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Antimicrobial Activity of *Moringa oleifera* Tea Leaves and Seeds Concentrated in Di Ethanol against *E. coli* Isolated from Ostrich Feces

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ABSTRACT

Ostriches are frequently infected with viral, fungal, and bacterial diseases. This disease does not require airborne transmission and does not involve the respiratory system. **Objective:** To determine the antimicrobial activity of *Moringa oleifera* tea leaves and seed against bacteria in Ostrich feces. **Methods:** Fecal samples were collected from captive ostriches at the W.A Apparel factory. *E. coli* was isolated after the samples were inoculated on EMB. The antimicrobial activity of *Moringa oleifera* seeds and tea leaves was investigated. The antimicrobial activity of Ostrich feces was tested against *E. coli*. **Results:** The results showed that tea extract had no antimicrobial activity against *E. coli*. *Moringa oleifera* seeds extract prepared in ethanol on the other hand, were effective against *E. coli*. **Conclusion:** *Moringa oleifera* seeds (di ethanolic extract) have the potential to be effective against *E. coli*.

INTRODUCTION

In Pakistan, ostrich farming is growing quickly as a result of a push to market the product and generate significant profits both domestically and abroad. One of the numerous viral, fungal, and bacterial diseases that affect ostriches is the bacterial disease known as Newcastle. This illness does not require airborne transmission and does not affect the respiratory system. The symptoms that have been noticed, like losing control of one's head, are neurological in nature. This illness doesn't have any macroscopic lesions [1]. *Escherichia coli* (*E. coli*) makes up a sizeable portion of the commensals in the gastrointestinal tracts of both humans and animals [2]. The majority of *E. coli* isolates are nonpathogenic and are believed to be indicators of fecal contamination of food, even though only 10 to 15% of isolates are pathogenic [3]. If the umbilicus is not cleaned, *E. coli* can infect it [4]. Neonatal chicks show symptoms like weakness and a quick demise within the first 10 days of birth. If the egg becomes infected, the chicks will hatch very weakly. An inflamed, reddened yolk sac in the abdomen, occasionally with strands of pus or milky pus, and bedding in the hatchery or neonatal-chick hut are pathological signs. *E. coli* (*E. coli* infection) is spread through the mouth and feces, and it is discovered using cloacal swabs [5]. The risk of *E. coli* infection in chicks is increased by the presence of an underlying viral or fungal infection, nutritional excess or deficiency, and a compromised immune system [4, 6]. *Moringa oleifera* seeds are well known for their coagulation properties for treating water and wastewater because they contain flocculent protein peptides [7, 8]. Antimicrobial properties have been found in *Moringa oleifera* seed extracts [9, 10]. Tropical tree *Moringa oleifera* is native to the western and

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sub-Himalayan region, India, Pakistan, Asia Minor, Africa, and Arabia [11, 12]. It has numerous economic uses and is simple to propagate. 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]. It is very nutritious and has many different medical uses. The various components of this plant are a good source of phenolics, vitamins, beta-carotene, amino acids, and protein. Additionally, they have a profile of important minerals [14]. The minerals calcium, copper, iron, potassium, magnesium, manganese, and zinc are all present in *Moringa oleifera*. The heart and circulatory system are stimulated as well as having anti-tumor properties by a number of plant parts, including the leaves, roots, seeds, bark, fruit, flowers, and immature pods [15]. The widespread use of plants to treat infectious diseases has been supported scientifically by numerous studies, and they may also be a source of novel, inexpensive antibiotics that are effective against pathogenic strains [16]. To clarify extremely murky water, *Moringa oleifera* seed powder works as a natural coagulant [17].

METHODS

The fecal samples were obtained in sterile polythene plastic bags from Youhanabad Lahore, Pakistan, where the ostriches were housed in captivity, and were taken from the top layer (0–15 cm). 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 were observed. 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 90 ml of sterile, distilled water. 50 ml samples were pipetted out using a micro-pipette from test tubes with a 10^{-2} and 10^{-4} after dilutions. Using a micro-pipette, 50 µl 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. Again, the growth of these plates was monitored during their 48–72-hour incubation at 37°C. Tea leaves and *Moringa oleifera* seeds were gathered from the Punjab University's agriculture department in Lahore, Pakistan. 50 ml of the solvent (Di-ethanol) and 10 grammes of *Moringa oleifera* tea were combined in a conical flask before the extract was allowed to sit for 8 days to dry. The extract was then further dried by being stirred for an hour on a magnetic stirrer. The extract was then filtered using Whatman filter paper No. 1 before being added to Eppendorf in a measured quantity to be used in subsequent steps. *Moringa oleifera* seeds (10 g) were ground into fine powder using a stainless-steel grinder and kept in 100% di-ethanol (50 ml) for overnight. Using sterile muslin cloth and sterile Whatman filter paper, the di-ethanol fraction was separated (no. 02). A rotary film evaporator was used to concentrate the filtered extract. On the EMB media, the morphological identification of the fecal isolated strains was seen. The isolated bacterial strains on the EMB had a green metallic sheen, and *E. coli* was recognized morphologically. Using the disc diffusion method, the antibacterial properties of the tea and seed extracts were identified. The test organisms were transferred from the pure cultures and kept in an aseptic environment under a laminar air cabinet. With the aid of the sterile inoculating loop, each test organism was moved from the subculture to the test tube containing 16 ml autoclaved media at 45 °C in an aseptic setting. To obtain a uniform suspension of organism, the test tubes were rotated to shake them. The bacterial suspensions were immediately added aseptically to the sterile Petri dishes. The Petri dishes were repeatedly turned, first in a clockwise direction and then an anticlockwise direction, to ensure that the test organisms were distributed uniformly. Both Sample discs and Standard discs were used for the antibacterial screening. Sample antibiotic discs (amoxicillin and erythromycin discs) were gently placed on the solidified agar plates that had just been seeded with the test organisms using sterile forceps to ensure complete contact with the medium surface. The zones of inhibition were kept from overlapping by spacing the discs so that they were no closer than 15 mm to the edge of the plate. The plates were then overturned, and they spent roughly 4 hours in a freezer at 4 °C. The substance had ample time to spread out into a sizable area of the medium as a result. After that, the plates were incubated at 37°C for 12 to 18 hours while upside down. The sample discs, antibiotic discs, and control discs were gently placed over the previously marked zones in the agar plates that had already been pre-inoculated with test bacteria. The materials on the discs were then given enough time to sufficiently diffuse into the surrounding agar medium by being placed on the plates, upside down in a 40 °C refrigerator for about 24 hours. After that, the plates were turned over and kept in the incubator at 37°C for 24 hours.

