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Determination of heavy metals, macro and trace elements in selected medicinal plants from Central Market of San Salvador, El Salvador

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Abstract: Medicinal plant's quality and safety are becoming a great interest topic worldwide, especially due to contamination with heavy metals. The main objective of this study is to determine the phytochemical composition and quantify the concentration of heavy metals, trace and macro elements in fourteen medicinal plants purchased in the Central Market of San Salvador. Samples were dried and fractionated, subsequently digested and analyzed at first by phytochemical screening and then by atomic absorption spectrometry. The concentration of twelve elements was determined, only Matricaria chamomilla exceeded the established World Health Organization limit for Cd and Cu. Acourtia nudicaulis and Turnera diffusa exceeded the permitted concentration of Ni. The concentration of these elements must be inspected in medicinal plants sold in the informal markets of El Salvador to ensure the safety and quality. To our knowledge, this is the first study of heavy metals in medicinal plants conducted in El Salvador.

Keywords: Medicinal plants; Heavy metals; Market; El Salvador; Trace element; Matricaria chamomilla.

1. Introduction

Traditional medicine plays an essential role in health care, estimated to be used by 60% of the worldŠs population [1]. The global market of medicinal plants has an annual growth rate of $5-15\%$, the market size in 2004 was \$62 billion and is expected to grow to \$5 trillion by 2050 [2]. Plants' preparations and infusions to treat illnesses have been widely practiced in Central America [3] In El Salvador, Mayan culture has transmitted the traditional uses of medicinal plants from generation to generation. A study made in 1992 found that 182,543 Salvadorans were using medicinal plants 1.56 kg/per capita (284,767 kg in total); it is considered, however, that the use of traditional medicine is stronger in the rural area [4]. The main market of medicinal plants in San Salvador City is the Central Market. Medicinal plants sold in informal Salvadoran markets have no traceability and regulations, so it is important to know they are safe.

There is a widespread misconception that medicinal plants are inherently safe, but it is well-known that some medicinal plants can accumulate heavy metals and elements when growing under natural conditions or when being contaminated by anthropogenic pollutants, inappropriate agronomic management, and storage [5,6]. In El Salvador environmental contamination by battery recycling factory has been reported and environmental emergency by lead contamination in 2010 has been decreed [7]. The crops around the affected area were contaminated. The World Health Organization (WHO) recommends determining the concentration of heavy metals because they have long biological half-lives and are potentially accumulated in body organs causing side effects [8], however, not only heavy metals can cause damage to the human body, but also a rouge concentration of other macro and trace elements can represent a risk for people who are consuming medicinal plants to treat chronic disease or have a metabolic condition where they are not able to process the element concentration.

The macro-elements are required for the correct function of the organism in an amount greater than 100 mg/day such as calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) [9]. Nickel (Ni), copper (Cu),

iron (Fe), and zinc (Zn) are trace elements because they are required in trace amounts, less than 100 mg/day [9]. Heavy metals are considering those elements that have a specific gravity greater than 4 g/mL [10], for example: lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). For this study, the European Medicines Agency (EMEA) elements classification will be used, because they classified the elements according to their toxicity [11].

This work aimed at monitoring the heavy metal, macro, and mineral elements concentration in the medicinal plants sold in the Central market to treat the disease that more affects the Salvadoran population in 2018 (respiratory disease, depression, anxiety, parasitism, Mellitus diabetes, digestive, hypertension, and headache) by atomic absorption spectrometry and phytochemical analysis [12].

2. Materials and Methods

2.1. Samples

Fourteen plant species, that were used to treat some diseases, that affected the Salvadorian population in 2018, were purchased at the Central Market (latitude: $14^0 23' 39''$ N, longitude: $89^0 23' 10''$ W) of San Salvador City, El Salvador Table 1. They were identified by the botanist Jenny Elizabeth Menjívar from the Natural History Museum of El Salvador, and the respective voucher specimens have been deposited in the herbarium at this museum.

Table 1. Medicinal plant species collected and results of phytochemical analysis.

