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Original article

Antibacterial activity of *Drynaria quercifolia* (L.) J. Smith and *Dryopteris cochleata* (Buch. Ham. ex. D. Don) against scale rot in pythons

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Abstract

Antibacterial activity of *Drynaria quercifolia* (L.) J. Smith and *Dryopteris cochleata* (Buch. Ham. ex. D. Don) was assessed in this study. The bacteria were isolated from scale rot conditions in captive Indian Rock Python (*Python molurus*) n=15 and Reticulated Python (*Python reticulates*) n=10 and cultured in suitable media and the bacterial isolates were identified as *Staphylococcus aureus* and *Escherichia coli*. Isolated bacterial strains were subjected to antibiotic sensitivity test by Kirby-Bauer's/disc-diffusion method and were found resistant to 6 antibiotics and sensitive to enrofloxacin, ciprofloxacin and gentamicin. Antibacterial activity of aqueous, ethanolic and petroleum ether of two fern extracts was monitored by agar-well diffusion method. Linezolid 30 µg/ml and imipenem 10 µg/ml as controls for gram-positive and gram-negative bacteria and 10% DMSO solution as negative control showed petroleum ether extract least zone of inhibition for both *E.coli* and *S.aureus*. Values of zone of inhibition were highest of ethanolic extracts. *E.coli* showed least zone of inhibition than *S.aureus*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the active solvent extracts were determined and showed MIC and MBC were least for ethanolic extract than aqueous and petroleum ether extract. Phytochemical studies of the collected ferns performed well with strong positivity for major phytoconstituents like tannin and phenol. The 11 phytoconstituents were well expressed during the qualitative analysis based on the order of solvent ethanol>aqueous>petroleum ether.

Key words: *Drynaria quercifolia* (L.) J. Smith, *Dryopteris cochleata* (Buch. Ham. ex. D. Don), *Escherichia coli*, *Staphylococcus aureus*, pythons

1. Introduction

Reptiles are ectothermic air breathing vertebrates, covered with scales or plates and are widely distributed in the Indian sub-continent. The pythons belong to the super family, Booidea and family, Pythonidae (Mader, 1996). Poor husbandry, malnutrition and lack of sanitary and hygienic procedures are major causes of disease in captive reptiles. These problems predispose to infectious disease, which is a major immediate cause of death in these animals (Marcus, 1981). Skin rot is a condition, caused by bacteria producing specific ulcerative and septic skin lesion in reptiles, because of crawling habit of these organism (Fowler, 1986).

Pteridophytes are primitive vascular plants, which can adapt well in terrestrial habitat. With the introduction of ethnobotany, many attempts have been made on the study of relationships of plants particularly for medicinal value of pteridophytes (Singh *et al.*, 2001). *Drynaria quercifolia* (L.) J. Smith belongs to the family Polypodiaceae and *Dryopteris cochleata* (Buch. Ham. ex. D. Don) belongs to the family Dryopteridaceae. Widespread use of antibiotics caused significant increase in antibiotic resistance of bacteria (Davies, 1994). Currently, these multidrug resistant bacteria have been emerging as one of the most important hospital and community pathogen worldwide. The emergence of these bacteria had caused a major concern and, thus there is a urgent need for new antibacterial agents (Spratt, 1994). Present study is an attempt to evaluate the antibacterial potential of these plants in various extracts of increasing polarity and to understand the phytochemical background of the extracts. The extracts were tested towards various skin rot conditions, caused by pathogenic bacteria in captive pythons.

2. Materials and Methods

2.1 Collection of plants sample and preparations of plant extracts

Rhizomes of *Drynaria quercifolia* (L.) J. Smith (Voucher specimen No. SPCH 1008) and *Dryopteris cochleata* (Buch. Ham. ex. D.

