

ANTIFUNGAL ACTIVITY OF *HYPNEA PANNOSA* J. AGARDH

Muhammad Ashraf & Salman Ahmed

Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

Hypnea pannosa J. Agardh (methanol extract of the whole alga) was subjected to antifungal screening. Extract showed good activity against *Trichophyton longifusus*, low activity against *Candida glabrata* and *Microsporum canis*. The extract was found to be inactive against *Fusarium solani*.

Keywords: *Candida*, *Hypnea*, *Microsporum*, *Trichophyton*, Antifungal Activity, Red Algae, Pakistan.

INTRODUCTION

Hypnea pannosa J. Agardh is a red marine alga, belongs to family Hypneaceae (Valeem & Shameel 2012). *H. pannosa* is distributed in Atlantic Islands: (Cape Verde Islands), North America: (Gulf of California and Mexico), Central America: (Costa Rica, El Salvador, México-Pacific), Nicaragua, Panama; South America: Brazil, Galápagos Islands; Africa: Eritrea, Madagascar, Mauritius, Senegal, Tanzania; Indian Ocean Islands: Aldabra Islands, Chagos Archipelago, Christmas Island, Diego Garcia Atoll, Laccadive Islands, Maldives, Réunion, Seychelles; South-west Asia: Bangladesh, India, Iran, Oman, Pakistan, Sri Lanka, Yemen; Asia: China, Japan, Korea, Taiwan; South-east Asia: Indonesia, Philippines, Singapore, Thailand, Vietnam; Australia and New Zealand: Queensland, Western Australia; Pacific Islands: Federated States of Micronesia, Fiji, French Polynesia, Hawaiian Islands, Mariana Islands, Samoa, Samoan Archipelago, Solomon Islands (Guiry & Guiry 2013). Amino acid such as alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, valine (Siddique *et al.* 2013) and brominated sesquiterpenes as 10-bromo-7, 12-dihydroxy- $\Delta^{3,4}$ -laurene, filiformin and filiforminol (Fig. 1) were the reported chemical constituents (Afaq-Hussain *et al.* 1991). *H. pannosa* possessed antibacterial (Shanmughapriya *et al.* 2008), analgesic, anti-emetic (Mazhar *et al.* 2011) and haemagglutinin activity (Alam & Usmanhani 1994).

Although the antifungal activity of *H. pannosa* against *Candida albicans* had been reported earlier (Shanmughapriya *et al.* 2008). Present study is the continuation of antifungal activity against *Candida glabrata*, *Fusarium solani*, *Microsporum canis* and *Trichophyton longifusus*.

MATERIALS & METHODS

Collection of plant materials

Hypnea pannosa was collected from Manora and Buleji the coastal areas of Karachi and dried under shade. Sample was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi for further reference.

Preparation of the extracts

The dried alga was crushed and soaked in methanol for seven days. The methanol extract was evaporated under reduced pressure at 35° C, following the protocol of Rizvi & Shameel (2005).

