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# *Comparative GC–MS and HPLC Analysis of Bioactive Compounds in Nerium oleander L. Leaf Extracts Obtained by Different Extraction Methods*

***Maryam S. Hussein[1],* Enas H. Al-Ani [1]and Abdel-Raheem, M. A.[2]***

[1] Department of plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq.
[2] Pests & plant protection Department, Agricultural and Biological Research Institute , National Research Centre , Egypt.

*Corresponding author: **marykom2092@gmail.com**

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## Abstract

Oleander (*Nerium oleander L.*) is a very toxic and biologically active decorative plant. *Nerium oleander* leaf extracts extracted in the presence of active ingredients: A focus study. Analytically, these compounds were analyzed through gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC). Analysis of the ethanolic extract by a GC-MS showed various bioactive compounds, specifically phytol, 5-hydroxymethylfurfural, N-hexadecanoic acid, squalene, and vitamin E. Phytol was the major constituent of this extract as represented by 27.44% of the total volume. In comparison, the aqueous extract contained predominantly N-hexadecanoic acid, oleic acid, and octadecanoic acid. HPLC characterization of the third extract showed three key excitations at retention times of 3.90, 5.11, and 6.08 min. Relation to standard samples also provided evidence of cardiac glycosides, especially oleandrin and nerlin. In the case of oleander leaf extracts, the study demonstrates that the extraction strategy plays a major role in determining their phytochemical composition. In addition the results have shown that the in-depth and precise characterization of bioactive compounds of the plant is obtained based on GC-MS together with HPLC techniques.

**Keywords:** *Nerium oleander L.*, HPLC, GC-MS, cardiac glycosides, extraction.

## Introduction

Plants constitute a major contributor of various secondary metabolites that are involved in the treatment and prevention of disease. The active constituents of different plants have been identified and characterized showing their pharmacological and biological activities. Most pharmaceuticals are obtained from secondary metabolites that occur in plants and compounds that derive from these metabolites. [3,4].

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Several literature studies have reported the ability of plant extracts and chemical constituents of plants products to prevent a wide range of pathological states or diseases. Many plant species in agriculture have protective properties against a wide variety of diseases, but the poisonous qualities mentioned previously are not researched and remain underexplored. The potential harmful effects of *Nerium oleander L*. on cancer were studied in this study while it has been shown to have a protective effect against cancer in previous studies. *Nerium oleander* species is a natural bioactive substance with an excellent diversity of bioactivity and it has strong antioxidant, anticancer, antimicrobial, and antidiabetic properties [5,6]. It grows normally as a shrub or small tree in warm and subtropical climate zones. It is part of Apocynaceae family and Nerium genus[7]. It indicates several biological and pharmacological activities of *Nerium oleander* because of its abundant phytochemical composition. Nerium consists of many nutrients with its leaves specifically rich in carbohydrates, flavonoids, alkaloids, steroids, cardiac glycosides, and tannins [8].

In this study, leaf samples of *N. oleander* have been collected and extracted. Phytochemical analysis and toxic effects were investigated. Toxic effects were associated with phytochemical content determined by qualitative analysis. Different extraction methods produced different results

## Materials and Methods

### Study design and sample collection:

Leaves of Oleander (*Nerium oleander*) were collected from Al-Nahrain University. The leaves were washed extensively with distilled water to remove dust and environmental contaminants as well as divided into equal parts for utilization in three extraction methods to compare the efficiency of these methods in extraction from the active constituents present in the plant. This allowed maceration, extraction from the fresh leaves, and solvent extraction.

### Ethanol Method

In this method, oleander leaves were washed and dried, then ground into a fine plant powder using a laboratory grinder. A known quantity of the plant powder was macerated using 70% ethanol as the extraction solvent.

A quantity of 100 g of the plant powder was macerated using 1000 mL of 70% ethanol as the extraction solvent. The mixture was left at room temperature for 12 hours in a shaker at the college laboratory to ensure maximum extraction of bioactive compounds."

