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CHEMICAL EXAMINATION AND BIOLOGICAL EVALUATION OF SOME MARINE COELENTERATES OF THE INDIAN OCEAN

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ABSTRACT

In the present study, ethyl acetate extracts of the two marine gorgonians Junceella juncea (JjE), Gorgonella umbraculum (GuE) and a soft coral sarcophyton trocheliophorum (StE) collected from the coasts of Indian Ocean were chemically examined and screened for anti-inflammatory (Evan's blue method), antibacterial (*Bacillus pumilis, Bacillus subtilis* and *Eschereichia coli* and *Proteus vulgaris*) and antifungal (Candida allricans and *Aspergillus niger*) activity. The structures of the new compounds isolated from the above mentioned extracts were elucidated by utilizing modern spectral and chemical techniques and were found to be spiroketal steroid, umbraculolide E (briarane diterpenoid) from GuE, Juncins I-M (briarane diterpenoid) from JjE, trocheliophorin (a novel sesquiterpenoid) from StE. All the extracts exhibited potent dose dependent anti-inflammatory activity (30 and 100 mg/kg) in the order JjE > StE > GuE when compared with that of the standard (ibuprofen). All the extracts and some available compounds isolated from them showed significant antibacterial (100 μg/mL) activity. JjE and GuE showed potent antibacterial activity against *E. coli*. GuE also exhibited highly significant antifungal activity against *Candida albicans*.

Key words: Gorgonella umbraculum, Junceella juncea, Sarcophyton trocholiophorum, Antifungal, Antibacterial, Anti-inflammatory.

INTRODUCTION

Marine organisms, especially in shallow tropical environments are known to produce bioactive secondary metabolites. The composition of the secondary metabolites are different to those isolated from terrestrial natural products, due to some factors such as

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aquatic medium, partial availability of sunlight and easy mobility of the nutrients both organic and inorganic. The lack of sunlight beneath the surface of the oceans may also be expected to cause the formation of unusual natural products[1].

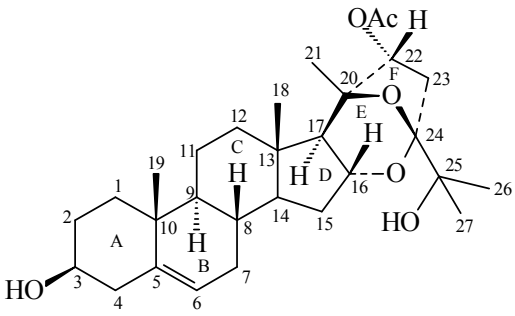
A large number of compounds, which were isolated from various marine organisms exhibited anticancer, antiviral, anti-inflammatory, antibacterial, antibiotic, cytotoxic and ichthiotoxic activities. Based on these reports, the authors isolated and elucidated products by chromatography and using modern spectral techniques, respectively.

Yields of the most of the pure compounds isolated from the three ethyl acetate extracts of the *Gorgonella umbraculum, Junceella juncea* and *Sarcophyton trocheliophorum* were found to be very low in quantities and were just enough to record the spectral data to establish their structural and hence, unavailable for pharmacological screening. Hence, the crude extracts of the above mentioned marine organisms and some available pure compounds isolated from them were screened for their antibacterial and antifungal activities.

