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**Research Article**

# Antibacterial activity of aqueous plant extracts against *Escherichia coli* and *Bacillus subtilis*

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## ABSTRACT

Spices are the key ingredients of the Indian food. Inspite of enhacing flavour of food, these are found to have medicinal importance too which is shown in our study. In this study, the antibacterial activity of aqueous leaf extracts of *Mentha arvensis* (Mint), *Curcuma longa* (Turmeric), *Piper nigrum* (Black pepper), *Zingiber officinale* (ginger) and *Azadirachta indica* (Neem) was evaluated against *Escherichia coli* and *Bacillus subtilis* by agar well diffusion method. The maximum antimicrobial activity expressed in terms of zone of inhibition was shown by aqueous extract of *Zingiber officinale* followed by *Curcuma longa* and *Azadirachta indica*. However, aqueous extract of *Piper nigrum* was only effective against *B. subtilis* and *Mentha arvnesis* did not show any activity against both the test organisms. This study scientifically validates the use of plants as a potent antibacterial agent.

***Keywords:*** Antibacterial activity, aqueous extracts, agar well diffusion method

## INTRODUCTION

Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. Herbs and spices are an important part of the human diet. They have been used for thousands of year to enhance the flavour, colour and aroma of food. In addition to boosting flavour, herbs and spices are also known for their preservative and medicinal value, which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices [1].

Medicinal and spice plants are renewable raw materials. Their production is an alternative to the overproduction of traditional crops in agriculture. They also have an increasing economic importance. Spices can be defined as any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground form that contribute flavour, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages [1]. Although as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances' being derived from a plant does not mean it is always harmless. One drug used for one ailment could actually be detrimental to the treatment of another. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, mutagens and abortifacient [2]. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethno pharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for photochemical and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobials. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations [3]. In lieu of the above justification, the present paper highlights the antibacterial activity of aqueous extracts of four plants namely, *Mentha arvensis* (Mint), *Curcuma longa* (Turmeric), *Piper nigrum* (Black pepper), *Zingiber officinale* (ginger) and *Azadirachta indica* (Neem) against *Escherichia coli* and *Bacillus subtilis*.

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## MATERIALS AND METHODS

### Collection of plant materials

The plant materials were collected from the agricultural field in Kurukshetra district. The samples were washed thoroughly to remove dirt particles present on the surface. The samples were then dried in oven. The samples were crushed into powdered form by mortar and pestle.

### Preparation of aqueous extracts

Twenty five grams of the material was soaked in 100 ml of hot sterile water and allowed to stand for 24 hrs followed by boiling until the volume was reduced to one-fourth ( 25ml). The crude extract was obtained by filtration and stored in a refrigerator at 4ºC [4].

**Isolation and maintenance of cultures**

*Esherichia coli* and *Bacillus subtilis* were extracted from food stuffs by serial dilution agar plate method. In this method, serial dilutions of samples obtained from food stuffs were prepared and aliquots from each dilution was added to the plates containing nutrient agar to allow the growth of microbes. All the bacterial isolates were identified by cultural, morphological biochemical characteristics (Gram and endospore staining) [5,6]. The plates were kept in an incubator at 37ºC. The slants were prepared from the pure cultures obtained and kept in the refrigerator at $4^0$C for further use.

**Standardization of inoculum**

The microbial inoculum was standardized at 0.5 McFarland. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. Original McFarland standards were made by mixing specified amounts of barium chloride and sulfuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$), with 9.95 ml of 1% sulfuric acid ($H_2SO_4$). The standard could be compared visually to a suspension of bacteria in sterile saline or nutrient broth [7].

**Agar well diffusion method**

Antibacterial activity of spices was tested using agar well diffusion method. 200µl of bacteria was aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hilton agar plates. A well of about 6.0mm diameter with sterile cock borer was aseptically punched on each agar plate. 50µl of the aqueous extracts namely *Mentha arvensis*, *Curcuma longa*, *Piper nigrum*, *Zingiber officinale* and *Azadirachta indica* were introduced into the wells in the plates. A negative control well was too made with 50µl of the sterial distilled water. A positive control was made by placing antibiotic disc (Ciprofloxacin) on agar plate. Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37ºC for 24 hours. Resulting zone of inhibition was measured using a Hi media zone scale [8]

