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Antibacterial Activity of Moringa oleifera Seed and Tea Leaves Extracts Prepared in Chloroform against Shigella strains Isolated from Ostrich Feces

Masoom Majid[1], Uzma Rafi[1*], Roheela Yasmeen[1], Syeda Shazia Bokhari[1] and Hafiz Shehzad Muzammil[2]

[1]Deparmtent of Zoology, Lahore Garrison University, Lahore, Pakistan
[2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

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***Corresponding Author:**
Uzma Rafi
Deparmtent of Zoology, Lahore Garrison University, Lahore, Pakistan
uzmazeeshan@lgu.edu.pk

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ABSTRACT

Shigella is the most common cause of the endemic form of shigellosis. The presence of bacteria such as *Shigella* is major threat to ostrich industry. **Objective:** To look for the presence of *Shigella* in Ostrich feces. **Methods:** The feces were collected from captive ostriches at the W.A Apparel factory. *Shigella* were isolated after the samples were inoculated on SS agar. The antimicrobial activity of *Moringa oleifera* seeds and tea leaves was investigated. Antimicrobial activity against *Shigella* isolated from ostrich feces was tested. **Results:** It was noticed that tea extract lacked antimicrobial activity against tested species. *Moringa oleifera* seeds, on the other hand, were effective against *Shigella*. **Conclusion**: *Moringa oleifera* seeds have been found to show inhibitive effect and are effective against *Shigella*.

INTRODUCTION

Salmonella, a bacterium that enters polluted areas by contact with rodents or wild bird reservoirs, is the cause of the bacterial illness salmonellosis. Salmonellosis is caused in animals older than 6 months, including cows, goats, and ostrich [1]. Salmonella serotypes *S. pullorum*, *S. gallinarum*, and *S. typhimurium* are often found in ostriches [2]. The birds may become prone to salmonellae infections due to inadequate shelter and nutrition [3]. Ostriches, particularly chicks, are sometimes severely injured by improperly built enclosures. Following an injury during loading, ostriches may potentially be exposed to microorganisms, especially if the loading ramp was poorly built. In abattoirs, strict cleanliness procedures make it exceedingly unlikely that carcasses may be contaminated [4-6]. *Shigella dysenteriae* and *Shigella boydii* are physiologically comparable to *Shigella flexneri* which is the rod-shaped bacteria. Shigellosis, an acute case of bloody diarrhea, is caused by it, making it significant. The most frequent cause of the endemic type of shigellosis is *Shigella flexneri (S. flexneri)*. It is a serious public health issue in underdeveloped nations. Shiga identified Shigella as the causative agent of bacillary dysentery in the 1890s [7]. In a study performed by Jin *et al.*, *Shigella flexneri* 2a strain 301 was isolated and sequenced. This bacterium was first discovered in a shigellosis patient in China in 1984. *Shigella* infection is a serious public health issue in underdeveloped nations with inadequate

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sanitation. Although other primates may get the disease, humans are more at risk. A broad range of foods may be infected even if no naturally occurring food items contain endogenous *Shigella* species. Shigellosis is transmitted orally via faeces. Other methods of transmission include contact with a contaminated inanimate item, some forms of sexual contact, and ingesting infected food or water (untreated wading pools, interactive water fountains). By physically transferring contaminated faeces, vectors like houseflies may transmit the illness. One reason is that pathogenic *Shigella* can resist gastric juice's low pH. For at least two hours, most *Shigella* isolates can withstand acidic treatment at pH 2.5. The incubation time, which ranges from 12 hours to 7 days but normally lasts 2-4 days, is inversely proportional to the number of germs that were consumed. It may take up to 4 weeks from the time of sickness until an infected individual excretes the organism in their faeces, at which point the condition is contagious. Within 4 weeks of the start of a disease, bacterial shedding normally stops; in rare occasions, it may continue for months. Carriage can be reduced by only a few days with antibiotic therapy [8]. As germs develop resistance to the antibiotics there is a need to switch to natural products. *Moringa oleifera* has antibacterial characteristics and its roots, flowers, bark, and stems, as well as seeds, have been studied for its medicinal benefits [9, 10]. The therapeutic and nutritional benefits of *Moringa oleifera* are astounding. A profile of significant minerals can be found in various portions of this plant, which is also a strong source of protein, vitamins, carotene, amino acids, and other phenolics [11]. Calcium, copper, iron, potassium, magnesium, manganese, and zinc are among the essential elements found in *Moringa oleifera*. In addition to acting as cardiac and circulatory stimulants, the plant's many parts—including the leaves, roots, seeds, fruit, blossoms, and immature pods—also have antitumor, antipyretic, antiepileptic, anti-inflammatory, and antiulcer properties [12, 13]. Numerous studies provide scientific support for the widespread use of plants against infectious disorders [14]. They may also be a source of novel, affordable medications to which pathogenic strains are not resistant. A natural coagulant, *Moringa oleifera* seed powder clarifies very murky water [15].

