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PHARMACOGNOSTIC, ANATOMICAL, AND ANTIMICROBIAL EVALUATION OF *NERIUM OLEANDER* (APOCYNACEAE) LEAF EXTRACT

M.M. ALJAF, S.A.R. HASAN, and M.A.H. AL-HADEETHI*

Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq
*Corresponding author's email: muazaz.a.h@ihcoedu.uobaghdad.edu.iq
Email addresses of co-authors: muhannad.m.a@ihcoedu.uobaghdad.edu.iq, sazan.a.h@ihcoedu.uobaghdad.edu.iq

SUMMARY

Nerium oleander L. (Apocynaceae), commonly known as oleander and rosebay, is a shrub cultivated worldwide in temperate and subtropical areas as an ornamental and landscaping plant. Oleander has a wide range of both internal and external medicinal uses. In the epidermis section, the stomata scattering on the lower surface is the type called unifacial, wherein the stomatal type is sunken. Distinguishing it is difficult in the surface view because of the epidermis and cuticle layer thickness. However, one can distinguish it in the cross-section of the leaf, which is an important taxonomic characteristic that separates this species from other species in the genus. The oleander plants varied in the cross-sectional shapes of their stems. Wild oleander plants showed higher contents of phenols, tannins, cardiac glycosides, and saponins than cultivated plants. Leaf extract evaluation serves as inhibitory agents for selected Gram-negative (*Acinetobacter baumannii* and *Escherichia coli*) and Gram-positive (*Staphylococcus aureus* and *Streptococcus faecalis*) pathogenic bacteria isolates. In *N. oleander*, the antibacterial efficacy of ethanolic and aqueous leaf extracts differed among bacterial genera. Both aqueous and ethanolic leaf extracts were effective against *E. coli*; however, they did not show any inhibitory activity against other bacterial strains, such as *A. baumannii*, *S. aureus*, and *St. faecalis*.

Keywords: Anatomical evaluation, antibacterial activity, Apocynaceae, aqueous and alcoholic leaf extract, oleander

Key findings: The latest research, comprising pharmacognostic and anatomy of the oleander (*N. oleander* L.), will help authenticate information about its medicinal values and species identification. Additionally, antibacterial activity investigation of *N. oleander* leaf extract against some pathogenic bacteria genera has also been successful.

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INTRODUCTION

Oleander (*Nerium oleander* L.) is the only species classified in the genus *Nerium*, belonging to the subfamily Apocynoideae of the family Apocynaceae. It is a highly poisonous plant with a wide range of cultivation that no precise region of origin has connected with it. Although its association is usually with the Mediterranean Basin, it extends to China, as well as Iraq, being grown everywhere and in every environment there (Shakya, 2016). The oleander plant flowers are multi-colored (white, red, pink, yellow, and orange), with no characteristic odors, and appear during March to April. Oleander is the plant of the low-lying, warm regions of the Middle East, characterized by its robust tolerance to weather fluctuations, drought, heat, and limited water. All parts of the plant are poisonous, primarily the stem and roots; hence, the need to warn children about it (Khare, 2004). In the Arabic world, the oleander has various strange names in different regions, such as “donkey's ear,” “donkey's rose,” and “false rose” (Zhao *et al.,* 2007).

Oleander is an evergreen shrub with many branches, reaching a plant height of about four meters. Its leaves are sharp lanceolate, reaching a length of about 10 cm, similar to almond leaves but longer and thicker. A child who chews one leaf could die, as children usually eat everything their fingers take hold of. Meanwhile, adults may die if they chew about 10 leaves. It is worth noting that most toxic components of this shrub are milky liquid that could come out when cutting any part of this shrub. The said shrub has elegant flowers with an elegant aroma of red, white, and crimson abounding in the Levant and some in Arab Gulf countries (Al-Snafi *et al.,* 2019).

