BIOLOGICAL EVALUATION OF MICROALGAE (*SPIRULINA PLATENSIS*) - A SUSTAINABLE LIVELIHOOD RESOURCE WITH UNEXPLOITED VALUE

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Abstract

Cyanobacteria are the one evolved before 3.5 billion years ago and they are the first type of bacteria that evolved that could fix atmospheric carbon dioxide into organic carbon compounds using water with the simultaneous evolution of oxygen. *Spirulina platensis* is a fresh water filamentous blue-green algae or Cyanobacterium which has been used as a food supplement since many years. It has been used as a complementary dietary ingredient feed for poultry and increasingly as a protein and vitamin supplement to aqua feeds. *Spirulina* possesses several health-promoting properties including the prevention of hyperglycaemia, alcoholic liver disease, and cancer. Therefore, the main purpose of the present study was to investigation the effects of different processing condition on the most important qualitative features of *Spirulina*. The condensed extracts were used for preliminary screening of phytochemical; estimation of biochemical composition was tested for carbohydrate, protein, and lipids. The compounds Isoquercetin and p-Coumaric acid was tested for Anti-cancerous activity, Ferulic acid and Caffeic acid were tested for Anti-oxidant activity, and Catechin and Apigenin was tested for Anti-inflammatory activity, using molecular docking approach.

Key words: *Spirulina*, phytochemical, biochemical, anti-cancerous, protein, anti-inflammatory and Molecular docking

Introduction

Agriculture is faced with multiple challenges in the 21st century: i) increase in food production for a growing world population, which is expected to increase by about 2.3 billion people over the next 40 years, mostly in upcoming countries, ii) insufficient fresh water supply together with land degradation, which causes losses in agricultural productivity, iii) increase of the production of feed- stock for bio energy, iv) adoption of more efficient and sustainable production methods and v) adaptation to climate change. It is a photosynthetic, filamentous Cyanobacterium, primarily found in salty lakes with alkaline pH and high concentrations of bicarbonate and carbonate, (Odgerel Bumandala, 2023).

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Thus, this multi beneficiary action of makes it an important natural product for the improvement of health of humans and animals. Blue-green algae are the evolutionary bridge between green plants and bacteria. It is rich in proteins (60-70 %), carbohydrates (20–30 %), lipids (5–15 %), and essential vitamins (e.g., B complex vitamins, β-carotene, and vitamin E). It characteristic photosynthetic pigment, phycocyanin (up to 18 %), is what lends it a distinctive blue colour and contributes to its numerous health benefits, including antioxidant, antimicrobial, anti-inflammatory properties. These attributes, along with its ability to provide a true-blue colour to foods and beverages without purple or green undertones, have made *Spirulina* a popular ingredient of dietary supplements.

Docking was performed using Lamarckian Genetic Algorithm with 10 independent runs per ligand with an initial population of 150 randomly placed ligand on the receptor binding site. A maximum of $2.5X10^5$ evaluations on the energy will be carried out for $27X10^3$ generations with a mutation rate of 0.02 and a cross over rate of 0.80. The local-energy-minimization algorithm was limited to 100 steps for 6% of the population. To explore the conformational space of ligands, the overall translation steps was set to 0.2 Å, and the overall rotation and torsion rotation step were set to 5 in the docking studies. The auto dock 4.0 program in ADT was executed and the docking scores were reported using binding free energy energies in kcal/mol.

Materials and methods

Study Area

Coimbatore is a city in Tamil Nadu, South India. It is the second largest city and urban agglomeration in the Indian state of Tamil Nadu after Chennai. It is the capital city in Kongu nadu region and is often been referred to as the Manchester of south India. The city is located on the banks of the Noyyal River surrounded by the Western Ghats and is administered by the Coimbatore municipal.

A B

Fig-1: A. Study Area. B. Location map

Collection of the selected sample

For the present study fresh sample of *Spirulina platensis* is purchased in the month of January, Kovai agro farm at singanallur, Coimbatore to analyse the preliminary phytochemical, biochemical, antioxidant, anticancer, anti-inflammatory, and molecular docking activities. The

collected *Spirulina* sample is undergone with various laboratory scale experiment pertaining to *Spirulina* production it is very important to have a nutrition farm. Sample was washed under running tap water air dried and then stored in airtight bottles in refrigerator.

