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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *ADENIUM OBESUM* (DESERT ROSE) LEAF

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ABSTRACT

The parts of *Adenium obesum* (Desert rose) plant has been known to possess a wide range of biological activity consisting stems and roots. The purpose of present study was to ensure the presence of antibacterial activity in aqueous and alcoholic extracts of leaves of *Adenium obesum.* Antibacterial sensitivity test was done against Gram positive bacteria (*Bacillus amyloliquefaciens* and *Staphylococcus aureus)* and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) by Agar well diffusion method. Inhibition length was calculated and found to be maximum in alcoholic extract. Phytochemical screening was done in order to check the presence of active components in leaves of *Adenium obesum*. Result showed that methanolic extract has effective antibacterial activity against Gram positive bacteria and ineffective against Gram negative bacteria. The study suggest that leaves of *Adenium obesum* (Desert rose) plant can be used as an antimicrobial agent and expected that leaves of *Adenium obesum* may be used as therapeutic agents for various diseases.

KEY WORDS: ***Adenium obesum,*** **Antibacterial sensitive test, Phytochemical screening**

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INTRODUCTION

Medicinal plants as herb are utilized by human being in universal phenomena. All drugs that were used in the past decades have shown various therapeutic impacts. Thus, medicinal plants may be defined as any plant that has some medicinal use. It has been recognized by World Health Organization that medicinal plant has shown important role in health care i.e. 80% of the world population in developing countries which are largely dependent on traditional medicines [1, 2]. The use of medicinal plant predates about the introduction of antibiotics and other modern drugs into African continent [3]. The previous studies states that different parts of plants have shown different antibacterial activity against Gram positive and Gram negative bacteria which have been a counterfeit to know which part should be used for pharmaceutical activities [2]. Phytochemical which belongs to several chemical classes has revealed the active components of medicinal plants that show inhibitory effect on all types of microorganism and also their bioactive extracts have shown sensitive activity against both Gram positive and Gram negative bacteria[4, 5]. There are various secondary metabolites that are present in plant which are actually responsible for numerous activities such as anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal, anti-oxidant and others. *Adenium obesum* which is also known as Desert Rose, belong to *Apocynaceae* family. *Adenium obesum* as *Allamanda*, *oleander*, *plumeria*, *periwinkle*, were recognized by United State Department of Agriculture Germplasm Resources Information Network. *Adenium obesum* is native to East Africa, Northeast Africa, and Arabian Peninsula. *Adenium obesum* is a short shrub or small tree that may have known as its plant types. Its main stem is short, thick and fleshy with distinct irregular swollen base, known as caudexes. Its branches are smooth, greyish-green to brown, upright and has irregular spaced. *Adenium obesum* is a slow growing plant with maximum observed heights of about 6 feet in South Florida but it is found smaller in size in many different countries. Leaves are spirally arranged and are mostly together at the end of branches[6, 7]. Stem of *Adenium obesum* has shown antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Nesisseria gonorrhea* and *Klebsiella pneumonia* and found MIC of 2×10^4µg/cm^3, No growth, 3×10^4µg/cm^3, 2×10^4µg/cm^3 and 3×10^4µg/cm^3 respectively [8]. Another study was done in order to determine the antibacterial activity of *Adenium obesum* extract and to compare it with an antibiotic drug Meropenem against *Acinetobacter baumannii* and not able to inhibit the growth of microorganism [9]. The acute toxicity of ethanol extract of *Adenium obesum* (stem bark) in Wister rats in relation to haemotological parameters was investigated and exposed rats did not show any sign of toxicity, morbidity and mortality [10]. As far as the ornamental flowers are concerned, flowers of *Adenium obesum* have shown the presence of secondary metabolites, phenolic content, radical scavenging activities and antibacterial activities of it. The chemical profiling of flowers of *Adenium obesum* on TLC has shown the presence of phenolic, alkaloid and terpenoids [11]. Synergistic activity of methanolic extract of stem bark of *Adenium obesum* against some clinic bacterial and found to be maximum antibacterial against both Gram positive and Gram negative bacteria more than individual one [12]. *Adenium obesum* (leaves) comes out to be a natural source as potential anticancer agents as ethnobotanical study of medicinal plant was done [13, 14]. The purpose of present study is to check the antibacterial activity of leaves of *Adenium obesum* plant against bacterial strain i.e. Gram positive bacteria (*Bacillus amyloliquefaciens* and *Staphylococcus aureus)* and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) by agar well diffusion method. Phytochemical screening was done in order to check the presence of secondary metabolites in the leaves of *Adenium obesum* plant and to know the presence of respective secondary metabolite that are responsible for inhibition of growth of microorganism and to provide a

scientific rationale for their use in traditional herbal remedies.

