MACRO ALGAE (*EUCHEUMA COTTONI* AND *SARGASSUM* SP.) ARE RESERVOIRS OF BIODIESEL AND BIOACTIVE COMPOUNDS

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ABSTRACT

Bioactive compounds such as phytochemicals and Fatty acid esters are screened from macroalgae *Eucheuma cottoni* and *Sargassum* sp. Ethyl acetate, DMSO and methanol extracts of algal species were tested for the presence of alkaloids, flavonoids, steroids, phenols, antioxidants and certain antifungal properties against fungi (*Candida sp., Fusarium sp.* and *Trichoderma* sp.) and antibacterial were tested against Gram +ve and Gram -ve bacteria (*Pseudomonas fluorescens, Klebsiella pneumonia, Staphylococcus aureus* and *E. coli*) based on Agar Well diffusion assay. Algal extracts were tested for antioxidant activity by using the methods of DPPH, Phosphomolybdenum and reducing power assay. The results showed that these two algal strain have the phytochemical properties. Macro algae are also considered as a very promising feedstock for biodiesel production. The oil contents of macroalgae was extracted by chemical extraction method and purified. The performance of the blended fuel (Algae oil +Diesel) was studied in four stroke single cylinder diesel engine and some improvements in break thermal efficiency for about 2 to 6%. Total fuel consumption of blended fuel is lesser than the diesel for about 0.1 to 0.32 kg hr^{-1}. Specific fuel consumption of blended fuel is lesser than the diesel for about 0.08 to 0.1 kg kW^{-1} hr^{-1}. Hydrocarbon emission of the blended fuel has decreased for about 1 to 3 ppm. CO, CO_2, Smoke emission of blended fuel was decreased significantly compared to Q-diesel, whereas NO emission of blended fuel was increased than Q-diesel. The emission characteristics of biodiesel blend percentage increased over varying engine loads. The algal biodiesel blends B5 and B10 showed slight decrease in specific fuel consumption but ECB5 showed higher brake thermal efficiency.

KEYWORDS: *Macroalgae; Phytochemical; Biodiesel; engine performance.*

1. INTRODUCTION

Marine macroalgae/seaweeds are widely used for many different purposes. They are consumed in some of the Asian countries, in western countries it is used as a source for phycocolloids, thickening and gelling agents for various applications, including food and pharmaceutical industries. Furthermore, they are also used for nutritional improvement in animal feed, cosmetics, herbal medicine, fertilizers, etc. Seaweeds are known as valuable sources of protein, elements, dietary fibers, vitamins, essential amino acids and essential fatty acids. Moreover, seaweeds also contain potential bioactive compounds which exhibit antibacterial, antiviral and antifungal properties (Marinho-Soriano, 2006). Macroalgae have been reported to contain more than 2400 natural products of commercial importance in pharmaceutical, biomedical, and nutraceutical industries (Saranya, 2013). They have also been extensively utilized as ingredients in human and animal food preparations owing to their high contents of polyunsaturated fatty acids (PUFAs) carbohydrates, vitamins, minerals and dietary fibers. Fat contents of seaweeds were found within the range of 1–6 g/100 g DW with high concentrations of long-chain polyunsaturated fatty acids (Ortiz, 2006). Fatty acids are important for human and animal health because they are precursors in the biosynthesis of eicosanoids, which are important bioregulators in many cellular processes (Gressler, 2010).The recent investigation of the use of alternative, non-food related feedstock such as oil from algae is becoming popular. Algae have the capability to convert carbon dioxide to biomass which can be further be downstream processed to produce biodiesel, fertilizer and other products (Sheehan, 1998) and also macroalgae have the low-cost cultivation and harvesting potential.

Biodiesel is non-toxic, readily biodegradable and increase lubricity of diesel fuels. Diesel engines are widely used as power sources in medium and heavy-duty applications because it has lower fuel consumption, carbon monoxide (CO) and hydrocarbons (HC) emission when compared to other gasoline engines (Hasimoglu, 2008). The use of vehicles all over the world especially in big cities and towns contribute the most in generating gaseous emission, hence cause the pollution of environment. Exhaust emission of diesel engines operating on neat biodiesel and its blends with diesel fuel have been reported in numerous studies (Kassaby, 2013). Many researchers investigate that increase in the percentage of biodiesel in blends will reduce the CO, SOx, HC, particulate matter (PM) emission and smoke. However, the emission of NOx depends on the biodiesel sources (Ahmed, 2010).

Eucheuma cottonii is an edible species of Pacific red seaweeds obtained from Malaysian North Borneo Sabah water which is a potential source of a variety of compounds like dietary fibers, vitamin C, α-tocopherol, minerals, fatty acid and protein (Matanjun, 2009). *E. cottonii* is a rich source of antioxidants (Matanjun, 2008), which can significantly prevent tissue damage by stimulating the wound healing process and also act as anti-inflammatory. *Sargassum* sp., one of the marine macro algae belonging to the class Phaeophyceae, is widely distributed in tropical and temperate oceans. It belongs to the marine family *Sargassaceae* and order Fucales. Wide range of bioactive properties of *Sargassum* has been reported (Devi, 2013). Due to its availability and bioactive property the current study was focused on some of the biomolecules for various medical and industrial applications.

