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GCMS analysis and *invitro* antibacterial and anti-inflammatory study on methanolic extract of *Thalassiosira weissflogii*

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ABSTRACT

Microalgae possess huge kind of natural bioactive compounds with potent values. In the present study, analysis of *invitro* anti inflammatory and anti bacterial activity from *Thalassiosira weissflogii* and anti inflammatory activity of extract was evaluated using albumin denaturation, membrane stabilization and proteinase inhibitory assay. Apart from this the methanolic extract of diatom *T. weissflogii* was subjected to antibacterial activity against different organisms such as *E. coli, S. aureus* and *B. subtilis* by agar well diffusion method. *T. weissflogii* against *E. coli, S. aureus* and *B. subtilis* was found to be 12 mm, 19 mm and 17 mm respectively. This was compared with inhibitory zone showed by 30 μg/ml of streptomycin that exhibit maximum of 18.6 mm for *E. coli,* 15.2 mm for *S. aureus* and 26 mm for *B. subtilis.* We found to contain various chemical constituents such as pigment, phenolic, tocopherol. The secondary metabolites of Diatom, *Thalassiosira weissflogii* was analysed and characterised using various analytical techniques such as UV–vis Spectroscopy and GCMS. In the present study, methanolic crude extract of *T. weissflogii* was extracted and examined for *in vitro* anti-inflammatory and antimicrobial activity. The future work will be determination of anti inflammatory activities by in vivo models.

1. Introduction

Natural products of higher plants may have another wellspring of antimicrobial compound with possibly novel mechanisms of action. They are useful in the treatment of infectious diseases whereas simultaneously mitigating numerous of the side effects that are often related with conventional antimicrobials (Bigoniya and Rana, 2010). Antimicrobial action shows that the occurrence of active constituents in the extractions of marine algae which can be exploited for the production of novel drugs for the benefit of the humanity. Among the marine flora and fauna, marine algae are rich sources of diverse bioactive compounds with a mixture of biological activities (Wijesekara and Kim, 2010). In recent times, their significance as a source of novel bioactive substances is rising rapidly and researchers have exposed that marine algal originated compounds reveal a variety of biological activities.

Inflammation is the reaction of living tissues to injury, infection or irritation. Lysosomal enzymes released during inflammation produce a variety of disorders which leads to the tissue injury by damaging the macromolecules and lipid peroxidation of membranes which are assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis etc. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane. HRBC or erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an *in vitro* measure of anti-inflammatory activity of the drugs or plant extracts (Chippada et al., 2011).

Nature is the first source of basic compounds and molecules, from which bigger molecules are being formed. Since early 50s, natural substrates have been a source of various products that can be applied in food, drug, cosmetic, textile, and energy (Chin et al., 2006) Microalgae are considered as a powerful mean for the production of biofuels and bioactive molecules like pigments, carotenoids, vitamins, proteins, phenolic compounds (Elumalai et al.,2014) which could be used widely for the production of nutraceuticals, pharmaceuticals, animal feed additives, and cosmetics (Borowitzka, 1995, 2013; Pulz and Gross, 2004; Spolaore et al., 2006; Mata et al., 2010; Barra et al., 2014; Enzing et al.,

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2014; Pérez-López et al., 2014). The natural pigments exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, antiobesity, anti-angiogenic and neuroprotective activities. Therefore, various natural pigments isolated from marine algae have attracted much attention in the fields of food, cosmetic and pharmacology (Pangestuti and Kim, 2011).

During the last years, many studies have been made on biological activities of the seaweed and identified as potential sources of natural antioxidants. Several authors studied the antimicrobial activities of marine algae in different parts of our country. In recent year seaweeds are widely used in several applications such as antimicrobial (Chiheb et al., 2009), antiviral (Bouhlal et al., 2010, 2011; Kim and Karadeniz, 2011), antifungal (De Felicio et al, 2010), anti-allergic (Na et al., 2005), anti-coagulant (Dayong et al., 2008), anti-cancer (Kim et al., 2011), anti-fouling (Bhadury and Wright, 2004) and antioxidant activities (Devi et al., 2011).

