***A Study on Nutritive role of Azadirachta indica leaves Extract (Neem)-Invitro approach and Antioxidant, antibacterial and antifungal activity***

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**ABSTRACT**

The neem tree (Azadirachta indica A. Juss) is a sizable evergreen tree with flavorful foliage and edible fruits. 350 kg of leaves can be produced anually by a mature tree. According to Jegede and Fagbenro (2007), neem has been used in traditional medicine throughout world for a variety of therapeutic purposes due to its anti-bacterial, anti-fungal, anti-viral, and anti-fertility properties. Phytochemical analyses revealed that the neem tree contains more than 100 different bioactive compounds, including sodium nimbinate, which may be used as a spermicide in animal care and even to control human fertility. The result of the study demonstrated how Azadirachta indica leaves extract contains biologically active ingredients such as alkaloids, flavonoids, saponins, triterpenoids and phenol which possess a wide array of pharmacological properties. Azadirachta indica extract was found to contain appreciable amounts of vitamins and minerals. The leaves extract was found to be antioxidant in nature which is evident from DPPH, ABTS assays. Also the results of antibacterial and antifungal potentials of the neem extract is well established.

**Keywords**: Neem, Azadirachta indica, anti-bacterial, anti-fungal, antioxidant.

**INTRODUCTION**

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as inserts, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10 %of the total. Herbal medicines don't differ greatly from conventional drugs in terms of how they work because chemical compounds in plants mediate their effect on the human body through processes that are identical to those that are already well understood for the chemical compounds in conventional drugs. Because of this, herbal medicines can have advantageous pharmacology, but they also have the same potential for negative side effects as traditional pharmaceutical drugs. Arizona India Inc. Since the dawn of civilization, medicinal plants have been an integral part of human society's efforts to fight disease. A.juus' Azadirachta indica (syn.

**AZADIRACHTA INDICA**

Medicinal plants are part and parcel of human society to combat disease, from the dawn of civilization. Azadirachta indica A.juus . For more than 2000 years, Malia azadirachta indica has been regarded in India and its surrounding nations as one of the most adaptable medicinal plants with a broad range of biological activity. Neem trees are known as "Arishtha" in Sanskrit, which means "reliever of sickness." In India, the tree is still referred to as the "village dispensary." Jessies [1] identified the neem tree as Azadirachta indica in 1830. More than 135 compounds have been isolated from various parts of neem since the early work by Siddiqu [2] on the isolation of nimbin, the first bitter compound, and several reviews have also been published on the chemistry and structural diversity of these compounds of neem extract have been demonstrated against streptococcus mutants and S.faecalis.

In addition to M. tuberculosis and strains resistant to streptomycin, the oil from the leaves, seeds, and bark exhibits a broad spectrum of antibacterial activity against Gram negative and Gram positive microbes. Ringworm, eczema, and scabies can all be treated with dried neem leaf extract, according to clinical trials. When applied locally, lotion made from neem leaves can treat certain dermatological conditions in their acute stages in 3–4 days or in their chronic stages in one night.

. There have been very few reports on the clinical trials done with bioactive compound isolated from neem. Sodium nimbidinate the sodium form of nimbidin, the primary bitter component extracted from neem seed, which has been demonstrated to have potent diuretic properties in a variety of clinical settings. Many different secondary metabolites that plants produce are utilised in the pharmaceutical industry as lead compounds or as direct precursors and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens.

However due to the idea that the use of traditional and herbal remedies lacks a scientifi c foundation, there has rarely been meaningful collaboration between traditional and western medical therapies. The World Health Organization states that the best place to get a range of drugs is from medicinal plants. To learn more about these plants' characteristics, safety, and effectiveness, further research should be done. A vast range of secondary metabolites, including tannins, terpenoids, alkaloids, flavonoids, are abundant in plants etc. That have been discovered to have therapeutic qualities in vitro consequently, our strategy comprised investigating the antibacterial properties of the medicinal herb Azadirachta indica and study its antimicrobial constituent.

