



## Phytochemical screening and biological studies of leaf extract of *Nerium oleander* L.

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**Abstract:** *Nerium oleander* L. has long been recognized for its healing properties among various ethnic groups and traditional healers and has a broad-spectrum of medicinal benefits, including cardio tonic, analgesic, antidiabetic, anti-inflammatory, antibacterial, antineoplastic, antifungal, antidepressant and antimutagenic. To begin the study, leaves extracts were prepared through maceration in non-polar solvent n-hexane and polar solvent ethanol. Total Phenolic Content and Total Flavonoid Content of the extracts were evaluated utilizing Folin-Ciocalteu method and standard chemical assay procedures respectively. To assess the antioxidant activity of the extract, a reducing power assay and a DPPH radical scavenging assay were employed. In order to evaluate the antibacterial activity, the borehole method using *Staphylococcus aureus* and *Escherichia coli* was performed. Furthermore, the GC-MS analysis of the ethanolic extract showed 23 major peaks, while the n-hexane extract contained 10 major peaks. The ethanolic leaf extract exhibited a Total Phenolic Content value of 87.1 mg GAE per gm and a Total Flavonoid Content value of 727.5 mg QE/g. Moreover, the reducing power assay demonstrated a linear graph, illustrating antioxidant properties of the plant. The DPPH radical scavenging assay showed an IC<sub>50</sub> value of 15.276 ug/mL, meanwhile the standard ascorbic acid had an IC<sub>50</sub> value of 6.72 ug/mL. Additionally, the ethanolic leaves extract displayed some antibacterial activity against *E. coli*.

**Keywords:** *Nerium oleander*, GC-MS, antibacterial

## INTRODUCTION

There has always been a special place for plants in the realm of medicine (Harvey et al., 2008). Regardless of the underlying philosophy, plants have been extensively utilized in major systems of medicine (Trease et al., 2009). In Nepal, for instance, traditional medicine plays a vital role in the healthcare system (Raut et al., 2011). The therapeutic benefits derived from plants have paved the way for the development of modern medicine (Kumar et al., 2014). Active compounds extracted from plants are now widely used in allopathic medicine, establishing a strong co-relation between their therapeutic and traditional uses of the plants from which they are derived (Ramandeep et al., 2015). In recent times, the search for natural antioxidants has gained momentum due to the increasing prevalence of diseases and the associated side effects of synthetic antioxidants (Zehiroglu et al., 2019). Scientists are exploring alternatives derived from natural sources that can effectively combat oxidative stress (Mohadjerani, 2012). Furthermore, the rise in infections and antibiotic resistance has fueled the need for new drugs. Effective antibiotics are crucial in addressing this global challenge. The emergence of antibiotic-resistant strains necessitates the continuous search and development of novel therapeutic agents (Chetwani et al., 2017).

Among the many medicinal plants, *N. oleander* stands out. This perennial shrub, with height of around four meters, beautifies gardens worldwide with its vibrant flowers (Garima et al., 2010). Extensive research has been conducted on this fascinating plant due to its complex pharmacological and toxicological characteristics. It has been revealed that the purified fractions of *N. oleander* leaves exhibit analgesic, anti-convulsant, reduced locomotor activity and as a whole CNS depressant action (Zia et al., 1995). Other investigations have found that methanolic extracts from *N. oleander* flowers possess strong antioxidant and hepatoprotective activity against liver damage caused by carbon tetrachloride in experimental animals (Singhal et al., 2012). Furthermore, it is reported that the leaves as well as roots of *N. oleander* have antimicrobial properties, effectively combating bacteria like *Bacillus pumilus*, *B. subtilis*, *Staphylococcus aureus* and *Escherichia coli*, as well as the fungus *Aspergillus niger* (Hussain et al., 2004). Additionally, it was suggested that extracts derived from *N. oleander* have prospects as an anticancer therapy for lung cancer patients, making them suitable for further investigation in phase II clinical trials (Montano et al., 2013). Moreover, the plant has been associated with other pharmacological activities such as antidiarrheals and cytotoxic (Hassan et al., 2011), effectiveness against larvae (Raveen et al., 2014), antihelmintics (Native et al., 2014), prevention of ulcers (Sabira et al., 1998) and anti-diabetics (Dey et al., 2015).

