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Full Length Research Paper

In vitro anti-fungal and anti-bacterial activity of *Drymoglossum piloselloides* L. Presl. against several fungi responsible for Athlete's foot and common pathogenic bacteria

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***Drymoglossum piloselloides* (DP) is a small epiphytic fern commonly seen on trunks of older trees. The antimicrobial activity of water, ethanol and chloroform extracts of DP was tested against *Trichophyton rubrum* ATCC 40051 and *Trichophyton mentagrophytes* ATCC 40004 which are the two most commonly cause of Athlete's foot, *Candida albicans* ATCC 14053, *Candida tropicalis* ATCC 14056, *Microsporum canis* (clinical isolate) and *Aspergillus fumigatus* ATCC 14109. The chloroform and ethanol extracts only had mild activity against the *Trichophyton* spp. and the water extract was devoid of any activity. The anti-fungal activity was statistically less potent than griseofulvin and fluconazole or itraconazole. The anti-bacterial activity of DP extracts was also tested against four bacterial strains using the disc diffusion and microdilution methods. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619, *Bacillus subtilis* and *Salmonella enteritidis* (clinical isolates). The antibacterial activity against gram positive bacteria was detected only in water extract minimum inhibitory concentration (MIC) 12.5 mg/ml against *S. aureus*. The minimum bactericidal concentrations of 25 mg/ml against *S. aureus*. This antimicrobial activity was compared to standard antibiotics (ampicillin and chloramphenicol). The water extract had statistically less potent anti-bacterial activity when compared to ampicillin and chloramphenicol. The anti-fungal and anti-bacterial activity of DP was minimal this may be due to the testing of crude extracts. Future studies of purified or semi purified samples may produce better results and may potentially be used in the treatment of Athlete's foot and the related secondary bacterial infections.**

Key words: *Drymoglossum piloselloides*, antifungal, antibacterial, *in vitro.*

INTRODUCTION

Drymoglossum piloselloides or "Paku Sisek Naga", "Paku Sakat Ribu-ribu" in Malay of the Polypodiaceae family is a small epiphytic fern is common in the wild of many Asian countries, commonly seen on trunks of older frees. The fern leaves (lamina) are thick, simple and numerous. This fern commonly mistaken for *Pyrrosia adnascens* (Sw.) Ching which is used in Chinese traditional medicine (Li, 1979).

Athlete's foot is the infection of the skin of the foot and most commonly due to *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Adejumo and Bamidele, 2009). Athlete's foot spreads easily by touching the toes or feet of an infected person. But most often, transmission is by walking barefoot near hot and humid area such as swimming pools or in locker rooms (Jain et al., 2010). Secondary bacterial infections also commonly observed in Athlete's foot that is not treated immediately.

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Bacteria such as *Staphylococcus aureus*, *Streptococcus* spp. and *Bacillus* spp. are common opportunistic pathogens that may cause secondary infections (Crum-Cianflone, 2008).

D. piloselloides contains sterols/triterpenes, phenols, flavonoids, tannin as major constituents (Dalimartha, 2008). It is used for parotitis, Tuberculosis, dysentery and urinary tract infections (Dalimartha, 2008). In Malaysia, *D. piloselloides* is used for the treatment of superficial bacterial infections, cough and gonorrhea (Abdul Rahman, 1996). To our knowledge, there is no previous study on the biomedical/medicinal properties of *D. piloselloides*. Therefore, the objective of this current study is to evaluate the antimicrobial property of *D. piloselloides* against several common bacteria and fungi.

MATERIALS AND METHODS

Plant material

Leaves of *D. piloselloides* were collected in the State of Selangor (Western Malaysia) and identified. A voucher specimen has been deposited at the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia. The leaves were washed, oven dried at 45°C overnight, then grounded into powder form and extraction using Soxhlet apparatus with chloroform, ethanol or distilled water as solvent for 12 h (Zakaria et al., 2005a). The solvent was concentrated under vacuum using a rotary evaporator (Zakaria et al., 2005b). The yields were 2.1, 4.5 and 10.2%, respectively. The solid residues were stored at -20°C prior to use.

Screening for antimicrobial activity

Sterile 6.0 mm diameter blank discs (Oxoid, UK) were used to impregnate different dilutions of the extracts as follow: 0, 10, 20 and 30 mg/ml extract. Discs were stored at –5°C prior to use. Tests were performed by the disc diffusion method (Somchit et al., 2010). Fungi: *Candida albicans* ATCC 14053, *Candida tropicalis* ATCC 14056, *T. rubrum* ATCC 40051 and *T. mentagrophytes* ATCC 40004, *Microsporum canis* (Clinical isolates and identified at the Department of Pathology and Microbiology, Universiti Putra Malaysia), *Aspergillus fumigatus* ATCC 14109 used in this study were from. Detailed method was published previously (Somchit et al., 2003).