Thalassiosira weissflogii is a unicellular microalgae found in marine environment which can grow in higher salinities. When observed under a microscope, it is short cylinder in shape and varies in size from 4 to 32 μm in diameter. The planktonic diatom *Thalassiosira weissflogii* can grow in varied habitat of marine environment and adapt to any conditions of salinity and mineral availability (Yvonne Lang et al., 2013). It also has the ability to accumulate mineral content from the environmental condition. The species can secrete varied secondary metabolites based on the culture condition. The growth is influenced by water quality, ionic concentration and salinity. It is primarily utilised as shrimp and shellfish feed. (Fryxell and Hasle, 1977; Dassow et al., 2006). Since this marine phytoplanktonic species was under explored for pharmacological activity and due to deficient literature pertaining to characterization and bioactivity of diatoms, in the present study, phenolics, Pigments and tocopherols were characterised in methanolic extracts of microalgae biomass. Anti-inflammatory and antimicrobial properties of methanolic extract of *Thalassiosira weissflogii* were also determined by various *invitro* methods.

2. Materials and methods

2.1. Cultivation and identified of T. weissflogii

T. weissflogii, collected from Marakkanam shrimp pond near Pondycherry. It was grown in laboratory and identified strain by Dr.S.Elumalai, Head, Department of Biotechnology, University of Madras, Gunidy Campus, Chennai.

2.2. Culture condition and preparation of the sample

The media compositions and culture procedures of Guillard (1983) were employed. One hundred mL of filtered seawater (Whatman GF/F, 0.7 μm) was poured into 125 mL Erlenmeyer flasks, and nutrient, metal and vitamin solutions were added. The flasks were plugged with non-absorbent cotton and autoclaved at 121 °C for 35 min. After cooling to room temperature, 3 mL of aged algae culture were added, and the suspension was stirred to homogeneity. The cultures were incubated under fluorescent light under 12:12, light: dark cycles. The sub-culture period for algal growth was one week.

Algae were collected by filtering the sample through a glass fibre membrane (Whatman GF/F, 0.7 μm, < 100 mmHg). For pigment extraction, 10 mL of acetone and the glass fibre membrane were placed in a 15 mL polypropylene (PP) centrifuge tube and were ground with a Teflon pestle. The tubes were then placed in a 4 °C incubator and shaken in darkness for 8 h. After high speed centrifugation, the supernatant was subjected to analysis.

Dry algal biomass weighing 10 g was dissolved in 100 ml of methanol, stirred continuously for 3 h and left undisturbed. After 24 h, the solution was evaporated to dryness and stored in clean bottle which was in turn stored at 15 °C for further studies (Ashasahalin et al., 2018).

2.3. Characterization of Thalassiosira weissflogii

2.3.1. UV–vis spectrophotometric analysis

Thalassiosira weissflogii dried sample was dissolved in methanol and the pigment profile was determined using a double–beam UV–Visible spectrophotometer at the wavelength range of 200–700 nm at room temperature (Zhoushuai Qin et al., 2013).

2.3.2. GC-MS analysis of bioactive compound from Thalassiosira weissflogii

Bioactive constituents like pigments, tocopherols, phenolic compound from *T. weissflogii* was determined using GC-MS analysis to confirm the presence of compounds (Narayani et al., 2016). The chromatogram was compared against NIST library for identification of bioactive compounds.

2.3.3. Antibacterial activity

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 h old - broth culture of *E. coli, S. aureus* and *B. subtilis* Wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of methanol extract of *T. weissflogii* was prepared with a different concentration (25, 50, 75 and 100 μg/mL). About 50 μl of methanol solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 hrs. Control experiments comprising inoculums without methanol extract were set up. The plates were incubated at 37 °C for 18–24 h for bacterial pathogens. Methanol (100%) without *T. weissflogii* extract was used as negative control and Streptomycin disc (30 μg) was used as the positive control. The diameter of the inhibition zone (mm) was measured. The experiment was repeated thrice, for each replicate the readings were taken in three different fixed directions and the average values were recorded (Bhuvana et al., 2018).

2.3.4. Anti-inflammatory activity

Inhibition of albumin denaturation method of was followed with minor modifications. The reaction mixture was consisting of test sample (100–500 μg/mL) and1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCl. The samples were incubated at 37 °C for 20 min and then heated at 51 °C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug of varied concentration 100–500 μg/mL. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows: % of inhibition = (Absorbance of control – Absorbance of sample)/Absorbance of control X 100 (Sakat et al., 2010; Vadivu & Lakshmi, 2008; Samanjit Kaur, et al, 2018).

