

# Antibacterial Activity and Phytochemical Screening of Kenyan *Nerium Oleander* (Apocynaceae) Crude Leaf Extracts

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**ABSTRACT:** Emergence of new infectious diseases, along with resurgence of several infections that initially appeared to have been controlled, have created the necessity for ethno pharmaceutical research studies. Optimization for screening methods used for the identification of antibacterial drugs from natural sources is of great value. Different plant parts such as leaves, barks and roots of *Nerium oleander* (Apocynaceae), have been used to treat skin diseases, relieve nasal congestion and cardio-tonic diseases. This study was aimed at determining the antibacterial activity with regard to *Staphylococcus aureus* and to conduct phytochemical screening of *Nerium oleander* leaf extracts. Experimental work was conducted by collection of plant material, preparation of organic extracts, phytochemical screening of crude leaf extracts, antibacterial susceptibility tests and determination of the zones of inhibition. The phytochemical analysis showed the presence of terpenoids, saponins, phenols, flavonoids, cardiac glycosides, alkaloids and tannins whereas anthraquinones were absent. Screening for antibacterial activity was done by the disc diffusion method. *Staphylococcus aureus* was found to be susceptible to the organic crude extracts. At 1000mg/ml concentration, the activity was highest; with a zone of 14.4mm compared to 62.5 mg/ml where the inhibition zone was 6.2mm in diameter. The results were compared with the zone of inhibition of amoxicillin(16mm) and were found to have identical activity at high concentrations as the standard antibiotic. The findings indicate the potential and relevance of rosebay leaves in pharmacology.

**KEYWORDS:** Antibacterial, *Nerium*, Phytochemical screening, zone of inhibition

## I. INTRODUCTION

In recent times, preparation of medicine has heavily relied on plants and natural compounds. This is an actual shift from the use of synthetic compounds that were predominantly manufactured by countries considered to be having leading edge technology<sup>[2]</sup>. Through research, the potential of plant extracts containing bioactive and therapeutic constituents has gradually been unleashed and tapped onto. Even though the synthetic compounds used in classical times came in handy in treating advanced stages of diseases, traditional herbs can be of remarkable value if they are well researched on. In rural settings of most developing countries, various plant parts have been used for a number of functions and ultimately, modern scientists have greatly appreciated and recognized the full worth of the plant kingdom. As the medical research studies progress, more antibiotics and antibacterial drugs have been produced to treat various diseases; and this has been an outstanding breakthrough in the health sector. However, during the last three decades, various bacteria have gained resistance to some of these drugs<sup>[34]</sup>. The World Health Organization in partnership with Drugs for Neglected Diseases initiative (DNDi) aims to come up with new treatments by improving existing antibiotics thus encouraging research and development<sup>[34]</sup>. It is approximated that 30000 to 70000 plant species globally have medicinal value<sup>[26]</sup>.

*Nerium oleander* is an upright shrub that is evergreen. It has a milky sap with clusters of very beautiful, lush, fragrant flowers and belongs to family Apocynaceae<sup>[33]</sup>. The pink cultivar is commonly known as rosebay, due to its flowers that resemble roses hence the name rosario; it blooms from May through to November while the white cultivar blooms the entire year<sup>[30]</sup>. The plant grows primarily in the subtropical biome. It is native to the Mediterranean region, Southern Asia spanning towards parts of China; distributed in the upper plain of Himalayas from Nepal westwards to Kashmir up to

1.95km extending to Afghanistan and found throughout India as fences and in gardens<sup>[18]</sup>. In fact, the history of *Nerium oleander* domestication can be traced back to ancient China when people wrote songs and poems about its beauty and breathtaking fragrance during the Song dynasty [1127-1279]. It has been naturalized and is now cultivated extensively across tropical regions in the world. It is distributed in Africa, Asia and Europe. It is easy to tend to, drought tolerant and can grow in poor soils provided it is not exposed to extremely cold temperatures thus it is easy to find it planted in gardens and parks. However, the plant parts may be toxic if ingested and for this reason, they are normally free from pests and diseases, and are untouched by grazers<sup>[29]</sup>. This study focuses on the antibacterial activity and phytochemical analysis of the leaf extracts of *Nerium oleander*.

