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Quality, Quantity Analysis of Cadmium, Lead, Nickel, Active Chemical Compounds and Cytotoxicity for Some Medicinal Plants in Southern Iraq

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ABSTRACT
Medicinal plants are a rich source of various chemical bioactive compounds and are utilized in the pharmaceutical and food industries. The aims of the study investigation of safety-consuming medicinal plants in local Iraqi markets, and measure levels of cadmium, lead, nickel, Manganese, Zanic, and copper. In chamomile, cinnamon, cumin, turmeric, hibiscus, cyperus, nettle, borage, colocynth, and sider. Plants were collected from local markets in Iraq, and prepared. Aqueous extract of all plants, qualitative tests were conducted for all active chemical compounds. Heavy and trace elements were analyzed and measured by atomic absorption spectroscopy. Then samples were subjected to cytotoxicity by hemolysis test. Results All medicinal plants gave positive results for active chemical compounds, showing highest cadmium contamination was in 0.32 mg.kg-1cinnamon (42%), while three plants were recorded as free of cadmium: Saad, Urtica, Borage, highest lead contamination was recorded 2.49 mg.kg-1, cinnamon by percentage (18%) and lowest contamination was 0.27 mg.kg-chamomile (2%). The highest concentration of nickel in Hibiscas was 15.13 mg.kg-1 (16%), lowest concentration was at cinnamon at 1.47 mg.kg-1 (2%). Manganese recorded 81.58 mg.kg-1 highest level in Sidr while the lowest level was 12.11(3%) in Hibiscas. Zinc recorded in Cumin was 27.88 (61%), and Urtica's lowest concentration was 0.09 mg.kg-1, Copper recorded at 14.70 mg.kg- in Al-Handal (17%), lowest concentration in Chamomile was 2.10 mg.kg-1 (2%). The highest hemolytic activity in the urtic plant was 23% at 100 µg/ml, and cinnamon was 11% at 100 µg/ml. The study concluded high levels of copper, lead, cadmium, and nickel are not permitted by the World Health Organization in cinnamon, Urtica, and Hibiscas also hemolytic activity of nettle and cinnamon plants was recorded by up 10% at 100 µg/ml. These plants cause toxicity for customers. The study recommends periodically medicinal plants in markets to determine concentrations of toxic heavy metals.
Keywords: Active compounds, Trace, Heavy, Cytotoxicity phytochemical

INTRODUCTION
Traditional medicinal plants are used in health care for about 80% of the world's population, so medicinal plants are the best alternative to laboratory-made drugs, because they contain high levels of biologically active chemical compounds, in addition to having few side effects, and they are relatively cheap and easy to obtain, and often provide the best solution for chronic diseases [1].

Part or all of the plant can be used directly or after extracting the active ingredient from it. There are several methods for extracting active substances (from plants, such as boiling extraction, cold extraction, or solvent extraction [2]. Medicinal plants currently occupy a large place in agricultural and industrial production and receive great attention in many producing countries as they are the main source of herbal medicinal drugs or the source of active substances that enter into the preparation of medicine in the form of extracts and are also used as a raw material for the production of important effective pharmaceutical chemical compounds such as cortisone, sex hormones [3, 4]. The value of annual global exports was estimated at 50.000 to 70.000 Medicinal plants with medicinal characterization cost an estimated USD 2.2 billion in 2012-2017, a potential global market for the plant. When used rationally, the probability of side effects [5].

Heavy and Trace Metals
Heavy metals are a term that refers to a group of naturally occurring mineral elements with densities greater than 5 g/cm 3 [6]. Heavy metals are one of the main natural components of the earth [7]. Heavy metals are present in different concentrations. Depending on the chemical and physical of soil and its adverse effects on human health, especially at high conc. Therefore, heavy metal pollution, in food requires wide attention. More toxic heavy metals are mercury, cadmium, lead, arsenic, copper, iron, zinc, and chromium [8, 9]. Mercury, cadmium, lead, and arsenic are largely unnecessary for humans and present in very small concentrations, while elements such as Cu, Zn, and Mg are essential trace elements. Heavy metals and trace, elements enter the environment during various chemical and petrochemical industries and their wastes, especially petroleum derivatives, paper, textiles, dyes, electroplating, tanning industries, and multiple human activities, mining, vehicle emissions, and chemical pesticides, fungicides, fertilizers, or agricultural activities. The natural source of heavy elements is emissions from volcanic eruptions, sea salt spray, forest, fires, erosion of rocks, biomass, and wind-borne soil particles [10]. Contamination of medicinal plants with heavy metals is considered an important health problem for consumers, as their accumulation and increased levels in the blood and various human tissues lead to health damage and damage to vital organs such as the liver, lungs, heart, kidneys, and problems with the reproductive system and nervous system [11].

Medicinal Plants under Study

Chamomile

Chamomile is one of the most popular and widely consumed medicinal plants [12]. The plant's name is derived from the Greek words chamois ('earth') and Melos ('apple') [13]. Chamomile is used to treat a variety of ailments, including colds, coughs, indigestion, liver disease and respiratory problems. It is used as a sedative and chamomile is also commonly used to treat aches and inflammations, eye, skin and mouth. Chamomile is a medicinal plant belongs to Asteraceae family, and its scientific name is Matric aria chamomile [14].

Cinnamon

The oldest active medicinal herb, used in China for 4000 years. Cinnamon is a tree, belonging to laurel family. Bark and leaves of cinnamon are most important parts used as spices and flavor additives in foods and many medical fields, bark and leaves of cinnamon are commonly used in cooking and its distilled essential oil is an important agent in food and beverage industries [15].

