

ANTIMICROBIAL STUDY OF NATURAL AND ARTIFICIAL SPICE EXTRACT ON VARIOUS STRAINS OF BACTERIA

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Abstract

The present study entitled “ANTIMICROBIAL STUDY OF NATURAL AND ARTIFICIAL SPICE EXTRACT ON VARIOUS STRAINS OF BACTERIA” was done to compare the antibacterial effects of natural and artificial sources of spices used for flavouring, coloring, or preserving food. Five different natural spices like Amchur (Dry mango powder), Dry ginger, Turmeric, Fenugreek, Red chili and artificial spices like Everest Amchur powder, Eastern Dry ginger powder, Eastern Turmeric powder, Eastern Fenugreek powder and Eastern Red chili powder were used. These spices used were tested on five different strains of bacteria of which three were gram positive (*Enterococcus faecalis*, *Staphylococcus aureus* and *Bacillus circulans*) and two were gram negative (*Klebsiella pneumoniae* and *Escherichia coli*). The method used for the study was Kirby-Bauer disc diffusion method. Among natural spices, Amchur exhibited the highest zone of inhibition (15mm) and Fenugreek with the least zone of inhibition. Among artificial spices, Everest Amchur powder exhibited the highest zone of inhibition (20mm) and Eastern Fenugreek powder showed no zones of inhibition against all the bacterial strains used. Natural spices were found to be most effective against the bacterial strains than the artificial spices. As a future work nutrigenomic study of spices should be conducted to find out the effects of spices on human health.

Keywords : Antimicrobial study, Zone of inhibition, Nutrigenomic study, Kirby-Bauer disk diffusion method, Adulterants.

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Introduction

A spice is a seed, fruit, root, or other plant substance primarily used for flavouring, coloring, or preserving food. Antimicrobial activity refers to the process of killing or inhibiting the disease-causing microbes. Antimicrobial agent can be antibacterial, antifungal, or antiviral. Many spices have antimicrobial properties. Therefore, spices are more commonly used in warmer climates, which have more infectious diseases. They have been used in our country cuisines since time immemorial. Their use was mainly due to health benefits as herbs and for the aroma they provided in the food. Today, they stand as Giants in Ayurvedic medicine with substances like turmeric, cloves and cardamom being used in every household. India contributes 75% of global spice production. Some of the commonly used spices in India are black cumin, cinnamon, coriander, red chili, Green cardamom, Fennel seed, asafoetida, white pepper, black pepper, bay leaf, dried ginger, turmeric etc.

Foodborne disease is an increasingly serious public health problem all over the world. The main cause is determined to be microorganisms. Spices and aromatic vegetable materials have long been used in food not only for their flavour and fragrance qualities and appetizing effects but also for their preservative and medicinal properties. Since the ancient times, they have been used for preventing food spoilage and deterioration and for extending the shelf life of foods. It has been extensively reported that the essential oils of spices have shown antimicrobial functions against foodborne pathogens. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions.

Spices can be classified as natural and artificial spices. Natural spices are the ones that are available locally, fresh, and dried or grinded into powder-form at home, whereas the artificial spices are the ones that are processed and produced on a large-scale in factory-mills of various spice-producing companies. The spices which are naturally produced in our homes are generally considered healthier as compared to the artificially produced spices as they are fresh, properly washed, and dried or grounded into powders for seasoning. The artificial ones are usually manufactured by grinding raw spices to make blended spices that are used for cooking purposes. Spices are an integral part of the Indian diet for centuries and they are used in vegetarian and non-vegetarian food and snack preparations. The busy lifestyle patterns have led people to rely more on these artificial spices, but these are most likely to be adulterated. The common adulterants which are added into these spices are sand, dirt, earth gritty matter, artificial color, starch, chalk powder, bark, stone, sawdust, lead chromate and argemone seed, etc. Turmeric powder can be adulterated by corn flour (starch), yellow-coloured talk or sawdust, lead chromate or melanin yellow dye. Red chili powder can be adulterated by brick powder, salt powder or talc powder and artificial color like Sudan Red. Food adulteration adversely affects the

nature, substance, and quality of the food. India produces a variety of spices and it is a must in every Indian kitchen. The risk of consuming adulterated spices is more if we use loose spices.