2. MATERIALS AND METHODS

2.1. Collection of macro algae: *Sargassum* sp. and *E. cottonii* are the two macro algae species collected from marine water on the coastal areas of Gagathapatnam, Mamelkudui Taluk, Pudukkottai District, Tamil Nadu, India (10° 3' 0" North, 79° 14' 0" East). Nearly 2 kg each of wet algae were collected, washed with fresh water to remove salt present over macro algae and the wet biomass was placed in a vacuum dryer to evaporate most of the moisture. After the complete removal of water the dry algae was grounded to 22 µm.

2.2. Lipid/Oil Extraction:

2.2.1. Oil Extraction with solvents: Approximately 0.5 g of each algal biomass was extracted with 3 different solvents namely acetone, ethyl acetate and methanol. This was done by primarily homogenizing the biomass with 10 ml of each solvent separately using mortar and pestle. This solution was then centrifuged at 5000 rpm for 7 min at 15 ºC according to Wang *et al*. (2006). The supernatant was collected and the remnant biomass was once again homogenized using mortar and pestle with 5 ml of the respective solvents. This was again centrifuged at 5000 rpm for 7 min at 15 ºC.

2.2.2. Soxhlet extraction: Dried macro algal powder of 100 g was taken with 180 ml of solvent in soxhlet apparatus. The mixture of chloroform and methanol was at 2:1 v/v ratio based on the principle developed by Folch, 1957. After 20 hr algal oil collected from round bottom flask was used for biodiesel production. Distillation was carried in order to separate the oil and solvent. The distilled algal oil is stored in glass bottle.

2.3. Antimicrobial activity by agar Well diffusion method: The wells (6mm diameter) were made by using gel puncher on Muller Hinton Agar plates. Overnight grown bacterial cultures of *Pseudomonas aeuroginosa, Klebsiella pneumoniae, E. coli, Staphylococcus aureus* and two days old fungal cultures of *Candida* sp., *Fusarium* sp. and *Trichoderma* sp., were swabbed on to the plates. The different concentrations of crude extracts were loaded in the wells. Methanol/acetone/ethyl acetate was used as negative controls and ampicillin was used as positive control. The plates were incubated at 37 ºC for a period of 16 hrs for bacterial strains and 27 °C for a period of 16 hrs for fungal strains. The extracts and supernatants containing antibacterial/fungal components developed distinct, clear, circular zones of inhibition around the wells and the diameters of clear zones were determined and used as an indication of antibacterial/fungal activity. The assay was carried out in triplicates and the results were recorded.

2.4. Antioxidant activity

2.4.1. DPPH Radical Scavenging assay: About 4 ml of 0.2 mM DPPH was added to 500 µl of each algal extracts , shaked vigorously and incubated in the dark for 30 min at room temperature. Control was prepared without the addition of algal extracts. Absorbance was measured at 517 nm and the scavenging activity was calculated as a percentage of the radical reduction. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula.

$$\% \text{ Radical scavenging activity} = \left(\frac{\text{Control OD-Sample OD}}{\text{Control OD}}\right) \text{ x } 100$$

2.4.2. Phosphomolybdenum assay: The antioxidant activity of the various algal extracts was evaluated by phosphomolybdenum method according to the procedure of Prieto et al (1999). An aliquot of 0.1 ml of algal extract was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). All the tubes were covered with aluminium foil and incubated at 95°C for 90 min. The tubes were cooled to RT and the absorbance of aqueous solution was measured at 695 nm. Ascorbic acid was used as standard.

2.4.3. Reducing power assay: The reducing power of the various algal extracts was determined by assay according to Yildirim *et al.* (2001). 2.5 ml of the various marine algal extracts was mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide and incubated at 50 °C for 20 min. This was then centrifuged for 10 min at 5000 rpm after addition of 2.5 ml of 10 % trichloroacetic acid. 2.5 ml aliquot of the supernatant was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1 % ferric chloride. After 10 min of incubation, the absorbance was measured at 700 nm.

2.5. Phytochemical screening

2.5.1. Detection of flavonoids: Flavonoids were detected by the solution of 10 % NaOH and concentrated HCl, appearance of yellow solution that turns colorless indicates the presence of flavonoids.

2.5.2. Detection of saponins: 1 ml of each algal extract was treated with 1 % lead acetate solution as described by Trease and Evans. Formation of white precipitate indicates the presence of saponins in the extracts.

2.5.3. Determination of total flavonoids: 5 ml of different algal extracts were taken in different test tubes and 5 ml of 2 % Aluminium chloride in metabolic solution was added. After ten minutes the OD was measured at 415 nm.

2.5.4. Determination of total phenolic content: The test was done to determine the total phenol content in the algal extracts based on the method described by McDonald, (2001). The total phenol content of the algal extracts was determined by the Folin-Ciocalteu method. To a 40 µL solution of the various algal extracts, 3.16 ml water and 200 µL Folin-Ciocalteu reagent were added and the mixture was shaken well. The mixture was incubated for 10 min at room temperature. To this solution 600 µL of a 0.25% sodium carbonate was added. This solution was further incubated at RT for 2 hrs and its absorbance was measured at 765 nm against the blank.

2.6. TLC analysis of the algal extracts

2.6.1. Identification of steroids using TLC: For the identification of steroids the stationary phase was the pre-coated silica sheets were cut into strips of 2 cm X 7.5 cm and mobile phase was chloroform/ethanol/water ratio of 18:8/1:2/1. 10 µl of Algal

extracts were added in the spot of TLC sheet (stationary phase) then placed into the TLC chamber which contain solvents (mobile phase). Mobile phase slowly rises up by the capillary action. The TLC strips were kept in the chromatography chamber for 40 min. Then strips were air dried till the solvent evaporates completely. The bands were detected under UV to observe the pink bands which correspond to steroids present in the extracts. Steroids were identified and Rf (Retention factor) value was calculated.