Systematic Position:

Kingdom : Eubacteria
Phylum : Cyanobacteria
Class : Cyanophyceae
Order : Oscillatorales
Family : Osellatorlaceae
Genus : *Spirulina*
Species : ***Spirulina platensis,* L.,**

A **B** **C**

Fig-2: A) Habit of *S. platensis* B) Dried *S.platensis* C) Powder of *S.platensis*

Plant description

Spirulina platensis, L.*,* is an unbranched, helicoidal, and filamentous blue-green algae or Cyanobacterium that has been found in various aquatic environments. Cultivated worldwide, *Arthrospira* is used as a dietary supplement or whole food. Thus, this multi beneficiary action of Spirulina makes it an important natural product for the improvement of health of humans and animals, (Siva Kiran *et al*., 2016).

Uses:

It is also used as a feed supplement in the aquaculture, aquarium, and poultry industries. It has been used as a complementary dietary ingredient of feed for poultry and increasingly as a protein and vitamin supplement to aqua feeds. This alga contains a diverse concentration of nutrients and has emerged as a wonder drug because of its varied uses.

Preparation of extract

Sample extracts were prepared by Soxhlet extraction method. 30 g of powdered material was uniformly packed into a thimble and extracted with 300 ml of methanol, ethanol, petroleum ether and acetone extract separately. The process of extraction must be continued for 24 hours or till the solvent in siphon tube of extractor become colourless. After that the extract was taken in a

beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC till future uses.

I. Phytochemical analysis, (Rohit Shankar mane *et al.*, 2018)

Test for Alkaloids - 2 ml of concentrated Hydrochloric acid (HCl) was added to 2 ml liquid sample extract. Then few drops Mayer's reagent was added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Terpenoids- 2 ml of chloroform along with concentrated Sulphuric acid were added to 0.5 ml of the liquid sample extract. Formation of reddish-brown colour at the interface indicates the presence of Terpenoids.

Test for Steroids- 2 ml of chloroform and 1 ml of sulphuric acid (H2SO4) were added to 0.5 ml of the liquid sample extract. Formation of reddish-brown ring at interface indicates the presence of steroids.

Test for Tannins-1 ml of ferric chloride (5% FeCl3) was added to 1 ml of the liquid sample extract. Formation of dark blue or greenish black colour indicates the presence of tannins.

Test for Saponins- 2 ml of distilled water was added to 2 ml liquid sample extract and shaken in graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Flavonoids-1 ml of 2N sodium hydroxide (NaOH) was added to 2 ml of liquid sample extract. Formation of yellow colour indicates the presence of flavonoids.

Test for Phenols -2 ml of distilled water followed by few drops of 10 % ferric chloride was added to 1 ml of the Spirulina platensis extract. Formation of blue or green colour indicates the presence of phenols.

Test for Coumarins- 1 ml of 10 % NaOH was added to 1 ml of liquid sample extract. Formation of yellow colour indicates the presence of Coumarins.

Test for Quinones- 1 ml of concentrated sulphuric acid (H2SO4) was added to 1 ml liquid sample extract. Formation of red colour indicates the presence of quinones.

Test for Glycosides-3ml of chloroform and 10% ammonium solution was added to 2 ml of the liquid sample extract. Formation of pink colour indicates the presence of glycosides.

II. Biochemical analysis (Ruma Arora Soni *et al.,* 2021.)

Carbohydrate Estimation

A solution of 1 ml of carbohydrate aliquot is easily blended with 3 ml of concentrated sulphuric acid and 30 s vortexed. The mixture starts boiling in a matter of seconds after adding acid. After removal, the solution was cooled in ice for a short time. Finally, the UV absorption amount at the wavelength of 315 nm is read on the spectrophotometer. Reference solutions are prepared after the same process as above, except that DDI (Distilled De-Ionised) water replaces the carbohydrate aliquot.

Lipid Estimation

The lipid was extracted from harvested algae and 0.3 g of dry biomass was ground to a fine powder in 1.80 ml water. Added to the mixture is a 22.72 ml mixture containing liquid chloroform and a 45.45 ml amount of methanol. Samples were sonicated at 9.9/5.0 s for 25 min on/off pulses.

Then, lysine cells were shaken with a magnetic stirring rod for 2 h followed by mixing and filtering through What man filter paper. The reagent used here was twice extracted as a preparatory step before use. The solution was evaporated in the rotary evaporator at a temperature of 40°C. The dried lipid samples were weighed gravimetrically.