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Don) (Voucher specimen No. SPCH 1009) were collected from Kolli hills, part of Eastern Ghats, Namakkal District, Tamil Nadu, India. The collected ferns were identified from Scott Christian College, Nagercoil, Tamil Nadu, India and a voucher specimen of herbarium was preserved in A.V.V.M Sri Pushpam College, Thanjavur District, Tamil Nadu, India. Collected rhizomes were shade dried and powdered and was stored in airtight polythene packs until use. One gram of shade dried powder of whole plant, *D. quercifolia* and *D. cochleata* were extracted with 20 ml ethanol, aqueous and petroleum ether (Merck, extra pure) for 1 min, using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature as stated (Kalpana Devi *et al.,* 2017). The sample was then filtered through Whatman No. 1 filter paper in a buchner funnel and evaporated under vacuum in a rotavator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100%. The solution was stored at 18°C under room temperature until use.

2.2 Isolation and identification of the bacteria

The bacteria were isolated from the clinical samples of naturally infected cases of skin rot conditions in captive pythons (n=25) from Arignar Anna Zoological Park ,Vandalur and Guindy Snake park Trust, Tamil Nadu, India during the period from June 2005 to May 2007. During the study period, wound swabs were collected from Indian Rock Python (*Python molurus*) n=15 and Reticulated Python (*Python reticulates*) n=10. Samples were cultured in suitable media and the bacterial isolates were identified by standard biochemical procedure, described previously for gram-negatives (Dubey *et al.,* 2012) and gram-positives (Dubey and Padhy, 2012). One gram-positive (*S. aureus*) and one gram-negative (*E. coli*) were isolated and were used in the study.

2.3 Antibiotic susceptibility test

All isolated bacterial strains were subjected to antibiotic sensitivity test by Kirby-Bauer's/disc-diffusion method, described previously (Sahu *et al.,* 2012). Nine antibiotics were used against gram-positives and gram-negatives.

2.4 Antibacterial activity test by agar-well diffusion method and determination of minimum inhibitory and bactericidal concentration

Antibacterial activity of three solvent extracts of *D. quercifolia* and *D. cochleata* was monitored by agar-well diffusion method, as described previously (Dubey *et al.,* 2012; Dubey and Padhy, 2012; Gireesha and Raju, 2016). The wells of 6 mm diameter were made on each plate with the help of cork borer and the wells were loaded with the samples tested. Linezolid 30 µg/ml and imipenem 10 µg/ml were used as controls for gram-positive and gram-negative bacterial work, respectively, and 10% DMSO solution was the negative control. The plates were incubated at 37°C for 24 h and the zone of inhibition was measured. The experiments were performed in triplicates. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the active solvent extracts were determined as described previously (Sahu *et al.,* 2012).

2.5 Phytochemical screening

Phytochemical studies of the collected ferns were carried out according to Harborne (1998). Different solvent systems were used for the separation of chemicals especially to study the anthelmintic property of the pteridophytes (*D. quercifolia* and *D. cochleata*), according to the polarity (aqueous, ethanol and petroleum ether), to identify the major natural chemical groups such as tannins, saponins, flavonoids, quinones, phenols, terpenoids, alkaloids, glycosides, cardio glycosides**,** coumarins and steroids.

3. Results

Skin rot condition in pythons (Figure 1) with 25 swab samples during the study period showed clinical isolates of *S.aureus* and *E.coli* and were found resistant to 6 antibiotics used (Figure 2). However, *S.aureus* and *E.coli* (Figures 3, 4) were sensitive to antibiotic enrofloxacin, ciprofloxacin and gentamicin. The antibiograms of the bacteria were recorded (Table 1). The extracts of *D. quercifolia* (Figure 5) and *D. cochleata* (Figure 6) showed petroleum ether extract least zone of inhibition for both, *E.coli* and *S.aureus*. Values of zone of inhibition were highest of ethanolic extracts. In general, *E.coli* showed least zone of inhibition than *S.aureus* among the three solvent extracts studied (Table 2).

MIC and MBC values of active rhizoid extracts of the two ferns were recorded with water, ethanol and petroleum ether as solvents as shown (Table 3). The phytochemical analysis of the two rhizoid extract using aqueous, ethanolic and petroleum ether solvent for the qualitative analysis for tannins, saponins, flavonoids, quinones, glycosides, cardio glycosides, terpenoids, phenol, coumarins, steroids and alkaloids as shown (Table 4). Among the three solvents used, ethanolic extract performed well to show strong positivity (++) for major phytoconstituents like tannin and phenol in these ferns studied. The 11 phytochemicals were well expressed during the qualitative analysis based on the order of solvent ethanol > aqueous > petroleum ether.

