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ORIGINAL ARTICLE **OPEN ACCESS**

Antimicrobial Activity and Phytochemical Analysis of Leaf, Stem and Flower Extracts of *Nerium oleander*.

***Jadhav A. V., Jadhav A.A. and Pathade G. R.**
Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth, "Deemed to be University", Karad

ABSTRACT

Phytochemicals are compounds produced by phytochemical plants to help them thrive or thwart competitors, predators or pathogens and can be used as poisons or traditional medicines. The present work is done to evaluate the phytochemical analysis of extracts of leaves, stem and flower of Nerium oleander. The antimicrobial activity of varying concentrations of solvent extracts of the plant against the pathogens Shigellasonei, Escherichia coli, Staphylococcus aureus, Proteus vulgaris and Salmonella typhi was investigated by the zone of inhibition in disc diffusion method. Phytochemical screening of the plant extract was done for steroid, tannin, saponin, anthocyanin, coumarin, alkaloids, proteins, amino acids, phytosterol, leucoanthocyanin, cardiac glycosides and flavonoids. The investigation showed that the plant extracts showed good antimicrobial activity against the selected pathogens and showed presence of certain useful phytochemicals.

***Key Words**: Nerium oleander, phytochemicals, disc diffusion method, antimicrobial activity.*

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INTRODUCTION

Phytochemistry deals with the study of chemicals derived from plants. Phytochemicals are chemical compounds produced by plants to thwart competitors, predators or pathogens by their antimicrobial activity. Phytochemicals are under research for their possible health effects. These can be extracted from the plants and isolated to define their structure and tested in the laboratory model systems to identify the specific role in given biological activity and its physiological action on the human body.

Nerium oleander, a frequently grown shrub in garden and public areas, contains numerous phytochemicals [1]. The plant contains cardiac glycosides- oleandrin and nerine that are significantly toxic and should be used with caution. It is used as rat poison and an insecticide. The decoction of leaves and flowers of *Nerium oleander* are cardiotonic, diuretic, anti-cancerous, diaphoretic, anti-inflammatory, anti-diabetic, antibacterial and antifungal in nature [2, 7]. The plant produces phytochemicals that are secondary metabolites such as alkaloids, flavonoids and steroids [3, 8]. Researchers have been exploring the potential of this plant for its therapeutic properties [4].So the aim of the present study was to explore the plant *Nerium oleander* for its antimicrobial activity on various pathogens and its phytochemical analysis.

MATERIAL AND METHODS

The stem, leaves and flowers of *Nerium oleander* were collected from Karad region. These were separated, washed, dried in shade and powdered. The solvents acetone, methanol, ethanol, hot water and cold water were used to extract the active agents from *Nerium oleander*. The extracts were made by mixing 5g of air-dried powder of leaves, stem and flowers in 20 ml solvents, leaving it overnight at 28^0C for evaporation and filtering it. This stock solution was used later on to make different solutions of varying concentrations [5]. The suspensions of 18 hrs old cultures of *E. coli, Staphylococcus aureus, Shigellasonei, Salmonella typhi* and *Proteus mirabilis* with turbidity adjusted to 108 Fu/mi were used to determine the antimicrobial activity of the extracts by disc diffusion method on Muller Hinton agar plate. After spreading 0.1ml of bacterial suspension on the plates, the disc of extracts were kept for diffusion for 5 min. These plates were incubated at 28^0C for 24 hrs and observed for zone of inhibition [6]. The phytochemical analysis of the extracts for steroids, tannin, saponin, anthocyanin, coumarin, alkaloids (Wagner test), proteins (Xanthoproteic test), amino acids (Ninhydrin test), phytosterol (Salkowskis test) phenol (ferric Chloride

test), leucoanthocyanin, cardiac glycosides (Keller-Killani test) and flavonoids (Alkaline reagent test) were done [4,9].

RESULTS AND DISCUSSION

Table 1 shows that the maximum inhibition was seen in 200mg/ml stem extracts of *Nerium oleander* in ethanol with zone of 11mm against *Staphylococuus aureus* and *Shigellasonei. Salmonella typhi* was inhibited by 200 mg/ml methanol extract showing inhibitory zone of 12mm. Table 2 shows the inhibition by 200mg/ml ethanol extract of leaves on *Shigellasonei* and *Proteus vulgaris* with a zone of 10mm and methanol extract of leaves with zone of 10mm against *Salmonella typhi*. Table 3 shows that the maximum inhibition was in 200mg/ml ethanol extract of flowers with zone of 12mm against *Shigellasonei*. *Escherichia coli* was inhibited by 200 mg/ml ethanol and acetone extract showing inhibitory zone of 10 mm. *Proteus vulgaris* was affected by 200 mg/ml of ethanol extract with an inhibitory zone of 11mm. Table 4 shows that cardial glycosides are present in all the extracts. Steroids, tannin, saponin, anthocyanin, coumarin, alkaloids and phytosterols are present in majority of the extracts.