*N. oleander* is known as 'Karabir' in Nepal. In south Asia, a decoction made from the leaves of this plant is used as traditional remedies for the treatment of scabies. It is applied externally to alleviate the symptoms of this irritating condition. Another application of this plant involves the use of its roots, which are transformed into a paste and applied as plasters to treat tumors. Additionally, the oil derived from the root bark is utilized for treating leprosy and other skin disorders characterized by scaling. (Garima et al., 2010; Ayouaz et al., 2023) While *N. oleander* has been recognized as a significant in traditional medicine and holds great potential for drug discovery, it unfortunately poses a constant risk of intoxication due to its narrow therapeutic index (Bavunoğlu et al., 2016). As per various pre-clinical and clinical reports, numerous cases of acute toxicity have been recorded due to intentional, accidental and suicidal oleander ingestion (Dey, 2020). According to previous studies, the median lethal dose (LD50) value of leaf extract was found to be 4 gm/kg in mice (Haeba et al., 2002) and the LD50 value for cattle was determined to be 50 mg/kg (Oryan et al., 1996). It is important to note that consumption of as little as 10-20 leaves can initiate adverse reactions in adults, while ingestion of a single leaf was found to be fatal to an infant or child (Garima et al., 2010).

All parts of the plant are poisonous and contain a range of toxic cardiac glycosides such as neriin, oleandrin, cardenolides, gentiobiosyl and odoroside (Ebrahimi et al., 2018). According to reports, certain glycosides like gentiobiosyl-oleandrin, gentiobiosyl-nerigoside, and gentiobiosyl-beaumontoside have been extracted from the leaves (Mallet et al., 1994). Additionally, it is reported that the lymph of this plant is rich in minerals and  $\alpha$ -tocopherol while there are also some mildly active cardenolides and inactive cardenolides, triterpenoids, resin, tannins, glucose, paraffin, ursolic acid, vitamin C and essential oils (Garima et al., 2010). In order to shed light on the scientific justification for its widespread traditional practices, this study explored the phytochemical and biological activities of *N. oleander* extract found in Nepal. Emphasizing its potential as an antimicrobial and antioxidant agent, we investigated the phytochemical constituents, total phenolic and flavonoid content, antioxidant as well as antimicrobial properties against both gram-positive and gram-negative microorganisms. This comprehensive study aims to strengthen the understanding of *N. oleander's* therapeutic potential by unraveling its beneficial compounds and assessing its biological activities. The plant *N. oleander* found in Nepal is shown in figure 1.

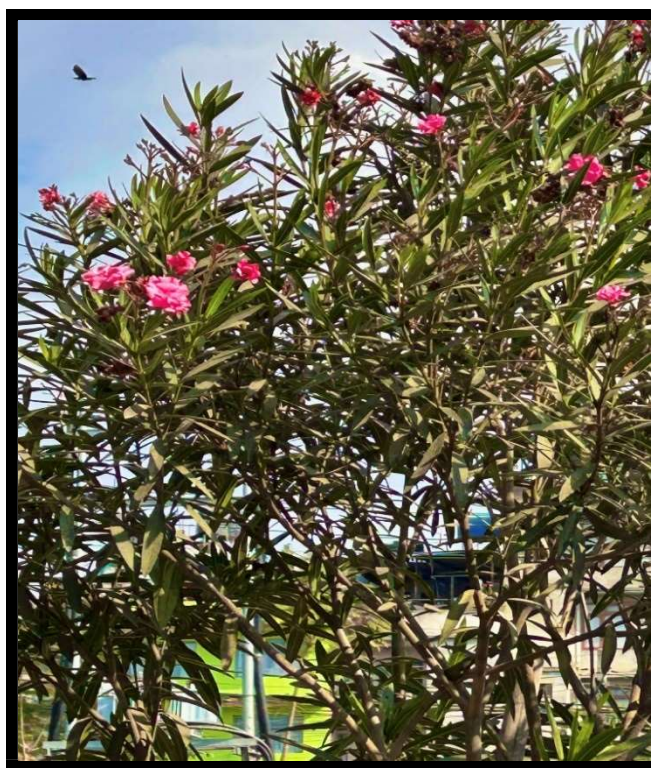


Figure 1: *Nerium oleander* L.

## MATERIALS & METHODS

**Plant material:** The collection site for *N. oleander* leaves was Kadaghari, located in the Kathmandu district of Bagmati zone, Nepal in October 2021 at an altitude of 1,315 meters. It was then identified by the National Herbarium and Plant Laboratories located in Lalitpur district, Nepal.

**Extract preparation:** A 100 grams of *N. oleander* leaves powder were measured and taken in a 500 mL conical flask. Then, it was extracted first using non-polar solvent n-hexane in a 1:3 ratio for a period of 72 hours. During this process, mixture was shaken occasionally. Once the 72 hours had passed, filtration of extract was carried out using Whatman filter paper No. 1 (Scientific Glass Works, Hyderabad, India). To concentrate the filtrates, a rotary evaporator set (Accumax India, Delhi, India) at 40°C was employed. The residue obtained from the n-hexane extraction was desiccated, weighed, and then reintroduced to extraction with polar solvent 70% ethanol in the same ratio for another 72 hours. Following the second extraction, the filtrates were again filtered followed by concentration with similar procedure carried out for n-hexane extract. The resulting extracts were stored in a refrigerator in airtight vials. To determine the extractive value, the weight after extraction was measured.

