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PHARMACOLOGICAL STUDIES OF NYCTANTHES ARBORTRISTIS AND NERIUM OLEANDER

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Nyctanthes arbortristis, Nerium oleander, Plant species, Macroscopy, Microscopy, Transverse section, Xylem, Phloem, Trichomes, Palisade cells, Bulliform cells, Collenchyma cells, Anatomical features, Morphology.

ABSTRACT

This study presents a comprehensive analysis of *Nyctanthes arbortristis* (Night-flowering Jasmine) and *Nerium oleander* (Oleander) through both macroscopic and microscopic studies. These two plants, belonging to the family Oleaceae and Apocynaceae, respectively, have garnered scientific interest due to their pharmacological and ecological significance. Macroscopic examinations encompassed morphological observations including leaf arrangement, flower structure, and overall plant architecture. Microscopic investigations delved into the anatomical features of various plant organs, such as leaf epidermis, trichomes, vascular bundles, and floral structures. The study employed light microscopy to explore cellular details and arrangement within tissues. Results revealed distinctive characteristics between the two species, aiding in taxonomical classification and providing insights into their ecological adaptations. Additionally, microscopic examinations unveiled intricate cellular structures that contribute to their diverse biological functions and potential medicinal properties. The findings of this study contribute to the botanical understanding of *Nyctanthes arbortristis* and *Nerium oleander*, shedding light on their ecological roles and pharmaceutical potential.

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INTRODUCTION

Nyctanthes arbortristis, commonly known as Night-flowering Jasmine, and *Nerium oleander*, referred to as Oleander, stand as botanical wonders with significant implications in the realm of medicine. These two plants have captured the attention of researchers and medical practitioners alike due to their profound pharmacological properties and potential therapeutic applications.

*Nyctanthes arbort*ristis, revered in traditional medicine systems for its anti-inflammatory, antipyretic and analgesic attributes, holds promise in the treatment of various ailments ranging from rheumatism to malaria[1-6]. Its traditional uses in various medicinal systems, particularly in Ayurveda, highlight its efficacy in combating microbial infections[7-9]. *Nyctanthes arbortristis* extracts have demonstrated antimicrobial activity against a range of pathogens, including bacteria, fungi, and viruses.

Research studies have reported the effectiveness of *Nyctanthes arbortristis* extracts against common pathogens such as Escherichia coli, Bacillus subtilis[10-11], Staphylococcus aureus[12], Candida albicans[13], and Herpes simplex virus[14]. These antimicrobial properties stem from the presence of bioactive compounds such as flavonoids, alkaloids and phenolic compounds found in different parts of the plant, including leaves, flowers, and stems[15]. The exploration of *Nyctanthes arbortristis'* antimicrobial potential underscores its significance in traditional medicine and its potential for future pharmaceutical applications in combating infectious diseases[16-19].

Nerium oleander is recognized for its multifaceted medicinal properties, including its potential as an anticancer agent[20-23]. Studies have revealed that *Nerium oleander* extracts contain bioactive compounds, such as oleandrin and nerioside, which exhibit cytotoxic effects against cancer cells[24,25]. These compounds have shown promise in inhibiting tumour growth and inducing apoptosis, making Nerium oleander a subject of interest in cancer research and drug development.

Furthermore, when combined with *Nyctanthes arbortristis*, *Nerium oleander* may enhance the antimicrobial efficacy[20-23] of *Nyctanthes arbortristis* extracts. This synergistic effect could result from the complementary action of bioactive compounds present in both plants, potentially leading to improved antimicrobial activity against a broader spectrum of pathogens[10,11].

The combination of *Nerium oleander* and *Nyctanthes arbortristis* represents a promising avenue for exploring novel therapeutic approaches, not only in cancer treatment but also in combating microbial infections. Further research into the synergistic interactions between these two botanical agents may unveil new insights into their medicinal potential and pave the way for the development of effective natural remedies against cancer and infectious diseases. Through centuries of exploration and contemporary scientific scrutiny, both *Nyctanthes arbortristis* and *Nerium oleander* have emerged as invaluable resources in the pursuit of novel medical treatments and pharmaceutical breakthroughs.

