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# Studies on antibacterial activity of four medicinal plants

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**ABSTRACT**

*The present investigation has been carried out to evaluate the antibacterial activity of ethanolic extracts of Acorus calamus rhizome, Alpinia galanga rhizome, Cinnamomum zeylanicum bark, Piper cubeba fruit, against two gram positive (Bacillus subtilis, Staphylococcus aureus) and two gram negative (Escherichia coli, Klebsiella pneumoniae) organisms respectively. Antibacterial activity was assessed by agar disc diffusion method. The activity of the extracts was measured by zone of inhibition and compared with a standard antibiotic Gentamicin. Among the various concentrations (50, 100, 150 µg/ml), 150µg/ml was found to be very effective. From the data of the results obtained in the present study, it can be concluded that, among the four plants selected, ethanol extracts of Acorus calamus and Alpinia galanga have given scopeful results against the microorganisms selected. Even though the extracts of Cinnamomum zeylanicum and Piper cubeba have antibacterial activity, at low concentration there was no appreciable activity.*

**Key words**: Antibacterial activity, *Acorus calamus* rhizome, *Alpinia galanga* rhizome, *Cinnamomum zeylanicum* bark, *Piper cubeba* fruit.

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## INTRODUCTION

Today the world finds itself in the midst of a multiplicity of problems particularly in the area of health care. The situation in this sector is alarming because of the emergence of new diseases. Consequently, the necessity of evolving new herbal remedies is on the ascendancy. In the present scenario where 80 percent of the world population has no access to the benefits of western medicines due to financial constraints, it is quite necessary to emphasize the relevance of traditional remedies which constitute a major part of the health care system in the developing countries and are also entering the therapeutics in the developed countries.

New efforts in the above area have been initiated globally and the newly emerging scientific discipline of Ethnopharmacology forms part of it. The use and search for drugs derived from plants have accelerated in recent years. While 25 to 50 percent of current pharmaceuticals are derived from plants, none are used as antimicrobial. Traditional healers have long been using plants to prevent or cure infectious diseases; western medicine also is moving in this direction. Plants are store houses of a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids which have demonstrated their antimicrobial properties *in vitro.*

Hence in the present study, four medicinal plants namely *Acorus calamus, Alpinia galanga, Cinnamomum zeylanicum, Piper cubeba* based on their traditional uses were selected for testing their antibacterial potential against reference bacterial strains of clinical importance.

It was evident from the literature survey that *Acorus calamus* has anti-fungal [1], antiulcer, cytoprotective, antitumour [2,3], antispasmodic [4], anti-inflammatory [5,6], anticonvulsant [7,8], anti-oxidant [9], broncho dilatory [10], and antidiabetic activities [11].

Various extracts of *Alpinia galanga have* antimicrobial [12,13], immunomodulatory, anti-oxidant [14], antidiabetic [15], antiplatelet and hypolipidemic activities [16]. Literature study reveals that *Cinnamomum zeylanicum* has antioxidant [17], anti-Inflammatory [18], neuroprotective [19], antidiabetic [20,21], antimicrobial [22], anticancer [23], cardio protective [24], cholesterol and lipid-lowering activities [25]. *Piper cubeba* has antiandrogenic [26], antibacterial [27], antioxidant [28], antifungal [29], metabolic [30], 5alpha-reductase inhibitory activities [31].

The present study was carried out to evaluate antibacterial activity of these four medicinal plants which may help to resolve the perennial problem posed by emerging resistant microbial strains.

## EXPERIMENTAL SECTION

### Collection of plant materials

Fresh *Acorus calamus* rhizome, *Alpinia galanga* rhizome, *Cinnamomum zeylanicum* bark, *Piper cubeba* fruit, were purchased from Ayurveda medical shop at Mannargudi, Thiruvarur Dt, Tamilnadu, India. The medicinal plant materials were cut into pieces and washed thoroughly 2-3 times with running water and once with sterile distilled water, then the plant material were air-dried on sterile blotter under shade.

### Extraction of plant material

Ethanolic extracts of selected plant materials were prepared according to the methodology of Indian pharmacopoeia [32]. The shade dried plant materials were subjected to pulverization to get coarse powder. The powdered materials were subjected to Soxhlet extraction separately with ethanol. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40º-50ºC). The ethanolic extracts of medicinal plants were put in air tight containers separately and stored in refrigerator till the time of use.

### Microorganisms

Microorganisms such as *Bacillus subtilis* (*B. subtilis*-MTCC 441), *Staphylococcus aureus* (*S. aureus* – MTCC 96), *Escherichia coli* (*E. coli*-MTCC 40), *Klebsiella pneumoniae* (*K. pneumoniae*- MTCC 109), were used as antibacterial test organisms. The bacteria were maintained on nutrient broth (NB).