2.6.2. Identification of alkaloids using TLC: For the identification of alkaloids, butane/acetic acid/water ratio of 4:4:1 was used as mobile phase. After 45 min the strips were air dried to evaporate the solvent and 2 % $FeCl_3$ in ethanol was sprayed to view the alkaloid bands present in the TLC chromatogram. The bands were detected under UV and Rf value was calculated.

2.6.3. Identification of flavonoids using TLC: For the identification of flavonoids the mobile phase was butanol/acetic acid/water ratio of 4:4:1. Then 1 % ferric chloride solution was dropped over the sheets and dried. Then the yellow spot of peaks were detected under UV and Rf value was calculated.

2.7. Biodiesel production

2.7.1. Transesterification: Sodium methoxide was used as a catalyst, for 25 ml oil 3% sodium methoxide in methanol was mixed by proper stirring for about 10 min. Incubate the mixture for three hours in orbital shaker, then transfer it into the separating funnel and kept for 24 hours to settle the biodiesel and sediment layers clearly. The biodiesel was collected and the glycerin was removed. The biodiesel was purified by washing 2-3 times gently with warm water to remove residual catalyst or soaps. Finally the biodiesel was dried by flash heating. The biodiesel was stored in an aluminum foil sealed container for analysis.

2.7.2. Blending: The algal oil was heated at 60 ^{o}C and stirred at 500 rpm. After that required amount of diesel was added and then stirred for about 60 min at 1000 rpm. Blending was done in the order of B5S (Diesel 95 % and *Sargassum* oil 5 %), B5E (Diesel 95 % and E. c*ottoni* oil 5 %), B10S (Diesel 90 % and *Sargassum* oil 10 %) and B10E (Diesel 95 % and E. c*ottoni* oil 5 %).

2.7.3. Performance: The engine performance was carried with single cylinder four stroke diesel engines. Decompression lever was kept in vertical position. Shaft was rotated with starting handle. When the flywheel pickup speed decompression lever into horizontal position, engine will pick up, starting handle was removed immediately and engine was allowed to warm up. The engine was started on 'No Load' and allowed to idle for some time. The time taken for 10 cc of fuel consumption was noted using stopwatch and fuel measuring burette. Now the engine was loaded gradually to the desired value. The engine was allowed to run at this load for some time in order to achieve steady state condition. The time taken for 10 cc fuel consumption was noted. Cut off the fuel supply by keeping the fuel governor lever in the other extreme position. The above procedure was followed for diesel and blended biodiesel.

2.8. Emission

2.8.1. Calibration procedure for testing of gas analysers: The probe was inserted into the exhaust pipe of the engine until there is no fluctuation in reading is obtained. The readings are displayed on the screen and it was printed. The emission measurements are carried out on dry basis. Before the second set of readings the probe was kept in atmospheric air for about 120 seconds after that the above procedure was repeated.

2.8.2. Calibration procedure for testing of smoke meters: The smoke meter was allowed to warm up for 5 min, the calibration of the meter should to be in zero. The value must lie within 0.1 m^{-1}. The meter shall have the standard accessories and checked that the sample hose, internal pipes etc are not deteriorated or damaged to ensure that there is no leakage. Free acceleration test was carried out using a vehicle and the print out details was checked.

3. RESULTS

3.1. Antimicrobial activity: The antibacterial and antifungal activities were measured with respect to the radius of the zones formed on the agar plates. The radii of the positive and negative controls were also measured and a graph was plotted to determine the maximum activity. Ampicillin (10 µg/ml) was used as the positive control and the solvent alone (ethyl acetate/ methanol/ acetone) was used as the negative control (Fig1a and 1b).

3.2. Antioxidant activity

3.2.1. Phosphomolybdenum assay: The absorbance of the end products of the various extracts were measured spectrophotometrically and plotted in a graph to analyse the percentage of activity and hence the percentage of antioxidants present in each extract could be calculated (Fig. 2a).

3.2.2. DPPH assay: The antioxidant amounts in the different extracts were measured spectrophotometrically using the DPPH assay. The results were calculated using the formula in section 2.4.1 and the percentages of antioxidants present in the various extracts were determined (Fig. 2b).

3.2.3. Reducing power assay: The reducing power and the antioxidant content of the extracts were measured spectrophotometrically. The concentration and the percentage of antioxidants present in the extracts were determined (Fig. 2c).

3.3. Phytochemical analysis

3.3.1. Test for flavonoids: Yellow color of extract on addition of HCl and disappearance of colour on addition of NaOH inferred the presence of flavonoids in the extracts. Methanol extracts showed mild indication whereas ethyl acetate extracts showed high level indication.

Table 1a. Test for Flavonoid content

Strains/Solvent	Methanol	DMSO	Ethyl Acetate
E. cottonii	+	-	+++
Sargassum Sp.	+	-	++

+ - less, ++moderate, +++ - high

3.3.2. Test for saponins: Formation of white precipitate indicated the presence of alkaloids in the extracts (Table 1b).

Table 1b. Test for saponin content

Strains/Solvent	Methanol	DMSO	Ethyl Acetate
E. cottonii	-	-	-
Sargassum Sp.	+	-	+

+ - less, ++moderate, +++ - high

3.3.2. Total phenols test: The concentration of the extracts mixed with Folin-Ciocalteu reagent and Na_2CO_3 was measured spectrophotometrically at 765 nm against the blank and the Table 1c. Shows the presence of phenols in the extracts.

