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# Pharmacological screening and antimicrobial evaluations of selected medicinal plants for new medicines

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**Abstract**
The search for new molecules, nowadays, has taken a slightly different route where the science of ethnobotany and ethnopharmacognosy are being used as guide to lead the chemist towards different sources and classes of compounds. It is in this context that the flora of the tropics by virtue of its diversity has a significant role to play in being able to provide new leads. In the present study, four selected Indian medicinal plants such as *Asparagus racemosus*, *Zinziber officinale*, *Piper nigrum*, and *Curcuma longa* were analyzed for phytochemical constituents and tested for antibacterial activity against pathogens. In most of the samples all the phytochemicals i.e. reducing sugar, Terpenoids, Flavonoids, Saponins, alkaloids, cardiac glycosides, carbohydrates and phytosterols were present. Methanol and aqueous extracts of the samples of all plants were evaluated for *in vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonasaeruginosa* by agar plate well diffusion method. All the plant extracts showed significant antibacterial activity against the tested organisms. However, methanol extracts showed maximum inhibitory effect from this *Zinziber officinale*, *Asparagus racemosus* and *Piper nigrum* were showed maximum bacterial inhibition than other plants.

**Keywords:** Medicinal plants; Phytochemical; antibacterial activity; pathogens

## Introduction
Antibiotics such as ampicillin, bacitracin, lincomycin, chlortetracycline and streptomycin promote growth because of an affect on the microflora in the gastrointestinal tract (Coates *et al*., 1963; deMan, 1975) [8, 10]. The phasing out of antibiotic growth promoters will affect the animal industry at large. To minimize the loss in growth, there is a need to find alternatives to antibiotic growth promoters. There are a number of non-therapeutic alternatives to antibiotic growth promoters, including enzymes, (in) organic acids, probiotics, herbs, immunostimulants and specific management practices (Mcewen and Fedorka-cray, 2002) [17].
Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity (Chang, 1995) [4]. More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials (Hsieh *et al*., 2001) [13]. The natural plant products have been reported to promote various activities like antistress, growth promoting, appetizing, tonic, immunostimulation, aphrodisiac and antimicrobials in the finfish and shrimp larviculture due to the active principle natures such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils. (Citarasu *et al*., 1998 [7]; Citarasu *et al*., 2002) [5]. Thus, in addition to enhancing the immune response, the antimicrobial effects of these herbs are beneficial to the host (Citarasu, 2012) [6]. The present study was conducted to determine the effect of different medicinal plant (herbs) extracts in diets as a possible alternative to antibiotic feed additives.
***Asparagus racemosus*** (Shatavari), has been described as a rasayana herb and has been used extensively as an adaptogen to increase the non-specific resistance of organisms against a variety of stresses. Besides use in the treatment of diarrhoea and dysentery, the plant also has antioxidant, immunostimulant, anti-dyspepsia and antitussive effects (Bopana and Saxena 2007) [2].

***Zingiber officinale*** (Ginger), has been shown to have antimicrobial activity (Srinivasan *et al*., 2001) [20]. Ethanolic extract of the rhizomes of *Z. officinale* showed significant inhibition of growth of both certain gram-positive and gram-negative bacteria. It also displayed antiinflammatory, analgesic, antipyretic and antimicrobial activities.

***Piper nigrum*** (Black pepper), is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus congestion, fever, intermittent fever, cold extremities, colic, gastric ailments and diarrhea. It has been shown to have antimicrobial activity (Dorman and Deans, 2000) [11].

***Curcuma longa*** (Turmeric), a perennial herb, has along tradition of use in the Chinese and Ayurvedic systems of medicine. Curcuminoids, a group of phenolic compounds isolated from the roots of *C. longa*, exhibited a variety of beneficial effects on health and has the ability to prevent certain diseases (Joe *et al*., 2004) [14]. In South Asia, the rhizomes from *C. longa*, are considered to have natural medicinal properties, including antibacterial, anti-inflammatory.

## Material and Methods
### *Selection of medicinal plants for this study*
Four medicinal plants including *Zinziber officinale* rhizomes (Ginger), *Asparagus racemosus* root (Shatavari), *Piper nigrum* fruits (Black Pepper), *Curcuma longa* rhizomes (Turmeric), were utilized in this studies. These plants have previously

been reported to have antibacterial activity against different bacterial strains.

***Preparation of Extracts***

**Processing *of the selected plant materials***

Collected plant root materials were shade dried with in temperature range of 28-35 °C. The drying process was continued to reduce moisture less than 14%. After drying, the plant materials were minced with wooden knife feeding into a grinder, minced materials were made into powder using teeth mills and sieved, and then the powder was stored in airtight container and kept at room temperature until further use.

***Extraction of selected plant material powder***

10g of dried powder was taken and boil at 100 °C for 2 hours, then filter the extracts, supernatant was collected and residues was discarded. The collected supernatant was condensed in water bath, and condensate was extracted again with methanol. The methanolic extract was concentrated in rotary evaporated under reduced pressure at room temperature 45-50 $^0$C, in order to avoid evaporation of plant metabolism. Aqueous extract was concentrated and stored at 4 $^0$C.