Protein Estimation - Kjeldahl's Method

Nitrogen, N is determined through the Titration Method. Approximately 1 gm of dried *Spirulina* sample was hydrolysed with 15 ml concentrated sulphuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block at 420°C for 2 h. After cooling, H_2O was added to the hydrolysates before neutralization and titration. The calculation of the protein based on the nitrogen content in the food is then carried out. Gel electrophoresis is usually used as a standard for the detection and quantification of proteins. Because the Kjeldahl's method does not measure the amount of protein, conversion factors (F) must be applied to figure out the protein content. A conversion factor of 6.25 is used, which corresponds to 0.16 g nitrogen per gram of protein. The protein content of foods and feeds is determined indirectly by determining the nitrogen content, which requires a nitrogen-to-protein conversion factor (NPCF). 6.25 were historically applied to all proteins based on two assumptions: (1) all proteins contained 16 percent nitrogen (100/16 = 6.25) and (2) all nitrogen was derived from protein. Due to variations in amino acid profiles and non-protein nitrogen, amino acid analyses revealed that a conversion factor of 6.25 overestimated the protein content of most foods.

III. Molecular Docking

The 2D structures of the compounds were retrieved from PubChem (Kim *et al*., 2023) in SMILES format. The Smiles format was translated into 3D structure PDB format using online Smiles Translator tool. The Molecular Weight, Hydrogen Bond Donors, Hydrogen Bond Acceptors and Log P, the Lipinski parameters along with Drug Likeness Score were calculated using Mol soft Online Software that can be accessed at. The receptor for the docking was retrieved from Protein Data Bank (Berman *et al.*, 2000). Molecular Docking of the identified receptor and synthesized ligands was performed using Auto dock Tools (ADT) 1.5.6, (Morris *et al.*, 2009).

Preparation of Receptor and Ligand files

Auto dock entails both the receptor and ligand in PDBQT format for assessing the binding affinity between them. PDBQT format restrains the atomic coordinates, partial charges, and atom types. Initially, the receptor file in PDB format obtained from Protein Databank was accessed in Auto dock Workspace. The water molecules in the receptor file were removed and implicit Hydrogen atoms were added. Finally, partial charges were added and the receptor file was saved in PDBQT format. Similarly, the ligand files in PDB format were retrieved by Auto dock and saved in PDBQT format.

Preparation of Grid and Dock Parameter files

Autogrid 4.2 program in ADT was used to perform the grid computation. The grips maps with a dimension of 60X60X60 and spacing of 0.375 Aº were cantered along the ligand binding site. The receptor and ligand files in PDBQT format along with the grid maps were saved as the

grid parameter file to execute the Autogrid program. After the Autogrid calculation, auto dock parameter file was created with the receptor, ligand, and selection of auto dock parameters.

Docking:

Docking was performed using Lamarckian Genetic Algorithm with 10 independent runs per ligand with an initial population of 150 randomly placed ligand on the receptor binding site. A maximum of $2.5X10^5$ evaluations on the energy will be carried out for $27X10^3$ generations with a mutation rate of 0.02 and a cross over rate of 0.80. The local-energy-minimization algorithm was limited to 100 steps for 6% of the population. To explore the conformational space of ligands, the overall translation steps was set to 0.2 Å, and the overall rotation and torsion rotation step were set to 5 in the docking studies. The auto dock 4.0 program in ADT was executed and the docking scores were reported using binding free energy energies in kcal/mol.

Molecular Interactions Visualization using PyMOL

The bound complex with the receptor and ligand was visualized using PyMOL molecular Visualization Software. PyMOL is a user-sponsored molecular visualization system on an open-source foundation, maintained and distributed by Schrödinger. PyMOL, a cross-platform molecular graphics tool, has been widely used for three-dimensional (3D) visualization of proteins, nucleic acids, small molecules, electron densities, surfaces, and trajectories. It is also capable of editing molecules, ray tracing, and making movies. (Schrodinger and DeLano, 2020).

Results and Discussions

Phytochemical screening of ten different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, Coumarins, quinones and glycosides) were tested in such as, ethanol, extracts of Spirulina platensis. The qualitative phytochemical analysis of ethanol, extracts of *Spirulina platensis* revealed that extract had better activity. Alkaloids, Terpenoids, Steroids, Saponins, Flavonoids, phenols, and Coumarins were present in the extracts of Spirulina platensis. Tannins quinones and glycosides were absent in the ethanolic extract.

Nutritional conditions are a critical factor affecting the growth and productivity of microalgae grown in mass cultures. This investigation was conducted with the primary objective of developing a simple and inexpensive method for lowering the cost of large-scale production. This objective was accomplished by substituting cheaper and locally available commercial fertilizers and chemicals for the nitrogen sources.

