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SHORT COMMUNICATION

Antimicrobial activity of leaves extracts of *Diplazium muricatum* and *Diplazium travancoricum* on poultry pathogens

Selvan Nallaiyan[1*], Umapathi Radhakrishnan[2], Haripriya Doraiswamy[2], Vijayakumar Palanisamy[2]

[1]PG and Research Department of Zoology & Biochemistry, Government Arts College (Autonomous), Kumbakonam, Tamil Nadu, India.
[2]Department of Biochemistry, Muthayammal College of Arts and Science, Rasipuram, Tamil Nadu, India.
E-mail: selvanbt@gmail.com

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ABSTRACT

The development of the Indian poultry industry in general, Namakkal, Tamil Nadu, layer industry in particular, is remarkably fast and it is growing on leaps and bounds with respect to poultry egg production. The layer birds in poultry were affected by bacterial pathogens. However, antimicrobial activities of *Diplazium muricatum* and Diplazium *travancoricum* were examined using agar diffusion method against *E. coli, Bacillus* and *Klebsiella* isolated from poultry litter collected from poultry farms. The experimental studies revealed, the crude extracts of *Diplazium muricatum* and *D. travancoricum* used in this study were inhibited the growth of microorganisms and zone of clearance ranged from 9 to 16 mm. The results indicated that *D. muricatum* and *D. travancoricum* had the potential antimicrobial compounds and great potential to use as a feed ingredient to prevent the pathogens.

Keywords: Poultry litter, Crude Extracts, *E. coli*, *Bacillus*, *Klebsiella*

INTRODUCTION

Many States in the world rely upon the poultry industry for a substantial portion of their agricultural income. The diseases of bacterial etiology present important factors in poultry production, therefore the sources of spreading the infection in poultry flocks and possible economic losses, which they induce, need to be investigated. Great agglomeration of poultry at poultry houses induce increased pathogenicity of some microbial agents, especially bacteria which could cause infection with high rate of morbidity and mortality.

Zoonotic potential of some microbial agents like *E. coli* present a special epidemiological problem in poultry breeding. Those agents pose a permanent risk for human health, mostly to people who work with poultry or to the consumers who eat contaminated poultry meat or eggs.

Processing of poultry litter is necessary for destruction of potential pathogens, improvement of handling and storage characteristics and maintenance or enhancement of palatability (Fontenot, 2000). Pathogenic microbial organisms gain access to the animal body through contaminated feed and water (Youdeowei *et al.*, 1999). The presence of these pathogenic microorganisms impact negatively on feed utilization and physiological functions with in the animal system (Ngodigha *et al.*, 2009). Poultry litter has useful properties as a fertilizer and soil amendment and has been used for many years in the production of a range of crops and products for human consumption. However concerns exist regarding litter contaminants including heavy metals and pathogens (Runge *et al.*, 2007).

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings (Bushra *et al.*, 2003). The abundance of plants on the earth's surfaces has led to an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar *et al.*, 2004). The two *Diplazium muricatum* and *Diplazium travancoricum* are among widely distributed in many countries. Bacterial infection of navel area is one of the most common causes of mortality in chicks during the first week after hatching (Pattison *et al.*, 2008). Several bacteria such as *Proteus*, *Enterobacter* spp., *Pseudomonas*, *Streptococcus*, *Clostridium* and *Bacillus* species have been isolated from yolksac infection of birds (Cotes *et al.*, 2004). *E. coli* is the most common contaminants of yolk sacs in chickens and about 70% of chicks with omphalitis has this bacterium in their yolk sacs (Saif *et al.*, 2008). *Escherichia coli* is a ubiquitous pathogen distributed throughout the world that causes significant economic losses to commercially produced poultry (Skyberg *et al.*, 2003). The sole species with in the large bacterial family *Bacillaceae* which is classified as being pathogenic for man and higher animals is *Bacillus*

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***Corresponding author**

anthracis, the etiological agent of anthrax. *Bacillus cereus*, however, has been shown to produce fatal infection when infected in large number in small laboratory animals (Clark, 1937). *Klebsiella pneumoniae* has been reported as one of the bacteria infecting the yolk sacs and causing embryos and chicks mortalities during their first week of life (Orajaka and Mohan, 1985).