***In vitro antibacterial studies***

***Antimicrobial susceptibility studies***

Inhibition of microbial growth was tested by using the paper disc agar diffusion method (Bauer-Kirby Method) while the MIC was determined by the dilution (both micro and macro) method (de Paiva *et al*., 2003) [9]. Standard aseptic microbiological methods were followed throughout this antibacterial study. Microorganisms such as *E. coli*, *P. aeruginosa* and *S. aureus* (Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam), were obtained.

***Screening by agar well diffusion method***

The fractions eluted from the column purification were screened by agar disc diffusion method (Bauer and Kirby, 1966) [1]. Disc of filter paper (5 MM) impregnated with herbal extracts were placed on an agar plate that was heavily and uniformly inoculated (lawn) with an actively growing culture of the organisms. The medium of choice was Muller Hinton Agar for *P. aeruginosa, S. aureus* and *E. coli*. The dynamics and timing of antimicrobial agent diffusion to establish a concentration gradient coupled with the growth of organisms over 16-24 hour duration is critical for reliable results.

***Minimum inhibitory concentration (MIC) and Minimal Bacterial Concentration (MBC)***

Of the 4 plants tested, only those that showed antibacterial activity against some of the selected pathogens were selected for further tests to calculate their MIC by dilution method. The Minimum bacterial concentration (MBC) (Jacobs and Demott. 1994) was determined by plating the culture from the tube without the growth of microorganisms. To determine the minimum bacterial concentration, aliquots of one loop full of the two lowest concentrations which inhibited bacterial growth, were streaked on petriplates containing Muller Hinton agar. After as overnight incubation at 37 $^0$C the plates were evaluated by comparing them with control plates containing bacteria without test compounds. The lowest concentration that gave no visible growth was taken as MBC.

**Results**

***Antibacterial screening***

The methanol extract of the medicinal plants *Zinziber officinale* rhizomes (Ginger), *Asparagus racemosus* root (Shatavari), *Piper nigrum* fruits (Black Pepper), *Curcuma longa* rhizomes (Turmeric) showed no antibacterial activity against *E. coli* at specific doses mentioned.

***Antibacterial screening for Agar well and Disc diffusion method.***

The antibacterial activities of the selected extracts (Zone of inhibition of mm diameter) were given in the Table 1. Among the different active extracts, *A. racemosus* controlled the pathogen of 12.30, 12 and 14 mm of zone of inhibition in *P. aeruginosa, S. aureus* and *E. coli* respectively. The *Z. officinale* extract controlled the range of 9 to 13 mm of zone of inhibition in *P. aeruginosa, S. aureus* and *E. coli* respectively. For the activity against, *P. aeruginosa, S. aureus* and *E. coli* of the plant *P. nigrum*, the activities are 13.30, 12 and 14 mm. The methanol extract of the medicinal plants *C. longa*, showed no antibacterial activity against *P. aeruginosa, S. aureus* and *E. coli.* at specific doses mentioned in Table 1 zone of inhibition respectively.

**Minimum Inhibitory Concentration (MIC) and Minimal Bacterial Concentration (MBC)**

The growth rate of the selected bacterial isolates at different hours were given in the Fig 1. The MIC and MBC of the above three extracts against the pathogens were given in the Tables 2a to 2c. The MICs' are 60, 70 and 50 µg in the three extracts against *P. aeruginosa.* For *S. aureus,* the MIC is 60 to 70 µg of the selected plant extracts. The MIC is further decreased to 40 to 60 µg against the *E.coli* of the selected extracts. The least minimum inhibitory concentration was detected against the *E. coli*.

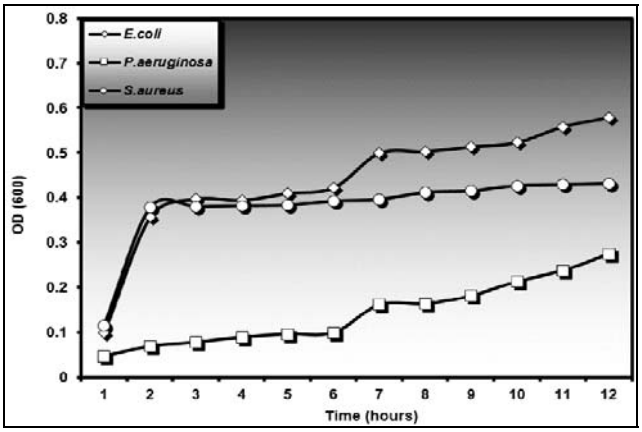


**Fig 1:** Growth rate of the selected bacterial isolates at different hours

**Table 1:** Antibacterial screening of the active fractions of different herbals extracts against pathogens.

| Sl. No. | Pathogens | Zone of inhibition (mm) of different active fraction of the herbal extracts | | | |
|---|---|---|---|---|---|
| | | ***Z. officinale*** | ***A. racemosus*** | ***P. nigrum*** | ***C. longa*** |
| 1 | *P.aeruginosa* | 12. 30±0.94 | 9.30±1.04 | 13.30±1.94 | 3.12±1.02 |
| 2 | *S. aureus* | 12.0±0.55 | 10.33±1.63 | 12.00±2.05 | 1.26±1.16 |
| 3 | *E. coli* | 14.0±0.95 | 12.4±0.63 | 14.00±1.50 | 2.08±0.14 |

