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The effect of extracts of *Selaginella involvens* and *Selaginella inaequalifolia* leaves on poultry pathogens

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ABSTRACT

Objective: To investigate the antimicrobial activity of some commonly available herbs against poultry pathogens such as *Escherichia coli (E. coli)* and *Pseudomonas* species isolated from poultry litter. **Methods:** The extracts of *Selaginella involvens* and *Selaginella inaequalifolia* were tested against *E. coli* and *Pseudomonas* isolated from poultry litter by the agar diffusion method. **Results:** Results indicated that different plant extracts showed inhibitory effects against *E. coli* (8–13 mm) and *Pseudomonas* (6.5–13 mm). The four different extracts of *Selaginella involvens* and *Selaginella inaequalifolia* showed similar levels of antimicrobial activity on *E. coli*. **Conclusions:** The antimicrobial activities of all the four plant extracts are comparable and their potential as alternatives in the treatment of infections by these microorganisms were present in the poultry litter. Susceptibility testing is conduced on isolates using drugs selected on the basis of their importance to human medicine and use in poultry production.

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care[1]. Medicinal plants are gifts of nature to cure limitless number of diseases among human beings. 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[2]. The antimicrobial activity of plant extracts are changeable acording to the other researchers findings[3]. These differnces are due to the genetic structure of plant species, ecological factors, biochemical constituents of plant extract, extraction solvents and tested microorganisms. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for few and re-emerging infectious diseases. Therefore , researches are increasingly turning their attention to folk medicine, looking for new leads to developed better drugs against microbial infections[4]. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant derived drugs is mainly due to current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects[5].

Selaginella plant species is considered as a strong anti-poison. In all kinds of poisonous affections, fronds ground with fresh rhizome of *Curcuma longa* L. (Manjal) is applied externally for one week for curing leucorrhea, fronds paste mixed with tender coconut water is administered twice

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a day for three days[6]. *Selaginella* plant species are also effective to reduce high fever[7].

The purpose of this study is to evaluate the potential antimicrobial activities on the *Escherichia coli (E. coli)* and *Pseudomonas* isolated from poultry litter, poultry wastes, Namakkal, Tamil Nadu, India (litter & excreta) which are generally added to the soil as a fertilizer[8]. *Salmonella*, *Staphylococcus*, *Pseudomonas*, *Escherichia*, *Campylobacter*, and *Clostridium*[9] are among the potentially pathogenic bacterial species previously isolated from litter.

Different strains of *E. coli* can cause a wide variety of disease syndromes in animals including septicemia, swollen head syndrome, cellulites and airsacculitis. Humans can be exposed to the bacteria by consuming improperly prepared eggs, meat, or milk from infected animals or from foods contaminated by the faeces of infected animals[10]. Bacterial infection of navel area is one of the most common causes of mortality in chicks during the first week after hatching[11]. *E. coli* is the most common contaminants of yolk sacs in chickens and about 70% of chicks with omphalitis had this bacterium in their yolk sacs. On the other hand, it is common to recover low numbers of *E. coli* from normal yolk sacs[12]. *E. coli* is one of the opportunist pathogen responsible for number of disease conditions such as yolk sacs infection, air sacs disease, perihepatitis, enteritis, omphalitis, coligranuloma, colibacillosis[13]. The ability of *Pseudomonas* spp. to cause infection of yolk sac of a chick is enhanced by its ability to degrade the proteins found in the yolk, creating the opportunity for other bacteria to multiply[14].

This study was therefore designed to evaluate the bacterial profile in poultry litter. To eradicate this infectious agents it is necessary to disinfect poultry houses before and after chicken arrival and during the production period, especially if health problems occur[15]. For these reasons we performed on experiment, which was aimed to determine the main bacterial contaminants at poultry litter and test the influence of the most utilized plant extracts and disinfectant to isolated pathogenic microorganisms.

2. Materials and methods

2.1. Collection of plant materials

The leaves were collected in large quantities from the forest of Tirunelveli hills, Kothayar, Tirunelveli hills, Western Ghates, Tamilnadu, India in mid February. The plant was then identified by Dr. M. Johnson of Department of Plant Biology & Plant Biotechnology, St. Xaveirs College (Autonomous), Tirunelveli, Tamil Nadu, India. A voucher specimen of the leaves was deposited at the herbarium of the Department of Plant Biology & Plant Biotechnology, St. Xaveirs College (Autonomous), Tirunelveli for future reference.

2.2. Preparation of crude extracts

The leaves of *Selaginella involvens* and *Selaginella inaequalifolia* were collected, shade dried and powdered. A total of 30 g of powdered leaves were extracted successively using non-polar to polar solvents viz petroleum ether, benzene, methanol, distilled water and aqueous using Soxhlet apparatus for 18 hours. After extraction they were kept for air dry and stored it in a refrigerator used for furher analysis. These extracts were dissolved in dimetyl sulphoxide (100 mg/mL) to make the final concentrations.

2.3. 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.

2.4. Isolation and identification of poultry bacterial pathogens

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[16]. One gram of each sample were serial diluted using 0.1% peptone water and 0.1 mL portions of each dilution were plated in sterile Eosin methylene blue (EMB) agar and Cetrimide agar. Plates were incubated for 24 hours at 37 ℃. After incubation, the colonies on EMB and Cetrimide agar were selected and confirmed by standard methods. Stock cultures of *E. coli* and *Pseudomonas* were grown in nutrient broth at 30 ℃ were subcultured and maintained in nutrient broth at 4 ℃.

