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Phytochemical Investigations of *Nerium Oleander* L. Leaves and Flowers

A. Ali Redha

Dept. of Chemistry, College of Science, University of Bahrain, Sakhir, Kingdom of Bahrain

Author's email id: ali96chem@gmail.com

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***Abstract*—** According to the qualitative phytochemical screening of oleander leaves and flowers done, both oleander leaves, and flowers contained phenols, tannins, flavonoids, coumarins, sterols, triterpenes, alkaloids and phlobatannins. Dried semi-powdered oleander leaves, and flowers were extracted with petroleum ether, chloroform, ethyl acetate, acetone, methanol, and water. The total phenolic content and flavonoid content of each extract was determined using ultra-violet/visible spectrophotometer. The alcoholic and aqueous extracts of oleander leaves and flowers contained the highest amount of phenolic compounds. The alcoholic extracts of oleander leaves and flowers contained the highest amount of flavonoids. The antioxidant power of each extract was also determined by measuring the radical scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH). The highest antioxidant power was of oleander flowers' alcoholic extract.

***Keywords*—** Phytochemicals; oleander; natural products; phenolic; antioxidant; DPPH

I. INTRODUCTION

Nerium oleander L., known as oleander, is an evergreen flowering plant belonging to the family of Apocynaceae [1]. Traditionally, oleander plant has been used in treating cancer, leprosy, and skin diseases in India [2]. It is rich in significant phytochemicals, including α-tocopherol, oleandrin, digitoxingenin, ursolic acid, quercetin, and vitamin C [3]. These compounds, which contain phenolic groups in their structure, show significant antioxidant activity. Phenolic compounds play a great role in the plant's defence system against pathogens and predators due to their antioxidant potential [4]. They are also one of the colour sources in plants and their products, they are responsible for the taste and flavour of plant products [4].
Researches have shown that oleander leaves and flowers have anti-nociceptive, anti-inflammatory, antimicrobial, anticancer, anti-leukemic, locomotor, diuretic, immunomodulating activity [3]. It has been reported that oleandrin, a cardiac steroid present in oleander, can increase intracellular calcium ion by inhibiting the sodium/potassium ions-ATPase pump and prompt the apoptosis of cancer cells [5]. It has also been reported that oleander extracts have inhibited the growth of bacteria such as *Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus subtilis* [2].

The medicinal properties of oleander have triggered phytochemical investigations of this plant. The aim of this research was to conduct phytochemical screening, determine the phenolic and flavonoid content and measure the radical scavenging activity of oleander leaves and flowers using different extracting solvents.

The current paper is organized as follows: Section I is includes background information about oleander plant and its pharmacological properties, Section II discusses previous research done with respect to the current research topic, Section III describes the methodology followed in the current research which involves the materials used in the experimental work, sample collection, extraction, phytochemical screening, phenolic content estimation, flavonoid content estimation and determination of antioxidant activity of oleander leaves and flowers. Section IV, illustrates and discusses the results and findings of the experimental work, while Section V sums up the findings of this research.

II. RELATED WORK

Mohadjerani has previously studied the total phenolic content and antioxidant activity of oleander leaves and flowers [6]. The extracting solvents were limited to water, methanol, water: methanol and acetone. In the current study, the range of solvents (i.e. polarity range of solvents) has been increased to explore the effect of extracting solvent on the phenolic content, flavonoid content, and antioxidant activity. According to the findings of Mohadjernai, the phenolic content of oleander flowers was greater than that of oleander leaves [6]. Similarly, the antioxidant activity of oleander flowers was found to be greater than that of oleander leaves [6].

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III. METHODOLOGY

Materials

Gallic acid, rutin and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Sample Collection & Preparation

Six healthy *Nerium oldeander* plants were identified in University of Bahrain's garden in Sakhir. Healthy green leaves and mature pink flowers were collected from the plants. The plants' leaves were washed with distilled water thoroughly. Leaves were cut into small pieces. Leaves and flowers were directly dried in a laboratory oven at 40˚C. The dried sample was crushed manually and kept in sealed plastic bags in dark and at room temperature until use.

Phytochemical Screening

An acid extract, aqueous extract, and methanolic extract of oleander were prepared according to Hassan *et al* [7]. The extracts were used to qualitatively determine the presence of phenols, tannins, flavonoids, coumarins, saponins, alkaloids, sterols and triterpenes, anthraquinone glycosides, and phlobatannins in oleander leaves and flowers according to Hassan *et al.* [7].