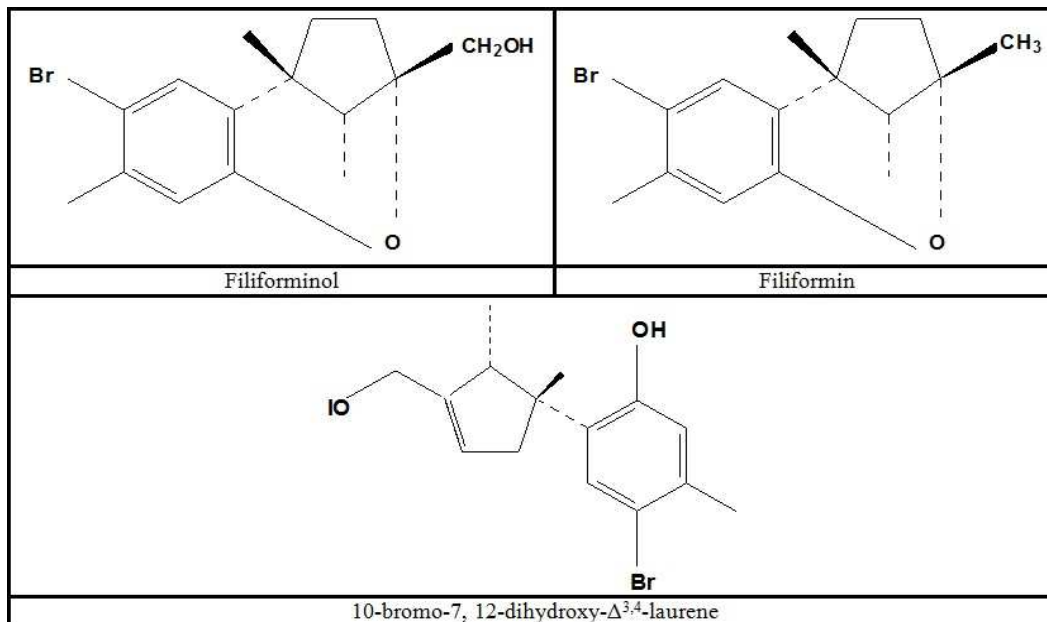


Fig. 1. Structure of constituents isolated from *Hypnea pannosa* (Afaq-Hussain *et al.*, 1991).

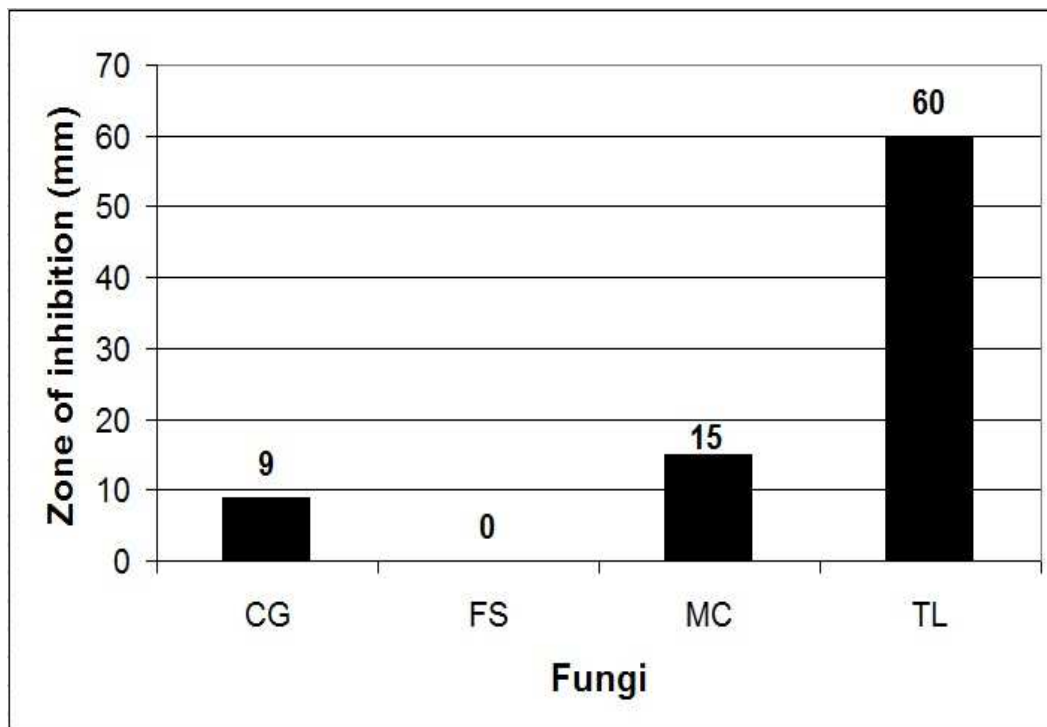


Fig. 2. Antifungal Activity of *Hypnea pannosa*. (CG= *Candida glabrata*, FS = *Fusarium solani*, MS= *Microsporium canis* and TS= *Trichophyton longifusus*).

Table I. Antifungal Activity of *Hypnea pannosa*.

