Himalayan Journal of Science and Technology (2017)

ISSN No: 2565-5132 (Print)

Original Research Article

Anti-Bacterial Effect of *Zanthoxylum armatum* Oleoresin Against Some Bacterial Isolates from Pork and Chicken Meat Sold in Dharan, Nepal

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Abstract

Zanthoxylum armatum is a medicinal plant found in the Himalayan range. The present study was carried out to unravel the anti-microbial activities of *Z. armatum* oleoresin against bacteria isolated from raw pork and chicken meat sold in Dharan sub-metropolitan city. Five bacterial species *Salmonella enterica* var Typhi, *Escherichia coli, Shigella dysenteriae, Bacillus cereus and Staphylococcus aureus* were isolated. The antimicrobial activity of oleoresin extracted from *Z. armatum* was tested by agar well diffusion method and MICs were compared with standard antibiotics against isolated bacteria. The MIC values of oleoresin were ranged from 25-75 µl/mL. Zone of inhibition for oleoresin extracted with acetone was 10 mm (25 µl/mL) against *Shigella dysenteriae* and *Staphylococcus aureus,* and 9.5 mm (25 µl/mL) against *Escherichia coli* respectively. Zone of inhibition of oleoresin extracted with chloroform was 12 mm (25 µl/mL) and 11 mm (25 µl/mL) against *Escherichia coli* and *Staphylococcus aureus* respectively, that of oleoresin extracted with cyclohexane was 10 mm (25 µl/mL and 50 µl/mL) against all the isolates, except *Bacillus cereus*. Zone of inhibition of oleoresin extracted with methanol was 10 mm (25 µl/mL) and 9.5 cm (25 µl/mL) against *Shigella dysenteriae* and *Bacillus cereus* and *Escherichia coli* respectively. Zone of inhibition of oleoresin extracted with petroleum ether was 10 mm (25 µl/mL and (50 µl/mL) against *Escherichia coli* and all the isolates except *Escherichia coli*. Increasing oleoresin concentrations showed greater antimicrobial effect on the isolates. *Bacillus cereus* was most affected; comparatively, *Salmonella enterica* var Typhi was least affected by all the antibiotics.

Key words: *Zanthoxylum armatum,* oleoresins, antibiotics, MIC

Introduction

Nepal is rich in diverse types of medicinal plants. Spices are parts of plants that are used for providing aroma, flavor or piquancy to foods and for seasoning the food (Oli, 2011). The spices contain essential oils and other chemical substances which give them fragrant, aromatic, pungent, acrid, bitter or other properties of aroma and taste (Parry, 1969). The essential oil is the fraction in spices which can be separated by steam or water distillation and is the major component contributing flavor but not the taste (Oli, 2011).

Zanthoxylum armatum is commonly known as *Timur* in Nepali and Nepal pepper or Prickly ash in English. The *timur* has fragrant, aromatic and pungent characteristic with biting and burning taste. In Nepal, ethnic people use *timur* widely. Berry of *timur* is used for the treatment of cold and in chutney preparation (ready to eat sauce) in Dumi Rai society (Rai, 2004). The seed powder can be taken orally with warm water to treat constipation, stomach pain, toothache and cold (Joshi and Edington, 1990; Manandhar, 1987). Traditionally, leaves and fruits are used as mouth freshener and in tooth care, while bark is used as ischiotoxic or piscicidal (Gaur, 1999).

Many spices possess significant antimicrobial activity; Gram positive bacteria are more sensitive than Gram negative bacteria, with the lactic acid bacteria being the most resistant among Gram positives (Jay, 1996). Increasing bacterial resistance is prompting resurgence in research on the antimicrobial role of herbs against resistant strains (Hemaiswarya et al, 2008; Alviano and Alviano, 2009).

Meats contain an abundance of all nutrients required for the growth of bacteria, yeasts and molds and adequate quantity of these constituents exist in fresh meats in an available form (Jay, 1996). Fresh meats such as beef, pork and lamb, as well as poultry, seafood and processed meats have high pH values. During bleeding, skinning and cutting, the main sources of micro-organisms are the hide, hooves, hair and intestinal tract (Frazier et al, 2009).

ISSN No: 2565-5132 (Print)

Knives, cloths, air, hands and clothing of the workers can serve as intermediate sources of contaminants. Contamination can come from carts, containers, other contaminated meat and personnel (Frazier et al., 2009). Bacteria of many genera are found in meat, among which some of the more important are *Pseudomonas, Acinetobacter, Moraxella, Alcaligenes, Micrococcus, Streptococcus, Sarcina, Leuconostoc, Lactobacillus, Proteus, Flavobacterium, Bacillus, Clostridium, Escherichia, Campylobacter, Salmonella* and *Streptomyces* (Frazier et al., 2009). Most of these isolates cause surface slime in meat. Hence this study was designed to study the antibacterial property of *Zanthoxyum armatum* against different bacterial species isolated from raw meat.

