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Antifungal property of marine sponge *Haliclona oculata* (Krikpatrick)

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ABSTRACT

Drug from marine resources is an area which offers an unprecedented opportunity for their pharmacological exploration and hence has received great attention during recent years for natural product chemistry, a promising new area of study. Thirty percent of all potential new natural drugs have been isolated from marine animals. About 75% of the recently registered and patented material to fight cancer comes from sponges. The methanol extract of the sponge showed promising antifungal activity against *Candida parapsilosis* and *Trichophyton mentagrophytes* (MIC 62.5 µg/ml). On fractionation of the methanolic extract, into four fractions, the antifungal activity was localized in chloroform soluble fraction against *Sporothrix schenckii* (MIC 62.5µg/ml), *Trichophyton mentagrophytes* (MIC 62.5µg/ml), *Aspergillus fumigatus* (MIC 62.5µg/ml), *Candida parapsilosis* (MIC 62.5µg/ml) and hexane soluble fraction *Sporothrix schenckii* (MIC 62.5µg/ml) *Candida parapsilosis* (MIC 62.5µg/ml) respectively. Both of these fractions were combined and chromatographed over a column of silica gel and six chromatographic fractions were evaluated for antifungal screening in *in vitro* models. Out of six fractions one showed promising antifungal activity against different models (Table-2), where as the others were found inactive. The active fraction-6 was found to be a mixture of four major alkaloids which were identified by LCMS analysis. The active fraction-6 was found to be a mixture of four major alkaloids which were identified by LCMS analysis. Further structure modifications of the identified alkaloids is required to enhance the antifungal activities in the semi-synthesized molecules. Modification of the structures of identified compounds from the active fraction may give enhanced activity and can be developed as a potential antifungal agent.

KEYWORDS

Antifungal;
Haliclona oculata;
Active principles mixture of four alkaloids.

INTRODUCTION

Marine sponges are incredible source of novel pharmacologically active compounds[25] which have earlier shown potent efficacy against various diseases. *Haliclona oculata*, a marine demospongiae belonging to order Haplosclerida, family Chalinidae is known to possess diverse pharmacological activity in several diseases such as cancer, neurodegeneration, type-2-diabetes, fungal and microbial infections[1,2,5,10,21,24]. These biological activities have been attributed to the presence of novel sterols, metabolites including ste-

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roids, terpenoids, alkaloids, cyclic peptides and unsaturated fatty acids[7,6,13,29]. *H. oculata* is soft rosy-brown to yellow brown branching sponge with small mouth like openings forming mammiform elevations, cigar-shaped oxeas, stylote or strongylote and laterally compressed branches. These branches may be isolated along the entire length (*H.oculata* Zeeland) or flabelliform (*Chalina oculata*) on a common stalk attached to the substratum with a small pedicel or foot. These colonies are attached on the dead coral stones in shallow water areas at the depth of 3-6 meter in subtidal region. These sponges are found in Vallai Island, Setukarai, Gulf of Mannar, Ramnathpuram and Tamil Nadu Coasts of India. We have recently reported antifilarial activity in *Haliclona exigua* and *H.oculata*[9,12].

Drug from marine resources is an area which offers an unprecedented opportunity for their pharmacological exploration and hence has received great attention during recent years for natural product chemistry, a promising new area of study. Secondary metabolites produced in marine organisms could be the

