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Metabolite Profiling of *Scenedesmus dimorphus* Grown on Dairy Wastewater and their Antioxidant and Anti-Diabetic Activity

MAHADEVI NARASANAGI[1], LINGAYYA HIREMATH[1*], SANGAPPA SHIVANADAPPA[1], NARENDRA KUMAR[1],

[1] Department of Biotechnology, R V College of Engineering, Bangalore, Karnataka, India

[*]Address For correspondence
Assistant Professor
Department of Biotechnology
R.V. College Engineering, Bangalore -560059
Phone no: 09886699277
Email: lingayah@rvce.edu.in

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ABSTRACT

In the present study, metabolites of *Scenedesmus dimorphus* grown in protease peptone medium containing dairy wastewater were compared with metabolites of control medium and their biological activity were assessed. *S. dimorphus* ($1.2x10^7$cells/ml) was grown in protease peptone medium (pH 7.0) containing 50% dairy wastewater and incubated at 20ºC with 12/12 hours Light/Dark at 15000lux intensity of light source for 28 days. After sufficient growth, the algal mat was recovered and extracted. Ethanol extract of *S. dimorphus* grown with 50% dairy wastewater indicated the presence of 11 bioactive compounds including two rare metabolites found at highest concentration which didn't match with any metabolites of GCMS library. Control extract of *S. dimorphus* revealed presence of 6-Octadecenoic acid (Z)- (28.6%) and Octadecanoic acid, 2-hydroxy-1,3-propanedi (26.85%). These bioactive molecules have been reported for antimicrobial, antioxidant, antidiarrheal, anti-inflammatory, anticancer, analgesic, anxiolytic, and anti-larvicidal properties. Further, the *S. dimorphus* extract showed significant anti-diabetic activity with an IC_{50} value of 177.8µg/ml versus the IC_{50} value of 77.9µg/ml of the metformin. The extract also revealed significant antioxidant activity with IC_{50} value of 68.3% versus 41.2% of ascorbic acid. Hence, *S. dimorphus* grown on dairy wastewater act as an ideal resource for the synthesis of various bioactive metabolites with several nutraceutical applications.

Keywords: *Scenodesmus dimorphus*, Dairy wastewater, Gas Chromatography-Mass Spectrometry, Nutraceuticals, anti-diabetic, antioxidant

INTRODUCTION

Freshwater unicellular microalgae *Scenodesmus dimorphus* belong to the class Chlorophyceae drawn considerable attention due to their possible use as a potential alternative to plant-origin biofuel, petroleum, and their products. Although *S. dimorphus* was formerly reported for the production of biodiesel as they contain high lipid content (34 and 35%), recently they are explored in several fields of science such as medicine, pharmaceutical, dairy, food industry, and nutraceuticals (Pushpakumari *et al.*, 2018). In addition to their lipid content, *S. dimorphus* also contains antioxidant pigments *viz.*, phycobiliprotein, astaxanthin, beta-carotene, lutein, insoluble fiber, beta-glycan, proteins, carbohydrates, vitamins, and minerals which are the main basis of utilizing the microalgae in arenas of the scientific world (Armaini *et al.*, 2020). Therefore, bioactive components purified from the *S. dimorphus* are explored as therapeutic molecules for the treatment of medically important diseases.

The active components of *S. dimorphus* play a significant role in nutraceuticals, food supplements, and the pharmaceutical industry. Molecules like beta-glucan help in the reduction of blood lipid through their antioxidant property and it is also an immunostimulator (Chu *et al.*, 2010). Pigments, vitamins, proteins, minerals, and lipid components *viz.*, α-linolenic acid, docosa hexanoic acid, eicosa pentanoic acid, poly-unsaturated fatty acid (PUFA), Omega-3, and Omega-6 fatty acids play important role in promoting lipid metabolism and reduction of obesity (Arun *et al.*, 2015; Chen *et al.*, 2017). As witnessed by improvement in RBCs, reticulocytes, haematocrit values, and haemoglobin in anemia mice, Aplastic anemia-associated bone marrow damage can be repaired using *S. dimorphus* as nutraceuticals (Armaini *et al.*, 2018).

The biosynthesis of cell components of *S. dimorphus* fluctuates based on the growth media on which the microalgae is cultivated. To achieve considerable biomass using economically feasible technique and to obtain diverse metabolites with wide applications, several industrial

wastes were exploited for the growth of *S. dimorphus*. Algal biofuel production, total nitrogen, and phosphorus removal were increased when *S. dimorphus* was cultivated in domestic secondary effluents compared to the synthetic BG11 medium (Zhang *et al.,* 2015). Likewise, owing to the high organic and easy availability, dairy wastewater was extensively explored for the cultivation of *S. dimorphus* for the production of biomass, biofuels, and other products. Several microalgae species such as *Scenedesmus abundans*, *Acutodesmus dimorphus*, *Chlorella pyrenoidosa*, *C. vulgaris*, *Anabaena ambigua,* and *Chlamydomonas reinhardtii* is have been cultured successfully in dairy wastewater. Using these microalgae several industrially important metabolites and high-value-added products that are explored in health supplements, animal feeds, pharmaceuticals, biofuels, cosmetics, and recombinant enzymes are produced (Gramegna *et al.*, 2020). Although, *S. dimorphus* has wide applications is various arena of science it has less explored for nutraceutical applications. Hence, in the present research metabolites of *S. dimorphus* stimulated by dairy waste were studies for their nutraceutical applications.

The current study focuses on the cultivation of *S. dimorphus* on protease peptone medium containing 50% dairy wastewater in a novel photobioreactor. The biomass of the microalgae recovered, dried, and crude metabolites were extracted. A phytochemical analysis of crude extract was conducted. GCMS results of crude extract of *S. dimorphus* grown on medium dairy wastewater were compared with the control medium containing no dairy wastewater. Finally, the antioxidant and anti-diabetic potential of the crude extract of *S. dimorphus* was determined.