Materials and Methods

The berries of *Z. armatum* were collected on September 2014 AD from upper region of Ward no 8 of Yangsila V.D.C. Similarly, 250 g of meat sample were collected in sterile polythene bags from 10 different sites of Dharan sub-metropolitan city. The samples were analyzed immediately within 2 h of collection at microbiology laboratory of Central Campus of Technology, Hattisar, Dharan.

Powdered *Timur* samples were kept in thimbles and extracted in soxhlet apparatus using different solvents, viz., acetone, chloroform, hexane, methanol, and petroleum ether. After extraction, the solvent was evaporated and the weight of oleoresin was taken (Oli, 2011).

All the isolates were isolated from raw pork and chicken meats being sold at the different locations of Dharan sub-metropolitan city by spread plate technique (Shah et al., 2013)).The isolates were sub-cultured on to their selective media for the maintenance of pure cultures (Fraser et al., 1996). After culturing, all the isolates were subjected to colony characterization (Fraser et al.,1996; Ananthanarayan & Paniker, 2000), Gram staining and bio-chemical tests such as gelatin liquefaction test, IMViC test, catalase test, oxidase test and urease test were performed (Shah et al., 2013). After identifying microbes, standard culture inoculums were prepared as per Srivastava et al., (2013).

For the assay of antibacterial activity, 3 wells were made aseptically at least 15mm from the edge of MHA media plate with a sterile cork borer. The agar plates were aseptically inoculated with test organisms. Different volumes of oleoresins (25, 50, 75, 100, 125 and 150 µl) and 50 µl of 1000 µg/mL standard antibiotics (viz; Ampicillin, Chloramphenicol, Streptomycin and Tetracycline) were poured separately into the wells using a sterile micropipette. After subsequent incubation for 24 h at 37°C, the minimum inhibitory concentrations (MICs) for all the isolates were obtained by Agar well diffusion method and compared. All the experiments were conducted in triplicate and statistically analyzed.

Results and Discussion

It revealed that the demand of meat in Dharan sub-metropolitan city is fulfilled by pork (50%), poultry meat (25%), male buffalo meat (20%) and mutton (5%). Fifty per cent of butchers cleaned their weapons with soap and water daily, 25% used only plain water, 20% used a piece of cloth and 5 % used stone to clean weapon by rubbing. Seventy per cent of them kept the leftover meat in refrigerator and in deep freezer, while the rest kept at room temperature.

Very interestingly, 70% of butchers disposed the solid waste by transporting in the city waste van, 20% dumped in the nearest jungle or drainage, 5 % used as feed for pig and 5% had their own dumping site for disposal. Thirty per cent of the butchers received training about meat quality. Ninety per cent of them did not have well managed slaughter house. Irrespective of the slaughter house and meat inspection Act 2055 and Food Act 2023 implemented in 1966, slaughtering, production and marketing is in awful condition which is not as per the guidelines. This may be the possibility of contamination of meat and transfer of many meat borne diseases to human being.

The maximum and minmum zone of inhibition and MIC values for oleoresin extracted with acetone was 10 mm (25 µl/mL) against *Shigella dysenteriae* and *Staphylococcus aureus,* and 9.5 mm (25 µl/mL) against *Escherichia coli* respectively. Oleoresin extracted with chloroform was 12 mm (25 µl/mL) and 11 mm (25 µl/mL) against *Escherichia coli* and *Staphylococcus aureus* respectively. Oleoresin extracted with cyclohexane was 10 mm (25 µl/mL and 50 µl/mL) against all the isolates, except *Bacillus cereus*.

Oleoresin extracted with methanol was 10 mm (25 µl/mL) and 9.5 cm (25 µl/mL) against *Shigella dysenteriae* and *Bacillus cereus* and *Escherichia coli* respectively. Oleoresin extracted with petroleum ether was 10 mm (25 µl/mL and (50 µl/mL) against *Escherichia coli* and all the isolates except *Escherichia coli*. Similarly, max and min zone of inhibition for Ampicillin sodium salt was 29 mm and 19 mm against *Bacillus cereus* and *Salmonella enterica* var Typhi respectively. Chloramphenicol showed 37 mm and 30 mm against *Shigella dysenteriae* and *Salmonella enterica* var Typhi respectively.

Streptomycin sulphate showed 33 mm and 18 mm against *Bacillus cereus* and *Shigella dysenteriae* respectively. Tetracycline hydrochloride showed 32 mm and 30.5 mm against *Bacillus cereus* and *Staphylococcus aureus* and *Shigella dysenteriae* respectively. Generally, increasing oleoresin concentrations showed greater antimicrobial effect on the isolates.

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Table 1: Effect of Oleoresin extracted with Acetone.