MATERIALS AND METHODS

Plant Samples

The plant of *Adenium obesum* was collected from Rajdhani Nursery, Jor Bagh, New Delhi and kept at Amity Institute of Biotechnology, Amity University Uttar Pradesh. Fresh leaves were used as plant sample.

Bacterial Strains

The bacterial strains of *Escherichia coli* (DH5α), *Bacillus amyloliquefaciens, Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Helix BioGenesis Pvt. Ltd., Noida, U.P. and were sub cultured freshly in Luria Broth (LB) medium and used further for research work.

Preparation of Antimicrobial Extracts

***Aqueous extract**:*

Leaves were washed, dried and crushed by pestle and mortal to make fine particles. The 5g of leaves were dissolved in 50ml of distilled water and it was boiled in water bath for 30 minutes at 100°C. The conical flasks of extract were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250rpm for 48hrs. After shaking they were filtered with muslin clothes and again filtered with filter paper twice. The filtered extracts were stored at 4°C[2, 15].

Alcoholic extracts

Leaves were washed, dried and crushed by pestle and mortal to make fine powder. The 5g of leaves were dissolved in 50ml of methanolic and its crude extracts were prepared by maceration method and equally by mixing in organic solvent by boiling at 50 - 65°C. The conical flasks of extract were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250rpm for 48hrs. After shaking they were filtered with muslin clothes and again filtered with filter paper twice. They were evaporated to dryness and extract amount were measured[2, 15].

Antimicrobial Sensitive Test Agar well diffusion method

LB agar media were prepared and autoclaved at 121°C for 15minutes at 15 Lbs and poured in sterile petri plates up to a uniform thickness of approximately 10-15minutes and the agar was allowed to set at ambient temperature. This method is suitable for organism to grow rapidly overnight at 35-37°C. The wells were made in medium after inoculation with microorganism. 200µl of inoculums were spread over LB agar plates using sterile spreader, after few minutes four wells were made in each Petri plated and loaded with 100µl of extracts and control (Kirby Bauer method). Plates were incubated at 37°C for 24hrs. Antimicrobial activity was observed by measuring zone of inhibition. The experiments were done in quadruplicates.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was done according to standardized protocol[15]. For this dried leaves were crushed with water and approximately 30 ml extracts were prepared by boiling in hot water bath for at half an hour and passing by the same through muslin cloth for filtrations [16].

Saponins

Foam test is used, whereas fraction of the 1 ml extract was vigorously shaken with water and observed for persistent foam.

Tannins

Few drops of 1% $FeCl_3$ solution was added to 2ml of extract the occurrence of blue, black, green ppt indicates the presence of tannins.

Flavonoids

2ml of 10% NaOH was added to 1ml of plant extract. The entire yellow colour was obtained on adding dilute HCl <1%> it changes to colourless. This indicates the presence of flavonoids.

Terpenoids

To 1ml of plant extract added 2ml chloroform and 2-3ml of concentrated H_2SO_4 carefully poured along side of test tube to form two

different layers. Formation of reddish violet colour indicated terpenoids.

Napthoquinone
To 1ml of extract add few drops of 10%KOH. Formation of blue black colour indicated napthoquinone.

Inulin
To 1ml of solution add α-Napthol and concentrated H_2SO_4 solution. Formation of brownish red colour indicated the presence of inulin.

Glycosides
To the extract add 5ml of H_2SO_4 and boil for 15minutes, cool and neutralize with equal volume of 10%NaOH, then Fehling's solution A and B < freshly prepared and mixed in ration of 1:1> was added. Brick red ppt of reducing sugar indicated the presence of glycosides.

Alkaloids
Wagner's Test is used where a fraction of the extract was treated with Wagner's reagent and observed for the formation of reddish brown precipitate.

Soluble Phenolic compound
A fraction of the extract was treated with 5% $FeCl_3$ solution and observed for the formation of deep blue colour.

