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Herbicidal Effects of Marine Animal, *Trochus tentorium* from Gulf of Mannar, Southeastern India

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ABSTRACT
The plants, animals and microorganisms of the marine environment with their wide range of chemical diversity are still an unexplored resource for the development of new agro-chemical agents. Works related to the herbicidal activity of the marine organisms are too scanty. Compared to the search for new pharmaceutical compounds, very little effort has been devoted to the exploration of agrochemical compounds from marine natural products. In the present study, the herbicidal activity of the crude extracts and partial purified fractions of *Trochus tentorium* was assayed using the duckweed, *Lemna minor* L. following a bench top bioassay. The crude acetone extract of *T. tentorium* decayed the fronds of *Lemna* plants at the concentration of 1000 mg mL^{-1} on the 4th day, while the results of same concentration of ethyl acetate, dichloromethane and methanol extracts showed decay of the plants on the 5th day of the experiment. This study reveals that the column-purified acetone fraction of the gastropod was able to decay *L. minor* to a better degree in comparison with the crude extracts. Hence, from the present study, it was noted that not only the 100% acetone fractions but also the 20:80 hexane: methanol and 80:20 acetone: methanol fractions showed a higher degree of activity against *L. minor*.

Key words: Marine mollusc, *Lemna minor* L., partial purification, herbicidal assay, Southeastern India

INTRODUCTION
An ideal herbicide should have potent activity against weeds, minimum toxicity against living things other than plants, high selectivity between crop plants and weeds and cause no damage to the environment by residual material. The weeds often form a green blanket on the water surface and highly reduce light penetration. Among the aquatic weeds, the duckweed, *Lemna minor* L. is considered as one of the important weeds. Thick populations of *L. minor* are often found in association with other aquatic plants, particularly with those that provide protection from wind and wave action (Vernon and Vandiver, 2005). Hence, the usage of herbicides for weed control against this weed has thus become apparent. Herbicides for practical use today are mostly synthetic compounds which inhibit the photosynthetic electron transfer and affect plant hormonal action. However, sooner or later weeds acquire resistance to the existing herbicides and thus the continued development of new and potent drugs to control the weeds is always required. Hence, the development of herbicides must always take into consideration the problem of environmental pollution (Okuda, 1992).

Compared to the search for new pharmaceutical compounds, very little effort has been devoted to the exploration of agrochemical compounds from marine natural products (Fenical, 1993). One of the major impediments to successful herbicide development is obtaining compounds capable of penetrating the cell membrane of target plant (weed) (Lyewellyn and Burnell, 2000). The plant, animals and microorganisms of the marine environment with their wide range of chemical diversity are still an unexplored resource for the development of new agro-chemical agents (Crombie, 1990). In the hunt for new agro-chemical agents, the plants, animals and microorganisms of the marine environment with their wide range of chemical diversity prove to be an unexplored resource. Works related to the herbicidal activity of the marine organisms are too scanty. However, there is relatively limited number of reports on the herbicidal activity of some marine natural products (Burnell *et al.*, 2000; Wagner *et al.*, 2002; Hylleberg and Kilburn, 2002; Omura *et al.*, 1990) and to date, research focused on isolating herbicidal prototype leads from marine origin has resulted in the report of about 40 active compounds. Many important herbicides are isolated from marine algae (Einhellig *et al.*, 1985). Of the marine organisms, in particular, the molluscs have always proven to be potential sources of marine natural products with vast array of diversified bio-activities. There are massive evidences to witness marine molluscs as potential supply of potent metabolites (Ganambal *et al.*, 2005; Hocklowski and Faulkner, 1983; Hubert *et al.*, 1996). In the present study, the crude as well as partial purified extracts of the gastropod, *Trochus tentorium* associated to corals was screened for herbicidal activity against *Lemna minor* (duckweed).

MATERIALS AND METHODS

Extraction of mollusc: The Gastropod, *Trochus tentorium* were collected by hand picking using SCUBA diving from the intertidal area at a depth of 3-5 m in Tuticorin coastal waters (Lat 8°45 and Long 78°13'E) of Gulf of Mannar, southeast coast of India. They were immediately brought to the laboratory and identified up to species level using standard keys (Hubert *et al.*, 1996; Riguera, 1997). The identified shells of *Trochus tentorium* were broke open by a hammer to remove the soft parts. The whole body of the samples (20 g) were cut in to small pieces and air-dried for 24 h at room temperature before extraction with solvents. Then the tissues were rinsed with sterile distilled water and the tissue samples were used for extraction using different solvents such as ethyl acetate, dichloromethane, acetone and methanol. The extracts were cold steeped overnight at -18°C and filtered with Whatman No. 1 filter paper. The filtrate was poured in previously weighed Petri plate and evaporated to dryness in rotary evaporator (Becerro *et al.*, 1994; Riguera, 1997; Wright, 1998). The dried crude extracts were used for herbicidal activity.

