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Fucoidan in Some Indian Brown Seaweeds Found along the Coast Gulf of Mannar

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Abstract: Fucoidan is a hetero-polymer of sulfated L-fucose extracted from brown seaweeds, sea urchin, sea cucumber etc. that display diverse bioactivity. In this investigation, yield, total carbohydrate, L-fucose and sulphate content of crude fucoidan extracted from 11 abundantly occurring brown seaweeds along the coast of Gulf of Mannar (Rameswaram regions), India were evaluated by adopting two different methods. A maximum amount of crude fucoidan was recorded from *Sargassum wightii* (7.15 % alga dry wt.) followed by *Dictyota dichotoma*, *Turbinaria ornata*, *T. conoides*, *T. decurrens*, *Sargassum myriocystum*, *S. ilicifolium*, *S. marginatum*, *Padina boergesenii*, *P. gymnospora* and *Stoechospermum marginatum* (2.13% alga dry wt.). Highest L-fucose content of 232.5 mg g^{-1} dry crude fucoidan (23.25% in crude fucoidan) was extracted in *Sargassum wightii* among the 11 seaweeds studied. Maximum sulphate content of 98.7 mg g^{-1} dry crude fucoidan (9.87% in crude fucoidan) was also recorded in *Sargassum wightii.* A method that yield maximum crude fucoidan constitute less L-fucose whereas the other method that extracted low crude fucoidan constitute high L-fucose content. Some of the brown seaweeds occurring along the coast of Gulf of Mannar contained potential yield of fucoidan. The yield and quality of fucoidan extracted from seaweeds were depending on the method followed for the extraction. The yield of fucoidan was varied among the brown seaweeds likely to be species specific.

Key words: Phaeophyceae, L-fucose, bioactivity, Rameswaram ,Tamilnadu, India

INTRODUCTION

Fucan is a hetero-polymer of sulfated L-fucose extracted from brown seaweeds, sea urchin (Pereira and Mulloy, 1999) and the sea cucumber (Mulloy *et al*., 2000). Since this hetero-polysaccharide was first isolated from the Fucales of *Phaeophyceae*, fucan of algal origin is known as fucoidan. They are located in the intercellular tissues as droplets that exude from the surface of the fronds (Doner and Whistler, 1973). This sulfated fucoidan proved to exhibiting wide range of bioactivities such as antiviral, anti-ulcer and anti-adhesive, anticoagulant, anti-inflammatory and antiproliferative and antitumoral properties (Schaeffer and Krylov, 2000; Nagaoka *et al*., 1999; Grauffel *et al*., 1989; Pereira and Mulloy, 1999). They known to inhibit vascular smooth muscle cells and fibroblast proliferation and can block sperm-egg binding in several species (Boisson-vidal *et al*., 1995). Structures of these polysaccharide vary among the species and sometimes among different parts of the plant (Chevolot *et al*., 1999). In contrast to animal fucans, fucoidans isolated from seaweeds have portions of other neutral sugars and uronic acids in addition to sulfated L-fucose in their structures. This structural complexity, chemical composition of fucoidan of algal origin may vary depending on the method of extraction (Grauffel *et al*., 1989). Thus, each new fucoidan of algal origin has unique compound with unique structural features may display varied bioactivity and would be a potentially a new drug. Even though extraction of fucoidans from brown seaweeds made in various parts of the world oceans and seas (Percival, 1968; Medcalf *et al*., 1978; Kloareg *et al*., 1986; Chevolot *et al*., 1999, 2001; Daniel *et al*., 1999, 2001; Berteau and Mulloy, 2003), except the work of Velayutham and Jayachandran (1991), fucoidans from the Indian brown seaweeds is very limited. Though luxuriant growth of brown seaweeds occurring along the 7200 km of Indian coastal line, evaluation of Indian brown seaweeds for the source of fucoidan not yet done. The Gulf of Mannar located in the southeast coast of India declared as a biosphere reserve with an area of 10, 500 km^2 considered as biodiversity hot spot supported with 3600 species of corals, crustaceans, mollusks, fin fishes, seaweeds, sea grasses, mangroves and other marine animals. Seaweeds occurring abundantly in the 21 islands of this Gulf region are exploited for their commercial utilization (Kumaraguru, 2006). Hence, in the present study, yield, total carbohydrate, L-fucose and sulphate composition of

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crude fucoidan were made from 11 brown seaweeds abundantly occurring along the coast of Gulf of Mannar (Rameswaram region), Tamil Nadu, India by using two different methods.