**Phytochemical screening:** Phytochemical screening was performed using the previously described standard procedure. The results were interpreted based on the color change observed during the qualitative test as shown in table 1 (Dahanayake et al., 2019).

Phytochemicals under screening	Test performed	Positive test coloration
Alkaloids	Dragendroff's test	Formation of reddish-brown precipitates
	Mayer's test	Cream colored precipitates
	Hager's test	Formation of yellow precipitates
	Wagner's test	Red brown precipitates formation
Tannins	Ferric chloride test	Brownish green or a blue-black coloration
Flavonoids	Shinoda test	Appearance of pink scarlet, crimson red or occasionally green to blue color
	Alkaline reagent test	Intense yellow color formation
	Zinc hydrochloride test	Red color formation
Carbohydrates	Molisch's test	Purple ring formation
Glycosides	Fehling's test	Red precipitate formation
Anthraquinone glycoside	Borntrager's test	Rose pink to red color in ammoniacal layer
Cardiac glycoside	Keller-Kiliani test	Violet ring formation
Terpenoids	Salkowski's test	Reddish brown coloration
Proteins	Biuret's test	Appearance of violet color
Amino acids	Ninhydrin test	Appearance of violet color
Saponins	Lead acetate test	Formation of white precipitates

Table 1: Test used for phytochemical screening of the extracts

**Gas chromatography-mass spectrometry (GC-MS) analysis:** GC-MS (Agilent Technologies, California, USA) analysis was conducted at NAST, Lalitpur, Kathmandu with the purpose of resolving the extract into its masses present. For the analysis, number of rinses with pre-solvent was 2 and the number of rinses with post-solvent and sample were 5. The suction plunger (Agilent Technologies, California, USA) speed was set to high, and the viscosity composition time was 0.2 seconds. The sample was pumped 5 times with a 0.3 second time interval. Both the injection plunger speed and syringe insertion speed were set to high while setting the injection mode to normal. For this analysis, the washing volume was 8  $\mu$ L and a single solvent vial was utilized.

**Total Phenolic Content (TPC):** The total polyphenolic content of the extracts was evaluated using Folin-Ciocalteu's reagent (Central Drug House, New Delhi, India). A mixture of 5 mL Folin-Ciocalteu's reagent (1:10 v/v dilution with distilled water), 4 mL of 7% aqueous sodium carbonate, and 1 mL of ethanolic extracts (1 mg/mL) was allowed to incubate for 30 minutes at 40°C in a water bath (Accumax India, Delhi, India). Following incubation, UV-Visible spectrophotometer (Electronics India, Himachal Pradesh, India) measured the absorbance of mixture at 760 nm. The TPC value was calculated by comparing the absorbance to a calibration curve prepared with various concentrations of gallic acid standards (10 to 200 µg/mL). Milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of the extract was the unit used to express the concentration of polyphenolic compounds in the extracts (Singleton and Rossi,1965).

**Total Flavonoid Content (TFC):** The total flavonoid content was studied using the application of aluminum trichloride reagent. A 4 mL of distilled water was taken in a volumetric flask having volume of 10 mL and mixed with a portion of 1 mL of various extracts dissolved in ethanol. At 0 minutes and 5 minutes, 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% AlCl<sub>3</sub> were introduced respectively. Likewise, 2 mL of 1 M sodium hydroxide was mixed at 6 minutes. The flask's volume was completed with 2.4 mL of distilled water and the contents were stirred thoroughly. The absorbance of the resulting pink-colored solution was measured at 510 nm relative to a blank that lacked the extracts. The TFC values were determined using a calibration curve prepared with various concentrations of quercetin standards (10 to 200 µg/mL) (Kamtekar et al., 2014). The unit used to express TFC in the extracts was milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g)

**Reducing power assay:** Ascorbic acid served as the standard in ferric reducing antioxidant power (FRAP) method carried out for reducing power assay. This technique involved mixing of different concentrations of the plant extracts in their corresponding solvents, 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide which was kept in a water bath for 20 minutes. The temperature of water bath was maintained at 50°C. To this solution, 2.5 mL of 10% trichloroacetic acid was poured after cooling and then centrifugation was carried out at 3000 rpm for 10 minutes. Next, 2.5 mL each of the top layer and distilled water were infused with 0.5 mL of freshly made ferric chloride solution. Then, UV-Visible spectrophotometer was operated at 700 nm and absorbance were noted. As a control, the same procedure was followed without the addition of the extracts. As for the standard, various concentrations of ascorbic acid were employed. A hike in absorbance depicted hike up in reducing power. The adjustment in extract concentration and contact time determined the reducing power, following the previously described method (Mohadjerani, 2012).

