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Phytochemical analysis and antimicrobial activity of *Nerium oleander* L.

[*] Alka Bameta, Anita Kumari, Dr. Anshulika Upadhyaya

Department of Biotechnology & Allied Science, M. B. Govt. P. G. College Haldwani, Nainital, Uttarakhand, India
Molecular Biology and Genetic Engineering Collage of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Abstract
Nerium oleander L. (Family: *Apocynaceae*) is commonly known as Arali found throughout India has been used in the treatment of a wide variety of malady conditions including, asthma, eczema, ringworm, abscesses, corns epilepsy, herpes, malaria, scabies, sores, and certain tumors. The present study is therefore undertaken to analyze its phytochemical constituents and antimicrobial activity using leaf and stem samples of plant. Dried samples were used to obtain various extract viz. methanolic, ethanolic, petroleum ether and chloroform. All the extracts were tested using Disc Diffusion Method against various Bacterial strains: *Bacillus subtilis* (gram +ve), *Staphylococcus aureus (*gram +ve), *Pseudomonas* (gram –ve), *Salmonella typhi* (gram -ve) and fungal strains: *Asparagus niger*, *Cryptococcus neoformans*, *Sacchromyces cerevisiae*, *Candida albicans*. In the case of bacterial and fungal culture used in this study methanolic extract showed the maximum amount of antimicrobial and antifungal activity than the other extract used. The phytochemical analysis showed the presence of phenol, steroids, saponins, tannins coumarin & reducing sugar in the solvent used in this study.

Keywords: antimicrobial activity, *nerium oleander*, phytochemicals and saponins

1. Introduction
Nerium Oleander belongs to the family Apocynaceae is a Mediterranean evergreen shrub with milky juice. In Hindi, it is commonly known as Kaner or Gandeera. It is widely grown as an ornamental plant in warm temperate and subtropical regions, due to its abundant and long-lasting flowering and moderate hardiness (Kingsbury, 1964; Hardinand Arena, 1974) [7, 2]. It has five species which occur from the Iberian Peninsula to Japan, (Hooker, 1895) [3]. About 155 genera and 2000 species distributed primarily in the tropics and sub tropics poorly represented in the temperate regions. *N. oleander* occurs in most countries around the Mediterranean seaboard (Portugal, Spain, Italy, Jugoslavia, Albania, Greece, Turkey, Lebanon, Syria, Israel, Jordan, Libya, Tunisia, Algeria and Morocco) and in all the larger Mediterranean islands, but is absent from the Canaries. Scattered populations may be found in the Sahara at `oueds' (seasonal watercourses) and other moist points, the species not occurring south from the desert (Ozenda, 1977) [10]. In India, the *oleander* is considered a native of the western Himalayas and west to Nepal. It is an urbanite plant widely used for ornamental purposes in streets, gardens, and hospitals. Some plants are utilized by certain heterotrophs and protected from others by their secondary toxic metabolites (Williams, 1970) [17]. *N. oleander* is one of these plants**.** Historical use of the *Nerium oleander* plant for medicinal applications has been reported in ancient texts and folklore for more than 1500 years. This species produces secondary metabolites (Paper & Franz, 1989) [11], some of which are of pharmacological interest. It used principally as an ornamental bush, its medicinal and toxicological properties have also been recognized. It is used in the treatment of a wide verity of maladies conditions including, asthma, dysmenorrheal, eczema, ringworm, abscesses, corns epilepsy, herpes, malaria, psoriasis, scabies, sores, and certain tumors (Langford and Boor, 1996) [8]. Drugs derived from *Nerium oleander* have been investigated as a treatment for cancer**.** Therapeutic use of oleander glycosides as cardiac drugs was assessed and documented in the 1930 (Shaw *et al*., 1979) [13]. In ethno botanical literature it is mentioned to be effective in the treatment of cardiac illnesses, asthma, corns, cancer and epilepsy (Fumiko, 1991; Siddiqui, 1997) [1, 14]**.** The present study focused on the antimicrobial activity and phytochemical analysis of different extracts.

2. Material and Methods
2.1 Collection of sample
The samples including leaves and stems of *N. oleander* were collected from Haldwani (Uttarakhand State) during February 2013, situated in the Nainital District of Kumaun region of Uttarakhand state (29.13°N 79.31°E) at an average elevation of 425 Mts above sea level.

