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# Evaluation of Antibacterial Activity of *Pongamia pinnata* L., *Curcuma longa* L. and *Mentha arvenis* L. Against *Staphylococcus aureus*

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**Abstract:** The climatic condition of India facilitates the growth of large variety of medicinal plants. The Millions of households in rural and urban localities consume traditional foods, make use of home remedies and follow health customs based on the principles of traditional systems of medicines. Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents. In recent years attempts have been made to investigate the indigenous drugs against infectious diseases may help to develop safer antimicrobial drugs. There is a continuous and urgent need to discover antimicrobial compounds with diverse chemical structure and noble mechanism of action because there has been an alarming increase in the incidence of new and re-emerging infectious disease. The aim of the present study is to explore the antibacterial activity of leaf extract of *Pongamia pinnata* L. *Curcuma longa L.* and *Mentha arvensis* L. against *Staphylococcus aureus* by using minimum inhibitory concentration (MIC) and zone of inhibition. The MIC is compared with control where is the zone of inhibition were compared with standard drug Gentamycin.
**Key words:** antimicrobial agents, infectious disease, minimum inhibitory concentration, zone of inhibition.

## Introduction

Plants produce some biomolecules which show anti-microbial activity. The 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. It was shown that phenolics and alkaloids are a significant group of bioactive compounds. Essential oils, propanoid, terpene are active on gram positive and gram negative bacteria. Many natural antimicrobial compounds derived from a wide variety of secondary metabolites can control infectious diseases[1].

*Staphylococcus aureus* is a facultative anaerobic gram positive bacterium which causes food poisoning usually grows on membrane and skin also found in gastro intestinal and urinary tract of warm blooded animals. In this context, the present work was undertaken to evaluate the antibacterial activity of ethanolic and aquous extract of *Pongamia pinnata* L., *Curcuma longa L.* and *Mentha arvensis* L. against staphylococcus aureus[2].

*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,

steroids, tannins, etc. *Pongamia pinnata* L. contains many alkaloids ex. glabrin, pinnatin, pongamal,fatty acids, sterol and disaccharides[3, 4, 5].

*Curcuma longa* is an Indian spice derived from the rhizomes of the plant and has a long history of use in Ayurvedic medicine as a treatment for inflammatory conditions. *C. longa* is a perennial member of the Zingiberaceae family. The fresh juice taken regularly on an empty stomach has been used to prevent stomach disorders. Turmeric has been shown to have anti-bacterial, anti-fungal, anti-oxidant, anti-ulcer, anti-inflammatory and possibly anti-cancer effects. *Curcuma longa* is comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin (Figure 1), as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins. The curcuminoid complex is also known as Indian saffron[6-8] .

*Mentha arvensis*, Pipertia is an important medicinal plant of family Lamiaceae. *Mentha arvensis* L. has high menthol content and also contains menthone and menthyl esters, particularly menthylacetate. Dried peppermint typically has 0.3-0.4% of volatile oil containing menthol (7-48%), menthone (20-46%), menthyl acetate (3-10%), menthofuran (1-17%) and 1,8.Mint contain minerals like calcium, potassium, sodium, magnesium, phosphorus and iron as well as vitamin A, C, K, folic acid, thiamine, riboflavin and niacin. Secondary metabolites like terpenoids and phenolic compounds also found[9].

## Materials and Methods

### Plant Extract

The plants used in this study i.e. *Pongamia pinnata*, *Curcuma longa* and *Mentha arvensis* were collected from local areas of Berhampur, Odisha. The taxonomical identification of the plant specimens was done at Central National Herbarium, Botanical Survey of India, Howrah. Voucher specimens were preserved in the Department of Botany and Biotechnology, Khallikote Autonomous College, Berhampur for further verification. The plant materials were washed after collection with tap water and air dried under shade, coarsely powdered and kept in airtight container.

The dried and powdered leaves (200 gm each) were separately soaked in 1000 ml of distilled water, methanol and ethanol. The mixture is stirred periodically using sterile glass rod up to 72 hours. Each solvent extract was collected separately and dried using rotary vacuum evaporator followed by lyophilizer and stored in dessicator until further use[10].

