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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of *Moringa oleifera* Seeds and Tea Leaves Extracts with Ethanol against *Shigella* Strains isolated from Ostrich Feces

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ABSTRACT

Shigella, the cause of bacillary dysentery, is a major public health concern in developing countries. The present study was performed to determine the antimicrobial activity of *Moringa oleifera* ethanolic extracts against *Shigella* isolated from feces of Ostrich. The fecal samples were collected from the ostrich kept in captivity at W.A Apparel factory. The samples were inoculated on SS agar and *Shigella* were isolated. *Moringa oleifera* seeds and tea leaves (di ethanolic extract) were evaluated for their antimicrobial activity. The antimicrobial activity was tested against *Shigella* isolated from feces of Ostrich. It was noticed that that tea extract did not show any antimicrobial activity against *Shigella* species. However, *Moringa oleifera* seeds prepared in ethanolic extract were effective against *Shigella*. It is concluded that *Moringa oleifera* seeds (ethanolic extract) have the potential to work against infection caused by *Shigella.*

INTRODUCTION

Severe infections that spread to the bloodstream, brain meningeal linings, or other deep tissue are also possible. For the treatment of invasive infections, it is essential. As antibiotic resistance has grown, finding effective antibiotics has grown more challenging. Antibiotic overuse, however ought to be avoided as it might result in the emergence of bacterial strains that are resistant to the drugs. Ostrich carcasses have also been found to contain *Salmonella* and *E. coli* (Akbarmehr, 2010). Samples of ostrich carcasses from eight Ohio and one Indiana slaughterhouse were processed by a researcher. *E. coli* was discovered in 91% (116/128) of the dressed carcasses tested. *Salmonella* was detected in 1 out of 152 carcass samples (1/152) (Bancessi *et al., 2020*). Both heat stable enterotoxin (ST) and heat labile enterotoxin (LT) tests on ostriches were negative (Behnamifar *et al*., 2020). One of the most common foodborne pathogens that infect both humans and animals is the *Salmonella* serovar (Bennett *et al.*, 2003). A rod-shaped bacterium called *Shigella flexneri* shares physiological characteristics with *Shigella dysenteriae* and *Shigella boydii*. Because it causes shigellosis, a severe case of bloody diarrhea, it is significant. The endemic form of shigellosis is most frequently caused by *Shigella flexneri*. In developing nations, *Shigella flexneri* (*S. flexneri* 2a) is a serious public health issue. In the 1890s, Shiga identified *Shigella* as the causative agent of bacillary dysentery (Broin *et al*., 2002; Bukar *et al.*, 2002). Additionally, they may be a source of new affordable antibiotics to which pathogenic strains are not resistant and numerous studies offer scientific justification for the widespread use of plants to treat infectious diseases (Chuang *et al.*, 2007). The *Moringa oleifera* seed powder acts as a natural coagulant to clarify extremely turbid water (Dhakad *et al.*, 2019). *In vitro* anti-fungal activities against dermatophytes like *Trichophyton rub rum, Trichophyton mentagrophytes, Epidermophyton floccosum,* and *Microsporum canis* have been reported, indicating that extracts could be used for the development of an anti skin disease agent in the future (Eilert *et al.*, 1981). Studies on crude Diethanol extract of dried *Moringa oleifera* seeds have suggested that it has anti-tumor promoting. Evaluating a plant's antimicrobial activity against food-borne microorganisms is a crucial step in screening it for sanitizing/preservative activity (Fahey, 2005; Guevara *et al.*, 1999). It has been determined that the seeds of *Moringa oleifera* contain the active antimicrobial compound 4(a-L-Rhamnosyloxy)benzyl isothiocyanate. Origins of M. In addition to this substance and benzyl isothiocyanate, oleifera do not also contain pterygospermin as was previously believed. Both species' defatted and shell free seeds contain 8–10% 4 (u-L-rhamnosyloxy)benzyl isothiocyanate, but this quantity is made from M. only when ascorbic acid is added while the oil is being extracted with water.

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The substance affects various bacteria and fungi. For *Mycobacterium phlei* and *Bacillus subtilis*, the minimal bactericidal concentration *in vitro* is 40 tmol/1 and 56 tmol/l, respectively (Gülhan *et al*., 2009; Jabeen *et al*., 2008; Jin *et al*., 2002).