RESULTS

The following experiment series is an initial effort in the field of microbial activity of leaves of *Adenium obesum*. In the present study, it was observed that aqueous extract of leaves of *Adenium obesum*has shown maximum antibacterial activity as an inhibition length against *Escherichia coli* i.e. 4.5mm, where as ineffective antibacterial activity was found against Gram positive bacteria such as *Bacillus amyloliquefaciens* i.e. inhibition length of 2mm. *Pseudomonas aeruginosa* and *Staphylococcus aureus* has shown inhibition length of 3 mm each as shown in Table 1. Methanolic extracts of leaves of *Adenium obesum* have shown maximum antibacterial activity against *Bacillus amyloliquefaciens* i.e. inhibition length of 9mm in comparition to *Escherichia coli* that has shown 6.5mm inhibition length as shown in Table 2. The resistance of methanolic extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was found to be inhibition length of 7mm and 5.5mm respectively. This antibacterial sensitive test also signifies that methanolic extract of leaves of *Adenium obesum*has maximum inhibitory effect on *Bacillus amyloliquefaciens* and aqueous extract of leaves of *Adenium obesum* has maximum inhibitory on growth of *Escherichia coli* as shown in Graph 1 and also shown in Figure 1. The zone of inhibition and inhibition length of antimicrobial activity of aqueous and methanolic extract of leaves of *Adenium obesum* against different bacteria is shown in Figure 1. The phytochemical screening of aqueous extract of leaves of *Adenium obesum* reveals the presence of various active components such as saponins, tannins, flavonoids, terpenoids, glycoside and alkaloid. However napthoquinone, inulin and soluble phenolic compounds were found to be absent in aqueous extract of leaves of *Adenium obesum* as shown in Table 3. This presence of secondary metabolites requires quantification and purification for further traditional use.

Table 1
Antibacterial activity of Aqueous extracts of leaves of Adenium obesum against Gram positive and Gram negative bacteria.

Bacterial Strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
Bacillus amyloliquefaciens	9	11	2
Staphylococcus aureus	9	12	3
Pseudomonas aeruginosa	9	12	3
Escherichia coli	9	13.5	4.5

Table 2
Antibacterial activity of Methanolic extracts of leaves of Adenium obesum against Gram positive and Gram negative bacteria.

Bacterial Strain	Well diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
Bacillus amyloliquefaciens	9	18	9
Staphylococcus aureus	9	16	7
Pseudomonas aeruginosa	9	14.5	5.5
Escherichia coli	9	15.5	6.5

Table 3
Preliminary Phytochemical screening of Aqueous extract of leaves of Adenium obesum.

S/No.	Name of the Phytoconstituents	Aqueous extract
1	Saponin	+
2	Tannin	+
3	Flavonoid	+
4	Terpenoid	+
5	Napthoquinone	-
6	Inulin	-
7	Glycoside	+
8	Alkaloid	+
9	Soluble Phenolic compound	-

(+) indicates the presence of the constituents while (–) indicates the absence of constituent

Antimicrobial Sensitive Test

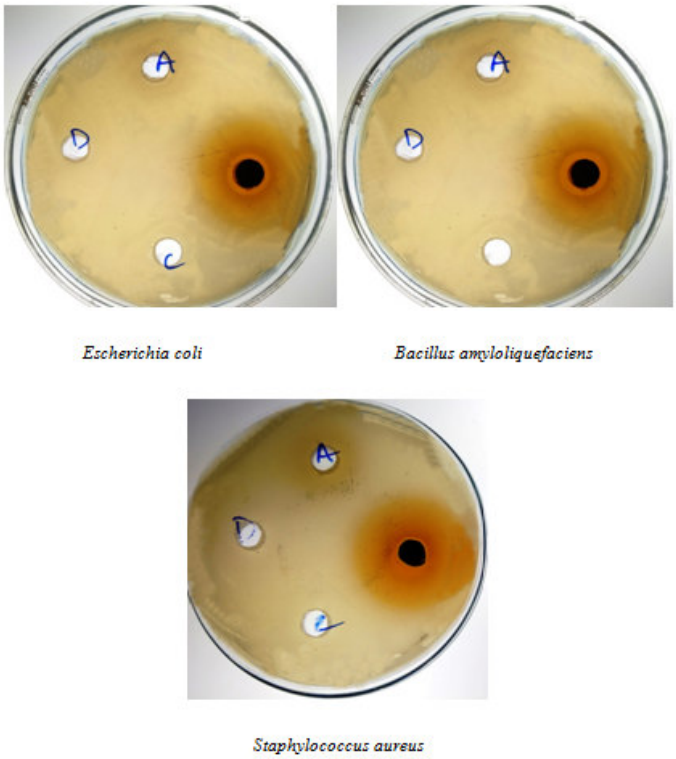
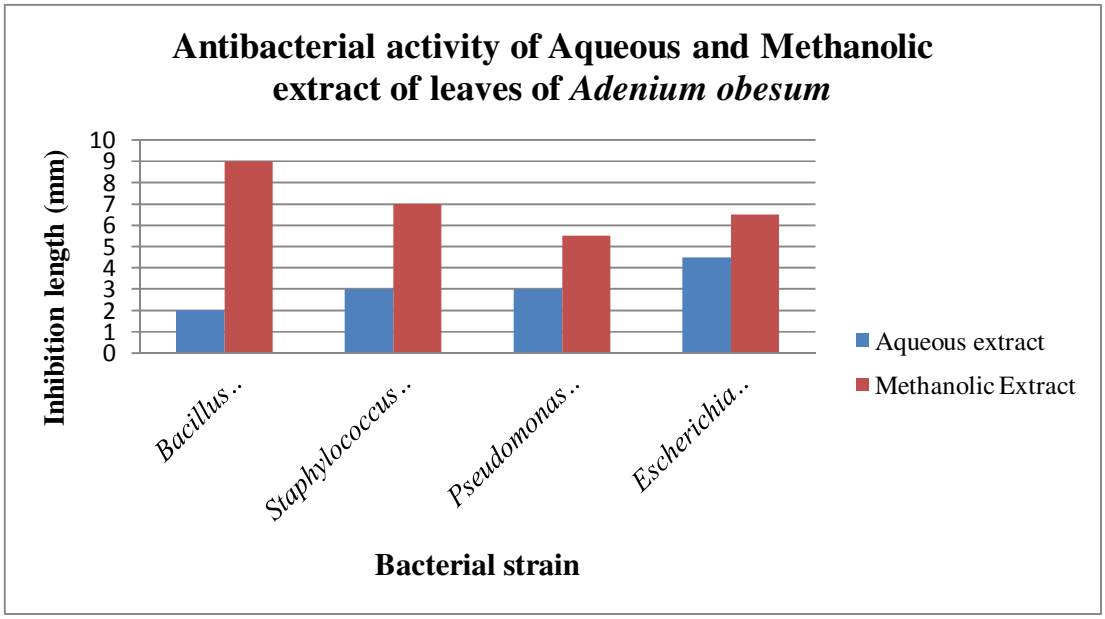