### Microorganism

The *Staphylococcus aureus* was obtained from Microbial type culture collection and gene bank, of Institute of Microbial Technology, Council of Scientific and Industrial Research, Chandigarh (MTCC code no.*1430). The preparation of Inoculums to get microorganism were from broth cultures containing approximately $5.10^5$ to $9.10^6$ colony forming units per milliliter (CFU/ml). Each diluted (1:50) inoculums was applied as a lawn with a micropipette calibrated to deliver 50ul containing around $9.10^6$ CFU. The discs impregnated with each extract were evaporated for 24 hours. Each disc containing appropriate plant extract was then deposited on the culture of micro-organisms. The plates were incubated for 20 h at 37°C.

### Drugs and Chemicals

Gentamycin was purchased from a local pharmacy. Nutrient agar and nutrient broth were purchased from Merck chemicals specialities pvt. Ltd., Navi Mumbai. Analytical graded chemicals were used for extraction process, phytochemical screening and other procedures.

### Phytochemical Screening

Qualitative analysis of groups of phytoconstituents of the above plant extracts were carried out based on standard protocols[11-13].

### Antibacterial activity of different plant extracts

Conventional broth dilution tests are used when only a few strains of bacteria need to be tested or when an accurate MIC estimation is required. A series of two-fold dilutions of the antibiotic under study is prepared in a volume of a suitable broth medium and standard inoculums of the test strain (commonly $10^5$ bacteria) is introduced into each tube. The test is incubated at 37º C overnight and the end-point is read as that concentration of antibiotic in which no turbidity can be seen. Un-inoculated tubes containing broth plus

antibiotic and broth alone act as sterility controls (an antibiotic-free tube inoculated with the test organism serves to indicate that the organism is viable in case the end-point is missed[14].

The agar diffusion assay technique is used to determine the antibacterial activity of extracts[15]. The antimicrobial effect may be inhibited or increased by extrinsic factors or contaminants. The agar type, salt concentration, incubation temperature and molecular size of the antimicrobial component influence results obtained with agar diffusion assays. The most widely used alternative technique in general microbial assay is serial dilution of the extract in a number of test tubes followed by the addition of the test organism to determine the MIC for the test organism using turbidity as an indication of growth that is dilution method[16]. To determine the MIC of the medicinal plants in the present study, the macrobroth dilution method was adopted. As per Beer and Lambert's law, concentration of standard solution (Plant extract) will show maximum optical density due to less inhibitory effect; whereas high concentration will reveal minimum optical density. For disc diffusion method, plates were incubated for 48 hours at 37°C then the inhabitation zone was observed. The MIC testing is obtained after 24 hours of incubation at 37°C. The readings were taken with the help of spectrophotometer.

## Results

### Preliminary phytochemical screening of different extracts

A summary of the results of preliminary phytochemical screening of methanolic, ethanolic and aqueous extracts of *Pongamia pinnata, Curcuma longa* and *Mentha arvensis* are furnished in the Table- 1.