After maceration, the mixture was filtered using Whatman filter paper to remove any remaining plant material. The filtrate was then concentrated to obtain the crude extract, which was subsequently dried by pouring it into glass Petri dishes and drying at 4°C. After drying, the material was scraped and stored in sterile containers until chemical analysis.[9]

### Aqueous Extraction Method

The fresh leaves of the *oleander* plant (*Nerium oleander*) were separated from the plant, then washed thoroughly with distilled water to remove dirt and contaminants, and then cut into small pieces, according to the method described by Yaaqoob et al.2022[10]. The aqueous filtrate was concentrated and then subjected to drying at room temperature in the shade to prevent the thermal degradation of heat-sensitive

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compounds. After complete drying, the residue was collected and stored in the refrigerator at 4°C until further analysis. Approximately 25 grams of fresh leaves were weighed and added to 100 mL of distilled water. The mixture was blended in an electric mixer for 15 minutes to obtain a homogeneous extract. This homogenization process enhances the consistency of the mixture and improves the extraction process. The extract was then filtered using Whatman No. 1 filter paper to remove plant tissue residues. The extract dissolved in water was distributed into 10 ml tubes, then placed in a centrifuge at 3000–5000 rpm to obtain the filtrate and precipitate. The filtrate was then carefully dried and kept in the refrigerator until it could be used in subsequent analyses[11].

### Methanol Extraction Method

The leaf samples were dried and then ground into a fine powder. One hundred grams of this powder was weighed and placed in a suitable glass container. One hundred and fifty milliliters of an extraction solution consisting of a 90:10 v/v mixture of methanol and water were added to the powder. The mixture was then subjected to intensive homogenization using a homogenizer for five minutes to ensure complete breakdown of plant tissues and release of the active ingredient. The resulting mixture was then filtered using Whatman No. 1 filter paper to separate the precipitate from the liquid. The extraction process was repeated on the solid residue to ensure the removal of all target compounds. The liquid extracts were then collected and evaporated using a rotary evaporator under reduced pressure and at 40°C. The concentrated extract was transferred to sealed bottles and stored in a refrigerator at 4°C until analysis[12].

## Results and Discussion

For each extraction method, the extract was analyzed to identify its active ingredients. The Ethanol and Aqueous Extraction methods were analyzed sequentially using a GC-Mass device, and the Methanol method using an HPLC device.

### 1. Ethanol extraction

A sample of the ethanol extract was analyzed using the Gas Chromatography-Mass Spectrometry technique to identify the active compounds in the extract. The chromatogram showed several peaks representing different compounds that were identified by comparing the mass spectra with the NIST library. Among the most important compounds detected were *phytol*, 5-*hydroxymethylfurfural*, and 2-*methoxy-4-vinylphenol*, as well as some fatty acids such as n-*hexadecanoic* acid and antioxidant compounds such as *squalene* and vitamin E. The predominant compound was *phytol*, comprising approximately 27.44% of the total peak area, indicating that the ethanolic extract contains a high percentage of terpenoid compounds. This compound is known to possess antioxidant and antimicrobial activities (de Moraes et al., 2014)[13] .The compound 5-*Hydroxymethylfurfural* was also detected at a relative peak area of 13.71%, which is a sugar-derived compound with antioxidant activities[13].

