ISOLATION, CHARACTERIZATION AND ACUTE TOXICITY STUDY OF DIOXOLO-PYRAN DERIVATIVE OBTAINED FROM MARINE ALGAE, CODIUM ELONGATUM.

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Abstract

Objective: The present study aims to isolate and characterize dioxolo-pyran derivative from marine green algae, *Codium elongatum*.

Method: The dioxolo-pyran derivative (DOPD) was isolated by aqueous extraction and purified by ethanol precipitation. The structure of DOPD was established with the help of infrared spectroscopy, nuclear mass spectroscopy and mass spectroscopy. An acute toxicity study of water extract and isolated DOPD was also conducted for the first time according to OECD (Organization for Economic Co-operation and Development) 423 guidelines.

Results: DOPD having IUPAC name: (3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-((((2*S*,5*S*,6*R*)-5-(benzyloxy)-6-((benzyloxy)methyl)-5,6-dihydro-2*H*-pyran-2-yl)oxy)methyl)-2,2,7,7-tetramethyltetrahydro-5 *H*-bis([1,3]dioxolo)[4,5- *b*:4',5'-*d*]pyran and chemical formula: $C_{32}H_{40}O_9$ has been isolated from *C.elongatum*. The findings were further supported by the results of IR, ESI-MS, ^{1}H-NMR and ^{13}C-NMR. Acute toxicity studies of DOPD and water extract showed no significant effect at the dose of 300 mg/kg and 2000. mg/kg**.**

Conclusion*:* This is the first paper to report the isolation, characterization and acute toxicity study of water extract and DOPD obtained from *Codium elongatum*.

Keywords*:* *Codium elongatum*, Dioxolo-pyran derivative, Green marine, Chlorophyta, Bryopsidales.

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INTRODUCTION.

The aquatic environment has gifted marine algae with the ability to withstand fluctuation in salinity, light intensity, temperature and tidal current (Jaswir and Monsur, 2011). These adaptations have an influence on algal morphological and biological properties (El Gamal, 2009; Bhakuni and Rawat, 2006). A wide array of metabolites has been reported from marine algae including chlorophyll, fatty acids, sterols, vitamins, amino acids, polysaccharides and xanthophylls (Yin *et al*., 2005; Schmid *et al.,* 2014; Lopez-Perez *et al*., 2017).

The three main divisions of marine algae were Chlorophyta, haeophyta and Rhodophyta. *Codium elongatum* is a marine alga that belongs to the group Chlorophyta and the family Codiaceae. Codium species were found to be widely distributed in the marine environments and least investigated among all Chlorophyceae (Akhila *et al*., 2007) Many researchers have investigated various extracts from *C.elongatum* for different biological activities. But till date, there is no evidence of any compound isolated from *C.elongatum*. This paper is the first to report the isolation, characterization and acute toxicity studies of a novel compound dioxolo-pyran derivative from green marine algae, *C. elongatum*

MATERIALS AND METHODS

Materials

All the solvents and chemicals used during the process of extraction and isolation are of analytical grade.

Plant material

Green algae, *C.elongatum* was collected from the Arabian Sea, Gulf of Kutch (the Marine National Park, Jamnagar) in January 2016. The specimen was deposited in the algal herbarium of CSIR-NBRI with serial no. MARIN-01 and accession no. 002610.

Extract preparation

The coarsely powdered algae (4gm) was macerated with 100 ml of water. Shaking was done frequently for the first 6 hours and then allowed to stand for 18 hours. It was filtered rapidly and the filtrate was concentrated with a rotary evaporator as per WHO guidelines (WHO,1992).