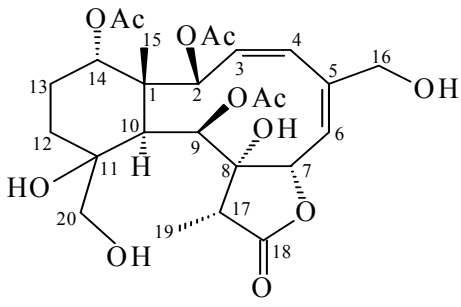
EXPERIMENTAL

Isolation and structural elucidation

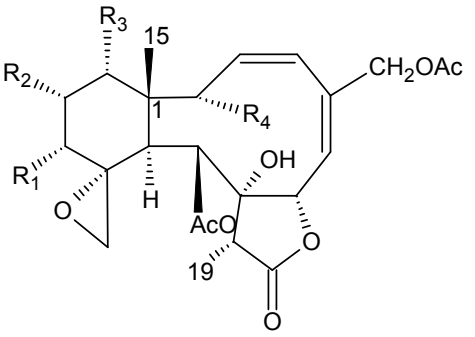
Extraction: The two gorgonians, *Gorgonella umbracullum* (Gu), *Junceella juncea* (Jj) and a soft coral *Sarcophyton trocholiophorum* (St) collected from Tuticorin coast were initially extracted with methanol. The ethyl acetate solubles of the initial methanolic extracts was concentrated under reduced pressure to produce a residue of Gu (30 g), Jj (35 g) and St (25 g).

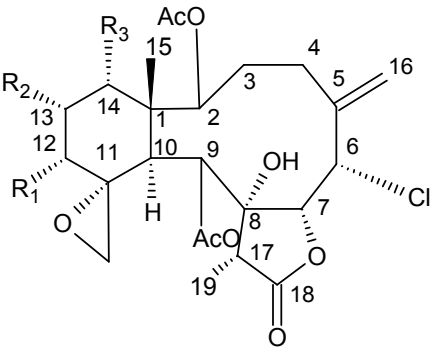


Spiroketal steroid



Umbraculolide E





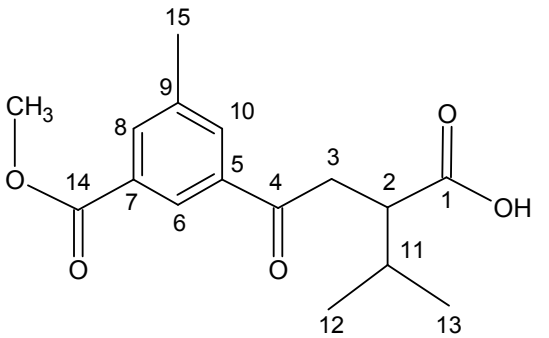
1. $R_1 = R_3 = R_4 = OAc$, $R_2 = OCOCH_2CH(CH_3)_2$

2. $R_1 = R_2 = OCOCH_2CH(CH_3)_2$, $R_3 = R_4 = OAc$

3. $R_1 = R_3 = OCOCH_2CH(CH_3)_2$, $R_2 = H$, $R_4 = OAc$

4. $R_1 = R_2 = OCOCH_2CH(CH_3)$, $R_3 = OAc$

5. $R_1 = R_3 = OCOCH_2CH(CH_3)_2$, $R_2 = H$



Trocheliophorin

The residues upon careful chromatography over silica gel column followed by further purification furnished two new compounds, spiroketalsteroid and umbraculolide E from *Gorgonella umbraculum*, and 5 new briarane diterpenoids, juncins I-M from *Junceela juncea* and one novel sesquiterpenoid, trocheliophorin from *Sarcophyton trocheliophorum*. The structures were established by the interpretation of spectral data (^{1}H NMR, ^{13}C NMR, UV, IR, Mass, 2D NMR-COSY, NOESY, HMBC and HMQC).

Antiinflammatory activity[2,3]: Adult mice (13 to 27 g) of either sex were used for anti-inflammatory activities. These animals were divided in 12 groups containing not less than four animals in each group. Group I served as control. Goup II & III were treated with ibuprofen 30 mg/kg & 60 mg/kg body wt. doses.

All the three extracts GuE, JjE and StE were administered to remaining groups each at three dose levels i.e. 10, 30 and 100 mg /kg body weight. Test samples are given as freshly prepared suspensions in 5% gum acacia. Two doses were given for each mice orally, one being given 24 hrs before anti-inflammatory agent and other being 30 minutes before it.