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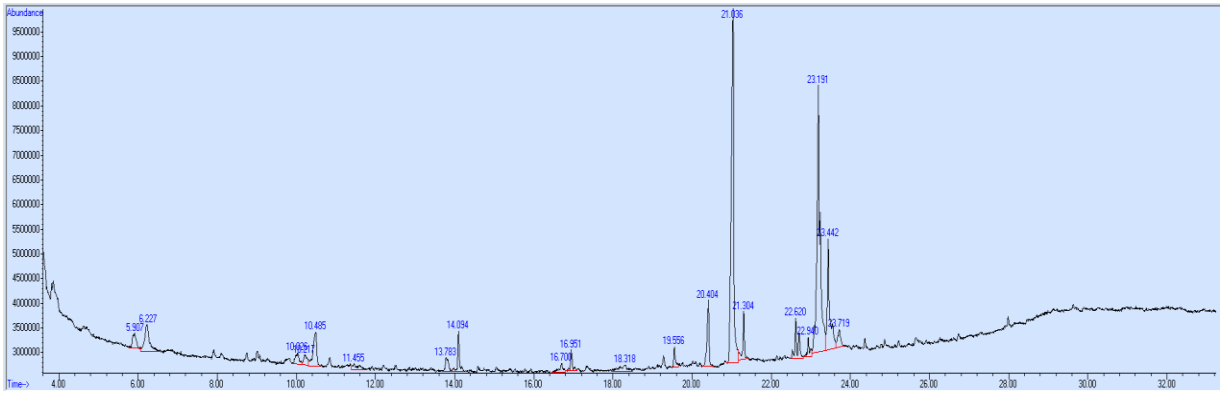

**Figure 1: GC–MS chromatogram of the ethanolic extract of *Nerium oleander* leaves.**

**Table 1: compounds identified in the ethanolic extract of *Nerium oleander* using Gas Chromatography–Mass Spectrometry**

| No | Compound | Retention Time (min) | Area % |
|---|---|---|---|
| 1 | *5-Hydroxymethylfurfural* | 9.10 | 13.71 |
| 2 | *2-Methoxy-4-vinylphenol* | 9.89 | 5.11 |
| 3 | *n-Hexadecanoic acid* | 20.39 | 3.53 |
| 4 | *Phytol* | 22.25 | 27.44 |
| 5 | *Squalene* | 30.26 | 2.47 |
| 6 | Vitamin E | 34.71 | 2.85 |

Based on these results, terpenoid compounds and bioactive compounds have been effectively extracted from oleander leaves with ethanol extraction.

**2. Aqueous extraction**

A sample of the extract was analyzed using GC-MS technology to identify the active ingredients present in it.

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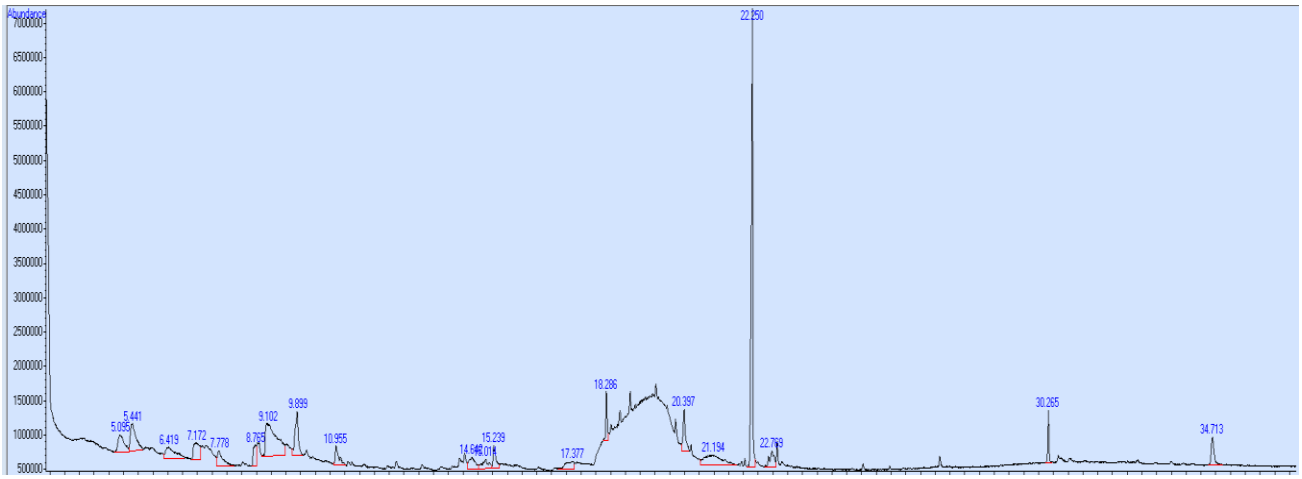