## II. LITERATURE SURVEY

### 1.1 Taxonomy

*Nerium oleander* is the only species classified in the genus *Nerium*<sup>[33]</sup>. The genus name comes from the classic green name 'nerion' meaning moist since it likes moist soils while the specific epithet is from the Italian 'oleandro' due its superficial resemblance to *Olive olea* plant even though they are actually unrelated<sup>[10, 26]</sup>. It is a member of tribe Nerieae of subfamily Apocynoideae in the family Apocynaceae, order Gentianales, class Magnoliopsida, division Tracheophyta. It is a shrub described using many scientific names<sup>[37]</sup> that are regarded as its synonyms: *Nerion oleandrum* St.-Lag, *Nerium carneum* hort ex Dum.Cours, *Nerium flavescens* Spino ex Roemer, *Nerium floridum* Salisbury, *Nerium indicum* Mill, *Nerium japonicum* Gentil, *Nerium glandiflorum* Desf, *Nerium odorum* Aiton, *Nerium lauriforme* Lamarck, *Nerium madonii* M. Vincent, *Nerium oleander* var, *Nerium mascatense* A.D.C, *Oleander indica* (Mill.) Medikus, *Oleander vulgaris* Medikus, *Nerium thyriflorum* Paxton. Some of the common names of *Nerium oleander* include; Adelfa[Spanish], Lal Karen, Kanher, Rosebay[English], Rosa Fransesa, Oleander [French, German], Oleandro[Portuguese]<sup>[30]</sup> and Afita [ Luo].

### 1.2 Botanical Description

*Nerium oleander* approximately grows up to 2-4 m tall in height. Its leaves are simple, narrow, linear-lanceolate and dark green<sup>[29, 31]</sup>. The leaf blades are oblong, truncated at the apex and rounded at the base. The midribs are green in colour. The petioles are either very slender or stouter depending on the cultivars. They grow in pairs or whorls of three (5-18 cm long), have an entire margin and reticulate venation. The young leaves are light green, glossy and mature to a dark green colour. The flowers are pink, tubular, fragrant (very nice scent), umbel, clustered, five-lobed fringed corolla. The flowers are 5 cm in diameter and they grow in clusters at the end of each branch. The fruits comprise of a narrow follicle; 8-16 cm long which ruptures open at maturity to release fluffy downy seeds. Filaments are hairy and seeds have brown feathery tips. Cut or broken branches exude a white sap.



(a) (b) (c) **Figure 1: *Nerium oleander*: (a) Simple, green lanceolate leaves, with entire margins, (b) Pink tubular, lobed, umbel flowers, (c) Oleander plant at a glance growing 3-4 meters tall (Achieng, 2023)**

### 1.3 Traditional uses of *Nerium oleander*

Even though it is recognized as a toxic plant, usage of oleander in traditional communities is well documented. Plant parts of oleander such as flowers, bark, the leaf juice and latex are of ethnomedicinal value. The flowers also make the plant an aesthetic. In India, several oleander plants grown in rows act as a live fence and road dividers because the plant can withstand heat, dust and it requires little water. Oleander contains potent cardiac glycosides which are used as arrow poisons in Sub-Saharan West Africa, particularly in Togo and Cameroon [25]. Ethnobotanical information shows that oleander is effective in treatment of cardiac illnesses, epilepsy, corns and wounds healing [18]. Ground leaves mixed with honey used to treat scabies [20]. The roots, boiled in water are effective in treating skin ailments. In Morocco, oleander plays an important role in treating malaria, hair loss, scabies and toothache [6]. Additionally, in folk medicine, oleander leaves were used to treat chest congestion, ringworm, inflammation and indigestion [13].

In Arab folk medicine, oleander was used in powder form and decoction to treat solid tumours and skin diseases [9]. The leaf extracts can be used for biological control of early blight disease in yams [7]. Wounds treated with *Nerium oleander* formulations showed increased tensile strength which is indicative of improved collagenation that contributes to better healing [19]. The leaves are a powerful repellent. A decoction of the leaves can be applied externally in the treatment of scabies and to reduce swellings. In traditional Chinese medicine, the flowers and leaves have been used to stimulate the cardiac muscles, relieve pain and eliminate blood stasis [9]. Some communities in Kenya use the leaves of this plant (ground and dried) to relieve nasal decongestion during common cold.

