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Medicinal Plant Extracts as Potential Green Antioxidants: Their Role in Environmental and Waste Management

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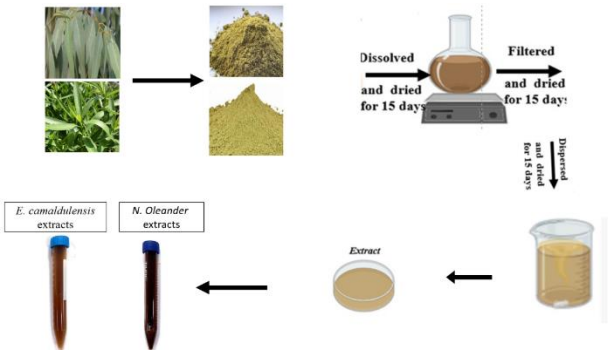
Graphical abstract



Abstract

Medicinal plants are rich in therapeutic compounds with strong potential for applications in the pharmaceutical industry. This study focused on analyzing the phytochemical composition, specifically phenols, flavonoids, and tannins, and their antioxidant properties in methanolic extracts from two selected medicinal plants: *Nerium oleander* (NO), and *Eucalyptus camaldulensis* (EC). The total contents of phenolic compounds, flavonoids, and tannins were quantified using standardized methods. Antioxidant activity was assessed through the DPPH radical scavenging assay. The analysis confirmed the presence of phenols, flavonoids, and tannins in all extracts. Notably, *E. camaldulensis* exhibited the highest concentrations of total phenolics and flavonoids (5.57 ± 0.22 mg GAE/g and 1.38 ± 0.06 mg QE/g, respectively). All extracts demonstrated strong antioxidant activity against DPPH and ABTS radicals, with IC_{50} values ranging from 0.55 to 49.43 µg/mL and 0.65 to 13.7 µg/mL, respectively. The findings suggest that these medicinal plant extracts could serve as valuable an antioxidant and anticoccidial agents for drug development.

Keywords: Extraction, pharmaceutical industry, antioxidant, biological activity, plants.

1. Introduction

The increasing interest in plant-based natural products for treating and preventing illnesses, as well as enhancing health, has attracted significant attention from both the scientific community and the general public in recent years. Medicinal plants are increasingly explored as cost-effective and low-risk sources for new drug development (Singh *et al.* 2023). Traditional plant-based medicine boasts a rich history that dates back to ancient civilizations, where plant components played a crucial role in the synthesis of medicines (Chaachouay and Zidane 2024). The rapid identification of pharmacologically active compounds from medicinal herbs has notably influenced contemporary medical practices (Fitzgerald *et al.* 2020). A diverse array of plant-derived molecules known as phytochemicals, recognized for their significant antioxidant properties, beneficial in treating various health conditions (Forni *et al.* 2019). Researchers have investigated numerous therapeutic plants for their ability to scavenge free radicals and provide antioxidant support (Jafri *et al.* 2023). Also, have identified a significant presence of reactive oxygen species (ROS) in the human body, including hydrogen peroxide (H_2O_2), molecular oxygen (O_2), and hydroxyl radicals (OH) (Madkour 2019). Approximately 5% of the oxygen inhaled by humans is converted into ROS due to univalent bonds (Kontoghiorghes and Kontoghiorghe 2019). These free radicals are implicated in various serious health conditions (Wang *et al.* 2013). To mitigate the harmful effects of these free radicals, researchers have utilized several enzymatic antioxidant defenses, including catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) (Khan *et al.* 2022). The excessive production of ROS can result from exposure to toxic substances, ultraviolet radiation, the mitochondrial electron transport chain, certain parasitic infections, and other factors.

Nerium oleander, a small evergreen shrub belonging to the Apocynaceae family, is widely recognized for its toxic properties, which limit its application in conventional medicine 9 (Balkan *et al.* 2018). Despite this, various folk medicinal practices have reported their use in treating conditions such as diabetes, rheumatism, and skin ailments (Anand 2022). Previous studies have indicated that different folk formulations of oleander flowers exhibit a range of beneficial effects, including cytotoxicity, anti-inflammatory, analgesic, antioxidant, cardioprotective, hepatoprotective, and neuroprotective properties (Balkan *et al.* 2018). Chemical analysis has revealed the presence of secondary metabolites such as cardenolides, triterpenes,

steroids, pregnanes, and flavonoids in various parts of *N. oleander* (Balkan *et al.* 2018).