Scientific name	Vernacular name	Plant part used	Prevalent disease	Phytochemical analysis
Asteraceae				
Gnaphalium viscosum	Gordolobo	Flowers	Respiratory diseases	Saponins, flavonoids, and tannins.
Tagetes lucida	Hierba de San Juan	Leaves	Depression, anxiety	Flavonoids, tannins,
				alkaloids, and sesquiterpene lactones.
Matricaria chamomilla	Manzanilla	Whole plant	Depression, anxiety	Flavonoids, tannins,
				and alkaloids.
Acourtia nudicaulis	Valeriana	Leaves	Depression, anxiety	Flavonoids.
Bignoniaceae				
Jacaranda mimosifolia	Gualanday	Leaves	Parasitism	Anthraquinone glycosides,
				tannins, and sesquiterpene lactones.
Chenopodiaceae				
Dysphania ambrosioides	Epazote	Aerial parts	Parasitism	Tannins and alkaloids.
Euphorbiaceae				
Croton guatemalensis	Copalchi	Leaves, stem bark	Mellitus diabetes	Flavonoids, tannins,
				alkaloids, and sesquiterpene lactones.
Moringaceae				
Moringa oleifera	Moringa	Leaves	Mellitus diabetes	Flavonoids, tannins,
				alkaloids, and sesquiterpene lactones.
Monimiaceae				
Peumus boldus	Boldo	Leaves	Digestive	Flavonoids, anthraquinone,
				glycosides, tannins,
				alkaloids, and sesquiterpene lactones.
Myrtaceae				
Syzygium jambos	Manzana rosa	Leaves	Mellitus diabetes	Tannins, anthraquinone
				glycosides, and sesquiterpene lactones.
Passifloraceae				
Turnera diffusa	Damiana	Leaves	Depression, anxiety	Flavonoids and tannins.
Passiflora platyloba	Pasiflora	Aerial parts	Depression, anxiety	Tannins.
Pentaphylacaceae				
Ternstroemia lineata.	Tilo	Flowers	Hypertension	Flavonoids, anthraquinone
				glycosides, tannins,
				and sesquiterpene lactones.
Verbenaceae				
Lantana camara	Cinco negritos	Leaves	Headache	Flavonoids, tannins,
				and sesquiterpene lactones.

2.2. Reagents and quality assurance

Standards of Pb, As, Hg, Cd, Cu, Fe, Zn, Ni, Ca, Mg, Na, and K (E. Merck, Germany) were used to prepare stock and working solutions. All reagents were analytical grade. HCl was supra pure quality was purchased from Sigma-Aldrich (USA) and ultrapure water (0.05μS) was produced using Barnstead GenPure Pro Water Purification System (Thermo Scientific, USA).

2.3. Extraction and phytochemical analysis

Ten grams of dried and ground plant material of each species were extracted with 95^0 ethyl alcohol (100 mL) in an ultrasonic bath VWR (USA, Model 97043-988) at environment temperature (25^0C) for 90 minutes. The phytochemical analysis was carried out according to the method previously described [13].

2.4. Digestion and metals quantification in plants

Two grams of each medicinal plant were weighed in a previously ignited and tared crucible, and gradually heated at 500^0C for 2 hours and cooled in a desiccator. 3 mL of bidistillated water and 2.5 mL of concentrated HCl (37%) were added, then filtered through Whatman filter paper No. 42 into A grade volumetric flasks. The final volume was made up to 50 ml with bidistilled water. The analysis was performed by using an atomic absorption spectrometer (Aanalyst 700, Perkin Elmer, USA) equipped with flame and graphite furnace following the Official Method of Analysis [14,15]. The operating parameters are summarized in Table 2. Analytical data quality of metals was ensured with repeated analysis samples ($n = 3$).

Table 2. Operating parameters.

	Method	wavelength	LOD[a] ($\mu g/g$)	LOQ[b] ($\mu g/g$)	linearity	Acetylene flow (L /min)	Air flow (L /min)	Argon flow (mL /min)
Pb	Graphite furnace	283.3nm	0.04	0.13	0.9991	–	–	250
As	Graphite furnace	193.7nm	0.001	0.03	0.9981	–	–	250
Hg[c]	Hydride generator	253.7nm	0.15	0.50	0.9991	–	–	250
Cd	Graphite furnace	228.8nm	0.004	0.013	0.9990	–	–	250
Ca	Flame	422.7nm	4.0	12.5	0.9997	2.0	17.0	–
Mg	Flame	285.2nm	0.4	1.2	0.9997	2.0	17.0	–
K	Flame	766.5nm	4.0	12.5	0.9996	2.0	17.0	–
Na	Flame	589.0nm	2.00	6.25	0.9997	2.0	17.0	–
Fe	Flame	248.3nm	8.00	25.0	0.9999	2.0	17.0	–
Zn	Flame	213.9nm	0.95	3.10	0.9999	2.0	17.0	–
Cu	Flame	324.nm	0.96	12.50	0.9999	2.0	17.0	–
Ni	Graphite furnace	232.0nm	0.15	0.50	0.997	–	–	250

