Phytochemical Investigation And Antibacterial Activity Of *Pongamia pinnata*(L). Against Some Multidrug Human Pathogens.

Madhuri M. Deshmukh1* and Navnath G.Kashid2

1*Research scholar, Dr. BAM University Aurangabad.
2Assistant professor, Department of Botany, Vasant Mahavidyalaya Kaij, Dist. Beed. Department of Botany, Vasant Mahavidyalaya Kaij Dist: Beed (MS). India.

Received: September 08, 2018                   Accepted: October 22, 2018

ABSTRACT

The present study aimed at evaluating the in vitro Phytochemical evaluation and antibacterial activity of Aqueous, Chloroform, Ethanol, Methanol, Petroleum Ether extracts of *Pongamia pinnata* against *Escherichia coli*, (ATCC 25922), *Salmonella Typhimurium*,(734 MTCC), *Staphylococcus aureus* (25923 ATCC) *Klebsella pneumonia* (MTCC) and *Pseudomonas aeruginosa* (ATCC 27953). The various phytochemicals Alkaloid, Flavonoid, Steroid, Phenol, Glyceroids, Triglyceroids, Amino acid and Proteins are present in tested plant, which showed Antibacterial Activity against selected human pathogens. The Ethanolic extract showed the highest anti-S. typhiactivity and was effective against all bacterial strains tested. Methanol, Chloroform, Aqueous extracts showed moderate activity and Petroleum Ether showed minimum inhibitory activity.

Keywords: medicinal plants, Phytochemicals, antibacterial activity.

INTRODUCTION: The medicinal plants proved bless for all living organisms for healing as well as for curing of human diseases. Due to the presence of Biochemical constituents Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are of two types, primary and secondary compounds. Chlorophyll, proteins, and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. Phytochemicals are the chemicals produces by various parts of the plants. These bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. These compounds have various activities such as antibacterial some have been reported to exhibit haemolytic and foaming activity reported by Feroz et al. The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening. Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings.

Plants have endless potential to synthesize economically important compounds such asphenolic compounds, Flavenoids, steroids. Amino acids, nitrogen containing compounds, vitamins and minerals which have anti-oxidant, anti-tumor, antimutagenic, anti-carcinogenic and diuretic activities. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance, search for plant resources has increased for their potential antimicrobial activity. According to world health organization medicinal plants would be the best source to obtain a variety of drugs in developed countries about 80% of plants are used in traditional medicine. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover muchmore potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings. The Current research in drug discovery from medicinal plants involves a multifaceted approach combining the phytochemical, botanical, biological, and molecular techniques. The medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including inflammation, cancer, HIV/AIDS, Alzheimer's, malaria, and typhoid, dysentery pain.

An effective collaboration between the traditional and western medical practitioners are due to the use of traditional and herbal medicines. Many species of plants synthesize and accumulate organic substances in quantities to be economically useful as chemical feed stocks or as raw materials for various scientific and commercial applications. The natural substances are applied, directly or indirectly, by a huge number of industries, and natural plant products. For example, phytochemicals are utilized to a large extent by the pharmaceutical, cosmetics, food and agrochemical industries. increasing incidence of drug resistant
pathogens have drawn attention of the pharmaceutical and scientific communities towards studies on the potential antimicrobial activity of plant derived substances. *Pongamia pinnata* L. is a species of family fabaceae and is a deciduous legume with soft shiny green leaves. The leaves are used for aliments. The plant extract contains flavonoids, carbohydrates, glycosides, steroid tannin etc. Meena Thomas et al.; Perez C. also checked antibacterial activity was checked using agar diffusion method. Sureshkumar et al., 2006 and Wahi A. K. 2002, also studied the hepatoprotective and antidibetic activity *Pergularia daemia* (Forsk) Chiov of Asclepiadaceae family it is used as anthelmitic, laxative, antipyretic, cures asthma, ulcers useful in eye troubles, uterine complaints and inflammations. Aerial parts this plant were reported to have the various pharmacological activities like hepataoprotective and anti-diabetic. The curative properties of medicinal plants are due to the presence of various phytochemicals.

1.1. *Pongamia pinnata* :- Given images shows the seeds and whole plant of *pongamia pinnata* tree.

