Antibacterial Activity of Garlic (Allium Sativum) And Cinnamon (Cinnamomum Verum) Against Isolated Bacteria

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Abstract- *The present study investigated the antibacterial activity of aqueous and ethanolic extracts of garlic (Allium sativum) and cinnamon (Cinnamomum verum) against isolated bacteria. The microorganisms from various sources were isolated and identified as E.coli, Staphylococcus, Klebsiella, Bacillus and Pseudomonas. Aqueous and ethanolic extracts of garlic and cinnamon were prepared and susceptibilities of isolates to extracts were tested using disc diffusion method. Both the extracts showed inhibitory effect against all isolates and it was observed that the aqueous garlic extract has more antibacterial activity than the aqueous cinnamon extract whereas ethanolic cinnamon extract has greater antibacterial activity than the ethanolic garlic extract. Aqueous garlic extract showed maximum inhibitory effect against E.coli (26mm) as comparision to aqueous cinnamon extract while ethanolic cinnamon extract showed maximum inhibitory effect against E.coli (28mm) as comparsion to ethanolic garlic extract.*

Keywords- Antibacterial activity, Cinnamon, Disc diffusion, Garlic.

I. INTRODUCTION

Spices have long been used in food not only for their flavor, fragrance qualities and appetizing effects but also for their preservative and medicinal properties. Naturally occurring compounds in spices such as sulfur compounds, terpenes and terpene derivatives, phenols, esters, aldehydes, alcohols and glycosides have shown antimicrobial functions [1]. Use of plant extracts for medicinal purposes has been common since ancient times [2]. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial function and could serve as a source of antimicrobial agents against food pathogens [3]. There has been increasing concerns of consumers about foods free or with lower level of chemical preservatives because these would be toxic for humans [4]. There have been many researches on antimicrobial activity of extracts of higher plants. Many plants have been used because of their antimicrobials traits which have been chiefly synthesized during secondary metabolism of plants [5].

Several scientific reports describe the inhibitory effect of spices on a variety of microorganisms, although considerable variation for resistance of different microorganisms to a given spice and of same microorganism to different spices has been observed [6]. Antimicrobial activities of spices depends on several factors such as kind of spice, composition and occurrence of spice, microbial species, substrate composition, processing conditions and its storage [7,8]. Recently there has been increasing interest in discovering new natural antimicrobials. Plants synthesize many compounds by secondary metabolism with complex chemical structures and some of them have related with antimicrobial properties found in plants [9]. Garlic and cinnamon are used for food preservation because of their bacteriocin based strategies and they are used as natural preservatives in poultry and meat products [10]. Certain spices prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity (11, 12). However, extent of inhibition depends on the combination of spice, microorganism and further storage factors like temperature, humidity, preservatives etc. [13]. The present study has investigated antibacterial activity of garlic (*Allium sativum)* and cinnamon (*Cinnamomum verum*) against isolated bacteria.

II. MATERIAL AND METHODS

2.1 Isolation of Microbes

Dry soil, wet soil, and sewage water were collected as samples from surrounding locality and microorganisms were isolated using serial dilution, spread plate and streak plate method. Non selective (nutrient agar) and Selective media (EMB, XLD, MSA, LBA, King's agar) were used for culturing microorganisms and their colony morphology was studied. Gram staining was used for microscopic examination. For identification, various biochemical tests (catalase test,

indole production test, citrate utilization test, triple sugar iron agar test, urease test) were performed for each isolate. Bacterial cultures were refreshed after every 3 to 4 days to avoid contamination. Inoculum was prepared by growing the bacterial culture in nutrient broth over night at 37°C.

2.2 Preparation of aqueous and ethanolic extracts of garlic and cinnamon

The spices including garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*) were purchased from local market. The spices were washed with distilled water thoroughly. Garlic (100gm) was peeled and washed first by distilled water and then surface sterilized by 95% ethanol. Garlic was homogenized aseptically using sterile mortar and pestle and then 200ml of sterilized distilled water was added in it. The homogenized mixture was filtered through sterile Whatman filter paper no. 1. Cinnamon (100 gm) was crushed and sieved through mesh cloth to get the fine powder and then soaked in 200ml of sterilized distilled water and were kept at room temperature for 24 hours, then were filtered using Whatman filter paper no. 1. These extracts were stored at 4°C in refrigerator for further use. The ethanolic extracts of garlic and cinnamon were prepared following same procedure with the exception of solvent which was 95% ethanol instead of sterilized distilled water.