Eucalyptus camaldulensis is a perennial evergreen tree from the Myrtaceae family. Known for its significant commercial and pharmacological potential, it ranks among the most widely distributed Eucalyptus species globally. Its dense wood is particularly valued for pulp production (Huang *et al.* 2022). Recent studies have highlighted the antibacterial, antioxidant, antifungal, anti-inflammatory, and anticancer properties of *E. camaldulensis* leaf extract. These effects are primarily attributed to the essential oils derived from the leaves (Saleem 2023 #1108).

The objective of this study is to assess the antioxidant activities (DPPH and ABTS) and quantify the phenolic compounds, flavonoids, and tannins present in methanolic extracts from two plants: *Nerium oleander (N. oleander),* and *Eucalyptus camaldulensis (E. camaldulensis).* Various bioanalytical methods will be employed for this analysis.

2. Manuscript Formatting

2.1. Collection of plant materials and preparation of extracts

The leaves of the herbaceous plants *Nerium oleander (N. oleander*) were collected from Riyadh garden, and *Eucalyptus camaldulensis (E. camaldulensis)* from Al-Qassim. A taxonomist from the Department of Botany at King Saud University confirmed the botanical identities of these plants. For extraction, 15 g of powdered leaves from N. oleander (NOE) and *E. camaldulensis* (ECE) were treated with methanol.

2.2. Preparation of the extract

Forty grams of the powdered materials were extracted separately with 70% methanol and placed on a shaker for three days. The resulting mixture was filtered through Whatman filter paper, and the filtrate was concentrated and dried using a rotary evaporator at 50 ºC, following Yang's method (Yang *et al.* 2016), until a thick dry substance was obtained. This powder was then dissolved in distilled water for subsequent laboratory experiments.

2.3. Chemical reagents

The following substances and standards were procured from Sigma Aldrich (St. Louis, MO, USA): DPPH, ABTS, linoleic acid, pyrogallol, gallic acid, Folin–Ciocalteu reagent, resorcinol, methyl gallate, catechin, 2-hydroxycinnamic acid, ellagic acid, quercetin, and cinnamic acid.

2.4. Total Phenolic Concentration Calculation

The total phenolic content of Lipo-Eucam was quantified using the Folin-Ciocalteu colorimetric test. In this method, 100 µL of either gallic acid or Lipo-Eucam ethanol solution was placed in 2 mL Eppendorf tubes. Then, 200 µL of a 10% Folin-Ciocalteu reagent was added, with distilled water used as a blank. After a brief vortexing, 800 µL of 0.7 M Na_2CO_3 solution was introduced, and the mixtures were incubated in the dark for two hours. The absorbance was measured at 765 nm, and a standard curve was created using absorbance values for gallic acid (0–0.1 mg/mL), enabling the determination of total phenolic content in Lipo-Eucam.

2.5. Total Flavonoid Concentration Calculation

The total flavonoid concentration was assessed using the aluminum chloride colorimetric assay. In this procedure, various concentrations of Lipo-Eucam or quercetin (30 µL) were mixed in 2 mL Eppendorf tubes with 160 µL of methanol. After adding 30 µL of freshly prepared 10% aluminum chloride in methanol and thorough mixing, 850 µL of distilled water and 30 µL of a 1 M sodium acetate solution were added. Following a vertexing step, the solutions were allowed to rest at room temperature for half an hour before measuring absorbance at 415 nm. The total flavonoid concentration was expressed as mg QE/g DW (milligrams of quercetin equivalent per gram of Lipo-Eucam).

2.6. Total Tannin Concentration Calculation

To determine the total tannin content (TTC) of the plant material, 0.1 mL of the extracted sample was combined with 1.5 mL of Milli-Q water and 1 mL of diluted Folin-Ciocalteu's reagent. After adding 0.8 mL of a 7.5% $NaHCO_3$ solution, the mixture was incubated at 45°C for 45 minutes. The components were mixed well and stored in a dark environment at room temperature for 20 minutes before measuring absorbance at a wavelength of 700 nm. The total tannin concentration was reported as mg TAE/g DW.