Among the nutrients required for algae growth, nitrogen is a critical component that is necessary for microalgae to maintain high production rates. Changes in the source of nitrogen and the number of cultivated media constrain microalgae's intensive growth and alter their pigment and biochemical composition. The present study examined the effect of various concentrations of two nitrogen sources on the productivity of S. platensis. Potassium nitrate and urea were chosen due to their lower cost (e.g., ammonium nitrate, sodium nitrate, or ammonium chloride). Nitrates contain one nitrogen atom (14%–16% nitrogen). Although urea has been recognized as a good source of nitrogen that algae can successfully metabolize. As can be seen from the biochemical composition, sample 2 had higher protein content due to the addition of urea. Additionally, it is used in proximate and elemental analysis. The nitrogen content of sample 2 was higher, while the

moisture and volatile matter content were lower. This clearly demonstrates that sample 2 produced superior results and that the nitrogen content of urea can be used in future studies

Protein, carbohydrate, lipid, and fats were estimated based on biochemical analysis. Based on the systematic evaluation, Spirulina contains protein, lipid, carbohydrates, aliphatic (C–H), carbonyl (esters and enzymes), carbonyl beta-unsaturated ketone amide (C=N), ester, symmetric C–H stretching vibration. The infrared spectral fingerprints of the product determine the significant element of the Spirulina powder as a protein quality based on the assessment of the Spirulina biomass. The various biochemical components of microalgae appear to be decomposed as follows: first, polysaccharides and proteins, then lipids.

From the literature search, it was found that *Spirulina platensis* has compounds such as Isoquercetin, p-Coumaric acid, Catechin, Apigenin, Ferulic acid, Caffeic acid, Gallic acid and Tyrosol. The compounds Isoquercetin and p-Coumaric acid were tested for Anti-Cancer activity, Ferulic acid and Caffeic acid were tested for Anti-Oxidant activity, Catechin and Apigenin were tested for Anti-inflammatory activity, using molecular docking approach.

Table-1 Qualitative analysis of phytochemical present in the ethanol extract of *Spirulina platensis*

S. No	Types of compounds	Ethanolic extract
1.	Alkaloids	++
2.	Terpenoids	++
3.	Steroids	++
4.	Tannins	-
5.	Saponins	++
6.	Flavonoids	++
7.	Phenols	++
8.	Coumarins	+
9.	Quinones	-
10.	Glycosides	-

(++ indicates strongly present, + indicate moderately present, and – indicates absent)

Table- 2 Analysis of biochemical composition

Sample	Carbohydrate	Protein	Lipid
Spirulina platensis	26%	43.7%	1.7%

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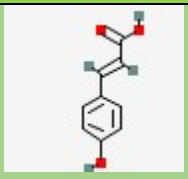
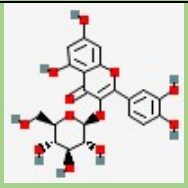
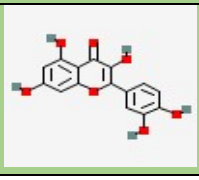
Table- 3 Molecular Docking (Anticancer Activity)

S. No	Compound	Binding energy (KJ/mol)	Ligand efficiency (KJ/mol)	Inhibitory constant	No of hydrogen bonds	Hydrogen bond	Chemical structure
1.	p-Coumaric Acid	-5.65	-0.47	71.76µM	3	His 165:HN::Lig:O His159:HE2::Lig:O Lig:O::Tyr207:O	
2.	Isoquercetin	-5.48	-0.17	96.84µM	5	Lig:H::Glu171:OE2 Lig:H::Asp128:OD1 Lig:H::Glu171:OE2 Asn172:HD21: Lig:O Lys169:HZ1:Lig:O	
3.	Quercetin	-7.18	-0.33	5.5 µM	2	Glu89:OE2:Lig:O Asp186:N::Lig:O	

PIM-1 Kinase is a drug target for Cancer (Tursynbay *et al*., 2016) is a Serine/Threonine Kinase that cellular functions such as Cell Cycle and Cell Survival. It is also a drug target for the Standard for Anti-Cancer Studies, Quercetin and thus the Phyto-compounds were docked against PIM-1 Kinase whose structure in PDB format was retrieved from Protein Data Bank (1xws).

