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Phytochemical Screening and Heavy Metals Contents of *Nicotiana glauca* Plant

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ABSTRACT

The phytochemical screening of *Nicotiana glauca* plant leaves and flowers extracts in water, ethanol, ethyl acetate, chloroform, and hexane indicated the presence of alkaloids, di-terpenes and cardiac glycosides. However, flavonoids were only found in aqueous extract. The heavy metals (Fe, Cu, Zn, Pb, Cd, and Mn) contents of *Nicotiana glauca* plant were determined. Among the considered metals, zinc and copper contents were the highest (69.18 ± 0.10 and 35.310 ± 0.004 ppm, respectively), while the contents of Pb, Mn and Cd were remarkably lower (1.780 ± 0.001, 1.070 ± 0.003, and 0.260 ± 0.001, respectively). According to World Health Organization (WHO), the content of Cd and Pb in the studied plant was found in the safe limits for medicinal plants. The obtained results impose that *Nicotiana glauca* plant from Gaser Khiar and Tripoli airport regions of Libya due to rather low content of heavy metals are appropriate for preparation of medicinal extracts.

INTRODUCTION

Natural products still the primary source of commercial medicines and drugs. A recent survey revealed that 61% of the 877 drugs introduced worldwide can be directly isolated or were inspired by natural products. Of the roughly 350,000 species of plants believed to exist, one-third of those have yet to be discovered. Of the quarter million that have been reported, only a fraction of them have been chemically investigated [1].

In folk medicine, most drugs of plant origin used in the form of extract of the whole plant material or part of it without identifying the exact effective compounds. These extracts showed important biological effects, including antiviral, antitumor, antimicrobial, insecticide and central nervous system effect. According World Health Organization (WHO), about 80 % of more than 4000 million people on earth rely chiefly on traditional medicine for their primary health care needs, using plant extract or their active principles mainly. The chemical constituents in medicinal plant usually explain the rational for the use of the plants in traditional medicine [2].

Heavy elements are those have a density of or more than 5 g cm^{-3}. Plants and living organisms need heavy metals in different concentrations, some of them are essential in very low concentrations because of their essential nutritious value but they may have harmful effects [3]. Contamination of plants with heavy metals may occur during growing, processing and handling. Contaminated medicinal herbs can be toxic, and should have high quality in order to protect users from contamination side effects [4]. The maximum permissible limits of toxic metals like arsenic, cadmium and lead in consumed medicinal herbs are 1.00, 0.30 and 10.00 ppm, respectively [5].

Nicotiana glauca (Figure 1) belongs to the plant family Solanaceae [6], in Libya known with local name Akkoz Musa while in some Arabic countries known with the name Massas. The plant is an evergreen, bluish green, erect, slender, sparsely-branched perennial, soft-woody shrub to small tree; usually up to 4 m tall or more which reproduces only by seeds [7]. The plant is native to South America but has widely been introduced to many parts of the world including South Africa, Kenya, Tanzania, Uganda, Asia and U.S.A. This plant however occurs mostly in warm areas because of its sensitivity to frost [8].

Figure 1. *Nicotiana glauca* plant

Nicotiana glauca is toxic to humans and animals [9] and this effect was noticed as early as the 1930's [10]. *Nicotiana glauca* extracts are widely used by traditional healers and showed remarkable biological activities such as Allelopathic activity [11]. Antifungal activity of acetone extracts of *Nicotiana glauca* against ten fungal phytopathogens was also reported [12]. *Nicotiana glauca* extracts also exhibit antibacterial and cytotoxic effects [13].

The aim of this study was to investigate the chemical components of *Nicotiana glauca* qualitatively using phytochemical screening and measuring heavy metals contents in the plant in order to give primary data about the pollution on the surrounding.

MATERIALS AND METHODS

Plant collection

The flowers and leaves of *Nicotiana glauca* were collected from two locations, Gaser Khiar and Tripoli airport road during spring 2015. The taxonomic identity of the plant was confirmed by Biology Department, Faculty of Education, Tripoli University.

Preparation of plant extracts

After collection, the fresh Leaves and flowers of *Nicotiana glauca* were washed with distilled water then left to dry in shade for one month to confirm complete dryness as the plant have large water content, then reduced using domestic blender (HOMMER, 220V, 250W) till very fine powder was obtained. To obtain fractions with different polarities, powdered plant material was extracted with water, ethanol, ethyl acetate, chloroform and hexane. Extraction with each solvent was done by boiling one gram of plant powder in 10.0 ml of suitable solvent for 15 minutes, extracts then were cooled down, filtered and stored in fridge until it was used for further analysis.

