Phytochemicals Analysis and Detection of Heavy metal contents in *Euphorbia nivulia* and Soil of Cholistan Desert by Atomic Absorption Spectrum

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Abstract:

Heavy metals can be defined as possessing some toxic activity and having an atomic weight greater than sodium. Heavy metals are part of different food chains in the ecosystem. They pose different harmful effects on the human body. The soil of the cholistan desert is very rich in compounds as evidenced by many research studies. These compounds are absorbed by native plants and can be isolated via phytochemical analysis. Euphorbia nivulia is native to the cholistan desert, Bahawalpur, and was used for different therapeutic purposes for many years. The soil of cholistan desert and *Euphorbia nivulia* were analyzed for the determination of heavy metals and phyto-constituents. Different parts of the plant contain several elements in varying concentrations such as cobalt, nickel, chromium, zinc, cadmium, copper, lead, and iron. The present study sought to determine the content of some heavy metals in *Euphorbia nivulia* and soil collected from the Cholistan desert. The mineral and heavy metal contents were analyzed using Atomic Absorption Spectrometry and phytoconstituents by standard qualitative procedures. This study revealed the presence of copper, chromium, iron, manganese, nickel, and

zinc. A considerable number of phytoconstituents such as alkaloids, glycosides, tannins, flavonoids, saponins, and phenolic contents were detected.

Introduction

Globally, use of alternative and complementary medicines is much appreciated in prevention and treatment of diseases (Song et al., 2021). Food condiments are first therapeutic approach for minor illness in addition to medicinal herbs. Attention towards quality issues and safety of phytopharmaceuticals are due to increasing trend of application of natural medicine (Hina et al., 2011). This application is widely utilized in different forms in various systems of medicine. As therapy of natural medicine is considered safe and less toxic, but plants also contains some toxic metabolites such as heavy metals which pose health risk issues when utilized for longer duration on one hand (Arpadjan et al., 2008; Itanna, 2002; Lasisi et al., 2006; Obi et al., 2006) and is beneficial also to some extent on other hand.

Heavy metals can be defined as possessing some toxic activity and having atomic weight greater than sodium (Adepoju-Bello et al., 2014). They are part of upper layer of earth and cannot be destroyed. Humans can intake them via medicine, drinking and eating, although they are also required in trace amounts and are part of body metabolism. Presence of more than enough concentration of heavy metals in human blood become toxic and damage vital organs such as brain, kidneys, liver and heart (Uddin et al., 2012).

Plants contain heavy metals of two catagories, essential and toxic. Essential heavy metals are necessary for growth, development and metabolism in living body and are required in minute quantities such as Co, Fe, Cr, Zn and Cu. Deficiency and excess of these heavy metals both lead to disturbances in body metabolism. Whereas toxic metals pose harmful effects in even low concentrations and are not required by body such as Hg, As, Cd and Pb (Abu-Darwish et al., 2009; Friberg et al., 1986; Islam et al., 2007; Singh and Garg, 2006). Heavy metals are part of different food chains in ecosystem (WHO 2007). Industrial residues, organic manure, lime and synthetic fertilizers contribute heavy metals in ecosystem in varying amounts (Annan et al., 2010; Kos et al., 1996; Martins et al., 2008). Due to unhygienic storage conditions in herbal shops, metals contaminants from environment continue to be added in these herbal parts. Different plants contains varying amounts of heavy metals such as canary seeds were found to contain more quantity of zinc and nickel as compared to wheat (Abdel-Aal et al., 2011).

Euphorbia nivulia contains multiple pharmacological properties such as anti-inflammatory, analgesic and antiseptic and is used in various diseases for therapeutic purposes such as worm infestations, boils, disorders of ear and skin. Presence of triterpenoids, tannins, alkaloids, glycosides, flavonoids and polyphenolics were found in plant.

Methodology

Acute toxicity assessment

Sprague-Dawley rats were used for acute toxicity of ENE. There were six (2 male and 4 female) rats in each group and these rats were orally administered (in the fasting conditions) with the extract at 150, 300, 500, 1000, 2000 mg/kg doses. After extracts administration, toxicity symptoms, mortality rates, behavioral patterns, physical appearance, injury, pain and illness signs, etc., were noticed daily for 2 weeks. Acute toxicity study was executed according to OEC 425 guidelines.

