4.0. RESULTS

4.1. FLUORESCENT ANALYSIS

4.1.1. Fluorescence analysis of treated powder of *S. wightii*

The observations made on fluorescent analysis of the treated powders of brown seaweed *S. wightii* were analyzed at Short-UV (254nm) and Long-UV (364nm) are recorded in the Table 1. *S. wightii* powder as such, treated with 1N NaOH (aq.) and 50% H$_2$SO$_4$ showed brownish green fluorescence at long-UV 364nm whereas the same powder when treated with IN NaOH (me.) and 50% HNO$_3$ exhibited reddish green fluorescence at long-UV 364nm. With 1N HCl, *S. wightii* powder showed brilliant green fluorescence at long-UV 364nm. In general, the seaweeds fluoresced at long-UV 364nm respectively.

4.1.2. Fluorescent analysis of *S. wightii* in various solvents

The results of the fluorescent analysis of the extracts of brown seaweed *S. wightii* in various solvents at Short-UV (254nm) and Long-UV (364nm) are depicted in the Table 2. In the case of *S. wightii*, showed brilliant green fluorescence in chloroform extract at long-UV 364nm. The acetone and distilled water extracts of *S. wightii* showed fluorescence green and in the case of ethanol the extract of *S. wightii* showed brownish dark green fluorescence at long-UV 364nm.

4.2. QUANTIFICATION OF PHYSICAL CONSTANTS

The physical constants such as, moisture content, total ash, water soluble ash and acid insoluble ash of brown seaweed *S. wightii* which were estimated are

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presented in Table 3. Total ash content was more in *S. wightii* when compared with water soluble ash and acid insoluble ash values.

### 4.2.1. EXTRACTIVE VALUE

Extractive values are also part of physical constants or standards with reference to pharmacological studies. The extractive value of brown seaweed *S. wightii* from five solvents was represented in Table 4. In the case of ethanolic solvent extract value was more followed by chloroform and remaining solvents showed lesser extractive values.

### 4.3. PHYTOCHEMICAL SCREENING

The results of the phytochemical screening are depicted in Table 5. The chloroform and hexane extract of brown seaweed *S. wightii* showed the presence of all the biochemicals analyzed. The ethanolic extract did not answer for both flavonoids and polyphenols while that of acetone extract for saponin and polyphenols.

### 4.4. QUANTIFICATION OF BIOCHEMICALS

Sharp variations in the quantity of many of the biochemicals in *S. wightii* were observed (Tables 6 - 8). The species *S. wightii* registered very high values for protein (232.21mgg⁻¹), carbohydrate (363.15mgg⁻¹) and organic carbon (332.20mgg⁻¹). The lesser values were obtained for lipid (81.32mgg⁻¹), calorific value (10.21KJb⁻¹) and fiber content (3.12%) (Table 6). The amount of tannin, phenol and anthocyanin present in *S. wightii* are depicted in the Table 7, respectively. Highest value was observed for tannin (41.23mgg⁻¹) followed by phenol (3.42mgg⁻¹) and anthocyanin (0.096mgg⁻¹). Results on the estimate of minerals and vitamins of *S. wightii* are presented in Table 8. Among the five inorganic minerals the sodium was found to be
maximum (90.20 mg g⁻¹) followed by that of magnesium (64.02 mg g⁻¹) and calcium (63.03 mg g⁻¹). Lesser amount of nitrogen (42.35 mg g⁻¹) and potassium (34.01 mg g⁻¹) was observed respectively. Among the four vitamins (A, B₃, C and E), vitamin C (6.22 mg g⁻¹) was found in large amount followed by that of vitamin E (2.10 mg g⁻¹), vitamin A (631.60 µg g⁻¹) and vitamin B₃ (0.657 mg g⁻¹) (Table 8).

4.5. HPTLC ANALYSIS OF S. WIGHTII

The results on the HPTLC analysis of S. wightii are presented in the form of peak tables (9-14), Chromatograms in the Figures 2, 6, 10, 14, 18 & 22 (a,b), densitogram (Fig. 3a,b, 4a,b, 5; 7a,b, 8a,b, 9; 11a,b, 12a,b, 13; 15a,b, 16a,b, 17; 19a,b, 20a,b, 21 & 23a,b, 24a,b, 25). These results confirmed the presence of one type of alkaloid, glycoside, three types of saponins, one type of steroid (stigmasterol) and one type of treprenoid in S. wightii. Whereas flavonoid was found to be absent in this brown seaweed S. wightii.

