

Invitro Antibacterial Activity of Ethanolic Extracts of Dietary Spices Against Clinical Isolates

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ABSTRACT: The *invitro* antibacterial activity of ethanolic extracts of three dietary spices *Coriandrum sativum* (Coriander), *Curcuma longa* (Turmeric) and *Capsicum annuum* (Red chili) were investigated by well diffusion method against clinical isolates which include Gram positive (*Enterococcus spp.*, *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Proteus spp.*, *Klebsiella pneumonia* and *Acinetobacter baumannii*) as well as by minimal inhibitory concentration and by microdilution method. Moreover, killing time of each extract was also calculated against *Escherichia coli*.

Keywords: Dietary spices, ethanolic, extracts, time kill assay.

INTRODUCTION

Spices are extracted from plants. They could be seeds, fruits, roots, barks, buds or other vegetable substance, considered as a core part to enhance flavor, color and aroma of food. Conventionally, flavor spices as well are used as cost effective preservatives as well. Additionally, they possess antimicrobial capabilities and medication values for treatment of common disturbances and ailments [1, 2]. To date various spices holding antibacterial activity towards pathogens are studied and documented [1, 2]. Furthermore, safest use of these dietary spices lead researchers to spot and study more active compounds [3-5]. In this modern era the interest in using natural substances has increased, due to biological functions and synergistic affects which are likely to shield the body against variety of infections. Spices are efficient anti-oxidants, anti-cancer agent, digestion facilitators and much more [6, 7, 9, 10].

Coriandrum sativum (Coriander) belongs to Family *Apiaceae*. Apart from being used as a spice it is widely used as a herb also. Its seed oil is one of the major oil in world market [6, 11-12].

Curcuma longa (Turmeric) belongs to Family *Zingiberaceae*, its polyphenolic compound 'Curcumin' has been recognized

as an antimicrobial and an anticancer agent [8, 10]. It holds potent anti-oxidant and anti-inflammatory characteristics [4].

Capsicum annuum (Red chili) belongs to Family *Solanacea*, it holds 'Capsaicin' that is medically very important and is used in preparation of ointments [13, 14]. This study explores the antimicrobial activity of ethanolic extracts from aromatic spices from available plants of Pakistan against clinical isolates.

MATERIALS AND METHODS

Microbial Strains: Eight clinical isolates were used in this study provided by the Clinical Laboratory. Of them two were Gram positive (*Enterococcus spp.*, *Staphylococcus aureus*) and six were Gram negative (*Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *Proteus spp.*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*).

Sample Purchasing And Authentication: All spices were identified and purchased from local market. Their voucher specimen numbers are deposited in the Department of Pharmacognosy, University of Karachi.

Preparation Of Extract: Spice Samples were soaked in ethanol separately at room temperature for fifteen days. After fifteen days all the samples were filtered and extracts were evaporated by using rotary evaporator to obtain semidried extracts.

Preparation Of Inoculum: Pure culture of test organisms were streaked onto Nutrient Agar Medium. Isolated Colonies

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of the test organisms were transferred in sterile PBS and turbidity of the suspensions were adjusted to 0.5

McFarland's Standard to get initial count of 10^8 cfu/ml and further diluted up to 10^6 cfu/ml.

Table 1. Zone of inhibition in diameter, (-) means no inhibition, *Berberis vulgaris* as a positive control, coriander versus control is significant correlation whereas Turmeric and Red chili is insignificant. *Staph. aureus*-*Staphylococcus aureus*; *P. aeruginosa*-*Pseudomonas aeruginosa*; *S. typhi*-*Salmonella typhi*; *A. baumannii*-*Acinetobacter baumannii*; *K. pneumoniae*-*Klebsiella pneumoniae*; *E. coli*-*Escherichia coli*.

