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Research Paper

Analysis of Oleandrin in Oleander Extract (*Nerium oleander*) by HPLC

Ghaleb Tayoub*, Huda Sulaiman, Malik Alorfi

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, Damascus, PO Box 6091, Syria

*Corresponding Author

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ABSTRACT

The purpose of this study was to determine the oleandrin concentrations in leaves, stems, flowers and roots of the wild-growing and cultivated *Nerium oleander* L. at the full flowering stage from seven wild population plants, collected from five sites located in Lattakia city (coastal region), and one site located in Damascus (semi-aride region; in addition to, a cultivated oleander in public gardens. Oleandrin was separated and quantified by high-performance liquid chromatography (HPLC) coupled with diode-array detector, on a reversed phase C_{18} column and employing water-acetonitrile (40:60) (v/v) as a mobile phase. HPLC results showed that, oleandrin concentrations varied by organ studied, location and planting region. The highst concentration of oleandrin was in the roots, followed by leaves, stems and flowers, respectively. Differences in oleandrin concentrations in relation to plant organ and location were statistically significant by LSD test at *P* values less than 0.05.

Keywords: Oleandrin; *Nerium oleander*; HPLC.

INTRODUCTION

Oleander (*Nerium oleander*, Linn), is an evergreen shrub belongs to Apocynaceae (Hadizadeh, et al., 2009); known locally in Syria as Defla, is widely distributed in Mediterranean and subtropical Asians regions (El-Shazly, et al., 1996; Begum, et al., 1997). This plant is also cultivated as an ornamental plant in tropical and subtropical parts of the world.

All parts of the plant, including the sap, either fresh, dried or boiled, are poisonous to humans, animals, certain insects, fish and birds, but now a day's numbers of pharmacological activities are determined by different scientists (Alfonso, et al., 1994; Langford and Boor, 1996; Adam, et al., 2001; Tiwari and Singh, 2003; Soto-Blanco, et al., 2006, Barbosa, et al., 2008; Gupta and Mittal, 2010., Omidi, et al., 2011). The leaves contain cardiac glycosides like oleandrin, oleandrigenin, digoxin, digitonin, digitoxigenin, nerizoside, neritaloside, odoroside (Trease and Evans, 2002; Tiwari and Singh, 2004).The flowers and leaves are used in folk medicine for the treatment of a wide variety of diseases including infection, malaria, autoimmunity,

abscesses, asthma, allergy, eczema, dysmenorrheal, epilepsy, anti-bacterial activity, antnociceptive activity, HIV and cancer (Newman, et al., 2001; Erdemoglu, et al., 2003). The root is bitter, aphrodisiac; tonic is good for chronic pain in the abdomen and pain in the joints, very poisonous, but an antidote to snake-venom and reveals antibacterial activity. (Hussain and Gorsi, 2004).

There have been numerous reports of poisoning and death from ingestion of oleander, oleander leaf tea, and its extract. It has killed adults, children, pets, and livestock. Even a small amount of oleander can cause death due to its effects on the heart (Bruneton, 1996; Aslani, 2004; Turan, et al., 2006). Inhaling the smoke from burning oleander or eating honey made from its nectar can produce poisonous effects. Extracts from any part of the oleander plant should not be used except under the careful observation and controlled conditions of a clinical trial according to the American Cancer Society (ACS, 2008).

Water-soluble extract from *N. oleander* called Anvirzel, is a compound that mainly consists of polysaccharides, proteins, two cardiac glycosides (Oleandrin and Oleandrigenin). The above glycoside has been used for many decades in treatment of heart failure and has been proved able to inhibit proliferation of tumor cells. According to literature data, Anvirzel™ reduces the cancer cells in pancreatic and prostate cancer. Recently, laboratory experimental data have also proven that Anvirzel™ has a wider field of action, also in other cancer types, such as breast, lung and colon cancer. It has been also proved that Anvirzel™ is more effective in low concentrations, thus avoiding the toxicity due to high concentrations of the drug (Apostolou, et al., 2011).

Oleandrin ($C_{32}H_{48}O_9$), a glycoside, is the main toxin, found in oleander. The concentration of oleandrin in plant tissues is approximately 0.08% (Schvartsman, 1979). Oleandrin is a promising agent for anti-cancer treatment. Studies showed potential *in vitro* effect on cancers of the colon, non-small cell lung cancer, leukemia, pancreas, melanoma and prostate (Smith, et al., 2001; Frese, et al., 2006; Turan, et al., 2006; Newman, et al., 2007; Felth, et al., 2009).

It may work as a cytotoxic agent, generating reactive oxygen species or inducing apoptosis, but has also shown to be synergistic with current chemotherapy. This may be due to its potential to inhibit P-glycoprotein. This transporter is responsible for phenotypes of cancer resistant to chemotherapeutic agents (Dean, 2002).

