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Minimum Inhibitory Concentration Analysis of *Nerium oleander* against Bacterial Pathogens

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ABSTRACT

Objective: In this present study, it is tried to find out the antimicrobial effect and Minimum Inhibitory Concentration (MIC) of *Nerium oleander* against *Bacillus subtilis* (IFO 3026), *Sarcina lutea* (IFO 3232), *Escherichia coli* (IFO 3007) and *Klebsiella Pneumoniae* (ATTC 10031). **Methods:** Powered leaves were prepared and used for extraction with various solvents, viz, the petroleum ether, and chloroform extract of the oleander. All the solvent extracts were evaporated to dryness. Using the disc diffusion method, the bacterial growth were inhibited, **Results:** Among the solvent extracts tested, petroleum ether extract inhibited the growth of all the tested bacteria having various degrees of inhibition zones. Highest inhibitory activity was observed against *E. coli* (1.9 cm) and minimum inhibitory concentration was observed 2 μg/ml also against *E. coli*. Both results were observed in case of petroleum ether extract. Petroleum ether extract also showed inhibitory zones of 1.8 cm, 1.4 cm and 1.5cm against *B. subtilis*, *S. lutea* and *K. pneumoniae*. On the other hand chloroform extract was observed to have inhibition zones of 1.2 cm, 1.6 cm, 1.8 cm and 1.5 cm against *B. subtilis*, *S. lutea*, *E. coli* and *K. pneumoniae* respectively. **Conclusions:** The study demonstrated that the petroleum ether extract of *N. oleander* is potentially good source of antibacterial agents. Further evaluation is necessary to identify the specific bioactive compounds, their mode of action and their nontoxic nature in vivo condition.

1. Introduction

The use of plant materials for medicines has a long history, since ancient times plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings as well as livestock[1]. Nature is a source of medicinal agents and these agents have been used for thousands of years and number of modern drugs has been isolated from natural sources[2]. Various medicinal plants have been used for years in daily life to treat diseases all over the world. Plants produce a diverse range of bioactive molecules. Higher plants as source of medicinal compounds to play a dominant role in the maintenance of human health since ancient times[3]. Infectious bacterial diseases are becoming serious threat in developing countries like Bangladesh where peoples are not aware of their primary healthcare. Due to the lack of proper treatment, indiscriminate use of antibiotics and also ignorance are the major problems to control such bacterial diseases. Nowadays, it is a common phenomenon that microorganisms are developing their resistance to many commercial antibiotics and that is the major cause of failure to treat various infectious diseases. Therefore, immense clinical problem in the treatment of infectious diseases has been raised[4]. Bangladesh possesses a rich flora of medicinal plants. Out of 5000 species of phanerogams and pteridophytes growing in this country more than a thousand are regarded as having medicinal properties. The herbal medicines may be in form of powders, liquids or mixtures, which may be raw or boiled, ointments, liniments and incisions[5].

Nerium oleander Linn. belongs to family Apocynaceae commonly known as Gandeera, is a large glabrous evergreen shrub with milky juice. Leaves in threes, shortly stalked, coriaceous, 10-15 cm long, linear-lanceolate, acuminate, tapering into the short, dark green and shining above, midrib stout; nerves numerous, spreading horizontally. Flowers are rose-coloured or white, fragrant. Calyx-lobes lanceolate. Corolla 3.8 cm. diam; fragrant, lobes rounded. Filaments hairy, appendages of anthers twice as long as the cells. Follicles 15-23 cm long, rigid, at length separating. Seeds about 13 cm long, tipped with a coma of light brown

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hairs[6].

Nerium oleander has many therapeutic uses in different traditional medicine of the world. In ethno botanical literature it is mentioned to be effective in the treatment of cardiac illnesses asthma, corns, cancer, epilepsy and also used as diuretic. The leaves and the flowers are cardio tonic, diaphoretic, diuretic, emetic, antibacterial, expectorant and have antiplatelet aggregation activity. Its various parts are reputed as therapeutic agents in the treatment of swellings, leprosy, eye and skin diseases shown that the same possibilities exist for stem of the plants[7]. Oleandrine is antiinflammatory, antitumoral, emollient and potentialises apoptosis. The hydroalcoholic and aqueous extract of the flowers is antinociceptive and cardiotonic[8]. The patented oleander extract Anvirzel™ is also used in Africa as a treatment for HIV-AIDS[9].

