received: 2019-05-23 DOI 10.19233/ASHN.2019.27

DETERMINATION OF HEAVY METAL CONTENT IN A *DAPHNE GNIDIUM* L. PLANT USING ATOMIC ABSORPTION SPECTROSCOPY

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ABSTRACT

The objective of this study was to determine the content of heavy metals in a Daphne gnidium *L. plant collected in the Middle Atlas (Ribat El Kheir). This plant is used in traditional medicine for its antioxidant effects and antigenotoxic*, *antiseptic and healing properties. This study is partly based on a differential thermal analysis (DTA), which indicated mass losses in three temperature regions. To explain this phenomenon, a sample of* D. gnidium *was heated in an oven at different temperatures: 100 °C, 300 °C and 600 °C for 6 hours, and after the calcination the sample was analysed by Fourier-transform infrared spectroscopy. The results of the study show that the plant* D. gnidium *contains several metals, with increased levels of Fe and Mg. These values are alarming because they are a major danger to the users of this plant.*

Key words: *Daphne gnidium*, heavy metals, AAS, DTA, GTA

DETERMINAZIONE DEL CONTENUTO DI METALLI PESANTI IN PIANTE DI *DAPHNE GNIDIUM* L. CON L'USO DI SPETTROSCOPIA DI ASSORBIMENTO ATOMICO

SINTESI

Lo studio si prefiggeva di determinare il contenuto di metalli pesanti in piante di Daphne gnidium *L. raccolte nel Medio Atlante (Ribat El Kheir). Questa specie è utilizzata nella medicina tradizionale per i suoi effetti antiossidanti e le proprietà antigenotossiche, antisettiche e curative. La ricerca in parte si basa su un'analisi termica differenziale (DTA), che ha indicato perdite di massa in tre regioni di temperatura. Per spiegare questo fenomeno, un campione di* D. gnidium è stato riscaldato in un forno a diverse temperature: 100 ° C, 300 ° C e 600 ° C per 6 ore, e *a seguito della calcinazione il campione è stato analizzato mediante spettroscopia in trasformata di Fourier. I risultati dello studio mostrano che* D. gnidium *contiene diversi metalli, con alti livelli di Fe e Mg. Questi valori sono allarmanti perché rappresentano un grave pericolo per l'utilizzo di questa specie a scopo curativo.*

Parole chiave: *Daphne gnidium*, metalli pesanti, AAS, DTA, GTA

INTRODUCTION

For a long time, people have used plants for their medicinal properties (Ivanova *et al.*, 2005), and in recent years the search for new substances based on natural products has increased. These can be beneficial to humans as natural cures for certain diseases (Roh *et al.*, 2004). The subject of this study is *Daphne gnidium* L., a spontaneous plant species common in semi-arid and subhumid areas of the Mediterranean region. The flowers appear during March–October. It is mainly used in traditional medicine as a remedy for hepatitis, toothache and skin diseases; the methanol extract of *D. gnidium* is known for its antibacterial and antifungal properties and good skin tolerance (Iauk *et al.*, 1996; Iauk *et al.*, 1997). Unfortunately, the industrial development and the introduction of new inputs into agriculture, such as fertilizers and pesticides, which can be transported by wind or water, have compromised the quality of these plants, some of which are now becoming unfit for human use due to the concentrations of heavy metals they contain. Today, much research is directed towards the study of the impact of heavy metals (Cd, Cu, Pb, Zn) on the germination and growth of plants. Mihoub *et al.* (2005) showed, during the germination of pea beans (*Pisum sativum* L.), that the cotyledons in stressed grains gradually accumulate Cd and Cu and retain high levels of Fe, Mg, and Zn. Remarkably, under conditions of heavy metal stress, some higher plants are able to focus the excess metal in certain organs (Sbartai *et al.*, 2012). Several factors may be responsible for the content of heavy metals in plants: the solubility of metals in the soil, soil properties (pH, redox potential), climate change, fertilizers, pesticides, and root systems (Kabelitz & Barbin, 1999). Khan *et al.* (2008) have shown that large amounts of heavy metals, such as Ni, Cd, Cr and Pb, are introduced into the soil through agricultural and industrial activities.

Fig. 1: Geographical map of the region of Ribat El Kheir.
Sl. 1: Zemljevid območja Ribat El Kheir.

Other studies have shown the influence of temperature on the release of Cd, Pb, Cu, and Zn from the branches and leaves of *Populus nigra* L. at temperatures ranging between 653 and 873 °K, in conditions of slow steam-assisted pyrolysis (Grottolaa *et al.*, 2019).

