Original Research Article

J. Funct. Mater. Biomol. 6(2) (2022) pp 563– 567

SACRED HEART RESEARCH PUBLICATIONS

Journal of Functional Materials and Biomolecules

Journal homepage: www.shcpub.edu.in

ISSN: 2456-9429

Phytochemical Profiling, Antioxidant and Antimicrobial Activity of Nerium Oleander Ethanolic Extracts

E. Ashwini[1] and G. Sivaelango[1*]

Received on 30 October 2022, accepted on 28 November 2022,
Published online on December 2022

Abstract

The paper deals with the phytochemical screening, antioxidant and antimicrobial activity of Nerium Oleander Linn. Flower and leaves using ethanolic extract. The phytochemical analysis shows the presence of Alkaloids, Carbohydrates, Quinones, Steroids, Tannins, and Terpenoids in the leaf, flower, leaf + flower extracts. Nerium oleander ethanolic extract of leaf + flower was most effective at 50µg /ml in terms of DPPH antioxidant activity, with a scavenging capability of roughly 99% compare to standard ascorbic acid 100%. Ethanolic extract Nerium oleander leaf + flower shows the maximum antibacterial activty found at 150 µg. Ethanolic extract Nerium oleander leaf + flower extract showed inhibition for E.Coli (21mm), S. Aureus (22mm), Salmonella spp. (22mm) and Streptococcus agalactiae (18mm) respectively. Ethanolic extract of Nerium oleander leaf + flower shows the maximum antifungal activty found at 200 µg. Ethanolic extract of Nerium oleander leaf + flower extract showed inhibition for Candida albicans (25mm) respectively.

Keywords: Nerium Oleander Linn, phytochemicals, antioxidant activity and antimicrobial activity.

1 Introduction

Antimicrobial activity that inhibiting the disease-causing microbes and different kind of antimicrobials that are used for this purpose. Antimicrobial may be antifungal or antibacterial activity [1]. The plant products are used to cure the many diseases and major source for the therapeutic agents [2]. The plants have more substances like medicinal values they are usually to cure the many diseases for long time. The usage of plants that have minimal or small side effect on the human beings [3]. The plants have rich source in antifungal agents and traditionally used for the treatment of infectious diseases. According to a report by the WHO (World Health Organization) for the medicinal purpose 20,000 plant species are currently used. The usage of plants in the pharmaceutical industries increases because they suggested to use as the remedy of diseases that would have some important ingredient [4]. The plants have rich biological resource of the traditional drugs and the different types of phytochemicals can obtain from the medicinal plant that benefit for the mankind and used in manufacturing of the drugs as well as in food supplements, medicines, raw materials, pharmaceutical intermediates for synthetic drugs, folk medicines, modern nutraceuticals [5].

The antimicrobial agent that has lot of chemical compounds that are biosynthetically or synthetically produced and destroy or suppress the growth of microorganism [6]. The antimicrobial agent that serves as a drug in different countries and the pharmacologically most important plant that present in large source of antimicrobial agent [7]. The leaves and flowers have more potency and they used in many medicinal fields. In Ayurveda and naturopathy flower used in Chinese therapies and play an important role in to treat the many diseases. The researcher is using the plant extract for the activities of antibacterial and antifungal. The plants are retard to the growth of microorganism that are investigated in many laboratories around the world since 1926 [8]. The current investigation is antimicrobial activity against the novel therapeutics. The medicinal plants have antioxidant that reducing the oxidative stress and induced tissue injury [9]. The *Nerium oleander* traditionally cure the ringworm, sores, asthma, tumor, skin cancer, epilepsy, warts and herpes. The numerous naturally present in antioxidant like phenolic compounds, carotenoids and ascorbic acid more effective [10]. They inhibit the lipid peroxidation by inactive lipoxygenase to free radicals and they activate oxygen by propagation reaction cycle and heavy metal ions. The plants have antioxidant activity and capable of protective against oxidative stress in biological system. The current study of *Nerium oleander* flower extract have natural and novel antioxidants. The ethanol flower extract of this plant shows antifungal activity against different fungal pathogen. The present study was undertaken to evaluate the leaves and flowers for their possible antimicrobial activity.

* Corresponding author: e-mail:biosiva90@gmail.com
[1]Department of Biochemistry, Sacred Heart College(Autonomous), Tirupattur Dt.Tamilnadu, India-635601.

