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Full Length Research Paper

Anti-fungal activity of *Ardisia crispa* (Thunb.) A.DC. against several fungi responsible for athlete's foot

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The body normally hosts a variety of saprotrophic micro-organisms that may cause infection. Athlete's foot causes scaling, flaking and itching of the affected skin. Blisters and cracked skin may also occur, leading to exposed raw tissue, pain, swelling and inflammation. Secondary bacterial infection can accompany the fungal infection. This work examined the anti-fungal activity of *Ardisia crispa* (AC) against common fungi that cause Athlete's foot and several other pathogenic fungi. The antimicrobial activity of water, ethanol and chloroform extracts of AC was tested against fungal strains using the disc diffusion method. This antimicrobial activity was compared to standard antifungal drugs (griseofulvin, fluconazole and itraconazole). Results revealed that chloroform extract of AC had potent anti-fungal activity against *Trichophyton rubrum* ATCC 40051 and *Trichophyton mentagrophytes* ATCC 40004 which are the two most commonly cause of Athlete's foot. Moderate activity was observed against *Candida albicans* ATCC 14053, *Candida tropicalis* ATCC 14056, *Microsporum canis* (clinical isolates and identified at the Department of Pathology and Microbiology, Universiti Putra Malaysia) and *Aspergillus fumigatus* ATCC 14109. The ethanol extract only had mild activity against the *Candidia spp* and the water extract was devoid of any activity. The anti-fungal activity of chloroform extract was statistically more potent than griseofulvin but less potent than fluconazole and itraconazole. Findings from current study support the use of AC in traditional medicine for the treatment of various fungal infections and may potentially be used in the treatment of athlete's foot.

Key words: *Ardisia crispa*, anti-fungal, athlete's foot.

INTRODUCTION

The genus *Ardisia* is the largest in the family Myrsinaceae and contain approximately 500 species of evergreen shrubs and trees are found throughout the tropical and subtropical regions of the world (Chen and Pipoly, 1996). *Ardisia crispa* and several other species are indigenous to Malaysia. It is locally known as "Akar Beluloh" or "Mata Pelanduk" in Malaysia and Indonesia. It is an evergreen shrub that grows to 1.2 to 2 m tall. It flowers from June to July, and the seeds ripen from September to December. Cultivars of three species (*A. crispa* A. D.C., *Ardisia japonica* Blume, and *Ardisia crenata* Sims) have been developed through breeding and subsequent selection in Japan (Kobayashi and de Mej´ıa, 2005). Although many *Ardisia* species have been used as sources of food and medicine, the detailed record on such usage is scarce and limited. The body normally hosts a variety of saprotrophic micro-organisms that may cause infection. Athlete's foot is a skin fungal infection and is medically referred to as 'tinea pedis'. Athlete's foot causes scaling, flaking and itching of the affected skin. Blisters and cracked skin may also occur, leading to exposed raw tissue, pain, swelling and inflammation. Therefore, the objective of this present investigation is to evaluate the anti-fungal properties of *A. crispa* (AC) extracts (Burkill, 1996).

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Table 1. Antifungal activity of *Ardisia crispa* extracts and standard antifungal drugs.

Samples	Concentration (mg/ml)	Fungi					
		T.r	T.m	C.a	C.t	M.c	A.f
Water	10	6.2±0.1[a]	6.5±0.2[a]	-	-	-	-
	20	7.6±0.2[a]	6.8±0.3[a]	-	-	-	-
	30	7.2±0.5[a]	10.2±0.3[b]	-	-	-	-
Ethanol	10	9.2±1.0[b]	7.2±0.2[a]	-	-	-	-
	20	11.6±0.9[c]	9.6±0.3[b]	-	-	-	-
	30	12.7±2.3[c]	10.2±0.3[b]	6.2±0.1[a]	-	-	-
Chloroform	10	11.2±0.9[c]	10.3±0.2[b]	7.2±0.2[a]	-	-	-
	20	13.6±1.3[d]	10.6±1.2[b]	9.6±0.3[b]	-	-	-
	30	16.2±0.6[e]	12.2±0.3[c]	10.2±0.3[b]	7.0±0.6[a]	7.3±0.1[a]	-
Griseofulvin	30	7.2±0.3[a]	6.5±0.3[a]	-	-	9.6±0.2[a]	-
Fluconazole	30	16.2±0.5[e]	12.5±0.2[c]	21.4±1.9[c]	12.1±1.2[b]	13.8±0.9[b]	-
Itraconazole	30	22.2±1.6[f]	16.5±0.8[d]	29.0±3.1[d]	14.0±1.6[b]	19.8±1.1[c]	20.5±0.2

T.r: *Trichophyton rubrum*, T.m: *Trichophyton mentagrophytes:* C.a, *Candida albicans*: C.t: *Candida tropicali*,; M.c: *Microsporum canis* and A.f, *Aspergillus fumigatus*. Values are mean ± sd (mm) of 3 separate experiments. No inhibition zone. [a-f] Means within a column with different superscripts differ significantly (p≤0.05) using ANOVA and Duncan multiple post test.