3. Result and discussion

3.1. Spectral characterization of active constituents

Diatoms are considered the most important species in marine food chains, and are among the most effective strains used in the nutrition of mollusk larvae because their content of lipids and PUFA, is considered the primary energy storage material. Diatoms generally contain high concentrations of w-3 PUFA including EPA and smaller proportions of DHA. Both of these PUFA are essential to the diets of many marine animals (Volkman et al., 1989; Thompson et al., 1992; Brown et al., 1996). Characterization of secondary metabolites provides an extensive resource for the isolation and identification of novel therapeutic agents for mankind.

The spectral analysis of pigments extracted from *Thalassiosira weissflogii* showed the presence of Diadinoxanthin and Chlorophyllide-a at the absorbance of 415 nm and 663 nm respectively (Fig. 1). Diadinoxanthin along with chlorophyll and fucoxanthin were the major pigments involved in light harvesting photosynthetic apparatus of

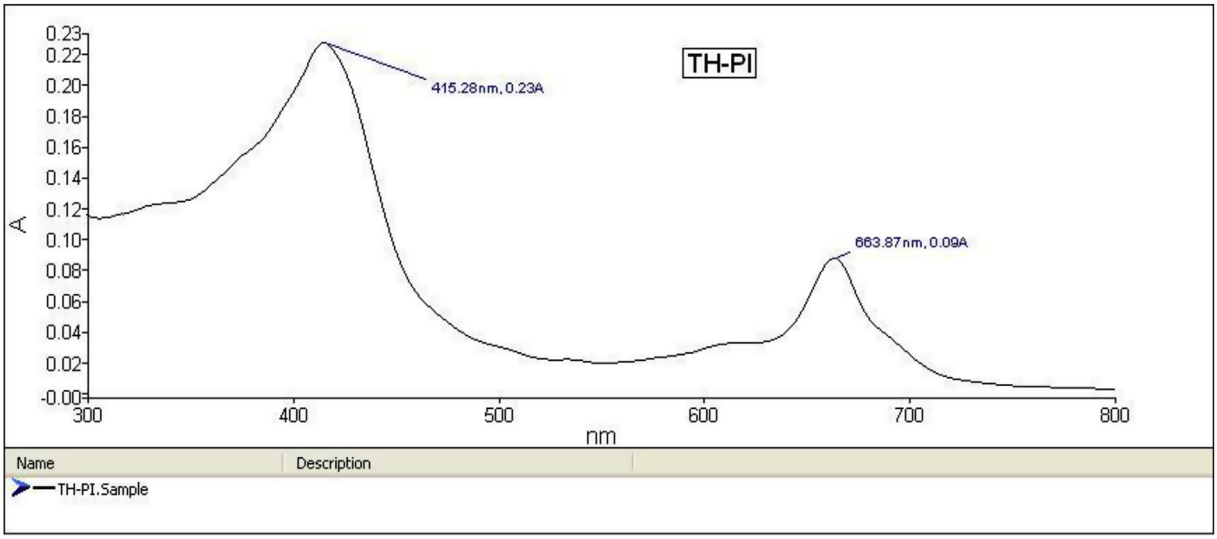

Fig. 1. Determination of Pigment in *Thalassiosira weissflogii* by UV–Vis spec.