Oleander, found in the coastal mountains, occurs wild; however, it has gained extensive growth as an ornamental plant, especially in parks and public streets. For medicinal purposes, the collection of leaves and other parts of the shrub happens in the fall before being dried. Noteworthy, these dried plant parts retain their high toxicity even after drying (Nagourney *et al.,* 2001). The leaves and peel contain cardiac glycosides, the most important of which are oleandrin, adilnerin, nirianthin, and nirin. These all belong to the steroid glycosides group, where the only glycoside with heart-strengthening qualities is rutin. The oleander seeds also contain unstudied glycosides and fixed oil. Past studies revealed the leaves contain the alkaloid curarin and flavonoid glycosides (Ali *et al.,* 2010).

The oleander leaf components are similar to those of digitalis leaves, which are used in treating heart diseases to strengthen the muscles and regulate the heartbeat. Thus, similar to digital preparation, their usual uses regulate heart rhythm and strengthen muscles. However, these components have a faster effect and weak cumulative properties and could also serve in cases of asystole accompanied by kidney failure (Redha, 2020). After diluting the leaf juice with water, they can be applicable as a medicine for controlling itching, sneezing, and scabies, as well as killing mice (Durhan *et al.,* 2022).

Oleander plant extracts are simple and eco-friendly antibacterial agents with considerable ecological advantages and highlight a compelling area for research. Green antimicrobial agents showed greater potential for ecological benefits because of the insufficient heavy metals and other harmful substances (Al-Obaidi, 2014; Shafiq *et al*., 2021). Numerous pharmaceutical companies have the opportunity to develop new antimicrobial formulations sourced from plants. Plant bioactive molecules act as secondary metabolites and defense mechanisms against herbivores, fungal attacks, microbial invasions, and viral infections. In the last decade, powerful agents have emerged to fight viral diseases. As a result, oleander plant extracts emerged to be rich in phytochemicals, with an increasing recognition as promising sources of viral inhibitors (Makia, 2017; Shafiq *et al*., 2021).

Identifying *Acinetobacter baumannii* as a key ESKAPE pathogen has become a priority for the World Health Organization (WHO) for the research and development of new treatments, owing to its extraordinary persistence and drug resistance (Scoffone *et al*., 2025). The *E. coli* usually lives in warm-blooded mammals. It is an essential indicator of fecal coliform contamination, as it is the most probable cause of urinary tract infections, known as uropathogenic *E. coli* (Hasan *et al*., 2021). It is the most common causative agent for uncomplicated and complicated urinary tract infections, as coliforms are typically effective as indicators of microbial water contamination (Hasan and Shakir, 2025).

Nosocomial pathogens, such as *Staphylococcus aureus* and *Streptococcus faecalis*, along with opportunistic organisms, proved to be linked to biofilm infections, including endocarditis, urinary tract infections, and chronic wound infections (Ch’ng *et al*., 2022). The presented research aimed to investigate the medical profile and plant anatomy of the oleander (*N. oleander* L.) that will help in authenticating information about its medicinal values and species identification. Additionally, the study sought to assess the antibacterial activity of *N. oleander* leaf extracts against some pathogenic bacteria genera.

MATERIALS AND METHODS

Collected plants

The collected oleander (*N. oleander* L.) wild plants came from the Goby-Qara Dagh, AL-Sulaymaniyah, Kurdistan Region, Iraq (between the latitude 35° 16.5740′ N and longitude 45° 21.2920′ E). The cultivated oleander plants were collections from the Herbal Garden, Ibn-AL-Haitham, University of Baghdad, Baghdad, Iraq.

Epidermis preparation

The scraping of the oleander leaf epidermis used a sharp dissecting lancet. After the scraping process, the sample underwent distilled water washing before being placed in a solution of sodium hypochlorite at 7% concentration for 10 min. Afterward, its washing with distilled water preceded its transfer to KOH 10% for 10–15 min, then replacing the solution with 70% ethyl alcohol for 10–15 min. Examination commenced with a KRÜSS compound microscope, followed by imaging done with an Am Scope (Model MU 1000) microscope-mounted camera, following the methodology of Al-Khazraji and Aziz (1989), with some modifications according to Al-Hadeethi *et al.* (2020).

