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Antibacterial effect of Moringa oleifera Tea Leaves and Seeds Extracts Prepared in Chloroform against E. coli Isolated from Ostrich Feces

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ABSTRACT

Ostrich farming is an important growing industry in Pakistan. Its business and importance is growing day by day. However, prevalence of bacteria is major threat to ostrich industry. **Objective:** To identify the dominant bacteria in the feces of ostriches. **Methods:** The ostrich that was kept in captivity at the W.A. Apparel factory provided the fecal samples. The samples were inoculated on EMB for the isolation of *E. coli*. Antibacterial effect of *Moringa oleifera* seeds and tea leaves with the use of chloroform as a solvent. The antibacterial activity was tested against *E. coli* using disc diffusion method. Amoxicillin and erythromycin were used as a control antibiotics. **Results:** It was noticed that tea extract did not show any antimicrobial activity against *E. coli*. However, *Moringa oleifera* seeds were effective against *E. coli*. **Conclusion:** It was concluded that *Moringa oleifera* seeds have the potential to work against *E. coli*.

INTRODUCTION

Ostriches only reproduce during specific times of the year because they are seasonal breeders. Although the timing and length of breeding can vary with latitude and altitude, it typically lasts between six and eight months annually [1]. The domesticated ostrich matures at two to three years old, whereas the wild ostrich does not reach sexual maturity until it is four to five years old. The female reaches sexual maturity a little sooner than the male. When fully grown, male ostriches develop their black and white coloring. Grayish-brown feathers are much duller in colour on females and young birds [2]. The largest egg laid by a living bird is by an ostrich. It can weigh up to 1900 g, be up to 17 to 19 cm long, and 14 to 15 cm wide [3]. Ostrich farming is a thriving industry that has grown significantly over the past few decades and is practiced all over the world. The first ostrich farm was established in South Africa in 1838, and the first commercial ostrich farm was established there in 1863. Ostriches produce large, high-quality eggs. A single ostrich can produce 1 kg of live weight for 4 kg of balance feed and 1 kg of fodder. The best FCR of any land animal is found in ostriches [4]. Pakistan, which is located in the Northern Hemisphere, experiences temperatures between (25 and 37°C) for the majority of the year, especially in the summer [5]. Ostriches are the best animal for extreme climatic conditions because their temperature tolerance ranges from -7 to 50 centigrade. Therefore, Pakistan's environment is favorable for ostrich farming. In Pakistan, ostriches are raised for their meat, skin, and feathers. Ostriches consume lucerne feed, which is widely accessible in Pakistan. The Government of the Punjab

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declared the ostrich to be a domestic bird of commercial interest in 2012 [6]. In the gastrointestinal tracts of both humans and animals, Escherichia coli (E. coli) plays a significant role as a commensal. 10 to 15% of some E. coli isolates are pathogenic, the majority of isolates are considered to be signs of fecal contamination of food [7]. Ostrich feces also contain other pathogens. The umbilicus becomes infected with E. coli when it is not cleaned [8]. Within the first 10 days after birth, neonatal chicks exhibit signs like weakness and a quick demise. If an infection develops in the egg, the chicks will hatch very frailly. One of the pathological symptoms is an inflamed, reddened yolk sac in the abdomen, occasionally with strands of pus or milky pus. Bedding in the neonatal-chick house or hatchery E. coli bacillosis (E. coli infection) spreads orally and through the faeces, and cloacal swabs are used to identify the pathogen [9]. Colibacillosis is a bacterial infection caused by the common, Gram-negative enterobacterium E. coli, which may not be harmful. Among the pathological lesions in a group of young ostriches with a high mortality rate were colibacillosis and the isolation of E. coli. Colibacillosis and Chlamydia spp. were shown to be related [10]. A chick's immune system may be weakened by an underlying viral or fungus infection, nutritional excesses or deficiencies, or other conditions [8]. At necropsy, tiny, yellowish-white nodules are found all over the hepatic parenchyma. Older lesions might be more similar to cheese than early lesions, which contain milk. Moringa oleifera (Moringa oleiferaceae), a tropical tree with many economic uses and ease of propagation, is a native of the western and sub-Himalayan region, India, Pakistan, Asia Minor, Africa, and Arabia [11, 12]. For food (leaves, green pods, flowers, and roasted seeds), spice (primarily roots), cooking and cosmetic oil (seeds), and medicinal use (all plant organs), the Moringa oleifera tree is grown [13–15].