2.7. DPPH Radical Scavenging Assay

The free radical scavenging activity of the extracts was evaluated using the DPPH radical scavenging assay, following methodologies established by (Bandonienė 2002 #1109). This assay assesses the ability of plant extracts to donate hydrogen atoms by decolorizing a methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl). A violet/purple color indicates DPPH presence, which fades to yellow upon interaction with antioxidants. A solution containing 0.1 mM DPPH in methanol was prepared and mixed with varying doses (12.5-150 µg/mL) of extract in methanol (2.4 mL DPPH solution combined with 1.6 mL extract). The reaction mixture was vortexed thoroughly and kept in darkness at room temperature for 30 minutes before measuring absorbance at 517 nm using a spectrophotometer; BHT served as a reference compound.

The percentage DPPH radical scavenging activity was calculated using:

$$\%\text{DPPH radical scavenging activity}=A_0\ (A_0-A_1) \times 100$$

where *A0* represents absorbance of the control and *A1* is that of the extract/standard. The percentage inhibition versus concentration graph allowed for IC_{50} calculation; experiments were repeated three times at each concentration.

2.8. ABTS Radical Cation Decolorization Assay

In this assay, color loss is measured when an antioxidant interacts with ABTS+ radical cations to convert them into ABTS and decolorize them. Kut *et al.*'s method outlines how to assess antioxidant activity through this process (Kut 2022 #1110). ABTS radical cations are generated using potassium persulfate (2.45 mM) combined with a water stock solution of ABTS (7 mM). The working solution is prepared by mixing equal parts of both stock solutions and incubating them for 16 hours at 25°C in darkness before

dilution with methanol to achieve an absorbance reading between 0.70 ± 0.2 units at 734 nm via spectrophotometry. Each experiment utilized fresh solvent with Trolox as an antioxidant standard; its calibration curve included concentrations ranging from 0 to 500 µM. In test tubes, diluted samples (1 mL) were mixed with an equal volume of ABTS+ radical cation solution; absorbance was measured after seven minutes at 734 nm to compute TEAC levels expressed as Trolox equivalents (in µM).

2.9. Statistical Analysis

The independent samples t-test was used to analyze the samples, the means and standard errors were computed respectively and the significance value ($p \leq 0.5$) was calculated.

3. Result

Table 1 displays the findings of the phytochemical study performed on the methanolic extracts of two medicinal plants (*N. leander and E. camaldulensis*). All plant extracts were found to include tannins, flavonoids, and phenols. Except for anthraquinone, all phytochemical components were present in the extract. Furthermore, saponins were found in every plant with the exception (**Table 1**).

Table 1. Phytochemicals in the methanolic extracts of two therapeutic plants.

Phytochemical groups	Plant extracts	
	N. oleander	***E. camaldulensis***
Phenols	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Triterpenes	+	–
Steroids	+	+
Anthraquinone	–	+
Alkaloids	+	+

+: presence of phytochemicals, –: absence of phytochemicals.

Total Phenolic, Flavonoid, and Tannin Contents

Table 2 displays the quantities of phenolic, flavonoid, and tannin contents found in medicinal plants. The extracts from *E. camaldulensis* exhibited the highest levels of total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC), measuring 63.56±0.55 mg GAE/g, 37.56±0.11 mg QE/g, and 34.80±1.7 mg GAE/g. In contrast, *N. oleander* extracts showed the lowest TFC and TTC values at 26.45±0.13 mg QE/g and 12.98±0.6 mg GAE/g, respectively (**Figure 1**).

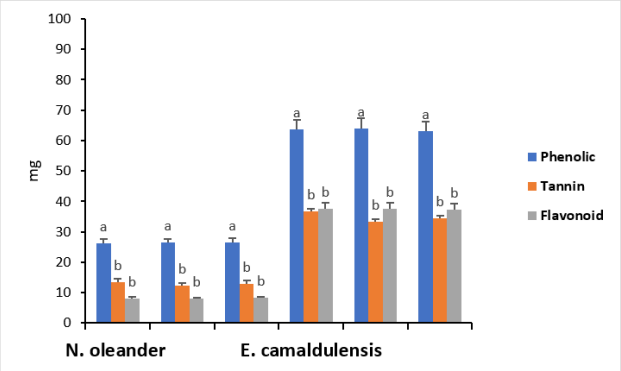


Figure 1. Total phenolic, flavonoid, and tannin concentrations in both *N. oleander and E. camaldulensis* extracts, (n = 3). Different superscript letters (a, b) indicate a significant difference at $p < 0.05$.