Table 1 c. Test for Phenols content

Strains/Solvent	Methanol	DMSO	Ethyl Acetate
E. cottonii	+	-	+++
Sargassum Sp.	+	++	+++

+ - less, ++moderate, +++ - high

3.3.3. Test for alkaloids: The appearance of bands in the after sprayed with 1 % $FeCl_3$ were observed for the presence of orange bands corresponding to alkaloids present in the extracts (Table 1 d).

Table 1 d. Test for alkaloids content

Strains/Solvent	Methanol	DMSO	Ethyl Acetate
E. cottonii	+	-	-
Sargassum Sp.	++	-	-

+ - less, ++moderate, +++ - high

3.3.4. Test for steroids: The bands on the pre-coated TLC strips corresponding to the various extracts were viewed under ultraviolet light. The pink colored bands corresponded to the various steroids present in the different algal extracts was observed (Table 1e).

Table 1 e. Test for alkaloids content

Strains/Solvent	Methanol	DMSO	Ethyl Acetate
E. cottonii	+	-	+
Sargassum Sp.	++	-	+++

+ - less, ++moderate, +++ - high

3.4. Thin layer chromatography

3.4.1. Identification of steroids using TLC: Algal extracts were tested for steroids identification using TLC. The bands were detected under UV. R_f (Retention factor) value was calculated as shown in Table 2a.

Table 2a. Rf values of algal extracts

S. No.	Strains	Rf Values of solvent extracts		
		Ethyl acetate	Methanol	DMSO
1.	*E. cottonii*	0.42	0.30	0.80
2.	*Sargassum* Sp.	0.69	0.76	*

* No result

3.4.2. Identification of flavonoids using TLC: Flavonoids are identified by TLC. Ferric chloride solution (1%) was dropped and sheets were dried. The yellow spot of peaks were detected under UV. R_f values were given in table 2b.

Table 2b. Rf values of algal extracts

S. No.	Strains	Rf Values of solvent extracts		
		Ethyl acetate	Methanol	DMSO
1.	*E. cottonii*	*	0.39	0.40
2.	*Sargassum* Sp.	0.95	*	*

* No result

3.4.3. Identification of alkaloids by TLC

Alkaloids identification was carried out based on TLC. The bands were detected under UV and Rf value was given in table 2c.

Table 2b. Rf values of algal extracts

S. No.	Strains	Rf Values of solvent extracts		
		Ethyl acetate	Methanol	DMSO
1.	*E. cottonii*	0.70	0.56	0.53
2.	*Sargassum* Sp.	*	0.52	*

* No result

3.5. Performance characteristics

3.5.1. Comparison of TFC: The specific gravity of blended algae oil is low when compared to the normal diesel. So the consumption rate was decreasing. However the consumption rate of B10 samples was increased with respect to B5 samples because of the decrease in calorific value of B10 samples. Biodiesel from *E. cottonii* sample has shown decrease in fuel consumption in spite of its low calorific value (Fig. 3a).

3.5.2. Comparison of SFC: The specific gravity of blended algae oil was low when compared to the diesel. So the consumption rate was decreasing. However the consumption rate of B10 samples was increased with respect to B5 samples because of the decrease in calorific value of B10 samples (Fig. 3b).

3.5.3. Comparison of Brake Thermal Efficiency: The total fuel consumption of the fuel is normally inversely proportional to the performance of the engine. So from the calculated value observed from Fig. 3c for the various load given to the engine the brake thermal efficiency of the blended algae oil is increased when compared to the normal diesel. EOB5 and EOB10 sample has showed increase in Brake Thermal Efficiency.

3.6. Emission characteristics

3.6.1. Comparison of HC: For every fuel, there is a decrease in HC emission on increase of the engine load (Fig. 3d). The higher combustion temperature at higher engine load contributes to the general decreasing trend. For biodiesel blended fuel, the HC emission is lower than that of diesel and decreases with increase of biodiesel in the fuel. However, the lower volatility of biodiesel compared with diesel contributes to the larger difference in HC emission at low engine loads.

