Original Article

Appraising the Neuroprotective Competence of Nitrogen-Enriched *Arthrospira platensis* in Comparison to Commercial Resource in Depressed Mice Models

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Abstract

Back ground: *Arthrospira platensis* encompasses vital nutrients and is commercialized globally. It comprises of imperative resource that has the potential to combat neurological deformities caused due to stress and anxiety. **Objective:** This work evaluates the neuroprotective effect of spirulina cultivated on nitrogen enriched medium and compares it with the commercial samples. **Materials and Methods:** The study is authenticated with an antioxidant assay and the vital compounds are relatively profiled by GC-MS study. Furthermore, a molecular docking analysis is implemented to investigate the therapeutic potentials of the phytocompounds against Monoamine Oxidase –A and establish them as inhibitors. The ethanolic extract of spirullina as are fed on depressed mice models to assay its neuroprotective effect and rehabilitation of brain cells by a histopathological study. **Results:** The antioxidant content of the augmented sample was consistent on par with the commercial sample. The *in silico* assay was performed with 10 extricated compounds of both the samples where, Butanoic acid, 3-hydroxy- furnished a minimum binding affinity energy value of -56.24 kcal/mol and dodecanamide was efficient to bind with the active site of the amino acid residue TYR 69 with a minimum energy of -87.8 kcal/mol. The histopathological examination upholds the refurbished parameter of vital phytocompounds with placid cellular edema and perivascular infiltration. **Conclusion:** There is a wide range of need to develop research against stress and anxiety and the study fortifies the restorative efficacy of the phytocompounds as a neuroprotective drug.

Keywords: *Arthrospira platensis, butanoic acid 3-hydroxy-, GC-MS, molecular docking, monoamine oxidase A, neuroprotectants*

Introduction

Arthrospira platensis (commonly known as spirulina) is a unicellular planktonic microalga that grows in aquatic habitat such as fresh, salt, and brackish bodies of water.[1] It is a free-floating cylindrically shaped filamentous photosynthetic species. It received its scientific term based on the unified morphology of septa arrangement and helical form. Spirulina [Figure 1] is renowned for its nutritional benefits and termed as a *super food* owing to the intense presence of vital nutrients such as vitamin B12. It is cultivated and marketed commercially worldwide both as a food supplement and as an active ingredient in functional foods.[2] It has attained considerable promotion in health sector and bestows pharmaceutical benefits with compounds such as carotene and omega 3 and 6 polyunsaturated fatty acids.[3]

Occurrences of stress-induced psychiatric disorders are prone more in the contemporary era of scientific developments that leads to stress-induced psychiatric disorders. Generalized anxiety disorder and depression (depressive episode) are commonly occurring psychiatric ailments that can overlap with similar symptoms. This menace is considered a threat and a burden that agonizes the world intensively.[4] Numerous psychotropic medications are designed to treat their pathology and symptoms. Monoamine inhibitors (MAO-I) are a class of antidepressants that subside deamination the action of monoamine oxidase and regulates the function of neurotransmitters.[5] Furthermore, the pathology of this mental ailment demands the nutritional condition of the body. There is an increased rate in the metabolism where the biochemical constitution of the body is utilized widely. There is a surplus change in the adrenal production and consumption of vitamins, minerals, and micronutrients which further accelerate metabolism of proteins, fats,

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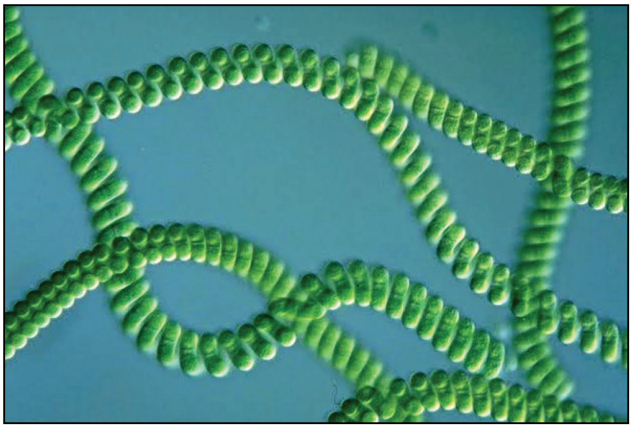

Figure 1: Pictorial representation of *Anthrospira plantensis* (Adapted from King Dnarmsa, Philippines, posted online)

and carbohydrates, producing quick energy to overcome this psychiatric condition.[6]

To manage this metabolic condition, there is a need of antioxidant defense in the biological system. Nonenzymatic antioxidants such as vitamin E (alphatocopherol), vitamin C (ascorbic acid), β-carotene, minerals, and trace elements can efficiently overcome the nutritional scarcity. The super food, spirulina, can facilitate this condition by bestowing its antioxidant potential. Furthermore, its credentials can be enhanced by culturing it in a nitrogen-enriched medium, as variation in its composition can increase its nutrient content and biomass.[7] This study persuades to evaluate the neuroprotective effect of spirulina cultivated on nitrogen-enriched medium and efforts a therapeutic remedy to hoist as monoamine inhibitors.