MATERIALS AND METHODS

Chemicals and reagents: Ethyl acetate/Ethanol, Conc.Hydrochloric acid (HCl), Ferric chloride ($FeCl_3$), Ammonia (NH_3) solution, Mayer's reagent, Conc. Sulphuric acid (H_2SO_4), Acetic anhydride, Chloroform ($CHCL_3$), Lead Acetate, Fehling's solution A and B, and 2,2-

diphenyl-1-picrylhydrazyl (DPPH), Metformin, Amylase, Starch, 3,5-Dinitrosalicylic acid (DNS), and Ascorbic acid were supplied by Sigma Aldrich.

Algal culture: A pure culture of *Scenedesmus dimorphus* with UTEX number 1237 was purchased from the Culture Collection of Algae, University of Texas Austin, USA.

Microbial Media: Protease peptone medium, Nutrient agar medium, and Muller Hinton Agar were purchased from Hi Media, Bangalore.

Dairy waste: With prior permission, in a sterile container dairy waste was collected from Bengaluru dairy.

Cultivation of *S. dimorphus* in a novel photo-bioreactor

The pure culture of *S. dimorphus* was sub-cultured on a sterile protease peptone agar medium. The medium was incubated for 4 weeks at 20ºC in a BOD (Biochemical Oxygen Demand) incubator with a 12/12 hours ratio of Light and Dark (L/D) at 3200 lux intensity of the light source. Once sufficient growth is noticed, seed culture was prepared by inoculating the fresh culture of *S. dimorphus* in 100ml of sterile protease peptone broth (pH 7.0) containing 50% dairy waste and inoculating with $1.2x10^7$cells/ml. The medium was incubated at 20ºC for 28 days at 15000lux intensity of the light source. After the incubation period, the mass culture of *S. dimorphus* was carried out in a specially designed novel photo-bioreactor containing 2.5liters of sterile protease peptone medium (pH 7.0). The medium in photo-bioreactor was added with 50% of dairy waste and inoculated with *S. dimorphus* inoculum containing $1.2x10^7$ cells/ml and incubated at 20ºC with 12/12 hours L/D at 15000lux intensity of the light source. The medium was incubated for 28 days to attain sufficient growth after which it is harvested.

Biomass recovery and extraction

Followed by the cultivation of biomass, the microalgae were recovered by filtration of the growth medium. The wet biomass of *S. dimorphus* was air-dried and used for successive solvent extraction using different organic solvents. The algal mat was made into powder using

a grinder mixer and the powder was successively extracted overnight in different solvents such as petroleum ether, chloroform, ethyl acetate, and ethanol to recover complete algal metabolites. All extracts were dried at room temperature by evaporating solvents and used for biological and antioxidant activity. *S. dimorphus* algal powder separated and extracted with ethanol overnight was used for chemical investigation and GC-MS analysis.

Chemical investigation of ethanol extract of *S. dimorphus*

Ethanol extract of *S. dimorphus* was used for chemical analysis of various bioactive components present in the microalgae (Kausar *et al.*, 2021).

Detection of Alkaloids

In 1ml of ethanol extract and a few drops of 0.1N HCl were added to form an aqueous layer. To the aqueous layer decanted, two drops of Mayer's reagent was added and the formation of white turbidity followed by precipitation indicates the presence of alkaloids. The addition of Wagner's reagent to the extract results in the reddish brown precipitate also indicates the presence of alkaloids.

Detection of flavonoids

In a test tube 1ml of algal extract was taken and a few drops of 10% ammonium hydroxide solution were added. The solution was mixed and the appearance of yellow colour indicates the presence of flavonoids. The 1ml extract treated with a few drops of 10% lead acetate solution showed the formation of a yellow colour precipitate which indicated the presence of flavonoids.

Test for tannins

Braymer's test: In 10ml of distilled water around 0.5g of algal powder was boiled and the solution was cooled down and filtered through filter paper. To 1ml of filtrate, 3ml of distilled water was added and 3 drops of 0.1% $ZeCl_3$ were added. The formation of brownish-green or blue-black reveals the presence of tannins. Gelatine test: Extract was dissolved in 5ml of

distilled water and 1% gelatin solution was added. To this mixture, 10% NaCl was added and the formation of a white precipitate indicates the presence of tannins.

Detection of phenols

Phenols in the extract were detected using the $FeCl_3$ test in which to the aqueous algal extract few drops of 5% $FeCl_3$ solution were added. Dark green or bluish-black colour formation indicates a positive test for phenols. In the lead acetate test, the extract was dissolved in 5ml of distilled water and 3ml of 10% lead acetate was added and the formation of a white precipitate shows the presence of phenols.

Test for carbohydrates

In 5ml of distilled water, 100mg of algal extract was dissolved and the filtrate was collected. To 1ml of filtrate 1ml of Fehling's solution A & B were added and boiled in a water bath. The formation of red precipitate shows a positive test. In Benedict's test, 0.5ml of Benedict's reagent was added to the 0.5ml of filtrate, and the mixture was boiled for 2 minutes. The formation of green colour indicates presence of carbohydrates.

Detection of terpenoids

Ethanol extract and $CHCl_3$ were taken in a container and mixed thoroughly and filtered. To the filtrate, a few drops of conc. H_2SO_4 was added and mixed thoroughly. The solution was allowed stand. The formation of a golden yellow colour ring at the bottom indicates the presence of terpenoids.

Test for oils and fats

A small quantity of algal extract was pressed between the filter paper. The formation of oil stain on the filter paper shows the presence of oils and fats.

Detection of proteins

Biuret method: to 2ml of algal filtrate, one drop of 2% copper sulphate solution was added and 1ml of 90% ethanol. To this solution, a few KOH pellets were added and the formation of red colour in the ethanol layer indicated the presence of protein.

