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Cytotoxic Potentials of Red Alga, *Laurencia brandenii* Collected from the Indian Coast

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Abstract: In the present study, red algae *Laurencia brandenii* from the southwest coast of India was extracted and fractionated in column chromatography using different solvent systems. The fractions were evaluated for brine shrimp cytotoxicity and hatchability assay using *Artemia salina*. The fraction eluted with petroleum:chloroform (6:4) exhibited excellent activity in both assays was subjected to GC-MS (Hewlett Packard) analysis. At a dose level 200 µg/ml the active fraction of algae elicited 100% hatching inhibition, whereas in toxicity assay it showed a LD_{50} value of 93 µg/ml. which might have cytotoxic activity. The fatty acid composition of active fraction revealed that the main acid was 9,12-Octadecadienoic acid (Z,Z)- (49.75 %) followed by n-Hexadecanoic acid (14.24 %) which might have functional role in biological activity.

Key words: *Laurencia brandenii* · *Artemia salina* · Cytotoxic activity · Brine shrimp hatchability assay

INTRODUCTION

According to Cragg *et al.* [1] drugs derived from unmodified natural products or drugs semi-synthetically obtained from marine natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994. This evidence contributes to support and quantify the significance of screening marine natural products. During the last decades there is a considerable increase of interest among the biotech fraternity in exploring the structurally diverse algal bioactive secondary metabolites. Marine algal community signifies a huge source of compounds endowed with ingenious structures and potent biological activities [2, 3]. These bioactive compounds are produced by biosynthetic routes quite different from those known for terrestrial plant metabolites. Many of the products presently used in the field of aquaculture, dietetics and cosmetics are produced by seaweeds or are derived from chemical modification of algal product [4].

The brine shrimp cytotoxicity assay was considered as a convenient probe for preliminary assessment of toxicity, detection of fungal toxins, heavy metals, pesticides and cytotoxicity testing of dental materials [5]. It can also be extrapolated for cell-line toxicity and anti tumor activity [6]. The brine shrimp assay is very useful for the isolation of biogenic compounds from plant extracts [7]. A number of studies have demonstrated the cytotoxic activity of macro algal secondary metabolites from different geographical region [8, 9, 10]. Southwest coast of India is endowed with rich diversity of marine algae with varies biological activities [11]. Studies regarding the antimicrobial property of *L. brandenii* from the Indian coast is already reported [12]. In light of this, the present study was therefore designed to evaluate cytotoxicity (using brine shrimps, *Artemia salina* lethality test) of *L. brandenii* collected from the southwest coast of India.

MATERIALS AND METHODS

Collection and Extraction of *L. brandenii* Bioactives: Seaweeds specimens were collected from the intertidal and subtidal habitat of Kollam (08° 54'N and 76° 38'E) area located in southwest coast of India. The collection were performed during December 2007 to February 2008 when red algal diversity remains dominant. Live and healthy plants were harvested manually and washed thoroughly in running water to remove epizoones, epiphytes, animal castings, sand, calcareous and other adhering detritus matters. Cleaned plant materials were shade dried under a stream of air flow for one week to prevent photolysis and thermal degradation. The completely dried material was weighed and ground coarsely in a mechanical grinder.

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For the extraction of bioactives, 1000.0 g of the dried *L. brandenii* powder was weighed and submerged in a flask containing 200 ml of methanol (purity grade 99 %) and placed at 35°C in a shaker at 120 rpm for 7 days for the extraction of active ingredients. The algal material was re-extracted with methanol in 1 L capacity round bottom flask in a water bath at 60°C for 3 h. The individual crude mixture were pooled and filtered using paper filter fitted with a Buchner funnel using suction pressure followed by centrifugation (Eppendorf) at 6000 xg for 5 min at 20°C. The supernatant was collected in a round-bottomed flask and the remaining solvent was concentrated up to 5-10 ml in a rotary vacuum evaporator (Yamato). The residue collected was evaporated to dry completely in a vacuum desiccator and stored in the refrigerator.