**Figure 2: GC–MS chromatogram of the aqueous extract of Nerium oleander.**

The results showed the presence of a number of fatty acids and their derivatives. Among the most prominent compounds discovered were n-Hexadecanoic acid at 27.95%, Oleic acid at 27.20%, and Octadecanoic acid at 9.11%. Most polar compounds in *Nerium oleander* possess high molecular weights and strong intermolecular bonding, which prevents them from vaporizing at the temperatures utilized in the GC injector without undergoing thermal degradation. Furthermore, without prior derivatization (to reduce polarity and increase volatility), these molecules exhibit poor affinity for the GC column phase. Consequently, Liquid Chromatography (HPLC) remains the gold standard for identifying these specific polar fractions, as it does not require sample volatilization.

These fatty acids are common compounds in plant extracts and many studies have shown that they possess antimicrobial and antioxidant activities[14].

**Table 2: compounds identified in the aqueous extract of *Nerium oleander* using GC–MS**

| No | Compound | Retention Time (min) | Area % |
|---|---|---|---|
| 1 | Hexadecanoic acid methyl ester | 20.40 | 4.03 |
| 2 | n-Hexadecanoic acid | 21.03 | 27.95 |
| 3 | Hexadecanoic acid ethyl ester | 21.30 | 2.67 |
| 4 | 9-Octadecenoic acid methyl ester | 22.62 | 3.41 |
| 5 | Oleic acid | 23.19 | 27.20 |
| 6 | Octadecanoic acid | 23.44 | 9.11 |

Oleic acid is known as one of the unsaturated fatty acids that possess anti-inflammatory properties and antibacterial activity[15].

These results show that Water extraction may lead to the extraction of polar compounds, including some fatty acid derivatives. Which is consistent with what (Azwanida ,2015)[9] stated about the effect of solvent polarity on the type of compounds extracted from medicinal plants. "Regarding the aqueous extract

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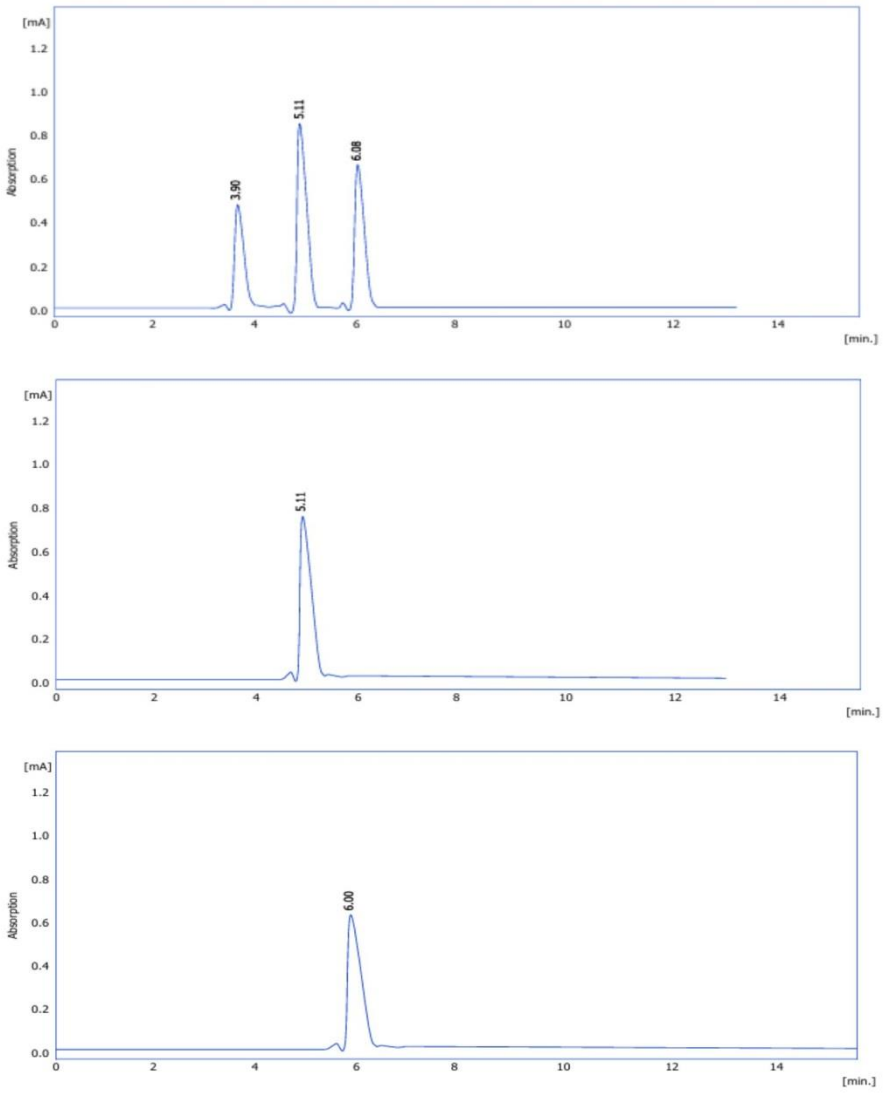
analysis, the absence of highly polar compounds such as flavonoids and alkaloids in the GC-MS chromatogram can be attributed to the inherent limitations of this technique. GC-MS is primarily effective for volatile and non-polar compounds. Highly polar molecules often require chemical derivatization to become volatile enough for GC analysis, or they should alternatively be characterized using HPLC, which is more suitable for such phytochemical classes."