MATERIALS AND METHODS

Plant materials

Leaves of AC 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. Leaves of AC were washed, oven dried at 45°C overnight, then grounded into powder form and extraction using Soxhlet apparatus with either chloroform, ethanol or distilled water as solvent for 12 h (Zakaria et al., 2005). The solvent was concentrated under vacuum using a rotary evaporator. The yields were 0.75, 1.3 and 2.9% 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., 2004a). Fungi: *Candida albicans* ATCC 14053, *Candida tropicalis* ATCC 14056, *Trichophyton rubrum* ATCC 40051 and *Trichophyton 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 that was published previously (Somchit et al., 2003). Standard antifungal drugs of griseofulvin, itraconazole and fluconazole diluted in dimethyl sulfoxide were impregnated onto sterile blank discs with the concentration of 30 mg/ml respectively.

Statistical analysis

Data was expressed as mean ± SD and analysed using analysis of variance. When interactions were significant, Duncan multiple post-test was performed. Values of p≤0.05 was considered significant.

RESULTS AND DISCUSSION

The antifungal activity of AC is in Table 1. The antifungal activity was positive against *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Candida albicans*, however this activity less potent than standard new antifungal drugs fluconazole and itraconazole. Interestingly, antifungal activity of AC against *T. rubrum* (Figure 1) and *T. mentagrophytes* (Figure 2) were more potent than griseofulvin, a commonly used antifungal drug. This plant has been used for generations to treat superficial fungal and several other bacterial infections (Burkill, 1996). This short investigation supports the use of this herb in traditional medicine. Clinical microbiologists are interested in the topic of antimicrobial plant extracts because firstly, it is very likely that these phytochemicals/herbs will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans (Cowan, 1999). The results form this current study revealed antimicrobial activity similar to some commercial antifungal agents. Interestingly, crude extracts of AC were potent against several fungi. This plant contains high levels of triterpines or terpenoids (Wang et al., 1992) and many studies have proven terpenoids are active against bacteria, fungi, viruses and protozoa (Cowan, 1999). This may be the possible mechanism of anti-microbial activity of AC. Indeed, many plants in Malaysia which we have tested have potent anti-microbial activity (Somchit et al., 2003,

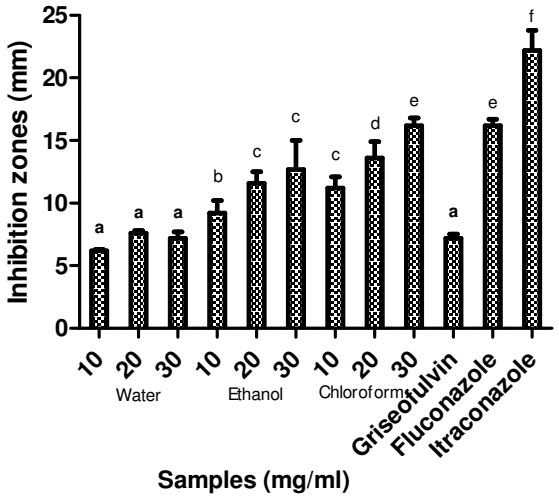

Figure 1. Antifungal activity of *Ardisia crispa* against *Trichophyton rubrum.* Values are mean ± sd (mm) of 3 separate experiments. [a-f] Means within a column with different superscripts differ significantly (p≤0.05) using ANOVA and Duncan multiple post test.

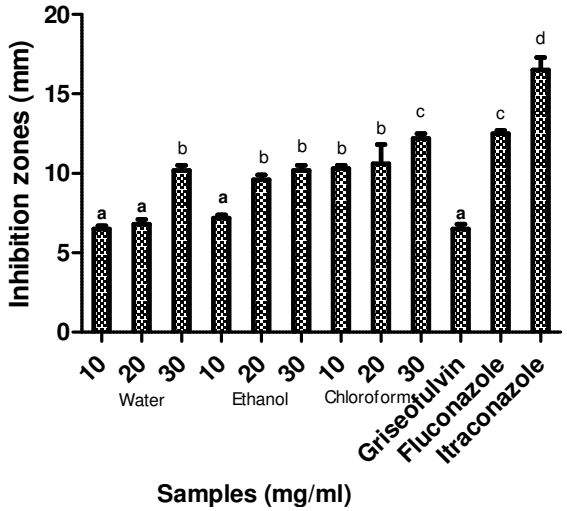

Figure 2. Antifungal activity of *Ardisia crispa* against *Trichophyton mentagrophytes.* Values are mean ± sd (mm) of 3 separate experiments. [a-d] Means within a column with different superscripts differ significantly (p≤0.05) using ANOVA and Duncan multiple post test.

2004a; Reezal et al., 2003; Elysha et al., 2005). The use of commercial synthetic anti-fungal drugs such as azole (fluconazole and itraconazole) may lead to many adverse drug reactions including rashes, hepatotoxicity (liver damage) and gastro-intestinal disturbances (Somchit et al., 2004b). These may limit their use; hence, traditional/ complementary medicine which is natural and less toxic to the system is preferred. Although athlete's foot causes only scaling, flaking and itching of the affected skin, with secondary bacterial infection, the sports performance of a person will be reduced.

Findings from current study support the use of AC in traditional medicine for the treatment of various fungal infections and may potentially be used in the treatment of athlete's foot. Studies are ongoing to identify these active phytochemicals and further elucidating the mechanism of action of this herb.

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