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Original Article

IN VITRO ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS TURMERIC, CHINNAMON, AND CLOVE AGAINST GM (+VE) AND GM (-VE) BACTERIA

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ABSTRACT

Objective: Various diseases are caused by different pathogenic microorganisms. Antibiotics are being used for treatment of these infectious diseases, yet unpredictable utilization of it leads towards antibacterial resistance. It is required to discover better approaches to battle against antibacterial resistance. Therefore, the study aimed to detect antibacterial sensitivity of ethanol extracts of *Curcuma longa* (turmeric), *Cinnamomum zeylanicum* (clove) and *Syzygium aromaticum* (cinnamon) against *Staphylococcus aureus* and *E coli*.

Methods: Prior to sensitivity testing, ethanol oils were extracted by an electric blender and each of the bacteria strains were cultured onto blood agar plate. Antibacterial activity was tested by agar well diffusion method where three different concentrations (50 µl, 75 µl and 100 µl) of selected plants extract were used so far as to measure the inhibition zone. Inhibition zone of the ethanol extract of these plants were calculated where three were found to be sensitive against *Staphylococcus aureus* and *E coli*.

Results: Greater inhibition zone 14.5 mm, 18.25 mm, 21.5 mm at100 µl against *Syaphylococcus aureus* in case of cinnamon whereas the least inhibition zone was showed by turmeric and it was 9.00 mm, 11.00 mm, and 12.75 mm at 100 µl extract against *E. coli.*

Conclusion: Overall, all the ethanol extracts were found to effective against these two bacteria but cinnamon can be used as more effective antibacterial agent in both human and veterinary field after the toxicological test.

Keywords: Medicinal plants, Turmeric, Cinnamon, Clove, Bacteria, Antibiotic resistance

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INTRODUCTION

Drug resistance is the most prime concern for both veterinary and human medicine expert despite the use of various types of antibiotics. Researchers are incessantly concerned with the high and growing number of multi-drug resistant bacteria caused due to uncontrolled use of antibiotics for treatment as well as to prevent different disease outbreaks. Consumers, food processors, and regulatory Agencies are also worried about the safety of foods containing antibiotic and its residual effect on human body. Therefore, there has been a growing interest in the identification and development of effective and nontoxic antimicrobial compounds using natural antibacterial compounds from medicinal plant [1-4].

Many medicinal plants around the world are being used as herbal remedies against many infectious diseases throughout the history of mankind [5]. Even today, medicinal plans are playing a sound role as therapeutic remedies for primary treatment in developing countries [6]. There are literally a large number of published research papers from around the world describing the antimicrobial activities of medicinal plants [7-10]. A study of the PubMed database (information from 1975 to 2005, accessible on the Internet) created around 1360 reports in the scientific and medicinal research that describing the antimicrobial activities of different plant species and their synthetic constituents. Moreover, a study of the Napralert database, the world's biggest medicinal database housed inside the University of Illinois at Chicago, demonstrates that of the 58,850 plant species recorded in the database, 6,550 species have been tested for antimicrobial activity, of which right around 4000 species had ethno medical information supporting the utilization of these plants to treat infectious diseases [7, 11]. The greater part of the plants had an action against a scope of microscopic part of organisms, parasites or Mycobacterium. Numerous medicinal plants have been tested against several strains of bacteria, the most widely recognized microbes utilized as a part of antibacterial tests include: *Escherichia coli, Enterococcus faecalis, Bacillus cereus, Bacillus subtillis, Chlamydia pneumonia, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa vancomycin resistant Enterococcus (VRE), and Helicobacter pylori* [12].

Among medicinal plant, spices have been used from the very beginning of the invention of fire as a food additive. However, it's being used as folk medicine and preservatives from ancient time [13]. Bacteriostatic and bactericidal or antioxidant activity are the reasons to be used the spices as folk medicine [14] and generally recognized them to be safe for human body, maybe for their traditional use or for lack of documentation about the toxicity [1]. But, we need to go out from this traditional use and should look forward to using widely both in human and animal medicine field. Several studies on antibacterial plant have been done all over the world with different other plans. But, there is a lack of generalized study with the selected plants. So, the study has done to screen the antibacterial activity of *Curcuma longa, Cinnamomum zeylanicum* and *Syzygiumaromaticum* against two common bacteria species *Escherichia coli* and *Staphylococcus aureus* and illustrate the comparative potential of medicinal plants.