Fungi	Linear growth (mm)		Zone of inhibition (mm) methanol extract 400 µg/mL	Std. Drug (Miconazole) MIC µg/mL
	Control	Methanol Extract		
<i>Candida glabrata</i>	100	91	9	110.8
<i>Fusarium solani</i>	100	100	0	73.25
<i>Microsporum canis</i>	100	85	15	98.4
<i>Trichophyton longifusus</i>	100	40	60	70

Antifungal Activity

The antifungal activity of methanol extract of *Hypnea pannosa* was performed using tube dilution method (Washington & Sutter 1980). Sabouraud dextrose agar was prepared by mixing 32.5 g sabouraud 4 % glucose and 4.0 g of agar-agar in 500 mL distilled water. It was steamed to dissolve and 4 mL was dispensed in screw capped tubes and autoclaved at 121° C for 15 min. Tubes were allowed to cool to 50° C. Stock solution of the extract was prepared by dissolving 24 mg in 1 mL DMSO. The stock solution was added to solidified sabouraud agar media to give 400 µg crude extract/mL of sabouraud dextrose agar. Tubes were allowed to solidify in slanting position and inoculated with 4 mm diameter piece of the inoculum removed from 7 days old culture of fungi. For non-mycelial growth, an agar surface streak was employed. DMSO and reference antifungal drugs were served as negative and positive control, respectively. The test tubes were incubated at 27-29° C for 7-10 days. Growth in the medium containing the extract was determined by measuring linear growth (mm) and zone of growth inhibition (mm) was calculated with reference to negative control. In that assay, Miconazole was used as standard antifungal drug.

RESULTS & DISCUSSION

Plants have been a source of medicinal compounds since pre-historic time. It has been a well established fact that all parts of plants are used in traditional system of medicine for centuries. Organic compounds from terrestrial and marine organisms had extensive past and present use in the treatment of many diseases and served as compounds of interest both in their natural form and templates for synthetic modification (Chin *et al.* 2006).

The antifungal activity of the methanol extract of the *Hypnea pannosa* (400 µg/mL) was carried out by tube dilution method. The antifungal activity was presented in Table I and Fig. 2. The methanol extract of *Hypnea pannosa* showed good activity against *Trichophyton longifusus* and low activity against *Candida glabrata* and *Microsporum canis*, whereas it was inactive against *Fusarium solani*. Brominated sesquiterpenes from red alga reported to possess anti fungal activity (Ji *et al.* 2007). Brominated sesquiterpenes was also reported from *Hypnea pannosa* (Afaq-Hussain *et al.* 1991). Therefore, it may be concluded that these sesquiterpenes play some role in anti fungal effect of *Hypnea pannosa*.

REFERENCES

- Afaq-Husain S, Shameel M, Usmanghani K, Ahmad M, Perveen S & Ahmad VU 1991 Brominated sesquiterpene metabolites of *Hypnea pannosa* (Gigartinales, Rhodophyta). *J Appl Phycol* 3(2): 111-113.
- Alam MT & Usmanghani K 1994 Studies on marine algae for haemagglutinic activity. *Pak J Pharm Sci* 7(2): 1-15.

-
- Chin YW, Balunas MJ, Chai HB & Kinghorn AD 2006** Drug discovery from natural sources. **The AAPS Journal** 8(2): E239-E253.
- Guiry MD & Guiry GM 2013** *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>; searched on 13 June 2013. Available: http://www.algaebase.org/search/species/detail/?species_id=2719
- Ji N-Y, Li X-M, Li K, Ding L-P, Gloer JB & Wang B-G 2007** Diterpenes, Sesquiterpenes, and a C₁₅-Acetogenin from the Marine Red Alga *Laurencia mariannensis*. **J Nat Prod** 70 (12): 1901-1905.
- Mazhar F, Hasan M, Azhar I, Ali MS, Zubair M, Zahid R & Akram M 2011** Some biological studies on *Hypnea pannosa* J. Ag. **Afr J Biotechnol** 10(61): 13313-13317.
- Rizvi MA & Shameel M 2005** Pharmaceutical Biology of Seaweeds from the Karachi Coast of Pakistan. **Pharm Biol** 43(2): 97-107.
- Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS & Natarajaseenivasan K 2008** Antimicrobial activity of seaweeds extracts against multi resistant pathogens. **Annals of Microbiol** 58(3): 535-541.
- Siddique MAM, Aktar M & Khatib MAM 2013** Proximate chemical composition and amino acid profile of two red seaweeds (*Hypnea pannosa* and *Hypnea musciformis*) collected from St. Martin's Island, Bangladesh. **J Fisher Sci** 7(2): 178-186.
- Valeem EE & Shameel M 2012** An account of fatty acid composition of algae growing in Pakistan. **Int J Phycol Phycochem** 8(2): 115-126.
- Washington JA & Sutter VL 1980** *Agar and Microbroth dilution Procedure*. **Amer Soc Microbiologu** Washington 3rd Ed 453-462 pp.
-