Materials and Methods

Drugs and reagents

All chemicals used were of analytical grade purchased from Rankam Chemical Co.

Strain procurement, culture development, and cultivation on nitrogen-enriched media

Spirulina (*Arthrospira platensis*) National Collection of Industrial Microorganisms (NCIM -5412) (on solid media) was procured from the National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, India. The strain was previously maintained on Zarrouk's agar media slants at 4°C. Loop full of spirulina culture was inoculated in a 50 mL flask containing 10 mL sterile standard medium (Modified Zarrouk's Medium) under sterile condition. Sodium carbonate was added after autoclaving, and pH was adjusted to 8.8–9.0. Growth and maintenance of the culture was done in an illuminated (4500 lux) growth room at 30 ± 2°C under 12/12 h light-dark cycles. Manual shaking of cultures was done periodically thrice every day. To study the effect of the inorganic nitrogen concentration on the growth of the strain NCIM-5412 in batch culture, a modified Zarrouk's medium was used where the concentration of sodium nitrate ($NaNO_3$) was 0.625, 1.250, 1.875, or 2.500 gL^{-1}

Preparation of ethanol extracts

The cultivated sample was shade dried and finely powdered. 10 g of powdered nitrogen-enriched sample (1) and the commercial sample obtained (2) were extracted using Soxhlet apparatus. The samples were placed in the thimble and air dried at room temperature for 18 h. Extraction was performed for 3 h using 100 mL solvent (95% ethanol) and the filtrate was consumed for further analysis.

Phytochemical determination

Determination of total phenolics

The total phenolic contents in the extracts were determined spectrophotometrically at an absorbance of 765 nm according to Folin-Ciocalteu method.[8] Tannic acid was used to set up the standard curve. The content of phenolic compounds of the samples was expressed as tannic acid equivalents in mg per gram dry weight. All the samples were analyzed in triplicates.

Determination of total flavonoids

Estimation of the total flavonoids in the extracts was carried out using the method of Ordon-Ez *et al.*,[9] where 0.5 mL of 2% $AlCl_3$ ethanol solution was added to 0.5 mL of sample. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoid, and the contents were expressed as quercetin equivalents in mg per gram dry material. All the determinations were performed in triplicates, and the mean absorption along with the regression value was assayed.

Determination of antioxidant activity

Reducing ability

The antioxidant capacity of the extracts was estimated spectrophotometrically (ferric reducing antioxidant power [FRAP] assay) following the procedure of Benzie and Strain.[10] The method is based on the reduction of Fe^{3+} tripyridyl triazine (TPTZ) complex (colorless complex) to Fe^{2+} TPTZ (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. FRAP reagent was prepared by mixing 300 mM acetate buffer, 10 mL TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in the proportion of 10:1:1 at 37°C. Freshly prepared working FRAP reagent was pipetted using 1–5 mL variable micropipette (3.995 mL) and mixed with 5 µL of the appropriately diluted plant sample and mixed thoroughly. An intense blue color complex was formed when ferric TPTZ (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 mL FRAP reagent + 5 µL distilled water) after 30 min incubation at 37°C. All the determinations were performed in triplicates. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass and compared with that of butylated hydroxytoluene, ascorbic acid, quercetin, and catechin.

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 17.0. Data obtained were expressed as mean ± standard deviation. One-way analysis of variance followed by multiple range test (Tukey post hoc test) was used to determine significant differences between the test groups and control. Differences at $P < 0.05$ were considered to be statistically significant.

Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC-MS) analysis of the samples was performed using a Perkin–Elmer Clarus 500 system comprising an AOC-20i auto-sampler. The GC is interfaced to a MS equipped with a column (Id: 250 µM) Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused extended to a length of 30 m. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min, and a sample injection volume of 1.6 µL was employed (a split ratio of 10:1). The inlet temperature was maintained at 280°C. The oven temperature was programmed initially at 60°C for 8 min, which further increased to 200°C for 5 min. This program extended at a rate of 20°C ending with 280°C for 15 min. Mass spectra were taken at 70 eV at a scan interval of 0.5 s and fragments from 45 to 450 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of phytocomponents

Interpretation of mass-spectrum was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained

Molecular docking

Graphical-Automatic Drug Design System for Docking, Screening and Post-Analysis program iGEMDOCK was used to gain the docking results of the listed compounds with the target. The binding site of the target was prepared and the energy-minimized compounds were imported.

Preparation of ligands

The three-dimensional structures of the phytocompounds considered as ligands were retrieved and downloaded in structural data format and converted to Protein Data Bank (PDB) format using the online tool smile translator (cactus.nci.nih.gov/translate). The compound structures were energy minimized and considered for docking studies

Preparation of protein target

The target protein monoamine oxidase (MAO A) was retrieved from PDB. Water molecules were removed, and a single chain was selected between two chains. Generally, all waters (except those coordinated to metals) are deleted, but water that connects between the ligand and the protein is sometimes retained. Problems in the PDB protein structure were repaired by adjusting the protein, metal ions, and cofactors. The structure-forming bonds from the ligand or a cofactor to a protein metal were deleted by adjusting the ligand bond orders and formal charges. The minimization was done to restrain the input protein coordinates by a selected root mean square deviation tolerance.

The docking protocol consisted of 25 generations per ligand and the population size of 100 random individuals. All the docking conformations were performed twice using genetic evolutionary algorithm, and the fitness of the docked structures were calculated. The hydrophobic and electrostatic preferences were set to 1.00. The binding site of the target was identified at a distance 8 Å. The empirical scoring function of iGEMDOCK was estimated as: Fitness = vdW + H bond + Elec.

Here, the vdW term is van der Waal energy; H bond and Elec terms are hydrogen bonding energy and electro statistic energy, respectively.

Animal study

All experiments of this study were performed in accordance with relevant guidelines and regulations of the institutional Ethical Committee for the Purpose of Control and Suspension of Experiments on Animals (CPCSEA) under the Ministry of Animal Welfare Division, with an approval ID: BDU/IAEC/2014/NE/39. Female Albino mice (5–6 weeks old, 22 ± 2 g) were housed in standard environmental conditions (12 h light-dark cycle, 50–70% humidity, and 20–25°C). Food and water were provided *ad libitum*. Seven mice were used in each group to minimize the number of experimental animals needed, while ensuring the validity of statistical analyses.

Experimental protocol and animal exposure

The aim of the study protocol was to induce depression and assay the neuroprotective effect by administering ethanol extracts of samples 1 and 2. The animals were randomly divided into four groups assigned as positive control (depressive without treatment), negative control (normal), and experimental groups 1 (nitrogen enriched [sample 1]) and 2 (commercial [sample 2]). The animals in positive control grouped as 1 were administered orally (p.o.) with saline as vehicle (1 mL of 0.9%). On injecting methyl isobutyl ketone (100 mg/kg weight, i.p.) the animals were depressed. This was regularly practiced for two weeks and proceeded by a treatment phase that sustained for a week. Eventually the animals were sacrificed for histopathological studies.

Brain histological assay

The mice were sacrificed by cervical dislocation 24 h after the final day of treatment phase. The brains were removed,

sectioned, and put in a fixative solution of 10% formalin at room temperature. The cortex region of the brain was sectioned and counterstained with alum hematoxyl. The slices were dehydrated with alcohol followed by xylene and processed for paraffin embedding as tissue blocks (mp 58–60.C). Transverse sections of 5-µm thickness were cut on a rotary microtome and were stained by Ehrlich's hematoxylin embedded with eosin in alcohol. The histopathological assay was executed under microscopic examination (DM 4000B, Leica Microsystems, Mumbai).

Results

Spirulina cultivation in nitrogen-enriched media and analysis of dry weight

Spirulina sp. NCIM-5412 was successfully cultured in the nitrogen-enriched modified media and its dry weight was determined. The dry weight of 0.23 g/L was achieved and considered significant to endure further product recovery studies.

Total phenolic and flavonoids contents

The phytochemical assay of both the samples of spirulina (sample 1: cultivated in nitrogen-enriched media; sample 2: commercial powder) was performed to expose the significance of phytocompounds and their applications in drug development. Results obtained in the present study showed an increased intensity of phenolic compounds in the enriched cultivated sample when compared with the standard, whereas the components of flavonoids were substantial in both the samples as shown in Table 1.

