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GC/MS analysis and In-vitro Antioxidant activity of methanol extract of Ulothrix flacca and its main constituent Dimethyl Sulfone.

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Abstract:*The determination of phytochemical constituents, total phenol, flavonoid contents and antioxidant assays of methanol extract of Ulothrix flacca and its main constituent dimethyl sulfone was studied. The mass spectra of the compounds were matched with the NIST library. The GC-MS analysis of methanol extracts of Ulothrix flacca showed sixteen peaks. Of all the sixteen chemical compounds revealed from the GC-MS analysis of Ulothrix flacca, Dimethyl Sulfone (C2H6O2S) (RT-8.9), 4-Bromobenzoic Acid, 2-Chlorophenyl Ester (C13H8BrClO2) (RT-12.642), Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester (C17H34O2) (RT-18.669) are the three major components. The methanol extracts of Ulothrix flacca possess phenolic and flavonoid content of (5.74 ± 0.45mg Gallic acid equivalent (GAE)/g Wt, and 12.58 ± 1.52mg quercetin eq/g wt) respectively. Antioxidant activity was determined using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical, for evaluating free radicle scavenging activity, ABTS radical cation scavenging activity, Ferric reducing antioxidant power (FRAP) assay, Phosphomolybdenum assay and Metal chelating activity using BHT, Rutin and Quercetin. The highest radicle scavenging activity was shown by dimethyl sulfone (15.156mg/ml), which is higher than the BHT and Rutin.* In vitro *antioxidant activity of methanolic extract of Ulothrix flacca and Dimethyl sulfone showed an increase with increasing concentration indicating positive association with the total phenolic and flavonoid contents of the extract, which could be considered for future applications in medicine, dietary supplements ,cosmetics or food industries.*

Keywords:*GC-MS analysis, Antioxidant assays, Ulothrixflacca, Dimethyl Sulfone.*

I. Introduction

Ulothrix flacca belongs to the family Ulothricaceae. The thallus is with unbranched filaments having single line of cells (uniseriate) to 5-7cm (2.5 inch) in length. The filaments generally forms in a thick mass and appear dark green on the rocks. The cells possess a single nucleus, and although narrow (15-35 m diameter), they are shorter (6-16 m long) than wide. The complete length of the cell is filled with collar shaped chloroplast. Reproductive cells appear swollenin the middle to 50 µm in diameter[1]. These species forms a very slippery coating on rocks in the mid to high intertidal zone in protected to semi-exposed areas in early summer.

During normal metabolism and energy production in the body, Oxygen derived free radicals or reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) includingsuperoxide (O_2), hypochlorous acid (HOCl), hydroxyl (.OH), nitrous acid (HNO_2), Peroxyl (ROO.), hydrogen peroxide (H_2O_2), nitroxide (NO.), peroxy nitrite (OONO.) and dinitrogen trioxide (N_2O_3) are generated[2]. Their production helps the normal healthy tissues perform physiological roles such as molecular signalling, signal transduction regulation, gene expression, activation of receptor mechanism and nuclear transduction among others[3]. When these ROS or RNS are in higher concentrations beyond the actual antioxidant capacity of a biological system, due to some metabolic and other environmental factors; such condition gives rise to an imbalance state known as oxidative or nitrosative stress; a situation that mediates damage to the biological molecules such as proteins, lipids, DNA and polysaccharides[3]. In the past decade, free radicles were implicated by showing the relevance of anti-oxidants in preventing various diseases[4]. The presence of antioxidants like flavonoids, phenolics, proanthocyanidins and tannins in plants and seaweeds may provide protection against a number of diseases; for example, the mortality from degenerative disorders has been inversely proportional to the ingestion of natural antioxidants [5].

Seaweeds are therefore being investigated for their antioxidant properties, and the demand for such natural antioxidants and food preservatives is increasing [6]. The oxidation processes of all living organisms are very intrinsic in the energy management, and are, therefore, should be kept under strict control by several cellular mechanisms [7]. Several types of extracts obtained from seaweeds have received special attention due to their potent pharmaceutical activities, including anticancer, immune-stimulant and antioxidant activities [8]. Seaweeds contain high amounts of polyphenols. For example, in the seaweed *Halimada* (Chlorophyceae), high

concentrations of polyphenols such as epicatechin, catechin, gallate, gallic acid and epigalloctechin are reported [9]. Algae generally has higher antioxidant activity due to a higher contents of nonenzymatic antioxidant components, such as ascorbic acid, phenols, reduced glutathione and flavonoids [10].

Dose dependent alterations were observed in Total Antioxidant Capacity, Total Oxidative stress levels and Nuclear Divison Index rates of *Ulothrixtenuissima* (Kurtz.). It was found to be non-genotoxic and caused sterility at higher concentrations due to oxidative stress [11]. However, in this seaweed, till date there are no detailed studies concerning the active components reported.Therefore,by using Gas Chromatography-Mass Spectrometry (GCMS) analysis, the present study was conducted to analyze the phytochemicals of unexplored seaweed, *Ulothrix flacca*. Thus the present study aimed to determine the Antioxidant potential with determination of its total phenolic and flavonoid contents, and GCMS analysis was performed for the identification of the phytochemical constituents.