Table 1: Antibiogram of clinically isolated bacteria in skin rot condition from Python (N=25) by the disc-diffusion method

Bacterium	Susceptibility to prescribed antibiotics								
	Chl	Tet	Sul	Gent	Amp	Amox	Doxy	Enro	Cipro
Escherichia coli	R	R	R	S	R	R	R	S	S
Staphylococcus aureus	R	R	R	S	R	R	R	S	S

R = Resistant; S = Sensitive

Table 2: Antimicrobial assay by agar-well diffusion method of different solvent extracts of ferns and antibiotics as reference control against isolated multidrug resistant bacteria (zone of inhibition in mm)

Strain	*D. quercifolia*			*D. cochleata*			Lz/Imp
	Aqueous	Ethanol	PE	Aqueous	Ethanol	PE	(µg/ml)
E.coli	23.000 ± 0.577	24.667 ± 0.667	17.000 ± 0.577	18.333 ± 0.333	21.333 ± 0.333	14.333 ± 0.882	25.333 ± 0.333
S. aureus	22.667 ± 0.333	28.667 ± 0.333	21.333 ± 0.882	21.667 ± 0.333	25.000 ± 0.577	16.667 ± 0.333	28.667 ± 0.333

PE = Petroleum ether; Lz = Linezolid 30; Imp = Imipenem 10

Table 3: Minimum inhibitory and bactericidal concentration of bioactive rhizome extracts of ferns against isolated multidrug resistant bacteria (mg/ml)

Strain	*Drynaria quercifolia*						*Dryopteris cochleata*					
	Aqueous		Ethanol		PE		Aqueous		Ethanol		PE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	12.50	25.0	3.125	25.0	25.00	50.0	25.00	50.0	6.250	25.0	25.00	50.0
Staphylococcus aureus	12.50	25.0	1.562	12.5	12.50	50.0	12.50	25.0	3.250	25.0	25.00	50.0

PE = Petroleum ether; MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration

Table 4: Preliminary phytochemical analysis of rhizome extracts of ferns with different solvents

Plants name	Solvents	Tannins	Saponins	Flavonoids	Quinones	Glycosides	Cardio glycosides	Terpenoids	Phenol	Coumarins	Steriods	Alkaloids
D.quercifolia	A	+	+	+	+	-	+	-	+	-	-	-
	E	++	++	+	++	-	+	+	++	+	+	-
	P	-	-	-	-	-	+	-	-	-	+	-
D.cochleata	A	++	+	+	++	-	+	-	+	+	-	-
	E	++	-	+	++	-	++	-	++	-	+	+
	P	-	-	-	-	-	-	-	-	-	-	-

A – Aqueous E – Ethanol P – Petroleum ether ++ Strong Positive + Positive - Negative