The binding energy between p-Coumaric Acid and PIM-1 Kinase, was -5.65 kcal/mol with a ligand Efficiency of -0.47 kcal/mol. 71.76 µM of p-Coumaric Acid is required to acquire half maximum Inhibition in PIM-1 Kinase protein. There are three Hydrogen bonds formed between the residues His 165, His 159 and Tyr 207 of PIM-1 Kinase and Oxygen atom of the ligand p-Coumaric acid. The binding energy between Isoquercetin and PIM-1 Kinase, was -5.48 kcal/mol with a ligand Efficiency of -0.17 kcal/mol. 96.84 µM of Isoquercetin is required to acquire half

maximum Inhibition in PIM-1 Kinase protein. There are 5 Hydrogen bonds formed between PIM-1 Kinase and Isoquercetin. Glu 171, Asp128, Asn172 and Lys 169 are the residues involved in the Hydrogen bond interaction.

The Docking of the Phytocompounds from *Spirulina Platensis* was compared with the Standard Quercetin and the binding energy between Quercetin and PIM-1 Kinase, was -7.18 kcal/mol with a ligand Efficiency of -0.33 kcal/mol. 5.5 µM of Quercetin is required to acquire half maximum Inhibition in PIM-1 Kinase protein. There are two Hydrogen bonds formed between OE2 of Glutamic acid 89 and Oxygen of Quercetin and another between Nitrogen of Aspartic acid 186 and Oxygen of Quercetin.Among the compounds that were docked, none of them showed better binding affinity with the Drug Target PIM-1 Kinase than the Standard Quercetin. However, they can be used as lead compounds for treating Cancer.

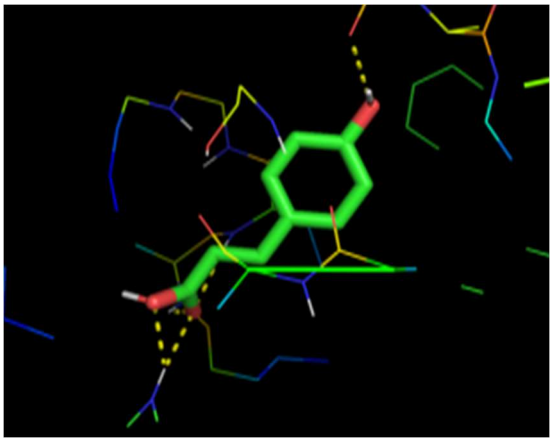
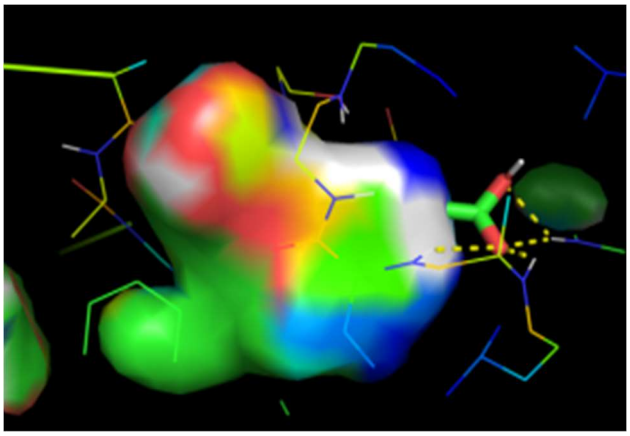
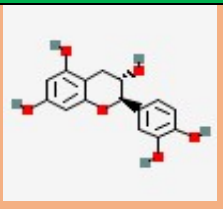
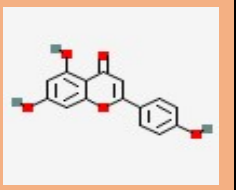
Docked conformation of p-Coumaric acid and PIM-1 Kinase	**Surface representation of Docked conformation between p-Coumaric acid and PIM-1 Kinase**
	

Table 4 Anti-Inflammatory Activity

S. No	Compound	Binding energy (KJ/mol)	Ligand efficiency (KJ/mol)	Inhibitory constant	No.Of hydrogen bonds	Hydrogen bond	Chemical structure
1.	Catechin	-8.53	-0.43	555.13nM	5	Asn537:HD22::Lig:O Val228:HN::Lig:O Lig:O::Gly553:O Arg376:H12::Lig:O Asn375:N::Lig:O	
2.	Apigenin	-7.82	-0.37	1.84 µM	7	Lig:H::Gly533:O Lig:H::Gly533:O Arg376:H::Lig:O Asn537:H::Lig:O Val228:H::Lig:O Asn375:H::Lig:O	