II. Materials And Methods

2.1 Chemicals and Reagents

All the analytical grade solvents and chemicals were obtained from Himedia chemicals, Mumbai, India. 2, 2 - azinobis 3-ethylbenzothiozolin-6-sulphonate (ABTS) was obtained from Sigma chemicals, USA. The other chemicals used were Gallic acid, quercetin, Rutin, 2,2- diphenylpicryl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), Ethylene diamine tetra acetic acid (EDTA), Trolox, Potassium persulphate.

2.2 Seaweed Collection

Ulothrix flacca is the green seeweed collected during the months of July and August, from the rocky platforms at Tenneti Park located at Vishakhapatnam coastal banks, Andhra Pradesh, India. Species identification was done by referring the printed journal (Y. Sarojini, P. Santharao, B. Sujatha K. Lakshminarayana. Distribution and diversity of marine macro algae in relation to environmental factors at Visakhapatnam coast. Seaweed Res. Utiln., 35 (1&2): 55-64, 2013 and authentified at Department of Botany, Andhra University, Visakhapatnam.

2.3 Methanol Extract Preparation

Seaweed sample was cleaned of epiphytes and extraneous matter, and necrotic parts were removed. Seaweeds were washed with seawater and then in fresh water. The seaweed was transported to the laboratory in sterile polythene bags at 0°C temperature. In the laboratory, sample was rinsed with sterile distilled water and were shade dried, cut into small pieces and powdered in a mixer grinder. The powdered sample was extracted in methanol thrice by soaking for overnight at room temperature. The extract from three consecutive soakings was pooled and evaporated under reduced pressure. The residue (crude extract) obtained was finally dried under vacuum pressure.

2.4 GC-MS Analysis

The seaweed *Ulothrix flacca*was subjected to GC-MS analysis for its structural characterization. GC-MS analysis was carried out using Clarus 600 gas chromatograph system. It is equipped with Claru600C mass spectrometer (PerkinElmer, USA). For GC–MS, an Elite-5MS fused silica capillary column coated with a 5% diphenyl/95% dimethyl polysiloxane stationary phase (60 m × 0.25 mm, film thickness 0.10 m; PerkinElmer precisely, USA) was used. For a total run time of 30 min, the injector temperature was kept at 200 °C whereas the oven temperature was programmed from 70 °C to 300 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. An appropriate blank was run from which the solvent delay was fixed to 4 min. The electron ionization mode with ion source temperature of 200 °C; ionization energy of 70 eV; scan interval of 0.2 s, GC interface temperature of 240 °C and fragments range from 50 to 600 m/z were set for the MS analysis. About 1 µL of the extract was injected manually in a split less mode.

2.5 Identification of Phytoconstituents

Using the database of National Institute Standard and Technology (NIST) having huge data with their fragmentation pattern, Interpretation on Mass-Spectrum of GC-MS was conducted. The spectrum of known components stored in the NIST library was used to compare the spectrum and fragmentation pattern of the unknown compounds. The name, structure and molecular weight of the components of the test materials were determined.

2.6 Determination of Total Phenolic Content

The level of total phenols in the crude extract of *Ulothrix flacca*was determined by using Folin–Ciocalteu reagent and external calibration with gallic acid. In this method 200 µL of seaweed extract was taken into a series of test tubes and made up to 1 mL with distilled water. Then, 500 µL of Folin – Ciocalteau Phenol reagent (1N) was added to all the test tubes including the blank and mixed thoroughly [12]. After 5 minutes, 2.5

mL of sodium carbonate solution (5%) was added to all the test tubes. All the test tubes were vortexed well to mix the contents and incubated in dark for 40 minutes. The formation of blue color in the incubated test tubes indicated the presence of phenolics. After incubation the absorbance was read at 725 nm against the reagent blank (distilled water) using spectrophotometer (Thermo Fisher Scientific, model 4001/4). The concentration of the total phenolics was calculated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve and the results were expressed as Gallic acid equivalents (GAE). The determination of total phenolic compounds in the fractions was carried out in triplicate and the results were averaged.

2.7 Determination of Total Flavonoid Content

Total flavonoid contents of *Ulothrixflacca*was determined according to the method described by Zhishen*et al.*,[13]. About 500 µL of seaweed extract was taken in series of test tubes and 2 mL of distilled water was added to each test tube. Then, 150 µL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation, 150 µL of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2 mL of 4% NaOH was added to all the test tubes and were made up to 5 mL using distilled water. After vortexing the contents in all the test tubes, they were allowed to stand for 15 minutes at room temperature. The development of pink color due to the presence of flavonoids was read spectrophotometrically at 510 nm against blank (distilled water). The total flavonoid compounds determination in the fractions was carried out in triplicate and the results were averaged. The results were expressed in Rutin equivalents (RE).

2.8 DPPH Radical Scavenging Activity

According to the method described by Braca*et al.*, the antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH[14]. Seaweed Sample and Dimethyl sulfone were taken with varying concentrations and the volume was adjusted to 100 µL with methanol. About 3 mL of a 0.1 mMmethanolic solution of $DPPH^{\bullet}$ was added to the aliquots of samples and standards (BHT and Rutin) was vortexed well. Negative control was prepared by adding 100 µL of methanol in 3 mL of 0.1 mMmethanolic solution $DPPH^{\bullet}$. The tubes were allowed to stand in dark for 15 minutes at room temperature. The absorbance of the samples were measured at 517 nm against the blank. Radical scavenging activity of the samples were expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of $DPPH^{\bullet}$ concentration. The antioxidant capacity was calculated using the following equation:

% Inhibition = $(A_{cotrol} - (A_{sample} - A_{blank}) / A_{cotrol}) \times 100$

Where the A_{sample} is the absorbance of the test sample (the sample test and DPPH solution), A_{cotrol} is the absorbance of the control (DPPH without sample), and the A_{blank} is the absorbance of the sample blank (Sample without the DPPH solution). Ascorbic acid was used as the positive control. The IC_{50} values were calculated by linear regression analysis and expressed as mean of three determinations.