In this study, we aimed to explore the antibacterial effect and minimum inhibitory concentration (MIC) analysis of *N. oleander* on *Bacillus subtilis* (IFO 3026), *Sarcina lutea* (IFO 3232), *Escherichia coli* (IFO 3007) and *Klebsiella Pneumoniae* (ATTC 10031).

2. Materials and methods

Collection of Plant Leaves

Leaves of *N. oleander* were collected from Kushtia, Bangladesh, and the collected leaves were air dried and powdered (100g).

Preparation of N. oleander Leaf Extracts

The preparations of various extracts were made from the shade dried and powdered part of *N. oleander* leaves. Ten grams of each of the dried and powdered materials were macerated separately with 50 ml of chloroform and petroleum ether for 48 hours. Leaf extracts were obtained by filtering with Whitman No. 1 filter paper and the solvents were recovered by rotary evaporator. Then it was weighed.

Serial Dilution, Disk Diffusion Method and Antimicrobial Bioassay

Individual solvents were serially diluted (up to 1 μl) as describ by Booth (2006)[10]. The leaf extract was taken into 2 test tubes and add 10 ml petroleum ether and 10 ml chloroform in each tube. Later the solutions were diluted to the 512 mg/ml and serially it was diluted to 512 μg/ml, 256 μg/ml, 128 μg/ml, 64 μg/ml, 32 μg/ml, 16 μg/ml, 8 μg/ml, 4 μg/ml, 2 μg/ml and 1 μg/ml and used for minimum inhibitory concentration analysis. Antibacterial activity of the test samples was tested by disc diffusion method according to Jorgensen and Fritsche (2007)[11]. Whatman no. 1 sterile filter paper disc (6 mm diameter) was impregnated with 10 μl of *Nerium oleander* leaf extracts of petroleum ether and chloroform. Briefly, the bacterial isolates namely *B. subtilis* (IFO 3026), *S. lutea* (IFO 3232), *E. coli* (IFO 3007) and *K. Pneumoniae* (ATTC 10031) were cultured in nutrient broth at 30℃ for 24 hours and the fresh inoculums (108-109 / ml) were taken for the test. Autoclaved nutrient agar medium was cold down to 40℃, and then poured into a sterile petri dish and allowed to set. 100 μl suspension of bacterial inoculums was spread to the media and then discs (each contains 10 μl test sample) were placed on the medium and incubated for 24 hours at 37℃. Commercial neomycin disc (10 μg/disc) was used as standard antibiotic. Negative controls were prepared using the same solvent employed to dissolve the samples. Standard reference antibiotic (10 μg/disc for each), were used as positive controls for the tested bacteria[12]. The antibacterial activity was determined by measuring the diameter of zone of inhibition by centimeter scale.

3. Results

The antimicrobial studies of chloroform and petroleum ether leaf extracts of *N. oleander* were performed against four identified bacteria namely, *B. subtilis* (IFO 3026), *S. lutea* (IFO 3232), *E. coli* (IFO 3007) and *K. Pneumoniae* (ATTC 10031). Zone of inhibitions (ZI) and minimum inhibitory concentrations (MIC) were determined (Table 1 & 2).

Petroleum ether extract: In case of *B. subtilis* petroleum ether extract showed 0.8cm inhibition zone at 8 μg/ml concentration. So, MIC is 8 μg/ml against *B. subtilis*. On the other hand MIC value of petroleum ether extract against *S. lutea* is 16 μg/ml. Whereas MIC is 2 μg/ml against *E. coli*. In addition against *K. pneumoniae* MIC is 4 μg/ml. So, highest inhibitory activity was observed against *E. coli* (1.9 cm) and minimum inhibitory concentration was observed 2 μg/ml also against *E. coli*. The positive control showed 2.7cm, 2.1cm, 2.5cm and 2.5cm inhibition zone against *B. subtilis, S. lutea, E. coli* and *K. Pneumoniae* respectively.

Chloroform extract: In case of *B. subtilis* chloroform extract found to show MIC value of 16 μg/ml. Whereas MIC values of 8 μg/ml, 4 μg/ml and 16 μg/ml were found against *S. lutea*, *E. coli* and *K. pneumoniae* respectively. Here also best result was observed against *E. coli* (0.2cm). The reference antibiotic disk which was used as positive control showed 2.6cm, 2.2cm, 2.5cm and 2.3cm inhibition zone against *B. subtilis, S. lutea, E. coli* and *K. pneumoniae* respectively.