2.2 Preparation of the extract
The plant materials were surface sterilized and shade-dried at ambient temperature (31 ºC) and were powdered using an electronic blender. The powdered mixture was then soaked in different solvents (ethanol, methanol, petroleum ether and chloroform) for 72 hrs. After filtering the contents using Whatman No 1 filter paper, the filtrate was then used for phytochemical analysis and antimicrobial activity.

2.3 Qualitative analysis of phytochemical constituents
The plant extracts methanol, ethanol, petroleum ether and chloroform were subjected to phytochemical analysis of the active compounds present in them. The compounds screened are reducing sugar, phenol, steroids, sugar, saponins, tannins and coumarin.

Table 1: Tests for analysis of phytochemical constituents

S. No.	Test	Observation	Inference
1	Test solution + minimum quantity of chloroform +3- 4 drops of acetic anhydride +1 drop sulphuric acid	Purple color changed to blue or green	Presence of steroid
2	Test solution+2ml Fehling's solution+3 ml of water	Red –orange color	Presence of reducing sugar
3	Test solution +small quantity of anthrone +few drops concentrated Sulphuric acid and heat	Green to purple color developed	Presence of sugar
4	Test solution +water + lead acetate	White precipitate formed	Presence of tannin
5	Test solution in the alcohol +1 drop of neutral ferric chloride.	Intense color formed	Presence of phenol
6	Test solution+ water and shake	Foamy lather formed	Presence of saponin
7	Test solution + 10 % NaOH	Yellow color developed	Presence of coumarin

2.4 Selection of microbial culture for antimicrobial activity

Bacillus subtilis (gram +ve), Staphylococcus aureus (gram +ve), Pseudomonas (gram –ve), Salmonella typhi (gram -ve), Aspergillus niger, Cryptococcus neoformans, Saccharomyces cerevisiae and Candida albicans were selected for the study of antibacterial and antimicrobial activity. The cultures were maintained on nutrient agar. A 24 hours broth culture of the selected species was used for the antimicrobial screening.

2.5 Preparation of Culture

Agar plates were prepared after sterilization by an autoclave. After sterilization, bacterial and fungal strains were inoculated into the media. 20 ml of the medium is poured into each Petri plates and allowed to solidify at room temperature.

2.6 Screening of Antimicrobial Activity-Disc diffusion method (Maruzzella & Henry, 1958) [9]

After solidification, the disc (5 mm) of Whatman no 1 filter paper was soaked in crude extracts in different solvents (methanol, ethanol, chloroform and petroleum ether) were placed carefully in the center of Petri-plates containing the solidified media. To compare the antimicrobial activity same concentration of the solvent using disc is placed in the plate which acts as a control to our crude solvents. The plates were incubated at 37 ^{0}C for 24 hrs for bacterial culture and 28^{0}C for 48 hrs for fungal strains. The antimicrobial activities were assayed by measuring the resultant 'zone of inhibition' with the help of the ruler.

3. Results and discussion

3.1 Phytochemical analysis

The preliminary phytochemical screening results of *Nerium oleander*, leaf and stem extract showed that stem contains more phytochemicals than leaves. The similar findings were obtained by Suganga *et al.,* (2012); Santhi (2011) [15, 12] while studying phytochemical and antimicrobial activity from *Nerium oleander*. They obtained the presence of various phytochemicals. Results of present phytochemical analysis of leaf and stem extract reported in Table 2 & 3 respectively. Out of seven phytochemical tests, different extracts of Leaf showed the presence of reducing sugar, phenol, steroid and coumarin. Negative results were recorded for saponin, sugar, and tannin. The methanolic and petroleum ether extract showed the presence of reducing sugar, steroids and coumarin while the other 2 extracts (chloroform & ethanol) showed the presence of reducing sugar and phenol respectively. In the case of stem extracts, all tests were positive. The ethanolic crude extracts showed maximum phytochemical constituents (phenol, saponin, tannin, coumarin) followed by methanol (saponin, coumarin) and chloroform (reducing sugar). While in the case of petroleum ether no phytochemical constituents were recorded.