Wet Digestion Procedure for Heavy Metals Analysis

5 grams of herbal sample was digested with 15 ml of 65% Nitric acid in 250 ml covered beakers to near dryness. After cooling to room temperature another 3 ml portion of 65% Nitric acid was added until the sample solutions became clear with bright brown color. Clear solutions were evaporated then 5 ml of Conc. HCl and water solution (1:1) was added then warmed to ensure complete digestion. After cooling, resulted solution was diluted to 250 ml with deionized water [15]. Three samples were prepared using same digestion protocol. The samples were analyzed using atomic absorption spectrometer Shimadzu AA-7000 (Figure 2).

Figure 2. Atomic Absorption Spectrophotometer AA-7000 (Flame Model)

Apparatus

Atomic absorption measurements were made using a Shimadzu AA-7000 with Deuterium lamp (D2-lamp) background correction and hollow cathode lamps. Air–acetylene flame was used for determination of all the elements. The working range, wavelength, slit, and detection limits are given in Table 1.

Table 1. The analytical characteristics used with AAS determination

Metal	Working range, ppm	Detection Limits, nm	Wavelength, nm	Slit, mm
Iron (Fe)	0.0-10.0	0.015	248.3	0.2
Copper (Cu)	0.0-1.0	0.007	213.9	1.0
Zinc (Zn)	0.0-5.0	0.021	324.8	0.5
Lead (Pb)	0.0-1.0	0.002	217.0	1.0
Cadmium (Cd)	0.0-1.0	0.003	228.8	0.5
Manganese (Mn)	0.0-2.0	0.005	279.5	0.2

Reagents

All the reagents used were of the analytical purity (Merck, Germany). The working solutions were prepared immediately before the analysis from the basic solution with 1000 mg/l concentration for all metals. For the preparation of standard solutions high purity Milli-Q water was used. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked overnight in 6 M HNO_3 solution and rinsed several times with ultra-pure water to eliminate absorbance due to detergent.

RESULTS AND DISCUSSION

Phytochemical screening applied on *Nicotiana glauca* extracts revealed presence of some secondary metabolites, such as flavonoids, alkaloids, cardiac glycosides while other of secondary metabolites were absent (Table 2).

Table 2. Phytochemical screening of *Nicotiana glauca*

	Water	EtOH*	EtOAc**	$CHCl_3$	Hexane
Alkaloids	+	+	+	+	+
Carbohydrates	-	-	-	-	-
Tannnins	-	-	-	-	-
Flavonoid	+	-	-	-	-
Protiens	-	-	-	-	-

Phenols	-	-	-	-	-
Saponins	-	-	-	-	-
C.Glycosides	+	+	+	+	+
Quinones	-	-	-	-	-
Amino Acids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Phytosterols	-	-	-	-	-
Diterpenes	+	+	+	+	+
Xanthoprotiens	-	-	-	-	-

[*]Ethanol

[**]Ethyl acetate

Flavonoids include a wide array of biological active compounds that are gaining interest due to their skeletal differences and number of members. Flavonoids biological activity have been reported to exhibit variety of biological effects such as anti-inflammatory, antibacterial, antiviral, antiallergic, cytotoxic antitumor, treatment of neurodegenerative diseases and vasodilatory action [16-19].

They exert these effects as antioxidants, free radical scavengers, antioxidant activity is the basis of many actions which explains the power of flavonoids in fighting many diseases [19].

Alkaloids found in all extracts indicating the richness of this plant with this vital type of natural products. Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, antibacterial and antihyperglycemic activities [20-24].

Cardiac glycosides also present in all *Nicotiana glauca* extracts. This type of glycosides are well known for their important role in treatment of congestive heart failure by increasing cardiac contractility via working as antiarrhythmic agents to control atrial fibrillation. In addition, certain cardiac glycosides were applied on prevention or treatment of malignant diseases [25].

The investigation also revealed the presence of diterpenes in the plant, naturally occurring diterpenes have shown many biological activities such as antibacterial, antifungal,

antimycobacterial, cytotoxic, antitumor, cardiovascular, antifeedant, insecticidal, antileishmanial [26]. Recently, many reports have extensively shown that several classes of diterpenoids exert significant cardiovascular effects [27].

Figure 2 shows the concentration of different heavy metals in our plant. The results of metals concentrations (Table 3) have revealed that the highest metal concentration was Zn with 69.18 ppm higher than permissible limit set by WHO for edible plants (27.60 ppm). Zinc is trace essential element and plays an important role in basic bio-processes for the plant and its deficiency could lead to serious growth problems.