Column purification of the active crude extracts: Partial purification of the crude extract of *T. tentorium* was carried out by the method of Chellaram *et al.* (2009). After initial screening, the higher activity was shown by acetone extract and it was fractionated by normal phase silica gel column chromatography by employing a step gradient solvent system from low to high polarity. Sequence of 100% hexane, 20% acetone: 80% hexane, 40% acetone: 60% hexane, 60% acetone: 40% hexane, 80% acetone: 20% hexane, 100% acetone, 80% acetone: 20% methanol, 60% acetone: 40% methanol, 40% acetone: 60% methanol, 20% acetone: 80% methanol and 100% methanol was used for elution. Each fraction thus obtained was once again evaporated, concentrated and assayed for herbicidal activity.

Features of duckweed *Lemna minor* (Arales: Lemnaceae): *Lemna minor* L. (duckweed) is a miniature aquatic monocot, *Lemna* plant, which consists of a central frond or mother frond with two attached daughter fronds and a filamentous root. Under normal conditions, the plant reproduces exponentially with budding of daughter fronds from pouches on the sides of the mother fronds. The sizes of the fronds generally range from 1.7 to 4.0 mm in length and from 0.80 to 4.0 mm in width. Fronds occur solitary or in groups of two or more. There is a single root extending from the base of each frond. Seen from a distance, it is difficult to distinguish individual plants in a body of water infested with duckweed because the surface of the water appears green. Earlier reports by Einhellig *et al*. (1985) provided the general guidelines for developing a *Lemna* bioassay to screen a large number of plant extracts and chemical substances for their effects on plant growth.

Herbicidal assay: The herbicidal activity of the crude extracts of *T. tentorium* was assayed against the duckweed, *Lemna minor* following a bench top bioassay described by McLaughlin *et al*. (1991). To assay the herbicidal activity, 20 mg of the dried crude extracts (*Trochus tentorium*) was dissolved in 20 mL of their respective solvents (ethyl acetate, dichloromethane, acetone and methanol). From this solution, 1000, 500, 100, 50 and 10 μL were pipetted into dram vials corresponding to 1000, 500, 100, 50 and 10 μg mL^{-1}, respectively in triplicates. Controls were added with appropriate solvents alone without the extracts. The solvents of the control and test vials were allowed to evaporate overnight and 2 mL of E-medium (consisting of KH_2PO_4 -680 mg, KNO_3 -1515 mg, Ca $(NO_3)_2.4H_2O$-1180 mg, $MgSO_4.7H_2O$ -492 mg, H_3BO_3 -286 mg, $MnCl_2.4H_2O$ -3.62 mg, $FeCl_2.6H_2O$ -5.40 mg, $ZnSO_4.7H_2O$ -0.22 mg, $CuSO_4.5H_2O$ -0.22 mg, $Na_2MoO_4.2H_2O$ -0.12 mg and EDTA -11.2 mg in one liter of distilled water) was added into each vial. A single healthy *L. minor* plant containing a rosette of three fronds was introduced into each vial and were placed in glass chamber with about 2-4 cm water at the bottom to maintain the moisture content of the chamber and sealed with glass plate. The fronds per vial were counted daily up to 6 days and symptoms of damage to the frond such as yellowing and decaying were also noted.

The column purified fractions were assayed for herbicidal activity and the bioassay was performed to find out which of the fractions were found to be more active in decaying the plant. The herbicidal activity of crude and column fractions of *T. tentorium* was tested at different concentrations 500, 300, 200, 100, 50, 25 and 10 mg and changes in the appearance of the fronds were noted.

RESULTS

The present study reveals that out of the different solvents, the acetone extract of *T. tentorium* decayed the fronds of *Lemna* plants at the concentration of 250 mg mL^{-1} on 6th day and ethyl acetate, dichloromethane and methanol extracts decayed the plants on 5th day of experiment at a concentration of 1000 mg mL^{-1} (Table 1). The acetone crude extracts of the gastropod, shown prominent herbicidal activities against *L. minor*. The column purified (100% acetone, 20:80 hexane: methanol and 80:20 acetone: methanol) fractions of *T. tentorium* shown complete decay of the Lemna plants (Table 2), however, 100% acetone fraction of the extract showed activity at a concentration of 100 mg mL^{-1} on the 5th day of the experiments. But the plants were found to be healthy till the 3rd day of experiment at a concentration of 10 mg mL^{-1}. The plants were decayed by all the fractions at a concentration of 500 mg mL^{-1} on the 4th day of the experiments. This study reveals that the column-purified acetone fractions of gastropod were able to decay *L. minor* to a better degree in comparison with the crude extracts. Hence from the present study, it was noted that not only the 100% acetone fractions but also the 20:80 hexane: methanol and 80:20 acetone: methanol fractions shown a higher degree of activity against *L. minor*.