E. coli was found to be more sensitive strain than the others. The least inhibitory effects were observed for *E. coli* (Table1 and 2). Some investigators noted that sensitivity of microorganisms to chemotherapeutics differs according to type of strain. Similiar results have been observed in our study.

Table 1

Minimum inhibitory concentration (MIC) values of leaf extract of *N. oleander* in petroleum ether.

Test strains	Petroleum ether extract of *N. oleander* leaves (mg/ml and μg/ml)											
	512 mg/ml	512 μg/ml	256 μg/ml	128 μg/ml	64 μg ml	32 μg/ml	16 μg/ml	8 μg/ml	4 μg/ml	2 μg/ml	Posit-ive	Nega-tive
B. Subtilis	1.8 cm	1.4 cm	1.0 cm	0.9 cm	0.8 cm	0.6 cm	0.6 cm	0.4 cm	–	–	2.7 cm	–
S. lutea	1.4cm	1.2 cm	1.0 cm	1.0 cm	0.9 cm	0.9 cm	0.8 cm	–	–	–	2.1 cm	–
E. Coli	1.9 cm	1.7 cm	1.4 cm	1.2 cm	1.0 cm	1.0 cm	0.9 cm	0.7 cm	0.5 cm	0.3 cm	2.5 cm	–
K. Pneumoniae	1.5 cm	1.2 cm	1.0 cm	1.0 cm	0.9 cm	0.8 cm	0.7 cm	0.7 cm	0.7 cm	–	2.5 cm	–

Table 2
Minimum inhibitory concentration (MIC) values of leaf extract of *N. oleander* in chloroform.

Test strains	Chloroform extract of *N. oleander* leaves (mg/ml and μg/ml)											
	512 mg/ml	512 μg/ml	256 μg/ml	128 μg/ml	64 μg/ml	32 μg/ml	16 μg/ml	8 μg/ml	4 μg/ml	2 μg/ml	Positive control	Nega-tive
B. Subtilis	1.2 cm	1.1 cm	1.0 cm	0.9 cm	0.8 cm	0.5 cm	0.3 cm	–	–	–	2.6 cm	–
S. lutea	1.6 cm	1.5 cm	1.2 cm	0.9 cm	0.7 cm	0.4 cm	0.3 cm	0.2 cm	–	–	2.2 cm	–
E. Coli	1.8 cm	1.3 cm	1.2 cm	1.0 cm	0.9 cm	0.7 cm	0.4 cm	0.3 cm	0.2 cm	–	2.5 cm	–
K. Pneumoniae	1.5cm	1.2 cm	1.1 cm	1.0 cm	0.6 cm	0.5 cm	0.3 cm	–	–	–	2.3 cm	–

4. Discussion

Antibiotics are valuable drugs for the treatment of several human diseases; however, no doubt their overuse has made worldwide antimicrobial resistance. Therefore, scientists are giving top most priority in search of alternative antimicrobial drugs from different parts of medicinal plants[4].The results of this research work indicated that extracts of the oleander leaves which was prepared using chloroform and petroleum ether, has a strong inhibitory activity on some pathogens. All of the extracts showed inhibition effect on tested bacterial strains. The petroleum ether and chloroform extracts of oleander showed the highest antibacterial activity against *E. coli*. MIC found in this study is much lower then other previous study[13,14]. Sawi *et al* (2010) showed that gram positive bacteria *B. subtilis* is more sensitive to *N. oleander* extracts than gram negative bacteria[14]. But our findings differ from that result showing *E. coli* was more sensitive strain than the others.

Oleander is one of the most poisonous plants and contains numerous toxic compounds; the most significant of these toxins are oleandrin and neriine, which are cardiac glycosides[15]. Oleandrin suppresses activation of nuclear transcription factor-κB, activator protein-1, and c-Jun NH2-terminal Kinase 1. Oleandrin can also block NF-κB activation, as determined by consensus DNA binding, IκBα degradation, and NF-κB-dependent reporter gene expression. And these could be the molecular mechanism how oleander extracts act against biological entity[16].

Further investigation is necessary to confirm the bioactive principles of the leaf of the plant. It is essential to do the quantitative analysis was done with the help of a chromatographer in gas phase and identify the metabolites responsible for antibacterial activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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