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Antimicrobial activity of Indian spices against pathogenic bacteria

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ABSTRACT

The present study investigated the antimicrobial potential of four Indian spices viz., black pepper, clove, cinnamon and turmeric against the human pathogenic bacteria. The ethanolic extract of all spices exhibited maximum antimicrobial potential. The ethanolic extract of all spices exhibited maximum antimicrobial potential. The ethanolic extract of clove showed highest potential against E. coli (25.0±0.81mm) while that of black pepper exhibited maximum activity against E. coli (22.3±0.56mm). The ethanolic extract of cinnamon exhibited maximum antibacterial property against E. coli (21.3±0.7mm) while that of turmeric showed highest potential against E. coli (29.3±0.47mm). The spices exhibited effective antimicrobial potential.

Key words: Antimicrobial potential, human pathogens, clove, cinnamon, black pepper, turmeric

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INTRODUCTION

The treatment of infectious diseases is becoming a serious concern due to increasing resistance against antibiotics amongst the pathogens is increasing at an alarming rate[1]. The antibiotics may also have adverse effects on the human body like effect on the normal flora and allergy [2]. This has led researchers to search for alternatives to drugs. The search is focused on medicinal plants which can prove to be the best alternative to antibiotics without any side-effects [3, 4,5]. The active component present in the medicinal plant extracts need to be purified and identified which can be developed as drug. The combinatorial synthesis approach can be applied to synthesize the compound which can mimic the natural component present in these medicinal plants with better efficacy. The spices used in Indian cooking have been used since ages for adding flavor and also for house-hold treatment of infectious diseases. It is imperative to study their antimicrobial activity against the common human pathogens so that the best spices can be further exploited to determine their active component which can be used for developing drugs. The present study was aimed at studying the antimicrobial activity of black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*) and turmeric (*Curcuma longa*) against the common human pathogens.

MATERIALS AND METHODS

2.1Bacterial culture

The pathogenic bacteria viz., *E. coli*, *Pseudomonas*, *Proteus*, *Salmonella*, *Alcaligenes*, *Staphylococcus* and *Klebsiella* were taken from culture collection center, department of microbiology, Dolphin (PG) Institute of Biomedical Sciences, Dehradun, India.

2.2 Acquisition of spices and preparation of extract

Black Pepper, clove, cinnamon and turmeric were procured from the local market. The spices were air dried at room temperature and grounded into fine powder. Three extracts *viz*., aqueous, ethanolic and methanolic were prepared. The extracts were prepared by dissolving spices in solvents in a concentration of 1:4 and keeping at room temperature for 24hrs in a sterile beaker covered with aluminium foil to avoid evaporation and then subjected to filtration through sterilized Whatman no. 1 filter paper. The solvent was dried and concentrated using orbital shaker at 40°C. The stock solutions of the extracts thus obtained were prepared by diluting the dried extracts with 50% of respective solvents.

2.3 Evaluation of antimicrobial activity of extracts

The antimicrobial activity of 12 crude extracts (aqueous, ethanolic and methanolic) of 4 spices against pathogenic bacteria was evaluated by using agar well diffusion method. The isolates were inoculated into 10mL of sterile Nutrient broth, and incubated at 37±1^{0}C overnight. The turbidity of culture was compared with Mac Farland standard number II. The cultures were swabbed on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab and allowed to dry for 3-5 minutes. Agar wells were prepared with the help sterilized borer with 10mm diameter. The extract of spices was diluted to give the final concentration 1000ppm, 2000ppm, 3000ppm and 4000ppm. 100 µl of different dilutions of the extracts was added to the wells of the inoculated plates. 50% ethanol and 50% methanol was used as control which was introduced into the well instead of the extract. The plates were incubated in an upright position at 37±1^{0}C for 24hrs. The zone of inhibition was measured and expressed in millimeters (mm).