Acetone extract	Test organisms (Zone of inhibition)				
	Salmonella enterica var Typhi (mm)	*Shigella dysenteriae* (mm)	*Escherichia coli* (mm)	*Bacillus cereus* (mm)	*Staphylococcus aureus* (mm)
25 µl	0	10	9.5	0	10
50 µl	0	10.5	9.5	10	10
75 µl	10	12	10.5	11.5	10.5
100 µl	13	15	11	12	14
125 µl	13.5	16	11.5	12.5	15
150 µl	14	16.5	12	13	17

Table 2: Effect of Oleoresin extracted with chloroform

Chloroform extract	Test organisms (Zone of inhibition)				
	Salmonella enterica var Typhi (mm)	*Shigella dysenteriae* (mm)	*Escherichia coli* (mm)	*Bacillus cereus* (mm)	*Staphylococcus aureus* (mm)
25 µl	0	0	12	0	11
50 µl	11.5	0	13	0	12
75 µl	12	10	14	10	12.5
100 µl	13	11.5	21	11	14
125 µl	14	13.5	25	14	15.5
150 µl	16	14	28	16	16

Table 3: Effect of Oleoresin extracted with cyclohexane

Cyclohexane extract	Test organisms (Zone of inhibition)				
	Salmonella enterica var Typhi (mm)	*Shigella dysenteriae* (mm)	*Escherichia coli* (mm)	*Bacillus cereus* (mm)	*Staphylococcus aureus* (mm)
25 µl	0	0	10	10	0
50 µl	10	10	11	11.5	10
75 µl	11.5	11	12	12	12
100 µl	14.5	12	12.5	13.5	13
125 µl	15.5	12	13	15	13.5
150 µl	16.5	12.5	13.5	16	14

Table 4: Effect of Oleoresin extracted with Methanol

Methanol extract	Test organisms (Zone of inhibition)				
	Salmonella enterica var Typhi (mm)	*Shigella dysenteriae* (mm)	*Escherichia coli* (mm)	*Bacillus cereus* (mm)	*Staphylococcus aureus* (mm)
25 µl	0	10	9.5	10	0
50 µl	11	10	10	11	10
75 µl	11.5	10.5	11.5	12.5	11.5
100 µl	12	12	12	13	12
125 µl	12.5	16	13.5	13.5	13
150 µl	13	18	14	14	14

Table 5: Effect of Oleoresin extracted with petroleum ether

Petroleum ether extract	Test organisms (Zone of inhibition)				
	Salmonella enterica var Typhi (mm)	*Shigella dysenteriae* (mm)	*Escherichia coli* (mm)	*Bacillus cereus* (mm)	*Staphylococcus aureus* (mm)
25 µl	0	0	10	0	0
50 µl	10	10	11	10	10
75 µl	11	10.5	11	10.5	10.5
100 µl	12	11	12	10.5	12
125 µl	12.5	11.5	15	11	13
150 µl	13.5	13	16.5	12	14

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Table 6: Effect of Standard Antibiotics

Antibiotics	*Test organisms*				
	Salmonella enterica var Typhi (mm)	*Shigella dysenteriae* (mm)	*Escherichia coli* (mm)	*Bacillus cereus* (mm)	*Staphylococcus aureus* (mm)
Ampicillin sodium salt	19	35	26	29	21
Chloramphenicol	30	37	32	33	33
Streptomycin sulphate	22	18	22	33	28
Tetracycline hydrochloride	31	30.5	31	32	32

When compared between the effect of oleoresins and standard antibiotics; *Salmonella enteric* var Typhi was relatively sensitive to Chloroform extract and Tetracycline. *Shigella dysenteriae* was sensitive to oleoresin extracted with acetone and methanol and sensitive to Chloramphenicol. *Escherichia coli* was sensitive to oleoresin extracted with chloroform and Chloramphenicol. *Bacillus cereus* was sensitive to oleoresin extracted with cyclohexane and methanol and sensitive to Chloramphenicol and Streptomycin sulphate; *Staphylococcus aureus* was sensitive to oleoresin extracted with chloroform and most sensitive to Chloramphenicol and Tetracycline. As stated by Hemaiswarya *et al*, 2008; Alviano and Alviano, 2009, the bacterial resistance against the drug has been a traumatic condition; led the investigation about the antimicrobial role of *Timur* oleoresin against bacterial strains.

Conclusions

The poor sanitation and hygienic conditions of meat shops are the primary reasons for meat contamination and transfer of diseases to human being, animals and birds by the isolates. Among the different oleoresins tested against 5 different isolates, oleoresin extracted with acetone and chloroform was found to the most effective and could be used as medicine and preservatives as well as in intervention of new drugs in future. The effect of *Z. armatum* oleoresin has shown the anti-microbial activities against the isolates. Hence, *Z. armatum* extracts might be an alternative to the antibiotics. It is also justified that the researches done by the various researchers about its use as medicines and preservatives by ethnic people of Nepal and other countries was significant. Therefore, the berries of *Z. armatum* now using only in household level by ethnic groups of Nepal can be materialized for the production of antibacterial agents. Likewise, the oleoresin extracts of *Z. armatum* can be used as medicine and preservatives and in intervention of new drug can be done in future.

Acknowledgements

First and foremost, the authors would like to express deepest sincere gratitude and thanks to Dean's Office, Institute of Science & Technology, Tribhuvan University for providing the research fund for this project and special thanks to Central Campus of Technology, Dharan for providing laboratories. Heartfelt thanks to Ain Bahadur Karki, Laxmi Prasad Shrestha and Mahesh Koirala for helping by giving every supports in the laboratories of Central Campus of Technology, T.U.

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