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Antioxidant activity of polysaccharide from Sargassum sp

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Abstract: Brown seaweeds are widely used in the medical field to treat tumor cells. In this present study brown seaweed Sargassum species was used to isolate polysaccharide and quantitative estimation of carbohydrate was done and the free radical scavenging activity of the isolated polysaccharide was determined using DPPH radical scavenging assay, Hydrogen peroxide scavenging activity Nitric oxide scavenging activity , Ferric reducing antioxidant Power (FRAP) Deoxyribose Radical Scavenging Activity, ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical cation Scavenging Assay, Superoxide radical scavenging activity, Estimation of lipid peroxidation using egg yolks, β carotene linoleic acid assay and SOD was performed. The obtained results gave a significant IC50 Value which indicates that the isolated polysaccharide from the Sargassum sp was able to inhibit the free radicals and can be used to treat tumour cells.

Keywords: Anticancer activity, Fucoidan, Fucose, polysaccharide, Sargassum sp

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I. INTRODUCTION

Brown Algae Contain Large Amounts of Cell-Wall Polysaccharides, Most of them are Sulfated Polysaccharide Fucoidans.(Asker et.al 2007[1]). Recently, several investigations have been focused on the isolation and function of the polysaccharides derived from different *Sargassum* species, which revealed multiple biological activities such as hepatic and renal protective activity (Josephine et al., 2007[2].)Brown seaweed or kelp has been harvested around the world for centuries. It is perhaps best known for its use in Japan and other Asian countries as sea vegetables. In the United States, kelp experienced a general interest in the mid-60's with its inclusion in many household products (e.g. toothpaste, fertilizer, pharmaceutical excipients, thickening agents, etc.). Like any other nutrient source, manufacturing processes have continued to improve both the yield and the quality of the raw compound. One such development for brown seaweed is the identification and isolation of fucoidans [3,4]. Fucoidans are sulphated polysaccharides with a fucose backbone found mainly in brown seaweed and account for more than 40% of the dry weight of the algal cell walls. The Harmful Action Of The Free Radicals Can, However, Blocked By Antioxidant Substances, Which Scavenge The Free radicals and detoxify the Organism (Balakumar et Al., 2010).[5] Furthermore, macroalgae have shown to provide a rich source of natural bioactive compounds with antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, hypercholesterolemia and hypolipidemic and antineoplasteic properties (El-Baroty GS et.al 2007) [6] Thus, there is a growing interest in the area of research on the positive effect of macroalgae on human health and other benefits (Sahera F et.al)[7]

II. MATERIALS AND METHOD.

2.1 *Sargassum sp* was collected from Mandapam coastal area, Rameshwaram. Collected sample was cleaned shade dried and then it was further dried using hot air oven. The dried seaweeds were subjected to grinding using mixer machine. The powdered *Sargassum sp* sample was stored in an air tight container and it was used for further analysis.

2.2 Extraction of Polysaccharide Using Soaked Water Method

A new extraction method of fucoidan from the soaked water of brown seaweed (Xiaolin Chen et.al February 2012)[8]was used for the extraction of fucoidan from *Sargassum sp* with a slight modification. 150gm of powdered seaweed sample was mixed with 2.4l of distilled water and it was kept in the shaker for 24hours. Then it was filtered and the filtrate was separated. From the filtrate 150ml of the filtered solution was taken and mixed with 1% chitosan.1% chitosan was prepared in 1% acetic acid solution. The mixed solution was kept for

12 hours in a shaker for agitation. The obtained mixture was centrifuged at 1500rpm for 5 minutes. Supernanent was discarded and the pellet was dried in the hot air oven at 50°.From the dried pellet,0.2176mg was weighed and it was dipped in 30ml of distilled water of PH 4 was adjusted and it was kept for 12 hours. After the period of 12 hours the mixture was agitated in room temperature for 2 hours and it was filtered. The filtrate was washed with ethanol for 15minutes and it was centrifuged and the pellet was removed, air dried and it was used for further analysis. Yield obtained is given in the equation. **(ref 2.2)**