Test for saponins

Foam test: in container, 0.5mg of algal extract was taken and 5ml of distilled water was added. The mixture was vigorously shaken and solution was left at room temperature for 15 minutes. The persistence of foam for 10 minutes revealed the presence of saponins**.**

Test for Terpenoids

Salkowski's test: in a test tube 0.2ml extract was taken, 2ml of chloroform was added followed by the addition of 3ml of Conc. H_2SO_4 from the side of test tube to form a layer. The formation of a red colour interface showed the presence of terpenoids.

GC-MS analysis of ethanol extract of *S. dimorphus*

Ethanol extract of *Scenedesmus dimorphus* was analyzed by using Perkin Elmer Turbo Mass Spectrophotometer (Clarus^{TM} SQ 8GCMS) that was accompanied by XLGC Perkin Elmer autosampler. GCMS utilizes the capillary column RTx5MS measuring 30m x 0.25mm ID and 0.25µm df. Helium was used as carrier gas at a flow rate of 1ml/minute. About 2ml of the test sample was injected in split mode using above-said column in the instrument. The initial temperature was set at 100ºC for 1 minute and then gradually temperature was increased to 300^0C for 20 minutes. The sample injected was run for the total run time of 40 minutes. Analysis was carried out the frequency of 2 scan/sec by mass spectra with a scanning range of 40-850m/z. GCMS spectra so obtained were compared with stored spectra of the NIST (Nationals Institute of Standards and Technology) library database. Based on the peak of the sample that matched with the peak of the NIST library the metabolites present in the ethanol extract of *S. dimorphus* were identified.

Biological activity of ethanol extract of *S. dimorphus*

Anti-diabetic activity of ethanol extract of *S. dimorphus*

Ethanol extract of *S. dimorphus* was evaluated for anti-diabetic potential in terms of alpha-amylase activity (Deepa *et al.*, 2020). To the sterile vials, 0.5ml of different concentrations *i.e.*, 50, 100, 150, 200 and 250 µg/ml of ethanol extracts were taken, and 1 ml of phosphate buffer saline (PBS) was added. Followed by this, 0.2ml of starch prepared at 5mg/ml and 0.2ml of 0.5mg/ml of amylase enzyme were added and vials were incubated at room temperature for 10 minutes. Metformin was used as a standard drug and solution without extract containing only starch and no enzyme was maintained as control. After incubation the reaction was halted by adding 0.4ml of DNS solution and kept in a boiling water bath for 5 minutes and the solution was cooled down and absorption was recorded at 540nm in a UV-visible spectrophotometer. The enzyme inhibition potential was calculated using the below formula

$$\% \text{ of } \alpha\text{-amylase inhibition} = [(Ac - As)/Ac] \times 100$$

Where, Ac- absorbance of the control, As – Absorbance of sample

Antioxidant activity by DPPH assay

The capacity of free radical scavenging activity of ethanol extract was evaluated using DPPH assay (Brand-Williams *et al.*, 1995). Different concentrations of 0.5ml of ethanol extracts such as 20, 40, 60, 80, and 100mg were taken in different sterile vials to which 1ml of 0.2mM of DPPH solution was added. Ascorbic acid was used standard drug and all vials were incubated in the dark at room temperature for 30 minutes. After incubation, absorbance of vials was recorded at 517nm in a pre-calibrated UV-VIS spectrophotometer. The percentages of antioxidant activity of vials were calculated using below-mentioned formula.

$$\% \text{ Antioxidant activity} = = [(Ac - As)/Ac] \times 100$$

Where, Ac- absorbance of control, As – Absorbance of sample

RESULTS AND DISCUSSION

The pure culture of *S. dimorphus* was cultivated in protease peptone media containing 50% of dairy waste and the culture was grown for 28 days under optimized growth conditions in a specially designed novel photo-bioreactor. Post cultivation, the wet algal mat was recovered, dried, and extracted by solvent. The ethanol extract was subjected to phytochemical investigation and GCMS analysis. The biological activity of *S. dimorphus* extract mainly antibacterial and antioxidant activity was performed.

Cultivation of *S. dimorphus* in a growth medium containing 50% dairy

The pure microalgae *S. dimorphus* was grown in an optimized growth medium mixed with 50% dairy waste and the medium was incubated at optimum growth conditions. On the 28th day of incubation green colour luxurious growth *S. dimorphus* was observed in a novel photo-bioreactor. As growth was monitored by intermittently withdrawing samples and observing under the microscope, no contamination was observed throughout the cultivation system. The growth of microalgae seen during cultivation in a photo-bioreactor and its microscopic view during cultivation has indicated in the Figure 1. The biomass production of *S. dimorphus* was also monitored using until it reaches maximum production after which is recovered by the filtration system.

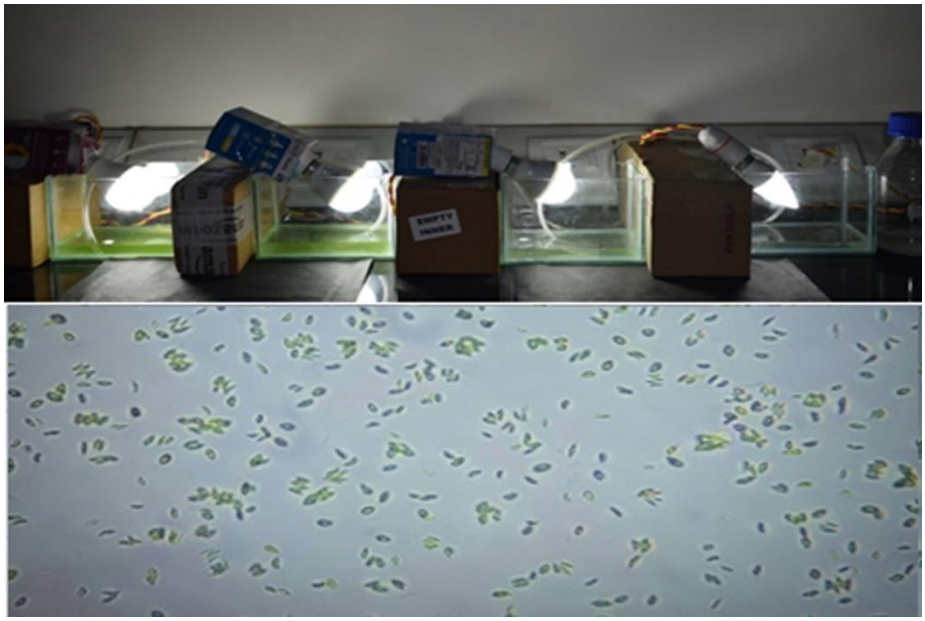

Figure 1: Mass cultivation of *S. dimorphus* in a photo-bioreactor under optimized growth conditions