**Table 2a:** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) of the active extract against the pathogen *Pseudomonas aeruginosa.*

| Con (µg) | Active Herbal Extract fractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Z. officinale*** | | ***A. racemosus*** | | ***P. nigrum*** | | ***C. longa*** | |
| | **MIC** | **MBC** | **MIC** | **MIC** | **MIC** | **MBC** | **MIC** | **MBC** |
| 10 | | ++++ | | ++++ | | ++++ | | ++ |
| 20 | | ++++ | | ++++ | | +++ | | + |
| 30 | | ++++ | | ++++ | | ++ | | - |
| 40 | | ++ | | ++++ | | ++ | | - |
| 50 | | ++ | | +++ | 50 | + | 10 | - |
| 60 | 60 | + | | ++ | | - | | - |
| 70 | | - | 70 | + | | - | | - |
| 80 | | - | | - | | - | | - |

No Growth; +: Minimum Growth; ++: Intermediate Growth; >+: Maximum Growth

**Table 2b:** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the active extract against the pathogen *Staphylococcus areus.*

| Con (µg) | Active Herbal Extract fractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Z. officinale*** | | ***A. racemosus*** | | ***P. nigrum*** | | ***C. longa*** | |
| | **MIC** | **MBC** | **MIC** | **MIC** | **MIC** | **MBC** | **MIC** | **MBC** |
| 10 | | ++++ | | ++++ | | ++++ | | + |
| 20 | | ++++ | | +++ | | +++ | | + |
| 30 | | ++++ | | +++ | | ++ | | - |
| 40 | | ++ | 60 | ++ | | ++ | | - |
| 50 | | ++ | | ++ | 60 | ++ | 10 | - |
| 60 | | ++ | | ++ | | + | | - |
| 70 | 70 | - | | - | | - | | - |
| 80 | | - | | - | | | | - |

No Growth; +: Minimum Growth; ++: Intermediate Growth; >+: Maximum Growth

**Table 2c:** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the active extract against the pathogen *E. coli.*

| Con (µg) | Active Herbal Extract fractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Z. officinale*** | | ***A. racemosus*** | | ***P. nigrum*** | | ***C. longa*** | |
| | **MIC** | **MBC** | **MIC** | **MIC** | **MIC** | **MBC** | **MIC** | **MBC** |
| 10 | | ++++ | | ++++ | | +++ | | + |
| 20 | | +++ | | ++++ | | +++ | | - |
| 30 | | ++ | | +++ | | ++ | | - |
| 40 | 40 | + | | ++ | | + | | - |
| 50 | | - | | + | 40 | - | 10 | - |
| 60 | | - | 60 | - | | - | | - |
| 70 | | - | | - | | - | | - |

No Growth; +: Minimum Growth; ++:Intermediate Growth; >+: Maximum Growth

**Discussion**

The antimicrobial effect of the medicinal plants is well documented (Valero and Salmeron, 2003) [21]. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al*., 2004) [16]. In this study, using the disk diffusion method it was observed that extracts of Shatavari and thyme produce antibacterial activity against both gram negative and gram positive pathogens. Results of this study confirmed the observation of earlier studies (Yuste and Fung, 2004) [22].

The Shatavari extract was found to be effective against *E. coli*, *P. aeruginosa*, and *S. aureus*. This effect is in agreement with other researchers regarding the antibacterial effect against *E. coli*, however there is a difference in the concentration of extract of Shatavari at which we found antibacterial activity (Rajpal 2002) [19]. Using the disk diffusion method, the concentration at which antibacterial activity was found was much higher than that of mentioned by the above mentioned authors. The MIC results of Shatavari by dilution method in our studies support an earlier finding by Rajpal 2002 [19]. In our studies the *Z. officinale* and *P.nigrum* extract showed antibacterial activity. This result supports the findings of many authors (Nevas *et al*., 2004) [18]. *Z. officinale* was found to be effective against *E. coli* and *P. aeruginosa* but not *S. aureus* by the disk diffusion method.

There was no antibacterial activity in extracts of *C. longa* against the tested pathogens at the specific dose. Our results are contradictory

with some researchers who reported antibacterial activity of above plants against gram positive and gram negative bacteria (Kalemba and Kunicka, 2003) [15]. This variation may be because of the dose used in this study, the method of extraction of medicinal plants, the method of antibacterial study, the genetic variation of plant, age of the plant or the environment. Our results suggest that gram-positive and gram negative bacteria are generally more sensitive to the spice and herb extracts. This was consistent with the previous studies on other spices and herbs (Ceylan and Fung 2004) [3].

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