2.3 Quantitative Estimation of carbohydrate using Phenol – Sulphuric acid method

Phenol - Sulphuric Acid Method is the most easiest and reliable method amongst the quantitative assays for carbohydrate estimation. It is mostly used in measuring neutral sugar content in oligosaccharides, proteoglycans, glycoproteins and glycolipids. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow-brown coloured product with phenol and has absorption maximum at 490 nm. This is one of the best methods to estimate total carbohydrate. **(ref Fig 2.3)**

2.4 Antioxidant and free radical scavenging activity

The total antioxidant and radical scavenging activity of the polysaccharide fucoidan extracts of the species, *Sargassum* was determined by standard protocol. *In vitro* assays include total phenolic, total flavonoid, DPPH assay, Hydrogen peroxide activity and Nitric oxide scavenging assay based on the procedure followed by (Suganya *et al.*, 2017)[9].

III. RESULT AND DISCUSSION

From the *Sargassum sp* polysaccharide was extracted and the yield obtained was calculated using the formula **(ref.Fig2.2)** and it was quantitatively estimated using Phenol and sulphuric acid method. **(ref Fig2.3),** after the estimation the extract was dissolved using the methanol and it was used to find the antioxidant and scavenging activities like DPPH activity **(ref. Fig2.4.1),** Hydrogen peroxide test **(ref.Fig2.4.2),** Nitric oxide scavenging activity**(ref. Fig2.4.3)**, ferric reducing antioxidant test **(ref.Fig2.4.4),**ABTS radical scavenging activity**(ref Fig2.4.5),** deoxyribose radical scavenging activity **(ref. Fig2.4.6**), Superoxide radical scavenging activity **(ref. Fig2.4.7),** estimation of lipid peroxide using egg yolk(**ref. Fig2.4.8**), β carotein linolic acid assay **(ref. Fig 2.4.9)** and SOD **(ref Fig 2.4.10**)

2.2 Yield obtained

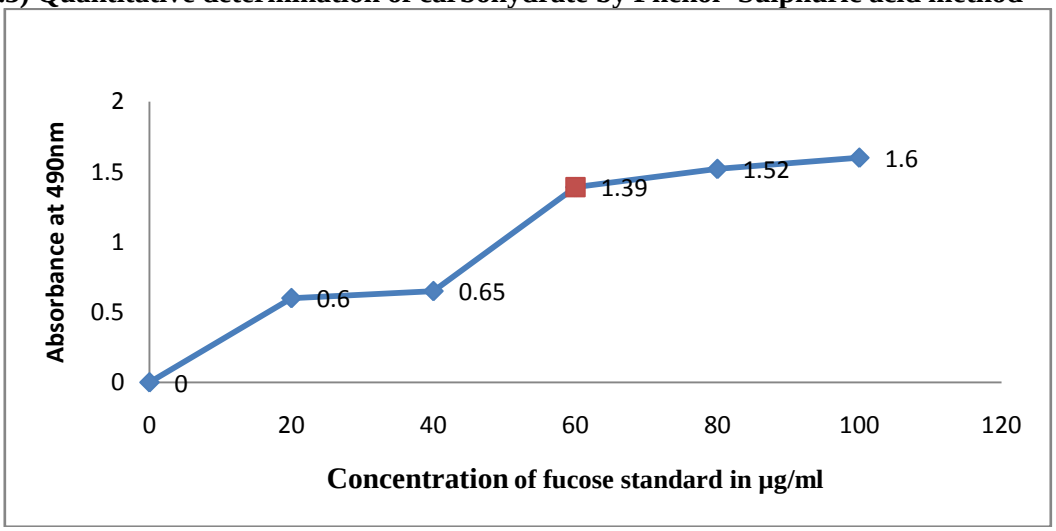
Total mass of the extracted polysaccharide was measured using the dry mass method

(Weight of the empty falcon tube - weight of the falcon tube along with the dried extract)

Total mass of the polysaccharide fucoidan extracted from 150 gm of the powered *sargassum sp*= 2.02mg