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MATERIALS AND METHODS

Plant Materials

The leaves of plants *Nyctanthes* and *Nerium* species were authenticated by Prof. A. Ravi Kumar, Ph.D., Nimra College of Pharmacy, Ibrahimpatnam, Vijayawada, India. They were collected from different areas of the Bapatla, Guntur, Krishna and Prakasham districts (coastal region) of Andhra Pradesh, India.

a. b.

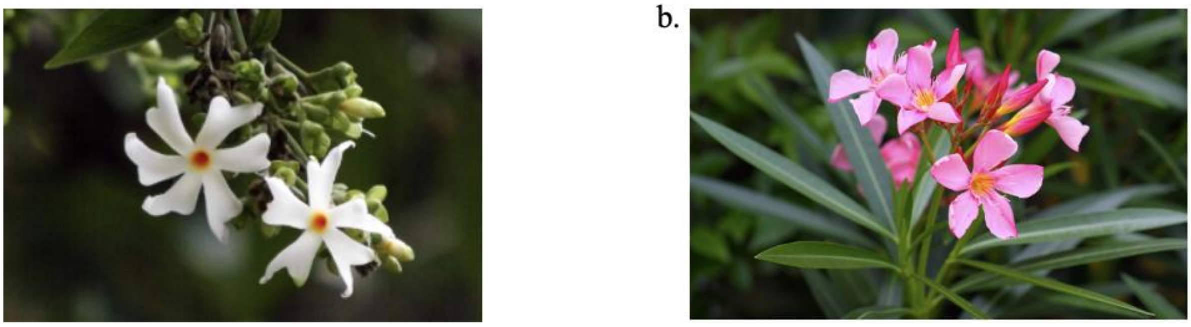

Figure 1: a. Image of *Nyctanthes arbortristis*, b. Image of *Nerium Oleander*.

Transverse Section of Leaves of *Nyctanthes* and *Nerium*

A fresh potato was cut into small rectangular pieces, and the mid rib of the leaf lamina was carefully excised with a blade. The sectioning process involved sandwiching the leaf and potato together, followed by slicing the section thinly with a blade. To collect the sections, a hairbrush was used to sweep them off the razor blade onto a watch glass containing water. From the collected sections, the most suitable thin section was selected and transferred onto a clean glass slide. Subsequently, two drops of phloroglucinol were added to the section and allowed to dry for one minute. Following this, two drops of hydrochloric acid (HCl) were added and dried for another minute. Finally, the prepared section was observed under a microscope to examine its cellular structure and any chemical reactions induced by the phloroglucinol and HCl treatments. This experimental procedure provides a systematic method for studying plant tissue morphology and composition at a microscopic level.

RESULTS AND DISCUSSION

Macroscopy of Nyctanthes Arbortristis:

Nyctanthes arbortristis typically grows to heights ranging from 15 to 25 meters, with an expansive spread of approximately 30 meters. Its imposing stature and wide canopy contribute to its presence in the landscape. The crown of exhibits a regular uniformity, forming an umbrella-shaped canopy that provides ample shade and aesthetic appeal. The leaves are alternately arranged along twigs and possess a notable swelling at the base of the petiole. Thread-like stipules accompany the leaves. These leaves

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are paripinnate, with each pinna bearing 6-16 diamond-shaped leaflets. The upper surface of the leaflets is shiny green, while the underside is dull and finely haired. The apical leaflets are particularly large, adding to the distinctive foliage of the plant[26].

Figure 2: Image showing leaves and stem of *Nyctanthes arbortristis*

Nyctanthes arbortristis produces tiny flowers characterized by their pinkish hue and bicolored stamens. The delicate beauty of its flowers adds to the plant's ornamental value. The fruit consists of black or brown oblong and lumpy pods. These pods are thick, straight, or slightly curved and belong to the non-dehiscing type, meaning they do not split open to release seeds. The bark is characterized by its colouration, which may range from black to brown. The texture of the bark may appear lumpy, contributing to the plant's overall macroscopic profile.

In summary, the macroscopic examination of *Nyctanthes arbortristis* reveals a combination of distinct growth characteristics, foliage attributes, floral features, fruit morphology, and bark appearance. These macroscopic features collectively define the botanical identity and visual appeal of *Nyctanthes arbortristis*, highlighting its significance in horticulture and landscaping.