Bacteria: *Escherichia coli* ATCC 25922 (American Type Culture Collection), *S. aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 49619, *Bacillus subtilis* and *Salmonella enteritidis* (Clinical isolates and identified at the Department of Pathology and Microbiology, Universiti Putra Malaysia). Detailed method was published previously (Somchit et al., 2003).

Commercial antibiotics disc which consists of chloramphenicol (30 mg/ml) and ampicillin (30 mg/ml) were used as reference. Standard antifungal drug griseofulvin, itraconazole and fluconazole diluted in dimethyl sulfoxide were impregnated onto sterile blank discs with the concentration of 30 mg/ml, respectively.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The extracts which had antimicrobial activity in the disc diffusion assay were subjected to the MIC assay based on the M27-T method of the National Committee for Clinical Laboratory Standards (Espinel-Ingroff, 1995). Briefly, for MIC of antibacterial activity, Muller Hinton broth (Difco Lab.) was used as culture medium. Ampicillin (Sigma) was used as antibiotic standard.

These experiments were repeated three times and results were expressed as an average value. The MIC endpoint readings were made visually. The MIC is considered the lowest drug concentration of antimicrobial agent inhibiting the total growth of microorganisms. The lowest concentration yielded no viable organisms is recorded as the MBC.

Statistical analysis

Results are expressed as mean ± S.D. and all data obtained were analysed using one-way ANOVA with Duncan post Hoc test using SPSS v. 17. Results with $p<0.05$ is considered significant.

RESULTS AND DISCUSSION

Table 1 expresses the results of the anti-bacterial activity of 3 extracts of *D. piloselloides*. Water extraction revealed potent anti-bacterial activity against all Gram positive and negative bacteria. However, this activity was more prominent against the Gram positive bacteria. These results were statistically similar to the standard anti-biotics, chloramphenicol and ampicillin. The antibacterial activity of extracts is illustrated in Figure 1, while the MIC and MBC data are presented in Table 2. The water extract had less potent anti-bacterial activity when compared to ampicillin. Findings from current study revealed that *D. piloselloides* had anti-bacterial activity.

Gram positive bacteria were more susceptible to *D. piloselloides* compared to gram negative bacteria. These results were similar to other medicinal plants that we tested such as *Ardisia crispa* (Somchit et al., 2011), *Acalypha indica* (Somchit et al., 2010) and *Cassia alata* (Somchit et al., 2003). The mechanism of action of extracts of *D. piloselloides* maybe due to the phytochemicals identified. *D. piloselloides* contains primarily sterols/triterpenes, phenols, flavonoids, tannin (Dalimartha, 2008). According to Shah et al. (1988), these major constituents are commonly identified in plants with high anti-microbial (antibacterial and anti-fungal) activity.

The chloroform and ethanol extracts only had mild activity against the *Trichophyton* spp. and the water extract was devoid of any anti-fungal activity (Table 3). The anti-fungal activity was statistically less potent than all standard anti-fungal drugs (griseofulvin and fluconazole or itraconazole) used. Figure 2 shows the anti-fungal activity of only chloroform extract of *D. piloselloides*. Commercial anti-fungal and anti-bacterial drugs may cause many adverse drug reactions such as severe hepatotoxicity (Hautekeete, 1995; Somchit et al., 2002; Somchit et al., 2004) which is life-threatening. Therefore, herbal remedy is the natural way for treating infections which is safer and it is estimated that 80% of the world's population presently uses herbal medicine for some aspect of primary health care (WHO, 2008).

Table 1. Antibacterial activity of *Drymoglossum piloselloides* extracts and standard antibiotics.

