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RESEARCH ARTICLE

PHYTOCHEMICAL ACTIVITY OF LEAVES OF *Selaginella involvens* AND *Selaginella inaequalifolia* EXTRACTS ON POULTRY PATHOGENS

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ABSTRACT

This may be the first report were two plants screened for its potential antimicrobial activity in poultry industry. In this experiment, evaluating antimicrobial activity, there are four solvents were used such as petroleum ether, benzene, methanol and water. The plants screened were *Selaginella involvens* and *Selaginella inaequalifolia*. Antimicrobial activity was tested against five bacterial strains; *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus* isolated from poultry farms by agar diffusion method or well diffusion method were used. The results showed that *Selaginella involvens* and *Selaginella inaequalifolia* extracts exhibited antimicrobial activities at a concentration of 100-300mg/ml. The zones of inhibition exhibited by *Selaginella involvens* extract ranged between 7-17mm while that of *Selaginella inaequalifolia* varied between 7- 15mm depending upon the solvents used. Therefore this can be selected for further investigation to determine its therapeutic potential. Its leaf extracts can also be used as a lead molecule in combating the diseases caused by the bacterial strains present in poultry industry.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. Medicinal plants represent a rich source of antimicrobial agents. Many of the plant material used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al.,* 2008). Furthermore the active components of herbal remedies have the advantages of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure components (Shariff, 2001). *Selaginella* plant species is considered as a strong anti poison. In all kinds of poisonous affections, fronds ground with fresh rhizome of *Curcuma langa L*. (Manjal) is applied externally for one week for curing leucorrhea, fronds paste mixed with tender coconut water is administered twice a day for three days (Udhayan *et al.,* 2008). *Selaginella* plant species are also effective to reduce high fever (Mannar *et al.,* 2008)

The purpose of this study is to evaluate the potential antimicrobial activities on the *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus* isolated from poultry litter, Namakkal, Tamil Nadu and India. Poultry litters are generally added to the soil as a fertilizer (El-Jalil *et al.,* 2008).

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Staphylococcus aureus is a very ubiquitous microorganism associated with omphalitis, yolk sac and liver infection in first week dead chicks and in- shell dead embryos (White, 2003). *Proteus* species has been associated with in-shell embryos mortality (Orajaka and Mohan, 1985). The egg shells were described as the major source of *Salmonella* species spread in hatcheries (Agabou, 2009). Salmonella enteritis or Salmonella typhimurium are among the major bacterial pathogens of poultry in the all world and most of their infections in humans result from the ingestion of contaminated poultry (Carli *et al.,* 2001). *S. aureus* is an important cause of a variety of diseases in humans and animals worldwide (Gilot and Leeuwen, 2004). *S. aureus* is a common cause of food poisoning in humans. *S. aureus* infections are common in poultry. Pullorum disease with *Salmonella,* pullorum represented a serious poultry health problem until huge resources were used to limit its expansion, but these efforts allowed the emergence of other *Salmonella* (Ammar *et al.,* 2010).

The sole species within the large bacterial family *Bacillaceae* which is classified as being pathogenic for man and higher animals is *bacillus 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 ingestions. 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). The results of present investigation clearly indicate that the antibacterial activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. In this study, we have described the antimicrobial activity of substances against bacteria and also the development of new drugs against poultry pathogens.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Selaginella involvens* and *Selaginella inaequalifolia* were collected in large quantities from the forest of Tirunelveli hills, Kothayar, Western Ghates, Tamil Nadu and India in mid March. The plants was then 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 herbarium of the Department of Plant Biotechnology, St. Xaveirs College (Autonomous), Tirunelveli for future reference.

Preparation of Crude Extracts

The leaves of *Selaginella involvens* and *Selaginella inaequalifolia* were collected, shade dried and powdered. 30g of powdered leaves were extracted successively using non-polar to polar solvents viz petroleum ether, benzene, methanol and Distilled water and aqueous Soxhlet apparatus for 18 hours. After extraction they were collected and these extracts were then poured into petridishes. Then kept for air dry and stored it in a refrigerator used for further analysis. These extracts were dissolved in dimetyl sulphoxide (50-150 mg/ml) to make the final concentrations which 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 gram of each sample were serial diluted using 0.1% peptone water and 0.1 ml portions of each dilution were plated in Sterile Staphylococcus Hi Veg agar (*Staphyllococcus*), Bismuth sulphite agar (*Salmonella*), Nutrient agar (*Proteus, Bacillus*) and Mac conkey agar (*Klebsiella*) medium. Plates were incubated for 24 hours at 37°C. After incubation, the colonies on the plates were selected and confirmed by standard methods. Stock cultures of *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus* were grown in nutrient broth at 30^{o}C were sub cultured and maintained in nutrient broth at 4^{o}C

Evaluation of Antimicrobial Activities

The agar diffusion method was used for the antimicrobial evaluations. Wells of 6mm diameter were punched into the sterile Mueller Hinton Agar with the test microorganisms and filled with 100-300mg/ml of plant extracts. The plates were incubated for 18h at 37°C. Antimicrobial activity was evaluated by measuring the inhibition zone in millimeter in diameter and recorded.