**DPPH radical scavenging activity:** The fraction's potential to scavenge free radical *in vitro* was assayed using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, following previously established procedure (Saeed et al., 2012). For this assay, reference sample was ascorbic acid and solvent used was methanol. Sample solutions of the extracts were made in solvent at different strengths (5, 10, 15, 20 and 25 µg/mL). Subsequently, 4 mL of a 0.1 mM DPPH produced in the solvent was fused with 1 mL of each strength of sample solution and reference sample solutions. For 30 minutes, the mixtures were concealed in the absence of light. Likewise, the control used was the mixture of 4 mL of 0.1 mM DPPH and 1 mL of methanol; kept in the absence of light for 30 minutes. After 30 minutes, UV-Visible spectrophotometer was operated at 517 nm and absorbance were recorded.

**Antimicrobial activity:** To assess the antimicrobial activity, the borehole method (Shresta et al, 2021) was employed and selected microorganisms susceptible to commercial-grade antibiotics. Isolated

bacteria were collected and stored in a refrigerator. The microorganisms under examination were gram-positive *Staphylococcus aureus* and gram-negative *E. coli*. A 50 µg/mL of gentamycin and 10 µg/mL of doxycycline, dissolved in a 1% DMSO (dimethyl sulfoxide) solution were utilized as standards. We prepared plant extracts solution in different concentrations (25, 50, 100, and 200 µg/mL) in the same DMSO solution. The manufacturer's instructions were followed in the preparation and sterilization of Mueller Hinton Agar (MHA). After being transferred into sterile petri plates in a sterile laminar hood, the agar was left to solidify. Using a 6 mm sterile well borer, boreholes were created. Using swab sticks that were freshly sterilized, the microorganisms were introduced into the plates. 50 µL of the extracts were transferred into the wells using sterile equipment. A total of 4 boreholes per plate were made, each containing a different concentration of the extract's solution. The same process was carried out again for the standard as well as for the blank. Following the incubation of plates for 24 hours, the zone of inhibition was measured.

## RESULTS & DISCUSSION

*Extractive value:* After drying, extracts were weighed and weight percentage of *N. oleander* were calculated. The result for extractive value (percentage yield of crude extract) is shown in the table 2.

Table.2. Extractive values of *N. oleander* extract

Extract	Yield %
Ethanol	26.02
N-hexane	0.9293

This value of leaves extracts indicated that the mass of polar component compound is in greater proportion. The extraction was first carried out in n-hexane. N-hexane is a non-polar solvent and does the extraction of non-polar bioactive compounds (Chavez et al, 2013). Since the crude extract yielded by n-hexane is only 0.9293%, this indicates that the mass of non-polar compound is in smaller proportion. It also revealed that ethanol is more efficient extraction solvent for *N. oleander* leaves.

*Phytochemical screening:* Tannins, alkaloids, flavonoids, terpenoid, saponins, glycosides, cardiac glycosides, carbohydrates and reducing sugar were detected in the ethanolic extract. The n-hexane extract revealed the presence of saponin only.

*GC-MS analysis:* N-Hexane extract of *N. oleander* showed the presence of 10 main compounds for which we recorded the masses. The mass obtained are shown in table 3. GC-MS analysis of Hydroethanolic extract led to the fragmentation of 23 main compounds. The mass obtained are tabulated in table 4 and the chromatogram is shown in figure 3.

Table 3: Analysis of n-hexane extract by GC-MS

Retention time	Peak area percentage	m/z
26.9116	5.7718	148.16
31.1192	6.7977	72.11
31.993	42.1382	208.21
38.0886	6.2487	256.42
39.2861	4.6992	248.4
40.8937	4.5759	759.4
41.2605	10.7402	280.4
41.3684	10.6594	264.4
45.0149	3.2756	452.7962
49.2872	2.6415	282.5475

Table 4: Analysis of ethanolic extract by GC-MS

Retention time	Peak area percentage	m/z
25.2069	7.6352	162.18
25.7787	0.8208	178.23
26.5231	0.7132	204.35
27.6991	2.9309	202.34
28.3248	1.2719	204.36
28.67	2.4665	192.21
29.5008	0.8237	208.25
30.0078	1.7697	202.33
30.1265	1.9904	220.35
31.0651	1.775	202.33
31.2809	11.5515	204.35
31.4535	1.3211	204.39
31.6477	1.4519	202.2
32.4461	1.5131	222.37
32.7266	1.3885	218.33
34.032	1.0914	188.26
34.1938	1.0292	134.22
34.5606	0.7635	216.19
36.7076	0.9784	320.5
38.0885	7.9815	256.42
41.2711	12.0796	280.4
41.3898	24.4347	278.4
41.7674	4.3558	284.5