MATERIALS AND METHODS

Fresh, matured, disease free and healthy brown seaweeds such as *Dictyota dichotoma* (Hudson) Lamouroux, *Padina boergesenii* Allender and Kraft, *P. gymnospora* (Kutzing) Sonder, *Stoechospermum marginatum* (C.Ag.) Kuetz., *Sargassum ilicifolium* (Turner.), *S. marginatum* (*C. agardh*) J. Agardh, *S. myriocystum* J. Agardh, *S. wightii* Greville, *Turbinaria conoides* Kuetzing, *T. decurrens* Bory and *T. ornata* J. Ag. occurring along the coast of Gulf of Mannar (Pamban, Rameswaram), Tamil Nadu, India collected during spring tide of January 2008 were washed thoroughly in seawater followed by tap water to remove the epiphytes and other extraneous materials. Then they were shade dried for 3 days. The shade dried samples were used for the extraction of crude fucoidan by adopting two different methods as described in the following as method I (Velayutham and Jeyachandren, 1991) and method II (Chotigeat *et al*., 2004). Crude fucoidans obtained from the two methods were estimated for their biochemical compositions such as total carbohydrate (Dubois *et al*., 1956), L-fucose (Dische and Shettles, 1948) and sulphate (Verma *et al*., 1977) contents.

Fucoidan yield was expressed in % dry weight of seaweed sample. Total carbohydrate, L-fucose and sulphate estimated from the dry crude fucoidan using both methods were expressed in % dry wt. of crude fucoidan. Experiments were done in triplicate and the mean values were expressed. Results on fucoidan obtained through method I and method II were statistically analyzed using one way ANOVA and expressed.

RESULTS

Results on yield, total carbohydrate, L-fucose and sulphate content of crude fucoidan extracted from 11 brown seaweeds by following the method of Velayutham and Jeyachandren (1991) as method I and Chotigeat *et al.* (2004) as method II are presented in the Table 1. Yield of crude fucoidan was more while extracted with method I than method II from all the 11 seaweeds investigated. Unlike yield, constituents of fucoidan such as total carbohydrate, L-fucose and sulphate were more in the crude fucoidan extracted by following the method II than method I in all brown seaweeds investigated. Trend in the results of yield and quality of fucoidan remained same in the 11 seaweeds. Yield and quality of fucoidan differed significantly between the two methods followed for extraction. Further, yield, total carbohydrate, L-fucose and sulphate of the crude fucoidan were recorded maximum in *Sargassum wightii* and minimum in *Stoechospermum marginatum* among 11 brown seaweeds evaluated.

Table 1: The yield and total carbohydrate, L-fucose and sulphate content of crude fucoidan extracted by method I (Velayutham and Jayachandran, 1991) and method II (Chotigeat *et al*., 2004) in some brown seaweeds occurring along the Coast of Gulf of Mannar (Rameswaram regions), Tamil Nadu, India

Seaweeds	Method	Fucoidan yield	Total carbohydrate in crude fucoidan	L-fucose in crude fucoidan	Sulphate in crude fucoidan
			---------------------------(mg g^{-1} dry wt.)---------------------------		
Dictyota dichotoma	I	67.2a	303.6a	168.7b	78.5b
	II	52.4b	358.3b	189.9a	82.3a
Padina boergesenii	I	36.3a	291.5a	112.6b	50.5a
	II	27.1b	271.4b	122.2a	53.2a
P. gymnospora	I	34.5a	253.2b	102.3b	48.4b
	II	22.3b	263.6a	115.6a	50.1a
Stoechospermum marginatum	I	21.3a	193.5a	63.1b	37.1b
	II	19.2a	172.3b	75.3a	48.2a
Sargassum ilicifolium	I	49.8a	321.3b	132.4b	58.6a
	II	45.3b	335.1a	138.8a	55.6a
S. marginatum	I	47.7a	291.5b	131.5b	56.2b
	II	41.6b	315.5a	146.6a	59.1a
S. myriocystum	I	55.2a	300.0b	127.9b	52.5b
	II	53.4a	348.9a	153.2a	56.3a
S. wightii	I	71.5a	432.6b	173.3b	89.1b
	II	67.2b	468.2a	232.5a	98.7a
Turbinaria conoides	I	58.3a	312.8b	142.1b	71.2b
	II	50.3b	333.1a	168.2a	79.1a
T. decurrens	I	57.2a	354.7a	155.2b	63.6b
	II	53.2a	348.1b	170.3a	71.3a
T. ornata	I	60.3a	350.9a	158.2b	66.4b
	II	57.7b	335.9b	172.1a	78.6a