**CLASSIFICATION**:

Common Name – Neem

Botanical Name – Azadirachta Indica

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Sapindales

Genus – Azadirachta

Species – A. indica

Family – Meliaceae

Locality : Arumbakkam , Chennais

Tamil : Veppai (வேப்பை)

**OBJECTIVES OF THE CURRENT STUDY**:

80% of the world's underdeveloped and developed nations still rely heavily on plant-based medicines for their main health care. Nearly two-thirds of all people on the planet relies on the curative power of plant based natural medicines for the reasons of their traditional use, belief, availability, accessibility and affordability. The traditional medicines are originated comes from plants that normally do not form the constituents of routine diet. However, most of the healing plants have not received proper scientific scrutiny. *Azadirachta indica* is one such plant traditionally used for medicinal purpose known to possess wide array of pharmacological actions and has been widely used for various ailments.

The current study's range of inquiry includes

* The Azadirachta indica leaf extract underwent phytochemical screening in order to conduct a qualitative investigation of the numerous bioactive components.
* Mineral content measurement to check for the presence of physiologically significant minerals Estimation of vitamin content in *Azadirachta indica*  leaves
* Vitamin content of Azadirachta indica leaves is estimated Analyzing Azadirachta indica's capacity to scavenge free radicals leaves extract by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Nitric Oxide (NO) and Superoxide radical scavenging assays.
* Determination of Antibacterial and antifungal potentials of *Azadirachta indica*  leaves extract

**PRELIMINARY PHYTOCHEMICAL SCREENING**

Azadirachta indica leaf extract's antibacterial and antifungal properties PRELIMINARY PHYTOCHEMICAL SCREENING Preliminary phytochemical screening of several plant elements was performed on the ethanolic extract of Azadrachta indica leaves (Harborne, 1998; Kokate, 2005).

* **TEST FOR ALKALOIDS**

1. Dragendorff”s Test
2. Wagner’s Test
3. Mayer’s Test

* **TEST FOR FLAVONOIDS**

1. Shinoda’s Test:
2. Alkaline Reagent Test

* **TEST FOR CARBOHYDRATES**

1. Molisch’s Test

* **TEST FOR GLYCOSIDES**

1. Legal’s Test
2. Borntrager’s Test

* **TEST FOR SAPONINS**
* **TEST FOR TANNINS**

1. Ferric Chloride Test
2. Lead Acetate Test

* **TEST FOR PHYTOSTEROL**

1. Libermann Burchard Test
2. Salkowski’s Test

* **TEST FOR TRITERPENOIDS**

1. Libermann Burchard Test
2. Noller Test

* **TEST FOR PROTEINS & AMINO ACIDS**

1. Ninhydrin Test
2. Biuret Test

* **TEST FOR ANTHRAQUINONES**
* **TEST FOR PHENOLS**

**DPPH RADICAL SCAVENGING ASSAY**

The free radical scavenging capacity of the DPPH was used to calculate the ethanolic extract of Azadirachta indica (Brand Williams et al., 1995). A 95% methanol solution of DPPH (500 M) was created. Five test tubes each contained 100, 200, 300, 400, and 500 g/ ml of the stock plant extract solution. The test medication was incubated with 0.5ml of newly made DPPH solution for 10 minutes, and the absorbance was measured using a spectrophotometer at 517 nm. Ascorbic acid standard was employed as a benchmark.

**ABTS ASSAY**

The ethanolic extract of Azadirachta indica was tested for its ability to scavenge ABTS radicals using the Re et al., 1999 method. ABTS stock solution (7 mM in water) and 2.45 mM potassium persulfate were combined to create the ABTS radical cation (ABTS•+), which was then let to sit in the dark at room temperature for 12–16 h before being used. Then, ABTS•+ using ethanol, the solution was diluted to have an absorbance of 0.7 at 734 nm. To 3.0 ml of diluted ABTS•+ solution, different concentration (200-1000 μg/ ml) of leaves extract in ethanol was added then, after 1 min the decrease in absorbance was measured spectrophotometrically at 734 nm.

**DETERMINATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY**

*MINIMUM INHIBITORY CONCENTRATION (MIC) MINIMUM BACTERICIDAL CONCENTRATION (MBC), MINIMUM FUNGICIDAL CONCENTRATION (MFC) ASSAYS*

The Clinical and Laboratory Standards Institute (CLSI) advised using a series of 2-fold macro-broth dilutions to estimate the MICs and MBCs of Azadirachta indica extract for the various tested bacterial suspensions (concentration) (Wikler, 2008). Azadirachta indica extract's minimal inhibitory concentration (MIC) against different fungal strains was calculated by broth microdilution method as described by the National Committee for clinical laboratory standards for fungi (M27-A2). The stock solutions of *Azadirachta indica* extract was diluted suitably as required from stock solution. The ranges should be prepared one step higher than the final dilution range required that if a final dilution range of 0.5, 1, 2, 4, 8, and 16 mg/ml is required then a range of 1, 2, 4, 8, 16 and 32 mg/ml should be prepared to compensate for the addition of an equal volume of inoculums. Two rows of 12 capped test tubes were arranged in the rack. For the first tube in each row, 8 ml of MH broth (bacteria) and 8 ml of SD broth (fungi) with the necessary concentration of Azadirachta indica extract were created from the appropriate stock solution already made and placed in a sterile 30 ml (universal) screw-capped container. A sterile pipette was used to combine the universal bottle's contents before transferring 2 ml to the first tube in each row. After adding 4 ml of broth to the remaining 4 ml in the universal bottle and thoroughly mixing it, 2 ml was transferred to the second tube in each row using a brand-new, sterile pipette. This method of dilution was carried out for up to 10 tubes. The final tube in each row received 2 ml of broth devoid of Leaves extract. By adding sterile distilled water as described above, the density of the bacterial suspension was changed (108 CFU/ml) to match that of the 0.5 McFarland standard. After being appropriately diluted (106 CFU/ml), the bacterial suspension was introduced to the tubes containing MH broth. By adding sterile distilled water as described above, the density of the fungal suspension was adjusted (3106 to 5106 CFU ml-1) to match that of the 0.5 McFarland standard. A positive control for bacteria was utilized, consisting of 30 g of chloramphenicol. The turbidity of the tubes was evaluated visually after 24 hours of incubation at 37oC in contrast to the uninoculated control.

Amphotericin B, 10 g/disc, was used as a positive control in the fungus assays. The turbidity of the tubes was visually evaluated after incubation at 28 oC for 42–78 h in comparison to the uninoculated control. The MIC is defined as the lowest concentration of the leaf extract required to support bacterial and/or fungal growth without evidence of post-incubation growth. Each experiment was performed three times. By sub-culturing 100 l from each tube from the MIC assay onto substance-free MH agar plates, the MBC was produced.

The MBC was determined as the lowest concentration of material that permits no discernible growth on the agar plate after the plates were incubated at 37oC for 24 hours. By plating a 100 l volume from the tubes displaying no discernible growth on SDA, the MFC was ascertained. The plates were incubated in MIC as previously mentioned. The MFC was defined as the lowest chemical concentration that prevented any observable growth on the agar plate.

**DETERMINATION OF ANTIMICROBIAL ACTIVITY**

The antibacterial activity of ethanolic extract of *Azadirachta indica* was tested against three Gram positive and three Gram negative bacteria. The inhibitory effect was assessed by well diffusion method. By using the serial dilution approach, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were also ascertained. The ethanolic extract of Azadirachta indica was tested for its antifungal abilities against popular pathogenic fungus strains. The disc diffusion method was used to evaluate the inhibitory impact. The serial dilution approach was also used to calculate the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC).

