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In vitro antidermatophytic activity and cytotoxicity of extracts derived from medicinal plants and marine algae

N.W. Sit [a,*], Y.S. Chan [a], S.C. Lai [a], L.N. Lim [a], G.T. Looi [a], P.L. Tay [a], Y.T. Tee [a], Y.Y. Woon [a], K.S. Khoo [b], H.C. Ong [c]

[a] Department of Biomedical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Bandar Barat, 31900 Kampar, Perak, Malaysia
[b] Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Bandar Barat, 31900 Kampar, Perak, Malaysia
[c] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

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ABSTRACT

Objectives. – This study was conducted to evaluate the antidermatophytic activity of 48 extracts obtained from medicinal plants (*Cibotium barometz*, *Melastoma malabathricum*, *Meuhlenbeckia platyclada*, *Rhapis excelsa*, *Syzygium myrtifolium*, *Vernonia amygdalina*) and marine algae (*Caulerpa sertularioides*, *Kappaphycus alvarezii*) against *Trichophyton rubrum* and *Trichophyton interdigitale* (ATCC reference strains), and the cytotoxicity using African monkey kidney epithelial (Vero) cells. Active plant extracts were screened for the presence of phytochemicals and tested against clinical isolates of *Trichophyton tonsurans*.

Methods. – Six different extracts (hexane, chloroform, ethyl acetate, ethanol, methanol and water) were obtained from each plant or algae sample using sequential solvent extraction. The antidermatophytic activity for the extracts was assessed using a colourimetric broth microdilution method. The viability of Vero cells was measured by Neutral Red uptake assay.

Results. – All the extracts (except the water extracts of *V. amygdalina*, *C. sertularioides* and *K. alvarezii*) showed antidermatophytic activity against *Trichophyton* spp. The minimum fungicidal concentration (MFC) ranges for the plant extracts against *T. rubrum* and *T. interdigitale* are 0.0025–2.50 and 0.005–2.50 mg/mL, respectively. The algae extracts exhibited lower potency against both species, showing MFC ranges of 0.08–2.50 and 0.31–2.50 mg/mL, respectively. The ethanol and methanol extracts from the leaves of *R. excelsa*, and the methanol and water extracts from the leaves of *S. myrtifolium* were highly active (MFC < 0.1 mg/mL) and with high selectivity indices (SI > 2.8) against reference strains of *T. rubrum* and *T. interdigitale*, and most of the clinical isolates of *T. tonsurans*. Phytochemical analysis indicates the presence of alkaloids, anthraquinones, flavonoids, saponins, tannins, phenolics and triterpenoids in the extracts.

Conclusions. – The medicinal plant extracts exhibited stronger antidermatophytic activity compared to the algae extracts. The leaves of *R. excelsa* and *S. myrtifolium* are potential sources of new antidermatophytic agents against *Trichophyton* spp.

1. Abbreviations

ATCC	American Type Culture Collection
CC_{50}	50% cytotoxic concentration
CLSI	Clinical and Laboratory Standards Institute
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration

2. Introduction

Dermatophytosis is known as "ringworm" or "tinea" due to the classical appearance of a circular lesion with an active border that develops on the skin of the infected person [1]. The disease is clinically classified, based on the sites of infection, as tinea pedis (feet), tinea cruris (groin), tinea corporis (trunk), tinea capitis (scalp), tinea faciei (face), tinea unguium/onychomycosis (nails) or tinea manuum (hands). Among the anthropophilic dermatophytes, *Trichophyton rubrum* is the leading pathogen causing tinea infections on skin and nails [2] while *Trichophyton interdigitale* (formerly known as *Trichophyton mentagrophytes*) ranks second

* Corresponding author.
E-mail address: sitnw@utar.edu.my (N.W. Sit).

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but is the most common infection-causing agent in some countries such as Croatia [3] and Venezuela [4]. In Malaysia, *Trichophyton tonsurans* is the most prevalent species among dermatophyte-induced onychomycosis in Hospital Kuala Lumpur, the largest government tertiary referral hospital in the country [5].

Dermatophytosis affects 20–25% of the world population [6], thus it poses a serious public health problem. The quality of life for the patients is sometimes impaired by the physical symptoms of dermatophytosis and associated psychological effects [7]. Several classes of antifungal drugs, such as imidazoles, triazoles and allylamines are available for the treatment of dermatophytosis. However, these drugs often have side effects, have a limited spectrum of activity and are costly. Resistance of *T. rubrum* to antifungal drugs terbinafine and griseofulvin has been reported [8]. These limitations justify the search for natural antifungal agents, which are safer, with higher efficacy or novel modes of action.

According to the new chemical entities registered for clinical use from 1981 to 2014, more than 60% of small-molecule anti-infective drugs are natural products or derivatives or mimics of natural products [9]. Terrestrial plants and marine algae are rich sources of bioactive molecules for drug discovery and development. Biosynthetic pathways in plants and marine algae enable them to produce an array of secondary metabolites with diverse chemical structures for protection against predations and infections, and to enhance the chances of survival in the harsh environments. The secondary metabolites are extracted using solvents of different polarity. Less polar solvents such as hexane, chloroform or ethyl acetate are usually used to extract alkaloids, fatty acids, flavonoids and terpenoids while more polar solvents such as ethanol, methanol or water yield anthocyanins, polypeptides, polyphenols, quinones, saponins, tannins and terpenoids [10].