2 Experimental

2.1. Collection of plant material

The leaf and flower of *Nerium oleander* were collected from nearby villages of Tirupattur district Tamil Nadu. The leaf and flower are washed with water and dried carefully in the absence of sunlight to remove the water molecules present in the leaf and flower. The dried leaf and flower are made into fine powder using blender. Then the fine powders are stored properly in an airtight container for future purpose.

Fig. 1 *Nerium oleander* Flower and leaves

2.2 Extraction of sample

About 40gm of the fine powder of the leaf and flower of *Nerium oleander* are taken in a thimble which is placed in a Soxhlet extractor for the purpose of extraction of phytochemicals present in the leaf and flower. The extraction is carried out using ethanol. The extracts obtained are collected separately and the solvents are evaporated using vacuum distillation and dried. The dried samples are stored in an airtight container for further analysis.

2.3 Qualitative Phytochemical Screening

Phytochemical screening:

The qualitative tests were carried out in leaf, flower and leaf + flower of *Nerium oleander* by adopting standard procedure (5-7). The ethanolic extract were screened for the presence of phytochemicals.

1. **Test for alkaloids**

Mayer's test: small portion of solvent free extract was stirred with few drops of diluted HCl and filtered. The filtrate was then tested for following colour test. (a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. (b) 5gms of potassium iodide was dissolved in 20 ml of distilled water (a) and (b) was mixed and the volume adjusted to 100ml with distilled water. Appearance of cream colour precipitate with Mayer's reagents showed the presence of alkaloids.

2. Test for flavonoids

Shinoda's test:5 ml of 20% sodium hydroxide was added to equal volume of the extract. A yellow solution indicates the presence of flavonoids.

3. **Test for steroids**

Liebermann Buchard test:A small amount of sample is treated with 2ml of acetic an-hydride followed by the addition of 3ml of H2SO4 Solution. Color changes from violet to
green or blue indicates the presence of steroids.

4. **Test for terpenoids**

Salkowski Test: To 1ml of extract add 0.5ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish-brown precipitate indicates the presence of terpenoids.

5. **Test for Saponins**

Froth test:5ml of extract is diluted with 20ml of distilled water and agitated for 10 minutes. Foam is formed which indicates the presence of saponins.

6. **Test for Carbohydrates**

Fehling test: Two milliliters of each plant extract were hydrolyzed with dilute HCl, neutralized with alkali, and then heated with Fehling's solution A and B. The formation of a red precipitate was an indication for the presence of a reducing sugar.

7. **Test for tannins and phenolic compounds**

Lead Acetate test:10% lead acetate solution, 0.5g of the extract was added and shaken to dissolved. A white precipitate observed indicate the presence of tannins and phenolic compounds.

8. **Test for glycosides:**

Keller-Killani test: To 2ml of extract, glacial acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Appearance of reddish-brown color at the junction of the two liquid layers indicates the presence of glycosides.

9. **Test for Quinones**

Sulfuric acid test: One drop of concentrated sulfuric acid was added to 5 ml of each extract dissolved in isopropyl alcohol. Formation of red color indicates the presence of quinones.

2.3.1 Quantitative Phytochemical Analysis

2.3.1.1 Determination of Total Tannin

The tannin content in the sample was estimated by the method of Fagbemi *et al.,*[11]. 1 mL of saturated sodium carbonate solution was added to 0.5 mL Folin-Denis reagent. The volume was made up to 10 mL with distilled water. After 30 min the tannins content was measured at 760 nm with the spectrophotometer against experimental blank adjusted to zero absorbance. Tannic acid was used as a standard compound**.**

2.4 Antioxidant Activity

2,2-Diphenyl-1-Picrylhydrazyl Free radical scavenging activity assay by Brand-Williams *et al.*[12].

The extracts were prepared in concentrations of 10, 20, 30, 40, and 50 µg/mL for this assay. First, 3 mL of extract of each concentration was mixed with 1 mL of the 0.1 mol/L DPPH solution prepared in methanol. Next, the tubes were incubated in the dark at room temperature for 30 min and then read at 517 nm using a UV-VIS

spectrophotometer. Solvent without extract was used as a negative control and AA was used as a positive control. The effect of antioxidant capacity was observed as the color change of purple DPPH to yellow/light-yellow and inhibition values of each extract were calculated using the following equation:

Inhibition (%)= [(control — blank) — (sample — blank] ×100/(control — blank)],

Where A control is the absorbance of the negative control and A sample is the absorbance of AA or extracts. Inhibitory concentration (IC50) values were calculated with inhibition rates using a four-parameter logistic regression model after sigmoidal curves were plotted. Each of the standards and the samples were measured in triplicate and mean values were used for the calculations.