Cross section of stem

The transverse sections’ preparation of the oleander stem proceeded by hand sectioning (Khazraji and Aziz, 1989). The samples’ cutting into small pieces preceded their placement in 1% hypochlorite AL sodium for 10 min to remove chlorophyll. Then placing the unstained sections on a slide with a cover slide continued. The samples’ examination under the microscope ensued being photographed with the camera.

Phytochemical compounds

For studying the phytochemical compounds following Kumar *et al.* (2023), the oleander leaves and flowers entailed drying in the oven at 40 °C for 48 h before crushing them into powder and extracting them by the maceration method using water. Then, taking 5 g of powder, the extraction continued in 100 mL of water in a shaking incubator for 15 h at room temperature, with the specimen filtered in Whatman No. 1 filter paper. Phytochemical analysis of the *N. oleander* leaf and flower extracts succeeded in finding the concentration of phenols, cardiac glycosides, saponins, and tannins.

Determination of phenolic contents

The adding of 2.0 mL of Na_2CO_3 (75%) to the extract preceded its incubation at 50 °C for 10 min. Afterward, when the sample was cool, the estimation of absorption used a Shimazu UV-1800 spectrophotometer at 765 nm against a blank without extract. Expressing the data of the outputs was as mg/g of gallic acid equals in mg per g [mg GAE/g] of dehydrated extract by the Folin-Ciocalteu reagent (Lee *et al.,* 2015).

Cardiac glycoside test

The cardiac glycoside extracts' determination employed the Keller-Killiani test. Adding 1 mL of acetic acid and two drops of ferric chloride to 2 mL of the oleander extract preceded the adding of 2 mL of concentrated sulfuric acid. The color change to reddish-brown is indicative of a positive test for cardiac glycosides (Archana *et al.,* 2012; Sati and Kumar, 2015).

Tannin test

When adding a few drops of lead acetate to 1 mL of oleander extract resulted in a huge white-brown precipitate appearance, it was a positive test for tannin (Archana *et al.*, 2012).

Saponin test

The 5 mL of distilled water added to 1 mL of oleander extract undergoes shaking for 14 min. The appearance of a froth column that did not disappear with the addition of HCl indicates a positive result for saponin (Savithramma *et al.,* 2011; Sati and Kumar*,* 2015).

Leaf extract preparation

The earlier obtained *Nerium oleander* leaves, after washing to remove dust and unwanted material, underwent drying of the leaves. Then, finely grinding the leaves continued before keeping it at laboratory temperature. The preparation of the aqueous extract (Ms) followed by placing 40 g of *N. oleander* ground powder of leaves in a flask (filled with 200 mL distilled water) and stirring with a magnetic blender (30 min) before centrifugation for 15 min. After centrifugation, the solution remained in an electric furnace at 35 °C, with the solutions prepared at 25%, 50%, 75%, and 100% mg/mL concentration**.** For further use, the extract with different ratios entailed storage at 4 °C.

Alcohol extract (Ks) resulted from putting 50 g of *N. oleander* mashed leaves in an extraction unit (Soxhlet), adding 350 mL of ethanol (80%), and heating to 40 °C. Then, a vacuum rotary evaporator operation ran for 12 h at 35 °C. Afterward, aqueous extract solutions followed (Al-Obaidi, 2014).

Pathogenic bacteria

Acquiring four bacterial genera, *Acinetobacter baumannii*, *Escherichia coli* (G-ve), *Staphylococcus aureus*, and *Streptococcus faecalis* (G+ve), the strain source was the University of Baghdad, Baghdad, Iraq. Their use helped evaluate the antibacterial activity of oleander *(N. oleander* L.) leaf extract. Bacterial proliferation was evident at 37 °C in peptone water and Mueller-Hinton agar media.

Antimicrobial test

Four to five selected colonies of bacteria emerged by mixing in sterile distilled water to create bacterial suspensions until the turbidity resembled McFarland 0.5 standards. The suspensions attained subsequent dilutions in sterile tubes to achieve a final concentration of 10^7. A distribution of 100 µL volume of the inoculum was successful onto Petri dishes with 20 mL of Mueller-Hinton agar before being incubated for 5 min (Mohammed *et al*., 2019).