Microscopy of *Nyctanthes arbortristis*:

Under microscopic examination, the upper epidermis of Nyctanthes arbortristis reveals a single layer of thin-walled, rectangular cells. These cells form the outermost protective layer of the leaf, helping to reduce water loss and protect the leaf from environmental stresses.

Mesophyll: Beneath the upper epidermis, the palisade tissue appears to be 3-4 layered. These layers consist of compactly arranged parenchyma cells optimized for photosynthesis. The palisade tissue efficiently absorbs light energy, facilitating the plant's metabolic processes.

Vascular Bundles: Prominent vascular bundles are observed within the leaf tissue of Nyctanthes arbortristis. These bundles contain both lignified xylem and non-lignified phloem. The xylem transports water and minerals from the roots to the rest of the plant, while the phloem transports organic

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nutrients synthesized during photosynthesis to various parts of the plant. Similar to the upper epidermis, the lower epidermis consists of thin-walled, rectangular cells. However, the lower epidermis is distinguished by the presence of specialized stomata known as anisocytic stomata. These stomata regulate gas exchange and water vapor release from the leaf, helping to maintain internal balance[27].

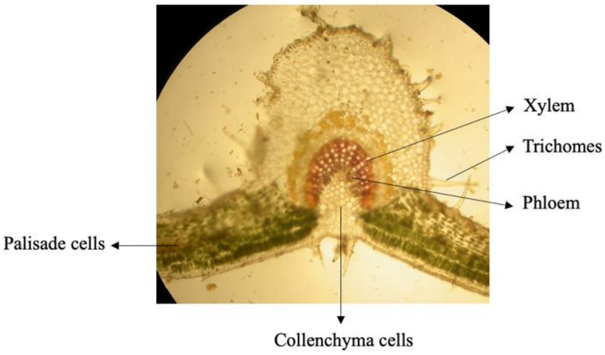

Figure 3: Transverse section of *Nyctanthes arbortristis.*

In summary, the microscopic examination of *Nyctanthes arbortristis'* leaf structure reveals adaptations for photosynthesis, gas exchange, and nutrient transport. The distinct tissue layers, vascular bundles, and specialized stomata contribute to the plant's efficient utilization of resources and maintenance of physiological processes.

Macroscopy of *Nerium Oleander*:

The *Nerium oleander*, a striking evergreen shrub or small tree, presents distinctive macroscopic features that contribute to its botanical identification and characterization. Typically reaching heights of 10-15 meters with a spread of approximately 2 meters, *Nerium oleander* exhibits a relatively tall and slender growth habit. The crown displays an irregular outline, often forming an umbrella-shaped canopy, which adds to its ornamental appeal and distinctiveness. The compound leaves are a defining characteristic, possessing a feathery appearance and a light, bright green coloration. These bi-pinnately compound leaves measure between 20-60 cm in length and are alternate in arrangement. Each leaf comprises 10-25 pairs of pinnae, ranging from 5-12 cm long, with numerous small oblong-obtuse leaflets attached. The leaflets themselves measure 0.5-2 cm in length and 0.3 cm in width, featuring a stalk-less attachment and rounded bases. Notably, the upper surface of the leaves is minutely hairy.

The flowers of *Nerium oleander* exhibit a range of colours, including pink, white, and red, adding to the plant's aesthetic allure. Although slightly fragrant, their visual appeal is a significant aspect of the plant's macroscopic identity. The fruit of Nerium oleander consists of elongated pods measuring between 30-75 cm in length and 3.8 cm in thickness. Initially green, these pods mature to a dark brown coloration. Nerium oleander typically features a large trunk with smooth bark characterized by a greyish-brown hue. The bark may exhibit slight cracking and numerous dots. Internally, the inner bark appears light brown in colour[28,29].

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Figure 4: Image showing leaves and stem of *Nerium oleander*.

An additional distinguishing feature of *Nerium oleander* is the presence of two compressed stipules located at the base of the leaf stalk. These stipules are adorned with long, narrow, comb-like teeth, further enhancing the plant's macroscopic profile. In summary, the macroscopic examination of *Nerium oleander* reveals a distinctive combination of growth habit, foliage, floral display, fruiting structures, bark characteristics, and unique stipular features, contributing to its botanical identity and recognition in various landscapes and environments.