Cumin (*Cuminum cyminum* L.) of the Apiaceae family is used as a traditional medicine and is native to the Mediterranean region Cumin is a short-leaved annual herbaceous plant (5-10 cm). The fruit of cumin contains a single yellow-brown seed. Cumin seeds have shown diuretic, gastrostomy, astringent, carminative, fungicidal and bactericidal properties

. Cumin (Cuminum cyminum L.) has broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria. It is an aromatic plant used in medicinal preparations [16].

Turmeric known as Arabic turmeric, curcuma in culture of Chinese, yellow, ginger jiang huang, and Japanese kyō, or okon) is a perennial rooted in Zingiberaceae family. It is cultivated annually in subtropical and tropical, regions of world [17]. India is the world's largest producer of turmeric; the most common species of the genus Curcuma is Curcuma longa. Other species of turmeric include, Curcuma zedoaria, Curcuma aromatic, Curcuma amada, and *Curcum rakataganta*. [18].

Hibiscus, commonly known as Chinese hibiscus, rose, Hawaiian hibiscus, and shoe dye plant, is a species of tropical hibiscus, a flowering plant in the genus Hibiscus of the Malvaceae family. It is widely cultivated in the western Pacific Ocean. Its species, Hibiscus cuperi and H. kaute. rosa-sinensis, are edible and used in salads by locals. The common name is "polishing plant". In Indonesia and Malaysia, these flowers are called "kembang sepatu" or "bunga sepatu", which literally means shoe flower [19].

Cyperus rotundus **(**also called coconut grass, nut grass, purple cyperus or Java grass, purple cyperus, reed cyperius, Khmer kravanh chruk) is a species of cyperaceae to and northern France and Austria and Asia. Word cyperus is derived from c. rotundus and many nessessary medina uses worldwide [20].

Nettle (*Urticaceae*), commonly known as nettle or common nettle

whole plant is used in traditional medicine to treatment of allergies, burns, anemia, skin rashes, internal infections, kidney stones, and stopped of bleeding. Pharmacological activities including antidiuretic, anti-inflammatory and antibacterial activity of U. dioica leaf extracts along with their toxicological evaluation [21].

Borage oil is extracted from the seeds of Borago officinal is. It contains high concentrations of essential fatty acids of the ω-6 series, which are essential for the skin. Linoleic acid found in borage oil has therapeutic effects in atopic dermatitis (AD). In traditional medicine using to treatment of skin infections, especially in children [22].

Citrullus colocynthis L.., a plant that grows in the deserts of Asia especially Middle East, North Africa, and southern Europe [23].

Citrullus colocynthis (*C. colocynthis*) has medicinal activity against a range of diseases, including diabetes, constipation, bronchitis, asthma, jaundice, joint pain, intestinal disorders, colic, gastroenteritis, hypertension, lung diseases, skin diseases, gynecological infections, and cancer [24].

Sidr included 40 species of small trees and shrubs belonging Rhamnaceae family. This genus found in reigns of have warm temperatures and climates worldwide [25]. Traditional medicine, Sidr have antibacterial activity, soothing, astringent activity, mouthwash, and toothache relief. It is used to treat a variety of medical conditions, including liver disease, digestive disorders, ant hyperglycemia, weakness, obesity, skin infections, urinary problems, bronchitis, pharyngitis, anemia, diarrhea and insomnia [26].

MATERIAL AND METHODS

Sample Collection

Ten random were collected of herbalists' shops and local markets for a group of unwrapped medicinal herbs that are commonly used by many people to treat some diseases as alternatives to chemical drugs. The experimental material consisted of 10 plants chamomile, cinnamon, cumin, turmeric, hibiscus, cyperus, nettle, borage, Citrullus colocynthis, and Ziziphus. Directly samples were purchased from markets spread in Basra and Thi Qar governorate.

Preparation of Sample

samples dried at 71 ˚C for one day, crushed and sieved (mesh = 1 00 µ00 to obtain a powder, kept in polyethylene bags until digestion and analysis.

Preparation of Aqueous Extract

50 grams from plant whole powder were refluxed with distilled water 250 ml for one day overnight, filtered by filter paper. The clear filtrate was concentrated by a rotary evaporator.

Phytochemical Screening Tests [27]

Carbohydrates test

Carbohydrates were tested using Molish's reagent.

Phenols Screen Test

Ferric Chloride (1%) Test
Flavonoids Screen Test
Flavonoids
(5N) Alcoholic KOH
Tannins Screen Test
1 % FeCl3
2 (2 %) per chloric acid test
Alkaloids Test
Wagner Test
Glycosides Screen Test
Triterpenes Screen Test

Heavy and Trace Metals Analysis

Heavy and trace elements cadmium (Cd), lead (Pb), nickel (Ni), nickel (Ni), Manganese (Mn), Zinc (Zn) and copper (Cu and Pb were digested according to method [28]. Sample preparation Glassware were washed with dist. Water, soaked overnight in 6 Normally HNO_3 solution and rinsed several times with pure distil. water, weighed samples (2.00 g) were transferred to a silica crucible and kept in a 450 °C oven for 3 hr. Then 5 ml conc. HNO_3 was added to crucible. crucible was placed on a hot plate and digested. Residue was dissolved added solution (2 ml HClO4 and 5 ml conc. HNO_3) continued heating to complete digestion process until dryness, then added 5ml HNO3 and H_2O (1:1), then added deionized distilled water to complete dissolution process, the contents were filtered with filter paper to remove un dissolved substances.