Table 2: Phytochemical analysis of leaf crude extract with different solvent system

S. No	Phytochemical constituents	Methanol	Chloroform	Ethanol	Petroleum ether
1	Reducing sugar	+	+	-	+
2	Phenol	-	-	+	-
3	Saponin	-	-	-	-
4	Sugar	-	-	-	-
5	Tannin	-	-	-	-
6	Steroid	+	-	-	+
7	Coumarin	+	-	-	+

Table 3: Phytochemical analysis of stem crude extract with different solvent system

S. No	Phytochemical constituents	Methanol	Chloroform	Ethanol	Petroleum ether
1	Reducing sugar	-	+	-	-
2	Phenol	-	-	+	-
3	Saponin	+	-	+	-
4	Sugar	-	-	-	-
5	Tannin	-	-	+	-
6	Steroid	-	+	-	-
7	Coumarin	+	-	+	-

3.2 Antimicrobial activity

Antimicrobial activity of various plant extracts of leaf and stem was studied by measuring the zone of inhibition formed around the disc in the agar plate and the results are given in table 4. Tannu *et al.*, (2011) [16] studied the antimicrobial activity of *N. oleander* stem extract and found that all the extracts showed good activity against all the bacterial strains used. Methanolic extract of *Nerium Oleander* had the maximum zone of inhibition i. e. 28mm (Jeyachandra *et al.* 2010) [5]. Same results were recorded in this study. Out of four leaf extracts in different solvents, methanolic extract showed potent activity against *S. aureus*, *B. subtilis* and *Pseudomonas*. The highest inhibitory zone was recorded in case of *Pseudomonas* (11mm) followed by *B.subtilis* (7mm) and *S. aureus* (5mm). The methanolic crude extract showed maximum activity followed by petroleum ether.While ethanol and chloroform was unable to show any antibacterial activity against any bacterial culture. None of the crude extracts were able to inhibit the growth of *S typhi.* Hussain and Gorsi (2004) [4] studied that root and leave ethanolic extract of *Nerium oleander* shows effective action against gram +ve and gram –ve bacteria and fungus. According to the Jude (2013)[6] *Nerium Oleander*, *Lippia nodiflora*, *Wattakaka volubilis* and *Weinmanna tinctoria* has possessed the highest zone of inhibition against *E. coli, K. pneumonia, S. typhi, P. vulgaris* and *P. mirabilis*. In our study Antifungal activity was recorded for four strains (*Asparagus niger*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, *Candida albicans*)

Table 4: Antimicrobial activity of plant extracts (Disc diffusion method)

Pathogenic microorganisms	Zone of inhibition (mm)							
	Leaf Crude Extracts				Stem Crude Extracts			
	MT	ET	CF	PE	MT	ET	CF	PE
Bacterial cultures								
B.subtilis	7	-	-	3	-	3	-	-
S.aureus	5	-	-	3	8	3	-	
Pseudomonas	11	-	-	3	9	-	-	-
S.typhii	-	-	-	0	3	-	-	-
Fungal cultures								
A.niger	-	-	-	-	-	-	-	-
C.neoformans	6	-	-	3	3	-	-	-
S.cerevisiae	4	2	-	-	4	2	2	-
C.albicans	3	-	3	-	3	-	-	-

(MT- Methanol, ET- Ethanol, CF- Chloroform and PE- Petroleum ether)

Maximum antifungal activity was found against *C. neoformance* (6mm) followed by *S. cerevisiae* (4mm) and *C. albicans* (3mm) in the case of leaf methanolic extract. Further investigation revealed that methanol crude extract was the potent antifungal agent followed by chloroform and ethanol crude extract, while petroleum ether crude extract was unable to inhibit the fungal growth. In the case of the stem, maximum antifungal activity was recorded against *C. neoformance* (4mm) followed by *S. cerevisiae* (3mm) and *C. albicans* (3mm). It is also found that methanolic crude extract was the potent anti fungal agent followed by ethanol crude extract, while petroleum ether and chloroform was unable to inhibit the fungal growth. Leaf, as well as stem crude extracts, did not show antifungal activity against *A. niger*.

4. Conclusion

The preliminary phytochemical screening results of *Nerium oleander*, leaf and stem extract showed that stem of *N. oleander* contain more phytochemicals than leaves. Our study indicates that the leaf and stem crude extracts of *Nerium oleander* has potential antimicrobial activity against some microbial species. So the use of the biologically active compounds from this plant could represent a natural alternative source of antibiotics.

5. References

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