2.9 ABTS Radical Cation Scavenging Activity

The total antioxidant activity of the samples were measured by ABTS radical cation decolourization assay according to the method described by Re *et al.,* [15]. 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) $ABTS^{\bullet+}$ was produced upon the reaction of 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 hours at room temperature. Prior to assay, this solution was diluted with methanol (about 1:89 v/v) and equilibrated at 30°C to produce an absorbance of 0.700 ± 0.02 at 734 nm. After the addition of 1 mL of diluted $ABTS^{\bullet+}$solution to 10 µL of the seaweed extract and Dimethyl sulfone all the test tubes were vortexed well and incubated in dark for 30 min at room temperature. Triplicate determinations were made at each dilution of the standards (BHT and Rutin), seaweed extract and Dimethyl sulfone and the absorbance was read against the blank at 734 nm. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µM TE/g of seaweed extract.

2.10 Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacities of samples were estimated according to the procedure described by Pulido *et al.* [16]. FRAP reagent (900 µL), prepared freshly and incubated at 37°C for 30 min, was mixed with 90 µL of distilled water and 30 µL of test samples or blank. The test samples and reagent blank were incubated at 37°C for 30 minutes in a water bath. The final dilution of the test samples in the reaction mixtures was 1/34. The FRAP reagent was prepared by mixing 2.5 mL of 20 mM $FeCl_3$. $6H_2O$, 2.5 mL of 20 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mMHCl and 25 mL of 0.3 M acetate buffer (pH-3.6). At the end of incubation, the absorbance readings were taken immediately at 593 nm against the reagent blank, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2000 µM, ($FeSO_4.7H_2O$) were used for the preparation of the calibration curve. Equivalent Concentration (EC) was defined as the concentration of antioxidant having a ferric-Tris(2-pyridyl)-s-triazine reducing ability equivalent to that of 1 mM $FeSO_4.7H_2O$.

EC was calculated as the concentration of the antioxidant giving an absorbance increase in the FRAP assay equivalent to that of the theoretical absorbance value of a 1 mM concentration of Fe (II) solution. Rutin and Quercetin were used as standards.

2.11 Phosphomolybdenum Assay

The antioxidant activities of extracts were evaluated by the formation of green phosphomolybdenum complex, according to the method of Prieto *et al.* [17]. About 100µL aliquots of the seaweed extract and dimethyl sulfone were added with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered with foil and then incubated in a water bath at 95°C for 90 minutes. The absorbance of the mixtures were measured at 695 nm against the reagent blank, after the samples had cooled to room temperature. The results were reported by means of mean value expressed as mg AAE /g extract. Rutin and Quercetin were used as reference standards.

2.12 Metal Chelating Activity

The ability of *Ulothrix flacca* seaweed extract and Dimethyl sulfone to chelate the ferrous ions were estimated by the method described by Dinis *et al.*, [18] with slight modifications. Initially, about 50µL of the extracts and BHT (Butylated hydroxyl toluene) were added to 100 µL solution of 2 mM $FeCl_2$. The reaction was initiated by the addition of 400 µL of 5 mMferrozine and the mixtures were made up to 3 mL using deionized water. The contents were shaken vigorously and left at room temperature for 10 minutes. Absorbance of the solutions were then measured spectrophotometrically at 562 nm against the blank. The percentage inhibition of ferrozine–Fe 2+ complex formation was calculated as [(A0- As)/ As] × 100, where As was the absorbance of the extract/ standard and A0 was the absorbance of the control. Na_2EDTA was used as positive control. EDTA was used as the standard metal chelating agent and the results were expressed in mg EDTA equivalents/ g extract.

Statistical Analyses

The experimental results were mean ± S.D. of three measurements and analyzed by SPSS 10 (SPSS Inc. Chicago, IL). Correlations were obtained by the Pearson correlation coefficient in bivariate correlations and *P* values <0.05 were regarded as significant.

III. Results

3.1 GC-MS analysis of *Ulothrix flacca* methanol extract

Gas Chromatography Mass Spectroscopy (GC-MS) was performed to identify the active constituents from the seaweed extract. The compounds identified are listed in Table 1.

Totally sixteen compounds were identified in methanolic extract of *Ulothrix flacca*extract by GC-MS analysis. The chromatogram is obtained by methanolic fraction of *Ulothrix flacca*. Name of the natural compound, retention time, molecular formula and molecular weight and area percentage were presented in table 7. The prevailing compounds were Dimethyl Sulfone, Phenol, 2-Nitro-, Benzaldehyde, 3-Bromo-, 4-Bromobenzoic Acid, 2-Chlorophenyl Ester, Benzoic Acid, 3-Bromo-, 2-Bromoethyl Ester, 3-Buten-2-One, 4-(2-Bromophenyl)-, Tetradecanoic Acid, Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester, Hentriacontane, Methanone, (4-Aminophenyl)(4-Bromophenyl)-, 2,5-Cyclohexadiene-1,4-Dione, 2,5-Diphenyl, Naphthalene, 2-Phenyl-, 2-Bromobenzoic Acid, 2,4,5-Trichlorophenyl Ester, Coumarin, 4-[1-(4-Nitrophenyl)-3-Oxopentylthio]-, Ergotaman-3',6',18-Trione, 9,10-Dihydro-12'-Hydroxy-2'-Methyl-5'-(Phenylmethyl, Acethydrazide, 2,2,2-Trifluoro-N2-(4-Bromobenzoyl)-.