Heavy elements and trace elements were measured by an atomic absorption spectrometer (AA6300, Shimadzu, Japan). The results were expressed as mg/kg (dry weight). The data were appropriately rounded according to the standard deviation value from the measurements in triplicate

Determination of Cytotoxicity of Studied Plants

Cytotoxicity were determined in vitro by hemolysis, inactivates red blood cells of human by some of extracts of plants. In current study were used of healthy and non-smoking blood from volunteer [29]. 30 µl of the aqueous. extract of plant at a conc. of (12.5, 25.0, 50.0, and 100.0 µg mL-1); 1.2 ml of distill. water was added as a positive control. and 1.2 ml of T.B.S as a negative control. mixtures were vortexes, then 2 h at room temperature, and then centrifuged at 4.000 x. g for 10 min at 4 C. Absorbance, of supernatants were measured at 541 nm in UV-Vis. Spectrophotometer, measurement, (%hemolysis) was calculated by following equation: Hemolysis percentage% = (AT–AS) / (A 100% H–AS) × 100%AT:

AT: Solution Absorbance
AS: Normal saline Absorbance
A.100%. H: Absorbance at 100 % hemolysis

RESULTS AND DISCUSSION

Classification of Plants

The plant classification process was carried out by specialists in plant taxonomy in the Department of Biology, shown in Table 1

Table 1 Types of medicinal plants and their classification used in the study

Medicinal plant name	family	The scientific name	Part use
chamomile	Compositae	*Matricaria chamomilla*	*Dried flowers*
cinnamon	Lauraceae	*Cinnamomun verum*	*bark*
Cumin	Umbeliferae	*Cumin cyminum*	*fruits*
turmeric	Zingiberaceae	*Curcuma longa*	*Tubers*
Hibiscas	Malvaceae	*Hibiscas sabdariffa*	*Dried flowers*
Saad ,	Cyperaceae	*Cyperus rotundus*	*Tubers*
Urtica	Urticaceae	*Urtica pilulifera*	*Leaves*
Borage	Boraginaceae	*Borango officinalis*	*Dried flowers*
Al-Handel	Cucurbitaceae.	Citrullus colocynthis	*fruits*
Sidr	Rhamneae	Zizyphus	*Leaves*

Qualitative Phytochemical Analysis of Medicinal Plants

Qualitative tests to detect active chemical compounds in aqueous extracts of ten medicinal plants commonly used in markets Iraq: chamomile, cinnamon, cumin and turmeric, Hibis, Saad, Urtica Borage Al-Handel, Sidr. The current study revealed the presence of medicinally effective chemicals results were in tests, carbohydrates, alkaloids, phenols, flavonoids, tannins, alkaloids, glycosides and terpenes were examined notes results Table 2.

Table 2 Preliminary qualitative phytochemical analysis of some

Medicinal plant	Phytochemical test						
	Carbohydrates	Phenols	Flavonoids	Tannins	Alkaloids	Glycosides	terpenes
chamomile	+	+	+	-	+	+	+
cinnamon	+	+	+	+	+	+	+

Cumin	-	++	++	+	+	+	+
turmeric	+	+	+	-	-	-	-
Hibiscas	++	+	-	-	-	++	-
Saad	+	++	+	+		++	++
Urtica	+	++	++	++	+	+	-
Borage	+	+	+	++	+	+	++
Al-Handel	-	+	++	++	++	-	++
Sidr	+	++	++	++	-	++	+

(+) indicates presence, (++) indicates presence in high levels, (-) indicates absence.

shows various results in aqueous solution. The medicinal value of plants lies in some of the chemicals that have an effect. First of all, physiological effects on the human body. Other phytochemicals have been found to have a wide range of actions. Which can cover against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect ant cholesterol and antibiotic properties Steroids and terpenoids show analgesic effects on the central nervous system flavonoids, steroids, saponins, tannins and terpenoids also have various medicinal values, such as anti-inflammatory, anti-cancer. Diabetic, analgesic and central nervous system activity. The importance of alkaloids, saponins and tannins in The use of several antibiotics in the treatment of common pathogenic strains has recently been [30].

The qualitative analysis of bioactive compounds (table2) showed that it is rich in chemical compounds that act as good antioxidants. Analysis of aqueous extracts contains nine phytochemicals (carbohydrates, phenols, flavonoids, tannins, alkaloids, glycosides and terpenoids). Some active chemical compounds did not give positive detection, may be present in other parts of the plant, or they may not be present due to the use of aqueous solvents in the extraction process. When other solvents such as ethanol and acetate are used, some active secondary metabolites may appear [31].

Concentrations of Trace Elements in Medicinal

valuate Content of Manganese (Mn), Zinc (Zn) and copper (Cu) in medicinal plants, herbs and spices samples. results showed highest concentration of manganese 81.58 in Sidr, percentage of manganese was (17%) 73.28 in Cumin, while lowest concentration of manganese were 12.11 in Hibiscas (3%) while anther plants had manganese concentrations of 56.66 cinnamon Cumin73.28, turmeric29.5, 25.09, Urtica 45.12, Borage 62.52, Saad.

The highest concentration of zinc was recorded at 78 in the cumin plant (61%), while lowest concentration was 0.09 in Urtica plant. zinc concentrations were 0.5, 27.88, 21.17, 0.36, 0.45, 0.12,0.39and 0.41 for the chamomile, cinnamon, turmeric, Hibiscas, Saad, Borage, Al-Handal, and Sidr plants respectively. While the results showed that highest concentration of copper were 14.70 in Al-Handel plant (17%), lowest concentration was 2.1 in chamomile (2%), results recorded 5.99,2.72,5.48,12.7,12.06,8.52,11.12 and 12.71 for plants cinnamon, Cumin, turmeric, Hibiscas, Saad, Urtica, Borage and Sidr respectively, Table3 and Figures1and 2.

