Viability of Herbal Treatments for Bacterial Wound Infections

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ABSTRACT

Objectives: This study was conducted to investigate whether the commonly used kitchen spices can be used as a potential source of antimicrobial agents or not.

Methods: The three spice extracts (aqueous, methanolic and ethanolic) of cinnamon, black pepper, turmeric, ginger and clove were prepared for their antimicrobial properties against common bacteria. Antimicrobial susceptibility testing was done by Well Diffusion Method on Muller Hinton Agar (MHA). Then the plates were incubated at 37°C for 24 hours. The zone of inhibition for each spice was measured. For Minimum Inhibitory Concentration (MIC), the crude extracts of ethanolic extract of clove were considered as having 100% concentration of antimicrobials. The extract was then diluted in Mueller Hinton broth serially. Standard Suspension of test organism was kept in each dilutions and incubated at 37°C for 24 hours. The highest dilution of extract showing inhibition of growth was considered as MIC.

Results: In comparison to all extract, the methanolic extract of ginger showed the largest zone of inhibition (3.6 cm) against *Escherichia coli*. Similarly, ethanolic extract of clove was the most effective against *E. coli* with the zone of inhibition (3 cm). In aqueous extraction, clove showed higher antimicrobial activity against *Staphylococcus aureus* (1.9 cm) and cinnamon was effective against *Klebsiella spp.* (1.9 cm). Among all the extracts, aqueous extract generally exhibited lower antimicrobial activity. The MIC of ethanolic extract of clove against *S. aureus*, *Bacillus spp., Klebsiella spp*. and *E. coli* found to be the dilutions 1: 0.78, 1: 0.048, 1:0.39 and 1:1.56, respectively.

Conclusion: This study concludes that clove and ginger extracts could be potential source of antimicrobials that could be used for treatment of bacterial wound infections.

Keywords: Spices, extracts, minimum inhibitory concentration, antimicrobial agents, Nepal.

INTRODUCTION

Wound refers to the damage of living tissue by the directly or indirectly interfering of pathogenic microorganisms rather than normal flora of skin or tissue which can cause acute or chronic infection (Hurlow & Bowler, 2022) Cure of acute wound infection depend on many factors like nature of injury, whereas chronic wound infection takes more time because of immune compromised, aging of disease, associated peripheral neuropathy, medication etc. (Fan et al. 2022). For the treatment of acute infection intravenous and orally both medications process is applied (Bassetti et al. 2019). Likewise, for the treatment of chronic wound ointment, antimicrobial creams are in used, these medications also used for the removal of bacteria and fungi that cause infection on subcutaneous tissue (Jamaledin et al. 2020). Electricity, heat, flame and radiation are one of the major reasons to cause burn infection (Norman et al. 2017), and also reason of causing more death all over about 180000 per year (Wang et al. 2018).

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Various microbes are found on the acute infection such pathogens are *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and many other microorganisms (Reid et al. 2011). From thousands of year herbal medicine are in used to treat the wound infection as an alternative medicine through their bioactive compound (Enioutina et al. 2017). Nowadays modern science is doing research on finding the properties of spices (Chaudhry & Tariq, 2006). Many microbes contaminated food causes illness from stomach pain to more severe symptoms. Natural bioactive component on spices have ability to control the pathogens and to protect the foods from contamination also used as food addictive (Chandarana et al. 2009).

All the spices contain many bioactive compounds which makes them as antimicrobial, preservative, addictive, antioxidants etc. (Shaikh et al. 2018). Clove has major component as eugenol, which have ability to effect amylase and protease production in Bacillus cereus, which can destroy the cell wall by cell lysis (Burt, 2004). Cinnamaldehyde present on cinnamon inhibits fungi and mycotoxins produced by some fungus (Dorri et al. 2018).

Similarly, ginger consists of phytonutrients which is able to kill the cancer cell present on the ovarian cell through apoptosis and auto phagocytosis (Rhode et al. 2007). Also, it is used to digest and act as anti-diarrheal, constipation etc. (Stewart et al. 1991). Black pepper has ability to control the androgen dependent as well as independent prostate cancer cell because of its Phytochemical properties (Samykutty et al. 2013). Turmeric contains more no. of bioactive components among them, curcuminoid and sesquiterpenoid plays significant role to shows threptic properties. Whereas, curcuminoid also helps to fight against HIV and cancerous cell as well as show antioxidants and anti-inflammatory property (Aggarwal & Sung, 2009).