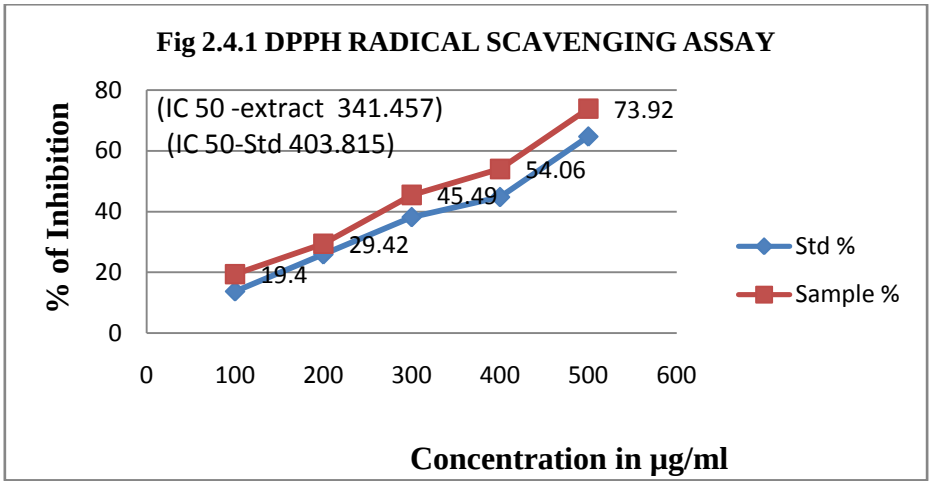
2.3 Fucose standard was used in different concentration 20, 40, 60, 80, 100, 120µg/ml and the fucoidan extract was used as a test sample and the total carbohydrate content of the test sample was found to be 60µg/ml

(Fig 2.3) Quantitative determination of carbohydrate by Phenol- Sulphuric acid method

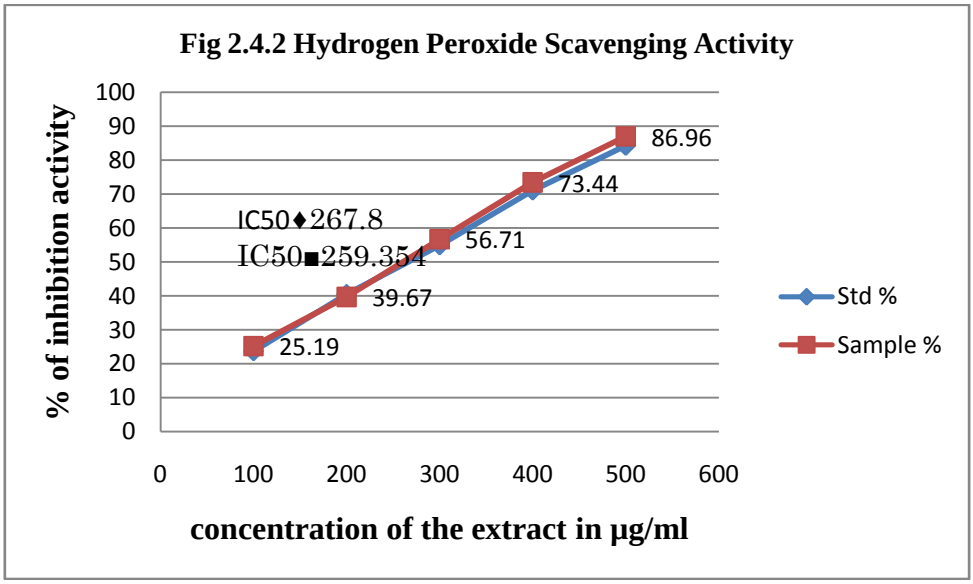


2.4.1 The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). (B. Thendral Hepsibha ,2010)[10]As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, e.g., a free

radical-scavenging antioxidant, the absorption strength is decreased and results in decolorization (yellow colour) with respect to the number of electrons captured (Blois, 1958)[11]. In the present study, the DPPH activity of ascorbic acid and methanol extract of the fucoidan was determined (ref **fig 2.4.1).** The samples of different concentration have the ability to scavenging DPPH at increasing percentages. The methanol extract showed the maximum DPPH radical scavenging activity (19.4% to 73.92%) With the IC 50 values 341.457µg/ml. The scavenging effect of standard ascorbic acid was found to be from 13.72 to 64.71 % with IC 50 values 403.815µg/ml.