**RESULT AND DISCUSSION**

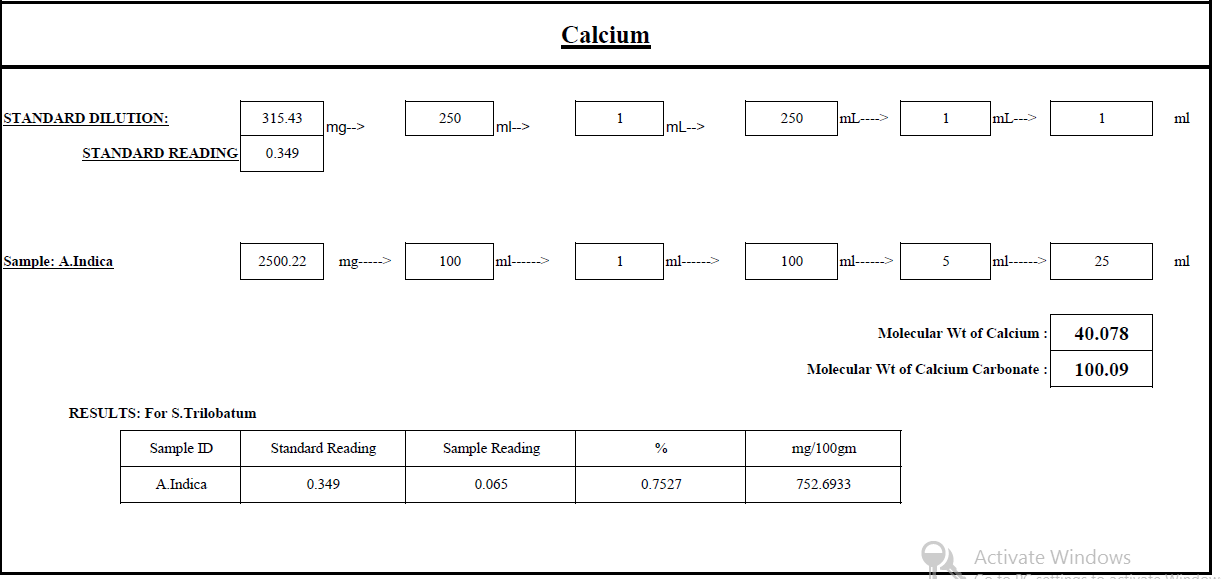
**Table 1: Phytochemical analysis of Azadirachta indica**

|  |  |
| --- | --- |
| **PHYTOCONSTITUENTS** | **INFERENCE** |
| Alkaloids | + |
| Flavonoids | + |
| Saponins | + |
| Tannins | + |
| Phytosterol | \_ |
| Diterpenes | \_ |
| Triterpenoids | \_ |
| Glycosides | + |
| Anthraquinones | \_ |
| Phenols | + |