The total phenolic, flavonoid, and tannin content were quantified using regression equations derived from the calibration curve. The phenolic compounds present in the methanolic leaf extract were calculated from the calibration curve equation ($y = 11.44x + 21.835$, $R^2 = 0.8066$) and expressed in terms of gallic acid equivalents (GAE). The results indicated contents of 26.45 ± 0.13, and 63.56 ± 0.55 for the respective compounds (**Figure 2**).

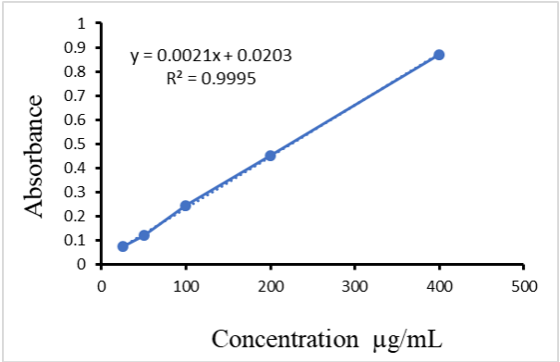


Figure 2. illustrates the standard calibration curve for the total phenol content of standard gallic acid.

The concentration of flavonoids (mg/g) in the methanolic leaf extract, expressed in chrysin equivalents, was determined using the regression equation from the calibration curve ($y = 4.611x + 6.795$, $R^2 = 0.1677$). The results were as follows: 8.13 ± 0.11 and 37.56 ± 0.11, respectively. **Figure 3** illustrates the standard calibration curves for chrysin.

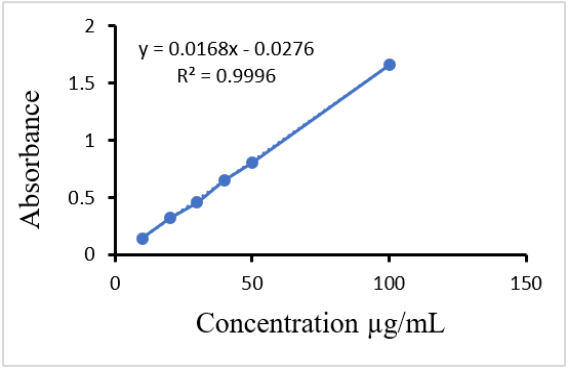


Figure 3. the standard calibration curve for the total flavonoid content for standard chrysin.

The concentration of tannins (mg/g) in the methanolic leaf extract was calculated in chrysin equivalents using the regression equation derived from the calibration curve (y = 0.0158x - 0.0266, R^2 = 0.9894). The figure depicts the standard calibration curves for chrysin (**Figure 4**).

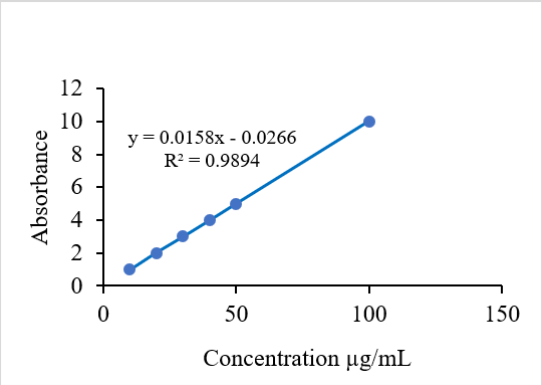


Figure 4. the standard calibration curve for the total tannins content of standard chrysin.

The antioxidant activity of methanolic leaf extracts from two plant species *N. oleander*, and *E. camaldulensis,* were assessed using the DPPH and ABTS radical scavenging assays. Results indicated that the scavenging ability against DPPH radicals increased with higher concentrations of the leaf extracts. This enhancement is attributed to the presence of phenolic compounds, including polyphenols, flavonoids, tannins, and phenolic terpenes, which are known to contribute significantly to the antioxidant effects of plant materials. Similarly, the ABTS radical scavenging activity also showed a positive correlation with the concentration of the methanolic extracts, with higher concentrations exhibiting greater scavenging activity (**Figures 5, 6**).