Antioxidant assay

Reducing ability (FRAP)

The reducing ability of both the extracts was in the range of 626.60–668.28 µM (Fe (II)/g) as shown in Table 2. The antioxidant potentials of both the extracts were estimated from their ability to reduce Ferric tripyridyltriazine (FeIII -TPTZ) complex to (FeII -TPTZ).

Table 1: Total phenolic and flavonoid contents of both the sample extracts

Compounds	Sample 1	Sample 2
	Cultivated	Commercial
Total phenolics[a]	15.48 ± 0.17*	13.45 ± 0.23
Total flavanoid[b]	0.91 ± 0.02	1.04 ± 0.05

[a]Expressed as mg tannic acid/g of dry plant material.
[b]Expressed as mg quercetin/g of dry plant material.
*Indicates that this value is significantly different from the other at $P < 0.05$

Table 2: Antioxidant assay both the sample extracts

Samples	FRAP*
Cultivated	626.60 ± 28.47
Commercial	668.28 ± 34.23
Ascorbic acid	962.02 ± 0.51

FRAP = ferric reducing antioxidant power.
*Expressed in units of µmol Fe (II)/g.

GC-MS studies

GC-MS chromatogram analysis of the ethanolic extract of cultivated sample [Figure 2] extricated 28 different compounds [Table 3], which indicates the assortment of different phytoconstituents. The ethanolic extract of commercial sample [Figure 3] imparted 20 different compounds which were detected and catalogued in parallel to the NIST library as predicted in Table 4. Furthermore, these compounds were compared with the GC-MS spectrum of commercial sample. Eight compounds, namely, butanoic acid, glycerin, nonanal,1,4:3, 6-dianhydro-a-d-glucopyranose, phenol,2,4-bis(1,1-dimethyl), heptadecane, octadecanoic acid, and dodecanamide, were observed to be common in both the GC-MS profiles.

Molecular docking studies of the phytocompounds against the target proteins MAO A

An *in silico* assay was done to determine the best compound by docking against the depression-inducing proteins MAO A. The docking study was performed using the program Gem Dock considering the vital phytocompounds as ligands. The active site associated with the protein (MAO A) were studies that composed of amino acid residues, such as Tyr69, Tyr197, Phe208, Glu216, Tyr407, Phe352, and Tyr444.[11] The phytocompounds drawn in the study were used as ligands to target the mental illness–inducing proteins and therefore aspire the compounds as antidepressant agents. The assay was performed by relating 10 extricated compounds of both the samples from the GC-MS assay. These compounds were selected based on their resemblance with the extracted compounds of commercial sample, medicinal assets, and high retention time during extrication. The poses of all compounds are depicted in Figure 4 and presented in Table 5. The predicted binding and docked energies are the sum of the intermolecular energy and the torsional free-energy penalty, and the docking ligand's internal energy, respectively, and the inhibition constant (Ki) is calculated in the software as follows: Ki = exp (ΔG/(R*T). However, the top ranked pose was generated with the phytocompound butanoic acid, 3-hydroxy-, which was isoenergetic against the proteins with a minimum energy score

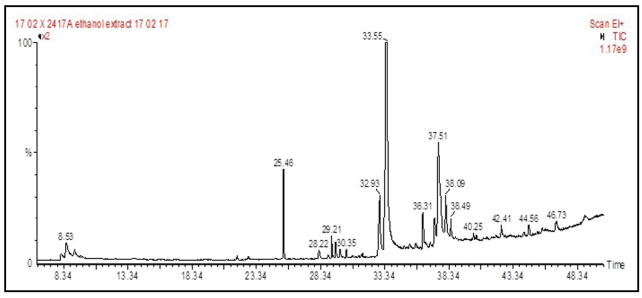

Figure 2: Pictorial representation of gas chromatography–mass spectrometry profile extricating phytocompounds from nitrogen-enriched cultivated sample of *Arthrospira platensis*

Table 3: List of extricated phytocompounds from nitrogen-enriched cultivated sample

