

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences

Research Article.....!!!

Received: 03-07-2012; Revised: 16-08-2012; Accepted: 20-08-2012

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *NERIUM INDICUM* LEAVES

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Keywords:

Bacillus subtilis, *Escherchia coli*, *Nerium indicum* and
Ofloxacin

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ABSTRACT

Aims: Traditional knowledge suggests that certain plants exhibits moderate antibacterial potential. *Nerium indicum* may also be such a plant which can show the antibacterial property and it is not scientifically been proved that *Nerium indicum* leaves having antibacterial potentials. Hence to explore this possibility we have design this work. **Materials and methods:** The antibacterial activity of *Nerium indicum* leaf extract had been seen by using cup plate method against *Bacillus subtilis*, and *Escherchia coli*. The antibacterial activity was compared with *Ofloxacin* in nutrient agar medium. **Results and Conclusions:** It was found that benzene extract was more effective and shown high zone of inhibition (14 mm) as compared to ethanolic extract which showed relatively less zone of inhibition (11 mm) for *Bacillus subtilis*. These results were compared with zone of inhibition shown by standard Ofloxacin (32mm). Both extract had shown negligible activity against Gram negative bacteria *E.coli*.

INTRODUCTION

Despite the huge number of antibiotics have produced by pharmacological industries, the new drugs are continuously searching due to increased resistance of microorganisms to these drugs. To overcome this problem, plants are being looked upon as an excellent alternate to combat the further spread of multidrug resistant microorganisms. In ayurvedic literature, drugs are not directly indicated as antimicrobial but are used to treat such medical conditions which has role of microorganisms as etiological factor. According to ayurveda, the antimicrobial activities of these plants may be due to their vyavayi, vikasi, tikshna and ushna virya properties and could be possible source to obtain new and effective herbal medicine to treat infection or multidrug resistance microorganisms. However it is necessary to conduct the toxicity study and controlled clinical trials to bring out safe and effective medicine¹.

Since the time immemorial, the mankind is suffering from several infections caused due to various microorganisms. Table 1 shows few examples of such bacteria and bacterial infection caused by it. Generally infections are treated by using various antibiotics of synthetic origin which may have severe side effects upon prolonged use.

Table 1: Bacteria and bacterial infection

Sr. no.	Name of Bacteria	Infection caused
1	<i>Salmonella Typhi</i>	Typhoid
2	<i>Pseudomonas aeruginosa</i>	Urinary Infection
3	<i>Candida albicans</i>	Oral Infection
4	<i>Escherchia coli</i>	Local Infection

Traditional knowledge suggests that certain plants exhibits moderate antibacterial potential. *Nerium indicum* may also be such a plant which can show the antibacterial property and it is not scientifically been proved that *Nerium indicum* leaves having antibacterial potentials. Hence to explore this possibility we have conducted the work.

Objectives of Studies

1. To explore the possibility of using the traditional medicines with proper chemical and pharmacological profiles.
2. To conduct the systemic phytochemical investigation of leaves of *Nerium indicum*.
3. To perform detailed study of antimicrobial activity of leaves of *Nerium indicum*.

Introduction to plant⁹:

Nerium indicum belongs to the Apocynaceae family, grown throughout India. *Nerium indicum* is an evergreen, smooth shrub with milky juice growing up to 4 meters in height. Leaves in three, shortly stalked, coriaceous, 10 to 15 centimeter long, tapering into short petiole, dark green and shining above. Seeds are about 1.3 cm long tipped with a coma of light brown hairs. It is basically leafy all year and the inflorescences bloom from June to October. It is found in area that is 6500 feet high above sea level. It is found all over India especially in Kashmir. *Nerium indicum* leaves and plant is shown in figure 1.



Figure 1: *Nerium indicum* leaves and plant

Botanical Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Proteales
Family	Nymphaeaceae
Genus	<i>Nerium</i>
Species	<i>Indicum</i>

Active Phytoconstituents

The plant contains Glycoside; tannin and volatile oil. The leaves contain two principles: neriin and oleandrin, both are cardiac glycosides with properties similar to digitalin. The seeds contain phytosterin and l-strophnathin. The bark contains toxic glycosides: rosaginin and nerlin, volatile oil, fixed oil. *Nerium indicum's* bark yielded two toxic bitter

principles neriodorin and neriodorein. Another toxic principle is karabin. Both karabin and neriodorin are probably resins, rather than glucosides. The pharmacological actions of neriin and oleandrin resemble those of digitalis glucosides.