The activity against pathogenic bacteria incurred assays in the agar-well diffusion method (ADM), suggested by the Kirby-Bauer method. The use of the said methodology measured the sensitivity of four bacterial genera, viz., *E. coli*, *A. baumannii*, *S. aureus*, and *St. faecalis* bacteria. The Mueller-Hinton agar usage tested the sensitivity of bacteria toward the oleander leaf extract. The cultured bacteria reached incubation at 37 °C for 24 h, with the diameter of inhibition measured after (Al-Kalifawi *et al*., 2015; Sadiq and Yildirim, 2024).

RESULTS AND DISCUSSION

The oleander (*Nerium oleander* L.) plant shape with different colors of flowers appears in Figure 1. The measured quantitative characteristics of the stomata are available in Table 1. In all the variety of oleander plants, the epidermis, in surface view, consisted of

Figure 1. Flower shapes of the cross section of stem in the oleander plants, E: epidermis, L: lenticels, C: cortex, V: vascular bundles, and P: pith.

Table 1. Quantitative characteristics measured (micrometers) of the stem cells in the oleander plants.

Sample	Cuticle thickness	Epidermis thickness	Cortex thickness	Vascular bundle thickness
Wild	5.2-5.8 (5.4)	45.5-50.2 (48.6)	110.2-116.1 (114.5)	213.1-223.6 (220.5)
Cultivated white rose	4.5-4.7 (4.6)	35.1-40.5 (37.3)	95.4-103.5 (101.3)	175.6-183.4 (179.5)
Cultivated red rose	4.1-4.6 (4.3)	33.1-38.4 (36.5)	88.5-97.3 (95.4)	155.9-169.3 (166.8)

ordinary epidermal cells almost straight on the upper and lower surfaces. The stomata appeared on the lower surface only, with this type of stomata called unifacial and the sunken type (Figure 2), which is the distinctive feature. Zhao *et al.* (2007) reported the distinguished features of stomata in the stems and twigs of *N. oleander*; however, one cannot differentiate them in the service view due to the thickness of the epidermis and cuticle layer that covers the adaxial and abaxial epidermises, but they are distinguishable in the cross section of the leaf (Figure 2).

In the oleander wild species, the measured length and width of stomata at the lower epidermis of oleander wild species were higher than the cultivated species. The stomatal index revealed the number of stomata, wherein the lower epidermis of wild species recorded a higher stomatal index than the cultivated species. Al-Khazraji and Aziz's (1989) studies revealed this was probably due to the differences in abundance of water and the existing environmental conditions.

The transverse section of the oleander leaf blade included the upper and lower epidermis, palisade, and spongy layers, with the trichomes distributed in the upper and lower epidermis and the druse crystals scattered in the leaf mesophyll. A difference in tissue thickness was also evident among the studied wild and cultivated species (Table 2, Figure 2), similar to Ataşlar's (2004) investigations on the *Saponaria kotschyi*. This can refer to the differences in the plant's environment and other factors affecting the

