

EXTRACTION, PHYTOCHEMICAL SCREENING, TLC AND *IN-VITRO* ANTI-OXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *NERIUM OLEANDER*

Ruchi Acharya*, Bhawna Sharma, Varsha Saratkar, Monika Amarghade, Jaswinder Mehta,
Peenu Mahendra Joshi

Department of Botany, Career College, Bhopal

*Corresponding Author's E mail: rch.acharya@gmail.com

Received 17 April 2021; Revised 22 April 2021; Accepted 11 May 2021, Available online 10 July 2021.



Cite this article as: Acharya R, Sharma B, Saratkar V, Amarghade M, Mehta R and Joshi PM. Acharya et al. Extraction, Phytochemical Screening, TLC and In-Vitro Anti-Oxidant Activity of Hydroalcoholic Extract of *Nerium Oleander*. Asian Journal of Pharmaceutical Education and Research. 2021; 10(3): 21-29.

<https://dx.doi.org/10.38164/AJPER/10.3.2021.21-29>

ABSTRACT

The objectives of this study are to screen the extraction, phytochemical screening, TLC and *In-vitro* anti-oxidant activity of hydroalcoholic extract of *Nerium oleander*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. Preliminary phytochemical studies of the extract were done according to the published standard methods. TPC of hydroalcoholic extract of *Nerium oleander* showed the content values of 0.475mg/100mg and followed by TFC were 1.081mg/100mg. DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity, the inhibitory concentration 50% (IC₅₀) value of *Nerium oleander* hydroalcoholic bark extract was found to be 85.89µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed. Thin-layer chromatographic profiling revealed quercetin and gallic acid present in herbal extract.

Keywords: *Nerium oleander*, Qualitative, Quantitative phytochemical, TLC, Antioxidant activity.

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc^{1,2}. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders³. Almost all organisms are protected up to some extent by free-radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids,

polyphenols, flavonoids and glutathione. Prior and Cao⁴, reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology⁵. *Nerium oleander* L. is an evergreen shrub or small tree in the dogbane family *Apocynaceae* and is planted throughout the tropical region of the world as garden and roadside trees. Previously cardiotonic, antibacterial, anticancer and anti platelet aggregation activities and also its depression in the central nervous system have been reported from this species⁶⁻⁷. Flavonoids are a class of compounds isolated in large quantities from genus of *Nerium*. Flavonoids account for approximately two thirds of the phenolics in our diet as well as a high percentage of the secondary metabolites in *Nerium oleander*⁸. There is also a report on antioxidant activity of essential oil of *Nerium oleander* flower⁹. Keeping this in view, the present study has been conducted to evaluate the extraction, phytochemical screening, TLC and *in-vitro* anti-oxidant activity of hydroalcoholic extract of *Nerium oleander*.

MATERIAL AND METHOD

Plant material

Leaves of *Nerium oleander* were collected from local area of Bhopal (M.P.) month of February, 2021. Plant material (Leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade.

Extraction by maceration method

124.13 gram of powdered leaves of *Nerium Oleander* were exhaustively extracted with hydroalcoholic solvent (methanol: aqueous: 80:20) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extract¹⁰.

Phytochemical screening

Phytochemical examinations were carried out extracts as per the following standard methods.

Thin layer chromatography

Extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples.

Detection and Calculation of R_f Value

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Total phenol content estimation

The total phenol content of the extract was determined by the modified folin-ciocalteu method¹¹. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method¹². 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the

estimation of flavonoids. 1 ml of 2% AlCl_3 solution was added to 3 ml of extract or each standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

***In-vitro* antioxidant activity of extract of *Nerium Oleander* using DPPH method**

DPPH scavenging activity was measured by the spectrophotometer¹¹. Stock solution (6 mg in 100 ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 $\mu\text{g/ml}$) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

RESULTS AND DISCUSSION

Preliminary phytochemical studies of the extract were done according to the published standard methods. Phytochemical analysis revealed the presence of phenols and flavonoids Table 1. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X + 0.011$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y = 0.032X + 0.018$, $R^2 = 0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance. TPC of hydroalcoholic extract of *Nerium oleander* showed the content values of 0.475 mg/100 mg and followed by TFC were 1.081 mg/100 mg Table 3. DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *Nerium oleander* hydro-alcoholic bark extract was found to be 85.89 $\mu\text{g/ml}$ as compared to that of ascorbic acid (17.68 $\mu\text{g/ml}$). A dose dependent activity with respect to concentration was observed Table 4. Thin-layer chromatographic profiling revealed quercetin and gallic acid present in herbal extract table 3.