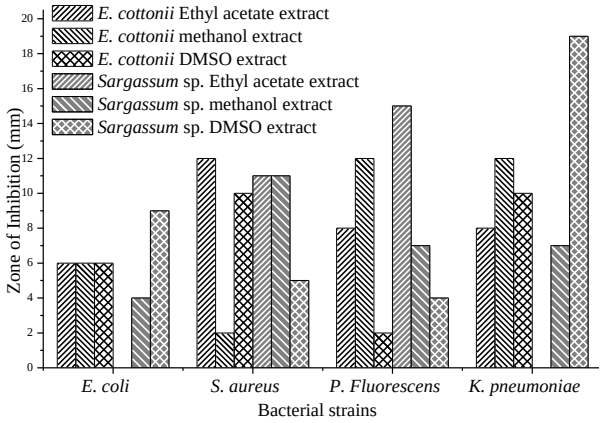

Fig. 1a

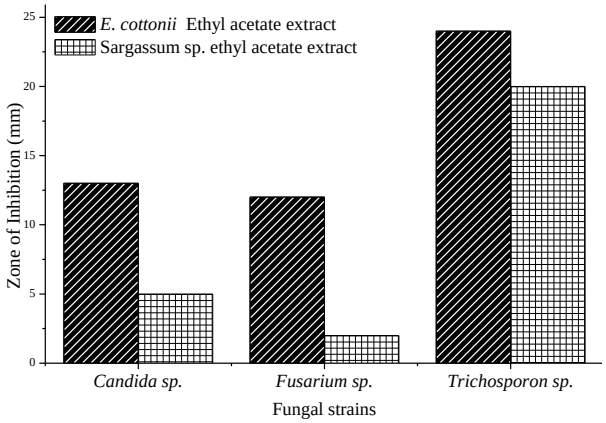

Fig. 1b

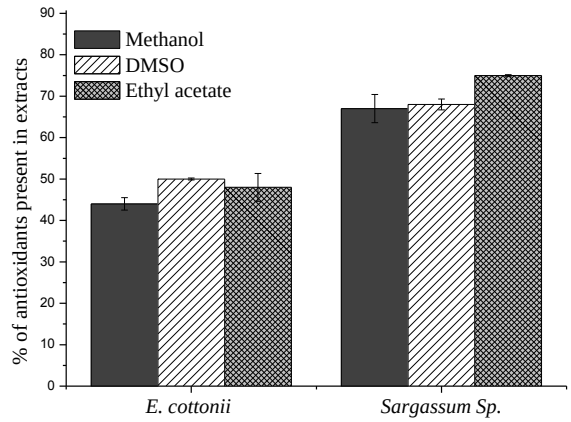

Fig. 2a

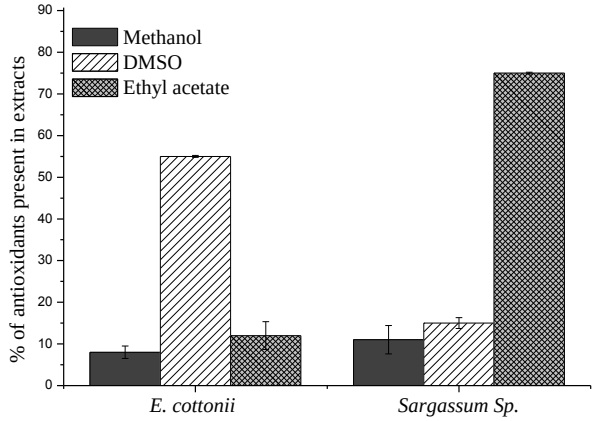

Fig. 2b

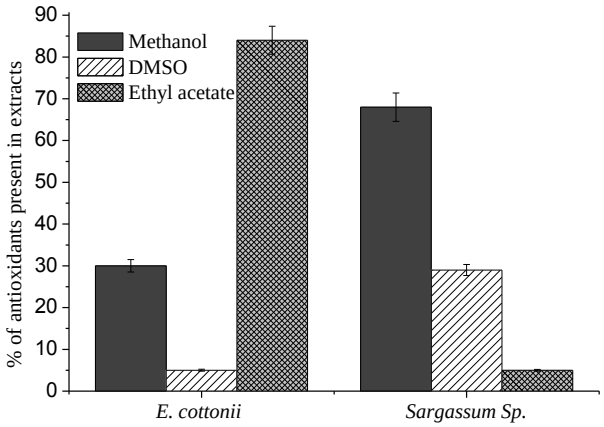

Fig. 2c

Fig.1a Antibacterial effect og algal extracts
Fig.1b Antifungal effects of algal extracts
Fig.2a DPPH assay activity of algal extracts
Fig.2b reducing power assay activity of algal extracts
Fig.2c Phosphomolebedum assay activity of algal extracts