Chromatography of *L. brandenii*: The methanolic extracts of *L. brandenii* (200 gm) was applied in a silica gel (60-120 mesh) column developed with petroleum ether and eluted with petroleum ether and chloroform (9:1 to 1:9 and 100% chloroform) followed by chloroform and methanol (9:1 to 1:9 and 100% methanol) yielded eleven fractions. The individual fractions were screened for bioassays. The fraction that was eluted using petroleum:chloroform (6:4) which exhibited activity was used for GC-MS (Hewlett Packard) analysis. The GC-MS electron ionization mode was 70 eV. Mass range was from *m/z* 45- 450 amu. Peak identification was carried out by comparison of the mass spectra with those available in the NIST Version 2.0 (2005).

Brine Shrimp Lethality Assay: About 1 g of *Artemia salina* (Linnaeus) cysts (Sanders Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was aerated in 1 L capacity glass cylinder (jar) containing filtered seawater. The air stone was placed in the bottom of the jar to ensure complete hydration of the cysts. After 24 hours incubation at room temperature (25-29°C), newly hatched free-swimming pink-coloured nauplii were harvested from the bottom outlet. As the cyst capsules floated on the surface, this collection method ensured pure harvest of nauplii. The freshly hatched free-swimming nauplii were used for the bioassay. The assay system was prepared with 2 ml of filtered seawater containing chosen concentration of extract in cavity blocks (Embryo cup). Parallel vehicle control (using 2 % methanol) and negative control (without vehicle) were included in the experimental setup. In each, 20 nauplii were transferred and the setup was allowed to remain for 24 h, under constant illumination. After 24 h, the dead nauplii were counted with a hand lens. Based on the percent mortality, the LD_{50} of the test compound was determined using probit scale [13].

Hatchability Assay: Brine shrimp hatchability assay was evaluated using the hatchability efficiency of *Artemia salina* against different concentration of *L. brandenii* fractions. About 2 mg of cyst was weighed and placed in 500 ml beaker containing different concentration of algal fraction dissolved in conditioned seawater and kept overnight with aeration. After 24h, the percentage of hatchability was calculated by comparing the number of newly hatched nauplii in chosen concentration with the number of nauplii in the control.

RESULTS AND DISCUSSION

Brine Shrimp Hatchability and Toxicity: In the brine shrimp hatchability assay, the degree of inhibition observed was directly related to the concentration of the active *L. brandenii* fraction. The dose level 200 µg/ml elicited 100% hatching inhibition after 24 h while at 100 µg/ml, the hatching rate was reduced to 27% (Table 1). At both concentrations, the toxicity level of algal fraction against the newly hatched nauplii was similar and caused 100% mortality during 6 h of exposure. It was observed that very low concentrations of algal fraction (50 µg/ml) were detrimental to brine shrimp eggs (Fig. 1). The inhibitory effect of the extract might be due to the toxic compounds present in the active fraction that possess ovicidal and larvicidal properties. This seaweed metabolites either affected the embryonic development or slays the eggs.

Active fraction of *L. brandenii* showed prominent result in brine shrimp cytotoxicity assay. The LD_{50} value was 93 µg/ml (Fig. 2). Literature related to cytotoxic activity of *L. brandenii* against *A. salina* is scanty. Cytotoxic activity of other red algae from the Indian coast is already reported [14]. Kladi *et al.* [15] reported the cytotoxicity of sesquiterpenes, isolated from the organic extract of the *Laurencia obtusa*, against several cell lines. Similarly, Zubia *et al.* [16] reported that *A. armata* had strong cytotoxic activities against cancer cell lines, Daudi and Jurkat cells.