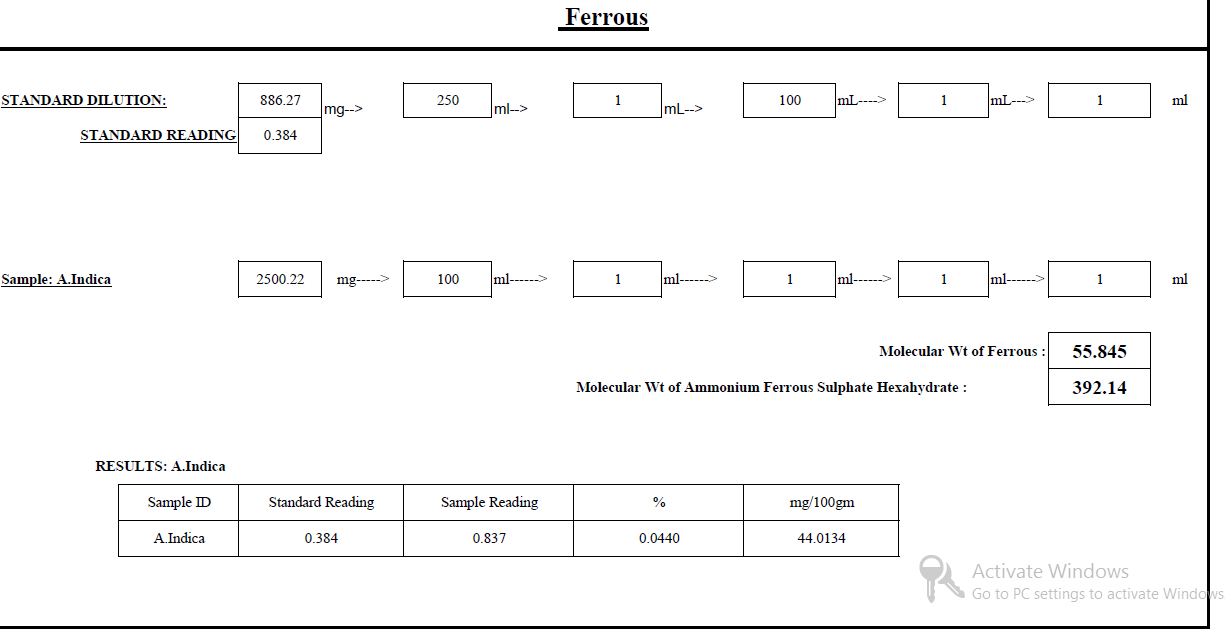
The above table shows the qualitative analysis of phytochemicals present in the ethanolic extract of Azadirachta indica *leaves extract*. From the preliminary phytochemical evaluation, it was found that the *Azadirachta indica leaves extract* extract has a positive response for the presence alkaloids, flavonoids, glycosides, saponins, Anthraquinones, Phenols tannins indicating the role of these phytochemicals in the observed effect.

The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. Phytochemicals include compounds with various biological properties (i.e. antioxidant, antiproliferative, DNA repair) which allow plants to cope up with environmental challenges including exposure to radiation and toxins. They are bioactive substances (secondary metabolites) that work with nutrients and dietary fibre to ward off disease. They are found in plants.Secondary metabolites such glycosides, alkaloids, and flavonoids have been reported to be present in the majority of plants with antidiabetic activities (Odetola et al., 2006). It has been demonstrated that many plants have strong antioxidant effects because of their phenolic components. According to an earlier research, this species' phytochemical screening revealed the presence of alkaloids, flavones, saponin, and flavonoids.From Azadirachta indica species leaves, alkaloids such soladunalinidine, solasonine, and solamargine have been identified.

**Table 2: Mineral analysis of Azadirachta indica**

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**Table 3: Minerals analysis of Azadirachta indica**

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The above tables represents the mineral content of Azadirachta indica leaves extract. The differences in the concentration of these elements are attributed to the soil composition and climate in which a plant grows. It has been found that the leaves of *Azadirachta indica* is rich in minerals like copper, magnesium, manganese, vanadium, chromium, calcium, zinc, sodium, and potassium which possess several pharamacological properties.

**Figure 1: Retention time for Vitamins (Standard)**



**Figure 2: Vitamin Content of Azadirachta indica Leaves extract**



The above figures represents the retention time for vitamin (Standard) and the vitamin content of Azadirachta indica leaves. The leaves content contain Ascorbic acid amounts. Metal as micronutrient is important for the normal functioning of vital organs and is present in many enzymes which activate them, thereby influence the biochemical processes that are required in our diet.

The present study has shown that the Azadirachta indica leaves examined have an appreciable content of contain Ascorbic acid. The leaves also contained good minerals with abundance of them in calcium, zinc, iron, manganese, and magnesium while they were least in potassium. The results suggest that the leaves if consumed in sufficient amount would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against several diseases.