METHODOLOGY

100 ml of distilled water through the measuring flask and 6.302 g of *Salmonella Shigella* Agar (SS agar) with the aid of a measuring balance are used to prepare the SS media. The media then left it on a hot plate to heat for 30 to 40 minutes. The surface layer (0–15 cm) was used to collect the fecal samples, which were then placed in sterile polythene bags. The fecal samples were obtained from the W. A. Apparel factory, which is a short distance from the ostriches' captivity in Youhanabad Lahore, Pakistan. The samples were taken in the early hours of the day. At the time of collection the weather conditions of the temperature, rain, humidity and wind by using thermometer, rain guage, hygrometer and anemometer were observed. To isolate the bacteria, the fecal samples were brought to the lab. Following a shake, 10 g of fecal sample was serially diluted to a concentration of 10(-6) with sterile distilled water. 50 µl samples from 10^{-2} and 10^{-4} test tubes were pipetted out using a micro-pipette from dilutions. Using a micro pipette, 50 µl of the samples were inoculated onto freshly prepared petri plates of SS Agar. For 48 to 72 hours, these Plates were placed in incubator and 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 gathered from the Punjab University in Lahore, Pakistan's Agriculture Department. Morphological identification of the fecal isolated strains of bacteria on the SS media was used to identify the organisms, which were *Shigella* spp. The pinkish colonies were seen, and *Shigella* spp. were recognized morphologically. 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. The growth of microorganisms will be inhibited if the test material exhibits any anti-microbial activity, creating a clear, defined zone known as the zone of inhibition. The diameter of the zone of inhibition in terms of millimeters is used to calculate the test agent's antibacterial activity. The experiments are run three times, and the mean reading is recorded each time. With the aid of sterile forceps, sample antibiotic discs (amoxicillin and erythromycin discs) were gently placed on the freshly seeded test organism filled agar plates that had solidified. The discs were placed so that they could not be more than 15 mm from the plate's edge and were spaced far enough apart to prevent the zones of inhibition from overlapping. Finally, the plates were incubated for 12 to 18 hours upside down at 37°C.

RESULTS

The *Moringa oleifera* seed extract was applied against isolated strains such as *Shigella spp.* of Ostrich. The Erythromycin and amoxicillin were used as a control. The antimicrobial activity of *Moringa oleifera* seed against *Shigella spp.* was recorded. Erythromycin showed inhibitory zone 14 mm.

The *Moringa oleifera* seed extracts (di ethanol) show zone of inhibition 5 mm against *Shigella* species.

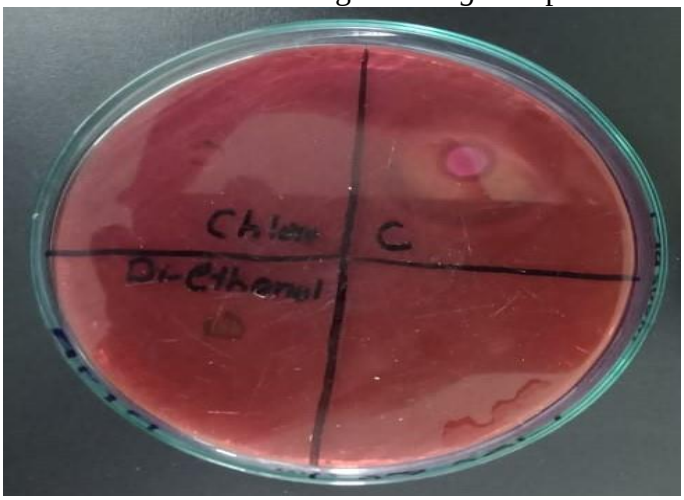

Figure 1: Petri plate showing disc diffusion and antimicrobial activity of *Moringa oleifera* seed with di ethanol extract against *Shigella.*

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Table 1: Antimicrobial activity of *Moringa oleifera* seed and tea (di ethanol extract) using disc diffusion method against *Shigella* species.

Tested bacteria	Diameter of Disc	Inhibition zone measurement	Inhibition zone measurement amoxicillin	Inhibition zone measurement erythromycin
***Moringa oleifera* seed (di ethanol extracts)**				
Shigella species	7 mm	5 mm	12 mm	14 mm
***Moringa oleifera* tea (di ethanol extracts)**				
Shigella species	7 mm	No zone	12 mm	14 mm

The *Moringa oleifera* tea (di ethanol extract) was applied against isolated strains of *Shigella spp.* from Ostrich.

No antimicrobial activity of *Moringa oleifera* tea against *Shigella spp.* was recorded. Erythromycin showed inhibitory zone 14 mm while amoxicillin showed activity of 12 mm against *Shigella spp* as shown in table 1 and figure 1.