METHODS

For preparation of EMB media, taking 100 ml distilled water through the measuring cylinder and 3.6g EMB agar with help of measuring balance in a conical flask. Then the media was autoclaved at 121°C and 15 psi. 10 fecal samples of Ostrich were collected from the W. E Apparel factory. Out of 10 samples, 5 samples were positive for the presence of E. coli. So, the prevalence percent rate of E. coli is 50% as 5 samples out 10 samples were found positive for the E. coli. The fecal samples were collected from the surface layer (0-15 cm) in sterile polythene plastic bags. The fecal samples were transported to the laboratory to isolate the bacteria. 10g of fecal sample was suspended in 90 ml of sterile distilled water, shaken and serially diluted to 10-6 with distilled water. After dilutions, 50 µl samples from 10-2 and 10-4 test tubes were pipetted out with micro-pipette. The 50 µl of the samples were inoculated onto freshly prepared petri plates of EMB Agar and SS Agar with micro-pipette. 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 gathered from the Punjab University in Lahore, Pakistan's Agriculture Department. The sample (10 g) was added to the chloroform (50 ml in each case) to make the tea extract, which was then allowed to sit at room temperature for 10 days. The extracts were filtered through sterile Whatman filter paper and separated using sterile muslin cloth (no. 02). Using a stainless steel grinder, 10 grammes of Moringa oleifera seed were reduced to a fine powder before being steeped overnight in 50 ml of 100% chloroform. Using sterile muslin cloth and sterile Whatman filter paper, the di-ethanol fraction was separated (no. 02). By using a magnetic stirrer, the filtered extract was concentrated. The organisms used were Escherichia coli. Morphological identification of the fecal isolated strains bacteria on the EMB media. The isolated strains of bacteria on EMB green metallic sheen were seen and E. coli were also morphologically identified. As, for molecular identification purified petri plates were sent to Islamabad sequencing. The tea extract was created by mixing the sample (10 g) with the chloroform (50 ml in each case), which was then left to sit at room temperature for 10 days. The extracts were separated with sterile muslin cloth and filtered through sterile Whatman filter paper (no. 02). 10 gram of Moringa oleifera seed were ground to a fine powder using a stainless steel grinder before being steeped for an entire night in 50 ml of 100% chloroform. The di-ethanol fraction was separated using sterile Whatman filter paper and muslin cloth (no. 02). The concentrated filtered extract was stirred with a magnetic stirrer. The antibacterial activity of the tea and seed extracts were determined using disc diffusion method. LB agar was poured in the petri plates and was swabbed with selected bacterial strains and discs were applied in their respective 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. Pharmaceuticals were used to obtain antibiotic powders. To create the stock solution, a known weight of antibiotic powder was dissolved in sterile, distilled water. The working solution was created by diluting the stock solution during the preparation of the disc. A paper disc with a diameter of 6 mm can hold 20 µl or 0.02 ml of solution. In ug/l, antibiotic solution concentrations were expressed.

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Using sterile forceps to ensure complete contact with the medium surface, sample antibiotic discs (amoxicillin and erythromycin discs) were placed gently on the solidified agar plates that had just been seeded with the test organisms. 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 18 to 24 hours upside down at 37 °C. The ability of the test agents to inhibit the growth of microorganisms around the discs, which results in a distinct zone of inhibition, serves as a gauge of their antimicrobial potency. Using a transparent scale, the diameter of the zones of inhibition in millimeters was measured after incubation (24 hours) to assess the antimicrobial activities of the test materials.

RESULTS

The Moringa oleifera seed extract was applied against isolated strains such as E. coli spp. isolated from Ostrich. The amoxicillin and erythromycin was used as a control. The antimicrobial activity of Moringa oleifera seed against E. coli spp. was recorded. However, E. coli spp. showed inhibitory zone of 07 mm. The Amoxicillin showed inhibitory zone of 12 mm while erythromycin showed inhibitory zone of 14 mm. The Moringa oleifera tea extract was applied against isolated strains such as E. coli spp. of Ostrich. The amoxicillin was used as a control. No antimicrobial activity of Moringa oleifera tea against E. coli spp. was recorded with no inhibitory zone (Table 1; Figure 1 and 2).

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

Table 1: Antibacterial activity of Moringa oleifera seed and tea (chloroform extract) disc diffusion method against E. coli.

