INTRODUCTION

Fire corals are the common inhabitants of fringing reefs. The name comes from the burning sensation inflicted by the nematocyst of the dactylozooid or defensive polyp. These corals have long been known to possess a stinging property. Divers have experienced intense pains and rashes on the face and the skin when coming in contact with such corals. The fire corals were identified as belonging to the genus *Millepora* (Darwin, 1851). Although nine species have been identified world-wide (Lewis, 1989), only two of these species have been observed along the coast of the Saudi Red Sea, i.e. *Millepora dichotoma* and *Millepora platyphylla*. The symbiotic algae were found to be *Gleodinium viscum* (Banazek et al., 1993). As the main constructive element of the coral reefs, corals are capable of com-
plex systems fostered by their close symbiotic relationship with phototrophic zooxanthellae. The interrelationship between the algae and coral determines the mode of feeding. Algae in the coral are responsible for oxygen production and some nutrient transfer (Schlinchter et al., 1995). Considerable amount of research work has been done on the biological aspects of these corals (Wittle et al., 1971, 1974; Frank et al., 1994; Hoegh et al., 1987; Mueller et al., 1983). The chemical characterisation of the protein constituents was carried out by a Japanese group of workers (Shiomi et al., 1989). They studied only the protein part of the fire corals. Recently, the lipid content and particularly the wax esters of the corals from Okinawa, has been reported (Yamashiro et al., 1999). Moreover, the lipid composition of the reef building coral eggs was studied by Arai et al., (1993). They found that the majority of the lipid content of eggs from Acropora millepora, A. tenuis and Montipora digitata was wax esters (69-81%) and ranged from C_{14:0} to C_{22:0}. Wax esters, however, are present widely in many marine phyla and species (Sargent, 1978). Sargent found that this type of lipid is abundant in organisms that experience short periods of food plenty followed by long periods of food shortage. Wakeham and Frew (1982) have also reported the presence of wax esters from marine particulate matter. They found a mixture of saturated and unsaturated wax esters over the range C_{28}-C_{42}. Also, several studies have identified individual saturated wax esters in different marine organisms. For example, hexadecanyl-hexadecanoate (C_{32}) has been identified in soft coral Sinularia microclavate from China (Zhujin et al., 1990), while hexadecanyl-octadecanoate (C_{36}) has also been found in soft coral Sarcophyton from China (Rosenheng et al., 1982). Bandara et al. (1987) found a saturated C_{36} wax ester from a marine organism from Sri-Lanka. Identification of wax ester structures using different mass spectrometry methods was established (e.g. Aasen et al., 1971; Wakeham and Frew, 1982). These studies concluded that each peak is composed of a number of wax esters differing in alkyl and acyl moieties but having the same total carbon number (Wakeham and Frew, 1982).

This study aimed to investigate the chemical constituents of the two species of Millepora from the Saudi Red Sea, firstly by extracting the constituents by normal solvent extraction and secondly by subjecting the coral to mild acid hydrolysis. The extracts and acid hydrolysate were fractionated and purified using chromatography techniques and examined by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy.