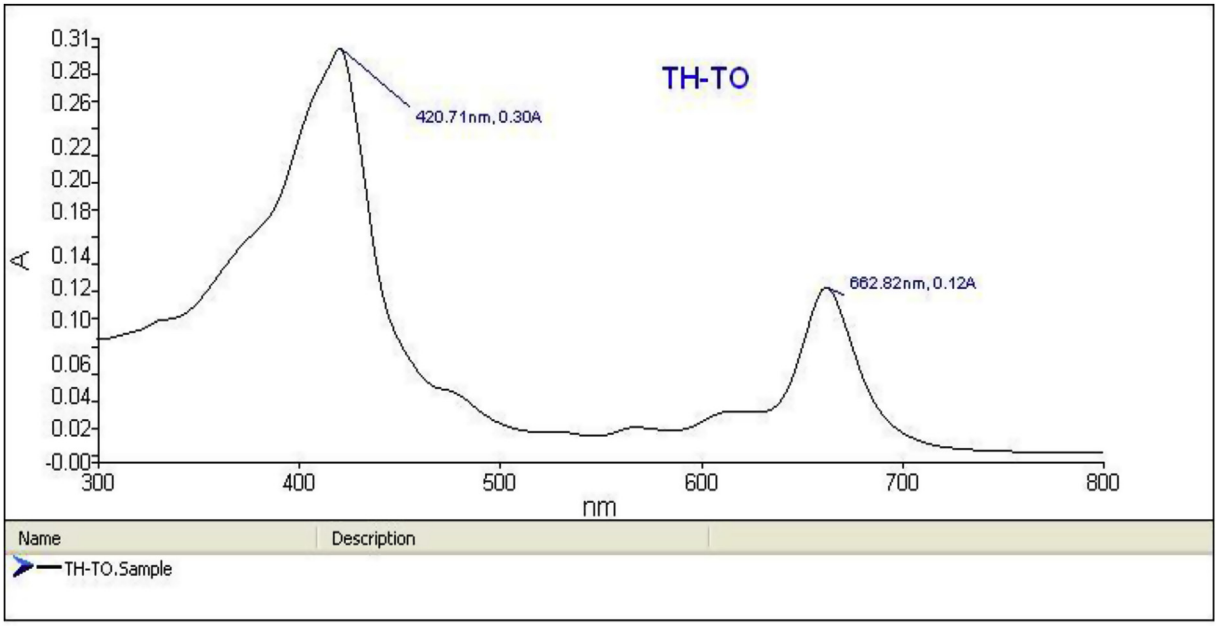

Fig. 2. Determination of Tocopherols in *Thalassiosira weissflogii by* UV–Vis spec analysis.

brown and diatomaceous algae (Albert et al., 1981). Similarly the sharp peak of tocopherol in 420.71 nm revealed the presence of β-carotene (Fig. 2). The presence of two peaks at 417 nm and 667 nm in the phenolic extract revealed the presence of phenolic acids, hydroxycinnamic acids (Fig. 3).

The major non volatile compounds can be identified by GC MS analysis. The crude methanolic extract revealed the high peak intensity compound predominant presence of major compound like Pentadecanoic acid, 14-methyl-, methyl ester (14.4393) and methyl-stearate among other derivatives. Minor compounds such, 10-methyl-, methyl ester, methyl tetradecanoate, tetradecanoic acid, 12- methyl-, methyl ester, 9-hexadecenoic acid, methyl ester, (Z)-, hexadecanoic acid, 14-methyl-, methyl ester, 10- octadecenoic acid, methyl ester, heptadecanoic acid, were also identified. These compounds are exhibited activities like antioxidant, cancer-preventive, hypercholesterolemic, nematicide, antifungal, antimicrobial. The advantage of GC-MS is its highest accuracy in the identification of derivatized compounds Fig. 4 & Table 1.

3.2. *In vitro antibacterial activity*

The methanolic extract of diatom *T. weissflogii* was subjected to antibacterial activity against different organisms such as *E. coli, S. aureus* and *B. Subtilis* by agar well diffusion method and the results was recorded in Table 1. The drug streptomycin which acts as the positive control against these organisms and negative control methanol was also tested and inhibitory zones were measured. From this study, it is clear that as the concentration of the test and positive drug increases, the zone of inhibition also tends to increase. At higher concentration (100 µg/mL), the maximum zone of inhibition by methanolic extract of *T. weissflogii* against *E. coli, S. aureus* and *B. subtilis* was found to be 12 mm, 19 mm and 17 mm respectively. This was compared with inhibitory zone showed by 30 µg/ml of streptomycin that exhibit maximum of 18.6 mm for *E. coli,* 15.2 mm for *S. aureus* and 26 mm for *B. subtilis*. The solvent methanol alone doesn't exhibit any zone of inhibition which confirms the antibacterial potential of *T. weissflogii*. Thus, the present investigation was to evaluate the antibacterial activity of *T.*