Preparation of Extracts for Quantitative Assays

An amount of 1 g of dried leaves or flowers of *Nerium oldeander* was weighed and macerated in 10 mL of petroleum ether, ethyl acetate, acetone, methanol, or water separately, for 24 hours. After 24 hours, the solutions were decanted, and the extracts were saved. To the plant material, another 10 mL of the same solvents were added. After 30 minutes, the solutions were filtered and mixed with previous extracts. The extracts were diluted to 25 mL using the same solvents. The extracts were stored at 5˚C until further use.

Determination of Total Phenolic Content

The total phenolic content of the extracts was determined according to Hassan *et al.* [7]. A volume of 0.5 mL of extract aliquot was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent and allowed to stand for 5 minutes. Following that, 2 mL of 7.5% sodium carbonate was added. The reaction was kept for 30 minutes, after that the absorbance of each sample was measured at 760 nm using PerkinElemer Lambda 25 UV/VIS spectrometer. A triplicate set of tests were conducted for each sample extract. The total amount of phenolic compounds of each extract was reported as gallic acid equivalents (GAE) milligram per gram.

Determination of Flavonoid Content

The flavonoid content of the extracts was determined according to aluminium chloride complex formation method followed by Hassan *et al.* [7]. A volume of 0.3 mL aliquot of each extract was diluted using 1.5 mL deionized water and mixed with 0.3 mL of 5% sodium nitrite. The reaction was kept standing for 10 minutes. Then, 0.45 mL of 10% aluminium chloride (in ethanol) was mixed with it and allowed to react for 5 minutes. A volume of 0.6 mL of 1 M sodium hydroxide was mixed with the solution, then the absorbance of each sample was measured at 510 nm using PerkinElemer Lambda 25 UV/VIS spectrometer. A triplicate set of tests were conducted for each sample extract. The total amount of phenolic compounds of each extract was reported as rutin equivalents (RE) milligram per gram.

Determination of Antioxidant Activity

Antioxidant activity was determined by measuring the radical scavenging power of extracts against DPPH. A volume of 50 µl of each sample extract were added to 2.95 mL DPPH solution (4.5 mg DPPH in 100 mL methanol) [8],[9]. After 30 minutes, the absorbance of each sample was measured at 517 nm using PerkinElemer Lambda 25 UV/VIS spectrometer [8],[9]. A triplicate set of tests were conducted for each sample extract. The percentage of scavenging activity of DPPH radical was measured using the following equation:

$$Percentage\ Radical\ Scavenging\ Activity\ (PRSA) = \left(\frac{Abs._{blank} - Abs._{sample}}{Abs._{blank}}\right) \times 100\%$$

IV. RESULTS AND DISCUSSION

Phytochemical Screening

Oleander leaves and flowers both contained phenols, tannins, flavonoids, coumarins, phlobatannins, sterols and triterpenes, and alkaloids as shown in Table 1. The presence of phenols, flavonoids, tannins, alkaloids, sterols and triterpenes will induce the antioxidant property of the oleander leaves and flowers. Saponins and anthroquinone glycosides were absent in oleander leaves and flowers.

Table 1. Phytochemical Screening of Oleander Leaves & Flowers

Tested Compounds	Oleander Leaves	Oleander Flowers
Phenols & Tannins	+	+
Flavonoids	+	+
Coumarins	+	+
Saponins	–	–
Alkaloids	+	+
*Sterols & Triterpenes**	+	+
Anthroquinone glycosides	–	–
Phlobatannins	+	+

*Both Liberman's test and Salkowski's test for sterols and triterpenes showed a positive result.

Phenolic Content of Oleander Leaves & Flowers

The phenolic compounds were mostly extracted by methanol and water as outlined in Table 2 and illustrated in Figure 1. Petroleum ether, acetone and ethyl acetate did not extract significant amount of phenols. The polar property methanol and water enabled the solvents to extract a higher amount of phenols which are usually polar. The strength of methanol may have caused to extract less polar phenolic compounds too. The results agree with previous research

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done, that the highest phenolic compounds were extracted by alcohol and water. The standard deviation of the results obtained can be considered low, suggesting the precision of results due to low random errors associated with experimental work. The difference between the phenolic content of the leaves and flowers was not significant at $p < 0.05$. The findings agree with the findings of Mohadjerani who reported that the phenolic content of oleander flowers was greater than that of oleander leaves [6].