Phytochemical studies

Extraction

10 kg dried powdered plant material (aerial parts) *Euphorbia nivulia* was macerated in 70% ethanol at room temperature for 15 days with occasional stirring. The macerated mixture was filtered three times with muslin cloth and then further filtration was done by Whatman Grade-1filter paper. The filtrate was then evaporated under reduced pressure (-760m Hg) and controlled temperature at 40^{o}C on the rotary evaporator. A thick and semisolid, dark brown gummy mass was obtained. The dried material was weighed, labelled and then stored at 4^{o}C in refrigerator in air tight container. The percentage yield was calculated. The condensed extract was used for the study (Annapurna et. al., 2004). Extract was dried, weighed, labelled and then stored at 4°C in refrigerator in air tight containers. Aqueous ethanolic extract was named as:

ENE = Ethanol extract of *Euphorbia nivulia* Buch.-Ham.

Preliminary qualitative phytochemical sreening

Preliminary qualitative phytochemical screening of ENE, ENH, ENC, ENB and ENA to identify the phytoconstituents like alkaloids, glycosides, flavonoids, tannins, saponins and phenols was carried out by using standard conventional procedures (WHO, 1998; Brain et al., 1975), (detail of various tests performed is summarised in Table 1).

Table 1: Tests performed for prelimmary qualitative phytochemical screening

Test for alkaloids	
Hager's test	50 mg extract + HCl (few ml) + filtration → 2ml filtrate+ 2 drops 50 mg extract + HCl (few ml) + filtration → 2ml filtrate + 2 drops Hager's reagent
Mayer's test	50 mg extract + HCl (few ml) + filtration → 2ml filtrate + 2 drops Mayer's reagent
Wagner's test	50 mg extract + HCl (few ml) + filtration → 2ml filtrate + 2 drops Wagner's reagent
Test for glycosides	
Keller Killiani test	500 mg extract in 5ml H_2O+2ml glacial acetic acid+1drop $FeCl_3$+ 1ml conc.H_2SO_4
Test for tannins	
$FeCl_3$ test	0.5gm extract+ H_2O +filtration→ filtrate + Aq.$FeCl_3$
Test for flavonoids	
Test with Alkali solution	500mg extract+Pet. ether (to eliminate fat) +30ml 80% ethanolic sol +filtration → filtrate + 4ml KOH (1%)
Test for saponins	
Froth test	500mg extract+ 20 ml hot boiling water, cooled, vigorously shaken for 15 min
Test for phenolic contents	
Fe Cl_3 test	500 mg extract+ 3/4 drops of $FeCl_3$

Figure 1: *Euphorbia nivulia* (Crowded and rosette leaves)

Figure 2: Hitachi Polarized Zeeman AAS, Z-8200, Japan

Atomic Absorption Analysis

In AAS, a solution containing the analyte was exposed to the light, produced by a hollow-cathode lamp, and electronic transitions from the ground state to excited electronic states

occurred. The light converted sample into free ground state atoms that could be excited. A lamp emitting light at a wavelength specific to the atoms was passed through the flame, and as the light energy was absorbed, the electrons in the atoms were elevated to an excited state. A monochromator was placed between the sample and the detector to reduce background interference. The detector measured the intensity of the beam of light and converted it to absorption data. The amount of light absorbed is measured and the concentration of the element in the sample can be calculated. There are two main sources of radiation available to AAS, namely, line source (LS) and continuum source (CS). CS is typically produced by deuterium lamps and emits light over a broad range of wavelengths, while LS, on the other hand, emits radiation at specific wavelengths, normally produced by a hollow cathode lamp.

When a magnetic field was applied to a sample, the light component polarized parallel to the magnetic field (P//) was absorbed by the atoms of the sample, while the light component polarized perpendicular to the magnetic field (P ⊥) was absorbed to a small extent by the atoms of the sample. On the other hand, background components, such as molecules and particles, were not affected by the applied magnetic field. Accordingly, both the light components, P// and P ⊥ , are attenuated by the background components by the same amount. As a result, the background contribution of absorption can be eliminated by taking the difference between the signals of two light components, thereby the desired atomic absorption signal is obtained. Since the same light source was used to measure both light components, P// and P ⊥, full double beam photometry was obtained, and accurate background correction was obtained throughout the entire wavelength range. With a continuous spectrum light source such as the D2 lamp, molecular absorption can be corrected only within a limited wavelength region, but neighboring line absorption cannot be corrected (Filho et al., 2011).