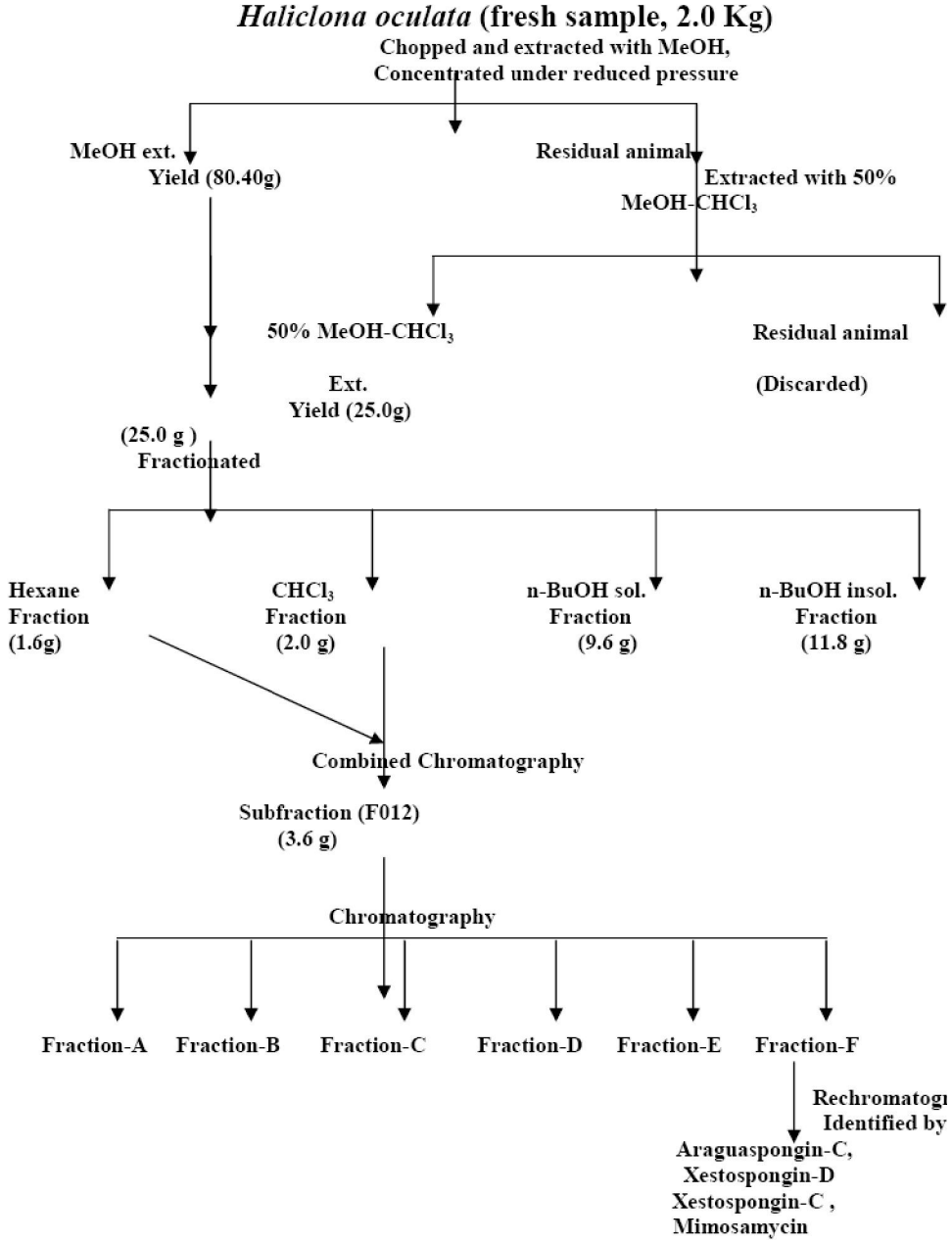


Figure 1

source of bioactive substance and useful in modeling compounds for drugs[8,11]. Marine microorganisms, whose immense genetic and biochemical diversity is only beginning to be appreciated, look likely to become a rich source of novel chemical entities for the discovery of more effective drugs. Marine sponges are shown to exhibit antibacterial, insecticidal, antiviral and antiplasmodial activities[28]. Antifungal activity of *Haliclona* spp. against *Aspergillus* strains has also been reported[1]. Some marine sponges are reported to possess antileishmanial activity. These include *Amphimedon viridis, Acanthostrongylophora* sp., *Neopetrosia* sp., *Plakortis angulospiculatus*, and *Pachymatisma johnstonii*[3,4,14,16,19,20]. Few researchers tried to isolate the chemical constituent of the *Haliclona exigua*[23,27]. The activity reported by the various workers in this sponge inhibited the rat brain nitric oxide synthase activity[26]. The present communication deals with the antifungal activity in the extract, fractions and pure compounds of *Haliclona oculata*, a marine sponge. The current manuscript reports on the antifungal efficacy of another species of this sponge, *H. oculata.*

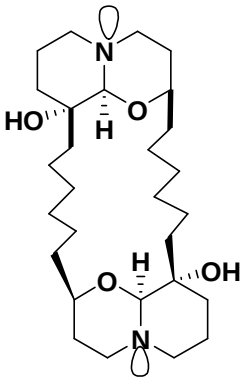
METHODS

Collection of material

Haliclona ocluta (Kirkpatrick) was collected from Tamil Nadu coast of India in the month of August and identified by P.A.Thomas, Fisheries Research Institute, Mandapam,India. Specimen sample (Voucher specimen No. 343) has been preserved in the Herbarium of Botany Division, CSIR-Central Drug Research Institute, Lucknow, India. Fresh sponges were filled in the steel containers containing n-butanol on Tamil Nadu coast of India and were transported to CDRI laboratory.

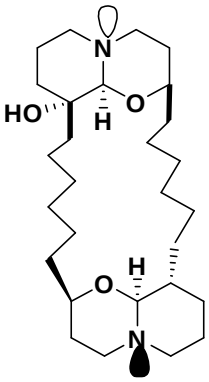
Extraction/fractionation/isolation procedures

Freshly collected *H.oculata* (2.0 kg) was cut into small pieces and extracted with methanol (4x4.0 L) at room temperature. The combined extract was filtered, concentrated under reduced pressure below 45^{0}C in a rotavapour to a viscous mass (45.0 g). The residual animal was further extracted with 50% methanol–chloroform (4x4.0 L) and the combined extract was filtered and concentrated under reduced pressure as above to a green viscous mass (35.0 g), the residual animal was rejected. Methanol extract (25.0 g) was fractionated into hexane (1.6 g), chloroform (2.0 g), n-