3. Results and discussion

Medicinal plants have been used in undeveloped countries as El Salvador to provide numerous remedies. The commercialized species at the Central Market to treat the common diseases of the Salvadoran population in 2018 are listed in Table 1. Samples were analyzed to determine the majoritarian secondary metabolites through a preliminary phytochemical analysis and the concentration of heavy metals, macro and trace elements. The results show that phenolic compounds (flavonoids, tannins, and anthraquinones) were present in almost all samples. It could be an auto defensive mechanism caused by toxic metals stress [16]. Eight of the studied species showed the presence of sesquiterpene lactones (see Table 1). Some of these species are traditionally employed to treat Mellitus diabetes; sesquiterpene lactones content might be related to the ethnoparmacological use of these plants [17,18]. Sympathetic stimulation acting directly on a- and SS-receptor, antipsychotic, anti-inflammatory, and hepatoprotective are some biological activities related to the presence of alkaloids [19]. The uses of T. lucida, M. chamomilla, D. ambrosioides, C. guatemalensis, M. oleifera and P. boldus could be related to the biological activities that alkaloids exert on the human body. From the analyzed samples, only G. viscosum was positive for saponin. Saponins are used in respiratory illness or disease which agrees with the traditionally reported use for G. viscosum [20,21]. Medicinal plant uses have already several regulations worldwide such as the European Pharmacopoeia, United State Food and Drug Administration, Pharmacopoeia of the People's Republic of China, etc [22]. Moreover, several countries have already developed their national guidelines to ensure satisfactory levels of heavy metals in medicinal plants [5]. A summary of heavy metal and trace elements limits are given in Table 3 [15,22,23]. In El Salvador, such regulations have not been established yet, however the established limits by WHO were used to compare the detected amounts of class 1, 2A and 3 elements. According to the EMEA, the Class 1 elements are human toxicants [11]. The results for this class are shown in Table 4.

Table 3. International regulation for arsenic and toxic metals content in medicinal plants.

			Reference values		Phytochemical analysis		
Element	Element classification EMEA	Oral Permitted Concentrations EMEA	Dietary reference intakes (mg/day)	WHO 2007	Us Food and Drug Administration	European Pharmacopoeia	Pharmacopoeia China
As	Class 1	1.5	ND	10	10	-	2
Cd	Class 1	0.5	ND	0.3	0.3	1	0.3
Hg	Class 1	3	ND	1	1	0.1	0.2
Pb	Class 1	0.5	ND	10	10	5	5
Ni	Class 2A	20	ND	10	-	-	-
Cu	Class 3	300	0.9	20	20	-	20
Fe	Other elements	NE	18-8	-	-	-	-
Zn	Other elements	NE	8 - 11	50	-	-	-
Ca	Other elements	NE	1,000 - 1,000	-	-	-	-
Mg	Other elements	NE	315 - 410	-	-	-	-
Na	Other elements	NE	1,500 - 1,500	-	-	-	-
K	Other elements	NE	2600 - 3400	-	-	-	-

Table 4. Heavy metal concentration in medicinal plants from the Central market of El Salvador.

Medicinal specie	Pba	Asa	Hga	Cda
G. viscosum	ND	0.05 ± 0.001	ND	0.23 ± 0.007
T. lucida	0.32 ± 0.002	ND	ND	0.04 ± 0.002
M. chamomilla	ND	0.04 ± 0.001	ND	2.87 ± 0.096
A. nudicaulis	0.14 ± 0.010	0.15 ± 0.006	ND	ND
J. mimosifolia	ND	0.09 ± 0.006	ND	ND
D. ambrosioides	ND	0.12 ± 0.006	ND	0.04 ± 0.002
C. guatemalensis	0.02 ± 0.002	0.06 ± 0.001	ND	0.01 ± 0.001
M. oleifera	ND	0.20 ± 0.001	ND	ND
P. boldus	ND	0.08 ± 0.001	ND	ND
S. jambos	ND	0.03 ± 0.001	ND	ND
T. diffusa	ND	0.110 ± 0.006	ND	ND
P. platyloba	ND	ND	ND	ND
T. lineata.	ND	ND	ND	ND
L. camara	ND	0.09 ± 0.006	ND	ND