**Table 1: Preliminary phytochemical investigation of different extracts**

| Group of Phytoconstituents | MPP | EPP | APP | MCL | ECL | ACL | MMA | EMA | AMA |
|---|---|---|---|---|---|---|---|---|---|
| Alkaloids | | | | | | | | | |
| Wagner's test | + | + | + | + | + | + | + | + | + |
| Mayer's test | + | + | + | + | + | + | + | + | + |
| Dragendorff's test | + | + | + | + | + | + | + | + | + |
| Hager's test | + | + | + | + | + | + | + | + | + |
| Carbohydrates | | | | | | | | | |
| Molisch's test | + | + | + | + | + | + | + | + | + |
| Benedict's test | + | + | + | + | + | + | + | + | + |
| Fehling's test | + | + | + | + | + | + | + | + | + |
| Iodine test | + | + | + | + | + | + | + | + | + |
| Glycosides | | | | | | | | | |
| General test | + | + | + | + | + | + | + | + | + |
| Cardiac glycosides | | | | | | | | | |
| Keller-Killiani test | + | + | + | + | + | + | - | - | - |
| Legal's test | + | + | + | + | + | + | - | - | - |
| Baljet's test | + | + | + | + | + | + | - | - | - |
| Anthraquinone glycosides | | | | | | | | | |
| Borntrager's test | + | + | + | + | + | + | - | - | - |
| Modified Borntrager's test | + | + | + | + | + | + | - | - | - |
| Saponin glycosides | | | | | | | | | |
| Foam test | + | + | + | + | + | + | + | + | + |
| Gums and mucilage | | | | | | | | | |
| Ruthenium Red Test | + | + | + | _ | _ | _ | _ | _ | _ |
| Molisch's Test | + | + | + | _ | _ | _ | _ | _ | _ |
| Swelling test | + | + | + | _ | _ | _ | _ | _ | _ |
| Proteins and Amino acids | | | | | | | | | |
| Biuret test | - | - | - | + | + | + | + | + | + |
| Ninhydrin's Test | - | - | - | + | + | + | + | + | + |
| Xanthoproteic's Test | - | - | - | + | + | + | + | + | + |
| Million's test | - | - | - | + | + | + | + | + | + |
| Tannins and phenolic compounds | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Test with heavy metals | + | + | + | + | + | + | + | + | + |
| Ferric chloride test | + | + | + | + | + | + | + | + | + |
| Nitric acid test | + | + | + | + | + | + | + | + | + |
| Gelatin test | + | + | + | + | + | + | + | + | + |
| Triterpenoids | | | | | | | | | |
| Tin and Thionyl chloride | + | + | + | + | + | + | - | - | - |
| Flavonoids | | | | | | | | | |
| Ferric chloride test | + | + | + | + | + | + | + | + | + |
| Shinoda test | + | + | + | + | + | + | + | + | + |
| NaOH test | + | + | + | + | + | + | + | + | + |
| Lead acetate test | + | + | + | + | + | + | + | + | + |
| Coumarins | | | | | | | | | |
| General test | + | + | + | + | + | + | - | - | - |
| Steroids | | | | | | | | | |
| Salkowski reaction | + | + | + | + | + | + | - | - | - |
| Liebermann-Burchard test | + | + | + | + | + | + | - | - | - |
| Fats and oils | | | | | | | | | |
| Spot test | - | - | - | - | - | - | + | + | + |

**(+) Sign indicates presence, (-) Sign indicates absence**

Where, MPP: Methanolic extracts of Pongamia pinnata, EPP: Ethanolic extracts of Pongamia pinnata, APP: Aqueous extracts of Pongamia pinnata.MCL: Methanolic extracts of Curcuma longa, ECL: Ethanolic extracts of Curcuma longa, ACL: Aqueous extracts of Curcuma longa, MAI: Methanolic extracts of MMA: Methanolic extracts of Mentha arvensis, EMA: Ethanolic extracts of Mentha arvensis, AMA: Aqueous extracts of Mentha arvensis

**Antibacterial activity of different plant extracts:**

The table- 2 reveals the MIC of *Pongamia pinnata* L. leaf extract against *Staphylococcus aureus*. Comparison of optical density indicates that ethanolic extract is more effective than that of methanolic and aqueous extract. . The MIC of *Curcuma longa* leaf extract against *Staphylococcus aureus*. The comparison of optical density indicates that ethanolic extract is more effective than that of methanolic and aqueous extract. The MIC of *Mentha arvensis* L. leaf extract against *Staphylococcus aureus*. Comparison of optical density indicates that methanolic extract is more effective than that of ethanolic and aqueous extract. The MIC standard of Gentamycine against *Staphylococcus aureous* is 0.001 mg/ml[17].

