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Extraction of fucoidan for the green synthesis of gold nanoparticles from *Sargassum polycystum* (C.Agardh, 1824) and its biopotentials

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ABSTRACT

The crude fucoidan was extracted from the brown alga *Sargassum polycystum* by hot water extraction. It was further purified by chromatographic techniques and preliminarily characterised using physical and chemical properties (colour, odour, taste, and texture), solubility, purity test and chemical analysis in combination of infrared (IR) spectroscopies. Further, the fucoidan assisted Gold nanoparticles (GNPs) were synthesised and characterised. Primarily the Nanoparticles (NPs) synthesis were confirmed by colour change and characterized by UV-vis spectroscopy. The biosynthesised nanoparticles were investigated for its therapeutic purposes.

Introduction

Seaweeds are the extraordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine. Seaweeds produce a variety of biologically active components with different structures and interesting functional properties (Kim and Bae, 2010). The bioactive components of seaweeds include polyphenols, peptides, polysaccharides, etc. Many of these active compounds are found to be useful functional ingredients with numerous health benefits. Among polysaccharides, fucoidans were particularly studied. Fucoidan is a group of marine sulfated polysaccharides of the cell-wall matrix of brown algae, containing large proportions of L-fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, glucose, mannose, uronic acids and rhamnose (Berteau and Mulloy, 2003). These acidic polysaccharides are known to exhibit a wide range of physiological and biological activities, medically useful activities (Boisson-Vidal *et al.,* 2000), such as anti-inflammatory (Ostergaard *et al.,* 2000), antiviral (Hoshino *et al.,* 1999), anticoagulant (Mourao 2004), antitumor (Riou *et al.,* 1996) and antiangiogenesis activities (Hahnenberger and Jakobson, 1991).

There are very scanty reports on fucoidan mediated synthesis of Gold Nanoparticles (F-GNPs) and developing such method would provide a cull to chemical method. Therefore, the current study was intended to synthesize, optimize and to characterize gold nanoparticles using the fucoidan extracted from *Sargassum polycystum* as reducing agent in a feasible way and further to explore its biopotentials as antioxidant and anticoagulant agents.

Materials and Methods

Collection

Sargassum polycystum C. Agardh (fresh weight) was collected from Arockiyapuram coast, Tamil Nadu, India. After thorough washing with seawater and manual sorting to remove epiphytes, the fresh biomass was exhaustively washed with tap water followed by distilled water, then shade dried and ground to pieces of diameter of approximately 1 mm. The alga was identified and authenticated by Dr. P. Anantharaman, Associate Professor, CAS in Marine Biology, Annamalai University, Cuddalore District, Tamil Nadu.

Depigmentation and deproteinizationof *Sargassum polycystum*

100 g of algal powder was soaked separately in an acetone solvent system (1:10) for two days in a shaker at 200

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rpm (Ammar *et al*., 2015). The polysaccharide mixture was deproteinized following the Sevag method (Luo and Fan, 2011.)

Extraction of Crude Sulfated polysaccharides

The depigmented *S. polycystum* (100 gm)seaweed was ground and sieved to pass through a 500 ìm sieve and 100 g of dried ground seaweed was extracted in 2 L of 0.03 M HCl with continuous stirring at 200 rpm for 4 h at 90°C water bath. The suspension was filtered, and precipitated using 60% ethanol. The precipitate was collected after centrifugation at 10,600 rpm for 10 min and the resulting pellet was freeze dried andlyophilized to get Fucoidan. The polysaccharide yield (%) was then calculated (Yang *et al.,* 2008)

Characterization of Polysaccharides

The Organoleptic evaluation refers to the evaluation of color, odour, shape, taste and special features which include touch and texture described by Kumar *et al.* (2011) and purity of polysaccharides by phytochemical analysis (Sofawara *et al.,* 1982). The approximate solubility of fucoidan extracted from *Sargassum polycystum* was checked for different common solvents depending on their polarity (Malviya, 2011).The IR spectrum was recorded with Perkin-Elmer model 297 IR Spectrophotometer for a range of frequencies between 400 and 4000 cm^{-1}. All samples were analyzed as KBr pellet (Edwards *et al*., 1958).