To calibrate an AAS, first a solution containing none of the element of interest was measured. This solution is called the 'blank' and determined the baseline absorption measurement. The absorption of solutions containing different known amounts of the element were measured. For example, 5, 10, 15, and 20 mg/L of Pb. From this data, a calibration curve was created. The calibration curve determined the relationship between the absorbance of the light and the concentration of the element in the solution. This curve follows the Beer Lambert Law.

FTIR analysis

FTIR spectrophotometer was used to record FTIR spectra. EN powder in minute quantity was placed directly on the germanium piece of the spectrometer with application of constant pressure. Omnic software (version 5.2) was used for analysis. The sample was scanned at room temperature at 400 to 400 cm^{-1} spectral range. To protect from signal-noise ratio, 100 interfero grams with spectral resolution of ± 4 cm^{-1} were averaged. Background spectra which were collected under identical conditions were subtracted from sample spectra. The procedure was performed in triplicate (Nivetha and Prasanna, 2016).

Results

Phytochemical Analysis of ENE

Results of phytochemical screening of ENE are given in table 2. The crude extract of plant was found to be positive for alkaloids, glycosides, tannins, flavonoids, saponins and phenols.

Table 2: Preliminary phytochemical screening of ENE

Class	**Test**	**Observation**	**Inference**
Alkaloids	Hager's test	Yellow ppt	Alkaloids present
	Mayer's test	Creamy ppt	Alkaloids present
	Wagner's test	Reddish brown ppt	Alkaloids present
Glycosides	Keller-Killani test	Brown ring (at junction)	Glycosides present
Tannins	$FeCl_3$ test	Blackish color	Tannins present
Flavonoids	Test with Alkali solution	Dark yellow color	Flavonoids present
Saponins	Froth test	Froth formation	Saponins present
Phenolic contents	$FeCl_3$ test	Blackish color	Phenolic contents present

Analysis of Heavy Metals in different Medicinal Plants

Phytoelements like Fe (1.484), Cu (0.072), Zn (0.384), Mn (0.173), Mg (0.204), Na (2.085) and Ca (1.031) are detected in ppm quantities from the ash sample of plant latex by using atomic absorption spectroscopy (Badgujar, 2011).

Table 3: Heavy metals contents of medicinal herbs/products (ppm) against permissible limits.

	Na	Fe	Mg	Ca	Cu	Zn	Ni	Mn	Cd	Hg
Permissible limits	51340 **	20	2000* **	614* *	10	50	1.5	200	0.3	0.1*
H. Integrifolia	8.07	1.40	2.01	5.26	0.07	0.20	0.02	0.00	0.04	0.06
D. regia	8.74	1.15	1.93	3.27	0.04	0.09	0.01	0.00	0.00	0.06
R. communis	7.95	0.36	2.02	3.37	0.03	0.09	0.01	0.02	0.00	0.37
C. equisetifolia	9.18	0.22	1.97	6.58	0.02	0.03	0.02	0.00	0.01	0.00
N. oleander	8.15	0.77	1.85	4.11	0.04	0.12	0.00	0.00	0.00	0.56
T. populnea	8.84	1.44	2.03	3.77	0.10	0.16	0.03	0.01	0.01	0.49
M.elengi	2.89	0.87	2.00	5.08	0.08	0.20	0.00	0.13	0.00	0.43
H. schizopetalus	0.00	0.47	1.90	3.55	0.05	0.09	0.00	0.00	0.00	1.30
P. pterocarpum	8.86	0.78	1.95	3.94	0.04	0.15	0.02	0.01	0.01	0.56
Malaysian Product	8.98	3.16	2.02	15.63	0.05	0.29	0.00	0.54	0.01	1.33

Niaz et al., 2013, * Maobe et al., 2012, **Khan et al., 2012, *** Omokehide et al., 2013

Analysis of Heavy Metals in Soil and ENE

Results of screening of ENE and soil for presence of heavy metals, are given in table 4. Soil contains Zn, Cu, Fe and Mn, whereas ENE contains all heavy metals in trace amounts.