N. oleander, which is known to contain oleandrin (Trease and Evans, 2002), is found in Syria. This plant grows wildly in coastal countryside were average rainfall is 700mm/year. And in Damascus region were average rainfall is 210 mm/year. It is also cultured in many Syrian regions, gardens and road sides, due to its ability to tolerate air pollution and drought.

The aim of this study was to determine oleandrin concentrations in different parts of *N. oleander* during flowering stage of plant life. Since there is no other study in available literature was performed.

MATERIALS AND METHODS

Sampling: *Nerium oleander* L., samples were harvested in May 2012 from seven wild and cultivated population plants: five of them were from sites located in Lattakia city (coastal region), namely Rodo, Wata Al-khan, Alboudi, Aldroukiate, Alqubeisah, and one cultivated oleander sample was taken from Public garden in Lattakia. The seventh samples were from Almouhamadia site located in Damascus (semi-aride region). The plants were identified by Prof. M. Oudat ((taxonomist, AECS). Voucher

specimens have been deposited in the laboratory of the plant biotechnology department at the Atomic Energy Commission of Syria (AECS). From each collection site three separate individual plants, (Height: 3-4m), were chosen to harvest mature green leaves, stems, flowers and roots. The raw materials were cleaned and oven-dried at 40°C, till constant weight. Samples then stored at -20°C until analysis.

Samples extraction: The samples extracted according to (Tittel and Wagner, 1981) method. Briefly, the dried samples were ground to fine powder with the aid of a mechanical grinder, then 100g of powder was extracted with 150ml of methanol water (90:10) using a homogenizer for 5 minutes. The mixture was then filtered through Whatman no.1 filter paper. The sample was re-extracted following the same above procedure. The extracts were collected, and evaporated by rotary evaporator to 10ml; the extract was stored in a refrigerator until analysis. All the samples were extracted in triplicate.

Chemicals, Standards solution: Oleandrin standard purchased from sigma-Aldrich, acetonitrile, methanol, water (Lichrosolv grade) were purchased from Merck. Olenadrin stock solution was prepared by dissolving 100 mg of oleandrin in 100 ml methanol and stored at 2-8°C. The standard working solutions used to build calibration curve were prepared by serial dilutions: 1, 4, 8, 10µg/ml of stock solution of oleandrin with methanol.

***HPLC-DAD system for analysis of Oleandrin*:** Chromatographic separation was achieved with LC system from Agilent (Infinity 1260) equipped with a diode array detector (DAD), using a reversed phase Eclipse C_{18}, (150 × 4.6 mm i.d.; 3.5µm) column from Agilent Co. the separation was conducted at 30°C. The mobile phase consisted of Acetonitrile:Water (60: 40). The flow rate was 1.5 ml/min, and the injection volume 20 µl, with UV detection at 220 nm.

***Statistical Analysis*:** Data were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was used to assess the significance of differences among groups. Multiple comparison test using the least significant difference LSD at *P* values less than 0.05 (SPSS, 17) was also applied. Microsoft Excel program was used to generate statistical histograms.

RESULTS AND DISCUSSION

Typical HPLC chromatograms of chemical standards and *N. oleander* are shown in Figure1. The retention times of A and B were 3.311-3.835 and 3.666 min, respectively. In the range of 1–10µg/ml, good correlation of linearity has been achieved (n=3; $R^2 = 0.9998$).

HPLC analysis results of oleandrin concentrations in leaves, roots, stems, flowers are given in table 1. The results showed that, oleandrin concentration in oleander during the flowering stage varies according to the organ studied, geographical location and region of planting. The maximum amount of oleandrin was in the roots, followed leaves, stems then flowers. Oleandrin concentration in roots was 1.5-2 times higher than that in leaves, 1.9-2.7 times higher than that recorded in stems, and in 4.1-6.9 times higher than that recorded in flowers, for both wild and cultivated oleander.

Oleandrin concentrations in plant from different locations ranged from 0.18mg/g dry weight (10%) to 0.31mg/g dry weight (18%) in leaves, and from 0.12mg/g dry weight (9%) to 0.23mg/g dry weight (20%), and from 0.34 (10%) to 0.64mg/g dry weight (18%) in roots. As for the flowers, the concentrations were generally low compared with other plant organs.