The present study aims to test the antibacterial activity of natural and artificial sources of spices. The natural sources of spices were brought as whole seeds and grinded in a mixer. The artificial sources of spices were brought as powdered forms of different spice companies from the nearby market. The companies chosen were EVEREST (Amchur powder) and EASTERN (Turmeric powder, Fenugreek powder, Dry ginger powder and Chili powder). The strains of bacteria used in this experiment are *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus circulans*.

Methodology

Preparation of Spice Extracts

The natural spices in its whole seed forms were grinded into powder form in a blender. Aqueous infusion of both natural and artificial spices was prepared by steeping 3g of each spice in 10ml distilled water in separate conical flasks. Each extract thus obtained was taken separately in a watch glass and marked for identification. The spice extracts were named according to the following abbreviations:

- i. NATURAL SPICES: Amchur (A), Dry ginger (D), Turmeric(T), Fenugreek(F), Red chilli (C).
- ii. ARTIFICIAL SPICES: Everest Amchur powder (EA), Eastern Dry ginger powder (ED), Eastern Turmeric powder (ET), Eastern Fenugreek powder (EF), Eastern Red chilli powder (EC).

Pure Culture Preparation

Bacterial pure culture was prepared by the streak plate method. A loop full of bacteria was taken from already existing culture and streaked onto a nutrient agar plate to eliminate any contaminant present and to isolate bacterial cultures. The isolated colonies thus obtained were maintained at 4°C in the refrigerator.

Preparation of Filter Paper Disc

Filter paper disc was prepared by using a punching machine and sterilized using an autoclave. The disc was then soaked in the extract for a specific time and was used for the anti-microbial sensitivity test.

Determining Antibiotic Sensitivity

It was done by the disc diffusion test, or agar diffusion test, or Kirby-Bauer test. It uses antibiotic discs to test the extend to which bacteria are affected by

antibiotics this method was used here to check the antibacterial effect of spices The diameter of the zone of inhibition was measured using a scale and recorded.

Results and Discussion

Antimicrobial sensitivity test of various natural and artificial spices was done to do a comparative study and to analyse their antibacterial effect. The natural spices such as Amchur, Dry ginger, Fenugreek, Turmeric, red chilli, and artificial spices such as Everest Amchur powder, Eastern Dry ginger powder, Fenugreek powder, Turmeric powder and red chili powder were used in the study against five strains of bacteria. The method used for the study was Kirby-Bauer disc diffusion method. The results obtained are given below in tables 1 and 2.

Bacterial strain	Zone of Inhibition (mm) of natural spices				
	Amchur	Dry ginger	Fenugreek	Turmeric	Red chilli
<i>S. aureus</i>	12	8.5	-	7	7
<i>B. circulans</i>	10	9.5	-	7.5	6.5
<i>E. coli</i>	10	9	6	8	9
<i>E. faecalis</i>	11	7	7.5	10	8
<i>K. pneumoniae</i>	15	10	-	7.5	6

Table 1 showing the zone of inhibition (in mm) exhibited by natural spice extracts.

Bacterial strain	Zone of Inhibition (mm) of artificial spices				
	Everest Amchur powder	Eastern Dry ginger powder	Eastern Fenugreek powder	Eastern Turmeric powder	Eastern Red chilli powder
<i>S. aureus</i>	10	8	-	7	-
<i>B. circulans</i>	15	8	-	8	6.5
<i>E. coli</i>	8	-	-	-	-
<i>E. faecalis</i>	13	-	-	5	6
<i>K. pneumoniae</i>	20	-	-	-	-

Table 2 showing the zone of inhibition (in mm) exhibited by artificial spice extracts.

Figure 1 showing the zone of inhibition exhibited by natural spice extracts against various bacteria.