Values with different letter(s) between two methods in each column showed significant different

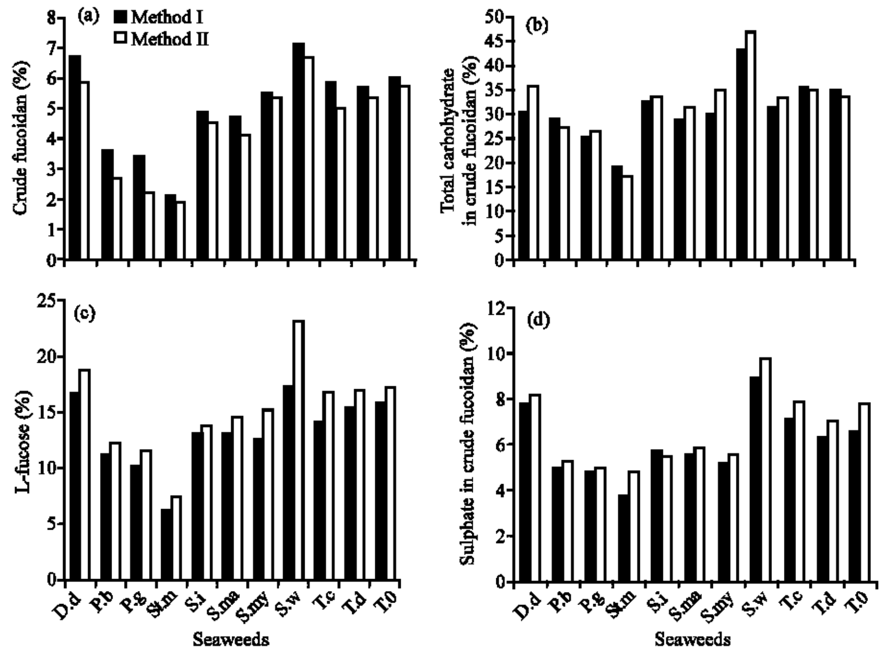

Fig. 1: Yield, total carbohydrate, L-fucose and sulphate content of crude fucoidan extracted by method I (Velayutham and Jayachandran, 1991) and method II (Chotigeat *et al*., 2004) in some brown seaweeds occurring along the coast of Gulf of Mannar (Rameswaram regions), Tamil Nadu, India. D.d: *Dictyota dichotoma*, P.b: *Padina boergesenii*, P.g: *P. gymnospora*, S.m: *Stoechospermum marginatum*, S.i: *Sargassum ilicifolium*, S.m: *S. marginatum*, S.my: *S. myriocystum*, S.w: *S. wightii*, T.c: *Turbinaria conoides*, T.d: *T. decurrens*, T.o: *T. ornate*

Yield of crude fucoidan: Significantly higher crude fucoidan was extracted in method I than in method II from all the 11 seaweeds. Maximum significant yield of 71.5 mg g^{-1} alga dry wt. and 67.2 mg g^{-1} alga dry wt. of crude fucoidan was extracted in the method I and II from *Sargassum wightii*, respectively. Other than *Sargassum* species, high significant yield of 67.2 mg g^{-1} alga dry wt. and 58.4 mg g^{-1} alga dry wt. of crude fucoidan was extracted by method I and II from *Dictyota dichotoma*, respectively. Of the three species of *Turbinaria*, a high significant yield of 60.31 mg g^{-1} alga dry wt. and 57.7 mg g^{-1} alga dry wt. of crude fucoidan was extracted from *Turbinaria ornata* by method I and method II, respectively. Among the *Padina* species, a high significant yield of 34.5 mg g^{-1} alga dry wt. and 22.3 mg g^{-1} alga dry wt. of crude fucoidan were extracted by method I and method II from *Padina gymnospora*, respectively. A minimum significant yield of 21.3 mg g^{-1} alga dry wt. and 19.2 mg g^{-1} alga dry wt. of crude fucoidan was extracted by method I and II from *Stoechospermum marginatum*, respectively among the 11 seaweeds investigated (Table 1).