RESULTS

All extracts of spices showed good antibacterial property (Table 1 to 4). The ethanolic extract of clove showed highest potential against *E. coli* (25.0±0.81mm), methanolic extract against *Alcaligenes* (18.3±0.24mm) and aqueous against *Proteus* (18.7±0.47mm).The ethanolic extract of black pepper showed highest potential against *E. coli* (22.3±0.56mm), methanolic extract against *Staphylococcus* (14.6±0.32mm) and aqueous against *E. coli* (19.5±0.47mm). The ethanolic extract of cinnamon exhibited maximum antibacterial property against *E. coli*(21.3±0.7mm) while methanolic extract against *Salmonella* (12.6±0.47mm) and aqueous extract against *Pseudomonas* (19.6±0.47mm). The ethanolic extract of turmeric showed highest potential against *E. coli*(29.3±0.47mm) while methanolicextract against *Alcaligenes*(12.6±0.47mm) and aqueous extract against *Pseudomonas* (18.3±0.47mm).

Table 1a: Antimicrobial activity of ethanolic extract of clove against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	10.0±1.4	16.6±0.9	21.5±0.54	25.0±0.81
Staphylococcus	9.56±1.24	11.4±0.81	14.3±0.47	17.6±0.47
Pseudomonas	8.56±0.47	10.3±0.94	13.3±0.94	15.3±0.45
Klebsiella	8.6±0.94	10.3±0.43	12.6±0.94	13.6±0.85
Proteus	7.6±0.45	9.5±0.34	11.4±0.56	14.3±0.49
Salmonella	7.6±0.32	9.3±0.37	12.3±0.47	14.5±0.47
Alcaligenes	6.3±0.47	8.3±0.35	11.5±0.35	15.2±0.37

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 1b: Antimicrobial activity of methanolic extract of clove against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	6.6±0.47	9.3±0.42	12.5±0.45	15.6±0.47
Staphylococcus	7.6±0.45	9.3±0.36	11±0.81	14.3±0.47
Pseudomonas	6.7±2.49	8.6±0.28	11.6±0.47	13.3±0.47
Klebsiella	9.6±0.45	10.6±0.56	11.4±0.54	13.6±0.47
Proteus	9.2±0.35	11.4±0.32	13.6±0.47	15.3±0.47
Salmonella	6.3±0.47	8.3±0.24	10.5±0.47	12.3±0.26
Alcaligenes	12.6±0.25	14.6±0.22	16.3±0.36	18.3±0.24

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 1c: Antimicrobial activity of aqueous extract of clove against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	6.6±0.47	9.6±0.27	14.5±0.42	17.2±0.42
Staphylococcus	6.8±0.33	10.2±0.42	14.0±0.25	17.5±0.47
Pseudomonas	6.5±0.24	9.6±0.47	13.3±0.94	17.2±0.47
Klebsiella	7.3±0.22	10.4±0.24	14.4±0.47	17.6±0.94
Proteus	6.6±0.26	10.3±0.17	14.3±0.24	18.7±0.47
Salmonella	17.3±0.24	20.3±0.33	23.3±0.25	25.3±0.22
Alcaligenes	11.6±0.33	15.3±0.47	19.4±0.47	24±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 2a: Antimicrobial activity of ethanolic extract of black pepper againstbacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	10.3±0.47	14.3±0.36	18.3±0.46	22.3±0.56
Staphylococcus	9.6±0.22	11.6±0.47	13.3±0.47	16.6±0.47
Pseudomonas	7.3±0.47	9.5±0.22	11.6±0.45	13.3±0.56
Klebsiella	7.6±0.94	8.3±0.45	9.6±0.35	10.3±0.47
Proteus	8.3±0.47	10.3±0.16	12.6±0.22	13.3±0.47
Salmonella	13.3±0.47	15.3±0.35	17.3±0.22	18.3±0.47
Alcaligenes	5.3±0.42	7.3±0.33	9.6±0.47	10.3±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 2b: Antimicrobial activity of methanolic extract of black pepper against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	8.5±0.34	10.5±0.32	12.3±0.32	14.3±0.24
Staphylococcus	7.3±0.22	9.6±0.25	12.3±0.24	14.6±0.32
Pseudomonas	4.5±0.14	5.3±0.24	6.5±0.33	7.3±0.47
Klebsiella	7.6±0.54	8.6±0.24	9.6±0.24	10.3±0.47
Proteus	5.3±0.24	7.3±0.25	9.6±0.32	11.3±0.34
Salmonella	6.3±0.18	8.5±0.24	10.3±0.34	12.3±0.32
Alcaligenes	1.6±0.10	2.5±0.27	4.3±0.17	5.3±0.22