MATERIALS AND METHODS

Selection of plant material

Three medicinal plants were selected in this research for detection of antibacterial activity under *in vitro* conditions in the laboratory. The scientific name, local name, part used in the study and its traditional application have shown in table 1. The plans were collected from different regions in Bangladesh based on their traditional application and claims. These plants were identified by Dept. of Pharmacology and Toxicology, Sylhet Agricultural University, Sylhet. These plants specimens have been deposited and preserved at the Laboratory of Microbiology, Sylhet Agricultural University, Sylhet for further procedure.

Table 1: Medicinal plants tested for antibacterial activity

No.	Scientific name	Local name	Part used in the study
1	*Curcuma longa*	Holud	Stem
2	*Cinnamomum zeylanicum*	Daruchini	Bark
3	*Syzygium aromaticum*	Lavang	Flower

Preparation of plant extract

The plant extract was prepared, according to Odey *et al.* (2012). Briefly, the medicinal plans after collection were thoroughly washed to remove debris and the earth remains. From these usable parts of the plans were separated and chopped into bits and allowed to dry under shade. The dried sample then blended for making them into powder form by electric Blender and preserved in airtight container. One hundred grams (100g) of each powder was weighed using an electronic measuring balance. Each powder was differently soaked in 400 ml of ethyl alcohol (80% BDH), at a ratio of 1:4 (powder/solvent and was agitated using an electric blender. After that every blended mixture was poured into air-tight plastic container and kept in the refrigerator at 4 °C for 48 h. The mixture then first filtered by cloth and then filtered by Whatman No 1 filter paper. The filtered solutions were stored in container and preserved in refrigerator for detection of antimicrobial activity.

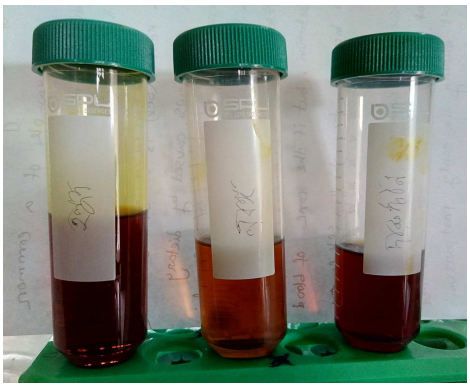

Fig. 1: Selected plant extracts

Bacteria and bacteria culture

Prior to sensitivity testing, *Escherichia coli* and *Staphylococcus aureus* strains were cultured onto Eosin Methylene Blue (EMB) and Mannitol Salt agar respectively and incubated for 24 h at 37 °C. A single colony was then cultured in 5 ml Nutrient Broth for 4 h at 37 °C. The density of bacteria culture required for the test was adjusted to 0.5 McFarland standard, (1.0 x 108 CFU/ml) measured using the Turbidometer (Oxoid, UK).

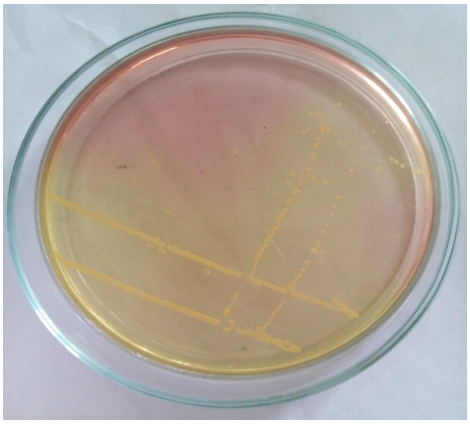

Fig. 2: *Staphylococcus aureus*

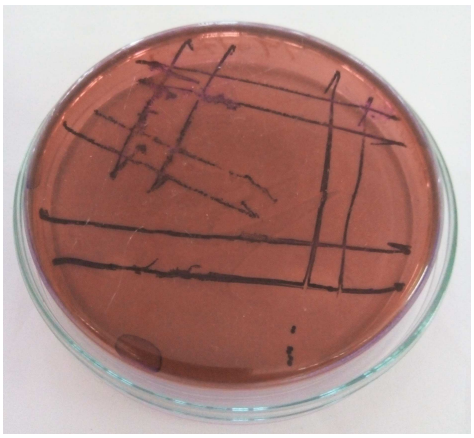

Fig. 3: *Escherichia coli*

Screening for antibacterial activity

Antibacterial activity was tested by agar well diffusion method where different concentrations of selected plant extract were used. The organisms were planted in sterile Petri plates using medium by softly mixing of 0.1 ml of the 24 h fresh cultures alone with 35 ml sterile liquid agar. 7 mm diameter wells were made using sterile borer after harding the agar of Petri plates. All wells were filled with 0.1 ml of extract and then incubated at 37 °C for 24 h. The diameter of the inhibition zone around of each well was measured to detect the antibacterial activity. The experiment was done in triplicate and mean diameter of inhibition zones were recorded.