Constituents of fucoidan: Unlike yield, constituents of fucoidan such as total carbohydrate, L-fucose and sulphate were more in the crude fucoidan extracted from the method II than method I in all the seaweeds studied. Total carbohydrate: Higher total carbohydrate was recorded in the crude fucoidan extracted by method II than method I. Among the seaweeds investigated, maximum of 46.82 and 43.26% of total carbohydrate was recorded in the crude fucoidan extracted by method II and method I from *Sargassum wightii*, respectively. In non-*Sargassum* species, 35.83 and 30.36% of total carbohydrate in crude fucoidan was extracted by method I and method II in *Dictyota dichotoma*, respectively. Of three species of *Turbinaria*, a high of 35.47 and 34.81% of total carbohydrate in crude fucoidan was extracted in *Turbinaria decurrens* by method II and method I, respectively. In the species of *Padina*, 29.15 and 27.14% of total carbohydrate were recorded in crude fucoidan extracted by method I and II in *Padina boergesenii*, respectively. Of the 11 brown seaweeds, minimum record of 19.35 % and 17.23 % of total carbohydrate in crude fucoidan was obtained in *Stoechospermum marginatum* extracted by method II and method I, respectively (Fig. 1a, b).

L-fucose is major sugar constituent of fucoidan. Higher amount of L-fucose was recorded in the crude fucoidan obtained in method II than method I in all 11 seaweeds investigated. Maximum of 23.25 and 17.33 % of L-fucose was recorded in crude fucoidan extracted by method II and I in *Sargassum wightii*, respectively. Among the non *Sargassum* species, high amount of 18.99 and 16.87% of L-fucose was found in the crude fucoidan extracted by method II and I in *Dictyota dichotoma*, respectively. Among the three species of *Turbinaria*, high amount of 17.21 and 15.82% of L-fucose was recorded in crude fucoidan extracted in *Turbinaria decurrens* by method II and method I, respectively. In *Padina*, 12.22 and 11.26% of L-fucose were recorded in crude fucoidan extracted by method II and method I in *Padina boergesenii*, respectively. Minimum recorded of 7.53 and 6.31% of L-fucose in crude fucoidan was obtained by method II and method I, respectively in *Stoechospermum marginatum,* among the 11 brown seaweeds studied (Fig. 1c).

Higher amount of sulphate was recorded in crude fucoidan obtained in method II than method I in all 11 seaweeds studied. Maximum of 9.87 and 8.91% of sulphate was recorded in crude fucoidan extracted by method II and I in *Sargassum wightii*, respectively among the 11 seaweeds investigated. In other seaweed species, high amount of 8.2 and 7.85% of sulphate was recorded in crude fucoidan obtained in the method II and I in *Dictyota dichotoma*, respectively. Of the three species of *Turbinaria*, high amount of 7.91 and 7.12% of sulphate was recorded in crude fucoidan extracted in *Turbinaria decurrens* by method II and method I, respectively. Among the species of *Padina*, a high of 5.32 and 5.05% of sulphate was found in the crude fucoidan extracted by method II and I in *Padina boergesenii*, respectively. Minimum of 4.82 and 3.71% of sulphate was recorded in crude fucoidan extracted by method II and method I, respectively in *Stoechospermum marginatum*, among the 11 seaweeds studied (Fig. 1d).