Figure 1
Antibacterial activity against Escherichia coli, Bacillus amyloliquefaciens and Staphylococcus aureus. (A) Aqueous extract, (B) Methanolic extract and (C & D) control.



Graph 1

Antibacterial activity of Aqueous and Methanolic extracts of leaves of Adenium obesum plant against Escherichia coli (DH5α), Bacillus amyloliquefaciens, Staphylococcus aureus and Pseudomonas aeruginosa.

DISCUSSION

Our Earth has been blessed with the billions of diverse plant species and these medicinal plants have been known to be useful to lots of people all over the world [9]. Plants as a source of large amount of drugs claimed to posses the antibiotic properties in the traditional system. These plants have some kind of secondary metabolites which exists as an active component that are responsible for their anti-bacterial, anti-fungal, anti-ulcer, anti-feedant, repellent, and pesticides and thus treat large number of diseases[17]. The following study is an initial effort in the field of microbial activity of leaves of *Adenium obesum*. Then antibacterial activity of aqueous and methanolic extract of leaves of *Adenium obesum* was observed against *Bacillus amyloliquefaciens* and was found to be less effective in comparison to previous report of antibacterial activity of *Adenium obesum* (Stem bark) against *Bacillus subtillis*[12]. As far as the *Staphylococcus aureus* is concerned, aqueous and methanolic extract of leaves of *Adenium obesum* has shown 3mm and 7mm inhibition length, respectively, as comparative to 17mm which was found in methanolic extract of stem of *Adenium obesum*[12, 8]. The antibacterial activity of aqueous and methanolic extract of leaves of *Adenium obesum* against *Pseudomonas aeruginosa* found to be 3mm and 5.5mm, respectively where as 10mm inhibition length was reported in methanolic extract of *Adenium obesum* (stem-bark) [12]. Resistance against *Escherichia coli* was observed to be 4.5mm and 6.5mm inhibition length in aqueous and methanolic extract as comparative to 12mm inhibition length which was reported in methanolic extract of *Adenium obesum* (stem-bark) [12]. It was also reported earlier that the minimum inhibitory concentration of methanolic extract of *Adenium obesum* (stem-bark) against *Escherichia coli* was 2×10^4µg/cm[38].

CONCLUSION

Authors concludes from the above research that leaves of *Adenium obesum* can be utilized as a good source of herbal drugs for various

microbial disease specifically bacterial diseases. It can be expected to use leaves of *Adenium obesum* as therapeutic agents because it has shown effective antimicrobial activity against *Bacillus amyloliquefaciens* and *Staphylococcus aureus*. After this study it has been found that bioactive extracts of leaves of *Adenium obesum* has shown comparative effect against both Gram positive as well as Gram negative bacteria because they consist large amount of secondary metabolites. Future work includes the purification and quantification of secondary metabolites to identify the active components involve in the antimicrobial activity.

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