Biosynthesis of gold nanoparticles

Aqueous solution (1mM) of Hydrogen tetrachloroaureate ($HAuCl_4$) was prepared and used for the synthesis of gold nanoparticles. Biosynthesis of gold nanoparticles was evaluated following the method of Rajathi *et al*. (2012). The reduction of metal ions was roughly monitored by visual observation of the solution (Fang *et al*., 2005). The bioreduction of $AuCl_4$ ions in solution was monitored by measuring the UV–vis spectra (Mie, 1908).

Antibacterial and Anticoagulation activity

Antibacterial activity of the fucoidan and synthesized gold nanoparticles were determined using the agar well diffusion method against selected pathogenic gram negative and gram positive bacteria *Staphylococcus aureus, Bacillus cereus, E. coli*. Amphicilin was used as a control antimicrobial agent. The formation of a clear zone around the cavity is an indication of antibacterial activity (Cappuccino and Sherman, 1996). Anticoagulant activity was determined by following the method of Mauray *et al*. (1995).The anticoagulant activity exhibited by fucoidan and GNPs on human blood clotting factors was evaluated through APTT and PT assays.

Results and Discussions

The total yield of fucoidan extracted from the brown alga *S. polycystum* at room temperature was 9.2%g/100 gm (Fig. 1A & B) which is almost same as that fucoidan polymer extracted from the brown seaweed *Undaria pinnatifida* (Yang *et al*., 2008). Chotigeat *et al.* (2004) reported that the yield of fucoidan extracted from *S. polycystum* was 2.74± 1.18g/100g dry weight. The yield was calculated on dry weight substratum of the sample. The Fucose 75.25%, Sulphate 11.74% is in close agreement with the results of fucoidan extracted from *Sargassum binderi* (Sinurat *et al*., 2015).

Physical properties of Fucoidan

One of the organoleptic characters of fucoidan is brown colour, odourless, tasteless, and irregular in shape with hard and rough surface in crude and powder form (Table-1). The solubility behaviour of the fucoidan indicates that it is readily soluble in water and forms viscous solution in warm water, sparingly soluble in cold water, whereas insoluble in ethanol, methanol, acetone and ether (Table-2). The purity of fucoidan was determined by prescribed phytochemical tests. This indicated the lack of alkaloids, steroids flavonoids, saponins, tannins and phenols in the sample. Only carbohydrates were found to be present, which confirms the purity (Table-3).

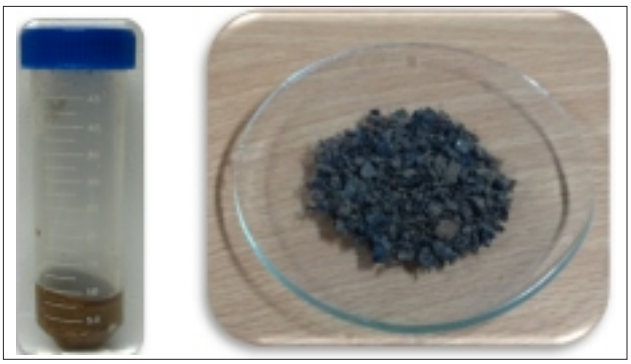

Fig. 1. *Precipitate of Fucoidan (A) & Fucoidan (B)*

Table-1. *Organoleptic evaluation of fucoidan Table 2.Solubility of fucoidan in different*