DISCUSSION

Bacterial contamination can spread from breeders to eggs vertically or horizontally. The eggs may become infected through the vertical route if bacteria are allowed to colonize the ovaries or oviducts. Despite the fact that in our study the type of isolated microbes in the mature female's cloaca and the content of infertile eggs were not comparable, the risk of microbial contamination spreading from the female birds reproductive system to eggs is very high (Khesorn, 2009). This study aimed at testing the antimicrobial activity of *Moringa oleifera* tea and seed against the *Shigella* isolated from Ostrich feces. The fecal samples were diluted and poured on SS Agar. Then bacterial growth was obtained, and the isolated colonies were streaked on SS agar. *Moringa oleifera* seed extract with ethanol was used against *Shigella*. The controls used were amoxicillin and erythromycin. Both controls were successful in showing the inhibitory zone of 12 mm and 14 mm thus limiting the growth of *Shigella*. The *Moringa oleifera* seed in ethanol extracts showed 5 mm zone of inhibition against *Shigella*. *Moringa oleifera* in vitro antibacterial activity using ethanolic extracts only significantly inhibited *Shigella* (Lar *et al.,* 2011). Additionally, the 95% ethanol extraction method yielded the most effective concentration of moringa extract 30 mg/ml. The results point to a useful method for using moringa, highlighting its significance as an eco-friendly alternative for water treatment in areas without a water supply system (Ley *et al.,* 2001). In this study ethanol was used against *Shigella*. The controls used were amoxicillin and erythromycin. Both controls were successful in showing the inhibitory zone of 12 mm and 14 mm thus limiting the growth of *Shigella* as shown in the tables above. Lar *et al*., 2011 reported that Di-ethanolic extract of *Moringa oleifera* seeds at 400mg/ml, 200 mg/ml and 100 mg/ml were inhibitory to *Shigella* were successful inhabit their growth. Although, our results were contrary to (Lar *et al*., 2011) findings but our results for *Moringa oleifera* tea leaves extract in Di-ethanol did not show any inhibitory zone with *Shigella.* Lar's extract shows inhibitory zone at 100-400 mg/ml (Mahbub *et al.,* 2011). This concludes that high concentration of extract works very well. Our extract concentration was just 5 mg/ml. The research conducted by Vaghasiya and Chanda, 2007 showed that *M. oleifera* crude Di-ethanol extracts had no or low activity against *Shigella. Our* results were showing similarity with this research conducted by Vaghasiya and Chanda, 2007 (Nato *et al.,* 2007). (Oluduro *et al.,* 2011) used Di-ethanolic extract of the *Moringa oleifera* seeds and found appreciable inhibitory effect against bacterial isolate. They reported that the extract had broad spectrum of activity. But our research was somehow parallel to work as our seed extract also showed inhibition against *Shigella* (Oluduro *et al*., 2011). (Khesorn *et al*., 2006 and Jabeen *et al.,* 2008) both reported that Di-ethanolic and purified dichloromethane extracts of the seeds had antibacterial action against gram negative organisms (*Shigella)* (Roy *et al.,* 2006; Vaghasiya

and Chanda, 2007). This report was also parallel to our work as our seed extract was also effective against *Shigella.*

CONCLUSIONS

It is concluded that *Moringa oleifera* seeds have inhibitory activity and can control pathogens such as *Shigella*. Therefore, the risk of *Shigella* prevalence can be decreased by feeding *Moringa* seeds to ostriches.

REFERENCES

Akbarmehr J. Isolation of Salmonella spp. from poultry (ostrich, pigeon, and chicken) and detection of their hilA gene by PCR method. African journal of microbiology research. 2010 Dec; 4(24):2678-81.

Bancessi A, Pinto MM, Duarte E, Catarino L, Nazareth T. The antimicrobial properties of *Moringa oleifera* Lam. for water treatment: a systematic review. SN Applied Sciences. 2020 Mar; 2(3):1-9. doi: 10.1007/s42452-020-2142-4

Behnamifar A, Rahimi S, Akhavizadegan MA, Karimi Torshizi MA, Maleki A. Isolation and Identification of Microorganisms in Eggs of a Commercial Ostrich Breeder Farm. J Anim Sci Res. 2020;4(3).