Downstream process of *S. dimorphus* biomass and extraction

Soon after attaining sufficient growth of biomass, *S. dimorphus* was recovered from the photo-bioreactor and biomass was collected by filtration technique. The biomass thus recovered was further weighed and shade dried. From 50 liters of cultivation medium, approximately 326.23g of wet biomass and 192.8 g of dried biomass were obtained. The dried algal biomass was divided into two parts in which one part was used for the whole extract by ethanol yielding 26.2g extract. Another part utilized for successive solvent extraction resulted in 1.5gm of petroleum extract, 10.34gm of chloroform extract, 15.2gm of ethyl acetate, and 6.26gm of ethanol extract. The results thus obtained were presented as a bar graph in Figure 2.

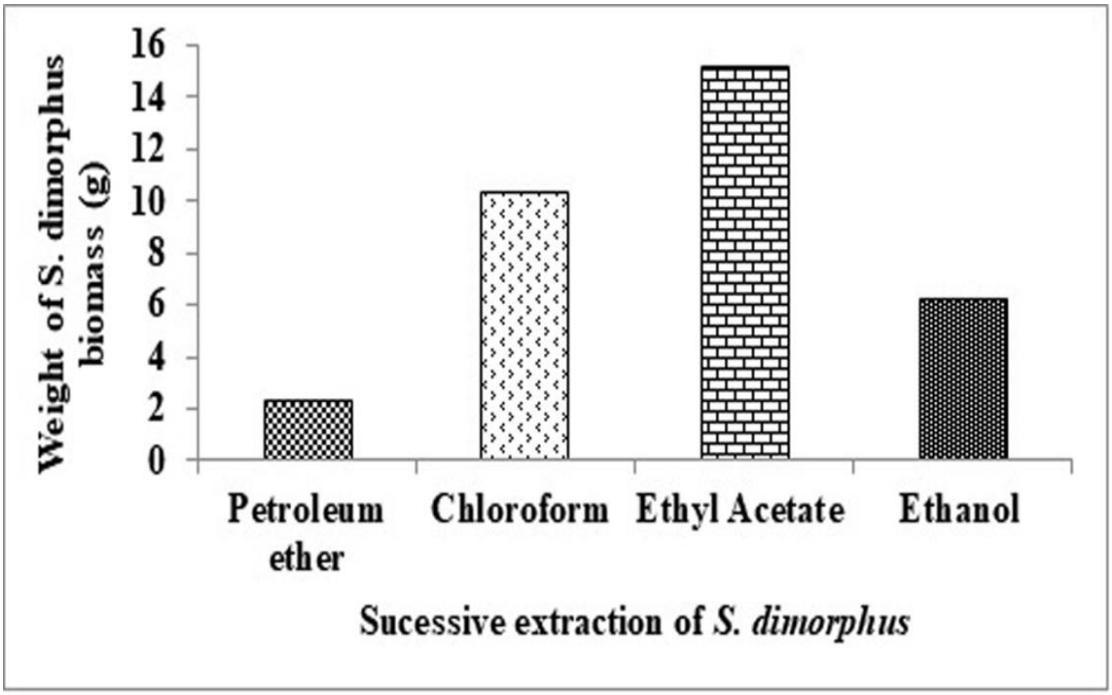

Figure 2: Weight of *S. dimorphus* extract obtained in successive extraction

Cell components of microalgae are found immense applications in health care sectors, the food industry, and as dietary supplements due to their high nutritional value. High content of protein, carbohydrates, amino acids, and 3 and 6 omega fatty acids, vitamins of microalgae play an important therapeutic role in several health issues like cancer, diabetes, arthritis, and cardiovascular disorders. Omega-3-fatty acids such as docosahexaenoic acid and Eicosapentaenoic acid produced from microalgae C*rypthecodinium*, *Schizachyrium*, *Spirulina*, and *Haematococcus* play an important role in the development of the brain and eyes in infants and preventing the blood coagulation, cardiovascular disease, maintenance of blood pressure, and functioning of the nervous system (Khavari *et al.*, 2021). Likewise, the various compounds of *S. dimorphus* found immense therapeutic applications, as pharmaceutical drugs, nutraceuticals, and food supplements (Armaini *et al.*, 2020). Although, several mechanisms responsible for the biosynthesis of biofuel in *S. dimorphus* have been proposed the molecular mechanisms for components responsible for other medical and nutraceutical applications are lacking (Sharma and Chauhan, 2016).

Chemical analysis of crude ethanol extract of *S. dimorphus*

The crude ethanol extract of *S. dimorphus* was subjected to chemical investigation by testing through various qualitative tests. The ethanol extract of microalgae indicated the presence of alkaloids, flavonoids, carbohydrates, proteins, oils, and fats. However, phenols, tannins, and saponins were detected in the ethanol extract of *S. dimorphus*. The algal extract also contains glycosides. The detail of the chemical components detected in the algal extract was shown in Table 1.