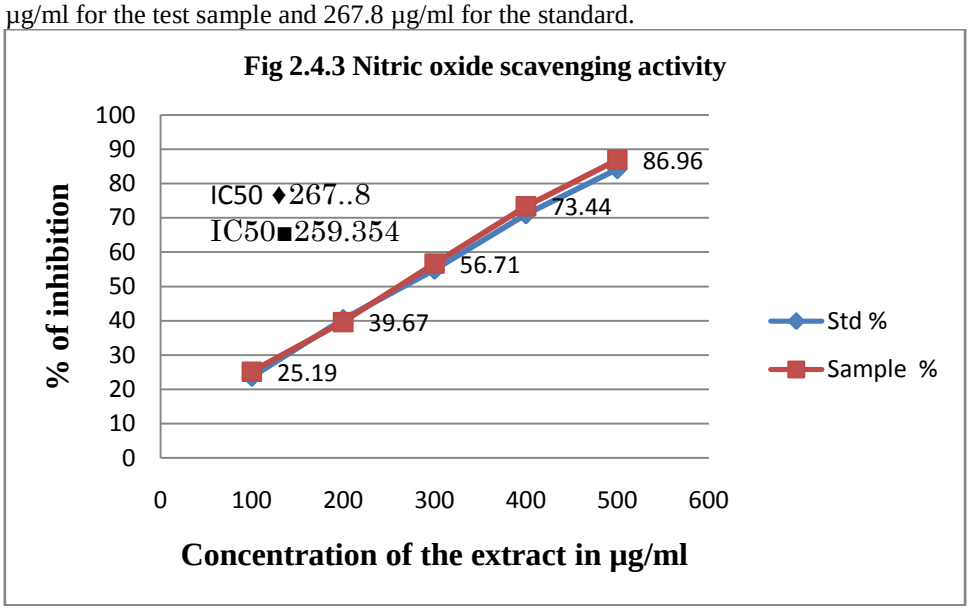


2.4.2 The hydrogen peroxide scavenging activity of test samples along with standard concentration was in the range of 100– 500µg/ml (**Fig2. 4. 2)** Maximum activity was shown by the sample extract in 100,300,400,500µg/ml of concentration and minimum scavenging activity was found in the concentration 200µg/ml when compared to the ascorbic acid standard at 230nm. It gave as IC 50 value 0f 267.8µg/ml for the standard and 259.354µg/ml for the sample in the methanol extract gave a better inhibition concentration when compared to the standard.



2.4.3 Nitric oxide ($NO^{\cdot}$) has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities (Nathan & Hibbs, 1991)[12]. Despite the possible beneficial effects of $NO^{\cdot}$, its contribution to oxidative damage is also reported. This is due to the fact that $NO^{\cdot}$ can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce $OH^{\cdot}$ and $NO^{\cdot}$ (Pacher et al.,2007)[13]. Large amounts of $NO^{\cdot}$ may lead to tissue damage. It would be interesting to develop potent and selective inhibitors of $NO^{\cdot}$ for potential therapeutic use (Nowakowska, 2007) [14].It was found that the test sample gave a increasing scavenging percentage from 25.19% to 86.96% (**ref fig**

2.4.3) while the standard ascorbic acid gave a percentage of 23.68% to 84.26%.This resulted in good IC50 value of 259.354 µg/ml for the test sample and 267.8 µg/ml for the standard.