**Kingdom:** Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Rosids

.Order:** Fabales

Family:** Fabaceae

Genus:** Millettia / Pongamia

*Pongamia Pinnata* plant

*Pongamia pinnata* is known as karanja is a mangrove plant belonging to genus pongamia and family Fabaceae. It is medium sized glabrous evergreen tree with short medium attaining height of around twenty five to thirty meter and its habitat is in the east Asia, Australia, Africa and widely distributed in India, Pakistan, Bangladesh, Philippine and Australia, Thailand. The leaves are soft, shiny burgundy in summer and mature to a glossy deep green as the season progress. Traditionally its bark is used in pile and various medicinal purposes. leave are effective as rheumatic pains and the seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis. Roots are used for cleaning gums, teeth, and ulcers also effective in gonorrhea. Flowers used for diabetes. In ayurveda and unani medicine, used as anti-inflammatory, antiplasmodial, anti-nonceptive, anti hyperglycemic, anti lipoxidative, anti diarrheal, anti- ulcer, anti-hyper ammonic and antioxidant. The phytochemical examinations of *Pongamia pinnata* plant have indicated the presence of different biocompounds furanoflavones, furanoflavonols, chromeno flavones, flavones, furanodiketones and glucosides.9,10,11,12

**Seed Description :-**

Pods of *Pongamia pinnata* L measures generally 4 to 5 cm long and 4 cm wide, thick walled and usually contain asingle or double seed. Seeds are 1-2 cm long, ellipticaland reniform, fig, oblong and light browncolour as shown in figure. The screening of plants for phytochemical compounds inorder to evaluate pharmacological effect hasbecome a random tool, very few vascularplants group with respect to antibacterial activity were studied. The present study is aimed to investigate phytochemicals and evaluate the antibacterial activity of *Pongamia pinnata* L. on hospitalized human pathogens, since this plant has been documented for several beneficial uses to mankind. The selected human pathogens are *Escherchia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (734 MTCC), *staphylococcus aerus* (MTCC) *Klebsella pneumonia* (MTCC). The pure culture of all these pathogen obtained from Department of Clinical Microbiology, Ghati Hospital Aurangabad.
**Escherichia coli** – It is commonly abbreviated *E. coli*. It is a gram-negative rod-shaped bacterium, commonly found in the intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes are pathogenic and can cause serious food poisoning in humans, and are occasionally responsible for product recalls.\(^{15,16}\)

**Phylum:** Proteobacteria  
**Class:** Gammaproteobacteria  
**Order:** Enterobacterales  
**Family:** Enterobacteriaceae  
**Genus:** *Escherichia*  
**Species:** *coli*

*Image - 1. Escherichia coli*  
are also responsible for a majority of cases of urinary tract infections. Food poisoning also caused by *E. coli* can result from eating unwashed vegetables or poorly butchered and undercooked meat and vegetables. O157:H7 is also notorious for causing serious and even life-threatening complications such as hemolytic-uremic syndrome. This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach. The O104:H4 strain is equally virulent and toxic which creates bloody diarrhea, but also is more enteroaggregative, means they stuck to intestinal layer.

**Image - 2. Urinary Tract Infection by Escherichia coli.**

*Salmonella typhi* :- is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family Enterobacteriaceae.

**Phylum:** Proteobacteria  
**Class:** Gammaproteobacteria  
**Order:** Enterobacterales  
**Family:** Enterobacteriaceae

*Salmonella* species are non-spore-forming, predominantly motile enterobacteria, with cell diameters between about 0.5 and 1 µm, lengths from 2 to 4 µm, and having flagella all around the cell body. They are also facultative aerobes. *Salmonella* species are intracellular pathogens. They causes disease named typhoid in human.

**Research Paper**  
IJRAR- International Journal of Research and Analytical Reviews
**Klebsella pneumoniae** – *Klebsiella* is a genus of nonmotile, they are also Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule like structure. The species are aerobic but some are facultatively anaerobic. Their ideal growth temperature is 35° to 37 °C, while their ideal pH level is about 7. *Klebsiella* organisms can lead to a wide range of disease states, mainly pneumonia, which attack on respiratory track of humans, urinary tract infections, meningitis, diarrhea, and soft tissue infections. The most notable human *Klebsiella* infections creating species is caused by *K. pneumoniae*, followed by *K. oxytoca*. Infections are more common in the very young, very old, and most infections spread because of non-sterile medical device. It shows threatening respiratory infection.