S. No	Parameters	Results
1.	Colour	Brown
2.	Odour	Odourless
3.	Taste	Tasteless
4.	Touch	Hard and Rough
5.	Texture	Powder

Table-2. *Solubility of fucoidan in different solvents*

S. No	Solvents	Solubility Behavior
1.	Cold water	Sparingly soluble
2.	Warm water	Quickly soluble
3.	Methanol	Insoluble
4.	Ethanol	Insoluble
5.	Acetone	Insoluble
6.	Diethyl ether	Insoluble
7.	H_2SO_4	Soluble
8.	HCl	Partially Soluble

Table-3. *Phytochemical analysis of fucoidan*

S. No	Phytochemicals	Results
1.	Alkaloids	-
2.	Saponoins	-
3.	Tannins	-
4.	Phenols	-
5.	Phlobatannins	-
6.	Flavonoids	-
7.	Steroids	-
8.	Carbohydrates	++
9.	Terpenoids	-

Fourier Transform Infrared spectroscopy

FT-IR analysis revealed the presence of bands at 3770, 3421, 2924, 2856, 2524, 2358, 1631, 1458, 1325, 1253, 1022, 813, 624,505, 462 cm^{-1}.FTIR spectrum of isolated fucoidan A1H (Fig. 2) having band at 1635/ cm indicate the presence of C=O stretching vibration of O-acetyl groups. The band at 1454/cm assigned for CH2 (galactose, xylose) and band at 1325/cm indicate the presence of CH3 (fucose, O-acetyls).The stretching at 1072/cm for symmetric O=S=O vibration of sulphate esters. Beside band at 613/cm indicates thepresence of C-O-S secondary axial sulphate at C-4 offucopyranose residue. The other major absorption band at 3433/cm indicates the presence of O-H stretching. The above characteristic features of FTIR spectrum (Fig. 2) show that the isolated polysaccharide was confirmed as fucoidan (Eluvakkal *et al.,* 2014).

Synthesis of gold nanoparticles from Fucoidan

The nanoparticles were primarily characterized by Visual observation and UV-vis spectralanalysis. After addition of *fucoidan* to the $HAuCl_4$ aqueous solution, the colour of the reaction mixture turned into purple colour after 4 h (Figure. 3 A&B). It is well known that GNPs exhibit a purple colour in aqueous solution due to the excitation in UV-visible spectrum. The appearance of a purple colour in solution containing the extract suggested the formation of gold nanoparticles (Mazhari and Agsar, 2016). Absorption spectra of gold nanoparticles showed a well defined Surface Plasmon's band centered at 550 nm.