Each mouse received through a tail vein 0.1 mL of an aqueous solution containing 0.5% Evans blue in 0.9% NaCl. Then immediately afterwards 0.05 mL of the solution of 4% formaldehyde in 0.9% saline was given subcutaneously in the dorsum of the right hind foot. The left hind foot received the same volume of saline, which served as control. After an hour, the accumulation of the blue dye in the test foot was compared with the control foot. The scores were given on the basis of the degree of accumulation of Evans blue in the right paw as described below.

0 – For no difference from the control foot.

1 – For slightly perceptible difference

2 – For a definite difference

3 – For a striking difference

Acute toxicity studies

Experimental animals – Albino mice of either sex weighing 20-25 g were obtained from M/s Ghosh Enterprises, Calcutta. The animals were stabilized for one week. They were maintained in standard condition at room temperature 60 ± 5% relative humidity and 12 hours light dark cycle. They had been given standard pellet diet obtained from National Institute of Nutrition, Hyderabad and purified water.

Procedure – The animals were divided into six groups; each group consisting of two animals. The extracts were weighed accurately and a suspension was prepared for each extract in 5% gum acacia solution. Each suspension was administered orally to animals in 300 and 1000 mg/kg body weight with all the three extracts Gu, Jj and St to all the six groups.

Antibacterial and antifungal activity : The antibacterial activity of the three extracts and some available pure compounds isolated from them were tested against *B. pumilis*, *B. subtilis, E. coli, P. vulgaris, Candida albicans* and *Aspergillus niger* using nutrient agar (Hi-Media) for antibacterial and potato dextrose agar medium (Hi-media) for antifungal activity.

The sterilized (auto claved at 120°C for 30 min) medium was inoculated (40-50°C) at 1% level using 18 hrs old culture of the test organisms and transferred into petri dishes to give a depth of 3-4 mm and allowed to set at room temperature for about 10 min and then refrigerated for 30 min. Solutions of the test compounds / extracts in conc. of 1 mg/mL were prepared in methanol and 0.05 mL of each solution was placed in cups by means of sterile micropipettes. One cup is used as control for methanol (0.05 mL) and another standard for ampicillin (1 mg/mL 0.05 mL) for antibacterial and itroconazole (1 mg/mL, 0.05 mL) for antifungal activity. The plates were preincubated for 1 h at room temperature and incubated at 37°C for 24 h and 48 h for antibacterial and antifungal activity respectively. The observed zone of inhibition is noted in Table 2.

RESULTS AND DISCUSSION

New spiroketal steroid from *Gorgonella umbraculum* yield 2.2 mg; mp 160-162°C, $[\alpha]_D$ = -20° (C, 0.01 in $CHCl_3$) analysed for $C_{29}H_{44}O_6$ was supported by the mass peak at m/z 429 $(M+H-60)^+$ ion its FAB mas spectrum. ν_{max} 1735 cm^{-1} ($OCOCH_3$). A study of ^1H and ^{13}C NMR spectral data was found that it is **22-acetoxy-3,25-dihydroxy-16,24,20-24-bisepoxy (3β, 16α, 20S, 22R, 24S)-cholesto-5-ene**[5].

Another new briarane diterpenoid from GuE yielded 8 mg; m.p. 190-191°C $[\alpha]_D$ – 21.6° (c, 0.1 in $CHCl_3$) and mol. formula $C_{26}H_{36}O_{12}$; m/z 1103 $(2M + Na^+)$, 563 $(M + Na^+)$ in its FAB mass pectrum. IR bands showed 1330 cm^{-1} (hydroxyls), 1773 cm^{-1} (γ-lactone) and 1738 cm^{-1} (ester). A study of ^1H and ^{13}C, NOESY, COSY, HMBC showed that it is a new briarane diterpenoid, **umbraculolide E**[6].

Five new diterpneoids **juncins I-M**[7] of the briarane skeleton have been isolated from Indian gorgonian *Junceela juncea*. The structures 1-5 were established by the interpretation of spectral data (^1H, ^{13}C NMR, COSY, NOESY, HMBC and HMQC).