Clinical Isolates	Coriander	Red Chili	Turmeric	B.Vulgaris Positive Control
<i>S. aureus</i>	11mm	-	13mm	21mm
<i>Enterococcus spp.</i>	12mm	-	-	16mm
<i>P. aeruginosa</i>	-	-	-	19mm
<i>E. coli</i>	11mm	10mm	8mm	15mm
<i>S. typhi</i>	14mm	12	10mm	15mm
<i>A. baumannii</i>	-	9mm	14mm	10mm
<i>K. pneumoniae</i>	9mm	16mm	-	11mm
<i>Proteus spp.</i>	10mm	11mm	9mm	11mm

Table 2. Minimum inhibitory concentration v/v so it is expressed in % and w/v in mg/ml, significant correlation for all three ethanolic extracts versus positive control. *S.aureus*-*Staphylococcus aureus*; *P. aeruginosa*-*Pseudomonas aeruginosa*; *S. typhi*-*Salmonella typhi*; *A. baumannii*-*Acinetobacter baumannii*; *K. pneumoniae*-*Klebsiella pneumoniae*; *E. coli*-*Escherichia coli*.

Clinical Isolates	Coriander	Turmeric	Red Chili	B.Vugaris Positive Control
<i>S.aureus</i>	2.5%	5%	5%	25%
<i>P.aeruginosa</i>	2.5%	5%	5%	6.25%
<i>S.typhi</i>	5%	5%	0.625%	6.25%
<i>E.coli</i>	2.5%	2.5%	0.625%	3.1%
<i>Enterococcus spp.</i>	1.25%	5%	0.625%	6.25%
<i>Proteus spp.</i>	2.5%	2.5%	0.625%	12.5%
<i>K.pneumoniae</i>	1.25%	5%	0.625%	1.5%
<i>A.baumannii</i>	0.625	5%	5%	3.1%

ANTIMICROBIAL ACTIVITY ASSAY

In vitro, antibacterial activity of each ethanolic extracts was investigated against the test strains. Two antibacterial sensitivity assays *i.e.*; Agar Well Diffusion Assay and Minimum Inhibitory Concentration were used to determine the growth inhibition [18, 19]. Additionally, killing time

assay of each extract in opposition to *Escherichia coli* was also investigated. *Berberis vulgaris* was used as a positive standard throughout the study. The stock solution of each extract was prepared in DMSO.

Agar Well Diffusion Assay: The screening of antibacterial activity of three selected spices was carried out using agar

Well Diffusion Assay. The adjusted culture was used to make lawn on Mueller Hinton Agar media plate (Oxoid®) using sterile cotton swab. 7mm sterile borer was used to make a well then 100 µl of 10% ethanolic extract was added to each well. The plates were incubated at 37°C for 24 hours. The sensitivity was determined on the basis of zone of inhibition around the well. This test was run in triplicate and repeated, therefore, n=6.

Minimum Inhibitory Concentration: To identify sensitivity of microbes towards ethanolic extracts, MIC was measured by microdilution method using 96 microtitre plate. The extracts were serially diluted in 1:2 dilutions according to CLSI recommendations. 10µl culture was added in each well, positive and negative controls were also run and plates were incubated at 37 °C for 24 hours. Microdilution Method was individually followed for all three samples against provided isolates. Plates were read by ELISA plate reader.

Time Kill Assay: This assay was performed using *Escherichia coli* to record the time taken to enforce the killing effects. The experiment was performed by inoculating test organism in nutrient broth and incubated at 37 °C for 24 hours. It was matched with 0.5 McFarland’s Index and broth culture was diluted to 10⁻³ dilution. 1ml of culture was added with 0.1ml of extract in cuvettes. Optical Density (OD) was measured at intervals of 0 min, 30 min, 60 min, 120 min, 240 min and 1440 min.

RESULT AND DISCUSSION

The antibacterial activities of ethanolic extracts in term of zone of inhibition and minimum inhibitory concentration are reported in Table 1 and 2.