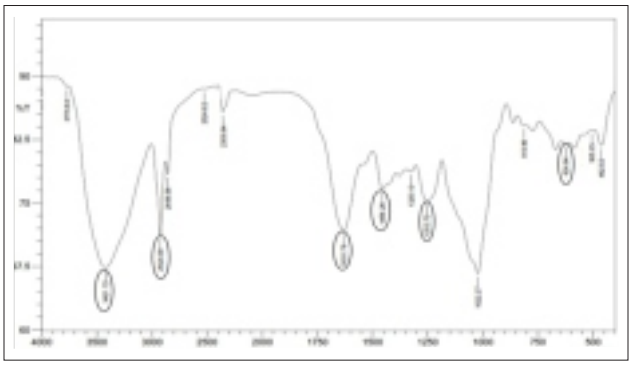

Fig. 2. *FTIR spectrum of fucoidan extracted from* S. polycystum

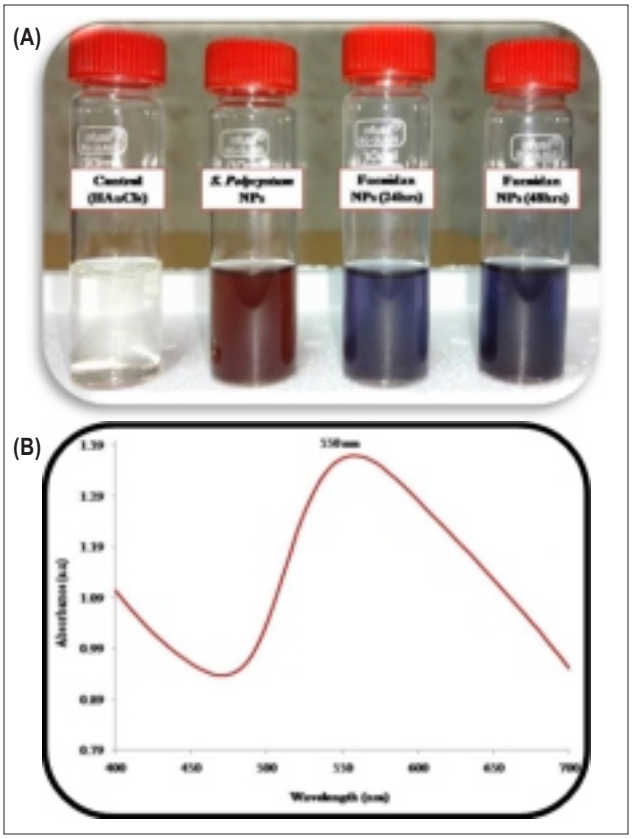

Fig. 3.*Visual observation (A) and UV-visible spectra (B) of gold nanoparticles synthesized by fucoidan of* S. polycystum

Antibacterial activity of Gold Nanoparticles

The maximum activity (24 mm) was obtained for *Staphylococcus aureus* and the minimum activity (19 mm) was obtained for *E.coli.* The study reveals that the antibacterial activity of gold nanoparticles synthesized from *S. polycystum* was effective against gram positive bacterium. Similarly,

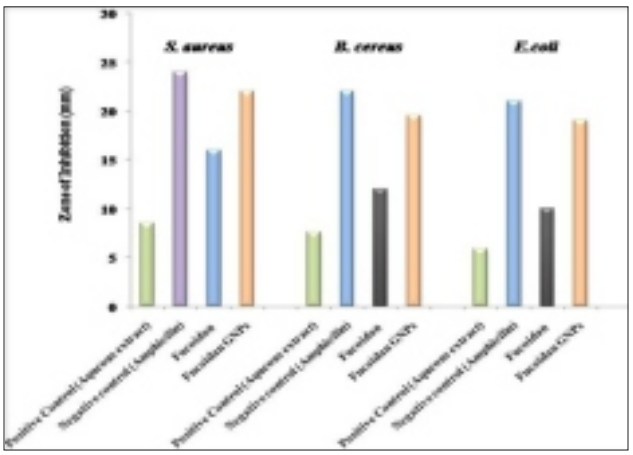

Fig. 4. Antibacterial activity of gold nanoparticles synthesized from fucoidan of *S. polycystum*

Table-4. *Anticoagulant activity of fucoidan and Fucoidan gold nanoparticles (F-GNPs)*

S.No	Compound	Anticoagulant assay	
		APTT (IU/mg)	PT(IU/mg)
1.	Fucoidan	64.4	12.5
2.	F- GNPs	75. 3	15.5

Chotigeat *et al.,* (2004) reported that the crude fucoidan from *Sargassum polycystum* showed the activity at 12 mg/ml against the *Staphylococcus aureus* (10mm).

The anticoagulant activity

In APTT test, the anticoagulant activity of fucoidan was determined as 64.4 IU whereas GNPs showed the inhibition of 75.3at mg/ml respectively. Subsequently in PT assay, the clotting was observed at 10.5 and 15.2 IU for fucoidan and F-GNPs (Table-4) as previously reported earlier (Eluvakkal *et al.,* 2014).

The anticoagulant and antibacterial activity showed that the fucoidan and fucoidan mediated gold nanoparticles (F-GNPs) exhibited potent anticoagulant and antibacterial activity. So, it can be developed as a promising anticoagulant for various thrombotic diseases and as a bactericidal agent.

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