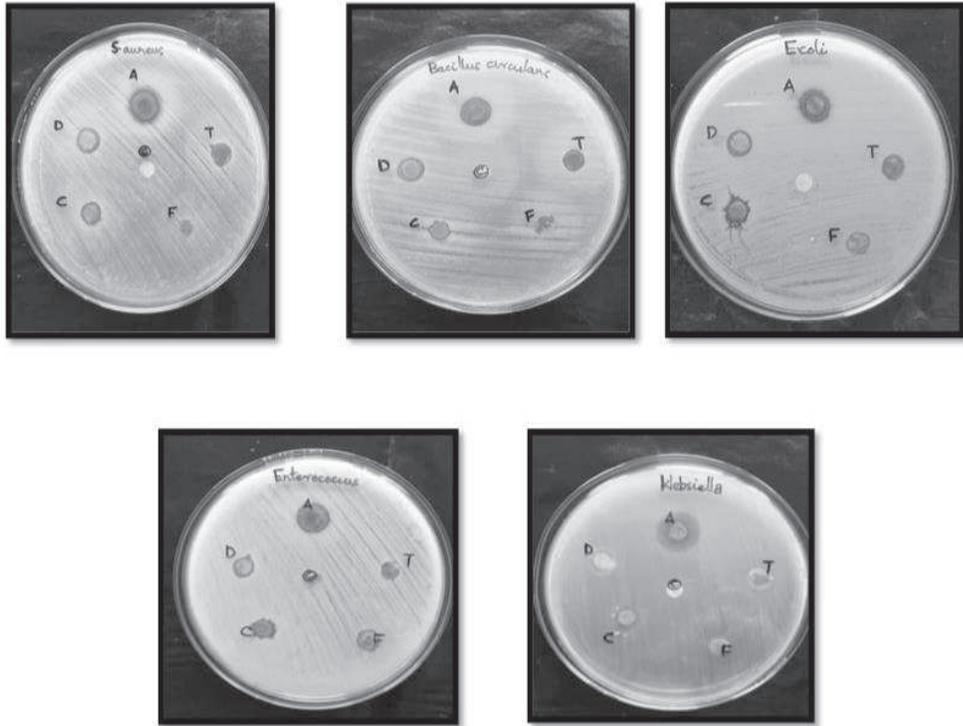
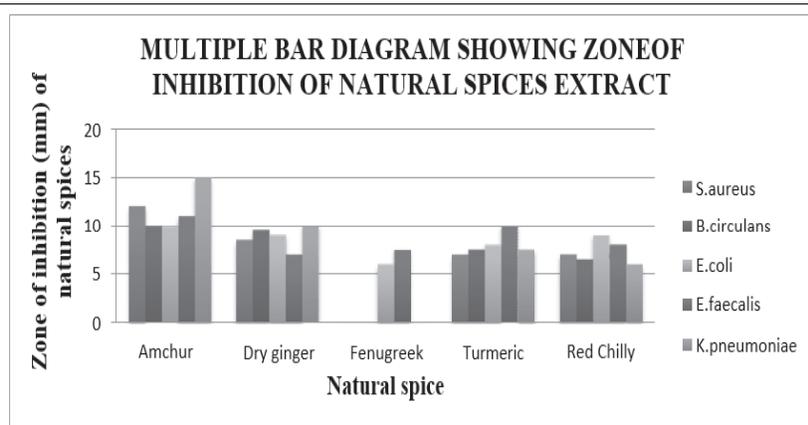


Figure 2 multiple bar diagram showing zone of inhibition of natural spice extracts.



