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EXTRACTION OF BIOACTIVE COMPOUNDS FROM SARGASSUM SP. AND THEIR BIOACTIVE POTENTIAL OF CYTOTOXICITY AND HEMOLYTIC ACTIVITIES.

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ABSTRACT:

INTRODUCTION: Seaweed from shallow maritime meadows, sargassum species are brown macroalgae from tropical and subtropical regions. These are a wholesome and abundant supply of bioactive substances, including vitamins, carotenoids, dietary fibers, proteins, and minerals. Additionally, a variety of Sargassum species were used to isolate a number of biologically active substances, including terpenoids. Numerous biological effects, including analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial, anti-tumor, fibrinolytic, immune-modulatory, anti-coagulant, hepatoprotective, and antiviral activity, are displayed by these isolated compounds.

AIM: The aim of the study is to evaluate cytotoxic and hemolytic activity of Sargassum.

MATERIALS AND METHODS: Preparation of the crude extract was done. Followed by the cytotoxic and hemolytic assay of the Sargassum sp using MTT assay.

RESULTS: When we compare the cytotoxic activity of Sargassum in different concentration, we find that at higher concentration the cell viability is the least. When we compare the hemolytic

activity of Sargassum at different concentrations we find that, at higher concentration the hemolysis is the highest.

DISCUSSION: Various researches have been done for cytotoxicity and hemolytic activity using natural and alternative compounds, Hep2 cell lines, in vitex stimulated digestive actions, sugars, etc.

CONCLUSION: Sargassum species can be used for their cytotoxic activity and hemolytic activity and can be used for further medical researches.

KEYWORDS: Sargassum sp, cytotoxic activity, hemolytic activity, assay, in vitro.

INTRODUCTION:

Seaweeds (macroalgae) form a diverse and ubiquitous group of photosynthetic organisms that play an essential role in aquatic ecosystems. These ecosystem engineers contribute significantly to global primary production and are the major habitat formers on rocky shores in temperate waters, providing food and shelter for aquatic life. Like other eukaryotic organisms, macroalgae harbor a rich diversity of associated microorganisms with functions related to host health and defense (1).Seaweeds are a source of novel bioactive compounds, such as phlorotannins and certain polysaccharides, that are not found in terrestrial plants but that may confer certain health-promoting properties. The consumption of seaweeds has been linked to a lower incidence of chronic diseases such as cancer, hyperlipidemia, and coronary heart disease (CHD), mainly on the basis of epidemiological studies comparing Japanese and Western diets (2).

Seaweed from shallow maritime meadows, sargassum species are brown macroalgae from tropical and subtropical regions. These are a wholesome and abundant supply of bioactive substances, including vitamins, carotenoids, dietary fibers, proteins, and minerals. Additionally, a variety of Sargassum species were used to isolate a number of biologically active substances, including terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, and pheophytine (3).

Numerous biological effects, including analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial, anti-tumor, fibrinolytic, immune-modulatory, anti-coagulant, hepatoprotective, and antiviral activity, are displayed by these isolated compounds. Therefore, the utilization of Sargassum species in the pharmaceutical and nutraceutical industries is highly promising (4).

MATERIALS AND METHODS:

PREPARATION OF CRUDE EXTRACT:

For preparing the crude extract of Sargassum, the weed has been air dried and made it into a powder sample. The powdered sample in then added with ethanol of 10ml and mixed using a shaker. Then the mixture is filtered using filter paper. The crude extract is ready.

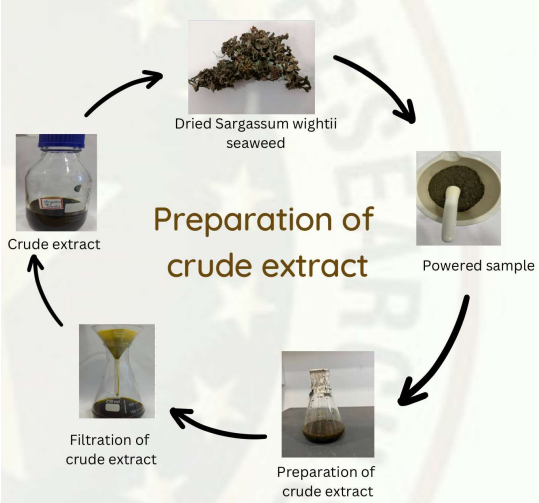

Fig 1. Preparation of the crude extract of dried Sargassum wightii.