Microscopy of *Nerium oleander*:

Under microscopic examination, the upper epidermis of Nerium oleander reveals a single layer of thin-walled rectangular cells covered with a protective cuticle. These cells form a continuous layer providing structural support and protection to the leaf tissue.

Mesophyll: The upper palisade layer consists of two compactly arranged layers of narrow parenchymatous cells. These cells are densely packed and primarily responsible for photosynthesis and light absorption. Beneath the upper palisade layer lies the spongy parenchyma, characterized by thin-walled, loosely arranged cells with large intercellular spaces. These spaces facilitate the exchange of gases such as carbon dioxide and oxygen during photosynthesis and respiration. The lower palisade layer consists of loosely arranged cells, smaller in size compared to those in the upper palisade layer. While still involved in photosynthesis, the lower palisade cells may have a different arrangement and density compared to their counterparts in the upper layer. Similar to the upper epidermis, the lower epidermis consists of a single layer of thin-walled rectangular cells covered with a cuticle. This layer provides additional protection to the leaf tissue and helps regulate gas exchange.

Midrib: The palisade parenchyma within the midrib appears as a single layer of cells, contributing to structural support and transport functions within the leaf.

Vascular Bundle: Within the vascular bundle, xylem tissue composed of lignified cells can be observed[28-30]. These cells are involved in the transport of water and minerals from the roots to the rest of the plant, providing essential support and nutrients.

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In summary, microscopy of Nerium oleander reveals a complex internal leaf structure optimized for photosynthesis, gas exchange, and nutrient transport. The arrangement and characteristics of various tissue layers and cell types contribute to the overall function and physiology of the leaf, highlighting the intricate adaptations of Nerium oleander to its environment.

CONCLUSION

The microscopic examination of the leaf structure of both *Nerium oleander* and *Nyctanthes arbortristis* reveals intricate adaptations tailored to optimize photosynthesis, gas exchange, and nutrient transport.

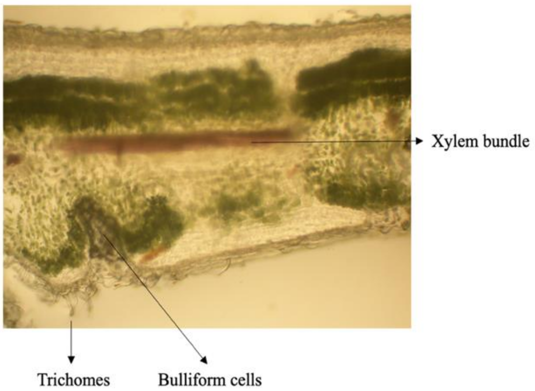

Figure 5: Transverse section of *Nerium oleander* midrib.

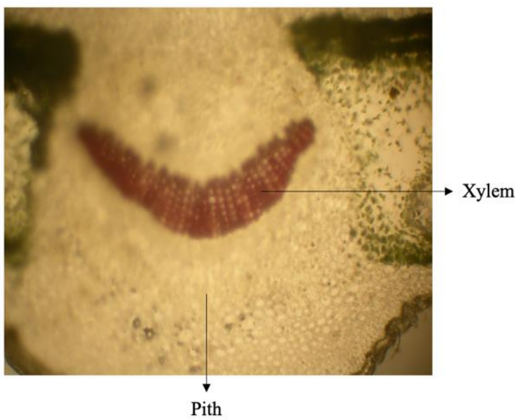

Figure 6: Transverse section of *Nerium oleander* midrib (enlarged).

Nerium oleander exhibits a well-organized leaf architecture characterized by thin-walled, rectangular cells forming the upper and lower epidermis, layered palisade tissue beneath the upper epidermis, prominent vascular bundles containing lignified xylem and non-lignified phloem, and specialized anisocytic stomata on the lower epidermis. These features contribute to efficient light absorption, water and nutrient transport, and regulation of gas exchange, highlighting the plant's adaptation to diverse environmental conditions. Similarly, *Nyctanthes arbortristis* displays a leaf structure comprising thin-

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walled, rectangular cells in the upper and lower epidermis, layered palisade tissue beneath the upper epidermis, prominent vascular bundles with lignified xylem and non-lignified phloem, and anisocytic stomata on the lower epidermis. These structural characteristics facilitate photosynthesis, gas exchange, and nutrient transport, enabling the plant to thrive in various ecological niches.