Due to those Phytochemical components, all spices show antimicrobial properties against certain bacterial strains which can be pathogens. So this study was done to assess capacity of these spices to kill the bacterial pathogens that can cause would infections. Study findings could help to determine the possibility of using these household spices as a source of potential antibacterial drugs.

METHODS

The study was conducted using powdered spices that was locally collected from Ason Market located at Kathmandu. The study was conducted between January to April 2024. The study was conducted in Microbiology Laboratory of Tri-Chandra Multiple Campus, Kathmandu, Nepal. The extract of spices was obtained from three different extractions i.e. methanolic, ethanolic and aqueous extraction. For the preparation of aqueous extract, 30gm of powdered form of selected five spices were suspended in 300ml of sterile distilled water in 5 different round bottom flasks. It was then incubated at room temperature with continuous shaking at 37°C for three days. After 3 days, it was then filtered using Whattman's No. 1 filter paper to obtained filtrate. The filtrate containing excess water was then removed by boiling at low flame. After some minutes, the crude extract was finally formed (Pandey et al. 2014). This crude extract was considered as 100% extract. Similar technique was applied to prepare both methanolic and ethanolic extracts of spices. The desired filtrate was then kept in different test tubes with covering its mouth in the refrigerator until the tests were done. The antimicrobial activities of spices were evaluated by using well diffusion method and procedure. Mueller Hinton Agar (MHA) was prepared using manufacturer's instructions. The isolated colonies of bacteria incubated in nutrient broth such as *Bacillus* spp., *Klebsiella* spp., *S. aureus* and *E. coli* were uniformly swabbed in MHA plate. Uniform wells of around 4 mm were cut using sterile cork borer. 20µl of the extracts were placed in wells using micropipette. The plates were then allowed to diffuse for 45 minutes. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition for each spices were measured. For determination of Minimum Inhibitory Concentration (MIC), the crude extracts of ethanolic extract of clove were considered as having 100% concentration of antimicrobials. The extract was then diluted in Mueller Hinton broth serially. Standard Suspension of test organism was kept in each dilutions and incubated at 37°C for 24 hours. The highest dilution of the extract showing inhibition of growth was considered as Minimum Inhibitory Concentration.

RESULTS

Among the extracts tested, ethanolic extract of clove showed highest activity against *E. coli* with zone of inhibition measuring 3.0 cm. The lowest activity was shown by aqueous extract of clove against *Bacillus* spp. with ZOI measuring 0.6 cm (Table 1).

As showin in Table 2, ethanol extract of cinnamon displayed greater ZOI against both *Bacillus* spp. and S. *aureus* measuring 2.6 cm. Cinnamon's aqueous extract showed the least activity against *Bacillus* spp. at 1.0 cm.

The methanolic extract of ginger showed the highest ZOI (3.6 cm) against *E. coli* and aqueous extract of ginger showed the lowest ZOI against three bacteria i.e. *Bacillus spp., E. coli* and *Klebsiella spp.* at 0.6 cm (Table 3).

Turmeric's ethanol extract displayed greater ZOI against gram positive bacteria (*S. aureus)*. On the other hand, aqueous extract of turmeric showed lower ZOI at 0.6 cm against both *E. coli* and *Klebsiella spp.* (Table 4).

As shown in Table 5, ethanolic extract of black pepper showed maximum ZOI at 2.3 cm against *E. coli* while the minimum ZOI was shown by aqueous extract against both *Bacillus spp*. and *E. coli* at 0.6 cm.

The MIC values of selected bacteria include *S. aureus*, *Bacillus spp*., *E. coli* and *Klebsiella spp*. were determined as: 1:0.78, 1:0.048, 1:1.56 and 1:0.39, respectivey (Table 6).