Lopez et al. [11] have compiled a list of antidermatophytic compounds isolated from non-volatile natural extracts belonging to several classes of secondary metabolites, i.e. alkaloids, coumarins, flavonoids, lignans, quinones, saponins and tannins. This highlights the potential of medicinal plants and marine algae for discovery of new antidermatophytic agents. This study was conducted in order to evaluate the *in vitro* antidermatophytic activity of 48 extracts obtained from six medicinal plants (*Cibotium barometz*, *Melastoma malabathricum*, *Meuhlenbeckia platyclada*, *Rhapis excelsa*, *Syzygium myrtifolium*, *Vernonia amygdalina*) and two marine algae (*Caulerpa sertularioides*, *Kappaphycus alvarezii*) from Malaysia against *T. rubrum* and *T. interdigitale*, and to test their cytotoxicity using African monkey kidney epithelial (Vero) cells. Selected active plant extracts were screened for phytochemicals and tested against clinical isolates of *Trichophyton tonsurans*.

3. Materials and methods

3.1. Medicinal plant and marine algae samples

Six species of medicinal plants and two species of marine algae were investigated in this study. The families, common names, parts used, collection sites and specimen voucher reference numbers are listed in Table 1. The plant samples were identified by Professor Hean Chooi Ong, an ethnobotanist affiliated with Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia while the identity for the two marine algae was confirmed by Dr. Kong Soo Khoo from the Faculty of Science, Universiti Tunku Abdul Rahman, Malaysia.

3.2. Preparation of sample extracts

After thorough cleaning, the fresh plant samples and the freeze-dried algae samples were extracted sequentially using hexane, chloroform, ethyl acetate, ethanol, methanol and distilled water. The maceration process was performed in two cycles for each solvent at room temperature, with agitation at 120 rpm. The water extract was lyophilised while other extracts were rotary-evaporated to dryness at 40 °C. All the dried extracts were kept at −20 °C prior to bioassay.

3.3. In vitro antidermatophytic assay

Two species of dermatophytes, *T. rubrum* (ATCC®28,188™) and *T. interdigitale* (ATCC®9533™) were used in the study. *T. rubrum* and *T. interdigitale* were grown at 30 °C for 5 days on oatmeal agar and potato dextrose agar respectively prior to the antidermatophytic assay. The inoculum preparation and incubation conditions for both species were carried out according to the M38-A2 guidelines published by Clinical Laboratory Standards Institute [12]. A colourimetric broth microdilution method using *p*-iodonitrotetrazolium chloride as the growth indicator [13] was used to determine the minimum inhibitory concentration (MIC) of each extract towards the dermatophytes. The final concentrations tested for each extract were ranged from 0.02 to 2.50 mg/mL, obtained by two — fold serial dilution in Roswell Park Memorial Institute — 1640 medium. Dilutions beyond 0.02 mg/mL were performed whenever necessary. Positive (griseofulvin antibiotic), growth (dermatophyte only), negative (extract only) and medium controls were included in each 96-well microplate. The minimum fungicidal concentration (MFC) of active extracts was subsequently determined by spread plate method using potato dextrose agar. The assay was conducted in triplicate.

Table 1
Details of medicinal plants and marine algae investigated.

Species	Family	Common name	Part used	Collection site	Specimen voucher reference number
Medicinal plants					
Cibotium barometz (L.) J. Sm	Cibotiaceae	Golden chicken fern	Rhizome hairs	Cameron Highlands, Pahang	UTAR/FSC/12/009
Melastoma malabathricum L	Melastomataceae	Malabar melastome	Leaves	Kampar, Perak	UTAR/FSC/13/007
Muehlenbeckia platyclada (F.J. Müll.) Meisn	Polygonaceae	Ribbon bush, Centipede plant	Stems	Cameron Highlands, Pahang	UTAR/FSC/12/011
Rhapis excelsa (Thunb.) Henry	Arecaceae	Broadleaf lady palm	Leaves	Kampar, Perak	UTAR/FSC/12/008
Syzygium myrtifolium Walp	Myrtaceae	"Kelat paya"	Leaves	Pagoh, Johor	UTAR/FSC/14/001
Vernonia amygdalina Delile	Compositae	Bitter leaf	Leaves	Ipoh, Perak	UTAR/FSC/12/004
Marine algae					
Caulerpa sertularioides (S. G. Gmelin) M. Howe	Caulerpaceae	Green feather alga	Whole	Port Dickson, Negeri Sembilan	UTAR/FSC/15/002
Kappaphycus alvarezii (Doty) Doty ex P. C. Silva	Solieriaceae	Elkhorn sea moss	Whole	Semporna, Sabah	UTAR/FSC/15/004

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