Table 1: Qualitative detection of compounds in the ethanol extract of S. dimorphus grown on dairy waste

S. No.	Qualitative test	Results
1	Test for Alkaloids	
	Mayer's test	Positive
	Wagner's test	Positive
2	Test for Flavonoids	
	Alkaline reagent test	Positive
	Lead acetate test	Positive
3	Test for tannins	
	Braymer's test	Negative
	Gelatine test:	Negative
4	Detection of phenols	
	Ferric chloride test	Negative
	Lead acetate test	Negative
5	Test of carbohydrate	
	Fehling's test	Positive
	Benedict's test	Positive
7	Test for oils and fats	Positive
8	Detection of proteins	Positive
9	Detection of saponins	Negative
10	Test for Glycosides	Positive

GCMS analysis of ethanol extract of *S. dimorphus*

The crude ethanol extract of *S. dimorphus* analysed by GCMS showed various peaks corresponding to different RT as indicated in the chromatogram. Each peak and its related compound in the NIST library were identified and the results thus obtained were presented in

Figure 3 and Table 2 Chromatogram revealed 42 peaks corresponded to 9 compounds and two peaks with RT values of 13.598 and 13.781 minutes were not matched to any of the compounds listed in the NIST library. Surprisingly, the highest peak area *i.e.*, 11446847, and with an 8.09% peak area was indicated by the unknown peak with an RT value of 13.781, and the second unknown peak was revealed the area of 1910853 (1.35% area). Followed by these, the next dominant peak area (RT 13.391 minutes) with 5.61% was attributed to the Hexadecane, and the peak with RT 18.27 minutes corresponded to Dibutyl phthalate with a peak area % of 5.56. Ethanol extract of *S. dimorphus* also revealed the presence of Eicosane as witnessed by a peak with RT 15.05 minutes corresponding peak area % of 5.39. The compound retained at 10.45 minutes RT was found to be Dodecane, 2,6,11-trimethyl- with peak area and area % of 5551845 and 3.92% respectively. Tetracosane was detected at an RT value of 20.56 minutes showing a peak area percentage of 3.51 and Decane, 3,7-dimethyl was eluted at an RT value of 7.4 minutes was corresponded to the 2.26% area. Some of the other components detected were Nonyl tetradecyl ether and Nonacosane at RT values of 19.4 and 20.52 minutes with peak area% of 0.77 and 1.06 respectively.

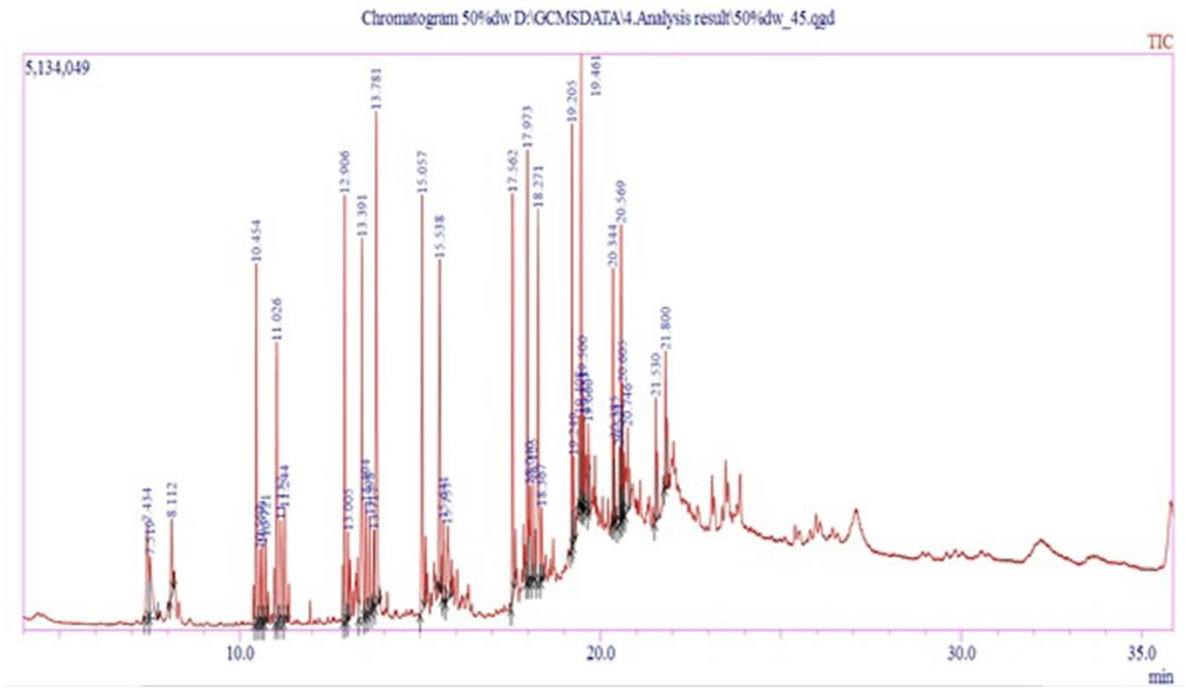

Figure 3: GC Chromatogram of ethanol extract of S. dimorphus grown on 50% dairy waste