The GC-MS analysis of *N. oleander* leaves showed the highest index of mass 208.21 with area percentage 42.1382 in n-hexane extract and mass of 278.4 with area percentage 24.4347 in ethanolic extract. Further identification of the masses using standards could help to confirm the identity of these compounds

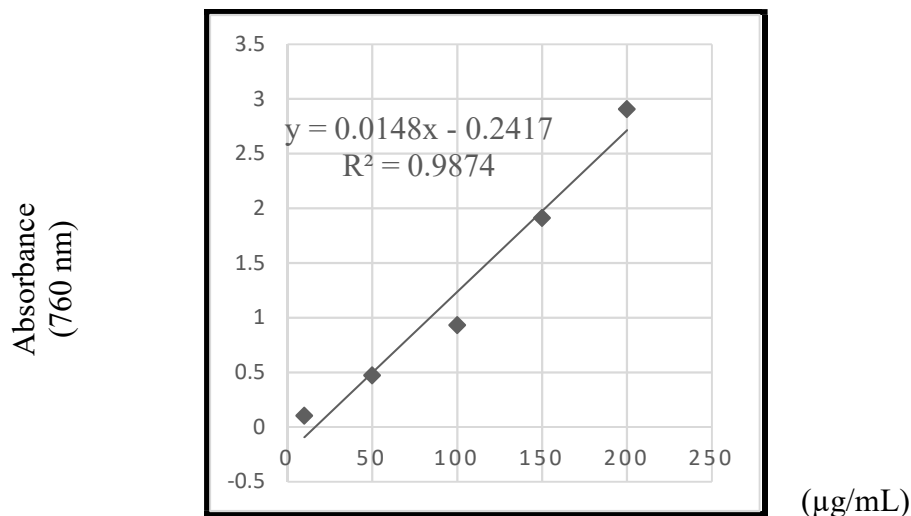


Figure 2: Standard gallic acid calibration curve

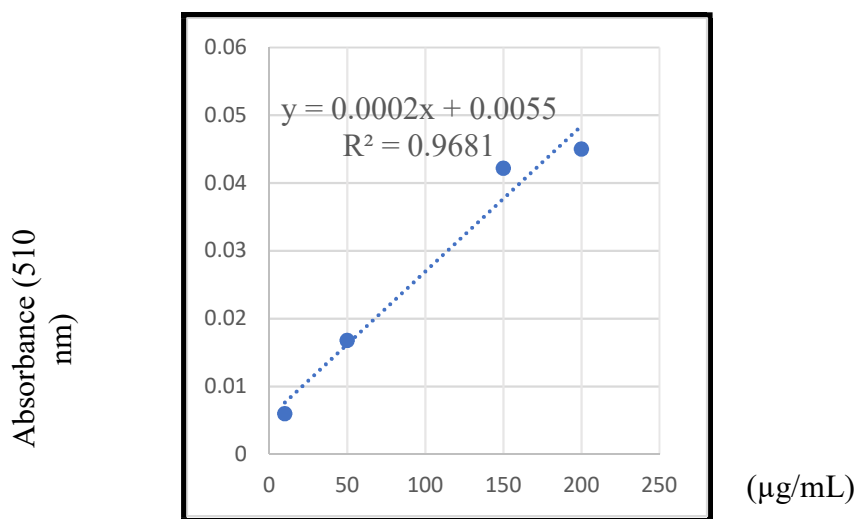


Figure 3: Standard quercetin calibration curve

**Total Phenolic Content (TPC) and Total Flavonoid Content (TFC):** The calibration curve was used to quantify the phenol content in the extract applying the regression equation  $Y = 0.0148X - 0.2417$ ,  $R^2 = 0.9874$ . The phenolic content of the hydroethanolic extract was obtained to be 87.1 mg GAE/gm of the extract. Likewise, the regression equation  $Y = 0.002X + 0.0055$ ,  $R^2 = 0.9681$  was applied to quantify the flavonoid content. Ethanolic extract of *N. oleander* shows 727.5 mg/g dry material TFC

in terms of quercetin equivalent. The standard gallic acid calibration curve is shown in figure 2 and the standard quercetin calibration curve is shown in figure 3.