**Table 2: MIC of different plant extract against *Staphylococcus aureus***

| **Concentration of leaf extract in (mg/ ml)** | **APP at 600 nm** | **EPP at 600 nm** | **MPP at 600 nm** | **ACL at 600 nm** | **ECL at 600 nm** | **MCL at 600 nm** | **AMA at 600 nm** | **EMA at 600 nm** | **MMA at 600 nm** |
|---|---|---|---|---|---|---|---|---|---|
| 0.31 | 0.85 | 0.81 | 0.73 | 0.94 | 0.95 | 0.91 | 0.93 | 0.87 | 0.86 |
| 0.62 | 0.53 | 0.67 | 0.65 | 0.82 | 0.28 | 0.82 | 0.81 | 0.63 | 0.77 |
| 1.25 | 0.38 | 0.52 | 0.54 | 0.69 | 0.19 | 0.64 | 0.73 | 0.49 | 0.59 |
| 2.5 | 0.20 | 0.31 | 0.32 | 0.38 | **0.05** | 0.42 | 0.42 | 0.37 | 0.32 |
| 5 | 0.35 | 0.29 | 0.37 | 0.29 | - | **0.09** | 0.59 | **0.12** | **0.10** |
| 10 | 0.28 | **0.14** | **0.16** | 0.07 | - | - | **0.23** | - | - |
| 20 | **0.19** | - | - | **0.12** | - | - | - | - | - |

Where, MPP: Methanolic extracts of Pongamia pinnata, EPP: Ethanolic extracts of Pongamia pinnata, APP: Aqueous extracts of Pongamia pinnata MCL: Methanolic extracts of Curcuma longa, ECL: Ethanolic extracts of Curcuma longa, ACL: Aqueous extracts of Curcuma longa, , MAI: Methanolic extracts of MMA: Methanolic extracts of Mentha arvensis, EMA: Ethanolic extracts of Mentha arvensis, AMA: Aqueous extracts of Mentha arvensis.

**Table 3: MIC summary of different plant extract against *Staphylococcus aureus***

| Plant Extract | | Concentration in (mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **0.31** | **0.62** | **1.25** | **2.5** | **5** | **10** | **20** |
| Pongamia pinnata | Aqueous extract | - | - | - | - | - | + | + |
| | Ethanolic extract | - | - | - | - | + | - | + |
| | Methanolic extract | - | - | - | - | + | + | + |
| Curcuma longa | Aqueous extract | - | - | - | - | + | + | + |
| | Ethanolic extract | - | - | - | + | + | + | + |
| | Methanolic extract | - | - | - | + | + | + | + |
| Mentha arvensis | Aqueous extract | - | - | - | + | + | + | + |
| | Ethanolic extract | - | - | - | + | + | + | + |
| | Methanolic extract | - | - | + | - | + | + | + |

(—)Sign indicates High turbidity, (+) Sign indicates less turbidity gives MIC value.

The table -3 is formed by taking optical density of different MIC concentration with different plant extracts against Staphylococcus aureus. The chart reveals the effect of different concentrations of plant extracts in different solvents. The negative sign indicates less effective value with high turbidity where as the positive sign indicates high effective value with less turbidity.