S. No.	Peak name	Retention time	Peak area	% Peak area
1.	Name: Propane, 1-(ethenyloxy)-2-methyl- Formula: $C_6H_{12}O$ MW: 100	3.51	308669	0.1052
2.	Name: Butanoic acid, 3-hydroxy- Formula: $C_4H_8O_3$ MW: 104	8.14	3870774	1.3190
3.	Name: Glycerin Formula: $C_3H_8O_3$ MW: 92	8.54	14050158	4.7877
4.	Name: (S)-3-Ethyl-4-methylpentanol Formula: $C_8H_{18}O$ MW: 130	9.66	384131	0.1309
5.	Name: Nonanal Formula: $C_9H_{18}O$ MW: 142	9.82	160317	0.0546
6.	Name: 1,4:3,6-Dianhydro-à-d-glucopyranose Formula: $C_6H_8O_4$ MW: 144	13.67	927729	0.3161
7.	Name: Nonanoic acid Formula: $C_9H_{18}O_2$ MW: 158	14.58	304483	0.1038
8.	Name: n-Decanoic acid Formula: $C_{10}H_{20}O_2$ MW: 172	16.97	104974	0.0358
9.	Name: Phenol, 2,4-bis(1,1-dimethylethyl)- Formula: $C_{14}H_{22}O$ MW: 206	20.99	110445	0.0376
10.	Name: 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- Formula: $C_{11}H_{16}O_2$ MW: 180	21.82	893521	0.3045
11.	Name: Dodecanoic acid Formula: $C_{12}H_{24}O_2$ MW: 200	22.70	552057	0.1881
12.	Name: Hexadecanal Formula: $C_{16}H_{32}O$ MW: 240	23.28	174897	0.0596
13.	Name: Heptadecane Formula: $C_{17}H_{36}$ MW: 240	25.46	13566568	4.6229
14.	Name: Tetradecanoic acid Formula: $C_{14}H_{28}O_2$ MW: 228	28.22	3136391	1.0687
15.	Name: 2-Heptadecenal Formula: $C_{17}H_{32}O$ MW: 252	28.96	1068988	0.3643
16.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	29.21	3506252	1.1948
17.	Name: 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- Formula: $C_{20}H_{40}$ MW: 280	29.31	302999	0.1032
18.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$ MW: 268	29.52	2944734	1.0034

Table 3: Continued

S. No.	Peak name	Retention time	Peak area	% Peak area
19.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	30.35	1594110	0.5432
20.	Name: Dodecanoic acid, methyl ester Formula: $C_{13}H_{26}O_2$ MW: 214	31.63	566611	0.1931
21.	Name: 9-Hexadecenoic acid Formula: $C_{16}H_{30}O_2$ MW: 254	32.93	21487156	7.3219
22.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	33.55	183636272	62.5752
23.	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	36.31	7798761	2.6575
24.	Name: 4-Hexadecen-6-yne, (Z)- Formula: $C_{16}H_{28}$ MW: 220	37.22	8795511	2.9971
25.	Name: 9,12-Octadecadienoic acid, methyl ester Formula: $C_{19}H_{34}O_2$ MW: 294	37.51	6921946	2.3587
26.	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	38.09	11170607	3.8065
27.	Name: Dodecanamide Formula: $C_{12}H_{25}NO$ MW: 199	38.49	4109407	1.4003
28.	Name: Heneicosane Formula: $C_{21}H_{44}$ MW: 296	40.25	1016375	0.3463

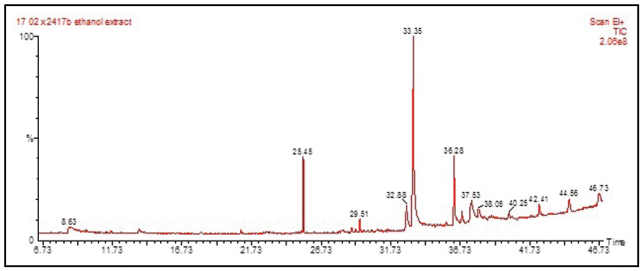

Figure 3: Pictorial representation of gas chromatography–mass spectrometry profile extricating phytocompounds from commercial sample of *Arthrospira platensis*

of −56.24 kcal/mol, whereas dodecanamide was efficient to bind with the active site of the amino acid TYR 69 with a minimum energy of −87.8 kcal/mol.

Histopathological examination

Histological observations after administration of methyl isobutyl ketone showed sustained cell damage due to the oxygen exhaustion in the neural cells. The majority of cells in the nitrogen-enriched source group remained intact and stained homogeneously [Figure 5]. Although the commercial sample upshot a reduced damage when compared with negative control, cell gliosis was relatively lower in the nitrogen-enriched sample.