Both plants exhibit specialized adaptations in their leaf anatomy, emphasizing their resilience and efficiency in utilizing resources for growth and survival. The microscopic examination provides valuable insights into the intricate mechanisms underlying the physiological processes of these plant species, underscoring their ecological significance and potential applications in various fields, including botany, agriculture, and medicine. Further research into the microscopic details of leaf structure and function may enhance our understanding of plant biology and inform conservation efforts aimed at preserving these valuable botanical resources.

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REFERENCES

1) Saxena RS, Gupta B, Saxena KK and Srivastava VK and Prasad DN.(1987) Analgesic, antipyretic and ulcerogenic activities of *Nyctanthes arbortristis* leaf extract. J Ethnopharmacol. *19*:193-200.
2) Amarite O, Bhuskat P, Patel N and Gadgoli. C. (2007) Evaluation of antioxidant activity of carotenoid from *Nyctanthes arbortristis*. Int J Pharmacol Biol Sci. 2007; *2*:57-59.
3) Omkar A, Jeeja T and Chhaya G.(2006) Evaluation of anti-inflammatory activity of *Nyctanthes arbortristis* and Onosma echiodes. Pharmacog.mag. *8*:258-260.
4) Agrawal, J., & Pal, A. (2013). *Nyctanthes arbor-tristis* Linn—A critical ethnopharmacological review. Journal of ethnopharmacology, *146*(3), 645-658.
5) Singh, J., Singh, A. P., & Singh, A. P. (2021). Nyctanthes arbor-tristis: a comprehensive review. World Journal of Current Medical and Pharmaceutical Research, 74-78.
6) Bhalakiya, H., & Modi, N. R. (2019). Traditional medicinal uses, phytochemical profile and pharmacological activities of *Nyctanthes arbortris*. *RJLBPCS*, *5*, 1003-1023.
7) Prajapati ND, Purohit SS, Sharma AK, Kumar A. Handbook of Medicinal Plants. India: Agrobios; 2003, 355-378.
8) Inamdar N, Edalat S, Kotwal VB, Pawar S. Herbal Drugs in Milieu of Modern Drugs. Int. J. Green Pharm. 2008; *2*(1): 2-8.
9) Sapna S, Ravi TK. Approaches towards Development and Promotion of Herbal Drugs. Pharmacognosy Review 2007; *1*(1): 180-184.
10) Kumar, A. R., & Yadav, C. S. P. D. (2013). Antibacterial activity of ethanolic extracts of *Nyctanthes arbortristis* and *Nerium oleander*. Indian Journal of Research in Pharmacy and Biotechnology, *1*(3), 310-313.
11) Yadav *et al*; (2013) Antibacterial activity of *Nyctanthes arbortristis Nerium oleander* and *Catharanthus roseus.* International Journal of Research in Pharmacy and Chemistry *3*(2):509-512.

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CrossRef DOI: https://doi.org/10.31426/ijrpb **Indexed in CAS and CABI, Impact Factor: 0.64**