### Antibacterial Activity

**Preparation of nutrient broth**

| | | |
|---|---|---|
| Beef extract | - | 0.3g |
| Peptone | - | 0.5g |
| Sodium Chloride | - | 0.5g |

The above ingredients were dissolved in 100ml of distilled water and sterilized by autoclaving at 121ºC for 15 minutes. After sterilization, one loopful of stock culture was inoculated into the sterilized medium. This transfer is made under aseptic condition. Then it was incubated at 37ºC for 24 hours. This culture was used for the further studies.

**Preparation of nutrient agar medium**

| | | |
|---|---|---|
| Beef extract | - | 3.0g |
| Peptone | - | 5.0g |
| Sodium Chloride | - | 5.0G |
| Agar | - | 15g |

The above ingredients were dissolved in 1000ml of distilled water and sterilized by autoclaving at 121ºC for 15 minutes. The pH of the medium was maintained at 7.2.

### Anti bacterial activity of plant extracts

The ethanolic extracts of *Acorus calamus*, *Alpinia galanga*, *Cinnamomum zeylanicum* and *Piper cubeba* were evaluated for anti bacterial activity by disc diffusion method. Different concentrations of the extracts (50, 100, 150

µg/ml) were prepared by reconstituting with water. The test microorganisms such as *B. subtilis, S. aureus, E. coli, K. pneumoniae* were seeded into respective medium by spread plate method 10µl ($10^6$ cells/ml) with the 24h cultures of bacteria grown in nutrient broth. After solidification, sterile filter paper discs (5mm in diameter) impregnated with the extracts were placed on test organism-plates. Gentamicin (30µg/ml) was used as standard antibiotic. The antibacterial assay plates were incubated at 37ºC for 24h. The diameters of the inhibition zones were measured in mm**.**

## RESULTS AND DISCUSSION

Infectious diseases are the world's leading cause of premature death, killing almost 50,000 people every day. Infectious pathogens include some viruses, bacteria, fungi, protozoa, multi cellular parasites, and aberrant proteins known as prions. These pathogens are the cause of disease epidemics, in the sense that without the pathogen, no infectious epidemic occurs. Transmission of pathogen can occur in various ways including physical contact, contaminated food, body fluids, objects, airborne inhalation, or through vector organisms [33].

Many bacteria gain access to the body by penetration of mucous membranes, lining of the respiratory tract, gastrointestinal tract, genitourinary tract. Most pathogens enter through the mucous membranes of the gastrointestinal and respiratory tracts are the easiest and most frequently travelled portal of entry for infectious microorganisms such as bacteria and fungi. Some diseases that are commonly contracted the respiratory tract include the common cold, pneumonia and tuberculosis.

*B. subtilis* is a gram positive, straight rod, 2 to 8µ long and 0.7µ wide, which grows in long chains. The organism is actively motile only in young cultures *B. subtilis* is frequently isolated from chronically infected wounds, and there are some reports of isolation from the blood stream.

*Staphylococcus aureus* is an aerobic, facultatively anaerobic and non motile gram positive bacterium. The disease spectrum in *Staphylococcus* food poisoning is due to its enterotoxin secretion and other lies in invasive burn infection with *Staphylococcus* and dissiminated abcessess. Toxic shock syndrome is also *Staphylococcal* infection in females, while new born infants and children develop superficial skin infections characterized by encrusted pustules [34]. It can cause pimples, impetigo, boils, cellulites, folliculitis, furuncles, carbuncles, and life threatening diseases such as pneumonia, meningitis, osteomylitis etc., [35].

The genus *Escherichia coli* consist of at least five species in which *E. coli* is the most frequently isolated. It is gram negative rod, often motile aerobic and facultative anaerobic bacteria. It is present in large number in gastrointestinal tract. It shows positive reaction for indole production from tryptophan, methyl red and calalase. It is frequently associated with bacterial sepsis, neonatal meningitis, infection of the urinary tract and gastroenteritis in travelers to countries with poor hygiene. It is also associated with diarrhoeal disease.

*K. pneumoniae* is a gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Although found in the normal flora of the mouth, skin and intestines, it can cause destructive changes to human lungs if aspirated. In the clinical setting, it is the most significant member of the *Klebsiella* genus of *Enterobacteriaceae*.

The discovery and development of antibiotics is the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However the rate of resistance of pathogenic microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency [36,37,38]. Isolation of microbial agents less susceptible to regular antibiotic and recovery of resistant isolates during antibacterial therapy is increasing throughout the world [39, 40]. In addition to this problem, antibiotics are sometimes associated with adverse side effects on the host, which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reaction [41].

The medicinal plants around the world contain may compounds with antibacterial activity [42]. Many efforts have been made to discover new antimicrobial compounds from various sources such as micro-organisms, animals and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds [43], The use of botanical medicines is generally on rise in many part of the world [44]. The screening of plants extract and plant products for antimicrobial activity has shown that plant represent a potential source of new anti-infective

agents [45,46,47]. Numerous experiments have been carried out to screen natural products for antimicrobial property [48,49,50,51,52,53,54,55,56]. Considering the above, it can be stated that plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the near future [57].