Isolation of dioxolo-pyran derivative

Algae (200 grams) was treated with 400 ml of 85% ethanol. After depigmentation algae was removed from the solvent and air dried, then it was treated with 0.2 N HCl (1:10 w/v) at 60^{o}C for 2 hours by stirring in water bath and left overnight. The mixture was vacuum filtered and algae was re-extracted with the same solvent for 1 hour. The extracts were combined and concentrated under reduced pressure at temperature not exceeding 40^{o}C. Three volume of ethanol was added to precipitate the DOPD. The precipitated DOPD was filtered, washed twice with 85% ethanol for 30 minutes each. The desalted compound was lyophilized and stored at 40^{o}C (Manilal *et al*., 2009; El-Rafie *et al*., 2013; Hernandez-Garibay *et al*., 20111; Barros *et al.*, 2013).

Infra-red spectroscopy

The Fourier transform IR spectra analysis was carried out using spectrum 400 FTIR spectrometers, Perkins Elmer, the USA having resolution 0.4 cm^{-1} and scan range 700 cm^{-1} to 30 cm^{-1} (Joshi *et al*., 2012).

Mass Spectroscopy

ESI-MASS analysis was carried out using Alliance 2795, Q-TOF Micromass Mass spectrometer, Waters Corporation, U.K. Mass range 50 to 2000 Dalton (De Souza *et al*., 2012).

Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR and ^{13}C NMR analysis was carried out using Avance NEO, 500 MHZ, Bruker, Switzerland with D_2O as a solvent. All Statistical calculations were done using EXCEL and Graph Pad Prism software. (Tabarasa *et al*., 2012).

Structure of isolated DOPD

Data from FT-IR, Mass spectroscopy and NMR spectroscopy were analyzed using Carbohydrate Structure Database System (CSDB), Glycosciences to obtain structure, IUPAC name and chemical formula of isolated DOPD, elemental analysis, molecular mass and

molecular weight were also recorded. (Egorova *et al*., 2015).

Acute toxicity study

Nulliparous non-pregnant female wistar rats aged around 8-12 weeks and weighing 180±20 grams were used in the study. As per OECD 423 guidelines, 3 animals were used in each step starting with 300 mg/kg (p.o.) to test substance of unknown toxicity. If 0 or 1 animal is found dead then the next dose of 2000 mg/kg (p.o.) was given. Animals were kept in close observation during the first 30 minutes. Thereafter, periodical observations were noted for 4 hr, 24 hr, 48 hr and then after regularly for 2 weeks animals were observed for any change in the skin, fur, eye, mucous membrane, circulatory, respiratory and nervous system. Behavioural pattern like severe pain, tremor or any sign of severe stress should also be taken into account (OECD guidelines, 2001).

RESULT AND DISCUSSION

The dioxolo-pyran derivative (DOPD) with chemical structure (Figure 1), IUPAC name: (3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-((((2*S*,5*S*,6*R*)-5-(benzyloxy)-6-((benzyloxy)methyl)-5,6-dihydro-2*H*-pyran-2-yl) oxy) methyl)-2,2,7,7-tetramethyltetrahydro-5 *H*-bis ([1,3] dioxolo) [4,5- *b*:4',5'-*d*] pyran and chemical formula: $C_{32}H_{40}O_9$ has been isolated from *C.elongatum*. The isolated DOPD accounts for 8 ± 0.5% of the algal dry weight.

The IR spectrum (Figure 2) interpretation of DOPD was shown in table 1. ESI-MS is used for obtaining molecular information about isolated dioxolo-pyran derivative. Aqueous extract was hydrolysed during the isolation process which makes it more viable for mass analysis (Xu *et al*., 2017). From ESI-MS spectra, the peak molecular weight was around 569.1 Da while the exact molecular weight of DOPD was 568.66 Da (Figure 3).

Chemical shift and signals of 1H NMR spectra (Figure 4) of DOPD was mentioned in table 2. Chemical shift and signals of 13C NMR spectra (Figure 5) of DOPD was mentioned in table 3.