METHODS

For preparation of SS media, 100ml distilled water taken through the measuring flask and 6.302 g SS agar with help of measuring balance in a conical flask. Then the media left it for heating at hot plate for 30-40 minutes. The prevalence percent rate of Shigella is 50% as 5 samples out 10 samples were found positive for the Shigella. In sterile polythene plastic bags, fecal samples were collected from the surface layer (0-15 cm). The fecal samples were collected from the W.A Apparel factory in Youhanabad, Lahore, Pakistan, where the ostriches were kept in captivity. The samples were collected in the early morning hours. At the time of collection, the temperature, precipitation, humidity, and wind were all monitored. To isolate the bacteria, the fecal samples were brought to the lab. Using distilled water, 10g of fecal sample was serially diluted to a concentration of 10-6 while suspended in 90ml of sterile, distilled water. 50 ml of samples from test tubes labelled 10-2 and 10-4 were pipetted out using a micro-pipette following dilutions. Using a micro-pipette, 50 ml of the samples were inoculated onto freshly made petri plates of EMB Agar and SS Agar. For 48 to 72 hours, these Plates were incubated at 37°C. There were numerous bacterial colonies found. The chosen bacterial colony, however, was picked and streaked using the streaking technique. Once more, these Plates were incubated for 48–72 hours at 37°C to watch their growth. Tea leaves and Moringa oleifera seeds were obtained from the Agriculture Department of Punjab University in Lahore, Pakistan. Shigella spp. were used as organisms. Morphological identification of bacteria isolated from feces on SS media. Shigella spp. were identified morphologically after observing the pinkish colonies. Using the disc diffusion method, the antibacterial properties of the tea and seed extracts were identified. The petri plates were filled with LB agar, swabbed with chosen bacterial strains, and then had discs placed in the appropriate sections. By measuring the diameter of the zone of inhibition, the antibacterial activity of the plates was evaluated after 18 hours of incubation at 37°C. Comparing the zones of inhibition of the various extracts allowed researchers to assess their antibacterial potential. From pharmaceuticals we obtain antibiotic powders (amoxicillin and erythromycin). To make the stock solution, a known weight of antibiotic powder was dissolved in sterile distilled water. To obtain the working solution, the stock solution was diluted during disc preparation. A 6mm diameter paper disc can absorb 0.02 ml or 20 ml of solution. Antibiotic solution concentrations were expressed in ug/ml. The sample, antibiotic, and control discs were gently placed on the previously marked zones of the agar plates pre-inoculated with test bacteria. The plates were then placed in an upside-down refrigerator at 40 °C for about 24 hours to allow the materials from the discs to diffuse into the surrounding agar medium. The plates were then inverted and placed in a 37°C incubator for 24 hours.

RESULTS

Antimicrobial activity of Moringa oleifera seed with chloroform extract using disc diffusion method was checked against Shigella. The Moringa oleifera seed

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extract was applied against isolated strains such as Shigella spp. of Ostrich. The erythromycin and amoxicillin were used as a control. No antimicrobial activity of Moringa oleifera tea against Shigella spp. was recorded. Erythromycin was showing zone of inhibition 14 mm. The Moringa oleifera tea extract was applied against isolated strains such as Shigella spp. of Ostrich. No antimicrobial activity of Moringa oleifera tea against Shigella spp. was recorded. Amoxicillin showed inhibitory zone 12 mm against Shigella as shown in table 1 and figure 1.