### 1.4 Bioassays using *Nerium oleander*

Different *Nerium oleander* extracts have been tested against *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* [29] with inhibition zones ranging from 8mm to 19mm [23]. These tested bacteria were found to be very sensitive to methanolic extracts and of moderate sensitivity to ethanolic extracts of *Nerium oleander* roots and leaves. Indeed, many studies have reported that Gram-negative bacteria are often more resistant to plant-derived antimicrobials, compared to Gram-positive [22]. *Nerium oleander* leaf extracts were active against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* with inhibition zones of 28.89 mm, 18.22 mm and 6.32 mm [11]. *Nerium oleander* organic leaf extracts inhibited *Escherichia coli*, *Bacillus subtilis*, *Sarcina lutea*, *Klebsiella pneumonia*, showing highest activity against *Escherichia coli* [17].

The common bioactive plant constituents include saponins, alkaloids, tannins, flavonoids and phenolic compounds [22]. The leaf contains phenols including cinnamic acid and catechin which physiologically aid the human body in immune responses, anti-aging and antibacterial properties. Aside from phenols, 2.3% of polysaccharides, galactose and galacturonic acid are present [33]. The polysaccharides exhibit anti-viral and anti-inflammatory activities in addition to being energy stores. The main glycosides in oleander are oleandrin and neriine [3]. Oleandrin is a saponin glycoside regarded as very potent for use in pharmaceuticals to provide solutions to respiratory ailments aside from having antiHIV and even anticancer properties. Neriine on the other hand is remarkably therapeutic.

Active components of *Nerium oleander* are triterpenes and cardenolides [4, 25, 30]. The entire oleander plant contains toxins; steroidal glycosidic cardenolides and pentacyclic terpenoids. However, the highest levels are in the roots and seeds. Studies [1, 23] found that Labdane diterpene, oleanderoic acid and a new triterpene - oleandren can be isolated from fresh, undried and uncrushed leaves of *Nerium oleander*. The leaves of *Nerium oleander* contain Heterosides (soluble sugar compounds) [18]. The medicinal value of plants is as a result of these chemical substances that produce particular physiological actions in the human body. *Nerium oleander* is also a source of folinerin [33], important for metabolism in specific biochemical reactions in the human body. The phytochemical screening of *Nerium oleander* leaf extracts was set to examine the presence of saponins, phenols, sterols, anthraquinones, flavonoids, glycosides and terpenoids.

## III. METHODOLOGY

### 2.1 Plant Material Identification and Collection

A complete sample of *Nerium oleander* was collected from The University of Nairobi Sports Complex, Kenya in March 2023. The leaves were plucked out from the plant, washed using distilled water to remove impurities and dried at room temperature. The leaves were 12cm to 14cm long and dark green in colour. They were ground into powder and sieved



using a mesh to obtain fine powder. The powdered products, 165.8g in weight, were stored in glass vials at 4°C ready for extraction, antibacterial activity tests and phytochemical screening.

## 2.2 Preparation of Plant Crude Extracts

According to standard protocols<sup>[14, 15, 32, 34]</sup>, 50g of the dry leaf powder sample was weighed and macerated in 500ml methanol: chloroform (1:1) in a corked conical flask; for 48 hours within which it was stirred twice at intervals of 24 hours to ensure complete extraction. The solution was decanted, filtered using Whatman filter paper and the resultant liquid collected in sterilized conical flasks. The extract was concentrated using a rotary evaporator at 40°C to obtain crude extracts which were stored at 4°C pending phytochemical screening and antibacterial tests.

## 2.3 Screening for Antibacterial Activity

28g of Nutrient Agar medium was suspended in 1000ml of distilled water. The mixture was stirred continuously to ensure the medium dissolved completely and then sterilized in an autoclave at 121°C for 15 minutes. 25 ml of the medium was poured into each Petri dish and left to solidify at room temperature. Once the medium solidified, the pure culture of the test organism; *Staphylococcus aureus* was inoculated using a sterilized cotton swab that was soaked in a bacterium suspension, thus allowing for uniform surface for bacterial growth. The inoculum was allowed to stick on the medium for 10 minutes ready for use in antibacterial sensitivity tests.