The molecular structures, nature and phytochemical activities of these compounds are tabulated in the Table 8. The GC-MS spectrum of *Ulothrix flacca*methanol extract is shown in the fig 1.

3.2 Total phenolic content

The total phenolic content of *Ulothrix flacca* extractwas determined by Folin–Ciocalteu method are reported as Gallic acid equivalents (Table 3). *Ulothrix flacca* methanol extract was containing 5.74% amount of phenolic compounds. The content of total phenolic was carried out based on the absorbance values of the various extract solutions, reacted with Folin–Ciocalteu reagent and compared with the standard solutions of Gallic equivalents as described above.

3.3 Total flavonoid content

The total flavonoid contents of *Ulothrix flacca* extractwas determined by aluminium chloride colorimetric method are reported as quercetin equivalents. *Ulothrix flacca* methanol extract was containing 12.58% amount of flavonoid compounds (Table 3).

3.4 DPPH scavenging activity

The ability of the sample to stabilize the DPPH radical by donating the electron, for this study a relatively unstable free radical, DPPH (1, 1-diphenyl, 2-picryl hydrazyl) was selected for the *invitro* assay method. DPPH was used as a substrate to evaluate the antioxidant activity of natural products from plants and microbial sources. The results were depicted in Fig 1.

From the figure 1, it was evident that DPPH scavenging activity was increased with increase in concentration of the extract. The 50% scavenging ability of the extract*Ulothrixflacca*and dimethyl sulfone were found to be at 9µg/mL and 15µg/mL respectively. And it was found that *Ulothrix flacca* is having comparatively equal inhibitory concentrations exerted by standard Rutin (Table 4).

3.5 ABTS assay

The ABTS assay is based on the inhibition of absorbance of the radical cation $ABTS^{+}$, that has a long characteristic wavelength absorption spectrum. The efficiency of ABTS radical scavenging potential of *Ulothrix flacca* seaweed extract and Dimethyl sulfone were estimated to identify the total reducing potential and were found to be 10905.6±159.73 µM TE/g of extract and 12755.3±185.52µM TE/g respectively and ABTS radical scavenging potential of *Ulothrix flacca* seaweed extract was found to be almost similar to the reducing potential of the natural antioxidant BHT and greater than that of Rutin. The ability of reducing capacity by standard, Dimethyl sulfone as well as the seaweed extract are described in the Table 4.

3.6 Ferric reducing antioxidant power assay (FRAP)

The ferric reducing ability of *Ulothrix flacca* seaweed extract and Dimethyl sulfone were estimated based on their ability to reduce TPTZ-Fe (III) complex to TPTZ- Fe (II). The reducing ability of the methanolic extract of*Ulothrix flacca*and Dimethyl sulfone by quenching the ions were compared with Rutin and Quercetin standards and the potentials were found to be 11.577±1.131mM Fe(II)E/mg extract and 18.157±1.056mM Fe(II)E/mg respectively. And the concentrations of antioxidant in the samples are given in the Table 4.

3.7 Phosphomolybdenum assay

The Phosphomolybdenum reduction potential (PMRP) of the seaweed extract refers to the ability of the extract to reduce phosphomolybdenum VI to phosphomolybdenum V, this reduction in turn reflects the reducing capacity of the methanol extract of *Ulothrix flacca*and Dimethyl sulfone. Among the varying concentrations of the seaweed extract *Ulothrix flacca*(60.66mg AAE/g extract) and Dimethyl sulfone(71.45±2.25 mg AAE/g), Dimethyl sulfone showed better reduction potential. Which were comparable with the value of natural antioxidants Rutin and Quercetin. Thus, the total antioxidant capacity observed for the extract of *Ulothrix flacca*and Dimethyl sulfone were correlated with its free radical scavenging activity equivalent to that of natural antioxidant ascorbic acid. The reducing potential of the samples were represented in the Table 4.

3.8 Metal chelating activity

The chelating effect on the ferrous ions by the methanol extract of seaweed*Ulothrix flacca*and Dimethyl sulfone were found to be 13.55mg EDTA/g of extract and 11.155±0.114mg EDTA/gm respectively. The chelating activities of samples were represented in Table 4.

IV. Discussion

Dimethyl sulfone or Methylsulfonylmethane (MSM) is the major component identified under the GC-MS analysis of *Ulothrix flacca*. It is an organic sulfur-containing natural compound having no reported toxicity. Dimethyl sulfone contains only eleven atoms and is found in foods, including fruits, vegetables, grains, and beverages [19]. It is a symmetric molecule with no isomeric forms. Dimethyl sulfone has also been detected in the human brain [20], blood plasma, and cerebrospinal fluid [21] by proton magnetic resonance spectroscopy. Dimethyl sulfone is volatile, easily lost during cooking, and is believed to be non-toxic [22], [23]. Dimethyl sulfone decreases arthritis pain and improves physical function of osteoarthritis human knees without major adverse events [24]. This compound has also been found to be effective for treating allergies [25], osteoarthritis pain [26], inflammation [27], repetitive stress injuries [28], and bladder disorders like intestinal cystitis [29]. Dimethyl sulfone can induce wound healing, contact inhibition, and can block the ability of cells to migrate through the extra-cellular matrix. Furthermore, in arbors seen in murine melanoma cell lines, Dimethyl sulfone can restore anchorage-dependent growth and irreversible senescence followed by arborization with melanosomes[30]. It inhibited the proliferation of HER2-positive and estrogen receptor-positive breast cancer cells in a dose-dependent manner [31].