Figure: 1 Skin rot in python

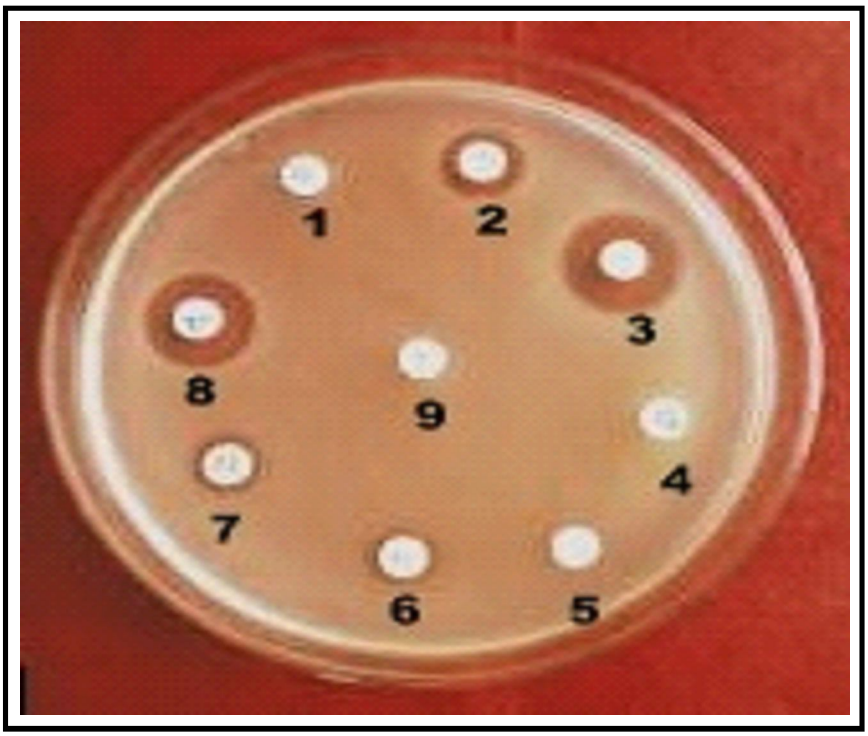

Figure 2: Antibiotic sensitivity test (ABST) antibiotics (µg/disc): 1): doxycycline 10; 2): enrofloxacin 5; 3): ciprofloxacin 5; 4): tetracycline 10; 5): sulphamnamide 30; 6): gentamicin 5; 7): ampicillin 10; 8): chloramphenicol 30; 9): amoxycillin 30.