While some metals may exist naturally in plants (Ca, Fe, Na, etc.), others are present in the plant heritage because of air pollution, agricultural activities and industrial discharges (Al, Cd, Pb). Several studies have been conducted with the prospect of their results proving useful to the development of phytoremediation techniques to decontaminate the soils of abandoned industrial sites (Garcia *et al.*, 2003). Although many metals, such as copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), and zinc (Zn), are essential for cells in low concentrations, but toxic in high amounts (Millaleo *et al.* 2010), there are also metals that have not been proven as necessary for the development of living organisms and are toxic even in very low concentrations (Hg, Pb, Cd) (Todeschini *et al.*, 2011).

MATERIAL AND METHODS

Plant material

The sample of *D. gnidium* was harvested from Ribat El Kheir (33° 49′ N, 4° 25′ W) located in the Middle Atlas of Morocco (Fig. 1). The climate in this region is varied, but experts consider it Mediterranean: average annual temperature 28 °C, maximum temperature close to 43 °C, minimum temperature between 4 and 6 °C, frosts observed in January and February. The annual rainfall average is 500 mm. Fog is infrequent. The prevailing winds are from east to southwest or from southwest to northeast.

DTA/TGA

Five milligrams of *D. gnidium* powder were used to conduct a differential thermal analysis (DTA) with a 60 Shimadzu workstation, performing simultaneously DTA/TGA at a heating speed of 20 °C/min for 29 min15s; note that this process is linear. DTA is generally associated with thermal gravimetric analysis (TGA), which measures mass loss versus temperature. This analysis determines the temperature intervals in which the sample undergoes changes due to the heat treatment applied. The results of DTA are presented in the form of a curve: DT (in microvolts) = f (T) (in °C).

Infrared

The same sample was analysed, in the presence of ground potassium bromide, with the Fourier-transform infrared spectroscopy (FTIR) technique, using a JASCO 4100 device and recording spectra over the range of 4000 cm^{-1} to 500 cm^{-1}.

Atomic absorption spectroscopy (AAS)

The ash recovered after the calcination of the plant at 600 °C in a muffle furnace, was mineralized with concentrated nitric acid and filtered with Whatman 0.45 mm filter paper to obtain a solution ready for the determination of metals by atomic absorption spectroscopy.

The AA-7000 series of atomic absorption spectrophotometers produced by the Japanese group Shimadzu is able to measure many different metals. It is composed of an atomizer, which prepares the sample for analysis, a radiant lamp, and a detector. The detection limit in this case was 0.03 ppb. The result of the AAS is presented in the form of a curve: A=f(C), where A = absorption of metal, and C = concentration of metal in ppm

RESULTS AND DISCUSSION

Before performing DTA, we analysed the raw plant by infrared spectroscopy (Fig. 2). The infrared spectrum showed a frequency around 3437 cm^{-1} dominated by the vibration n(OH) of aromatic and aliphatic structures, and a band around 2925 cm^{-1} corresponding to the valence vibration strip n(C-H). The spectrum also showed a defined band at 1630 cm^{-1} that could be assigned to the valence vibration n(C=C) of the aromatic structure. A remarkable band at a frequency of 1382 cm^{-1} was attributed to the n(C-O-C) vibration of aryl-alkyl ether. The band that appeared around 1038 cm^{-1} indicated the presence of a (Si-O) group (Fig. 2).

In order to understand the molecular composition of the plant, 5 mg of *D. gnidium* were used for a thermal analysis (DTA). The results of this analysis are shown in Figure 3. The DTA allowed us to identify three temperature intervals at which we observed significant mass loss.

As shown in Table 1, significant losses of mass were observed from 160 °C onwards, and all of the organic material was lost at 600 °C.

To explain this weight loss at different temperature ranges, the sample was heated in a muffle furnace at different temperatures: 110 °C, 300 °C and 600 °C for