**Reducing power assay:** As sample and standard concentrations hiked up, so did their reducing powers for the hydro alcoholic extracts and standard. Both the sample extract  $R^2 = 0.9538$  and the standard  $R^2 = 0.9988$  indicated a satisfactory linear relationship in the reducing power assay. The sample's graph was found to be linear as, indicating its antioxidant property. The reducing power assay of the extract and the standard is represented in figure 4.

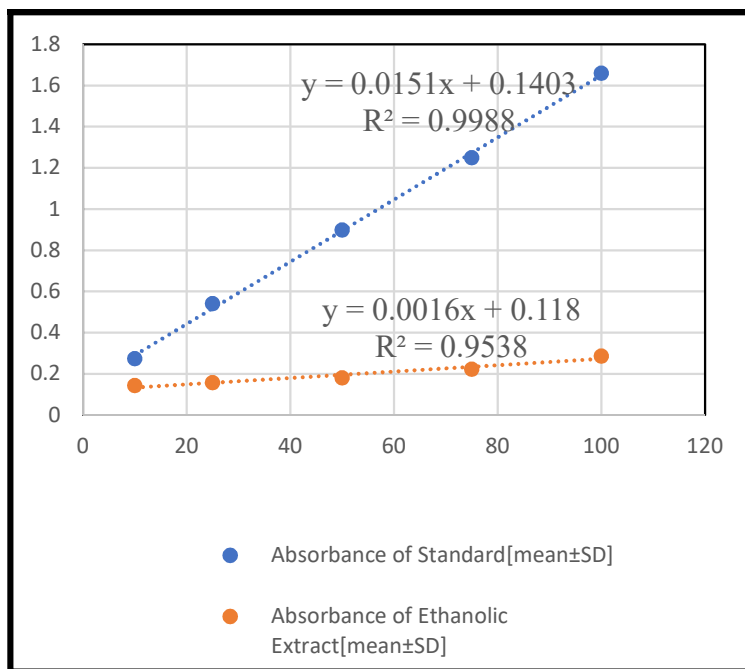
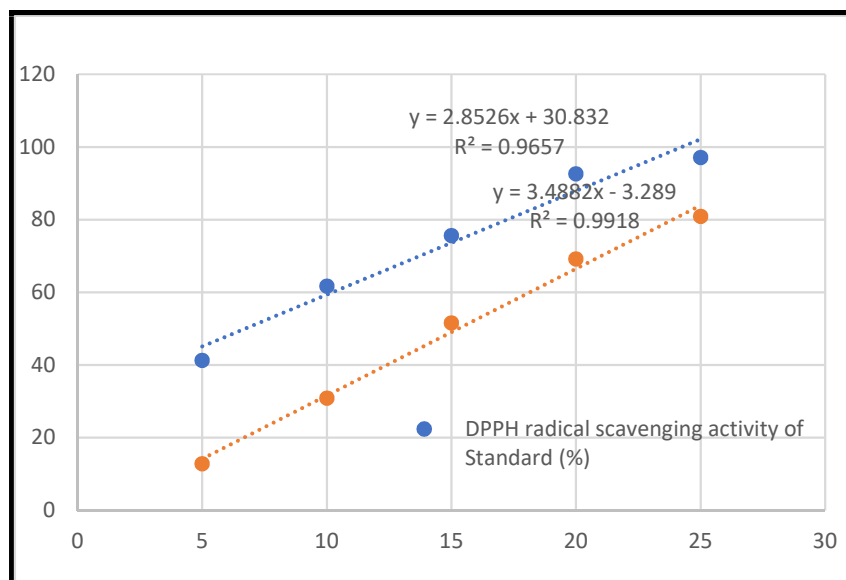


Figure 4: Ferric reducing power assay of extract and standard

**DPPH free radical scavenging activity:** The varying concentrations of *N. oleander* that was tested (5, 10, 15, 20, 25  $\mu\text{g}/\text{mL}$ ) and the standard ascorbic acid demonstrated comparable antioxidant activity. For every tested concentration, the percentage of antioxidant activity increased in a dose-dependent manner. The concentration of standard used that is ascorbic acid was ranged from 5 to 25  $\mu\text{g}/\text{mL}$ . As the concentration of ascorbic acid increased, there was gradual increase in percentage of inhibition. The  $\text{IC}_{50}$  value for ascorbic acid was determined to be 6.78  $\mu\text{g}/\text{mL}$  and the  $\text{IC}_{50}$  value for ethanolic extracts was determined to be 15.276  $\mu\text{g}/\text{mL}$ . A lower  $\text{IC}_{50}$  value indicates stronger scavenging abilities. Although the values are not as low as those of ascorbic acid, the experiment found that the ethanolic extracts of this plant exhibit DPPH radical scavenging activity. The antioxidant activity determination by DPPH method is represented in figure 5.



