Phytochemical analysis & Antibacterial activity of *Nerium oleander*

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**Abstract:**

*Nerium indicum* [Family: Apocynaceae] is commonly known as Arali [Tam] found throughout India, and has been used in the treatment of cancer, cardiotonic, leprosy and skin diseases. Plant parts such as root, bark and leaves are used. The present study is therefore undertaken to analyse its phytochemical constituents in solvents like Benene, Chloroform and Alcohol and to screen its antibacterial activity. The dried leaf sample is extracted with solvents by cold maceration. The phytochemical analysis showed the presence of Alkaloids, Terpenoids, Cardiac glycosides, Saponins, Tannins & Carbohydrates in all the solvents. All the extracts were screened for antibacterial activity by Disc Diffusion Method. Out of the cultures used *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* showed better zone of inhibition which is 10mm, 9mm & 7mm respectively.

**Introduction:**

Medicinal plants are of great importance to the health of individuals and community.

Its medicinal value lies in certain chemical substances that has its physiological action on the human body. Various medicinal plants have been used for years in daily life to treat disease all over the world. Most important bioactive constituents of plants are alkaloids, flavanoids, glycosides and tannins (Hill, 1952)\(^1\). The knowledge of these chemical
constituents of plants would further be valuable in discovering the actual value of folkoric remedies. Pharmaceutical industries have started manufacturing increasing number of new phytomedicines.

_Nerium oleander_ (common name: Arali) belonging to the family Apocynaceae is widely distributed in temperate regions throughout the world. It is an evergreen shrub with milky latex with flowers red, pink and white colored, fragrant. More than 400 varieties have been introduced so far. Generally the parts used are roots and leaves. This plant which is known to contain active cardiac glycosides is used in the treatment of cardiac asthma. It may have positive effects in patients with leimyosarcoma and prostate / breast cancer. It is also used as diuretic, anti-inflammatory agent, anti-parasitic and for neurological disorders and cardiac abnormalities. The present study focused on the phytochemical analysis and antibacterial activity of selected extracts of _Nerium oleander._

**Methodology:**

1. **Collection of the plant materials:**

   Nerium oleander plants were collected during the months of September and October from Bhavani singh herbal garden, Yercaud, Tamilnadu.

2. **Preparation of the extract:**

   The fresh leaves were shade dried for 15 days and then powdered using a mechanical grinder. The coarsely powdered leaves of the plant were then extracted completely by cold maceration. The solvents used for maceration were benzene, chloroform and ethanol (50%). Cold maceration of the samples were carried out by soaking 2g of the sample in 15ml solvent and then allowing them to stand with intermittent shaking for three days. The filtrate obtained was then used for phytochemical analysis and antibacterial studies.

3. **Qualitative analysis of phytochemical constituents:** (Hayashi.T et al., 1993, Harborne, 1973)

   The plant extracts (benzene, chloroform and ethanol) were subjected to phytochemical studies to qualitatively analyze the active components present in them. The compounds screened are terpenoids, cardiac glycosides, flavanoids, alkaloids, tannins, saponins, phenolic compounds, carbohydrates and phlobatannins.

4. **Selection of bacterial species.**

   _Bacillus subtilis_, _Staphylococcus aureus_, _Salmonella typhimurium_, _Klebsiella pneumoniae_, _Pseudomonas aeruginosa_, _Proteus vulgaris_, _Escherichia coli_ were selected for the study of antibacterial activity. The bacterial culture were maintained on nutrient agar plate.

   A 24 hour broth culture of the selected species were used for antibacterial screening.

5. **Antibacterial screening - Disc diffusion method** (Bauer.L.V, Staden.J.V)
Muller Hinton Agar (MHA) plates were prepared and sterilised by an autoclave. In an aseptic room, 20 ml of the medium is poured into each petridish and allowed to solidify at room temperature. After solidification, the test organisms were inoculated with the help of a sterile cotton swab soaked in a bacterial culture or suspension, providing an uniform surface growth of bacterium. It is used for antibacterial sensitivity studies. Then sterile whatman filter paper discs (5 mm) were immersed in crude extract and different dilutions were plated on MHA plates. They were then incubated at 37 °C for 16 to 18 hours.