2.4.4 In Ferric oxide test standard gave a percentage of (0.012 to 0.062%) was recorded at concentration 100 – 500 µg/ml which Showed lesser activity than test sample extract .Percentage for the test sample was (0.0018 to 0.095%)

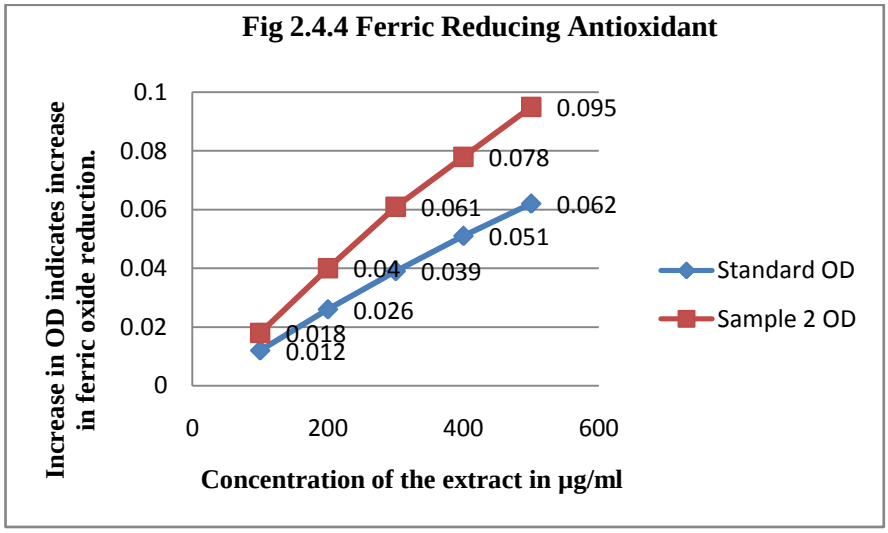


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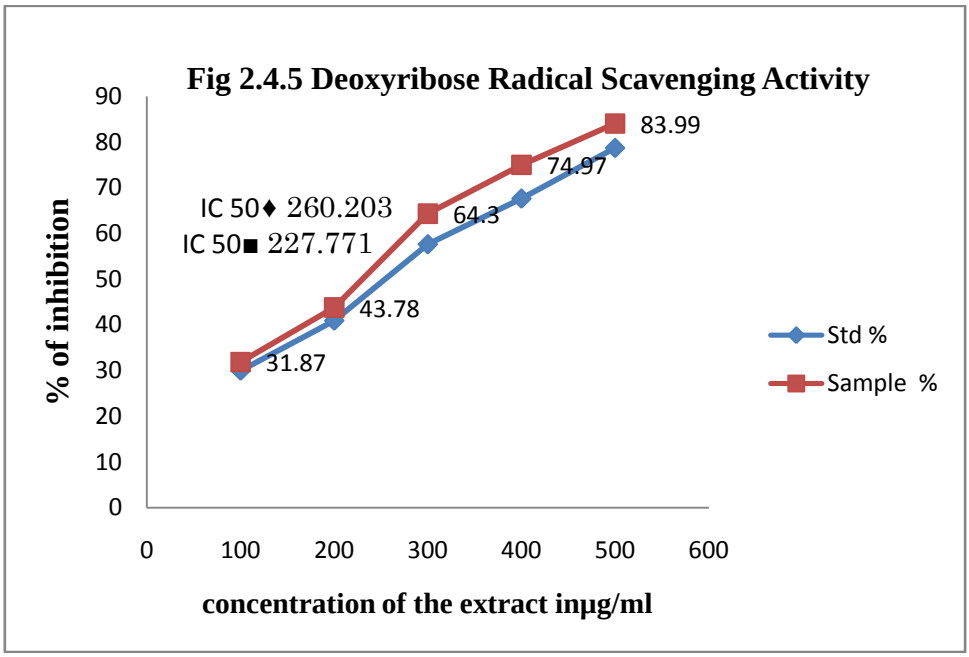
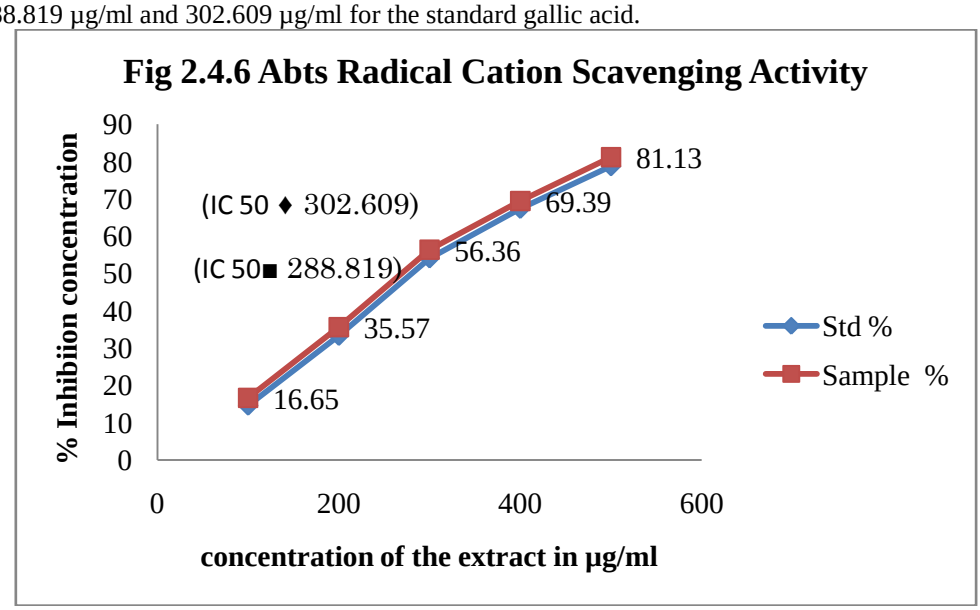