In the present study, ethanol extracts of four medicinal plants namely *Acorus calamus*, *Alpinia galanga*, *Cinnamomum zeylanicum*, *Piper cubeba* were evaluated for antibacterial activity against two gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two gram negative (*Escherichia coli*, *Klebsiella pneumoniae*) organisms. Extracts at three different concentrations (50, 100, 150µg) were used. The antibacterial activity of the extracts was compared with a standard antibiotic Gentamicin (30µg).

**Table 1: Antibacterial activity of ethanolic extract of *Acorus calamus***

| S. No. | Bacteria | Zone of Inhibition (diameter in mm) | | | |
|---|---|---|---|---|---|
| | | **C** | **50µg** | **100µg** | **150µg** |
| 1 | *Bacillus subtilis* | - | 07 | 09 | 15 |
| 2 | *Staphylococcus aureus* | - | 07 | 08 | 28 |
| 3 | *Escherichia coli* | - | 06 | 09 | 14 |
| 4 | *Klebsiella pneumoniae* | - | 09 | 09 | 26 |

**Table 2: Antibacterial activity of ethanolic extract of *Alpinia galanga***

| S. No. | Bacteria | Zone of Inhibition (diameter in mm) | | | |
|---|---|---|---|---|---|
| | | **C** | **50µg** | **100µg** | **150µg** |
| 1 | *Bacillus subtilis* | - | 10 | 12 | 17 |
| 2 | *Staphylococcus aureus* | - | 10 | 12 | 25 |
| 3 | *Escherichia coli* | - | 07 | 08 | 13 |
| 4 | *Klebsiella pneumoniae* | - | 14 | 16 | 21 |

**Table 3: Antibacterial activity of ethanolic extract of *Cinnamomum zeylanicum***

| S. No. | Bacteria | Zone of Inhibition (diameter in mm) | | | |
|---|---|---|---|---|---|
| | | C | 50µg | 100µg | 150µg |
| 1 | *Bacillus subtilis* | - | 08 | 10 | 13 |
| 2 | *Staphylococcus aureus* | - | 08 | 08 | 18 |
| 3 | *Escherichia coli* | - | - | 07 | 08 |
| 4 | *Klebsiella pneumoniae* | - | 10 | 12 | 17 |

**Table 4: Antibacterial activity of ethanolic extract of *Piper cubeba***

| S. No. | Bacteria | Zone of Inhibition (diameter in mm) | | | |
|---|---|---|---|---|---|
| | | **C** | **50µg** | **100µg** | **150µg** |
| 1 | *Bacillus subtilis* | - | 07 | 09 | 13 |
| 2 | *Staphylococcus aureus* | - | - | - | 28 |
| 3 | *Escherichia coli* | - | - | - | 11 |
| 4 | *Klebsiella pneumoniae* | - | 08 | 10 | 17 |

**Table 5: Antibacterial activity of Gentamicin**

| S. No. | Bacteria | Zone of Inhibition (diameter in mm) |
|---|---|---|
| | | 30µg |
| 1 | *Bacillus subtilis* | 19 |
| 2 | *Staphylococcus aureus* | 20 |
| 3 | *Escherichia coli* | 12 |
| 4 | *Klebsiella pneumoniae* | 20 |

*Acorus calamus* was found to exhibit maximum zone of inhibition for *Staphylococcus aureus* (28 mm/150µg). It has shown antibacterial activity in a dose dependent manner. The activity was least against *E. coli* (14 mm/150µg). The antibacterial activity of ethanolic extract of *A. calamus* was more for all the tested organisms than the standard antibiotic except *B. subtilis* (Table 1).

In the case of *Alpinia galangal,* maximum activity was obtained against *Staphylococcus aureus* (25 mm/150µg). The extract was effective in controlling the growth of bacteria at all the three concentrations tested (Table 2). Except

*Bacillus subtilis*, for the other three organisms it has produced inhibition zone more than the standard antibiotic (Table 5).

*Cinnamomum zeylanicum* extract was found to be less effective than *Acorus calamus* and *Alpinia galanga*. The least activity was recorded against *E.* coli (8 mm/ 150µg). There was no activity against *E.* coli at a concentration of 50µg (Table 3). Like the other two plants, *Cinnamomum zeylanicum* also shown maximum inhibition of growth against *S. aureus*.

Among the four plants, ethanolic extract of *Piper cubeba* was found to be least effective in controlling the growth of bacteria selected. At concentrations of 50µg and 100µg it has not shown any activity against *Staphylococcus aureus* and *Escherichia coli*. It has revealed maximum activity against *S. aureus* at 150µg (Table 4).

## CONCLUSION

From the data of the results obtained in the present study, it can be concluded that, among the four plants selected, ethanol extracts of *Acorus calamus* and *Alpinia galanga* have given scopeful results against the microorganisms tested. Even though the extracts of *Cinnamomum zeylanicum* and *Piper cubeba* have antibacterial activity, at low concentration there was no appreciable activity.

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