Food- producing animals acquire these pathogens by ingestion. Contamination of animal feed before arrival it and while on the farm contributes to infection and colonization of food-producing animals with these pathogens. Pathogens can then be transmitted through the food chain to humans and cause human food borne illness (John *et al.*, 2002).

Medicinal plants have been used since centuries to treat various diseases in man and animals. It is not surprising, therefore, that several herbal agents have been empirically used in poultry birds and other animals (Jahan *et al.*, 2008). The present study was carried out with the objective to evaluate the growth promoting efficacy of some medicinal plants and their influence on the poultry industry performance.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Diplazium muricatum* and *Diplazium travancoricum* were collected in large quantities from the forest of Tirunelveli hills, Kothayar, Western Ghates, Tamil Nadu and India in mid March. The plants were identified by Dr. M. Johnson of Department of Plant Biology and Plant Biotechnology, St. Xaveirs College (Autonomous), Tirunelveli, Tamil Nadu, India. A voucher specimen of the leaves was deposited at the hebarium of the Department of Plant Biotechnology, St. Xaveirs College (Autonomous), Tirunelveli for future reference.

Preparation of crude extracts

The leaves of *Diplazium muricatum* and *Diplazium travancoricum* were shade dried and powdered. A 30 g of powder was used to extraction of antimicrobial compounds using non-polar to polar solvents *viz* acetone, isopropanol, ethanol, distilled water and aqueous Soxhlet apparatus for 18 h. After extraction they were collected and these extracts were then poured into Petri dishes. Then kept for air dry and stored it in a refrigerator used for further analysis. These extracts were dissolved in DMSO (100%), (200-300 mg/mL) to make the final concentrations and kept in refrigerator till used.

Collection of poultry litter sample

Litter samples were collected from various poultry farms in Namakkal, Tamil Nadu, India, then pooled into a sterile plastic bag. To prevent cross contamination, surgical shoe and latex gloves were used. Samples were placed in an ice chest with ice packs during transport to the laboratory.

Plating and Identification

Each poultry litter sample was mixed in the sealed plastic bag by vigorously agitating the bag by hand for 1 min. Five samples of litter were then placed in 45 mL of 0.1% peptone water in a sterile 50 mL polypropylene conical tube, and vortexed for 1 min (Islam *et al.*, 2004). One mL of each sample was serially diluted using 0.1% peptone water and 0.1 mL portions of each dilution were plated in Sterile Eosin methylene blue agar (EMB), Nutrient agar and MacConkey (MC) agar medium. Plates were incubated for 24 h at 37 °C. After incubation, the colonies on EMB, MC agar and nutrient agar were selected and confirmed by standard methods. Stock cultures were isolated from poultry litter of *E. coli*, *Bacillus* sp. and *Klebsiella* sp. were subcultured and maintained in nutrient broth at 4 °C.

Evaluation of Antimicrobial Activities

The agar diffusion method was used for the antimicrobial evaluations. Wells of 6 mm diameter were punched into the sterile Mueller Hinton agar with the test microorganisms and filled with 200-300 mg/mL of plant extracts. The plates were incubated for 24 h at 37 °C (Haripriya *et al.*, 2010). Antimicrobial activity was evaluated by measuring the inhibition zone in millimeter in diameter and recorded.

RESULTS AND DISCUSSION

The strains of *E. coli*, *Bacillus* sp. and *Klebsiella* sp. were isolated from poultry litter and identified by standard microbiological method as shown in Table 1. A total of four extracts from two plant species were investigated against these isolated poultry pathogens. The antimicrobial activities of the crude extracts of *Diplazium muricatum (D. muricatum) and Diplazium travancoricum (D. travancoricum)* against the test pathogen were shown in Table 2 and Table 3. As Tables 2 indicating, the extracts of acetone of *D. muricatum* showed higher antimicrobial activity to *E. coli, Bacillus* sp. and *Klebsiella* sp. (11-16 mm, 11-15 mm, 9-11 mm). The isopropanol extracts of *D. muricatum* also showed increased antimicrobial activity against *E. coli*, *Bacillus* sp. and *Klebsiella* sp. (12-16 mm, 11-14 mm, 11-13 mm).