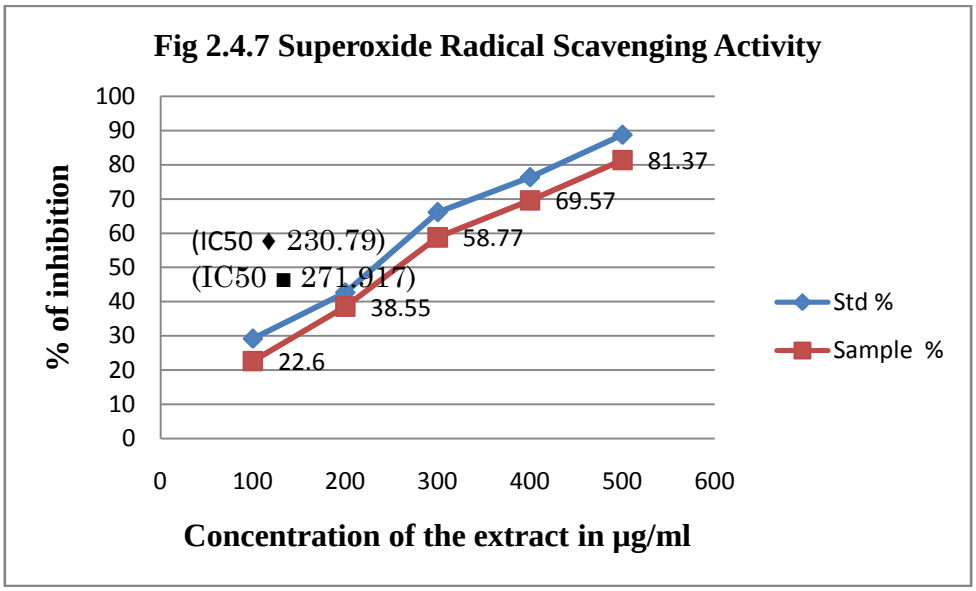
Fig 2.4.5 In this Deoxyribose radical scavenging activity polysaccharide extract of *Sargassum sp* gave a increasing inhibition concentration from 31.87% to 83.99% while the standard gave a comparatively lesser inhibiton concentration ranging from 29.96% to 78.75% this gave a significant inhibiton concentration value for the test sample 260.203 µg/ml and 227.771 µg/ml for the standard .