Table 3 levels of Trace elements in medicinal plants

medicinal plants	Concentration of Trace elements ($mg.kg^{-1}$) Mean± SD (n =3)		
	Mn	Zn	Cu±
chamomile	44.42 ± 9.07	0.53 ± 0.38	2.10 ± 0.32
cinnamon	56.66 ± 3.27	27.88 ± 3.10	5.99 ± 0.67
Cumin	73.28 ± 1.11	78 ± 3.34	2.72 ± 0.98
turmeric	29.5 ± 1.28	21.17 ± 2.21	5.48 ± 1.69
Hibiscas	12.11 ± 0.41	0.36 ± 0.07	12.7 ± 2.81
Saad	25.09 ± 3.20	0. 45 ± 0.16	12.06 ± 1.04
Urtica	45.12 ± 4.09	0.09 ± 0.033	8.52 ± 1.17
Borage	62.52 ± 7.91	0.12 ± 0.62	11.12 ± 1.20
Al-Handel	49.55 ± 1.81	0.39 ± 0.04	14.70 ± 2.00
Sidr	81.58 ± 5.0	0.41 ± 0.25	12.71 ± 1.02

Standard deviation.SD

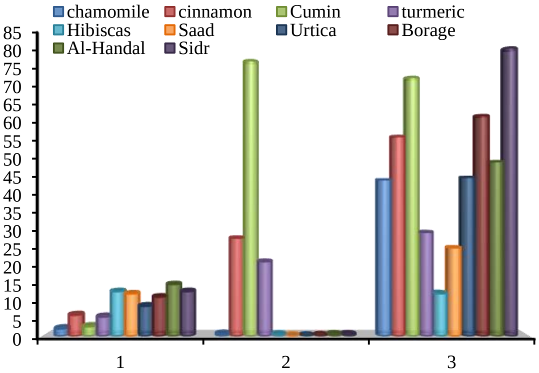


Fig. 1 Concentrations of Trace elements in medicinal

Copper plays a role in oxidative defense system, on the other hand, chronic copper toxicity can lead to severe toxicity. Zinc is acted of cofactor for more 199 enzymes involved in metabolic pathways, but its high levels in human body can be toxic due to its interference with copper metabolism [32]. Therefore, dietary zinc intake should be adequate. Zinc is acted of cofactor for more 195 enzymes specially metabolism bio reactions, high levels of zinc in human body toxic because Zn acted with copper in some metabolic pathways [32] dietary zinc intake should be adequate. Zinc content in all samples analyzed was relatively low, all samples examined were found to contain acceptable amounts of two elements, copper and zinc. WHO recommended limit for zinc in medicinal plants is 50 mg/kg while the daily intake is 11 mg/day [33]. The results revealed that zinc concentrations were below the recommended limit in all the plants studied except Cumin. The safe concentration of Cu in medicinal herbs about 20.00 to 150.00 mg/kg, present article reported copper levels below permissible limit. The results are in agreement with Maobe et al. reported that the concentrations ranged from 3.05 mg/k.g - 14.4 mg/kg [34] also within the limits of the World Health Organization (2006). Copper is an essential trace element that the body requires in small amounts and is involved in the functioning of many enzymes, especially oxidation-reduction enzymes, hemocyanin & metabolism in cell, high concentrations of copper lead to anemia and live cells damage & kidney problems. Patients with Wilson's disease have greater risk of health from excessive copper exposure [35, 36].