Discussion

The neuroprotective capability of spirulina cultivated on nitrogen-enriched medium is endorsed in this study by an *in silico* assay substantiated with an *in vivo* analysis. Appearance of the cultivated culture was dark green in color when compared with the commercial sample that depicts the increasing cell mass. Furthermore, the influence of nitrogen in the media would increase the concentration of phenolic compounds. Miranda *et al.*[12] studied the antioxidant activity of carotenoids, phenolics, and tocopherols extracted from *Spirulina maxima* and found that the phenolic compounds are responsible for the antioxidant properties.

Therefore the study substantiates phenolic compounds as vital source in the mechanism of reduction among intermediate chain reaction. Polyphenolic compounds are known to have antioxidant activity and are sourced due to their redox properties, which play a significant role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[13] This infers an immense advantage in increasing the therapeutic efficacy against several human ailments.[14] The phytochemical studies endorse this by an elevated intensity in the presence of phenols when evaluated with the commercial sample, and this was reported by Parvin *et al.*[15] Hence this study

Table 4: List of extricated phytocompounds from commercial sample

S. No.	Peak name	Retention time	Peak area	% Peak area
1.	Name: Hexanal Formula: $C_6H_{12}O$ MW: 100	3.52	155076	0.3286
2.	Name: Butanoic acid, 3-hydroxy- Formula: $C_4H_8O_3$ MW: 104	4.80	238873	0.5062
3.	Name: Glycerin Formula: $C_3H_8O_3$ MW: 92	8.63	3509723	7.4378
4.	Name: Nonanal Formula: $C_9H_{18}O$ MW: 142	9.82	116878	0.2477
5.	Name: 1,4:3,6-Dianhydro-à-d-glucopyranose Formula: $C_6H_8O_4$ MW: 144	13.65	613097	1.2993
6.	Name: Phenol, 2,4-bis(1,1-dimethylethyl)- Formula: $C_{14}H_{22}O$ MW: 206	20.97	303487	0.6431
7.	Name: 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- Formula: $C_{11}H_{16}O_2$ MW: 180	21.83	134552	0.2851
8.	Name: Heptadecane Formula: $C_{17}H_{36}$ MW: 240	25.45	4444861	9.4195
9.	Name: 7-Tetradecene, (E)- Formula: $C_{14}H_{28}$ MW: 196	28.03	102875	0.2180
10.	Name: E-2-Tetradecen-1-ol Formula: $C_{14}H_{28}O$ MW: 212	28.94	371003	0.7862
11.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	29.21	202478	0.4291
12.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$ MW: 268	29.51	883725	1.8728
13.	Name: E-9-Tetradecenoic acid Formula: $C_{14}H_{26}O_2$ MW: 226	32.88	3430156	7.2691
14.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	33.53	18770122	39.7774
15.	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	36.28	5783280	12.2558
16.	Name: 9,12-Octadecadienoic acid, methyl ester Formula: $C_{19}H_{34}O_2$ MW: 294	37.51	4291294	9.0940
17.	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	38.05	1250945	2.6510
18.	Name: Dodecanamide Formula: $C_{12}H_{25}NO$ MW: 199	38.49	209908	0.4448

Table 4: Continued

S. No.	Peak name	Retention time	Peak area	% Peak area
19.	Name: Hexadecane Formula: $C_{16}H_{34}$ MW: 226	40.25	551115	1.1679
20.	Name: Heptadecane, 2,6,10,15-tetramethyl- Formula: $C_{21}H_{44}$ MW: 296	44.56	1824496	3.8664