Bennett RN, Mellon FA, Foidl N, Pratt JH, Dupont MS, Perkins L, *et al*. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L.(horseradish tree) and Moringa stenopetala L. Journal of agricultural and food chemistry. 2003 Jun; 51(12):3546-53. doi: 10.1021/jf0211480

Broin M, Santaella C, Cuine S, Kokou K, Peltier G, Joet T. Flocculent activity of a recombinant protein from *Moringa oleifera* Lam. seeds. Applied microbiology and biotechnology. 2002 Oct; 60(1):114-9. doi: 10.1007/s00253-002-1106-5

Bukar A, Uba A, Oyeyi TI. Phytochemical analysis and antimicrobial activity of Parkia biglobosa (Jacq.) Benth. extracts againt some food--borne microrganisms. Advances in Environmental Biology. 2010 Jan:74-80.

Chuang PH, Lee CW, Chou JY, Murugan M, Shieh BJ, Chen HM. Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. Bioresource technology. 2007 Jan; 98(1):232-6. doi: 10.1016/j.biortech.2005.11.003

Dhakad AK, Ikram M, Sharma S, Khan S, Pandey VV, Singh A. Biological, nutritional, and therapeutic significance of *Moringa oleifera* Lam. Phytotherapy Research. 2019 Nov;33(11):2870-903. doi: 10.1002/ptr.6475.

Eilert U, Wolters B, Nahrstedt A. The antibiotic principle of seeds of *Moringa oleifera* and Moringa stenopetala. Planta medica. 1981 May;42(05):55-61.

Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom state of Nigeria. Journal of Ethnopharmacology. 2008 Feb; 115(3):387-408. doi: 10.1016/j.jep.2007.10.021

Fahey JW. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for life Journal. 2005 Dec; 1(5):1-5.

Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, *et al*. An antitumor promoter from *Moringa oleifera* Lam. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 1999 Apr; 440(2):181-8. doi: 10.1016/S1383-5718(99)00025-X

Gülhan T, Ilhan Z, Aksakal A, Solmaz H. Escherichia coli Strains Isolated from Healthy Animals. Journal of Animal and Veterinary Advances. 2009; 8(10):2058-62.

Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pak J Bot. 2008 Aug; 40(4):1349-58.

Jin Q, Yuan Z, Xu J, Wang Y, Shen Y, Lu W, Wang J, *et al*. Genome sequence of *Shigella* flexneri 2a: insights into pathogenicity through comparison with genomes of Escherichia coli K12 and O157. Nucleic acids research. 2002 Oct; 30(20):4432-41. doi: 10.1093/nar/gkf566

Khesorn N. Antibacterial activity of the capsules of *Moringa oleifera* Lam.(Moringaceae). J. Ethnopharmacol. 2009;36:233-7.

Lar PM, Ojile EE, Dashe E, Oluoma JN. Antibacterial Activity on *Moringa oleifera* Seed Extracts on Some Gram Negative Bacterial Isolates.

Ley EC, Morishita TY, Brisker T, Harr BS. Prevalence of Salmonella, Campylobacter, and Escherichia coli on ostrich carcasses and the susceptibility of ostrich-origin E. coli isolates to various antibiotics. Avian Diseases. 2001 Jul:696-700.

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Mahbub KR, Hoq MM, Ahmed MM, Sarker A. In vitro antibacterial activity of Crescentia cujete and *Moringa oleifera*. Bangladesh Res Pub J. 2011; 5(4):337-43.

Nato F, Phalipon A, Nguyen LP, Diep TT, Sansonetti P, Germani Y. Dipstick for rapid diagnosis of *Shigella* flexneri 2a in stool. PLoS One. 2007 Apr; 2(4):e361. doi: 10.1371/journal.pone.0000361

Oluduro OA, Idowu TO, Aderiye BI, Famurewa O, Omoboye OO. Evaluation of antibacterial potential of crude extract of *Moringa oleifera* seed on orthopaedics wound isolates and characterization of phenylmethanamine and benzyl isothiocyanate derivatives. Research Journal of Medicinal Plant. 2012; 6(5):383-94. **doi :** 10.3923/rjmp.2012.383.394

Roy P, Purushothaman V, Koteeswaran A, Dhillon AS. Isolation, characterization, and antimicrobial drug resistance pattern of Escherichia coli isolated from Japanese quail and their environment. Journal of applied poultry research. 2006 Oct; 15(3):442-6. doi: 10.1093/japr/15.3.442

Vaghasiya Y, CHANDA S. Screening of methanol and acetone extracts of fourteen Indian medicinal plants for antimicrobial activity. Turkish Journal of Biology. 2007; 31(4):243-8.