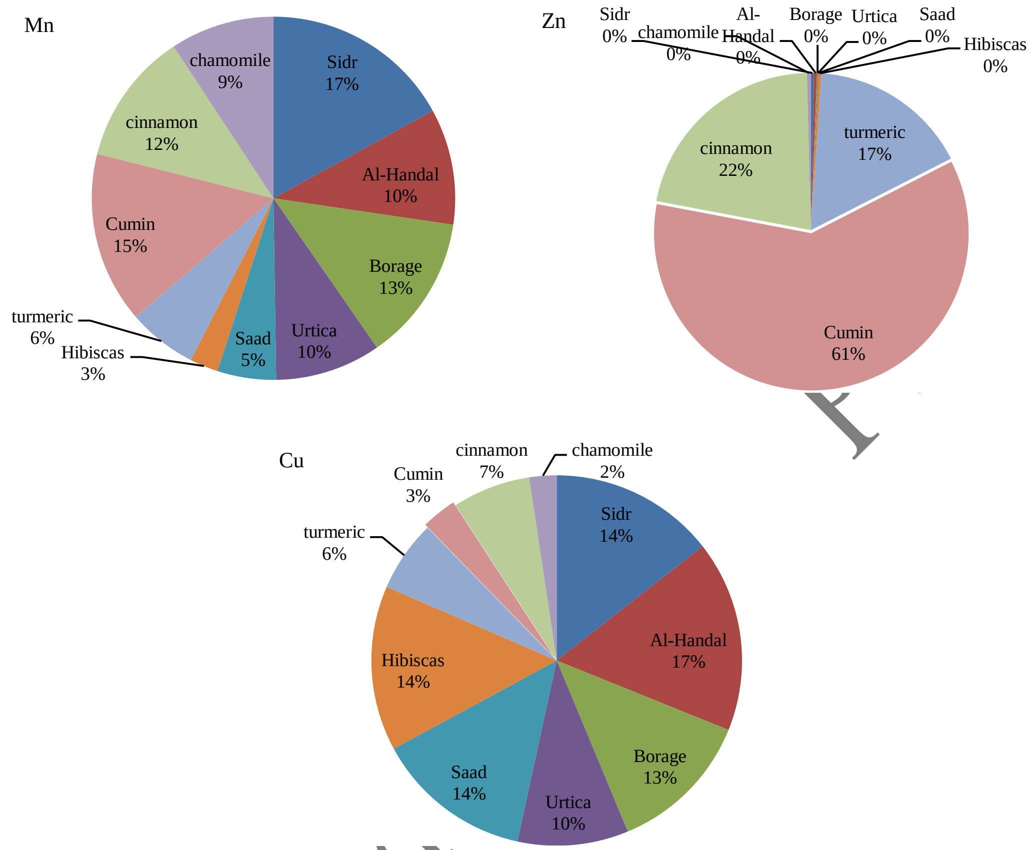


Fig. 2 percentage of Mn, Zn and Cu in medicinal plants

Concentrations of Heavy Metals in Medicinal

The results recorded concentrations of heavy elements cadmium Cd, lead Pb and nickel Ni in plants under study in unit ($mg.kg^{-1}$); highest level of cadmium were 0.32in cinnamon (17%), while the lowest concentration of cadmium was 0.01 in Al-Handel (1%), While the concentration of cadmium in plants Saad, Urtica and Borage are below detection limit (BDL). levels of Cd in chamomile was 0.18 in cinnamon, 0.32 in cumin, 0.05 in turmeric0.02 in Hibiscas 0.03 and sider 0.14. The highest concentration of Pb was recorded at Urtica 2.59, percentage (19%) while the lowest concentration was 0.27 in chamomile (2%). The concentrations of Pb were 2.49, 2.10, 0.88, 0.82, 1.95, 1.32, 0.92 and 0.49 for cinnamon, Cumin, turmeric, hibiscus, saad, Borage, Al-Handel and sidr plants, respectively. While results showed highest concentration of Nickel was 15.13 in Hibiscas (16%), lowest level was 1.47 in cinnamon (2%), also results were recorded as 5.17, 11.87, 9.19, 11.04, 7.18, 8.16, 13.91 and 9.77 for chamomile, Cumin, turmeric, Saad, Urtica, Borage, Al-Handel and sidr plants, respectively. Table.4 and Figure 3 and 4.

Table 4 levels of Heavy metals in medicinal plants

medicinal plants	concentration of Heavy metals (mg.kg-1) ± SD (n =3)		
	Cd	Pb	Ni
chamomile	0.18 ± 0.1	0.27 ± 0.13	5.17 ± 0.03
cinnamon	0.32 ± 0.31	2.49 ± 1.01	1.47 ± 0.12
Cumin	0.05 ± 0.09	2.10	11.87 ± 0.26
turmeric	0.02 ± 0.10	0.88 ± 0.01	9.19 ± 0.76
Hibiscas	0.03 ± 0.02	0.82 ± 02	15.13 ± 2.72
Saad	BDL	1.95 ± 0.42	11.04 ± 6.26
Urtica	BDL	2.59 ± 0.81	7.18 ± 1.25
Borage	BDL	1.32 ± 0.1	8.16 ± 1.21
Al-Handal	0.01 + 0.001	0.92 ± 0.01	13.91 ± 0.70
Sidr	0.14 ± 0.01	0.49 ± 0.07	9.77 ± 0.20