Traditional Uses

- Roots are bitter, acrid, astringent, anthelmintic, aphrodisiac, stomachic, febrifuge, diuretic, emetic, expectorant, cardi tonic and anticancer. Useful in cardiac asthma, renal and vesical calculi, chronic stomache, joint pains, leprosy and ulcers.
- Leaves are powerful repellent. A decoction of the leaves has been applied externally in the treatment of scabies and to reduce swellings.
- Root, locally and internally, used for abortion.
- Fresh leaves applied to tumors to hasten suppuration.
- In traditional Chinese medicine, the flowers and leaves have been used to stimulate the cardiac muscles, relieve pain and eliminate blood stasis.

MATERIALS AND METHODS

Chemicals, reagents and solvents:

All the chemicals, reagents and solvents used were of analytical grade obtained from Finar Chemicals, Mumbai.

Collection of plant materials:

Fresh leaves of *Nerium indicum* were collected from medicinal plant garden of Satara College of Pharmacy, Satara, and were authenticated by Surywanshi J. S., Dept. of Pharmacognosy and Phytochemistry. S.C.O.P., Satara. The leaves were shade dried and pulverized to a coarse powder.

Materials for Antibacterial Screening:

Alcoholic and benzene extract of collected *Nerium indicum* leaves prepared in laboratory by soxhlt extraction method. Suspension of *E coli* and *Bacillus subtilis*, Nutrient Agar medium and Sterilized Petri plates were obtained from Dept. of microbiology S.C.O.P., Satara. Standard antibiotic Ofloxacin obtained by Okasa Pharma, Satara.

Extraction of plant materials

The dried plant powder of *Nerium indicum* was extracted *successively* by using petroleum ether (40 – 60°C), benzene and ethanol (90%).

Preparation of petroleum ether (40 – 60⁰C) Extract

About 200 gm dried powder of *Nerium indicum* leaves were extracted with 500 ml of petroleum ether (40–60⁰C) at 40 to 50⁰C. The extraction was continued until the solvent in thimble becomes clear. Then extract was filtered, the solvent was distilled off and the extract was concentrated on water bath till an greasy extract was obtained.

Preparation of benzene Extract

The dried mark obtained from the previous extraction by using petroleum ether was subjected to *soxhation* with benzene. The extract then filtered and solvent was evaporated on water bath to get a solid sticky extract.

Preparation of Ethanol (90%) Extract

The dried mark obtained from the previous extraction by using benzene was subjected to soxhlation with ethanol (90%). The extract then filtered and solvent was evaporated on water bath to get a solid powdery extract.

Preliminary phytochemical screening¹⁰

Petroleum ether, Benzene and Ethanol extracts were subjected to Preliminary phytochemical screening by using standard reagents. For each extract the test solution was prepared by dissolving 1gm extract in its individual solvent and same was used for primary detection. Following phytoconstituents screened using standard test methods reported.

Test for carbohydrates

- Molish's test (General test)

Test for Anthraquinone Glycosides

- Borntrager's test for Anthraquinone Glycosides
- Modified Borntrager's test for C-Glycosides

Test for Cardiac Glycosides

- Baljet's test
- Legal's test (Test for cardenoloids)
- Test for deoxysugars (Keller-Killani test)

Test for Tannins and phenolic compounds

Color reaction with 5% FeCl₃ solution, Lead acetate solution, Gelatin solution, Bromine water and dilute Iodine solution

Test for Alkaloids

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute HCl. Shake well and filter, perform following tests:

- *Dragendroff's test*
- *Mayer's test*
- *Hager's test*
- *Wagner's test*

Test for Flavonoids

- *Shinoda test:* To dry powder or extract, add 5 ml. 95% ethanol, few drops conc. HCl and 0.5 g magnesium turnings → Pink color observed.
- To small quantity of residue, add lead acetate solution. → Yellow colored precipitate is formed.
- Addition of increasing amount of sodium hydroxide to the residue shows yellow coloration, which decolorize after addition of acid.