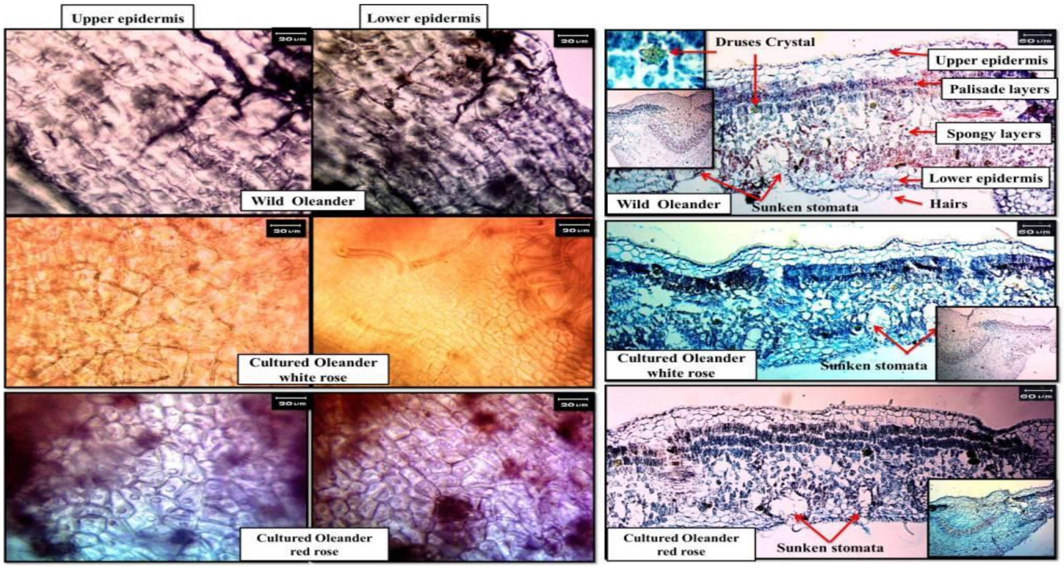

Figure 2. Surface view appearances of the shape and type of stomata in the epidermis of leaves and the cross section of leaf blade and leaf midrib in the oleander plants.

Table 2. Quantitative characteristics measured (micrometers) of the stomata cells in the oleander plants.

Sample	Upper epidermis		Stomatal index	Lower epidermis		Stomatal index
	Stomata length	Stomata width		Stomata length	Stomata width	
Wild	-	-	-	53.1-59.3 (58.5)	37.6-43.2 (41.5)	20
Cultivated white rose	-	-	-	48.3-54.4 (52.5)	37.5-44.3 (42.1)	25
Cultivated red rose	-	-	-	43.1-49.6 (47.5)	36.1-41.2 (37.5)	25

plants. Likewise, Figure 2, showing the cross section of the midrib, denotes the shape of vascular bundles in the species that appeared crescent-shaped, located in the middle of the cortex, differing among the plants in their thickness (Table 2). Branislava *et al.* (2007) also reported the same anatomical features of the leaf appearance, with some hairs and four layers of epidermal cells and druse crystals. These features were xeromorphic and imply that such types of plants prevail in dry places.

The oleander stem cross-section showed differences in the stem's characteristics among wild, red-flowered, and white-flowered plants, and some variations were greatly significant for taxonomic evaluation in isolating and diagnosing these species (Table 3, Figure 1). The results revealed oleander plants varied in the cross-sectional shapes of the stems. The wild-collected plants had semi-circular stems, while the cultivated plants with white flowers had heart-shaped stems, and the cultivated oleander plants with red flowers had circular stems. The epidermis layer comprised a single row of oval-shaped cells. The cortex represents the layer that follows the epidermis, comprising two tissues, angular collenchyma tissue, and the sections also differed in the number of rows of collenchyma tissue. The collenchyma tissue rows reached 3–5 in the wild plants, the cultivated with white rose had 3–4, and the cultivated with red rose had 2–3 rows. The vascular bundles represent the center of the stem and all appeared in the secondary growth stage. The pith was in the

Table 3. Concentrations of the biochemical compounds in the oleander plants.

a. Phenol	
Wild plants	41.53±4.40 mg
Cultivated white rose	39.57 ±3.35 mg
Cultivated red rose	40.55 ± 4.36 mg
b. Cardiac glycoside	
Wild plants	170.87 ± 0.22 mg SE/g
Cultivated white rose	125.46 ± 0.13 mg SE/g
Cultivated red rose	130.49 ± 0.15 mg SE/g
c. Tannin	
Wild plants	460.90 ± 0.40mg TE/g
Cultivated white rose	457.85 ± 0.35mg TE/g
Cultivated red rose	460.88 ± 0.38mg TE/g
d. Saponin	
Wild plants	30.44 ± 0.015mgDE/g
Cultivated white rose	25.36 ± 0.010mgDE/g
Cultivated red rose	26.39 ± 0.013mgDE/g