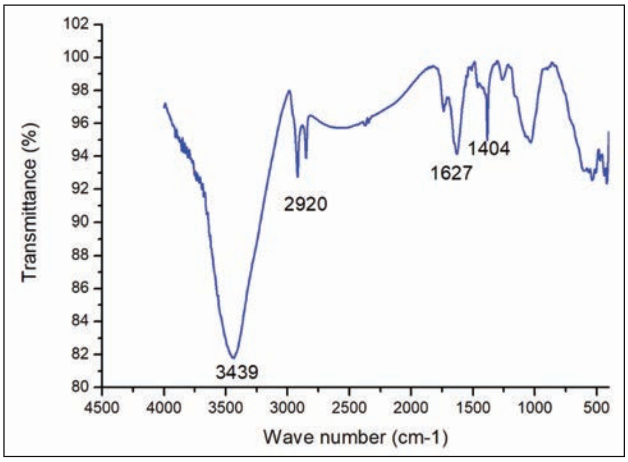

***Fig. 2: IR spectrum of** D. gnidium.*
***Sl. 2: IR spekter vrste** D. gnidium.*

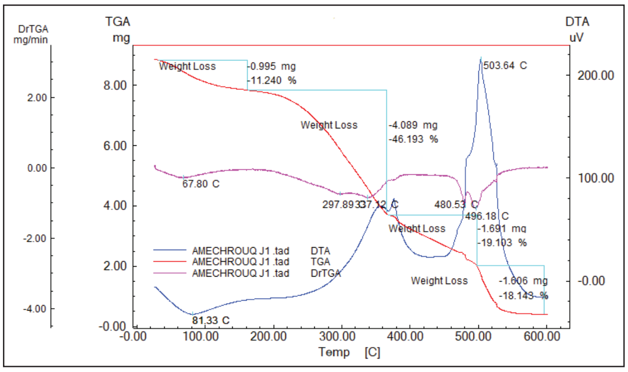

Fig. 3: DTA/TGA spectrum of** D. gnidium**.
Sl. 3: DTA/TGA spekter vrste** D. gnidium**.

calcination. After cooling in a desiccator, the samples were analysed by IR spectroscopy (KBr). Table 2 shows mass loss at each temperature range.

Tab. 1: Mass losses of** D. gnidium **in DTA.
Tab. 1: Masne izgube vrste** D. gnidium **na diferencialni termalni analizi (DTA).

Temperature (°C)	Mass losses in %	Total losses (%)
0-160 °C	11.24%	11.24
160-365 °C	46.19%	57.43
365-600 °C	18.14%	94.68

The results of the calcination confirmed those of the DTA. We noted that more than half of the organic material was lost at 300 °C, and another important loss occurred at 600 °C.

Figure 4 shows the superposed IR spectra of the calcined samples. At 110°C, we noted the persistence of vibration valence bands of the n(OH), n(C-H), and n(C=C) bonds. At 300°C, we noted the disappearance of (OH), the appearance of a vibration band of the

Tab. 2: Mass losses in the calcining** D. gnidium **(m_i: the initial mass, m_f: the final mass).
Tab. 2: Masne izgube pri žganju vrste** D. gnidium **(m_i: začetna masa, m_f: končna masa).

Temperature (°C)	m_i (g)	m_f (g)	Loss (%)
110	5.44	4.66	14.34
300	5.32	2.20	58.64
600	10.73	1.11	89.65

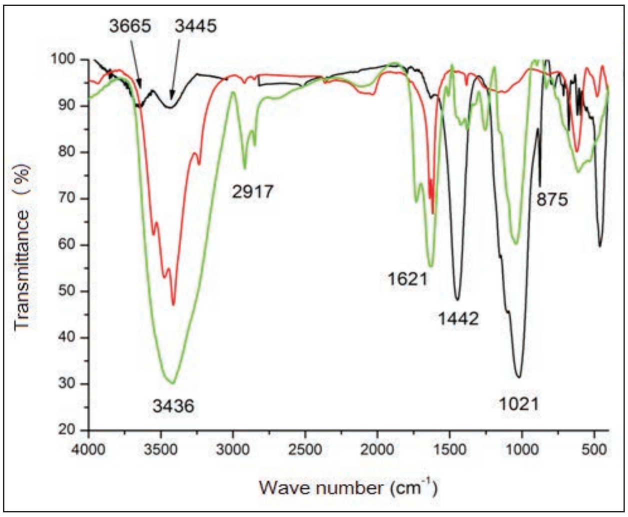

Fig. 4: Superposed IR spectra of the samples carried on ignition.
Sl. 4: Krovni IR spekter žganih vzorcev.

n(N-H) bond at 3436 cm^{-1} corresponding to secondary amines, this latter was accompanied by the appearance of a deformation valence band of the d(N-H) bond. The spectrum also recorded decreases in band intensities of n(C-H) and n(C=C) bonds, which indicated the beginning of disappearance of the organic matter.

At 600 °C, we noticed the disappearance of the vibration band of the n(N-H) bond and the band related to organic matter, and bands related to other mineral elements, such as kaolinite, smectite, quartz, and calcite

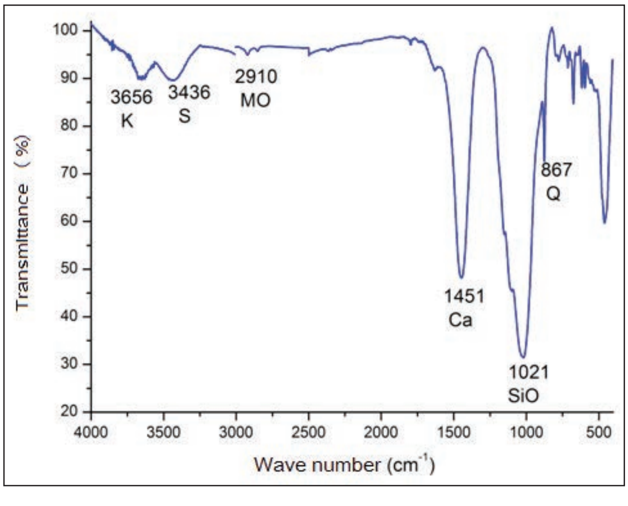

Fig. 5: IR spectrum of the sample calcined at 600 °C (K: kaolinite, S: smectite, CA: calcinite, Q: quartz, MO: organic material).
Sl. 5: IR spekter vzorca segretega na 600 °C (J: kaolinit, S: smektit, CA: kalcinit, Q: kremen, MO: organska snov).