2.5 Antimicrobial Activity

Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Various antimicrobial agents are used for this purpose. Antimicrobial may be anti-bacterial, anti-fungal activity. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plant extracts [1,13].

2.5.1 Antibacterial Activity

Agar Well Diffusion Method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similar to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then a hole with a diameter of 6mm is punched aseptically with a sterile cork borer or a tip, and a volume (50–150 µl) of the ethanolic extract of *Nerium oleander* leaf, flower and mixture at desired concentration is introduced into the well. And the positive control tetracycline disc kept in the agar surface. Then agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [1,13].

2.5.2 Antifungal Activity

Agar Well Diffusion Method

Similar to the procedure used in disk-diffusion method, the Sabouraud dextrose agar (SDA) plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then a hole with a diameter of 6mm is punched aseptically with a sterile cork borer or a tip, and a volume (50–150 µl) of the ethanolic extract of *Nerium oleander* leaf, flower and mixture extract at desired concentration is introduced into the well. And the positive control is used as a fluconazole drug. Then agar plates are incubated for 25-30 Celsius for 5-7 days. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [1,13].

3 Results and Discussion

3.1 Phytochemical Analysis of *Nerium Oleander*

Qualitative phytochemical analysis of ethanolic extract of *Nerium oleander* leaf, flower, leaf +flowerwas assessed. To identify the presence of various phytochemicals like carbohydrates, steroids, tannins, alkaloids, flavonoids, glycosides, phenol, quinones, saponins, and terpenoids. In the table-1 shows the Presence of Alkaloids, Carbohydrates, Quinones, Steroids, Tannins, and Terpenoids in the leaf, flower, leaf + flower extracts. The Flavonoids, Glycosides, Phenols and Saponins are absent in the leaf, flower, leaf + flower extracts.

Table 1: Phytochemical profiling of Ethanolic extract of *Nerium oleander* leaf, flower and leaf + flower.

S. No	Phytochemicals	Ethanolic extract of *Nerium oleander* leaf	Ethanolic extract of *Nerium oleander* Flower	Ethanolic extract of *Nerium oleander* leaf + flower
1.	Carbohydrates	+	+	+
2.	Alkaloids	+	+	+
3.	Flavonoids	-	-	-
4.	Glycosides	-	-	-
5.	Phenols	-	-	-
6.	Steroids	+	+	+
7.	Quinones	+	+	+
8.	Saponins	-	-	-
9.	Terpenoids	+	+	+
10.	Tannins	+	+	+

(Symbol (+) indicate positive and (-) indicate negative)

3.1.1 Quantitative Phytochemical Analysis

The qualitative analysis of phytochemicals using a standard method for to identify the major phytochemicals like tannins, Alkaloids, Steroids, Carbohydrates, Terpenoids and Quinones. The quantitative analysis shows the result that have more concentration in tannin from leaf + flower extract and also this compared to Leaf and Flower extract.

Table 2: Quantitative Phytochemical Analysis

Phytochemicals	Ethanolic extract of *Nerium oleander* leaf	Ethanolic extract of *Nerium oleander* flower	Ethanolic extract of *Nerium oleander* leaf + flower
Total Tannins content	0.435 ± 0.00	0.432 ± 0.00	0.469 ± 0.00

Mean ± Standard deviation (n=3)

3.2 Antioxidant Activity

In the ethanolic extract of leaf, flower, leaf + flower, the DPPH radical scavenging and lowering power is shown in Figures 2. DPPH, a stable radical, can be used to investigate the antioxidant capabilities of bioactive substances. *Nerium oleander ethanolic* extract antioxidant activity was measured using ascorbic acid as a control by measuring the absorbance of the DPPH radical in the

sample at 517 nm. *Nerium oleander* ethanolic extract of leaf + flower was most effective at 50µg /ml in terms of DPPH antioxidant activity, with a scavenging capability of roughly 99% compare to standard ascorbic acid 100%.