A stock solution of the organic extract was diluted to concentrations of 100% (1000mg/ml), 50% (500mg/ml), 25% (250mg/ml), 12.5% (125mg/ml) and 6.25% (62.5 mg/ml). Five sterile test tubes were arranged on a rack. From the stock solution, 1ml of the extract was transferred to the first test tube. To the second test tube, using 500ml of extract and 500ml of the diluent (distilled water) as the starting volume, a successive two-fold serial dilution of the extract was conducted.

The disc diffusion method was used to assess antibacterial activity of the crude extracts. The set concentrations of the organic extracts were pipetted on each sterilized Whatman filter paper disc of 6 mm in diameter. 20 µl of each dilution was inoculated such that 5 µl of the extract was spotted alternately on both sides of the disc and allowed to dry before the next 5µl is spotted to ensure precise inoculation. The discs were dried and placed aseptically on the Nutrient Agar plates with 1ml of the growth test microorganism overnight. Amoxicillin, a standard antibiotic, was used as a positive control whereas a disc containing water only was used as a negative control. All discs were fully dried before application on the agar plate containing the bacterium. The *Staphylococcus aureus* culture with the discs were incubated at 30°C for 24 hours. Bacterial growth was determined by measuring the diameter of the zone of inhibition using a transparent ruler in millimeters. The Minimum Inhibitory Concentration was determined by measuring the lowest concentration in which the growth of *Staphylococcus aureus* was inhibited.

## 2.4 Phytochemical Screening

The compounds that were screened according to standard procedures<sup>[15,16]</sup> were; Alkaloids, Flavonoids, Tannins, Saponins, Terpenes, Phenols, Cardiac glycosides and Anthraquinones.

### 2.4.1 Test for Alkaloids

1ml of the plant extract was placed in a test tube. To which, 1 ml of potassium bismuth iodide solution was added and shaken. An orange red precipitate formed would indicate the presence of alkaloids.

### 2.4.2 Test for Flavonoids

1ml of the extract was placed in a test tube, few drops of sodium hydroxide solution were added and shaken. An intense yellow colour that turns colourless after adding dilute acid would show that flavonoids are present.

### 2.4.3 Test for Tannins

1% of gelatin solution containing sodium chloride was added to 1ml of the extract in a test tube and shaken. Appearance of a white precipitate would indicate that tannins are present.

### 2.4.4 Test for Saponins

5 ml of distilled water was added to 1ml of the plant extract, shaken and heated in a boiling tube for 5 minutes. Formation of a stable layer of foam that persists for 15 minutes would indicate presence of saponins.

#### 2.4.5 Test for Sterols

Concentrated sulphuric acid was slowly added along the wall of the test tube containing 1ml of the plant extract. An effervescence followed by appearance of a clear reddish brown colour at the interface indicated a steroidal ring.

#### 2.4.6 Test for Terpenes

To 1ml of the extract in a test tube, 2ml of chloroform was added, shaken and filtered. 2 drops of concentrated sulphuric acid were added, shaken then allowed to stand. A yellow- golden precipitate indicated the presence of terpenes.

#### 2.4.7 Test for Phenols

To 1ml of the extract in a test tube, a few drops of freshly prepared ferric chloride solution separately were added and shaken, a bluish black colour showed the presence of phenols.

#### 2.4.8 Test for Cardiac Glycosides

To 1ml of the extract, 1.5ml of lead acetate was added, shaken and filtered. The residue was dissolved in glacial acetic acid. A few drops of Ferric chloride were then added and the mixture placed in a test tube containing 2ml of sulphuric acid. Emergence of a reddish brown layer that turns bluish green would imply the presence of cardiac glycosides.

#### 2.4.9 Test for Anthraquinones

1ml of the test sample was dissolved in 5 ml of dilute HCl. 5ml of 5% ferric chloride solution was added, shaken and placed over a water bath for 10 minutes, cooled and filtered. It was then extracted with benzene. The upper lipophilic layer was separated and treated with 4ml of dilute ammonia solution. A colour change of the lower layer to violet, then pink would indicate the presence of anthraquinones.