TABLE 2: COMPARISON OF GCMS PROFILE OF ETHANOL EXTRACT OF S. DIMORPHUS WITH AND WITHOUT DAIRY WASTE

Peak#	Retention time (minutes)	Peak Area	Area%	Compound name	
				Extract with dairy waste	Control extract
1	7.434	3194905	2.26	Decane, 3,7-dimethyl-	-
	7.438	3144954	0.50	-	Decane, 3,7-dimethyl-
2	7.519	2778411	1.96	Dodecane	-
	8.115	2220518	0.35	-	Decane, 3,7-dimethyl-
3	8.112	1737758	1.23	Decane, 3,7-domethyl-	-
	10.459	6537866	1.03	-	Dodecane, 2,6,11-trimethyl-
4	11.034	5480521	0.86	-	Dodecane, 2,6,11-trimethyl-
	10.454	5551845	3.92	Dodecane, 2,6,11-trimethyl-	-
5	11.147	2802127	0.44	-	Dodecane, 2,6,11-trimethyl-
	10.557	1260700	0.89	Dodecane, 2,6,11-trimethyl-	-
6	10.630	1211908	0.86	Dodecane, 2,6,11-trimethyl-	-
	11.253	2584172	0.41	-	Dodecane, 2,6,11-trimethyl-
7	10.721	1302026	0.92	Dodecane, 2,6,11-trimethyl-	
	12.915	7317822	1.15	-	Hexadecane
8	11.026	4656928	3.29	Dodecane, 2,6,11-trimethyl-	
	13.397	8632822	1.36	-	Hexadecane
9	11.137	2418489	1.71	Dodecane, 2,6,11-trimethyl-	
	13.502	2052577	0.32	-	Hexadecane
10	11.244	2258036	1.60	Dodecane, 2,6,11-trimethyl-	-
	13.606	2055854	0.32	-	Hexadecane
11	12.906	6487644	4.58	Hexadecane	-
	13.802	8773608	1.38	-	2,4-Di-tert-butylphenol
12	13.005	1228984	0.87	Hexadecane	-
	15.068	7899520	1.24	-	Eicosane
13	13.391	7942521	5.61	Hexadecane	-
	15.547	6837709	1.08	-	Hexadecane
14	13.494	1955195	1.38	Hexadecane	-
	17.575	6709728	1.06	-	Eicosane
15	13.598	1910853	1.35		
	17.985	8890310	1.40		Eicosane
16	13.717	1549965	1.10	Hexadecane	-
	18.060	2715123	0.43	-	Eicosane
17	13.781	11446847	8.09		
	18.140	3356723	0.53	-	Eicosane
18	15.057	7624525	5.39	Eicosane	-
	18.209	2131262	0.34	-	Eicosyl isopropyl ether
19	15.538	6144531	4.34	Hexadecane	-
	18.304	8384641	1.32	-	Dibutyl phthalate
20	15.641	1809167	1.28	Hexadecane	-
	19.223	5881156	0.93	-	Eicosane
21	15.757	2297756	1.62	Eicosane	-
	19.485	21585775	3.40	-	Tetracosane
22	15.562	6841732	4.83	Eicosane	-
	19.753	181814091	28.65	-	6-Octadecenoic acid, (Z)-
23	17.562	6503719	4.60	Eicosane	-
	19.820	170388358	26.85	-	Octadecanoic acid, 2-hydroxy-1,3-propanedi
24	18.040	2159377	1.53	Eicosane	-
	20.096	111009737	17.49	-	Heptane, 2,6-diphenyl-3-methyl-
25	18.125	1707682	1.21	Eicosane	-
	20.37	4 14611461	2.30	-	Tetracosane
26	18.271	7872985	5.56	Dibutyl phthalate	-

	20.594	5451530	0.86	-	Tetracosane
27	18.367	1465400	1.04	Eicosane	-
	21.150	9253697	1.46	-	9-Octadecenoic acid (Z)-, oxiranylmethyl ester
28	19.205	5526068	3.90	Eicosane	-
	21.547	3847270	0.61	-	2-Methylhexacosane
29	19.249	1599916	1.13	Eicosane	-
	21.822	1777122	0.28	-	Tetracosane
30	19.408	1096507	0.77	Nonyl Tetradecyl ether	-
	24.319	10476237	1.65	-	cis-13-Docosenoyl chloride
31	19.461	5698677	4.03	Eicosane	-
32	19.500	1568526	1.11	Eicosane	-
33	19.553	1196056	0.85	Tetracosane	-
34	19.660	1193055	0.84	Eicosane	-
35	20.344	3709471	2.62	Tetracosane	-
36	20.385	1235064	0.87	Eicosane	-
37	20.521	1503732	1.06	Nonacosane	-
38	20.569	4966703	3.51	Tetracosane	-
39	20.605	1697935	1.20	Tetracosane	-
40	20.746	2629983	1.86	Eicosane	-
41	21.530	2215231	1.57	Tetracosane	-
42	21.800	2366725	1.67	Tetracosane	-

In contrast to GC-MS components of *S. dimorphus* grown on 50% dairy waste, the control extract of *S. dimorphus* showed the highest concentration of 6-Octadecenoic acid, (Z)- and Octadecanoic acid, 2-hydroxy-1,3-propanedi corresponding to 28.65% and 26.85% respectively. Followed by these components, 17.49% of Heptane, 2,6-diphenyl-3-methyl- was detected in the *S. dimorphus* extract. Additionally, some other compounds detected only in the control extract of *S. dimorphus* with less than 2% includes Eicosyl isopropyl ether, 9-Octadecenoic acid (Z)-, oxiranylmethyl ester, 2-Methylhexacosane, cis-13-Docosenoyl chloride, and 2,4-Di-tert-butylphenol and results shown in Figure.4. On the contrary, the only molecules detected in the extract of *S. dimorphus* grown on dairy waste were Dodecane, Nonyl Tetradecyl ether, and Nonacosane.