Samples	Conc. (mg/ml)	Bacteria				
		E. c	***S. e***	***S. a***	***S. p***	***B. s***
Water	10	9.2 ± 0.3^{a}	6.2 ± 0.1^{a}	11.6± 0.7^{b}	10.1± 0.2^{b}	10.2± 0.9^{b}
	20	11.6 ± 1.5^{b}	8.1 ± 0.2^{b}	19.2± 2.5^{c}	12.1± 1.5^{c}	16.4± 1.5$_{c}$
	30	15.6 ± 1.1^{c}	8.3 ± 0.7^{b}	23.6± 2.0^{d}	18.2 ± 3.1^{de}	19.2± 1.2^{c}
Ethanol	10	-	-	7.3 ± 0.5^{a}	6.1 ± 0.0^{a}	7.1 ± 0.5^{a}
	20	-	-	8.1 ± 0.4^{a}	7.2 ± 0.3^{a}	7.2 ± 0.4^{a}
	30	9.1 ± 0.5^{a}	-	11.1± 0.2^{b}	9.1 ± 0.6^{b}	8.1 ± 1.0^{a}
Chloroform	10	-	-	-	-	-
	20	-	-	-	-	-
	30	-	-	7.2 ± 0.1^{a}	-	7.0 ± 0.1^{a}
Ampicillin	30	20.1 ± 1.2^{d}	23.1 ± 1.3^{d}	26.7 ± 3.2^{de}	16.3± 1.1^{d}	20.3± 1.9^{d}
Chloramphenicol	30	15.2 ± 0.3^{c}	17.2 ± 0.5^{c}	30.2± 3.9^{e}	20.2± 1.3^{e}	17.1± 2.6^{c}

E.c, *Escherichia coli*; S.e, *Salmonella enteriditis*; S.a, *Staphylococcus aureus*; S.p, *Streptococcus pneumoniae*; B.s, *Bacillus subtilis.* Values are mean ± sd (mm) of 3 separate experiments. – No inhibition zone. $^{a-e}$ Means within a column with different superscripts differ significantly ($p < 0.05$) using ANOVA and Duncan multiple post test.