### IV. EXPERIMENTAL RESULTS

#### 4.1 Antibacterial Activity and Minimum Inhibitory Concentration of *Nerium oleander* crude leaf extracts.

As illustrated in table 1, at 100% concentration, the extract showed the largest diameter of zone of inhibition (14.4 mm) which was 1000mg/ml of *Nerium oleander* extract while 62.5mg/ml (6.25%) concentration showed the minimum diameter (6.7 mm) of the zone of inhibition. A gradual reduction in diameter of the zone of inhibition was observed with decreasing concentration; at 500mg/ml (10mm), 250mg/ml (9.2 mm), 125mg/ml (8mm) and 6.7 mm for 62.5 mg/ml. The standard antibiotic showed a zone of 16mm.

**Table 1: Diameter of the zone of inhibition of *Nerium oleander* crude leaf extracts against *Staphylococcus aureus* at different concentrations.**

Concentration (%)	Diameter of clearing zone (mm) against <i>Staphylococcus aureus</i>
100	14.4mm
50	10mm
25	9.2mm
12.5	8mm
6.25	6.7mm
Positive Control	16mm

The zone of inhibition showed a gradual decline (14.4mm,10mm,9.2mm, 8mm, 6.2mm) with an increasing dilution of the extract as illustrated in Figure 2 below. At different concentrations, the inhibitory effects of the extracts were very close and quite identical in magnitude, compared to amoxicillin (16mm); the standard antibiotic.

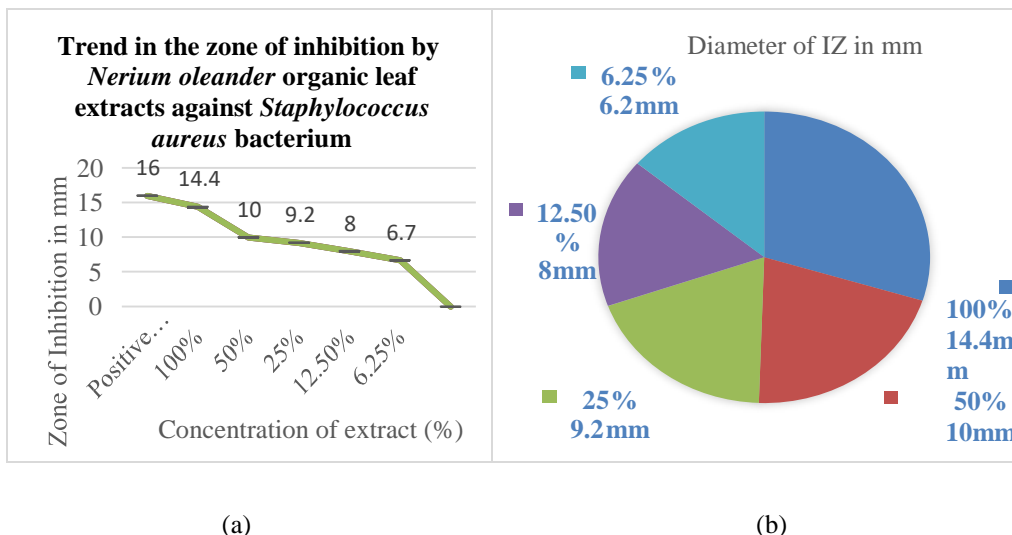


Figure 2: (a)The declining trend in the zone of inhibition at different concentrations of *Nerium oleander* crude organic extracts against *Staphylococcus aureus* bacterium (b) Pie chart showing the zones of inhibition of *Nerium oleander* organic leaf extracts

#### 4.2 Phytochemical screening of *Nerium oleander* crude leaf extracts

Phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins, sterols, phenols, cardiac glycosides and terpenes. Anthraquinones were absent. (Table 2)

Table 2: Phytochemical compounds present in *Nerium oleander* crude leaf extracts