## 3. Methanol Extraction Method

An extract was analysed by HPLC for active substances present in *Nerium oleander*.

**Figure 3: HPLC chromatogram showing the detected peaks at retention times 3.90, 5.11, and 6.08 min.**



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The chromatogram showed three main peaks at different retention times, indicating the presence of several compounds in the plant extract. The peaks appeared at 3.90, 5.11 and 6.08 minutes with area percentages of 25%, 40% and 35% respectively. Analysis of *Oleandrin* Chromatographic separation was achieved with a diode array detector (DAD), using a reversed phase Eclipse C18, (250 mm × 4.6 mm) The mobile phase consisted of Acetonitrile: Water (60: 40). The flow rate was 1.5 ml/min, and the injection volume 100 µl, with UV detection at 220 nm.

| Peak No | Retention Time (min) | Area % | Proposed compound |
|---|---|---|---|
| 1 | 3.90 | 25 | |
| 2 | 5.11 | 40 | *Oleandrin* |
| 3 | 6.08 | 35 | *nerlin* |

**Table 3: HPLC chromatographic peaks of *Nerium oleander* using High-Performance Liquid Chromatography**

## Conclusion:

The conclusion drawn from this research is that oleander leaves contain a diversity of biochemical compounds with biological significance based on chemical profiles of them, including terpenoids, fatty acids and cardiac glycosides, as determined by chromatographic techniques. It is evident that the solvent extraction mode was highly relevant and has an important role to play in identifying the chemical identity of the extracted extract; since ethanol has high selectivity to attract terpenoid and antioxidant compounds, and aqueous extract had a significant concentration of fatty acids. HPLC was also performed and the results were further strengthened through the identification of cardiac glycosides, particularly the compound "Oleandrin" the hallmark of oleander plant.

On the whole, these findings support the fact that this plant is indeed a naturally generated resource abundant in promising biomolecules that serve to extend the possibilities in pharmaceutical and biochemical applications.

## Author's Contribution Statement

- Maryam S. Hussein: Conducted some experiments, data rearrangement and drafted the initial manuscript.
- Enas H. AL-Ani: Contributed to the conception and design of the study, and conducted some characteristics of the products.
- Abdel-Raheem, M. A. : Contributed to the conception and design of the study.

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