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# "Antimicrobial Activity of Leaves Extract of *Plumbago Zeylanica* Plant against Known Drugs"

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**Abstract:** *Antimicrobial properties of leaves extracts of P.zeylanica plant against known drugs. Because different part of this Plant ( such as :-leaf, root, flower, fruit ), are traditionally, claimed to be used for the treatment of ailment including Anti-fungal, Anti tumor disease of Heart Rheumatic pains, Liver diseases, Fever, Diabetes, and Kidney diseases. This plant are use in various diseases treatment, so that i will use P. zeylanica plant for analysis of Anti-microbial activities.*

## 1. INTRODUCTION

The P. *Zeylanica* is commonly know as Ceylon, Leadwort, Chitra, Chitra, and Chitramoolam. P. Zeylanica, *(Plumbaginaceae)* is perennial, sub-scandant strub one of the common plant used in Indian traditional system of medicine. Is a tripical strub, it grow wild as a garden plant in India.

The leaves & root *of P. zeylanica* are widely used medicinally in India (Bhattacharjee 1998, chen et, al; 2011) its leaves are simple, alternate, ovate, lanceolate and acute. The root is cylindrical and irregularly bent having transverse shallow fissures at bent. Plumbogin administered intratumorally and orally at 2mg/kg. deacreed tumor growth by 70% and 60% respectively in rates with methyl chelanthrence indused tumor it's ED so was 0.75 mg /kg. Plumboagin was active against p388 lymphotic, eukeamia at 4mg/kg. And showed Antibacterial and Antifungal activity against awide variety of bacteria and fungi**.** (M.P.singh & himadri panda 2005)**.** *P.zeylanica* is belived to kill intestinal parasites, and it is used clinically to Rheumatism, Intestinal parasites, Anemia, due to **"**stagnant blood**",** external and Internal Trauma, Toxin, Swelling, Itching, Ringworm and Mali Gnant Furunculous Scabies**.** (Jiangsu 1979)**.** In India it is usually used to treat fever of Malaria. Pharmacological studies have indicated that, *P.zeylanica* extract has Antiplasmodial (Simonsen *et, al*; 2001**),** Antimicrobial (Ahmad et, al; 2000), Antifungal (Mehmood et, al; 1999), Anti inflammantory (Oyedapo 1996)**,** Anti hyperglycemic (Alaguniju *et, al;* 1999), hypolipidaemic and Anti atherosclerotic Activities. (Sharma *et, al;* 1991).

P.zeylanica root is powerfully poisonous and its internal use is attended with great danger, it couses obortion. The root is sometimes given internally but more commonly it is employed as a local irritant to the us uteri. It is also used as an irritant to be skin by malingeners or to support false chatges. It enters into the composition of several India preparation used as caustic or abortifiacients, root reduced to a past is opening them, with milk, vinager or salt and water the past may be applied in leprosy and other obstinate skin diseases, unhealthy ulcers scabies ets. Milky juice is also unuseful application. In Ayurvada root & leaves is useful in dyspepsia, piles, anasarca, diarrhoca skin diseases ets. A tinclare the root bark is employed as an antiperiodic. A favourite medicine for platulence is a powder called shadhkarana yoga recommended by susruta.

Root has a beneficial effect on piles, in these cases it is given in vireos combination. E.g. an earthen jar or pot of which the inside is lines with a past of the root is uses for preparing curds (Dadhi or Kanjica) which is given to persons suffering from haemorrhoids and prurigo.

Root & leaves was employed in the treatment of intermiltent fever by Dr.Oswald it act as a powerful sudoriffic first pound it with ghee and add powders previously made add powders previously made and pound them again with ghee and convent into pills of 6 grain each. Dose is 1 to 4 pills as after active and tonic useful in nervous and rheumatic effection and in reducing obesity. (M.P.singh *et.al* 2005).

**Material: Simple Collection** (1) Plant material (2) Micro organisms (3) Antibiotic

**Laboratory Media:** (a) Nutrient agar media (b) sabouraud agar/potato dextrose agar media

## 2. MATERIAL AND METHOD

### 2.1. Sample Collection

Fresh leaves of the medicinal plant *P.zeylanica* **(*plumbaginaceae*)** were collected from the Institute of Medicinal Plant,

### 2.2. Sample Preparation

Fully growth fresh leaves of *P.zeylanica* were weight (2k.g) .The leaves were shade- dried at room temperature for 10 days & powdered in grinder. The dried leaves were crushed in to a coarse powder with the help of an electrical grinder. The powder so obtained was stored in air tight, the glass bottles for use even required.