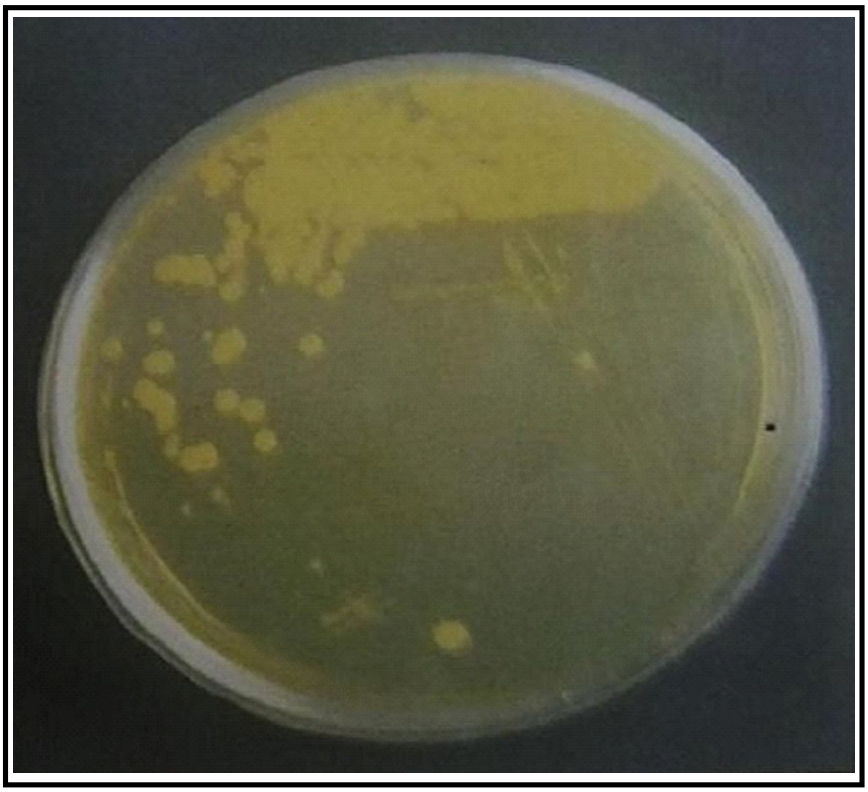

Figure 3: *S. aureus*

Figure 4: *E. coli*

Figure 5 : ***D. quercifolia***

Figure 6: *D. cochleata*

4. Discussion

Mader (1996) stated gram-positive bacteria are not considered pathogenic in reptiles and are common inhabitants, especially of the skin. However, some gram-positive bacteria, *Staphlyococci* are usually pathogenic, has a 95 % correlation. Similarly, gram-negative bacteria are the most common bacterial pathogens and it was in confirmation with the present study, where *S.aureus* and *E.coli* clinical isolates from natural infection of skin rot condition in these pythons. Occurrence of bacterial infections affecting snakes as found out in this study was in agreement with the findings of Marcus (1981) who quoted that culture for ruling out bacteria, should be taken from the lesions that were not responding to symptomatic therapy, such as daily bathing, use of antiseptic solution, *etc.* Encountering various infections affecting the skin (Beynon and Cooper, 1991) detailed on the occurrence of ventral dermal necrosis or scale rot condition, which was specifically seen in snakes. However, though the *Pseudomonas* sp. and *Aeromonas* sp. were often related with such conditions, the current study in snakes revealed evidences of *E. coli* and *S. aureus* organism in the culture. Occurrence of bacterial infections affecting skin (Wallach and Boever, 1983) on the incidence of infections with *Micrococcus* sp. and *Bacterium* sp. which were isolated from the sub-cutaneous abscess in reptiles and also emphasized that the differential diagnosis in such cases should associate the detailed microbacterial infections and culture was required for specific differentiation, as carried out with in this study programme. Encountering the *E. coli* and *S. aureus* organism during the culture tests carried out was in agreement with the report furnished by Rosenthal (1997), isolated *Pseudomonas aeruginosa, Corynebacterium xerosis, Streptococcus* sp. and *Citrobacter freundii* from a necrotizing skin lesion of a rattle snake.

In all the cases that revealed evidence of bacterial infections affecting the skin in snakes, the antibiotic sensitivity test revealed enrofloxacin and ciprofloxacin as sensitive drugs. Usage of gentamicin in bacterial infection of snakes was recommended by Shah *et al.* (1991). Apart from the sensitivity to *E. coli* and *S. aureus*, organism as found in this study. Shah *et al.* (1991) opined that gentamicin was found to be more sensitive to *Pseudomonas aeruginosa* organisms also in pythons. Lawrence (1987) also opined that antibiotic therapy as with gentamicin had to be initiated at the dose rate of 2.5 mg/kg body weight every three days and this usually proved effective. Usage of fluoroquinolone compounds, which were also the sensitive drugs to the bacterial infection in the snakes under study, was quoted by Devasena and Adilaxmamma (2001). They also opined that in addition to enrofloxacin, other drugs suggested in case of bacterial dermatitis were amikacin and ceftazidime. Administration of fluoroquinolones was further recommended in snakes by Fowler (1986). Mader (1996) quoted that fluroquinolones (enrofloxacin and ciprofloxacin), aminoglycosides, cephalosporin, metronidazole and pencillin were the antimicrobials, recommended for treatment of common bacterial isolates pathogenicity and suggested aminoglycosides for *E.coli* and fluroquinolones for *S.aureus* coagulase positive organism was in confirmation to this study.

Various studies have been reported on the antibacterial activity of fern extracts. Nayar *et al.* (2013) depicted the antibacterial activity of terrestrial fern, *Lygodium fexuosum* (L.) Sw. against multidrug resistant enteric and uropathogenic bacteria. Similarly, Thomas *et al.* (2012) stated the *in vitro* antibacterial activity of *Acrostichum aureum* Linn. against isolated skin pathogens of human beings. Mithraja *et al.* (2012) isolated urinary tract bacterial pathogens for antibacterial activity, using *D. quercifolia*. Parihar *et al.* (2010)

performed *in vitro* antibacterial activity of some important pteridophytes fronds against isolated skin pathogen. However, the present study in captive pythons is the first reported study of clinical isolates against skin rot conditions against recurrent multidrug resistant drug infection and suitable ethnomedical values of pteridophytes with these multi resistant drugs.

It is to be recommended that the substrate in the floor may be treated accordingly inside the serpentine cage, in order to avoid the recurrence of skin infections in case of snakes. If not, there were more probabilities for the recurrence of skin infections and snakes must be kept at a relatively constant temperature during antibiotic therapy after performing antibiotic sensitivity test (ABST) with rotational therapy of allopathic and ethnomedicine to overcome bacterial resistant in these serpentine species.

5. Conclusion

Reptiles are ectothermic poikilotherms, where captive rearing of the species favor stressful conditions, harbor various bacterial infections producing skin and mouth rot conditions in these serpentine species, which are a common problem prevailing in zoo conditions. Overcome antibiotic resistance, herbal therapy alternative to allopathic medications holds good in treatment and control measures.

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Conflict of interest

We declare that we have no conflict of interest.

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