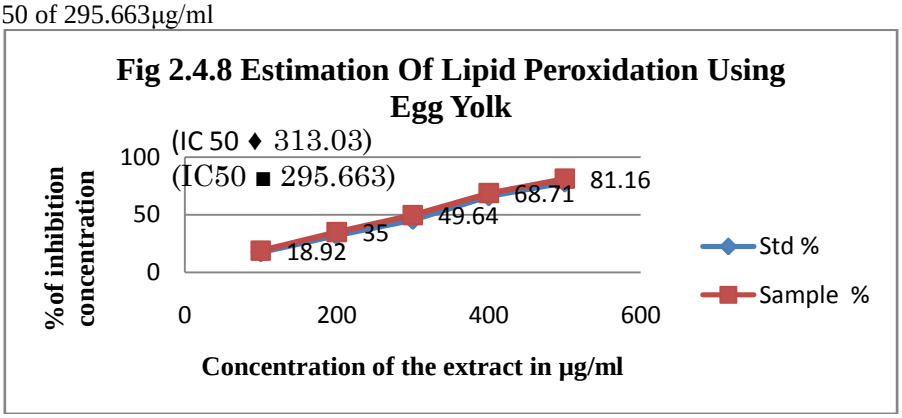
2.4.6 In this Abts radical scavenging activity polysaccharide extracted from the *Sargassum sp* gave a increasing inhibition concentration from 16.65% to 81.13% while the standard gave a comparatively lesser inhibiton concentration ranging from 14.53% to 78.75% this gave a significant inhibiton concentration value for the test sample 288.819 µg/ml and 302.609 µg/ml for the standard gallic acid.



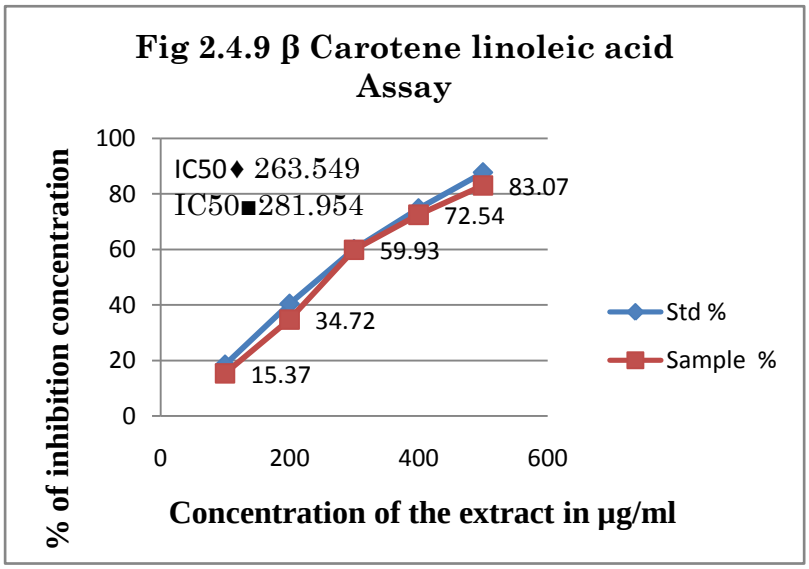
2.4.7 In this superoxide radical scavenging activity polysaccharide extracted from *Sargassum sp* gave a lesser inhibition concentration from 22.6% to 81.37% while the standard gave a comparatively higher scavenging concentration ranging from 29.14% to 88.70% this gave a non significant inhibiton concentration value for the test sample 230.790 µg/ml and 271.917 µg/ml for the standard.