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 2c: Antimicrobial activity of aqueous extract of black pepper against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	11.3±0.41	14.3±044	16.3±0.47	19.5±0.47
Staphylococcus	9.6±0.22	10.6±0.17	13.3±0.37	16.6±0.47
Pseudomonas	11.6±0.24	13.3±0.21	14.3±0.33	16.6±0.41
Klebsiella	8.6±0.24	10.6±0.26	12.3±0.25	13.3±0.21
Proteus	8.3±0.23	10.3±0.32	12.6±0.14	13.3±0.34
Salmonella	15.5±0.33	16.4±0.27	17.3±0.18	18.3±0.23
Alcaligenes	9.6±0.17	11.3±0.22	13.3±0.47	15.3±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 3a: Antimicrobial activity of ethanolic extract of cinnamon against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	10.6±0.32	14.3±0.23	17.5±0.15	21.3±0.7
Staphylococcus	3.5±0.30	5.3±0.32	7.6±0.36	9.3±0.42
Pseudomonas	5.3±0.24	7.5±0.40	9.5±0.32	13.3±0.47
Klebsiella	11.6±0.35	13.3±0.47	15.6±0.94	16.3±0.7
Proteus	4.6±0.32	6.3±0.27	8.4±0.23	10.3±0.42
Salmonella	5.6±0.34	6.6±0.23	7.6±0.32	9.6±0.47
Alcaligenes	7.6±0.24	8.3±0.35	9.6±0.47	10.3±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 3b: Antimicrobial activity of methanolic extract of cinnamon against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	3.6±0.21	5.3±0.24	7.5±0.33	9.6±0.32
Staphylococcus	5.6±0.16	7.3±0.47	9±0.81	11.3±0.47
Pseudomonas	2.3±0.12	4.3±0.21	6.3±0.32	8.3±0.37
Klebsiella	3.6±0.7	5.3±0.47	7.3±0.47	8.6±0.94
Proteus	5.6±0.47	6.3±0.23	8.4±0.24	10.3±0.47
Salmonella	6.6±0.16	9.6±0.21	10.5±0.32	12.6±0.47
Alcaligenes	6.5±0.21	7.3±0.32	8.6±0.35	10.3±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 3c: Antimicrobial activity of aqueous extract of cinnamon against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	6.6±0.47	9.3±0.47	11.5±0.33	12.6±0.32
Staphylococcus	3.0±0.81	5.3±0.47	6.3±0.47	7.3±0.47
Pseudomonas	7.6±0.47	10.6±0.32	15.3±0.44	19.6±0.47
Klebsiella	5.6±0.31	7.6±0.32	9.3±0.33	10.3±0.47
Proteus	7.6±0.47	9.3±0.23	11.4±0.24	13.3±0.47
Salmonella	11.6±0.47	13.6±0.32	16.6±0.25	18.6±0.47
Alcaligenes	6.6±0.47	9.3±0.32	11.6±0.34	13.3±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 4a: Antimicrobial activity of ethanolic extract of turmeric against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	11.6±0.15	16.3±0.25	21.3±0.43	29.3±0.47
Staphylococcus	8.6±0.34	9.3±0.35	10.7±0.81	13.6±0.47
Pseudomonas	3.6±0.47	4.3±0.47	5.4±0.23	6.6±0.47
Klebsiella	8.6±0.94	10.3±0.47	12.6±0.94	14.3±0.47
Proteus	9.3±0.24	11.6±0.32	12.6±0.22	13.3±1.24
Salmonella	7.4±0.47	9.6±0.34	11.6±0.43	12.5±0.94
Alcaligenes	3.3±0.47	4.3±0.21	5.3±0.47	7.3±0.32