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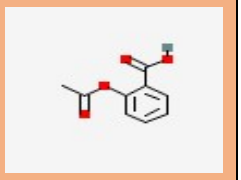
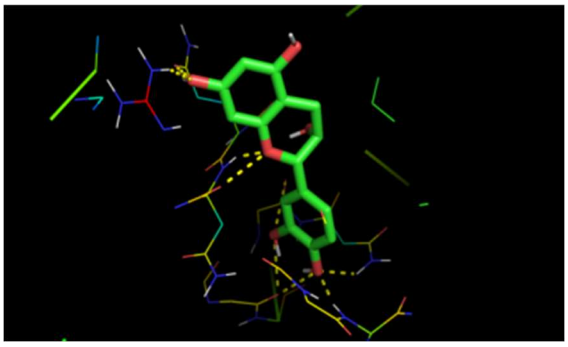
						Lig:H::Gln374:O	
3.	Aspirin	-5.93	-0.46	45.08 µM	4	Val538:NH::Lig:O Gly533:O::Lig:O Val228:NH::Lig:O Asn537:HD22::Lig:O	

Cyclooxygenases play a vital role in inflammation and are responsible for the production of prostaglandins and is the widely used drug target for Anti-Inflammation study (Zhiran et al., 2022). The Drug Target was chosen as Cyclooxygenase and the structure was downloaded from PDB with PDB Id, 5f19.

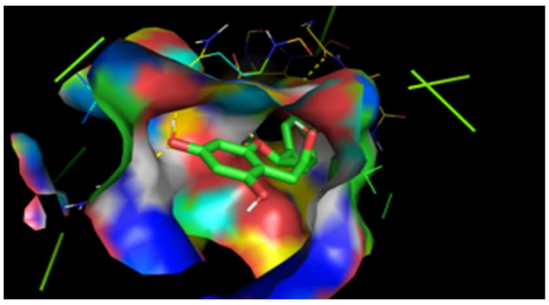
The binding energy between Catechin and Cyclooxygenase, was -8.53 kcal/mol with a ligand Efficiency of -0.45 kcal/mol. 555.13nM of Catechin is required to acquire half maximum Inhibition in Cyclooxygenase. There are 5 Hydrogen bonds formed between Cyclooxygenase and Catechin.

The binding energy between Apigenin and Cyclooxygenase, was -7.82 kcal/mol with a ligand Efficiency of -0.37 kcal/mol. 1.84 µMof Apigenin is required to acquire half maximum Inhibition in Cyclooxygenase. There are 7 Hydrogen bonds formed between Cyclooxygenase and Catechin. The Docking of the Phytocompounds was compared with the Standard Aspirin and the binding energy between Aspirin and Cyclooxygenase, was -5.93 kcal/mol with a ligand Efficiency of -0.46 kcal/mol. 45.08 µM of Aspirin is required to acquire half maximum Inhibition in Cyclooxygenase. There are four Hydrogen bonds formed between Aspirin and Cyclooxygenase. Both the compounds from *Spirulina Platensis* show better affinity with the drug target and can be a better alternative to treat inflammation.

Docked conformation of Catechin and Cyclooxygenase



Surface representation of Docked conformation between Catechin and Cyclooxygenase



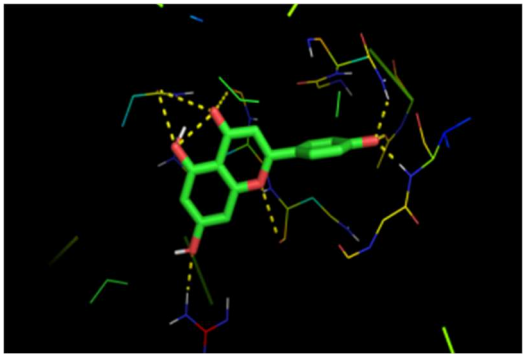
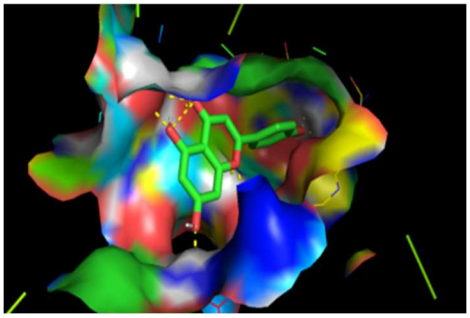
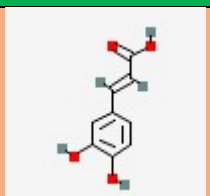
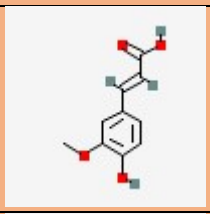
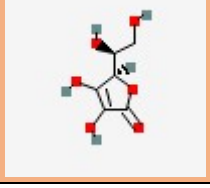
Docked conformation of Apigenin and Cyclooxygenase	Surface representation of Docked conformation between Apigenin and Cyclooxygenase
	