Based on the yield, total carbohydrate, L-fucose and sulphate of crude fucoidan extracted by the method I and II, 11 seaweeds are hierarched as follows: *Sargassum wightii*, *Dictyota dichotoma*, *Turbinaria conoides*, *Turbinaria ornata*, *Turbinaria decurrens*, *Sargassum ilicifolium*, *Sargassum marginatum*, *Sargassum myriocystum*, *Padina boergesenii*, *P. gymnospora* and *Stoechospermum marginatum.*

DISCUSSION

Fucoidan mainly constituted with sulfated L-fucose easily extracted from the cell wall of brown seaweeds (*Phaeophyceae*) with hot water (Percival and Ross, 1950) or acid solution (Black, 1954) accounted more than 40% of the dry weight of isolated cell walls (Kloareg, 1984). From crude fucoidan, fucose was quantified by the cysteine-sulfuric method (Winzler, 1955) and content of fucoidan was 1.18 to 2.74 per 100 g dry weight of *Sargassum polycystum* and *Pelvetica canaliculata* (Colliec *et al*., 1994). In the present investigation, yield, total carbohydrate, L-fucose and sulphate content of crude fucoidan extracted by two methods such as Velayutham and Jeyachandren, 1991 (method I) and Chotigeat *et al*. (2004) (method II) from 11 brown seaweeds occurring along the coast of Gulf of Mannar (Rameswaram regions), Tamil Nadu, India. Of the two methods followed, higher fucoidan yield was recorded in method I (Velayutham and Jeyachandren, 1991) than method II (Chotigeat *et al*., 2004). A maximum amount of crude fucoidan was recorded from *Sargassum wightii* (7.15% alga dry wt.) followed by *Dictyota dichotoma*, *Turbinaria ornata*, *T. conoides*, *T. decurrens*, *Sargassum myriocystum*, *S. ilicifolium*, *S. marginatum*, *Padina boergesenii*, *P. gymnospora* and *Stoechospermum marginatum* (2.13% alga dry wt.) when extracted by method I, in the present study. Though both extraction methods involved acid extraction, yield of crude fucoidan was more when the seaweeds extracted by method I. Velayutham and Jeyachandren (1991) reported 4.2% (alga dry wt.) of crude fucoidan in *Sargassum ilicifolium* whereas by following the same method in the present investigation, 7.15% (alga dry wt.) of crude fucoidan was extracted in *Sargassum wightii.* This present investigation supported that yield of fucoidan in the brown seaweeds depending on the method followed for extraction and species of seaweeds used. Wang and Zhae (1985) extracted 3.9% of crude fucoidan from *Sargassum horneri.* Fucoidan extracted from *Utricularia aurea* was 1.3% of dry weight and comprised of 62.5% of glucuronic acid and 4.98% of fucose in the crude extract and 28.74% of the fucose was sulfated (Choosawad *et al*., 2005). High yield of crude fucoidan was achieved in seaweeds extracted method I (Velayutham and Jeyachandren, 1991) whereas constituents of fucoidan was high in the crude extracted by method II ,in the present study. This suggested that quality of fucoidan was achieved by following the method II (Chotigeat *et al*., 2004). In the present study, total carbohydrate in crude fucoidan was ranged from 43.26% (*Sargassum wightii)* to 17.23% (*Stoechospermum marginatum*) among the 11 brown seaweeds evaluated. Percival and Ross (1950) described fucoidan in common brown seaweed *F. vesiculosus* as a polysaccharide constituted with L-fucose containing α (1>2) glycosidic bonds sulfated at 4th position with branches of sulfated fucose at every five units. This model supported by O'Neill (1954) and Cote (1959). Fucoidan consisting of

L-fucose, sulfate and acetate in a molar proportion of 1:1:0.1 with small amounts of xylose and galactose isolated from the brown seaweed *Fucus evanescens* (Bilan *et al*., 2002). In the present study, maximum L-fucose in crude fucoidan was recorded in *Sargassum wightii* (23.25%) and minimum was recorded in *Stoechospermum marginatum* (7.53%). Fucoidan extracted from seaweed *Utricularia aurea* was 1.3% in alga dry weight comprised of glucuronic acid 62.5% and fucose 4.98% in crude fucoidan of which 28.74% of the fucose was sulfated (Choosawad *et al*., 2005). In the present investigation, higher amount of sulphate was recorded in crude fucoidan obtained in method II than method I in all 11 seaweeds studied.

In conclusion, brown seaweeds occurring along the coast of Gulf of Mannar contained potential yield of fucoidan. The yield and quality of fucoidan extracted from seaweeds were depending on the method followed for the extraction. The yield of fucoidan was varied among the brown seaweeds likely to be species specific.

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