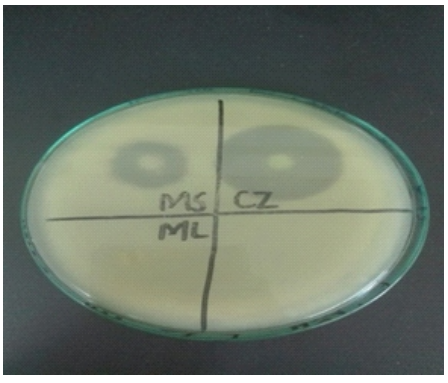

Figure 1: Petri plates showing disc diffusion and antimicrobial activity of Moringa oleifera seed with chloroform against E. coli.

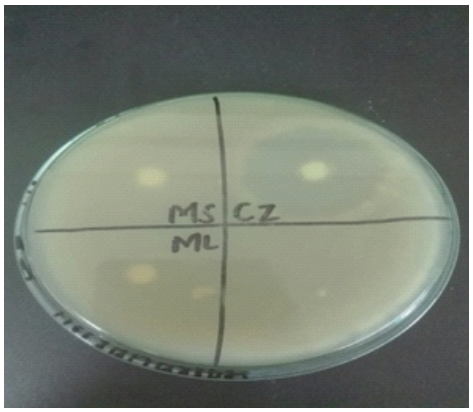

Figure 2: Petri plate showing disc diffusion and antimicrobial activity of Moringa oleifera tea with chloroform against E. coli.

DISCUSSION

aThis study aimed at testing the antimicrobial activity of Moringa oleifera tea and seed against the E. coli isolated from Ostrich feces. The fecal samples were collected from the W.E Apparel factory located near Youhanabad, Lahore Pakistan. The fecal samples were diluted and poured on EMB Agar. Then bacterial growth was obtained and the isolated colonies were streaked on EMB agar plates. Moringa oleifera tea extract with chloroform was used against E. coli. The controls used were Amoxicillin and Erythromycin were successful in showing the inhibitory zone of 12 mm, and 14 mm respectively thus limiting the growth of E. coli (table 1). The Moringa oleifera tea was not successful in limiting the growth of E. coli. According to Bukar et al., Moringa oleifera leaf chloroform was effective against S. typhimurium (10 mm) E. coli (08mm), and S. typhi (07mm) at 50–200 mg/ml concentration. As the extract showed no inhibition zone against the E.coli our research was in opposition to Bukar et al., [16]. Given the extract's low extract concentration, this might be feasible. At 50-200 mg/ml, Bukar's extract displayed an inhibitory zone. Only 5 mg/ml of extract were present in our sample. Moringa oleifera tea extract combined with chloroform demonstrated activity on E. coli, according to Arzai (2008). P. aeruginosa, E. coli, and S. aureus and S. typhi. Our research was in contrary to this (Arzai 2008) as our Moringa oleifera tea chloroform extract showed no result with the pathogens (E. coli) [17]. This may also be due to low concentration of the extract as we used 5g/ml of the extract. In comparison to M. ovalifolia seeds and bark powder extracted with the same solvent, M. oleifera seeds and bark powder has higher antibacterial activity, according to Shailemo et al., study. Testing was done on E. coli and other pathogens using methanol-based extracts of M. ovalifolia and M. oleifera seeds. According to Dorothea et al., the inhibition zone of M. oleifera seed extract (methanol) against E. coli had an inhibition zone of roughly 6

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mm. Our study's findings were somewhat similar to those of Shailemo et al., 2016 because our Moringa oleifera seed extract (chloroform) also displayed a 7 mm inhibition zone against E. coli. Shailemo et al., reported that seed extract (methanol) of M. ovalifolia also showed inhibitory zone of 6mm with E. coli [18]. Patel et al., reported that Moringa oleifera is high nutritional and medicinal value due to phenolic content [19]. According to Madsen et al., it can be used against enteric pathogen like E. coli [20].

CONCLUSIONS

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

REFERENCES

[1] Shanawany MM. The importance of light for ostriches. Ostrich Update. 1994; 3:52-4.

[2] Gandini GC and Keffen RH. Sex determination of the south-african ostrich (struthio-camelus). Journal of the South African Veterinary Association. 1985 Dec; 56(4):209-10.