HEMOLYTIC ASSAY:

Hemolytic activity was determined by incubating suspensions of human red blood cells with serial dilutions of each selected peptides. Red blood cells were rinsed several times in PBS by centrifugation for 3 min at 3,000 g until the OD of the supernatant reached the OD of the control (PBS only). Red blood cells were counted by a hemocytometer and adjusted to 7.7610660.36106 cells/mL. Red blood cells were then incubated at room temperature for 1 h in 10% Triton X-100 (positive control), in PBS (blank), or with amphipathic peptides at concentrations of 0.4, 0.8, 1.6, 3.1, 6.2, 12.5 and 25 mM, only for Pin2 [14] and Pin2 [17] the 50 and 100 mM concentrations were evaluated. The samples were then centrifuged at 10,000 g for 5 min, the supernatant was separated from the pellet, and its absorbance measured at 570 nm. The relative optical density compared to that of the suspension treated with 10% Triton X-100 was defined as the percentage of hemolysis.(5)

INVITRO CYTOTOXIC ASSAY:

Brine shrimp lethality assay method was used to meas- ure the cytotoxic effects of the biosynthesized AgNPs. Brine shrimp eggs were hatched in a glass tank using sea water and an aerator. The larvae were visibly alive and moving towards light source 72 h after placing in the water and were subsequently used. In three differ- ent test tubes, around 10 shrimp larvae were added along with 10-ml sea water. One milliliter of 0.1, 0.5, and 1 µg/mL of all three samples of AgNPs was added to each test tube. A control test tube was pre- pared by omitting AgNPs. After 24 h, the numbers of dead and alive larvae were counted and percentage mortality was calculated. (6).

RESULTS:

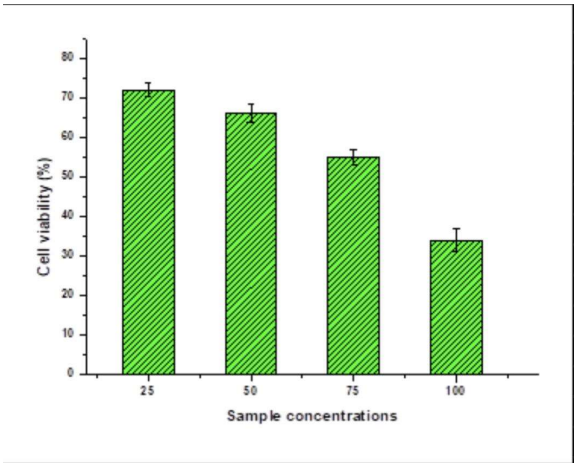

Fig 2. Cytotoxicity activity - MTT assay MCF cell line.

Sample concentration		
µg/ml	24Hrs	SE
25	75.2	1.8
50	68.2	2.2
75	56.3	1.8
100	35.6	2.8

Fig 3. Sample concentration for evaluating Cytotoxic activity of the seaweed.

When we compare the cytotoxic activity of Sargassum in different concentration, we find that at higher concentration the cell viability is the least.

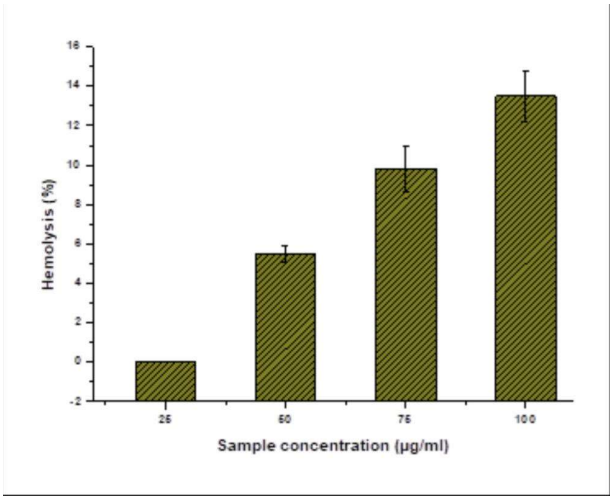

Fig 4. Hemolytic activity.

Sample concent	RBC cell lysis	St.Er
25	0	0
50	5.5	0.4
75	9.8	1.2
100	13.5	1.3

Fig 5. Sample concentration for Hemolytic assay of the seaweed.

When we compare the hemolytic activity of Sargassum at different concentrations we find that, at higher concentration the hemolysis is the highest.