MATERIALS AND METHODS

The coral species were collected from out side Sharm Obhur, 35 km north of Jeddah, in order to avoid the suspicion of contamination. The two coral species were collected from the fringing reef of Jeddah coast at a depth of about 10 m (Fig. 1). The collection was made during the months of September-December 1999; the average temperature of the surface seawater was 29°C, salinity 39.6% and pH 8.16. The specimens were washed with seawater and ground in a mortar to about 1 mm pieces. Lipid was extracted from the two species using chloroform and methanol (1:1). The samples of the two species were soaked in the mixture and kept overnight in a shaker. The supernatant was filtered and the solvent was removed using a rotary evaporator under vacuum at 40°C. The residue of the organic matter was dried under a stream of nitrogen and finally weighed using a microbalance. In anoth-
er experiment, the residue of the fire coral was dried, weighed and then subjected to acid hydrolys-
es. An aliquot of the fire coral residue was treated
with 2 N hydrochloric acid. The acid was gradually
added at intervals till the effervescence of carbon
dioxide ceased. The solution was heated on a hot
plate at 80°C for 30 minutes. It was cooled and neu-
tralised. Then, it was partitioned with ether (3 x 200
ml), and the ether extract was dried over anhydrous
sodium sulphate and evaporated under vacuum at
40°C to a crude gummy material. The concentrate
of solvent extraction and the acid hydrolysate were
separately chromatographed with TLC on silica gel
using benzene: ethyl acetate, (9:11) as the solvent
system. The spots were distinctly visible under
ultra-violet (UV) light. The top spot was scratched
from the TLC plate, and dissolved in chloro-
form/methanol and then filtered. The filtrate was
evaporated under a nitrogen atmosphere. The frac-
tion was further chromatographed on TLC using
hexane : chloroform (85:15) as the solvent system.
Again the top spot was scratched from the TLC
plate and the compounds were extracted and then
analysed using GC-MS. The GC-MS analyses were
made on a Shimadzu GC-17A gas chromatograph
equipped with a spilt/splitless injector, and a DB
fused silica column (25 m x 0.3 mm i.d., 0.17 µm
DB-1% phenyl/ 99% methyl-silicone) using helium
carrier gas. The GC conditions were 40-300°C at
5°C min⁻¹ then isothermal for 5 min., and the injec-
tor temperature was 250°C. The end of the GC col-
umn was introduced into the electron impact (EI)
source of a Shimadzu QP-5000 Quadrupole mass
spectrometer. Typical mass spectrometer operating
conditions were as follows: transfer line 230°C, ion
source 250°C, electron energy 70 eV. All samples
were analysed in full data acquisition (SCAN)
mode by scanning from 50-500 daltons at 1 cycle/s.
Identification of the compounds was based on com-
parison of retention indices and mass spectra of the
analytes with literature data (e.g. Hites, 1992;
Aasen et al., 1971; Wakeham and Frew, 1982) and
in certain cases by co-injection with authentic com-
pounds. The high-resolution mass spectrometry and
NMR analyses of the fatty acid esters were per-
formed at the H.E.J. Research Institute of Chem-
istry, University of Karachi, Pakistan. The condi-
tions of the NMR (Bruker-500 MHz) were MI=0.5
cm, MAX=1000 cm, pc=1.0, and the solvent used
was CDCl₃.

![Mass spectra](image)

**Fig. 2.** – Mass spectra (background corrected) of the wax esters isolated from the two species of fire coral from Saudi Red Sea Coast. (A) a
mixture of 14/16 and 12/18 (B) 16/16.
RESULTS AND DISCUSSION

The extractable organic matter of the two species of fire coral was fractionated using TLC and three spots were distinctly visible under the UV light. The top layer (high Rf) of the second TLC plate was analysed using GC-MS. Four compounds were identified as long-chain wax esters (Figs. 2 and 3). The structural identification was confirmed by nuclear magnetic resonance analyses. It would be very interesting to compare and contrast the results of our studies with those of other groups workers (Aasen, 1971; Wakeham and Frew, 1982). The latter group have isolated all these four wax esters, but from particulate matter, and have stated that each one of these existed in three isomeric forms. In the present study, the GC-quadrupole spectra were carefully examined to calculate the diagnostic ions, \( R' \) being the alkyl group in the alcohol moiety and \( R \) being the alkyl group in the acid moiety. The relative

<table>
<thead>
<tr>
<th>M*</th>
<th>Wax Ester</th>
<th>[R' - 1]+</th>
<th>CO₂R*</th>
<th>CH₂=C⁻</th>
<th>OH⁻</th>
<th>CH₂=C⁻</th>
<th>OH⁻</th>
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<tr>
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<td>Alcohol</td>
<td>Acid</td>
<td>Mass</td>
<td>Int.</td>
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<tr>
<td>452</td>
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<tr>
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Fig. 3. – Mass spectra (background corrected) of the wax esters isolated from the two species of fire coral from Saudi Red Sea Coast. (A) a mixture of 16/18 and 18/16 (B) 18/18.