2.3 Antibacterial activity Test

Antibacterial activity of garlic and cinnamon extracts was tested against different bacterial isolates at different concentration of extracts (5,10,15µl) using the disc diffusion method. Bacterial lawn was prepared on Mueller-Hinton agar plates using sterile cotton swab and 24h old broth culture of bacteria. Discs were prepared from Whatman filter paper No. 1 and sterilized. Sterilized discs were dipped in garlic extract (in different concentrations as 5µl, 10µl, 15µl) and placed onto the Mueller-Hinton agar plates swabbed with bacterial cultures. The plates were incubated at 37^0C for 24h. This procedure was repeated for each organism with garlic and cinnamon extract. Zones of inhibition were observed and measured for each microorganism.

III. RESULTS

3.1 Identification of bacteria

Colony morphology of different bacterial isolates was studied on nutrient agar on the basis of form, color, size, elevation, opacity and surface after incubation at 37°C for 24-48 hrs (Table 1). Microscopic examination was performed by Gram staining (Table 2). Colony morphology was also studied on selective media (Table 3). Different bacterial isolates were characterized on the basis of biochemical test (table 4). Bacterial isolates identified from different samples were *E.coli*, *Staphylococcus*, *Klebsiella*, *Bacillus* and *Pseudomonas* (Table 5).

Table 1: Colony morphology of isolated microorganisms on nutrient agar

S.No.	Form	Color	Size	Elevation	Surface	Opacity
1	Circular	Creamy-white	Large	Raised	Smooth	Opaque
2	Circular	Golden-yellow	Small	Raised	Smooth	Opaque
3	Circular	Grayish	Small	Raised	Smooth	Translucent
4	Circular	Dirty-white	Small	Raised	Dry	Opaque
5	Irregular	Cream-white	Large	Flat	Dry	Opaque
6	Circular and irregular	Dirty-white	Large	Flat	Smooth	Translucent

Table 2: Microscopic examination of recovered isolates

S.No.	Arrangement	Shape	Gram Staining
1	Single	Rod	Gram –ve
2	Cluster	Cocci	Gram +ve
3	Single	Rod	Gram +ve

Table 3: Morphological study of microorganisms on selective media

S.No.	Media used	Size	Color	Surface	Microorganism
1	EMB	Small	Metallic green	Smooth	*E.coli*
2	MSA	Small	Yellow	Smooth	*Staphylococcus*
3	XLD	Small	Light pink	Rough	*Klebsiella*
4	LBA	Small	Cream	Rough	*Bacillus*
5	KA	Small	Light yellow	Smooth	*Pseudomonas*

Table 4: Biochemical characterization of bacterial isolates

S.No.	Catalase test	Indole test	TSI test	Citrate test	Urease test	Organism confirmed
1	+	+	+	-	-	*E.coli*
2	+	-	+	-	-	*Staphylococcus*
3	+	-	+	+	+	*Klebsiella*
4	+	-	-	+	-	*Bacillus*
5	+	-	-	+	-	*Pseudomonas*

Table 5: Different bacterial isolates identified from various samples

Samples	Bacterial isolates
Dry soil	*E.coli, Bacillus*
Wet soil	*E.coli, Staphylococcus, Klebsiella*
Sewage water	*Pseudomonas*

3.2 Antibacterial activity:

Aqueous and ethanolic extracts of garlic and cinnamon showed antibacterial activity against all the isolated bacteria. The sensitivity of the previously mentioned bacteria gradually increased with the increase in concentrations of

extract. It was observed that the aqueous garlic extract has more antibacterial activity than the aqueous cinnamon extract whereas ethanolic cinnamon extract has greater antibacterial activity than the ethanolic garlic extract.

The results of antibacterial activity are summarized in table-6,7. At 15µl concentration, aqueous garlic extract showed maximum inhibitory effect against *E.coli* (26mm) as compared to aqueous cinnamon extract that showed maximum inhibitory effect against *E.coli* (20mm). Ethanolic cinnamon extract showed maximum inhibitory effect against *E.coli* (28mm) as compared to ethanolic garlic extract that showed maximum inhibitory effect against *Staphylococcus* (25mm). Minimum inhibitory effect was shown by aqueous cinnamon extract against *Pseudomonas* (10mm) while aqueous garlic extract showed minimum inhibitory effect against *Klebsiella* (12mm). Ethanolic cinnamon extract showed minimum inhibitory effect against *Bacillus* (13mm) while ethanolic garlic extract showed minimum inhibitory effect against *Klebsiella* (20mm).

Table 6: Antibacterial activity of different concentrations of aqueous garlic and cinnamon extract against isolated bacteria.