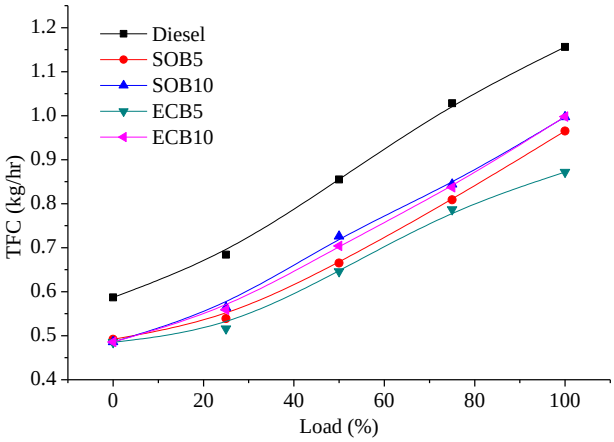

Fig. 3a

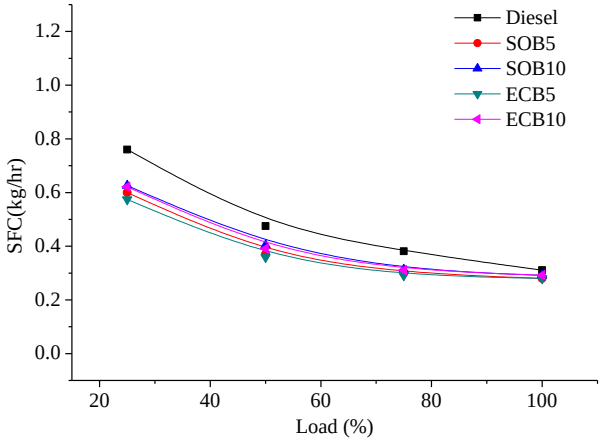

Fig. 3b

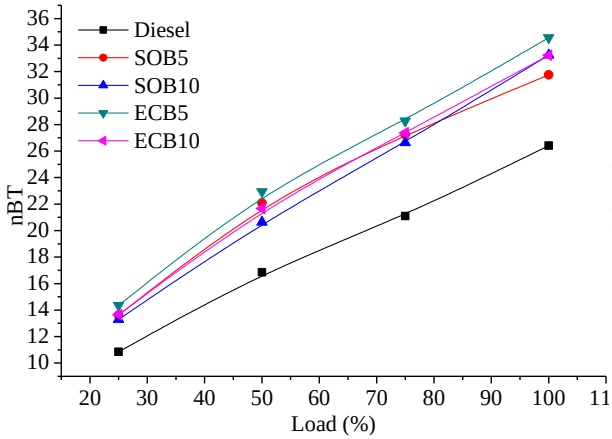

Fig. 3c

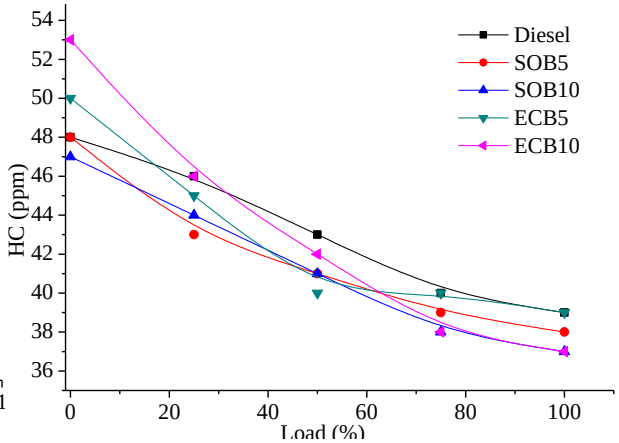

Fig. 3d

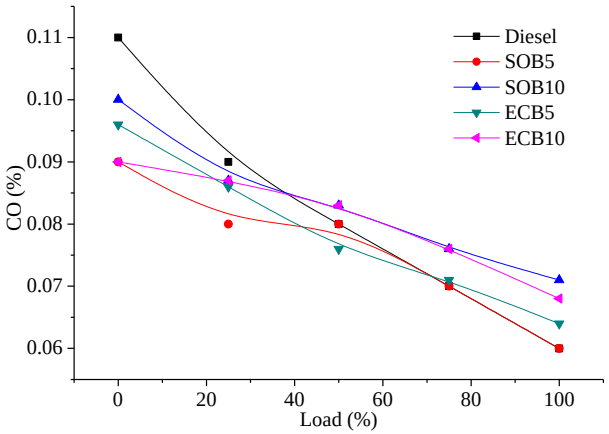

Fig. 3e

Fig. 3a TFC
Fig. 3b SFC
Fig. 3c Brake thermal efficiency
Fig. 3d HC emission
Fig. 3e CO emission

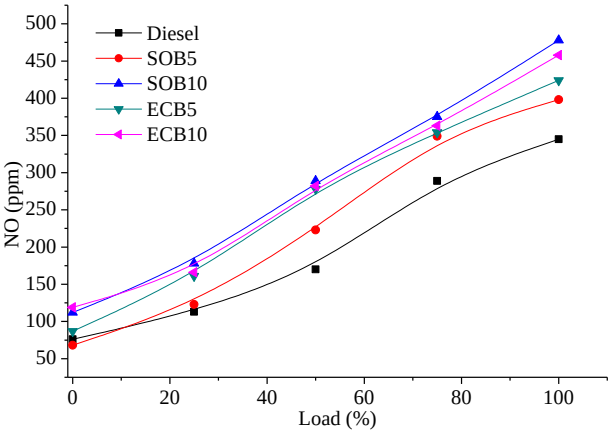

Fig. 3f

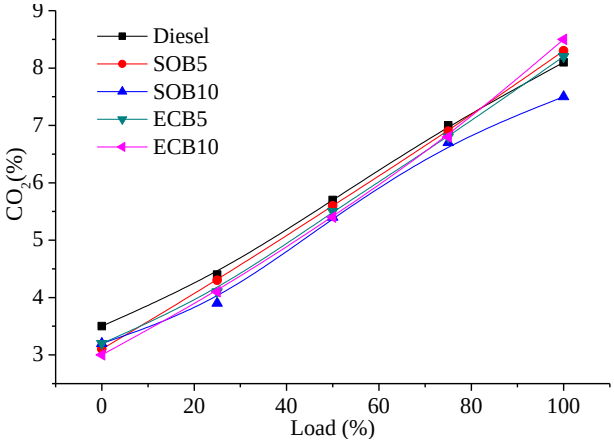

Fig. 3g

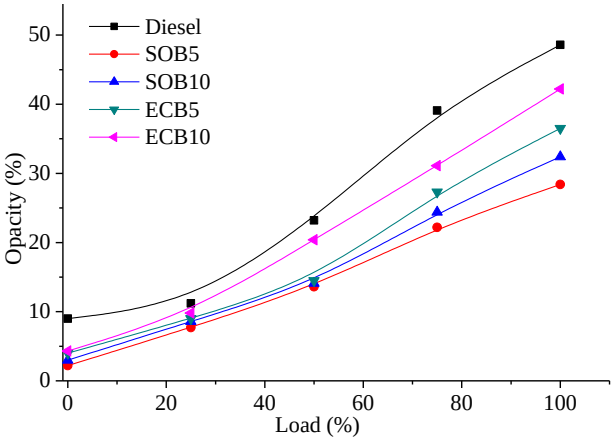

Fig. 3h

Fig. 3f NO emission

Fig. 3g CO_2 emission

Fig. 3h Opacity

3.6.2. Comparison of CO: Higher combustion temperature at higher engine load contributes to the decrease in CO emission with the addition of biodiesel, CO emission also decreases (Fig. 3e). It is possible that the oxygen contained in the fuel enhances complete combustion in the cylinder and reduces CO emission.