2-Nitrophenol is a water soluble, yellow, crystalline material, moderately toxic, low melting point (45° C). When heated to decomposition 2-Nitrophenol emits toxic fumes of oxides of nitrogen. In molten form violent reaction with strong alkali (85 % potassium hydroxide). Thesenitrophenols are blood poisons,but they alsoact onthenervoussystem andirritate theskin and

mucousmembranes, leading to dermatitis and chronic eczema.Themain use of*ortho*-and*para*-nitrophenolsis in the production of *ortho*- and *para*-aminophenols.The permeation of nitrophenols through epidermal cells was studied from newborn rat skin cultured on type IV collagen-coated Millipore filters with positive results[32].

Acethydrazide, 2,2,2-Trifluoro-N2-(4-Bromobenzoyl)- is the chemical component that has been showed up in the seaweed extract. Relative compounds are having Antituberculosis and antiinflammatory activities. Alpha-[5-(5-Nitro-2-furyl)-1,3,4-oxadiazol-2-ylthio]**acethydrazide**, alpha-[5-(5-nitro-2-furyl)-1,3,4-oxadiazol-2 ylthio]acetamide, delta-allyl-1-[(5-(5-nitro-2-furyl)-1,3,4-oxadiazol-2-ylthio]acety) thiosemicarbazide, and other related compounds weresynthesised for testing against Mycobacterium tuberculosis [33]. 2-Methylindole-3-**acethydrazide** was reacted with arylisothiocyanate to give the corresponding 4-arylthiosemicarbazides 2a-d.

Ergotaman-3',6',18- Trione, 9,10- Dihydro-12'- Hydroxy-2'- Methyl-5' (Phenylmethyl), is the chemical compound that was identified in the GC-MS analysis of *Ulothrix flacca.* Ergotamine damages the peripheral epithelium as well as produces vasoconstriction peripherally. Ergotamine is conducive to vascular stasis, thrombosis, and gangrene in high doses. It can be used to increase uterine contractivity and occasionally as a post-partum to decrease uterine bleeding. Ergotamine continues to be prescribed for migraines. The common name of prescription is Cafergot which is a combination of caffeine and ergotamine. Contraindications include Raynaud's Syndrome, Coronary Artery Disease, Pregnancy, Pruritus, Hepatic disease, Atherosclerosis, Buerger's syndromeand Renal Disease [34].

Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester is the compound that was identified in *Ulothrix flacca.*Tetradecanoic acid or Myristic acid (14 carbon atoms) was discovered in the seed of the tropical tree *Myristicafragrans*, from which its name.It is a saturated fatty acid member of the sub-group called long chain fatty acids (LCFA), from 14 to 18 carbon atoms. It occurs, as glycerol ester, in most animal and vegetable fats and oils. Tetradecanoic acid presence was registered from seven different types of date fruits when investigating their Physical and chemical properties, antioxidant activity, total phenol and mineral profile [35]. The fatty acid composition of date seed oil revealed the presence of five dominant fatty acids, where Myristic acid is one of them [36]. Myristic acid is used as a surfactant, cleansing, opacifying& thickening agent in cosmetics products. Has also emulsifying properties. Mostly all kinds of personal care products including soaps, cleansing creams, lotions, hair conditioners, shaving products contains myristic acid [37]**.**

Coumarin, 4-[1-(4-Nitrophenyl)-3-Oxopentylthio] - is the compound that was identified in *Ulothrix flacca.*Natural coumarins comprise five major subtypes-simple coumarins, linear furanocoumarins, angular furanocoumarins, linear pyranocoumarins, and angular pyranocoumarins. Several natural coumarins exhibit strong antifungal activities and can be examined as potential lead compounds for developing novel antifungal agents [38]. Synthesized coumarin derivatives showed significant anti-inflammatory and anticancer activity, which can be regarded as the promising drug candidates for development of anticancer drugs targeting cancer-related inflammation (Thomas *et. al.,* 2016). According to Singhla and Piplani (2016)[39], a synthesized coumarin related compound displayed significant antiamnesic activity, antioxidant activity in comparison to donepezil and AChE inhibitory activity.

The key role of phenolic and flavonoid compounds in free radical scavenging and/or reducing systems was supported by the data obtained from the total phenolic and flavonoid methods. The way of determination of the level of total phenolics is based on the compounds chemical reducing capacity relative to Gallic acid but not based on absolute measurements of the amounts of phenolic compounds. It is important to note that; there is a positive relationship between antioxidant activity and the amount of phenolic compounds of the crude extracts. The phenolic derivative compounds tend to be the vital antioxidants which are responsible to exhibit scavenging efficiency on the free radicals. Reactive oxygen species are numerous and widely distributed in the plant kingdom [40]. Total phenolic and flavonoid contents of the chlorophyceae member*Ulothrixflacca* showed positive correlations with the DPPH radical scavenging activity. The extract of *Ulothrix flacca*was found to be the less potent scavenger in the tested samples. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants [41]. Ascorbic acid was usedas the reference antioxidant in this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a deep purple color. The DPPH is decolorized when DPPH accepts an electron donated by an antioxidant compound, which can be quantitatively measured from the changes in absorbance. The IC50 values of all the samples have been furnished in the Table 4. Scavenging of DPPH radical was found to rise with increasing concentration of the samples. The redox properties of the phenolic compounds are responsible for the antioxidant activity, which plays an important role in adsorbing and neutralizing the free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [42].