ANTIBACTERIAL SCREENING⁸

The antibacterial activity of *Nerium indicum leaf extract* had been seen by using *cup plate method*. For antibacterial screening firstly the bacterial suspension was collected from microbiology laboratory. Nutrient agar weighed and dissolved in distilled water. It sterilized by autoclaving. After sterilization it has been allowed to cool and then poured in to petri plates in sufficient quantity aseptically. Petri plates were kept as it is for 5 minute at room temperature for solidification of agar medium. For antibacterial screening cup had been made in solidified agar medium by using borer of 8 mm diameter. Four cups were created. Two petri plates were prepared, one for showing activity against Gram positive bacteria *Bacillus subtilis*, and one for showing activity against Gram negative bacteria *Escherchia coli*.

The extracts were dissolved in 1% dimethyl sulphoxide (DMSO) and concentration used of extract was 100 mg/ml. The antibacterial activity was compared with *Ofloxacin* (100 mg/ml) in nutrient agar medium. The petri plates impregnated with solidified agar medium get sprayed with bacterial suspension by using glass spreader. Out of these four cups first two were impregnated with both extracts Benzene (A) and Ethanolic (B)

extract, respectively; third with standard drug Ofloxacin (C) and fourth with control (D). The plates were then incubated at 37 ± 2 °C temperature for 24 hours. The zone of inhibition was observed as shown in figures 2.

RESULTS AND DISCUSSION

The result of preliminary phytochemical screening for Petroleum ether, Benzene and Ethanol extracts is shown in table 2.

Table 2: Result of Preliminary Phytochemical Screening

Sr. No	Phytoconstituents	Name of Extract		
		Petroleum Ether	Benzene	Alcohol
1	Carbohydrate	-	-	-
2	Anthraquinone glycosides	-	-	-
3	Cardiac glycosides	-	+	+
4	Tannins and Phenolic compounds	-	+	+
5	Alkaloid	-	+	+
6	Flavonoids	-	+	+

(+) for presence of Phytochemical and (-) for absence of Phytochemical

The result of antibacterial activity of ethanolic extract and benzene extract of leaves of *Nerium indicum* have been summarized in table 3.

Table 3: Result for antibacterial activity of ethanolic and benzene extracts of leaves of *Nerium indicum*

Sr. No.	Test Sample	Bacterial Species	Zone of Inhibition (in mm)
1	ethanolic extract	<i>Bacillus subtilis</i>	16
		<i>Escherchia-coli</i>	04
2	Benzene extract	<i>Bacillus subtilis</i>	10
		<i>Escherchia-coli</i>	02
3	Ofloxacin	<i>Bacillus subtilis</i>	32
		<i>Escherchia-coli</i>	10

From the result obtained in table 3 it was found that ethanolic extract was more effective and shown high zone of inhibition for *bacillus subtilis* than benzene extract. Both extract had shown negligible activity against Gram negative bacteria *E-coli*. The results of present study prove the traditional use of *Nerium indicum* leaves in various infectious diseases as mentioned earlier. This activity may be due to the presence of alkaloids, cardiac glycosides, flavonoids and tannins as active phenolic phytoconstituents in the plant as observed in earlier phytochemical screening.



Figure 2: Benzene extract (A), Ethanolic extract (B), Ofloxacin (C) and control (D) showing activity against *Bacillus subtilis*

CONCLUSION

Traditional knowledge suggests that *Nerium indicum* has the antibacterial property and it is not scientifically been proved that *Nerium indicum* leaves having antibacterial potentials. Present work was initiated with exploring possibility that leaves of *Nerium indicum* have antibacterial activity. After the results obtained by experiments outcomes it has been concluded that leaves of *Nerium indicum* possess antibacterial activity in selected extracts of benzene and ethanol against Gram positive microorganism *Bacillus subtilis* but negligible activity against Gram negative microorganism *E-coli*. Further comparative study for antibacterial activity of both extracts with standard antibiotic Ofloxacin showed relatively higher zone of inhibition for ethanolic extract than benzene extract therefore ethanolic extract possesses potential antibacterial activity over benzene extract against *Bacillus subtilis*.

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