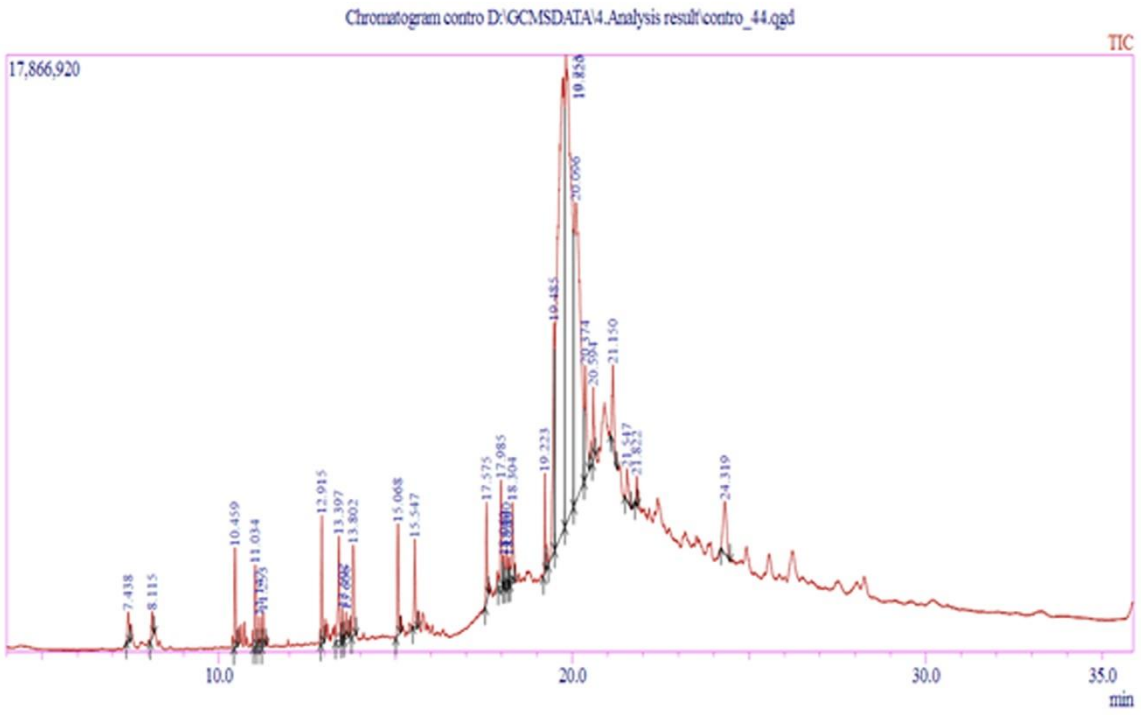

Figure.4: GC-MS analysis of ethanol extract of S. dimorphus grown on optimized medium (Control)

The essential oil present in the extract of *Trianthema decandra* L. viz., 3,7-dimethyldecane, 2,4-Di-tert-butylphenol, Hexadecane, Eicosane, Tetracosane, and Nonacosane showed promising antioxidant and antimicrobial activity against 12 isolates of bacteria and fungi which was almost equivalent to activity of standard antibiotics such as Chloramphenicol and nystatin (Geethalakshmi and Sarada, 2013). Dodacane main ingredient in petroleum and diesel isolated from various plant oils possess potential antioxidant and antimicrobial activities. It is also used as an anti-adhesion, an excipient in the drug industry, and to control candidemia (Ortansa *et al.*, 2020). The molecule 2,6,11-trimethyldodecane reported from Urtica dioica and rhizosphere of forest tree showed analgesic properties and plant pathogen-suppressing activity respectively (Dhouibi *et al.*, 2018; Yu *et al.*, 2022). Methanolic extract of Cycas pectinata containing Eicosyl isopropyl ether has reported for various pharmacological profiles such as antidiarrheal, anti-inflammatory, analgesic, thrombolytic anxiolytic and antioxidant properties (Tareq *et al.*, 2020).

Dibutylphthalate is an active secondary metabolite isolated from *Begonia malabarica* Lam. Herb and stem extract of Ipomoea carnea revealed potential antibacterial, anticancer, and anti-larvicidal activity against vectors such as Culex quinquefasciatus and Aedes aegypti vectors that anticipate in the transmission of several tropical diseases like Malaria, dengue fever, and Filaria (Shobi and Viswanathan, 2018). Ethyl acetate extract of Streptomyces chumphonensis containing 6-Octadecenoic acid, (Z)- showed antibacterial activity against a set of Gram-positive and Gram-negative bacteria and anticancer activity breast cancer activity (Manikandan et al., 2019).

Biological activity of ethanol extract of *S. dimorphus*

The anti-diabetic potential of *S. dimorphus* extract

The extract of *S. dimorphus* showed dose-dependent anti-diabetic potential as witnessed by increased inhibition of α-amylase activity by extract used in the range of 50 - 250µg/ml as shown in Figure 5. The highest anti-diabetic activity was noticed at 250µg/ml of extract corresponding to 71.99% of enzyme inhibition. However, the metformin was used at 250µg/ml conc. revealed 85.94% α-amylase inhibition activity. The IC_{50} value of ethanol extract of the algal sample was 177.88µg/ml in contrast to the IC_{50} value of 77.9µg/ml. Hence, the algal extract showed significant anti-diabetic activity in a dose-associated manner and would play important role control of diabetes.