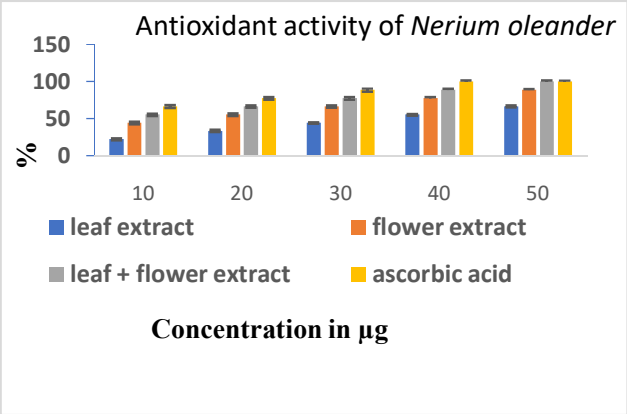

Figure 2: Antioxidant Activity of Ethanolic Extracts of *Nerium oleander* leaf, flower, leaf + flower.

3.3 Antimicrobial Activity

3.3.1 Antibacterial Activity

Antibacterial (both gram positive and gram negative) ethanol extract of *Nerium oleander* is depicted in Table 3 and (Figure 3(a), (b), (c) & (d).

Table 3: Antibacterial Activityof ethanol extract of *Nerium oleander*

Name of the Microbes	Ethanolic Extract of *Nerium oleander* leaf (mm)			Ethanolic Extract of *Nerium oleander* flower (mm)			Ethanolic Extract of *Nerium oleander* leaf + flower (mm)		
Bacteria	Concentration in µg								
	50	100	150	50	100	150	50	100	150
	Inhibition zone (mm)								
Staphylococcus aureus	11	17	19	11	20	21	11	12	22
Escherichia coli	15	16	19	16	17	20	17	13	21
Salmonella spp.	13	15	19	11	12	21	15	16	22
Streptococcus agalactiae	10	14	16	11	14	16	13	14	18

Ethanolic extract *Nerium oleander* leaf + flower shows the maximum antibacterial activty found at 150 µg. Ethanolic extract *Nerium oleander* leaf + flower extract showed inhibition for *E.Coli* (21mm), *S. Aureus* (22mm), *Salmonella spp*. (22mm) and *Streptococcus agalactiae* (18mm) respectively. The ethanolic extarct *Nerium oleander* possess wide range of antibacterial activity. Ethanolic extract *Nerium oleander* leaf + flower showed singinifiant inhibitory potential against both gram positive and garm negative bacteria.

Figure: 3(a)

Figure: 3(b)

Figure: 3(c)

Figure: 3(d)

3.3.2 Antifungal Activity

Antifungal activity of ethanol extract of *Nerium oleander* is depicted in Table 4 and (Figure 4(a), (b) & (c). Ethanolic extract of *Nerium oleander* leaf + flower shows the maximum antifungal activty found at 200 µg. Ethanolic extract of *Nerium oleander* leaf + flower extract showed inhibition for Candida albicans (25mm) respectively. The ethanolic extarct *Nerium oleander* posses wide range of antifungal activity. Ethanolic extract *Nerium oleander* leaf + flower showed singinifiant inhibitory potential against fungi.

Table 4: Antifungal Activityof ethanol extract of *Nerium oleander*

Name of the Mcro bes	Ethanolic Extract of *Nerium oleander* leaf (mm)				Ethanolic Extract of *Nerium oleander* flower (mm)				Ethanolic Extract of *Nerium oleander* leaf + flower (mm)			
fungi	Concentration in µg											
	50	100	150	200	50	100	150	200	50	100	150	200
	Inhibition zone (mm)											
Candida albicans	11	12	14	16	11	15	14	20	12	13	20	25

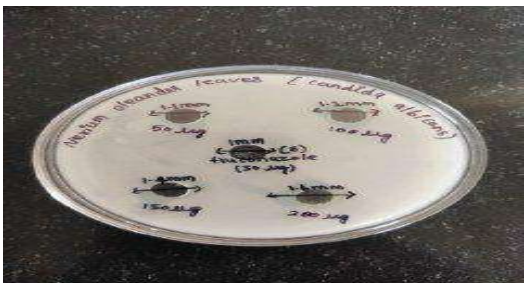

Figure: 4(a)