Table 1: Brine Shrimp hatchability assay of *L. brandenii* against *A. salina*

	% of Mortality in various concentration µg/ml			
L. brandenii	50	100	150	200
	18.6±3.5	27.7±5.7	77±4.2	100±0.0

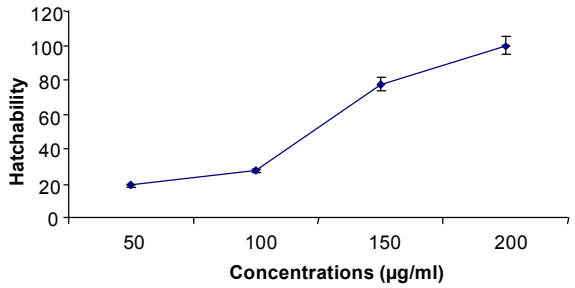

Fig. 1: Brine Shrimp hatchability assay of *L. brandenii* against *A. salina*

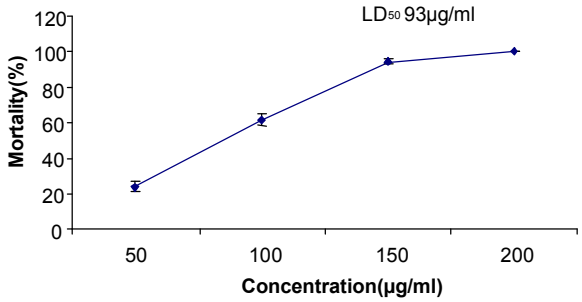

Fig. 2: Brine shrimp lethality of *L. brandenii* against *A. salina*

Cytotoxic property by plant material is due to the presence of antitumor compounds [17]. Many of the secondary metabolites produced by the marine red algae are well known for their cytotoxic property. As noted by Harada and Kamei, [18] the extract from a red alga, *Amphiroa zonata* exhibited strong cytotoxicity to human leukemic cell line. El-Baroty *et al.* [19] demonstrated the cytotoxic activities of powdered *Asparagopsis taxiformis* and its water extract on *Daphna magna*. Halogenated monoterpenes, isolated from *Plocamium cartilagineum* exhibited cytotoxic activity [20]. Bromophenols from *Polysiphonia lanosa* possesses in-vitro cytotoxic activities against DLD-1 cells [21]. Similarly, aqueous extract of *Scinaia okamurae* showed potent activity against B-16 cells [22]. The present study supports that brine shrimp bioassay as a reliable method for the assessment of bioactivity of seaweeds and lends support for their use in pharmacology.

GC-MS Analysis of Active Fraction: A high resolution mass spectrum equipped with a data system in combination with Gas Chromatography (Hewlett Packard) was used for the chemical analysis of active fraction. Chemical characteristics of active fraction on the basis of spectral data by GC-MS were found to be a mixture of fatty acids. A total of 22 peaks were observed with

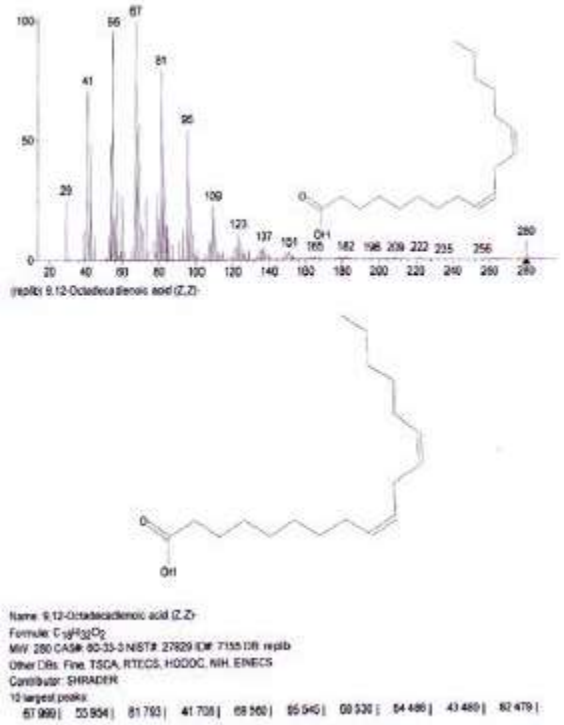

Fig. 3: MS spectrum profile of the 9,12-Octadecadienoic acid (Z,Z)- analyzed from NIST library