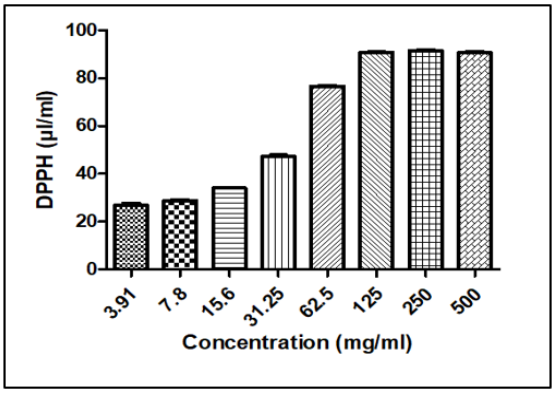


Figure 5. DPPH estimation in N. oleander extracts: the presented data are the mean values derived from three replicates ± SD.

Figures 7 and 8 present the IC_{50} values, indicating the extract concentration required for 50% radical inhibition of DPPH and ABTS radicals. A lower IC_{50} value indicates a higher antioxidant capacity. The findings illustrated that *E. camaldulensis* exhibited the highest scavenging activity with an IC_{50} value of 76.286 ± 0.22 mg/mL, and *N. oleander* (60.814 ± 0.37 mg/mL). The significantly lower IC_{50} value for *E. camaldulensis* ($p < 0.05$) suggests it possesses a greater concentration of antioxidants compared to *N. oleander* (**Figures 7, 8**).

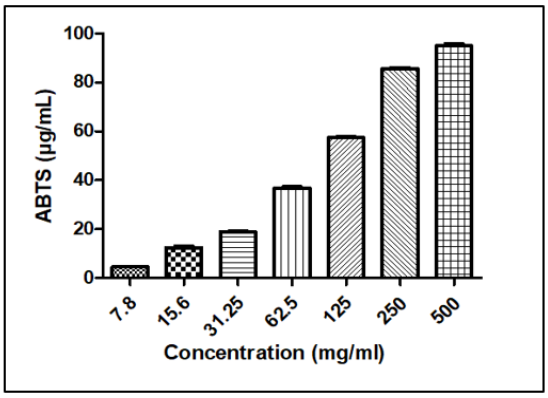


Figure 6. ABTS estimation in N. oleander extracts: the presented data are the mean values derived from three replicates ± SD.

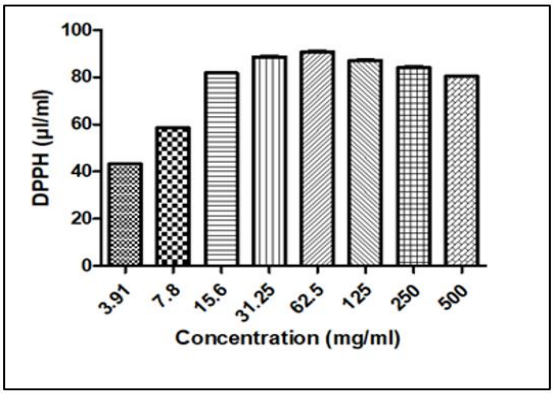


Figure 7. Concentration of DPPH in *E. camaldulensis* extracts: the presented data are the mean values derived from three replicates ± SD.

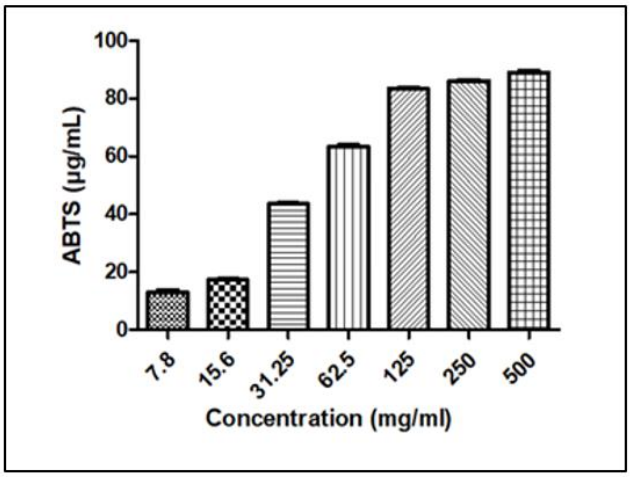


Figure 8. Concentration of ABTS in *E. camaldulensis* extracts: the presented data are the mean values derived from three replicates ± SD.

The extracts *E. camaldulensis* presented the highest DPPH (76.286 ± 0.22 and 67.426 ± 0.46, respectively). While the extracts of *N. oleander* presented the lowest in DPPH and ABTS (60.814 ± 0.37 and 44.340 ± 0.37, respectively), (**Table 2**).