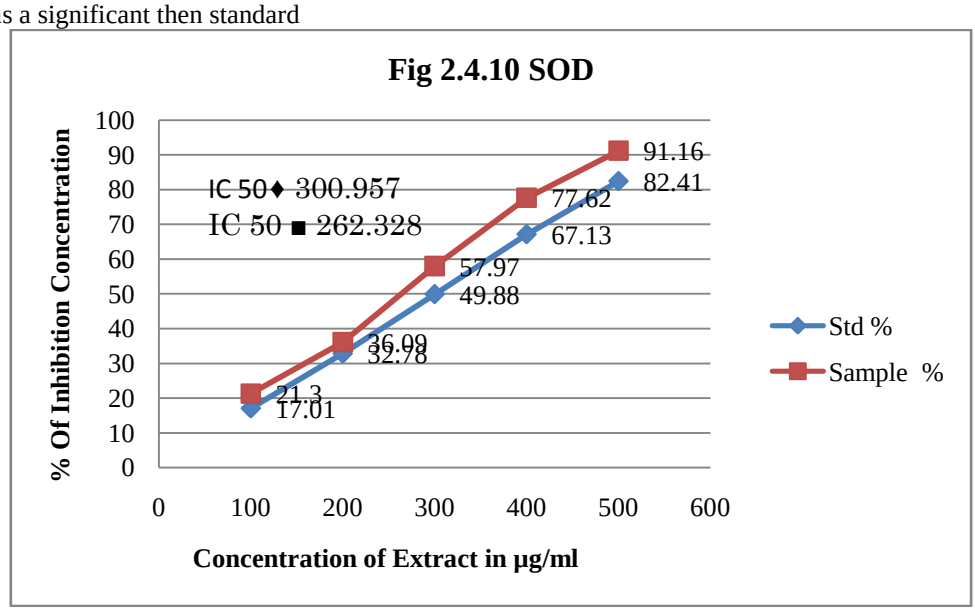
2.4.8 Percentage of inhibition by standard BHT ranged from 17.70% to 88.70% which is slightly lesser than the test sample 18.92 to 81.16%. Similarly, the IC 50 value of standard was 313.030µg/ml and the test sample gave significant IC 50 of 295.663µg/ml



2.4.9 Percentage of inhibition by standard BHT ranged from 18.48S% to 87.74% which is slightly higher than the test sample OD15.37 to 83.07%. Similarly, the IC 50 value of standard was 265.549µg/ml and the test sample IC 50 value of 281.954 µg/ml.



2.4.10 The percentage of inhibition of the standard was recorded 17.01% to 82.41% while the test sample polysaccharide extracted from *Sargassum sp* gave 21.3% to 91.16% and the inhibition concentration of the test sample was a significant then standard



IV. CONCLUSION

In this current study it is found that polysaccharide extracted from the *Sargassum sp* using the soaked water method is one of easiest and time consuming method. The fucoidan extracted was quantitatively determined using the standard procedure of phenol and sulphuric acid method to confirm the presence of polysaccharide called fucoidan using the standard fucose. Fucoidan are sulphated polysaccharide with fucose back bone it was first isolated by kylin in 1913(Guangling Jiao et.al 2011) [15].

Plant derived polysaccharide compounds have multitask effects exerting influence on different levels and via different mechanisms. There is strong evidence supporting the positive role of medicinal plants in oncology, and that they affect all phases of the cancer process (Manojkumar.K et.al 2013) [16] Plants have played an important role as a source of effective anti-cancer agents and it is significant that over 60% of currently used anti-cancer agents are derived from natural sources including plants, marine organisms and micro-organisms (Cragg et al., 1997) [17].

A large number of herbal and non-herbal plants possess various chemical compounds which exhibits antioxidant properties (Syed Ali Mohammed Yacoob et.al.,2018)[18] In many research articles flavanoids, carotenoids (Suganya V et.al Nov 2017)[9] and phenolic compounds (B. Thendral Hepsibha et.al May 2017)[10] and polysaccharides (Manojkumar.K et.al)[16] are found to a good source of anticancer activity. Natural antioxidants plays potential role in protecting the cells against free radical induced damage. The diet rich in antioxidants from natural source can prevent and protect the cells in combating several diseases.(Anuradha V et.al)[19]Astaxanthin in esterified form can be used as potent drug for various diseases associated with cellular damage and oxidative stress. Further, it could also be explored for anti-inflammatory and other pharmacological studies (Suganya.V et.al.,2017)[20]

From the current study also it is found that the polysaccharide extracted from the *Sargassum sp* exhibited a significant anticancer and scavenging activity except in β carotene linoleic acid assay and superoxide radical scavenging activity. This indicates that the polysaccharide (fucoidan) can be used in the therapeutic studies in *invivo* condition to treat the cancer cell lines.

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