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 4b: Antimicrobial activity of methanolic extract of turmeric against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	2.6±0.15	3.3±0.25	4.3±0.43	5.6±0.47
Staphylococcus	7.3±0.22	8.3±0.25	9.6±0.42	10.6±0.43
Pseudomonas	5.3±0.47	7.5±0.32	8.7±0.47	10.3±0.47
Klebsiella	7.4±0.47	8.6±0.34	9±0.43	10.6±0.94
Proteus	3.3±0.24	5.6±0.32	6.6±0.42	8.3±0.45
Salmonella	8.4±0.47	9.6±0.34	10.6±0.43	11.5±0.94
Alcaligenes	6.3±0.15	8.3±0.47	10.6±0.33	12.6±0.47

** Concentration in ppm.*
Values are mean ± SD of three replicates

Table 4c: Antimicrobial activity of aqueous extract of turmeric against bacterial pathogens

Name of organism	Zone of inhibition (mm)			
	1000	2000	3000	4000
E. coli	4.3±0.47	6.6±0.43	8.3±0.23	10.3±0.27
Staphylococcus	5.6±0.47	7.5±0.22	10.3±0.26	12.3±0.47
Pseudomonas	13.6±0.22	15.3±0.21	17.3±0.17	18.3±0.47
Klebsiella	3.3±0.47	6.6±0.23	9.3±0.47	12.6±0.47
Proteus	4.3±0.34	6.5±0.36	8.6±0.32	10.3±0.24
Salmonella	9.3±0.47	12.6±0.47	15.6±0.23	17.3±0.47
Alcaligenes	7.3±0.24	9.5±0.47	11.3±0.33	13.6±0.35

** Concentration in ppm.*
Values are mean ± SD of three replicates

DISCUSSION

The resistance to multiple drugs has become a common feature in which most of the organisms associated with diarrhoea and other enteric diseases [6, 7, 8], urinary tract infection [9, 10], neonatal infection [11, 12] and wound infection [13, 14, 15].Spices are an important part of the human diet in India. They have been used for thousands of years to enhance the flavour, color and aroma of food. Spices are well known for their preservative and medicinal value in households. It is however in recent years that the spices have drawn the attention of researchers due to increasing resistance against antibiotics amongst pathogens [16, 17, 18].

The present work was conducted to evaluate the antimicrobial potential of Indian spices namely, *Syzygium aromaticum* (clove), *Cinnamomum zeylanicum* (cinnamon), *Piper nigrum* (black pepper) and *Curcuma longa* (turmeric). The spices were purchased in dried form and grinded before subjecting to crude phytochemical extraction. In the present study, three solvents namely water, ethanol and methanol were selected for extraction. The ethanolic extract of the four spices exhibited the maximum antimicrobial activity against pathogens. The aqueous and the methanolic extract showed less activity against the isolates.

The most potent antimicrobial constituents in many spices are aromatic phenolic compounds. It can thus be concluded that the antimicrobial efficacy of clove is due to eugenol while that of cinnamon is due to eugenol and cinnamic aldehyde [19, 20]. Piperine in black pepper is the active constituent [21] while curcumin is the active constituent of turmeric [22].

The active constituent of spices may exhibit their antimicrobial effect either by degradation of cell wall, disruption of cytoplasmic membrane, leakage of cellular components, damage protein, interfere with the enzymatic activities inside cell, affect synthesis of DNA and RNA, affect electron transport and nutrient uptake, leakage of cellular components, impair the energy production inside cell, change fatty acid and phospholipid constituents [23].

From this study, it may be concluded that the ethanolic extract of spices can be used as a potential source of natural antimicrobial compound against pathogenic bacteria. This preliminary study can be further extended in determining the active component of the spices so that effective medicinal preparations can be made. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments [24, 25].

CONCLUSION

The present study is a preliminary research work focusing on the investigation of antimicrobial potential of Indian spices on pathogenic bacteria. Turmeric was found to be the most potent spice exhibiting maximum antimicrobial potential followed by clove, black pepper and cinnamon. The spices exhibited a very strong antimicrobial potential.

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