The ethanolic extract of *Curcuma longa* (Turmeric) showed antibacterial activity against *Escherichia coli* with zone diameter of 8mm and MIC 2.5%, *Acinetobacter baumannii*

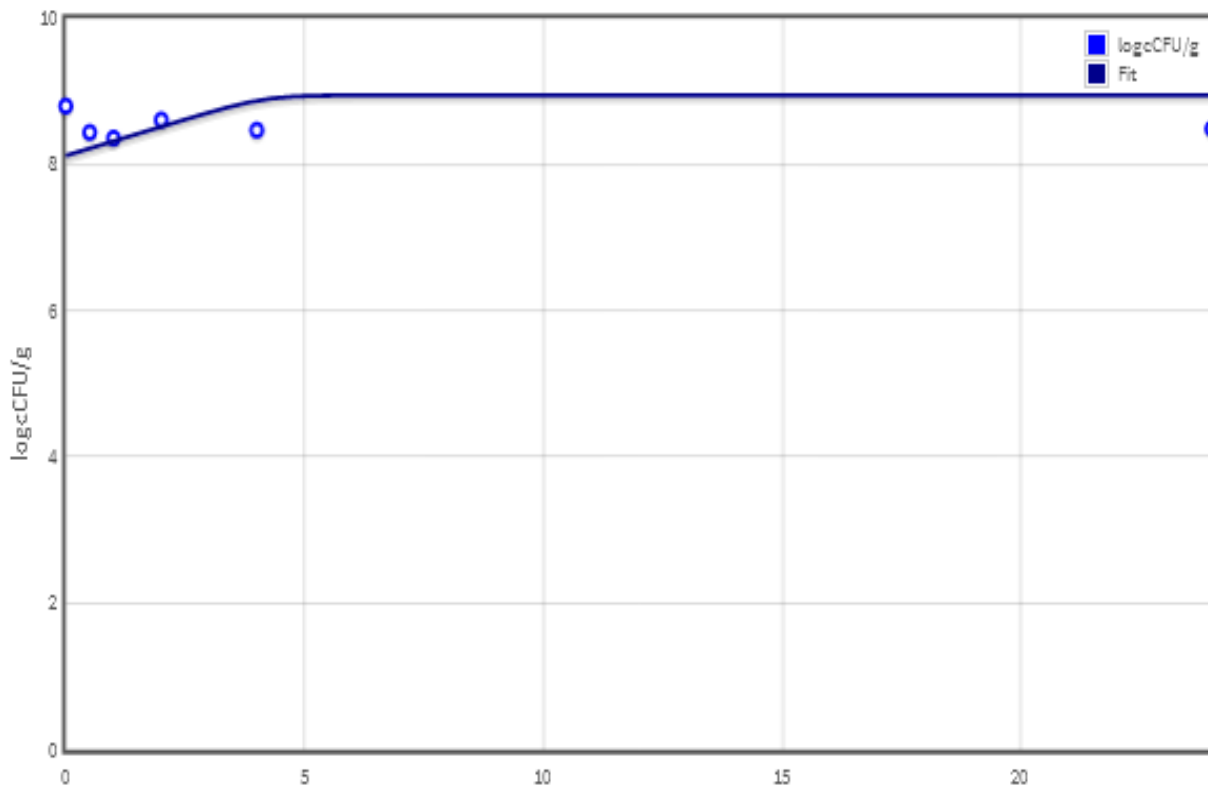


Fig. (1). Time kill of *E.coli* in ethanolic extract of Red chili fitted in DmFit (Dynamic modeling fit) R2= 0.701 and SE of fit 0.208.

14mm with MIC 5%, *Staphylococcus aureus* 13mm with MIC 5%, *Pseudomonas aeruginosa* 10mm with MIC 2.5% and *Salmonella typhi* 10mm MIC 5%, while *Klebsiella pneumoniae* and *Enterococcus spp.* were not sensitive towards Turmeric.

Coriandrum sativum (Coriander) showed antimicrobial activity against all organism except *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, *Staphylococcus aureus* 11mm

with MIC 2.5%, *Salmonella typhi* 14mm with MIC 5%, *Escherichia coli* 11mm with MIC 2.5% *Enterococcus spp.* 12mm MIC 1.25%, *Proteus spp.* 10mm MIC 2.5%, *Klebsiella pneumoniae* 9mm MIC 1.25%.