**Figure 5:** Anti-oxidant activity determination by DPPH method

*Antimicrobial activity:* Extract of 200 µg/mL showed zone of inhibition of 13 mm against *E. coli* while no any zone of inhibition was seen for *S. aureus* at any experimental concentration. The present study indicated that zone of inhibition by plant extract of *N. oleander* was found to be against *E. coli* i.e. 13 mm at concentration 200 µg/mL.

### CONCLUSION

The studied plant *N. oleander* has the potential to exhibit beneficial therapeutic properties as it shows notable TPC, TFC value along with considerable antioxidant and antimicrobial activity. Given these findings, it may be useful to conduct more studies on this plant for exploration of new compounds to combat the contemporary issues of antibiotic resistance and need for natural antioxidants. In light of these possibilities, *N. oleander* emerges as a promising candidate for drug discovery.

### ACKNOWLEDGMENT

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### DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

### DECLARATION OF HONOUR

We declare on our honour that our results are not fake and made up.

## REFERENCES

- Aboud AS (2015) Antimicrobial activities of aqueous and ethanolic extracts from *Nerium oleander* used in the treatment of burns infections isolates. *Journal of Pharmaceutical, Chemical and Biological Sciences*. 2(4):248-258.
- Ayouaz S, Arab R, Mouhoubi K, Madani K (2023) *Nerium oleander* Lin: A review of chemical, pharmacological and traditional uses. *Journal of Biomedical Research and Environmental Sciences*. 4(4):641-650
- Bavunoğlu I, Balta M & Türkmen Z (2016) Oleander poisoning as an example of self-medication attempt. *Balkan Medical Journal*. 33(5):559-562.
- Chaudhary K, Prasad DN & Sandhu BS (2015) Preliminary pharmacognostic and phytochemical studies on *Nerium oleander* Linn. (White cultivar). *Journal of Pharmacognosy and Phytochemistry*. 4(1).
- Chetwani K, Agnihotri RK, & Chaturvedi P (2017) Aqueous, acetone and ethanolic extract of *Nerium indicum* L. as potential antibacterial agent against *Pseudomonas aeruginosa*. *International Journal of Applied Environmental Sciences*. 12(9):1721-1732.
- Dahanayake JM, Perera PK, Galappatty P, Perera HDSM, Arawwawala LDAM (2019) Comparative phytochemical analysis and antioxidant activities of tamalakyadi decoction with its modified dosage forms. *Evidence-based Complementary and Alternative Medicine*. 6037137.
- Dey P (2020) The pharmaco-toxicological conundrum of oleander: Potential role of gut microbiome. *Biomedicine & Pharmacotherapy*. 129:110422.
- Dey P, Saha MR, Chowdhuri SR, Sen A, Sarkar MP, Halder B & Chaudhuri TK (2015) Assessment of anti-diabetic activity of an ethnopharmacological plant *Nerium oleander* through alloxan induced diabetes in mice. *Journal of Ethnopharmacology*. 161:128-137.
- Ebrahimi F, Nohooji GM & Miri SM (2018) Agronomic and pharmacological aspects of *Nerium oleander*: an important medicinal plant. *The First National Congress and International Fair of Medicinal Plants and Strategies for Persian Medicine that Affect Diabetes*. 9-11.
- Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*. 4:177.
- Elvin-Lewis M (2001) Should we be concerned about herbal remedies? *Journal of Ethnopharmacology*. 75(2-3):141-164.
- Fabricant DS & Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*. 109(1):69-75.
- Farooqui S & Tyagi T (2018) *Nerium oleander*: It's application in basic and applied science: A Review. *International Journal of Pharmaceutical Sciences*. 10(3):1-4.
- Garima Z & Amla B (2010) A review on chemistry and pharmacological activity of *Nerium oleander* L. *Journal of Chemical and Pharmaceutical Research*. 2(6):351-358.
- Haeba M, Mohamed A, Mehdi A, Nair GA (2002) Toxicity of *Nerium oleander* leaf extract in mice. *Journal of Environmental Biology*. 23(3):231-237
- Harvey AL (2008) Natural products in drug discovery. *Drug Discovery Today*. 13(19-20):894-901.
- Hase GJ, Deshmukh KK, Murade VD, Pokharkar RD, Phatanagre ND, Hase DP, ... & Gosavi AB (2016) Phytopharmacology of *Nerium oleander* L. A review. *International Journal of Phytopharmacology*. 7(2):0975-9328.
- Hussain MA & Gorski MS (2004). Antimicrobial activity of *Nerium oleander* Linn. *Asian Journal of Plant Sciences*. 13(4):8-16.
- Javed H, Khan MM, Khan A, Vaibhav K, Ahmad A, Khuwaja G, & Islam F (2011) S-allyl cysteine attenuates oxidative stress associated cognitive impairment and neurodegeneration in mouse model of streptozotocin-induced experimental dementia of Alzheimer. *Brain Research*. 1389:133-142.
- Kamtekar et. al. (2014) Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *Journal of Applied Pharmaceutical Science*. 4(09):61-65.
- Kumar P & Fartyal M (2014) Evaluation of antimicrobial efficacy of Flavonoids, Alkaloids and Steroids of *Nerium oleander* Linn against some pathogenic bacteria. *International Journal of Drug Discovery and Research*. 6(3):119-127
- Mallet JF, Cerrati C, Ucciani E, Gamisans J, Gruber M (1994) Antioxidant activity of plant leaves in relation to their alpha-tocopherol content. *Food Chemistry*. 49(1):61-65.
- Mohadjerani M (2012) Antioxidant activity and total phenolic content of *Nerium oleander* L. grown in North of Iran. *Iranian Journal of Pharmaceutical Research: IJPR*. 11(4):1121-1126.
- Montaño JM, Morón E, Orta ML, Mateos S & Lázaro M (2013) A hydroalcoholic extract from the leaves of *Nerium oleander* inhibits glycolysis and induces selective killing of lung cancer cells. *Planta Medica*. 79(12):1017-1023.
- Mouhcine M, Amin L, Saaid A, Khalil H & Laila B (2019) Cytotoxic, antioxidant and antimicrobial activities of *Nerium oleander* collected in Morocco. *Asian Pacific Journal of Tropical Medicine*. 12(1):32.