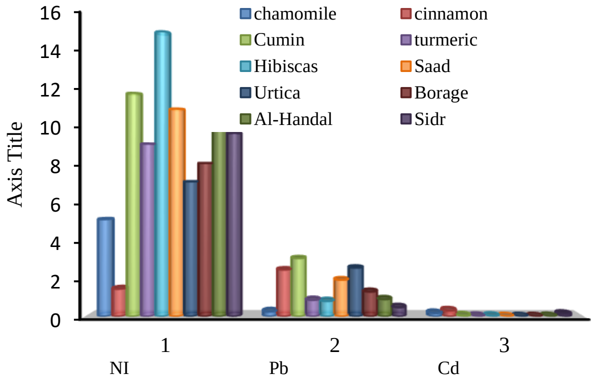


Fig. 3 Concentrations of Heavy metals in medicinal

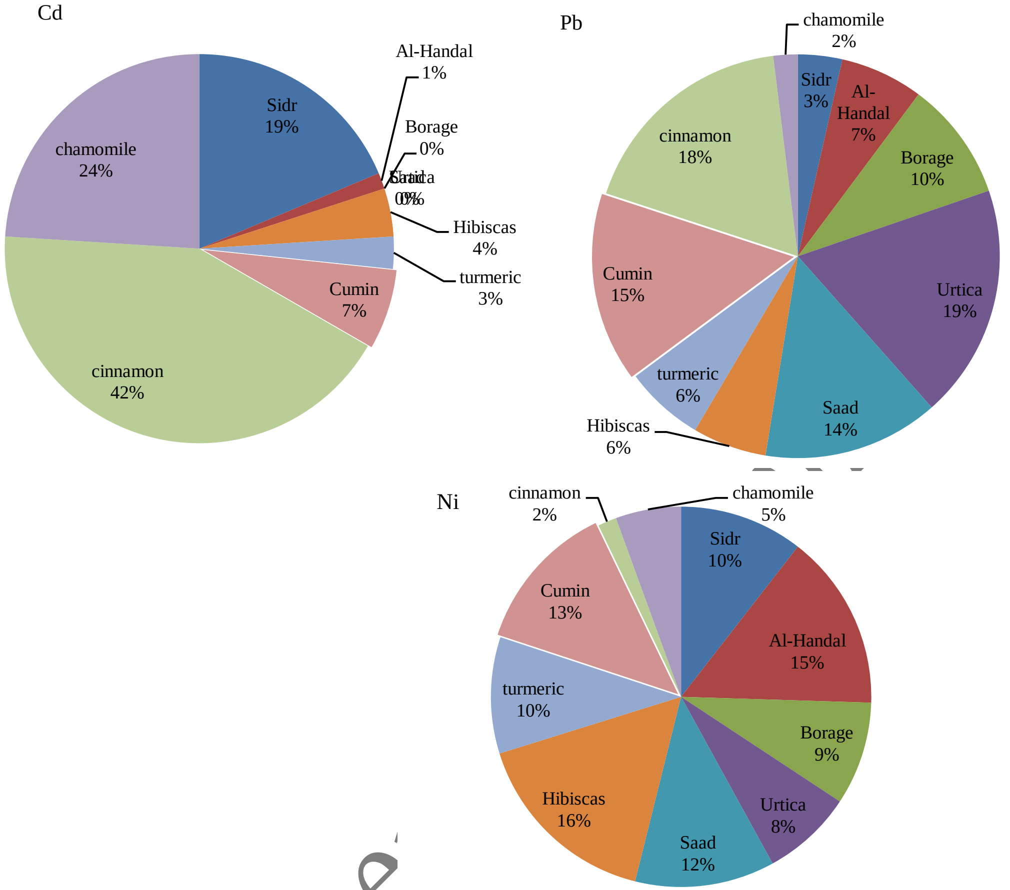


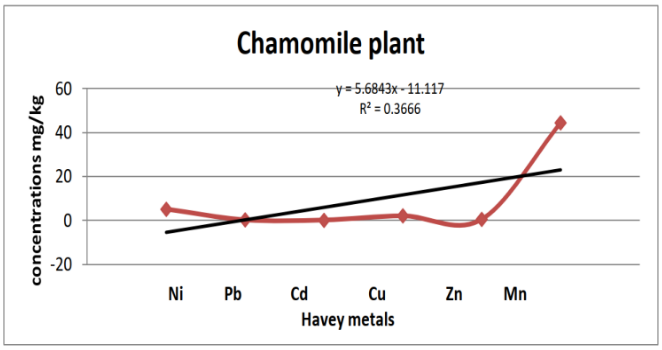
Fig. 4 percentage of Cd, Pb and Ni in medicinal plants

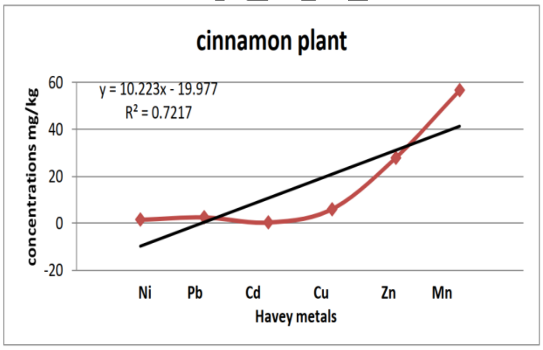
lead (Pb) is the most common and stable heavy metal in nature, and is a dangerous cumulative toxicant to the body. If lead levels exceed the permissible limits, the use of these contaminated plants may lead to toxicity characterized by colic, anemia, chronic kidney inflammation, headache spasms, and damage to the brain and central nervous system [37]. Cadmium (Cd) is a non-essential trace metal causes hypertension, liver & kidney diseases Cadmiumoisoning causes diseases lead to weak bones, special types of anemia, kidney problems, and increased accumulation of cadmium may lead to death [38]. The maximum allowable limit of Cd in raw herbs (0.2 mg.Kg -1) stated in the Ph. Euro (2008) [38]. The problem with heavy metals is when they accumulate in important organs of the body, either through feeding or medical treatment with plants contaminated with them, causing two metabolic disorders. Cadmium causes rickets and kidney inflammation, and lead causes cancerous tumors and kidney disease [39], Copper is less toxic than cadmium and lead, but they all become dangerous when their concentration exceeds the limits [40].

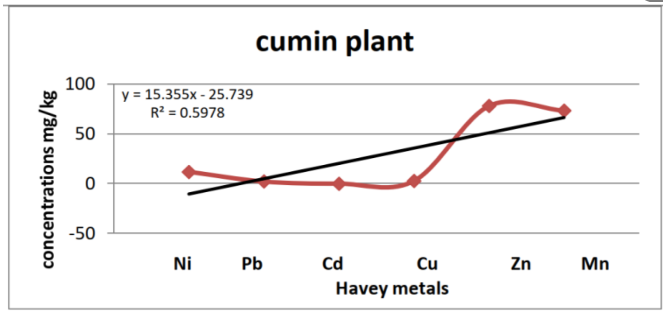
It is within the permissible limits according to (WHO/FAO). Lead reaches the soil through car exhausts, damaged batteries, and heavy water, which leads to its accumulation in plants due to the absorption of lead from contaminated soil by plant roots, thus entering the food chain, causing disorders and diseases in the nervous system of children. Lead (Pb) is one of most common toxic environmental pollutants. Pb chemical reacted with some biomolecules and adversely affects nervous, reproductive, digestive, immune, renal, cardiovascular, and muscular systems as well as growth processes. [41] Cadmium appears to mimic zinc, and to a lesser extent calcium and cadmium compounds are classified as carcinogenic to humans [42]. Therefore, an increase in the content of cadmium in food is harmful. E.P.A (Environmental Protection Agency) recommended an intake of less than one milligram/day considered toxic [43]. The permissible limit for nickel in medicinal plants consumed is 1.5 mg/kg, while daily requirement for humans is 1 mg/day. From results obtained in this study, all concentrations were more than WHO limits (2005). Nickel is an essential element for animals and plants, but in low concentrations, nickel is necessary in lipid metabolism and important in process of erythrocytes producing in human. However, high levels of nickel cause toxicity and cause serious diseases such as blindness, results of study Grath and Smith confirmed increased concentrations of cadmium lead to kidney and liver disorders [44] The researchers examined content of lead, cadmium, zinc and chromium in herbal spices. These authors found high levels of lead and cadmium in some medicinal plants cinnamon plant 6.25 mg/kg, 0.22 mg/kg respectively, in basil 2.26 mg/kg and 0.48 mg/kg respectively and marjoram plant 1.29 mg/kg and 0.40 mg/kg respectively. These results are consistent with the results of present study. The increased levels of lead and cadmium in spices suggest that plants are likely exposed to accumulations of elements from environment. [45] Pb levels are generally higher in leaves than in other parts from plant, possibly because lead contamination occurs mainly through the atmosphere, while the ability of plants to absorb lead is generally low The results of our present study are also consistent with the results of [46, 47]. Confirmed content of heavy metals in some vegetable s and spices found excess amounts of lead, cadmium and zinc in tested mixed spices. Abu Arab and Abu Dunya and Murphy *et al.* confirmed that the heavy metal content in spices varies depending on origin, environmental pollution, plant part and technological processes [48]. Germany and similarly Austria, reference concentrations for leafy plants are fixed at 0.80 mg/kg lead and 0.20 mg/kg cadmium on a fresh weight basis [45]. As in the European Union, heavy metal levels in food are regulated by a Commission Regulation (eC/466/2001), which sets limits for lead (0.30 mg/kg) and cadmium (0.20 mg/kg) in leafy plants and fresh herbs. On the other hand, WHO recommendations are less restrictive. Permissible limits in medicines and herbal products are 10.00 mg/kg and 0.300 mg/kg of lead and cadmium, respectively [49,50] as shown in Table 5.