All the analyzed species were below the WHO established limit for heavy metals (Hg, Pb, Cd, and As), except M. chamomilla, which showed a Cd concentration of $2.870 g/g$ overtaking the established limit $(0.3 g/g)$. Toxic metals accumulation varied between medicinal plants and primarily depends on species, soil quality, and its inherent controls [24]. M. chamomilla has been reported as a bioindicator of heavy metal pollution for its capacity to accumulate certain metals, especially Cd [25–28]. Furthermore, it has been demonstrated that plants growing in traffic areas or polluted areas from battery factories tend to accumulate metals as Cd [25,29]. Medicinal plants, that are sold in the Salvadoran market, are indistinctly collected from rural or urban areas. Cd has not reported functions in a human body, animals or plants and it can be accumulated in tissues [9]. Chronic exposure to Cd can cause pulmonary disease, metabolic syndrome, an increase in inflammatory biomarkers, organ injuries like liver and kidney, affect the vascular and immune system, and could cause cancer [30,31]. The results for Class 2, 3 and other elements are given in Table 5.

Ni and Cu are Class $2A$ and 3 elements respectively, they are less dangerous in comparison to Class 1 elements. A. nudicaulis and T. diffusa exceeded the WHO limit for Ni $(10 g/g)$ with concentrations of 45.480 and $27.350 g/g$ respectively. A. nudicaulis do not have previous reports of phytochemical and element trace analysis. T. diffusa has been previously reported as a Ni hyperaccumulator plant with concentrations of 56 ţg/g [32]. According to the WHO, Ni is possibly carcinogenic and represent health risks to humans [33]. Cu is an essential element required for the proper functioning of many important enzyme systems and helps maintain the strength of blood vessels, skin, epithelial and connective tissue in the human body [30,33]. In general, the concentration of Cu in plants is within the range of $20 - 600$ ppm [34]. Nevertheless, the limit concentration established by the WHO in medicinal plants is $20 \mu g/g$. Only M. chamomilla exceeded this limit with a concentration of $101.487 \mu g/g$. Sadhu et al., (2015) express that elements like Cu have low transfer coefficients and only a minimum amount is transferred from soils to plants. It could mean that external contamination occurred. When Cu is absorbed in the human body it can produce fever with flu-like symptoms, hair and skin discoloration, dermatitis, irritation of the upper respiratory tract, and nausea [30]. Medicinal plants sold in popular markets in El Salvador are not subject to health control by regulatory authorities, therefore, it is necessary to test them for metal contamination.

For the ŞOther elementsŤ as the EMEA classified, there is no established limit however, dietary reference intake values were taken into account to compare the founded values in analyzed samples [23]. Fe plays an important role in plant photosynthesis, DNA synthesis and is a component of hemoglobin [9,24]. The recommended amount of Fe is 8 and 11 mg/day for females and males aged between $19 - 50$ respectively [23]. An excess of Fe in the human body can cause severe damage to the stomach and homeostasis [24]. All analyzed plants were below these values; the maximum Fe concentration was found in A. nudicaulis $(2,62 g)$ and the minimum in T. lineata $(52.49 \mu g)$. Regarding Zn, it is usually present in plants between 20 and $100 mg/kg$ [25]. Zn is an essential element and has an important role in tissue repair and wound healing, digestion, cellular metabolism, and bone formation [9,30]. The detected concentrations for Zn in the studied plants were between 5.52 and $81.54 \mu g/g$ for $T.$ lineata and D. ambrosioides, respectively. It is reported that Zn cannot be taken up by plant from the soil and high concentration could come from an anthropogenic source [35]. Negative effects of Zn are vomiting, diarrhea, bloody urine, icterus, liver, and kidney failure [9].

Table 5. Trace and macro-element concentrations in medicinal plants from the Central market of El Salvador.