**Figure – 4. Klebsella pneumonia.**

**Figure – 5. Klebsella pneumonia Respiratory Tract infection.**

**Staphylococcus aureus** - *Staphylococcus aureus* is a gram-positive, round-shaped bacteria that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. *S. aureus* has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue. Pocket of infection that forms at the site of injury. Usually filled with pus. Area surrounding the abscess is usually red, painful and swollen and the skin surrounding the abscess can feel warm to the touch.

**Figure – 6. Staphylococcus aureus.**
**Figure 7.** Staphylococcus aureus skin infection.

**Pseudomonas aeruginosa** :- It is a common Gram-negative, rod-shaped bacterium that can cause disease in humans, that causes severe acute and chronic infections at different sites within the body such as urinary tract, skin (burn or surgical wounds), and the respiratory tract. It occurs in burnt patient injury and that’s why patients injury turns bluish green pigmentation.

![Pseudomonas aeruginosa](image)

**Domain**- Bacteria
**Phy**- Proteobacteria
**Class**- Gammaproteobacteria
**Order**- Pseudomonadles
**Family**- Pseudomonaceae
**Genus**- *Pseudomonas*
**Species**- *P. aeruginosa*

**Figure 7.**

**Figure 8.** Pseudomonas aeruginosa

**MATERIALS AND METHODS :-**

**Collection of samples** The seeds of *Pongamia pinnata* were used for the experiment. The plant seeds were collected from nearby areas of chhawani Aurangabad. The taxonomical identification of the plant specimens was done by taxonomist Dr. Dhabe Sir, HOD Dr. BAM University Aurangabad. Accession number **642**.

**Apparatus Used** :- soxhlet apparatus, Rotary vacuum evaporator (UGC-MRP 2009Buchi Labortechnik AG), Water bath, Autoclave, Laminar air flow, Incubator, Magnetic stirrer (2 MLH. petridishes, beakers, round bottom flask, whatmen filter paper, cork borer 6 mm diameter, micropipette, etc)
Chemicals used: Methanol, Ethanol, Petroleum Ether, Chloroform. Tests reagents which are used for preliminary phytochemical testing.

Preparation of extracts

Macerration: 500 grams of plant seeds of *Pongami pinnata* was collected and shade dried for about fifteen days. It got crushed in coarse powder and stored in an airtight glass jar for further use. The seed powder of approximately 100 gram soaked in aqueous and kept in a conical flask for about twenty four hours. It got filtered through whatmns filter paper no.1. Remaining filtrate make semi solid by using Rotary evaporator.

Soxhlet Extraction:

Each solvent Ethanol, Methanol, Petroleum Ether, Chloroform added with 100 gram of pongamia pinnata powder. It got extracted by using Soxhelt apparatus, by taking six cycles of each solvent. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude semisolid extracts were stored in refrigerator, for further use.

Phytochemicals analysis:

The extracts prepared were analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the Khandelwal Practical Book.

Test for alkaloids:

The extract of the crude semisolid or solid extract of each solvent was evaporated to dryness in water bath. The residues were dissolved in Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer’s reagent, one portion was treated with equal amount of Dragendorff’s reagent and the third portion was treated with equal amount of Wagner’s reagent respectively. The appearance of creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids.

Test for saponins:

About 1ml of plant seed extract was vigorously shaken with water in a test tube and then heated to boil. Froth was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for tannins:

About 1 ml of plant seeds extract was added was in of water in a test tube and filtered. A few drops of ferric chloride was added and observed for brownish green or black coloration.

Test for steroids:

Acetic anhydride was added to 1ml of plant leaf extract of each sample along with few drops of sulphuric acid. The colour changed from violet to blue green indicate the presence of steroids.
Test for flavonoids
1ml of extract solution was treated with methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange -red colour for flavones.