Capsicum annum (Red chili) showed antibacterial activity against *Salmonella typhi* with zone diameter 12mm MIC 0.625%, *Escherichia coli* 10mm MIC 0.625%, *Proteus spp.* 11mm MIC 0.625%, *K. pneumoniae* 16mm MIC 0.625% and

Acinetobacter baumannii 9mm with MIC 5%. Some of the isolates failed to show results in well diffusion assay whereas, their minimum inhibitory concentration was determined so it may be due to improper diffusion of extracts in media and might be human error. Pearson's Correlation of zone of inhibition and minimum inhibitory concentration of each ethanolic extracts was calculated versus positive control. Zone of inhibition of coriander extract indicated significant correlation whereas turmeric and red chilli showed insignificant correlation while minimum inhibitory concentration correlation was significant for all three ethanolic extracts.

Time Kill Assay: The assay was performed using *Escherichia coli* to analyze the time taken by test compound to exert its killing effect. Killing time of each extract was compared with the positive control, *Berberis vulgaris* Fig. (4). Graphs were plotted by using DMFit (dynamic modeling fit) [20]. In the presence of test compound *Capsicum annuum* (Red chili) the log CFU/ml was 8.80 at 0min. The organism started to grow but compound started exerting its effect and slight decrease was observed after 30min till 1440min as shown in Fig. (1). In the presence of *Curcuma*

longa (Turmeric) the log CFU/ml was 8.68 at 0min later on a decrease was seen in viable counts of *Escherichia coli* as shown in graph Fig. (2). While *Coriandrum sativum* (Coriander) extracts, initially had log CFU/ml was 8.86 which started decreasing after 30min till end as shown in Fig. (3).

In vitro activities of these plant extracts showed good antibacterial activity not in favor of pathogenic microorganism. It can be claimed that folk medicine can be used as effectively as modern medicine to combat infectious microbes. [15] *Pseudomonas aeruginosa*, a notorious organism known for its resistance to various antibiotics and *Acinetobacter baumannii*, [16].

Multi drug resistant (MDR), is an important cause of nosocomial infections which is also affected by ethanolic extracts of these spices and witnessed greater antibacterial activity. Although there is a great achievement and advancement in medicine technology but people are realizing to use natural products to ensure safety [17]. Interest in using natural products has been revived as a result of antimicrobial resistance to various drugs.