Table 1: Inhibitory Zone Diameter of Stem Extracts of *Nerium oleander.*

Name of test organisms	Diameter of Zone Inhibition (mm) width													
	Ethanol extract (mg/ml)				Methanol extract (mg/ml)				Acetone extract(mg/ml)				Hot water	Cold water
	Control	50	100	200	Control	50	100	200	Control	50	100	200	-	-
Staphylococcus aureus	5	7	9	11	5	7	9	10	4	6	7	8	-	-
Escherichia coli	5	6	8	9	3	6	7	8	4	5	6	8	-	-
Shigellasonei	5	5	9	11	5	6	8	9	4	6	7	8	-	-
Proteus vulgaris	5	5	9	10	5	7	9	10	3	6	7	8	-	-
Salmonella typhi	4	5	7	10	4	6	10	12	4	7	9	11	-	-

Table 2: Inhibitory Zone Diameter of Leaves Extracts of *Nerium oleander.*

Name of test organisms	Diameter Of Zone Inhibition (mm) width													
	Ethanol extract (mg/ml)				Methanol extract (mg/ml)				Acetone extract(mg/ml)				Hot water	Cold water
	Control	50	100	200	Control	50	100	200	Control	50	100	200	-	-
Staphylococcus aureus	5	6	6	8	5	6	6	6	4	5	5	5	-	-
Escherichia coli	5	9	9	9	3	5	5	5	4	6	6	6	-	-
Shigellasonei	5	10	10	10	5	6	7	7	4	5	5	5	-	-
Proteus vulgaris	5	8	9	10	5	6	7	7	3	7	7	7	-	-
Salmonella typhi	4	5	7	7	4	10	10	10	4	7	7	7	-	-

Table 3: Inhibitory Zone Diameter of Flower Extracts of *Nerium oleander.*

Name of test organisms	Diameter of zone inhibition (mm) width													
	Ethanol extract (mg/ml)				Methanol extract (mg/ml)				Acetone extract (mg/ml)				Hot water	Cold water
	Control	50	100	200	Control	50	100	200	Control	50	100	200	-	-
Staphylococcus aureus	5	6	7	8	5	5	7	9	4	4	7	9	-	-
Escherichia coli	5	5	7	10	3	4	5	7	4	4	7	10	-	-
Shigellasonei	5	7	10	12	5	6	7	8	4	5	6	8	-	-
Proteus vulgaris	5	7	9	11	5	5	6	9	3	5	7	8	-	-
Salmonella typhi	4	5	6	10	4	4	5	8	4	6	8	9	-	-

Table 4: Results Of Phytochemical Analysis of Stem, Leaves and Flowers Extracts in Ethanol, Methanol and Acetone, Hot and Cold Water of *Nerium oleander.*

Phytoconstituents	***Nerium oleanderr* extracts**								
	Ethanol			Methanol			Acetone		
	Leaves	Stem	Flower	Leaves	Stem	Flower	Leaves	Stem	Flower
Steroids	+	+	-	+	+	+	+	+	+
Tannin	+	+	+	+	+	-	+	+	+
Saponin	+	+	+	+	+	+	-	+	+
Anthocyanin	+	+	+	-	+	+	+	+	+
Coumarin	+	+	+	+	+	+	+	+	-
Alkaloids	+	+	+	+	+	+	+	-	+
Proteins	-	-	+	+	-	+	-	+	+
Amino acids	-	+	-	-	+	+	-	-	+
Phytosteroid	+	+	+	+	+	+	+	+	-
Phenol	+	+	+	-	-	+	+	+	+
Leucoanthocyanin	-	-	-	-	+	+	-	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	-	-	+	+	+

(+) sign indicates positive results (-) sign indicates negative results

CONCLUSION

The stem, leaves and flower extracts of *Nerium oleander* showed antimicrobial activity against the pathogens *Escherichia coli, Staphylococcus aureus, Shigellasonei, Salmonella typhi* and *Proteus vulgaris.* The stem, leaves and flower extracts in hot and cold water showed no antimicrobial activity. Maximum inhibition was seen in 200mg/ml stem extracts in methanol with a zone diameter of 12mm and acetone with a zone diameter of 11mm against *Salmonella typhi*. *Shigellasonei* and *Staphylococuus aureus* were inhibited by 200 mg/ml ethanol extract of stem showing a zone of with zone of 11mm. The leaves extracts showed maximum inhibition was in 200mg/ml ethanol extract with zone of 10mm against *Shigellasonei* and *Proteus vulgaris*. *Salmonella typhi* was inhibited with a zone diameter of 10mm by methanol extract of leaves. The flower extracts showed maximum inhibition in 200mg/ml in ethanol extract with zone of 12mm against *Shigellasonei*. *Proteus vulgaris* was inhibited by 200 mg/ml ethanol extract of flowers showing inhibitory zone of 11 mm. *Escherichia coli* and *Salmonella typhi* were inhibited by 200 mg/ml of ethanol extract of flowers showing an inhibitory zone of 10mm diameter. The leaves extract in acetone with 200 mg/ml inhibited *Escherichia coli* showing an inhibitory zone of 10mm. Phytochemical investigation of *Nerium oleander* in various extracts revealed the presence of many active phytoconstituents like cardial glycosides, steroids, tannin, saponin, anthocyanin, coumarin, alkaloids and phytosterols.

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