Organisms	Aqueous extract	Zone of inhibition (mm) at different concentrations		
		5 µl	10µl	15 µl
E.coli	Garlic	22	24	26
	Cinnamon	14	15	20
Staphylococcus	Garlic	18	20	24
	Cinnamon	9	11	14
Klebsiella	Garlic	6	9	12
	Cinnamon	8	11	14
Bacillus	Garlic	18	20	23
	Cinnamon	11	14	18
Pseudomonas	Garlic	14	16	20
	Cinnamon	4	7	10

Table 7: Antibacterial activity of different concentrations of ethanolic garlic and cinnamon extract against isolated bacteria.

Organisms	Ethanolic extract	Zone of inhibition (mm) at different concentrations		
		5µl	10µl	15 µl
E.coli	Garlic	15	18	22
	Cinnamon	21	24	28
Staphylococcus	Garlic	18	22	25
	Cinnamon	12	14	17
Klebsiella	Garlic	13	16	20
	Cinnamon	8	12	14
Bacillus	Garlic	16	20	22
	Cinnamon	7	10	13
Pseudomonas	Garlic	18	22	24
	Cinnamon	10	13	16

IV. DISCUSSION

In the present research work, antibacterial activity of aqueous and ethanolic extracts of garlic and cinnamon were tested against some isolated pathogenic bacteria (*E.coli, Klebsiella, Staphylococcus, Bacillus and Pseudomonas*) by using the disc diffusion method. According to results aqueous garlic extract showed maximum inhibitory effect against *E.coli* (26mm) as compared to aqueous cinnamon extract while ethanolic cinnamon extract showed maximum inhibitory effect against *E.coli* (28mm) as compared to ethanolic garlic extract.

The antimicrobial activity of a plant is due to specific photochemical or essential oils present in it [14]. Various garlic and ginger preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus,* and *Clostridium* [15,16,17,18,19]. The main factors that determine the antimicrobial activity of plants are the type and composition of the plant, the amount or concentration of the active ingredient used, pH, temperature and the type of microorganism. Other factors that determine how effective a herb will be may include the type of environment (climate, bugs, soil quality) in which a plant grew, when it was harvested, stored and processed, species or variety and whether or not there are contaminants such as heavy metals and pesticides in the plant [20]. Ethanolic extract of cinnamon was found to be effective against *Escherichia coli*, *Pseudomonas aeroginosa*, *Staphyllacocus aureus* and inhibition zones observed were 27mm, 19mm, 16mm respectively while for ehanolic garlic extract, inhibition zones observed were 20mm, 27mm, 27mm for these bacteria [21].

The antibacterial activity of aqueous and ethanolic extract of garlic, cinnamon and turmeric was tested against *B. subtilis* (DSM 3256) and *E.coli* (ATCC 25922) at different concentration by using disc diffusion method. Garlic showed the best inhibitory activity showing maximum zone of 26mm against *B. subtilis* and a zone of 22mm against *E. coli*. The aqueous extracts of garlic were more effective than ethanolic extract. In the case of cinnamon, the ethanolic extracts were more effective exhibiting zones of 16mm against *B. subtilis* and 17mm against *E.coli* [17]. Antibacterial activity of aqueous extract of garlic, ginger, clove, cinnamon was tested against four test microorganisms *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi* by using agar diffusion method. On observation, it was found that all the spices showed inhibitory effect and garlic showed maximum inhibition on *E. coli*. Inhibition zones observed for aqueous garlic extract were 29mm against *B. subtilis*, 26mm against *S. aureus*, 31mm against *E. coli*, 25mm against *S. typhi* and for aqueous cinnamon extract were 19mm against *B. subtilis*, 16mm against *S. aureus*, 17mm against *E. coli*, 13mm against *S. typhi* [22].

Growth inhibitory effect of alcoholic extract of garlic was studied on *S. aureus*. Results showed that the effect increased with increasing the concentration of alcoholic extract. Maximum zone of inhibition observed was 23mm at

100mg/ml [23]. Antibacterial effect of aqueous and ethanolic extracts of ginger and garlic has been determined toward six clinical bacterial isolates (*S. aureus*, *S. epidermidis*, *S. pyogens* (G+ve) and *P. aeruginosa*, *K. pneumonia*, *P. mirabilis* (G-ve)) by disc diffusion method. The outcomes of susceptibility experiment depicted that ethanolic extract of garlic and ginger showed more inhibitory effect than aqueous extract. Ethanolic garlic extract showed maximum zone of 20mm against *K. pneumonia* while aqueous garlic extract also showed maximum zone of 11mm against *K. pneumonia*. Aqueous extract of garlic gave no inhibitory effect against *S. epidermidis*, *S. pyogens* and *P. aeruginosa* and ethnolic extract of garlic does not gave inhibitory effect against *S. pyogens* [24].

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