A novel rearrangemened sesquiterpenoid[8], **trocheliophorin** has been isolated from the soft coral *Sarcophyton trocheliophorum* is a colorless oil (8 mg), $[\alpha]_D$ + 0.356° (c, 0.04 in $CHCl_3$) and mol. formula : $C_{16}H_{20}O_5$; m/z 310.2 $(M + NH_4^+)$, 602.5 $(2M + NH_4^+)$ in its +

FAB mass spectrum. IR spectrum showed peaks at 3430 (COOH) and 1738 (ester) and strong UV absorption at 226 nm and weak absorption at 291 nm. The structure of the novel acid was elucidated by spectral data (^{1}H, ^{13}C, APT, COSY, HMQC, HMBC and Mass).

Acute toxicity studies: The oral LD 50 for GuE was found to be at 1000 mg/kg body weight. The LD 50 for JjE and StE were found to be above 1000 mg/kg body weight. The study indicated anti-inflammatory activity of the extracts at sublethal doses indicating the presence of potential compounds for such activity.

Anti-inflammatory activity: JjE produced significant activity at a concentration of 30 and 100 mg/kg body wt. StE showed highly significant activity at 30 mg/kg and 100 mg/kg body wt. GuE produced comparable activity as that of standard.

Table – 1. Anti-inflammatory activity of the extracts – GuE, JjE, StE scores obtained one hour after 4% formalin and Evan's blue administration, which was followed after two doses of test substance.

	Mouse No.			
	1	**2**	**3**	**4**
Group 1 - Control	3	3	3	3
Group 2 - Ibuprofen (30 mg / kg)	3	1	2	2
Group 3 - Ibuprofen (60 mg / kg)	1	1	0	0
Group 4 - GuE (10 mg / kg)	3	2	1	3
Group 5 - GuE (30 mg / kg)	2	2	2	2
Group 6 - GuE (100 mg / kg)	0	Dead	1	0
Group 7 - JjE (10 mg / kg)	0	1	0	2
Group 8 - JjE (30 mg / kg)	1	1	1	1
Group 9 - JjE (100 mg / kg)	1	0	2	0
Group 10 - StE (10 mg / kg)	1	1	2	2
Group 11 - StE (30 mg / kg)	2	2	1	1
Group 12 - StE (100 mg / kg)	0	0	0	1

Antimicrobial activity: The ethyl acetate extract of *Junceella juncea* (JjE),

Gorgonella umbraculum (GuE) showed significant activities against *B. pumilis* and *E. coli*, while the compounds GuP_1, GuP_2, JjP_2 and StE showed moderate activity against *B. subtilis, B. pumilis, E. coli* and *P. vulgaris*.

Antifungal activity : GuE exhibited significant activity against *Candida albicans* and other compounds / extracts do not possess activity against *C. albicans* and *A. niger*.

Table 2 Antibacterial and antifungal activity of the extracts and pure compounds

Compound/ extract Code No.	Inhibition zone diameter (mm)					
	B. subtilis	***B. pumilis***	***E.coli***	***P.vulgaris***	***C. allbicans***	***A. niger***
Gup1	14	14	15	14	10	8
Gup2	14	12	14	15	8	8
Jjp1	15	17	18	15	9	8
Jjp2	12	13	14	13	8	8
GuE	12	14	18	14	29	10
JjE	18	17	18	15	10	8
StE	15	16	13	16	8	8
Control methanol	8	8	8	8	8	8
Ampicillin (1 µg/ mL)	17	18	17	16	24	21

Cup diameter = 8 mm Volume of the solution = 0.05 mL per cup
Solvent: Methanol

CONCLUSIONS

The marine organisms once again proved to be a good source for new compounds, which are biologically active. Further studies have to be conducted on other models to establish the mechanism of action for the compounds/extracts having significant anti-inflammatory activity.

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