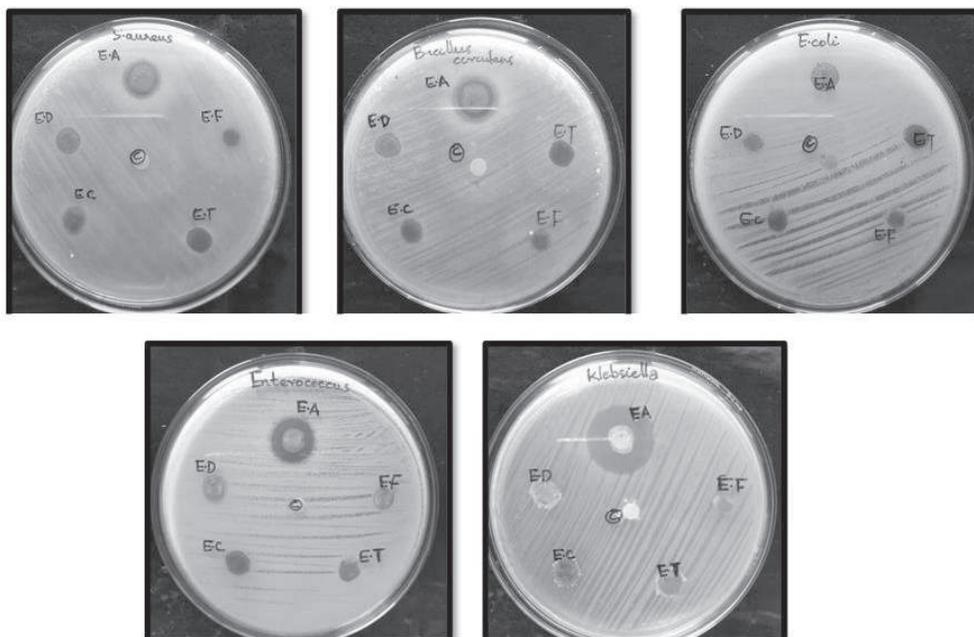
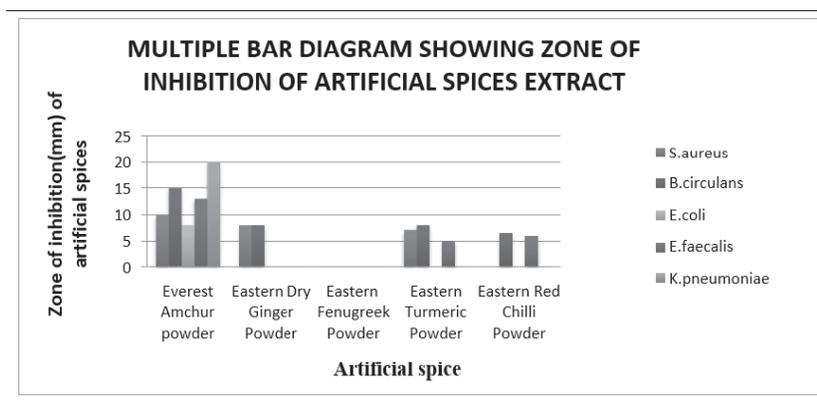


Figure 3 showing the zone of inhibition exhibited by artificial spice extracts against various bacteria.

Figure 4 multiple bar diagram showing zone of inhibition of artificial spice extracts.



Most of the natural spices were found to be effective against all the test organisms. Amchur exhibited the highest zone of inhibition against all the bacterial strains used (Zone of inhibition of 15mm). Gram-negative bacteria like *K. pneumoniae* and Gram-positive bacteria like *S. aureus* were found to be more susceptible to Amchur. It showed greater antibacterial activity than Everest Amchur powder except

for which *K. pneumoniae*, *B. circulans* and *E. faecalis* were most susceptible to Everest Amchur than natural Amchur. This result is of great significance as Everest Amchur powder is cheap and easily available in the market. Further, it also accounts for the fine, and good quality mangoes employed by Everest to produce Amchur.

In a study conducted by V. Srivastava and M. Musaddiq in 2011 in which aqueous extracts of different spices were studied, Amchur were found active against gram- positive as well as gram-negative bacteria. The unboiled aqueous extract of Amchur was effective against *Staphylococcus aureus* with a zone of inhibition of 10.5 mm.

Dry ginger exhibited antimicrobial activity against all the five strains of bacteria used. *K. pneumoniae* was the most susceptible with a zone of inhibition of 10 mm. Whereas Eastern Dry ginger powder exhibited antimicrobial activity against only two strains of bacteria which were *S. aureus* and *B. circulans* with a zone of inhibition of 8 mm. The present study showed that natural dry ginger exhibit antibacterial activity against *E. coli* (zone of inhibition of 9mm) which are in accordance with Laohakunjit et al., (2008), who showed that Ginger can inhibit an antibiotic-resistant strain of *E. coli*, a bacterium that causes intestinal and food-borne illness. Turmeric showed greater antimicrobial activity against all the five strains of bacteria used. Eastern Turmeric showed antimicrobial activity against only three strains of bacteria namely *S. aureus*, *B. circulans* and *E. faecalis*. These results agree with the studies of Gul et al., in 2015 in which the antibacterial activities of different turmeric extracts were tested against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans* by disc diffusion method. Water extracted samples of turmeric stored at room temperature inhibited the growth of *Escherichia coli* and *Salmonella typhi* while aqueous extract autoclaved at 121°C for 30 min reduced the growth of *Escherichia coli* and *Staphylococcus aureus*.