The ethanol extracts *D. muricatum* of also exhibiting good antimicrobial activity against poultry pathogens as *E. coli, Bacillus* sp. and *Klebsiella* sp. (11-14 mm, 11-15 mm, 11-13 mm). The water extracts of *D. muricatum* also had higher inhibitory effects on three microorganisms (11-14 mm, 9-12 mm, 10-13 mm, 11-13 mm) in this study.

All the four extracts such as acetone, isopropanol, ethanol and water of *D. travancoricum* showed also good activity against *E. coli, Bacillus* sp. and *Klebsiella* sp. from the Tables 3. The acetone extracts showed 12-16 mm, 12-16 mm, and 10-11 mm; isopropanol extracts showed 11-14 mm, 12-15 mm and 10-11 mm against *E. coli*, *Bacillus* sp. and *Klebsiella* sp. The ethanol extracts showed 12-16 mm, 12-16 mm and 10-12 mm; water

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Table 1: Identification of poultry bacterial pathogens

Biochemical Test	*E. coli*	*Bacillus* sp.	*Klebsiella* sp.
Cultural Characteristics	Green colony	White Waxy	Slimy White
Gram stain	-	+	-
Shape	Short rod	Rod	Rod
Catalase Test	+	-	+
Citrate Utilization	-	-	+
Oxidase Test	-	+	-
Indole Production	+	-	-
Motility	+	+	-
MR Reaction	+	-	-

+: Positive reaction; -: Negative reaction

Table 2: Antimicrobial activity of plant extracts of *D. muricatum* against *E. coli, Bacillus* sp. and *Klebsiella* sp.

Solvents	Zone of inhibition (mm) (SD)					
	E. coli		*Bacillus* sp.		*Klebsiella*sp.	
	X	B- D	X	B- D	X	B- D
Acetone	-	13.66±0.14	-	13±0.0	-	10±0.0
Isopropanol	-	14±0.0	-	12.66±0.14	-	10±0.0
Ethanol	-	12.33±0.1	-	13±0.0	-	12±0.0
Water	-	12.33±0.1	-	11.33±0.1	-	12±0.0

X: Control; B: 200 mg/mL; C: 250mg/mL; D: 300mg/mL (Plant extracts)

Table 3: Antimicrobial activity of plant extracts of *D. travancoricum* against *E. coli, Bacillus* sp. and *Klebsiella* sp.

Solvents	Zone of inhibition (mm) (SD)					
	E. coli		*Bacillus* sp.		*Klebsiella*sp.	
	X	B- D	X	B- D	X	B- D
Acetone	-	14±0.0	-	14±0.0	-	10.33±0.1
Isopropanol	-	12.33±0.14	-	13.33±0.1	-	10.33±0.1
Ethanol	-	14±0.0	-	14.66±0.14	-	11±0.0
Water	-	10.66±0.14	-	12.33±0.14	-	11±0.0

X: Control; B: 200mg/mL; C: 250mg/mL; D: 300mg/mL (Plant extracts)

extracts showed 9-12 mm, 11-14 mm and 10-12 mm against *E. coli*, *Bacillus* sp. and *Klebsiella* sp.

This is the first study on this plant regarding poultry pathogens. Medicinal plants compete with the synthetic drugs. As the world is becoming more advanced, new diseases are emerging in animals and human beings by irrational use of antimiocrobial growth promoters. Finally we conclude that the effect of *D. muricatum and D. travancoricum* plant extracts revealed that reduce pathogenicity isolated from poultry farms.

Further evaluation of the antimicrobial properties of these extracts against a more extensive panel of microbial agents is warranted. Likewise, purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents. Whilst the extracts examined in this report appear promising.

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