Table 1: Result of Phytochemical screening of *Neriumoleander*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	Hager's Test:	-ve
2.	Glycosides	
	Legal's Test:	-ve
3.	Flavonoids	
	Lead acetate Test:	+ve
4.	Diterpenes	
	Copper acetate Test:	+ve
5.	Phenol	
	Ferric Chloride Test:	+ve
6.	Proteins	
	Xanthoproteic Test:	+ve
7.	Carbohydrate	
	Fehling's Test:	+ve
8.	Saponins	
	Froth Test:	+ve

Table 2: Calculation of R_f Value of hydroalcoholic extract of *Neriumoleander*

S.No.	Compound	R _f Value
1.	Quercetin	0.59
2.	Hydroalcoholic extract	0.27, 0.44, 0.51, 0.61, 0.66,
	Long UV	0.77, 0.92
	Short UV	0.33, 0.46, 0.57, 0.64, 0.77,
	Normal light	0.92
		0.57, 0.61, 0.75, 0.92
3.	Gallic acid	0.45
4.	Hydroalcoholic extract	0.47, 0.58, 0.63, 0.80, 0.94
	Long UV	0.32, 0.45, 0.67, 0.81, 0.94
	Short UV	0.63, 0.81, 0.96
	Normal light	

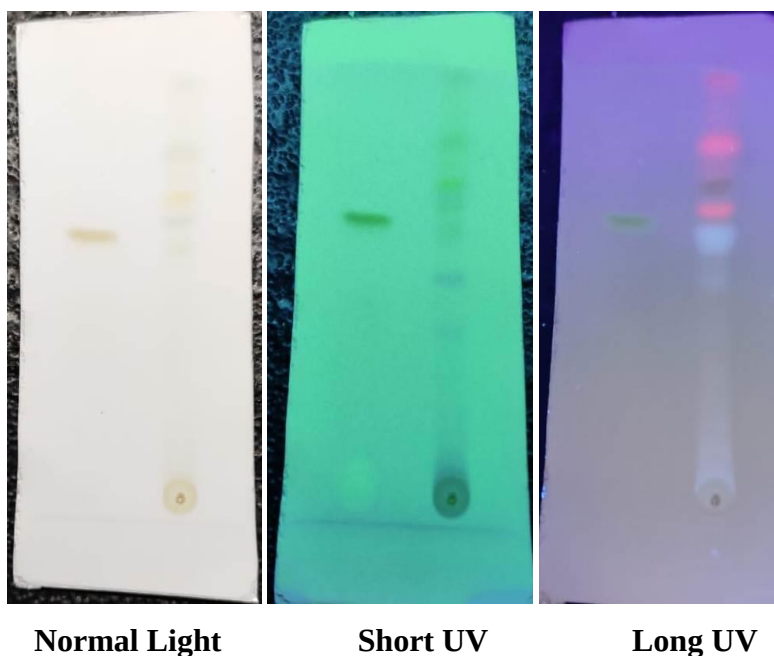


Fig. 1: Spot-1= Quercetin, Spot-2=hydroalcoholic extract of *Neriumoleander*

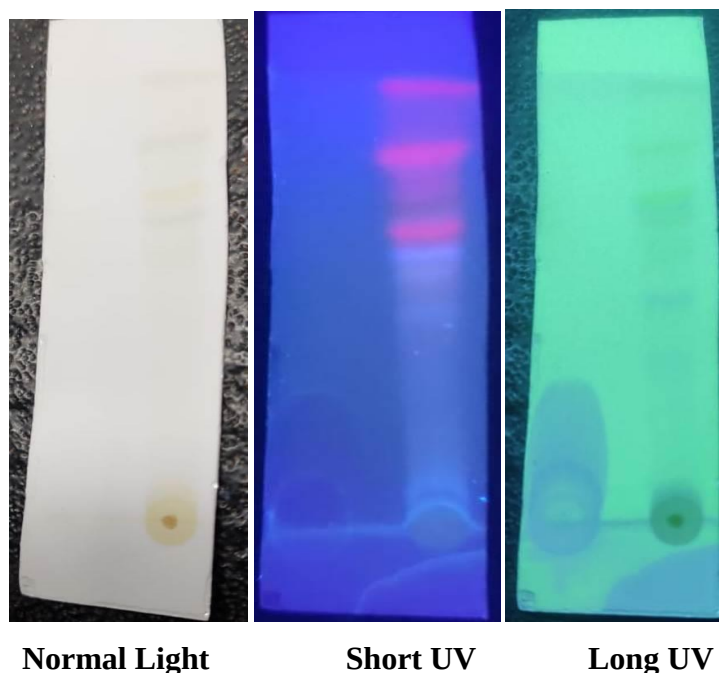


Fig. 2: Spot-1=Gallic acid, Spot-2=hydroalcoholic extract of *Neriumoleander*

Table 3: Estimation of total flavonoids and phenolcontent ofhydroalcoholic extract of *Nerium Oleander*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.475	1.081