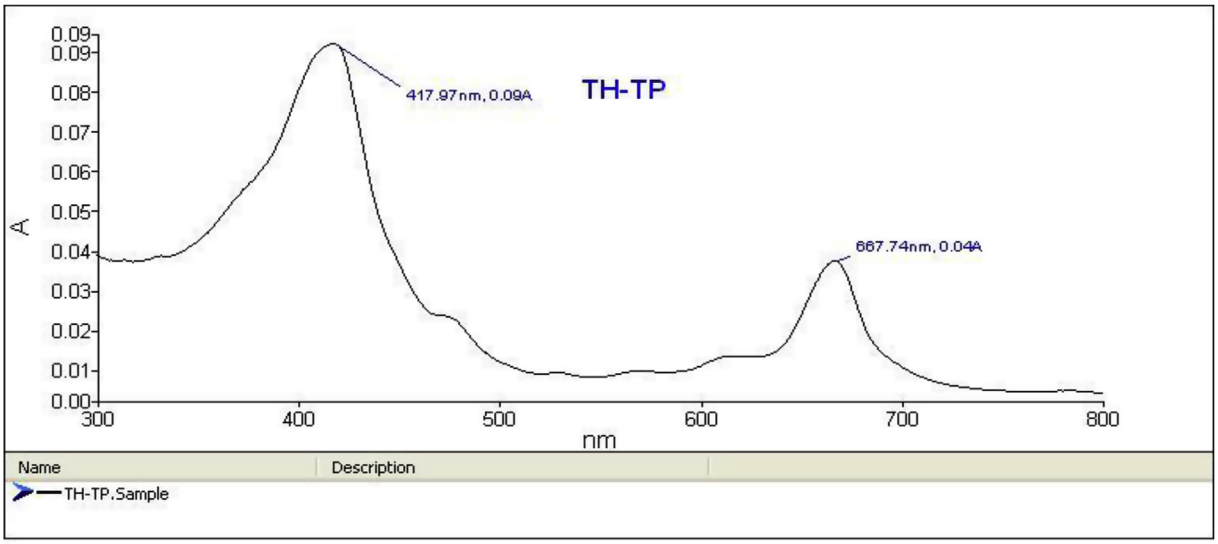

Fig. 3. Determination of Total Phenolic in *Thalassiosira weissflogii by* UV vis spec analysis.

weissflogii diatom. Several researchers have been reported that seaweeds are excellent sources of different bioactive components which exhibit different biological activities (Rodríguez-Peña et al., 2010; Bhacuni and Rawat, 2005, Priyadharshini et al., 2011). Based on the solubility and polarity, the methanolic extract of *T. weissflogii* reveals the strongest antibacterial activity against three different organisms. Similarly, the strongest antibacterial activity was also exhibited by the methanol extract by *Sargassum polycystum* reported by Kausalya and Narasimha Rao (2015). Many studies proved that methanol have higher antibacterial activity than that of extracts obtained from different solvents (Kumar et al., 2008; Seenivasan et al., 2010; Lavany and Veerappan, 2011). Based on several examinations it is concluded that the methanol extract of seaweeds contains phenolics, alkaloids and amino acids which may responsible for the antimicrobial activity (Devi et al., 2008; Meenakshi et al., 2009; Cox et al., 2010; Srivastava et al., 2010). Qin et al. (2013) studies also investigates the antimicrobial activities of a marine diatom, *T. rotula* against microorganisms including three Gram-stain positive and six Gram-stain negative bacteria and one species of yeast. The results found that extracts of *T. rotula* inhibited the growth of *Vibrio harveyi, Staphylococcus aureus, Micrococcus luteus and*