12) Singh, A. K., & Solanki, S. (2022). Antimicrobial activity of Nyctanthes arbor-tristis against Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa and Salmonella typhi. Int. Jr. Pharm. Sci. and Research, *13*(7), 1000-1009.
13) Khan, Z. K., Manglani, A., Shukla, P. K., Puri, A., Saxena, R. P., & Tandon, J. S. (1995). Immunomodulatory effect of plant extracts and iridoid glucosides from Nyctanthes arbortristis against systemic candidiasis in mice. International journal of pharmacognosy, *33*(4), 297-304.
14) Jadhav, P., Kapoor, N., Thomas, B., Lal, H., & Kshirsagar, N. (2012). Antiviral potential of selected Indian medicinal (ayurvedic) plants against herpes simplex virus 1 and 2. North American journal of medical sciences, *4*(12), 641.
15) Yadav, C. S. P. D., Bharadwaj, N. S. P., Yedukondalu, M., Methushala, C. H., & Kumar, R. (2013). Phytochemical evaluation of Nyctanthes arbortristis, Nerium oleander and Catharathnus roseus. Indian Journal of Research in Pharmacy and Biotechnology, *1*(3), 333.
16) Kusum S and Akki. (2009) Phytochemical investigation and in vitro evaluation of *Nyctanthes arbortristis* leaf extract for antioxidant property. J Pharm Res. *2*(4):752-755.
17) Tandon JS, Srivastava V and Guru PY.Iridoids (1991) A new class of leishmanicidal agents from *Nyctanthes arbortristis*. J Nat Prod. *4*:1102-1104.
18) Hukkeri VI, Akki KS, SUreban RR, Gopalakrishna B, Byahatti VV and Rajendra SV.(2006) Hepatoprtective of the leaves of *Nyctanthes arbortristis* Linn. Indian J Pharm Sci, *68*(4):542-543.
19) Vats M, Sharma N and Sardana S,(2009) Antimicrobial Activity of stem bark of *Nyctanthes arbortristis* linn. (Oleaceae), Int. J Pharmacognosy and Photochemical Research. *1*(1):12-14.
20) Shawuti, S., Bairam, C., Beyatlı, A., Kariper, İ. A., Korkut, I. N., Aktaş, Z., ... & Kuruca, S. E. (2021). Green synthesis and characterization of silver and iron nanoparticles using Nerium oleander extracts and their antibacterial and anticancer activities. Plant Introduction, *2*(91/92), 36-49.
21) Lokesh R,Leonard Barnalas, Madhuri P,Saurav K,Sundar K,(2010) Larvicidal activity of *Trigonella*, foenum and *Nerium oleander* Linn. Current research journal of biological sciences *2*(3):154-160.
22) Ayouaz, S., Koss-Mikołajczyk, I., Adjeroud-Abdellatif, N., Bartoszek, A., Arab, R., Mouhoubi, K., ... & Madani, K. (2023). Anticarcinogenic and antioxidant activities of leaves and flowers hydroalcoholic extracts of Nerium oleander L.: PCA analysis and phytochemical content by FTIR spectroscopy. The North African Journal of Food and Nutrition Research, *7*(15), 1-8.
23) Farkhondeh, T., Kianmehr, M., Kazemi, T., Samarghandian, S., & Khazdair, M. R. (2020). Toxicity effects of Nerium oleander, basic and clinical evidence: A comprehensive review. Human & experimental toxicology, *39*(6), 773-784.
24) Seher, A., Hanif, M. A., Ayub, M. A., Jilani, M. I., & Mahomoodally, M. F. (2020). Oleander. In Medicinal Plants of South Asia, 525-539. Elsevier.
25) Shanthi R, Lakshmi G, Priyadarshini A. M, Anandaraj L. (2011). Phytochemical screening of *Nerium oleander* Linn. leaves and *Momordica charantia* leaves. International research journal of pharmacy 2(1) 131-135.
26) Jain, P. K., & Pandey, A. (2016). The wonder of Ayurvedic medicine-Nyctanthes arbortristis. *Int J Herb Med*, *4*(4), 9-17.

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CrossRef DOI: https://doi.org/10.31426/ijrpb **Indexed in CAS and CABI, Impact Factor: 0.64**

27) Bijauliya, R. K., Kannojia, P., Mishra, P., Singh, P. K., & Kannaujia, R. (2021). Pharmacognostical and Physiochemical Study on the Leaves of Nyctanthes arbor-tristis Linn. Journal of Drug Delivery and Therapeutics, *11*(4), 30-34.
28) Abdalla, M. M., Eltahir, A. S., & El-Kamali, H. H. (2016). Comparative morph-anatomical leaf characters of Nerium oleander and Catharanthus roseus family (Apocynaceae). European Journal of Basic and Applied Sciences Vol, *3*(3), 68-73.
29) Sargin, S. A. (2021). Micro-Morphological, Anatomical and Statistical Correlative Evaluation Between Different Altitudes and Leaf Structural Features of Nerium oleander L.(Apocynaceae), Growing in the Middle-West Taurus, Turkey. Botany Research Journal, *14*(1).
30) Bashir, K., Sohail, A., Ali, U., Ullah, A., Ul Haq, Z., Gul, B., ... & Asghar, M. (2020). Foliar micromorphology and its role in identification of the Apocynaceae taxa. Microscopy Research and Technique, 83(7), 755-766.