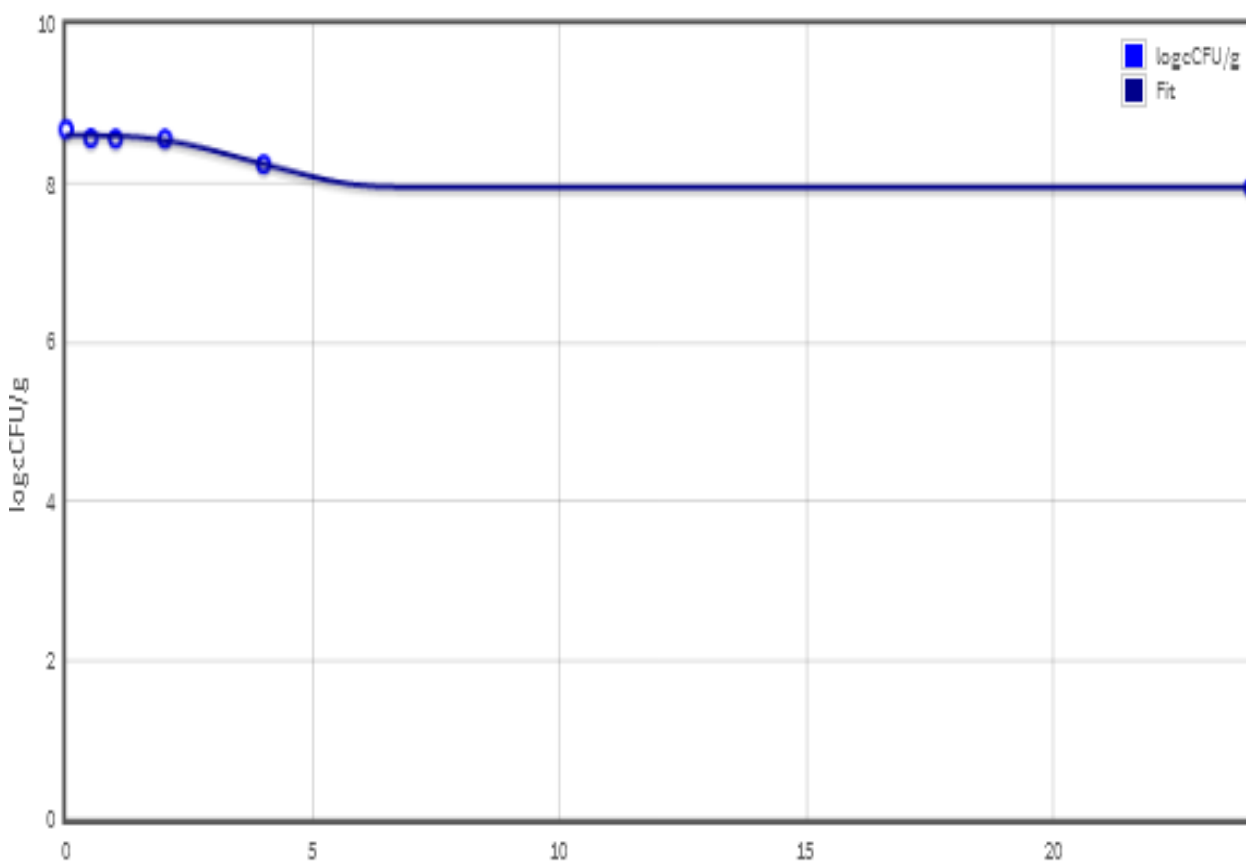


Fig. (2). Time kill of *E. coli* in ethanolic extract of turmeric fitted in DmFit (Dynamic modeling fit) $R^2 = 0.947$ and SE of fit 0.0638.

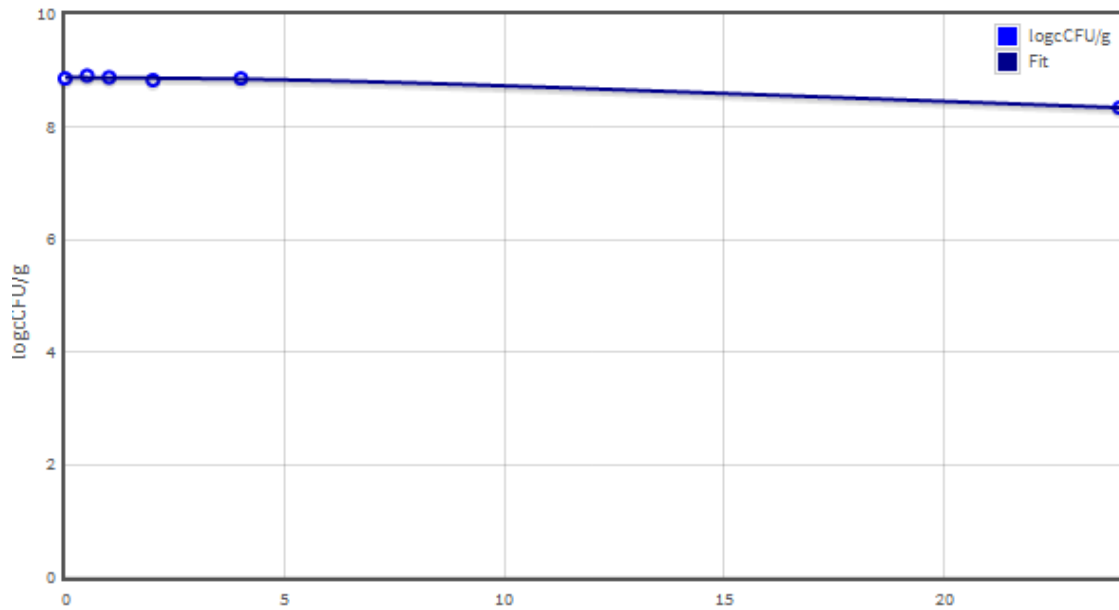


Fig. (3). Time kill of *E.coli* in ethanolic extract of Coriander fitted in DmFit (Dynamic modeling fit) R2= 0.978 and SE of fit 0.032.