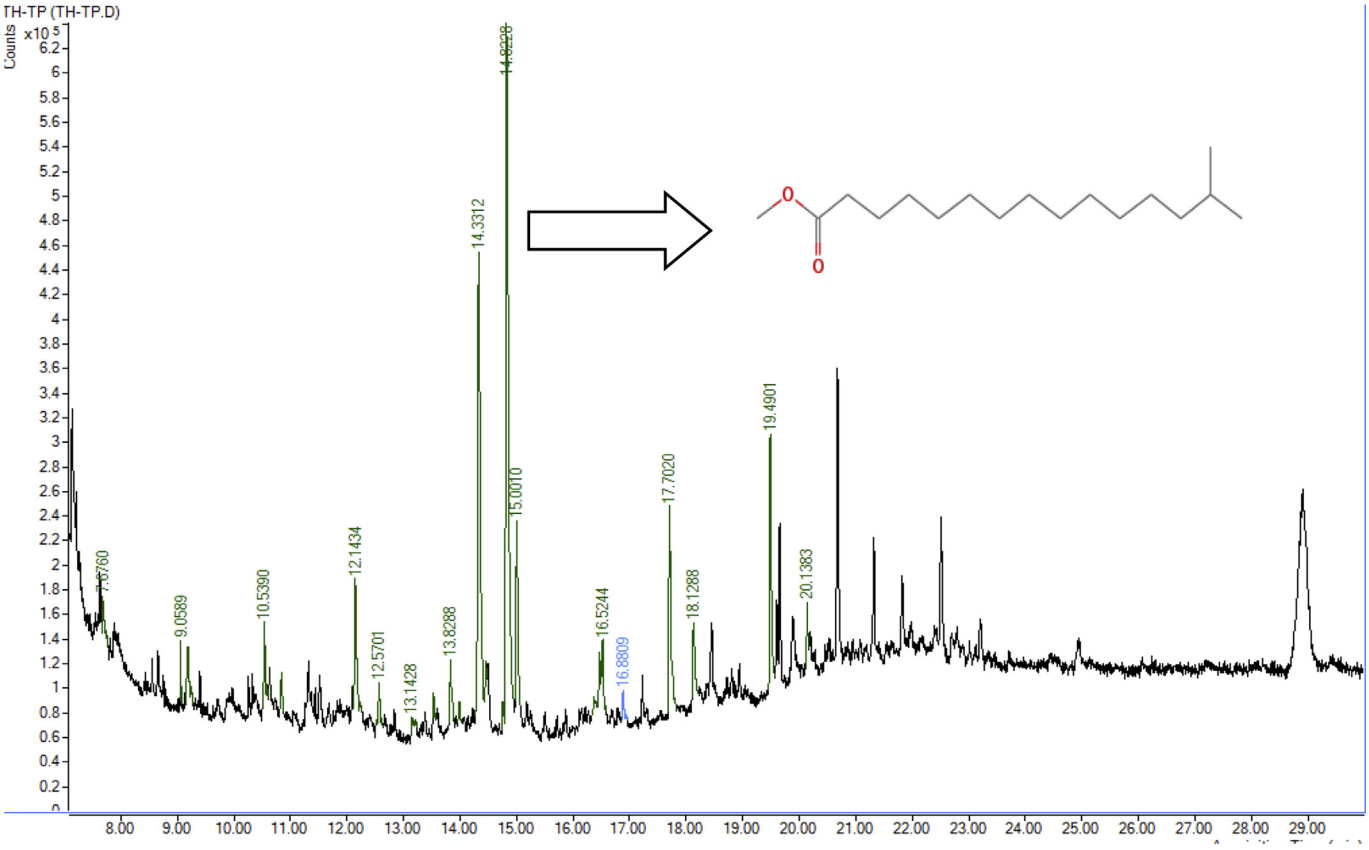

Fig. 4. GC-MS chromatogram of metanolic extract of *Thalassiosira weissflogii*.

Table 1
Compounds identified by GC MS.

Compound RT	Name	Mol. Weight	Area %
10.6958	2,4-Di-tert-butylphenol	206.167	0.154
11.2522	4-Heptafluorobutyryloxyhexadecane	438.237	0.318
12.3542	Methyl tetradecanoate	242.225	1.171
12.997	Pentadecanoic acid, methyl ester	256.24	0.27
13.0942	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	196.11	0.879
13.5156	1-Octadecyne	250.266	2.13
13.7695	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296.308	0.644
13.8073	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278.152	1.239
13.9748	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296.308	1.05
14.2287	7-Hexadecenoic acid, methyl ester, (Z)-	268.24	1.547
14.3097	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334.214	7.938
14.4393	Pentadecanoic acid, 14-methyl-, methyl ester	270.256	22.215
14.5366	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292.204	1.961
14.8067	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334.214	13.323
14.9849	1,2-Benzenedicarboxylic acid, butyl octyl ester	334.214	5.749
15.174	Phthalic acid, monodecyl ester	306.183	0.664
16.2544	11-Octadecenoic acid, methyl ester	296.272	0.868
16.3084	11-Octadecenoic acid, methyl ester	296.272	1.165
16.3624	Phytol	296.308	2.314
16.5083	Methyl stearate	298.287	20.161
29.3866	Campesterol	400.371	1.036

Table 2
Antibacterial activity of methanolic extract of *Thalassiosira weissflogii* by agar well diffusion method.