Table 4: Heavy metal contents detected in soil and ENE by AAS against 0.99 ppm standard besides critical limits and permissible respectively in parentheses.

S. no.	**Heavy Metal**	**Quantity detected in Soil (ppm)**	**Quantity detected in ENE (ppm)**
1	Zinc	2.715(<1.0)	0.69(50.0)
2	Copper	1.39(<0.4)	0.46(10.0)
3	Iron	6.42(<4.5)	7.74(20.0)
4	Manganese	8.565(<2.0)	0.38(200)
5	Nickel	-	0.20(1.50)
6	Chromium	-	0.02(5.0)

Atomic absorption spectrophotometery of EN cr

Elements in EN cr were determined by atomic absorption spectrophotometer following the conditions described in AOAC (1990). The instrumental operating conditions were as follows (Table 5).

Table 5: Operational conditions for determination of heavy metals by AAS

Elements	**Wave length (nm)**	**Slit wdth (nm)**	**Lamp crrent (mA)**	**Burner head**	**Flame**	**Burner hight (mm)**	**Oxidant gas pressure (Flow rate) (kpa)**	**Fuel gas pessure (Flow rate) (kpa)**
Copper	324.8	1.3	7.5	Standard type	Air-C_2H_2	7.5	160	7
Chromium	359.3	1.3	7.5					12
Iron	248.3	0.2	10					6
Manganese	279.6	0.4	7.5					7
Nickel	232.0	0.2	10.0					7
Zinc	213.9	1.3	10.0					6

FTIR analysis

In the FTIR spectrum the broad peak at 3328 cm^{-1} may be due to phenolics (hydrogen bonding). The peaks at 2919, 2850 cm^{-1} indicates saturated hydrocarbons like CH_3, CH_2, CH. The peak at 1732 cm^{-1} is due to ketonic group where as the peak at 1598 cm^{-1} due to conjugated double bond. The peak at 1417 cm^{-1} is due to C-H bending vibrations. Prominent peak at 1026 cm^{-1} indicates C-O group (figure 3).

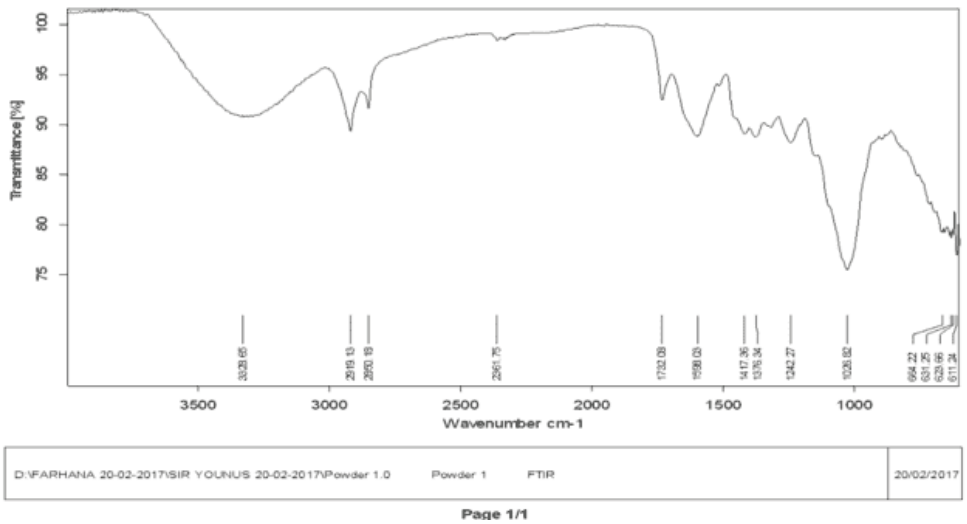

Figure 3: FTIR spectrum of *Euphorbia nivulia* (powder)