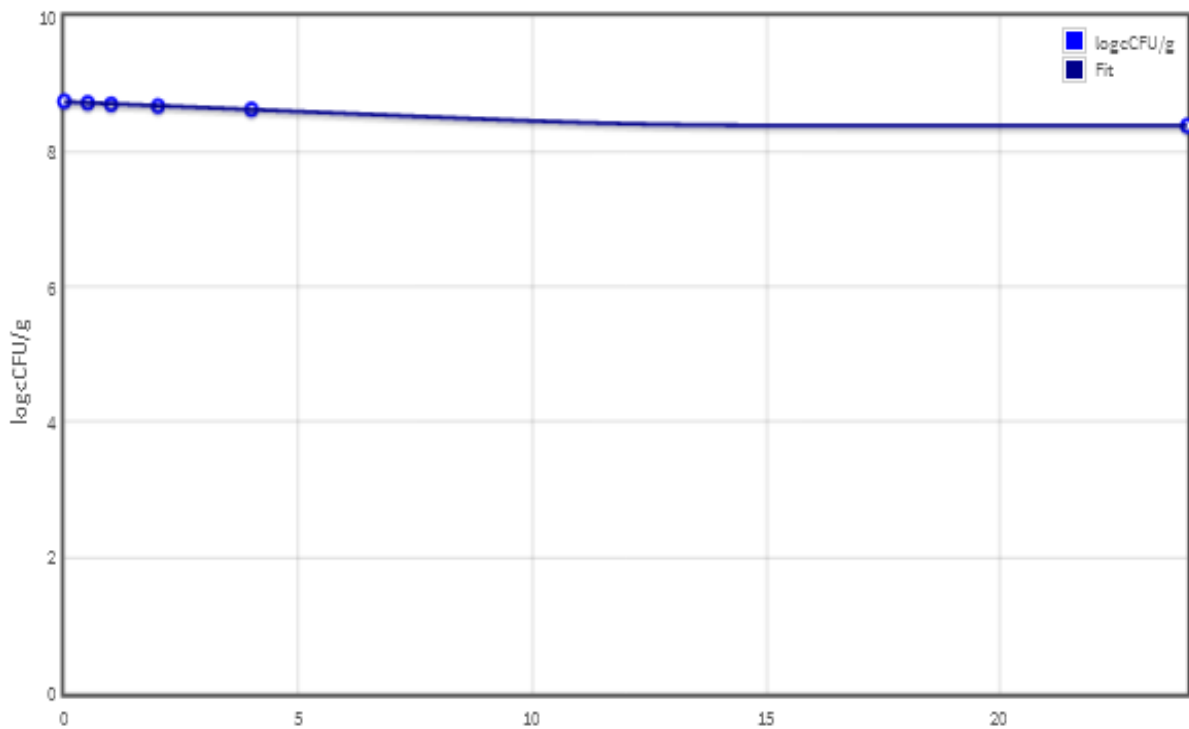


Fig. (4). Time kill of *Berberis vulgaris* positive control fitted in DmFit (Dynamic modeling fit) R2= 0.997 and SE of fit 0.00748.

CONCLUSION

This study witnessed the antimicrobial potential of *Coriandrum sativum* (Coriander), *Curcuma longa* (Turmeric) and *Capsicum annuum* (Red chili). Their effective use can be

made for treatment of certain infections and it is exposed that they have a significant scope towards development of herbal antimicrobial agents.

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CONFLICT OF INTEREST

Declared none.

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