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Research Article

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ANTIMICROBIAL ACTIVITY OF NERIUM INDICUM

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ABSTRACT

Present investigations are aimed towards the antimicrobial activity of ethanolic and aqueous extracts of *Nerium indicum* against *Klebsiella pneumonia, Staphylococcus aureus* and *Streptococcus pyogenes.* The investigation reveals that the alcoholic extract of *Nerium indicum* possess a strong antimicrobial activity against these organisms with a maximum zone of inhibition of 34 mm with *Streptococcus pyogenes,* 23 mm with *Staphylococcus aureus* and 22 mm with *Klebsiella pneumonia* in 100 mg of alcoholic extract impregnated discs. The results were compared with Chlorampnenicol impregnated discs. Present investigations reveal the antimicrobial potential of *Nerium indicum* which is freely available plant in Libya and may be explored

as an alternative for the treatment of many microbial infections.

KEYWORDS: Nerium indicum, Chlorampnenicol, microbial infections.

INTRODUCTION

The Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents, research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means, such as species-specific phages. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant. Therefore, there is a need to look for substances from other sources, especially Plants, with proven antimicrobial activity.

Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. ^[1-5] More recently, medicinal plant extracts were developed and

proposed for use as natural antimicrobials. ^[6-8] However, little or no work has been done on the effects of plant extracts as a potent antimicrobial agent. The present study was conduced to determine the effect of different extracts of medicinal plant as a possible alternative to antibiotics. In the present study, *Nerium indicum* was screened against multi-drug resistant bacteria including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Streptococcus pyogenes*.

MATERIAL AND METHODS

The leaves of *Nerium indicum* were collected from Derna, Libya, dried in shade for about 10 days, powdered and passed through the sieve. The coarse powder was used for the extraction.

Preparation of Alcoholic Extract

Extraction of plant material was carried out by cold maceration method. About 100g of powder was weighed and macerated in about 400ml of alcohol (methanol) and kept for about four days at room temperature for the complete extraction of the plant material. The alcoholic extract was filtrated and evaporated to dryness.

Preparation of Aqueous Extract

About 100g of powder was weighed and macerated in about 400ml distilled water and kept for about four days at room temperature for the complete extraction of the plant material. The aqueous extract was filtrated and evaporated to dryness.

Preparation of Samples

Sample 1: contained 50 mg of alcoholic extract in 10ml of ethanol.
Sample 2: contained 100 mg of alcoholic extract in 10ml of ethanol.
Sample 3: contained 100 mg of aqueous extract in 10ml of distilled water.
Standard: Discs impregnated in Chloramphenicol were used as standard (CT0012B, C10 mcg Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, England).

Preparation of culture media and inoculation of bacteria

The required quantity of nutrient agar powder was weight and put it in 500 ml conical flask. Sufficient amount of distilled water was added and heated on water bath to melt the media. The melted media was autoclave at 121°C for 20 minute. It was cooled to 55°C (to prevent excess condensation of moisture when the liquid agar solidified in the Petri dish). Agar was poured into the plates, lowering the cover into place immediately and gently tilted the plate from side to side (to distribute the agar uniformly over the bottom). Agar medium was allowed to harden, undisturbed, until it was thoroughly solidified.

Preparation of Blood Media: It is nutrient agar containing 5-10% of blood.

Inoculation of bacteria and determination of antimicrobial activity

The culture media containing following different microorganisms were used in the present experimentation.

- Klebsiella pneumonia
- Staphylococcus aureus
- Streptococcus pyogenes

RESULT & DISCUSSION

In the present investigations, antimicrobial activity of methanolic and aqueous extracts of *Nerium indicum* against *Klebsiella pneumonia, Staphylococcus aureus* and *Streptococcus pyogenes* were investigated and the observations are recorded in Table.

The investigations reveal that the alcoholic extract of *Nerium indicum* possess a strong antimicrobial activity against *Streptococcus pyogenes* with a maximum zone of inhibition of 34 mm and 17 mm in 100 mg and 50 mg concentrations respectively as compared to standard Chloramphenicol impregnated discs where the zone of inhibition was found to be 9 mm. The aqueous extract could not show any zone of inhibition in 100 mg concentration even after 48 hours of incubation.

In case of *Staphylococcus aureus*, alcoholic as well as aqueous extracts both showed antimicrobial activities against the said organism. The maximum zone of inhibition was recorded in 100 mg concentration (23 mm), followed by 50 mg concentration (18 mm) and aqueous extract (14 mm) as compared to Chloramphenicol (15 mm).

The aqueous extract did not show any activity against *Klebsiella pneumonia* but the alcoholic extracts in 100 mg and 50 mg concentrations showed 22 mm and 15 mm of zone of inhibitions respectively as compared to the standard in which 22 mm zone of inhibition was recorded. It was also observe that the growth of microorganisms restarted in *Streptococcus pyogenes* in 50 mg concentration of alcoholic extract and *Klebsiella pneumonia* in both the concentrations of alcoholic extracts.

Table: Determination of Antimicrobial activity of different extracts of Nerium indicum(n=5).

Microorganism	Zone of inhibition (mm)			
	Sample 1	Sample 2	Sample 3	Standard
Klebsiella pneumonia	15	22	-	22
Staphylococcus aureus	18	23	14	15
Streptococcus pyogenes	17	34	-	9





Figure (1): Effect of methanolic and aqueous extract of *Nerium indicum* on *Staphylococcus aureus*.



Figure (2): Effect of methanolic and aqueous extract of *Nerium indicum* on *Klebsiella pneumonia*.

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Figure (3): Effect of methanolic and aqueous extract of *Nerium indicum* on *Streptococcus pyogenes*.

SUMMARY AND CONCLUSION

The Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents, research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means.

In the present investigations, antimicrobial properties of ethanolic and aqueous extracts of *Nerium indicum* against *Klebsiella pneumonia, Staphylococcus aureus* and *Streptococcus pyogenes* were investigated. The investigation reveals that the alcoholic extract of Nerium possess a strong antimicrobial activity against these organisms with a maximum zone of inhibition of 34 mm with *Streptococcus pyogenes,* 23 mm with *Staphylococcus aureus* and 22 mm with *Klebsiella pneumonia* in 100 mg of alcoholic extract impregnated discs. The 50 mg alcoholic extract was less effective while the aqueous extract was active only against *Staphylococcus aureus* with a zone of inhibition of 14 mm. The results were compared with Chlorampnenicol impregnated discs.

Nerium indicum is a freely available plant in Libya possessing a number of medicinal properties. Present investigations reveal the antimicrobial potential of *Nerium* which may be explored as an alternative for the treatment of many microbial infections.

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