To assess the antioxidant potential of the plant extracts, we analyzed the IC_{50} curve in relation to the positive control. Overall, the IC_{50} values for the methanolic extracts of *N. oleander* and *E. camaldulensis* showed an increase as the concentrations ranged from 3.91 µg/mL to 500 µg/mL (**Figures 9 and 10**).

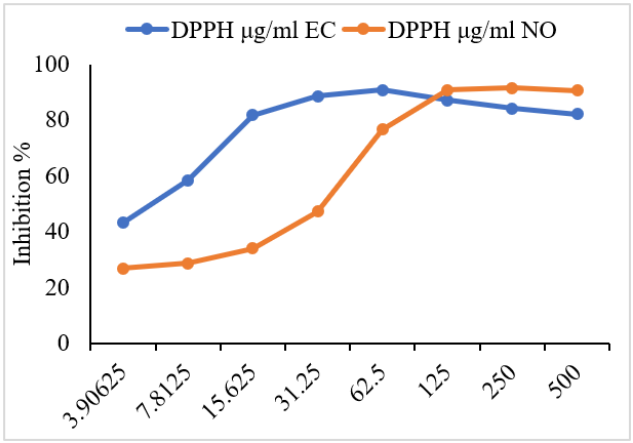


Figure 9. DPPH scavenging activity of curry *N. oleander* and *E. camaldulensis* methanolic extracts compared with standard.

The antioxidant effects of plant extraction are mostly due to the radical-scavenging abilities of phenolic components. It was found that the IC_{50} values for the *N. oleander* and *E. camaldulensis* were different from one extract to another. The *N. oleander* extract had the highest CI_{50} values in DPPH (32.0982 ± 13.098 µg/mL) and ABTS (111.531 ± 6.01681 µg/mL). The *E. camaldulensis* extract had the highest CI_{50} values in DPPH (8.9209 ± 0.0908 µg/mL) and ABTS (38.6515 ± 1.5697 µg/mL) (**Table 3**).

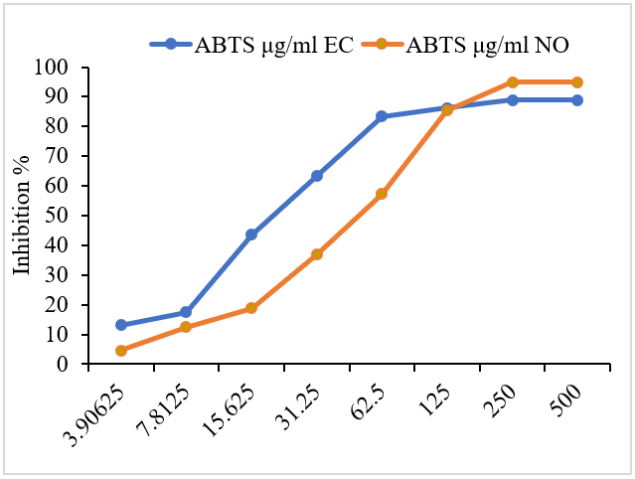


Figure 10. ABTS scavenging activity of curry *N. oleander* and *E. camaldulensis* extracts compared with standard.

Table 2. Antioxidant activity of concentrations with DPPH and ABTS assay of *N. oleander,* and *E. camaldulensis.*

Extracts	Inhibition%	
	DPPH (µg/mL)	ABTS(µg/mL)
N.oleander	60.814± 0.37[b]	44.340 ± 0.37[b]
E. camaldulensis	76.286 ± 0.22[a]	56.529 ± 0.37[a]

Table 3. The IC_{50} values for DPPH and ABTS assays to *N. oleander,* and *E. camaldulensis.*

Extracts	IC_{50} (µg/mL)	
	DPPH	ABTS
N. oleander	32.0982 ± 13.098	111.531 ± 6.01681
E. camaldulensis	8.9209 ± 0.0908	38.6515 ± 1.5697

4. Discussion

The phytochemical analysis revealed that both *N. oleander* and *E. camaldulensis* methanolic extracts contain a diverse array of secondary metabolites, including phenols, flavonoids, tannins, saponins, steroids, and alkaloids. Notably, *E. camaldulensis* uniquely contained anthraquinones, while *N. oleander* exhibited triterpenes. These compounds are well-documented for their biological activities, particularly antioxidant properties, aligning with the observed results (Bhatti *et al.* 2022). Antioxidant capacity is often a key criterion for evaluating bioactive and functional components (Kurek *et al.* 2022). Phenolic and flavonoid compounds are particularly significant due to their diverse biological activities (Karak 2019).