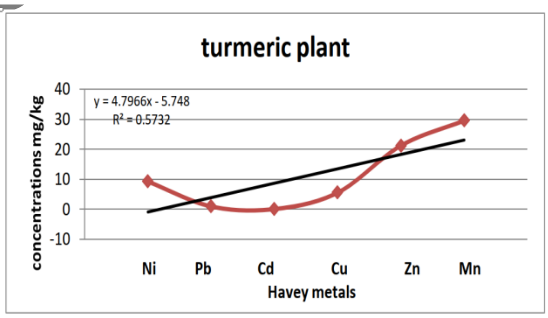
Table 5 Safety limit for metals in medicinal plants WHO, FDA and European Pharmacopoeia (Ph. Euro)

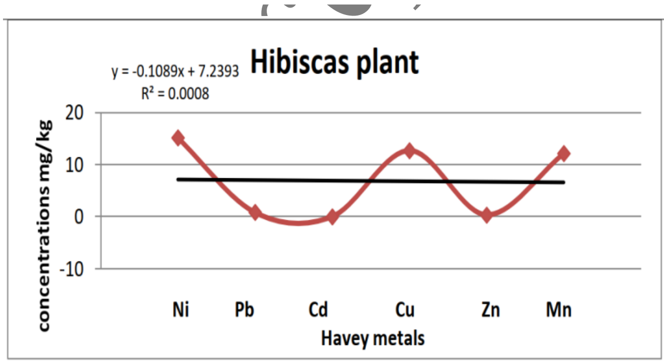
Metal	WHO/FDAPermissible limit	Ph. Eur. Permissible limit(mg kg-1)	FAO/WHO PTWI(mg kg-1)
Cd	0.3	0.5	7
pb	10	5	25