### 2.3. Crude Soxhlet Extraction

The apparatus, first described by FRANZ VON SOXHLET in 1879. Is was originally designed for the extraction of a lipide from a solid material.

Principle- A soxhlet method is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent,

The basic procedure calls for a solid sample to be placed in a porous container and allowing continuously solvent to extract continuously.

### 2.4. Bacteria Strains & Fungus Strains

*E.coli* (gram-negative), Staphylococcus aureus, bacillus cereus (gram- positive) and candida fungus.

### 2.5. Preparation of Inoculums

Bacterial strain preserved in nutrient agar at 4c were revived in nutrient broth and incubated at 37 +1c overnight, and fungus strain preserved in sabouraud dextrose agar at 4c were revived in sabouraud broth and incubated at 28= 24 houres.

### 2.6. Selection of Reference Antibiotics

Reference antibiotics ciprofloxacin (bacteria), co-trimoxazole (fungus) was obtained from authorized medical shop Bilaspur (C.G), the purity of the antibiotic is 99.8.

### 2.7. Dilution and Inoculums Preparation

The dried plant extracts *Plumbago zeylanica* leaves and antibiotic (ciprofloxacin, & co-trimoxazole) were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 5 to 10 mg/ml.

## 3. WELL DIFFUSION METHOD

The antimicrobials present in the plant extract are allowed to diffuse to out into the interact in a plate freshly seeded with the test organisms the resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres. The inhibition zone produced by a commercial antibiotic- Ciprofloxacin (bacteria), and Co-trimoxazole (fungus) was used as positive control, and the solvent (aqueous, methanol, & acetone) as the negative control.

**Nutrient Agar Medium:** - the medium was prepared by dissolving 33.9 g of the commercially available nutrient agar medium in 1000ml of distilled water. The dissolved medium was the dissolved medium was autoclaved at 15 Ibs pressure at 121°C for 15 minutes .the autoclaved medium was mixed well and poured on to 100ml petreplets (25-30ml/plats) while still molten.

**Nutrient Broth :-( 1liter)** 1 l of nutrient broth was prepared by dissolving 13g of commercially available nutrient medium (Hi midia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autocaving at 15 Ibs pressure (120°C-121°C) for 15 minutes.

## 4. AGAR DISC DIFFUSION METHOD

Paper discs impregnated with specific antibiotics or the test substances are placed on the surface of the nutrient agar medium, and sabaroued dextrose agar media inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard .the plates are incubated and the zones of inhibition around each dish are measured.

## 5. OBSERVATION

Antimicrobial properties test were then carried out by well diffusion method & disc diffusion method after incubation of nutrient agar media, sabouraud dextrose plates with bacterial/fungal strain, positive control (antibiotics ), negative control (solvent) and plant extracts for 24 hrs at 37° c and 48 hrs at 28° c respectively inhibition zone were observed. As the concentration of antibiotics and plant extracts the inhibition zone obtain for both method in each microorganisms.

## 6. RESULT & DISCUSSION

In study aqueous extract, methanol extract, and acetone extract of *Plumbago zeylanica L*. leaves were test various bacteria (gram +Ve, & gram –Ve bacteria) and fungus, spectra showing inhibition in millimetre. Antibiotics are using as positive control. The result is found in such a way that the aqueous extract is more effective in all four micro-organism as compared to methanol extract and acetone extract.

The use of medical plant of *Plumbago zeylanica* play a large role in covering the basic health needs in developing countries, these plant may after new sources of antibacterial , antifungal, and antiviral agents with significant activity against infective micro-organisms (Munoz-monger et al 2003, Coelho de souza *et al* 2004 ). The antimicrobial activity of medicinal plant against various pathogenic bacteria has been demonstrated, (tadhani and subhash, 2006, Lino and Deogracious, 2006, Doughari, 2006, Abere et al 2007). The in vitro antimicrobial activity and the minimum inhibitory concentration of crude extract of P.zeylanica leaves and antibiotics were assessed by the well diffusion method and disc diffusion method. The results were compared with the standard antibiotics (ciprofoxine from bacteria & cotrimozole from fungus, the result showed revealed significantly higher bacteria and fungus then that of plant extract and standard antibiotics.