Table 1: Antimicrobial activity of cloves extract against different bacterial pathogens

Extract	Zone of Inhibition in cm			
	Bacillus spp.	***E. coli***	***Klebsiella spp.***	***S. aureus***
Aqueous	1.0	1.6	1.9	1.8
Ethanol	2.6	2.1	1.2	2.6
Methanol	1.6	2.2	1.6	1.9

Table 2: Antimicrobial activity of different extraction of cinnamon against test bacterial strains

Extract	Zone of Inhibition in cm			
	Bacillus spp.	***E. coli***	***Klebsiella spp.***	***S. aureus***
Aqueous	1.0	1.6	1.9	1.8
Ethanol	2.6	2.1	1.2	2.6
Methanol	1.6	2.2	1.6	1.9

Table 3: Antimicrobial activity of different extraction of ginger against test bacterial strains

Extract	Zone of inhibition in cm			
	Bacillus spp.	***E. coli***	***Klebsiella spp.***	***S. aureus***
Aqueous	0.6	0.6	0.6	0.8
Ethanol	2.3	1.7	0.8	0.8
Methanol	1.7	3.6	1.6	2.5

Table 4: Antimicrobial activity of different extraction of turmeric against test bacterial strains

Extract	Zone of inhibition in cm			
	Bacillus spp.	***E. coli***	***Klebsiella spp.***	***S. aureus***
Aqueous	0.7	0.6	0.6	1.0
Ethanol	1.5	2.2	1.6	3.1
Methanol	1.7	1.7	0.8	1.8

Table 5: Antimicrobial activity of different extraction of black pepper against test bacterial strains

Extract	*Zone of inhibition in cm*			
	Bacillus spp.	***E. coli***	***Klebsiella spp.***	***S. aureus***
Aqueous	0.6	0.6	1.6	0.6
Ethanol	1.6	2.3	2.1	0.7
Methanol	1.0	1.3	0.9	1.7

Table 6: Determination of MIC of ethanolic extract of clove against test bacterial strains

Bacteria	**Dilution in percentage**
S. aureus	1:0.78
Bacillus spp..	1:0.048
Klebsiella spp..	1:0.39
E. coli	1:1.56

DISCUSSION

The study found that ethanolic extract of clove exhibits notable antimicrobial activity against *E. coli* with the zone of inhibition at 3 cm and least significant against *Klebsiella* spp. Likewise, in methanolic extract of clove the largest zone of inhibition was observed in *Klebsiella* spp. (1.9 cm) which shows the good antimicrobial activity and least at *Bacillus* spp. Furthermore, cloves aqueous extract results larger zone of inhibition at 1.9 cm opposed to *S. aureus* indicating some effectiveness and lower activity in *Bacillus* spp. This result supports the observation obtained by (Rawat & Rawat, 2015), which showed the ethanolic extract of clove has highest potential against Gram negative bacteria *E. coli* with the maximum ZOI (25.0± 0.81 mm). Similarly, the result from the study by (Pandey et al. 2014), the maximum ZOI was found to be 11 mm against *K. pneumoniae* in the methanolic extract of clove.

The present study shows, the ethanol extract of cinnamon highlights the strong potential as antimicrobial agent by displaying highest activity against both *S. aureus* as well as *Bacillus spp.* at 2.6 cm ZOI where least activity against *Klebsiella* spp. Likely the cinnamon's methanol extract displayed largest zone of inhibition against *E. coli* at 2.2 cm and least activity against both *Klebsiella* spp. and *Bacillus* spp. with 1.6 cm ZOI. And finally, the aqueous extract of cinnamon showed good potential against *Klebsiella* spp. with a ZOI of 1.9 cm and low potential against Bacillus spp. at 1cm zone of inhibition.

This result supports the study conducted from Maharjan et al (2011), the crude extract of cinnamon was effective against *S. aureus* with zone of inhibition 10 mm and 15 mm at 50 and 100 µl, respectively. Similarly, this result supports the study conducted by (Pandey et al. 2014), in which the extract of cinnamon showed largest zone of inhibition 10, 12, 13 and 14 mm at 100, 150, 200 and 250 µl/ml concentration respectively against *S. aureus*. Similar finding was observed from the study conducted by (Gupta et al. 2008), cinnamon extract was effective against *S. aureus* with a ZOI 16mm which shows the good potential of cinnamon against Gram positive bacteria *S. aureus*. The result obtained from the study of (Patel et al. 2022) matches our result as the extract of cinnamon was sensitive at different level of concentrations against bacteria involving *S. aureus, B. cereus*, *Listeria monocytogenes* and *E. coli.*

From this study, it was found that methanolic extract of ginger showed the largest zone of inhibition (3.6) against gram negative bacteria E. *coli.* Whereas the least activity was seen against *Klebsiella spp*. In case of ethanolic extract of ginger, it was most effective against *Bacillus* spp. with a zone of inhibition (2.3cm) whereas least activity was seen against *Klebsiella* and *S. aureus.* On the other hand, ginger's aqueous extract exhibits activity against *S. aureus* with zone of inhibition below 1cm (0.8cm) which result limited effectiveness with all bacteria.