***Invitro antioxidant potential of Azadirachta indica***

The free radical scavenging potential of natural products can be assessed by several assays. Among them, DPPH, ABTS, assays are routinely practiced for the assessment of antioxidant properties of different natural compounds, as they are easy, affordable and reliable. In the present study, the antioxidant potential of Azadirachta indica leaves extract is examined by DPPH, ABTS assays.

The ability of natural compounds to scavenge the DPPH radical can be expressed as its magnitude of antioxidative ability. DPPH radical in alcoholic solution is deep purple in colour with an absorption peak at 515 nm. DPPH assay is based on the principle that DPPH radical on accepting a hydrogen atom from the scavenger molecule i.e. antioxidant, results in reduction of unpaired valence electron at one atom of nitrogen bridge in DPPH leading to the change of purple color to yellow with concomitant decrease in absorbance at 515 nm . The change in colour from deep purple to yellow or the decrease in intensity signifies the antioxidant potential of the test compound.

ABTS assay is used for the screening of antioxidant activity of both water and lipid soluble compounds. The assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS, generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of antioxidants. The change in color intensity is proportional to the antioxidant efficiency of compounds.

**Figure 3: DPPH radical scavenging potential of Azadirachta indica (Ethanolic extract)**

**% inhibition**

**Concentration (µg)**

**Figure 4: DPPH radical scavenging potential of Azadirachta indica (Hexane extract)**

**% inhibition**

**Concentration (µg)**

The above figures shows the dose dependent effect of Azadirachta indica on the percentage inhibition of DPPH and ABTS radicals present in the reaction mixtures. The ethanolic extract of Azadirachta indica scavenges DPPH and ABTS radical in a concentration dependent manner. The antioxidants react with DPPH, a purple colored stable free radical and convert it into a colorless α-α-diphenyl-β-picryl hydrazine. The amount of DPPH reduced could be quantified by measuring a decrease in absorbance at 517 nm. ABTS assay is used for the screening of antioxidant activity of both water and lipid soluble compounds. The assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS, generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of antioxidants .The change in color intensity is proportional to the antioxidant efficiency of compound. The leaves extract of Azadirachta indica significantly and concentration dependently reduced DPPH and ABTS radicals. The dose dependent free radical scavenging effect of ethanolic as well as hexane extract is shown in However at a concentration of 500µg/ml, the ethanolic extract significantly scavenged 80.31 % of DPPH radicals, hexane extract scavenged 75.47% of DPPH radicals and ethanolic extract (500µg/ml) scavenged 79.10 % and hexane extract scavenged 75.83%ABTS radicals indicating the free radical scavenging potential of the extract.

The worldwide increase in resistance of pathogenic microorganisms to time-honored antibiotics necessitates the search for alternative strategies preferably from plant origin. Many secondary metabolites produced by plants, including flavonoids, alkaloids, and tannins, have long piqued human curiosity. However, there are very few studies on the pharmacological effects of medicinal plants, and only a small portion of the earth's 40,000 plant species have been carefully examined for their antimicrobial effects (Shokeen et al., 2009). There are currently no experimental scientific investigations that can prove the potential antibacterial qualities of many of these medicines, despite screening of Indian medicinal plants having indicated various degrees of antimicrobial activity against pathogenic and opportunistic microbes. A natural biological reaction of bacteria to the selection pressure of an antimicrobial medication is antimicrobial resistance.

Since antibiotic use became widespread 50 years ago, microorganisms have relentlessly developed resistance (Martínez and Baquero, 2002). The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient (Nascimento et al., 2000). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). This *in vitro* study demonstrated that folk medicine can be as effective as modern allopathic medicine to treat pathogenic microorganism. The use of *Leaves* in folk medicine suggests that it represent an economic and safe alternative to treat common infectious diseases. Detailed investigations in to the active components responsible for the observed antimicrobial activity may open new avenues for drug development and control of antibiotic resistant pathogenesis (Quave, 2008). Plant based antimicrobials represent a vast untapped source for medicines and further exploration of their usefulness is necessary.