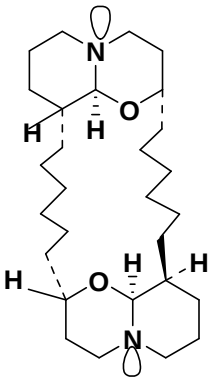


x

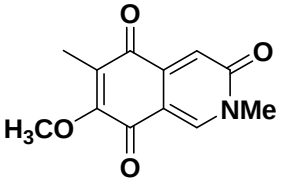
Araguaspongin-C MW 479 (M^++ H)



Xeatospongin-D MW 463 (M^++ H)



Xeatospongin-C MW 447 (M^++ H)



Mimosamycin MW 232 (M^+- H)

Figure 2

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butanol soluble (9.6 g) and n-butanol insoluble fractions (11.8 g). When all the fractions were screened for antifungal testing, the chloroform fraction was found showing promising results. The hexane fraction was also active in few models therefore both the fractions were combined and was resolved into six fractions by column chromatography, of which only one was found to possess potent antifungal activity. This chromatographic fraction was found to be a mixture of 4 major compounds which were identified by LCMS as Mimosamycin,[22] Xestospongin-C[15] Xestospongin-D[15] and Araguspongin-C,[27] with some minor compounds as shown in Figure 1.

Methodology of in vitro antifungal testing

The antifungal activity of the sample was determined by two fold micro broth dilution method as per guide lines of[17,18]. Briefly, the compounds were dissolved in

TABLE 1 : In vitro evaluation of antifungal activity of *Haliclona oculata*

Fungi Fluconazole (µg/ml)	MeOH MIC (µg/ml)	50% MeOH-$CHCl_3$ MIC (µg/ml)	Hexane fraction MIC (µg/ml)	Chloroform fraction MIC (µg/ml)	n-butanol soluble fraction MIC (µg/ml)	n-butanol insoluble fraction MIC (µg/ml)
Candida albicans 0.5	125	500	125	125	500	-
Cryptococcus Neoformans 1.0	250	250	500	250	500	250
Sporothrix schenckii 1.0	125	125	31.2	31.2	500	500
Trichophyton mentagrophytes 2.0	31.2	125	62.5	62.5	250	250
Aspergillus fumigatus 2.0	500	-	-	250	-	-
Candida parapsilosis 1.0	62.5	62.5	31.2	31.2	125	125

Fungi	Chrom. fraction MIC (µg/ml) (A)	Chrom. fraction MIC (µg/ml) (B)	Chrom. fraction MIC (µg/ml) (C)	Chrom. fraction MIC (µg/ml) (D)	Chrom. fraction MIC (µg/ml) (E)	Chrom. fraction MIC (µg/ml) (F)
Candida albicans	-	-	-	250	500	31.2
Cryptococcus neoformans	-	-	-	500	-	31.2
Sporothrix schenckii	500	500	500	-	-	500 -
Trichophyton mentagrophytes	-	500	-	15.6	500	15.6
Aspergillus fumigatus	-	-	-	-	-	62.5 -
Candida parapsilosis	-	-	-	125	-	7.8

DMSO (10%) to get a stock (10 mg/ml) solution. Minimum inhibitory concentrations of standard antifungal (Ketoconazole) and the compounds were measured in 96% Well tissue plate using RPMI 1640 media buffered with MOPS (3-[N-Morpholino] propane sulphonic acid) (Sigma Chemical Co.). Starting incubated at 35 ^{0}c in a moist, dark chamber and MIC were recorded spectrophotometrically.

RESULTS AND DISCUSSION

The methanol extract of the sponge showed promising antifungal activity against *Candida parapsilosis* and *Trichophyton mentagrophytes* (MIC 62.5 μg/ml). On fractionation of the methanolic extract, into four fractions, the antifungal activity was localized in chloroform soluble fraction against *Sporothrix schenckii* (MIC 62.5μg/ml), *Trichophyton mentagrophytes* (MIC 62.5μg/ml), *Aspergillus fumigatus* (MIC 62.5μg/ml), *Candida parapsilosis* (MIC 62.5μg/ml) and hexane soluble fraction *Sporothrix schenckii* (MIC 62.5μg/ml) *Candida parapsilosis* (MIC 62.5μg/ml) respectively. Both of these fractions were combined and chromatographed over a column of silica gel and six chromatographic fractions were evaluated for antifungal screening in *in vitro* models. Out of six fractions one showed promising antifungal activity against different models (TABLE 1), where as the others were found inactive. The active fraction-6 was found to be a mixture of four major alkaloids which were identified by LCMS analysis. Since the marine environment is an exceptional reservoir of bioactive natural products, which produced several novel structures with unique biological properties, which may not found in terrestrial natural products. The ocean environment is massively complex, consisting of extreme variations in pressure, salinity, temperature, and biological habitats. Among the groups of marine organisms, sponges are the most diverse and abundant, due to their soft bodies and sedentary life styles. These marine invertebrates have evolved chemical defense mechanicsms against other invading organisms, which involve the production of secondary metabolites. Further structure modifications of the identified alkaloids is required to enhance the antifungal activities in the semi-synthesized molecules for the development of a new antifungal agent.

CONCLUSIONS

Modification of the structures of identified compounds from the active fraction may give enhanced activity and can be developed as a potential antifungal agent.

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