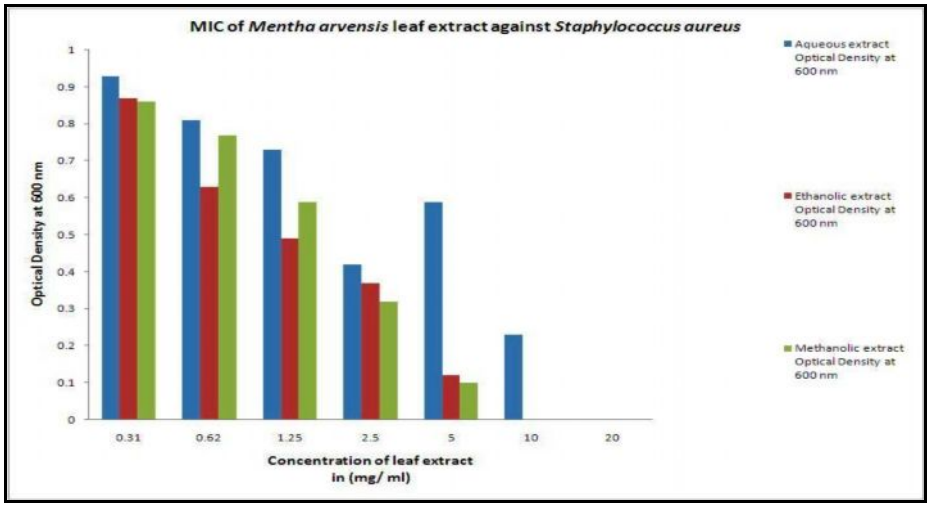


**Figure 1: MIC of *Mentha arvensis* leaf extract against *Staphylococcus aureus***

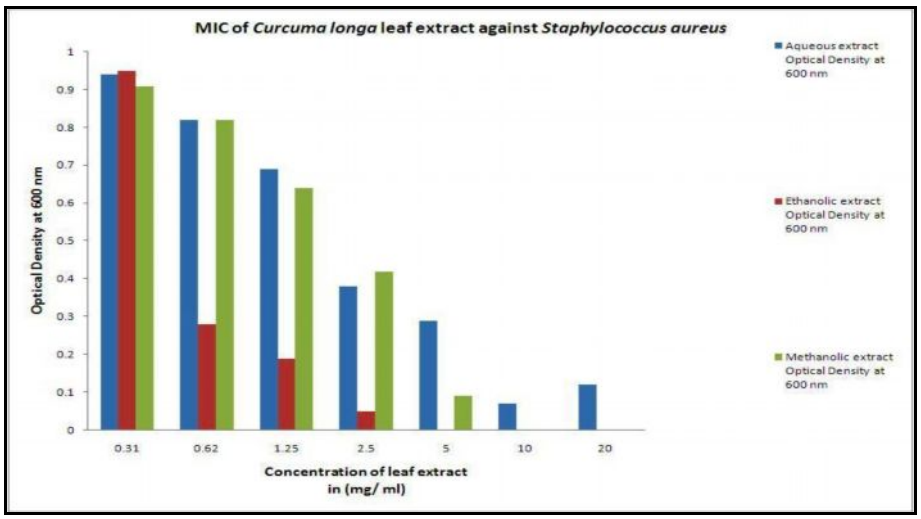


**Figure 2: MIC of *Curcuma longa* leaf extract against *Staphylococcus aureus***

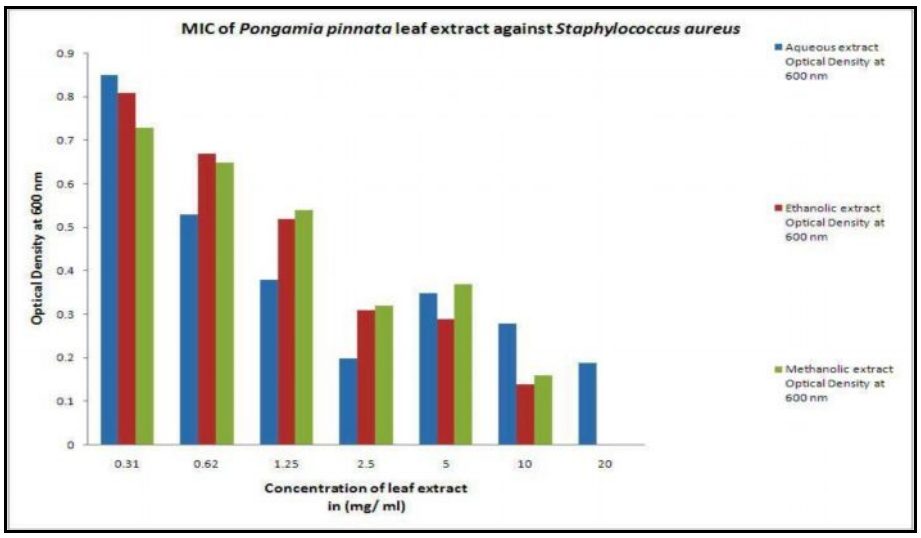


**Figure 3: MIC of *Pongamia pinnata* L. leaf extract against *Staphylococcus aureus***

The Figure- 1 reveals the MIC of *Pongamia pinnata* L. leaf extract against *Staphylococcus aureus*. The graph indicates that 10 mg/ml of *Pongamia pinnata* (ethanolic extract) is the MIC against *Staphylococcus aureus.*