At least 130 medications are currently in use worldwide, albeit some of them are now being produced synthetically for financial reasons (Newman et al., 2000). These drugs are all single chemical entities taken from higher plants and modified further synthetically. As a result, research into the antibacterial and antifungal properties of leaves, a common medicinal plant that has been widely utilized in traditional medicine in one form or another for its advantageous pharmacological action, was explored on a global scale.

**Table 4: Antibacterial activity of *Azadirachta indica* extract - Zone of inhibition in diameter (mm).**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No.** | **Bacterial species** | **Control** | **50 μg** | **100 μg** | **150 μg** | **200 μg** | **250 μg** | **Chloramphenicol  (30 μg)** |
| **Gram Positive** | |  | | | | | | |
| 1 | *Staphylococcus epidermidis* | - | 2.0 | 6.0 | 15.0 | 18.0 | 22.0 | 20 |
| 2 | *Bacillus subtilis* | - | 3.0 | 8.0 | 16.0 | 23.0 | 22.0 | 25 |
| **Gram Negative** | |  | | | | | | |
| 3 | *Klebsiella pneumoniae* | - | 4.0 | 9.0 | 13.0 | 21.5 | 25.0 | 28 |
| 4 | *Salmonella typhi* | - | 1.0 | 7.0 | 15.0 | 18.5 | 21.0 | 25 |

The above table shows the antibacterial activity of ethanolic extract of *Leaves. Leaves* four different Gram positive and Gram negative bacterial strains. The antibacterial potency of *Leaves* extract was evaluated by the presence or absence of inhibition zones and zone diameters (mm). The results of the present study indicate that the ethanolic extract of *Leaves* showed a maximum inhibitory zone in a dose dependant manner. However, there was no significant difference between the levels of zone of inhibition at the concentration of 200 µg and 250 µg. Among the Gram positive bacteria, *Bacillus subtilis* showed a larger diameter of clearance than that of other Gram positive bacteria used in this study. Among the Gram negative bacteria, *Klebsiella pneumoniae* than that of other Gram negative bacteria.The zone of clearance achieved by *Leaves. Leaves* extract is comparable to that of standard antibiotic, chloramphenicol.

**Table 5: MICs and MBCs of *Azadirachta indica* extract on Gram positive and Gram**

**negative bacteria**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bacterial species** | **Minimum Inhibitory Concentration (MIC)** | | **Minimum Bactericidal Concentration (MBC)** | |
| ***Azadirachta indica* extract (mg/ml)** | **Chloramphenicol (µg/ml)** | ***Azadirachta indica* extract extract (mg/ml)** | **Chloramphenicol (µg/ml)** |
| **Gram positive** | | | | |
| *Staphylococcus epidermidis* | 4 | 3 | 2 | 4 |
| *Bacillus subtilis* | 3 | 2 | 2 | 3 |
| **Gram negative** | | | | |
| *Salmonella typhi* | 3 | 2 | 5 | 3 |
| *Klebsiella pneumoniae* | 2 | 2 | 2 | 4 |

The minimum inhibitory concentration and minimum bactericidal concentration of Leavesextract as well as the standard antibiotic, chloramphenicol is shown inthe abovetable**.** The MIC value of *Leaves* extract against both Gram positive and Gram negative bacterial strains varies from 1 mg to 5 mg and the results are comparable with the standard antibiotic, chloramphenicol. The highest MIC values were shown by *Enterococcus faecalis* in Gram positive bacteria and by *Salmonella typhi* in gram negative bacteria. The lowest MIC values were displayed by *Bacillus subtilis* in Gram positive bacteria and *K.pneumoniae* in gram negative.