Data analysis

The data were analyzed as the means and the standard deviation of the means (means±SE) with analysis of variance (ANOVA) and means were separated using Duncan grouping in a statistical system (SAS-2007) software of computer program.

RESULTS AND DISCUSSION

Evaluation of the antibacterial activity of *Curcuma longa* (Turmeric)

The antibacterial effect of *Curcuma longa* (Turmeric) on *E. coli* and *Staphylococcus aureus* has been described in table 2. We used three different doses for each bacteria to measure the inhibition zone in the media. From the table we found that at 75 µl level of ethanol extract of turmeric showed the highest inhibition zone in case of both bacteria. That it had been reduced in using the extract at 100 µl where the inhibition zone was 10.75 mm and 9.5 mm respectively for E. coli and Staphylococcus aureus. There was no significant difference (p>.05) in antibacterial activity of turmeric. At 50 µl turmeric showed 9.00 mm inhibition zone in the culture of *E. coli* wherein *Staphylococcus aureus* it was 8.5 mm. The antimicrobial activity of *C. longa* has been reported in previous study. It was found that the C. longa has inhibitory effect against *Pseudomonous aeruginosa* [15, 16], *Aeromonas hydrophila*, *Listeria monocytogenes* and *Salmonella typhimuriaum* DT104 and methicillin-resistant *Staphylococcus aureus* [17]. In this study, the results showed antibacterial activity of *C. longa* ethanol extract against *E. coli* and *Staphylococcus aureus*. The result from this study may supported the antimicrobial activity and somehow the confirmation of antimicrobial activity of *C. longa*. Moreover, it may support the use of C. longa for antimicrobial treatment disease or prevention of bacteria growth.

Table 2: Evaluation of antibacterial activity of *Curcuma longa* (Turmeric)

Concentration plant extract	*E. coli*	*Staphylococcus aureus*
	Zone of inhibition (mean±SD) mm	
50 µl	9.00[b]±1.4142	8.5[a]±5.8023
75 µl	11.00[a]±2.1602	10[a]±1.1547
100 µl	10.75[c]±2.63	9.5[b]±6.6081
P-Value (At p<.05)	.389459	.915656

Evaluation of the antibacterial activity of *Cinnamomum zeylanicum (*Cinnamon)

Table 3 illustrated the effect of cinnamon on two different bacteria named *E. coli* and Staphylococcus aureus. It is clear from the table that cinnamon worked mostly on *Staphylococcus aureus* where there gradual increase through the rising the dose of the extract starting 14.5 mm inhibition zone at 50 µl that reached to 21.5 mm inhibition zone at 100 µl. On the other hand, *E. coli* also showed sensitivity to cinamon though it is comparatively lower than *Staphylococcus aureus*. The inhibition zone, in that case, is 12.25, 13.5 and 15.75 respectively. There found significance difference (p<.05) in both bacteria.

The findings of this work were observed to be steady with the work done by [18] who indicated distinctive concentration of basic oil of cinnamon against *Staphylococcus aureus*. Moreover, [19] who discovered that cinnamon oil was powerful against E. coli (gram negative) and in his experiment proved that the fundamental oil of cinnamon produce inhibition zone against *Staphylococcus aureus*. These discoveries are additionally very comparable with the result of [20] revealing that cinnamon bark oil completely suppresses the development of some gram-positive and gram-negative microscopic organisms, growths [21]. As the agent, cinnamaldehyde, has ended up being especially powerful against a few types of gram-positive and gram-negative microbes [22-24]. It has been suggested that cinnamaldehyde and eugenol restrain generation of a basic compound by bacteria or potentially cause harm to the cell mass of microscopic organisms [25]. Along these lines, the high antibacterial activity of cinnamon oil is because of the presence of a high amount of cinnamaldehyde.