## 7. LIST OF TABLE

**Table1.** *Inhibition zone of plant extract –well diffusion method*

| microorganisms | Aqueous extract | | | Methanol extract | | | Acetone extract | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1ml | 2ml | 3ml | 1ml | 2ml | 3ml | 1ml | 2ml | 3ml |
| *E.coli* | 7mm | 11mm | 4mm | 8mm | 8mm | 5mm | 4mm | 5mm | 3mm |
| *Staphylococcus* | 10mm | 11mm | 5mm | 8mm | 8mm | 3mm | 6mm | 8mm | 4mm |
| *Bacillus* | 8mm | 6mm | 4mm | 6mm | 7.2mm | 3mm | 6mm | 6mm | 3mm |
| *Candida* | 7mm | 10mm | 5mm | 5mm | 7mm | - | 7mm | 5mm | - |

**Table2.** *Inhibition zone of antibiotics- well diffusion method*

| MICROORGANISMS | INHIBITION ZONE OF ANTIBIOTICS | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Aqueous extract | | | Methanol extract | | | Acetone extract | | |
| | 1ml | | | 2ml | | | 3ml | | |
| *E.coli* | 7mm | 8mm | 6mm | 7mm | 6mm | 6mm | 5mm | 5mm | 4mm |
| *Staphylococcus* | 8mm | 9mm | 6mm | 6mm | 6mm | 4mm | 6mm | 7mm | 5mm |
| *Bacillus* | 6mm | 6mm | 5mm | 5mm | 6mm | 3mm | 6mm | 6mm | 2mm |
| *Candida* | 9mm | 7mm | 5mm | 6mm | 7mm | 5.6mm | 4mm | 5mm | 3mm |

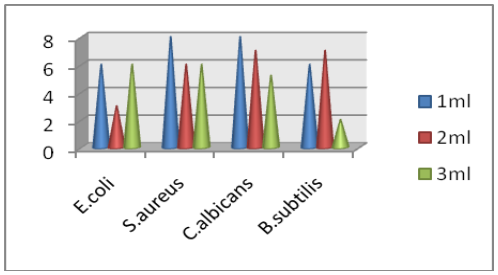
**Table3.** *Inhibition zone of plant extract- disk diffusion method*

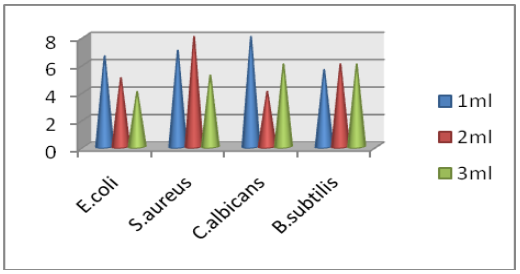
| Micro organism | aqueous | | | Methanol | | | Acetone | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1ml | 2ml | 3ml | 1ml | 2ml | 3ml | 1ml | 2ml | 3ml |
| *E.coli* | 11mm | 7mm | 7mm | 6mm | 6mm | 4mm | 6.6mm | 5mm | 4mm |
| *staphylococcus* | 8mm | 8mm | 7mm | 7mm | 6.5mm | 3.3mm | 7mm | 8mm | 5.2mm |
| *Bacillus* | 6mm | 9mm | 5mm | 5mm | 6mm | 4.2mm | 5.6mm | 6mm | 6mm |
| *candida* | 5mm | 7mm | 4mm | 8mm | 6mm | 5mm | 8mm | 4mm | 6mm |

**Table4.** *Inhibition zone of antibiotics – disk diffusion method*

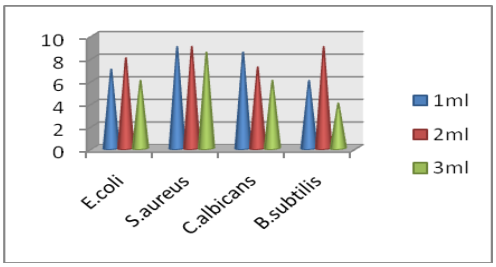
| Micro-organisms | Aqueous extract | | | Methanol extract | | | Acetone extract | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1ml | 2ml | 3ml | 1ml | 2ml | 3ml | 1ml | 2ml | 3ml |
| *E.coli* | 7mm | 8mm | 6mm | 6mm | 9mm | 5mm | 6mm | 3mm | 6mm |
| *Staphylococcus* | 9mm | 9mm | 8.5mm | 5mm | 8mm | 8mm | 5mm | 6mm | 6mm |
| *Bacillus* | 6mm | 9mm | 4mm | 8mm | 4mm | 5.2mm | 6mm | 7mm | 2mm |
| *Candida* | 8.5mm | 7.2mm | 6mm | 7mm | 7mm | 5mm | 8mm | 7mm | 5.2mm |