Serial No.	Phytoconstituents	Test	Expected outcome	observation
1	Alkaloids	Drangedroff's test	Reddish brown precipitate	+
2	Flavonoids	Alkaline Reagent	Intense yellow colour	+
3	Tannins	Gelatin test	White precipitate	+
4	Saponins	Foam test	Stable layer of foam	+
5	Sterols	Salkowski's test	Effervescence followed by a reddish brown colour	+
6	Terpenes	Lead Acetate test	Yellow golden colour	+
7	Phenols	Ferric Chloride Test	Bluish black colour	+
8	Glycosides	Keller Killani Test	Blue- green colour	+
9	Anthraquinones	Bontrager's Test	Pink colour	-

Key: + present - absent

#### V. DISCUSSION

The susceptibility of *Staphylococcus aureus* bacterium to different concentrations of *Nerium oleander* organic leaf extracts was identical in magnitude when compared to the standard antibiotic. Simultaneously increasing the

concentrations of the extracts notified the trend in activity. The highest concentration (1000mg/ml) showed the highest value for zone of inhibition against *Staphylococcus aureus* while the least activity was shown by (62.5mg/ml).

Thus, active components present in organic extracts of *Nerium oleander* leaf extract can be used in treating diseases caused by bacteria. *Nerium oleander* leaf extracts showed a zone of inhibition of 13mm against *Staphylococcus aureus* [18]. It is also recorded [22], that methanol leaf extracts are active compared to Ciprofloxacin as a standard. The highest activity of this plant might be due the presence of terpenoids, saponins and amino acids. Further studies [18] showed that methanol extracts of *Nerium indicum* demonstrated antibacterial activity against multidrug resistant bacteria such *Escherichia coli*, *Pseudomonas aeruginosa* and MRSA with MIC values of between 0.3 to 4 mg/ml. Strains of *Staphylococcus aureus* and *P. aeruginosa* that are highly considered to be multidrug resistant to antibiotics such as quinolones, macrolides, rifampin and beta- lactams were found to be inhibited at 0.65 and 0.75 mg/ml MIC values.

From table 2, alkaloids, phenols, saponins, terpenes, tannins, terpenes and cardiac glycosides are present in *Nerium oleander* leaves. Terpenes and its derivatives have a number of chemical functions including antibacterial activity against pathogens [1]. At a concentration of 0.12 mg/ml, terpenes exhibit antibacterial effects on the growth of *Staphylococcus aureus*. Phenolic compounds on the other hand, have aromatic rings comprising one or more hydroxyl groups that play a role in antioxidant properties [3]. Epigallocatechin, a phenolic compound and quercetin, a polyphenolic compound inhibits growth of *Mycobacterium tuberculosis* and *Klebsiella pneumoniae* at MIC values of 35– 250 µg/ml. This compound(Phenol), binds to the Adenosine Triphosphate binding site of the DNA gyrase subunit thus preventing bacterial growth.

Flavonoids are active against Gram-positive bacteria such as *Staphylococcus aureus* and also against Methicillin resistant *Staphylococcus aureus* and *S. haemolyticus*. Growth of Gram negative bacteria can also be inhibited by flavonoids: for example, *Escherichia coli*. Cardiac glycosides have an anti- adhesion property which is responsible for preventing bacteria from binding and proliferating. They have bacterial receptors(colonic). Tannins and flavonoids in plant extracts are effective in inhibiting bacteria by mechanisms such as cell wall disruption, inhibiting production of bacterial enzymes and by binding to surface proteins on the bacterial cell. Sterols are important in pharmacology due to their role in hormone regulation and membrane protein functions.

## VI. CONCLUSION AND RECOMMENDATIONS

*Nerium oleander* crude leaf extracts show different antibacterial activities at varying concentrations. It contains alkaloids, phenols, flavonoids, saponins, cardiac glycosides, terpenes and tannins which attributed to the antibacterial activity against *Staphylococcus aureus*. Thus, *Nerium oleander* can potentially provide an excellent alternative source of antibacterial drugs that are naturally obtained for use in commercial production of medication. To clarify how phytochemicals act on various bacteria it is recommended that further investigation be conducted to evaluate the action of *Nerium oleander* leaf extracts using experimental models to confirm their actual efficacy for use in product development. These products can be used in the biomedical, cosmetic and pharmaceutical industries. Detailed trials on toxicology of *Nerium oleander* leaf extracts can be conducted to find out their safety and improve their quality.

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