ABTS is a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids and plasma antioxidants. The radical monocation of ABTS [2,2-

azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] is generated by the oxidation of ABTS with potassium persulfate and is reduced in the presence of hydrogen-donating antioxidants. Both the duration of reaction and concentration of antioxidant on the inhibition of the radical cation absorption are taken into consideration when determining the antioxidant activity. This assay improves the original TEAC assay (the ferryl myoglobin/ABTS assay) for determining the antioxidant activity in two ways. First, the chemistry of direct generation of the ABTS radical monocation without the involvement of an intermediary radical. Second, is a decolorization assay; rather than the generation of the radical taking place continually in the presence of the antioxidant, the radical cation is formed prior to addition of antioxidant test systems. In the present study Dimethyl sulfonewas found to have the highest total reducing capacity than the *Ulothrix flacca* extract.

Ferric reducing antioxidant power (FRAP) table 4, shows the reducing power capabilities of the samples compared to ascorbic acid. The samples displayed good reducing power which was found to rise with increasing concentrations of the samples. In reducing power assays, the presence of antioxidants in the samples can reduce the oxidized form of iron (Fe3+) to its reduced form (Fe2+) by donating an electron. So it can be assumed that the presence of reductants (antioxidants) in the chlorophyceae extract and Dimethyl sulfone causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, the Fe2+ complex can be measured by the formation of Perl's Prussian blue at 700 nm. A higher absorbance indicates greater reducing power ability [43]. Significantly Dimethyl sulfone showed better reducing capacity than the *Ulothrix flacca*methanol extract.

The total antioxidant capacity (TAC) depends on the reduction of Mo(VI) to Mo(V) by the sample and subsequent formation of green phosphate/Mo(V) complex at acid pH. It can evaluate both water-soluble and fat-soluble antioxidants. It was observed that the Dimethyl sulfone possesses significant total antioxidant capacity when compared to the seaweed extract and lesser activity to the standards Rutin and Quercetin (table 4).

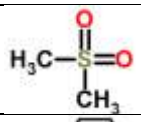
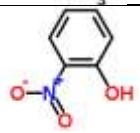
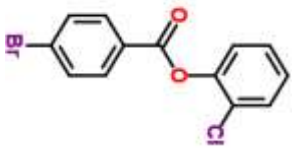
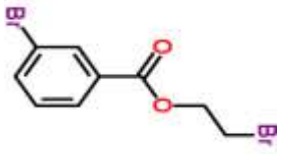
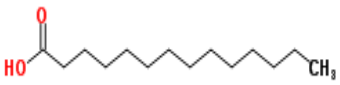
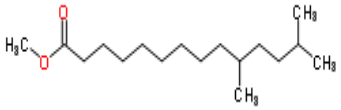
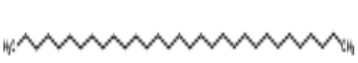
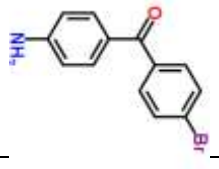
The chelating of Fe^{2+} by extract was estimated by the method of Dinis et al. (1994). Transition metal ions in biological system could catalyse the Haber-Weiss and Fentontype reactions, resulting in the generation of hydroxyl radicals (OH*). These transition metal ions could form chelate with the antioxidants, which result in the suppression of OH* generation, and inhibit the peroxidation process of biological molecules. Ferrozine can quantitatively form complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction allows the estimation of the chelating activity of the coexisting chelator. Fe^{2+}, the transition metal ion possess the ability to move single electrons which can form and propagate many radical reactions, even starting with relatively non-reactive radicals [44]. Dimethyl sulfone, the most active sample followed by *Ulothrix flacca* extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The chelating effect of other extracts on Fe^{2+} and ferrozine complex formation is shown in Table 4.

V. Figures And Tables

TABLE 1: The active compounds in *Ulothrix flacca* methanol extract analyzed by GC-MS analysis