Table 5: ANTI-OXIDANT ACTIVITY

S. No	Compound	Binding energy (KJ/mol)	Ligand efficiency (KJ/mol)	Inhibitory constant	No. Of hydrogen bonds	Hydrogen bond	Chemical structure
1.	Caffeic Acid	-5.34	-0.41	121.41 µM	3	Ser138:H::Lig:O Lys44:H::Lig:O Lig:H::Asp135:O	
2.	Ferulic Acid	-4.99	-0.36	221.2 µM	2	Lig:H::Leu45:O Ser138:H::Lig:O	
3.	Ascorbic acid	-4.64	-0.39	397.52 µM	4	Arg43:O::Lig:O Gly47:NH::Lig:O Ser134:HG::Lig:O Leu45:O::Lig:O	

Potential target for anti-oxidant activity is NADPH oxidase (Nox enzymes), that play a central role because they can regulate other enzymatic sources of ROS (Tomasz and David, 2006). Hence the Drug Target was chosen as NADPH oxidase and the structure was downloaded from PDB with PDB Id, 7u8g.

The binding energy between Caffeic acid and NADPH oxidase, was -5.34 kcal/mol with a ligand Efficiency of -0.41 kcal/mol. 121.41 µMof Caffeic acid is required to acquire half maximum Inhibition in NADPH oxidase. There are 3 Hydrogen bonds formed between Cyclooxygenase and Caffeic acid.

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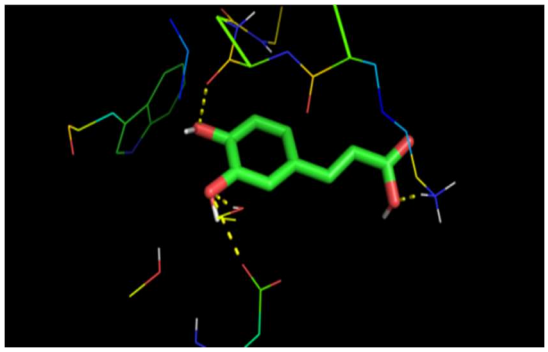
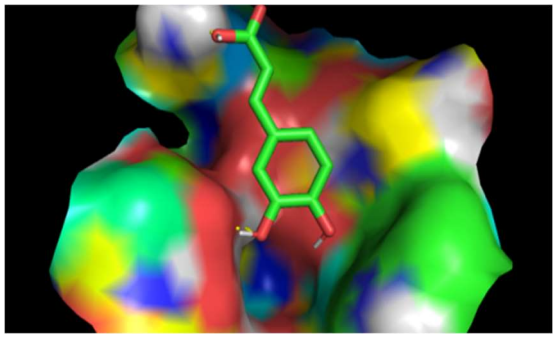
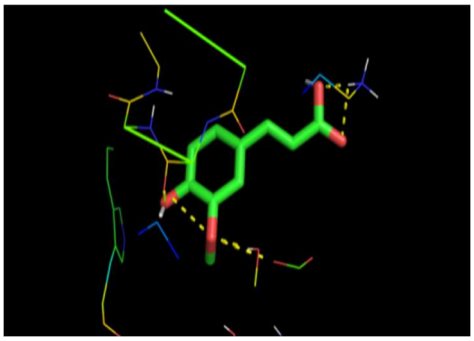
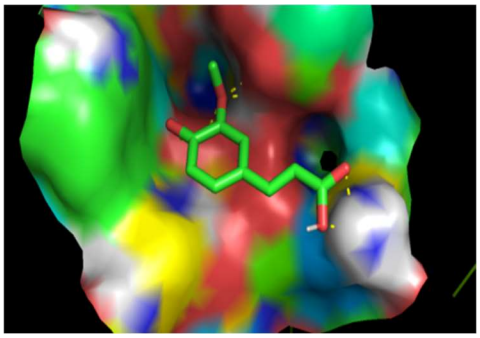
The binding energy between Ferulic acid and NADPH oxidase, was -4.99 kcal/mol with a ligand Efficiency of -0.36 kcal/mol. 221.2 µMof ferulic acid is required to acquire half maximum Inhibition in NADPH oxidase. There are 2 Hydrogen bonds formed between Cyclooxygenase and Caffeic acid.