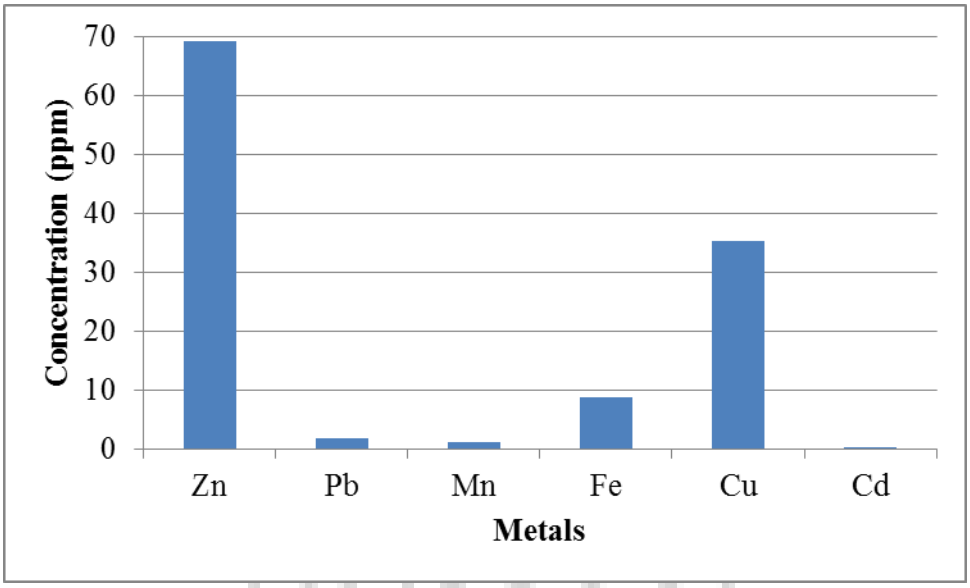

Figure 2. Concentrations of heavy metals in *Nicotiana glauca* plant

Cadmium concentration was 0.260 ppm; the least between all measured metals (less than 0.30 ppm); the WHO limit for medicinal herbs. The increase of cadmium level in human body could cause serious problems in kidney and liver, vascular, and immune system [28]. Copper level was found 35.310 ppm, no limits values were set by WHO for cupper in medicinal plants, although China and Singapore have permissible limits 20 ppm and 150 ppm respectively [4].

Table 3. The content of heavy metals in *Nicotiana glauca* plant

Metal	Zn	Pb	Mn	Fe	Cu	Cd
Content, ppm	69.18 ± 0.10	1.780 ± 0.001	1.070 ± 0.003	8.730 ± 0.001	35.310 ± 0.004	0.260 ± 0.004

Toxicity with copper could cause hair and skin discoloration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea. Copper deficiency is rare but it causes anemia , malnourished infants can have copper deficiency [29].

Iron is very important for humans and animals. The importance attributed to his role in formation of hemoglobin, oxidation of proteins, carbohydrates and fats then controlling weight loss and diabetes [29]. Iron concentration was 8.730 ppm which is lower than WHO limit for medicinal plants (20 ppm) [30].

Manganese is one of the micronutrients and very important for plant growth functions such as photosynthesis, it works as a catalyst, Mn also plays an important role in rapid germination and some enzymatic processes in seeds and new tissues. Manganese deficiency symptoms in plants include stunting of growth and reduced flower formation [31]. Manganese concentration in the studied plant was 1.070 ppm. No toxic limit was set by WHO for Mn level in medicinal plants. However, comparing with studies carried out on different medicinal plants in Egypt and Kenya [28], the level of *Nicotiana glauca* is very low.

Lead is non-essential trace and highly poisonous element. Pb induces various toxic effects in humans at low doses and mainly targeting nervous system in both adults and children [5]. The permissible limit set by FAO / WHO in edible plants was 0.43 ppm while in WHO herbal medicine is 10 ppm [5]. Lead concentration was found 1.780 ppm which is lower than WHO limit.

CONCLUSION

It has been concluded that the *Nicotiana glauca* plant showed the presence of alkaloids, di-terpenes and cardiac glycosides. However, the analysis of chosen plant and their extracts showed

the presence of Fe, Cu, Zn, Pb, Cd, and Mn. The zinc concentration in the plant was rather high and Pb, Mn, and Cd concentrations were significantly low.

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