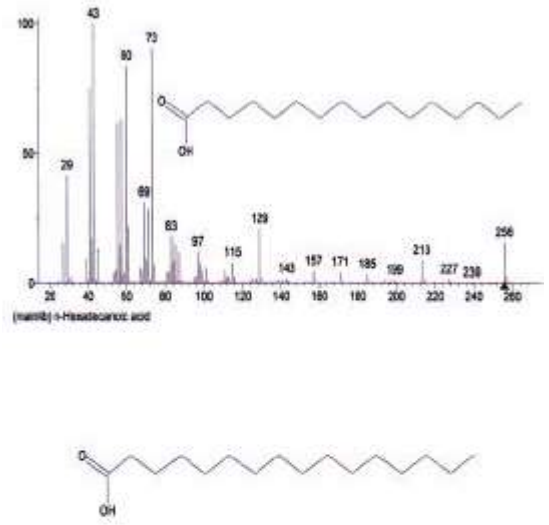

Fig. 4: MS spectrum profile of the n-Hexadecanoic acid analyzed from NIST library

Table 2: GC-MS data of active fraction of *L. brandenii*

No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
1	5.01	Cyclohexasiloxane - dodecomethyl	$C_{12}H_{36}O_6Si_6$	444	1.07
2	8.20	Trisiloxane 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethyl	$C_{12}H_{36}O_4Si_5$	384	0.89
3	9.36	3,5-Dimethyl-5-hexan-3-ol	$C_{18}H_6O$	128	0.11
4	11.64	3,5-Dimethyl-5-hexan-3-ol	$C_{18}H_6O$	128	0.11
5	13.59	4-Dodecanol	$C_{12}H_{26}O$	186	0.08
6	16.04	2-Decanone. 5,9-dimethyl-	$C_{12}H_{24}O$	184	0.13
7	18.65	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.24
8	20.90	9-Dodecanoic acid, methyl ester,(E)-	$C_{13}H_{24}O_2$	212	0.48
9	21.11	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354	0.40
10	21.85	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	49.75
11	24.33	Cyclohexanecarboxylic acid, decyl ester	$C_{17}H_{32}O_2$	268	2.70
12	25.96	9,12-Octadecadienoyl chloride (Z,Z)-	$C_{18}H_{31}ClO$	298	3.50
13	28.26	Oxalic acid, allyl pentadecyl ester	$C_{20}H_{36}O_4$	340	1.23
14	28.75	cis- 9,10-Epoxyoctadecan-1-ol	$C_{18}H_{36}O_2$	284	0.61
15	29.79	Heptanoic acid, 9-decen-1-yl ester	$C_{17}H_{32}O_2$	268	0.65
16	30.63	9-Octadecenal	$C_{18}H_{34}O$	266	5.91
17	34.00	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl	$C_{25}H_{38}O_2$	370	0.44
18	34.51	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354	1.11
19	37.64	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	3.49
20	38.61	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	254	3.01
21	40.00	7,11-Hexadecadienal	$C_{16}H_{28}O$	236	5.99
22	40.40	Thunbergol	$C_{20}H_{34}O$	290	4.11

retention times and relative percentage as presented in Table.1. It is possible that bioactive compounds primarily consisting of 9,12-Octadecadienoic acid (Z,Z)- (49.75 %) may be involved in antimicrobial activity. The fatty acid composition of active fraction revealed that the main acid was octadecadienoic acid (49.75 %) followed by n-Hexadecanoic acid (14.24 %) (Fig. 3 and 4). Seaweeds exhibit a high level of fatty acid diversity and many of which possess potential bioactivity. Therefore in the present study, biological activity of *L. brandenii* might be attributed due to the presence of fatty acid, octadecadienoic acid (49.75 %) in higher percentage.

Based on the GC-MS results, the chemical constituents of main active fraction was extrapolated as low molecular weight lipophilic compound composed of mixture of volatile metabolites and fatty acids. It was found that the main constituent of the purified fraction correspond to octadecadienoic acid (49.75 %).

CONCLUSION

In conclusion, the results of the present study revealed that the marine red alga, *L. brandenii* is a potential producer of cytotoxic secondary metabolites. Therefore, this alga might be utilized for the development of novel anticancer drug leads. Thus, the mechanism of selective cytotoxicity is needed on further studies.

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