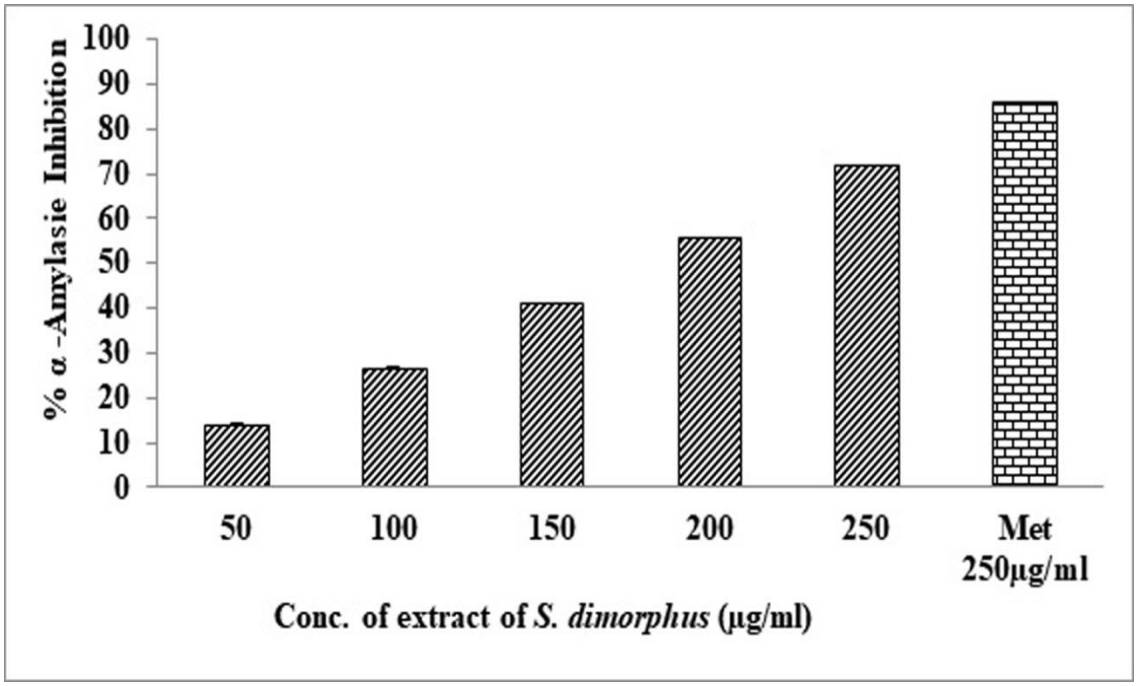

Figure 5: Alpha-amylase activities of different concentrations of *S. dimorphus* extract

The free radical scavenging capacity of *S. dimorphus*

Ethanol extract of *S. dimorphus* increased substantial antioxidant capacity when tested from 20 – 100µg/ml and results indicated in Figure 6. The percentage of free radical scavenging potential was increased as the tested dose of ethanol extract increased showing 15.6%, 28.2%, 45.2%, 58.1%, and 72.5% of antioxidant activity at 20, 40, 60, 80, and 100µg/ml of extract respectively. The IC_{50} value of algal extract was determined as 68.37% in contrast to the 41.2% IC_{50} value of the standard vitamin C. The maximum activity *i.e.,* 72.5% was noticed at 100µg/ml compared to the 86.94% of antioxidant potential of standard drug Ascorbic acid used at the same concentration. The algal extract of *S. dimorphus* indicated a significant free radical scavenging activity.

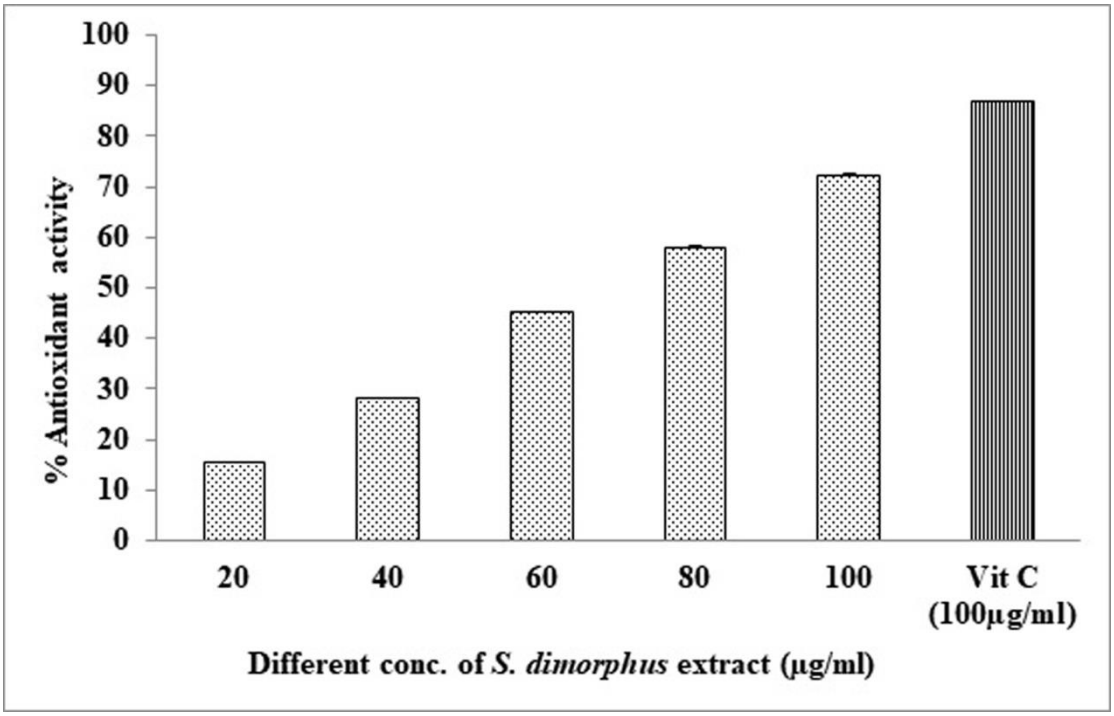

Figure 6: Determination of Antioxidant potential of *S. dimorphus* extract

CONCLUSION

The metabolites profiling of *S. dimorphus* on protease peptone medium containing 50% dairy wastewater revealed the production of several novel metabolites in high concentration. The components present in the dairy wastewater would have stimulated the production of these rare molecules. Phytochemical investigation and GC-MS analysis of a crude extract of *S. dimorphus* showed several medically important metabolites possessing antimicrobial, antioxidant, anti-inflammatory, antidiarrheal, anticancer, and antioxidant potential. Therefore, dairy wastewater plays a significant role in stimulating *S. dimorphus* to produce medically important metabolites and the control of important illnesses.

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ABBREVIATIONS

PUFA: poly-unsaturated fatty acid; L/D: Light and Dark; BOD: Biochemical Oxygen Demand; GCMS: Gas chromatography–mass spectrometry; NIST: Nationals Institute of Standards and Technology; DNS: 3,5-Dinitrosalicylic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl.

AUTHOR CONTRIBUTIONS

Substantial contributions were made by all authors in conducting research, interpretation of data, and preparing manuscripts.

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No financial support is obtained to report.

CONFLICT OF INTEREST

The Authors declare no conflict of interest.

DATA AVAILABILITY

Present research article includes all the data generated and analysed.

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