Similarly, in the study conducted by (Rahman et al. 2020), ethanolic extract of ginger showed the sensitivity against *S. aureus* with the zone of inhibition 8,13 and 19 mm at 25,50 and 100 µg/ml. However, the study concluded by (Shrestha, 2022) recognized that crude extract of ginger was effective against *S. aureus* with highest zone of inhibition (11.67 mm). Similar finding was observed from the study by (Sawant et al. 2021) the crude extract of ginger results the largest zone of inhibition 43 and 44 mm for 50 and 100%DMSO concentration respectively against gram negative bacteria *E. coli.*

In the study, it was found that the ethanolic extract of turmeric showed the largest zone of inhibition against *Klebsiella* spp. at 2.1 cm and least against *S. aureus* at 0.7 cm. Likewise turmeric's methanol extract exhibits limited effectiveness comparing to other spices, with highest ZOI at 17 cm against *S. aureus* and least effective against *Klebsiella* spp. with 0.9 cm zone of inhibition. And the aqueous extract of turmeric showed minimal antimicrobial activity with largest zone of inhibition at 1.6 cm against *Klebsiella* spp.

According to the study conducted by (Selvam et al. 2012), turmeric showed the good inhibitory activity against *E. coli* with a ZOI ranging from 7-15 mm. Similar findings was observed from the study by (Sylveste et al. 2015) showed that extract of Java turmeric is highly effective against all resistance *K. pneumoniae* with a ZOI ranging from 8.67-10 mm. A similar study done by (Nwinee et al. 2022) reveals that extract of turmeric is effective against *S. aureus* with zone of inhibition 15, 24 and 30 mm at 100, 200 and 300 mg/ml respectively. A study that is conducted by (Niamsa & Sittiwet, 2009) had shown inhibitory activity against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *K. pneumoniae* ATCC and *S. epidermidis*
ATCC.

From this study, it displayed significant antimicrobial activity with a largest ZOI 3.1 cm particularly against *S. aureus* whereas least significant against *Bacillus spp*. with 1.5 cm ZOI which highlights the potential of black pepper as strong antimicrobial agent. In addition, methanol extract of black pepper showed the largest ZOI against *S. aureus* at 1.8cm and least against *Klebsiella* spp. (0.8cm). And aqueous extract showed low antimicrobial activity compared to other extract with highest ZOI against *S. aureus* at 1 cm.

Similar, result was obtained from the study by (Pundir & Jain, 2010), aqueous extract of black pepper showed good antimicrobial activity against *S. aureus* with the ZOI ranging from 25 mm to 30 mm. Moreover, this result somehow matched with the result observed by (Karsha & Lakshmi, 2010) in which black pepper showed largest ZOI against *S. aureus* at 20 mm indicating to the highest potential as antimicrobial agent. Similar, finding was observed by the study (Rani et al. 2013), result with largest ZOI (18 mm) against Gram positive bacteria *S. aureus* and minimum against Gram negative bacteria *E. coli.*

In this study MIC of ethanolic extract of cloves were determined against bacteria such as *S. aureus*, *Bacillus spp*., *Klebsiella* spp. and *E. coli*. The MIC indicates the minimum amount of cloves ethanolic extract need to hinder the growth of disease causing bacteria. In comparison to all selected bacterial pathogens, *Bacillus spp*. was found to be sensitive in highest dilution 1:0.048. According to previous study (Singh & Pandey, 2011), the MIC of clove's extract was found to be 0.385 mg/ml against *S. aureus* and 2.31 mg/ml against *E. coli.* Furthermore, the study conducted by (Liu et al. 2021) the MIC value of ethanolic extract of clove was found to be 64 µg/ml for both *E. coli* and *K. pneumoniae*.

Conclusion

This study concludes that the extracts from clove and ginger have viability to be used as source of therapeutic agents to treat bacterial wound infections.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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