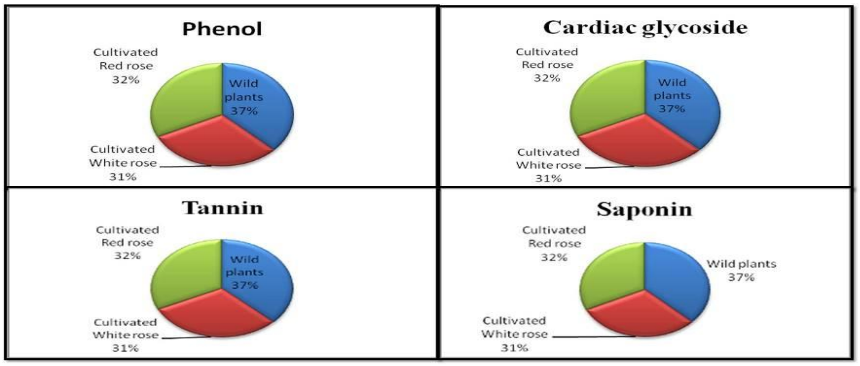

Figure 3. Diagram showing the phenols, cardiac glycoside, tannin, and saponin in the oleander plants.

center surrounded by vascular bundles, and the pith shape was triangular in the wild and white flower rose plants and elliptical in the plants with red flowers. Guidoti *et al.* (2015) also reported morpho-anatomic characterization of the stem and leaves in the *Tabernaemontana catharinensis* belonging to the same family, Apocynaceae.

The specimens obtained by water extracts of *N. oleander* leaves and flowers underwent the qualitative-phytochemical analysis. Favorable effects resulted in the appearance of phenols, cardiac glycosides, tannins, and saponins (Table 3, Figure 3). Al-Snafi (2020) and Al-Snafi *et al.'s* (2019) studies comprising analysis of the phenolic and flavonoid contents in the medicinal plants shared the importance of these bioactive compounds in treating numerous diseases.

The antibacterial activity of *N. oleander* leaf extracts against four bacteria genera, *E. coli, A. baumannii, S. aureus,* and *St. faecalis,* appears in Table 4. The oleander leaf water extracts exhibited almost no activity at the 25%, 50%, and 75% concentrations for the bacterial isolates, except for *E. coli*. The inhibition diameters were 16, 13, and 10 mm, ranked within that order, while the other three bacteria genera (*A. baumannii, S. aureus,* and *St. faecalis*) showed no inhibitory effects. The oleander leaf water extract with 100% concentration indicated a zero-diameter inhibition zone for all the tested bacteria as compared to the control (Figure 4A).

Despite the inhibitory action of the alcohol extract, this was due to the presence of flavonoids, tannins, and some phenolic compounds with a biological influence on

Table 4. Effect of aqueous and alcoholic extract of *Nerium oleander* leaves in different concentrations as inhibitors on pathogenic (G+ve and G-ve) bacteria growth.

Extract concentration	Inhibition zone diameter			
	Staphylococcus aureus	*Streptococcus faecalis*	*E. coli*	*Acinetobacter baumannii*
Ms (100%)	N.A.	N.A.	N.A.	N.A.
Ms (75%)	N.A.	N.A.	10	N.A.
Ms (50%)	N.A.	N.A.	13	N.A.
Ms (25%)	N.A.	N.A.	16	N.A.
Ks (100%)	N.A.	N.A	N.A.	N.A.
Ks (75%)	N.A.	N.A.	10	N.A.
Ks (50%)	N.A.	N.A.	12	N.A.
Ks (25%)	N.A.	N.A.	14	N.A.

Ms: aqueous extract, Ks: alcoholic extract, N.A.: No growth.