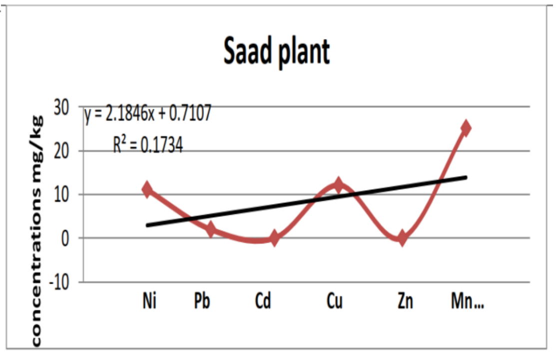












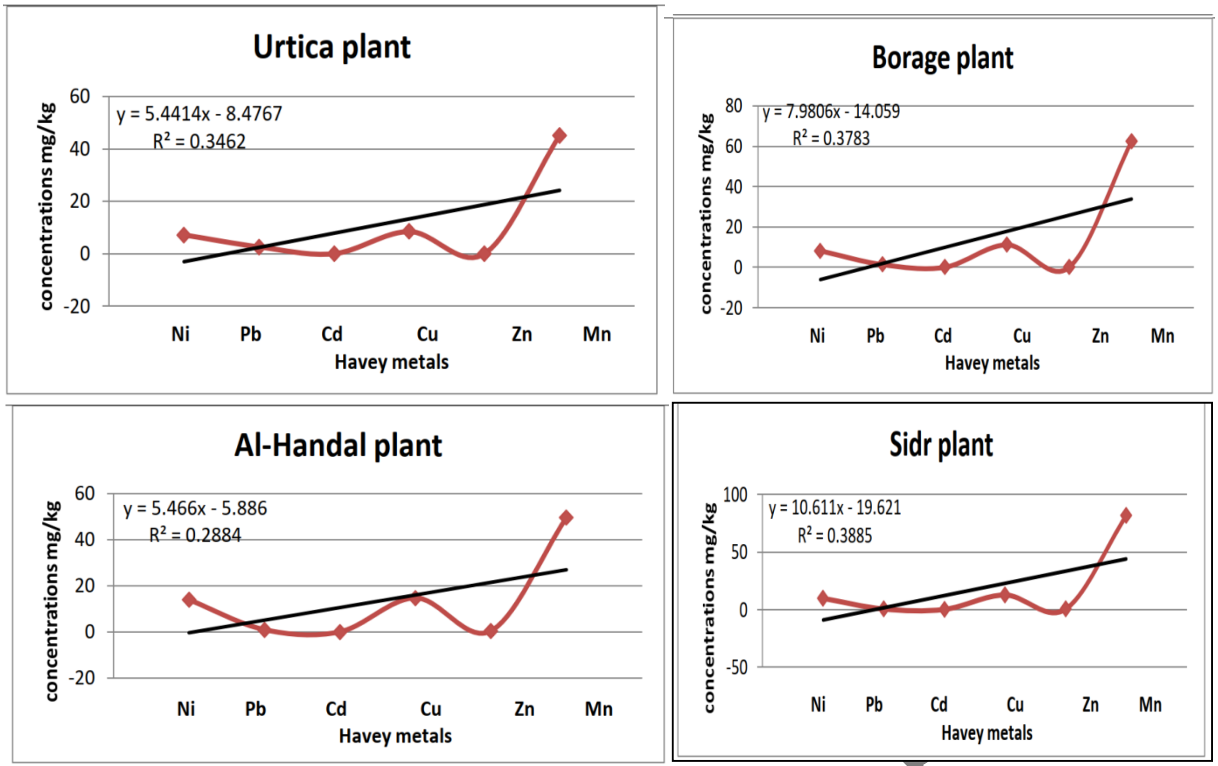


Fig. 5 Correlation's relationship each plant with all heavy metals

Cytotoxicity for Aqueous Extracts

The results of hemolysis activity in current study for all aqueous extracted plants under study recorded that hemolysis activity increases with increasing concentration of extract 12.5 µg/ml 25µg/ml, 50 µg/ml and 100 µg/ml, where the highest percentage of hemolysis activity (cytotoxicity) was at 100 µg/ml hemolysis activity in Utica plant was 23% at 100 µg/ml while cinnoma plants recorded 11% at 100µg/ml. The remaining plants recorded hemolysis results less than 10% at all concentrations show Table 6**.**

Table 6 percentage cytotoxicity of aqueous extracts of medicinal plants

Medicinal plants	Hemolytic activity % (concentration µg/ml)			
	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
chamomile	2%	2%	3%	6%
cinnamon	3%	4%	8%	11%
Cumin	4%	3%	2%	6%
turmeric	7%	7%	2%	2%
Hibiscas	1%	3%	8%	8%
Saad	4%	4%	2%	3%
Urtica	4%	5%	7%	23%
Borage	2%	4%	4%	5%
Al-Handal	4%	5%	5%	9%
Sidr	4%	6%	3%	6%

Toxic effect of hot aqueous extracts of studied plants on human blood cells was determined. Hemolysis is an important measure of safety of ingestion and consumption of medicinal plants along with other toxicity tests. The amount of hemoglobin secreted can be measured quantitatively to determine risk of erythrocytes destruction. When hemolysis rate less than 10%, hemolysis of red blood cells depends on concentration of plant extracts, incubation period and temperature. Red blood cell destruction occurs due to breakdown of red blood cell membrane due to combined toxicity of toxic substances and groups present in basic structure of proteins. Red blood cells are the more abundant cells in body and have own biological and morphological properties. They contain polyunsaturated fatty acids (PUFA) in the composition of their cell membrane and due redox reaction oxygen. transport property, as a result, phospholipids and proteins in red blood cell membrane are oxidized, biochemical changes are accompanied by factors such as a deficiency of antioxidants, continuous exposure to radiation, high contamination with transition metals, oxidizing drugs and hemoglobin diseases. Hemolysis rate increases when erythrocytes exposed to free radicals ROS and RNS such as hydrogen peroxide, singlet oxygen, and pollutants cause peroxidation of membrane phospholipids. [51] Hemolysis is related with concentration of extract, presence of active chemical compounds and extent of its contamination with heavy metals. Medicinal plants under study were evaluated for cytotoxic activity by hemolysis test to reach toxicity profile of plants in vitro using aqueous extracts at different concentrations and fresh blood from volunteers (healthy subjects). Average values of hemolytic activity (%) extracts of studied plants presented in Table 6. As a result, studied plants showed highest hemolytic activity of 23% in nettle, while cinnamon also showed the maximum activity of 11% in the aqueous extract. The results of the study are consistent with de Freitas et al 2008 which found that polyphenols present in aqueous extracts of medicinal plants protect red blood cell oxidation and hemolysis. Anti-hemolytic activity of Cyperus was due to phytochemicals present as flavonoids present in Cyperus and other medicinal plants were reported to exhibit anti-hemolytic effects and positively affected the stability of red blood cell membrane. The anti-hemolytic activity of thyme extract may be related to its content of polyphenols, flavonoids and other secondary metabolites [52].

CONCLUSION

All plants under study contain most active chemical compounds: carbohydrates, phenols, flavonoids, alkaloids, tannins, glycosides and terpenes. Heavy metal content approximately 2%-4% of some samples contained high levels of lead, nickel and cadmium, highest cadmium content in cinnoma, while highest lead content in Utica and highest nickel level in al-handle. Zinc, copper and manganese content in most of studied plants within permissible limits and met appropriate safety standards. Urtica and Hibiscas also hemolytic activity of nettle and cinnamon plants was recorded up than 10% at 100 μg/ml. Consumption of plants under study in large quantities and frequent a daily basis may cause poisoning due to accumulation of lead, nickel and cadmium in the body

Recommendations

recommend studying and determining levels of heavy and trace elements for all medicinal plants available in local and imported markets and conducting a cytotoxicity test to ensure their safety for human consumption or not, because many plants are used as extracts or spices or are consumed fresh randomly.

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