PEAK	R.TIME	NAME OF THE COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT	AREA %
1	8.9	Dimethyl Sulfone	$C_2H_6O_2S$	94	23.46
2	10.626	Phenol, 2-Nitro-	$C_6H_5NO_3$	139	9.59
3	11.541	Benzaldehyde, 3-Bromo-	C-H_5BrO	184	0.1
4	12.642	4-Bromobenzoic Acid, 2-Chlorophenyl Ester	$C_{13}H_8BrClO_2$	310	16.41
5	14.002	Benzoic Acid, 3-Bromo-, 2-Bromoethyl Ester	$C_9H_8Br_2O_2$	306	0.36
6	15.693	3-Buten-2-One, 4-(2-Bromophenyl)-	$C_{10}H_{10}BrNO$	224	1.89
7	16.868	Tetradecanoic Acid	$C_{14}H_{28}O_2$	228	0.59
8	18.669	Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester	$C_{17}H_{34}O_2$	270	14.29
9	22.446	Hentriacontane	$C_{31}H_{64}$	436	0.56
10	22.896	Methanone, (4-Aminophenyl)(4-Bromophenyl)-	$C_{13}H_{10}BrNO$	275	0.84
11	24.256	2,5-Cyclohexadiene-1,4-Dione, 2,5-Diphenyl	$C_{18}H_{12}O_2$	260	6.59
12	24.491	Naphthalene, 2-Phenyl-	$C_{16}H_{12}$	204	2.49
13	24.947	2-Bromobenzoic Acid, 2,4,5-Trichlorophenyl Ester	$C_{13}H_6BrCl_3O_2$	378	0.83
14	25.637	Coumarin, 4-[1-(4-Nitrophenyl)-3-Oxopentylthio]-	$C_{20}H_{17}NO_5S$	383	8.73
15	26.202	Ergotaman-3',6',18-Trione, 9,10-Dihydro-12'-Hydroxy-2'-Methyl-5'-(Phenylmethyl	$C_{34}H_{41}N_5O_8S$	583	0.91
16	27.147	Acethydrazide, 2,2,2-Trifluoro-N2-(4-Bromobenzoyl)-	$C_9H_6BrF_3N_2O_2$	310	12.36

TABLE 2: Molecular structures, nature and their biological activities of the phytochemical compounds of methanol extracts of *Ulothrix flacca* showed by GC-MS analysis.

S NO	COMPOUND NAME	MOLECULAR STRUCTURE	COMPOUND NATURE	ACTIVITY
1	Dimethyl Sulfone		Sulfur compound	Antibiotic, Antiinflammatory , Anticancerous.
2	Phenol, 2-Nitro-		Phenol	Fungicide, Dyes, Rubber chemicals.
3	Benzaldehyde, 3-Bromo-		Aldehyde	Flavouring substance, Cosmetics.
4	4-Bromobenzoic Acid, 2-Chlorophenyl Ester		Ester	Anticancerous, Pesticides, Anticognitive
5	Benzoic Acid, 3-Bromo-, 2-Bromoethyl Ester		Ester	Unknown
6	3-Buten-2-One, 4-(2-Bromophenyl)-		Indole	Unknown
7	Tetradecanoic Acid (Myristic acid)		Fatty acid	Cosmetics
8	Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester		Fatty acid Ester	Cosmetics, Antioxidant, Hypocholesterolemic.
9	Hentriacontane		Hydrocarbon	Diuretic, Antiinflammatory .
10	Methanone, (4-Aminophenyl)(4-Bromophenyl)-		Ketone	Antimicrobial, Insect antifeedant

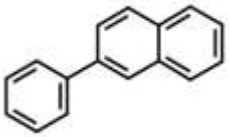
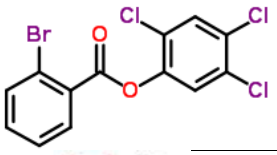
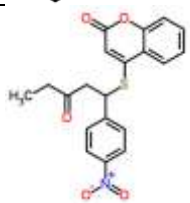
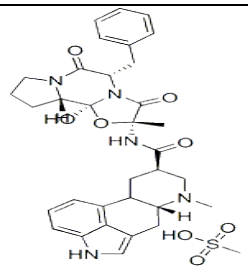
11	2,5-Cyclohexadiene-1,4-Dione, 2,5-Diphenyl		Quinone	Antiproliferative, Immunosuppressive
12	Naphthalene, 2-Phenyl-		Aromatic hydrocarbon	Antineoplastic, Antiinflammatory, Antifertility
13	2-Bromobenzoic Acid, 2,4,5-Trichlorophenyl Ester		Ester	Antioxidant, Cytotoxic.
14	Coumarin, 4-[1-(4-Nitrophenyl)-3-Oxopentylthio]-		Benzopyrone	Anticoagulant, Hepatotoxicity, Perfumes, Edema modifier, Appetite suppressive.
15	Ergotaman-3',6',18-Trione, 9,10-Dihydro-12'-Hydroxy-2'-Methyl-5'-(Phenylmethyl		Alkaloid	Antimigraine, Analgesic, Vasoconstrictor
16	Acethydrazide, 2,2,2-Trifluoro-N2-(4-Bromobenzoyl)-		Hydrazide	Antituberculosis, Antiinflammatory.

TABLE 3: Total phenols and flavonoids content of*Ulothrix flacca* methanolic extract.

Methanol extracts	**Phenolics %(w/w)** mg GAE/g.	**Flavonoids %(w/w)** mg RE/g
Ulothrix flacca	5.74 ± 0.45	12.58 ± 1.52