To evaluate the toxic character of a substance determination of the LD_{50} (lethal dose causing the death of 50% of animals in the test group) value is the initial step. It is the basic assessment to obtain information about toxicity of a substance, dose of a new compound and mode of the toxic action of a compound (Akhila *et al*., 2007)**.**

The result of two weeks observations of acute toxicity study was mentioned in table 4. Animals involved in acute toxicity studies show no relevant changes at 300 mg/kg and 2000 mg/kg**.**

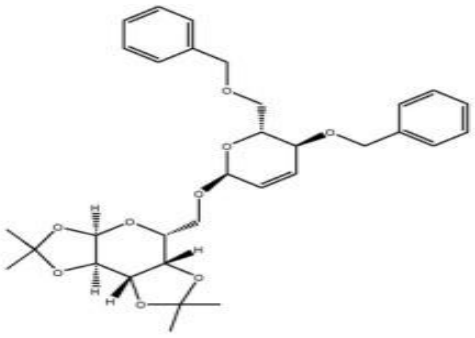

Fig. 1: Chemical structure of DOPD

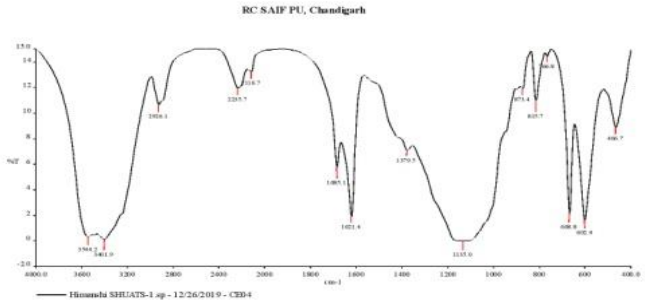

Fig. 2: Infrared spectra of DOPD

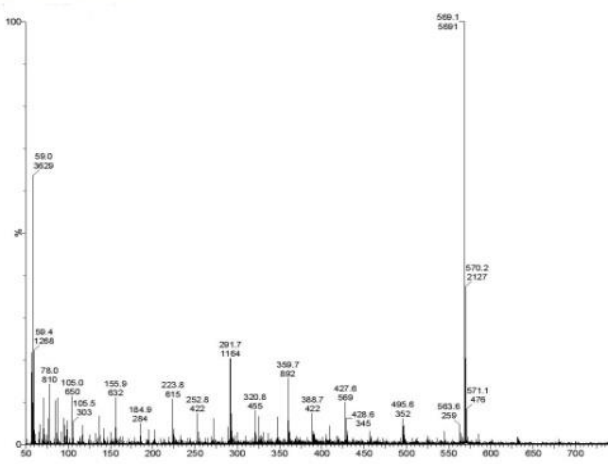

Fig. 3. Mass spectra of DOPD

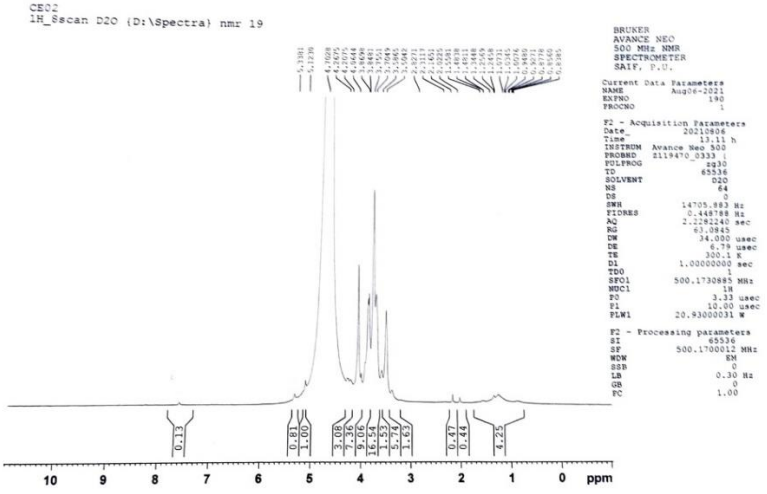

Fig. 4: ^{1}H NMR of DOPD

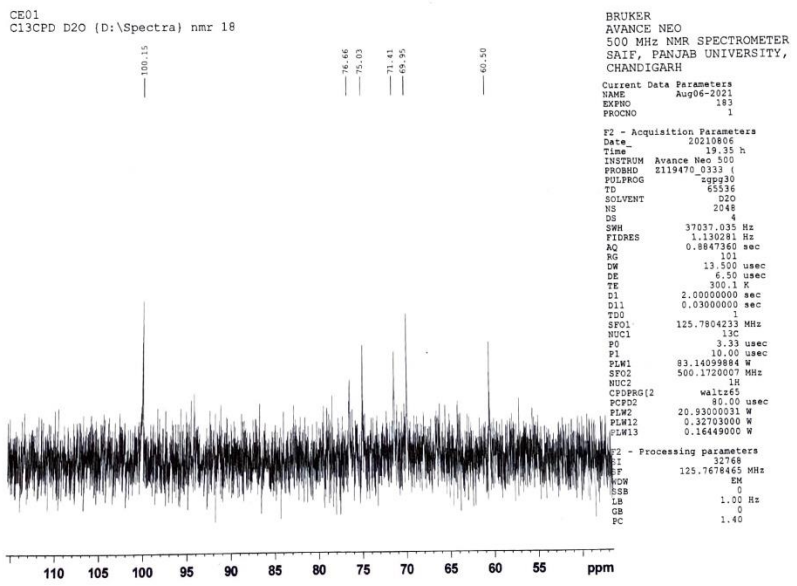

Fig. 5: ^{13}C NMR of DOPD

Table 1. IR spectrum interpretation of DOPD

Functional group	**Frequency (cm^{-1})**
-CH. stretch of benzene ring	3544-3401
-CH- stretch of alkane	2926.1
-CH- stretch of tetrahydro-pyran ring	2235.7
-CH- stretch of pyran	2116.7
-CH- stretch of –H-C=C-H-C-	1621.4
-CH- stretch of ether	1379.5
-CH- stretch of diethyl ether	1135.0
-CH- bend	873.4, 815.7, 766.8
-CO- bend	466.7

Table 2. 1H NMR chemical shift of DOPD