S.No.	Concentration µg/ml	Zone of inhibition (mm)		
		E.coli	*S. aureus*	*B. subtilis*
1	25	3 ± 0.11	6 ± 0.14	8 ± 0.03
2	50	4 ± 0.23	9 ± 0.23	10 ± 0.07
3	75	9 ± 0.46	14 ± 0.10	12 ± 0.17
4	100	12 ± 0.02	19 ± 0.13	17 ± 0.31
5	Streptomycin (30 µg)	18.6 ± 0.25	15.2 ± 0.23	26.0 ± 0.30
6	Methanol	–	–	–

The values are mean ± SD of triplicate values.

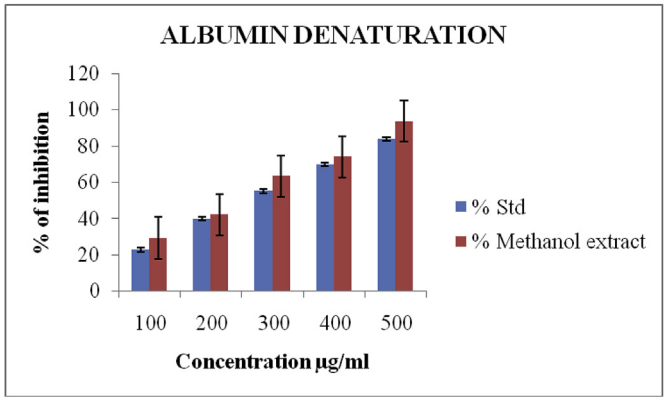

Fig. 5. In vitro Anti-inflammatory activity by Inhibition of Albumin denaturation method.

Bacillus pumilus. Thus, the present research was concluded to have better antibacterial activities as compared with previous investigations.

3.3. In vitro antiinflammatory activity

Albumin Denaturation is a process in which most biological proteins lose their biological function when denatured. Denaturation of proteins is a predictable cause of inflammation (Suganaya et al., 2017; Prasanth et al., 2013; Leelaprakash and Mohan Das, 2011). As part of the investigation on the mechanism of the anti-inflammation activity, the

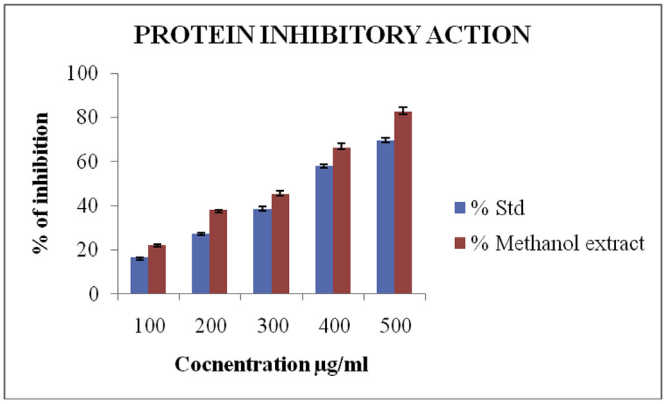

Fig. 6. In vitro Anti-inflammatory activities by Proteinase inhibitory action.