TABLE 1. – Intensities of characteristic ions containing the alcohol moiety in mass spectra of saturated wax esters of fire coral from Saudi Red Sea Coast
intensities of $[R'H]^+$; $CO_2R'+; CH=COH-O-R'$ and $CH=COH_2-O-R'$ were determined (Table 1). Similarly the intensities of $(RCO_2H-propyl)^+; (RCO)^+; (RCO_2H)^+ \text{ and } (RCO_2H_2)^+$ were calculated (Table 2). From these tables (1 and 2) it can be concluded that for ester 30:0 two isomers were possible, 14:0/16:0 and 12:0/18:0, while for the 32:0 wax ester one structure was confirmed, 16:0/16:0. However, two isomers were observed for 34:0 and were 16:0/18:0 and 18:0/16:0. The last compound, 36:0, existed in 18:0/18:0 form. The other isomeric wax esters mentioned by Wakeham and Frew (1982) were not detected in the spectra of the present study. It is very likely that the lack of these isomers in the coral may be attributed to the specific metabolic pathways. It is noteworthy that the number and nature of isomers of wax esters of the fire coral extract show some variations compared with similar wax esters reported earlier. The NMR spectrum supported the proposed structures. The spectrum was taken in CDCl$_3$, and 500 MHz indicated the following signals 0.86 (t, CH$_3$), 1.24 (m, CH$_2$), 2.27 (t, -CH$_2$-CO) and 4.04 (t, -OCH$_2$). The presence of wax esters in marine organisms has been reported by several groups of workers (e.g. Sergant, 1978; Wakeham and Frew, 1982; Arai et al., 1993).

### Table 2. Intensities of characteristic ions containing the acyl moiety in mass spectra of saturated wax esters of fire coral from Saudi Red Sea Coast.

<table>
<thead>
<tr>
<th>M+ Mass</th>
<th>Wax Alcohol</th>
<th>Ester Acid</th>
<th>RCO$_2H$-propyl Mass</th>
<th>RCO$^+$ Mass</th>
<th>RCO$_2H^+$ Mass</th>
<th>RCO$_2H_2^+$ Mass</th>
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**Fig. 4.** (A) Mass spectrum (background corrected) of the diphenylamine isolated from the two species of fire coral from Saudi Red Sea Coast. (B) Mass spectrum of the authentic diphenylamine.
Many corrosive compounds are phenolic in nature (Harborne, 1984). Therefore *M. dichotoma* and *M. platyphylla* were subjected to acidic hydrolysis to isolate any phenolic compounds if present. It was observed that three fractions were obtained when it was subjected to acidic hydrolysis. The top fraction was separated by preparative layer chromatography. It was further resolved by running in a different solvent system (hexane : chloroform 85:15). A spot was scratched from the TLC plate that had a similar Rf value to that of authentic diphenylamine (Sigma Chemical Ltd.). There was no change in the Rf value when authentic diphenylamine was mixed with the one isolated from coral in two different solvent systems on TLC (hexane: chloroform, 85:15, Rf 0.35) and with (benzene: methanol, 60:40, Rf 0.81). It was also noticed that the mass spectrum pattern was similar to that of the authentic diphenylamine (Fig. 4). The extract was co-injected with the authentic diphenylamine to yield a single peak. Moreover, the mass spectra patterns of both diphenylamines (isolated from fire coral and authentic) were identical to those described in the literature (Hites, 1992). The mass spectrum of diphenylamine exhibited a molecular ion peak, a base peak at non-even m/z 169, and the presence of a moderate peak at m/z 168 (relative intensity 61%), confirming the loss of hydrogen from the amino group. The relatively abundant fragment at m/z 142 is an important feature that is derived from M-HCN. In addition, the mass spectrum showed a series of peaks m/z 51, 65 and 77 indicating a phenyl group. It is interesting to note that this compound has not yet been reported from other marine organisms (MarinLit Database, 1996). It is to be noted that diphenylamine is harmful to skin and causes rashes and a burning sensation. It was found during the toxicity test of the extract in guinea pigs that LD$_{50}$ was 30 mg/kg of body weight, indicating a high sensitivity to this compound (Leng, 1987).

**CONCLUSIONS**

Four long-chain wax esters with a total number of 32-36 carbon atoms were observed in fire coral extract. All these have been previously reported from other sources. The isomeric compounds of these wax esters were to some extent present in a different distribution to those reported previously. It is normally known that wax esters are waxy in nature and in *M. dichotoma* and *M. platyphylla* they act as an energy reserve for nutrient transfer and also may act as a protective coating of the defense system (nematocyst polyps) of this type of coral. However, much work is needed to study the biochemical pathways of these compounds and also to verify, if possible, the position of these materials in fire coral. The presence of diphenylamine in acid hydrolysate of the two species of fire coral could be considered as one of the mechanisms used by fire coral to possess its stinging property.

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