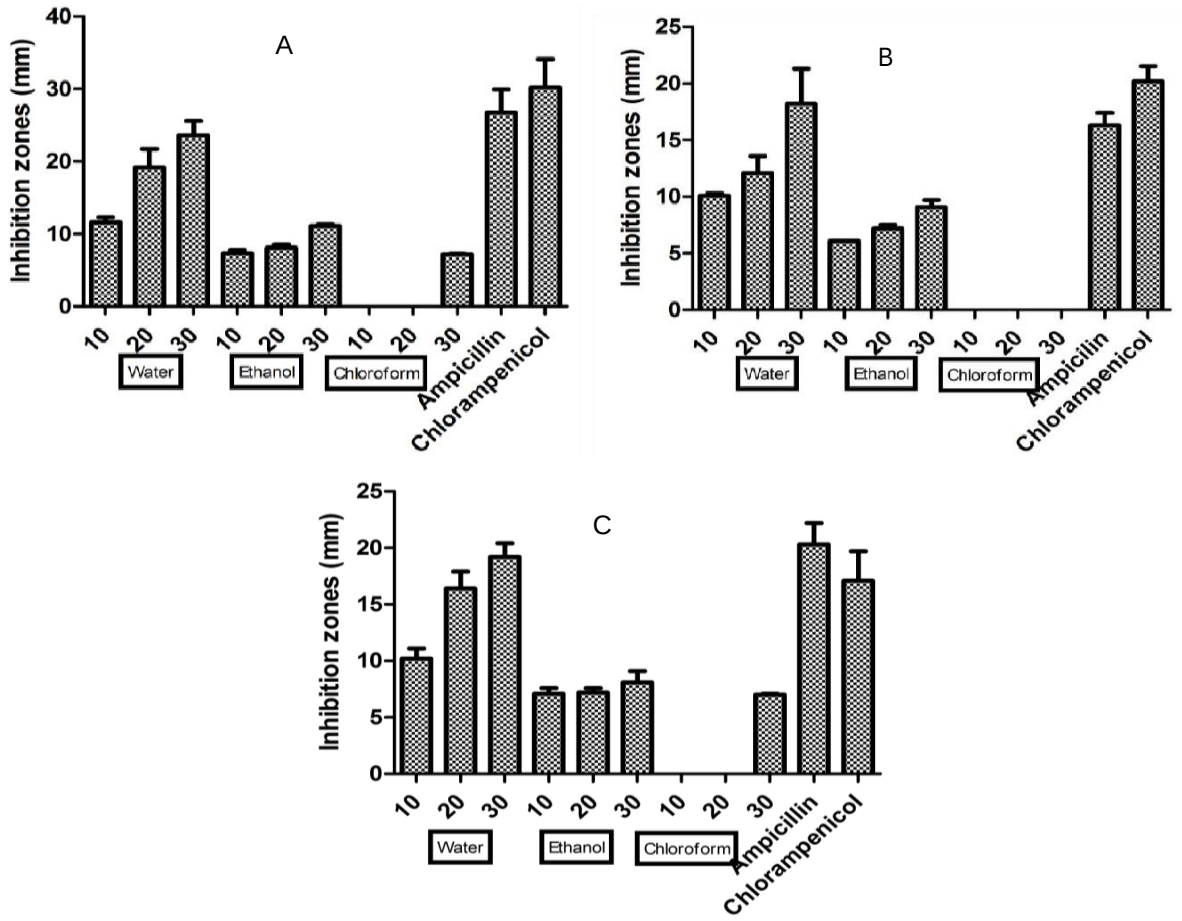

Figure 1. Antibacterial activity of *Drymoglossum piloselloides* extracts (A) Against *Staphylococcus aureus* (B) Against *Streptococcus pneumonia* (C) Against *Bacillus subtilis*

B

Table 2. MIC and MBC of different solvent extracts of *Drymoglossum piloselloides.*

Bacteria	MIC (mg/ml)[a]				MBC (mg/ml)[a]		
	Water	Ethanol	Chloroform	Ampicillin	Water	Ethanol	Chloroform
S. aureus	0.8	3.1	-	0.8	3.1	25	-
B. subtilis	1.6	12.5	-	6.2	3.1	25	-
S. enteridiidis	-	-	-	6.2	-	-	-
E. coli	12.5	25	-	12.5	100	>100	-
S. pneumoniae	1.6	12.5	-	1.6	6.2	50	-

[a] Average of 3 separate assay and - not determined.

Table 3. Antifungal activity of *Drymoglossum piloselloides* extracts and standard antifungal drugs.

Samples	Conc. mg/ml)	Fungi					
		T. r	*T. m*	*C. a*	*C. t*	*M. c*	*A. f*
Water	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-
Ethanol	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	-	-
Chloroform	10	7.2 ± 0.2	-	-	-	-	-
	20	9.6 ± 0.3	-	-	-	-	-
	30	10.2 ± 0.3	12.3 ± 1.2	-	-	-	-
Griseofulvin	30	7.2 ± 0.3	6.5 ± 0.3	-	-	9.6 ± 0.2	-
Fluconazole	30	16.2 ± 0.5	12.5 ± 0.2	21.4 ± 1.9	12.1 ± 1.2	13.8 ± 0.9	-
Itraconazole	30	22.2 ± 1.6	16.5 ± 0.8	29.0 ± 3.1	14.0 ± 1.6	19.8 ± 1.1	20.5 ± 0.2

T.r Trichophyton rubrum; T.m Trichophyton mentagrophytes C.a, *Candida albicans*; C.t, *Candida tropicalis*; M.c, *Microsporum canis*; A.f, *Aspergillus fumigatus*. Values are mean ± sd (mm) of 3 separate experiments. – No inhibition zone.

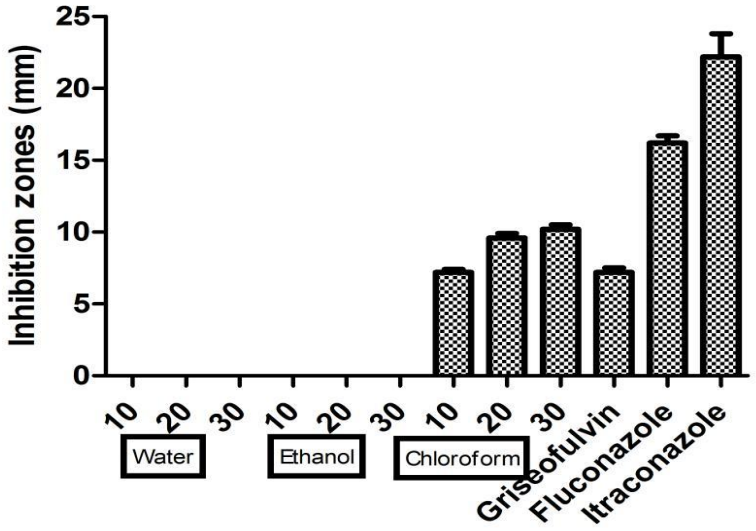

Figure 2. Antifungal activity of *Drymoglossum piloselloides* extracts against *Trichophyton rubrum.*

The anti-fungal activity of *D. piloselloides* was minimal, and this may be due to the testing of crude extracts. Future studies of purified or semi purified samples may produce better results and may potentially be used in the treatment of Athlete's foot, other fungi or bacterial infections.

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