Red Chilli powder showed greater antimicrobial activity against all the five strains of bacteria used. Eastern Red Chilli showed antimicrobial activity against only two strains of bacteria namely *B. circulans* and *E. faecalis*. Koffi-Nevry et al. in 2012 showed that the aqueous and methanol extract of *Capsicum annum* showed encouraging and interesting results against bacterial activities because it is found to be active against *Staphylococcus aureus*, *Salmonella typhimurium*, and *vibrio cholera*. From the present study, Fenugreek was the least effective against all strains of bacteria used. It exhibited antibacterial activity against only two strains of bacteria, namely *E. coli* and *E. faecalis*, among which *E. faecalis* was the most susceptible with a zone of inhibition of 7.5mm. On the other hand, Eastern Fenugreek Powder showed no zones of inhibition against all the bacterial strains.

This result was following a study conducted by Walli et al., (2015) in which Crude extracts of the plant materials (seeds and powder) obtained in aqueous and alcoholic solvents were tested against six different microorganisms including *E.*

coli, *C. albicans*, and *P. vulgaris*, *St. aureus*, *St. pidermis*, and *St. saprophyticus*. The results show that only the boiling water extraction of fenugreek seeds has an inhibitory effect on the growth of *St. aureus* by the two different testing methods, agar disc diffusion and agar well diffusion. This may be explained by that only the boiling water extract contains the antimicrobial active ingredients of fenugreek that are responsible for the antimicrobial effect, while both cold-water extract and methanol are not suitable for such purposes.

Thus, it can be concluded that the study showed that the various types of spice extract used for the study have potential antibacterial activity against most of the pathogenic bacteria used in the study.

Conclusion

The present study revealed that most of the natural and artificial spices used for the study showed antimicrobial activity against the five strains of bacteria tested namely *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus circulans*, *Escherichia coli* and *Enterococcus faecalis*.

Of all the spices used, Amchur exhibited the highest antibacterial activity against all the test organisms used. Natural Amchur showed greater antibacterial activity than Everest Amchur powder except for which *K. pneumoniae*, *B. circulans* and *E. faecalis* were most susceptible to Everest Amchur than natural Amchur. This accounts for the finest quality and purity of the mangoes used by Everest. Thus, this result confirms the good product quality of Everest Amchur powder which is easily available and cheap. Therefore, Amchur with many health benefits and little or no risk factors, which is prominently used in the northern parts of India can be often included in our diet to a wide range of dishes, salads, beverages, etc. as well as for medicinal purposes.

Natural sources of Dry ginger, Turmeric and Red chili also demonstrated greater antibacterial effect. Fenugreek showed the least zone of inhibition. On the other hand, Eastern Turmeric powder, Dry ginger powder and red chili powder also exhibited significant antibacterial effect. Eastern Fenugreek powder showed no zone of inhibition against all the bacterial strains. Natural spices were found to be most effective against the bacterial strains than the artificial spices as it is free from adulteration and some of the artificial spices showed no zones of inhibition for certain bacterial strains for which the natural spices exhibited antibacterial activity. Spices are eco-friendly, safe and can be used as natural alternatives to avoid health hazards of chemically antimicrobial agents, As majority of the investigations are concentrating on the antimicrobial properties of spices against various pathogenic bacterial, fungal, and viral species, as a future work nutrigenomic study of spices should be conducted to find out the effects of spices on human health to provide a molecular understanding of how the chemical and biological components of common spices in

our diet can affect the health by altering the expression of genes and the structure of the human genome.

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