Type of carbon	**Chemical shift (ppm)**
-CH_2- of dioxolo-pyran amd tetrahydro-pyran	4.7033-4.0073
-CH-O- of dioxolo-pyran	5.1190
=CH- (bridge)	3.98215-3.3870
=CH- attached with dioxolo-pyran ring	5.3381-5.1230
-CH- of dioxolo ring	1.21
-CH- of phenyl group	7.13

Table 3. 13C chemical shift of DOPD

Type of carbon	**Chemical shift (ppm)**
Phenyl	100.15
Ether	71.41
-CH- bridge	69.95
Carbon at bridge between oxygen and phenyl group	71.41
Tetrahydro-pyran ring	76.66
Pyran ring	75.03
Dioxolo ring	100.15

Table 4: Acute toxicity study of *Codium elongatum*

Treatment	Doses (mg/kg BW)	0.5 hr	4 hr	24 hr	48 hr	1 week	2 week
Water (control)	----	N	N	N	N	N	N
Water extract	300	N	N	N	N	N	N
	2000	N	N	N	N	N	N
DOPD	300	N	N	N	N	N	N
	2000	N	N	N	N	N	N

N=nil

CONCLUSION

For the first time a novel compound, dioxolo-pyran derivative was isolated from *C.elongatum* and characterized by FT-IR, ESI-MS and NMR. The LD_{50} value of water extract and DOPD was calculated as more than 300 mg/kg and less than 2000 mg/kg of body weight. Further, studies need to be done to establish the pharmacological activity and mode of action of isolated compound.

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CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding present study

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