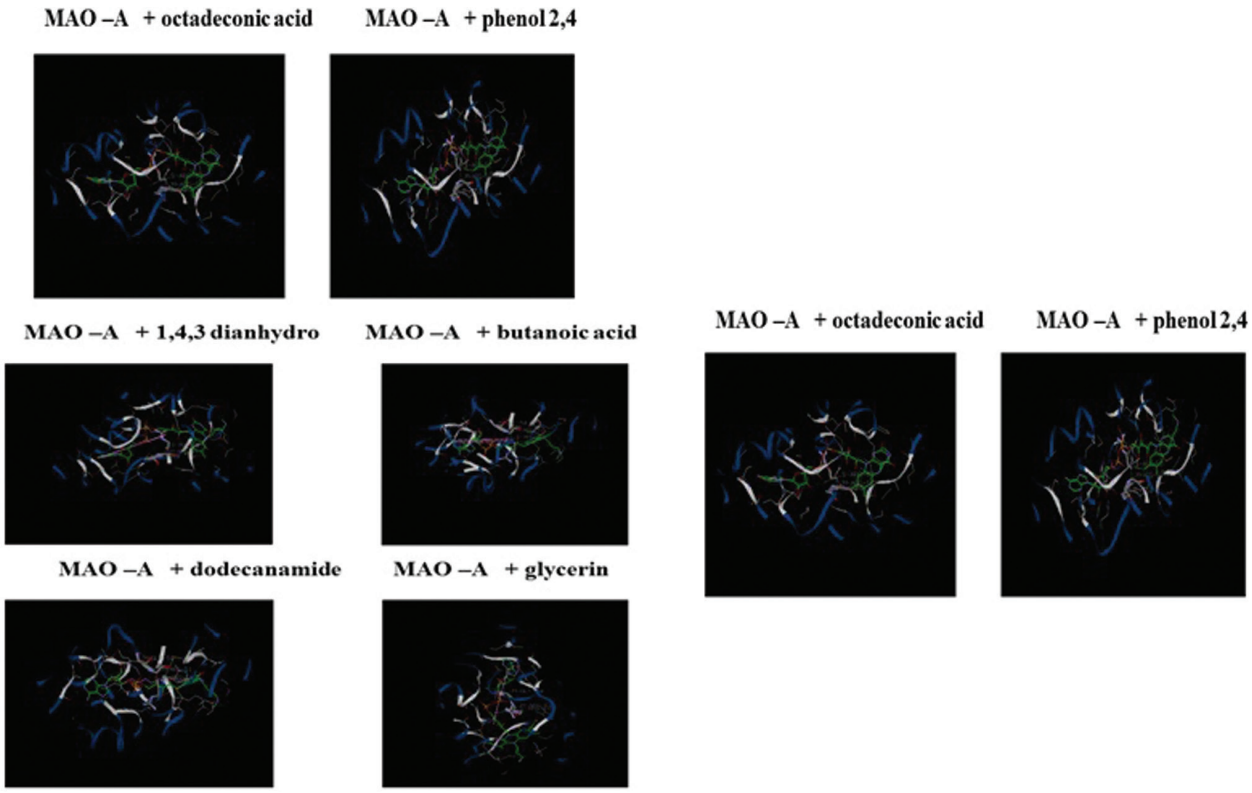

Figure 4: Pictorial representation of the test compounds interaction with its targeted protein

Table 5: Hits and docking score of phytocompounds against the targeted protein monoamine oxidase A

S. No	Compounds	Energy	vdW	H Bond	Elec
1	1,4:3,6-dianhydro-a-d-glucopyranose	−75.78	−65.4	−10.39	0
2	Buatonic acid,3 hydroxy	−56.24	−28.87	−25.13	−2.24
3	Dodecanamide	−87.83	−72.3	−15.52	0
4	Glycerin	−57.82	−29.07	−28.75	0
5	Heneicosane	−119.85	−119.85	0	0
6	Heptadecane	−87.34	−87.34	0	0
7	Hexadecanel	−83.95	−83.95	0	0
8	Nonanal	−65.84	−62.34	−3.5	0
9	Octadeconic acid	−126.08	−119.08	−7	0
10	Phenol 2,4-bis	−83.31	−77.31	−6	0

attests Miranda *et al.*[12] and accounts the augmentation of phenol compounds in nitrogen-enriched medium that functions as a good antioxidant. The FRAP values for the ethanol extracts of both the samples were significantly lower than that of the standard source ascorbic acid and can be enhanced further with improved cultural conditions. The FRAP is a widely used technique that evaluates the antioxidant activity exhibited by the presence of phytocompounds. The flavonoids and phenolic compounds exhibit a strong potency of binding with the metal complex, particularly iron and copper. Hence the reducing capacity of the extracts is based on the principle of increase in the absorbance of the reaction mixtures, and as the absorbance increases, the antioxidant activity also increases.[16] GC-MS is an incredible technique used for the analysis of nonpolar and volatile components.[17]