The quantitative analysis demonstrated significantly higher TPC (63.56 vs. 26.45 mg GAE/g), flavonoid (TFC: 37.56 vs. 8.13 mg QE/g), and TTC (34.80 vs. 12.98 mg GAE/g) contents in *E. camaldulensis* compared to *N. oleander*. Phenolic compounds, including tannins and flavonoids, are potent antioxidants due to their ability to donate hydrogen electrons, neutralizing free radicals, as previously reported by (Bakir Çilesizoğlu 2022; Rosendal 2020). The superior antioxidant DPPH activity in both *E. camaldulensis* (IC_{50}: 8.92 µg/mL and ABTS IC_{50}: 38.65 µg/mL) compared to *N. oleander* (IC_{50}: 32.10 µg/mL and ABTS IC_{50}: 111.53 µg/mL), strongly correlates with its higher phenolic content. This aligns with established literature linking phenolic-rich extracts to enhanced radical scavenging capacity, supporting findings from (Kumar 2017; Abdul-Sahib 2023).

Although *E. camaldulensis* lacks triterpenes, its antioxidant performance remained high, highlighting the dominant role of phenolics. The presence of anthraquinones may have also contributed to this effect. The significantly lower IC_{50} values ($p < 0.05$) for *E. camaldulensis* underscore its potential as a natural antioxidant, surpassing *N. oleander* in potency. These findings align with studies (Mani 2021; Ouattara 2024), *E. camaldulensis* species as rich reservoirs of bioactive phenolics.

The results indicate that phenolic compounds are primarily responsible for antioxidant activity, followed by tannins and flavonoids. Additionally, the reducing power activities and hydrogen peroxide scavenging capabilities of *E. camaldulensis* extract showed a positive correlation with phenolic content and tannins at varying significance levels (Nasr *et al.* 2019). A study (Sani *et al.* 2014) reported that *E. camaldulensis* leaves, stem-barks, fruits, seeds, and roots yielded substantial amounts of phenols in methanol extracts (Sani *et al.* 2014). The antioxidant properties of *E. camaldulensis* position it as a viable candidate for food, cosmetic, and nutraceutical industries (Syukri 2024; Sánchez-Loredo 2024; Mahmoud Dogara 2024).

The production of plant-based antioxidants or anticoccidial agents necessitates efficient waste management systems, particularly in the pharmaceutical and agricultural sectors. The antioxidant properties of *E. camaldulensis* position it as a viable candidate for the food, cosmetic, and nutraceutical industries. Recent advancements in AI provide robust frameworks for this purpose. Gandhimathi *et al.* (2024) proposed biomedical waste classification through deep learning, optimizing safer disposal. Similarly, Madeshwaren *et al.* (2025) proposed an ecowaste framework to enhance the accuracy and sustainability of urban biomedical waste management. Furthermore, Vairavel *et al.* (2025) employed bio-composites to transform waste from extraction processes into raw materials, thereby completing the economic cycle. Future research should explore synergies between phytochemical profiling, material science, and AI to develop integrated, sustainable systems. Through the chemical analysis of the plants and the regional specificity of Saudi Arabia, the study moves from a general phytochemical analysis to a significant contribution that highlights the outstanding value of Saudi plant resources.

5. Conclusions

The findings suggest that phytochemical analysis revealed significant bioactive compounds, including phenols, flavonoids, and tannins, in the methanolic extracts of *N. oleander and E. camaldulensis. Notably, E. camaldulensis* exhibited higher total phenolic, flavonoid, and tannin contents than *N. oleander*. The identified compounds showed a strong correlation with antioxidant activity, as confirmed by DPPH and ABTS assays. *E. camaldulensis* showed superior radical scavenging capacity, with significantly lower values for DPPH and ABTS than N. oleander, indicating its stronger antioxidant potential. The *E. camaldulensis* is a more promising source of natural antioxidants, likely due to its richer phytochemical profile. Overall, both plants demonstrate medicinal value, with E. camaldulensis standing out for its antioxidant efficacy. This study provides the first new characterization of the unique phenolic signatures *of N. oleander and E. camaldulensis* in the Saudi desert environment, offering valuable insights for science, local applications, and environmental conservation.

Ethics approval and consent to participate

Not applicable for that section.

Consent for publication

The authors declare that there are no conflicts of interest.

Availability of data and material

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

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