Table 2. Phenolic Content of Oleander Leaves & Flowers as Gallic Acid Equivalents (GAE)

Extracts	Oleander Leaves (mg GAE/ g)	Oleander Flowers (mg GAE/ g)
Petroleum ether	N.D.	N.D.
Acetone	0.19 ±0.03	0.68 ±0.03
Ethyl acetate	2.07 ±0.04	0.13 ±0.03
Methanol	4.70 ±0.15	5.94 ±0.11
Water	4.44 ±0.05	4.58 ±0.04

N.D. = Not detected

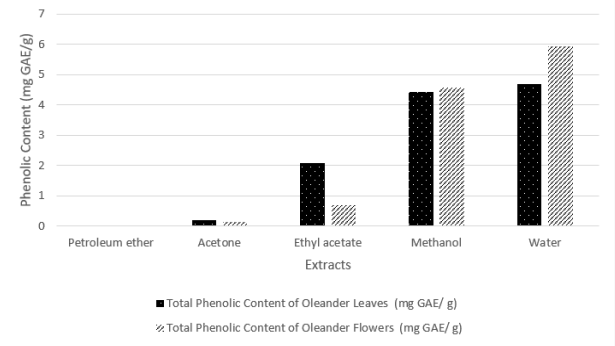

Figure 1. Phenolic Content of Oleander Leaves & Flowers as Gallic Acid Equivalents (GAE)

Flavonoid Content of Oleander Leaves & Flowers

As the phenolic compounds, flavonoids were mostly extracted by methanol and water as outlined in Table 3 and illustrated in Figure 2. The amount of flavonoids extracted by petroleum ether, acetone and ethyl acetate were not detectable. The polar property methanol and water enabled the solvents to extract a higher amount of flavonoids which are usually polar. The strength of methanol may have caused to extract less polar flavonoids compounds too.

The standard deviation of the results obtained can be considered low, suggesting the precision of results due to low random errors associated with experimental work. The difference between the flavonoid content of the leaves and flowers was not significant at $p < 0.05$.

Table 3. Flavonoid Content of Oleander Leaves & Flowers as Rutin Equivalents (RE)

Extracts	Oleander Leaves (mg RE/ g)	Oleander Flowers (mg RE/ g)
Petroleum ether	N.D.	N.D.
Acetone	N.D.	N.D.
Ethyl acetate	N.D.	N.D.
Methanol	32.87 ±0.10	40.04 ±0.09
Water	25.00 ±0.04	7.10 ±0.02

N.D. = Not detected

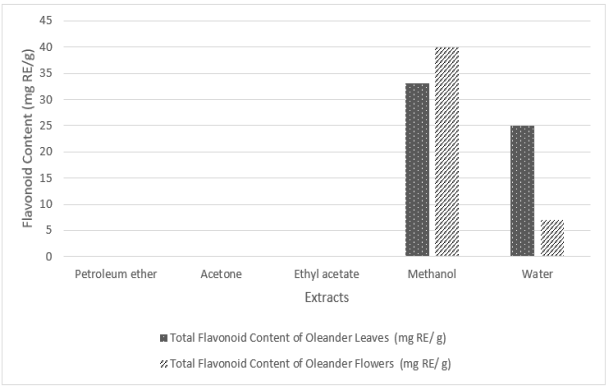

Figure 2. Flavonoid Content of Oleander Leaves & Flowers as Rutin Equivalents (RE)

Antioxidant Activity of Oleander Leaves & Flowers

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable and free radical that can be reduced to form DPPH-H. DPPH can resemble the toxic peroxide radicals, which form inside the human body as byproducts of oxygen metabolism, which can cause severe damages to body cells, tissues, and organs. As mentioned earlier, phenolic compounds have an antioxidant potential which can avoid the oxidation caused by radicals in the body by oxidizing themselves and allowing the radicals to reduce by their electrons. The percentage scavenging activity of DPPH radical by oleander leaves and flower extracts were determined by calculating the difference percentage of absorbance of a DPPH free form sample and a DPPH sample that was introduced with the respective extracts.