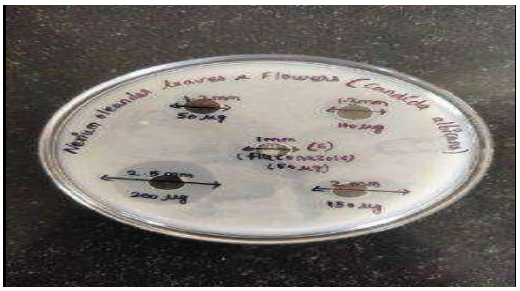

Figure: 4(b)

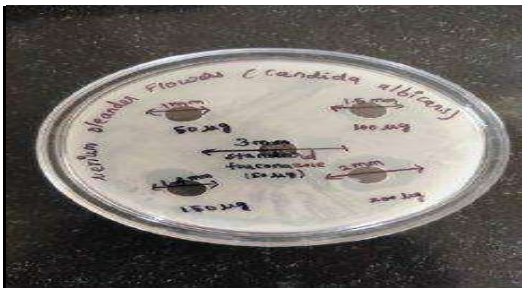

Figure: 4(c)

4 Conclusions

In the current study of ethanolic extract of *Nerium oleander* possess various important biologically important secondary metabolites such as alkaloids, tannins, steroids, and carbohydrates. Ethanolic extract was shown to possess strong antioxidant activity using *in vitro* method. The *in vitro* antioxidant potential of ethanolic extract may be due to its effect free radical-scavenging property. Total tannin content study revealed that ethanolic extract possess significant amount of these compounds. The antimicrobial study of ethanolic extract conclude that *Nerium oleander* possess significant antimicrobial activity against wide range of microbes with includes both gram-positive, gram-negative bacteria and fungus. The potent antioxidant, and antimicrobial properties of ethanolic extract of *Nerium oleander* may be mainly attributed by various phytochemicals presents in these plants. Current study revealed that, the ethanolic extract of *Nerium oleander* may be considered as alternative to treatment for various ailments like oxidative stress wide range of microbial infections.

References

[1] Magaldi S., Mata-Essayag S., Hartung de Capriles C. Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis. 8 (2004) 39-45.

[2] Joshi, B., L. Sunil and S. Anuja. Antibacterial property of different medicinal plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. Kathmandu University Journal of Science, Engineering and Technology. 5(1) (2009) 143-150.

[3] Doughari, J.H. Antimicrobial activity of Tamarindus indica Linn. Tropical J. Pharma. Res.5(2) (2006) 597-603.

[4] Nostro, A., M.P. Germano, V.D. Angelo, A. Marino and M.A. Cannatelli. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology. 30 (2000) 379-384.

[5] Tumwine, W. Implementation of the framework convention on Tobacco control in Africa: Current Status of Legislation. Int. J. Environ. Res. and Public Health. 8 (2011) 4312-4331.

[6] Lavanya, G. and G.P. Brahmaprakash. Phytochemical screening and antimicrobial acyivity of compounds from selected medicinal and aromatic plants. Int. J. Sci. &Nature. 2(2) (2011) 287-291.

[7] Mahesh, B. and S. Satish. Antimicrobial activity of some important medicinal plant against plant and human pathogens. WorldJ. Agri. Sci.4(S) (2008) 839-843.

[8] Bakht, J., Azra and M. Shafi. Antimicrobial activity of Nicotiana tabacum using different solvents extracts. Pak. J. Bot. 44(1) (2012) 459-463.

[9] Pourmorad, F., S.J. Hosseinimehr and Shahabimajd, N. Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. S. Afr. J. Biotechnol. 5 (2006) 1142-1145.

[10] Duh, P.D., Y.Y. Tu and Yen, G.C. Antioxidant activity of aqueous extract of Harnjyur (Chrysanthemum morifolium Ramat). Lebensmwiss Technol. 32 (1999) 269-277.

[11] Fagbemi TN, Oshodiu AA, Pinmoroti KO Processing Effects on some Anti-Nutritinal Factors and in Vitro Multi Enzyme Protein Digestibility (IVPD) of Three Tropical Seeds:Breadnut (Artocarpus altilis),Cashewnut (Anarcardium occidentale) and Fluted Pumpkin (Telfairia occidentalis). Pakistan Journal of Nutrition. 4(4) (2005) 250-256.

[12] Brand-Williams, W., Cuvelier, M. E., & Berset, C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technologie. 28 (1995) 25–30.

[13] Valgas, S.M. De Souza, E.F.A. Smânia, et al., Screening methods to determine antibacterial activity of natural products, Braz. J. Microbiol. 38 (2007) 369-380.