RESULTS

The strains of *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus* 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 activity of the dried extracts of *Selaginella involvens* and *Selaginella inaequalifolia* against the test pathogen were shown in Table 2 and Table 3. As table 2 indicating, the extracts of petroleum ether, benzene, methanol and water of *S. involvens* and *S. inaequalifolia* showed higher antimicrobial activity against *bacillus species*. The zone of clearance ranged from 7-17mm and 7-15mm. *Selaginella involvens* and *Selaginella inaequalifolia* of all the extracts exhibit good antimicrobial activity against *Klebsiella* species and ranged from 11-13mm and 11-14mm. The petroleum ether extracts of *Selaginella involvens* and *Selaginella inaequalifolia* having no inhibitory effects on *Proteus* species. But the benzene, methanol and water extracts of *Selaginella involvens* and *Selaginella inaequalifolia* showed less activity against *Proteus* species ranged from 8-10mm and 7-9mm. The petroleum ether, benzene and methanol extracts of *Selaginella involvens* having absence of inhibitory concentration, but there is water extract of *Selaginella involvens* showed limited effect against *Staphylococcus species* (8-11mm). The petroleum ether, benzene and methanol extracts of *Selaginella inaequalifolia* were observed result as, 7-15mm, the water extract having no results on *Staphylococcus* species. *Selaginella involvens* in petroleum ether and benzene extracts having less activity against Salmonella species (7-11mm). Then the methanol and water extracts of *Selaginella involvens* did not inhibit *Salmonella.* The petroleum ether, benzene and methanol extracts of *Selaginella inaequalifolia* also showed less activity against *Salmonella* species. (9-12mm). The water extracts of *Selaginella inaequalifolia* no inhibitory effect on *Salmonella* species. Selaginella 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 (Joo *et al.,* 2008). The results of present study revealed that medicinal plants especially *Selaginella involvens* and *Selaginella inaequalifolia* can be used as medicinal drugs in the poultry farms with better production and performance.

Table 1. Identification of poultry bacterial pathogens

Biochemical Test	*Bacillus*	*Klebsiella*	*Salmonella*	*Staphylococcus*	*Proteus*
Cultural Characteristics	White waxy	Slight pinkish	Grayish	Pure White	Slimy white
Gram stain	+	-	-	+	-
Shape	Rod	Rod	Rod	Coccus	Rod
Catalase Test	-	+	+	-	+
Oxidase Test	+	-	-	+	-
Citrate utilization	-	+	+	-	-
Lactose fermentation	-	AG	-	-	-
Indole production	-	-	-	-	+
Motility	+	-	+	-	+

(+) = Positive reaction; (-) = Negative reaction; AG = Acid and Gas production

Table 2. Antimicrobial activity of plant extracts of *Selaginella involvens* against *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus species*

Pathogens	Zones of inhibition in diameter (100 – 300 mg/ml)												
	Control	Petroleum Ether			Benzene			Methanol			Water		
	X	A	B	C	A	B	C	A	B	C	A	B	C
Bacillus	-	10	12	12	12	16	17	9	10	10	12	13	14
Klebsiella	-	12	12	13	12	12	13	12	12	13	11	11	12
Salmonella	-	9	10	11	7	7	7	-	-	-	-	-	-
Staphylococcus	-	-	-	-	-	-	-	-	-	-	8	10	11
Proteus	-	-	-	-	8	8	8	9	9	9	10	10	10

Control – Dimethyl Sulphoxide; A – 100mg ; B- 200mg; C- 300mg of plant extracts.

Table 3. Antimicrobial activity of plant extracts of *Selaginella inaequalifolia* against *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus species.*

Pathogens	Zones of inhibition in diameter (100 – 300 mg/ml)												
	Control	Petroleum Ether			Benzene			Methanol			Water		
	X	A	B	C	A	B	C	A	B	C	A	B	C
Bacillus	-	11	12	15	10	12	13	8	9	10	7	7	7
Klebsiella	-	11	11	12	12	12	12	12	12	13	12	13	14
Salmonella	-	10	10	10	9	9	9	10	11	12	-	-	-
Staphylococcus	-	7	7	7	8	8	8	12	13	15	-	-	-
Proteus	-	-	-	-	9	9	9	7	7	7	9	9	9

Control – Dimethyl Sulphoxide; A – 100mg ; B- 200mg; C- 300mg of plant extracts.

DISCUSSION

This may the first report regarding these two plants in poultry pathogens. The effect of plant extracts, use as antimicrobial agents to control pathogens in poultry industries was proved. The petroleum ether, benzene, methanol and water extracts of the leaves of *Selaginella involvens* and *Selaginella inaequalifolia* were subjected to a preliminary screening for antimicrobial activity against *Klebsiella, Salmonella, Staphylococcus, Proteus* and *Bacillus* isolated from poultry litter. Our study clearly indicate that the antibacterial activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs, to control the loss incurred by poultry industry by bacterial pathogens.

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