Discussion

Presence of toxic metals in soil or plants is much harmful when consumed by human body as evidenced by study conducted in Karachi, Pakistan (Hina et al., 2012). This study revealed prevalence of respiratory tract infections such as nasal congestion, chest congestion, allergy, asthma, tonsillitis, pharangitis, bronchitis, cough (dry or productive) and common cold due to presence of heavy metals. Many herbal formulations are present in market that deals with such types of ailments for therapeutic purposes. These formulations are available in freeze dried extract, pill and syrup forms. Although herbal physicians claim no side effects in these herbal formulations but it was estimated that some herbal preparations contain toxic metals such as arsenic, cadmium, mercury and lead in concentrations from 10-100%. Presence of inter current diseases, nutritional deficiency, poor dietary status and weak immune system results in increased absorption of heavy metals in human body, which can lead to complicated conditions. Toxicity of metals depends on quantity of dosage, number and duration of doses taken for therapeutic purposes. It was also observed that absorption rate of heavy metals was more in syrup formulations as compared to solid doses. Other complications include lung cancer, bronchopneumonia, pneumonitis, irritability, extreme restlessness, chest pain, vomiting, headache, cough, dryness of mucus membranes and even death in case of poisoning of cadmium and lead. Other complications of cadmium poisoning include tubular necrosis, glycosuria, aminoaciduria, nephrotoxicity, proteinuria, osteomalacia, osteoporosis and symptoms related to calcium metabolism. Decreased amount of iron and other essential nutrients in body can

stimulate increased absorption of cadmium. Toxic levels of lead can cause gastrointestinal and nervous system problems. Arsenic poisoning can cause fetotoxicity, hepato-renal damage, immunosuppression, lung cancer, peripheral neuritis, skin changes and nasal septum (Hina et al., 2012).

Phytochemical analysis of ENE revealed presence of various compounds such as phenols, saponins, flavonoids, tannins, glycosides and alkaloids, confirmed by various tests such as Hager's test, Mayer's test and Wagner's test for presence of alkaloids, Keller-Killani test for glycosides, $FeCl_3$ test for tannins and phenolic contents, test with alkali solution for flavonoids and froth test for saponins. Presence of iron, copper, zinc, manganese, nickel and chromium was found in plant extract; whereas zinc, copper, iron and manganese were detected in soil of Cholistan Desert, lacking nickel and chromium by using atomic absorption spectroscopy. FTIR analysis revealed peaks and bonding patterns of these metals.

AAS is an analytical technique used to determine how much certain elements are in a sample. It uses the principle that atoms (and ions) can absorb light at a specific, unique wavelength. When this specific wavelength of light is provided, the energy (light) is absorbed by the atom. Electrons in the atom move from the ground state to an excited state. The amount of light absorbed is measured and the concentration of the element in the sample can be calculated.

An electron is excited from the ground state to higher energy level by absorbing energy (light) at a specific wavelength. In atomic absorption spectroscopy, the wavelength of absorbed light is determined by the type of atom (which element it is) and the energy levels the electrons are moving to. How much light is absorbed is determined by the concentration of the element in the sample.

The Beer Lambert law describes the relationship between light absorption and concentration of the element. According to the law, the amount of light absorbed is proportional to the number of atoms excited from the ground state in the flame

Atomic absorption spectrophotometers are used for measuring numerous elements, especially metallic elements, in solutions at concentrations from mg/L (ppm, 1/1 million) to µg/L (ppb, 1/1 billion). Forty years of ongoing technological development has produced improvements in the sensitivity of the Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer, which is now sensitive enough to measure elements, particularly arsenic, at concentrations of 1 µg/L, 1/10 of the standard value for tap water. AAS finds wide application in fields that vary from mining to

pharmaceuticals, environmental control and agriculture. Most heavy metals are toxic and should be avoided as far as possible. It helps to ensure that the drug is free from the catalysts like palladium or platinum used to make them was performed by an AAS (Lewen, 2011).

Conclusion

As most of the Pakistani herbal medicinal products widely used to treat and prevent various respiratory tract ailments contained detectable levels of mercury, arsenic, cadmium and lead. Similarly, some heavy metals were also found in extract of *Euphorbia nivulia* and soil of Cholistan desert which can cause metal toxicity in body. However metal hazards related to the use of these herbal formulations can be eradicated by minimizing the metal contamination to these medicines. Awareness should be made to the physicians, general public and local healers about the toxicities of metals to the human health when treating the patients.

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