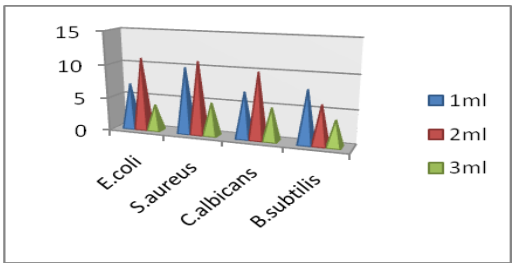
*Inhibition zone of plant extract:* *Inhibition zone of antibiotics*



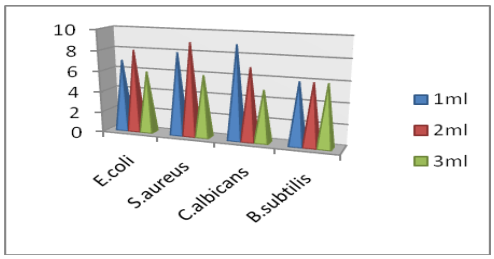



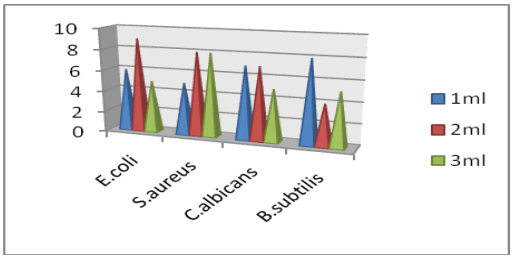

*Inhibition zone of plant extract:* *Inhibition zone of antibiotics*



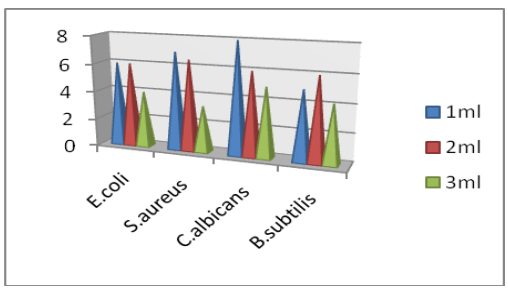



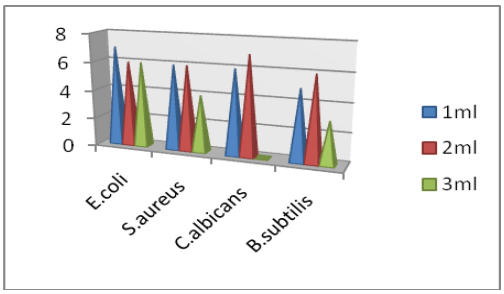

*Inhibition zone of plant extract:* *Inhibition zone of antibiotics*



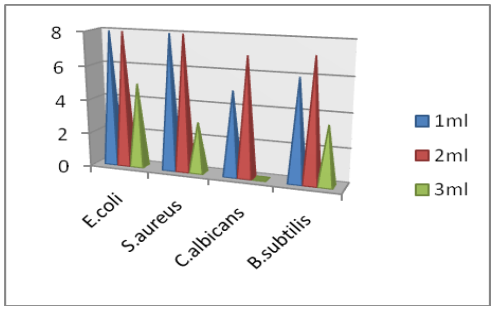




*Inhibition zone of plant extract:* *Inhibition zone of antibiotics*




*Inhibition zone of antibiotics*



## 8. Inhibition Zone of Plant Extract and Antibiotic by Well Diffusion and DISE Diffusion Method


## 9. Conclusion

*Plumbago zeylanica* is used for centuries in ayurvedic medicine to increase longitivitly and vitality, it is the most important medicinal plant extensively used in herbal formulation and antimicrobial activity. In this study we have antimicrobial properties of Plumbag zeylanica from against known drugs .from the results of antimicrobial properties three solvent (name as:-aqueous, methanol, acetone) used in this studies. Aqueous extract solution was exhibited best antibacterial & antifungal activity. The micro-organisms using –E-coli,

*Staphylococcus aureus, bacillus and canedida* (fungus). And the antibiotics (ciprofloxacin for using bacteria & co-trimoxazole for using fungus ) are exhibited maximum zone of inhibition bacterial growth and fungal growth. Plumbago zeylanica leaves can exhibit varying degrees of therapeutic values in treatment of fungal, malaria, and bacterial infection including cancer. Plumbago zeylanica leaves extract has been very good antimicrobial properties.

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