroelements and heavy metals in the studied plant with concentrations expressed as mg of analyte per kg of sample, and also some results of previous studies. The highest concentrations were observed for macroelements (Na, K, Ca, Mg, and P). In case of heavy metals, the highest value noticed was for Cu (35.96 mg/kg) and the smallest was for Zn (18.39 mg/kg). The levels of major and minor metals in the tested medicinal plant are presented in Figures (7) and (8).

Table 7. Major and minor metal levels in Ocimum Basilicum

Metal	Found Content (mg/kg)	Reported Content (mg/kg)
Ca	5560.12 ± 109.01	965 – 1774 [42]
Mg	8457.22 ± 81.14	610 – 3778 [42]
Na	2824.41 ± 54.72	-
K	58288.33 ± 370.32	7500 – 19300 [43]
P	4669.19 ± 73.38	3000 – 5560 [43]
Fe	32.25 ± 0.96	185.73 –1101.23 [40] 4.90 – 107.4 [42]
Cu	35.96 ± 0.60	1.44 –18.87 [40] 7.4 – 29.2 [41] 3.84 – 14.05 [42]
Zn	18.39 ± 0.37	15.22 –112.19 [40] 7.6 – 34.2 [41] 90.65 [42]

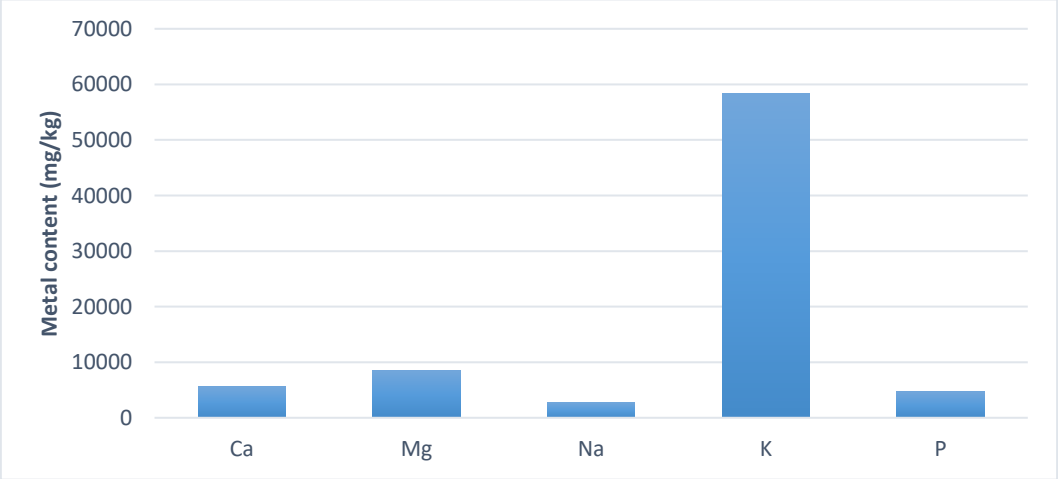


Fig. 7. Levels of calcium magnesium, sodium, potassium, and phosphorous in Basilicum

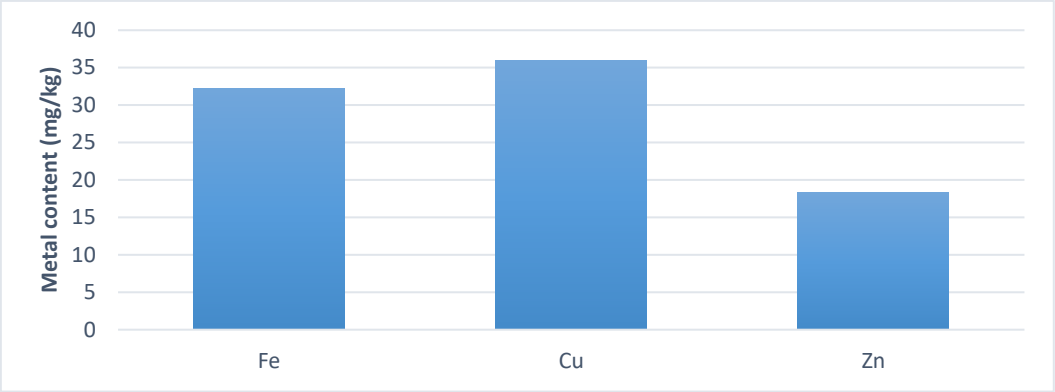


Fig. 8. Levels of Iron, Copper, and Zinc in Basilicum

The highest metal level was found to be potassium, with a content of 58288 mg/kg in the tested plant. Potassium is a major mineral and, unlike sodium, is a primary mineral found primarily within body, cells. Magnesium and calcium had the second highest metal levels, measuring 8457 and 5560 mg/kg, respectively. Calcium content in ten medicinal plants may aid in the prevention of degenerative and inflammatory diseases such as heart disease, skin infections, arthritis, gout, and respiratory tract infections [44]. The maximum micro-minerals (or heavy metals) monitored were copper and iron and their levels were 35.96 and 32.25mg/kg, respectively. The proper concentration of Fe in all plant species is critical for both plant health and nutrient supply to humans and animals. Iron is also a micronutrient required by almost all organisms [45]. Zn and Cu are essential elements for human, animal, and plant growth, and they play important roles in various metabolic processes.

Conclusion

Based on the findings, it can be concluded that crude extracts of Ocimum Basilicum can be prepared using ethanol and distilled water. Both plant extracts contained coumarins, alkaloids, flavonoids, tannins, saponins, phenols, carbohydrates, and proteins. Our findings show that Ocimum Basilicum (leaves) is an important source of various minerals, with significant differences between micro- and macro-elements. Phosphomolybdenum and Folin-Ciocalteu methods were used to determine the antioxidant activities (AA) and total phenolic content (TPC) of Ocimum Basilicum. There was a strong correlation between AA and TPC and extraction concentration. The antioxidant properties of Ocimum Basilicum from Libya were confirmed in this study.

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