RESULTS

The *Moringa oleifera* seed extract was applied against isolated strains such as *E. coli* of Ostrich. The amoxicillin and erythromycin were used as a control. The antimicrobial activity of *Moringa oleifera* seed extracts against *E. coli* show 06 mm of inhibitory zone. The, amoxicillin showed inhibitory zone of 12 mm. The *Moringa oleifera* tea (di

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ethanol extract was applied against isolated strains of *E. coli.* of Ostrich. No antimicrobial activity of *Moringa oleifera* tea against *E. coli* was recorded. The *E. coli* showed no inhibitory zone, while erythromycin showed inhibitory zone of 14 mm as shown in Table 1 and Figure 1.

Tested bacteria	Diameter of Disc	Inhibition zone measurement	Inhibition zone measurement amoxicillin	Inhibition zone measurement erythromycin
***Moringa oleifera* seed (di ethanol extracts)**				
E. coli	7 mm	6 mm	12 mm	14 mm
***Moringa oleifera* tea (di ethanol extracts)**				
E. coli	7 mm	No zone	12 mm	14 mm

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

Figure 1: Petri plate showing disc diffusion and antimicrobial activity of *Moringa oleifera* seed and tea leaves with Di-ethanol against *E. coli*

DISCUSSION

Moringa oleifera tea extract with ethanol was used against pathogens *E. coli*. The controls used were amoxicillin and erythromycin. Both controls were successful in showing the inhibitory zone of 12mm and 14 mm thus limiting the growth of *E. coli*. The *Moringa oleifera* tea did not successfully stop pathogen growth. According to Napolean *et al.*, *M. oleifera* tea Di-ethanol extract can be toxic to some types of bacteria, including *Enterobacter spp., S. aureus, P. aeruginosa, S. typi, and E. coli,* at concentrations of 50 to 200 mg/ml [18]. Our research was contrary to Napoleon *et al.*, as the extract showed no inhibition zone against the *E. coli.* This might be possible due to the low concentration of extract. Nepolean's extract showed inhibitory zone at 50-200mg/ml. Our extract concentration was just 05 mg/ml. Arzai *et al.*, also reported that *Moringa oleifera* tea extract with Di-ethanol showed activity towards *P. aeruginosa, S. aureus, E. coli, and S. typhi.* Our research was also contrary to this research as our *Moringa oleifera* tea extract with Di-ethanol showed no result with the pathogens *E. coli* [19]. This may also be due to low concentration of the tea extract as we used 5g/ml of the extract. Mohamed *et al.*, reported that *Moringa oleifera* leaf extract with ethanol showed inhibitory zone of 11mm with *E. coli* [20]. Our research was contrary to this research as our tea extract (di-ethanol) *Moringa oleifera* showed no activity against *E. coli*. Other studies have demonstrated that a substance produced in the seed is connected to the antibacterial activity of *Moringa oleifera* seeds [21, 22]. Burns, insect bites, and rashes are just a few of the minor skin conditions that the *Moringa oleifera* seed oil can treat because it has antiseptic and anti-inflammatory properties. It has been claimed that adding crushed Moringa *oleifera seeds to soiled, bacterial-filled water has the power to remove impurities. The* most widely used water purifiers, such as aluminum sulphate, are thought to be less effective than *Moringa oleifera* seeds because they may be toxic [23]. According to the findings of our study, the extract of *Moringa oleifera* performed better at low or moderate temperatures (4 °C or 37 °C). High temperatures (70°C or higher), however, caused the activity to cease. Extracts were tested in this study inhibited the growth of *E. coli*.

CONCLUSIONS

It is concluded that *Moringa oleifera* seeds (ethanolic extracts) have inhibitory activity and can control pathogens such as *E. coli*. So, by including *Moringa* seeds in the diet of Ostrich, they can reduce their risk of infection caused by *E. coli*.

Conflicts of Interest

The authors declare no conflict of interest.

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