(Fig. 5) appearing instead. These results were in good agreement with the literature (Hachi *et al.*, 2002).

The sample that was calcined at 600 °C and treated with concentrated nitric acid was subsequently analysed by AAS. The results showed a very high Fe and Mg content (1207.7906 mg/kg). These values are alarming, indicating a serious health threat to potential users of this plant (Koumolou *et al.*, 2012). Sathiamoorthy *et al.* (2003) conducted comparative studies on 42 medicinal plants from the Negev desert. The highest iron concentrations were recorded in *Gundelia tournefortii* (3020 ppm) and *Anchusa strigosa* (2485 mg/kg). The concentration of Cu in *D. gnidium* was 0.2738 mg/kg. This value is below the limit concentration in raw plant material provided by the World Health Organization (WHO), which is 150 mg/kg (World Health Organization, 2007). The value of Li was 0.0083 mg/kg. These values show that the copper and lithium levels in the plant posed no risk for its users.

In relation to Zn, the limit value in *D. gnidium* was 1.7683 mg/kg), which is within the standards (1 to mg/kg). This means that in terms of Zn, the plant was not a significant threat to its consumers, who use it as an antiseptic or healing agent (Koumolou *et al.*, 2012). Not so the leaves of *Populus euphratica* and *Z. geslini* from the mentioned comparative study, which registered Zn concentrations well above the norm: 113 mg/kg and 119.10 mg/kg respectively (Sathiamoorthy *et al.*, 2003).

While the concentration of Pb in *D. gnidium* was 0.5148 mg/kg – below the WHO limit (10 mg/kg) (Boulila Zoghlami *et al.*, 2006), Cd concentration was 0.3787 mg/kg – exceeding the WHO tolerance limit (0.3 mg/kg) by 26.23% (World Health Organization, 2007). Regarding Al, atomic absorption spectroscopy showed that it was not present in *D. gnidium,* while the value for Ca was 32.5020 mg/kg. Studies conducted by Boulila Zoghlima *et al.* (2006) on tomato (*Lycopersicon esculentum*) showed that the negative impact of cadmium on certain growth and development processes can be mitigated by an adequate calcium intake in the culture medium, and that the addition of Ca to a culture medium containing Cd (growth inhibitor) improves the production of plant biomass.

CONCLUSIONS

In the present study, we have tried to assess the toxicity of the plant *D. gnidium* through the determination of the heavy metals in it that could harm the health of the plant's consumers. The results of the analyses conducted revealed high concentrations of Fe, Mg, and Cd, thus confirming the concerns which had prompted this study. This contamination by heavy metals could be the cause of many diseases among the population consuming this plant, hence the need to seek the origin of these metals (soil, water, agricultural activities) and of the process of contamination, especially since *D. gnidium* bark powder is also used orally in the treatment of syphilis and venereal diseases.

DOLOČEVANJE VSEBNOSTI TEŽKIH KOVIN V VOLČINU VRSTE *DAPHNE GNIDIUM* L. Z UPORABO ATOMSKE ABSORBCIJSKE SPEKTROSKOPIJE

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POVZETEK

Cilj raziskave je bil določiti vsebnost težkih kovin v volčinu vrste Daphne gnidium *L., nabranem v osrednjem Atlasu (Ribat El Kheir). To rastlino uporabljajo v medicini kot antioksidant in zaradi antigenotoksičnih, antiseptičnih in zdravilnih učinkovin. Raziskava delno temelji na diferencialni termalni analizi (DTA), ki pokaže izgubo mase v treh temperaturnih regijah. Da bi razložili ta pojav so vzorec vrste* D. gnidium *segrevali v pečici na različnih temperaturah: 100 °C, 300 °C in 600 °C za 6 ur, in ga po segrevanju analizirali s Fourierjejevo infrardečo spektroskopijo. Rezultati so pokazali, da vsebuje volčin številne kovine, še posebej visok nivo Fe in Mg. Te vrednosti so alarmantne, saj lahko povzročijo nevarnost pri uporabnikih.*

Ključne besede*:* *Daphne gnidium*, težke kovine, AAS, DTA, GTA

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