[3] Jarvis MJ, Jarvis C, Keffen RH. Breeding seasons and laying patterns of the southern African ostrich Struthio camelus. Ibis. 1985 Oct; 127(4):442-9. doi: 10.1111/j.1474-919X.1985.tb04840.x

[4] Cooper RG and Horbañczuk JO. Anatomical and physiological characteristics of ostrich (Struthio camelus var. domesticus) meat determine its nutritional importance for man. Animal Science Journal. 2002 Jun; 73(3):167-73. doi: 10.1046/j.1344-3941.2002.00024.x

[5] Anjum FM and Walker CE. Grain, flour and bread-making properties of eight Pakistani hard white spring wheat cultivars grown at three different locations for 2 years. International Journal of Food Science and Technology. 2000 Aug; 35(4):407-16. doi: 10.1046/j.1365-2621.2000.00400.x

[6] Abbas G, Mahmood S, Sajid M, Ali Y. Ostrich Farming: A New Turn in Poultry Industry of Pakistan. Advances in Zoology and Botany. 2017; 5(3):33-8.

[7] Silva N, Igrejas G, Gonçalves A, Poeta P. Commensal gut bacteria: distribution of Enterococcus species and prevalence of Escherichia coli phylogenetic groups in animals and humans in Portugal. Annals of Microbiology. 2012 Jun; 62(2):449-59. doi: 10.1007/s13213-011-0308-4

[8] Foggin CM. Veterinary problems of ostriches. The Topaz introduction to practical ostrich farming. 1992.

[9] Tully TN and Shane SM. Husbandry practices as related to infectious and parasitic diseases of farmed ratites. International Office of Epizootics. 1996 Mar; 15(1):73-89. doi: 10.20506/rst.15.1.916

[10] Kolb J, Kankondi R, Hübschle OJ. Isolation of Chlamydia spp. from ostriches (Struthio camelus). DTW. Deutsche Tierarztliche Wochenschrift. 1993 Nov; 100(11):454-.

[11] Mughal MH, Ali G, Srivastava PS, Iqbal M. Improvement of drumstick (Moringa pterygosperma Gaertn.)–a unique source of food and medicine through tissue culture. Hamdard Med. 1999; 42(1):37-42.

[12] Somali MA, Bajneid MA, Al-Fhaimani SS. Chemical composition and characteristics ofMoringa peregrina seeds and seeds oil. Journal of the American Oil Chemists' Society. 1984 Jan; 61(1):85-6. doi: 10.1007/BF02672051

[13] Rebecca HS, Sharon M, Arbainsyah A, Lucienne D. Moringa oleifera: medicinal and socio-economic uses. International Course on Economic Botany. National Herbarium Leiden, Netherlands. 2006 Sep: 2-6.

[14] Stevens GC, Baiyeri KP, Akinnnagbe O. Ethno-medicinal and culinary uses of Moringa oleifera Lam. in Nigeria. Journal of Medicinal Plants Research. 2013; 7(13):799-804.

[15] Oliveira JT, Silveira SB, Vasconcelos IM, Cavada BS, Moreira RA. Compositional and nutritional attributes of seeds from the multiple purpose tree Moringa oleifera Lamarck. Journal of the Science of Food and Agriculture. 1999 May; 79(6):815-20. doi: 10.1002/(SICI)1097-0010(19990501)79:6<815::AID-JSFA290>3.0.CO;2-P

[16] 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.

[17] Arzai A H. Detection of β-lactamase producing microorganisms and their susceptibility to selected antibiotics and medicinal plant extracts. PhD thesis submitted to the Department of Biological Sciences, Bayero University, Kano. 2008.

[18] Shailemo DH, Kwaambwa HM, Kandawa-Schulz M, Msagati TA. Antibacterial activity of Moringa ovalifolia and Moringa oleifera methanol, N-hexane and water seeds and bark extracts against pathogens that are implicated in water borne diseases. Green and Sustainable Chemistry. 2016; 6(02):71. doi: 10.4236/gsc.2016.62006

[19] Patel S, Thakur AS, Chandy A, Manigauha A. Moringa oleifera: a review of there medicinal and economical importance to the health and nation. Drug invention today. 2010 Jul; 2(7):339-42.

[20] Madsen M, Schlundt J, El Fadil EO. Effect of water coagulation by seeds of Moringa oleifera on. Journal of tropical medicine and hygiene. 1987; 90:101-9.

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