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Hypoglycemic Activity and Brine Shrimp Lethality of (7E,11E,1R,2S,3R,4R,14S)-14-Acetoxy-3,4-epoxycembra-7,11,15-triene-17,2-olide: A Metabolite of *Lobophytum crassum*†

ALLURI V. KRISHNARAJU, TAYI V.N. RAO, MULABAGAL VANISREE and GOTTUMUKKALA V. SUBBARAJU*
Laila Impex R&D Centre, Unit-I, Phase-II
Jawahar Autonagar, Vijayawada- 520 007, India
E-mail: subbaraju@nettlinx.com

Hypoglycemic activity and brine shrimp lethality of ethyl acetate soluble fraction of *Lobophytum crassum* and its active constituent (7E,11E,1R,2S,3R,4R,14S)-14-acetoxy-3,4-epoxycembra-7,11,15-triene-17,2-olide were tested. Both ethyl acetate fraction and cembranoid diterpene exhibited moderate hypoglycemic activity whereas cembranoid diterpene exhibited potent activity in brine shrimp lethality assay.

Key Words: *Lobophytum crassum*, *Artemia salina*, Indian Ocean, Hypoglycemic activity, Sucrose tolerance test.

INTRODUCTION

Alcyonaceans (soft corals; phylum Coelenterata) of the genus *Lobophytum* have been known to accumulate metabolites, which are structurally unique and pharmacologically diverse[1]. The terpenoid content of alcyonaceans, particularly *Lobophytum* species, vary considerably based on geographical location and season of collection. These soft corals release eggs along with considerable quantity of toxic cembranoid diterpenes which provide chemical defense for the eggs and colonies and confer significant ecological advantage to soft corals[2]. Soft corals are also known to exhibit a wide spectrum of pharmacological activities. In continuation of our studies on bioactivity evaluation of alcyonaceans[3,4], we have carried out hypoglycemic activity and brine shrimp lethality of ethyl acetate extractives of *Lobophytum crassum*, and its active constituent cembranoid diterpene. The results obtained from these studies have been described in this paper.

The utilization of simple bioassays for the evaluation of extracts, fractions and compounds obtained from marine organisms is one of the emerging approaches to find active ingredients from natural resources. Brine shrimp lethality (BSL) assay is a simple bench top bioassay developed by McLaughlin *et al.*[5] and Sathaye

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et al.[6] The results obtained by this assay have been reported to be corroborative with the cytotoxicities determined in 9KB and 9PS cell lines[7, 8].

EXPERIMENTAL

Sucrose, NaOH, gum acacia, anesthetic ether and other reagents are of AR grade and were procured from Qualigens Fine Chemicals, Mumbai, India. Enzymatic GOD/POD glucose test kit was obtained from E. Merck Limited, Mumbai, India. Brine shrimp (*Artemia saliva* cysts) eggs were obtained from Argent Chemical Laboratories, Redmond, WA, USA. Podophyllotoxin and *Salacia* extract were supplied by Laila Impex. A Varian (model Cary 50 Conc) UV-Vis spectrophotometer was used for measuring optical density.

Animals used: Albino wistar rats of both sexes, weighing between 170–250 g. The animals were supplied by Mahaveera Enterprises, Hyderabad, India, and were housed in polycarbonate cages in a temperature and humidity controlled environment. They were fed with standard rodent feed supplied by Amrut Laboratory Animal Feeds, Hyderabad, India. Water was provided *ad libitum* and a 12 h light and 12 h dark cycle was maintained.

Collection of the soft coral: Specimens of the soft coral were collected at the Rameswaram coast of the Indian Ocean during June 2000. Freshly collected specimens (10 kg wet wt.) were washed with fresh water to remove salt deposits and other adhering materials, cut into thin slices and soaked in aqueous ethanol (95%). The specimens were identified as *Lobophytum crassum* by Dr. Phil Alderslade (Northern Territory Museum of Arts and Sciences, Darwin, Australia). Voucher specimen was deposited at NTM of Australia (NTMC 13105).

Extraction: The specimens of the soft coral were cut into thin slices and soaked in aqueous ethanol (95%) at the site of collection. After soaking the soft coral for a month in aqueous ethanol, the ethanolic solution was decanted. The coral was extracted six times more with methanol, solvent was removed from the extract under vacuum and the combined concentrate was partitioned with water and ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulphate, which on concentration gave a dark green residue (105 g).