4.6. HPLC ANALYSIS OF S.WIGHTII FOR AMINO ACIDS AND FATTY ACIDS

In total, 21 amino acids were detected (Fig. 26a,b) and quantified by using HPLC technique (Table 15). Glycine was found to the maximum extent followed by threonine and cystine. The lowest amount was recorded for the amino acid tryptophan. Among 21 amino acids, nine amino acids which are regarded as essential for humans (essential amino acids) namely phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine and histidine were found in quantifiable amounts in this brown seaweed Sargassum wightii. Total score on essential amino acids was found to be 4.5169% while that of non-essential amino acids 5.9095%.
In total, 6 fatty acids were detected (Fig. 27a,b) and quantified by using HPLC technique (Table 16). Two of them were saturated fatty acid (SFA) namely palmitic acid and stearic acid, one monounsaturated fatty acid (MUFA) oleic acid and three poly unsaturated fatty acid (PUFA) viz, linolenic acid, alpha linolenic acid and moroctic acid. The saturated fatty acid content score (2.655%) and poly-unsaturated fatty acid content score (2.1266%) was higher than the mono-unsaturated fatty acid score (0.1121%).

4.7. ANTIDIABETIC PROPERTY OF S.WIGHTII (ANTIOXIDANT & HYPOGLYCAEMIC ACTIVITY)

4.7.1. Antioxidant activity of S.wightii

Whole blood glycosylated haemoglobin levels were found to be significantly elevated in alloxan-induced diabetic rats as compared to normal-control rats and 25 & 50 mg/kg/p.o., S. wightii treated rats exhibited significant (P<0.05) level of glycosylated haemoglobin (Fig. 28). Serum creatine phosphokinase (CPK) activity enhanced significantly in alloxan-induced diabetic rats as compared to normal-control rats and S.wightii (25 and 50 mg/kg/p.o) treated rats exhibited significant (P<0.05) level of creatine phosphokinase (CPK) in alloxan-induced diabetic rats (Fig. 28).

Alloxan-induced diabetic rats exhibited significant (P<0.01) elevation in heart (cardiac) enzymes superoxide-dismutase (SOD) and catalase (CAT) as well as in malonaldehyde (MDA) levels (Fig. 29). In the case of kidney (renal) and livers (hepatic), both the enzyme SOD and CAT were significantly decreased in alloxan-induced diabetic rats along with the significant elevation in MDA levels (Fig. 30 and Fig. 31). In case of erythrocyte, MDA levels were significantly increased in alloxan-
induced diabetic rats without significant alteration in the antioxidant enzyme (SOD and CAT) as compared to normal-control rats (Fig. 32). With repeated treatment of *S. wightii* (25 and 50 mg/kg/p.o) for 21 days, the MDA levels were significantly reduced comparable to the normal rats (Fig. 32).

### 4.7.2. Hypoglycaemic activity of *S. wightii*

The effect of *S. wightii* extract on fasting blood glucose was assessed in normal-control rats for 30m, 60m and 120m. The percentage reduction in blood glucose levels after 12m (2h) in the *S. wightii* (25 & 50 mg/kg/p.o) treated rats showed P<0.001 level of significance when compared to that of normal-control rats (Fig. 33).

#### 4.7.2.1. Effect on oral glucose tolerance

Results of the glucose tolerance test conducted on normal-control rats fed with extracts of *S. wightii* (25 & 50 mg/kg/p.o) are shown in Fig. 34. Thirty minutes after feeding glucose, the blood sugar rose in normal-controls rats. Treatment with the extract of *S. wightii* produced maximum reduction in blood sugar level at 60, 120 min in comparison to normal-controls rats. The effect was less pronounced at 30m indicating a late onset of the effect.

#### 4.7.2.2. Effect on alloxan induced diabetic rats

The effect of *S. wightii* extracts in alloxan-induced diabetic rats is given in Fig. 35. Alloxan-induced (60 mg/kg/i.p) led to the elevation of blood glucose at (P<0.001) levels of significance, which were maintained over a period of 3weeks (21days). After 3weeks (21days) of daily treatment with extract (25 & 50 mg/kg/p.o) led to a dose dependent fall in blood glucose level at (P<0.01) levels of significance.
4.8. ANTIOBESITIC ACTIVITY OF S. WIGHTII

The present investigation was carried out to evaluate the effect of *S. wightii* extract on lipid content in the serum, liver (hapatic), kidney (renal), heart (cardiac) and pancreas tissues in normal-control rats, alloxan-induced diabetic rats and *S. wightii* (25 & 50 mg/kg/p.o) treated rats (Figures 36-41). In serum lipid content, cholesterol, free fatty acids, phospholipids, and triglycerides were significantly higher in the alloxan-induced diabetic rats when compared to that of normal-control rats. The continuous treatment with *S. wightii* (25 & 50 mg/kg/p.o) extract brought down the cholesterol, free fatty acids, phospholipids, and triglycerides in the diabetic-treated rats to almost normal (Fig 36).