Medicinal specie	Ni	Cu	Fe	Zn	Ca	Mg	Na	K
G. viscosum	0.95 ± 0.045	11.86 ± 0.137	511.07 ± 6.16	35.46 ± 0.104	8,474.8 ± 24.3	21,460.1 ± 34.1	165.45 ± 7.65	16,373.2 ± 485.5
T. lucida	0.73 ± 0.038	5.69 ± 0.080	83.03 ± 0.68	26.24 ± 0.629	20,854.5 ± 358.4	2,569.6 ±57.7	108.96 ± 5.21	15,409.2 ± 368.6
M. chamomilla	0.72 ± 0.025	101.48 ± 0.080	101.49 ± 0.97	63.07 ± 0.440	10,867.7 ± 216.4	2,014.2 ± 54.6	609.47 ± 26.05	33,376.6 ± 691.4
A. nudicaulis	45.48 ±1.899	5.50 ± 0.015	2,620.39 ± 29.64	20.36 ± 0.117	11,275.8 ± 165.8	4,205.7 ± 53.5	175.32 ± 7.75	19,400.6 ± 549.5
J. mimosifolia	6.40 ± 0.298	7.07 ± 0.159	140.02 ± 2.35	7.24 ± 0.163	11,699.3 ± 260.9	4,075.9 ± 52.3	117.45 ± 4.89	4,029.7 ± 155.8
D. ambrosioides	ND	5.77 ± 0.105	140.99 ± 0.07	81.54 ± 0.335	36,230.9 ± 314.1	8,037.9 ± 174.9	1,839.68 ± 52.23	37,988.6 ± 652.9
C. guatemalensis	ND	3.11 ± 0.122	86.91 ± 1.58	19.78 ± 0.205	61,997.3 ± 325.8	2,973.4 ±96.9	195.39 ± 6.48	19,137.0 ± 608.7
M. oleifera	ND	4.70 ± 0.025	498.15 ± 5.28	16.23 ± 0.833	36,743.3 ± 182.0	3,940.2 ± 49.5	385.07 ± 9.84	12,894.4 ± 459.5
P. boldus	0.56 ± 0.036	0.963 ± 0.015	109.11 ± 2.18	9.47 ± 0.026	17,656.0 ± 393.1	1,908.0 ± 35.8	72.68 ± 2.48	2,618.5 ± 125.2
S. jambos	ND	1.597 ± 0.025	77.52 ± 0.67	11.29 ± 0.012	1,136.3 ± 45.2	2,904.3 ± 76.9	580.93 ± 19.65	1,996.6 ± 41.5
T. diffusa	27.35 ± 1.290	6.460 ± 0.098	587.91 ± 5.88	27.82 ± 0.192	15,324.2 ± 208.5	5,397.2 ± 139.9	208.65 ± 11.02	6,917.0 ± 315.3
P. platyloba	1.17 ± 0.059	4.273 ± 0.114	125.96 ± 2.90	14.28 ± 0.117	28,608.9 ± 195.4	1,216.1 ± 31.7	203.63 ± 10.05	16,630.0 ± 781.2
T. lineata.	2.69 ± 0.101	5.763 ± 0.167	52.49 ± 0.68	5.52 ± 0.072	6,406.0 ± 88.3	3,501.6 ± 57.5	197.16 ± 9.82	11,949.2 ± 429.3
L. camara	0.71 ± 0.026	5.110 ± 0.089	130.72 ± 4.06	22.52 ± 0.061	33,788.2 ± 609.2	5,626.9 ± 148.4	190.35 ± 8.69	14,258.6 ± 564.8

All the studied plants did not exceed the Zn recommended intake amount for males and females, however, it is important to monitor these medicinal plants that are used to treat chronic disease.

Ca and Mg are important elements for a large number of body functions such as for the activity of the immune system, growth, bone formation, and reduce the risks of osteoporosis [36]. Doses up to 1,500 and 400 mg/day for Ca and Mg supplement would not expect adverse effects for a healthy person [9]. Na and K are the main cations in intracellular fluid, regulating muscle contraction, conduction of nerve impulse, and acid-base balance [9,37]. All the studied species were below the recommended value for Ca, Mg, Na, and K. The detected concentration for those minerals resulted in agreement with the reported values for [9].

4. Conclusion

Traditional medicine is the sum of ancestral knowledge transmitted from generation to generation to treat diseases. In El Salvador, the Mayan culture was one of the most dominant cultures, which had a lot of knowledge in medicinal plants use. Based on the fact that natural medicine does not pose a potential health risk, medicinal plants have been used indiscriminately and without regulation for many years. However, the global increase in industrialization, the use of pesticides and microorganisms has led to an increase in soil contamination, which has increased the risk for plants to store substances that are harmful to health. Some of these pollutants can be avoided using good agricultural and good storage practices, however, in El Salvador there are no regulations so far. The heavy metals concentration, found in medicinal plants sold at Central Market, did not exceed the allowed limit, except for Matricaria chamomilla which presented a concentration almost 10 times higher than the Cd allowed concentration. This could be due to contaminated soils by the battery industry. For macro and trace elements, the studied plants have an important source of them, but also had a lower amount in a gram, than the dietary reference intake. It is always important to take this value into account when people who are consuming the medicinal plants have a metabolic disease or kidney pathologies.

Except for M. chamomilla, A. nudicaulis and T. diffusa the analyzed plants contained safe levels of heavy metals, macro, and trace elements and, hence, may not represent side effects associated with elements toxicity. As for M. chamomilla is recommend to continue studying the concentration of Cd in different markets and places. This is the first study of elements determination in medicinal plants in El Salvador and will be the baseline to other studies finding the efficacy and safety.

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