The results of the study indicated that *Leaves.* Leaves extract showed effective inhibitory activity against Gram-positive bacteria, *Bacillus subtilis* and gram negative bacteria *Klebsiella pneumonia. Bacillus subtilis* showed a larger diameter of clearance than that of other Gram positive bacteria used in this study. Similarly, *Leaves. Leaves* extract showed a maximum zone of clearance in the Gram negative bacteria, *Klebsiella pneumoniae* than that of other Gram negative bacteria.

Minimum inhibitory concentrations are considered the “gold standard” for determining the susceptibility of microorganisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing (Andrews, 2001). A lower MIC number means that less medication is needed to stop an organism's growth, making lower MIC antimicrobials more effective antimicrobial agents. The highest MIC and MBC values were shown by *Enterococcus faecalis* in Gram positive bacteria and by *Salmonella typhi* in gram negative bacteria. The lowest MIC and MBC values were displayed by *Bacillus subtilis* in Gram positive bacteria and *K.pneumoniae* in gram negative.

**Table 6: Antifungal activity of *Azadirachta indica* extract against fungal species tested by disc diffusion assay.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No.** | **Strains** | **Control** | **0.175 mg/disc** | **0.375 mg/disc** | **0.75 mg/disc** | **1.5 mg/disc** | **3 mg/disc** | **Amphotericin B** |
| **1** | *Saccharomyces cerevisiae* | **\_** | **\_** | 7.0 | 11.5 | 13.0 | 15 | 20 |
| **2** | *Penicillium chrysogenum* | **\_** | 8.0 | 10.0 | 15 | 20 | 22 | 24 |

The above table shows the antifungal activity of ethanolic extract of *leaves and* eight different fungal species. The antifungal potency of *leaves and* extract was evaluated by the presence or absence of inhibition zones and zone diameters (mm). It is evident that the ethanolic extract of *Leaves* showed a maximum inhibitory zone in a dose dependant manner. However, there was no significant difference between the levels of zone of inhibition at the concentration of 1.5 mg and 3 mg/disc. The antifungal potency of *leaves* the *C. albicans* showed a larger diameter of clearance than that of other strains. Moreover, the zone of clearance achieved by *leaves and* extract is comparable to that of standard drug, Amphotericin B.

The minimum inhibitory concentration and minimum fungicidal concentration of *leaves* extract as well as the standard antifungal drug, Amphotericin B is depicted in Table 22. The MIC value of *leaves* extract against fungal strains varies from 1 mg to 7 mg and the results are comparable with the standard antifungal agent, Amphotericin B. The highest MIC values by *Saccharomyces cerevisiae.*

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries (Portillo et al., 2001). In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. Although new drugs have been introduced to combat this problem, the development of resistance to antifungal drugs has become increasingly apparent, especially in patients who require long-term treatment or who are receiving antifungal prophylaxis, and there is growing awareness of shifts of flora to more-resistant species.

The fungal strains used in the present study were selected on the basis of their clinical importance. Agar disc diffusion method was performed in the present study to investigate the antifungal activity ofleaves extract. The highest activity (diameter of zone of inhibition 25 mm) was demonstrated by the ethanolic extract of *leaves* *C. albicans* while the lowest activity was observed against *S. cervisiae*. The results of the *in vitro* antifungal assay revealed that the growths of fungal strains were affected by the *leaves* extract by forming clear inhibition zones.

The MICs and MFCs showed that *S. cerevisiae* has the highest MIC (7mg/ml) and MFC (7mg/ml) while the lowest MIC of 2 mg/ml was demonstrated by *C. albicans*. The fungistatic or fungicidal effect of natural products and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelium germination inhibition and it is also reported that plant lytic enzyme act in the fungal cell wall causing breakage of β-1,3 glycan, β-1,6, glycan and chitin polymer (Brull, 1999). The observed antifungal effect of the extract might be due to the presence of biologically important ingredients present in the leaves.

The remarkable bactericidal, fungicidal effects of *leaves* extract suggest that the leaves a may be a useful source for the development of novel antibacterial, antifungal agent against pathogenic bacteria and fungi.

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