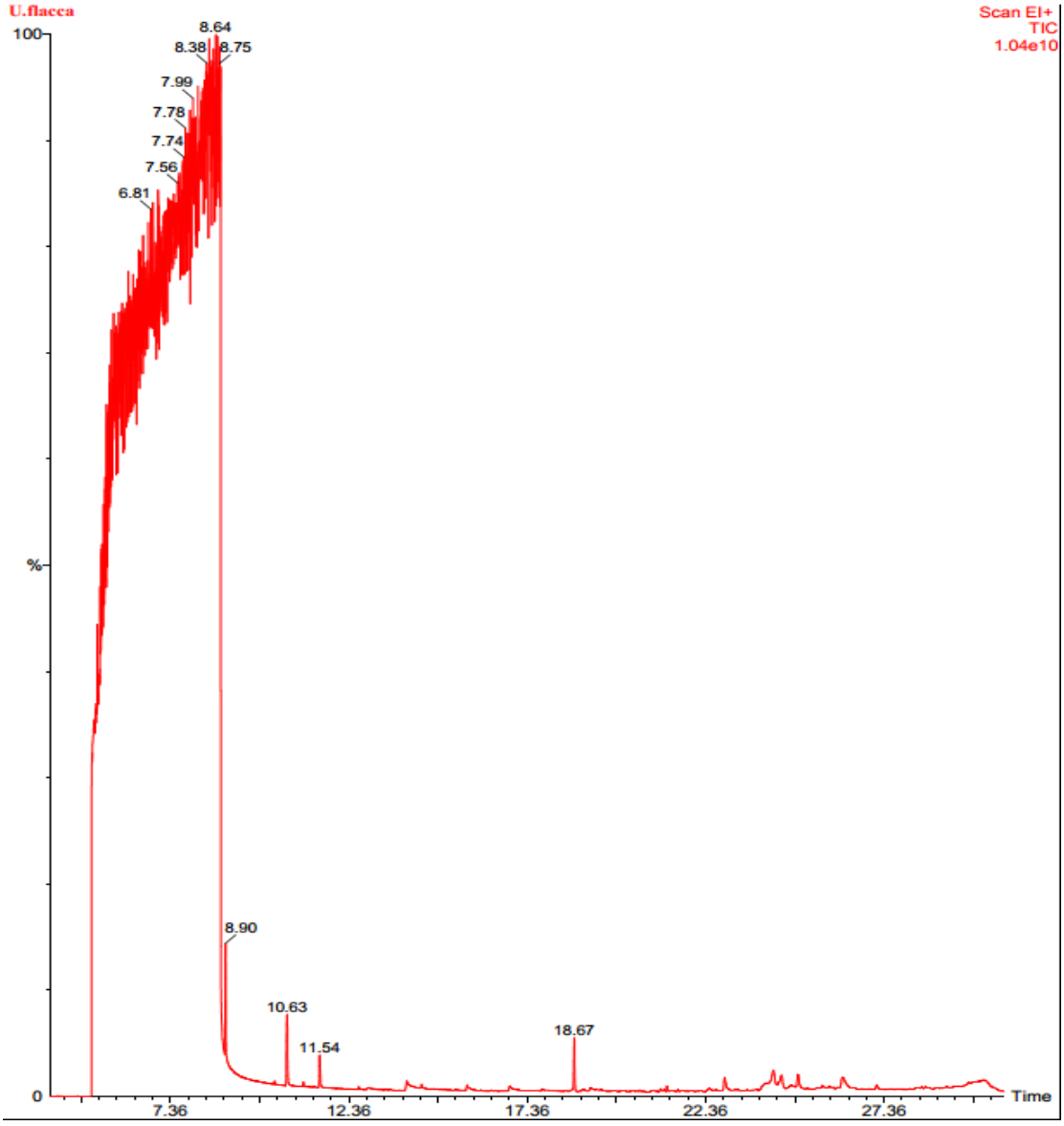
The values are means ± SD of three replicates.

TABLE 4: The table represents the reducing potential of methanol extract of *Ulothrix flacca* and Dimethyl sulfone by DPPH, ABTS, FRAP, PMRP and Fe^{2+} chelating activities.

Methanol Extracts	DPPH Ic50 values (mg/ml)	$ABTS^{+}$ (µM TE/g extract)	FRAP (mM Fe(II)E/mg extract)	PMRP (mg AAE/g extract)	Fe^{2+} chelating activity (mg EDTA/g extract)
Dimethyl sulfone	15.156±0.525	12755.3±185.52	18.157±1.056	71.45±2.25	11.155±0.114
Ulothrix flacca	9.052±1.035	10905.6±159.73	11.577±1.131	60.66±7.68	13.55±1.156
BHT	6.35±1.008	9423.65±66.82	–	–	-
Rutin	9.73±0.56	8942.79±119.76	109.28±5.29	397.18±5.39	-
Quercetin	-	-	287.57±86.53	457.52±9.53	-
EDTA	-	-	-	-	25.562±0.895

Values are mean of triplicate determination (n=3) ± standard deviation
TE – Trolox Equivalents; AAE – Ascorbic Acid Equivalents; Fe(II)E - Fe(II) Equivalents

Fig 1: GC-MS chromatogram of *Ulothrix flacca* methanol extract.



VI. Conclusion

In conclusion, the results of this study suggest that the methanolic extracts of *Ulothrix flacca* have strong anti-oxidative effects which might be contributed by some major bioactive active compounds such as Dimethyl sulfone, Acethydrazide, 2,2,2-Trifluoro-N2-(4-Bromobenzoyl), Coumarin, 4-[1-(4-Nitrophenyl)-3-

Oxopentylthio]-, Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester , 4-Bromobenzoic Acid, 2-Chlorophenyl Ester and phenol 2-nitro. The anti-oxidative effects of the dimethyl sulfone were better than the methanolic extract which can be used to investigate the in vivo anti-oxidative and anti-cancerous effects in the further studies. Additionally, findings of this study further support the use of the *Ulothrix flacca* methanol extracts as an anti-oxidative medicine.

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