Results:

Phytochemicals:

Results of the phytochemical constituents of *Nerium oleander* reported in Table 1.

The phytochemical screening showed the presence of terpenoids, alkaloids, glycosides, saponins, tannin and carbohydrates. Negative results were obtained for phenolic compounds, flavanoids and phlobatanins.

Antibacterial activity:

Positive results were established by the presence of clear zones of inhibition around active extracts. The zone of inhibition were measured and regarded immediately using a zone reader (Udaykumar and Hazeena Begum, 2002). The results obtained were compared with that of zone of inhibition produced by standard antibiotic discs.

Out of different dilution tried, a dilution of 6:1 gave better results. The results are tabulated in Table 2.

Discussion:

Previous reports from (Rahila et al, 1994 and Gill L.S, 1992) also showed that plants like *Euphorbia heterophylla* and *Scorparia dulcis* contains tannins, alkaloids and terpenoids. Also *Euphorbia sp.* are used in the treatment of cough, asthma and hay fever. Some of the phytochemical compounds detected for example: glycosides, saponins, tannins, flavanoids, terpenoids and alkaloids have variously been reported to have antimicrobial activity. (Leven et al, 1979) Since the plant of the present study, *Nerium oleander* was also found to possess phytochemicals responsible for antimicrobial activity, an antibacterial assay was carried out to find whether it has got the activity against selected bacterial species. Out of the seven bacterial species analysed for antibacterial effect, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* showed better zone of inhibition (10, 9, 7 mm respectively). Out of the extracts tried, ethanolic extract gave better results. This showed that the natural products from the leaves of *Nerium oleander* has certain active principles responsible for antibacterial activity. Since the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and
Salmonella typhi murium is controlled by Nerium oleander, it indicates that they could inhibit the activity of bacteria which causes diarrhoea, polymixin and typhoid respectively.

**Conclusion:**

Since the selected plant Nerium oleander has got certain active principles and is effective in controlling the growth of

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**Table 1: Results of Phytochemical analysis of plant extracts**

| Tests                  | Benzene | Chloroform | Ethanol |
|------------------------|---------|------------|---------|
| Terpenoids             | ++      | ++         | ++      |
| Cardiac glycosides     | ++      | ++         | ++      |
| Alkaloids              | +       | +          | +       |
| Flavanoids             | _       | _          | _       |
| Saponins               | +       | +          | +       |
| Tannins                | +       | +          | +       |
| Phenolic compounds     | _       | _          | _       |
| Phlobatanin            | _       | _          | _       |
| Carbohydrates          | +       | +          | +       |

++ - Good result      + - Moderate result  — - Negative result

**Table 2: Antimicrobial activity of plant extracts (Disc diffusion method)**

| Bacterial cultures | Benzene (mm) | Chloroform (mm) | Ethanol (mm) |
|--------------------|--------------|-----------------|--------------|
| 1.a                | 2            | 5               | 7            |
| b                  | _            | 5               | 7            |
| 2.a                | 2            | 4               | _            |
| b                  | 1            | 6               | 7            |
| 3.a                | _            | 2               | 4            |
| b                  | _            | 2               | 4            |
|   | 4.a | b | 5.a | b | 6.a | b | 7.a | b | Positive control | Negative control |
|---|-----|---|-----|---|-----|---|-----|---|-----------------|------------------|
|   |     |   |     |   |     |   |     |   | (Ampicillin)    | (solvent)        |
|   | _   | _ | 3   | 3 | 2   | 3 |     | 1 | 16              | _               |
|   | 4   | 2 | 3   | 3 | 4   | 3 | 5   | 3 |                 |                 |
|   | 9   | 9 | 7   | 7 | 7   | 7 | 1   | 2 |                 |                 |

1. *Salmonella typhimurium*  5. *Klebsiella pneumoniae*
2. *Escherichia coli*  6. *Staphylococcus aureus*
3. *Proteus vulgaris*  7. *Bacillus subtilis*
4. *Pseudomonas aeruginosa* a. crude extract; b. dilution (6:1)

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