Hypoglycemic activity: Hypoglycemic activity was tested by the inhibition of sucrose induced raise in serum glucose levels (SGL), by the test substances in Albino wistar rats[5, 6]. The procedure involves fasting the rats for overnight at *ad libitum* water, numbered, weighed and randomly divided into groups of six animals each. Prior to treatment blood samples were drawn from sinus orbital plexus of all animals using heparin coated glass capillaries under mild ether anesthesia. The blood samples were tested for serum glucose levels using enzymatic GOD/POD method[7, 8]. Optical densities were measured at 500 nm. SGL was calculated as follows: SGL = (test OD/Standard OD) × 100 and the results were expressed in mg/dL. All the groups were treated orally with corresponding test substances, standard and vehicle (5% gum acacia). After 30 min, all animals were given 20 mL/kg of 20% sucrose solution orally using gastric tube. 1 h after treatment, blood samples were drawn again under mild ether anesthesia and tested for serum glucose levels using the same procedure as

described above for initial serum glucose estimation. The data was subjected to statistical treatment using *t*-test and inhibitory rate was calculated by comparing the mean increase in serum glucose levels of control as well as treated groups. *Salacia* extracts which were known to possess hypoglycemic activity were used as positive control.

Brine shrimp lethality assay: Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical vessel (1 L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from the brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in vials each containing 4.5 mL brine solution and added various concentrations of drug solutions and volume was made up to 5 mL, using brine solution and maintained at 37°C for 24 h under the light of incandescent lamps and surviving larvae were counted. Each experiment was conducted along with control (vehicle), different concentrations of the test substance in a set of three tubes for each dose. The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{50} values were obtained from the best-fit line plotted concentration (μg) *vs.* percentage lethality. Podophyllotoxin was used as a positive control.

RESULTS AND DISCUSSION

Ethyl acetate soluble fraction of *Lobophytum crassum*, and pure compound isolated were tested for hypoglycemic activity in albino wistar rats. Ethyl acetate fraction exhibited moderate hypoglycemic activity and its active constituent cembranoid diterpene showed potent activity in comparison to the *Salacia* standard tested. These results are summarized in Table-1.

TABLE-1
HYPOGLYCEMIC ACTIVITY RESULTS

S.N.	Test substance	Oral dose (mg/kg)	ISGL (mg/dL) (mean ± SE)	FSGL (mg/dL) (mean ± SE)	Inhibitory rate
1.	Control (vehicle)	—	82.24 ± 5.08	142.57 ± 1.98	—
2.	Standard (*Salacia*)	100	82.81 ± 2.39	101.72 ± 4.01	28.65*
3.	EtOAc fraction	200	82.40 ± 3.81	112.07 ± 5.38	23.85*
4.	Cembranoid diterpene	50	82.58 ± 4.29	115.32 ± 6.46	19.11*

*P is greater than 0.01.
ISGL = Initial serum glucose levels. FSGL = Final serum glucose levels.

Ethyl acetate fraction and its active constituent cembranoid diterpene from the soft coral, Lobophytum crassum were tested for brine shrimp lethality and results are summarized in Table-2. The results are expressed in LC_{50} (concentration at which the test substances produce 50% mortality) obtained by best-fit line plotted concentration in μg against percentage lethality.

TABLE-2
BRINE SHRIMP LETHALITY ASSAY DATA*

Test substance	Concentration (μg/mL)	0	100	250	500	750	1000	LC_{50} (μg/mL)
Ethyl acetate fraction	Mean viable larvae	9.33	9.33	8.66	8.00	4.00	2.67	760
	% Lethality	C[a]	0	7.14	14.28	42.00	71.42	
	Concentration (μg/mL)	0	1	2.5	5	10	25	
Cembranoid diterpene	Mean viable larvae	8.00	7.33	6.67	4.33	2.33	0.33	5.45
	% Lethality	C[a]	8.33	10.66	45.83	70.83	95.83	
Podophyllotoxin	Mean viable larvae	8.33	—	5.6	4.8	1	0	3.10
	% Lethality	C[a]	—	32.8	42	94	100	

Values are mean of three tubes. [a]Considered as zero per cent lethality.

Ethyl acetate soluble fraction showed moderate activity in brine shrimp lethality assay, but weak compared to that of the podophyllotoxin standard. Cembranoid diterpene showed potent activity in brine shrimp lethality assay and is equipotent to that of the podophyllotoxin. The potent brine shrimp lethality is corroborative to the fact that the cembranoid diterpenes are toxic metabolites of soft corals, released along with eggs to provide chemical defense and protect their eggs. The results obtained from the present experiments support further the biomedical potential of soft corals of the genus *Lobophytum*.

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