Table 3: Evaluation of the antibacterial activity of *Cinnamomum zeylanicum (*Cinnamon)

Concentration plant extract	*E. coli*	*Staphylococcus aureus*
	Zone of Inhibition (mean±SD) mm	
50 µl	12.25[a]±1.5	14.5[a]±2.3805
75 µl	13.5[a]±1.291	18.25[c]±2.7538
100 µl	15.75[a]±1.2583	21.5[b]±3.1091
P-Value (At p<.05)	.015475	.01847

Evaluation of antibacterial activity of *Syzygium aromaticum* (Clove)

Table 4 provide us the information about the antibacterial sensitivity of *E. coli* and *Staphylococcus aureus* against clove. The table showed that the highest sensitivity found at 100 µl dose against *Staphylococcus aureus* whilst the least in the case of E. coli in case of 50 µl of ethanol extract. Comparatively *Staphylococcus aureus* is more sensitive to clove than *E. coli* to clove. There were significant differences (p<.05) in the case of both bacteria.

This finding is in agreement with those of other workers [27-28]. They reported that clove extracts showed activities in the range (concentrations) from 20 to 250 µg/ml. The present data showed the effect of using clove oil both *E. coli* and *Staphylococcus aureus* bacteria. This data are agreed with some studies [29-32] and reported that a synergistic effect was observed for *P. aeruginosa*, which is resistant to 19 different antibiotics. This occurred during the association of antibiotics with extracts from clove, jambolan, pomegranate and thyme. Bisset (1994) reported that this effect was also observed for *K. pneumoniae* when 20 µg/ml of clove extract was combined to ampicillin. Also, the study [30] reported that the growth of *Proteus* spp. was inhibited when either clove extract (10 µg/ml) or eugenol (5 µg/ml) was combined to tetracycline.

Table 4: Evaluation of the antibacterial activity of *Syzygium aromaticum* (Clove)

Concentration plant extract	*E. coli*	*Staphylococcus aureus*
	Zone of inhibition (mean±SD) mm	
50 µl	9.75[a]±1.7078	10.5[b]±1.7078
75 µl	10.75[a]±1.5	13.25[c]±1.2583
100 µl	13.5[c]±2.0817	15.00[a]±1.8257
P-Value (At p<.05)	.022848	.01847

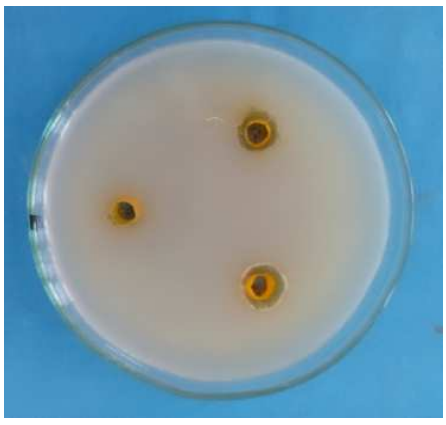

Fig. 4: Inhibition zone of *Curcuma longa*

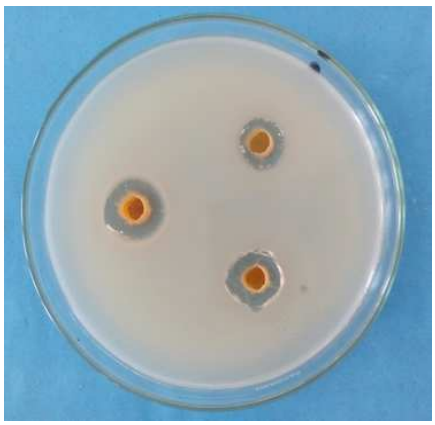

Fig. 5: Inhibition zone of *Cinnamomum zeylanicum*

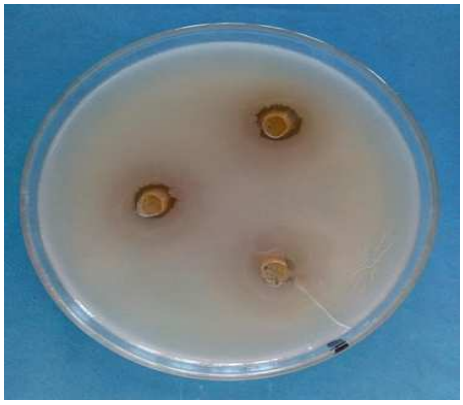

Fig. 6: Inhibition zone of *Syzygiumaromaticum*

CONCLUSION

Inhibition zone of the ethanol extract of these plants was calculated where three were found to be sensitive against *Syaphylococcus aureus* and *E coli*. However, the ethanol extract of *Cinnamomum zeylanicum* was found to have more effective antimicrobial activity showing its maximum efficacy for both bacteria. Data from the literature, as well as our results, reveal the great potential of plant oils as antibacterial agent, in spite of the fact that they have not been completely investigated, more studies need to be conducted. Therefore, our results revealed the importance of plant oils when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

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