3.6.3. Comparison of NO_X: The NO_x concentration increases with increase of engine load for all the fuels (Fig. 3f). Compared with diesel, NO_x emission of the biodiesel blended fuel increases slightly at all tested engine loads and the increase is more

obvious at higher engine loads. The increase in the NOx emission for the blended algae oil over the diesel is significant. So it was observed that of the four samples SOB10 shows a significant increase in NO.

3.6.4. Comparison of CO_2: The CO_2 emission increases with increase in load, as expected. The lower percentage of biodiesel blends emits very low amount of CO_2 in comparison with diesel. SOB5 emits very low level of CO_2 emissions (Fig. 3g). But, its emission level is lower than that of the diesel mode. More amount of CO_2 in exhaust emission is an indication of the complete combustion of fuel. This supports the higher value of exhaust gas temperature. The increase in the Carbon-di-oxide emission for the blended algae oil over the diesel is significant.

3.6.5. Comparison of smoke opacity: The Smoke opacity emission increases with increases in load, as expected. The smoke opacity percentage determines the emission of the smoke in the exhaust. The higher percentage of biodiesel blends emits very low amount of smoke in comparison with diesel (Fig. 3h). As the percentage of blend increases there is a decrease in smoke emissions.

4. DISCUSSION

The evidence of algal bioactive compounds and their antimicrobial activity has been established through many studies. Algae contain different chemical compounds and different extract show different biological activities. Alkaloids are used for the preparation of a wide range of less toxic synthetic pesticides and also acts as antibiotics. Alkaloids, possessing antimitotic and allergic effects at cellular level, are being studied widely. They are also used in local anesthesia and to as a pain relievers. Such alkaloids were viewed here by the method of Trease and Evans (2002). The bands viewed on spraying $FeCl_3$ revealed the presence of alkaloids in the extracts according to the method of Trease and Evans (2002).

Flavonoids are anti-allergic, anti-inflammatory, anti-microbial, and anti-cancer agent. Their retention time showed the presence of alkaloids in algal extracts. Ethyl acetate extracts of *Sargassum* sp. showed highest Rf value compared to methanol and DMSO extracts of *E. cottonii*. Thus the flavonoids are responsible for antimicrobial activity and antioxidant activity of the algal extracts. Saponins are structurally diverse compounds, they act as antioxidants, antifungal and antiviral agents. The white precipitates obtained in the algal extracts showed the presence of saponins in methanol and ethyl acetate extract of *Sargassum* sp. Steroids constitute an important class of hormones. They are used to reduce inflammation, to treat autoimmune diseases, to gain weight and to prevent anaemia. Such steroids are present widely in most green and even more in brown algae. These steroids present in the algal extracts under study have been identified by thin layer chromatography and viewed as pink bands under ultraviolet light at 224 nm, corresponding to the presence of steroids in the samples. DMSO extract of *E. cottonii* showed the highest value.

The phenolic compound and its derivatives, including simple phenols, flavonoids, phenyl proponoids, tannins, lignins and many other substances, contain aromatic rings and hydroxyl groups that will determine the radical scavenging power of the compound (Dziedziz and Hudson, 1983). Folin-Ciocalteu reagent uses the minimum volume of reagents and almost eliminates wasted reagent. Sodium carbonate was used as a neutralization reagent. The highest phenol content and activity was measured in the ethyl acetate extracts of both the strains.Components with antioxidant activities can be found in only a few species of algae. There are a number of reports on the evaluation of antioxidant activity in microalgae and cyanobacteria, *E. cottoni* (Matanjun, 2008), *Sargasam* sp. (Zahra, 2007). These studies concluded that several microalgal genera contain potent antioxidants. Free radicals contribute to more than one hundred disorders in human. Due to negative effects of synthetic antioxidants nowadays, much attention has been placed on phytoconstituents. Many of the phytoconstituents are beneficial and many of them are acting as natural antioxidants. The results of the present investigation are suggestive of the potential of solvent extracts in scavenging free radical. The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex, which has absorption at 695 nm. Maximum antioxidant activity was observed in DMSO extract of *E. cottonii*.

The radical scavenging activity of the extracts could be related to the nature and amount of phenolics, flavonoids and their hydrogen donating ability (Shimada, 1992). The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of $Fe_3+(CN-)_6$ to $Fe_2+(CN-)_6$ [19]. The product was visualized by forming the intense Prussian blue color complex and then measured at λ700 nm. The maximum antioxidant activity was observed in methanol extract of *Sargassum* sp. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species which contributes to the absorbance variation.

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule which does not absorb at 519 nm. The reduction capacity of DPPH radical was determined by the increasing its percentage inhibition. It is visually noticeable as a change in color from purple to yellow. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stoichiometrically related to the number of electrons gained. From the DPPH assay, the ethyl acetate extract of *Sargassum* sp. showed highest percentage (74 %). The pathogenic bacterial strains were resistant to some of the extracts whereas, *E. cottonii* showed highest inhibitory activity against *Trichoderma* sp. among all the three fungal strains. Maximum inhibition zone radii for bacteria was observed for *P. fluorescens*

in ethyl acetate extract of *Sargassum* sp., DMSO extract of *Sargassum* sp. Showed maximum inhibitory activity against *K. pneumoniae*

Sodium methoxide reduces the problems of saponification (reaction for soap formation) caused by the free water produced when sodium hydroxide is used as catalyst. The use of biodiesel in a conventional diesel engine results in significant reduction of unburned hydrocarbons, carbon monoxide and particulate matter. Biodiesel has no sulfur or aromatics.