The effect of *S. wightii* (25 & 50 mg/kg/p.o) extracts on serum HDL-C, LDL-C, VLDL-C and total lipids in control, induced diabetic rats and *S. wightii* (25 & 50 mg/kg/p.o) treated rats is shown in Fig, 41. Serum HDL-C levels were significantly lowered in alloxan-induced diabetic rats. But LDL-C, VLDL-C and total lipids were significantly elevated in the alloxan-induced rats compared to those in normal-control rats. Treatment with *S. wightii* (25 & 50 mg/kg/p.o) extract lowered these lipoproteins in the diabetic-treated rats to nearly normal levels.

4.9. CARDIOPROTECTIVE PROPERTY OF S. WIGHTII

4.9.1 Effect of *S. wightii* on diagnosis marker enzymes during isoproterenol induced myocardial infarction in rats

The activities of lactate-dehydrogenase, alanine-transferease and aspartate-transferease (Fig. 42 a,b) in heart (cardiac) in control, isoproterenol-induced cardiac infarcted rats and *S. wightii* (300 & 400 mg/kg/p.o) treated rats were analyzed. A
significant decrease in myocardial enzyme lactase-dehydrogenase in heart (cardiac) tissue was observed in *S. wightii* (300 & 400 mg/kg/p.o) treated rats for 7, 11 and 15 days when compared to that of isoproterenol-induced rats but aspartate-transeferase increased when compared to that of alanine-transeferase in control, induced and *S. wightii* (300 & 400 mg/kg/p.o) treated rats.

The levels of Vitamin C & E (non-enzyme) in myocardial-infracted rats were almost normal in the pretreated ethanolic extract of *S. wightii* (Fig. 43). The enzyme superoxide-dismutase (SOD) and catalase (CAT) activities in heart (cardiac) in control, isoproterenol-induced diabetic rats and *S. wightii* (300 & 400 mg/kg/p.o) treated rats are represented in the Fig. 44. A significant (P<0.05) decrease in the SOD and CAT enzyme in heart (cardiac) tissue was observed in isoproterenol-induced rats. The level of SOD and CAT enzyme activities are almost similar to that of normal-control rats.

The reduced glutathione and glutathione peroxidase activities in heart (cardiac) tissue of control, isoproterenol-induced diabetic rats and *S. wightii* (300 & 400 mg/kg/p.o) treated rats are presented in (Fig. 45). A significant (P<0.05) decrease in the glutathione peroxidase and reduced glutathione activities in heart (cardiac) was observed in isoproterenol-induced rats. The activities were near normal in the ethanolic extract of *S. wightii* (300 & 400 mg/kg/p.o) treated in isoproterenol-induced rats near normal. Glutathione is the substrate for the enzyme glutathione peroxidases, which metabolized hydrogen peroxides. The activities of lipid peroxidase and glutathione reductase also increased in isoproterenol-induced rats when compared with
control rats, which are reverted in *S. wightii* (300 & 400 mg/kg/p.o) ethanolic extract treated rats (Fig. 46).

**4.9.2. Histopathological studies of cardiac tissue on isoproterenol induced rat**

Histological findings confirmed the induction of myocardial infarction by isoproterenol. The following observations were made in the heart (cardiac) tissue of normal-control, isoproterenol-Hcl induced myocardial infarcted rats and *S. wightii* (300 & 400 mg/kg/p.o) treated (Plate-6&7). The control rat heart showed normal architecture. In the isoproterenol-induced myocardial infarcted rat heart showed severe cardiac damage with large areas of endocardial fibre degeneration with Foci of necrosis and inflammatory infiltrate in the myocardium (Plate-6, Fig. a,b). The rats given 300 mg/kg of *S. wightii* extract alone did not show any abnormal changes in the architecture of the heart (Plate-7, Fig a). The administration of 400 mg/kg of *S. wightii* extract treated rats exhibited normal endocardial and myocardial fibre with occasional focus of few inflammatory cells only (Plate-7, Fig b).