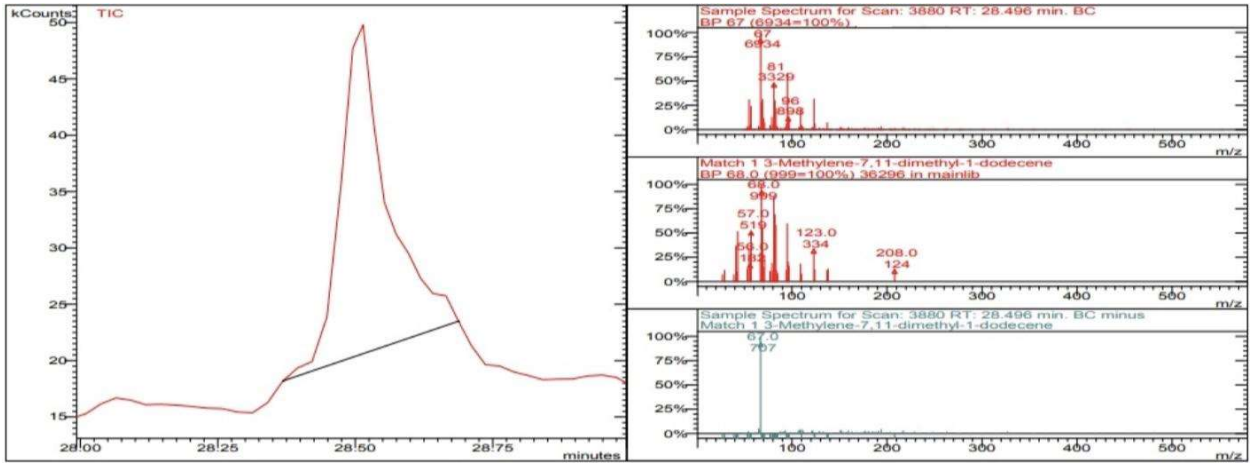

Fig 6. Sample spectrum

DISCUSSION:

The extracts of three *Sargassum* seaweeds from Persian Gulf, Iran were screened for their antioxidant, cytotoxic and phytochemical analysis. Phytochemical and cytotoxic results were similar with few differences in all species. *Sargassum angustifolium* showed the highest antioxidant activity. These seaweed extracts and their active components could emerge as natural and alternative antioxidants or serve as starting points for synthesizing more effective cytotoxic drugs. (7)

One way analysis shows significant ($p<0.05$) difference for the various concentration of Sargassum sp. extract against Hep2 and MCF-7 cell lines. Present study infers that the Sargassum sp extract exhibit effective antitumor activity and seems to have no side effects. They are less cost effective, easy in production and purification. (8).

This study is the first to examine the influences of in vitro simulated digestive model on antidiabetic and cytotoxic potentials of *Sargassum* spp. All tested samples expressed remarkable

α-amylase inhibitory and cytotoxic properties at the final stage of digestion.The two major compounds palmitic acid and fucosterol may principally contribute to antidiabetic and cytotoxicity on human multiple myeloma U266 cell properties of fractions from *Sargassum* spp. Advanced spectroscopic techniques should be used to identify other potent bioactive compounds of the brown seaweed (9).

Anticoagulant activity was observed to be high in the precipitate which correlated with the increased polyphenols and total sugars respectively. There was 2.6–3.9-folds increase in anticoagulant activity in the final purified fractions, with a maximum activity in case of sample fermented with *Enterococcus faecium* (10).

From this study we found, that effect on bleeding time after 14 days treatment of Sargassum extract in vivo prolonged the clotting and bleeding time significantly ($p < 0.05$). Prothrombin time (PT) and activated partial thromboplastin time (APTT) tests on plasma confirm these research. From this study we conclude, that the flavonoid compound in *Sargassum cristaefolium* exhibited an anticoagulant activity.(11).

CONCLUSION:

After analyzing the activity of Sargassum species according to its concentration we come to a conclusion that Sargassum can be used as a cytotoxic and hemolytic element. The higher the concentration of the Sargassum extract, the more would be a positive outcome of the better results. To draw any conclusion on cytotoxic activity of Sargassum more research and data would be needed.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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DURATION OF THE STUDY:

The study was conducted for 3 months.

ETHICAL CLEARANCE NUMBER:

As it is an in vitro study, ethical clearance number is not required.

SOURCE OF FUNDING:

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