- Namian, P, Talebi T, Germi KG & Shabani F (2013) Screening of biological activities (antioxidant, antibacterial and antitumor) of Nerium oleander leaf and flower extracts. *American Journal of Phytomedicine and Clinical Therapeutics*, 10(11):378-384.
- Oryan A, Maham M, Rezakhani A, Maleki M (1996) Morphological studies on experimental oleander poisoning in cattle. *Journal of Veterinary Medicine*. 43(1-10):625-634
- Ramandeep K, Vikas G, Francis CA, & Parveen B (2015) Scope and bottlenecks in clinical trials of herbal drugs-present scenario. *Journal of Pharmaceutical Research*. 14(1):26-32.
- Raut B & Khanal DP (2011) Present status of traditional healthcare system in Nepal. *International Journal of Research in Ayurveda and Pharmacy*. 2(3):876-882.
- Saeed N, Khan MR & Shabbir M (2012) Antioxidant activity, total phenolic and total flavonoid contents of the whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*. 12(1):221.
- Samatha Talari Shyamsundarachary (2012) Quantification of Total Phenolic and Total Flavonoid contents in extracts. *Academic Sciences Asian Journal of Pharmaceutical and Clinical Research*. 5(13):201-2013.
- Shrestha S, Bhandari S, Aryal B, Marasini BP, Khanal S, Poudel P, Rayamajhee B, Adhikar B, Bhattarai BR & Parajuli N (2021) Evaluation of phytochemical, antioxidant and antibacterial activities of selected medicinal plants. *Nepal Journal of Biotechnology*. 9(1):50-62.
- Singhal KG & Gupta GD (2012) Hepatoprotective and antioxidant activity of methanolic extract of flowers of Nerium oleander against CCl<sub>4</sub>-induced liver injury in rats. *Asian Pacific Journal of Tropical Medicine*. 5(9):677-685.
- Singleton VL & Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 16(3):144-158.
- Stankovi, Milan S (2011) Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science*. 33:63-72.
- Trease GE & Evans WC (2009) *Plants in medicine: The origins of pharmacognosy*. Pharmacognosy. Edinburg, London, NY, Philadelphia, St. Louis, Sydney, Toronto: W. Saunders. 3-4.
- Vábková et.al (2012) Determination of total phenolic content, total flavonoid content and FRAP in culinary herbs in relation to harvest time. *Acta Universitatis Agriculturae ET Silviculturae Mendellanae Brunensis*. LX (1):167-172.
- Yuan H, Ma Q, Ye L & Piao G (2016) The traditional medicine and modern medicine from natural products. *National Library of Medicine*. 21(5):559.
- Zehiroglu C, & Sarikaya SB (2019) The importance of antioxidants and place in scientific and technological studies. *Journal of Food science and Technology*. 56(11):4757-4774.
- Zia A, Siddiqui BS, Begum S, Siddiqui S & Suria A (1995) Studies on the constituents of the leaves of Nerium oleander on behavior pattern in mice. *Journal of Ethnopharmacology*. 49(1):33-39.