Test for anthraquinones
About 1 ml of extract was taken in a dry test tube and chloroform was added and shaken.. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides
1 ml of extract was dissolved glacial acetic acid containing ferric chloride solution. This was then added with conc. sulphuric acid. A brown ring obtained showed the presence of cardioids.

Test for Proteins
To 1ml of extract 70% NaOH solution and 2 to 3 drops of 1% CuSO4 solution was added. A violet colour indicated the presence of protein.

Test for Amino Acids
To 1 ml of sample was added to Ninhydrin reagent and kept in water bath for few minutes appearance of purple colour indicated the presence of amino acids.

Test for Tri-Terpenoids
1 ml of each extract was added to chloroform and few drops of sulphuric acid to form reddish brown colour represent presence of tri-terpenoids.

Test for Reducing Sugar
To 1 ml of extract, and few drops of Molisch’s reagent was added and shaken well. 1 ml of conc. sulphuric acid was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Antibacterial assay :- A fixed inhibitory concentration of 150 μg/ml of seed extract was tested by using Agar well Diffusion method and Disc diffusion method and compared with the antibiotic streptomycin as a positive control at equal concentration. Each extract was dissolved in DMSO and wells filled with 1 ml in each well. Petri plates kept in incubator at 37 ºc for about 24 hours. Then result obtained as mentioned in table chart.

RESULT AND DISCUSSION :- The paper describes antibacterial activity of pongamia pinnata seed of different selected organic solvents and aqueous solvent as given in table. Ethanol extract shows more inhibitory action against selected gram positive and gram negative bacteria.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Methanol extract</th>
<th>Chloroform Extract</th>
<th>Petroleum ether extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Flavonoids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Quinones</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Betacyanins</td>
<td>++</td>
<td>_</td>
<td>+ +</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Anthocyanins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>8.</td>
<td>Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Terpenoids</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>Phenols</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>Amino acid</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>protein</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>15.</td>
<td>anthraquiones</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>16.</td>
<td>triterpenoid</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Whereas:+++ : Strongly present, ++ : Mildly present , + Present and – Absent.
Antibacterial Activity of *Pongamia pinnata* against selected Human pathogen

<table>
<thead>
<tr>
<th>Name of solvent extracts</th>
<th>Name of used Bacteria and zone of inhibition in (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Escherchia coli</em></td>
</tr>
<tr>
<td>Ethanol</td>
<td>17</td>
</tr>
<tr>
<td>Methanol</td>
<td>16</td>
</tr>
<tr>
<td>Pet. Ether</td>
<td>10</td>
</tr>
<tr>
<td>Chloroform</td>
<td>16</td>
</tr>
<tr>
<td>Aqueous</td>
<td>12</td>
</tr>
</tbody>
</table>

From above checked antibacterial activity of pongamia pinnata ethanol extract antibacterial activity is 17 mm followed by methanol 16mm, chloroform 16mm, aqueous 12mm, and pet. Ether 10mm of inhibition zone. The all extract are more effective against salmonella typhi bacterium but less effective against other bacteria. It has presence of secondary metabolites or phytochemicals which are effective against certain human pathogen but not all.

Fig. 11. Antibacterial Activity of *Pongamia pinnata* (L)

Fig. 12. Antibacterial Activity of *Pongamia pinnata* against *S. aerus* and *S. typhi*.
CONCLUSION:
It is concluded by our finding that ethanol extract of Pongamia pinnata seed is more effective than other tested extract. Also it showed potent effect against salmonella typhi. It showed distinct zone of inhibition against salmonella typhi as that of the standard streptomycin. It was also proved that this plant can be used for further pharmaceutical production of antibacterial drugs. But there is need to study about concentration of each phytochemical quantitatively by using advanced technique. It can firstly used on a animal model for confirmation.

FUTURE PLAN :
To test quantitative analysis of pongamia pinnata extract by using HPTLC.

ACKNOWLEDGEMENT :
The authors express their sincere gratitude to Department of Chemical Technology Dr.BAM University Aurangabad for giving opportunity to carry out initial part of our research work. Authors also thankful to Prof. Dhape for authentication of plant. Dr. N. G. Kashid for their gracious guidance.

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