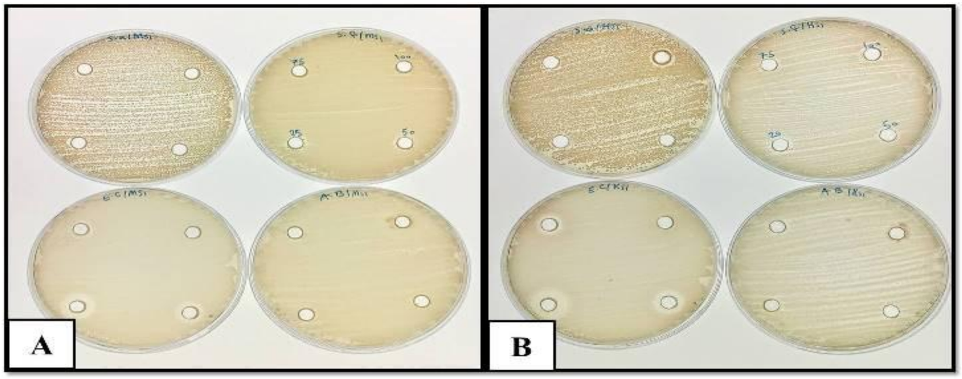

Figure 4. A: Antibacterial activity of the aqueous extract of *N. oleander* against *Escherichia coli, Acinetobacter baumanii, Staphylococcus aureus,* and *Streptococcus faecalis* at 25%, 50%, 75%, and 100% concentrations, and B: the antibacterial activity of the alcoholic extract of *N. oleander* against the same isolates (*Escherichia coli, Acinetobacter baumannii, Staphylococcus aureus,* and *Streptococcus faecalis)* at 25%, 50%, 75%, and 100% concentrations.

several bacterial strains through their hydroxyl groups (Hasoon, 2018). The alcoholic extract showed no inhibitory effect on the tested isolates at the 100% concentration; however, at the other three concentrations, only the *E. coli* exhibited this effect, measuring 14, 12, and 10 mm, respectively (Figure 4B). Phenolic compounds can coagulate bacterial cell proteins and destroy enzymes that produce essential amino acids to promote cell growth and division.

The antibacterial test of *N. oleander* revealed that both ethanolic and aqueous extracts exhibited a moderate antibacterial effect, restricted solely to Gram-positive bacteria (Mohammed *et al.*, 2019). This could refer to the presence of the cell membrane, which could be non-permeable to the tested plant extracts, restricting their penetration into the bacteria. Derwich *et al.* (2010) discovered that *N. oleander* extracts were more effective against Gram-negative bacteria (*E. coli*, and *P. aeruginosa*) than to Gram-positive bacteria (*S. aureus)*. The variation in perspective primarily arises from the solvent's capacity and polarity in extracting a wider array of active, non-polar compounds, including flavonoids, tannins, and phenols, from the plant material (Al-Manhel and Niamah, 2015).

The methanolic extract demonstrated a pronounced antibacterial effect, while the ethanolic extract exhibited moderate antibacterial activity against all the tested bacteria (Sharma *et al*., 2010). Conversely, as also reported by Namian *et al.* (2013), notable antibacterial effects of hexanic, dichloromethane, and methanolic extracts from both leaves and flower extracts occurred against *E. coli*, *E. carotovora*, *S. aureus, B. cereus,* and *B. pumilus*. These showed inhibition zones between 8 and 19 mm (Figure 4B). The varying antibacterial effects of *N. oleander* leaf extract emphasize the biochemical differences among the plant samples and the extraction solvents utilized (Mohammed *et al*., 2019).

CONCLUSIONS

The oleander (*N. oleander* L.) plants of the sunken type of stomata displayed scattering on the lower surfaces, which was unifacial. Such a type is difficult to distinguish in the surface view due to the epidermis thickness. The cross-section of the stem showed variations in stem characteristics of the wild and cultured samples, and these differences were of taxonomic importance in isolating and diagnosing these variations. The antibacterial effectiveness of ethanol (Ks) and aqueous (Ms) oleander leaf extracts revealed both extracts proved active against *E. coli*; however, they were not effective against other G+ve and G-ve bacterial isolates (*A. baumannii*, *S. aureus*, and *St. faecalis*).

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