The Figure- 2 reveals the MIC of *Curcuma longa* leaf extract against *Staphylococcus aureus*. The graph indicates that 2.5 mg/ml of *Curcuma longa* (ethanolic extract) is the MIC against *Staphylococcus aureus.*

The Figure- 3 reveals the MIC of *Mentha arvensis* leaf extract against *Staphylococcus aureus*. The graph indicates that 5 mg/ml of *Mentha arvensis* (methanolic extract) is the MIC against *Staphylococcus aureus.*

## Discussion

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens[18, 19]. The main objective of the present study was to evaluate the ability of the plants extract to inhibit the growth of pathogenic bacteria with and without antibiotics and non-antibiotics drugs and to determine their ability to enhance the activity of antibiotics or non-antibiotics drugs. Antimicrobial activity was recorded with the zone of inhibition and MIC.

In the present study, against *Staphylococcus aureus*, aqueous extract of *Mentha arvensis* is more effective than the methanolic and ethanolic extracts; ethanolic extract of *Pongamia pinnata* is more effective than the aqueous and methanolic extracts; ethanolic extract *Curcuma longa* leaf extract is more effective than that of methanolic and aqueous extracts.

Phytochemical screening of crude extract of *Mentha arvensis* L. indicated the presence of alkaloids, carbohydrates, flavonoids, fats, oils. Glycosides, coumarins and steroids were absent in the test sample. Court *et al.*, (1993) reported the presence of menthol, menthone, menthofuran in mint oil. Small amounts of additional compounds like limonene, pulegone, pinene are present in peppermint oil. All the extracts of *Pongamia pinnata* L. contain alkaloids, anthraquinone glycosides, flavonoids, coumarins and carbohydrates and steroids. Karangin, pongamol, pinnatin are the main chemical constituents found in *Pongamia pinnata* L. *Curcuma longa* L. Singh *et al.*, (2013) reported that aqueous extract of *Curcuma longa is more effective against Staphylococcus aureus* than methanolic extract. Result of the present study does not agree with this. Clinical isolates of *Staphylococcus aureus* showed more sensitive than the standard bacteria. *Curcuma longa* has curative potential for skin disease like acne, boils and leprosy. Curcumin may be tropically applied to treat inflammation and associated irritation of the skin and allergies of the skin. *Pongamia pinnata* L. Aqueous methanol extracts from bark, leaves and seeds indicated the presence of protocatechuic, ellagic. Ferulic, gallic acid in the bark; sorbic, fenulic, salicyclic and p-coumaric acids in the leaves. Antimicrobial activity of *Pongamia pinnata* leaf extract is stronger than reported in some earlier studies[20-23].

Plants are rich source of phytochemicals produced as secondary metabolites. More than 400, 000 tropical flowering plants have medicinal values[24]. Flavonoids may be bacteriostatic or bacteriocidal. It may be inhibiting DNA synthesis and RNA synthesis in gram positive *Staphylococcus aureus*[25]. Many microbial enzymes are inhibited by tannins or combination of tannin with other compounds tannins. It may disrupt the membrane. Increased *Staphylococcus aureus* activity by combination of β lactam antibiotics and tannic acid has been observed[26]. Coumarins are highly toxic to rodents. So they should be cautiously used. Coumarins intercalate with DNA of viruses. The terpenes are active against both gram positive and gram negative bacteria. Alkaloids have effect on RNA polymerase, topoisomerasases, nucleic acid[27].

In the study the major importance of phytochemical was that the broad groups of phytochemical compounds present were able to be detected and this followed the biological activities associated. It would be exciting to screen all the other known compounds given the resources and from there it would be necessary to identify the actual compounds. This phytochemicals help in cureing of many diseases.

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