Table 4: % Inhibition of ascorbic acid and extract of *Nerium Oleander*using DPPH method

S. No.	Concentration(μ g/ml)	Ascorbic acid	Hydroalcoholic extract
1	10	44.65	16.52
2	20	48.62	23.54
3	40	65.34	38.54
4	60	69.65	40.55
5	80	77.41	47.69
6	100	84.13	53.12
	IC 50	17.68	85.89

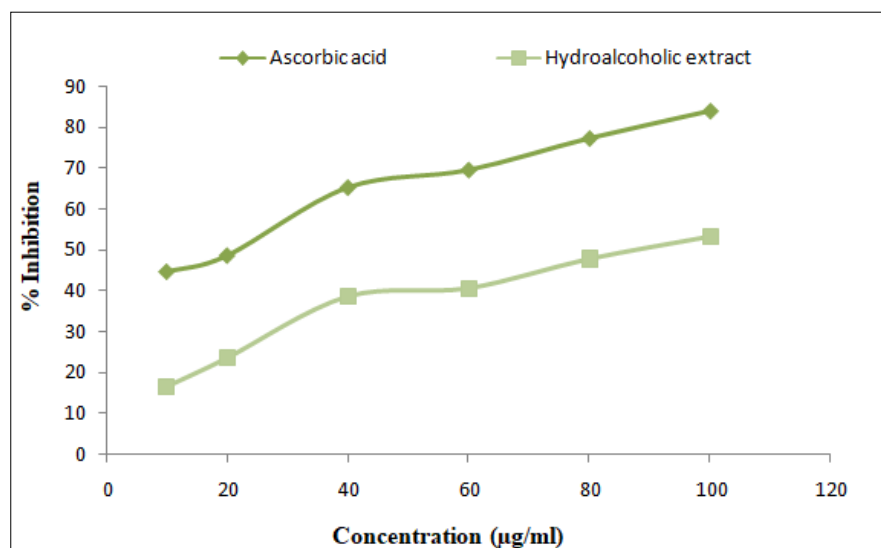


Figure 3: % Inhibition of ascorbic acid and extract of *Nerium Oleander* using DPPH method

CONCLUSION

In conclusion, we showed that *Nerium oleander* possesses an effective antioxidant activity, which includes free radical scavenging and reducing power. The Hydroalcoholic extract contains the highest amount of phenolic and flavonoid contents and also exhibited the strongest antioxidant capacity. These findings are useful for further research to identify, isolate and characterize the specific compound which is responsible for the higher antioxidant activity.

References

1. Halliwell B and Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and diseases. *Biochem J* 1984; 219:1-4.
2. Maxwell SR. Prospect for the use of antioxidant therapies. *Drugs* 1995; 49:45-361.
3. Rice-Evans CA, Miller NJ and Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 1996; 20:933-56.
4. Prior RL and Cao G. Variability in dietary antioxidant related natural product supplements: The need for methods of standardization. *J Am Nutraceutical Assoc* 1999;2:46-56.
5. Dhawan BN. Centrally acting agents from Indian plants. In: Koslow SH, Murthy RS, Coelho GV, editors. *Decade of the Brain: India/USA Research in Mental Health and Neurosciences*. Rockville: National Institute of Mental Health; 1995. p. 197-202.
6. Huq MM, Jabbar A, Rashid MA and Hasan CM. A novel antibacterial and cardiac steroid from the roots of *Nerium oleander*. *Fitoterapia*. 1999;70:5-9.

7. Burrows GE and Tyrl RJ. Toxic Plants of North America. Ames: Iowa State University Press; 2001. pp. 78–82.
8. De Majie G, Castano-Tostado E and Loarca-Pina G. Antimutagenic effects of natural phenolic compounds in beans. *Mutat. Res. Gen. Tox. En.* 1999; 441:1–9.
9. Slinkard K and Singleton VL. Total phenol analysis; automation and comparison with manual methods. *Am. J. Enol. Vitic.* 1977; 28:49–55.
10. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
11. Parkhe G and Bharti D. In Vitro Antioxidant Activity, Total Phenolic and Flavonoid Contents of Hydroalcoholic Extract of Leaves of *Lagerstroemia Parviflora* Roxb. *Journal of Drug Delivery and Therapeutics.* 2019; 9: 708-711.
12. Parkhe G and Bharti. Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of *Vitex trifolia* Linn, *Journal of Drug Delivery and Therapeutics.* 2019; 9(4-A):705-707