

Biological investigations of Indian Phaeophyceae : 17. Seasonal variation of antibacterial activity of total sterols obtained from frozen samples of *Sargassum johnstonii* Setchell et Gardner

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ABSTRACT

From lipid fraction of frozen samples of *Sargassum johnstonii*, unsaponifiable part was extracted with diethyl ether to isolate total sterols. The extracted sterols were obtained for a period of nine months and tested against test bacteria. The extracts from June to October months showed antibacterial activity while the samples from November to January and May did not show any activity over the test bacteria *Proteus vulgaris* a gram-negative organism which has shown high sensitivity towards the samples from June and October.

Introduction

Lipids of the seaweeds possess triglycerides, sterols, fatty acids, antibiotics, growth factors, hydrocarbons, vitamins, toxins and peptides (Fogg, 1962; Lefevre, 1964., Sieburth, 1969; Hellebust, 1974 and Venkataraman, 1974). The biological activity of many of the lipid constituents from marine algae has been documented. (Martinez-Nadal *et al.*, 1963; Rao and Parekh, 1981; Padmini *et al.*, 1986, 1987, 1989). Padmini (1992 a,b, 1995) studied and reported on several aspects of *Sargassum*. In the present paper an attempt has been made to investigate the bioactive of total sterols extracted from frozen samples of *Sargassum johnstonii* studied over a period of nine months.

Materials and Methods

Frozen algal samples were collected from Okha, Gujarat Coast of India from June to May. Algal samples were washed, dried, and ground (Padmini *et al.*, 1986). Total lipids were extracted as per the method given by Orcutt and Patterson (1975). Lipid extraction followed by saponification and later isolation of total sterols from unsaponifiable portion was obtained by the method given by Padmini *et al.*, (1986).

The algal sterols were screened in duplicate and tested against six test bacteria namely *Staphylococcus aureus*, *S. citreus*, *Bacillus megatherium* (gram-positive), *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhosa para B* (gram-negative). Controls were run by respective solvent. Presence of inhibition

zones appeared after 24 hours around the disc and indicated the sensitivity of the bioactive substances present in sterols extracted from *Sargassum johnstonii*.

Results

Table 1 shows the antibacterial activity obtained from total sterols extracted, from nine monthly sample collections. Extracts from June to October months possess good antibacterial activity while the extracts from November to January and May months have no effect over the test bacteria. Among the test bacteria tested, *Proteus vulgaris*, a gram negative bacteria which causes urinary tract infections was highly sensitive to the sterol fractions obtained from the samples collected from October and June months. *Bacillus megatherium* a gram-positive bacteria was sensitive to July and August samples. In general gram-negative organisms were more sensitive to sterols than gram-positive bacteria.

Discussion

Species belonging to Dictyotales and Fucales were found to be active (Nakazava *et al.*, 1976; Cacamese *et al.*, 1985; Yamamoto *et al.*, 1973, 1981 & 1983; Ma Jin Wei and Tan Wei-Ci, 1984; Padmini, 1989). These studies support the results from the present investigation showing that *Sargassum johnstonii* also possesses compounds with pharmaceutical importance.

Padmini (1992 a, b) reported that unsaponifiable portion from lipid extract obtained from dry samples of *Sargassum johnstonii* exhibited good antibacterial activity than the unsaponifiable part of frozen samples from the present study. The reasons for this difference in activities is due to the accumulation of bioactive compounds in the dry algal samples while the same get diluted because of high water content in frozen samples.

Table 1. Seasonal variation in antibacterial activity of sterols obtained from frozen samples of *Sargassum johnstonii*

Different months	(Inhibition zone in mm)					
	Test Bacteria					
	(Gram-positive)			(Gram-negative)		
Staphylococcus aureus	Staphylococcus citrus	Bacillus megatherium	Proteus vulgaris	Pseudomonas auruginosa	Salmonella typhosa para B	
June	+	+	++	+++	+	++
July	T	+	++	-	-	T
August	T	+	T	-	-	T
September	++	++	T	+	++	+
October	+	T	T	+++	-	T
November	-	-	-	-	-	-
December	-	T	-	-	-	T
January	-	T	-	-	-	T
May	-	-	-	-	-	-

- = No activity

+ = Moderate activity

T = Trace activity

++ = Good activity

+++ = Very Good activity

Crude extracts obtained by using diethyl ether with frozen samples of *Sargassum merrifieldii* and *S. cinctum* (Padmini 1995), the former sample exhibited moderate antibacterial activity against four test bacteria and no activity against *Salmonella typhosa para B* and *Escherichia coli*. Similar results were obtained from the present algal samples showing confirmity about the pharmaceutical properties by *Sargassum johnstonii* and even in higher degrees.

Shelat (1979) from *Sargassum plagiophyllum* and *S. tenerrimum*, Naqvi *et al.*, (1981) and Pesando and Caram (1984) obtained best antimicrobial activities against several test bacteria. Yamamoto *et al.*, (1981 & 1983) tested the extracts obtained from *Sargassum* spp. to check and to control solid tumour and leukemia. Thus the present findings are agreeing with the views from the above mentioned works.

Hornsey and Hide (1974) classified four main types of seaweeds based on the seasonal variation in their antibacterial activity. According to them the seaweeds exhibit optimum degrees of antibacterial activities when they attain maturity. *Sargassum johnstonii* starts growing from June and reaches its maturity during October - November when it possesses peak activity during winter season. Finally it reaches to extinction stage by April and May. In the present investigation the samples proved the above mentioned theory to be true showing highest activity in October and there was no activity afterwards as the alga started deterioration. Hence the bacteria were not inhibited.

Padmini *et al.*, (1987, 1989) and Padmini (1992 a & b) reported that a gram-negative bacteria, *Proteus vulgaris* inhibited maximum than the remaining test bacteria when investigated unsaponifiable part and crude extracts obtained with diethyl ether.

This fact also agrees with the present results. Large scale screening of red, green and brown seaweeds carried out by Rechelt and Borowitz (1984) showed that majority of brown seaweed extracts exhibited very good antibacterial activities. Thus the above mentioned reports confirm the results of the present investigation.

It has been proved that *Sargassum* species possess bioactive compounds in different degrees. The amount of activity in marine algae depends on several factors such as status of seaweed sample, month and season in which the seaweed sample collected and put for extraction purpose, type of solvent used for extraction, the age of the algae, the type of test bacteria used and the temperature the sterols was extracted. Hence the author strongly suggests that Indian Algalogists should concentrate on studies related to algal bioactive substances to combat dreadful diseases to save mankind.

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