Table 1: Herbicidal activity of the crude extracts of *T. tentorium* against the *Lemna minor*

Days	1						2				3				4				5				6	
Solvents used conc. (mg mL^{-1})	EA	A	DM	M	EA	A	DM	M	EA	A	DM	M	EA	A	DM	M	EA	A	DM	M	EA	A	DM	M
1000	G	LY	G	G	LY	Y	LY	LY	Y	Y	LY	LY	Y	D	Y	Y	D	D	D	D	D	D	D	D
500	GH	G	GH	GH	G	LY	G	G	G	Y	G	G	LY	Y	Y	LY	LY	D	LY	LY	Y	D	Y	Y
250	GH	GH	GH	GH	GH	G	GH	GH	GH	LY	GH	GH	G	LY	G	G	LY	Y	LY	LY	LY	D	LY	LY
100	GH	GH	GH	GH	GH	GH	GH	GH	GH	G	GH	GH	GH	LY	GH	GH	LY	LY	G	G	LY	Y	LY	LY
50	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	G	GH	GH	G	G	G	G	G	LY	G	G
25	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	G	G	GH	G	LY	G	G
10	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	G	GH	GH	GH	GH	GH	GH
Control	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH

Note: A- Acetone; EA- Ethyl acetate; DM- Dichloromethane and M- Methanol. GH- Green and healthy; G- Green; LY- Light yellow; Y- Yellow and D- Dead

Table 2: Herbicidal activity of the 100% acetone column fractions of *T. tentorium* against the *Lemna minor*

Days		1			2			3			4			5			6	
Solvents used (%)	H:M	A	A:M	H:M	A	A:M	H:M	A	A:M	H: M	A	A:M	H:M	A	A:M	H:M	A	A:M
conc. (mg mL^{-1})	20:80		80:20	20:80		80:20	20:80		80:20	20:80		80:20	20:80	80:20	20:80	20:80		80:20
500	Y	Y	Y	Y	D	Y	Y	D	Y	D	D	D	D	D	D	D	D	D
300	GH	GH	GH	Y	Y	Y	Y	D	Y	Y	D	Y	D	D	D	D	D	D
200	GH	G	GH	LY	Y	LY	Y	Y	Y	Y	D	Y	Y	D	D	D	D	D
100	GH	G	GH	G	LY	GH	G	Y	LY	LY	Y	LY	Y	D	Y	Y	D	Y
50	GH	GH	GH	GH	G	G	LY	LY	LY	LY	LY	LY	LY	Y	LY	Y	Y	Y
10	GH	GH	GH	GH	GH	GH	G	LY	G	G	LY	G	LY	LY	LY	LY	Y	LY
Control	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH

Note: A- Acetone, H- Hexane and M- Methanol. GH- Green and healthy; G- Green; H- Healthy; LY- Light yellow; Y- Yellow and D- Dead

DISCUSSION

The *Lemna* assay data agreed with antitumour activities. McLaughlin *et al.* (1991) have reported that using *Lemna* assay, the search for biodegradable herbicides may be extended to include natural compounds and this is a simple screen for such activity. Reports are available on the herbicidal activities of 9 purified algal metabolites (Fenical, 1997). Lyewellyn and Burnell (2000) had stated that the number of free growing plants is low on coral reefs; therefore, the chemicals produced by these coral reefs can be widely developed as herbicides. Peng *et al.* (2003) identified 18 structurally diverse marine-derived compounds and examined for herbicidal activities and it was concluded that several new classes of compounds have been shown to be herbicidal in nature. In the present study, the acetone extracts of *Trochus tentorium* were found to be efficient in decaying the fronds of *Lemna minor* at a concentration of 1000 mg mL^{-1} on the 4th day of the experiment. Chellaram *et al.* (2004) observed that different solvents like ethyl acetate, acetone and dichloromethane were used for the extraction of the winged oyster, *Pteria chinensis*, however, the acetone extract was able to cause decaying of the fronds of *Lemna minor* at 5th day at a concentration of 1000 mg mL^{-1} which is same concentration to the present study and so the extracts of *T. tentorium* are assumed to be potent producers of novel metabolites. Anand (2002) isolated bacterial strains from the surface of sponges, seaweeds, crabs, ascidians and cephalopod eggs and reported that they were able to bring forth 90% inhibition against *L. minor*.

In conclusion, the results indicate that the crude and column fractions (100% acetone, 20:80 methanol: acetone and 80:20 acetone: hexane) of *T. tentorium* screened for herbicidal activity and extracts of 100% acetone may possess some biologically active compounds. The activity of the column purified fractions was found to be prominent in comparison to the crude counterparts. So the partially purified fraction of the 100% acetone *T. tentorium* undeniably provides a tool for the development of novel herbicides.

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