Tested bacteria	Diameter of Disc	Inhibition zone measurement	Inhibition zone measurement erythromycin	Inhibition zone measurement amoxicillin
***Moringa oleifera* seed**				
Shigella	7 mm	7 mm	14 mm	12 mm
***Moringa oleifera* tea**				
Shigella	7 mm	No zone	14 mm	12 mm

Table 1: Antibacterial activity of Moringa oleifera seed and tea (chloroform extract) against Shigella using disc diffusion method

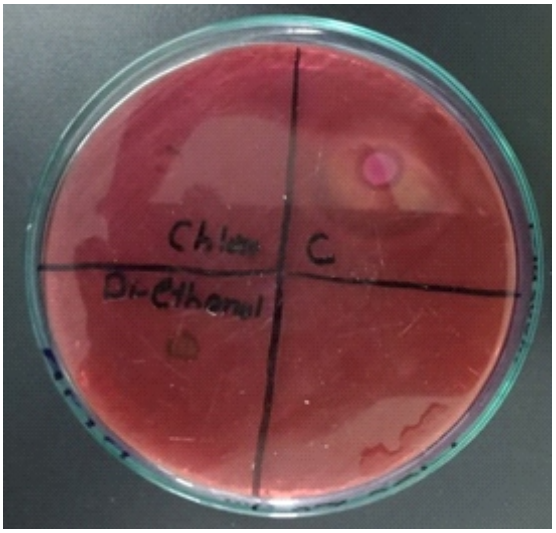

Figure 1: Petri plate showing disc diffusion and antimicrobial activity of Moringa oleifera seed and leaves tea with chloroform extract against Shigella.

DISCUSSION

There are millions of infections reported each year from the endemic disease shigellosis. The disease's rapid spread may be explained by the bacterium's low infectious dose, direct person-to-person transmission, tainted food and water transmission, and low susceptibility to stomach acids [16]. The purpose of this study was to test the antimicrobial activity of Moringa oleifera tea and seed against Shigella isolated from ostrich feces. The fecal samples were collected from the W.E Apparel factory in Lahore, Pakistan, near Youhanabad. The feces were diluted and placed on SS Agar. After obtaining bacterial growth, the isolated colonies were streaked on SS agar. Moringa oleifera seed extract with chloroform was used against pathogens Shigella. The controls used were amoxicillin and erythromycin. Both controls were successful in showing the inhibitory zone of 12 mm thus limiting the growth of Shigella as shown in the tables above. Moringa oleifera seed chloroform was demonstrated by Bukar et al. to be active on S. aureus, Enterobacter spp., and E. coli (09 mm) at concentrations of 50–200 mg/ml. Shigella spp., S. aureus, P. aeruginosa, and S. Typhi were insensitive to all of the tested concentrations. The seed extract demonstrated an inhibitory zone against the Shigella (7 mm) in Bukar et al., findings, which were in accordance with one of our studies [17]. In 2011, Lar et al., studied the antibacterial efficacy of Moringa oleifera aqueous and ethanolic extracts against various gram-negative bacteria (Escherichia coli, Shigella flexneri and Salmonella typhi). Between 50 mg/ml and 400 mg/ml of extract were utilized. According to the inhibition zones created by the extract, Shigella lailexneri and Escherichia coli were both inhibited by the ethanolic extract at 400 mg/ml, 200 mg/ml, and 100 mg/ml. Both the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) for the two species were 100 mg/ml. According to the findings of the Lar et al., research, Moringa oleifera seeds are effective against the diarrheal agent Shigella flexneri, and their range of use as a water purifier and water treatment agent has been expanded. This suggests that Moringa oleifera seeds could be helpful in treating certain gastro intestinal illnesses and wound infections brought on by gram-negative bacteria. The MIC for Moringa oleifera to suppress the action of pathogens was reported to be 100 mg/ml by Lar et al. The use of too little extract may have been a contributing factor in the inability to generate an inhibitory zone [18]. Nikon et al., observed that In vitro antibacterial activity against Shigella boydii, Shigella dysenteriae, and Staphylococcus aureus was present in a chemical isolated from ethanol extract rather than crude chloroform extract [19]. According to Delelegn et al., seed powder and extract may prevent and control bacterial infections [20].

CONCLUSIONS

It is concluded that Moringa oleifera seeds are capable of showing inhibitory activity and can control pathogens like Shigella. So, if Moringa seeds are fed to the Ostriches in their diet, the prevalence risk of Shigella can be reduced.

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