The alcoholic extracts (methanol extracts), followed by aqueous extracts, showed the highest antioxidant capacity compared to the other extracts as outlined in Table 4 and illustrated in Graph 3. The antioxidant power of petroleum ether, acetone and ethyl acetate extracts were not considerable as outlined in Table 4 and illustrated in Figure 3. The percentage scavenging activity of extracts can be related to the total phenolic content of each extract, as phenolic compounds are considered one of the sources of the antioxidant potential of many plants.

According to calculated values, there is an overall weak positive correlation between the total phenolic content and radical scavenging power of oleander leaves and flowers' extracts. Although phenolic compounds were absent in the petroleum ether extracts, but they showed a radical scavenging activity which can be due to the presence of non-polar terpenes, which acted as radical scavengers. The R^2-value of the graph of total phenolic content of oleander leaves and their radical scavenging power is higher than that of oleander flowers' extracts. The R^2-value of the first is 0.877, while that of the second is 0.639, the closer the value to 1 suggests a stronger relationship between the two variables.

For oleander leaves, the odd result which may have led to a less desirable R^2-value, might be that of ethyl acetate extract, which contained a desirable amount of phenolic

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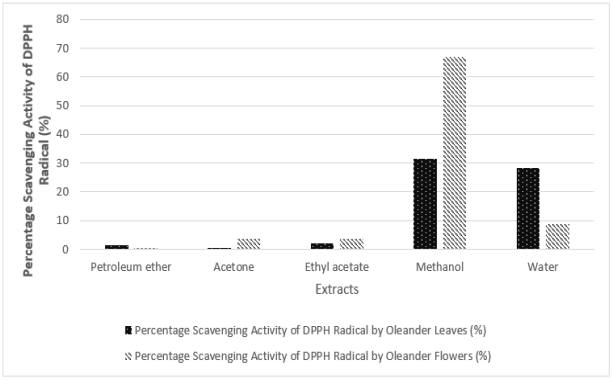
content but the extract did not show the expected radical scavenging power. For oleander flowers, the odd result which may have led to a low R^2-value, might be that of aqueous extract, which contained a desirable amount of phenolic content but the extract did not show the expected radical scavenging power. The phenolic compounds of those two extracts might have not had the power to reduce DPPH radical. For further evaluation, a different radical can be used to determine the radical scavenging potential of oleander extracts.

Table 4. Percentage Scavenging Activity of DPPH Radical by Oleander Leaves & Flowers

Extracts	Percentage Scavenging Activity of DPPH by Oleander Leaves (%)	Percentage Scavenging Activity of DPPH by Oleander Flowers (%)
Petroleum ether	1.30 ±1.33	0.48 ±0.67
Acetone	0.19 ±0.16	3.58 ±0.81
Ethyl acetate	2.07 ±1.77	3.70 ±0.23
Methanol	31.64 ±2.36	67.12 ±45.36
Water	28.15 ±0.81	8.80 ±0.87



Graph 3. Antioxidant Activity of Oleander Leaves & Flowers

V. CONCLUSION AND FUTURE SCOPE

According to the phytochemical screening done for oleander leaves and flowers, they both contained phenols, tannins, flavonoids, coumarins, phlobatannins, sterols and triterpenes, and alkaloids. The phenolic and flavonoid content of different extracts of oleander leaves and flowers varied according to the polarity of solvent. Alcoholic and aqueous extracts of oleander leaves showed relatively significant amount of phenolic and flavonoid content. The antioxidant capacity of oleander extracts was evaluated by DPPH radical scavenging potential. The alcoholic extract of oleander flowers showed the highest activity. For future work, the antioxidant power of oleander leaves and flowers may be evaluated using different radicals and methods.

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AUTHOR PROFILE

Mr. A. Ali Redha received his Bachelor of Science in Chemistry from University of Bahrain, Kingdom of Bahrain in 2018. As an undergraduate, he focused on phytochemical investigations and medicinal studies of a wide range of plants. Ali then pursed his postgraduate Masters of Science degree in Analytical Chemistry and graduated with a distinction from Loughborough University, United Kingdom in 2019. Since September 2019, Ali is a chemistry instructor at Department of Chemistry, University of Bahrain, Kingdom of Bahrain.

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