**RESULTS AND DISCUSSION**

In the present study, antimicrobial activity of five aqueous plant extracts of turmeric, mint, black pepper, ginger and neem was evaluated against *E. coli* and *B. subtilis* was evaluated by agar well diffusion method. Ciprofloxacin was used as positive control and distilled water was used as negative control. As revealed from Table 1, the turmeric was found to be effective against both *Escherichia coli* with zone of inhibition of 7mm and *Bacillus subtilis* with zone of inhibition ranging between 8 to 10mm. The aqueous extract of mint was ineffective against both the test organisms. The black pepper aqueous extract was effective against *B. subtilis* but not against *E. coli*. The data presented in table 1 also reveals that ginger and neem were effective against both the test organisms. Okemo *et al*. [9] assessed the crude extracts of the neem plant *Azadirachta indica* A. Juss which were previously determined to have strong antibacterial activity were investigated for their rate and extent of bacterial killing. In this study, both *E. coli* and *P. aeruginosa* which had previously shown an MIC of 8 mg/ml/ were not killed by the extracts even after 24hrs at any of the concentrations tested. Hoque *et al*. [10] determined the antibacterial activity of neem extracts against 21 strains of food borne pathogens. Neem extracts showed higher antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria. None of the extracts showed antimicrobial activity against *E. coli* O157:H7 and *Salmonella* enteritidis. Neogi *et al*. [11] assayed the antibacterial effects of crude ethanol and aqueous extracts of *Allium sativum*, *Zingiber officinale*, *Curcuma longa* and *Azadirachta indica* against *Staphylococcus aureus*,*Eschirichia coli* and *Salmonella typhi*. The antibacterial activity was determined only by agar disc diffusion method. The highest inhibition zone of 15 mm was found with synergistic combinations of garlic and turmeric on *Staphylococcus aureus*. *E. coli* was more resistant than other both organisms. Results of these kinds herald an interesting promise of designing a potentially active antibacterial synergized agent of plant origin.

**Table 1. Antimicrobial activity of aqueous extracts against *Escherichia coli* and *Bacillus subtilis*.**

| Plant extract | Zone of growth inhibition (mm) | |
|---|---|---|
| | *Escherichia coli* | *Bacillus subtilis* |
| *Azadirachta indica* | 7±0.37 | 7±0.81 |
| *Zingiber officinale* | 8±0.37 | 9±0.37 |
| *Piper nigrum* | 0 | 8±0.81 |
| *Mentha arvensis* | 0 | 0 |
| *Curcuma longa* | 7±0.57 | 9±0.37 |
| Positive control (Ciprofloxacin) | 22±0.37 | 32±0.81 |
| Negative control (Distilled Water) | 0 | 0 |

± Standard deviation

There might be several reasons for the lower antibacterial activity shown by the plant extracts as suggested by Parekh and Chanda [12], either the plant part used or the type of extraction might have resulted in the lower activity in this study, or the time of collection of herbal material and climate, which might, inturn, affect the amount of active constituents in the plant material. The water was found to be least effective in extracting the active antimicrobial components present in the herbs in the present study. The results agree with the observations reported by Arora [13] who evaluated the antimicrobial activity of aqueous extracts of ginger and garlic on *Escherichia coli* and *Salmonella typhi*. The antimicrobial action of the aqueous extracts could be ascribed to the anionic components such as thiocyanante, nitrate, chlorides and sulphates besides other water soluble components which are naturally occurring in the plant material [14]. The difference in potency shown by the plant extracts may be due to different sensitivity of food associated strains, difference in concentrations, methods of extraction used in the study and the little diffusion properties of these extracts in the agar as suggested by El- Astal *et al*.[15]. Although the plant extracts in the present study have shown lower antimicrobial activity, they still might be considered as potential drug agents for curing infectious diseases.

**CONCLUSIONS**

Spices and herbs are one of the most commonly used natural antimicrobial agents and have been used traditionally for thousands of years by many cultures. The antimicrobial activity of spices and herbs may differ between strains within the same species. Moreover, the antimicrobial properties of spices and herbs may differ depending on the form of spices added, such as fresh, dried, or extracted forms and

also differ depending on the harvesting seasons and between geographical sources. In the present study, the plant extracts have shown antimicrobial activity against human pathogens, which is lower in terms of zone of growth inhibition of Food-associated microorganisms . Further studies are required to enhance the activity of plant extracts and to identify the active components actually responsible for the antimicrobial activity.

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