2.5. 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 140 μL of plant extracts. The plates were incubated for 18 h at 37 ℃. Antimicrobial activity was evaluated by measuring the inhibition zone in millimeter in diameter and recorded.

3. Results

The strains of *E. coli* and *Pseudomonas* were isolated from poultry litter and identified by standard microbiological method as shown in Table 1. A total four extracts from 2 plant species were investigated against these isolated poultry pathogens. The antimicrobial activities of the dried extracts of *Selaginella involvens* (*S. involvens*) and *Selaginella inaequalifolia* (*S. inaequalifolia*) against the test pathogen were shown in Table 2 and Table 3. As Table 2 indicating, the extracts of petroleum ether of *S. involvens* showed higher antimicrobial activity to *E. coli* (12–13 mm) and *Pseudomonas* (10–13 mm). The benzene extracts of *S. involvens* showed high activity against *E. coli* (8–11 mm) and

Peudomonas showed less activity (6.5–9 mm). The methanol extracts of *S. involvens* exhibited high activity against *E. coli* (11–13 mm) and *Pseudomonas* exhibited low activity (6.5–9 mm). Water extracts of *S. involvens* also had higher inhibitory effects on the both microorganisms (10–12 mm) in this study.

Table 1
Identification of poultry bacterial pathogens.

Biochemical test	*E. coli*	*Pseudomonas*
Cultural characteristics	Green smooth colony	Yellow-green pigment
Gram stain	−	−
Shape	Short rod	Rod
Catalase test	+	+
Citrate utilization	−	+
Lactose fermentation	+	−
Indole production	+	−
Motility	+	+

(+) = Positive reaction and (−) = Negative reaction.

On the other hand, *S. inaequalifolia* plants also had good activity against *E. coli* and *Pseudomonas* organisms as can be seen from Table 3. Petroleum ether extracts showed (11–12 mm) and (9–10 mm), benzene extracts showed (11–13 mm) and (6.5–11 mm). The methanolic and water extracts of *S. inaequalifolia* showed high activity against test organism *E. coli* isolated from poultry litter showed (11–13 mm, 11–13 mm) and *Pseudomonas* having (8–11 mm, 7–10 mm), respectively.

This study indicated that petroleum ether, benzene, methanol and water extracts of *S. involvens, S. inaequalifolia* showed similar antimicrobial effects on the microorganism *E. coli* isolated from the poultry litters. Also *Pseudomonas* had low antimicrobial activity compared to *E. coli*. Most of the bacteria isolated in this study were commonly experienced by man and animals in their day to day exposure and their bodies had developed some degree of relative resistance[17].

Therefore, our results revealed the importance of plant extracts to control bacteria, which were becoming a threat to human health. The results in the study suggested that plant extracts reduce pathogenicity of some microbial agents, especially bacteria which could cause infection with high rate of morbidity and mortality.

Table 2
Antimicrobial activity of plant extracts of *S. involvens* against *E. coli* and *Pseudomonas* (mm).

Solvent used	Zones of inhibitionin diameter							
	E. coli				*Pseudomonas*			
	A	B	C	D	A	B	C	D
Petroleum ether	–	13.0	12.0	12.0	–	13.0	11.0	10.0
Benzene	–	11.0	9.0	8.0	–	9.0	8.0	6.5
Methanol	–	13.0	12.0	11.0	–	9.0	7.0	6.5
Water	–	12.0	11.0	10.0	–	12.0	11.0	10.0

A: Control, B: 140 μL, C: 120 μL, D: 100 μL of extract samples (100 mg/mL).

Table 3
Antimicrobial activity of plant extracts of *S. inaequalifolia* against *E. coli* and *Pseudomonas*(mm).

Solvent used	Zones of inhibition							
	E. coli				*Pseudomonas*			
	A	B	C	D	A	B	C	D
Petroleum ether	–	12.0	11.0	11.0	–	10.0	9.0	9.0
Benzene	–	13.0	12.0	11.0	–	11.0	9.0	6.5
Methanol	–	13.0	12.0	11.0	–	11.0	9.0	8.0
Water	–	13.0	12.0	11.0	–	10.0	8.0	7.0

A: Control, B: 140 μL, C: 120 μL, D: 100 μL of extract samples (100 mg/mL).

4. Discussion

To discuss the effect of plant extracts for use as antimicrobial agents to maintain the plant ingredients activity, the petroleum ether, benzene, methanol and water extracts of the leaves of *S. involvens*, *S. inaequalifolia* were subjected to a preliminary screening for antimicrobial activity against *E. coli* and *Pseudomonas* isolated from poultry litter.

S. involvens may be safe anti-acne source in the therapeutics application of the treatment of acne development by reducing the chance of non specific initiation and angmentation phase of the inflammatory response[18].

Future research on this study can however focus on optimizing clinically the chemotherapeutic processes of *S. involvens* and *S. inaequalifolia* plant species for treatment and control of these common poultry infections and other parts of the country where the plants can be easily cultivated or otherwise commercialized for pharmaceutical purpose.

Conflict of interest statement

We declare that we have no conflict of interest.

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