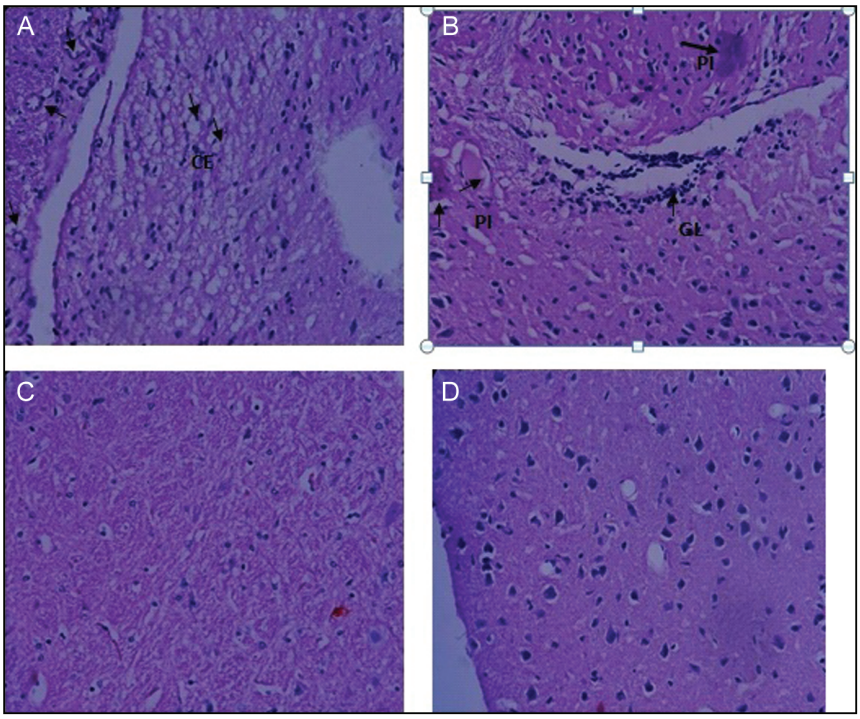

Figure 5: Histopathological examination in cerebral cortex regions of the (A) depression-incited mice showing cellular edema (CE), (B) depressionincited mice showing perivascular infiltration (PI) with increased gliosis (GL), (C) normal mice, and (D) treated mice with nitrogen-enriched ethanol extract of *Anthrospira plantensis* (x400)

The study slots in two samples namely, ethanolic extract of cultivated sample and commercial powder. The target protein MAO A was counteracted by 10 different phytocompounds efficiently through amino acid residues. Flexible docking of all data sets used for the computational study was carried out on the active site of the protein. The lowest energy and maximum number of conformations per cluster were set as the criteria to predict the binding modes of the compounds. Based on the procedure explained in the experimental section, the binding affinity of the docked molecules was evaluated by binding energy, docked energy, calculated inhibition constants (calc.), and hydrogen bonds in addition to the hydrophobic interactions at the channel pocket, where ΔG is the docking energy, Rcal is 1.98719, and TK is 298.[18-20] The synchronized neuroprotective effect of the vital phytoconstituents was further assayed by an *in vivo* study that included histopathological analysis. Persistent diminution in cerebral blood flow and brain energy metabolism causes progressive dysfunction of neurons, resulting in degenerative changes in glial cells. This damage was characterized by loose and disordered arrangements of cells and swelling deformations in cell shape resulting in shortening and disappearance of apical dendrites. The restraint in blood flow further cause microvascular constriction and glucose and oxygen delivery [21,22] that can develop cellular edema and perivascular infiltration [Figure 2]. These are considered as neural deformity that symbolizes pathological condition due to the stress induced to the animals, which reduced cognitive abilities with an increase in anxiety. Subsequently, damage was much less than that seen in treated samples.

Conclusion

This study has an optimistic result that concludes with the potential of spirulina (*Anthrospira platensis*) as an effective antioxidant stress reliever that can act as a neuroprotectant. The variation in the culture conditions with nitrogen-enriched media increases the biomass content, and the considerable total phenolic content substantiates the antioxidant efficacy. Furthermore, the GC/MS assay reveals the specific phytocompounds present in the plant sample. Eight phytocompounds were common to the commercial sample, and this depicts that there was no deleterious change in the extrication with ethanol. The docking study delivers a note that the compounds can act as monoamine inhibitors. Butanoic acid, 3-hydroxy- exhibited a minimum binding energy score of −56.24 kcal/mol and dodecanamide furnished a value of −87.8 kcal/mol in the targeted active site. The central nervous system has a high oxygen consumption and low antioxidant defense activity. This study bestows a blend of vital compounds whose interactions are substantiated by *in silico* assay, which is endorsed by an *in vivo* study that examined the restoration ability of oxidative stress caused by the brain damage. Conversely, imperative research can elevate these compounds as psychoactive drugs with preclinical studies that can target various other mental ailments and pathological conditions. Downstream processing, purification, and standardization would bring in superior standard and claim its demand commercially.

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Conflicts of interest

There are no conflicts of interest.

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