The Docking of the Phytocompounds was compared with the Standard Ascorbic acid and the binding energy between Ascorbic acid and NADPH oxidase, was -4.64 kcal/mol with a ligand Efficiency of -0.39 kcal/mol. 397.52 µM of Ascorbic acid is required to acquire half maximum Inhibition in NADPH oxidase. There are four Hydrogen bonds formed between O of Arginine 43 and Oxygen of Ascorbic acid and another Hydrogen bond between Glycine 47 and Oxygen atom of Ascorbic acid. The third Hydrogen bond is formed between Serine 134 of NADPH Oxidase and Oxygen of the Standard Ascorbic acid and the last hydrogen bond between Leucine 45 and Oxygen of Ligand.

Both Caffeic acid and ferulic acid show better affinity for the drug target NADPH oxidase and hence can be tested as better alternatives for anti-oxidant activity.

Docked conformation of Caffeic acid and NADPH Oxidase	**Surface representation of Docked conformation between Caffeic acid and NADPH Oxidase**
	

Docked conformation of Ferulic acid and NADPH Oxidase	**Surface representation of Docked conformation between Ferulic acid and NADPH Oxidase**
	

Summary and Conclusion

The various medicine is used at present day for human disease have been commonly originated from the naturally available plants and algae. In addition to its rich mineral content, vitamin and amino acids composition, antiviral and anti-inflammatory action, *Arthrospira platensis* has also a regulatory effect on cholesterol level, oxidative stress, mitochondrial dysfunction, and neurodegenerative disorders.

Phytochemical screening of ten different chemical compounds (alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones and glycosides) were tested in such as, ethanol, extracts of Spirulina platensis. The qualitative phytochemical analysis of ethanol, extracts of Spirulina platensis revealed that extract had better activity. Alkaloids, Terpenoids, Steroids, Saponins Flavonoid, and phenol coumarins were present in the extracts of Spirulina platensis. Tannins quinones and glycosides were absent in the ethanol extract.

The bio chemical study was Protein, carbohydrate, lipid, and were estimated based on biochemical analysis. Based on the systematic evaluation, spirulina contains protein, lipid, carbohydrates, aliphatic (C–H), carbonyl (esters and enzymes), carbonyl beta-unsaturated ketone amide (C=N), ester, symmetric C–H stretching vibration. The infrared spectral fingerprints of the product determine the significant element of the spirulina powder as a protein quality based on the assessment of the spirulina biomass. The various biochemical components of microalgae appear to be decomposed as follows: first, polysaccharides and proteins, then lipids.

There are two Hydrogen bonds formed between OE2 of Glutamic acid 89 and Oxygen of Quercetin and another between Nitrogen of Aspartic acid 186 and Oxygen of Quercetin. Among the compounds that were docked, none of them showed better binding affinity with the Drug Target PIM-1 Kinase than the Standard Quercetin. However, they can be used as lead compounds for treating Cancer.

The Docking of the Phytocompounds was compared with the Standard Aspirin and the binding energy between Aspirin and Cyclooxygenase, was -5.93 kcal/mol with a ligand Efficiency of -0.46 kcal/mol. 45.08 μM of Aspirin is required to acquire half maximum Inhibition in Cyclooxygenase. There are four Hydrogen bonds formed between Aspirin and Cyclooxygenase. Both the compounds from *Spirulina Platensis* show better affinity with the drug target and can be a better alternative to treat inflammation.

The third Hydrogen bond is formed between Serine 134 of NADPH Oxidase and Oxygen of the Standard Ascorbic acid and the last hydrogen bond between Leucine 45 and Oxygen of Ligand. Both Caffeic acid and ferulic acid show better affinity for the drug target NADPH oxidase and hence can be tested as better alternatives for anti-oxidant activity.

It is a good source of protein and many nutrition-like amino acids, vitamins, and minerals. It is a very low-cost production. It will make an evolution in the food industry. Innovative formulations are further needed to fortify conventional foods with *Spirulina* based protein content system. Further efforts should be made to increase of yield and outcome of economy to teach in the villagers and student communities about its value-added products like energy drinks and traditional products. It serves as source of income for the tribal women and will improve their

livelihood. It improves their health conditions. This will provide employability by linking Self Help group of the region and all over the world.

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