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ANTIFUNGAL ACTIVITY OF *HYPNEA PANNOSA* J. AGARDH AGAINST *ASPERGILLUS FLAVUS*

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ABSTRACT

The methanol extract of the whole alga *Hypnea pannosa* J. Agardh was subjected to antifungal screening. It showed low activity against *Aspergillus flavus* Link *ex* Gray 1821.

Keywords: *Hypnea pannosa*, Red algae, Antifungal activity, *Aspergillus flavus*, Pakistan.

INTRODUCTION

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

Aspergillus flavus Link *ex* Gray 1821 caused a broad spectrum of diseases, ranging from hypersensitivity reactions to invasive infections in humans, associated with angioinvasion. The infections caused by the fungus were allergic bronchopulmonary aspergillosis and allergens, *Aspergillus osteomyelitis* following trauma, aspergillus rhinosinusitis, chronic cavitary pulmonary aspergillosis and aspergilloma, craniocerebral aspergillosis, cutaneous aspergillosis wound infections and osteomyelitis following trauma, endocarditis, myocarditis and pericarditis, keratitis and endophthalmitis (Roberts *et al.* 1984, Burke *et al.* 1991, Talbot *et al.* 1991, Clancy *et al.* 1998, Panjabi & Shah 2011). Hedayati *et al.* (2007) reported that *Aspergillus flavus* produced aflatoxins, the most toxic and potent hepatocarcinogenic natural compounds. Shanmughapriya *et al.* (2008) found seven species of seaweeds, which were highly bioactive on the multiresistant pathogens. The purpose of present study is to report the antifungal activity of *Hypnea pannosa* J. Agardh. against *Aspergillus flavus*. Although the antifungal activity of *Hypnea pannosa* against *Candida albicans* (Shanmughapriya *et al.* 2008) *Candida glabrata, Fusarium solani, Microsporum canis* and *Trichophyton longifusus* (Ashraf & Ahmed 2013) has been reported earlier. The present study is the further extension of its antifungal activity against *Aspergillus flavus.*

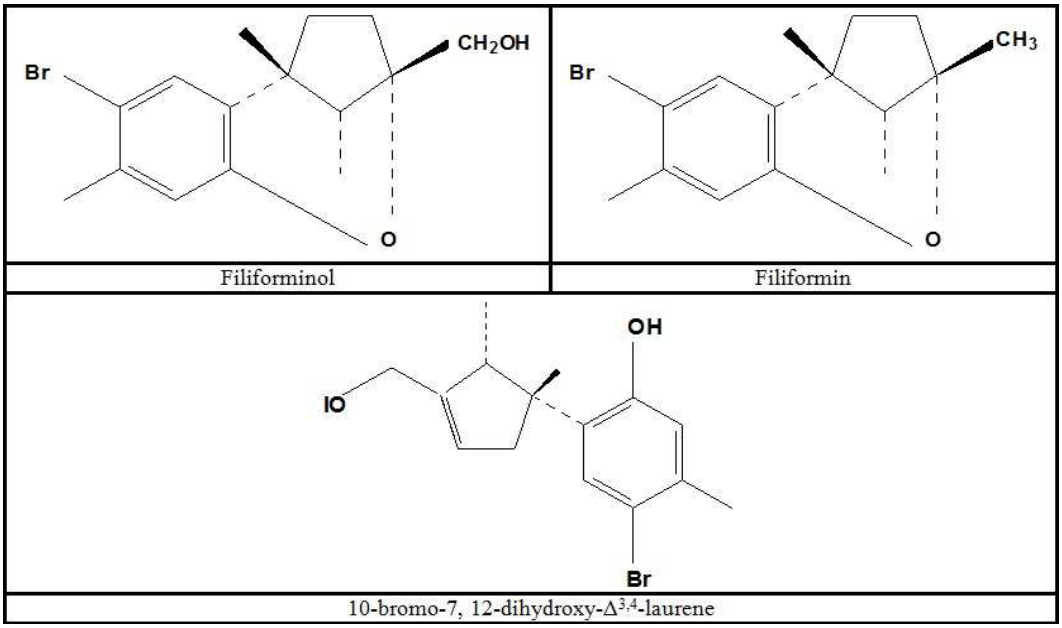

Fig. 1. Structure of constituents isolated from *Hypnea pannosa* (Afaq-Hussain *et al.* 1991).

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).

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 then 4 ml is dispensed in screw capped tubes and autoclaved at 121° C for 15 minutes. 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 incolum removed from 7 days old culture of fungi. For nonmycelial growth, an agar surface streak was employed. DMSO and reference antifungal drugs 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, Amphotericin B was used as standard antifungal drug.

Table I. Antifungal Activity of *Hypnea pannosa* J. Agardh.

Fungus	Linear growth (mm)		Zone of inhibition (mm) methanol extract 400 µg/mL	Standard Drug (Amphotericin B) MIC µg/mL
	Control	Methanol Extract		
Aspergillus flavus	100	73	27	20

RESULTS & DISCUSSION

The antifungal activity of the methanol extract of the *Hypnea pannosa* (400 µg/mL) was carried out by tube dilution method. The antifungal activity is presented above (Table I). The methanol extract of *Hypnea pannosa* showed low activity against *Aspergillus flavus***.** Some investigators reported isolates of *Aspergillus flavus* resistant to Amphotericin B *in vitro* but that was not universally accepted (Hedayati *et al.* 2007). Hypersensitivity myocarditis, an autoimmune reaction was often related to recently initiated medication (Burke *et al.* 1991). The antimicrobial principle from seaweed was found to be a lipophilic compound. The compound was stable over a wide range of temperature (30-60° C). The active principles of highly active seaweeds *Acrosiphonia orientalis* and *Stocheospermum marginatum* were bactericidal (Shanmughapriya *et al.* 2008). Brominated sesquiterpenes from red alga reported to possess anti fungal activity (Ji *et al.* 2007). Afaq-Hussain *et al.* (1991) also reported brominated sesquiterpenes from *Hypnea pannosa*. Therefore, it can be concluded that the sesquiterpenes might play some role in anti fungal effect of *Hypnea pannosa*.

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