5. CONCLUSION

Screening of bioactive compounds from marine algae *E. cottonii* and *Sargassum* sp. was studied and their extracts of Methanol, Ethyl acetate, and DMSO have antibacterial and antifungal activity. Presence of Alkaloids, flavonoids, saponins, phenol and steroids were observed in both the strains by preliminary phytochemical analysis. Alkaloids, flavonoids and steroids were confirmed by TLC profiling of the algal extract in different solvent system. The performance of the blended fuel with diesel showed improvement in the efficiency in brake thermal efficiency. TFC and SFC of blended fuel were lesser than the diesel. HC, CO, CO_2 and Smoke emission was decreased compared to diesel. Engine could be run without any difficulty using algae oil blends so there is no further modification is needed for diesel engines. Thus algal oil blended diesel can be effectively employed as a suitable alternative fuel in existing diesel engine.

REFERENCES

Ahmed AS, Khan S, Hamdan S, Rahman R, Kalam A, Masjuki HH, Mahlia TMI, Biodiesel Production from Macro Algae as a Green Fuel for Diesel Engine, Journal of Energy & Environment,2 (1), 2010, 1-5.

Devi JAI, Balan GS, Periyanayagam K, Pharmacognostical study and phytochemical evaluation of brown seaweed *Sargassumwightii*, Journal of Coastal Life Medicine 1(3), 2013, 199-204.

Dziedzic SZ, Hudson BJF, Hydroxyisoflavones as antioxidants for edible oils, Food Chemistry 11, 1983, 161-166.

Folch J, Lees M, Stanley GHS, A simple method for the isolation and purification of total lipids from animal tissues, Journal of Biological Chemistry, 226,1957, 497–509.

Gressler V, Yokoya NS, Fujii MT, Colepicolo P, Mancini J, Torres RP, Pinto E, Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food Chem, 120. 2010, 585-590.

Hasimoglu C, Ciniviz M, Ozsert I, Icingur Y, Parlak A, Salman MS, Performance characteristics of a low heat rejection diesel engine operating with biodiesel, Renew Energy, 33, 2008,1709–1715.

Kassaby MEL, Allah MAN, Studying the effect of compression ratio on an engine fuelled with waste oil produced biodiesel/diesel fue,l Alexandria Engineering Journal, 52(1),2013, 1–11.

Matanjun P, Mohamed S, Mustapha NM, Muhammad K, Ming CH, Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo, J ApplPhycol, 2008, DOI 10.1007/s10811-007-9264-6

Matanjun P, Mohamed S, Mustapha NM,Muhammad K, Nutrient content of tropical edible seaweeds, Eucheumacottonii, Caulerpalentillifera and Sar- gassumpolycystum, Journal of Applied Phycology, 21,2009, 75–80.

Matanjun P, Muhamed S, Mustapha NM, Muhammad K, Antioxidant activities and phenolics content of eight species of sea weeds from north Borneo, J ApplPhycol, 20, 2008, 367-373.

McDonald S, Prenzler PD, Antolovich M, Robards K, Phenolic content and antioxidant activity of olive extracts, Food Chemistry, 73, 2001, 73–84.

Ortiza J, Romeroa N, Roberta P, Arayab J, Hernándezc JL, Bozzoa C, Navarretea E, Osorioa A, Riosa A, Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulvalactuca* and *Durvillaea Antarctica* Food Chemistry 99(1), 2006, 98–104 DOI: 10.1016/j.foodchem.2005.07.027

Prieto P, Pineda M and Aguilar M, Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E, Analytical Biochemistry, 269, 1999, 337-341.

Saranya C, Girija K, Estimation of major pigment content in seaweeds collected from Pondicherry coast, The Experiment, 9(1), 2013, 522-525.

Sheehan J, Dunahay T, Benemann J, Roessler P, A Look back at the U.S. Department of Energy's aquatic species program: Biodiesel from algae. Golden, Colorado: National Renewable Energy Laboratory, 1998.

Shimada K, Fujikawa K, Yahara K, Nakamura T,Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion, J Agr Food Chem, 40, 1992, 945–948.

Soriano ME, Fonseca PC, Carneiro MAA, Moreira WSC, Seasonal variation in the chemical composition of two tropical seaweeds. Bio- resource Technology, 97, 2006, 2402–2406.

Trease GE, Evans WC, Pharmacognosy. 15th Ed. London: Saunders Publishers; 2002. pp. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.

Wang W, Vignani R, Scali M, Cresti M, A universal and rapid protocol for protein extraction from recalcitrant plant tissues for proteomic analysis, Electrophoresis, 27, 2006, 2782–2786

Yildirim A, Mavi A, Kara AA, Determination of antioxidant and antimicrobial activities of Rumexcrispus L. extracts, Journal of Agricultural and Food Chemistry, 49, 2001, 4083-4089.

Zahra R, Mehrnaz M, Farzaneh V, Kohzad S, Antioxidant activity of extract from a brown alga, Sargassumbovenum,Afr J Biotechnol, 6(24), 2007, 2740–2745