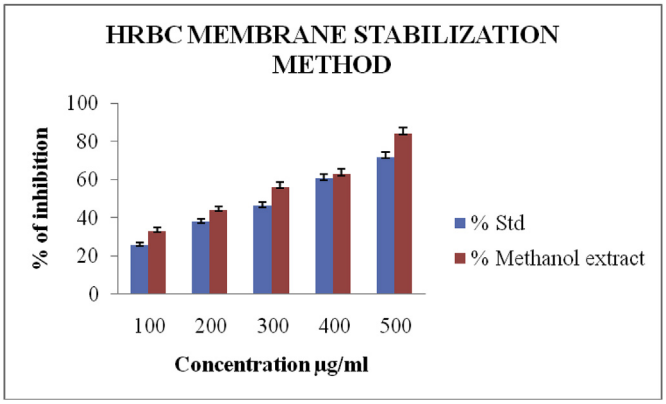

Fig. 7. *In vitro* Anti-inflammatory activities by HRBC membrane stabilization.

ability of methanolic extract of *T. weissflogii* to inhibit protein denaturation was premeditated. It was effective in inhibiting albumin denaturation with maximum inhibition of 83.72% observed at 500 µg/mL. The standard anti-inflammation drug Aspirin, showed the maximum inhibition 93.53% at the same concentration (Fig. 5).

Based on previous statement it is concluded that proteinase play a significant role in the development of tissue damage during inflammatory reactions and important level of protection was provided

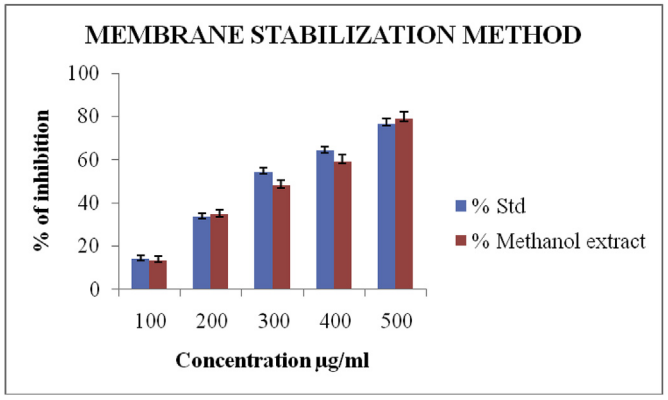

Fig. 8. *In vitro* Anti-inflammatory activity by Heat induced haemolysis.

by proteinase inhibitors (Das and Chatterjee, 1995). Methanolic extract of *T. weissflogii* exhibited considerable antiproteinase activity at different concentrations from 100 to 500 µg/ml which is shown in Fig. 6. The test sample showed maximum inhibition of 69.48% and Aspirin showed the maximum inhibition of 82.46% at 500 µg/ml.

The HRBC membrane stabilization method was used to determine the *in vitro* anti inflammatory activity since the erythrocyte membrane is corresponding to the lysosomal membrane. Stabilization of lysosomal is essential in limiting the inflammatory reaction by preventing the release of lysosomal constituents. During inflammation, the release of lysosomal enzymes produces a various disorders (Gandhidasan et al., 1991; Shenoy et al., 2006). In this study, HRBC membrane stabilization method for methanolic extract of *T. weissflogii* was determined to test the *in vitro* anti-inflammatory activity which was represented in Fig. 7. At lower concentration i.e. 100 µg/ml, the higher activity was exhibited by standard drug (14.37%) than the test sample (13.37%) but at concentration 500 µg/ml higher activities was found by the test sample that exhibit 78.84% than the standard drug that possesses 76.65%.

The methanolic extract of *T. weissflogii* was efficient in inhibiting the heat induced haemolysis at different concentrations such as 100, 200, 300, 400 and 500 µg/ml. The results showed that test sample at concentration 500 µg/ml shows significant inhibition of 84.52% than the standard drug Diclofenac which produce only 72.05%. The IC_{50} values of test sample and standard was found to be 247.759 and 311.818 µg/mL respectively (Fig. 8).

4. Conclusion

The analytical characterization of methanolic extract of *Thalassiosira weissflogii* aids in the identification of active metabolites and revealed the presence of. An *in vitro* study was used to establish the potent anti-inflammatory and antibacterial activity of *Thalassiosira weissflogii*. Form the present study, it is confirmed that *Thalassiosira weissflogii* can be used as potent anti-inflammatory drug in various disorders like cancer, neurological disorder and aging and also for the treatment of various bacterial infections. Further, the extract has to be examined through *invivo* experiments and to confirm their mechanism of action as novel therapeutic agent.

Conflicts of interest

No conflict of interest to be declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101148.

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