Analysis of variance using LSD test at *P* values less than 0.05 showed statistically significant differences in oleandrin concentration in the roots, stems and leaves. While the flowers showed no statistically significant differences in oleandrin concentrations between the various locations ($P>0.05$). Comparing our results with a previous study on the leaves of oleander plant (Abdul Latif, 2008) showed that the concentration of oleandrin varies depending on the sampling date and location, we did not find any study on concentration of oleandrin in this plant. It should be noted that the indirect environmental factors (high annual rainfall, nature of the soil, geographic location, and high altitude above sea level) play a large role on oleandrin concentration. Therefore we attribute the differences in the concentration of oleandrin to environmental conditions surrounding each studied location. Even though, the roots of both natural and cultivated *N. oleander* can be considered as good oleandrin sources, results showed that flowers have lower oleandrin concentrations and not to be used for oleandrin isolation.

CONCLUSION

Oleandrin concentrations in *N. oleander* parts were in root> leaf> stem> flower. Plants from Rodo (Lattakia) contained the highest concentrations of oleandrin in roots, leaves and flowers. Where as in plants from Al-qubeisah (Lattakia) the highest concentrations of oleandrin were in the stem. *N. Oleander* roots and leaves might be considered a cheap and wealthy source of oleandrin from plants in the flowering stage. Studying the relationship between the stage of plant life and the concentration of oleandrin in plants from different locations might be useful in determining the best period and the best plant population to be used for medicinal and pharmaceutical purposes.

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Table-1: Amount of oleandrin determined in different tissues of *N. oleander* (mg/g) dry weight.

Region	Location	Leaf	Flower	Stem	Root
Lattakia	Rodo	0.31±0.03 (18%)	0.13±0.04 (19%)	0.23±0.03 (16%)	0.64±0.05 (18%)
	Wata Al-khan	0.26±0.04 (14%)	0.09±0.03 (14%)	0.19±0.03 (13%)	0.42±0.04 (13%)
	Al-qubeisah	0.30±0.03 (17%)	0.12±0.04 (18%)	0.28±0.03 (20%)	0.62±0.03 (19%)
	Al-boudi	0.29±0.04 (16%)	0.08±0.02 (12%)	0.21±0.04 (15%)	0.55±0.03 (17%)
	Al-droukiate	0.20±0.04 (11%)	0.07±0.02 (11%)	0.16±0.03 (11%)	0.34±0.04 (10%)
	cultivated oleander	0.18±0.03 (10%)	0.07±0.03 (11%)	0.12±0.02 (9% value)	0.35±0.03 (11%)
Damascus	Al-mohammedia	0.26±0.04 (14%)	0.1±0.02 (15%)	0.22±0.03 (16%)	0.41±0.04 (12%)

- Oleandrin concentration expressed as mg/ g derived from the average of three independently extraction replicates.

r^2= 0.9998

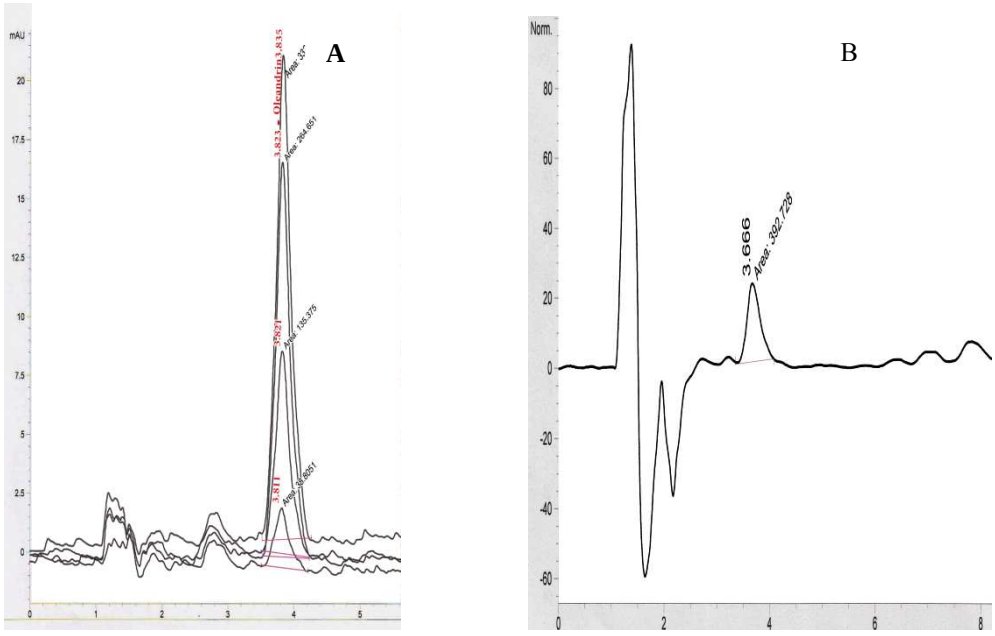

Figure-1:. Typical HPLC chromatograms of chemical standards and samples.

- **A**: chemical standards; **B**: Chromatogram of oleandrin in the leaves of *N. oleander* ((t_R = 3.666 min).

